THIRD EDITION

FUNDAMENTALS OF Pharmacognosy AND Phytotherapy

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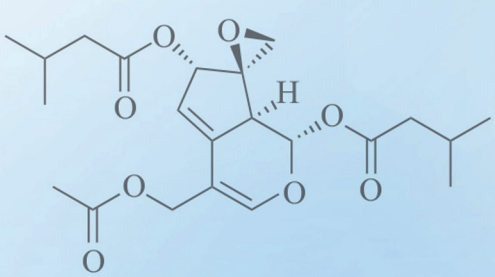
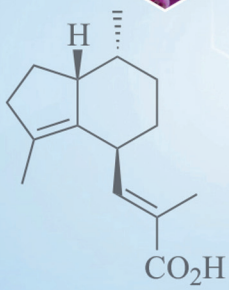

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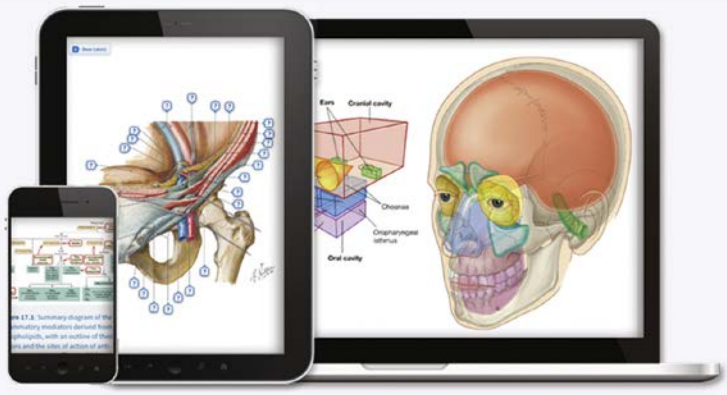

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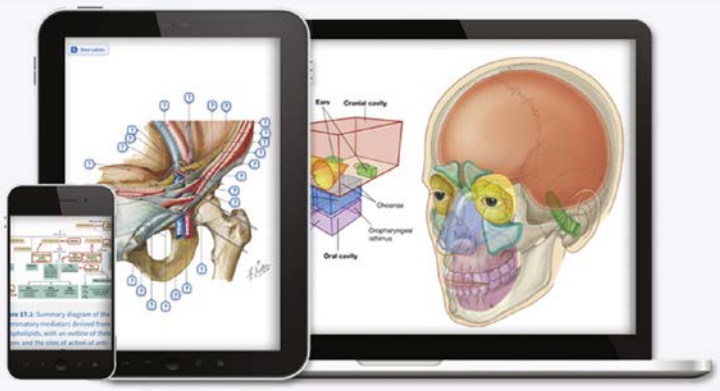

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AND Phytotherapy

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FUNDAMENTALS OF Pharmacognosy AND Phytotherapy

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Dedication

To our families, for their unfailing support throughout the preparation of this book, and to our many colleagues around the world who are interested in medicinal plants/ethnopharmacology, pharmacognosy and phytotherapy.

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Foreword

A. DOUGLAS KINGHORN

Worldwide, drugs derived from organisms continue to be important for the treatment and prevention of many diseases. Pharmacognosy, which is defined in this book as 'the science of biogenic or nature-derived pharmaceuticals and poisons', has been an established pharmaceutical science taught in institutions of pharmacy education for well over a hundred years.

The subject area has changed considerably since its initiation, having metamorphosed from a largely descriptive botanical and mycological field in the late 19th and early 20th centuries, to having much more of a chemical and biological focus within approximately the last 60 years. Today, pharmacognosy embraces the scientific study of mainly small-molecule organic compounds from plants, animals and microbes, of both terrestrial and marine origin. It has been estimated that almost 50% of new drugs introduced into medicine in Western countries over the last 70 years were either obtained directly from an ever more diverse range of organisms, or were otherwise derived from natural products. Even in an age of biotechnology, computer chemistry and refinements in chemical synthesis, there continues to be a steady stream of new natural product-derived drugs approved by the U.S. Food and Drug Administration. Most of the new natural product-derived drugs that have reached the market over the last decade and a half have been obtained from terrestrial microbe sources, but there are several other examples of both higher plant and marine animal origin. In the United States, the first two examples of 'botanical drug products' (sinecatechins from green tea – *Camellia sinensis* (L.) Kuntze and crofelemer from *Croton lechleri* Müll. Arg.) have received official approval within the last 10 years. Natural products continue to be widely utilized as laboratory probes for many different targets to help better understand cellular processes.

Pharmacognosy has evolved relatively recently to include the topics of phytotherapy and nutraceuticals. Also, the teaching of pharmacognosy has become more relevant than previously over the last 20 years, as a result of a substantially increased use of herbal remedies (phytomedicines) by the public, particularly in Europe, North America and Australasia. If the United States is taken as an example, community pharmacists have to deal with the availability of a rather bewildering array of thousands of 'botanical dietary supplement' products, of which many were introduced soon after the passage of the Dietary Supplement Health and Education Act of 1994. There are now many publications in the biomedical literature describing the biological properties and mechanistic parameters of purified constituents of dietary supplements of natural origin. Therefore, societal interest in pharmacognosy is likely to increase in the future as the biochemical role of phytomedicines, nutraceuticals and drugs of natural origin in general becomes more clearly defined.

The third edition of this volume, *Fundamentals of Pharmacognosy and Phytotherapy*, by Michael Heinrich, Joanne Barnes, José Prieto Garcia, Simon Gibbons and Elizabeth Williamson, aims to provide a contemporary perspective of natural product drugs providing an introduction into this field. The book is organized into two major parts, 'Fundamentals of pharmacognosy' (Part A) and 'Important natural products and phytomedicines in medicine and pharmacy' (Part B). Part A is divided into five sections, dealing, in turn, with: the history and importance of pharmacognosy and phytotherapy in pharmacy and medicine; relevant principles of botany and ethnobotany; the chemistry and analysis of secondary metabolites of organisms pertinent to drug therapy; the characterization and standardization of phytomedicines and nutraceuticals; and the use of

medicinal plants in Oriental and South Asian systems of traditional medicine, as well as in Western complementary and alternative medicine. Part B provides coverage of the use of phytomedicines in various therapeutic categories, affecting, respectively: the gastrointestinal system and biliary system; the cardiovascular system; the respiratory system; the central nervous system; infectious diseases; the endocrine system (including effects on diabetes); the reproductive tract; the musculoskeletal system and bones; the skin; the eyes; the ears, nose and orthopharynx; weight loss supplements; and miscellaneous supportive therapies. Some of these categories are new for this third edition of the book.

This comprehensive pharmacognosy textbook integrates very effectively the various traditional elements of pharmacognosy and phytotherapy. The five talented coauthors have been successful in this endeavour in large part because they have contributed their collective and complementary technical expertise in several diverse areas, including ethnobotany and ethnopharmacology, classical botanical pharmacognosy, natural product and analytical chemistry, phytochemistry, phytotherapy and clinical pharmacy.

This new edition may be confidently recommended for purchase by undergraduate and professional doctoral students in pharmacy, as well as beginning graduate students in programmes in the pharmaceutical sciences and related areas. It will also be of great interest for use in continuing education courses for pharmacists, dentists, nurses and physicians. In addition, all those with a scientific interest in herbalism and traditional medicine will find the content of value. The book will moreover serve as a reliable source of information on natural product drugs for the interested lay reader. The previous editions of *Fundamentals of Pharmacognosy and Phytotherapy* have proven to be very highly regarded by readers. This updated and partially reorganized volume will be especially warmly welcomed by educators of future pharmacists and of other healthcare professionals.

Foreword

MARK BLUMENTHAL

Consumer interest in and use of herbs, teas, medicinal plants, phytomedicines, plant-derived ingredients and so-called 'nutraceuticals' has been growing worldwide over the past four decades. Scientific and clinical research on the chemistry, pharmacology, toxicology, and clinical applications of medicinal plants and related products is accelerating at a significant rate. The growth of publications on such research increased almost 700% in a 30-year period from 1977 to 2007, and if such growth in research publications were measured today (2017), the rate of the continued increase might be even higher.

Accordingly, the corresponding need for reliable professional educational materials on these increasingly popular natural products has never been more compelling.

Consumers continue to purchase and utilize herbs and medicinal plant products in growing numbers. They are regulated in a variety of ways, either as a form of food or as various types of medicines: dietary supplements in the US and food supplements in the European Union (EU), so-called Traditional Herbal Medicines in the EU, Natural Health Products in Canada, and other names for a wide spectrum of product formulations that are regulated under a variety of regulatory regimes.

Pharmacognosy is the study of drugs of natural origin, whether they are derived from plants, bacteria, fungi, or animals. Many modern drugs are derived directly from plants – a truism that is commonly known among pharmacists, physicians, researchers, and even many educated consumers. Classic examples include the still-employed cardiac drug digoxin (from the toxic foxglove plant, *Digitalis purpurea* and *D. lanata*), the anti-inflammatory drug colchicine (from the also toxic Mediterranean autumn crocus, *Colchicum autumnale*) and the analgesic and anodyne opiates – codeine and morphine – from the opium poppy (*Papaver somniferum*). The latter (morphine) is the very first plant-based drug (isolated in 1804). In relatively rapid succession, in the early 19th century other plant-derived single-chemical entity drugs were isolated from their plant sources: strychnine (from *Strychnos nux-vomica*), the antimalarial quinine (from the South American cinchona bark, *Cinchona officinalis*), and numerous others, giving rise to the modern pharmaceutical industry.

In recent years there has been some debate among researchers as to whether the search for new pharmaceutical drugs from plants is a worthwhile endeavour. Chemists normally prefer the purity of single-chemical entity (SCE) drugs, whether natural or synthetic, and chemical synthesis has often been the preferred route of discovery, when appropriate, usually due to the lower cost and chemical simplicity of SCEs, i.e. when compared to the chemically complex botanical materials – roots, leaves, barks, flowers, seeds, etc., and their extracts.

Investigations into the long history of the use of medicinal plants in indigenous cultures – the sciences of ethnobotany and ethnopharmacology – has often produced excellent leads for the development of new medicines. Although now dated, the most-cited study of this process was published by the World Health Organization in 1979 in which 119 modern plant-derived drugs were listed (including, of course, all those mentioned above). Not surprisingly, the modern applications of 74% of these drugs correlated directly with the traditional ethnobotanical uses of their source plants.

Lest anyone think that the ethnobotanical approach to drug discovery is an archaic and/or futile endeavour, one only need review the blockbuster news in

the medical plant community in 2015 that Chinese researchers were granted the Nobel Prize in Physiology or Medicine for discovery of the highly successful antimalarial drug artemisinin, derived from the traditional Chinese herb *quinghao* (*Artemisia annua*, sometimes referred to as sweet wormwood). Traditional uses of this plant for reducing intermittent fevers led to its being investigated by Chinese scientists in the 1970s when seeking leads for the development of a new antimalarial drug.

Further interest is generated by the recent development and official United States government approval of two new plant-derived chemically complex medicines, a tannin-rich extract of green tea (*Camellia sinensis*) in an ointment for the topical treatment of human papilloma virus and an oligomeric proanthocyanidin-rich extract of the exudate of the bark of a South American tree sangre de drago (*Croton lechlerii*, dragon's blood) for symptomatic relief of diarrhoea in HIV/AIDS patients on antiretroviral therapy. Approval of new chemically complex plant-derived medicines is probably inevitable and may constitute a new era in pharmacy and medicine.

Medicinal plants have played and will continue to play a significant role in the development of modern medicines, and will continue to be used by the public as self-selected medicines and supplements. Correspondingly, a growing number of licensed health professionals also recommend or prescribe medicinal plant-based supplements and related natural products.

Of particular interest is the increased consumer acceptance and use of herbs and medicinal plant products in industrialized nations. In the U S market, retail sales of herbs and other plant-based dietary supplements in 2015 reached almost US$7 billion – a new record for sales in the United States. These sales data do not reflect similar growth in other countries. This does not reflect the additional sales volume related to the use of herbal teas or the growing use of botanical ingredients in cosmetics and, more recently, in conventional foods, e.g. nutrition bars.

What should be immediately clear from examining a list of top-selling herbs (at least as it represents consumer demand in the United States) is the presence of many botanicals that have a long history of food use, either as spices and food flavourings and/or as conventional foods.

Popular spices such as turmeric root and rhizome (*Curcuma longa*), the top-selling supplement in the United States in the natural food store channel for the past 4 years, garlic (*Allium sativum*), ginger (*Zingiber officinale*), and red pepper (*Capsicum annuum*) are used by consumers for a variety of traditional and clinically documented health benefits. Conventional foods, such as cranberry (*Vaccinium macrocarpon*), bilberry (*Vaccinium myrtillus*, an anthocyanin-rich type of blueberry), pomegranate (*Punica granatum*), soy (*Glycine max*), and/or their extracts have gained significant popularity as dietary supplements and phytomedicines in various countries, again based on the growing amount of clinical research demonstrating their obvious safety, as well as their activity/efficacy in published clinical trials.

This brings a relatively new spectrum of plants to the pharmacognosist for study of their chemistry, pharmacology, toxicology, and various ways that these substances in raw, dried, powdered form, or as extracts (and essential oils) can be formulated for optimal consumption by a growing consumer base interested in safe, effective, reliable natural products.

With the increased acceptance of the health-promoting benefits of many food items, one can forecast that future pharmacognosy educational materials will recognize the field of nutritional biochemistry as an equally important area of research. The growing trend of 'food as medicine' is rife with opportunities for pharmacists, physicians, and dietitians/nutritionists to share vitally important information for the benefit of health consumers.

While consumer demand for natural health products continues to grow and the market responds with an almost dizzying array of natural products, one of the most important recent developments is the growing concern of botanical experts regarding the quality and identity of botanical raw materials, extracts, and essential oils.

Quality issues related to herbal ingredients have come under increased scrutiny due to increased reports of problems related to the first issue noted above, i.e. the adulteration of the raw materials, extracts, or essential oils with undisclosed lower-cost ingredients. While adulteration with another plant can be a result of accidental misidentification of the plant in the field or in processing and/or inadequate training of harvesting and processing personnel, reliable reports continue to confirm the highly lamentable practice of intentional adulteration of botanical materials used as ingredients in consumer products meant for health effects, e.g. dietary/food supplements. Adulterated botanical materials are also found in cosmetic products and other items for topical use.

There are generally two areas of concern when it comes to medicinal plant quality: (1) identity and authenticity, and (2) purity. With respect to the former,

the first priority in any effective quality-control system for herbs and medicinal plants is ensuring the proper identity of the raw plant material, whether it is to be used as simply raw material for a consumer product (e.g. a herbal tea or capsule), or whether it is to be used as the starting material for the creation of a botanical extract (made with one or more solvents) or distilled into an essential oil. The second priority of purity deals with ensuring that the raw material does not contain any excessive levels of other plant materials (pharmacopeias usually allow up to 2% of foreign organic material in herbal starting materials used for medicinal purposes), excessive levels of pesticides or other agricultural chemicals or excessive levels of heavy metals (often derived from soil or water), and that there is no microbial contamination.

Adulteration of foods, spices, botanical drugs, and medicinal products is not a recent phenomenon. Recent publications document the problem of substitution with undisclosed lower-cost materials and/or dilution with such ingredients going as far back as 2000 years, and probably even earlier. It was for this reason that the first pharmacopeia was initiated in the late 15th century – as a means to compile methods to ensure the proper botanical identity of herbal drugs and to detect known adulterants.

As a section of this book demonstrates, botanical authentication is a necessary first step in any robust and effective quality-control system used in the various areas of the supply chain and manufacturing in the botanical industry. Plant species identification and authentication is conducted using various techniques – macroscopic and organoleptic, microscopic, chemical and genetic. Industry and nonprofit groups continually offer training programmes on these techniques in order to serve the increasingly specialized quality-control needs of the global botanical industry. Accordingly, there is a growing need in the burgeoning botanical industry for well-qualified technicians and scientists with adequate training in medicinal plant identity, authentication, and other means of appropriate qualification, e.g. botanists, pharmacognosists, natural product chemists, and others. This textbook presents as an excellent reference contributing to such education and training.

While earlier pharmacognosy texts tended to be organized on a phytochemical basis (e.g. alkaloids, glycosides, tannins, etc.), this text takes the rational approach of organizing the content based on a therapeutic and clinical basis – physiological systems upon which the natural medicines have their primary actions and uses (e.g. cardiovascular system, nervous system, digestive system, etc.). The authors, all experts in pharmacognosy, also provide essential and rational information for the appropriate use of medicinal plants in therapy, especially as practiced by licensed health professionals.

This book, the third edition of a work that has gained significant prominence and respect as a leading textbook, contains highly valuable and authoritative information for anyone who is a student of pharmacy and/or pharmacognosy. It is also a valuable reference for industry quality-control personnel, herbalists, natural product researchers, and others in the health professionals who wish to learn about the growing popularity of herbs and medicinal plants and how natural botanical preparations can provide a growing range of safe and effective health benefits for consumers worldwide.

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Preface

In the last few decades, pharmacognosy (the study of drugs of natural origin) as an academic discipline, and its application in healthcare, has changed remarkably but still focuses on the quality of products and the development of new medicines Anagnostou and Heinrich, 2017. There has been a revival of interest in natural products as sources of new drugs, phytotherapy and herbal medicines highlighting the responsibilities of healthcare professionals. This has created the need to provide teaching and learning in these topics for students of pharmacy, medicine, nursing and other health professions, including medical herbalism. Knowledge about plant-derived medicinal products is essential in all areas of healthcare, not only because these forms of treatment are a popular healthcare choice (often used as self-medication) but because of the importance of them in many traditional medical systems globally. This text aims to provide a *contemporary, therapeutics-oriented perspective on plants as medicines*, as well as a general overview of the topic that non-specialist readers will find interesting and useful. This book is not a guide to treatment, rather, it is a textbook presenting the scientific principles and summarizing the traditional, preclinical and clinical evidence underpinning the use of herbal- and other plant-derived medicines. The content has arisen, in part, from new lecture courses developed by the authors, as well as from developments in the analysis, evidence-based practice and regulation of herbal medicines. The book covers all fundamental aspects of pharmacognosy, as well as topics relating to the therapeutic use of plant drugs, known as phytotherapy. The text is unique in covering the subject of medicinal plants as an important element of contemporary healthcare, an approach that reflects the current public interest in it.

The book combines sections on the scientific study of plant drugs – phytochemistry, ethnopharmacy and botany – with descriptions of traditional medicine systems, such as Western medical herbalism, traditional Chinese medicine (TCM), and Ayurvedic medicine. For this edition, a new chapter covering the analysis of complex plant mixtures has been added (phytochemical analysis) and summaries of Australian Aboriginal medicine, Rongoā Māori (Aotearoa New Zealand), Kampo (Japan), Unani (India, Pakistan, Middle East), Jamu (Indonesia, Malaysia) and the traditional medicine of the Americas, have also been included. This new material is introduced and supported by a new overview of medicinal plants in healthcare systems. As in previous editions, a separate chapter covers 'integrative' or 'complementary/alternative' therapies, such as aromatherapy, homoeopathy and others, if it involves the use of plant-derived substances.

The fully revised and updated Part B continues to provide a comprehensive summary of the use of and evidence for herbal medicines and pure natural substances in different medical conditions, organized into therapeutic categories based on the British National Formulary (BNF) classification. Numerous medicinal plants and a new section on superfoods have been added. Natural product-derived drugs include those produced by biotechnology and from animal and microbial sources; these are vast and important subjects, so comprehensive coverage was not possible in this text.

Our intention is to equip the reader with the information and knowledge necessary to understand the role of natural products in the drug discovery process, and to enable the assessment of potential benefits and harms of plant-based medicines when advising patients who wish to use them. We aim to provide a

strong foundation in the scientific principles of plant-derived drugs, focusing on their chemistry, with chemical structures where necessary and appropriate.

In this new edition, we have increased the references and further reading material in each section, so the reader can delve further into the subject, and consult the original work from which our information was taken. We thank all those who have contributed advice, suggestions and support, including our colleagues, both present and former, and the wider Pharmacognosiaceae. Finally, from our plant family to our own families, as ever, our deepest and warmest thanks are for you.

Last, but not least, *Dr José Prieto Garcia* has joined the team of authors.

Michael Heinrich
Joanne Barnes
José M. Prieto Garcia
Simon Gibbons
Elizabeth M. Williamson

Reference

Anagnostou, S., Heinrich, M., 2017. Pharmacognosy – from Pharmacographica to DNA-based techniques'? Planta Medica. doi.org/10.1055/s-0043–108999.

PART A

Fundamentals of pharmacognosy

Why are plants and their extracts still important in pharmacy and medicine? In 2015, the scientific importance of pharmacognosy and natural product research was highlighted globally. Prof. Youyou Tu (* 1930, China) was awarded the Nobel Prize in Physiology or Medicine '*for her discoveries concerning a novel therapy against malaria*' and more specifically for the discovery and development of artemisinin, one of the most important antimalarial drugs we have today (Tu 2016) and to Prof. William C. Campbell (* 1930, Northern Ireland / USA) and Prof. Satoshi Ōmura (* 1935, Japan) '*for their discoveries concerning a novel therapy against infections caused by roundworm parasites*'. These two scientists led the development of avermectins, fungal metabolites from *Streptomyces avermitilis* and their derivatives (Campbell 1991, Ōmura and Shiomi 2007).

Historically, plants have yielded some of our most important drugs, but, with the great advances in medicinal chemistry of the last century, in many drug discovery programmes synthetic drugs superseded them as the main focus of research. However, the development of drugs using natural products as 'lead' molecules continues, and many plant-derived pure compounds (or **natural products**) are used in modern, conventional medicine; other compounds are potentially useful to humans or are of toxicological relevance.

There has also been a huge rise in the use of phytopharmaceuticals and herbal medicines in recent years, especially in North America, Europe and Australasia. Traditional medicine, which uses many plant remedies, remains an important (and in some cases, the only) form of treatment in many developing countries but is increasing throughout the world. People in many countries now want to cure minor health problems with something 'natural' and ageing populations have an increasing demand for medicines and foods ('nutraceuticals') to help combat the symptoms and problems of ageing. This public demand is an enormous challenge for all health professionals, many of whom have little specialist knowledge of natural medicines.

This book is divided into two parts. The first part addresses the concepts that help in the understanding of the pharmacognostical basis of such medical products, including pharmaceutical, pharmacological, toxicological and phytochemical aspects. The second part is devoted to important plant-derived medicines, which are arranged in therapeutic categories.

Part A is devoted to the *basic scientific principles* underlying the use of medicinal plants, and the extracts and pure compounds (sometimes referred to as 'natural products') derived from them. This part is selective, and highlights those aspects most relevant to everyday practice. In the first chapter, a general introduction to the scientific field of pharmacognosy, and one of its main applications, phytotherapy, is given. Chapter 2 provides an overview of the historical development of plants in pharmacy and medicine, showing how the modern use of medicinal plants has evolved. In Chapters 3–5 the botanical basis of the discipline is summarized, covering classification and the use of plants by people with little or no access to modern medicine – known as ethnobotany and ethnopharmacy. Chapters 6–8 deal mainly with phytochemistry. Here, the types of compounds that may be present are discussed, together with their isolation and identification, using chromatographic and spectroscopic techniques. In Chapter 9 a very short overview of the process is given, from agricultural production or collection from the wild, to the processing of the pharmaceutical product or health-food supplement. Phytomedicines have particular attributes not encountered using synthetic drugs or single

compounds, in that the botanical drug or an extract derived from it may be combined with other herbal drugs or extracts. This may involve synergistic and other interactions; this is discussed in Chapter 10. Some ancient written traditions, such as Chinese and Ayurvedic medicine, have been passed on for centuries, and their practical use and philosophical basis are presented in Chapter 11. Chapter 12 looks at the complementary and alternative therapies that are currently popular in Europe, Australasia and North America. These are non-science-based approaches to healing, to which pharmacists and members of the medical professions are now being more frequently exposed. More detailed information on these topics can be found in the further reading sections of each chapter. Due to lack of space, there is no section on biotechnology (e.g. fermentation and tissue culture), which is a more specialized area and of less relevance to practising pharmacists and medical doctors.

Section 1

Phytotherapy and pharmacognosy

SECTION CONTENTS

Chapter 1

Importance of plants in modern pharmacy and medicine

AIMS AND DEFINITIONS

This introductory textbook aims to provide a scientific basis for the use of plants in pharmacy (pharmacognosy) and also to describe the main characteristics of herbal medicines (herbal medicinal products, herbal remedies, phytomedicines) and their clinical use [herbal medicine (UK), Phytotherapy (Continental Europe)]. There is also an overview of some historical aspects of medicinal plants use by different societies (ethnobotany, ethnopharmacology) and on the role of plants in a variety of popular 'non-scientific' medical systems (traditional medicine).

Pharmacognosy (derived from *pharmakon*, 'remedy', and *gignosco*, 'knowledge') is the science of biogenic or nature-derived pharmaceuticals and poisons. It deals with all medicinal plants, including those yielding complex mixtures, which are used in the form of crude herbs (comminuted herbal substance) or extracts (phytotherapy), pure compounds such as morphine, and foods having additional health benefits only in the context of having preventive effects (**nutraceuticals**).

TYPES OF DRUGS DERIVED FROM PLANTS

HERBAL DRUGS, DERIVED FROM SPECIFIC PARTS OF A MEDICINAL PLANT

Botanical drugs that form the basis for herbal remedies or phytomedicines include, for example:

- The aerial parts of St John's wort (*Hypericum perforatum*), used in the treatment of mild to moderate depression.
- The leaves of *Ginkgo biloba*, used for cognitive deficiencies (often in the elderly), including impairment of memory and affective symptoms such as anxiety.
- The flower heads of chamomile (*Chamomilla recutita*), used for mild gastrointestinal complaints and as an anti-inflammatory agent.
- The leaves and pods of senna (*Cassia* spp.), used for constipation.

From the perspective of pharmacognosy and rational phytotherapy, such products lie alongside, and in some cases are, conventional pharmaceutical medicines. Herbal medicines are often considered to be part of complementary and alternative medicine (CAM), and the use of herbal medicinal products (HMPs) has increased alongside the increasing utilization of CAM across the developed world.

NATURAL PRODUCTS OR COMPOUNDS ISOLATED FROM NATURE

These are pure chemical entities, often used in the form of licensed medicines. They are sometimes produced synthetically and referred to as 'nature identical' (if that is the case), but were originally discovered from plant drugs. Examples include:

- Morphine, from opium poppy (*Papaver somniferum*), used as an analgesic.
- Digoxin and other digitalis glycosides, from foxglove (*Digitalis* spp.), used to treat heart failure.
- Taxol, from the Pacific yew (*Taxus brevifolia*), used as an anticancer treatment.
- Quinine, from *Cinchona* bark (*Cinchona* spp.), used in the treatment of malaria.
- Galanthamine from *Galanthus* and *Leucojum* species, used in the management of cognitive disorders.

NUTRACEUTICALS OR 'FUNCTIONAL FOODS'

Many foods are known to have beneficial effects on health. Examples include:

- Garlic, ginger, turmeric and many other herbs and spices.
- Anthocyanin- or flavonoid-containing plants such as bilberries, cocoa and red wine.
- Carotenoid-containing plants such as tomatoes, carrots and many other vegetables.

USE OF HERBAL MEDICINES

Globally, the WHO (World Health Organization) now advocates universal health coverage and the integration of safe and effective traditional providers and complementary services into self-care practices and health service delivery, with a particular focus on herbal medicines. The use of these remedies is extensive, increasing and complex. In several surveys 20–33% of the UK's population claimed to regularly use complementary and alternative medicine (CAM) alone or in addition to orthodox or conventional medicine and treatments. In the UK, usage is particularly frequent amongst those who are over-the-counter-medicines users. There is not, on the whole, a wide understanding of what herbal medicines are (or are not) (IPSOS-MORI 2008). Healthcare professionals and students also commonly use such products. Forty-three percent of students at a University School of Pharmacy reported using at least one type of CAM during the last 12 months (Freymann et al 2006).

In the United States, approximately 38% of adults and approximately 12% of children are using some form of CAM (NIH/NCCAM). Kennedy et al (2008) showed that in the preceding 12 months about 38 million adults in the United States (18.9% of the population) used herbal medicines or supplements, but that only one-third revealed this use to their physician. Data for other regions are even more limited, but the usage of herbal medicines is widespread in countries like India, Indonesia, Australia and China, to name just a few.

In addition, market research data reveal high levels of expenditure on herbal medicines, although it is difficult to obtain precise figures for sales of such products since some are classed as food supplements and are sold through numerous outlets. For similar reasons, it is usually not possible to compare properly the estimates for expenditure on herbal medicines using different studies and in different countries. For 2009, it was estimated that the total value of the global market of herbal medicines was around $83 billion. In 2009 in the United States alone, consumers spent a total estimated $5 billion on herbal dietary supplements. In the UK in 2007, the market for herbal medicines was estimated to be almost £700 million, which, compared with many other European countries, is rather low. The European market for herbal supplements and herbal medicines is currently worth about $7.4 billion. Germany is the largest European market, with a 27% share, followed by France (24%), Italy (12%) and the UK (9%). The Indian healthcare market is valued at $7.3 billion, 60% of which is controlled by pharmaceutical drug manufacturers, while 30% is controlled by Ayurvedic medicine manufacturers, and the Chinese market is worth around $8 billion (Gruenwald 2008).

In most continental European countries, such phytomedicines are licensed medicinal products and are used under medical supervision. However, the widespread use of herbal medicines by the general public raises several important issues. Some of these relate to how individuals, whether consumers or healthcare professionals, perceive and use these preparations; other concerns relate to the quality, safety and efficacy of the herbal medicines themselves.

As part of the primary healthcare team, pharmacists, as well as nurses and general practitioners, need to be competent in advising consumers on the safe, effective and appropriate use of all medicines, including herbal medicines. Healthcare professionals also need to be aware that patients may be using products and making healthcare choices without their knowledge.

There are many reasons for the increased use of herbal medicines. These may range from the appeal of products from 'nature' and the perception that such products are 'safe' (or at least 'safer' than conventional medicines, which are often derogatorily referred to as 'drugs'), to more complex reasons related to the philosophical views and religious beliefs of individuals.

In developed countries, most purchases of HMPs are made on a self-selection basis from pharmacies and health-food stores, as well as from supermarkets, by mail order and via the Internet. **Normally, with the exception of pharmacists, there is no requirement for a trained healthcare professional to be available on the premises to provide information and advice** and, in any case, most HMPs can be sold or supplied without the involvement of a healthcare professional. Several studies have confirmed that many individuals do not seek professional advice before purchasing or using such products, even when purchased from a pharmacy (Barnes et al 1998, Gulian et al 2002). Rather, consumers of HMPs

tend to rely on their own (usually limited) knowledge, or are guided by advice from friends and relatives or the popular media. Consumers who do seek professional advice (e.g. from their pharmacist or general practitioner) may find that they are not able to have their question(s) answered fully. In some cases this may be because the information simply is not available, but it is also recognized that, at present, many healthcare professionals are not adequately informed about herbal medicines, particularly with regard to their quality, safety and efficacy. This book attempts to redress that omission.

Herbal medicines are used for general health maintenance, as well as for treating disease and this includes serious conditions such as cancer, HIV/AIDS, multiple sclerosis, asthma, rheumatoid arthritis and osteoarthritis. Older patients, pregnant and breastfeeding women, and children also take HMPs, and this raises concerns because, as with conventional medicines, precautions need to be taken. For example, few medicines (whether conventional or herbal) have been established as safe for use during pregnancy and it is generally accepted that no medicine should be taken during pregnancy unless the benefit to the mother or foetus outweighs any possible risk to the foetus. Similarly, HMPs should be used with caution in children and the elderly, who, as with conventional medicines, differ from adults in their response, metabolism and clearance of drugs. The use of herbal medicines by patients who are already taking prescribed medicines is of particular concern as there is the potential for drug–herb interactions to occur. For example, important pharmacokinetic and pharmacodynamic interactions between St John's wort (*Hypericum perforatum* L.) and certain conventional medicines have been documented (Williamson et al 2009) and mechanisms for such interactions have been identified. Generally, information on interactions between HMPs and conventional medicines is limited, although potential drug–herb interactions can sometimes be identified based on the known phytochemistry and pharmacological properties of the herbs involved.

These issues illustrate once more the need for healthcare professionals, and especially pharmacists, to be knowledgeable about HMPs, and professional bodies are increasingly mindful of their responsibilities regarding herbal medicines and have taken steps to address the issue. It is now recognized by the UK Committee on Safety of Medicines and the Medicines and Healthcare Products Regulatory Agency that pharmacists have an important role to play in pharmacovigilance regarding HMPs; this involves reporting suspected adverse reactions and disseminating information to patients and the public about safety concerns. Calls for healthcare professionals to be competent with regard to herbal medicines and other 'complementary' therapies are now coming from outside the professions.

In summary, the use of herbal products continues to be a popular healthcare choice among patients and the general public. Most pharmacies sell herbal medicines and it is likely that pharmacists will be asked for advice on such products or that they will have to consider other implications of herbal product use, such as interactions with conventional medicines. This book provides the scientific background to the use of plants as medicines.

SOME FUNDAMENTAL ASPECTS OF THE REGULATION OF HERBAL MEDICINES*

The regulation of herbal medicines is complex, varies greatly and is constantly changing. These diverse regulatory frameworks form an essential basis for the activities of all healthcare professionals and for research on such products. For example, in the UK, until recently *Ginkgo biloba* was considered a food and now is, as in other European countries, regulated as a traditional herbal medical product. It is a herbal medical product in Germany and a food supplement in the USA. In the UK echinacea may be a traditional herbal medical product or a food supplement or a registered medicine. It is classed as a dietary supplement in the USA and in general as a medicine in Germany. Therefore a brief and selective overview of the regulation of herbal medicines in key English-speaking countries is given here, excluding the regulation of the professions involved in their production, prescription and dispensing. We also include a short overview of the regulation of traditional Chinese medicines in the three key political entities where they are used widely: Hong Kong, People's Republic of China and Taiwan.

UNITED KINGDOM

In essence today's regulatory framework in the UK is very similar to those in other countries of the European Union (based on the regulation as of 2017). Historically, in the UK, many of the concerns regarding herbal medicinal products have arisen from the lack of regulation of such products. Consequently, such products lacked

*Acknowledgement: We gratefully acknowledge the input from many colleagues and their teams who provided information on the regulatory situation in individual countries: Eric Brand (TCM), Pulok Mukherjee (India), Hans Wohlmuth (Australia), Johannes v. Staden (South Africa) and Udoamaka Ezuruike (Nigeria).

evidence for acceptable standards for quality, safety and efficacy. A range of safety problems arose as a result of the use of unlicensed herbal preparations of inadequate pharmaceutical quality.

The basis for the current regulatory regime for the licensing of medicines in the UK is set out by the 1968 Medicines Act and other regulations that have arisen from the implementation of relevant European Commission legislation, namely Directive 65/65/EEC, now revised as Directive 2001/83/EC. Under this legislation, which has been in place since 2004, manufacturers of products, including herbal remedies, which are classed as medicinal products must hold a marketing authorization (MA, or product licence, PL) for that product unless it satisfies the criteria for exemption from the requirement for a MA. In essence, medicinal products are defined as those that are medicinal by presentation *or* (not *and*) by function. Manufacturers of products comprising new chemical entities, including isolated constituents from plant or other natural sources, are required to submit applications for MAs for those products, based on the full dossier of chemical, pharmaceutical, pharmacological, toxicological and clinical data.

Herbal products are available on the UK market as:

- Licensed herbal medicines.
- Traditional herbal medical products registered under the European 'Traditional Herbal Medicinal Product Directive' (THMPD)
- Herbal medicines exempt from licensing.
- Unlicensed herbal products, sold as food or dietary supplements.
- Prescription-only medicines (POM); these potentially hazardous plants may only be dispensed by order of a prescription by a registered doctor.
- Pharmacy-only medicines (P); certain others may only be supplied by a registered pharmacist, or may be subject to dose (but not duration of treatment) and route of administration restrictions.

Licensed herbal medicines

Most licensed herbal products in the UK were initially granted a product licence of right (PLR) because they were already on the market when the licensing system was introduced in the 1970s. When PLRs were reviewed, manufacturers of herbal products intended for use in minor self-limiting conditions were permitted to rely on bibliographic evidence to support efficacy and safety, rather than being required to carry out new controlled clinical trials. So, many licensed herbal medicinal products have not necessarily undergone stringent testing. The 'well-established use' directive (99/83/EC) was intended to allow greater flexibility on the use of bibliographic data to demonstrate safety and efficacy, and it was hoped that this legislation would provide a regulatory framework for the many unlicensed herbal products on the market. Unfortunately, interpretations of the provisions of the directive vary between EU member states and this directive is not widely accepted in the UK.

Traditional herbal medical products

The Traditional Herbal Medicinal Products Directive (THMPD) 2004/24/EC (see http://www.mhra.gov.uk) is a regulatory process established to provide a mechanism whereby manufacturers of good-quality herbal medicines can register their products as medicinal products, and this allows them to make (restricted) medicinal claims on the packaging and the patient information leaflet (PIL):

- Evidence that a corresponding herbal product (i.e. one derived from the same botanical drug and prepared in a similar way) has been used traditionally for at least 30 years (15 years non-EU and 15 years in the EU, or more than 30 years in the EU).
- Bibliographic data on safety with an expert report.
- A quality dossier specifying how the company complies with the quality guidance requirements of the regulators.
- Details on the PIL, packaging, naming and labelling.

Products registered under this directive can only be used for minor, self-limiting conditions. Overall, it provides an assurance that the patient is receiving not only a good-quality product, but also more reliable advice on its use.

Unlicensed herbal medicines

HMPs still exempt from licensing are those 'compounded and supplied by herbalists on their own recommendation' [specified under Section 12(1) of the Medicines Act 1968] and those consisting solely of dried, crushed or comminuted (fragmented) plants. They must not contain any non-herbal 'active' ingredients and are sold under their botanical name and with no written recommendations for use [specified under Section 12(2) of the Medicines Act]. The exemptions

were initially intended to give herbalists the flexibility to prepare remedies for their patients, although, at present, there is no statutory regulation of herbalists in the UK (this is under review).

Traditional medicines used by ethnic groups include traditional Chinese medicines (TCM) and Ayurvedic medicines. These products are subject to the same legislation as 'Western' herbal medicines. Some toxic species, like *Aristolochia* sp., are banned. Adulteration and poor-quality problems are, therefore, generally limited to unregulated products (mostly supplements).

There still remain problems associated with some imported medicines: in addition to containing non-herbal ingredients such as animal parts and/or minerals, some manufactured ('patent') 'herbal' products have been found to contain conventional drugs that may have POM status in the UK (e.g. dexamethasone and glibenclamide) or that are banned. Non-herbal active ingredients of any type (chemically synthesized, animal products) cannot legally be included in herbal remedies, and inclusion of drugs with POM status represents an additional infringement of European and US legislation. Some ingredients, such as certain species of plants, are also restricted under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

AUSTRALIA

In Australia, Western herbal medicine is one of the most popular forms of CAM and a range of ethnic medicines, especially TCM, are increasingly becoming popular. All medicines, including herbal and other complementary medicines, are covered by the Therapeutic Goods Act (1989) and regulated by a federal agency, the Therapeutic Goods Administration (TGA, http://www.tga.gov.au/). A statutory expert committee, the Advisory Committee on Complementary Medicines (formerly the Complementary Medicines Evaluation Committee) provides the TGA with advice on the regulation of complementary medicines. The Australian regulatory guidelines for complementary medicines (http://www.tga.gov.au/docs/html/argcm.htm) provide information to help producers and distributors of complementary medicines to meet their obligations under therapeutic goods legislation.

Australia adopts a two-tiered, risk-based approach to the regulation of all medicines. Low-risk medicines, including most herbal and complementary medicines, are included in the Australian Register of Therapeutic Goods (ARTG) as listed medicines and identified by a unique AUST L number of the label. Medicines deemed to be of higher risk are entered on the register as registered medicines and identified by an AUST R number. While registered medicines undergo full pre-market safety and efficacy evaluation, listed medicines are not evaluated for efficacy, but product sponsors must hold evidence to support the claims they make about the product. Random and targeted post-market audits of this evidence are carried out by the regulator. Indications and claims for listed medicines are limited to health maintenance, health enhancement or non-serious, self-limiting conditions and may be supported by evidence from traditional use or scientific evidence. Both listed and registered medicines must be made to pharmaceutical good manufacturing practice (GMP) standards, and herbal ingredients must conform with the relevant BP (also EurPhar) monograph, if one exists.

Medicines extemporaneously compounded for a specific patient following consultation are exempt from inclusion on the ARTG; this allows herbalists and other practitioners to compound individualized formulae for their patients.

CANADA

In Canada herbal medicines are classified as 'natural health products' that require a product licence before they can be marketed. The relevant agency is 'Health Canada'. Since 2004 this is regulated in the 'The Natural Health Products Regulations'. The system is intended to find an equilibrium between openness towards various health paradigms (e.g. traditional Chinese medicine, Ayurveda, Western traditional herbalism, etc.) and scientific rigor. Hence, specific health claims are allowed on the basis of a variable evidence base that becomes more stringent with the severity of the condition treated with a product. A manufacturer has to submit detailed information on the product to Health Canada, including: medicinal ingredients, source, potency, non-medicinal ingredients and recommended use(s). Once a product has been assessed and granted market authorization by Health Canada, the product label will bear an eight-digit product licence number preceded by the distinct letters NPN (Natural Product Number), or, in the case of a homeopathic medicine, by the letters DIN-HM (Homeopathic Medicine Number).

This number on the label will inform consumers that the product license application has been reviewed and approved by Health Canada to meet the standards in terms of safety, efficacy and quality. GMPs must be guaranteed in the product's production in order to ensure the

product's quality and thus its safety. In addition, the system requires that all Canadian producers and importers of natural health products are licensed.

Similar to the UK, an Adverse Reaction Reporting System for natural health products is in place and is used to warn the public (http://www.hc-sc.gc.ca/dhp-mps/prodnatur/about-apropos/index-eng.php).

INDIA

India has an ancient heritage of traditional medicine (see Chapter 11) with a well-recorded and widely practiced knowledge of herbal medicine. The Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) within the Ministry of Health & Family Welfare focuses on its regulation and on the improvement of standards in the areas of quality control and standardization of drugs, improving the availability of raw material, research and development, education/training of professionals and wider outreach about these traditional medical systems.

The **Traditional Herbal Medicines Act, 2006** regulates the sale of the traditional herbal medicines, which are marketed without any license and control on the basis of being from ancient texts. According to the act, every retailer or seller of traditional herbal medicines needs to have a license from the Authority to sell traditional herbal medicines. Every manufacturer of traditional herbal medicine needs to work under the principles of GMP and has to list the ingredients of each medicine on the packaging of the medicine along with their accurate quantity. Side effects and warnings of contraindications need to be stated on the package. Pharmacopoeia Committees have been established to develop quality standards for the main groups of therapeutically relevant drugs of Ayurveda, Unani, Siddha and homeopathy (Mukherhee et al 2007).

The Indian Government also established an independent body – the 'National Medicinal Plants Board' under the Ministry of Health and Family Welfare. It is responsible for co-ordinating all matters relating to the development of medicinal plants, including policies and strategies for conservation, proper harvesting, cost-effective cultivation and marketing of raw material in order to protect, sustain and develop this sector. Uniquely, the Indian government has established programmes for the documentation of traditional Indian knowledge, which is already available in the public domain. The political goal is to safeguard the sovereignty of this traditional knowledge and to protect it from being misused in patenting on non-patentable inventions. The **Traditional Knowledge Digital Library (TKDL)** is an original proprietary database, which is fully protected under national and international laws of Intellectual Property Rights and is maintained and developed by the government. TKDL also allows automatic conversion of information from Sanskrit into various languages. The information includes names of plants, Ayurvedic descriptions of diseases under their modern names and therapeutic formulations (Mukherhee et al 2007).

NEW ZEALAND

In New Zealand (NZ) herbal medicines and other natural health products (NHPs; or 'complementary medicines', CMs) are largely unregulated. Herbal and homeopathic remedies are defined in the NZ Medicines Act 1981, but are exempt from requirements to obtain pre-market approval with respect to quality, safety and efficacy. Other exemptions in the Medicines Act allow 'natural-health' practitioners (not defined) to make 'individualised' herbal remedies for specific patients in response to a consultation. Most CMs are regulated as 'dietary supplements' under the Dietary Supplement Regulations 1985 and the Food Act 1981. These regulations do not require adherence to GMP nor pre-market approval of products, but do provide some restrictions on ingredients of dietary supplements, and don't allow therapeutic claims to be made.

Initial plans (2010) followed by a detailed draft proposal (2015) to regulate NHPs/CMs on the NZ market were released by the NZ Ministry of Health for a Natural Health and Supplementary Products Bill. The regulations will relate only to manufactured NHPs/CMs sold in 'over-the-counter' settings; 'natural health' practitioners preparing products for specific patients will not be affected, as this is covered under the current Medicines Act exemption (although the Medicines Act itself is due to be replaced by a new Therapeutic Products Bill).

NIGERIA

Manufactured herbal medicinal products such as processed plant extracts in capsules or compressed as tablets, as well as liquid preparations containing a specified dose, are the only forms of herbal medicine that currently are subject to regulation in Nigeria by the National Agency for Food and Drug Administration and Control (NAFDAC). This applies to both those manufactured locally and those that are imported, including homeopathic products. A registration status provides a permit for it to be sold and distributed outside the locality of production. The quantitative list of ingredi-

ents either by their botanical names or common names, evidence of efficacy and safety, as well as the indications and contraindications of the product are some of the requirements for registration. Products that meet the above requirements are assigned a NAFDAC registration number, which is labelled on the package. A 'listing status' is initially given to manufactured herbal medicinal products for a period of two years, which precludes the need for rigorous assessment of good manufacturing practices or laboratory evaluations of efficacy. This is only valid for two years. A full registration licence, which is valid for five years, is then given after a reassessment of the safety of the product based on post market surveillance and laboratory evaluations of safety and efficacy (NAFDAC 2016).

Unfortunately, NAFDAC regulations do not apply to 'extemporaneous preparations' that are made by herbalists themselves and directly sold to the consumers. However, these types of preparations constitute a greater proportion of herbal medicines being sold in Nigeria, particularly in herbal trade fairs around the country, more so because of the huge expenses often required for product registration. There is therefore an absence of an extensive regulatory framework encompassing most of the herbal medicines utilized in Nigeria. Consequently, the current system is ill-suited for pharmacovigilance monitoring.

SOUTH AFRICA

South Africa has a long history of exploiting its rich and diverse plant resources for herbal medicines. The extensive use of traditional medicines in South Africa has led to the blossoming of an informal herbal product industry that mainly produces semi- and full-processed mixtures. The production and trade in these unprocessed indigenous herbal products remain largely unregulated both in terms of Good Agricultural Practice (GAP) and GMP [as defined in the World Health Organization (WHO) guidelines]. However, herbal medicines can go through the full drug evaluation procedure in the Medicines Control Council (MCC), according to internationally accepted efficacy and safety standards. In South Africa, GAP guidelines, which would constitute the first step in quality assurance in the cultivation and collection of medicinal plants, have not been developed. As of 2016, the South African Bureau of Standards (SABS) is in the process of developing standards for traditional medicines in line with GMP, to ensure that pharmaceutical products are manufactured to a consistent quality and for the appropriate intended use.

The regulatory framework also includes recognition of the importance of traditional healers within the primary healthcare system and the need to conserve plant biodiversity. The Traditional Health Practitioners Act (No. 22 of 2007) provides a regulatory framework to ensure the efficacy, safety, and quality of traditional healthcare services, and for control over the registration, training and conduct of traditional health practitioners.

Although many studies have recommended cultivation, the majority of medicinal plants are still harvested from wild populations. The National Management Environmental: Biodiversity Act No. 10 of 2004 is a regulatory framework for the use of indigenous biological resources in a sustainable manner and the fair and equitable sharing of benefits arising from bioprospecting involving indigenous biological resources. The extent to which these regulatory policies are enforced remains unclear. Overall, a plethora of herbal medicines, which have not been formally evaluated by the Medicines Control Council for quality, efficacy and safety, are being produced and distributed in the largely unregulated South African market.

UNITED STATES OF AMERICA (USA)

In the USA, herbal medicines are generally regulated as 'dietary supplements'. The U.S. Food and Drug Administration (FDA, www.fda.gov/Food/DietarySupplements/) is in charge of these comparatively loose regulations (http://nccam.nih.gov/health/supplements/wiseuse.htm). Some key characteristics stand out:

- Prior to marketing, dietary supplements do not have to be assessed for safety and effectiveness. Limited therapeutic claims may be made, e.g. that a dietary supplement addresses a nutrient deficiency, supports health, or is linked to a particular body function (e.g. immunity). However, this requires supportive prior research. Such a claim must be followed by the words 'This statement has not been evaluated by the U.S. Food and Drug Administration (FDA). This product is not intended to diagnose, treat, cure, or prevent any disease'.
- Since 2008 GMPs are expected in order to ensure that dietary supplements are processed consistently and meet quality standards.
- Once a dietary supplement is on the market, the FDA monitors the claims made and the product's safety. If inappropriate claims are made the manufacturer receives a warning letter or is required to remove the product from the marketplace. If a product is found to be unsafe, the FDA takes similar action against the manufacturer and/or distributor.

REGULATION OF TCM IN HONG KONG, THE PEOPLE'S REPUBLIC OF CHINA AND TAIWAN

Hong Kong

Herbal medicines in Hong Kong are regulated by the 'Chinese Medicine Ordinance', which governs the licensing of herbal traders as well as the registration of proprietary Chinese medicines. Additionally, a licensing system is established for Chinese medicine practitioners wishing to engage in the clinical practice of herbal medicine.

Chinese herbal medicines are classified into Schedule 1 and Schedule 2 substances; Schedule 1 substances include 31 toxic herbal, animal, and mineral materials that are subject to special restrictions. Proprietary Chinese medicines must be registered prior to sale, and include any proprietary product that is composed of Chinese herbal medicines, formulated in a finished dose form, and known or claimed to be used for the diagnosis, treatment, prevention, or alleviation of any disease or symptom of a disease in human beings, or for the regulation of functional states of the human body.

People's Republic of China

In the PR China, herbal medicines are regulated as medicines by the State Food and Drug Administration (SFDA), although 84 individual Chinese herbal medicines are also permitted for use as food products under regulations governing 'dual-use foods and medicines'. The identity and quality-control requirements of herbal medicines are established in the Chinese Pharmacopoeia (latest edition 2015), which includes official specifications for crude drugs and decoction pieces derived from herbal, animal, and mineral sources, as well as compound formulas.

Separate regulations apply to 'decoction pieces' (dried sliced herbal materials that are dispensed for decoctions) and prepared formulas (often described as 'patent medicines'). Requirements for GMP are specified for factories that prepare decoction pieces as well as finished products, and additional requirements for herbal processing facilities are also established. Additionally, modern delivery methods such as granular extracts of individual Chinese medicines are increasingly used in hospitals; however, only six factories are currently licensed to produce these products nationwide, and pharmacopoeia standards specific to granule extracts have not yet been formally implemented.

Taiwan

The Department of Chinese Medicine in the Ministry of Health and Welfare establishes regulations related to Chinese herbal medicines. Licensed Chinese medical doctors may prescribe herbal medicines, and the National Health Insurance scheme has covered granular extracts of Chinese herbal medicines since 1995. The Taiwan Herbal Pharmacopoeia specifies standards for the identity and quality of individual herbs, and manufactured herbal products must follow GMP requirements. Over 300 compound formulas and over 300 single herb extracts are available by prescription as granular extracts; bulk herbs are also widely used as a decoction but they are not covered by the National Health Insurance. Manufacturers can generally apply to produce Chinese herbal formulas that were recorded in traditional texts prior to the early 20th century without filing a new investigational drug application, which is necessary for novel formulas that lack a historical precedent.

References

Barnes, J., Mills, S.Y., Abbot, N.C., et al., 1998. Different standards for reporting ADRs to herbal remedies and conventional OTC medicines: face-to-face interviews with 515 users of herbal remedies. British Journal of Clinical Pharmacology 45, 496–500.

Campbell, W.C., 1991. Ivermectin as an antiparasitic agent for use in humans. Annual Review of Microbiology 45, 445–474. http://dx.doi.org/10.1146/annurev.mi.45.100191.002305.

Freymann, H., Rennie, T., Bates, I., Nebel, S., Heinrich, M., 2006. Knowledge and use of herbal medical products and other CAM among British undergraduate pharmacy students. Pharmacy World and Science 27, 13–18.

Gruenwald, J., 2008. The global herbs and botanicals market. Nutraceutical World July/Aug 2008.

Gulian, C., Barnes, J., Francis, S.-A., et al., 2002. Types and preferred sources of information concerning herbal medicinal products: face-to-face interviews with users of herbal medicinal products. International Journal of Pharmaceutical Practice 10 (suppl), R33.

IPSOS – Mori, 2008. Public Perceptions of Herbal Medicines General Public Qualitative & Quantitative Research. IPSOS-Mori, London, UK.
Kennedy, J., Wang, C.-C., Wu, Ch-H., 2008. Patient disclosure about herb and supplement use among adults in the US. Evidence Based Complementary and Alternative Medicine (eCAM) 5, 451–456.
Mukherjee, P.K., Venkatesh, M., Kumar, V., 2007. An overview on the development in regulation and control of medicinal and aromatic plants in the Indian system of medicine. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas (BLACPMA) 6, 129–136.
Ōmura, S., Shiomi, K., 2007. Discovery, chemistry, and chemical biology of microbial products. Pure and Applied Chemistry 79, 581–591. http://dx.doi.org/10.1351/pac200779040581.
Tu, Y.Y., 2016. Artemisinin—a gift from traditional Chinese medicine to the world (Nobel Lecture). Angewandte Chemie International Edition 55, 10210–10226.
Williamson, E.M., Driver, S., Baxter, K. (Eds.), 2009. Stockley's Herbal Medicines Interactions. Pharmaceutical Press, London.

Further reading

Barnes, J., Phillipson, J.D., Anderson, L.A., 2009. Herbal medicines. A guide for healthcare professionals, third ed. Pharmaceutical Press, London.
Barnes, J., McLachlan, A.J., Sherwin, C.T., Enioutina, E.Y., 2016. Herbal medicines: challenges in the modern world. Part 1: Australia and New Zealand. Expert Review of Clinical Pharmacology 9 (7), 905–915.
Bruneton, J., 1995. Pharmacognosy, phytochemistry, medicinal plants. Springer-Verlag, Berlin.
Cavaliere, C., Rea, P., Lynch, M.E., Blumenthal, M., 2010. Herbal supplement sales rise in all channels in 2009. HerbalGram 86, 82–85.
Evans, W.C., 2009. Trease and Evans's pharmacognosy, sixteenth ed. WB Saunders, London.
Hänsel, R., Sticher, O. (Eds.), 2010. Pharmakognosie – Phytopharmazie. Springer, Berlin.
McEwen, J., 2004. What does TGA approval of medicines mean? Australian Prescriber 27, 156–158.
Mills, S., Bone, K., 2000. Phytotherapy. Principles and practice. Churchill Livingstone, London.
NAFDAC, 2016. http://WWW.NAFDAC.GOV.NG/IMAGES/GUIDELINES/HERBALS/GUIDE34_GUIDELINES%20FOR%20REGISTRATION%20%20LISTING%20OF%20HERBAL%20MEDICINES%20AND.pdf (accessed 7/6/2016).
Robbers, J.E., Speedie, M.K., Tyler, V.E., 1996. Pharmacognosy and pharmacobiotechnology. Williams & Wilkins, Baltimore.
Ross, I., 1999. Medicinal plants of the world, vol. I. Humana Press, Totawa, NJ.
Ross, I., 2000. Medicinal plants of the world, vol. II. Humana Press, Totawa, NJ.
Schulz, V., Haensel, R., Tyler, V., 1998. Rational phytotherapy. Springer-Verlag, Berlin.
Thomas, K.J., Nicholl, J.P., Coleman, P., 2001. Use and expenditure on complementary medicine in England: a population based survey. Complementary Therapies in Medicine 9, 2–11.
Williamson, E., 2003. Potter's herbal cyclopedia. CW Daniels, Saffron Walden.

Chapter 2

Pharmacognosy and its history: people, plants and natural products

The history of pharmacy was for centuries identical to the history of pharmacognosy, or the study of **materia medica,** which were obtained from natural sources – mostly plants, but also minerals, animals and fungi. While European traditions are particularly well known and have had a strong influence on modern pharmacognosy in the West, almost all societies have well-established customs, some of which have hardly been studied at all. The study of these traditions not only provides insight into how the field has developed, but it is also a fascinating example of our ability to develop a diversity of cultural practices. The use of medicinal plants in Europe has been influenced by early European scholars, the concepts of lay people and, more recently, by an influx of people and products from non-European traditions. This historical overview covers only Europe and the most well-known traditions of Asia: **traditional Chinese medicine** (TCM), **Ayurveda** and **Jamu**. TCM and Ayurveda will be discussed further in a separate chapter, because they are still used widely today.

SOURCES OF INFORMATION

The sources available for understanding the history of medicinal (as well as nutritional and toxic) plant use are archaeological records and written documents. The desire to summarize information for future generations and to present the writings of the classical (mostly early Greek) scholars to a wider audience was the major stimulus for writing about medicinal plants. The traditions of Japan, India and China were also documented in many early manuscripts and books (Mazar 1998, Waller 1998). No written records are available for other regions of the world either because they were never produced (e.g. in Australia, many parts of Africa and South America, some regions of Asia) or because documents were lost or destroyed by (especially European) invaders (e.g. in Meso-America). Therefore, for many parts of the world the first written records are reports by early travellers who were sent by their respective feudal governments to explore the wealth of the New World. These people included missionaries, explorers, salesmen, researchers and, later, colonial officers. The information was important to European societies for several reasons, such as poisoned arrows posing a threat to explorers and settlers, as well as the prospect of finding new medicines.

EARLY ARABIC AND EUROPEAN RECORDS

Humans have always used plants in a multitude of ways in a tradition spanning human evolution. The selection of medicinal plants is a conscious process that has led to an enormous number of medicinal plants being used by the numerous cultures of the world.

An early European example is medicinal mushrooms, which were found with the Austrian/Italian 'iceman' of the Alps of Ötztal (3300 BCE). Two walnut-sized objects were identified as the birch polypore [*Piptoporus betulinus* (Bull. ex Fr.) P. Karst. = *Fomitopsis betulina* (Bull.) B.K. Cui, M.L. Han & Y.C. Dai], a bracket fungus common in alpine and other cooler environments. This species contains toxic natural products, and one of its active constituents (agaric acid) is a very strong and effective purgative, which leads to strong and short-lasting diarrhoea. It also has antibiotic effects against mycobacteria and toxic

effects on diverse organisms (Capasso 1998). Since the iceman also harboured eggs of the whipworm (*Trichuris trichiuria*) in his gut, he may well have suffered from gastrointestinal cramps and anaemia. The finding of *Piptoporus betulinus* points to the possible treatment of gastrointestinal problems using these mushrooms. Also, scarred cuts on the skin of the iceman might indicate the use of medicinal plants, since the burning of herbs over an incision on the skin was a frequent practice in many ancient European cultures (Capasso 1998).

THE DOCUMENTS OF SHANIDAR IV

The earliest documented record, which presumably relates to medicinal (or ritual) plants, dates from 60,000 BCE and was found in the grave of a Neanderthal man from Shanidar IV, an archaeological site in Iraq. Pollen of several species of plants was discovered (Leroi-Gourhan 1975, Lietava 1992, Solecki 1975):

Centaurea solstitialis L. (knapweed, Asteraceae).
Ephedra altissima Desf. (ephedra, Ephedraceae).
Achillea sp. (yarrow, Asteraceae).
Althea sp. (mallow, Malvaceae).
Muscari sp. (grape hyacinth, Liliaceae/Hyacinthaceae).
Senecio sp. (groundsel, Asteraceae).

These species were possibly laid on the ground and formed a carpet on which the dead were laid. These plants could have been of major cultural importance to the people of Shanidar IV. Whether they were used as medicine cannot be determined, but it seems likely. Today, these species are important medicinal plants used for a range of indications. However, others have criticized these reports, because:

- Detailed archaeobotanical descriptions of the pollen were never published.
- Normally, pollen does not survive well in the Near East.
- There is good evidence that ants often hoard pollen in a similar context (Sommer 1999).

Thus, although this may be a finding with no direct bearing on the culture of Shanidar, these species (or closely related ones from the same genus) are still important today in the phytotherapy of Iraq and are also known from other cultural traditions. These species may well be typical for the Neanderthal people, and may also be part of a tradition for which Shanidar IV represents the first available record.

CLASSICAL ARABIC, GREEK AND ROMAN RECORDS

The oldest written information in the European-Arabic traditions comes from the Sumerians and Akkadians of Mesopotamia, thus originating from the same area as the archaeological records of Shanidar IV. Similar documents have survived millennia in Egypt. The Egyptians documented their knowledge (including medical and pharmaceutical) on papyrus, which is sort of paper made from *Cyperus papyrus* L., an aquatic sedge (also called papyrus) found throughout southern Europe and northern Africa. The most important of these writings is the Ebers Papyrus, which originates from around 1500 BCE. This document was reputedly found in a tomb, and bought in 1873 by Georg Ebers, who deposited it at the University of Leipzig and two years later published a facsimile edition. The Ebers Papyrus is a medical handbook covering all sorts of illnesses and includes empirical as well as symbolic forms of treatment. The diagnostic precision documented in this text is impressive. Other papyri focus on recipes for pharmaceutical preparations (e.g. the so-called Berlin Papyrus).

Greek medicine has been the focus of historical pharmaceutical research for many decades. The Greek scholar Pedanius Dioscorides (Fig. 2.1) from Anarzabos (1st century BCE) is considered to be the 'father of [Western] medicine'. His works were a doctrine governing pharmaceutical and medical practice for more than 1500 years, and which heavily influenced European pharmacy. He was an excellent pharmacognosist and described more than 600 medicinal plants. Other Greek and Roman scholars were also influential in developing related fields of healthcare and the natural sciences. Hippocrates, a Greek medical doctor (ca. 460–375 BCE) came from the island of Kos, and heavily influenced European medical traditions. He was the first of a series of (otherwise largely unknown) authors who produced the so-called *Corpus*

Fig. 2.1 Pedanius Dioscorides. Reproduced with permission from The Wellcome Library, London.

Hippocraticum (a collection of works on medical practice). Importantly, the Hippocratic authors started to differentiate between food and medicine (cf. Totelin 2015) and thus laid the foundation for one of the key differentiations of natural resources used by humans. The Graeco-Roman medical doctor Claudius Galen (Galenus) (130–201 CE) summarized the complex body of Graeco-Roman pharmacy and medicine, and his name survives in the pharmaceutical term 'galenical'. Pliny the Elder (23 or 24–79 CE, killed in Pompeii at the eruption of Vesuvius) was the first to produce a 'cosmography' (a detailed account) of natural history, which included cosmology, mineralogy, botany, zoology and medicinal products derived from plants and animals.

CLASSICAL CHINESE RECORDS

Written documents about medicinal plants are essential elements of many cultures of Asia. In China, India, Japan and Indonesia, writings pointing to a long tradition of plant use survive. In China, the field developed as an element of Taoist thought: followers tried to assure a long life (or immortality) through meditation, special diets, medicinal plants, exercise and specific sexual practices. The most important work in this tradition is the *Shen nong ben cao jing* (the 'Drug treatise of the divine countryman'), which is now only available as part of later compilations (Waller 1998; see also Chapter 11, p 172 et seq). This 2200-year-old work provides the earliest treatise of Chinese medicine theory and is one of the four classical sources on Chinese traditional medicine including 365 drugs, most of botanical origin. For each, the following information is provided:

- Geographical origin.
- Optimum period for collection.
- Therapeutic properties.
- Forms of preparation and dose.

These scholarly ideas were passed on from master to student, and modified and adapted over centuries of use. Unfortunately, in none of the cases do we have a surviving written record. Table 2.1 summarizes some of the Chinese works that include important chapters on drugs.

In the 16th century the first systematic treatise on (herbal) drugs using a scientific method was produced. The *Ben Cao Gang Mu* ('Drugs', by Li Shizhen, 1518–1593) contains information about 1892 drugs (in 52 chapters) and more than 11,000 recipes are given in an appendix. The drugs are classified into 16 categories (e.g. herbs, cereals, vegetables, fruits). For each drug the following information is provided (Waller 1998):

- Definition of the drug.
- Selected commentaries.
- Classification according to the four characteristics of temperatures and the five types of taste.
- Uses (detailed information on uses according to the criteria of Chinese medicine).
- Corrections of previous mistakes.
- Methods of preparing the drug.
- New features.
- Examples of recipes.

The recognition of the need to further develop the usage of a plant, to correct earlier mistakes and include new information is particularly noteworthy. However, the numerous medicopharmaceutical traditions of the Chinese minorities were not included in these works and we therefore have no historical records of their pharmacopoeias. Today, TCM has become a medical system used in many countries and the governments of the People's Republic of China and Taiwan actively promote it. However, there also are many concerns with regards to quality, therapeutic benefits, safety and appropriateness (Booker 2015).

OTHER ASIAN TRADITIONAL MEDICINE

Overall, the written records on other Asian medicines are less comprehensive than for Chinese medicine. The oldest form of traditional Asian medicine is Ayurveda, which is basically Hindu in origin and which is a sort

Table 2.1 Chinese works that include important sections on drugs (after Waller 1998)

YEAR	AUTHOR IF KNOWN	TITLE
200 BCE	Various	*Shen nong ben cao jing* (the drug treatise of the divine countryman)
2nd century	Zhang Zhongjing	*Shang han za bing lun* (about the various illnesses caused by cold damage)
6th century	Tao Hongjing	*Shen nong ben cao jingji zhu* (collected commentaries on *Shen nong ben cao jing*)
1082 (Song dynasty)	Tang Shenwei	*Jing shi zheng lei bei ji ben cao* (classified materia medica from historical classics for emergency)
10th to 11th centuries	Su Song	*Ben cao tu jing* (Illustrated materia medica)
16th century	Li Shizhen	*Ben cao gang mu* (information about medicinal drugs: a monographic treatment)

of art-science-philosophy of life. In this respect, it resembles traditional Chinese medicine, and like TCM it has influenced the development of more practical, less esoteric forms of medicine, which are used for routine or minor illnesses in the home. Related types of medicine include Jamu, the traditional system of Indonesia, which will be described briefly below. All these forms of traditional medicine use herbs and minerals and have many features in common. Naturally, many plants are common to all systems and to various official drugs that were formerly (or still) included in the British Pharmacopoeia (BP), European Pharmacopoeia (Eur. Ph.) and US Pharmacopeia (USP).

Ayurveda

Ayurveda is arguably one of the most ancient of all recorded medicinal traditions. It is considered to be the origin of systemized medicine, because ancient Hindu writings on medicine contain no references to foreign medicine, whereas Greek and Middle Eastern texts do refer to ideas and drugs of Indian origin. Dioscorides (who influenced Hippocrates) is thought to have taken many of his ideas from India, so it looks as though the first comprehensive medical knowledge originated there. The term 'Ayurveda' comes from *ayur* meaning 'life' and *veda* meaning 'knowledge' and is a later addition to Hindu sacred writing from 1200 BCE called the *Artharva-veda*. The first school to teach Ayurvedic medicine was at the University of Banaras in 500 BCE and the great Samhita (or encyclopaedia of medicine) was written. Seven hundred years later another great encyclopaedia was written and these two together form the basis of Ayurveda. The living and the non-living environment, including humans, is composed of the elements earth (*prithvi*), water (*jala*), fire (*tejac*), air (*vaju*) and space (*akasa*). For an understanding of these traditions, the concept of impurity and cleansing is also essential. Illness is the consequence of imbalance between the various elements and it is the goal of treatment to restore this balance (see Chapter 11 for details).

Jamu

Indonesian traditional medicine, Jamu, is thought to have originated in the ancient palaces of Surakarta and Yogyakarta in central Java, from ancient Javanese cultural practices and also as a result of the influence of Chinese, Indian and Arabian medicine. Carvings at the temple of Borobudur dating back to 800–900 CE depict the use of *kalpataruh* leaves ('the tree that never dies') to make medicines. The Javanese influence spread to Bali as links were established, and in 1343 an army of the Majapahit kingdom of eastern Java was sent to subjugate the Balinese. Success was short-lived and the Balinese retaliated, regaining their independence. After Islam was adopted in Java and the Majapahit Empire destroyed, many Javanese fled, mainly to Bali, taking with them their books, culture and customs, including medicine. In this way, Javanese traditions survived in Bali more or less intact, and the island remained relatively isolated until the conquest by the Dutch in 1906 and 1908. Other islands in the archipelago use Jamu with regional variations.

There are a few surviving records, but often those that do exist are closely guarded by healers or their families. They are considered to be sacred and, for example, those in the palace at Yogyakarta, are closed to outsiders. In Bali, medical knowledge was inscribed on *lontar* leaves (a type of palm) and in Java on paper. Consequently, they are often in poor condition and difficult to read. Two of the most important manuscripts – *Serat kawruh bab jampi-jampi* ('A treatise on all manner of cures') and *Serat Centhini* ('Book of Centhini') – are in the Surakarta Palace library. The former contains a total of 1734 formulae made from natural materials and indications as to their use. The *Serat Centhini* is an 18th century work of 12 volumes and, although it contains much information and advice of a general nature and numerous folk tales, it is still an excellent account of medical treatment in ancient Java.

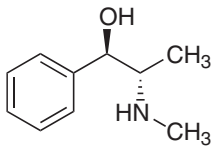
The status of Jamu started to improve ca. 1940 with the Second Congress of Indonesian Physicians, at which time it was decided that an in-depth study of traditional medicine was needed. A further impetus was the Japanese occupation of 1942–1944, when the Dai Nippon government set up the Indonesian Traditional Medicines Committee; another boost occurred during Indonesia's War of Independence when orthodox medicine was in short supply. President Sukarno decreed that the nation should be self-supporting, so many people turned to the traditional remedies used by their ancestors (see Beers 2001).

Jamu contains many elements of TCM, such as treating 'hot' illnesses with 'cold' remedies, and of Ayurveda, in which religious aspects and the use of massage are very important. Remedies from Indonesia such as clove (*Syzygium aromaticum*), nutmeg (*Myristica fragrans*), Java tea [*Orthosiphon stamineaus* (=*O. aristatus*) and *Orthosiphon* spp.], jambul (*Eugenia jambolana*) and galangal (*Alpinia galanga*) are still used around the world as medicines or culinary spices.

Kampo

Kampo, or traditional Japanese medicine, is sometimes referred to as low-dose TCM. Until 1875 (when the medical examination for Japanese doctors became restricted to Western medicine), the Chinese system was the main form of medical practice in Japan, having arrived via Korea and been absorbed into native medicine. Exchange of scholars with China meant that religious and medical practices were virtually identical; for example, the medical system established in Japan in 701 was an exact copy of that of the T'ang dynasty in China. In the Nara period (710–783), when Buddhism became even more popular, medicine became extremely complex and included facets of Ayurveda as well as of Arabian medicine. Native medicine remained in the background and, after concerns that it would be subsumed into Chinese medicine, the compendium of Japanese medicine, *Daidoruijoho*, was compiled in 808 on the orders of the Emperor Heizei. In 894, official cultural exchange with China was halted, and native medicine was temporarily reinstated. Knowledge gained from China, however, continued to be assimilated, and in 984 the court physician Yasuyori Tamba compiled the *Ishinho*, which consisted of 30 scrolls detailing the medical knowledge of the Sui and T'ang dynasties. Although based entirely on Chinese medicine, it is still invaluable as a record of medicine as practised in Japan at that time.

In 1184, the framework began to change when a reformed system was introduced by Yorimoto Minamoto in which native medicine was included, and by 1574 Dosan Manase had set down all the elements of medical thought, which became a form of independent Japanese medicine during the Edo period. This resulted in Kampo, and it remained the main form of medicine until the introduction of Western medicine in 1771, by Genpaku Sugita. Although Sugita did not reject Kampo, and advocated its use in his textbook *Keieiyawa*, it fell into decline because of a lack of evidence and an increasingly scholastic rather than empirical approach to treatment. Towards the end of the 19th century, despite important events such as the isolation of ephedrine (Fig. 2.2) by Nagayoshi Nagai, Kampo was still largely ignored by the Japanese medical establishment. However, by 1940, a university course on Kampo was instituted, and now most schools of medicine in Japan offer courses on traditional medicine integrated with Western medicine. In 1983, it was estimated that about 40% of Japanese clinicians were writing Kampo herbal prescriptions and today's research in Japan and Korea continues to confirm the validity of many of its remedies (Takemi et al 1985).



(–)-Ephedrine

Fig. 2.2 (-)-Ephedrine from *Ephedra* spp.

MEDICINE AT THE CENTRE OF THE AMERICAS – AZTECS AND OTHER CULTURES

While we have numerous written documents relating to the medicines used in Asia and Europe, very little has been documented in writing for the Americas. Until 1492, American medicine was truly traditional, without links with the 'Old World'. Under Spanish rule, interest in plants known to Amerindians produced manifold contributions to the world's medicine and science.

The first written document dates from 1552 and is by Martín de la Cruz the *Libellus de Medicinalibus Indorum Herbis* (Little Book of the Medicinal Herbs of the Indians). It is written in Nahuatl, the Aztec's language and was translated into Latin by Juan Badiano. The book describes the medicinal properties of plants used in the highlands of México with colour illustrations. This first illustrated and descriptive text on American traditional plant-based medicine gave rise to pioneering works on American medical botany including Fray Bernadino de Sahagún's famous *Codex Florentino* (ca. 1570), and Francisco Hernandez's *'History of the Plants of New Spain'* (1571–1576), providing a basis for bioscientific assessment of the documented effects desired by the Aztecs. Similar compilations in the former Inca empire (De Acosta 1588, Monardes 1568), responded more to current medical interests in Europe. Over four centuries, plants from the Americas were heralded as potential panaceas. Quinine (from the bark of *Cinchona officinalis* L.) and D-tubocurarine (from arrow poisons) both derived from South American plants altered biomedicine and world history (see p. 25; Heinrich et al 2014).

THE EUROPEAN MIDDLE AGES AND ARABIA

After the conquest of the southern part of the Roman Empire by Arab troops, Greek medical texts were translated into Arabic and adapted to the needs of the Arabs. Many of the Greek texts survived only in Arab transcripts. Ibn Sina, or Avicenna from Afshana (980–1037), wrote a monumental treatise *Qânûn fi'l tibb*

('Canon of medicine'; ca. 1020), which was heavily influenced by Galen and which in turn influenced the scholastic traditions especially of southern Europe. This five-volume book remained the most influential work in the field of medicine and pharmacy for more than 500 years, together with direct interpretations of Dioscorides' work. While many Arab scholars worked in eastern Arabia, Arab-dominated parts of Spain became a second centre for classical Arab medicine. An important early example is the *Umdat at-tabîb* ('The medical references') by an unknown botanist from Seville. Similarly, the pharmacist, botanist, and physician Ibn al-Baytar (1197–1248) systematically recorded Islamic physicians' contributions to medicine during the Middle Ages, and most importantly about 300–400 new medicinal preparations from this period.

Thanks to the tolerant policies of the Arab administration, many of the most influential representatives of Arab scholarly traditions were Jews, including Maimoides (1135–1204) and Averroes (1126–1198). In Christian parts of Europe, the texts of the classical Greeks and Romans were copied from the Arabian records and annotated, often by monks. The Italian monastery of Monte Cassino is one of the earliest examples of such a tradition; others developed around the monasteries of Chartres (France) and St Gall (Switzerland).

A common element of all monasteries was a medicinal plant garden, which was used both for growing herbs to treat patients and to teach the younger generation about medicinal plants. The species included in these gardens were common to most monasteries and many of the species are still important medicinal plants today. Of particular interest is the *Capitulare de villis* of Charles the Great (Charlemagne, 747–814), who ordered that medicinal (and other plants) should be grown in the King's gardens and in monasteries, and specifically listed 24 species. Walahfri(e)d Strabo (808 or 809–849), Abbot of the monastery of Reichenau (Lake Constance), deserves mention because of his *Liber de cultura hortum* ('Book on the growing of plants'), the first 'textbook' on (medical) botany, and the *Hortulus*, a Latin poem about the medical plants grown in the district. The *Hortulus* is not only famous as a piece of poetry, but also as a vivid and excellent description of the appearance and virtues of medicinal plants. Table 2.2 lists the plants reported in the *Capitulare de villis* and in some other sources of the 10th and 11th centuries. Today, many of reported in the *Capitulare de villis* and

Table 2.2 Species of plants listed in the *Capitulare de villis*

BOTANICAL NAME*	FAMILY	ENGLISH NAME	GEOGRAPHICAL ORIGIN
Achillea millefolium[a,b]	Asteraceae	Milfoil	Northern hemisphere
Agrimonia eupatoria[a,b]	Rosaceae	Agrimony	Europe, south-eastern Asia
Allium ascalonicum	Alliaceae	Shallot	Western Asia
Allium cepa	Alliaceae	Onion	Western Persia
Allium ampeloprasum (?)	Alliaceae	Leek	Western Mediterranean
Allium sativum	Alliaceae	Garlic	South-eastern Asia
Allium schoenoprasum	Alliaceae	Chives	Southern Europe
Althaea officinalis	Malvaceae	Marsh mallow	Eastern Mediterranean
Anethum graveolens	Apiaceae	Dill	Western Asia, southern Europe
Anthriscus cerefolium (L.) Hoffm.[a]	Poaceae	Chervil	Western Asia, south-eastern Europe
Apium graveolens[a]	Apiaceae	Celery	Western Asia, southern Europe
Artemisia abrotanum[a]	Asteraceae	Southernwood, old man	Eastern Europe, western Asia
Artemisia absinthium[a,b]	Asteraceae	Wormwood	Europe, Asia
Beta vulgaris	Chenopodiaceae	Beetroot	Mediterranean and Atlantic Coast
Brassica oleracea	Brassicaceae	Kale, borecole	Mediterranean and Atlantic Coast
Brassica oleracea var. *gongyloides*	Brassicaceae	Kohlrabi	Mediterranean and Atlantic Coast
Castanea sativa Mill.	Fagaceae	Sweet chestnut	Southern Europe, Africa, south-eastern Asia
Cichorium intybus	Asteraceae	Chicory	Europe, Asia

Table 2.2 Species of plants listed in the *Capitulare de villis*—cont'd

BOTANICAL NAME*	FAMILY	ENGLISH NAME	GEOGRAPHICAL ORIGIN
Coriandrum sativum	Apiaceae	Coriander	Orient
Corylus avellana	Betulaceae	Hazel	Europe, Asia
Cucumis melo[a]	Cucurbitaceae	Melon	Africa, southern Asia
Cucumis sativus	Cucurbitaceae	Cucumber	Western India
Cuminum cyminum	Apiaceae	Cumin	Turkey, eastern Europe
Cydonia oblonga	Rosaceae	Quince	Western Asia
Ficus carica	Moraceae	Fig	Western Mediterranean
Foeniculum vulgare Mill.[a]	Apiacae	Fennel	Mediterranean
Iris x *germanica*[a]	Iridaceae	Iris	South-eastern Europe
Juglans regia	Juglandaceae	European walnut	Western Asia, eastern Europe
Juniperus sabina	Juniperaceae	Juniper	Alps, southern Europe
Lactuca sativa	Asteraceae	Lettuce	Western Asia, southern Europe
Lagenaria siceraria (Molina) Standl.[a]	Cucurbitaceae	Calabash, bottle gourd	Africa, Asia (America)
Laurus nobilis	Lauraceae	(Bay) laurel	South-eastern Europe, south-western Asia
Lepidium sativum	Brassicaceae	Pepperwort	Orient
Levisticum officinale W.D.J.Koch [a]	Apiaceae	Lovage	Persia/Iran
Lilium candidum[a]	Liliaceae	Lily	Western Asia
Malus pumila (Mill.)	Rosaceae	Apple	Europe, western Asia
Malva neglecta Wallr.	Malvaceae	Mallow	Europe, Asia
Marrubium vulgare[a,b]	Lamiaceae	Horehound	Mediterranean
Mentha spicata subsp. *spicata* (syn.: *Mentha crispa)*	Lamiaceae	True spearmint	Mediterranean
Mentha pulegium[a]	Lamiaceae	Pennyroyal	Mediterranean
Mentha spp.[a]	Lamiaceae	Mint	Southern Europe, Mediterranean
Mespilus germanica	Rosaceae	Medlar	South-eastern Europe, western Asia
Morus nigra	Moraceae	Mulberries	Western Asia
Nepeta cataria[a]	Lamiaceae	Catnip	Eastern Mediterranean
Nigella sativa	Ranunculaceae	Nigella, black cumin	Western Asia, southern Europe
Papaver somniferum[a]	Papaveraceae	Opium poppy	Mediterranean
Pastinaca sativa	Apiaceae	Parsnip	Europe, Caucasus
Petroselinum crispum (Mill.) Fuss	Apiaceae	Parsley	South-eastern Europe, western Asia
Prunus avium (L.) L./P. cerasus	Rosaceae	Wild cherry, mazzard	Europe, Asia, Persia
Prunus domestica	Rosaceae	Plum	Western Asia
Prunus amygdalus Batch [=P. dulcis (Mill.) D.A.Webb]	Rosaceae	(Sweet) almond	Western Asia
Prunus persica (L.) Batsch	Rosaceae	Peach	China
Pyrus communis	Rosaceae	Pear	Central and southern Europe, south-western Asia
Raphanus raphanistrum subsp. sativus (L.) Domin (syn.: *Raphanus sativus)*[a]	Brassicaceae	Radish	Western Asia
Rosa gallica[a]	Rosaceae	French rose	Southern Europe

Continued

Table 2.2 Species of plants listed in the *Capitulare de villis*—cont'd

BOTANICAL NAME*	FAMILY	ENGLISH NAME	GEOGRAPHICAL ORIGIN
Rosmarinus officinalis	Lamiaceae	Rosemary	Mediterranean
Ruta graveolens[a]	Rutaceae	Rue	South-eastern Europe
Salvia officinalis[a]	Lamiaceae	Sage	South-eastern Europe, Mediterranean
Salvia sclarea[a]	Lamiaceae	Clary (sage)	Mediterranean
Satureja hortensis	Lamiaceae	Summer savoury	Western Mediterranean
Sorbus domestica	Rosaceae	Service tree	Central and southern Europe, south-western Asia
Stachys officinalis (L.) Trevis. (Betonica officinalis L.)[a,b]	Lamiaceae	Betonica	Western Europe, Mediterranean
Tanacetum balsamita[a]	Asteraceae	Balsamite, costmary	South-eastern Europe
Tanacetum vulgare	Asteraceae	Tansy	Europe, Caucasus

*– unless stated otherwise, the species was first and validly described by Carl v. Linné and the acronym is 'L'.
[a]– Species listed by Walahfried Strabo in his *Hortulus* [information based on Vogellehner (1987)].
[b]– Not in the *Capitulare de villis* but in other sources from the period.

in some other sources of the 10th and 11th centuries. Today, many of these plants are still important medicinally or in other ways. Many are vegetables, fruits or other foods. The list shows not only the long tradition of medicinal plant use in Europe, but also the importance of these resources to the state and religious powers during the Middle Ages. Although these were not necessarily of interest as scholarly writings, they were at least a practical resource.

A plan (which was not executed) for a medicinal herb garden for the Cloister of St Galls (Switzerland), dating from the year 820, has been preserved and gives an account of the species that were to be grown in a cloister garden. In general, pharmacy and medicine were of minor importance in the European scholastic traditions, as shown for example by the fact that in the Monastery of St Gall there were only six books on medicine, but 1000 on theology. Scholastic traditions, influenced by Greek-Arab medicine and philosophy, were practised in numerous European cloisters. In Arab-dominated Sicily, the first medical centre of medieval Europe was established in Salerno (12th century). Until 1130, before the Council of Clermont, the monks combined medical and theological work, but after this date only lay members of the monastery were permitted to practise medicine. Simultaneously, the first universities (Paris 1110, Bologna 1113, Oxford 1167, Montpellier 1181, Prague 1348) were founded which provided training in medicine.

The climax of medieval medico-botanical literature was reached in the 11th century with *De viribus herbarum* ('On the virtues of herbs') and *Macer floridus*, a Latin poem from around 1070 CE, presumed to be by Odo of Meune (Magdunensis), the Abbot of Beauprai. In this educational poem, 65 medicinal plants and spices are presented. Other frequently cited sources are the descriptions of the medical virtues of plants by the Benedictine nun, early mysticist and abbess Hildegard of Bingen (1098–1179). In her works *Physica* and *Causae et curae* she included many remedies that were popularly used during the 12th century. Her writings also focus on prophetic and mystical topics. The works of both scholars are only available as later copies in other texts, which unfortunately gives a rather distorted idea of the originals, as they are heavily re-interpreted.

PRINTED REPORTS IN THE EUROPEAN TRADITION (16TH CENTURY)

For over 1500 years the classical and most influential book in Europe had been Dioscorides' *De materia medica*. Until the Europeans' (re-)invention of printing in the mid-15th century (by Gutenberg), texts were hand-written codices, which were used almost exclusively by the clergy and scholars in monasteries. A wider distribution of the information on medicinal plants in Europe began with the early herbals, which rapidly became very popular and which made the information about medicinal plants available in the languages of lay people. These were still strongly influenced by Graeco-Roman concepts, but during the 16th century many other sources began to have an influence (Table 2.3).

Table 2.3 Examples of early European herbals from the 15th and 16th centuries (based on Leibrock-Plehn 1992 and Arber 1938)

YEAR	AUTHOR	TITLE	LANGUAGE
1478	Dioscorides	*De materia medica*	Latin
1481	Anon.	The Latin Herbarius	Latin
1485	Anon.	The German Herbarius (*Gart der Gesundheit*)	German
1525	Anon.	Herball [*Rycharde Banckes' Herball*]	English
1526 (ca.)	Anon.	*Le grand herbier en francoys*	French
1530	Otto Brunfels	*Herbarium vivae eicones ad naturae imitationem*	Latin
1530–1574	Nicolás Monardes	*Historia medicinal de las cosas que se traen de nuestras Indias Occidentales que sirven en medicina*	Spanish
1532	Otto Brunfels	*Contrafayt Kreüterbuch*	German
1533	Eucharius Rösslin/Adam Lonitzer (1946)	*Kreüterbuch von allen Erdtgewächs*	German
1534	Various	*Ogrod zdrowia* ('The garden of health')	Polish
1541	Conradus Gesnerus	*Historia plantarum et vires ex Dioscorides*	Latin
1542	Leonhard Fuchs (Fig. 2.3)	*De historia stirpium commentarii insignes*	Latin
1543	Leonhard Fuchs	*New Kreüterbch*	German
1546	Hieronymus Bock	*Kreüterbuch*	German
1546	Dioskorides	*Kreüter Buch* (translated by J Dantzen von Ast)	German
1548	William Turner	*Libellus de re herbaria novus, in quo herbarium*	Latin
1554	Remibertus Dodonaeus	*Cruÿterboeck*	Flemish
1554	Pietro A Mattioli	*Commentarii, in libros sex pedacii Dioscoridis Anazarbi*	Latin/Italian
1560 (ca.)	(Pseudo) Albertus Magnus	*Ein neuer Albert Magnus*	German
1563	Garcia ab Horto (Orto/d'Orta)	*Orto/coloquios dos simples, e drogas he cousas medicinais da India* (Portuguese d'Orta; first published in Goa, India)	Portuguese
1576	Carolus Clusius	*Rariorum aliquot stirpium per hispanias observatum historia*	Latin
1588	Jakob Theodor (Tabernae montanus)	*Neuw Kreüterbuch*	German
1596	Casparus Bauhinus	*Phytopinax*	Latin
1596	John Gerard	*General historie of plantes* (or The 'Herball')	English
1597	Antoine Constantin	*Brief traicté de la pharmacie provinciale...*	French

Herbals were rapidly becoming available in various European languages and in fact many later authors copied, translated and re-interpreted the earlier books. This was especially so for the woodcuts used for illustration (Fig. 2.4); these were often used in several editions or were copied. The herbals changed the role of European pharmacy and medicine and influenced contemporary orally transmitted popular medicine. Previously there had been two lines of practice: the herbal traditions of the monasteries and the popular tradition, which remains practically unknown. Books in European languages made scholastic information much more widely available and it seems that the literate population was eager to learn about these medico-pharmaceutical practices. These new books became the driving force of European 'phytotherapy', which developed rapidly over the following centuries.

The trade in botanical drugs increased during this period. From the East Indies came nutmeg (*Myristica fragrans* Houtt., Myristicaceae), already used by the Greeks as an aromatic and for treating gastrointestinal problems. Rhubarb (*Rheum palmatum* L. and *Rh. officinale* Baill., Polygonaceae) arrived in Europe from India in the 10th century and was employed as a strong purgative. Another important change at this time was the discovery of healing plants with new properties, during the exploration and conquest of the 'New Worlds' – the Americas, as well as some regions of Asia and Africa. For example, 'guayacán' (*Guaiacum sanctum*, Zygophyllaceae), from Meso-America, was used against

syphilis, despite its lack of any relevant pharmacological effects.

Nicolás Monardes was particularly important in the dissemination of knowledge about medicinal plants from the New World. His principal work, *Historia medicinal de las cosas que se traen de nuestras Indias Occidentales que sirven en medicina* ('Medical history of all those things which are brought from our Western India and may be used as medicines') was published in 1574. Some parts had appeared as early as ca. 1530. Another influential scholar during this period was Theophrastus Bombastus of Hohenheim, better known as Paracelsus (1493–1541). His importance lies less in the written record he left but more in his medical and pharmaceutical inventions and concepts. He rejected the established medical system and, after a fierce fight with the medical faculty of Basel in 1528, fled to Salzburg. According to some sources, he had publicly burned the 'Canon of medicine' by Avicenna. He introduced minerals into medical practice and called for the extraction of the active principle from animals, plants or minerals, a goal that was not achieved until the beginning of the 19th century (see below). He regarded the human body as a 'microcosm', with its substances and powers needing to be brought into harmony with the 'macrocosm' or universe. According to Paracelsus, healing was due to 'the power of life, which is only supported by the medical doctor and the medicine'. Although some of his ideas anticipated later ones, at the time they were largely rejected. The first pharmacopoeias were issued by autonomous cities, and became legally binding documents on the composition, preparation and storage of pharmaceuticals.

Fig. 2.3 Leonhard Fuchs. Reproduced with permission from The University Library, Tübingen

THE FIRST PHARMACOPOEIAS

- *Ricettario Fiorentino* (Florence, Italy), 1498.
- *Pharmacopoeia of Nuremberg* (Frankonia, Germany) or *Pharmacorum omnium*, 1546.
- *Pharmacopoeia Londiniensis* (UK), 1618, one of the most influential early pharmaceutical treatises.

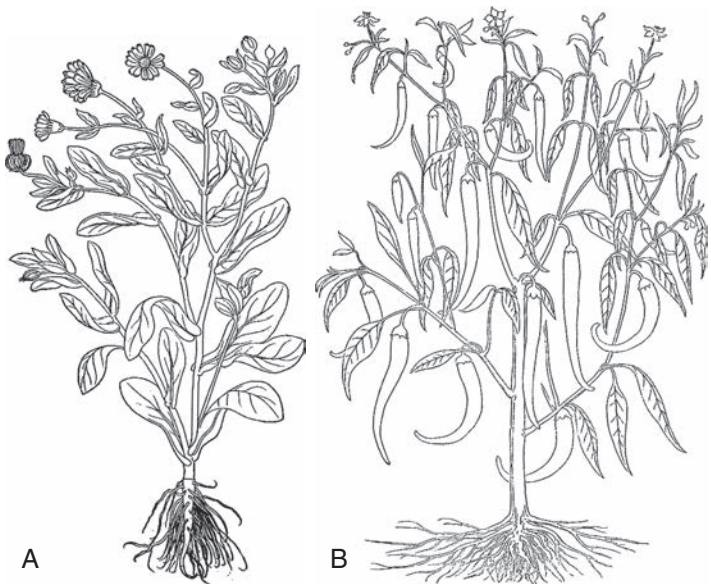


Fig. 2.4 Examples of early woodcuts: (A) marigold or Ringelblume (*Calendula officinalis* L.), one of the most important medicinal plants in historical and modern phytotherapy; (B) capsicum (chili pepper; *Capsicum frutescens* L.). Reproduced with permission from The University Library, Tübingen

These pharmacopoeias were mainly intended to bring some order into the many forms of preparation available at the time, the varying composition of medicines and to reduce the problems arising out of their variability.

Another development was the establishment of independent guilds specializing in the sale of medicinal plants, even though apothecaries had practiced this for centuries. In 1617, the Worshipful Society of Apothecaries was founded in London, and in 1673 it formed its own garden of medicinal plants, known today as the Chelsea Physic Garden (Minter 2000). One of the most well-known English apothecaries (and astrologers) of the 17th century is Nicholas Culpeper (1616–1654), best known for his 'English physician' – more commonly called 'Culpeper's herbal'. This is the only herbal that rivals in popularity John Gerard's *General historie of plantes*, but his arrogant dismissal of orthodox practitioners made him very unpopular with many physicians. Culpeper describes plants that grow in Britain and which can be used to cure a person or to 'preserve one's body in health'. He is also known for his translation *A physicall directory* (from Latin into English) of the London Pharmacopoeia of 1618 published in 1649 (Arber 1938).

MEDICAL HERBALISM

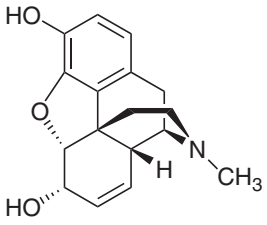
The use of medicinal plants was always an important part of the medical systems of the world, and Europe was no exception. Little is known about popular traditions in medieval and early modern Europe and our knowledge starts with the availability of written (printed) records on medicinal plant use by common people. As pointed out by Griggs (1981, p. 88), a woman in the 17th century was a 'superwoman' capable of administering 'any wholesome receipts or medicines for the good of the family's health'. A typical example of such a remedy is foxglove (*Digitalis purpurea*), reportedly used by an English housewife to treat dropsy, and then more systematically by the physician William Withering (1741–1799; Fig. 2.5). Withering transformed the orally transmitted knowledge of British herbalism into a form of medicine that could be used by medical doctors. Prior to that, herbalism was more of a clinical practice interested in the patient's welfare, and less of a systematic study of the virtues and chemical properties of medicinal plants.

EUROPEAN PHARMACOGNOSY AND NATURAL PRODUCT CHEMISTRY IN THE 18TH AND 19TH CENTURIES

In the 17th and 18th centuries, knowledge about plant-derived drugs expanded, but attempts to 'distillate' the active ingredients from plants were unsuccessful. The main outcome during this period was detailed observations on the clinical usefulness of medicinal products, which had been recorded in previous centuries or imported from non-European countries. The next main shift in emphasis came in the early 19th century when it became clear that the pharmaceutical properties of plants are due to specific molecules that can be isolated and characterized. This led to the development of a field of research now called **natural product chemistry** or, specifically for plants, **phytochemistry**. Pure chemical entities were isolated and their structures elucidated. Some were then developed into medicines or chemically modified for medicinal use. Examples of such early pure drugs include:

- **Morphine** (Fig. 2.6) from opium poppy (*Papaver somniferum* L., Papaveraceae), which was first identified by FW Sertürner of Germany (Fig. 2.7) in 1804 and chemically characterized in 1817 as an

Fig. 2.5 William Withering. Reproduced with permission from The Wellcome Library, London.



Morphine

Fig. 2.6 Morphine from opium poppy (*Papaver somniferum*).

alkaloid. The full structure was established in 1923, by JM Gulland and R Robinson, in Manchester.

- **Quinine** (Fig. 2.8), from cinchona bark (*Cinchona succirubra* Vahl and others), was first isolated by Pierre Joseph Pelletier and Joseph Bienaime Caventou of France in 1820; the structure was elucidated in the 1880s by various laboratories. Pelletier and Caventou were also instrumental in isolating many of the alkaloids mentioned below.
- **Salicin**, from willow bark (*Salix* spp., Salicaceae), was first isolated by Johannes Buchner in Germany. It was derivatized first (in 1838) by Rafaele Pirea (France) to yield salicylic acid, and later (1899) by the Bayer company, to yield acetylsalicylic acid, or **aspirin** – a compound that was previously known but which had not been exploited pharmaceutically (Fig. 2.9).

Fig. 2.7 FW Sertürner. Reproduced with permission from Wood Library-Museum of Anesthesiology, Park Ridge, IL.

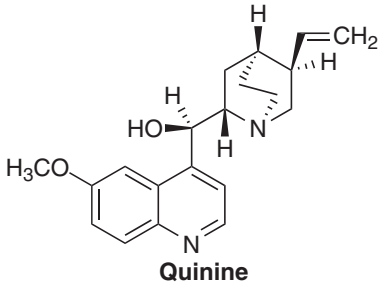


Fig. 2.8 Quinine from cinchona bark (*Cinchona succirubra*).

EXAMPLES OF PURE COMPOUNDS ISOLATED DURING THE EARLY 19TH CENTURY

Atropine (1833), from belladonna (*Atropa belladonna* L., Solanaceae), was used at the time for asthma.

Caffeine (1821), from the coffee shrub (*Coffea arabica* L. and *C. canephora* Pierre ex A. Froehner, *Rubiaceae*); its structure was elucidated in 1882.

Coniine, a highly poisonous natural product, was first isolated in 1826 from hemlock (*Conium maculatum* L., Apiaceae). Its properties had been known for years (Socrates used hemlock to commit suicide) and it was the first alkaloid to have its structure elucidated (1870). Some years later it was synthesized (1889).

Emetine (1817), from ipecacuanha (*Carapichea ipecacuanha* (Brot.) L. Andersson, syn.: *Cephaelis ipecacuanha* (Brot.) Willd., Rubiaceae), was fully characterized as late as 1948 and used as an emetic as well as in cough medications.

Strychnine (1817), from *Strychnos* spp. (Loganiaceae), was used as a tonic and stimulant (Sneader 1996).

Also, early in the 19th century, the term 'pharmacognosy' was coined by the Austrian professor Johann Adam Schmidt (1759–1809) and was included in his posthumously published book *Lehrbuch der Materia Medica* (1811). This period thus saw the development of a well-defined scientific field of inquiry, which developed rapidly during the century.

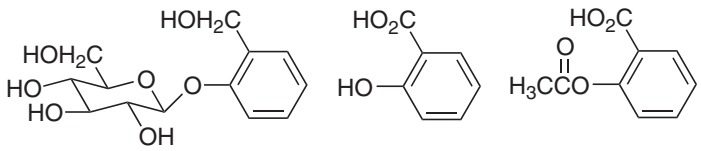


Fig. 2.9 Salicin and salicylic acid from willow bark (*Salix* spp.) and aspirin (acetyl salicylic acid).

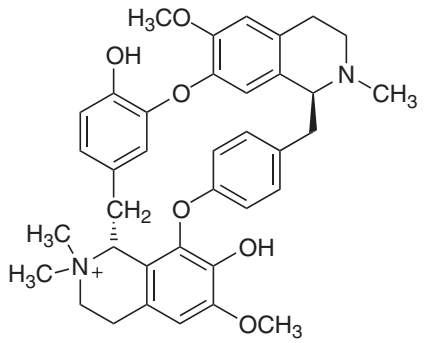
One of the main achievements of 19th century science in the field of medicinal plants was the development of methods to study the pharmacological effects of compounds and extracts. The French physiologist Claude Bernard (1813–1878), who conducted detailed studies on the pharmacological effects of plant extracts, must be considered one of the first scientists in this tradition. He was particularly interested in curare – a drug and arrow poison used by the American Indians of the Amazon, and the focus of research of many explorers. The ethnobotanical story of curare is described further in Chapter 5 (von Humboldt, 1997).

Bernard noted that, if curare was administered into living tissue directly, via an arrow or a poisoned instrument, it resulted in death more quickly, and that death occurred more rapidly if dissolved curare was used rather than the dried toxin (Bernard 1966:92). He was also able to demonstrate that the main cause of death was by muscular paralysis, and that animals showed no signs of nervousness or pain. Further investigations showed that, if the blood flow in the hind leg of a frog was interrupted using a ligature (without affecting the innervation) and the curare was introduced via an injury of that limb, the limb retained mobility and the animal did not die (Bernard 1996:95–96, 115, [orig. 1864]). One of the facts noted by all those who reported on curare is the lack of toxicity of the poison in the gastrointestinal tract and, indeed, the Indians used curare both as a poison and as a remedy for the stomach.

Bernard went on to say:

> *In our physiological studies we were able to identify the effect of the American arrow poison curare as one on the nervous motoric element and subsequently to determine a mechanism which results in death, which is an inert ability of this poisoned substance, but do we have to stop here and have we reached the border which our current [19th century] science allows us to reach? I do not think so. One has to separate the active principle of curare from the foreign substances, with which it is mixed, and one also has to study which physical and chemical changes the toxic substance imprints onto the organic element [i.e. the body] in order to paralyse its activity.*
>
> ***[Bernard 1966:121 (orig. 1864), translation MH]***

Later, the botanical source of curare was identified as *Chondrodendron tomentosum* Ruiz et Pavon, and the agent largely responsible for the pharmacological activity first isolated. It was found to be an alkaloid, and named D-tubocurarine because of its source, 'tube curare', so-called because of the bamboo tubes used as storage containers. In 1947 the structure of this complex alkaloid, a bisbenzylisoquinoline, was finally established (Fig. 2.10). The story of this poison is one of the most fascinating examples of transforming a drug used in an indigenous culture into a medication and research tool, and, although D-tubocurarine is now used less frequently for muscular relaxation during surgery, it has been used as a template for the development of newer and better drugs.



Tubocurarine

Fig. 2.10 D-tubocurarine from the American arrow poison curare.

The 19th century thus saw the integration of ethnobotanical, pharmacological and phytochemical studies, a process that had taken many decades but which allowed the development of a new approach to the study and the pharmaceutical use of plants. Ultimately, herbal remedies became transformed into chemically defined drugs.

THE 20TH CENTURY

One of the most important events that influenced the use of medicinal plants in the Western world in the last century was the serendipitous discovery of the antibacterial properties of fungal metabolites such as benzylpenicillin, by Florey and Fleming in 1928 at St Mary's Hospital (London). These natural products changed forever the perception and use of plant-derived metabolites as medicines by both scientists and the lay public. Another important development came with the advent of synthetic chemistry in the field of pharmacy. Many of these studies involved compounds that were synthesized because of their potential as colouring material (Sneader 1996). The first successful use of a synthetic compound as a chemotherapeutic agent was achieved by Paul Ehrlich in Germany (1854–1915); he successfully used methylene blue in the treatment of mild forms of malaria in 1891. Unfortunately, this finding could not be extended to the more severe forms of malaria common in the tropics. Many further studies on the therapeutic properties of dyes and of other synthetic compounds followed.

The latter part of the 20th century saw a rapid expansion in knowledge of secondary natural products, their biosynthesis and biological and pharmacological effects. A large number of natural products or their derivatives were introduced as medicines including many anti-cancer agents (paclitaxol, the vinca alkaloids; see Chapter 9, pp. 148–151), the anti-malarial agent artemisinin, and the anti-dementia medication galanthamine, to name just a few (Heinrich 2010, Heinrich and Teoh 2004, Newman and Cragg 2012). Numerous examples of drugs that are natural products, their derivatives or a pharmacophore based on a natural product have been introduced. There is now a better understanding of the genetic basis of the reactions that give rise to such compounds as well as the biochemical (and in many cases genetic) basis of many important illnesses. This has opened up new opportunities and avenues for drug development. This culminated in 2015 with the Nobel Prize in Physiology or Medicine being awarded to Youyou Tu (2011), William C. Campbell and Satoshi Ōmura (see p. 1, Intro to Part A) for their seminal contribution to the discovery and development of novel nature-derived medicines for treating parasitic diseases.

References

Arber, A., 1938. Herbals. Their origin and evolution. A chapter in the history of botany 1470–1670. Cambridge University Press, Cambridge.

Beers, S.-J., 2001. Jamu. The ancient art of Indonesian herbal healing. Periplus Editions (HK) Ltd, Singapore.

Bernard, C., 1966. Physiologische Untersuchungen über einige amerikanische Gifte. Das Curare. In: Bernard, C., Mani, N. (Eds.), Ausgewählte physiologische Schriften. Huber Verlag, Bern, pp. 84–133. [orig. French 1864].

Booker, A., 2015. Chinese medicine: contentions and global complexities. In: Heinrich, M., Jaeger, A.K. (Eds.), Ethnopharmacology. Wiley, Chichester.

Capasso, L., 1998. 5300 years ago, the ice man used natural laxatives and antibiotics. Lancet 352, 1894.

Griggs, B., 1981. Green pharmacy. A history of herbal medicine. Norman & Hobhouse, London.

Heinrich, M., 2010. Ethnopharmacology and drug development. In: Mander, L., Lui, H.-W. (Eds.), Comprehensive Natural Products II Chemistry and Biology, vol. 3. Elsevier, Oxford, pp. 351–338.

Heinrich, M., Leonti, M., Frei-Haller, B., 2014. A perspective on natural products research and ethnopharmacology in México. The eagle and the serpent on the prickly pear cactus. J Nat Prod 77, 678–689.

Heinrich, M., Teoh, H.L., 2004. Galanthamine from snowdrop – the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. J Ethnopharmacol 92, 147–162.

Leibrock-Plehn, L., 1992. Hexenkräuter oder Arznei: die Abtreibungsmittel im 16 und 17 Jahrhundert. Heidelberger Schriften zur Pharmazie- und Naturwissenschaftsgeschichte, Bd 6. Wissenschaftliche Verlagsgesellschaft, Stuttgart.

Leroi-Gourhan, A., 1975. The flowers found with Shanidar IV, a Neanderthal burial in Iraq. Science 190, 562–564.

Lietava, J., 1992. Medicinal plants in a middle paleolithic grave Shanidar IV. J Ethnopharmacol 35, 263–266.

Mazar, G., 1998. Ayurvedische phytotherapie in Indien. Zeitschrift für Phytotherapie 19, 269–274.

Minter, S., 2000. The Apothecaries' Garden. Sutton Publications, Stroud.

Newman, D.J., Cragg, G.M., 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 75, 311–335.

Sneader, W., 1996. Drug prototypes and their exploitation. Wiley, Chichester.

Solecki, R.S., 1975. Shanidar IV, a Neanderthal flower burial in Northern Iraq. Science 190, 880–881.

Sommer, J.D., 1999. The Shanidar IV 'flower burial': a re-evaluation of Neanderthal burial ritual. Cambridge Archaeological Journal 9 (1), 127–137.

Takemi, T., Hasegawa, M., Kumagai, A., Otsuka, Y., 1985. Herbal medicine: Kampo, past and present. Tsumura Juntendo Inc., Tokyo.

Totelin, L., 2015. When foods become remedies in ancient Greece: the curious case of garlic and other substances. J Ethnopharmacol 67, 30–37.

Tu, Y., 2011. The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. Nature Medicine 17, 1217–1220.

Vogellehner, D., 1987. Jardines et verges en Europe occidentale (VIII–XVIII siècles). Flaran 9, 11–40.

von Humboldt, A., 1997. Die Forschungsreise in den Tropen Amerikas. [Studienausgabe Bd 2, Teilband 3]. Wissenschaftliche Buchsgesellschaft, Darmstadt.

Waller, F., 1998. Phytotherapie der traditionellen chinesischen Medizin. Zeitschrift für Phytotherapie 19, 77–89.

Further reading

Adams, M., Berset, C., Kessler, M., Hamburger, M., 2009. Medicinal herbs for the treatment of rheumatic disorders—A survey of European herbals from the 16th and 17th century. J Ethnopharmaco 121, 343–359.

Burger, A., Wachter, H., 1998. Hunnius pharmazeutisches Wörterbuch, 8 Aufl. Walter de Gruyter, Berlin.

Harvey, A.L., Edrada-Ebel, R.A., Quinn, R.A., 2015. The re-emergence of natural products for drug discovery in the genomics era. Nat Rev Drug Discov 14, 111–129.

Section 2

Basic plant biology

SECTION CONTENTS

Chapter 3

General principles of botany: morphology and systematics

The chapters in this section provide a short introduction to the bioscientific basis for all aspects of the use of plants in pharmacy required for understanding herbal medicines and pure natural products.

The following case study shows not only that knowledge about medicinal plants is relevant, because pharmacy uses many pure natural products derived from plants, but also that pharmacists can and should advise patients about common medicinal plants.

A (HYPOTHETICAL) CASE STUDY BASED ON G HATFIELD'S RESEARCH ABOUT THE USAGE OF MEDICINAL PLANTS IN NORFOLK

While you are working as a locum pharmacist, a patient informs you that his general practitioner is worried about unexplained low levels of potassium (hypokalaemia). Among other things, the patient is complaining of chronic constipation and requests several pharmaceuticals. He also reports that he uses a 'herbal tea', which he prepares from the plant he calls 'pick-a-cheese' and grows in his back garden. This tea helps him to overcome the problem of constipation.

How do you react? Is the patient using a little known, but unproblematic, herbal product? Further inquiry about the case tells you:

- 'Pick-a-cheese' may be a widely distributed garden plant and weed known also as 'common mallow', which has the botanical name of *Malva sylvestris*, or it may be some other botanical species known under the same common name.
- Upon your request the patient brings you a branch of the plant and with the help of the scientific (botanical) literature you make a positive identification of this plant as *Malva sylvestris*. The identification is based on the features of the plant (leaves, fruit, flowers) that you are able to observe.
- In checking the active constituents (especially polysaccharides) you come to the conclusion that the plant is unlikely to contribute to the symptoms as they were reported by the patient. The plant is widely used as a local food item (also, for example, in Mediterranean France) and as a household remedy. Toxic natural products seem to be absent. Therefore, the search for a cause of the hypokalaemia continues...

[For further information on Norfolk country remedies readers are referred to Hatfield G 1994 Country remedies. The Boydell Press, Woodbridge.]

PLANTS AND DRUGS

Pharmacognosy is the study of medical products derived from our living environment, especially those derived from plants and fungi. From the botanical point of view, the first concern is how to define a pharmaceutical (or medical) plant-derived drug.

In the context of pharmacy a botanical drug is a product that is either:

- Derived from a plant and transformed into a drug by drying certain plant parts, or sometimes the whole plant, or
- Obtained from a plant, but no longer retains the structure of the plant or its organs and contains a complex mixture of biogenic compounds (e.g. fatty and essential oils, gums, resins, balms).

The term 'drug' is linguistically related to 'dry' and is presumably derived from the Middle Low German *droge* ('dry') (American Heritage Dictionary 1997).

Isolated pure natural products such as the numerous pharmaceuticals used in pharmacy are thus not 'botanical drugs', but rather chemically defined drugs

derived from nature. Botanical drugs are generally derived from specific plant organs of a plant species. The following plant organs are the most important, with the Latin name that is used, for example in international trade, in parentheses:

- Aerial parts or herb (herba).
- Leaf (folia).
- Flower (flos).
- Fruit (fructus).
- Bark (cortex).
- Root (radix).
- Rhizome (rhizoma).
- Bulb (bulbus).

The large majority of botanical drugs in current use are derived from leaves or aerial parts.

Botanically speaking, a plant-derived drug should be defined in terms of not only the species from which it is obtained, but also the plant part that is used to produce the dried product. Thus, a drug is considered to be adulterated if the wrong plant parts are included (e.g. aerial parts instead of leaves).

In the following sections of this chapter a brief overview of botanical taxonomy is given, then the higher plants are discussed on the basis of their main organs, function, morphology and anatomy. Since most of the pharmaceutical products derived from plants are from the higher plants (or Magnoliopsida), little reference is made here to other plants such as lichens, mosses, algae, or to mushrooms or micro-organisms.

Microscopic characteristics play an important role in identifying a botanical drug. Although microscopy is now only rarely used in everyday pharmaceutical practice, there is a large number of features that allow the identification of botanical material. Since classical textbooks provide an extensive description of such features, microscopic identification is only occasionally discussed in this introductory textbook.

These days, drug identification is achieved using a combination of methods, including thin-layer chromatography, high-performance liquid chromatography and microscopic methods. In large (phyto-) pharmaceutical companies, near-infrared spectroscopy has become an essential tool.

TAXONOMY

The **species** is the principal unit within the study of **systematics**. Biological diversity is subdivided into >500,000 discontinuous units (the botanical species) and >2 million zoological species. The species is thus the basic unit for studying relationships among living organisms. Systematicists study the relationships between species.

Taxonomy is the science of naming organisms and their correct integration into the existing system of nomenclature. Each of these names is called a **taxon** (pl. taxa), which thus stands for any named taxonomic unit. In order to make this diversity easier to understand, it is structured into a series of highly hierarchical categories, which ideally should represent the natural relationship between all the taxa.

The opium poppy, *Papaver somniferum* L.

Binomial: this is the genus and species names, plus the authority. Thus, in this example, *Papaver somniferum* is the binomial (the basic unit of taxonomy and systematics). It is followed by a short acronym (in this case 'L.'), which indicates the botanist who provided the first scientific description of the species and who assigned the botanical name [in this example, 'L.' stands for Carl von Linnaeus (or Linné), a Swedish botanist (1707–1778) who developed the binomial nomenclature].*

Species: *somniferum*, here meaning 'sleep-producing'.

Genus: *Papaver* (a group of species, in this case poppies, which are closely related).

Family: Papaveraceae (a group of genera sharing certain traits, named after one of the genera).

Order: Papaverales.

Class: Magnoliatae.

Subphylum: Magnoliphytina (seed-bearing plants with covered seeds).

Division (=Phylum): Spermatophyta (seed-bearing plants).

Kingdom: Plantae (the plants), one of three kingdoms, the others being the animals and fungi.

*In some cases, there is first a name in parentheses, followed by a second name not in parentheses. For example, in the case of the common aloe, *Aloe vera* (L.) Burm. f., the name in parentheses indicates the author (Linnaeus) who first described the species but assigned it to a different genus. The second name in this case, Burm. f., stands for the 18th century botanist Nicolaas Laurens Burman; f. stands for *filius* (son), since he is the son of another well-known botanist who provided numerous first descriptions of botanical species.

In this textbook we provide the full name of a plant species wherever possible, this includes the plant's binomial name (i.e. genus and species) as well as the abbreviated name of the person who described this taxon in a validated way. This is essential for exactly defining what species is used medicinally.

A species is generally characterized as having morphologically similar members and being able to inbreed. Since Carl Linnaeus, the names of species are given in binomial form: the first part of the name indicates the wider taxonomic group, the **genus**; the second part of the name is the **species**. Plant species must be reported in a taxonomically correct way and a wide range of Internet resources exist including the 'Medicinal Plant Name Service' (http://www.kew.org/kew-science/people-and-data/resources-and-databases/medicinal-plant-names-services) and 'The Plant List' (http://www.theplantlist.org).

In order to better understand biological diversity, the species are arranged into clusters of varying degree of similarity, forming a hierarchy. The basic classification of the plant kingdom into divisions circumscribes the main groups of plants, including:

- Algae, including the green algae (Chlorophyta) and the red algae (Rhodophyta).
- Mosses (Bryophyta).
- Ferns (Pteridophyta).
- Seed-bearing plants (Spermatophyta).

As mentioned above, only a few algae, mosses and ferns have yielded pharmaceutically important products and will therefore only be discussed very cursorily.

THE IMPORTANCE OF TAXONOMY

The exact naming (taxonomy) and an understanding of the species' relationship to other species is an essential basis for pharmacognostical work. Only such endeavour allows the correct identification of a botanical drug, and consequently is the basis for further pharmacological, phytochemical, analytical or clinical studies.

MORPHOLOGY AND ANATOMY OF HIGHER PLANTS (SPERMATOPHYTA)

FLOWER

The flower (Fig. 3.1) is the essential reproductive organ of a plant. It is frequently very showy in order to attract pollinators, but in other instances the flowers are minute and difficult to distinguish from the neighbouring organs or from other flowers.

For an inexperienced observer, two characteristics of a flower are particularly noteworthy: the size and

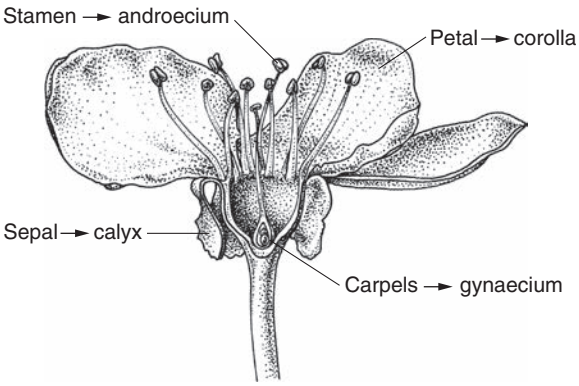


Fig. 3.1 Schematic line drawing of a flower.

the colour. Although these are often good characteristics of a species, others are more important from a botanical point of view.

Such characteristics include the form of the various parts of a flower, whether these parts are fused (joined) or separate (free), how many of each of these structures normally exist per flower, whether or not all flowers on a plant (or in a group of plants of the same species) are similar. Morphologically speaking, many parts of the flower are modified leaves, which during the development of higher plants have taken on specific functions for reproduction:

- The **calyx**, with individual sepals, generally serves as an outer protective cover during the budding stage of the flower. It is often greenish in colour, can be either fused or separate, and may sometimes drop off at the beginning of the flowering phase (e.g. *Chelidonium majus* L., greater celandine).
- The **corolla**, with individual petals, serves as an important element to attract the pollinator in animal-pollinated flowers. It is either fused or separate and may be very reduced, for example, in plants pollinated with the help of the wind. Most commonly, the number of petals is regarded as a key feature and can vary from a well-defined number (e.g. four, five or six) to a large number that is no longer counted (written as ∞). The colour of the petals is not a good characteristic generally, since it may vary within a genus or even within a species. All of these features – i.e. the number and form of the petals, whether they are fused or not and their size – are important pieces of information for identifying a plant.
- The **androecium**, with its individual **stamens** (also known as 'stamina') which produce the pollen, forms a ring around the innermost part of the

flower. In some species, the anther is restricted to only some of the flowers on a plant (whereas the others only have a gynaecium). In other species, androecium-bearing flowers are restricted to some plants, whereas the others bear flowers with only a gynaecium. Again, their number is important for identifying a plant.

- **Gynaecium** (pl. gynaecia; also called gynoecium) with individual carpels. This develops into the fruit (i.e. the seed covered by the pericarp) and includes the ovules (the part of the fruit bearing the reproductive organs which develop into the seeds).
- The **stigma** and **style** – together with the gynaecium – form the **pistil.** Their size and form are important differences between species.

Another essential aspect of the flower's morphology is the position of the gynaecium with respect to the position of the corolla on the pistil: i.e. epigynous (the corolla and other elements of the flower are attached to or near the summit of the ovary), or hypogynous (the corolla and other elements of the flower are attached at or below the bottom of the ovary).

Inflorescences

The way in which flowers are arranged to form an inflorescence is another useful feature for recognizing (medicinal) plants, but this is beyond the scope of this introduction (Heywood 1993).

Drugs

Although the flowers are of great botanical importance, they are only a minor source of drugs used in phytotherapy or pharmacy. A very important example is:

- Chamomile, *Matricaria recutita* L. (Matricariase flos).

Other examples include:

- Calendula, *Calendula officinalis* L. (Calendulae flos).
- Arnica, *Arnica montana* L. (Arnicae flos).
- Hops, *Humulus lupulus* L. (Humuli flos).

FRUIT AND SEED

The development of seeds occurred relatively late in the evolution of plants. The lower plants, such as algae, mosses and ferns, do not produce seeds. Gymnosperms such as the maiden hair tree (*Ginkgo biloba* L.) (see below) were the first group of organisms to produce seeds, from which the angiosperms or fruit-bearing plants evolved. The gymnosperms are characterized by seeds that are not covered by a secondary outer protective layer, but only by the testa – the seed's outer layer.

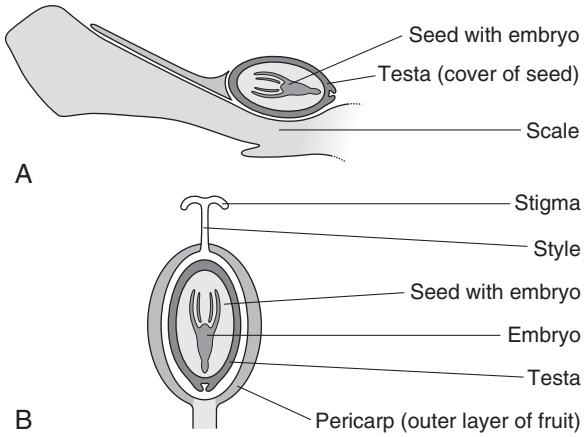


Fig. 3.2 Simplified schematic representation of (A) a gymnosperm seed sitting on a scale (as in a fir tree) and (B) an angiosperm fruit, covered by both the testa and the pericarp.

In the angiosperms, the ovule and later the seed are covered with a specialized organ (the carpels), which in turn develops into the pericarp (Fig. 3.2). This, the outer layer of the fruit, can either be hard as in nuts, all soft as in berries (dates, tomatoes), or hard and soft as in a drupe (cherry, olives). Drugs from the fruit thus have to be derived from an angiosperm species.

The morphology of a fruit provides important information as to the identity of a plant species or medicinal drug. Another distinction of fruits is based on the number of carpels and gynaecia per fruit, which may be:

- Simple (developed from a single carpel).
- Aggregate (several carpels of one gynaecium are united in one fruit, as in raspberries and strawberries).
- Multiple (gynaecia of more than one flower form the fruit).

Drugs

Fruits and seeds have yielded important phytotherapeutic products, including:

Fruit

- Caraway, *Carum carvi* L. (Carvi fructus).
- Fennel, *Foeniculum vulgare* Miller (Foeniculi fructus).

- Saw palmetto, *Serenoa repens* (Bartram) Small (Sabal fructus).
- Schizandra/schisandra, *Schisandra chinensis* Baillon (Schisandrae fructus).

Seed

- (White) mustard, *Sinapis alba* L. (Sinapi semen).
- Horse chestnut seeds, *Aesculus hippocastanum* L. (Hippocastani semen).
- Ispaghula, *Plantago ovata* Forssk. and *Plantago* spp. (Plantago ovatae semen), and psyllium, *Plantago afra* L. (=*P. psyllium*, Psylli semen).

LEAVES

The leaves arise out of the stem; their key function is the assimilation of glucose and its derivative, starch, from water and carbon dioxide (photosynthesis) using energy provided by sunlight.

PHOTOSYNTHESIS

The net photosynthetic reaction is:

$$6CO_2 + 6H_2O \xrightarrow{h\nu} C_6H_{12}O_6 \text{ (glucose)} + 6O_2$$

This process is key, not only to the survival of all plants, but also in providing the energy and, ultimately, the basic building blocks for the secondary metabolites, which are used as pharmaceuticals.

The function of the leaves, as collectors of the sun's energy and its assimilation, results in their typical general anatomy with a petiole (stem) and a lamina (blade). In many cases, the petiole is reduced and may be missing completely. Plants have adapted to a multitude of environments and this adaptation is reflected in the anatomical and morphological features of the leaf. For example, adaptation to dry conditions gives rise to leaves that conserve moisture, which may be fleshy or possess a thick cuticle. These are termed xerophytic leaves, and include oleander (*Nerium oleander* L.).

The lower surface of the leaf is generally covered with stomata, pores that are surrounded by specialized cells and that are responsible for the gaseous exchange between the plant and its environment (uptake of CO_2 and emission of water vapour and O_2).

The nodes (or 'knots') are the parts of the stem where the leaves and lateral buds join; the intermediate area is called the internodium. A key characteristic of a species is the way in which the leaves are arranged on the stem. For example, they may be (Fig. 3.3):

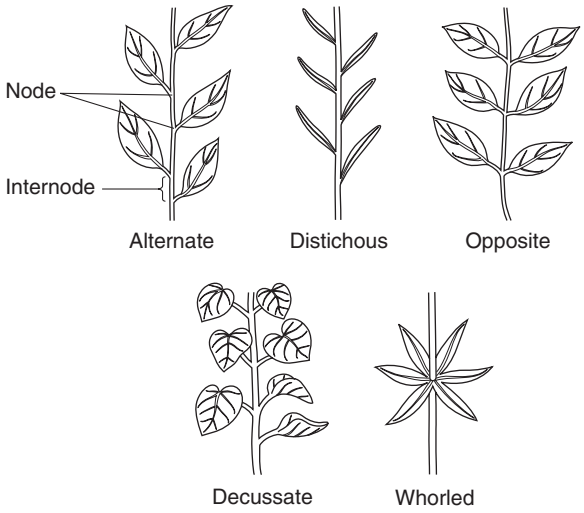


Fig. 3.3 Types of arrangements of leaves.

- **Alternate**: the leaves form an alternate or helical pattern around the stem, also called spiral.
- **Distichous**: there is a single leaf at each node, and the leaves of two neighbouring nodes are disposed in opposite positions.
- **Opposite**: the leaves occur in pairs, with each leaf opposing the other at the nodes.
- **Decussate**: this is a special case of opposite, where each successive pair of leaves is at a right angle to the previous pair (typical for the mint family).
- **Whorled**: three or more leaves are found at one node.

Another important characteristic is the form of the leaves. Typically, the main distinction is between simple or compound. Simple leaves have blades that are not divided into distinct morphologically separate leaflets, but form a single blade, which may be deeply lobed. In compound leaves, there are two or more leaflets, which often have their own small petioles (called petiolules). The form and size of leaves are essential characteristics (Fig. 3.4A). For example, leaves may be described as oval, oblong, rounded, linear, lanceolate, ovate, obovate, spatulate or cordate. The margin of the leaf is another characteristic feature. It can be entire (smooth), serrate (saw-toothed), dentate (toothed), sinuate (wavy) or ciliate (hairy) (Fig. 3.4B). Also, the base and the apex often have a very characteristic form.

Microscopic characteristics of leaves include the form and number of stomata, the inner structure of the leaves, specialized secretory tissues including trichomes (glandular hairs), covering trichomes or

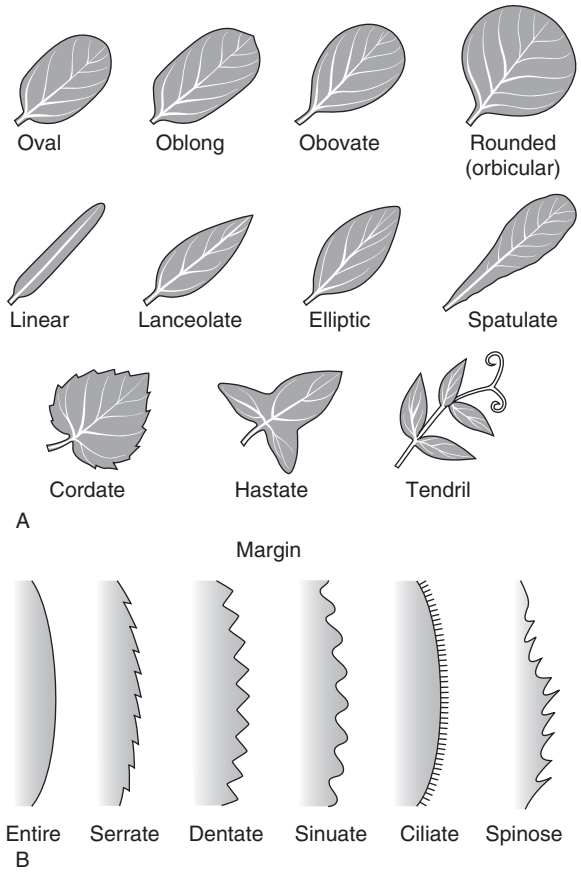


Fig. 3.4 (A) Characteristic shapes of leaves. (B) Characteristic margins of leaves.

bristles, and the presence of calcium oxalate structures, which give a characteristic refractive pattern under polarized light.

The powdered leaves of several members of the nightshade family (Solanaceae), which yield some botanical drugs that are important for the industrial extraction of the alkaloid atropine, cannot be distinguished using normal chemical methods since they all contain similar alkaloids. On the other hand, they can easily be distinguished microscopically by the presence of different forms of crystals formed by the different species and deposited in the cells (Fig. 3.5).

Drugs

Numerous drugs contain leaf material as the main component. Some widely used ones include:

- (Common) balm, *Melissa officinalis* L. (Melissae folium).
- Deadly nightshade, *Atropa belladonna* L. (Belladonnae folium) (and other solanaceous species).

None of these are used in phytotherapy, but rather for the extraction of alkaloids, of which they have a high content.

- Ginkgo, *Ginkgo biloba* L. (Ginkgo folium).
- Green tea, *Camellia sinensis* (L.) Kuntze (Theae folium).
- Peppermint, *Mentha* × *piperita* L. (Menthae folium).
- (Red) bearberry, *Arctostaphylos uva-ursi* (L.) Spreng. (Uvae ursi folium).

SHOOTS (=STEM, LEAVES AND REPRODUCTIVE ORGANS)

An essential differentiation needs to be made between herbaceous ('herbs') and woody plants (trees and shrubs). In both cases, the function of the stem is to provide the physical strength required for positioning the leaves/flowers and fruit in the most adaptive way. The stem is a cylindrical organ that, together with the root, forms the main axis of a plant. Herbaceous species are generally short-lived and often grow rapidly and the distinction between the outside and the inner stem can only be made by detailed examination. Woody species, on the other hand, show a clear distinction between the bark and the (inner) wood.

In the stem, the transport of water and inorganic nutrients (upward transport) is achieved in the **xylem**, which only occurs in the inner parts of the stem and forms an essential part of the wood. The **phloem**, on the other hand, is the plant part responsible for the transport of assimilates (sugars and polysaccharides), which generally occurs from the leaves downwards. Between the wood and the bark is the cambium, the tissue that gives rise to new cells, which then differentiate and form the outer (bark) and inner (wood) parts of a secondary stem. The fine structure of a bark or wood is an important diagnostic criterion for identifying a drug. The bark as an outer protective layer frequently accumulates biologically active substances; for example, several of the pharmaceutically important barks accumulate tannins.

Drugs: stem

Stem material is often part of those drugs that are derived from all above-ground parts (herb or herba). No

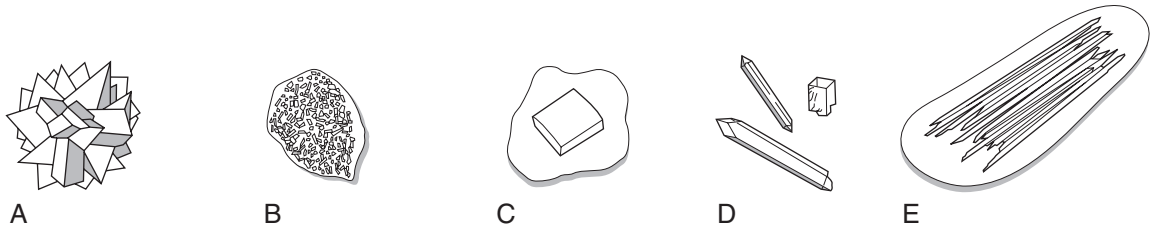


Fig. 3.5 Calcium oxalate crystals, main forms: (A) rosette (e.g. *Datura stramonium*, Solanaceae); (B) sand (e.g. *Atropa belladonna*, Solanaceae); (C) monoclinic prism (e.g. *Hyosyamus niger*, Solanaceae); (D) needles (e.g. *Iris germanica*, Iridaceae); (E) raphides (e.g. *Urginea maritima*, Hyacinthaceae).

stem-derived drug is currently of major importance. Some underground organs used as drugs (rhizome of tormentil) or food (potato) are in fact modified stems that have taken on specific new functions (storage, spreading of the plant) (see below).

Drugs: bark

- Frangula, *Frangula alnus* Mill. (syn. *Rhamnus frangula* L.) (Frangulae cortex).
- Red cinchona, *Cinchona pubescens* Vahl (syn.: *Cinchona succirubra* Pav. ex Klotzsch), *C. calisaya* Wedd. (the main cultivated species in southern Asia) and *Cinchona* spp. (Cinchonae cortex).
- Oak, *Quercus petraea* (Matt.) Liebl. and *Qu. robur* L. (Quercus cortex).
- Willow, *Salix alba* L. and *Salix* spp. (Salicis cortex).

Drugs: aerial parts (=stem, leaves plus flowers/fruit)

- Ephedra, *Ephedra sinica* Stapf (Ephedra herba).
- Hawthorn, *Crataegus monogyna* Jacq. and *C. laevigata* (Poir.) DC. (syn. *C. oxycantha*) (Crataegi herba or Crataegi folium cum flore).
- Passion flower, *Passiflora incarnata* L. (Passiflora herba).
- Wormwood, *Artemisia absinthium* L. (Absinthii herba); in Africa and Asia, sweet or annual wormwood (*Artemisia annua* L.) is used in the treatment of malaria.

The substitution of leaves with aerial parts of the same species is a common problem with cheap phytopharmaceuticals used as 'health food supplements'. These adulterated drugs often contain fewer and/or other active constituents and this points to the need to define not only the species, but also the plant part to be used pharmaceutically.

ROOT

Three functions of a typical root are of particular importance to a plant:

- It provides an anchor in the ground or any other substrate and thus allows the development of the plant's above-ground organs (anchorage).
- It is the main organ for the uptake of water and inorganic nutrients (absorption and conduction).
- It often serves to store surplus energy, generally in the form of polysaccharides such as starch and inulin (storage).

The root is generally composed of an outer layer (the bark of the root including the hypodermis) and an inner cylinder, containing the xylem and the phloem. The two organs of the root are separated by the endodermis, an inner protective layer. Water and inorganic nutrients are transported upwards in the xylem; assimilates are transported in the phloem.

Very young plants have a primary root, which during development soon becomes thicker and adds layers of secondary tissue. It is the secondary roots – often roots or rootstocks with a special storage function – that are used in pharmacy.

ROOTSTOCK AND SPECIALIZED UNDERGROUND ORGANS

Some underground organs can be distinguished on botanical grounds from the root. Although they may have some functions similar to roots, botanically they are derived from other parts of the plants; they are therefore a separate group of plant organs and yield another group of botanical drugs. They include rhizomes and tubers (generally, both are morphologically a stem) and underground bulbs (morphologically derived from parts of the leaves).

Rhizome and root (radix) drugs

Underground organs of only a few species have yielded pharmaceutically important drugs. Examples include:

- Devil's claw, *Harpagophytum procumbens* (Burch.) DC. ex Meisn. (Harpagophyti radix, thickened roots).
- Korean ginseng, *Panax ginseng* C. A. Mey. (Ginseng radix).
- Tormentill, *Potentilla erecta* (L.) Raeusch. (syn. *Potentilla tormentilla* Stokes, Potentillae radix).
- Echinacea, *Echinacea angustifolia* DC., *E. pallida* Nuttall and *E. purpurea* (L.) Moench (Echinacea radix).
- Siberian ginseng, *Eleutherococcus senticosus* Maximimowicz (Eleutherococci radix).
- Kava-kava, *Macropiper methysticum* (G.Forst.) Miq. (better known under its synonym: *Piper methysticum* G.Forst.; Rhizoma Piperis Methystici – kava-kava rhizome).
- Chinese foxglove root, *Rehmannia glutinosa* (Gaertn.) DC. (Rehmannia radix).
- Rhubarb, *Rheum palmatum* L. and *Rh. officinale* Baill., as well as their hybrids (Rhei radix, thickened roots).
- Sarsaparilla, *Smilax ornata* Lem. *Smilax regelii* Killip & C.V.Morton, and *Smilax* spp. (Sarsaparillae radix).

DIVERSE AND UNSPECIFIED BOTANICAL DRUGS

Some drugs are derived from the whole plant or from specialized organs (e.g. the bulbs in the case of garlic, *Allium sativum* L.). The exudates of *Aloe vera* (L.) Burm. f. (syn. *Aloe barbadensis* Mill.) leaves are used as a strong purgative.

References

(For further references see Chapter 4).

American Heritage Publishing Company, 1997. American heritage dictionary, third ed. Houghton Mifflin Harcourt, Boston.

Heywood, V.H., 1993. Flowering plants of the world. Batsford Ltd., London.

Chapter 4

Families yielding important phytopharmaceuticals

Systematics has always been an important tool in pharmacognostical practice and research. Related families often contain similar types of compounds and, therefore, may have similar beneficial or toxic effects. Consequently, an understanding of the systematic position of a medicinal plant species allows some deductions to be made about the (biologically active) secondary natural products from the species. For example, many members of the mint family are known to contain essential oil.

In this chapter, the pharmaceutically most important families are highlighted, especially those that have yielded many, or very important, botanical drugs. Since a species may yield several botanical drugs (e.g. from the flowers and the leaves), these are not included in this chapter, but can be found in Part B. Here, 20 families (out of a total of more than 200 recognized families) have been selected as being particularly important or interesting and are presented in alphabetical order within the groupings angiosperms and gymnosperms. The families are not classified further; more detailed information on the systematic position of these families can be found in relevant botanical textbooks (see p. 51 and in 'Angiosperm Phylogeny Group' 2016).

ANGIOSPERMS (MAGNOLIPHYTA)

These are the plants commonly known as 'fruit-bearing plants', i.e. the seed is covered by closed carpels. The fruit are sometimes very large and yield many of the economically important botanical products used because of their nutritional properties. An important characteristic of these plants is double fertilization, in which cells other than the egg unite during the fertilization to give a triploid endosperm. This then develops into the fruit, which may also include other parts of the flowers. The flowers are typically fertilized by animals (i.e. zoogamous; mostly insects, but also birds, bats and spiders). Many species of this huge group have secondarily lost this trait and are fertilized with the help of the wind (e.g. oak, birch). At least 240,000 species of angiosperm are known, making it the largest group of plants. Many estimates, however, are much higher.

The taxon was originally split into two large groups – the Dicotyledoneae and the Monocotyledoneae – distinguished, *inter alia*, by the different number of cotyledons (primary leaves), but modern systematic classifications reject this division into only two groups.

ALLIACEAE ('MONOCOTYLEDONEAE')

Allium is the only important genus of this family, which includes not only important food plants, such as the common onion (*Allium cepa* L.), leek (*A. porrum* L.) and chives (*A. schoenoprasum* L.), but also the medicinal plant garlic (*A. sativum* L.). The genus is often included in the Liliaceae (i.e. the broadly defined lily family).

Important medicinal plant from the family

- *Allium sativum* L. (garlic, see Chapter 14).

Morphological characteristics of the family

These perennial herbs have underground storage organs (onions), which are used for hibernation. Typically, the flowers are composed of a perianth of two whorls of three with the sepals and petals having identical shape (i.e. the calyx and corolla are indistinguishable),

six stamens and three superior, fused gynaecia. This is, in fact, the typical composition of the flowers of many related families that were previously united with the Liliaceae. The leaves are simple, annual, spirally arranged, parallel-veined and often of a round shape. The fruit is a capsule.

Distribution

The 700 species of family are found in northern temperate to Mediterranean regions.

Chemical characteristics of the family

The genus *Allium* is particularly well known for its very simple sulphur-containing compounds, especially alliin and allicin (Fig. 4.1), which are thought to be involved in the reported pharmacological activities of the plant as a bactericidal antibiotic, in the treatment of arterial hypertension and in the prevention of arteriosclerosis and stroke.

APIACEAE (ALSO CALLED UMBELLIFERAE)

Important medicinal plants in the family (see also various monographs in Chapter 20)

- *Carum carvi* L. (caraway), a carminative and also important as a spice.
- *Coriandrum sativum* L. (coriander), a carminative and also important as a spice.
- *Foeniculum vulgare* Mill. (fennel), a mild carminative.
- *Levisticum officinale* W.D.J.Koch (lovage), a carminative and antidyspeptic.
- *Pimpinella anisum* L. (anise-fruit, wrongly called 'seed'), an expectorant, spasmolytic and carminative.

Morphological characteristics of the family

This family of nearly exclusively herbaceous species is characterized by hermaphrodite flowers in a double umbel (Fig. 4.2); note that the closely related Araliaceae have a simple umbel. Typical for the family are the furrowed stems and hollow internodes, leaves with a sheathing base and generally a much-divided lamina. The flowers are relatively inconspicuous, with two pistils, an inferior gynaecium with two carpels, a small calyx and generally a white to greenish corolla, with free petals and sepals.

Distribution

Members of this family, which has about 3000 species, are mostly native to temperate regions of the northern hemisphere.

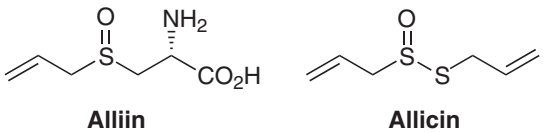
Chemical characteristics of the family

Unlike the Araliaceae, members of this family are often rich in essential oil, which is one of the main reasons for the pharmaceutical importance of many of the apiaceous drugs (see above). Also common are 17-carbon skeleton polyacetylenes, which are sometimes poisonous, and (furano-)coumarins, which are responsible for phototoxic effects (e.g. in *Heracleum mantegazzianum* Sommier and Levier, hogweed). Some species accumulate alkaloids (e.g. the toxic coniine from hemlock, *Conium maculatum* L.).

ARALIACEAE

Important medicinal plants from the family

- *Hedera helix* L. [(common) ivy], used as a cough remedy.
- *Panax ginseng* C. A. Mey. (ginseng), used as an adaptogene (a very ill-defined category) and to combat mental and physical stress [and sometimes replaced by *Eleutherococcus senticosus* (Rupr. and



Alliin **Allicin**

Fig. 4.1

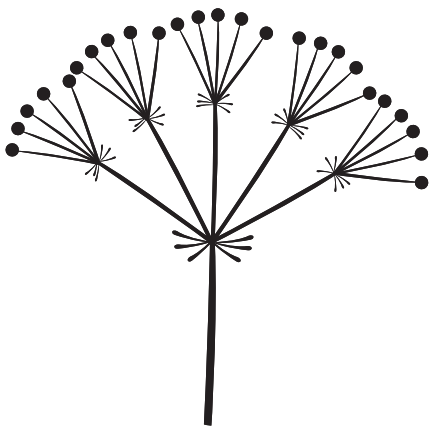


Fig. 4.2 Double umbel.

Maxim.) Maxim. (syn.: *Acanthopanax senticosus* (Rupr. & Maxim.) Harms) from the same family].

Morphological characteristics of the family

This family consists mostly of woody species, characterized by hermaphrodite flowers in a simple umbel (see the closely related Apiaceae with a double umbel). The leaf lobes are hand-shaped, and the flowers relatively inconspicuous with two pistils, an inferior gynaecium, a small calyx and generally a white to greenish corolla, with free petals and sepals.

Distribution

This family of >700 species is widely dispersed in tropical and subtropical Asia and in the Americas. *Hedera helix* is the only species native to Europe.

Chemical characteristics of the family

Of particular importance from a pharmacognostical perspective are the saponins, triterpenoids and some acetylenic compounds. The triterpenoids (ginsengosides) are implicated in the pharmacological effects of *Panax ginseng*, while saponins (hederasaponins) are of relevance for the secretolytic effect of *Hedera helix*.

ASPHODELACEAE ('MONOCOTYLEDONEAE')

This family is often included in the Liliaceae (lily family).

Important medicinal plants from the family

- *Aloe vera* (L.) Burman f. (syn. *Aloe barbadensis*, Barbardos aloe) and A. *ferox* Miller (Cape aloe), both strong purgatives (see p. 238, Chapter 20).

Morphological characteristics of the family

Members of this family are generally perennials, and, in the case of *Aloe*, usually woody, with a basal rosette and the typical radial hermaphrodite flower structure of the Liliales. The petals and sepals are identical in form and colour, and composed of 3+3 free or fused, 3+3 free stamens and three fused superior carpels.

Distribution

This family, with about 600 species, is widely distributed in South Africa (a characteristic element of the Cape flora); some species occur naturally in the Mediterranean (*Asphodelus*).

Chemical characteristics of the family

Typical for the genus *Aloe* are anthranoids and anthraglycosides (aloe-emodin), which are responsible for the species' laxative effects, as well as polysaccharides accumulating in the leaves. Contrary to other related families, the Asphodelaceae do not accumulate steroidal saponins.

ASTERACEAE – THE 'DAISY' FAMILY (ALSO KNOWN AS COMPOSITAE)

This large family has kept botanists busy for many centuries and still no universally accepted classification exists. All members of the family have a complex inflorescence (the capitula), which gave rise to the older name of the family: Compositae (=inflorescence composed of many flowers). In other features, the family is rather diverse, especially with respect to its chemistry.

Important medicinal plants from the family

- *Arnica montana* L. (arnica), used topically, especially for bruises.
- *Artemisia absinthum* L. (wormwood or absinthium), used as a bitter tonic and choleretic.
- *Calendula officinalis* L. (marigold), used topically, especially for some skin afflictions.
- *Cnicus benedictus* L. (cnicus), used as a cholagogue (a bitter aromatic stimulant).
- *Cynara scolymus* L. (artichoke), used in the treatment of liver and gallbladder complaints and several other conditions.
- *Echinacea angustifolia* DC., *E. pallida* Nuttall and *E. purpurea* (L.) Moench (Cone flower), now commonly used as an immunostimulant.
- *Matricaria recutita* L. (chamomille/camomille; several botanical synonyms are also commonly used, including *Chamomilla recutita* and *Matricaria chamomilla*) (see Chapter 20, pp. 240–241).
- *Tussilago farfara* L. (coltsfoot), a now little used expectorant and demulcent.

Morphological characteristics of the family (Fig. 4.3)

The family is largely composed of herbaceous and shrubby species, but some very conspicuous trees are also known. The most important morphological trait is the complex flower head, a flower-like structure, which

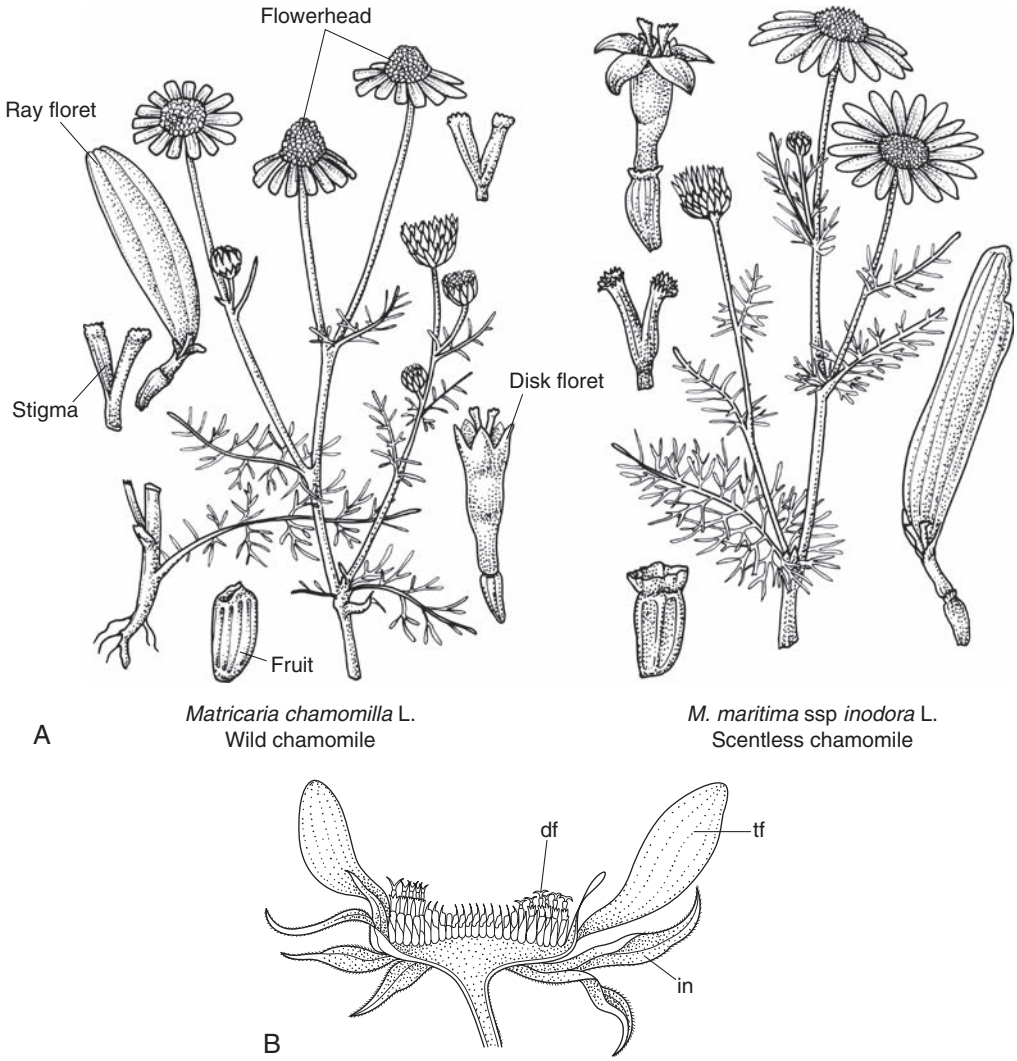


Fig. 4.3 (A) Two members of the genus *Matricaria*. (Left) *Matricaria chamomilla* L. is aromatic and used medicinally. (Right) *Matricaria maritima* L. subsp. *inodora* Schultz [=*Tripleurospermum perforatum* (Mérat) Wagenitz], also known as *Matricaria inodora*, is not aromatic and is not used medicinally. The illustration shows typical morphological differences in these two species, such as the form of the flower heads and the fruit, but it also shows how similar the two species are in many other characteristics. From Fitch (1924). (B) Schematic of typical flower heads (a capitulum) of the Asteraceae (compositae). df, disk flowers; tf, tubular flowers; in, involucre. From Brimble LFJ 1942 Intermediate Botany. Macmillan, London.

may in fact be composed of a few or many flowers (**capitulum** or **pseudanthium**). In some sections of the family (e.g. the subfamily Lactucoideae, which includes lettuce and dandelion), only ligulate (tongue-shaped) or disk (ray) florets are present in the dense heads. In the other major segment (subfamily Asteroideae), both ligulate and radiate/discoid flowers are present on the same flower head, the former generally forming an outer, showy ring with the inner often containing large amounts of pollen. The flowers are epigynous, bisexual or sometimes female, sterile or functionally male. The (outer) calyx has five fused sepals and in many instances later develops into a pappus (featherlike in dandelions, in other instances more bristly), which is used as a means for dispersing the fruit; it is lacking in many other taxa. The fused petals (generally five) form a tubus or a ligula. The two gynaecia are epigynous and develop into tiny, nut-like fruits (achene or cypsela). The leaves are generally spirally arranged, simple, dissect or more or less compound.

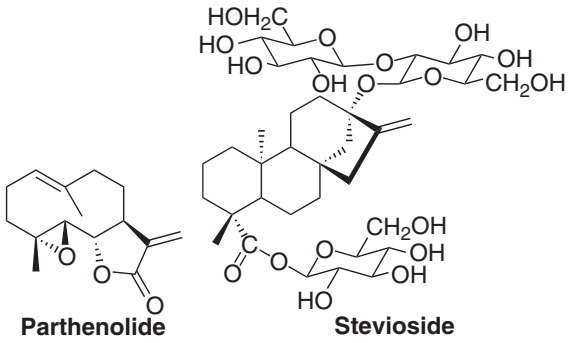


Fig. 4.4

Distribution

More than 21,000 species are known from practically all parts of the world, with the exception of Antarctica, and the family has found niches in a large variety of ecosystems. The family is particularly well-represented in Central America and southern North America (Mexico).

Chemical characteristics of the family

A typical chemical trait of this family is the presence of polyfructanes (especially inulin) as storage carbohydrates (instead of polysaccharides) in perennial taxa. Inulin-containing drugs are used for preparing malted coffee (e.g. from the rootstocks of *Cichorium intybus*, chicory). In many taxa, some segments of the family accumulate sesquiterpene lactones (typically with 15-carbon atoms such as parthenolide; Fig. 4.4), which are important natural products responsible for the pharmacological effects of many botanical drugs such as *Chrysanthemum parthenium* (feverfew) and *Arnica montana* (arnica). Polyacetylenic compounds (polyenes), and essential oil, are also widely distributed. Some taxa accumulate pyrrolizidine alkaloids, which, for example, are present in *Tussilago farfara* (coltsfoot) in very small amounts. Many of these alkaloids are known for their hepatotoxic effects. Other taxa accumulate unusual diterpenoids; the diterpene glycoside stevioside (see Fig. 4.4), for example, is of interest because of its intensely sweet taste.

CAESALPINIACEAE

This family was formerly part of the Leguminosae (or Fabaceae) and is closely related to two other families: the Fabaceae (see below) and the Mimosaceae (not discussed). Many contain nitrogen-fixing bacteria in root nodules. This symbiotic relationship is beneficial to both partners (for the plant, increased availability of physiologically usable nitrogen; for the bacterium, protection and optimal conditions for growth).

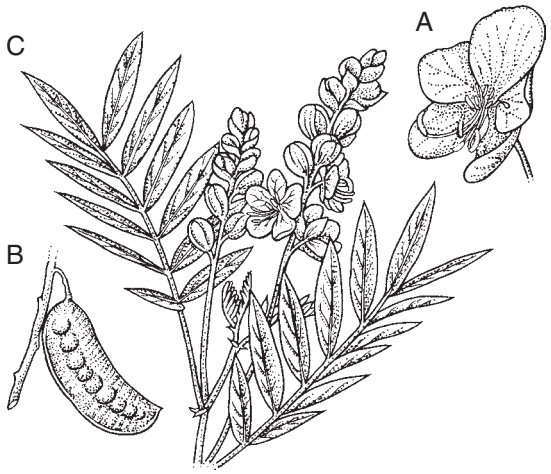


Fig. 4.5 *Cassia angustifolia*, a typical Caesalpiniaceae: (A) typical zygomorphic flower (yellow in its natural state); (B) fruit (one of the botanical drugs obtained from the species); (C) flowering branch showing the leaves composed of leaflets, and the inflorescence. Modified after Frohne & Jensen (1998).

Important medicinal plants from the family

- *Cassia senna* L. and *C. angustifolia* Vahl (Senna), used as a cathartic.

Morphological characteristics of the family (Fig. 4.5)

Nearly all of the taxa are shrubs and trees. Typically, the leaves are pinnate. The free or fused calyx is composed of five sepals, the corolla of five generally free petals, the androecium of ten stamens, with many taxa showing a reduction in the number of stamens (five) or the development of staminodes instead of stamens. The flowers are zygomorphic and have a very characteristic shape if seen from above, resembling a shallow cup.

Distribution

The 2000 species of this family are mostly native to tropical and subtropical regions, with some species common in the Mediterranean region. The family includes the ornamental *Cercis siliquastrum* L. (the Judas tree), native to the western Mediterranean, which, according to (very doubtful) legend, was the tree on which Judas Iscariot hanged himself.

Chemical characteristics of the family

From a pharmaceutical perspective, the presence of anthranoides with strong laxative effects is of particular interest. Other taxa accumulate alkaloids, such as the diterpene alkaloids of the toxic *Erythrophleum*.

FABACEAE

This family is also classified together with the Mimosaceae and the Caesalpiniaceae as the Leguminosae (or Fabaceae, s.l.; see note under Caesalpiniaceae). One of its most well-known characteristics is that many of its taxa are able to bind atmospheric nitrogen.

Important medicinal plants from the family

- *Cytisus scoparius* (L.) Link (common or Scotch broom), which yields sparteine (formerly used in cardiac arrhythmias, as an oxytoxic, and in hypotonia to raise blood pressure).
- *Glycyrrhiza glabra* L. (liquorice), used as an expectorant and for many other purposes.
- *Melilotus officinalis* L. (melilot or sweet clover); the anticoagulant drug warfarin was developed from dicoumarol, first isolated from spoiled hay of sweet clover.
- *Physostigma venenosum* Balfour (Calabar bean), a traditional West African arrow poison, which contains the cholinesterase inhibitor physostigmine, used as a myotic in glaucoma, in postoperative paralysis of the intestine and to counteract atropine poisoning.

Morphological characteristics of the family

This family is characterized by a large number of derived traits. Most of the taxa of this family are herbaceous, sometimes shrubby and only very rarely trees. Typically, the leaves are pinnate and sometimes the terminal one is modified to form a tendril, used for climbing. Bipinnate leaves are not found in this family. The five sepals are at least basally united. The corolla is formed of five petals and has a very characteristic butterfly-like shape (papilionaceous), with the two lower petals fused and forming a keel-shaped structure, the two lateral ones protruding on both sides of the flower and the largest petal protruding above the flower, being particularly showy. The androecium of ten stamens generally forms a characteristic tubular structure with at least nine out of ten of the stamens forming a sheath. Normally, the fruit are pods, containing beans (technically called legumes) with two sutures, which open during the drying of the fruit (Fig. 4.6).

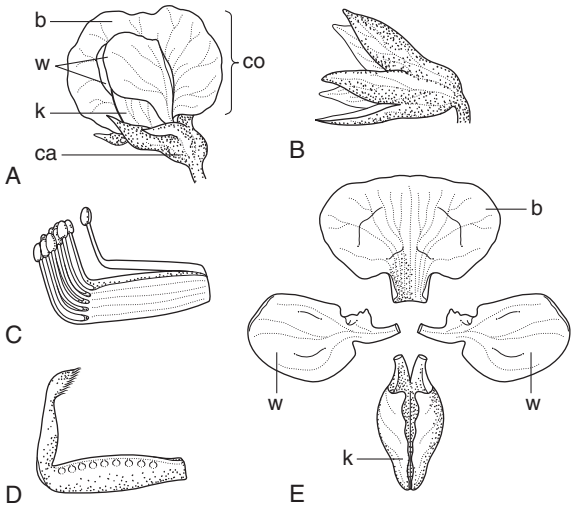


Fig. 4.6 Flower of *Pisum sativum* (common pea, Fabaceae, sensu stricto): (A) entire flower showing the various elements of the corolla (CO; b, banner; w, wing (two); k, keel; ca calyx); (B) calyx; (C) stamens (nine fused and one free); (D) gynaecium; (E) the four petals of the corolla. Modified after Frohne D, Jensen U 1998 Systematik des Pflanzenreichs, 5 Aufl. Wissenschaftliche Verlagsgesellschaft, Stuttgart.

Distribution

This is a cosmopolitan family with about 11,000 species, and is one of the most important families. It includes many plants used as food: for example, numerous species of beans (*Phaseolus* and *Vigna* spp., *Vicia faba* L.), peas (*Pisum sativum* L.), soy [*Glycine max* (L.) Merrill], fodder plants (*Lupinus* spp.) and medicines (see above).

Chemical characteristics of the family

This large family is characterized by an impressive phytochemical diversity. Polyphenols (especially flavonoids and tannins) are common, but from a pharmaceutical perspective the various types of alkaloids are probably the most interesting and pharmaceutically relevant groups of compounds. In the genera *Genista* and *Cytisus* (both commonly called broom) as well as *Laburnum*, quinolizidine alkaloids, including cytisine and sparteine (Fig. 4.7), are common. The hepatotoxic pyrrolizidine alkaloids are found in this family (e.g. in members of the genus *Crotolaria*).

Other important groups of natural products are the isoflavonoids, known for their oestrogenic activity, and the coumarins used as anticoagulants (see *Melilotus officinalis* above). *Glycyrrhiza glabra* L. (licorice) is used because of its high content of the triterpenoid glycyrrhic acid, which, if joined to a sugar, is called

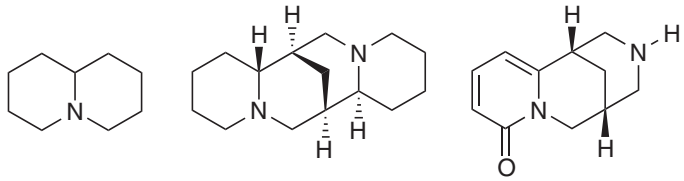


Fig. 4.7

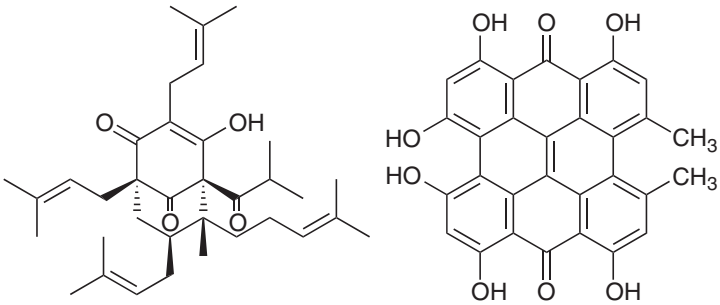


Fig. 4.8

glycyrrhizin (a saponin) and is used in confectionery as well as in the treatment of gastric ulcers (controversial). Last, but not least, the lectins must be mentioned. These large (MW 40,000–150,000), sugar-binding proteins agglutinate red blood cells and are a common element of the seeds of many species. Some are toxic to mammals, for example phasin from the common bean (*Phaseolus* spp.), which is the cause of the toxicity of uncooked beans.

HYPERICACEAE

This small family was formerly part of the Guttiferae and is of pharmaceutical importance because of St John's wort, which in the last decade of the 20th century became one of the most important medicinal plants in Western medicine.

Important medicinal plant from the family

- *Hypericum perforatum* L. (St John's wort) has clinically well-established effects in mild forms of depression. It has also been employed topically for inflammatory conditions of the skin.

Morphological characteristics of the family

The leaves are opposite, often dotted with glands. A characteristic feature of this family is a secondary increase in the number of stamens (polyandrous flowers). The fruit are usually capsules, but berries may occur in some species.

Distribution

This family, with about 900 species, has its main area of distribution in the tropics and in temperate regions.

Chemical characteristics of the family

The former name Guttiferae is an important indicator of a characteristic chemical feature: the presence of resins, balsam and other glands containing excretory products. For example, the hypericin glands, with a characteristic red colour, are present especially in the flowers and contain naphthodianthrones, including hypericin (Fig. 4.8) and pseudohypericin, which are characteristic for some sections of the genus. Typical of the family in general are also xanthones (found nearly exclusively in this family and in the Gentianaceae). The genus is known to accumulate flavonoids and their glycosides (rutoside, hyperoside), as well as hyperforin (see Fig. 4.8) and its derivatives, which are derived from the terpenoid pathway.

LAMIACEAE

The Lamiaceae is a family yielding a high number of medicinal taxa, especially due to their high content of essential oil.

Important medicinal plants from the family

- *Lavandula angustifolia* Miller (lavender), a mild carminative and spasmolytic.
- *Melissa officinalis* L. (balm), a mild sedative, carminative and spasmolytic.
- *Mentha arvensis* L. var. *piperascens* Malinvand (Japanese mint), yields a commonly used essential oil (e.g. for respiratory problems).
- *Mentha* × *piperita* L. (peppermint), a commonly used carminative and spasmolytic and a hybrid between *M. spicata* L. and *M. aquatica* L. (see Chapter 20, pp. 245–246)
- *Mentha spicata* L. (spearmint), commonly used in toothpaste and chewing gum, with mild carminative effects.
- *Rosmarinus officinalis* L. (rosemary), a carminative and spasmolytic.
- *Salvia officinalis* L. (sage), used as a topical antiseptic (gargling) and orally as a carminative and spasmolytic.
- *Thymus vulgaris* L. (thyme), a carminative and spasmolytic.

Morphological characteristics of the family (Fig. 4.9)

Most of the taxa in this family are herbs or small shrubs with the young stems often being four-angled. All of them have opposite simple or rarely pinnate leaves. The zygomorphic flowers, with very characteristic shortstalked epidermal glands, are very typical. They are bisexual, with five fused sepals, five generally zygomorphic petals, four or two stamens and two very characteristic fused gynaecia each divided into two partial units developing into a nut with a secondary division into nutlets.

Distribution

This important family with 5600 species is cosmopolitan and has a centre of distribution spanning from the Mediterranean to Central Asia.

Chemical characteristics of the family

Essential oil in the epidermal glands is very common. Some segments of the family are known to accumulate monoterpenoid glycosides (iridoids). Many species also accumulate rosmarinic acid and other derivatives of caffeic acid. Rosmarinic acid (Fig. 4.10) is of some pharmaceutical importance because of its non-specific complement activation and inhibition of the biosynthesis of leukotrienes (leading to an anti-inflammatory effect), as well as its antiviral activity.

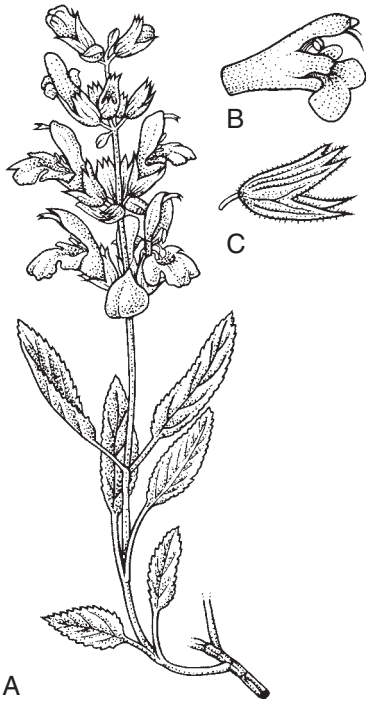
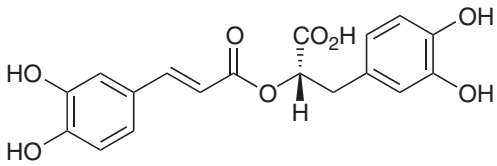


Fig. 4.9 *Salvia officinalis* (sage, Lamiaceae): (A) flowering branch showing typical leaves and the inflorescence; (B) corolla; (C) calyx. After Frohne D, Jensen U 1998 Systematik des Pflanzenreichs, 5 Aufl. Wissenschaftliche Verlagsgesellschaft, Stuttgart.



Rosmarinic acid

Fig. 4.10

PALMACEAE (ARECACEAE, PALMAE, 'MONOCOTYLEDONEAE')

The palms are particularly important because they include many species widely used as food, but in recent years at least one has become medically important.

Important medicinal plant from the family

- *Serenoa repens* (Bartram) Small (saw palmetto, sabal), for difficulty in micturition in benign prostate hyperplasia in the early stages.

Morphological characteristics of the family

These are generally unbranched, mostly erect, trees with primary thickening of the stem and a crown of large, often branched, leaves. The flowers are generally unisexual and radial, consisting of two whorls with three perianth leaves and six stamens. The three-lobed carpels may be free or united and develop into a berry, drupe or nut.

Distribution

The family, with about 2700 exclusively evergreen woody species, is widely distributed in the tropics and subtropics.

Chemical characteristics of the family

The accumulation of polyphenols, some relatively simple alkaloids (especially pyridine derivatives) and steroidal saponins, as well as fatty acids [coconut (*Cocos nucifera* L.) and oil palm (*Elaeis guineensis* Jacq.)] is typical. The pharmaceutical use of *Serenoa repens*, on the other hand, seems to be due to the presence of relatively large amounts of the ubiquitous triterpenoid β-sitosterol.

PAPAVERACEAE

This rather small family has yielded a multitude of pharmaceutically or toxicologically important genera (e.g. *Chelidonium, Eschholzia, Glaucium, Papaver*), and natural products from two of its representatives are particularly widely used.

Important medicinal plants from the family

- *Chelidonium majus* L. (greater celandine), which yields the alkaloid chelidonine, sometimes employed as a cholagogue.
- *Papaver somniferum* L. [(opium) poppy], which yields a multitude of pharmacologically active alkaloids and is a well-known and dangerous narcotic.

Morphological characteristics of the family (Fig. 4.11)

This family of (generally) herbs or sub-shrubs typically has spirally arranged leaves that are entire or lobed or dissected. The generally large flowers are bisexual, have an inferior gynaecium, a reduced number of sepals (two to occasionally four), often four petals and numerous stamens. The fruit is a capsule (e.g. *Papaver somniferum*, which is lanced to obtain opium), with valves or pores for seed dispersal.

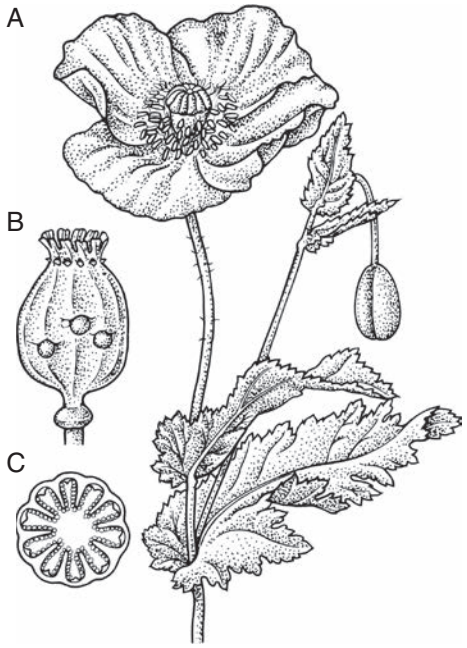


Fig. 4.11 *Papaver somniferum* (Papaveraceae). Botanical line drawings showing: (A) a flowering shoot; (B) the fruit (a capsule), with the latex shown at the lanced parts; (C) a cross-section of the fruit. After Frohne D, Jensen U, 1998. Systematik des Pflanzenreichs, 5 Aufl. Wissenschaftliche Verlagsgesellschaft, Stuttgart.

Distribution

This small family with about 200 species is mostly confined to the northern temperate regions of the world.

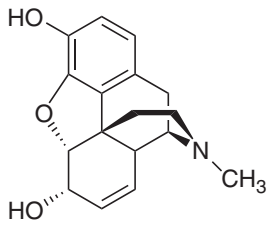
Chemical characteristics of the family

The laticifers (or latex vessels) are rich in isoquinoline alkaloids, including morphine (Fig. 4.12), papaverine, codeine, thebaine and noscapine. Some of these alkaloids are typical benzylisoquinoline alkaloids (papaverine, noscapine); others are chemically modified and have two additional ring systems (morphinane-type skeleton).

PIPERACEAE

Important medicinal plants from the family

- *Piper methysticum* Forster f. (kava-kava), traditionally used as a mild stimulant in Oceania and now used for conditions of nervous anxiety; recent reports of liver toxicity have resulted in withdrawal in many countries.
- *Piper nigrum* L. (black and white pepper), occasionally used in rubefacient preparations and as a spice.



Morphine

Fig. 4.12

Fig. 4.13 *Piper nigram* (black pepper, Piperaceae). Line drawing of a fruiting shoot showing the typical leaves and the fruiting inflorescence. After Frohne D, Jensen U 1998 Systematik des Pflanzenreichs, 5 Aufl. Wissenschaftliche Verlagsgesellschaft, Stuttgart. http://www.wissenschaftliche-verlagsgesellschaft.de/service/widerrufsbelehrung.html.

Morphological characteristics of the family (Fig. 4.13)

This family of shrubs and herbs or small trees generally has simple, spirally arranged, leaves. The flowers are drastically reduced and sit in dense fleshy spikes.

Distribution

The family, with about 2000 species, is restricted to the tropics. The most important genera are *Piper* (including black pepper and kava-kava) and *Peperomia*. Some species are epiphytic (grow on other plants).

Chemical characteristics of the family

Pungent acidic amides, such as piperine, are known from several members of this family, and sometimes essential oil is present. The α-pyrone derivatives (e.g. kavain) from *Piper methysticum* are another group of commonly found compounds known from species of *Piper*.

POACEAE ('MONOCOTYLEDONEAE')

The 'grass' family is not very important with respect to their bioactive contents, but many pharmaceuticals contain starches derived from corn, rice or wheat as excipients.

Important medicinal plants from the family

- *Zea mays* L. (maize, corn) and other cereals, a common staple food; starches are also used in antidiarrhoeal preparations.

Morphological characteristics of the family

Most are herbs, often with rhizomes, and sometimes perennial. The leaves are distichous (with a single leaf at each node and the leaves of two neighbouring nodes disposed in opposite positions), elongate with parallel main veins, often with a characteristic sheath at the base. A typical feature of the family is the wind-pollinated flowers, which form spike-like inflorescences (panicles).

Distribution

This cosmopolitan family has about 9000 species. Many of the economically important food staples are from this family and are grown all over the world.

Chemical characteristics of the family

Members of this family often accumulate silicates and some members have fruits rich in polysaccharides (starch) and proteinaceous tissue, mostly in the endosperm.

RHAMNACEAE

Important medicinal plants from the family

- *Rhamnus purshiana* DC. (American cascara) and *Rhamnus frangula* L. (syn. *Frangula alnus*, European alder, buckthorn), both used as strong purgatives.

Morphological characteristics of the family

This family mostly consists of trees with simple leaves, which are arranged spirally or opposite. The flowers

are small and unisexual with an epigynous gynaecium and four or five small petals. The fruit is a drupe (fleshy exo- and mesocarp with a hard endocarp).

Distribution

A relatively small cosmopolitan, mainly tropical and subtropical, family, with about 900 species.

Chemical characteristics of the family

The family is best known pharmaceutically because some taxa accumulate anthraquinones. Also, alkaloids of the benzylisoquinoline type and the cyclo-peptide type are known from many taxa.

RUBIACEAE

The family yields one of the most important stimulants, coffee (*Coffea arabica* L. and *C. canephora* Pierre ex Froehner) and one of the first and most important medicinal plants brought over from the 'New World', cinchona bark (see below).

Important medicinal plants from the family

- *Cinchona succirubra* Weddell, *C. calisaya* Weddell and *Cinchona* spp. (cinchona, Peruvian bark), used as a bitter tonic, febrifuge and against malaria.

Morphological characteristics of the family

This family consists mostly of trees or shrubs, with some lianas (climbing plants) and herbs. It has simple, entire and generally decussate leaves, which are nearly always opposed and which usually have connate stipules (sometimes as large as the leaves themselves, e.g. *Gallium*). The usually bisexual and epigynous, insect-pollinated flowers have four to five petals and four to five sepals, and five (or four) stamens and two gynaecia. The type of fruit varies (berry, drupe, capsule).

Distribution

This is a large cosmopolitan family with more than 10,000 species, particularly prominent in the tropical and warmer regions of the world.

Chemical characteristics of the family

The family is known for a large diversity of classes of natural products, including iridoids (a group of monoterpenoids), alkaloids (including indole alkaloids such as quinine from *Cinchona* spp.), methylxanthines such as caffeine, theobromine and theophylline, and anthranoids in some taxa (e.g. the now obsolete medicinal plant *Rubia tinctorum*, which was withdrawn because of its genotoxic effect).

RUTACEAE

The family includes some of the most important fruit-bearing plants known: the genus *Citrus* with orange, lemon, lime, mandarin, grapefruit, etc.

Important medicinal plants from the family

- *Pilocarpus jaborandi* Holmes and *Pilocarpus* spp. (pilocarpus), for the isolation of pilocarpine, which is used in ophthalmology.
- *Ruta graveolens* L. (rue), formerly widely used as an emmenagogue and spasmolytic, shows strong phototoxic side effects.
- Many species (especially of the genus *Citrus*) are aromatic and used as foods as well as in pharmacy and perfumery.

Morphological characteristics of the family

Most members of this family are trees or shrubs with spirally arranged, three pinnate or foliate (rarely simple) leaves. The bisexual flowers generally have five sepals and petals, 5+5 stamens and four or five hypogynous gynaecia.

Distribution

There are approximately 1700 species of this family distributed all over the world, but the tropics are particularly rich in them.

Chemical characteristics of the family

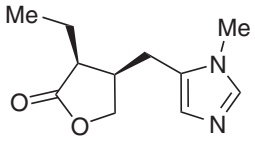
Essential oil is common in many taxa (*Citrus, Ruta*) and can be found in lysigenous secretory cavities in the parenchyma and pericarp. Alkaloids are also frequently found, especially benzyltetrahydroisoquinoline, acridone and imidazole types (pilocarpine; Fig. 4.14). The acridone alkaloids have so far only been reported from the Rutaceae. Other groups of natural products typically encountered are furano- and pyranocoumarins (e.g. bergapten from *Citrus aurantium* subsp. *bergamia*, used to flavour Earl Grey tea), as well as simple coumarins.

SOLANACEAE

This family includes one of the most important staples *Solanum tuberosum* (potato) and many medicinal and toxic plants known for their highly active natural products.

Important medicinal plants from the family

- *Atropa belladonna* L. (deadly nightshade, atropa), *Datura stramonium* L. (stramonium) and *Hyoscyamus niger* L. (henbane), which yield alkaloids with spasmolytic and anticholinergic properties; atropine is used in ophthalmology.



Pilocarpine

Fig. 4.14

Morphological characteristics of the family (Fig. 4.15)

The usually simple, lobed or pinnate/three-foliate leaves of these shrubs, herbs or trees are generally arranged spirally. The taxa have bisexual, radial flowers with five fused sepals, mostly five fused petals, five stamens and two gynaecia, which generally develop into a berry or a capsule.

Distribution

This family of about 2600 species is particularly well represented in South and Central America, but is widely distributed in most parts of the world.

Chemical characteristics of the family

Typical for the family are alkaloids, especially of the tropane, nicotine and steroidal types (Fig. 4.16). Many taxa are characterized by oxalic acid, which often forms typical structures (e.g. sand-like in *Atropa belladonna*, irregular crystals in *Datura stramonium*).

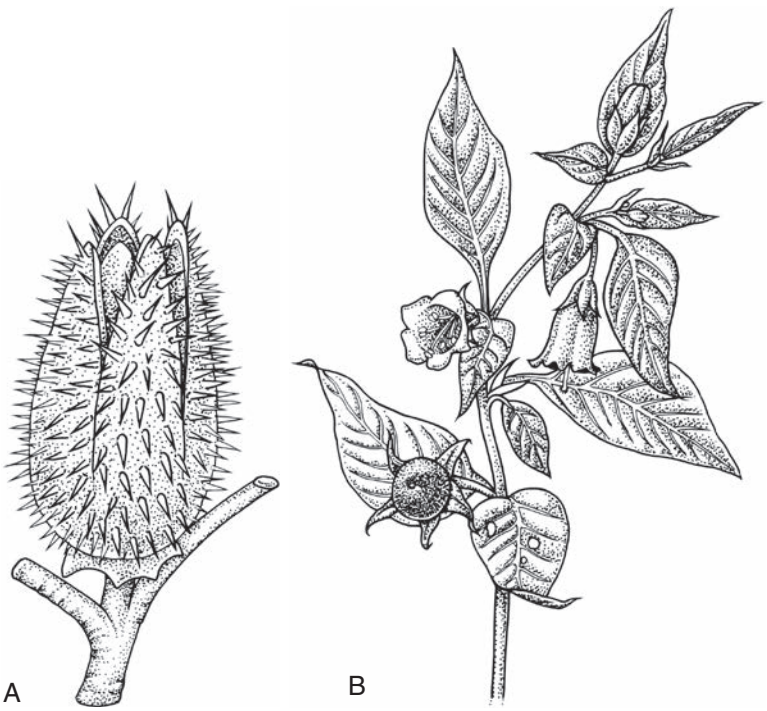


Fig. 4.15 (A) *Datura stramonium* (datura, Solanaceae), showing capsule enclosing the highly toxic seeds. (B) *Atropa belladonna* (deadly nightshade, atropa, Solanaceae), showing a flowering and fruiting branch with the violet-brown (outside) and dirty yellow (inside) flowers and the shiny black berries (highly toxic). After Frohne D, Jensen U 1998 Systematik des Pflanzenreichs, 5 Aufl. Wissenschaftliche Verlagsgesellschaft, Stuttgart. http://www.wissenschaftliche-verlagsgesellschaft.de/service/widerrufsbelehrung.html.

ZINGIBERACEAE ('MONOCOTYLEDONEAE')

In terms of pharmaceutical usage, this family is the most important of the former class Monocotyledoneae (which includes the Liliaceae, Palmaceae and Poaceae). Many members of this family are native to the Indo-Malayan region and are thus particularly important in Asian medical systems.

Important medicinal plants from the family

- *Curcuma zanthorrhiza* Roxburgh (Temu lawak, Javanese turmeric).
- *Curcuma longa* L. (syn. *C. domestica*, turmeric), a commonly used spice and popular remedy used, for example, for inflammatory and liver diseases, and in most Asian medical systems for a large variety of illnesses.
- *Elettaria cardamomum* (L.) Maton (cardamom), which is mostly used as a spice but also as a medicine.
- *Zingiber officinale* Roscoe (ginger), used for a large variety of illnesses, including travel sickness, respiratory and gastrointestinal disorders.

Morphological characteristics of the family

Generally, the species of this family are aromatic herbs with very prominent thickened rhizomes. The latter are often rich in essential oil, stored in typical secretory cells. The leaves are arranged spirally or are distichous with a sheath around the stem (similar to the grasses). However, these sheaths are arranged in such a way that they form a stem-like structure, which supports the real, rather weak, stem. The zygomorphic and bisexual flowers are often very large and prominent and are pollinated by birds, bats or large (often nocturnal) insects.

Distribution

The family is distributed throughout the tropics, but many species are native to Asia (Indo-Malayan region).

Chemical characteristics of the family

This family is one of the few families of the former Monocotyledons, which is rich in essential oil with terpenes such as borneol, camphor and cineole (all oxygen-containing monoterpenes), camphene, pinene (monoterpenes) and zingiberene (a sesquiterpene), as well as phenylpropanoids (cinnamic acid derivatives) (Fig. 4.17). Typically, these compounds accumulate in oil cells, an important microscopical characteristic of the rhizomes of the Zingiberaceae.

GYMNOSPERMS

This much smaller group of seed-bearing plants differs from the angiosperms in not having the seeds enclosed in carpels (the seeds are naked) and in not having double fertilization. The gymnosperms are generally fertilized with the help of the wind and are often characterized as having needles instead of broad leaves (the most important exception being *Ginkgo biloba*, the Chinese maidenhair tree). Only about 750 species are known, but some species are extremely important in the production of timber [European fir (*Abies* spp.), spruce (*Picea* spp.), Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco), all Pinaceae] and some yield medically important essential oil. The most important medicinal plant is *Ginkgo biloba*.

GINKGOACEAE

This is one of the most ancient families of the seedbearing plants and was widely distributed during the Mesozoic (180 million years ago). Only one species survives today.

Important medicinal plant from the family

- *Ginkgo biloba* L. (Chinese maidenhair tree), used for its memory-improving properties.

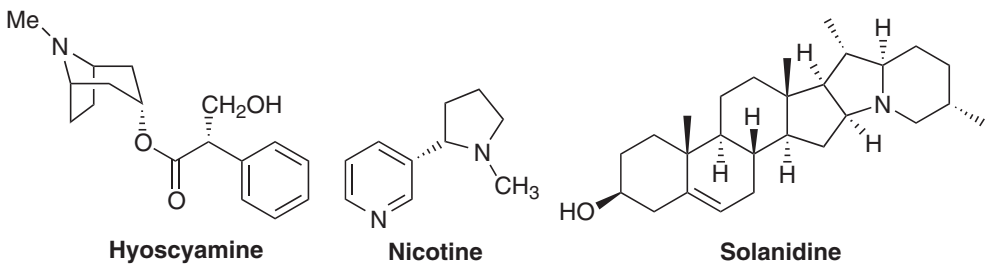


Fig. 4.16

Borneol **Camphor** **α-Pinene**

Fig. 4.17

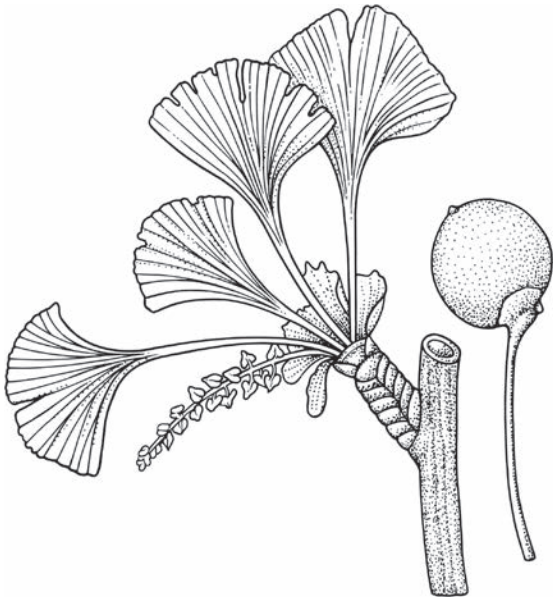
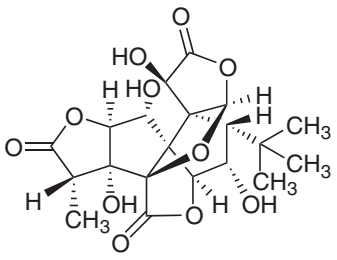


Fig. 4.18 *Ginkgo biloba* (Chinese maidenhair tree, Ginkgoaceae) showing the typical fan-shaped young leaves and a seed (sometimes wrongly called fruit). After Frohne D, Jensen U 1998 Systematik des Pflanzenreichs, 5 Aufl. Wissenschaftliche Verlagsgesellschaft, Stuttgart. http://www.wissenschaftliche-verlagsgesellschaft.de/service/widerrufsbelehrung.html.

Morphological characteristics of the family (Fig. 4.18)

The characteristic fan-shaped leaves, which often have an indention at the apex, are well known. The tree does not bear fruit but has a pseudo-fruit, with the outer part of the seed (testa) developing into a fleshy cover, which has a strong unpleasant smell of butyric acid and a hard inner part. Fertilization is not by pollen but by means of microspermatozoids, a characteristic of less well-advanced plants. Another typical aspect is the separation of the deposition of the microspermatozoids on the gametophytes and fertilization (the unification of macro- and microspermatozoid). Thus, the seeds found on the ground during the autumn are not yet fertilized, but will be during the winter.



Ginkgolide C

Fig. 4.19

Distribution

Ginkgo biloba is native to a small region in south-eastern Asia and is now widely planted in many temperate regions of the world.

Chemical characteristics of the family

The most important, and unique, group of natural products are the ginkgolides (Fig. 4.19), which are unusual two-ringed diterpenoids with three lactone functions. Biflavonoids and glycosylated flavonoids are other groups of typical natural products.

PINACEAE

Important medicinal plants from the family

- *Abies* spp. (fir).
- *Picea* spp. (spruce).

Morphological characteristics of the family (Fig. 4.20)

The trees of this family (conifers) are evergreen and usually have opposed or whorled branches. Typically, the leaves of this family are needle-shaped and linear ('pine needles'). The pollen- and gynoecium-producing flowers are separate, but on one plant (monoecious). The pollen-producing cones are small and herbaceous. They produce large amounts of pollen, which is transported by the wind. The female cones are usually woody with spirally arranged scales, each usually with two ovules on the upper surface, and subtended by a more or less united bract. There are usually two winged, wind-distributed seeds per scale.

Distribution

This small family (about 200 species) is widely distributed in the north temperate regions of the world

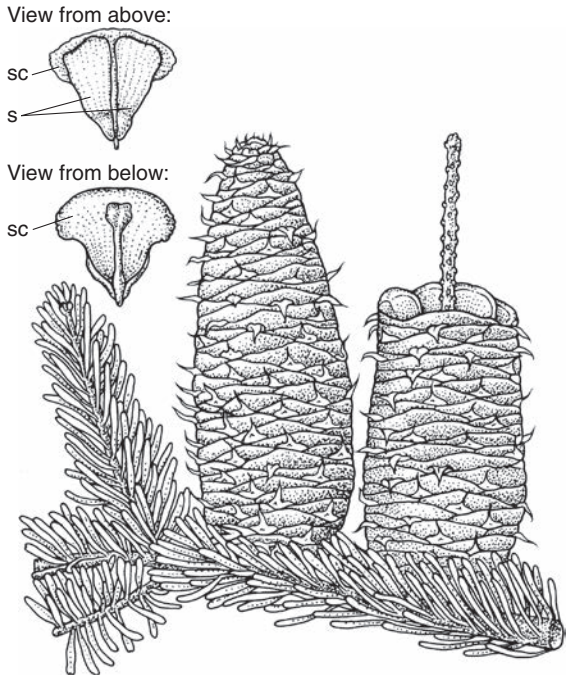


Fig. 4.20 *Abies alba* (fir tree, Pinaceae), showing a branch with cones, and a single scale viewed from above and below. s, seed; sc, scale. After Frohne D, Jensen U 1998 Systematik des Pflanzenreichs, 5 Aufl. Wissenschaftliche Verlagsgesellschaft, Stuttgart. http://www.wissenschaftliche-verlagsgesellschaft.de/service/widerrufsbelehrung.html.

[including regions with long annual periods of extreme frost, such as high mountains (Alps), the northernmost parts of Western Europe and the Asian tundra] and extends into the warmer regions of the northern hemisphere. Many members of this family are accordingly very frost- and drought-resistant and form large tree- or shrub-dominated zones of vegetation.

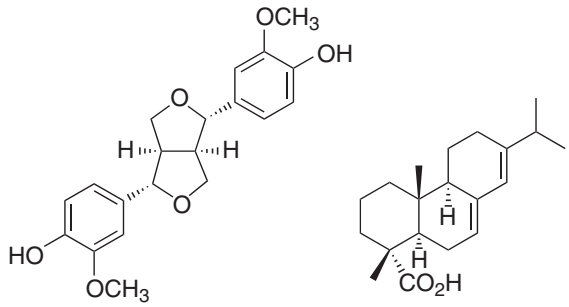


Fig. 4.21

Chemical characteristics of the family

The best known pharmaceutical products from this family are essential oils and balsams, which are typically found in schizogenic excretion ducts of the leaves as well as in excretion pores of wood and bark. Both are rich in monoterpenoids, such as α-pinene and borneol. Mixtures of oil and resin from these species are called **turpentine**, while the resinous part is called **colophony** and is particularly rich in terpenoids (including diterpenoids such as abietic acid). Other widely reported groups of compounds from members of this family are flavonoids, condensed tannins and lignans (e.g. pinoresinol) (Fig. 4.21).

Further reading

Angiosperm Phylogeny Group (2016), "An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV" (PDF), Botanical Journal of the Linnean Society, 181 (1): 1–20.

Brimble, L.F.J., 1942. Intermediate botany. Macmillan, London.

Evans, W.C., 2000. Trease and Evans' pharmacognosy, fifteenth ed. WB Saunders, London.

Fitch, W.H., 1924. Illustrations of the British flora. Reeve, London.

Frohne, D., Jensen, U., 1998. Systematik des Pflanzenreichs. 5 Aufl. Wissenschaftliche Verlagsgesellschaft, Stuttgart.

Heywood, V.H., 1993. Flowering plants of the world. Batsford, London.

Judd, W.S., Campbell, C.S., Kellogg, E.A., et al., 2002. Plant systematics. A phylogenetic approach, second ed. Sinauer, Sunderland, MA.

Mabberly, D.J., 1990. The plant book. Cambridge University Press, Cambridge.

Raven, P.H., Evert, R.F., Eichhorn, S.E., 1999. Biology of plants, sixth ed. WH Freeman, New York.

Robbers, J.E., Speedie, M.K., Tyler, V.E., 1996. Pharmacognosy and pharmacobiotechnology. Williams & Williams, Baltimore.

Sitte, P., Ziegler, H., Ehrendorfer, F., Bresinsky, A., 1997. Lehrbuch der Botanik ('Strasburger'). 34 Aufl. Gustav Fischer Verlag, Stuttgart.

Chapter 5

Ethnobotany and ethnopharmacy

Many drugs that are commonly used today (e.g. aspirin, ephedrine, ergometrine, tubocurarine, digoxin, reserpine, atropine) came into use through the study of indigenous (including European) remedies – that is, through the bioscientific investigation of plants used by people throughout the world. Table 5.1 lists just a few of the many examples of drugs derived from plants. As can be seen, most plant-derived pharmaceuticals and phytomedicines currently in use were (and often still are) used by native people around the world. Accordingly, our information is derived from local knowledge as it was and is utilized throughout the world, although European and Mediterranean traditions have had a particular impact on these developments. The historical development of this knowledge is discussed in Chapter 2. This chapter is devoted to traditions as old as or older than the written records but which have been passed on orally from one generation to the next. Some of this information, however, may have not been documented in codices or studied scientifically until very recently.

Today such 'traditional medicine' (TM; for details see Chapters 14-18) has become a priority of pharmaceutical and medical research in many countries. The WHO (2013) has developed a comprehensive strategy for developing it. The World Health Assembly resolution on traditional medicine (WHA62.13) has defined the following goals for all states:

> *harnessing the potential contribution of TM to health, wellness and people centred health care;*
>
> *promoting the safe and effective use of TM by regulating, researching and integrating TM products, practitioners and practice into health systems, where appropriate.*
>
> ***(WHO 2013:11)***

Ethno*pharmacology* (Heinrich and Jaeger 2015) is an interdisciplinary field of research that looks specifically at such traditional medical systems and other empirical knowledge systems used throughout the world. Foods, toxins and other useful substances are often included in ethno*botanical* studies, which thus cover the whole range of local knowledge of and practice with useful plants. In the context of ethnopharmacology the potential health benefits and (as with all drugs) the potential toxicological risks associated with such remedies are of particular importance. Empirical knowledge was sometimes recorded in herbals and other texts on *materia medica*. Written traditions are obviously better documented and easier to access, but both written and oral forms of indigenous phytotherapy are important factors influencing the use of medicinal plants in the Western world. Each year new plants become popular with some sections of the population (and often are as quickly forgotten again). Only a few are sufficiently well studied scientifically and can be recommended on the basis of bioscientific and/or clinical evidence.

ETHNOBOTANY

Shortly before the start of the 20th century (1896), the American botanist William Harshberger coined the term 'ethnobotany' – the study of plant use by humans.

Ethnobotany studies the relationship between humans and plants in all its complexity, and is generally based on a detailed observation and study of the use a society makes of plants, including all the beliefs and cultural practices associated with this use.

Table 5.1 Botanical drugs used in indigenous medicine or of importance in the development of modern drugs

BOTANICAL NAME	ENGLISH NAME	INDIGENOUS USE	ORIGIN (REGIONAL AND ETHNIC)	USES IN BIOMEDICINE	RELEVANT BIOLOGICALLY ACTIVE COMPOUNDS
Aesculus hippocastanum L. (Hippocastanaceae)[a]	Horse chestnut	Anti-inflammatory	South-eastern Europe	Chronic inflammatory conditions, circulatory problems	Aescin (a saponin mixture)
Ammi visnaga (L.) Lam. (syn.: *Visnaga daucoides* Gaertn., Apiaceae)	Visnaga	Inflammatory and infectious conditions of the mouth, diuretic, 'palpitations of the aorta'	Northern Africa	Increase of cardiac activity	Khellin, and development of cromoglycate
Ananas comosus (L.) Merr. (Bromeliaceae)	Pineapple	Anthelmintic, expectorant, abortifacient	South America (today pantropical)	Anti-inflammatory	Bromelain
Atropa belladonna L. (Solanaceae)	Deadly nightshade	Pain relief, asthma, inflammatory conditions	Europe, Middle East	Parkinsonism, anti-emetic	(–)-Hyoscyamine
Camptotheca acuminata Decne. (Nyssaceae)	–	?	South and South-eastern Asia	Cancer chemotherapy (inhibitor of topoisomerase 1)	Camptothecin (under development)
Carapichea ipecacuanha (Brot.) L.Andersson [syn.: *Cephaelis ipecacuanha* (Brot.) Tussac, Rubiaceae]	Ipecacuanha	Amoebiasis, expectorant, emetic	Tropical regions of South America	Expectorant, emetic, amoebiasis	Emetine
Catharanthus roseus (L.) G. Don (Apocynaceae)	Madagascar periwinkle	Diabetes mellitus	Madagascar (today a pantropical garden plant)	Cancer chemotherapy	Vincristine, vinblastine
Chondrodendron tomentosum Ruiz & Pav. (Menispermaceae)	–	Arrow poison	Brazil, Peru	Muscular relaxation (during operations)	D-Tubocurarine (Fig. 5.1) (and derivatives)
Cinchona pubescens Vahl (syn.: *C. succirubra* Pav. ex Klotzsch) and spp. (Rubiaceae)	Jesuits' bark	No indigenous uses were recorded during the 16th and 17th centuries	Northern South America	Malaria, cardiac arrhythmia	Quinine
Colchicum autumnale L. (Colchicaceae)	Meadow saffron	Poison	Europe	Gout	Colchicine
Combretum caffrum (Eckl. & Zeyh.) Kuntze (Combrataceae)	Bushwillow tree	An ingredient of arrow poisons?	South Africa	Cancer chemotherapy	Combretastatin A-4
Cryptolepis sanguinolenta (Lindl.) Schltr. (Asclepiadaceae)	Cryptolepis / Ghana quinine	Various symptoms, which may possibly be associated with diabetes	West Africa (e.g. Ghana)	Diabetes	Cryptolepine
Curcuma xanthorrhiza Roxb. and spp. (Zingiberaceae)	Turmeric (curcuma)	Cholagogue, stomachic, carminative	India (?), today widely distributed in the tropics	Hepatic disorders	Curcumin, essential oil

Continued

Table 5.1 Botanical drugs used in indigenous medicine or of importance in the development of modern drugs—cont'd

BOTANICAL NAME	ENGLISH NAME	INDIGENOUS USE	ORIGIN (REGIONAL AND ETHNIC)	USES IN BIOMEDICINE	RELEVANT BIOLOGICALLY ACTIVE COMPOUNDS
Datura metel L./*D. innoxia* Mill. (Solanacaeae)	Thorn-apple	Hallucinogen	Africa and Asia, Middle America	Travel sickness, preoperative medication	Scopolamine (hyoscine)
Digitalis spp. (Scrophulariaceae)	Foxglove	Dropsy	Europe	Cardiac arrhythmia, atrial fibrillation	Digitalis glycosides
Drimia maritima (L.) Stearn (syn. *Urginea maritima* (L.) Baker (Asparagaceae / Hyacinthaceae)	Sea onion	Dropsy, emetic, diuretic	Mediterranean	Coronary insufficiency	C_{24}-steroidal cardiac glycosides
Echinacea angustifolia DC. and *E. purpurea* (L.) Moench (Asteraceae)[a]	Echinacea	Pain relief, anti-inflammatory, wounds	North America	Immunostimulant	Combined effect of several groups of compound
Ephedra sinica Stapf (Ephedraceae)	Ephedra	Chronic cough	China	Cough suppressant	Forskolin
Filipendula ulmaria (L.) Maxim. (Rosaceae)	Meadowsweet	Various uses including as diuretic, kidney problems	Europe, northern Asia	Pain	Acetylsalicylic acid
Frangula purshiana (DC.) Cooper (syn.: *Rhamnus purshiana* DC.) and other *Frangula* and *Rhamnus* spp. (Rhamnaceae)[a]	Cascara sagrada	Widely used as a laxative	Western North America	Purgative	Anthraquinones
Galanthus nivalis L. and related spp. (Amaryllidaceae)	Snowdrop	According to Fuchs (1543), not used pharmaceutically (?)	Warmer regions of Europe	Dementia, including Alzheimer's disease	Galanthamine
Ginkgo biloba L. (Ginkgoaceae)[a]	Ginkgo	Asthma, anthelmintic (fruit)	Eastern China, today widely cultivated	Dementia, cerebral deficiencies, cerebral circulatory problems	Ginkgolides
Harpagophytum procumbens (Burch.) DC. ex Meisn. (Pedaliaceae)[a]	Devil's claw	Fever, unspecified illnesses of the blood, pain relief (especially after parturition), inflammatory conditions, as digestive	Southern Africa	Pain, especially rheumatism	Harpagoside (?), caffeic acid derivatives
Hyoscyamus niger L. (Solanaceae)	Henbane	Pain relief (topical as ointment and plaster), fever, respiratory illnesses	Europe	Anticholinergic	Hyoscyamine

Table 5.1 Botanical drugs used in indigenous medicine or of importance in the development of modern drugs—cont'd

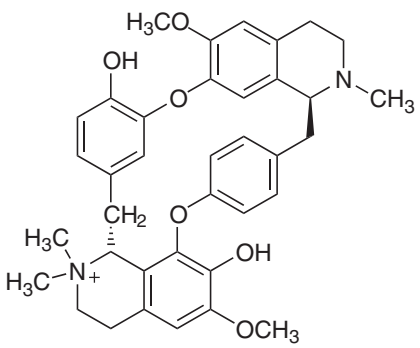
BOTANICAL NAME	ENGLISH NAME	INDIGENOUS USE	ORIGIN (REGIONAL AND ETHNIC)	USES IN BIOMEDICINE	RELEVANT BIOLOGICALLY ACTIVE COMPOUNDS
Hypericum perforatum L. (Hypericaceae)[a]	St John's wort	Very diverse uses including wounds, rheumatism, gout, menstrual problems	Europe	Mild forms of depression, topical for inflammatory conditions (oil)	Hyperforin, flavonoids, hypericin
Justicia adhatoda L. (syn. *Adhatoda vasica* Nees, Acanthaceae)	–	Antispasmodic, antiseptic, anti-asthmatic, fish poison, insecticide	India, Sri Lanka	Antispasmodic, oxytocic, cough suppressant	Vasicin (model for expectorants bromhexin and ambroxol)
Macropiper methysticum (G.Forst.) Miq. *(syn.: Piper methysticum* G. Forst.; Piperaceae)[a]	Kava-kava	Ritual stimulant and tonic	Polynesia	Anxiolytic, mild stimulant	Kava pyrones and others
Papaver somniferum L. (Solanaceae)	(Opium) poppy	Pain relief, tranquillizer, hallucinogen	Western Mediterranean	Pain (P), cough (C), antispasmodic (S)	Morphine (P), Codeine (C), Papaverine (S)
Physostigma venenosum Balf. (Fabaceae sensu stricto)	Calabar bean	Poison ('ordeal' and arrow poison)	Tropical West Africa (Sierra Leone–Democratic Republic of Congo)	Glaucoma	Physostigmine
Pilocarpus joborandi Holmes (Rutaceae)	Jaborandi	Poison	Africa	Parasympathomimetic, glaucoma	Pilocarpine
Podophyllum peltatum L. (Berberidaceae)	May apple	Especially as laxative, also for skin infections	North-eastern North America	Cancer chemotherapy, warts	Podophyllotoxin (and other lignans)
Prunus africana (Hook, f.) Kalkman (Rosaceae)[a]	African plum	Laxative (veterinary) and diverse other uses	Tropical Africa	Prostate hyperplasia	Especially sitosterol
Psoralea corylifolia L. [= *Cullen corylifolium* (L.) Medik., Fabaceae (sensu stricto)]	–	Stomachic, various skin infections	Asia	Psoriasis	Psoralen
Rauvolvia spp.	Snake root	Emetic, cholera	Widely distributed in the tropics	Cardiac arrhythmia (A), high blood pressure (B)	Ajmalin (A), reserpine (B)
Salix spp. (Salicaceae)[a]	Willow	A variety of uses, especially for chronic and acute inflammatory conditions	Europe, Asia, North America	Various types of pain (lower back pain), chronic inflammatory conditions	Salicin and derivatives (model for aspirin)
Senna alexandrina Mill. *(syn.: Cassia senna* L.) and related spp. (Caesalpiniaceae)	Senna	Laxative	North-eastern Africa, Middle East	Laxative	Sennoside anthraquinones

Continued

Table 5.1 Botanical drugs used in indigenous medicine or of importance in the development of modern drugs—cont'd

BOTANICAL NAME	ENGLISH NAME	INDIGENOUS USE	ORIGIN (REGIONAL AND ETHNIC)	USES IN BIOMEDICINE	RELEVANT BIOLOGICALLY ACTIVE COMPOUNDS
Strophanthus gratus (Wall. & Hook.) Baill. and *Strophanthus* spp. (Apocynaceae)	–	Arrow poison	Tropical Africa	Coronary insufficiency	Strophantin, ouabain
Syzygium aromaticum (L.) Merr. & L.M.Perry (Myrtaceae)[a]	Clove	Stomachic, digestive, antidiarrhoea, oil for toothache and rheumatism	Molucca Islands (formerly Spice Islands)	Toothache	Eugenol
Taxus brevifolia Nutt. (Taxaceae)	Californian yew	Very diverse uses including for 'cancer' (Tsimshian)	Western USA	Cancer chemotherapy (induction of tubulin aggregation)	Paclitaxel

[a]Examples of plants commonly used in European phytotherapy. In general, the others are not used as an extract, but as pure compounds, or served as a model for developing semi-synthetic drugs or as standardized (normalized) extracts.



Tubocurarine

Fig. 5.1

It is usual for ethnobotanists to live with indigenous people, to share the everyday life of their community and, of course, to respect the underlying cultures. Ethnobotanists have a responsibility both to the scientific community and to the indigenous cultures. According to the above definition, ethnobotany focuses not only on medicinal plants, but also on other natural products derived from nature, such as:

- Food.
- Plants used in rituals.
- Colouring agents.
- Fibre plants.
- Poisons.
- Fertilizers.
- Building materials for houses, household items, boats, etc.
- Ornamentals.
- Oil plants.

This broad definition is still used today, but modern ethnobotanists face a multitude of other tasks and challenges (see below). Medicinal plants have always been one of the main research interests of ethnobotany and the study of these resources has also made significant contributions to the theoretical development of the field (Berlin 1992); however, the more anthropologically oriented fields of research are beyond the scope of this introductory chapter.

ETHNOPHARMACOLOGY

Ethnopharmacology as a specifically designated field of research has had a relatively short history. The term was first used in 1967 in the title of a book on hallucinogens (Efron et al 1970). The field is nowadays much more broadly defined.

The observation, identification, description and experimental investigation of the ingredients, and the effects of the ingredients, and the effects of such indigenous drugs, is a truly interdisciplinary field of research that is very important in the study of traditional medicine. **Ethnopharmacology** is here defined as 'the interdisciplinary scientific exploration of biologically active agents traditionally employed or observed by man' (Bruhn and Holmstedt 1981:405–406).

This definition draws attention to the bioscientific study of indigenous drugs but does not explicitly address the issue of searching for new drugs. Medicinal plants are an important element of indigenous medical systems in many parts of the world, and these resources are usually regarded as part of the traditional knowledge of a culture. Europe has for many years profited from the exchange of ideas with other continents, and many of the natural products and phytomedicines used today are derived from plants used in indigenous cultures. Examples of 18th century explorers who described indigenous plant use in detail are Richard Spruce (British), Hipolito Ruiz (Spanish) and Alexander von Humboldt (German), who co-discovered curare.

THE STORY OF CURARE

An interesting example of an early ethnopharmacological approach is provided by the study of the botanical origin of the arrow poison curare, its physiological effects and the compound responsible for these effects. Curare was used by certain, wild, tribes in South America for poisoning their arrows and many early explorers documented this usage. The historical aspects of the scientific investigation of curare by Bernard (1966) are outlined in Chapter 2, but the detailed descriptions made by Alexander von Humboldt in 1800, of the process used to prepare poisoned arrows in Esmeralda on the Orinoco River, are equally interesting. Von Humboldt had met a group of native people who were celebrating their return from an expedition to gather the raw material for making the poison and he described the 'chemical laboratory' used to prepare the poison (von Humboldt 1997:88, from the original text published 1800):

> *He [an old Indian] was the chemist of the community. With him we saw large boilers (Siedekessel) made out of clay, to be used for boiling the plant sap; plainer containers, which speed up the evaporation process because of their large surface; banana leaves, rolled to form a cone-shaped bag [and] used to filter the liquid which may contain varying amounts of fibres. This hut transformed into a laboratory was very tidy and clean.*

The botanical source of curare was finally identified as the climbing vine *Chondrodendron tomentosum* Ruiz and Pavón. Other species of the Menispermaceae (*Curarea* spp. and *Abuta* spp.) and Loganiaceae (*Strychnos* spp.) are also used in the production of curares of varying types (Bisset 1991). Von Humboldt then eloquently described one of the classical problems of ethnopharmacology:

> *We are unable to make a botanical identification because this tree [which produces the raw material for the production of curare] only grows at quite some distance from Esmeralda and because [it] did not have flowers and fruit. I had mentioned this type of misfortune previously, that the most noteworthy plants cannot be examined by the traveller, while others whose chemical activities are not known [i.e. which are not used ethnobotanically] are found covered with thousands of flowers and fruit.*

THE ROLE OF THE ETHNOBOTANIST

The role of the ethnobotanist in the search for new drugs was important until the second half of the 20th century, when other approaches became more 'fashionable'. More recently, the study of ethnobotany has again received considerable interest in the media and in some segments of the scientific community. Also, the 'Western' use of such information has come under increasing scrutiny, and the national and indigenous rights to these resources have become acknowledged by most academic and industrial researchers. These developments result in a considerable challenge to (and increasing responsibilities for) ethnobotanists and ethnopharmacologists. Simultaneously, the need for basic scientific investigation of plants used in indigenous medical systems is becoming ever more relevant. The public availability of research results is as essential for further developing and 'upgrading' indigenous and traditional medicine as it is for any other medical or pharmaceutical system.

ETHNOPHARMACOLOGY AND THE CONVENTION ON BIOLOGICAL DIVERSITY (CONVENTION OF RIO)

None of the studies discussed so far took the benefits for the providers (the states and their people) into account. This has changed in recent years. Ethnopharmacological and related research using the biological

resources of a country are today based on agreements and permits, which in turn are based on international and bilateral treaties. The most important of these is the Convention of Rio or the Convention on Biological Diversity (see http://www.biodiv.org/chm/conv.htm), which looks in particular at the rights and responsibilities associated with biodiversity on an international level:

> *The objectives of this Convention, to be pursued in accordance with its relevant provisions, are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to technologies, and by appropriate funding.*

The basic principles of access are regulated in article 5:

> *States have, in accordance with the Charter of the United Nations and the principles of international law, the sovereign right to exploit their own resources pursuant to their own environmental policies, and the responsibility to ensure that activities within their jurisdiction or control do not cause damage to the environment of other States or of areas beyond the limits of national jurisdiction.*

The rights of indigenous peoples and other keepers of local knowledge are addressed in article 8j:

> *Subject to its national legislation, respect, preserve and maintain knowledge, innovations and practices of indigenous and local communities embodying traditional lifestyles relevant for the conservation and sustainable use of biological diversity and promote their wider application with the approval and involvement of the holders of such knowledge, innovations and practices and encourage the equitable sharing of the benefits arising from the utilization of such knowledge, innovations and practices.*

This and subsequent treaties significantly changed the basic conditions for ethnopharmacological research. Countries that provide the resources for natural product research and drug development now have well-defined rights. This specifically includes the sharing of any benefits that may accrue from the collaboration. Access to resources is addressed in article 15, which is crucial for an understanding of this and any other activity that may yield economically important products:

> *15.1. Recognizing the sovereign rights of States over their natural resources, the authority to determine access to genetic resources rests with the national governments and is subject to national legislation.*
>
> *15.5. Access to genetic resources shall be subject to prior informed consent of the Contracting Party providing such resources, unless otherwise determined by that Party.*
>
> *15.7. Each Contracting Party shall take legislative, administrative or policy measures...with the aim of sharing in a fair and equitable way the results of research and development and the benefits arising from the commercial and other utilization of genetic resources with the Contracting Party providing such resources. Such sharing shall be upon mutually agreed terms.*

In the case of ethnopharmacological research, the needs and interests of the collaborating community become an essential part of the research, and in fact there is an inextricable link between cultural and biological diversity. This principle was first formulated at the 1st International Congress on Ethnobiology held in Belem (Brazil) in 1988. No generally agreed upon standards have so far been accepted, but the importance of obtaining the 'prior informed consent' of the informants has been stressed by numerous authors (e.g. Posey 2002, Secretariat of the Convention on Biological Diversity 2001).

After this first Convention the mutual obligations were developed further, including most recently the Nagoya Protocol of 2010 (https://www.cbd.int/abs/about/), and today a complex set of international policies and guidelines exists. Other relevant agreements include trade-related aspects of intellectual property rights (TRIPS) and World Trade Organization (WTO) agreements (cf. www.wto.org). While the implementation is complex and time consuming, in major academic circles it is the general consensus that their implementation is an essential foundation of any form of ethnopharmacological and related research.

BIOPROSPECTING AND ETHNOPHARMACOLOGY

Studies dealing with medicinal and other useful plants and their bioactive compounds have used many concepts and methodologies. These are interdisciplinary or multidisciplinary studies, combining

Table 5.2 Ethnopharmacology and bioprospecting compared

ETHNOPHARMACOLOGY	BIOPROSPECTING
Overall goals	
(Herbal) drug development, especially for local uses	Drug discovery for the international market
Complex plant extracts (phytotherapy)	Pure natural products as drugs
Social importance of medicinal and other useful plants	–
Cultural meaning of resources and understanding of indigenous concepts about plant use and of the selection criteria for medicinal plants	–
Main disciplines involved	
Anthropology	–
Biology (ecology)	Biology including (very prominently) ecology
Pharmacology/molecular biology	Pharmacology and molecular biology
Pharmacognosy and phytochemistry	Phytochemistry
Number of samples collected	
Very few (up to several hundred)	As many as possible, preferably several thousand
Selected characteristics	
Detailed information on a small segment of the local flora (and fauna)	Limited information about many taxa
Database on ethnopharmaceutical uses of plants	Database on many taxa (including ecology)
Development of autochthonous resources (especially local plant gardens, small-scale production of herbal preparations)	Inventory (expanded herbaria) economically sustainable alternative use to destructive exploitation (e.g. logging)
Pharmacological study	
Preferably using low-throughput screening assays, which allow a detailed understanding of the local or indigenous uses	The assay is not selected on the basis of local usage, instead high-throughput screening systems are used
Key problem	
Safety and efficacy of herbal preparations	Local agendas (rights) and compensation to access

such diverse fields as anthropology, pharmacology, pharmacognosy or pharmaceutical biology, natural product chemistry, toxicology, clinical research, plant physiology and others. In order to analyse their strengths and weaknesses, and especially the outcomes of research, two different but closely related approaches can be distinguished: **bioprospecting** and **ethnopharmacology** (Table 5.2).

Bioprospecting focuses on the development of new drugs for the huge markets of the northern hemisphere. Potentially highly profitable pharmaceutical products are developed, based on the biological and chemical diversity of the various ecosystems on Earth; this requires an enormous financial input. The research starts with the collection of biogenic samples (plants, fungi, other micro-organisms and animals), progresses through analysis of the chemical, biological and pharmacological activities and ends with the development of drug templates or new drugs. A key process in this search is high-throughput screening systems such as those that have been established by major international pharmaceutical companies. Huge libraries of compounds (and sometimes extracts) are screened for biological activity against specific targets. Active natural products are only one of the many sources of material for these batteries of tests but serve as a starting point for drug development. Currently, some companies envision screening 500,000 samples a week against a single target; thus, it becomes essential to have an enormous number of chemically diverse samples available.

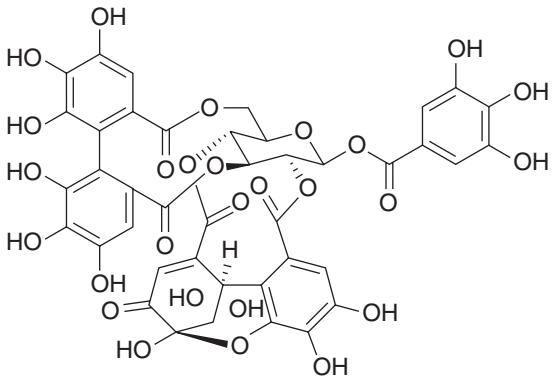
The other approach may best be termed ethnopharmacological. Ethnobotanical studies generally result in the documentation of a rather limited set of well-documented useful plants, mostly medicinal, but

also those known to be toxic or used in nutrition. In ethnopharmacology, an important goal is the development of improved preparations for use by local people. Thus, it is essential to obtain information on the bioactive compounds from these plants, their relative contribution to the effects of the extract (including, for example, synergistic or antagonistic effects), the toxicological profile of the extract and its constituents. By restricting ethnopharmacology to the bioscientific study of indigenous uses, attention is drawn to the need for improving indigenous phytomedical systems, especially in developing countries. This requires research strategies for studying indigenous medicinal plants and their uses.

The importance of conserving such nature-derived products in the healthcare of the original keepers of such knowledge must be the main goal of truly interdisciplinary research. Ethnopharmacology may contribute to the development of new pharmaceutical products for the markets of the northern hemisphere, but this is only one of several targets. Truly multidisciplinary research on medicinal plants requires the inclusion of other methodologies from such fields as medical or pharmaceutical anthropology or sociology. Not only do we need a detailed understanding, incorporating social scientific and bioscientific methods, but we also need to support all means available of making better use of these products. It has been pointed out that the two approaches – ethnopharmacology and biodiversity prospecting – are not mutually exclusive and the two concepts as they are outlined here are rarely realized in such extreme forms. Instead, any discussion should specifically draw attention to the particular strengths and roles of both approaches. In bioprospecting programmes, which are directed specifically towards infectious diseases, the use of 'ethnobotanical' information is very useful and promising (Lewis 2000). However, this is not necessarily the case in cancer chemotherapy, for example, where highly toxic plants are not used in traditional medicine because the dose cannot be controlled sufficiently well to ensure safety.

EXAMPLES OF MODERN ETHNOPHARMACOLOGICAL STUDIES

A project focusing on the bioscientific study of indigenous uses of Ghanaian medicinal plants leading to the identification of some bioactive constituents in *Phyllanthus muellerianus* (Kuntze) Exell (Phyllantaceae) is



Geraniin

Fig. 5.2

a good example (Hensel et al 2015). An ethnopharmacological field study in the Ashanti region of Ghana was initiated and the binational collaboration between Ghanaian and German researchers identified 104 plant species traditionally used for wound healing. *In vitro* studies of extracts from the most prominent candidates were conducted using primary human fibroblasts and human keratinocytes. A high degree of concordance with the traditional use was observed.

In case of the aqueous extract from the dried leaves of *P. muellerianus* a total tannin content of 14% was found. The ellagitannin geraniin (Fig. 5.2) with two isomers was identified as a major constituent. The compounds identified help to explain the rationale behind the indigenous uses. The tannins are known to have antibacterial activity, which was also corroborated in the studies by Hensel et al (2015), and anti-inflammatory effects contribute to the therapeutic benefits (which, however, have not yet been studied clinically). Specifically, the stimulation of the epidermal keratinocyte barrier and the formation of extracellular matrix markers from the fibroblasts of typical skin cells were identified as key mechanisms. The extract also showed antiviral activity (in *Herpes simplex* virus 1 – HSV-1). While this research (Hensel et al 2015) is unlikely to result in new drug leads for a wider (global) commercial use as a licensed medicine, it offers a foundation for local uses of this species.

The second example is drawn from ethnobotanical fieldwork in Eastern Guatemala with a Mayan-speaking people, the Chorti. The Chorti use the fruit of *Ocimum micranthum* Willd. (Lamiaceae) in the treatment of infectious and inflammatory eye diseases (Kufer et al, unpublished). The fruits are

approximately 1 mm in diameter and hard, and several of these are applied directly into the eye. At first glance this seems an unlikely remedy for eye problems, but the rationale behind it becomes evident when considering the morphological and chemical make-up. The fruits are covered with a mucilaginous layer containing complex polysaccharides, which form a soft layer around the fruit if it is put into water (Heinrich 1992). This layer may well have a cleansing effect, and polysaccharides are known to be useful in the treatment of inflammatory conditions and bacterial or viral infections. Although there are no pharmacological data from experimental studies available to corroborate this use, information on the histochemical structure of the fruit makes it likely that the treatment has some scientific basis.

The above two examples demonstrate the relevance of ethnopharmacology in relation to the scientific study of indigenous medical products. However, ethnopharmacology, as the science bridging the gap between natural sciences and anthropology, should also look at symbolic and cognitive aspects. People may select plants because of their specific pharmacological properties, but also because of the symbolic power they may believe the plant to hold. Understanding these aspects requires cognitive and symbolic analysis of field data. Another example from field studies with the Mixe in Mexico can be used. At the end of a course of medical treatment, the patient is sometimes given a petal of *Argemone mexicana* L. (known to the Mixe as San Pedro Agats, Papaveraceae). This plant is known to contain a large number of biologically active alkaloids, including protopine. However, the yellow petals are presumably used, not because they exert a pharmacological effect (at this dose) but because they symbolize the bread of the Last Supper according to Christian mythology. Thus, they are a powerful symbol for the end of the healing process (for other examples of symbolic and empirical forms of plant use see Heinrich 2013). And this species, which has become (an often noxious) weed in many countries, has been studied in great detail for potential antimalarial effects. This is based on uses to treat the symptoms of malaria in Mali and some clinical evidence has become available (Graz et al 2015)

The role of ethnopharmacology can be extended beyond that defined previously. It looks not only at empirical aspects of indigenous and popular plant use, but also at the cognitive foundations of this use. Only if these issues are to be included will it be a truly interdisciplinary field of research (Bruhn and Holmstedt 1981:406). The key tasks of pharmaceutical researchers in this interdisciplinary process will be to:

- Study the pharmacological effects of the most widely used species (for selection criteria for the ethnopharmacologically most important taxa, see Heinrich et al 1998a).
- Further develop local ethnopharmacopoeias.
- Characterize the relevant constituents.
- Formulate improved (but relatively simple) galenical preparations.

This will result in a truly ethnopharmaceutical approach to medicinal plants that encompasses all sub-disciplines of pharmacy and medicine. The value of integrating ethnobotanical with phytochemical and pharmacological studies has been clearly demonstrated. While, for example, the likelihood of developing new therapeutic agents for use in biomedicine is relatively low (although it is certainly much higher than with many other approaches), such studies confirm the therapeutic value and contribute to our general scientific knowledge about medicinal plants (Heinrich 2013).

Another key development in recent decades has been the study of the use of herbal medicines and foods by migrants or in urban contexts. Such research is not primarily relevant for 'documenting and evaluating traditional knowledge' but often the focus is on understanding how people cope with the changes migration brings about and how such plant use helps to maintain and develop links with the country of origin. Importantly, it can contribute to improving primary health services for such populations and as such it has direct public health implications.

Some plants have many side effects or are highly toxic. In an example from the Highlands of Mexico, Bah et al (1994) showed that a species popularly used there contains hepatotoxic pyrrolizidine alkaloids, which pose potential health risks. Although this information is available to the scientific community, the general public may not be aware of these risks. Such data must be summarized appropriately and made available to local people in regions where the plants are used. It is now essential to develop partnerships with institutions capable of translating these findings into an effective strategy.

CONCLUSION

With the increasing importance given to local and traditional medicines, especially herbal medicines, and, for example, to the development of a global strategy on traditional medicines, the study of indigenous, orally transmitted medical systems has become a priority in many countries, for example, in Asia, Africa and Southern America. It illustrates that the pharmaceutical sciences will profit in many ways by including such approaches. It is also important to further consolidate local knowledge. Ethnopharmacology in this context will provide patients in developing countries in Africa, South and Central America, Asia and South-East Asia with access to some evidence-based forms of their own 'traditional' medicine.

The term '**ethnopharmacy**' may well be the most appropriate to stress the breadth of such an approach, since it encompasses all the relevant disciplines: pharmacognosy, pharmacology, pharmaceutics (especially relating to herbal extracts), drug delivery, toxicology, bioavailability and metabolism studies, as well as pharmacy practice and policy development. This would allow the development of local resources into elements to be used in primary healthcare.

References

(NB: This selection of sources covers the complexity of this multidisciplinary topic and provides further reading due to the brevity of this introduction. Heinrich and Jaeger (eds, 2015) provide a detailed overview on many aspects of ethnopharmacology.).

Bah, M., Bye, R., Pereda-Miranda, R., 1994. Hepatotoxic pyrrolizidine alkaloids in the Mexican medicinal plant *Packera candidissima* (Asteraceae: Senecioneae). J. Ethnopharmacol. 43, 19–30.

Berlin, B., 1992. Ethnobiological classification. Principles of categorization of plants and animals in traditional societies. Princeton University Press, Princeton, NJ.

Bernard, C., 1966. Physiologische Untersuchungen über einige amerikanische Gifte. Das Curare. In: Bernard, C., Mani, N. (Eds.), Ausgewählte physiologische Schriften. Huber Verlag, Bern [orig. French 1864], pp. 84–133.

Bisset, N.G., 1991. One man's poison, another man's medicine. J. Ethnopharmacol. 32, 71–81.

Bruhn, J.G., Holmstedt, B., 1981. Ethnopharmacology: objectives, principles and perspectives. In: Beal, J.L., Reinhard, E. (Eds.), Natural products as medicinal agents. Hippokrates Verlag, Stuttgart, pp. 405–430.

Efron, D., Farber, S.M., Holmstedt, B., et al., 1970. Ethnopharmacologic search for psychoactive drugs. Public Health Service Publications no. 1645. Government Printing Office, Washington, DC (reprint, orig. 1967).

Graz, B., Willcox, M., Elisabetsky, E., 2015. Retrospective treatment-outcome as a method of collecting clinical data in ethnopharmacological surveys. In: Heinrich, M., Jaeger, A.K. (Eds.), Ethnopharmacology. ULLA Book series, Wiley, Chichester, pp. 251–261.

Heinrich, M., 1992. Economic botany of American Labiatae. In: Harley, R.M., Reynolds, T. (Eds.), Advances in labiatae science. Royal Botanical Gardens, Kew, pp. 475–488.

Heinrich, M., 2013. Ethnopharmacology and drug discovery. In: Reedijk, J. (Ed.), Elsevier reference module in chemistry, molecular sciences and chemical engineering. Elsevier, Waltham, MA.

Heinrich, M., Jaeger, A.K. (Eds.), 2015. Ethnopharmacology. ULLA Book series, Wiley, Chichester.

Heinrich, M., Ankli, A., Frei, B., et al., 1998a. Medicinal plants in Mexico: healers' consensus and cultural importance. Soc. Sci. Med. 47, 1863–1875.

Hensel, A., Kisseih, E., Agyare, C., Lechtenberg, M., Petereit, F., Asase, A., 2015. From ethnopharmacological field study to phytochemistry and preclinical research: the example of Ghanaian medicinal plants for improved wound healing. In: Heinrich, M., Jaeger, A.K. (Eds.), Ethnopharmacology. ULLA Book series, Wiley, Chichester, pp. 179–197.

Lewis, W., 2000. Ethnopharmacology and the search for new therapeutics. In: Minnis, P.E., Elisens, W.J. (Eds.), Biodiversity and native America. University of Oklahoma Press, Norman, OK.

Posey, D.A., 2002. Kayapó ethnoecology and culture. In: Plenderleith, K. (Ed.), Studies in environmental anthropology, vol. 6. Routledge, London.

Secretariat of the Convention on Biological Diversity, 2001. Handbook of the convention on biological diversity. Earthscan, London.

von Humboldt, A. (Ed. H. Beck), 1997. Die Forschungsreise in den Tropen Amerikas. Studienausgabe Bd 2, Teilband 3. Wissenschaftliche Buchgesellschaft, Darmstadt.

WHO, 2013. WHO traditional medicine strategy: 2014–2023. World Health Organization Geneva.

Further reading

Brett, J., Heinrich, M., 1998. Culture perception and the environment. J. Appl. Bot. 72, 67–69.

Cotton, C.M., 1997. Ethnobotany. Wiley, Chichester.

Etkin, N.L., 1985. Ethnopharmacology: biobehavioral approaches in the anthropological study of indigenous medicines. Annu. Rev. Anthropol. 17, 23–42.

Etkin, N.L., Harris, D.R., Prendergast, H.D.V., et al., 1998. Plants for food and medicine. Royal Botanical Gardens, Kew.

Heinrich, M., 1994. Herbal and symbolic medicines of the Lowland Mixe (Oaxaca, Mexico): disease concepts, healers' roles, and plant use. Anthropos 89, 73–83.

Heinrich, M., 2001. Ethnobotanik und Ethnopharmakologie. Eine Einführung. Wissenschaftliche Verlagsgesellschaft, Stuttgart.

Heinrich, M., Robles, M., West, J.E., et al., 1998b. Ethnopharmacology of Mexican Asteraceae (Compositae). Annu. Rev. Pharmacol. Toxico. 38, 539–565.

Moerman, D.E., 1998. Native American ethnobotany. Timber Press, Portland, OR.

Neuwinger, D., 2000. African traditional medicines. Medpharm, Stuttgart.

Okpako, D.T., 1989. Principles of pharmacology: a tropical approach. Cambridge University Press, Cambridge.

Okpako, D.T., 1999. Traditional African medicine: theory and pharmacology explored. Trends. Pharmacol. Sci. 20, 482–485.

Schultes, R.E., 1962. The role of the ethnobotanist in the search for new medicinal plants. Lloydia 25, 257–266.

Schultes, R.E., 1983. Richard Spruce: an early ethnobotanist and explorer of the Northwest Amazon and Northern Andes. J. Ethnobiol. 3, 139–147.

Schultes, R.E., Thenen, Nemry von, de Jaramillo-Arango, M.J., 1998. The journals of Hipólito Ruiz: Spanish botanist in Peru and Chile 1777–1788. Timber Press, Portland, OR.

Schultes, R.E., Raffauf, R., 1990. The healing forest. Medicinal and toxic plants of the Northwest Amazonia. Dioscorides Press, Portland, OR.

Sofowara, A., 1979. African medicinal plants: proceedings of a conference. University of Ife Press, Nigeria.

Section 3

Natural product chemistry

SECTION CONTENTS

Chapter 6

Natural product chemistry

This chapter looks briefly at the chemistry of natural products. This is the study of chemicals produced by the many diverse organisms of nature, including plants, microbes (fungi and bacteria), marine organisms and more exotic sources such as frog skins and insects.

Natural products are defined here as organic compounds in the molecular weight range 100–2000. In a broader sense, the term natural products can also be applied to bulk substances from nature, such as crude plant material, foodstuffs, resins and exudates from plants (e.g. myrrh and frankincense) or extracts of plant material (water or alcoholic extracts). The treatment of this subject will be confined to pure **single chemical entities** (i.e. chemicals with a well-defined structure and purity).

Historically, natural products have formed the basis of medicines and, even now, many of the compounds that are pharmaceutically and medicinally important are derived from natural sources. The reasons for this are complicated, but probably stem from the ability of nature to produce a fantastic array of **structurally complex** and **diverse** molecules. A number of theories have been proposed as to why these compounds are produced and, although we can only theorize as to why these products occur in nature, it is highly likely that many of them are produced as part of a chemical defence system to protect the producing organism from attack. Examples of this defence include the synthesis of antimicrobial compounds by plants that are infected by bacteria and fungi (compounds known as **phytoalexins**), or the synthesis of highly toxic natural products in the skins of Central American frogs to deter predation by other animals. Whatever the reasons for the presence of these compounds in nature, they are an invaluable and under-exploited resource that can be used to find new drug molecules.

There are many different ways to classify the enormous chemical diversity of natural products. In this book we chose to follow a mixed criterion to facilitate the task: all natural products but not including alkaloids are grouped by their **biosynthetic origin**. This consists of grouping them by end products of the same plant metabolic pathway. These end products may undergo further glycosylation, which results in important changes in their solubility, reactivity, bioavailability and pharmacology. Therefore, we will discuss their glycosides separately. Finally, we will present you with the most pharmaceutically relevant alkaloids classified mainly by their heterocyclic structure.

THE POLYKETIDES

Polyketide natural products form an immense group of therapeutically important compounds comprising many antibiotics (macrolides and tetracyclines), fatty acids and aromatic compounds (anthrone purgative glycosides and anthracyclic antitumour agents).

Polyketides are mainly acetate (C_2) derived metabolites and occur throughout all organisms (as fatty acids and glycerides), but it is the microbes, predominantly the filamentous bacteria of the genus *Streptomyces*, that produce structurally diverse types of polyketides, especially as antibiotic substances. The biosynthesis of these compounds begins (Fig. 6.1) with the condensation of one molecule of **malonyl-CoA** (CoA is short for coenzyme A) with one molecule of **acetyl-CoA** to form the simple polyketide acetoacetyl-CoA. In this reaction

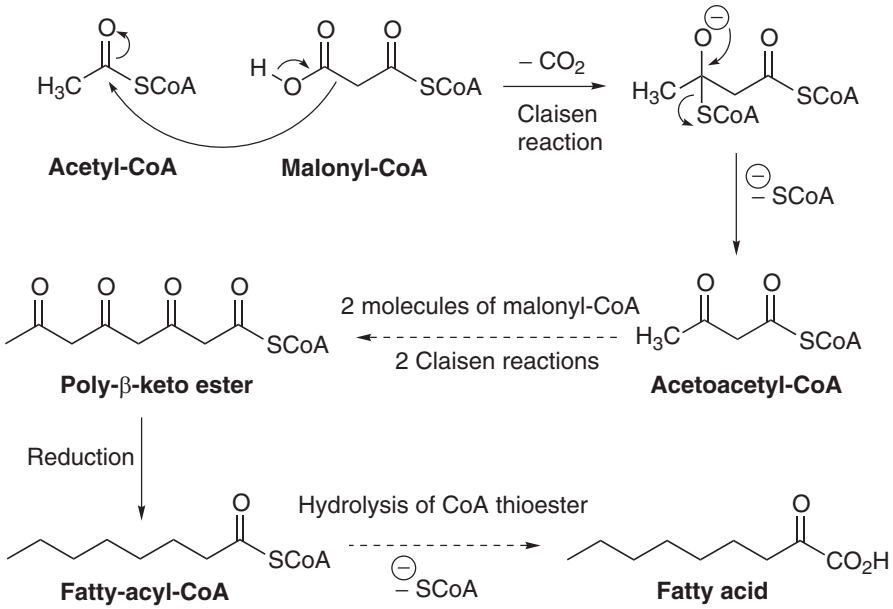


Fig. 6.1

(Claisen reaction), one molecule of CO_2 and one molecule of HSCoA are generated. The reaction occurs because the carbon between both carbonyl groups of malonyl-CoA (the acidic carbon) is nucleophilic and can attack an electropositive (electron-deficient) centre (e.g. the carbon of a carbonyl group).

The curved arrows in Fig. 6.1 indicate the movement of a pair of electrons to form a bond. Further condensation reactions between another molecule of malonyl-CoA and the growing polyketide lead to chain elongation, in which every other carbon in the chain is a carbonyl group. These chains are known as **poly-β-keto esters** and are the reactive intermediates that form the polyketides. Using these esters, large chains such as fatty acids can be constructed and, in fact, reduction of the carbonyl groups and hydrolysis of the -SHCoA thioester leads to the fatty acid class of compounds. The expanding polyketide chain may be attached as a thioester to either CoA or to a protein called an acyl-carrier protein. Multiple Claisen reactions with additional molecules of malonyl-CoA can generate long-chain fatty acids such as stearic acid and myristic acid.

The poly-β-keto ester can also cyclize to give aromatic natural products, and the way in which the poly-β-keto ester folds determines the type of natural product generated (Fig. 6.2). If the poly-β-keto ester folds as **A1**, then loss of a proton, followed by an intramolecular **Claisen reaction** of intermediate **A2** (by attack of the acidic carbon on the carbonyl), would result in the formation of a cyclic polyketide enolate **A3,** which will rearrange to the keto compound with expulsion of the SCoA anion, resulting in the ketone **A4**. This ketone would readily undergo keto-enol tautomerism to the more favoured aromatic triphenol **A5 (phloroacetophenone)**.

Should the poly-β-keto ester fold as **B1**, then an aldol reaction on intermediate **B2** will occur by attack of the carbonyl by the acidic carbon, and, with the addition of a proton, an alcohol is formed, resulting in intermediate **B3**. This alcohol can then dehydrate to the conjugated alkene **B4**, which can also tautomerize and, via hydrolysis of the thioester-SCoA, the aromatic phenolic acid **orsellinic acid (B5)** is formed.

The reactive nature of poly-β-keto esters gives rise to many useful pharmaceuticals, and, because they are oxygen-rich starting precursors, the final natural products are generally rich in functional group chemistry. Ketone groups are often retained, but reduction to alcohols and the formation of ethers is common and many polyketides, particularly certain antibiotics and antitumour agents, also occur as glycosides.

FATTY ACIDS AND GLYCERIDES

This group of polyketides is widely distributed and present as part of the general biochemistry of all organisms, particularly as components of cell membranes. They are usually insoluble in water and soluble in organic solvents such as hexane, diethyl ether and chloroform. These natural products are sometimes referred to as fixed oils (liquid) or fats (solid), although these terms are imprecise as both fixed oils and fats

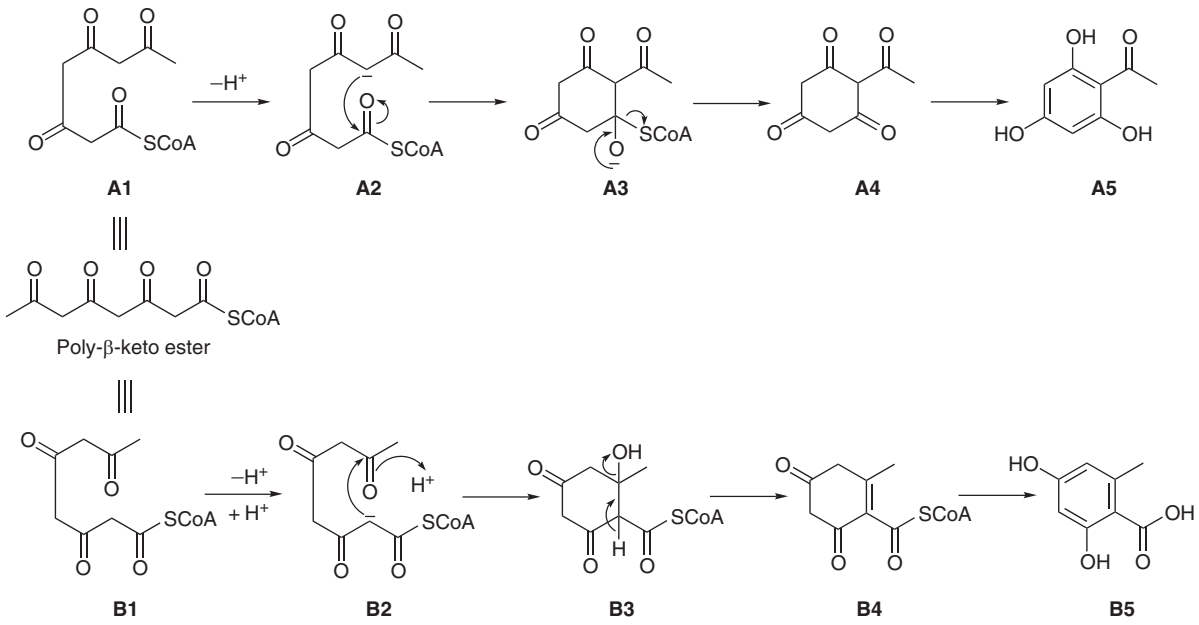


Fig. 6.2

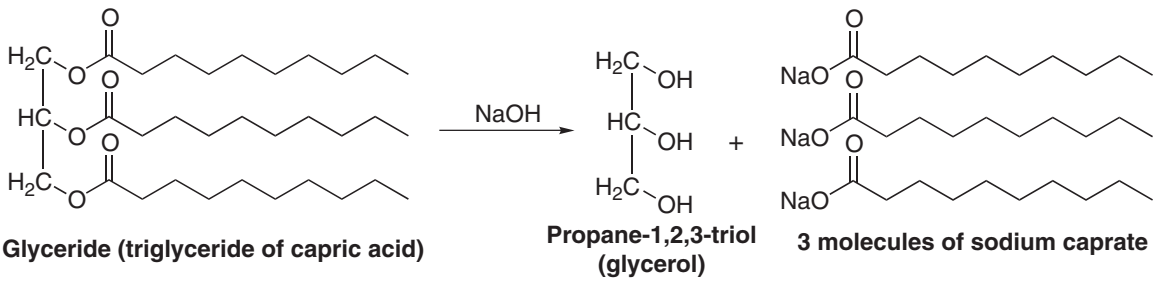


Fig. 6.3

contain mixtures of **glycerides** and free fatty acids and the state of the compound (i.e. liquid or solid) will depend on the temperature as well as the composition. Glycerides are fatty acid esters of **propane-1,2,3-triol (glycerol)**. They are sometimes referred to as saponifiable natural products, meaning that they can be converted into soaps by a strong base (NaOH). The term saponifiable comes from the Latin word *sapo* meaning 'soap'. Saponification of fatty acids and glycerides with sodium hydroxide results in the formation of the sodium salts of the fatty acids (Fig. 6.3).

Glycerides can be very complicated mixtures as, unlike the example given in Fig. 6.3, the substituents on the glycerol alcohol may be different from each other, and it is not uncommon for lipophilic plant extracts to contain many types of glycerides.

Fatty acids are very important as formulation agents and vehicles in pharmacy and as components of cosmetics and soaps. Table 6.1 lists the common names, chemical formulae, sources and uses of the more common fatty acids.

The **saturated** fats are widespread in nature. The three most common (**myristic, palmitic** and **stearic acids**) differ in two methylene groups and contain no double bonds.

The **unsaturated** fatty acids contain a varying number of double bonds. This, together with the length of the carbon chain, is indicated after the name of the fatty acid. For example, **oleic acid** (18:1), which is widespread in plants and is a major component of olive oil from the olive tree *Olea europaea* (Oleaceae), has an 18-carbon chain and one double bond. **α-Linolenic acid** (18:3) is a constituent of linseed oil from *Linum usitatissimum* (Linaceae) and is used in liniments and as a highly valued additive in oil-based paints. The related acid, **γ-linolenic acid** (18:3), is found in evening

Table 6.1 Common fatty acids

COMMON NAME	FORMULA	OIL AND SOURCE	OIL USE
Arachidic	$CH_3(CH_2)_{18}CO_2H$ (20:0)	Peanut oil, butter, *Arachis hypogaea* (Fabaceae)	Lubricant, food, emollient
Behenic	$CH_3(CH_2)_{20}CO_2H$ (22:0)	Carnauba wax, *Copernicia prunifera* (Arecaceae)	Polish for coated tablets
Butyric	$CH_3(CH_2)_2CO_2H$ (4:0)	Butter fat, cow, *Bovus taurus*	Food oil
Caproic	$CH_3(CH_2)_4CO_2H$ (6:0)	Coconut oil, *Cocos nucifera* (Arecaceae)	Manufacture of flavours
Caprylic	$CH_3(CH_2)_6CO_2H$ (8:0)	Coconut oil, *Cocos nucifera* (Arecaceae)	Dietary supplement, perfumes
Capric	$CH_3(CH_2)_8CO_2H$ (10:0)	*Cuphea viscosissima, C. lanceolata* (Lythraceae)	Artificial flavours, perfumes
Docosahexaenoic	$CH_3CH_2(CH{=}CHCH_2)_6CH_2CO_2H$ (22:6) all *cis*	Cod liver oil and halibut liver oil	Dietary supplement
Eicosapentaenoic	$CH_3CH_2(CH{=}CHCH_2)_5(CH_2)_2CO_2H$ (20:5) all *cis*	Cod liver oil and halibut liver oil	Dietary supplement
Erucic	$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}CO_2H$ (22:1) *cis*	Rapeseed oil, *Brassica napus* var. *oleifera* (Brassicaceae)	Food
Lauric	$CH_3(CH_2)_{10}CO_2H$ (12:0)	Coconut and palm kernel oil, *Cocos nucifera, Elaeis guineensis* (Arecaceae)	Food, soaps, shampoos
Linoleic	$CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6CO_2H$ (18:2) all *cis*	Vegetable oils, soybean, corn, *Glycine max* (Fabaceae)	Essential fatty acid
α-Linolenic	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)7CO_2H$	Linseed oil, *Linum usitatissimum* (Linaceae)	Liniments, paints, food
γ-Linolenic	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_4CO_2H$	Evening primrose oil, *Oenothera biennis* (Onagraceae)	Dietary supplement
Myristic	$CH_3(CH_2)_{12}CO_2H$	Coconut and palm kernel oil, *Cocos nucifera, Elaeis guineensis*	Food, soaps, shampoos
Nervonic	$CH_3(CH_2)_7CH{=}CH(CH_2)_{13}CO_2H$ (24:1) all *cis*	Honesty oil, *Lunaria annua* (Brassicaceae)	Supplement for multiple sclerosis patients
Oleic	$CH_3(CH_2)_7CH{=}CH\ (CH_2)_7CO_2H$ (18:1) *cis*	Olive oil, *Olea europaea* (Oleaceae)	Food, emulsifying agent
Palmitic	$CH_3(CH_2)_{14}CO_2H$ (16:0)	Coconut and palm kernel oil, *Cocos nucifera, Elaeis guineensis*	Food, soaps, candles
Ricinoleic	$CH_3(CH_2)_5CH(OH)CH_2CH{=}CH(CH_2)_7CO_2H$ (18:1) *cis*	Castor oil, *Ricinus communis* (Euphorbiaceae)	Soap manufacture
Stearic	$CH_3(CH_2)_{16}CO_2H$ (18:0)	Olive oil, *Olea europaea* (Oleaceae)	Suppositories, tablet coatings

primrose oil from *Oenothera biennis* (Onagraceae) and is widely used as a dietary supplement. This acid (like α-linolenic acid) is an essential fatty acid and is a precursor to the prostaglandins, which are involved in many biochemical pathways. Evening primrose oil has gained increasing popularity as an aid to alleviating symptoms associated with multiple sclerosis and premenstrual tension. **Ricinoleic acid** is the main purgative ingredient of castor oil from the seeds of *Ricinus communis* (Euphorbiaceae), which was used as a domestic purgative but is now used as a source of the oil for the manufacture of soap and as a cream base.

The **polyunsaturated** fatty acids contain three or more double bonds and are particularly beneficial in the diet as antioxidants. A number of health-food supplements are available as oils or capsules containing fish liver oils from **cod** and **halibut**, which are rich in polyunsaturated fats.

Natural oils that are high in fatty acids and glycerides are also used as components of oral formulations

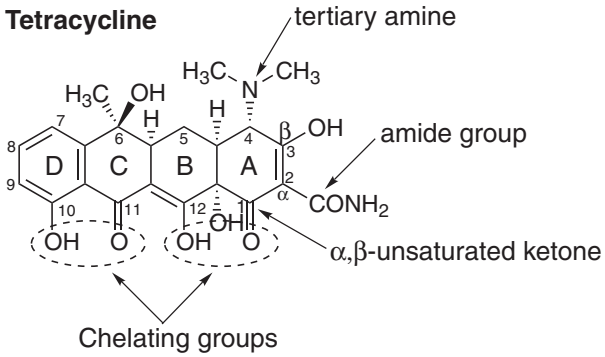
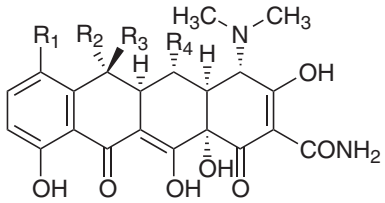


Fig. 6.4



Tetracycline, $R_1 = H, R_2 = CH_3, R_3 = OH, R_4 = H$
Oxytetracycline, $R_1 = H, R_2 = CH_3, R_3 = OH, R_4 = OH$
Doxycycline, $R_1 = H, R_2 = H, R_3 = CH_3, R_4 = OH$
Minocycline, $R_1 = N(CH_3)_2, R_2 = H, R_3 = H, R_4 = H$

Fig. 6.5

and vehicles for injections of pharmaceuticals. Some of the most common oils used in oral preparations include cocoa, olive, almond and coconut oils.

In man, the saturated fats are precursors for the biosynthesis of cholesterol, high serum levels of which are implicated in heart disease through the formation of atherosclerotic plaques in arteries. By reducing fat intake or by consuming foods that are high in unsaturated fats (particularly polyunsaturated fats), the risk of heart disease is reduced.

THE TETRACYCLINES

These polyketide-derived natural products are tetracyclic (i.e. have four linear six-membered rings, from which the group was named) and were discovered as part of a screening programme of extracts produced by filamentous bacteria (Actinomycetes), which are common components of soil. The most widely studied group of actinomycetes are species of the genus *Streptomyces*, which are very adept at producing many types of polyketide natural products of which the antibiotic **tetracycline** (Fig. 6.4) and the **anthracyclic** antitumour agents (see Chapter 8) are excellent examples.

The key features of this class of compound are shown in Fig. 6.4. Although tetracycline has numerous functional groups, including a tertiary amine, hydroxyls, an amide, a phenolic hydroxy and keto groups, it is still possible to see that tetracycline is a member of the polyketide class of natural products by looking at the lower portion of the molecule. C_{10}, C_{11}, C_{12} and C_1 are oxygenated, indicating that the precursor of this compound was a poly-β-keto ester. C_{10} and C_{11} and C_{12} and C_1 form part of a chelating system that is essential for antibiotic activity and may readily chelate metal ions such as calcium, magnesium, iron or aluminium and become inactive. This is one of the reasons why oral formulations of the tetracycline antibiotics are never given with foodstuffs that are high in these ions (e.g. Ca^{2+} in milk) or with antacids, which are high in cations such as Mg^{2+}. This group of antibiotics has been long known and they have a very broad spectrum of activity against Gram-positive and Gram-negative bacteria, spirochetes, mycoplasmae, rickettsiae and chlamydiae. **Tetracycline** comes from mutants of *Streptomyces aureofaciens*, and the related analogue **oxytetracycline** from *S. rimosus* (Fig. 6.5). These antibiotics are widely used as topical formulations for the treatment of acne, and as oral/injection preparations.

Griseofulvin

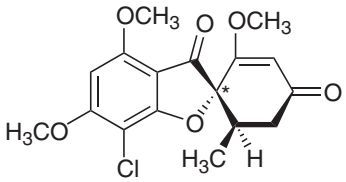


Fig. 6.6

Minocycline and **doxycycline** are produced semi-synthetically from natural tetracyclines. Minocycline has a very broad spectrum of activity and is recommended for the treatment of respiratory and urinary tract infections and as a prophylaxis for meningitis caused by *Neisseria meningitides*. Doxycycline (Vibramycin) is used in the treatment of chest infections caused by *Mycoplasma* and *Chlamydia* and is also used prophylactically against malaria in regions where there is a high incidence of drug resistance.

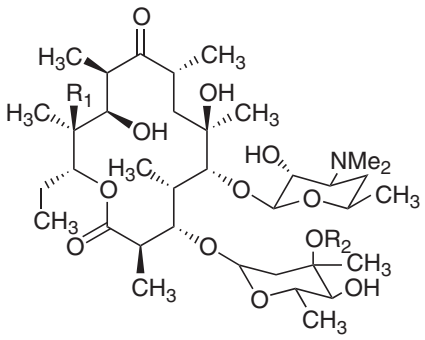
GRISEOFULVIN

Another polyketide antibiotic is griseofulvin (Grisovin) from the fungus (mould) *Penicillium griseofulvum* (Fig. 6.6). This compound was originally isolated by researchers at the London School of Hygiene and Tropical Medicine.

Griseofulvin is a **spiro** compound; that is, it has two rings that are **fused** at one carbon. Initially, the compound was used to treat fungal infections in animals and plants, but it is now recommended for the systemic treatment of fungal dermatophytic infections of the skin, hair, nails and feet caused by fungi belonging to the genera *Trichophyton, Epidermophyton* and *Microsporum*. Its main use is in veterinary practice for the treatment of ringworm in animals; it is marketed as Fulcin and Grisovin.

ERYTHROMYCIN A

Erythromycin A is a complex polyketide from *Saccharopolyspora erythrea* (Actinomycetes), which is a filamentous bacterium, originally classified in the genus *Streptomyces*. This compound is a member of the natural product class of **macrolide** antibiotics; these can contain 12 or more carbons in the main ring system. The term macrolide is derived from the fact that erythromycin is a large ring structure (**macro**) and is also a cyclic ester referred to as an **olide** (a lactone). As can be seen from Fig. 6.7, erythromycin A has the best features of natural products, being highly chiral (possessing many stereochemical centres) and having



Erythromycin A, R_1 = OH, R_2 = CH_3
Erythromycin B, R_1 = H, R_2 = CH_3
Erythromycin C, R_1 = OH, R_2 = H

Fig. 6.7

many different functional groups, including a sugar, an amino sugar, lactone, ketone and hydroxyl groups.

The therapeutic antibiotic is marketed as a mixture containing predominantly erythromycin A with small amounts of erythromycins B and C. Erythromycin is used to treat Legionnaire's disease and for patients with respiratory tract infections who are allergic to penicillin. A number of semi-synthetic analogues (including **clarithromycin** and **azithromycin**) have been made and there has been interest in the genetic manipulation of *Saccharopolyspora erythrea* to produce 'un-natural' natural products. These compounds could potentially be analogues of the erythromycin class and might also have antibiotic activity.

THE STATINS

A further group of polyketide-derived natural products is the statins, so named for their ability to lower (bring into stasis) the production of cholesterol, high levels of which are a major contributing factor to the development of heart disease. The rationale behind the use of these compounds is as inhibitors of the enzyme hydroxymethylglutaryl-CoA (HMG-CoA) reductase, which catalyses the conversion of HMG-CoA (Fig. 6.8) to mevalonic acid, one of the key intermediates in the biosynthesis of cholesterol. HMG-CoA reductase became a target for the discovery of the natural product inhibitor **mevastatin**, which was initially isolated from cultures of the fungi *Penicillium citrinum* and *Penicillium brevicompactum* (Fig. 6.8).

Following this discovery, the methyl analogue **lovastatin** was isolated from *Monascus ruber* and *Aspergillus terreus* and is also an inhibitor of HMG-CoA reductase. **Simvastatin** (Zocor) is the dimethyl analogue of

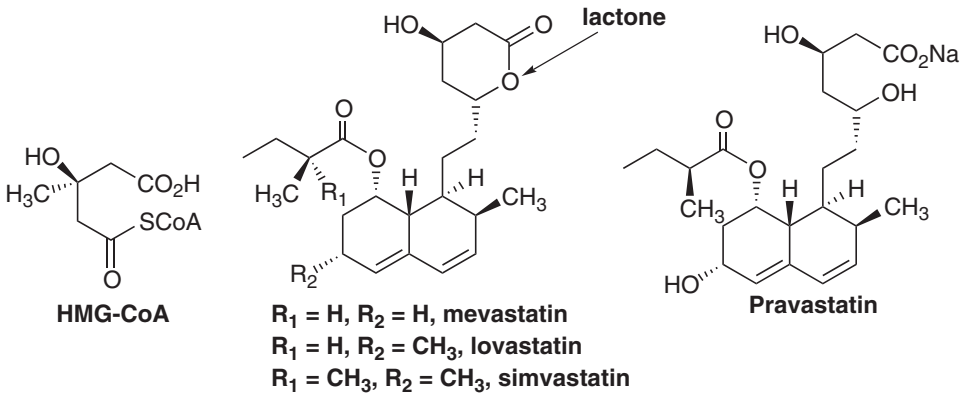


Fig. 6.8

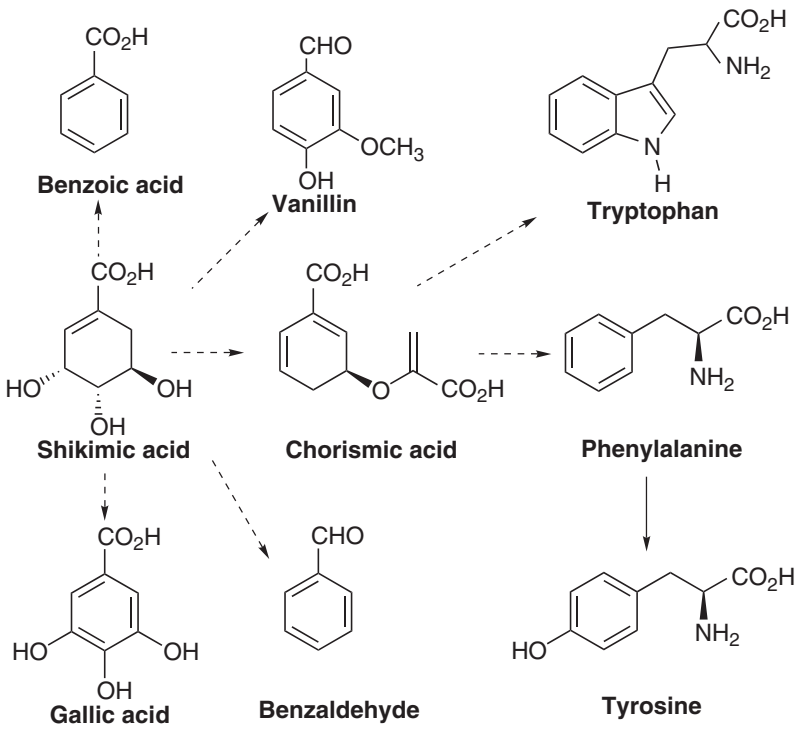
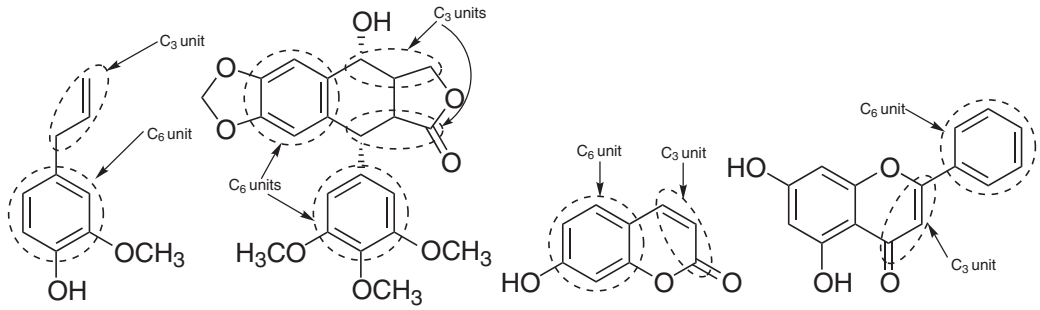


Fig. 6.9

mevastatin and all three compounds are prodrugs, being activated by the hydrolysis (ring opening) of the lactone ring to β-hydroxy acids by liver enzymes. These acids are similar in structure to HMG-CoA and are inhibitors of the reductase enzyme. **Pravastatin** (Lipostat) is semi-synthetically produced by microbial hydroxylation of mevastatin by *Streptomyces carbophilus*. Unlike the previous examples, the lactone ring is opened to form the β-hydroxy acid, which is then converted into the sodium salt, increasing its hydrophilic water-soluble nature.

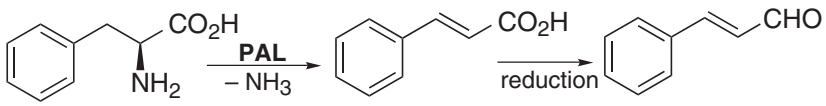
SHIKIMIC-ACID-DERIVED NATURAL PRODUCTS

Shikimic acid, sometimes referred to as shikimate, is a simple acid precursor for many natural products and aromatic amino acids, including phenylalanine, tyrosine, tryptophan, the simple aromatic acids that are common in nature (e.g. benzoic and gallic acids) and aromatic aldehydes, such as vanillin and benzaldehyde that contribute to the pungent smell of many plants (Fig. 6.9).



Eugenol (phenylpropene) **Podophyllotoxin (lignan)** **Umbelliferone (coumarin)** **Chrysin (flavonoid)**

Fig. 6.10



Phenylalanine **Cinnamic acid** **Cinnamaldehyde**

PAL = phenylalanine ammonia lysase

Fig. 6.11

A number of natural product groups can be constructed from the amino acid phenylalanine, in particular the **phenylpropenes, lignans, coumarins** and **flavonoids**, all of which possess a common substructure based on an aromatic 6-carbon ring (C_6 unit) with a 3-carbon chain (C_3 unit) attached to the aromatic ring (Fig. 6.10). Many reactions can occur to this 9-carbon unit, including oxidation, reduction, methylation, cyclization, glycosylation (addition of a sugar) and dimerization, all of which contribute to the value of natural products as a resource of biologically active compounds and enhance the qualities of structural complexity with the presence of chirality and functionality.

PHENYLPROPENES

The phenylpropenes are the simplest of the shikimic-acid-derived natural products and consist purely of an aromatic ring with an unsaturated 3-carbon chain attached to the ring. They are biosynthesized by the oxidation of phenylalanine by the enzyme phenylalanine ammonia lyase, which through the loss of ammonia results in the formation of cinnamic acid. Cinnamic acid may then undergo a number of **elaboration reactions** to generate many of the phenylpropenes. For example, in Fig. 6.11, cinnamic acid is reduced to the corresponding aldehyde, **cinnamaldehyde**, which is the major component of cinnamon oil derived from the bark of *Cinnamomum zeylanicum* (Lauraceae) and used as a spice and flavouring. Cinnamon has a rich history, being used by the ancient Chinese as a treatment for fever and diarrhoea and by the Egyptians as a fragrant ingredient in embalming mixtures.

Cinnamon leaf also contains **eugenol**, the major constituent of oil of cloves derived from *Syzygium aromaticum* (Myrtaceae). Clove oil was used as a dental anaesthetic and antiseptic, both properties of which are due to eugenol, and the oil is still widely used as a short-term relief for dental pain. These phenylpropenes may have many different functional groups (e.g. OCH_3, O-CH_2-O, OH) and the double bond may be in a different position in the C_3 chain (e.g. eugenol versus **anethole**) (Fig. 6.12). They are common components of spices, have highly aromatic pungent aromas and many are broadly antimicrobial, with activities against yeasts and bacteria. Some members of this class can also cause inflammation.

Myristicin is a component of nutmeg (*Myristica fragrans*, Myristicaceae) and is thought to be the hallucinogenic component when this spice is ingested in large quantities. This phenylpropene is very lipophilic due to the presence of methylenedioxy and methyl ether substituent groups and it has been proposed that *in vivo* the double bond of this compound is aminated (an amino group is added), resulting in the formation of an 'amphetamine-like' compound.

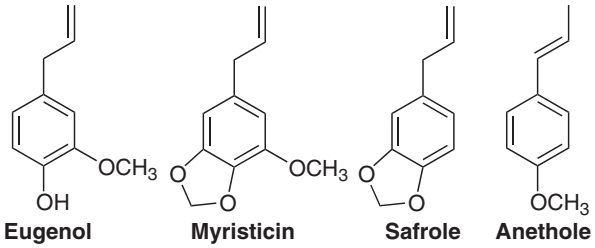


Fig. 6.12

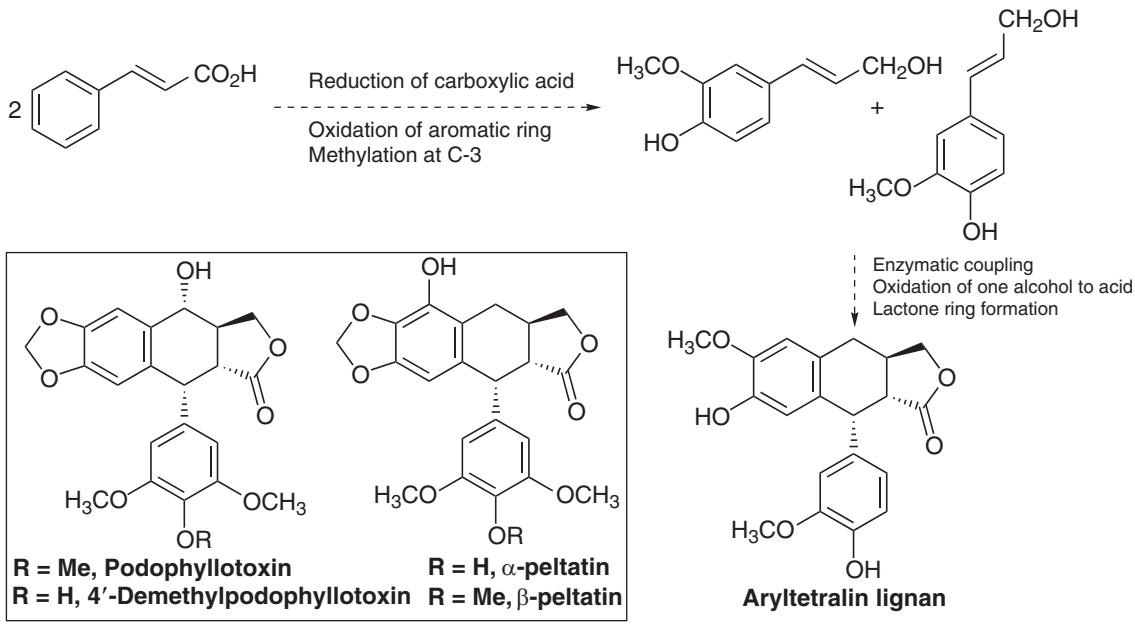


Fig. 6.13

It should be noted, however, that high doses can be fatal and the ingestion of large amounts of nutmeg should be avoided. **Safrole**, and particularly ***trans*-anethole**, are the major components of anise-flavoured essential oils from star anise (*Illicium verum*, Illiciaceae), aniseed (*Pimpinella anisum*, Apiaceae) and fennel (*Foeniculum vulgare* var. *vulgare*, Apiaceae). These oils are components of popular Mediterranean beverages such as anisette, ouzo and raki. When water is added to these drinks a cloudy white suspension results, which is attributable to a decrease in the solubility of these phenylpropenes as they are more soluble in ethanol than in water.

The phenylpropenes are generally produced by **steam distillation** (see Chapter 7) of plant material (e.g. cloves) to produce an essential oil, which is normally a complex mixture of phenylpropenes and other volatile natural products such as **hemiterpenes, monoterpenes** and **sesquiterpenes** (see below). The steam distillation procedure involves boiling the plant material with water and trapping the vapour in a distillation apparatus. The condensed liquid is transferred to a separating funnel and, as the oils are immiscible with water and form a less dense layer, they can be readily removed.

LIGNANS

Lignans are low-molecular-weight polymers formed by the coupling of two phenylpropene units through their C_3 side-chains (Fig. 6.13) and between the aromatic ring and the C_3 chain. A common precursor of lignans is cinnamyl alcohol, which can readily form free radicals and enzymatically dimerize to form aryltetralin-type lignans of which the compounds **podophyllotoxin, 4′-demethylpodophyllotoxin** and **α-** and **β-peltatin** (from *Podophyllum peltatum* and *Podophyllum hexandrum*, Berberidaceae) are examples (Fig. 6.14).

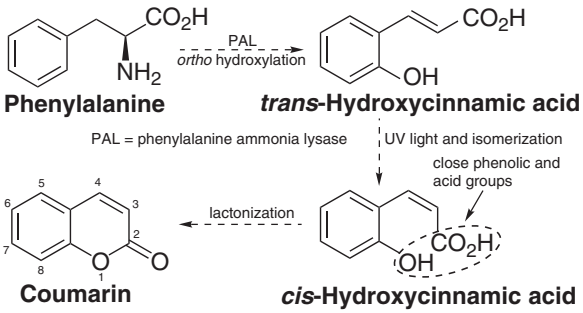


Fig. 6.14

This class of compound is common in the plant kingdom, especially in the heartwood and leaves and as major constituents of resinous exudates from roots and bark. The resin obtained from the roots of *P. peltatum* has long been used as a treatment for warts by North American Indians, and some preparations still exist that contain podophyllin, which is an ethanolic extraction of the resin rich in podophyllotoxin. This lignan is a dimer of two 9-carbon (C_6–C_3) units and is **polycyclic** (possessing more than one ring). This natural product has a 5-membered lactone ring or cyclic ester and there are many examples of these types of lignans possessing this functional group.

Much work has been done on the podophyllotoxin (aryltetralin) class of lignans, the major active cytotoxic principle, podophyllotoxin, being isolated in the 1940s. This compound inhibits the enzyme tubulin polymerase, which is needed for the synthesis of tubulin, a protein that is a vital component of cell division (mitosis). Podophyllotoxin is highly toxic and not used clinically for the treatment of cancers, but this class of compounds was an excellent template on which to base the semisynthetic analogues **etoposide** and **teniposide** (see Chapter 9).

COUMARINS

The coumarins are shikimate-derived metabolites formed when phenylalanine is deaminated and hydroxylated to *trans*-hydroxycinnamic acid (Fig. 6.14). The double bond of this acid is readily converted to the *cis* form by light-catalysed isomerization, resulting in the formation of a compound that has phenol and acidic groups in close proximity. These may then react **intramolecularly** to form a lactone and the basic coumarin nucleus, typified by the compound **coumarin** itself, which contributes to the smell of newly mown hay.

The majority of the coumarins are oxygenated at position C_7, resulting from *para* hydroxylation of cinnamic acid to give coumaric acid prior to further *ortho* hydroxylation, isomerization and lactone formation.

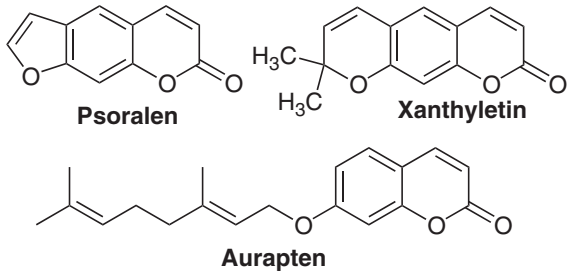


Fig. 6.15

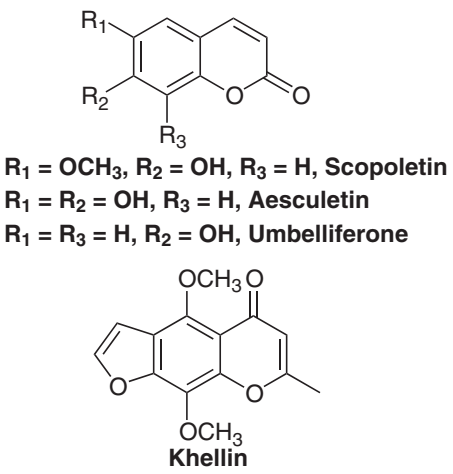
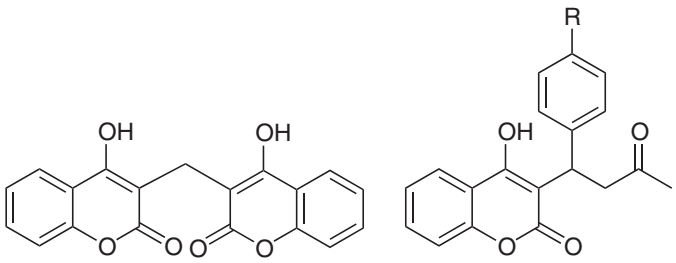


Fig. 6.16

Coumarins have a limited distribution in the plant kingdom and have been used to classify plants according to their presence (chemotaxonomy). They are commonly found in the plant families Apiaceae, Rutaceae, Asteraceae and Fabaceae and, as with all of the natural products mentioned so far, undergo many elaboration reactions, including hydroxylation and methylation and, particularly, the addition of terpenoid-derived groups (C_2, C_5 and C_{10} units) (Fig. 6.15).

Some coumarins are **phytoalexins** and are synthesized *de novo* by the plant following infection by a bacterium or fungus. These phytoalexins are broadly antimicrobial; for example, **scopoletin** is synthesized by the potato (*Solanum tuberosum*) following fungal infection. **Aesculetin** occurs in the horse chestnut (*Aesculus hippocastanum*) and phytotherapeutic preparations of the bark of this species are used to treat capillary fragility. *Hieracium pilosella* (Asteraceae), also known as mouse ear, contains **umbelliferone** and was used to treat brucellosis in veterinary medicine and the antibacterial activity of this plant drug may in part be due to the presence of this simple phenol (Fig. 6.16). **Khellin** is an **isocoumarin** (**chromone**) natural product



Dicoumarol

R = H, Warfarin
R = NO_2, Nicoumalone

Fig. 6.17

from *Ammi visnaga* (Apiaceae) and has activity as a spasmolytic and vasodilator.

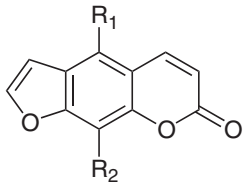
It has long been known that animals fed sweet clover (*Melilotus officinalis*, Fabaceae) die from haemorrhaging. The poisonous compound responsible for this adverse effect was identified as the bishydroxycoumarin (hydroxylated coumarin dimer) **dicoumarol** (Fig. 6.17).

Dicoumarol is used as an anticoagulant in the USA. It is used alone or in conjunction with heparin in the prophylaxis and treatment of blood clotting and to arrest gangrene after frostbite. A number of compounds have been synthesized based on the dicoumarol structure and include salts of **warfarin** and **nicoumalone**, which are widely used as anticoagulants. These agents interfere with vitamin K function in liver cells; this vitamin is necessary for the synthesis of 'normal' prothrombin. A deficiency of vitamin K leads to abnormal prothrombin synthesis and a reduction in activity of the blood-clotting mechanism. Warfarin has also been used as a rat poison.

Warfarin and nicoumalone are examples of fully synthetic agents that have been developed from a natural product template.

The **psoralens** are coumarins that possess a furan ring and are sometimes known as **furocoumarins** or **furanocoumarins** because of this ring. Examples are **psoralen, bergapten, xanthotoxin** and **isopimpinellin** (Fig. 6.18).

Because of the extended chromophore of these compounds, they readily absorb light and fluoresce blue/yellow under long-wave ultraviolet light (UV-A, 320–380 nm). These compounds may be produced by the plant as a protection mechanism against high doses of sunlight and some coumarins are formulated into sunscreens and cosmetics for this purpose. The psoralens are typical of the citrus (Rutaceae) and celery (Apiaceae) families. Some plants of these groups are known as 'blister bushes' as the psoralens they contain are known to cause phototoxicity. This can prove difficult



R_1 = R_2 = H, Psoralen
R_1 = OCH_3, R_2 = H, Bergapten
R_1 = H, R_2 = OCH_3, Xanthotoxin
R_1 = R_2 = OCH_3, Isopimpinellin

Fig. 6.18

for farmers who clear large amounts of giant hogweed (*Heracleum mantegazzianum*) and come into contact with sap from the plant, which is rich in psoralens and, in the presence of sunlight, can cause inflammation and, in severe cases, blistering of the skin. Other species that are known to be phototoxic include hogweed (*Heracleum sphondylium*), rue (*Ruta graveolens*) and some *Citrus* spp., particularly essential oils from bergamot (*Citrus aurantium* subsp. *bergamia*, Rutaceae) of which a major constituent is **bergapten**. A number of apiaceous herbs that have culinary significance, such as celery (*Apium graveolens*), parsley (*Petroselinum crispum*), parsnip (*Pastinaca sativa*) and angelica (*Angelica archangelica*), may even cause phototoxicity due to the presence of furanocoumarins.

The mechanism of this phototoxicity has yet to be fully elucidated, but it is known that the psoralens are carcinogenic and mutagenic due to the formation of adducts with pyrimidine bases of DNA, such as thymine, via cycloaddition (Fig. 6.19). This reaction can occur with one (monoadduct) or two (di-adduct) pyrimidine bases and may result in cross-linking of DNA.

Preparations using apiaceous and rutaceous plants containing psoralens have been used to promote skin pigmentation in the disease vitiligo, a disease that is

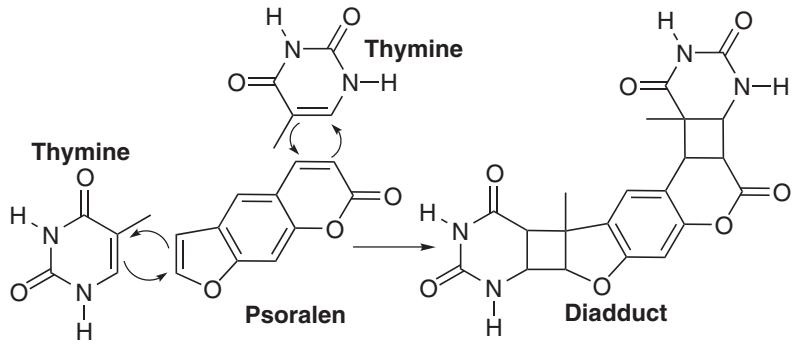


Fig. 6.19

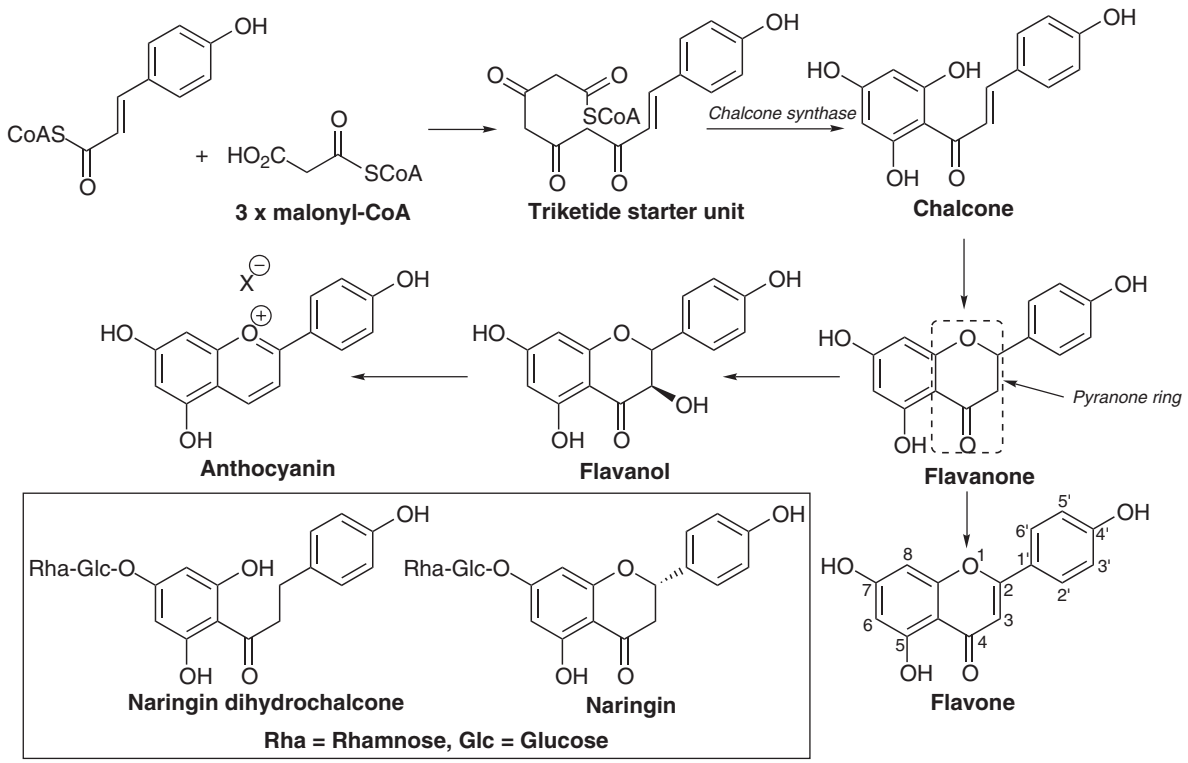


Fig. 6.20

common in the Middle East and results from patches of skin that are deficient in the pigment melanin. Pure **xanthotoxin** is used to treat severe vitiligo and psoriasis, and is given orally in combination with UV-A. This results in coloration and pigmentation of non-pigmented skin areas and an improvement in the psoriatic skin by reducing cell proliferation. The treatment is not without risks and requires careful regulation to prevent skin cancer or cataract formation. The therapy is referred to as PUVA (psoralen+UV-A) or photodynamic therapy in which a drug is activated by the application of UV light.

FLAVONOIDS

The flavonoids are derived from a C_6–C_3 (phenylpropane) unit, which has as its source shikimic acid (via phenylalanine) and a further C_6 unit that is derived from the polyketide pathway. This polyketide fragment is generated by three molecules of malonyl-CoA, which combine with the C_6–C_3 unit (as a CoA thioester) to form a triketide starter unit (Fig. 6.20). Flavonoids are, therefore, of mixed biosynthesis, consisting of units derived from both shikimic acid and polyketide pathways.

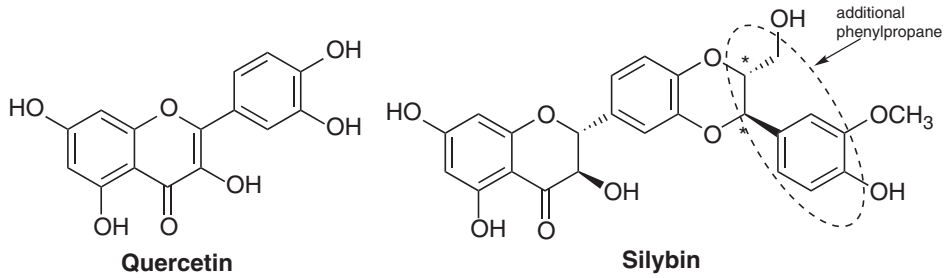


Fig. 6.21

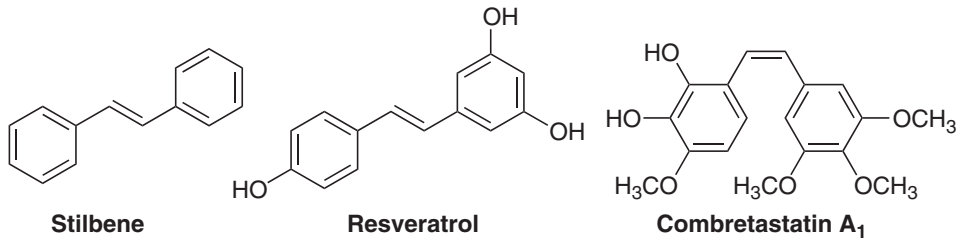


Fig. 6.22

The triketide starter unit undergoes cyclization by the enzyme chalcone synthase to generate the **chalcone** group of flavonoids. Cyclization can then occur to give a pyranone ring-containing **flavanone** nucleus, which can either have the C_2–C_3 bond oxidized (unsaturated) to give the **flavones** or be hydroxylated at position C_3 of the pyranone ring to give the **flavanol** group of flavonoids. The flavanols may then be further oxidized to yield the **anthocyanins**, which contribute to the brilliant blues of flowers and the dark colour of red wine. The flavonoids contribute to many of the other colours found in nature, particularly the yellow and orange of petals; even the colourless flavonoids absorb light in the UV spectrum (due to their extensive chromophores) and are visible to many insects. It is likely that these compounds have high ecological importance in nature as colour attractants to insects and birds as an aid to plant pollination. Certain flavonoids also markedly affect the taste of foods; for example, some are very bitter and astringent such as the flavanone glycoside **naringin** (Fig. 6.20), which occurs in the peel of grapefruit (*Citrus paradisi*). Interestingly, the closely related compound **naringin dihydrochalcone** (Fig. 6.20), which lacks the pyranone ring of naringin, is exceptionally sweet, being some 1000 times sweeter than table sugar (sucrose).

It is likely that the flavonoids have important dietary significance because, being phenolic compounds, they are strongly antioxidant. Many disease states are known to be exacerbated by the presence of free radicals such as superoxide and hydroxyl, and flavonoids have the ability to scavenge and effectively 'mop up' these damaging oxidizing species. Foods rich in this group have, therefore, been proposed to be important in ameliorating diseases such as cancer and heart disease (which can be worsened by oxidation of low-density lipoprotein); **quercetin** (Fig. 6.21), a flavonoid present in many foodstuffs, is a strong antioxidant. Components of milk thistle (*Silybum marianum*), in particular **silybin** (Fig. 6.21), are antihepatotoxins; extracts of milk thistle are generally known as **silymarin** and are used to reduce the effects of poisoning by fungi of the genus *Amanita*, which produces the deadly peptide toxins the amanitins. The mechanism of action of these antihepatotoxins is not entirely clear, but it has been proposed that they protect liver cells by reducing entry of the toxic peptides through the cell membrane and by acting as broad-spectrum antioxidants by scavenging the free radicals that can lead to hepatotoxicity. Silybin is a flavanol that has an additional phenylpropane unit joined to it as a di-ether and it exists in the extract as a mixture of enantiomers at one of the positions where this additional unit is joined (* in Fig. 6.21).

The **stilbenes**, sometimes referred to as **bisbenzyls** or **stilbenoids**, are related to the flavonoids and have the basic structure C_6–C_2–C_6 (Fig. 6.22) arising from the loss of one carbon (as CO_2) from the triketide starter unit. The simplest member of this class is **stilbene**. There is much interest in this class of compounds, especially in **resveratrol**, a component of red wine that has antioxidant, anticancer and anti-inflammatory activity. There is a low incidence of heart disease among the French

population where large concentrations of fatty acids are sometimes present in the diet. It has been suggested that this low rate of heart disease is due to the consumption of red wine, which is rich in resveratrol and other flavonoids, and that the presence of these antioxidant compounds is cardioprotective. This phenomenon is known as 'the French paradox' and cardiologists advise patients who have a history of heart disease to consume a glass of red wine per day. Another group of stilbenoids of current interest is the **combretastatins**. For example, **combretastatin A_1**, which is a cytotoxic drug lead, is a potent inhibitor of microtubule assembly and thought to have antitumour activity as a result of specifically targeting the vasculature of tumours. Combretastatin A_1 is derived from *Combretum caffrum* (Combretaceae) and there has been much work on the Combretaceae to look for other biologically active members of this class.

TANNINS

In addition to the flavonoids, another class of natural products that gives rise to the astringency and bitterness in plants and food is the **tannins**. This group comprises water-soluble polyphenolic compounds, which may have a high molecular weight. They are broadly divided into two groups: the **hydrolysable** tannins, which are formed by the esterification of sugars (e.g. glucose) with simple phenolic acids that are shikimate-derived (e.g. gallic acid), and the **non-hydrolysable** tannins, which are sometimes referred to as **condensed** tannins, that occur due to polymerization (condensation) reactions between flavonoids (Fig. 6.23).

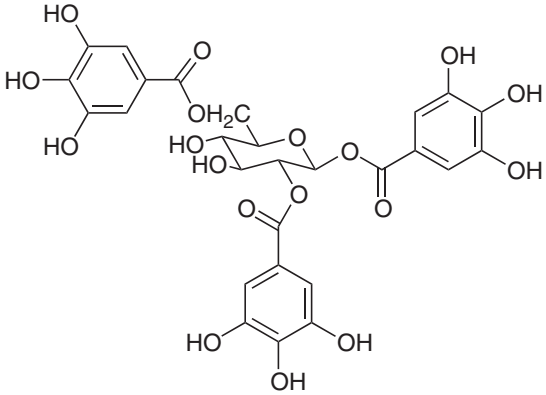
As their name suggests, the hydrolysable tannins may be hydrolysed with base to simple acids and sugars. A key feature of tannins is their ability to bind to proteins, and they have been used to tan leather, clarify beer and as astringent preparations in pharmacy. They have a very wide distribution in the plant kingdom and may be produced by a plant as a feeding deterrent, as their binding to proteins may reduce the dietary value of the plant as a food.

Tannic acid is a mixture of gallic acid esters of glucose and is obtained from nutgall, which is an abnormal growth of the tree *Quercus infectoria* produced by insects. These growths (galls) are harvested and extracted with solvents (ether and water); the aqueous layer is collected and evaporated to yield tannic acid, which is further purified and used as a topical preparation for cold sores.

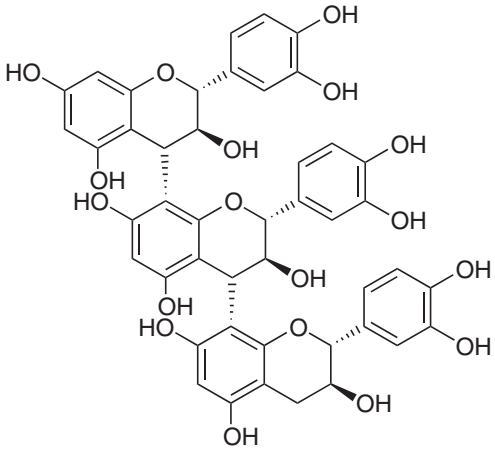
THE TERPENES

The terpenes are very widespread in nature and occur in most species, including man. They are sometimes referred to as **isoprenes** because a common recurring motif in their structure (the branched repeating C_5 unit, the **isopentane** skeleton) is similar to isoprene (Fig. 6.24). Terpenes (**hemiterpenes, monoterpenes** and **sesquiterpenes**) contribute to many of the aromas associated with plants and range in complexity from simple C_5 units (**hemiterpenes**) up to the **polyisoprenes**, which include latex, leaf waxes and rubber. Terpenes are derived from a number of extensive reactions between two C_5 units [**dimethylallyl pyrophosphate (DMAPP**) and **isopentenyl pyrophosphate (IPP)**] (Fig. 6.24); the products of these reactions will, therefore, have multiples of five carbons. DMAPP and IPP are biosynthesized from two sources (**mevalonic acid** or **deoxyxylulose phosphate**).

The terpenes are a perfect example of a natural product class that is highly structurally diverse, has



Hydrolysable tannin (trigalloyl glucose)



Non-hydrolysable tannin (flavonoid trimer)

Fig. 6.23

many members that are chiral and have extensive functional group chemistry. The simplest are the hemiterpenes (C_5) produced by modification reactions to either DMAPP or IPP and include simple acids such as the structural isomers **tiglic acid** and **angelic acid** (Fig. 6.24), which form esters with many natural products. The monoterpenes (C_{10}), sesquiterpenes (C_{15}), **diterpenes** (C_{20}), **triterpenes** and **steroids** (C_{30}-derived) and the **tetraterpenes** (**carotenoids**, C_{40}) are all important medicinally and thus will be dealt with in more detail.

MONOTERPENES (C_{10})

Together with the phenylpropenes, the **monoterpenes** are major constituents of the volatile oils that are common in plants and which contribute to their aroma. This group of compounds has highly characteristic odours and tastes and is used widely in the food and cosmetic industries in flavourings and perfumes. Monoterpenes are present in the leaf glands of plants and in the skin and peel of fruit (in particular *Citrus* spp.). The reasons for the presence of these compounds in the exterior organs of the plant are due to the many complex interactions that plants have with other organisms: some monoterpenes are insect attractants (to aid pollination), others have a broad spectrum of antimicrobial activity to inhibit growth and invasion by bacteria and fungi (e.g. **thymol**). Volatile oils in plants are highly complex and their analysis by gas chromatography (GC) can show the presence of hundreds of individual components, many of which are monoterpenoid. These oils are highly prized in the perfume industry; plants such as jasmine are cultivated and the monoterpene-rich oils harvested for the production of popular fragrances. Monoterpenes may be either aliphatic (**acyclic** or straight chain) or **cyclic** (saturated, partially unsaturated or fully aromatic) compounds. These natural products usually possess functional groups such as ethers, hydroxyls, acids, aldehydes, esters or ketone moieties, and are generally highly volatile and fat-soluble (lipophilic).

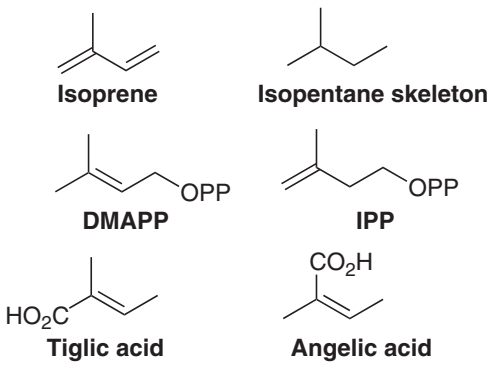


Fig. 6.24

Biosynthetically, the monoterpenes are produced by the reaction between DMAPP and IPP in the presence of the enzyme **prenyltransferase** (Fig. 6.25). The first step of this reaction is thought to be the ionization of DMAPP to a cation (through the loss of pyrophosphate), which is then attacked by the double bond of

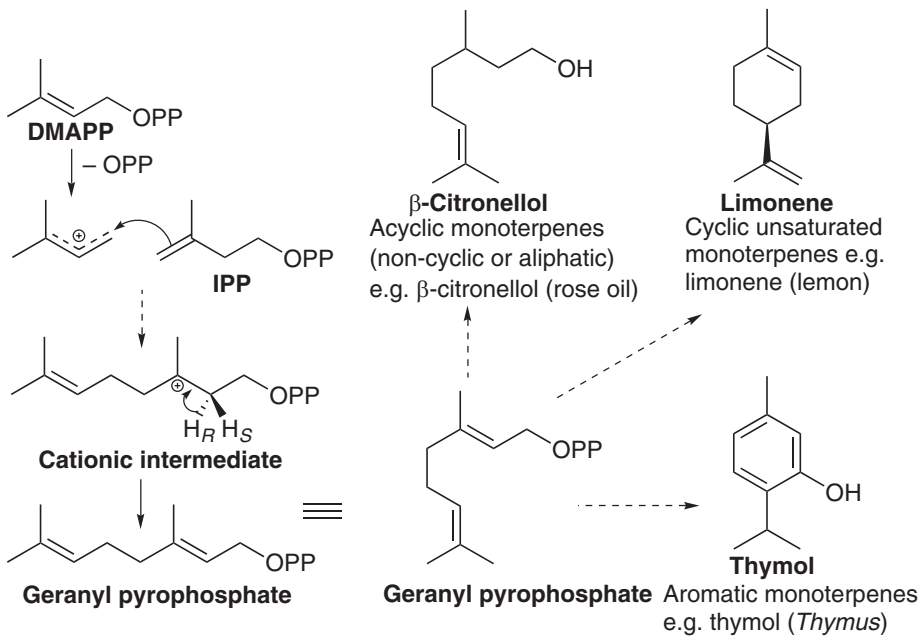


Fig. 6.25

IPP to generate a further cationic intermediate. Loss of a proton from the carbon neighbouring the cation (resulting in double bond formation) occurs in a stereospecific fashion (the R proton is lost) and this generates **geranyl pyrophosphate** (a C_{10} unit).

Geranyl pyrophosphate can then undergo many reactions to generate the variety of monoterpenes observed, such as simple modification to give the acyclic monoterpene **β-citronellol**, which is a component of rose oil. Geranyl pyrophosphate can be cyclized to give cyclic monoterpenes, which may be fully saturated, partially unsaturated or fully aromatic products of which **menthol, piperitone** and **carvacrol** are examples, respectively (Fig. 6.26).

As with the polyketides, some key features of monoterpenes (and terpenes in general) are the presence of stereochemical centres (chiral centres) and wide-ranging functional group chemistry. The extensive structural diversity of this group is astounding considering that all of the monoterpenes are derived from just one C_{10} unit, geranyl pyrophosphate.

Linalool, a major constituent of coriander oil (*Coriandrum sativum*), is used as a flavouring and carminative. **Myrcene**, which is present in hop oil, is also used as a flavouring. Tea tree oil (from *Melaleuca alternifolia*) was used by the indigenous peoples of Australia as a treatment for skin infections; a main ingredient of this volatile oil is the tertiary hydroxylated monoterpene **α-terpineol**. **1,8-Cineole**, the structurally related ether, also has antibacterial properties and comes from species of *Eucalyptus* that are in the same plant family as *Melaleuca*, the Myrtaceae. **Menthol** and **menthone** are major constituents of oils of plants belonging to the genus *Mentha* (Lamiaceae); in particular, peppermint (*Mentha* × *piperita*) is used as a flavouring and carminative tea, and menthone is included in some pharmaceutical preparations as a nasal decongestant. **Thujone** has a cyclopropane ring as a functional group and is a constituent of *Artemisia absinthium*, an extract of which was used as an anthelmintic by the French army, hence the common name for this plant, wormwood. The liqueur absinthe was prepared by making an alcoholic extract of wormwood; this was highly popular amongst artists and literati in 19th century France. Unfortunately, high doses of this beverage induce hallucinations and the drink is addictive (not just the alcohol), and these effects led to the term 'absinthism' to describe the side effects associated with absinthe. Due to these problems, the production of absinthe was banned in 1915. **Carvone** is derived from dill (*Anethum graveolens*) and caraway oils (*Carum carvi*), which have use as calming ingredients in gripe water preparations. **α-Pinene**, which has a cyclobutane ring system, is the major constituent of juniper oil (*Juniperus communis*), which is antiseptic and used in aromatherapy and as a flavouring. Oil from *Cinnamomum camphora* (Lauraceae) is produced by steam distillation of the wood and is rich in **camphor**, which is antiseptic and used in soaps.

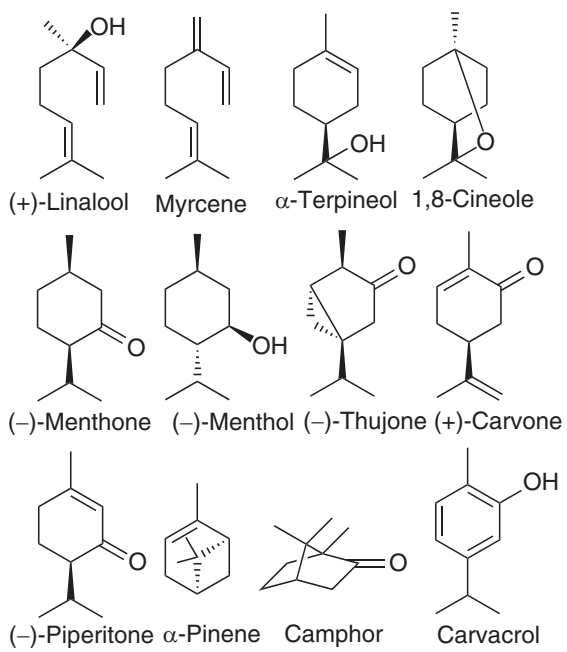


Fig. 6.26

Although oils from plants such as caraway, coriander, dill, peppermint and eucalyptus are widely used as flavouring agents and perfumes for many preparations (including foods, cosmetics and pharmaceuticals), at present not a great deal is known about the biological activity of the monoterpene components present in these complex mixtures. Natural oils have a very specific aroma, which accounts for the preference for these complex natural mixtures rather than cheaper synthetic alternatives. They are produced by **steam distillation** (see Chapter 7) and, unless much is known about the stability of the oil components, care must be taken using this technique as some monoterpenes are **thermolabile** (i.e. they decompose on heating). The analysis of these complex mixtures is usually performed by gas chromatography (GC) or the combined technique of GC and mass spectrometry (GC–MS), which utilizes the separating power of GC with MS to yield the molecular ions of components of a mixture, and in some cases fragmentation information, which can aid in determining the structure of these components.

The perfume industry has a great interest in monoterpene mixtures and uses preparative GC to separate and

isolate individual components, which a highly qualified perfumer then smells to find compounds with a distinctive, novel or unusual aroma that can be blended with other volatiles to give a popular fragrance.

The **iridoids** are monoterpenes derived from the **iridane** skeleton, which is derived from geranyl pyrophosphate and, when oxidized, produces the iridoid skeleton (Fig. 6.27). These natural products are normally esterified and are common in the plant families Lamiaceae, Gentianaceae and Valerianaceae. The compounds are highly oxygenated and the esters are often derived from hemiterpenes; for example, valeric acid is esterified to form **valtrate** and **didrovaltrate**.

These compounds come from valerian (*Valeriana officinalis*, Valerianaceae), which was used as a sedative for the treatment of 'shell shock', a condition with which troops serving in the First World War were afflicted following extensive barrage by high-explosive shells. This class of iridoids is often referred to as the **valepotriates**; they are highly functional, possessing isovalerate esters and an epoxide group that is possibly responsible for the *in vitro* cytotoxicity of valtrate and didrovaltrate. It is still not known exactly which class of compounds is responsible for the sedative activity, although the iridoids are widely regarded as the active components. However, it has been suggested that γ-aminobutyric acid (GABA), which is present in aqueous extracts of valerian, contributes to the sedative activity. Valerian also contains a number of small acids, such as isovaleric acid, that are structurally similar to GABA; these may, therefore, contribute to the sedative action of this herb extract. Valerian is commonly found in herbal remedies to improve sleep and is often used in conjunction with extracts from hops (*Humulus lupulus*) (e.g. in the preparation Valerina Night-Time®).

SESQUITERPENES (C_{15})

These natural products have properties similar to those of the monoterpenes, are constituents of many of the volatile oils and in some cases are broadly antimicrobial and anti-insecticidal, therefore contributing to the overall chemical defence of the producing organism. The starting unit for these compounds is **farnesyl pyrophosphate** (FPP), which is produced by the reaction of GPP (the monoterpene precursor) with a molecule of IPP (Fig. 6.28). The reaction is analogous to that for the formation of the monoterpenes in which a cationic intermediate is formed that reacts with IPP with elimination of a hydrogen ion.

As with the monoterpenes, FPP can cyclize to form linear (acyclic) and cyclic sesquiterpenes. A key feature of these metabolites is their ability to undergo extensive elaboration chemistry, where they are highly functionalized, thus giving rise to the high structural diversity seen within this group of natural products. It is not always easy to see that these complex, functional, cyclic chiral compounds are derived from FPP due to these elaboration reactions. However, if the C_{15} **skeleton** of FPP is compared to **arteannuin B**, it can be seen how even complex structures are constructed (Fig. 6.29).

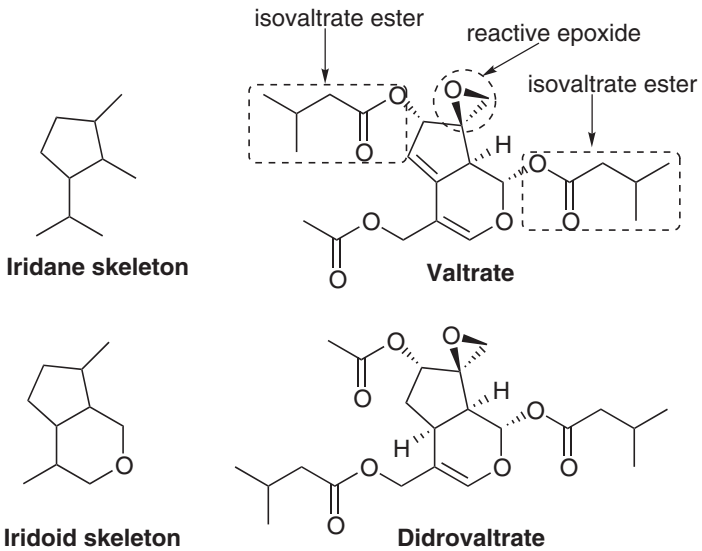


Fig. 6.27

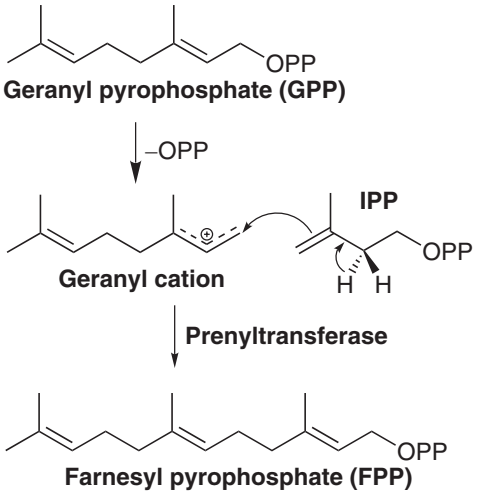


Fig. 6.28

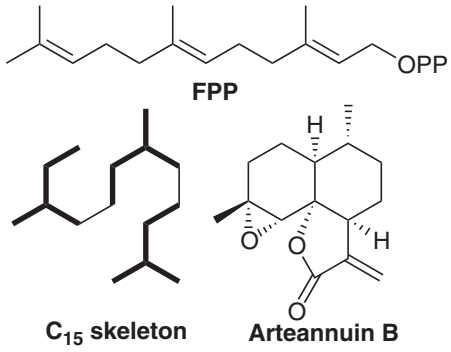


Fig. 6.29

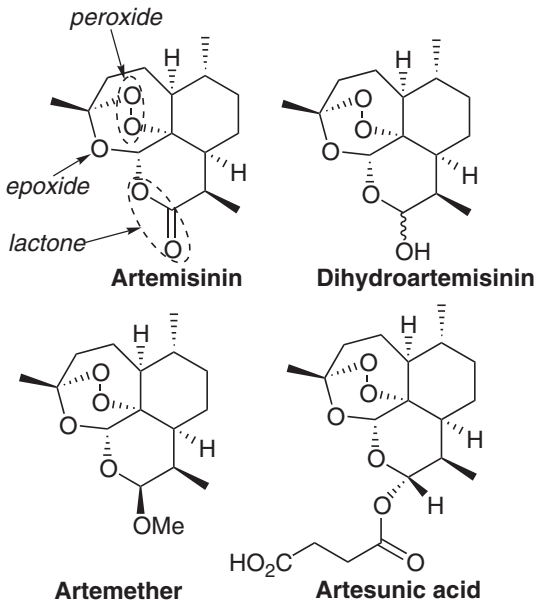


Fig. 6.30

The most important sesquiterpene from the pharmaceutical perspective is the antimalarial product **artemisinin** (Fig. 6.30) from sweet wormwood (*Artemisia annua*, Asteraceae). This herb is widely distributed throughout Europe but also has a long history of use for the treatment of fevers and malaria in China where the drug is known as Qinghao. Artemisinin has a number of interesting features, including an ether, a lactone (cyclic ester) and an unusual peroxide functional group.

The peroxide is essential for the antimalarial activity and much work has been done to enhance the solubility of the compound whilst retaining the biological activity. **Artemether**, the methyl ether of **dihydroartemisinin** (which possesses an acetal functional group), is used for the treatment of chloroquine-resistant and multidrug-resistant *Plasmodium falciparum* under the trademark Paluther. **Artesunic acid** (a succinic acid derivative marketed as Artesunate) is more water-soluble than artemether and is hydrolysed *in vitro* to dihydroartemisinin. These compounds are very lipid-soluble, are rapidly absorbed into the central nervous system (CNS) and, therefore, may have potential in treating cerebral malaria. It has been proposed that these peroxides complex to the iron atom of haem (which is produced by the degradation of haemoglobin) resulting in the formation of oxy radicals. These radicals may then re-arrange to generate carbon-centred radicals, which can attack biomolecules such as DNA and proteins leading to parasite death.

Interestingly, another Chinese medicinal plant used for treating malaria, *Artabotrys uncinatus* (Annonaceae), also contains a series of sesquiterpene peroxides (typically, **yingzhaosu A**; Fig. 6.31), which are responsible for the antimalarial activity.

In China, studies have been conducted into cottonseed oil (*Gossypium hirsutum*), which has been shown to have contraceptive effects and restrict fertility in men and women when incorporated into the diet. In men, the oil has been shown to alter sperm maturation, motility and inhibit enzymes necessary for fertilization. In women, inhibition of implantation has been observed. The active component is the **bis-sesquiterpene** (**sesquiterpene dimer**) **(–)-gossypol**, which exists in the plant with the (+)-isomer. These compounds are optically active due to restricted rotation around the bond that joins the two naphthalene ring systems. Studies show that the antifertility effect is reversible after stopping administration, provided that the treatment has not been prolonged.

DITERPENES

There are few examples of C_{20} diterpenes as drugs, but a former best-selling antitumour agent, paclitaxel,

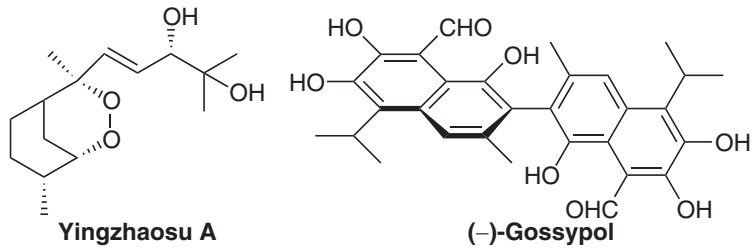


Fig. 6.31

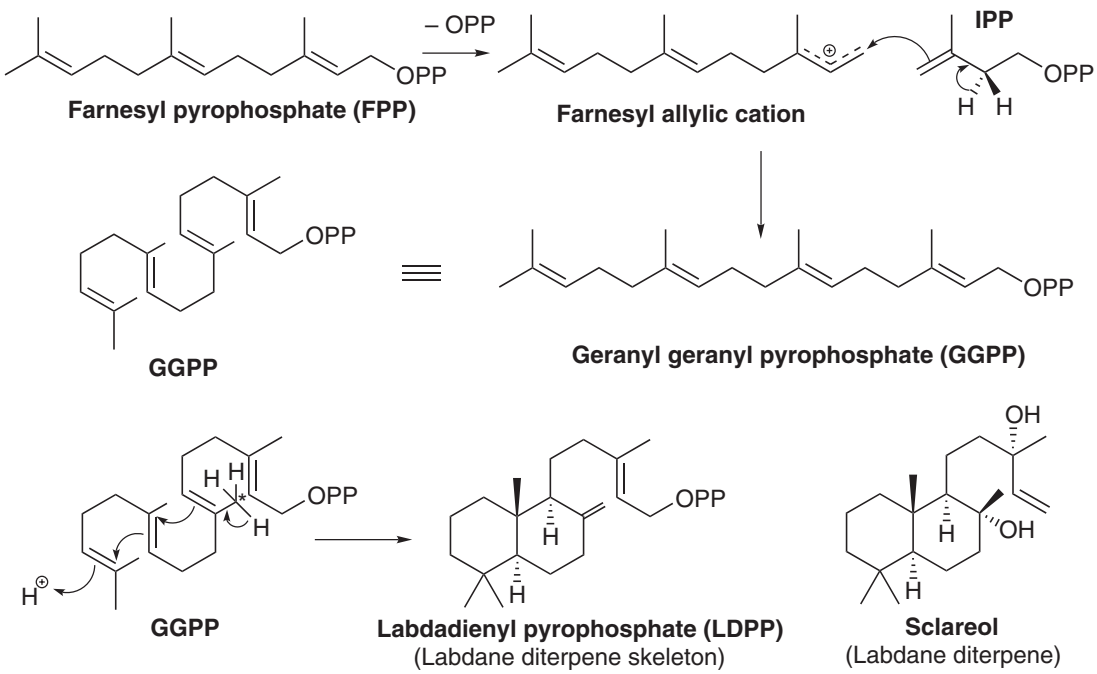


Fig. 6.32

is based on this class of natural products. These compounds are complex in structure and, until the use of multidimensional nuclear magnetic resonance (NMR) spectroscopy, the structure elucidation of these compounds (along with other higher terpenes, e.g. triterpenes) was not routine. NMR has made the structure determination of these compounds readily achievable, even if only 1–2 mg of natural product is available, and it is likely that more examples of this class will become drug candidates in the future. Historically, plants producing diterpenes that contain a nitrogen atom (the so-called **diterpene alkaloids**), such as *Aconitum* sp. and *Delphinium* sp., have been used for a number of illnesses, including decongestants; however, these compounds (e.g. **aconitine**) are highly toxic and preparations containing these plants are no longer used.

Members of the diterpene class are formed by the reaction of farnesyl pyrophosphate (FPP), a C_{15} unit, with isopentenyl pyrophosphate (IPP), the C_5 unit that is the common building block for all of the terpenes. The first step of this reaction is the formation of a **farnesyl allylic cation** (analogous to the other examples of terpenes seen), which then reacts with IPP with stereospecific loss of a proton, resulting in the formation of **geranyl geranyl pyrophosphate** (GGPP). Depending on how GGPP folds and cyclizes, a very large number of products may result (Fig. 6.32).

Loss of a proton from an allylic methyl (* in Fig. 6.32) and migration of bonds to form a bicyclic structure results in the formation of **labdadienyl pyrophosphate** (LDPP), which is a member of the **labdane** class of diterpenes of which **sclareol** from the clary sage (*Salvia sclarea*, Lamiaceae) is widely used in the perfumery industry. Sclareol is generated by hydrolysis of LDPP. If the *exo*methylene of LDPP reacts with a proton to form a cationic intermediate, this may undergo a series of **Wagner–Meerwein** hydride and methyl shifts (Fig. 6.33).

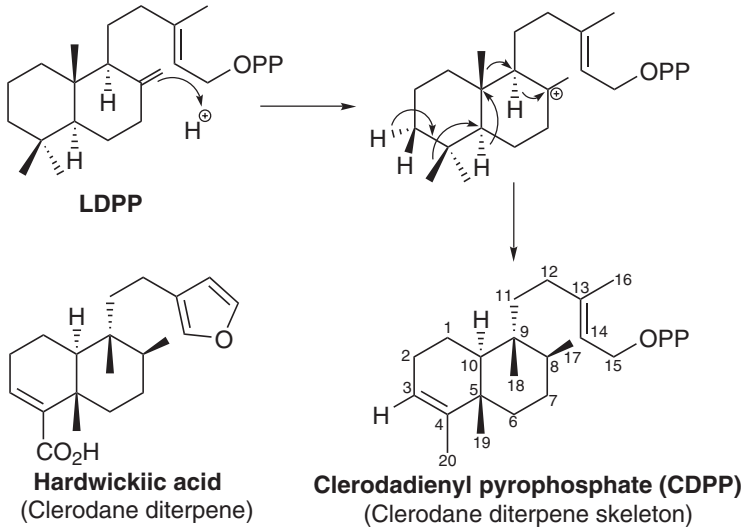


Fig. 6.33

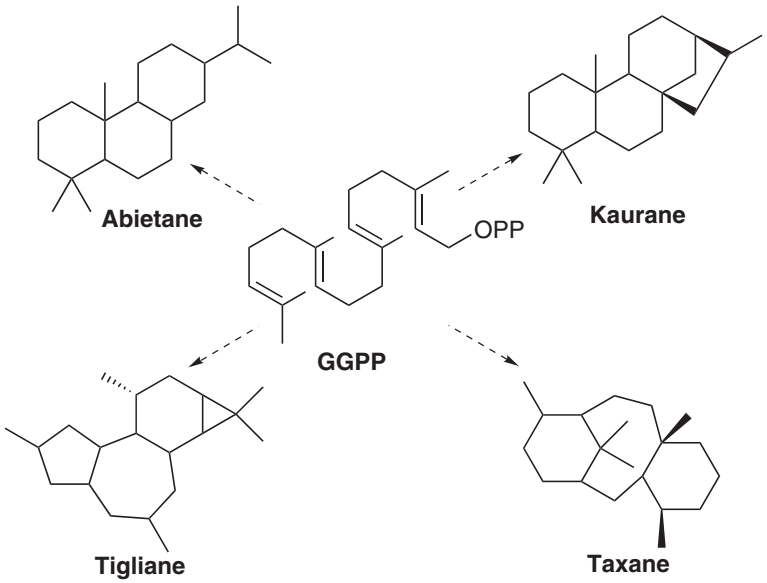
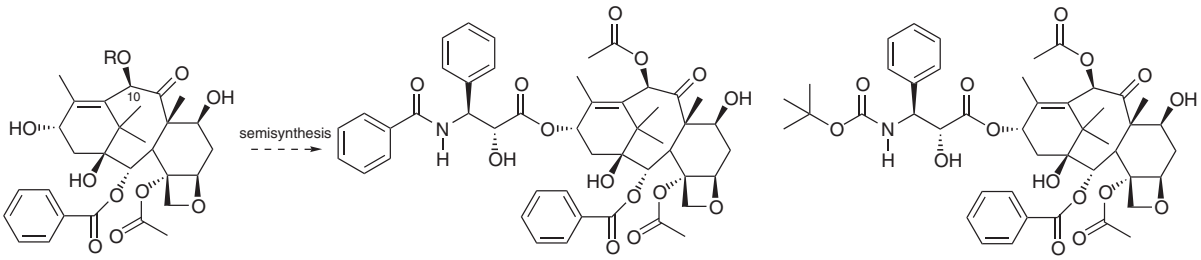


Fig. 6.34

These reactions are sometimes referred to as **1,2-shifts** (indicating a movement of a group from a position to a neighbouring carbon) or **NIH shifts** (after the National Institutes of Health, where this reaction was studied). The hydride on C_9 migrates to C_8, the methyl on C_{10} migrates to C_9, the hydride on C_5 migrates to C_{10}, the β-methyl on C_4 migrates to C_5 and, finally, a proton is lost at C_3 resulting in the formation of a C_3–C_4 double bond. This series of migrations yields **clerodadienyl pyrophosphate** (CDPP; a **clerodane** diterpene) with many members of this class; for example, **hardwickiic acid,** which possesses a furan ring (produced by oxidation and cyclization of the six-carbon side-chain at C_9) and a carboxylic acid (produced by oxidation of C_{20}). An important facet of these Wagner–Meerwein shifts is the inversion of stereochemistry at the chiral centres where migration has occurred. For example, in LDPP, the methyl at C_{10} is β (coming up out of the plane of the page), whereas the corresponding group in CDPP is an α hydrogen (going down into the plane of the page).

GGPP can cyclize to give an extraordinarily wide range of diterpene groups, some of which are shown in Fig. 6.34.



R = acetyl, baccatin III
R = H, 10-deacetylbaccatin III

Taxol (Paclitaxel)

Docetaxel (Taxotere)

Fig. 6.35

It is important to understand that, once a simple skeleton has been produced, a wide array of further elaboration reactions can occur, resulting in the highly complicated natural products of this class (e.g. paclitaxel; Fig. 6.35). This antitumour diterpene was discovered in 1971 by Monroe Wall and Mansukh Wani at the Research Triangle Institute as part of a programme funded by the National Cancer Institute. This compound is dealt with in further detail in Chapter 8. It was not until the 1980s that further work on the mode of action of this compound prompted its development and release onto the US market in 1993 under the trade name Taxol for the treatment of ovarian cancers.

Paclitaxel is present in the bark of the Pacific yew (*Taxus brevifolia*, Taxaceae), a slow-growing tree from the forests of north-west Canada and the USA that takes 100 years before it can be exploited for processing. The wood of *T. brevifolia* is not suitable for timber production and was in danger of replacement by faster growing conifers, but this practice has been stopped. The yield of paclitaxel is also low (0.01–0.02%) as it takes three 100-year-old trees to produce 1 g of the drug. Thus, with a course of treatment being 2 g, it was quickly realized that the supply of paclitaxel had to come from another source. *Taxus brevifolia* produces a wide range of taxane diterpenes, and related compounds are also found in the common English yew, *Taxus baccata*. Paclitaxel belongs to a small class of taxanes that possess a four-membered ether (also called an oxirane) and a complex nitrogen-containing ester side-chain; both of these functional groups are essential for antitumour activity. The solution to the problem of low concentration of the drug came from the knowledge that related compounds, such as **baccatin III** and **10-deacetylbaccatin III** (Fig. 6.35), were present in greater concentrations than paclitaxel and could be converted to paclitaxel by simple reactions.

Most importantly, 10-deacetylbaccatin III is also present in the needles (leaves) of the faster growing English yew (*T. baccata*) at a higher concentration (0.1%) and, unlike the bark, the needles can be harvested without destroying the tree. This is an example of a **renewable resource**, which is an important concept in natural product chemistry, for, if a biologically active compound is developed into a drug, then large-scale production is always necessary. This is not problematic if a compound from a plant can be synthesized (semi- or fully synthesized) or produced by cell culture. Another route to this compound is to extract a mixture of taxanes and use enzymes that specifically cleave ester groups from the taxane nucleus, resulting in a higher concentration of 10-deacetylbaccatin.

It has also been shown that *Taxomyces andreanae*, a fungus that lives in close association with the yew tree, produces small concentrations of paclitaxel in fermentation culture. It is possible that the fungus has inherited the gene from the tree (or vice versa), which allows the organism to produce paclitaxel. Another fungus that has been isolated from the Himalayan yew tree (*Taxus wallachiana*) is *Pestalotiopsis microspora*, which produces higher concentrations of paclitaxel than *T. andreanae*. Taxol is now produced by large-scale plant cell culture fermentation.

Docetaxel (Taxotere) (Fig. 6.35), a related semi-synthetically produced taxane diterpene, is also used clinically for the treatment of ovarian cancers and has a modified side-chain to that of paclitaxel.

TRITERPENES

The triterpenes are C_{30}-derived terpenoids with an exceptionally wide distribution, including man, plants, fungi, bacteria, soft corals and amphibia. The triterpenes include some very important molecules, such as the **steroids** (e.g. **testosterone**), which are degraded triterpenes with many important functions in mammals, notably as sex hormones. Other types include the **sterols** (e.g. **β-sitosterol**), which are common tetracyclic steroidal

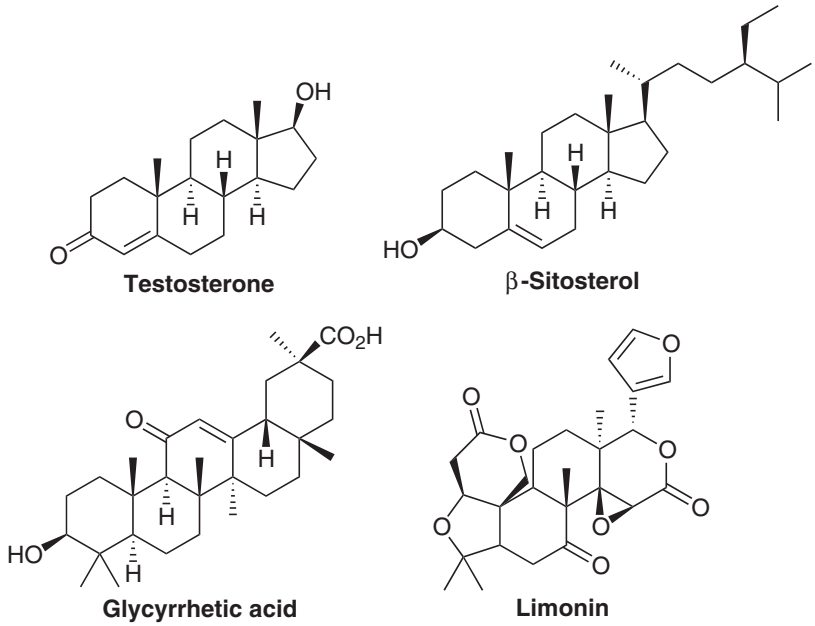


Fig. 6.36

alcohols with ubiquitous distribution in plants, the **pentacyclic triterpenes** such as **glycyrrhetic acid** found in liquorice and the **limonoids** (e.g. **limonin**), which are highly oxidized bitter principles present in the *Citrus* plant family (Rutaceae) (Fig. 6.36).

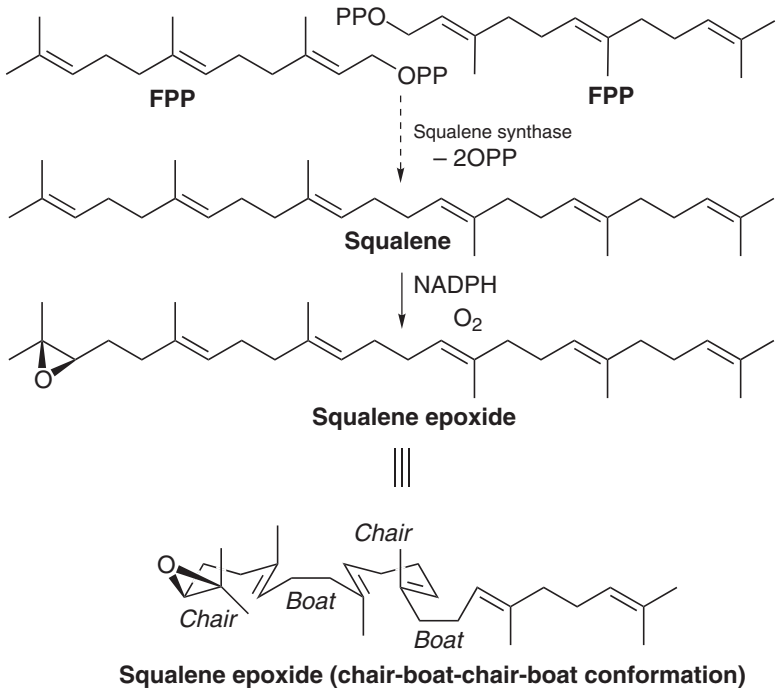
Triterpenes are also components of **resins** and resinous exudates from plants (e.g. frankincense and myrrh); myrrh is derived from the Arabic word for bitter, a characteristic which many triterpenes display. These resins are common from trees belonging to the plant family Burseraceae (which includes the myrrh-producing *Commiphora* sp.) and are produced following damage to the tree as a physical barrier to attack by fungi and bacteria. Additionally, many of the terpenoid components of these resins have high antimicrobial activity, killing potentially invasive microbes, slowing their growth until the tree has repaired the damage or providing a physical barrier toward further invasion.

Their biosynthesis starts with the reaction between two molecules of farnesyl pyrophosphate (FPP) to form the true precursor of all triterpenes, **squalene** (Fig. 6.37). Squalene is then enzymatically epoxidized to **squalene epoxide** which, when folded in a particular conformation such as the 'chair-boat-chair-boat' conformation, can cyclize to give **sterol intermediate 1,** which is the precursor of the steroids and sterols (Fig. 6.38). This intermediate can undergo a series of Wagner–Meerwein shifts to give **lanosterol**, a common component of plants and of wool fat.

Oxidation and loss of methyls at positions C_4 and C_{14}, introduction of a C_5–C_6 double bond (oxidation) and loss of two double bonds (one at C_8–C_9 and one in the side-chain) would result in the formation of **cholesterol**. Cholesterol is the main animal sterol, a component of cell membranes and gallstones, and control of the levels of this sterol is important in the management of heart disease. The basic steroid nucleus and numbering of the ring system depicting the A, B, C and D rings is given for cholesterol (Fig. 6.39).

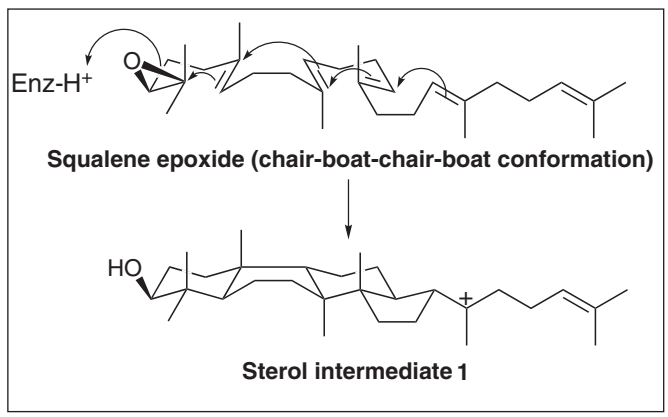
Other common sterols include the **phytosterols** (plant sterols) **β-sitosterol** and **stigmasterol** (which differs from β-sitosterol only by the presence of a double bond at position C_{22}–C_{23}), which are widespread in plants, and **ergosterol**, which is ubiquitous in fungi as a cell-wall component (Fig. 6.39).

There is a great need for steroids in the pharmaceutical industry and this is met by using the plant sterol **diosgenin** from the wild yam (*Dioscorea* sp.). Diosgenin also occurs naturally as a glycoside (a sugar is attached at the hydroxyl position) and without the sugar the compound is referred to as a **genin**. Unlike the other plant sterols mentioned, the side-chain that is normally present at position C_{17} has been formed into two ring structures. Diosgenin can be converted into **progesterone** via a chemical process known as the marker degradation, which gives access to many important steroids such as **testosterone** (a male sex hormone) and **oestradiol** (a female sex hormone),



Squalene epoxide (chair-boat-chair-boat conformation)

Fig. 6.37



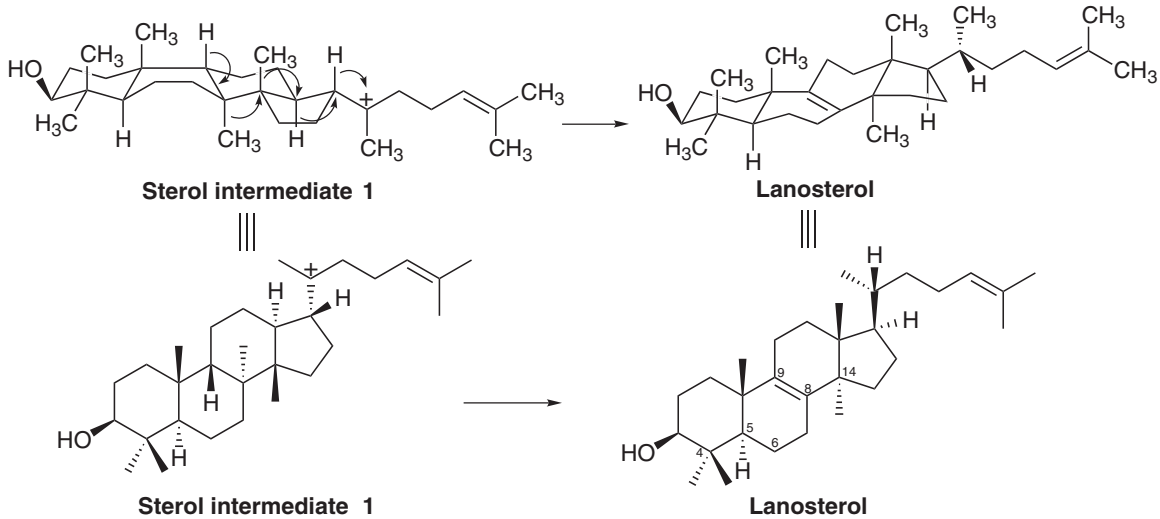


Fig. 6.38

which has had the A ring aromatized, resulting in the loss of a methyl group from C_{10} (Fig. 6.40).

Another semi-synthetic compound that lacks this methyl is the oral contraceptive **norethisterone**, which has an unusual acetylene group at position C_{17}. One of the most widely used steroids in pharmaceutical preparations is the anti-inflammatory drug **hydrocortisone** (cortisol). This compound has an hydroxyl group at C_{11} that is introduced into the molecule in a stereospecific manner in fermentation culture using fungi of the genus *Rhizopus*.

If squalene is folded in a different conformation (chair-chair-chair-boat), then cyclization mediated by a cyclase enzyme results in the formation of a different intermediate, **sterol intermediate II**, which is the precursor of the pentacyclic triterpenes (Fig. 6.41).

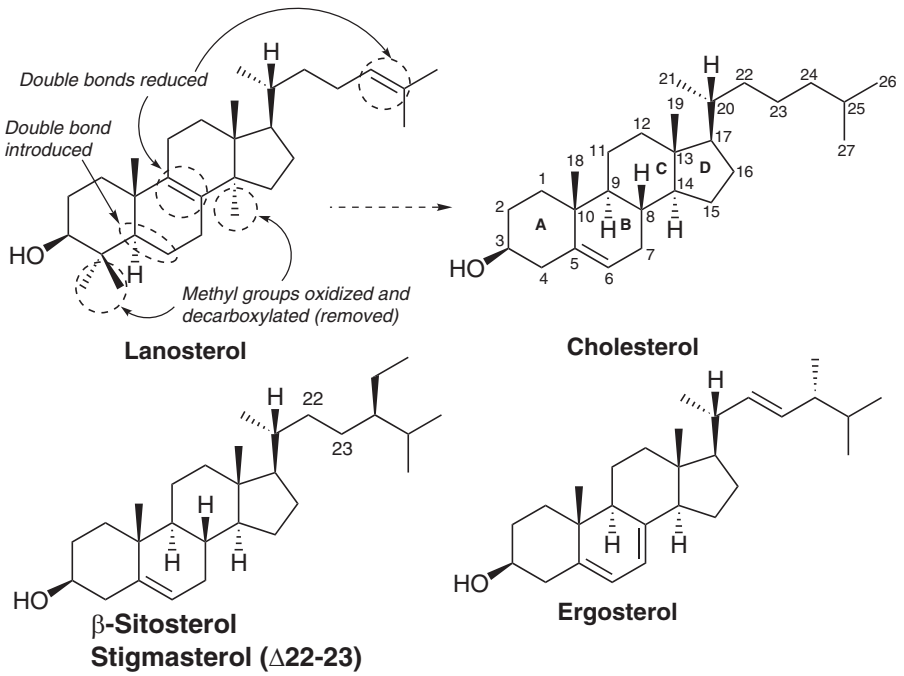


Fig. 6.39

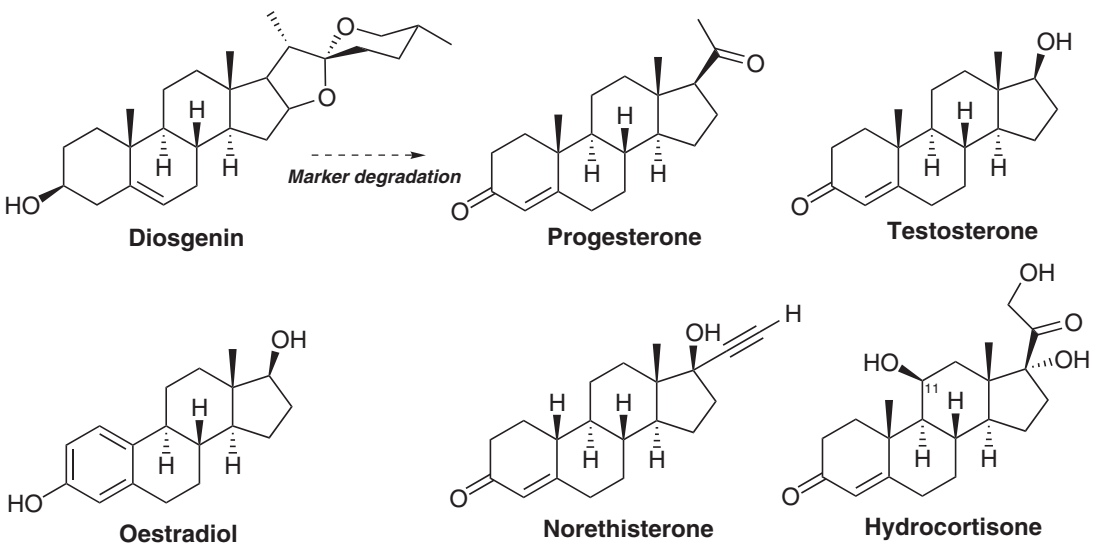


Fig. 6.40

Migration of the C_{16}–C_{17} bond to satisfy the positive charge results in the formation of **sterol intermediate III**. This may undergo several rearrangements to give different triterpene skeletons. Pathway 1 involves formation of a bond between C_{18} and C_X, resulting in a positive charge on C_Y (through removal of one pair of electrons from the double bond to form the C_{18}–C_X bond). This may be satisfied by a series of Wagner–Meerwein methyl and hydride shifts with loss of a proton from C_{12} resulting in a C_{12} double bond. This pathway gives us the **ursane**-type triterpenes of which **α-amyrin** is an example, possessing a double bond in position C_{12} (referred to as a Δ^{12}-ursene) (Fig. 6.41).

Pathway 2 occurs through the formation of a C_{18}–C_Y bond, which leaves a positive charge on C_X which is stabilized by the two methyls attached to it. This intermediate may then lose a hydrogen ion from one of these methyls to form a neutral double bond and the **lupane** skeleton (pathway a), or the bond between C_Y and C_Z may migrate to C_X, giving a carbocation at C_Y. Wagner–Meerwein migrations and loss of a hydrogen ion from C_{12} forming a double bond gives the **oleanane** triterpene skeleton, of which **β-amyrin** is typical, again possessing a double bond at C_{12}. This compound may be referred to as a Δ^{12}-oleanene.

Pentacyclic triterpenes are common in plants and herbal remedies such as horse chestnut (*Aesculus hippocastanum*) and liquorice (*Glycyrrhiza glabra*). Examples such as **protoaescigenin, baringtogenol** (both from horse chestnut) and **glycyrrhetic acid** (liquorice) (Fig. 6.42) have a high degree of functionality and chirality, and usually occur in the plant material in the form of glycosides.

Horse chestnut is used as an anti-inflammatory and antibruising remedy and liquorice has a long history of use as an anti-inflammatory (anti-ulcer) agent.

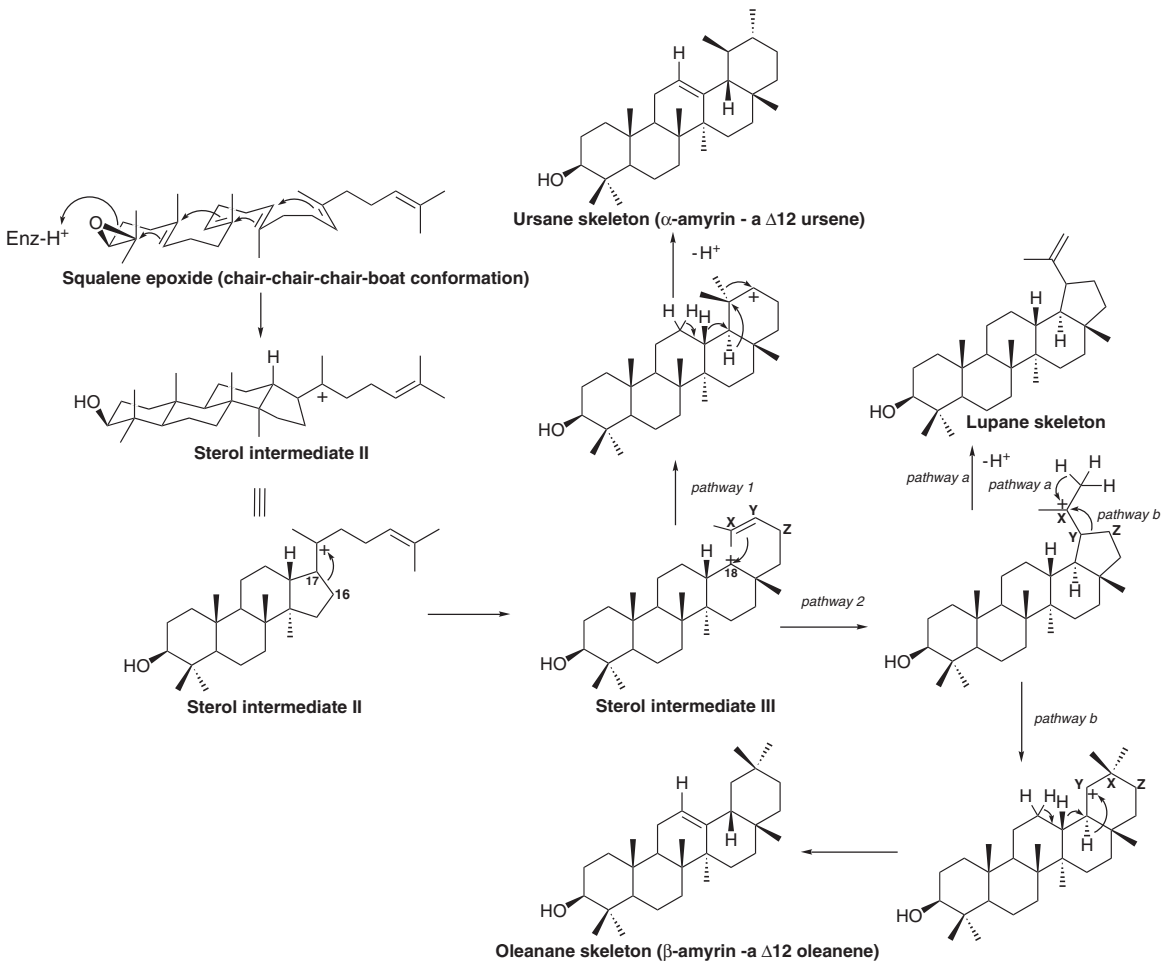
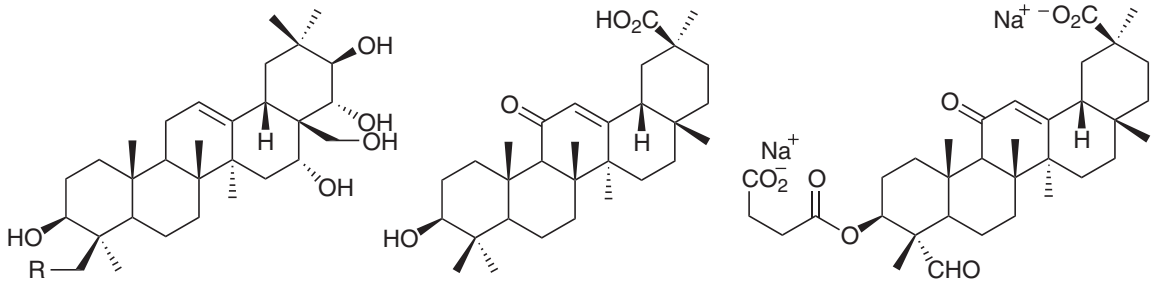


Fig. 6.41

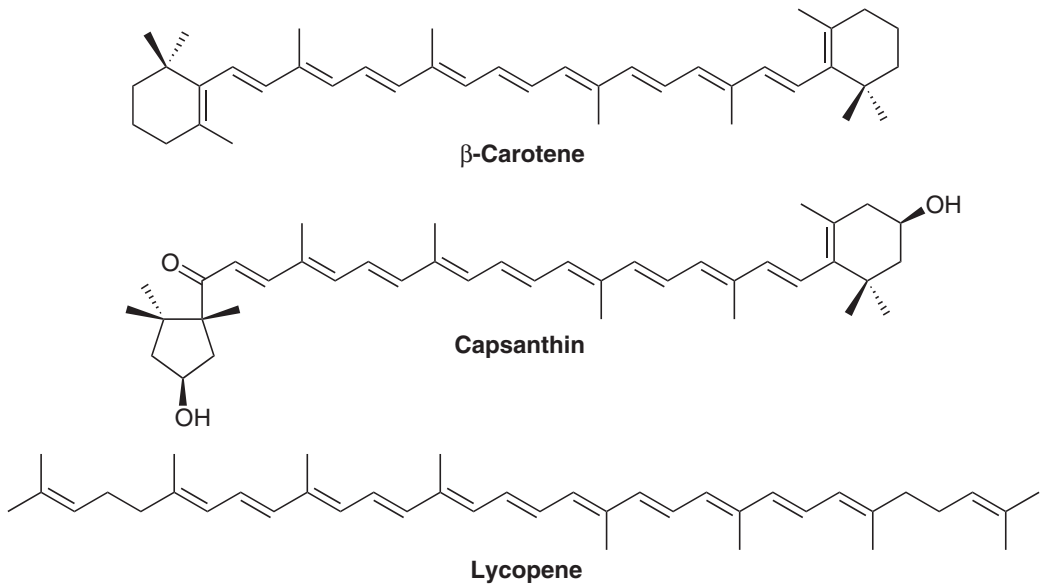


R = OH, Protoaescigenin
R = H, Barringtogenol

Glycyrrhetic acid

Carbenoxolone sodium

Fig. 6.42



β-Carotene

Capsanthin

Lycopene

Fig. 6.43

Carbenoxolone sodium is a semi-synthetic derivative of glycyrrhetic acid that is widely prescribed for the treatment of gastric ulcers.

TETRATERPENES (C_{40})

The final class of terpenoids that will be dealt with are the **tetraterpenes**, which are C_{40} natural products derived from the reaction of two molecules of geranyl geranyl pyrophosphate (C_{20}). Members of this class are sometimes referred to as **carotenes** or **carotenoids** because of their occurrence in the carrot (*Daucus carota*). As with the flavonoids, the tetraterpenes are highly pigmented natural products and are responsible for the very bright colours of certain plants, in particular the orange of carrots due to **β-carotene**, and the brilliant red colour of tomatoes (*Lycopersicon esculentum*) and peppers (*Capsicum anuum*), which is due to **lycopene** and **capsanthin**, respectively (Fig. 6.43). These compounds are highly conjugated and strongly UV light absorbing, and are involved in photosynthesis as light accessory pigments. They are widely distributed in plants and may also act as a protection factor against UV light damage. Because of their high colouration they are employed as colouring agents in foods, pharmaceuticals and cosmetics.

The tetraterpenes are strong antioxidants, being preferentially oxidized over biological molecules such as nucleic acids and proteins. It is thought that many disease states such as certain cancers and heart disease are exacerbated by species that cause oxidation; therefore, the presence of these compounds may retard the development of such diseases. The presence of lycopene in the diet has been shown to reduce the incidence of prostate cancer in men and it is likely that the tetraterpenes have high dietary significance and are important in cancer chemoprevention.

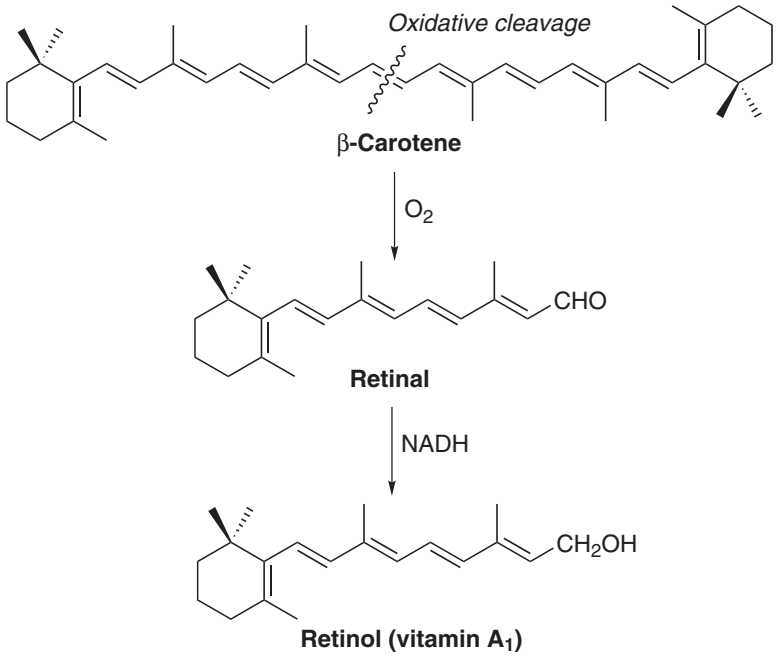
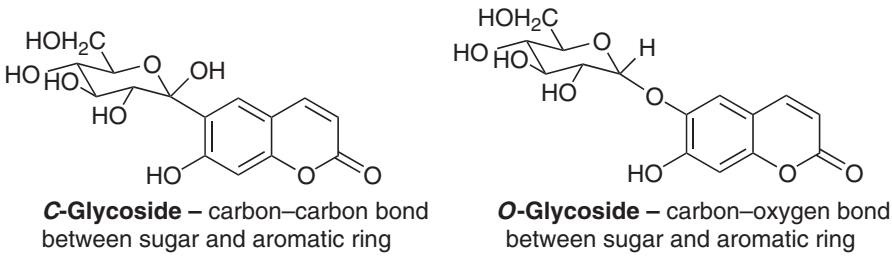


Fig. 6.44



***C*-Glycoside –** carbon–carbon bond between sugar and aromatic ring

***O*-Glycoside –** carbon–oxygen bond between sugar and aromatic ring

Fig. 6.45

The tetraterpenes are precursors of **vitamin A_1** (**retinol**), a deficiency of which results in a reduction in sight efficiency through changes to the cornea and conjunctiva. Vitamin A_1 occurs naturally in fish liver oils, carrots, green and yellow vegetables, and dairy products. It is biosynthesized by the oxidative cleavage of β-carotene to **retinal**, which is then reduced to **retinol** (**vitamin A_1**) (Fig. 6.44).

Vitamin A preparations are also used to treat nappy rash, skin irritations and minor burns; vitamin A acid (retinoic acid) and vitamin A palmitate are used as treatments for acne.

THE GLYCOSIDES

The glycosides are discussed in a separate section here as they enhance the structural diversity of other natural product classes. The term glycoside is a generic term for a natural product that is chemically bound to a sugar. Thus the glycoside is composed of parts: the **sugar** and the **aglycone**. The aglycone may be a terpene, a flavonoid, a coumarin or practically any other natural product. If the aglycone is a triterpene, it is sometimes referred to as a **genin** (e.g. protoaescigenin; Fig. 6.42). Glycosides are very common in nature and provide extra chemical diversity and structural complexity in natural products.

There are two basic classes of glycosides: the ***C*-glycosides**, in which the sugar is attached to the aglycone through a carbon–carbon bond, and the ***O*-glycosides** in which the sugar is connected to the aglycone through an oxygen–carbon bond (Fig. 6.45).

Glycosides are usually more polar than the aglycone, and glycoside formation generally increases water solubility. This may allow the producing organism to transport and store the glycoside more effectively.

CYANIDE GLYCOSIDES

Some glycosides are undoubtedly used by plants as a chemical defence and this is certainly so with the cyanide glycosides. These compounds, in the presence of enzymes such as **β-glucosidase**, lose their sugar portion to form a **cyanohydrin,** which, in the presence of water, can undergo hydrolysis to give **benzaldehyde** and the highly toxic **hydrogen cyanide** (HCN) (Fig. 6.46).

Cyanide glycosides such as **amygdalin** (see Fig. 6.50) are present in many species of the genus *Prunus*, which includes commercially important fruit such as peaches, cherries, plums and apricots. Fortunately, the enzymes that convert these compounds to the cyanohydrins are localized in different parts of the plant or are absent. In the case of sweet almonds (*Prunus amygdalus* var. *dulcis*), the enzymes are present but there are no cyanide glycosides present.

Cassava (*Manihot esculenta*) is consumed widely in Africa as a food-stuff and both the enzymes and cyanide glycosides are present, although extensive boiling of the cassava before eating results in the removal of the toxic HCN. Some cassavas are eaten raw, but it is highly likely that these are chemical races of the plant that lack either the glycosides or the enzymes, so raw cassava should certainly be avoided if there is doubt about the presence of these compounds.

GLUCOSINOLATES

The plant family Brassicaceae includes cabbages, sprouts and the mustards and produces a group of glycosides known as **glucosinolates**. These are sulphur- and nitrogen-containing glycosides previously referred to as nitrogen mustards. A common example of this group is **sinalbin** from white mustard (*Sinapis alba*), which in the presence of the enzyme **myrosinase** is converted into a **thiohydroximate**, which rearranges with the loss of a hydrogen sulphate salt to the **isothiocyanate**, **acrinylisothiocyanate** (Fig. 6.47).

These isothiocyanates are exceptionally pungent and impart a strong aroma to mustards, which can be described as hot or even acrid to the taste. In black mustard (*Brassica nigra*), the simple glucosinolate **sinigrin** is converted in the same fashion to **allylisothiocyanate** (see Fig. 6.51), which is an oil and far more volatile than acrinylisothiocyanate. The oils derived from mustards are rich in these isothiocyanates and are mildly irritant; they are used medicinally as externally applied treatments for muscular pain.

CARDIAC GLYCOSIDES

Many plants contain **cardioactive** or **cardiac glycosides**, which have a profound effect on heart rhythm. They are commonly found in the genera *Convallaria, Nerium, Helleborus* and *Digitalis*. The aglycone portion is steroidal in nature and is sometimes referred to as a **cardenolide**, being **card**ioactive and possessing an alkene and an **olide** (a cyclic ester) (Fig. 6.48).

Being 'steroid-like', the aglycone (genin) portion is derived from the triterpenes and these compounds may have a wide variety of sugars attached to the steroid portion. The most widely studied plant that

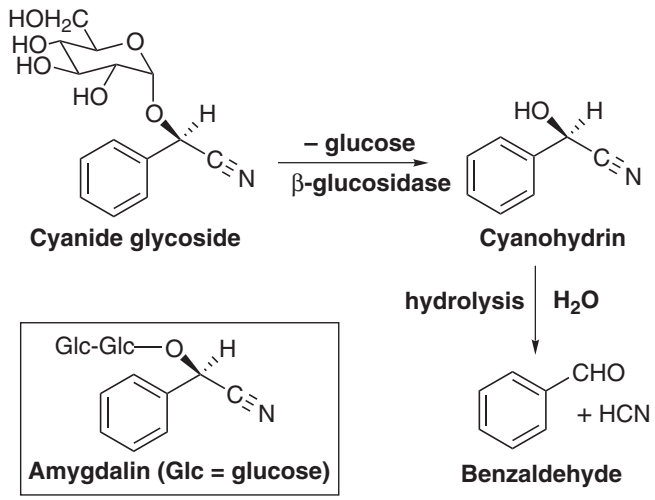


Fig. 6.46

contains these compounds is the foxglove (*Digitalis purpurea*) of the plant family Scrophulariaceae, which was used as long ago as the 18th century for the treatment of heart disease described as 'dropsy'. The basis of this use was well founded as this plant contains the medicinal agents **digoxin** and **digitoxin** (Fig. 6.48). Digoxin is the most widely used cardiac glycoside in congestive heart failure and is now produced by isolation from the related species *Digitalis lanata*. Related cardiac glycosides, which because they are very fast-acting compounds are used in emergencies via the intravenous route, are **lanatoside C** and **deacetyl-lanatoside C**.

Triterpene glycosides have widespread distribution in plants and are sometimes referred to as **saponins** as they have soap-like properties and readily

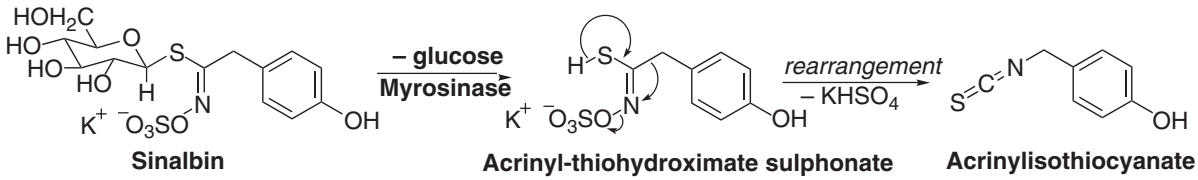


Fig. 6.47

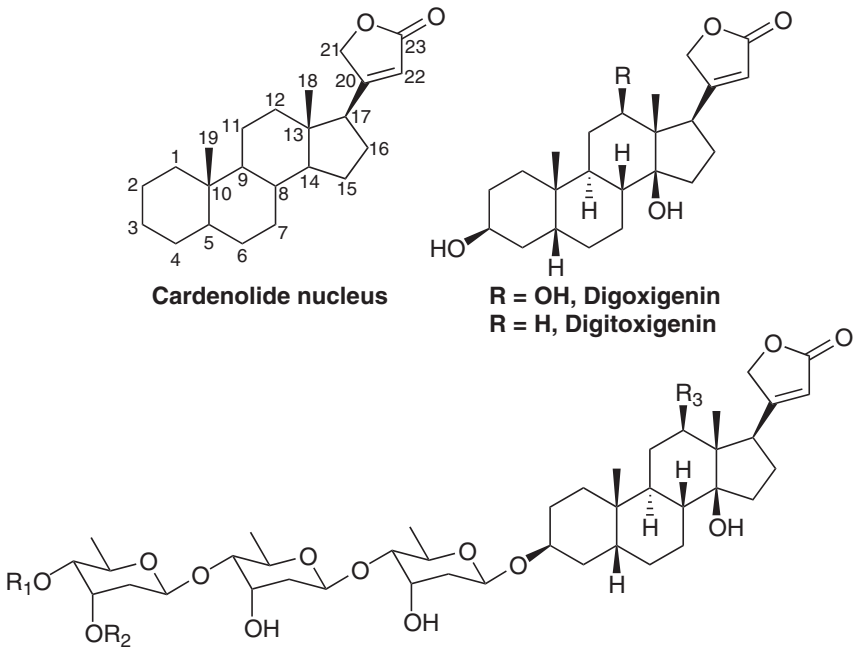


Fig. 6.48

form foams. Medicinally important examples include **glycyrrhizic acid** from liquorice (*Glycyrrhiza glabra*) (Fig. 6.49), which is used as a treatment for stomach ulcers and the salts of which are intensely sweet. The sugars in Fig. 6.49 are of the **glucuronic acid** type and are shown as their Fischer projections.

Triterpene glycosides are steroid-like in structure and overuse can lead to similar symptoms associated with steroid overuse such as hypertension and thrombosis.

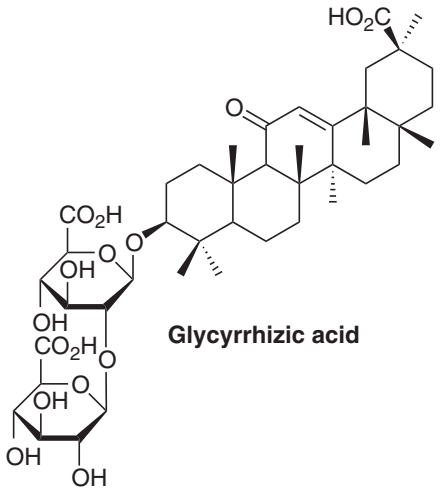


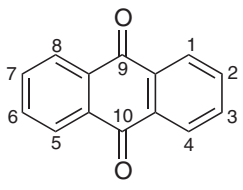
Fig. 6.49

ANTHRAQUINONE GLYCOSIDES

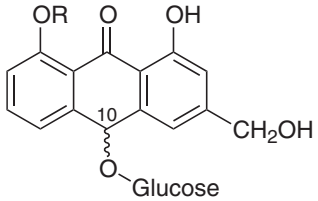
A number of plants that contain **anthraquinone** or **anthrone glycosides** (Fig. 6.50) have long been known for their laxative properties. They include **cascara** (*Rhamnus purshiana*), **aloe** (*Aloe vera*) and **senna**; the latter is divided into two species (*Cassia angustifolia*, known as **Tinnevelly senna**, and *Cassia senna*, known as **Alexandrian senna**). Aloe is used as a laxative as well as a treatment for minor burns. It contains a mixture of anthraquinone glycosides of which **barbaloin** is the major component and is a mixture of 10*R* and 10*S* isomers; the purified components are referred to as **aloin A** and **B**. The gel or mucilage from aloe is rich in polysaccharides and these anthraquinone glycosides, and is incorporated into creams and ointments to treat abrasions, burns and skin irritation.

Cascara was in use in the late 19th century as a laxative by the preparation of the bark of the tree. The main active principle is the diglucoside **cascaroside**, which, in common with barbaloin, exists as a mixture of epimers at position C10 as **cascaroside A** (10*S*) and **B** (10*R*).

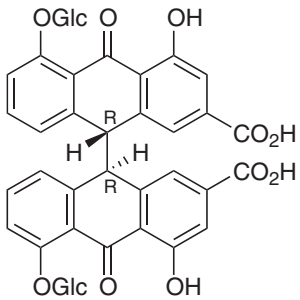
There is little difference in the chemistry of the two senna species. The active constituents are **sennosides A** and **B** (Fig. 6.50). These natural products are **dianthrones** (dimers) of the **anthrone** skeleton. The fresh leaves of senna contain glycosides with additional sugar groups present and these are naturally hydrolysed to sennosides A and B. *In vivo*, the sennosides are then hydrolysed to the dianthrones (lacking the glucose



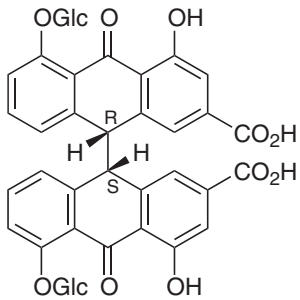
Anthraquinone nucleus



R = H, Barbaloin
R = Glucose, Cascaroside



Sennoside A



Sennoside B

Fig. 6.50

sugars). Senna is widely prescribed for constipation; an example of a marketed product is Senokot.

THE ALKALOIDS

No other group of natural products has contributed more to medicines and pharmaceutical preparations than the alkaloids. As a group, they display an exceptionally wide array of biological activities and have an equally wide distribution, being present in plants, fungi, bacteria, amphibia, insects, marine animals and man. Plants and fungi rich in these natural products were used by early man to relieve pain, as recreational stimulants or, in religious ceremonies, to enter a psychological state to achieve 'communication' with his ancestors or God. The German pharmacist Karl Friedrich Wilhelm Meissner first coined the term 'alkaloid' in 1818, to describe substances that had alkaline (hence alkaloid) properties. Many alkaloids are, indeed, alkaline in nature (Fig. 6.51) as they possess either a **primary, secondary** or **tertiary amine** functional group and the alkaline (basic) properties of these groups may be exploited to aid their extraction and purification (see Chapter 7). However, some alkaloids exist as quaternary amine salts in which a lone pair of electrons from the nitrogen atom is used to form a bond with another group (e.g. methyl) and, therefore, a positive charge resides on the nitrogen making this group essentially neutral (neither basic nor acidic). Care must, therefore, be taken with the alkali or base definition of alkaloids as some are neutral, especially the amides (Fig. 6.51), and some alkaloids possess phenolic groups, which actually contribute to the acidity of the molecule.

Alkaloids may also naturally exist as salts, which are the product of a reaction of a base (alkaloid) and an acid (e.g. sulphuric acid to give the sulphate, or hydrochloric acid to give the hydrochloride). A further definition of this group is that they are heterocyclic natural products containing nitrogen, but in our definition we will include compounds that contain nitrogen in an aliphatic chain (e.g. the **phenyl-alkylamines**; see below). Biosynthetically, the alkaloids are produced from several different amino acids thereby giving rise to a diverse group of fundamental structures (Fig. 6.52). A biosynthetic treatment of this class

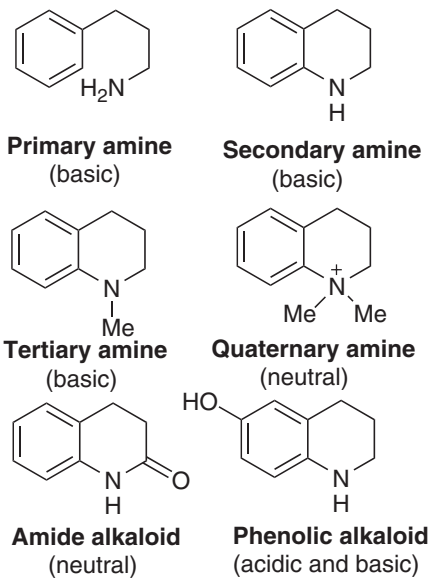


Fig. 6.51

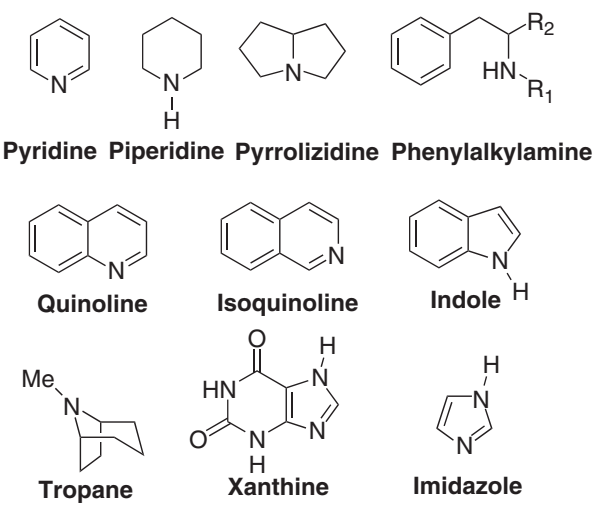


Fig. 6.52

is outside the scope of this chapter; consequently, this group of natural products will be dealt with by alkaloid class.

PYRIDINE, PIPERIDINE AND PYRROLIZIDINE ALKALOIDS

The most widely studied member of the pyridine class is **nicotine**, the stimulant alkaloidal component of tobacco (*Nicotiana tabacum*, Solanaceae) (Fig. 6.53), which is responsible for the addictive nature of cigarettes and other tobacco preparations. Nicotine is used as a model for addiction to other drugs such as **heroin**. The compound has a pyrrole ring attached to the pyridine ring. Pharmaceutically, nicotine is formulated into chewing gum as an aid to cessation of smoking in products such as Nicorette.

The European plant hemlock (*Conium maculatum*, Apiaceae) produces the highly poisonous piperidine alkaloid **coniine**, which has an alkyl (C_3) side-chain at the 2-position of the piperidine ring. This plant is famous as it was used to execute the Greek philosopher Socrates who was found guilty of treason and forced to drink a preparation of hemlock. Occasional poisoning with this plant occurs when children use the hollow stems as 'pea shooters' and ingest small quantities of the poison.

In the Indian subcontinent, large quantities of betel nuts (*Areca catechu*, Arecaceae) are consumed by farm workers for their stimulant properties to alleviate fatigue. The nuts are red (due to the presence of tannins), which causes staining of the teeth. These nuts are addictive, the active stimulant component being the piperidine alkaloid **arecoline**. Like nicotine, arecoline binds to the nicotinic receptors and has a stimulant effect on the CNS.

Lobeline is found in the leaves and tops of *Lobelia inflata* (Campanulaceae), which is also known as wild tobacco or pukeweed. It has similar effects to those of nicotine and arecoline and has been used as a smoking deterrent. Much work has been done to find alkaloids with activity against HIV of which **castanospermine** from *Castanospermum australe* (Fabaceae) is exceptional. This compound is an inhibitor of α-glucosidase, an enzyme involved in glycoprotein processing, which is important in the formation of viral coating, abnormalities of which stop infection of white blood cells. Castanospermine is a **polyhydroxylated alkaloid** (PHA) and is in fact a sugar analogue (compare with glucose in Fig. 6.53), which explains its activity against the glucosidase enzymes involved in the formation of glycoproteins. The compound is sometimes classified as an indolizidine alkaloid, but, as it also has

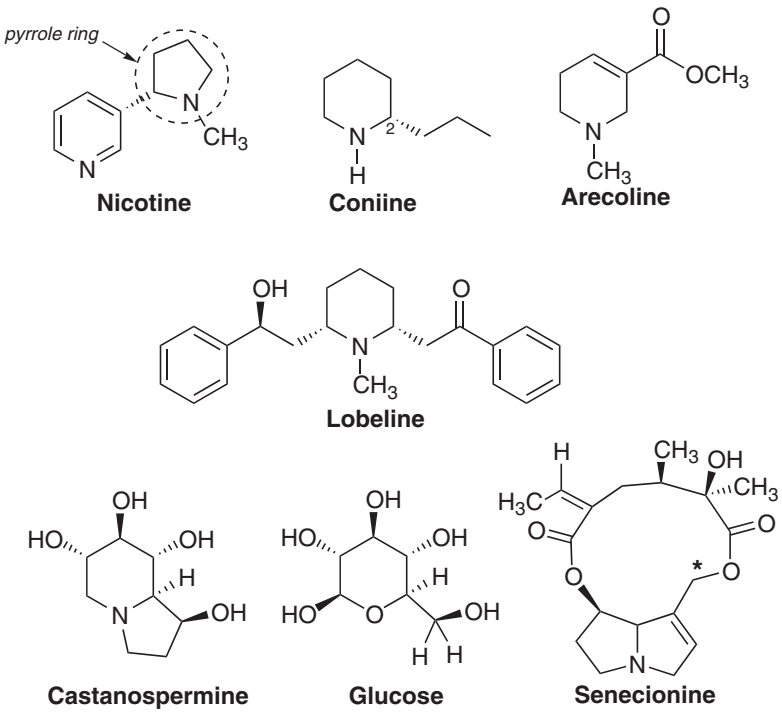


Fig. 6.53

a piperidine ring system, it is included in this section for convenience.

Senecionine is a member of the pyrrolizidine class of alkaloids, which have gained notoriety due to their hepatotoxic properties. These compounds possess a reactive carbon (* in Fig. 6.53) that is readily alkylated by reactive thiol groups present in many enzymes found in the liver. This accounts for the withdrawal of comfrey (*Symphytum officinale*, Boraginaceae), which has a long history of use as a medicinal plant but also contains these toxic alkaloids. Senecionine occurs in groundsel (*Senecio vulgaris*, Asteraceae), which is problematic in farms and paddocks where it can cause poisoning of livestock and horses.

PHENYLALKYLAMINE ALKALOIDS

The natural products of this group do not have a cyclic nitrogen atom but have either a free amine or an alkyl-substituted amine. In Chinese medicine, Ma Huang (*Ephedra sinica*, Ephedraceae) has a long tradition of use as a treatment for colds, asthma and other bronchial conditions. The biologically active component of this species is **ephedrine** (Fig. 6.54), which possesses CNS stimulatory, vasoconstrictive and bronchodilatory properties. These effects are similar to those of the natural hormone **adrenaline** (**epinephrine**), which is structurally similar (Fig. 6.54). Ephedrine has two stereogenic (chiral) centres and, therefore, has four possible isomers. Injections of (–)-ephedrine are used for severe asthma and life-threatening anaphylactic shock. Another isomer of ephedrine, (+)-**pseudoephedrine**, is used in cough preparations such as Sudafed for its bronchodilatory properties. Herbal *Ephedra* has recently gained notoriety as 'herbal ecstasy', with a number of sources selling plant material over the Internet and in magazines. Claims of the stimulant's 'ecstasy-like' properties are not unfounded due to the high similarity in structure of ephedrine and **ecstasy** (**methylenedioxymethylamphetamine**, **MDMA**). These herbal preparations are dangerous and should therefore be avoided.

The indigenous peoples of central and north Mexico and the south-western USA ingest the dried heads ('buttons') of the cactus (*Lophophora williamsii*, Cactaceae) as part of their religious ceremonies. This plant material, known as **peyote**, induces vivid dreams and hallucinations; the biologically active natural product responsible is **mescaline**, a trimethoxylated phenylethylamine. Ingestion of pure mescaline fails to give the same response as consumption of peyote, which is possibly due to the contribution of other compounds present in the plant material.

A compound that is included in this section for convenience is **colchicine**, an alkaloidal amine from the autumn crocus (*Colchicum autumnale*, Colchicaceae). This plant was known by the Greek physician Dioscorides and has been widely used on the Arabian Peninsula for centuries in the treatment of gout and it is still used today for this purpose. However, it is highly cytotoxic and antimitotic, being an inhibitor of microtubule formation.

QUINOLINE ALKALOIDS

The Spanish conquistadors who invaded Peru in the latter part of the 16th century discovered that the

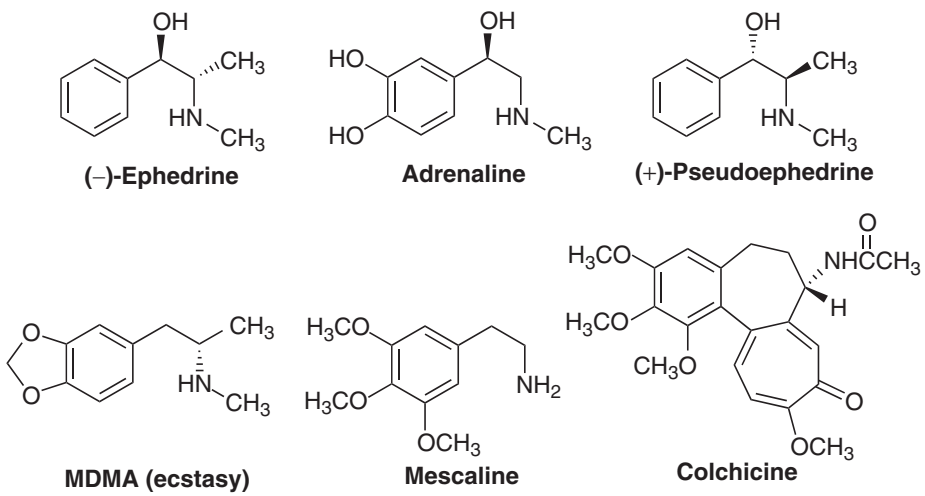


Fig. 6.54

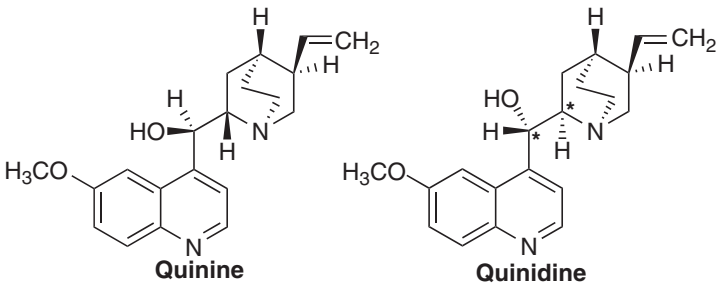


Fig. 6.55

indigenous Incas of this area used a preparation of the bark of a rain-forest tree to treat fevers, especially malaria. The Jesuit priests accompanying the invading force collected large amounts of this bark and used it to prevent and treat malaria. The bark was shipped back to Europe where it became known as **Jesuit bark** or Peruvian bark and gained great fame as a treatment for malaria. The trees responsible for this biological activity are of the genus *Cinchona* (Rubiaceae), which produce the quinoline alkaloid **quinine**, first isolated in 1820 by the French pharmacists Pelletier and Caventou (Fig. 6.55). The structure of this compound was not known, however, until 1908 and total synthesis was only achieved in the mid-1940s. The pure compound was used extensively as an antimalarial and was a template for synthetic antimalarials such as **quinacrine, chloroquine** and **mefloquine**. Resistance to these agents, particularly chloroquine, has become increasingly widespread, in particular through removal of the antimalarial from the cell by plasmodial membrane-bound efflux mechanisms, resulting in a low intracellular (ineffective) concentration of the drug. Interestingly, quinine is active in many cases against chloroquine-resistant malaria and there has been increased use of this drug. It is thought that quinine and other quinoline antimalarials exert their effects by binding to haem, a degradation product of haemoglobin. This haem–quinoline conjugate is toxic and leads to death of the parasite. In the absence of quinine, haem is converted into a polymeric form known as haemozoin or malaria pigment which is non-toxic. Plasmodia are highly adaptable organisms and at present there is a need for new antimalarials to counter multidrug resistance in *Plasmodium falciparum*.

Quinine also has a use as a treatment for night cramps in the elderly and is added to Indian tonic water where it imparts a bitter taste and a brilliant fluorescence under UV light.

Quinidine is an isomer of quinine and has a different configuration at the positions marked * in Fig. 6.55. It was observed that patients suffering from malaria who also had atrial fibrillation were cured of arrhythmias by quinine and quinidine. Quinidine is used to treat type I cardiac arrhythmias.

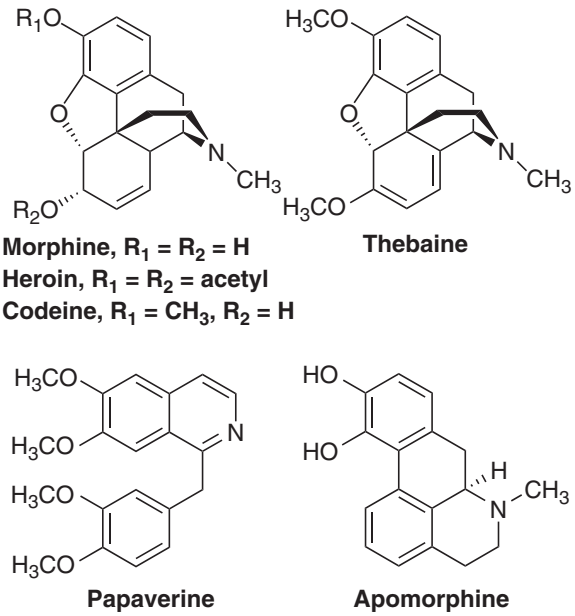
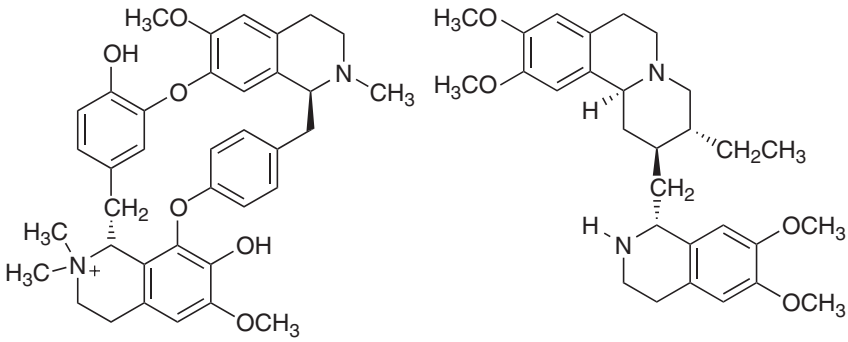


Fig. 6.56

ISOQUINOLINE ALKALOIDS

Within the alkaloids as a group, the isoquinolines have had a profound effect on human society as agents for pain relief and as drugs of abuse. In particular, **opium**, which is rich in **morphinane-**type isoquinolines, has been used for millennia in the treatment of pain and as a narcotic substance and, arguably, no other substance has caused so much human misery.

Opium is the gummy exudate of the unripe capsules of the opium poppy (*Papaver somniferum*, Papaveraceae) and contains more than 30 alkaloids, of which the major components are **morphine**, **codeine**, **thebaine**, **papaverine** and **noscapine** (Fig. 6.56).



Tubocurarine **Emetine**

Fig. 6.57

The majority of opium, which is produced for illegal drug use, now originates in Afghanistan.

When the British conquered the area of Bengal (now eastern India and Bangladesh) in the late 18th century, they discovered an area rich in opium fields and, as at that point in time there was a huge demand for Chinese tea, the opium was therefore used as a form of currency. Unfortunately, the addictive nature of opium was not well known and many Chinese became addicted through smoking the crude drug in opium dens (which were also a part of London life in the 19th century). This generated a huge social problem and resulted in war (the **Opium Wars**) between Britain and China, resulting in China having to cede land (including Hong Kong) to the British.

Morphine, derived from the name for the Greek god of sleep *Morpheus*, possesses both a basic tertiary amine and an acidic phenol functional group. These groups allow morphine to be readily purified by acids and bases; pure morphine was produced in the 1880s and was rapidly recognized as an excellent analgesic when injected (despite its addictive properties). Morphine is readily converted into the drug of abuse, **heroin** (**diamorphine**), by acetylation of both hydroxyl groups using acetic anhydride. Much has been written on the destructive nature of heroin as a drug of abuse, but this agent is highly useful in the management of pain, particularly in patients with terminal cancer.

Why morphine should dramatically affect analgesia in humans was a mystery until the discovery that we also produce a natural **end**ogenous mo**rphine**-like substance (**endorphin**), which acts at the same site as morphine and is a pentapeptide (Tyr-Gly-Gly-Phe-Met). This molecule, named **met-enkephalin** (met being the terminal methionine residue, and enkephalin being derived from the Greek for 'in head') has a portion that shows striking similarity to morphine and explains why both molecules bind to the opiate receptor. Morphine is used as a centrally acting analgesic and as a smooth muscle relaxant.

Codeine is the phenolic methyl ether of morphine and is widely used as an over-the-counter analgesic and a cough suppressant. It is formulated with other analgesic agents such as aspirin and paracetamol. Both morphine and codeine are the most important analgesics for the management of moderate to severe pain. A number of semi-synthetic morphinanes have been produced as analgesics and cough suppressants; these include **pholcodine** and **dihydrocodeine**. Morphine was also used as a template for other analgesic agents including **pethidine**, which is one of the most widely used synthetic opiates.

Thebaine is the starting point for the synthesis of many agents, including codeine and veterinary sedatives such as **etorphine**.

Papaverine is an antispasmodic and is formulated with some analgesics such as aspirin. It is also used as a treatment for male impotence, and its activity as a Ca^{2+} channel blocker led to the development of **verapamil**. **Apomorphine** is prepared by heating morphine with concentrated hydrochloric acid and has recently been shown to be of use in the treatment of Parkinson's disease as this compound is a dopamimetic. **Papaveretum** is a total alkaloid extract of opium (containing 85% morphine, 8% codeine and 7% papaverine) from which the minor alkaloid **noscapine** has been removed as it is genotoxic. Papaveretum is used as a premedication.

Indigenous peoples of South America use a variety of arrow poisons for hunting purposes, of which **curare** is one and acts as a strong muscle relaxant. This poison is prepared from plants of the family Menispermaceae, notably *Chondrodendron tomentosum*, which kills by paralysis of the muscles required to breathe. The major active component of this species is the isoquinoline alkaloid **tubocurarine**, so named because the curare poison was carried in bamboo 'tubes' prior to use (Fig. 6.57).

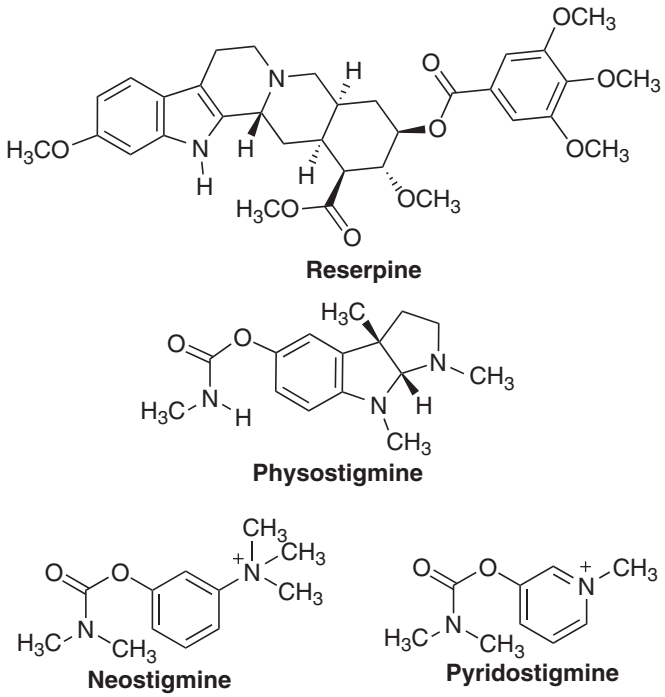


Fig. 6.58

Tubocurarine is a quaternary salt and as a chloride has found use as a muscle relaxant in surgical procedures. The compound was also a template for the development of other muscle relaxants of which **atracurium** (**Tracrium**) is an excellent example.

Ipecac (*Caephaelis ipecacuanha*, Rubiaceae) is a shrub indigenous to Brazil and produces rhizomes (underground stems) that were used by the indigenous peoples to treat diarrhoea. The main alkaloidal components of this species are **emetine, psychotrine** and **cephaeline**. Ipecac was used to treat amoebic dysentery, but the side effects (vomiting, nausea and severe gastrointestinal disturbance) stopped its use. However, it is used as an emetic in the form of a syrup to induce vomiting after poisoning and drug overdose. In addition to its emetic and amoebicidal properties, emetine (Fig. 6.57) is an expectorant and is added to many cough medicines.

INDOLE ALKALOIDS

Like the isoquinolines, the indole alkaloids are a very important source of bioactive compounds. Snake root (*Rauvolfia serpentina*, Apocynaceae) is a shrub common to the Indian subcontinent; it has been used as a panacea in the Ayurvedic system of medicine, with uses described for the treatment of snakebite and madness. **Reserpine**, the major component of this species, was used as an antihypertensive agent, but due to side effects (neurotoxicity, cytotoxicity and depression) it is now not in use (Fig. 6.58).

British missionaries working in the Calabar coast area of West Africa (Nigeria and Cameroon) reported that criminal trials were conducted using the Calabar bean (*Physostigma venenosum*, Fabaceae). The beans of this plant are highly toxic and, when an individual was accused of a crime, they were forced to consume an extract of the bean. This practice was 'trial by ordeal' and accounts for the other name for the Calabar bean, the 'ordeal bean'. Should the individual live then they were innocent of the crime, but death indicated guilt. The margin between innocence and guilt was probably a result of the completeness or incompleteness of extraction of the toxic chemicals from the plant! The toxic component of this species is **physostigmine** (Fig. 6.58), which is an inhibitor of acetylcholinesterase, resulting in an enhancement of the activity of acetylcholine (which is degraded by acetylcholinesterase). There is interest in this compound in the treatment of Alzheimer's disease in which a low concentration of acetylcholine in the brain is observed. Synthetic compounds based on physostigmine include **neostigmine** and **pyridostigmine**, which are used to treat myasthenia gravis, a rare disease characterized by severe muscle weakness.

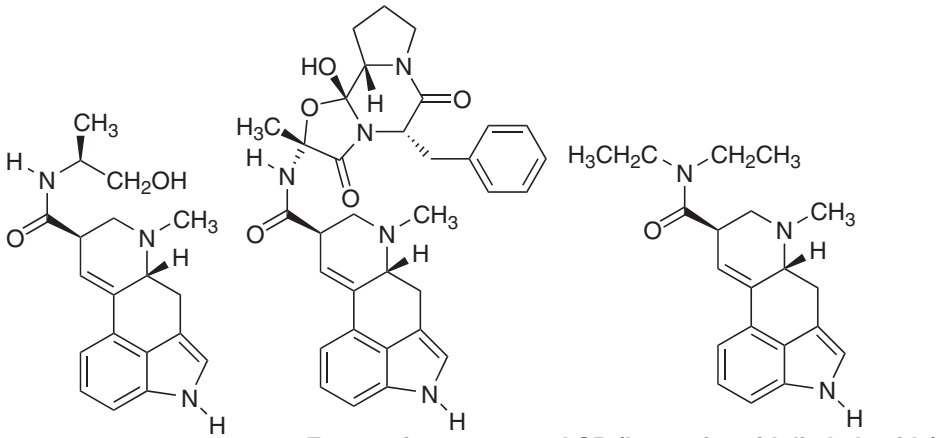


Fig. 6.59

Poisoning through contamination of rye grain by fungi, in particular by *Claviceps purpurea*, has been described since the Middle Ages. This fungus produces dark-coloured structures (sclerotia) known as **ergot** on rye plants; these structures are rich in indole alkaloids. The poisoning from ingestion of bread made from contaminated grain is highly unpleasant, with victims complaining of burning, 'fire-like' sensations throughout their extremities and of vivid highly coloured hallucinations. These poisons can cause massive constriction of blood vessels, leading to 'blackened' limbs and gangrene. This condition became known as St Anthony's fire after the saint who spent much of his life meditating in the fire-like heat of the Sinai desert. Because bread was the staple diet in the Middle Ages, it is likely that this condition was widespread, especially as the damp surroundings in which grain was kept are conducive to the growth of the fungus. Ergot was used as an obstetric preparation in the 1500s to shorten labour during childbirth. It contains several groups of indole alkaloids such as the **ergometrine** type, which have simple amide side-chains, and the **ergotamine** group, which possess complex amino-acid-derived side-chains (Fig. 6.59).

Ergometrine is an oxytocic used to expel the placenta after childbirth or to increase contractions. This compound also acts on the pituitary as well as on the uterine muscles. Ergotamine was first used in the 1920s for the relief of migraine and is still used today. It reduces vasodilation, which can occur in throbbing migraine headache. The ergot alkaloids were used as a template for the semi-synthesis of **bromocriptine, pergolide** and **cabergolide**, which have use in neurological disorders such as Parkinson's disease. Ergot can cause hallucinations, and the hallucinogenic drug of abuse **LSD** (**lysergic acid diethylamide**) is structurally related to these compounds (Fig. 6.59).

Many of the psychoactive compounds (including LSD) are structurally related to **tryptamine**, as are the **harmine** and **harmaline** alkaloids from *Peganum harmala* (Syrian Rue, Nitrariaceae) and the yahé or ayahuasca preparations (*Bansiteriopsis caapi* and *B. inebrians*, Malpighiaceae), which are prepared by Amazonian shamen. Ayahuasca is used as part of the community rituals of some Peruvian groups to preserve their traditional ways and to promote bonding and the establishment of social order. **Ibogaine**, from iboga (*Tabernanthe iboga*, Apocynaceae), is hallucinogenic and anticonvulsant, and has recently been studied as a treatment for heroin addiction. Psychoactive indole derivatives are even found in amphibia, notably in the skin of species of the genus *Bufo*, which produce **bufotenin** (Fig. 6.60).

Mushrooms of the genera *Psilocybe, Panaeolus, Conocybe* and *Stropharia* are known to produce psychoactive substances such as **psilocybin**, which is a phosphate salt in the fungi and is converted into **psilocin** *in vivo* (Fig. 6.60). The Aztecs of Mexico revered certain fungi (*Psilocybe mexicana*, Strophariaceae) as the 'flesh of the Gods' and gave it the name Teonanactl. The reverence for these mushrooms is presumably attributed to the profound hallucinogenic effects they exert, and, in Europe, many related species such as the liberty cap (*Psilocybe semilanceata*) are collected illegally for recreational abuse. These fungi are colloquially referred to as 'magic mushrooms', but as

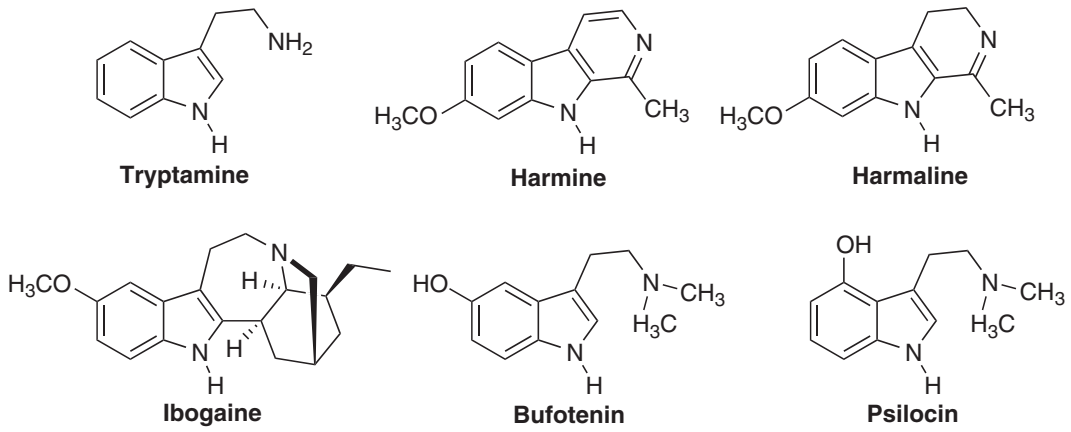


Fig. 6.60

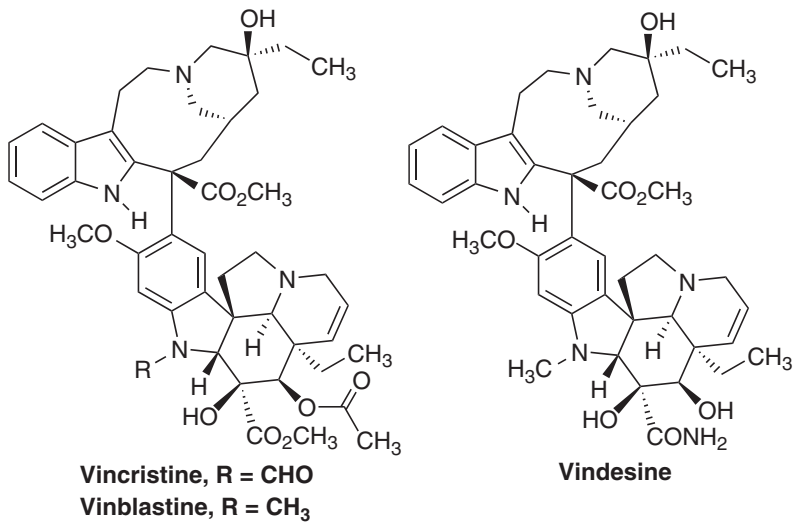


Fig. 6.61

fungal taxonomy is highly complex there are risks of collecting poisonous species and the outcome may not be 'magic' at all.

The most important alkaloids of the indole group are the anticancer agents **vincristine** and **vinblastine** from the Madagascar periwinkle (*Catharanthus roseus*, Apocynaceae). These are complex **bisindole** (dimeric indole) natural products present in small quantities in the plant material. **Vindesine** is a semi-synthetic derivative which is also used clinically. These compounds are used for the treatment of Hodgkin's lymphoma, acute leukaemia and some solid tumours (Fig. 6.61) and are dealt with in further detail in Chapter 8.

Strychnine and **brucine** (Fig. 6.62) are intensely bitter indoles from the seeds of nux-vomica or 'vomiting nut' (*Strychnos nux-vomica*, Loganiaceae),

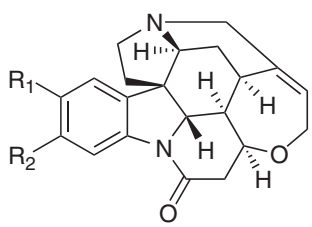


Fig. 6.62

which is a tree indigenous to India. Preparations of nux-vomica were used as a stimulant tonic until the middle of the 20th century. However, these compounds are highly poisonous (strychnine is used as a rodenticide) and they are responsible for occasional

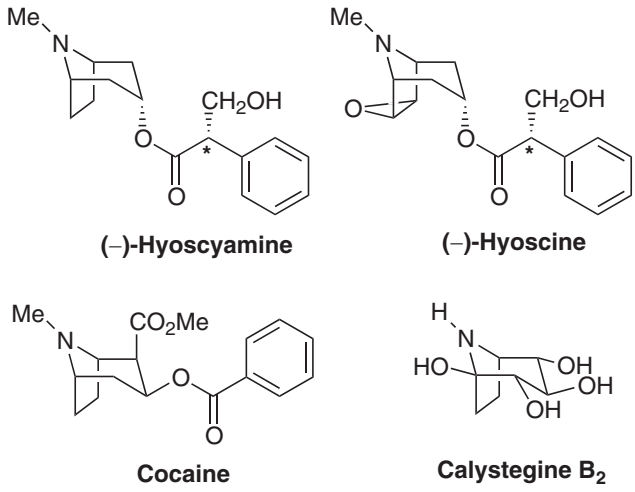


Fig. 6.63

poisoning incidents. They are of historical interest only in pharmacy and are now used as research tools.

TROPANE ALKALOIDS

The European plant deadly nightshade (*Atropa belladonna*, Solanaceae) produces **hyoscyamine** (Fig. 6.63), which occurs in the plant as a racemic mixture [(+) and (–) isomers, sometimes denoted (±)] at the chiral centre denoted * in Fig. 6.63. This mixture is often referred to as **atropine**. The generic name of the plant refers to Atropos, the ancient Greek Fate who, in mythology, cut the thread of life, and belladonna, meaning beautiful lady in Italian and refers to the use of the juice of the berries of this plant by ladies in the 16th century to dilate the pupils of their eyes, which was considered an attractive feature. Hyoscyamine is an anticholinergic and also has been used to treat acute arrhythmias and to dilate the pupil of the eye (a mydriatic) for ophthalmic examinations. Semi-synthetic derivatives are also used (such as **tropicamide**) that are less longer-acting. Hyoscyamine also occurs in other species of Solanaceae, notably henbane (*Hyoscyamus niger*) and thornapple (*Datura stramonium*), together with **hyoscine**, also known as **scopolamine**, which is the epoxide derivative of hyoscyamine. Hyoscine is widely used as a premedication prior to operations to dry up secretions produced by inhalant anaesthetics and reduce nausea caused by the opiates. It is also a component of many travel (motion) sickness preparations.

The drug of abuse **cocaine** comes from the South American plants *Erythroxylum coca* and *E. truxillense* (Erythroxylaceae), which grow at high altitudes in the Andes in Colombia, Peru and Bolivia. As with heroin, this drug causes much misery and is a highly addictive CNS stimulant. Medicinally, cocaine has limited use as a local anaesthetic in ear, nose and throat surgery, and in the control of severe pain for patients with terminal cancer.

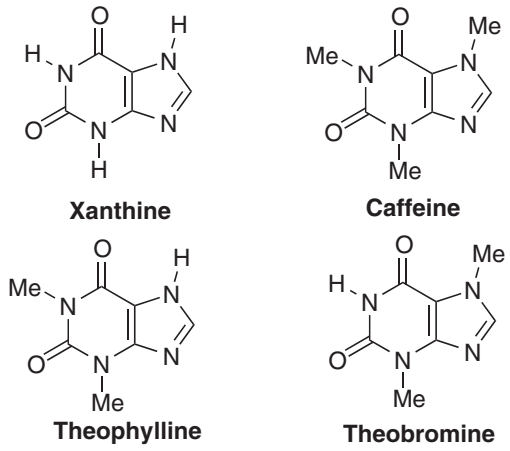
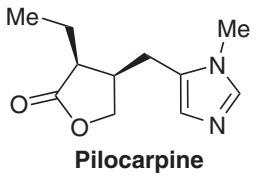


Fig. 6.64

The calystegines, typically **calystegine B_2**, are *nor*-tropane alkaloids ('nor' meaning lacking a carbon), which lack the *N*-methyl group of the tropanes. These compounds are widely distributed in the plant kingdom, particularly in the plant families Solanaceae and Convolvulaceae, which include a number of fruit and vegetables (e.g. tomatoes). The calystegines are currently of interest as inhibitors of glycosidase enzymes and they may have potential toxicity when ingested.

XANTHINE ALKALOIDS

The xanthine alkaloids are probably the most widely known (and used) group of alkaloids, being constituents



Pilocarpine

Fig. 6.65

of popular daily beverages such as tea (*Camellia sinensis*, Theaceae) and coffee (*Coffea arabica*, Rubiaceae). Coffee contains the **xanthine** (or **purine**) alkaloid **caffeine** (1–2%) (Fig. 6.64); typically a cup of instant coffee contains approximately 50 mg of caffeine. The caffeine content is appreciably higher in Turkish or Arabic coffees, which are highly concentrated and may contain up to 300 mg of caffeine per cup. Caffeine is a CNS stimulant and is a component of Proplus, a highly popular product amongst students to counter fatigue and drowsiness. It is also a diuretic and is used in combination with analgesics.

Together with caffeine, **theophylline** and **theobromine** (Fig. 6.64) are minor components of tea; theobromine also occurs in cocoa (*Theobroma cacao*, Malvaceae). All three alkaloids differ only in the number and position of methyl substituents around the xanthine ring system. Theophylline is a diuretic and its derivatives (e.g. **aminophylline**) are used to relax the smooth muscle of the bronchi for relief of asthma.

IMIDAZOLE ALKALOIDS

The only member of this class that is of pharmaceutical merit is **pilocarpine** from jaborandi (*Pilocarpus jaborandi*, Rutaceae), a tree common to South America (Fig. 6.65). Pilocarpine is a cholinergic agent and is used to stimulate muscarinic receptors of the eye in the treatment of glaucoma. In the eye, this compound and its derivatives (salts such as the hydrochloride and nitrate) cause pupillary constriction (miosis) and relieve eye pressure by facilitating better ocular drainage. Currently, there is interest in this class of alkaloid as muscarinic agonists in the treatment of Alzheimer's disease.

Further reading

Appendino, G., Fontana, G., Pollastro, F., 2010. Natural products drug discovery. In: Mander, L., Lui, H.W. (Eds.), Comprehensive natural products II, vol. 3. Elsevier, Oxford, pp. 205–236.

Bruneton, J., 1995. Pharmacognosy, phytochemistry, medicinal plants. Springer-Verlag, Berlin.

Dewick, P.W., 2009. Medicinal natural products: a biosynthetic approach, third ed. Wiley, Chichester.

Evans, W.C., 2002. Trease and Evans' pharmacognosy, fifteenth ed. Baillière Tindall, London.

Luckner, M., 1990. Secondary metabolism in microorganisms, plants and animals, third ed. Springer-Verlag, Berlin.

Mann, J., 1994. Chemical aspects of biosynthesis. Oxford chemistry primers. Oxford University Press, Oxford.

Robbers, J.E., Speedie, M.K., Tyler, V.E., 1996. Pharmacognosy and pharmacobiotechnology. Williams & Wilkins, Baltimore.

Samuelsson, G., Bohlin, L., 2010. Drugs of natural origin: a treatise of pharmacognosy. Taylor & Francis, London.

Thadani, M.B., 1996. Medicinal and pharmaceutical uses of natural products. Cantext Publications, Winnipeg.

Torsell, K.B.G., 1997. Natural product chemistry: a mechanistic, biosynthetic and ecological approach, second ed. Swedish Pharmaceutical Press, Stockholm.

Chapter 7

Methods in natural product analytical chemistry

This chapter reviews the different approaches for the extraction of natural compounds from the **botanical drug** or more generally **biomass**. This yields a '**crude extract**' which may be analysed chemically to ascertain the presence of the different classes of primary or secondary metabolites described in Chapter 6. This can be achieved by classic, wet chemical methods – **phytochemical screening** – or modern, instrumental methods based on the combination of suitable **chromatography and detection techniques**. Their fundamental principles, operating procedures, common applications to medicinal plants, interpretation of the results and inherent limitations will be systematically discussed.

PREPARATION AND LIQUID EXTRACTION

Whether samples are plants, microbes (**fermented** or **solid phase**), marine organisms (corals, slugs, tunicates) or insects, in this context they are referred to as **biomass**. In the case of plants, following their identification and classification by a field botanist into a species and family, samples are collected from the aerial parts (leaves, stem and stem bark), the trunk bark and roots or, in the case of large trees, the timber or the heartwood (inner timber). These samples are then gently air-dried, although this can be problematic in highly humid environments such as rainforests and coastal regions. Better control is achieved in the laboratory using drying cabinets or lyophilizers (freeze-driers), although biomass must be dried quickly to avoid degradation of components by air or by microbes. Care must be taken with lyophilizers as they utilize a high vacuum, which can remove volatile components that may have interesting biological activities.

Once biomass has been dried, it is ground into small particles using either a blender or a mill. Plant material is often milled twice, first using a coarse mill and then a fine mill to generate a fine powder. The grinding process is important as effective extraction depends on the size of the biomass particles; large particles will be poorly extracted, whereas small particles provide an overall higher surface area and, therefore, will be extracted more efficiently.

Selection of the **solvent extraction** approach is very important. For herbal medicines used in commercial preparations water and ethanol combinations are used most commonly. If a plant is under investigation from an ethnobotanical perspective, then the extraction should mimic the traditional use. For example, if an indigenous people use a specific extraction protocol such as a water extract, a cold/hot tea, alcohol or alcohol–water mixtures, then an identical or at least a very similar method should be used in the laboratory so that the same natural products are extracted. Failure to extract biomass properly may result in loss of access to active compounds. Additionally, using an inappropriate extraction method, such as strong heating of biomass with a solvent, may result in degradation of natural products and consequent loss of biological activity.

Numerous extraction methods are available, the simplest being **cold extraction** (in a large flask with agitation of the biomass using a stirrer) in which the ground dried material is extracted at room temperature sequentially with solvents of increasing polarity: first hexane (or petroleum ether), then chloroform (or dichloromethane), ethyl acetate, acetone, methanol and finally water. The major advantage of this protocol is that it is a **soft extraction method** as the extract is not heated and there is little potential degradation of natural products. The use of sequential solvents of

increasing polarity enables division of natural products according to their solubility (and polarity) in the extraction solvents. This can greatly simplify an isolation process. Cold extraction allows most compounds to be extracted, although some may have limited solubility in the extracting solvent at room temperature.

In **hot percolation**, the biomass is added to a round-bottomed flask containing solvent and the mixture is heated gently under reflux. Typically, the plant material is 'stewed' using solvents such as ethanol or aqueous ethanol mixtures. The technique is sometimes referred to as **total extraction** and has the advantage that, with ethanol, the majority of lipophilic and polar compounds is extracted. A concentration equilibrium between compounds in solution and in the biomass is established, resulting in moderate extraction of natural products. Heating the extracts for long periods may also degrade labile compounds; therefore a pilot experiment should first be attempted and extracts assessed for biological activity to ascertain whether this extraction method degrades the bioactive natural products. Care should be taken, as extraction is never truly total; for example, some highly lipophilic natural products are insoluble in polar solvents (e.g. the monoterpenes).

Supercritical fluid extraction utilizes the fact that some gases behave as liquids when under pressure and have solvating properties. The most important example is carbon dioxide, which can be used to extract biomass and has the advantage that, once the pressure has been removed, the gas boils off leaving a clean extract. Carbon dioxide is a non-polar solvent but the polarity of the supercritical fluid extraction solvent may be increased by addition of a modifying agent, which is usually another solvent (e.g. methanol or dichloromethane).

The most widely used method for extraction of plant natural products is **Soxhlet extraction** (Fig. 7.1). This technique uses continuous extraction by solvents of increasing polarity. The biomass is placed in a Soxhlet thimble constructed of filter paper, through which solvent is continuously refluxed. The Soxhlet apparatus will empty its contents into the round-bottomed flask once the solvent reaches a certain level. As fresh solvent enters the apparatus by a reflux condenser, extraction is very efficient and compounds are effectively drawn into the solvent from the biomass due to their low initial concentration in the solvent. The method is the best extraction method for the recovery of large quantities of extract but suffers from the same drawbacks as other hot extraction methods – the possible degradation of products. If the biological activity is not lost on heating, the technique can be used in drug lead discovery.

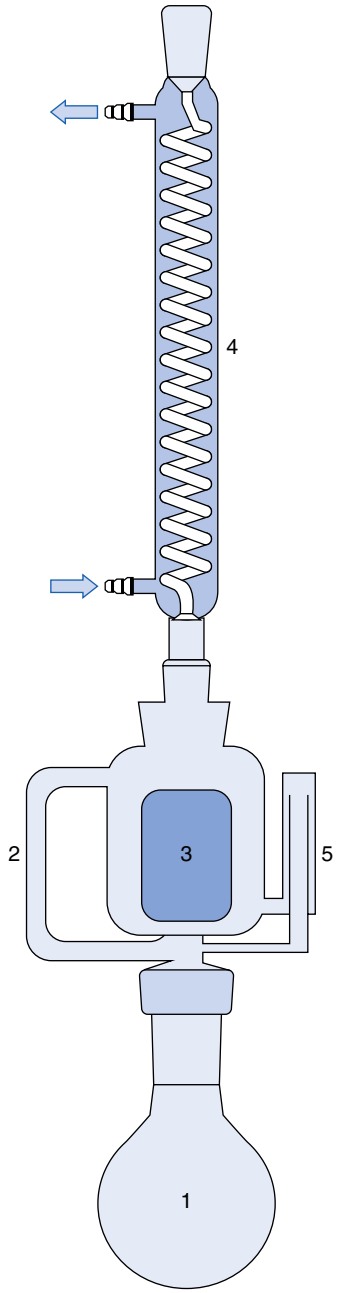


Fig. 7.1 Soxhlet extraction apparatus. (1) The flask containing the extractive solvent is heated up and the solvent vapours are directed through (2) into the extraction chamber, which contains the plant material in a thimble (3). The solvent vapours condense in (4) and drop over the plant material producing a liquid extract. When this reaches a certain level, it is syphoned through (5) down into the flask (1) and the cycle repeats. © Jose M Prieto Garcia.

In general terms, regardless of the extraction process used, extracts are of two types: **lipophilic** ('fat-loving'), resulting from extraction by non-polar solvents (e.g. petrol, ethyl acetate, chloroform, dichloromethane), and **hydrophilic** ('water-loving'), produced by extracting biomass with polar solvents (e.g. acetone, methanol, water).

Due to the variable solubility of the components in different solvents, the chemical complexity of the biomass is simplified in the respective extract. This can greatly simplify the isolation of an active compound from the extract. Additionally, certain classes of compounds may have high solubility in a particular solvent (e.g. the monoterpenes in hexane), which again can simplify the chemical complexity of an extract and help with the isolation process.

Regardless of the extraction technique used, extracts are concentrated under vacuum using rotary evaporators for large volumes of solvent (> 5 ml) or 'blown down' under nitrogen for small volumes (1–5 ml), ensuring that volatile components are not lost. Removal of solvent should be carried out immediately after extraction, as natural products may be unstable in the solvent. Aqueous extracts are generally freeze-dried using a lyophilizer. Dried extracts should be stored at –20°C prior to screening for biological activity as this will decrease the possibility of bioactive natural product degradation.

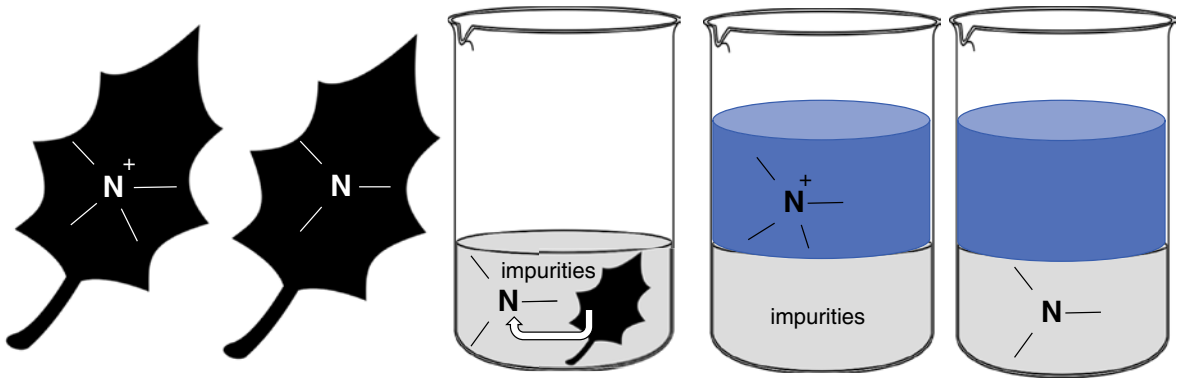
If it is known that certain classes of compounds, such as acids or bases, are present in the biomass, they can be extracted using a tailored protocol. The most common group of natural products that are extracted in this manner is the alkaloids. These basic compounds are often present in plant material as salts:

1. Alkaloids can be recovered from their salts by making the dry powdered plant material alkaline with aqueous ammonia. This leaves the alkaloids as **free bases** that are no longer ionic salts and are much more soluble in organic solvents such as dichloromethane or ethyl acetate.
2. This increased solubility in organic solvents allows **partitioning** of the free bases into ethyl acetate or dichloromethane, which can then be separated from the aqueous ammonia layer in a separating funnel as these solvents form immiscible layers.
3. The dichloromethane solution will contain the free bases, which can be extracted with aqueous acid, for example by extracting three times with 2 M hydrochloric acid, and the alkaloids will transfer from the organic phase to the aqueous phase as hydrochloride salts. The remaining dichloromethane layer can be tested using a specific colour test for alkaloids (e.g. Dragendorff's reagent) to ensure that all of the alkaloids have been transferred to the acidic aqueous layer.
4. The acidic layer can then be basified, which results in the precipitation of the alkaloids (which are no longer salts and therefore no longer soluble in aqueous media) and can be extracted back into an organic solvent (ethyl acetate or dichloromethane).

This extraction method (illustrated in Fig. 7.2) generates a mixture of alkaloids that are essentially free of neutral or acidic plant components and is specific for compounds that are basic (able to form free bases). However, care should be taken with alkaloid extractions as the acids and bases employed may destroy active natural products that have functional groups which are readily susceptible to degradation (e.g. glycosides, epoxides and esters). Additionally, the stereochemistry of a molecule may be affected by the presence of these strong reagents. The most important factor to consider is whether the biological activity has been retained following the extraction protocol.

STEAM DISTILLATION

Essential oils are special extracts from **aromatic plants**. They consist of a mixture of **volatile** (low molecular mass) plant secondary metabolites. A typical essential oil may contain over a hundred compounds, mainly terpenes and phenylpropenes. These volatile chemicals are usually extracted by steam distillation and recovered after condensation by a water-cooled condenser. To this end, the plant is either submersed in water and boiled or the steam is passed through the dry plant material to directly vaporize the volatile compounds. The condensed liquid contains two immiscible phases: one is the **essential oil** and the other is the **hydrosol**, which is water containing some of the most polar volatiles. Essential oils are usually less dense than water and therefore present as the upper phase of the distillate but a few essential oils are denser than water and will have to be recovered as the lower phase. There is only one notable exception to the use of steam distillation: the peel of *Citrus* sp. fruits (lemon, orange, grapefruit, lime), in which the peel is mechanically pressed in cold conditions to yield the pharmaceutical grade essential oil.



Plant Material (Fresh or dry)	Plant Material 'wet' with a base, e.g. ammonia	Plant Material Extracted with non-polar solvents, e.g. Dichloromethane	Partition with Aqueous acid solution, e.g. 2 M Hydrochloric acid	Acidic layer is basified, and can be extracted back into an organic solvent, e.g. Dichloromethane
Alkaloids in the form of salts	Alkaloids become basic	Alkaloids extracted from the plant material together with other non-polar impurities (Fats, waxes, terpenes)	Alkaloids in the form of salts extracted Other non-polar impurities (Fats, waxes, terpenes) remain in the non-polar solvent which is discarded	Alkaloids become basic and get extracted into a fresh aliquot of the non-polar solvent of choice which can be easily evaporated

Fig. 7.2 Classical scheme of the extraction and purification method of the alkaloids contained in dry plant materials using L/L partition. © Jose M Prieto Garcia.

ANALYTICAL TECHNIQUES IN NATURAL PRODUCTS CHEMISTRY

They consist of a vast array of different techniques commonly referred as 'Phytochemical Analysis'. These methods can be used to study the chemical composition of plants (and other organisms) or – in a more applied approach – to identify medicinal active metabolites. Phytochemical analyses may be used in the quality assessment of medicines from natural sources and – in this case – they are usually complemented with some specific methods described together in pharmacopoeia as 'pharmacognostical methods'. These complementary methods ascertain the quality of the 'herbal drug' by macroscopic and microscopic examination of the plant material as well as some basic analytical methods that are not based on separation and/or identification of specific secondary metabolites. Aspects of the fundamentals and application of these methods are described in Chapter 3 (General principles of botany: morphology and systematics) and Chapter 10 (Production, quality control and standardization of herbal materials). The exact protocols are described in every national or international pharmacopoeia.

Phytochemical analyses encompass classical, wet chemical methods as wells as modern, instrumental techniques. Classical quantitative analysis uses mass, colour or volume changes to quantify their amount. Instrumental methods typically separate samples into their components using selective extraction, chromatography or electrophoresis. Then qualitative and quantitative analysis can be performed often with detectors attached to the same instrument and may use light interaction, heat interaction, electric fields or magnetic fields.

PHYTOCHEMICAL SCREENING

Classical qualitative or quantitative methods such as precipitation, extraction, and/or distillation – commonly referred to as phytochemical screening methods – may be used to identify the presence of certain classes of secondary metabolites by exploiting differences in colour, odour, behaviour or reactivity. The presence of a certain group of natural compounds sharing similar physical behaviour, reactivity of chemical functionality can be easily and conveniently identified and/or quantified in this way. These techniques represent the start of phytochemistry as a discipline within pharmacognosy and were vastly developed and refined during the 19th century. Nonetheless, they required substantial amounts of sample to be accurate. Table 7.1 show some of the general tests used for phytochemical screening. However, such methods are now more and more often replaced by more advanced techniques.

Table 7.1 Some of the general tests used in phytochemical screening

ALKALOIDS	EXTRACTS ARE DISSOLVED IN DILUTE HYDROCHLORIC ACID AND FILTERED	
	Filtrates are treated with:	Positive result:
Dragendroff's Test	Potassium bismuth iodide	Formation of red precipitate
ANTHRANOL GLYCOSIDES	EXTRACTS ARE HYDROLYSED IN DILUTE HYDROCHLORIC ACID AND FILTERED	
	Filtrates are treated with:	Positive result:
Modified Borntrager's	Ferric chloride solution and immersed in boiling water for 5 min. The solution is cooled and extracted with equal volumes of chloroform. The chloroform layer is separated and treated with ammonia solution	Formation of rose-pink colour in the ammoniacal layer
FLAVONOIDS	PLANT MATERIALS ARE EXTRACTED WITH DISTILLED WATER AND FILTERED	
	Filtrates are treated with:	Positive result:
Alkaline Reagent Test	A few drops of sodium hydroxide solution	Formation of intense yellow colour, which becomes colourless on addition of dilute acid
PHENOLS	PLANT MATERIALS ARE EXTRACTED WITH DISTILLED WATER AND FILTERED	
	Filtrates are treated with:	Positive result:
Ferric Chloride Test	3–4 drops of ferric chloride solution	Formation of bluish-black colour
PHYTOSTEROLS	PLANT MATERIALS ARE EXTRACTED WITH CHLOROFORM AND FILTERED	
	Filtrates are treated with:	Positive result:
Liebermann Burchard's Test	A few drops of acetic anhydride, boiled and cooled. Then conc. sulphuric acid is added	Formation of a brown ring at the water-chloroform interphase
SAPONINS	EXTRACTS ARE DILUTED WITH DISTILLED WATER ACID AND FILTERED	
	Filtrates are:	Positive result:
Foam Test	Shaken with 2 ml of water	Foam produced persists for ten minutes
TANNINS	PLANT MATERIALS ARE EXTRACTED WITH DISTILLED WATER AND FILTERED	
	Filtrates are treated with:	Positive result:
Gelatin Test	1% gelatin solution containing sodium chloride	Formation of white precipitate

There are a number of more specific reactions to differentiate between certain subclasses of secondary metabolites. For example, tropane alkaloids (such as atropine or hyoscine, see Fig. 6.63) will test positive with the Dragendorff's test but will also form a purple colour when treated with fuming nitric acid followed by addition of a solution of dimethylformamide and a few drops of concentrated tetraethylammonium hydroxide. However, alkaloids without the characteristic tropane ring will not usually yield this colour.

In the field of forensic sciences, natural substances used as drugs of abuse are often preliminarily identified using such specific screening methods. If they test positive, their identity is further confirmed by more refined techniques such as immunoassays or instrumental chromatography.

INSTRUMENTAL (CHROMATOGRAPHY-BASED) ANALYTICAL TECHNIQUES

With the advent of **chromatography** in the mid-20th century it was possible to **separate** minute amounts of complex mixtures of chemicals ('crude extracts') allowing for the detection and/or identification of their single compounds:

Sample (a mixture of compounds) → Chromatography → Compound *1* + Compound *2* + (…) + Compound *n*

Chromatography is a physical method of separation in which the components to be separated **are distributed between two phases**, one of which is stationary (**stationary phase**) while the other moves in a definite direction (the **mobile phase**). In chromatography, a solute molecule moves along a small channel

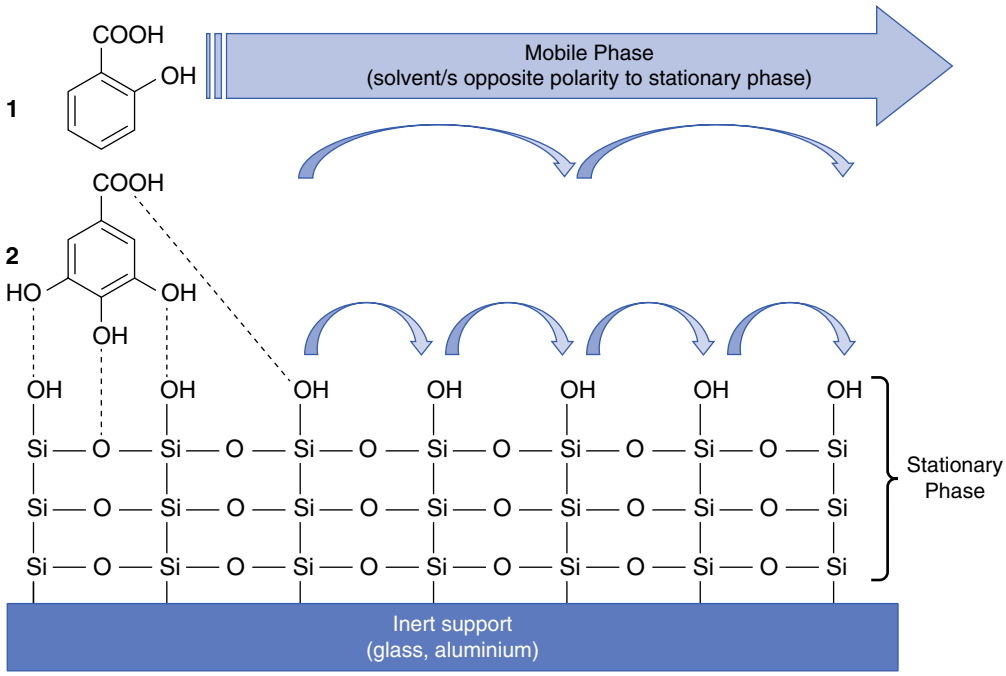


Fig. 7.3 Physicochemical mechanisms underlying chromatography. Different degrees of interaction between the phytochemicals salicilic acid (1) and gallic acid (2), stationary phase (silica gel, acting as a polar adsorbent) and mobile phase (a less polar solvent, providing partition) result in one molecule (1) eluting faster than the other (2). © Jose M Prieto Garcia.

(made up by the stationary phase) the remainder of which is filled with solvent molecules (mobile phase). The solute is subjected to two forces acting in different directions: the impelling force provided by the solvent's flow and the retarding force consisting of interactions with the stationary phase, which are governed by weak intermolecular forces such as **London forces** and **hydrogen bonds**, among others.

Each interaction of the solute with the stationary phase will slow down the compound. Millions of interactions occur in chromatography, thus resulting in an efficient separation in a short time. Each solute may have more or less interactions depending on their functional groups, resulting in different elution speed or elution times (Fig. 7.3).

Many different types of stationary phases have been developed over the years to offer different chemical selectivity. Initially, chromatography was based on materials such as cellulose, kieselguhr and aluminium oxide. Although they are not entirely phased out, silica gel and its derivatives (e.g. reverse phase, cyano, diol and amide) are nowadays preferred due to their superior reproducibility and versatility (Fig. 7.4). All these stationary phases operate on adsorption/partition principles. Other stationary phases operating on different principles are size exclusion and ion-chromatography, which will be described in p. 125 of Chapter 8.

The detection of the individual compounds can be achieved by chemical (usually destructive) or physical (usually non-destructive) means. The latter mostly comprise **spectroscopic** and **spectrometric** techniques that are performed with the help of a **detector**. Detectors are placed at the exit of a **chromatography instrument** – usually liquid or gas chromatography – in order to provide information regarding the single separated compounds and to help in their **identification**:

Sample (a mixture of compounds) → Separation → Detection of each compound

Theoretically we can **hyphenate** (= link sequentially) as many detectors as technically possible. The separated compounds (or eluate) may pass through several detectors, one detector at a time. This is called linear hyphenation and helps to accumulate structural information to aid with the identification of the components within the sample:

Sample (a mixture of compounds) → Separation → Detector *1* → Detector 2 → (…) → Detector *n*

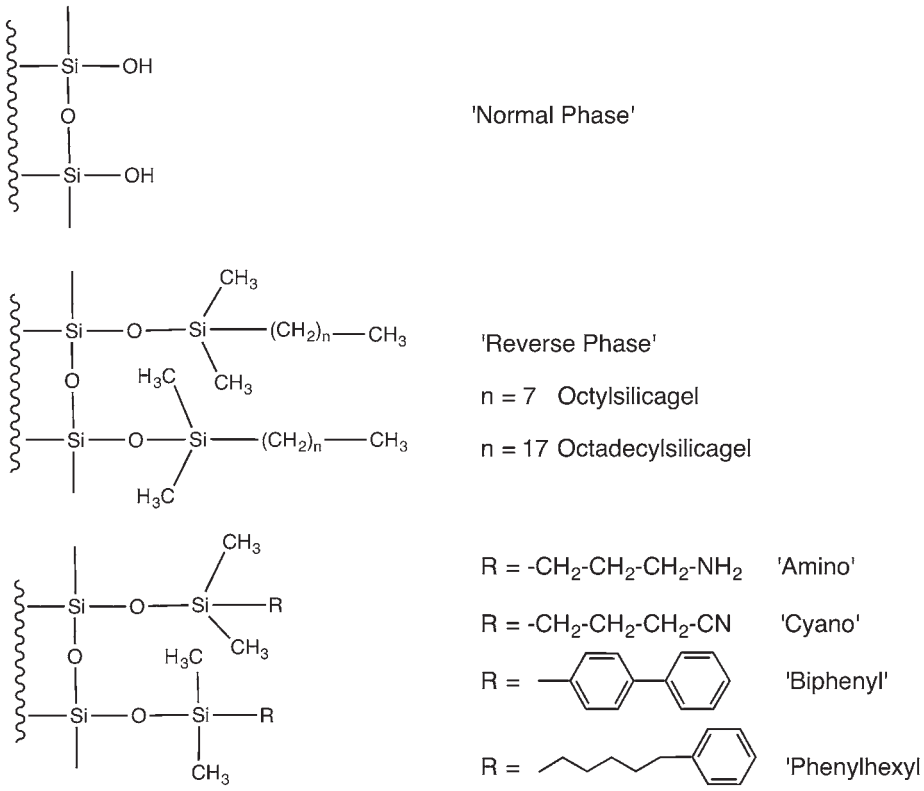


Fig. 7.4 Common stationary phases used in adsorption/partition chromatography. © Jose M Prieto Garcia.

When more than one detector is used it is mandatory to start with all non-destructive detectors before finishing with a destructive one. Examples of common **linear series of detectors** include ultraviolet-light scattering, ultraviolet-mass spectrometry – or ultraviolet-nuclear magnetic resonance-mass spectrometry. If we **split** the eluate we can **detect in parallel**. Here the only limitation is the quantity of the eluate and/or the sensitivity of the detectors. For example, a liquid chromatograph can be coupled in parallel with ultraviolet-mass spectrometry and light scattering, whilst a gas chromatograph eluate may be split to both combustion and mass spectrometry detectors. Technical details on these detectors are presented later in this chapter.

THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography (TLC) is one of the most widely used and easiest analytical methods for the fingerprint and/or quality control of crude extracts. This method employs glass or aluminium plates that are pre-coated with sorbent (e.g. silica gel) of varying thickness dependent on the amount of material to be loaded onto the plates. The coating on analytical plates is generally of 0.2 mm thickness. The compound mixture is loaded at 1–2 cm from the bottom edge of the plate as a spot or small band. The plate is then lowered into a tank containing a predetermined solvent system that will migrate up the plate by capillarity and separate the compound mixture according to the polarity of the components.

This technique allows micrograms of material to be separated and has been the workhorse in pharmacopoeial and forensic analyses since its invention in the 1950s. Although it is still the first choice for many protocols, it is being progressively replaced by more accurate and/or HPLC protocols. Samples such as medicinal plant extracts (e.g. ginkgo tinctures) or drugs of abuse (e.g. cannabis resin) may be compared with **reference substances** (e.g. bilobalide and tetrahydrocannabinol, respectively) for quick identification.

Although any type of stationary phase is commercially available in convenient pre-coated plates, silica gel 60 is still the most universal one and can achieve almost every type of separation if used with a suitable choice of mobile phase. There are many such solvent systems already optimized for the separation with good resolution for almost every class and subclass of

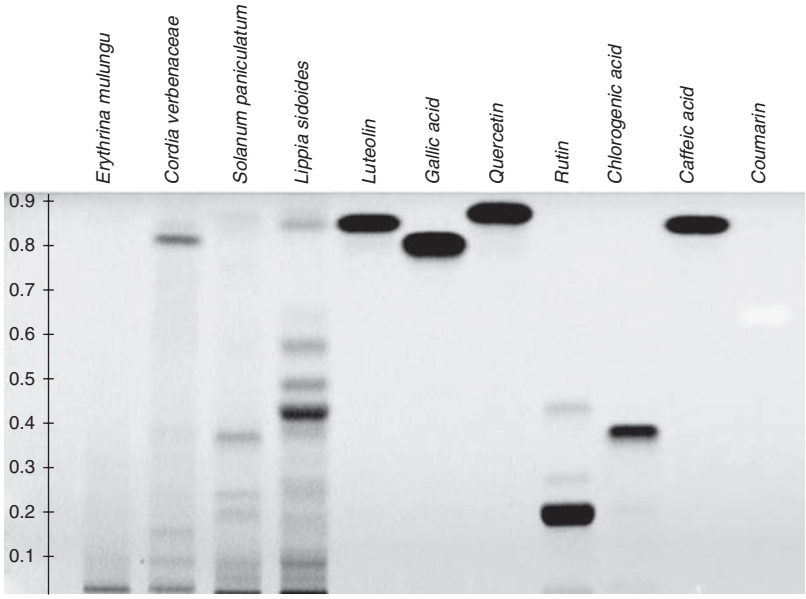


Fig. 7.5 Thin-layer chromatography plate under UV light at 254 nm before derivatization. The natural products absorbing at a similar short-wave UV light will appear as black spots on a green background generated by a fluorescent indicator (F254).

natural products. For example, the use of a solvent system made up of ethyl acetate–formic acid–acetic acid–water in the proportions 100:11:11:27 gives an excellent separation of flavonoid glycosides only. Under these conditions, **less polar** non-glycosylated compounds elute with the solvent front whilst the **more polar** flavonoid glycosides such as rutin and chlorogenic acid – containing quinic acid, which is a cyclic polyol similar to sugars – are well resolved in the middle zone of the plate, allowing their individual identification (Fig. 7.4).

The preparation of mobile phases is no trivial thing, as they have to be mixed ensuring that every solvent is slowly (sometimes drop by drop) and sequentially added with frequent stirring and/or shaking until the final result is one transparent phase only. They need to be used fresh as they continuously change their composition over time, usually towards an enrichment of the less volatile components.

Sorbent-coated plates often incorporate a fluorescent indicator (F_{254}) so that natural products that absorb short-wave UV light (e.g. 254 nm) will appear as black spots on a bright green background (Fig. 7.5). Under long-wave UV light (e.g. 365 nm), certain compounds may emit a brilliant fluorescence on a dark background (Fig. 7.6). Both UV absorbance and fluorescence properties may be used to monitor the separation of compounds on a TLC plate.

The result of the TLC analyses is more often monitored after the **chemical development** of the plate. The plate is quickly immersed or carefully sprayed with chemical reagents followed by air drying at room temperature or heating at a certain temperature in an oven or on a hot surface. Development reagents can be unspecific or specific for a certain class of natural products. Examples of the most common developing reagents used in phytochemistry are shown in Table 7.2.

For each spot/band a retention factor (*Rf*) is calculated as the ratio of the distance travelled on the adsorbent by a given compound to that travelled by the leading edge of the solvent (or mobile phase), both measured from the point of application of the test substance. This parameter varies between 0 and 1 and has no units. If a spot or band shows the same *Rf* and reactivity as the developing reagent of a reference substance in at least three very different conditions, then they are considered as chemically equivalent and may well be the same compound/s. **Reference substances** (sometimes called standards) may consist of crude extracts from botanically authenticated herbal drugs or pure (95–99%) single chemicals.

Resolution of very complex mixtures of chemically similar components may be achieved by bi-dimensional TLC. One sample only is applied close to the bottom left corner of a 20 × 20 cm TLC plate. Then, it is developed once in a suitable mobile phase. The plate is dried, turned 90° degrees to the left and developed

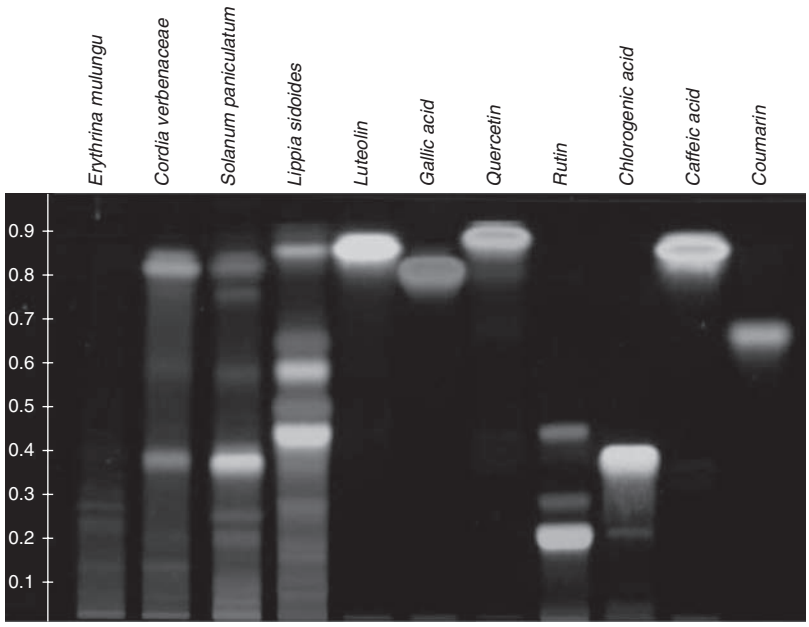


Fig. 7.6 Thin-layer chromatography plate under UV light at 365 nm before derivatization. The natural products that absorb at similar short-wave UV light will appear as spots with various colours depending on their structures on a dark background.

Table 7.2 Four common developing reagents used in thin-layer chromatography analyses

REAGENT	COMPOSITION	RESULT/NATURAL PRODUCT DETECTED
p-Anisaldehyde – H_2SO_4	Spray with a solution of freshly prepared 0.5 ml *p*-anisaldehyde in 50 ml glacial acetic acid and 1 ml 97% sulphuric acid. Heat to 105°C until maximum visualization of spots	Results: phenols, terpenes, sugars, and steroids turn violet, blue, red, grey or green
Ninhydrin	Spray with a solution of 0.2 g ninhydrin in 100 ml ethanol and heat to 110°C until spots appear	Results: reddish spots appear. For detection of amines (alkaloids) but also amino acids, amino sugars
Natural substance-polyethylene glycol reagent (NST/PEG)	Spray a 1% methanol solution of natural substance reagent A (diphenylboric acid β-aminoethyl ester) and a 5% ethanol PEG 4000 solution, one after the other onto the TLC-plate (approx. 10 ml and 8 ml). Adding PEG will lead to an increase in detection sensitivity	Results: Intensive fluorescent colours will appear immediately or after 15 min in UV 365 nm. For detection of flavonoids
Vanillin – H_3PO_4	Spray plate with 1 g vanillin in 100 ml 50% aqueous H_3PO_4. Heat 5–30 min at 110°C	Results: Chars all natural products resulting in brown, grey or black spots. Coloured or fluorescent spots (at 254 and 360 nm) may be seen

again with a second solvent system. Using this technique, for example, flavonoids from the marigold flower (*Calendula officinalis* L.) can be better separated in microcrystalline cellulose plates developed with a first run in *tert*-butanol:acetic acid:water (3:1:1) followed by a second orthogonal run in 15% aqueous acetic acid (Ćetković et al 2003).

There are a number of advantages to TLC for the analysis and isolation of natural products:

- Cost-effective compared with instrumental methods and requires little training or knowledge of chromatography
- Easy scale-up from analytical to preparative mode with quick isolation of milligram to gram amounts of product
- Flexibility of choice of mobile and stationary phases

- A separation may be readily optimized to 'zero in' on one component and methods may be quickly developed
- Practically any separation can be achieved with the correct mobile and stationary phases
- A large number of samples may be analysed or separated simultaneously.

The major disadvantages of TLC are that:

- Loading and speed are poor compared with instrumental chromatography
- There is poor detection and control of elution compared with high-performance liquid chromatography.

HIGH-PERFORMANCE THIN-LAYER LIQUID CHROMATOGRAPHY

High-performance thin-layer liquid chromatography (HPTLC) is the automatization of all necessary steps in TLC analyses. A robotic instrument executes the application of exact volumes of samples on specially produced TLC plates. These are then developed within automated tanks under controlled temperature, humidity and solvent saturation conditions. Development of the plates by immersion in the chosen reagent is also done by a robotic arm and reproducible drying is achieved on hot plates at the exact required temperature.

The power of this instrumental technique is only fully exploited if the plates are coated with improved stationary phases. Typically, they consist of ultra-pure silica with spherical particles of 7 μm, significantly lower than the 10–12 μm particle size in classical precoated TLC plates. This results in very compact spots that lower detection limits and running times. The reproducibility achieved using HPTLC instruments allows for fully quantitative protocols if the plates are scanned under UV or visible light followed by densitometry analyses using calibrated imaging systems and suitable software. HPTLC analyses can be used for routine quality control in the pharmaceutical industry provided that the software supports documentation under international quality standards.

GAS CHROMATOGRAPHY

Gas chromatography (GC) is an instrumental adsorption/partition chromatography technique in which the mobile phase is a gas (referred to as the **carrier gas**). The chromatographic columns are of very small diameter and are often called '**capillary columns**'. The stationary phases are either packed columns (e.g. in the case of adsorbent materials such as silica) or coating the interior of the column as a thin film (e.g. when partition is required with a reverse phase). However, the main factor in the elution order of the compounds is their molecular mass, with the lighter molecules eluting ahead of heavier ones. The substance is heated until vaporized immediately after injection into the system. This is then carried by the gas stream (e.g. high-purity helium, hydrogen or nitrogen) through the column and every component of the sample undergoes distribution between the gas and liquid or solid stationary phase in a similar manner to that in other forms of chromatography. The choice of gas is mainly dictated by the detector system used. Optimal elution of the components is achieved by a balance of the pressure of the gas acting as mobile phase and temperature of the column, which is placed inside a thermostatic oven. Carrier gas flow rates are typically between 1 and 10 ml/min and the temperature may vary from 50 to 300°C. Using electronic pressure control allows the instrument to compensate for changes in gas pressure as the column oven temperature is increased. Fig. 7.7 shows the scheme of a GC chromatograph; it can be run in fully automated mode, and with carousel autosamplers it is possible to analyse tens to hundreds of samples.

Detection in GC has been classically based on **combustion type detectors**, with flame ionization detection (FID) being among the most frequently used in natural products analyses. Combustion type detectors require the addition of a 'fuel gas' (usually hydrogen) and an 'oxidizing gas' (usually dry air). Non-combustion detectors such as **electrochemical type detectors** (e.g. ECD) do not require any added gas but may often use argon or methane as a '**make-up**' gas to increase detector sensitivity. The sensitivity of the ECD, invented by the British scientist James Lovelock in the late 1960s, is impressive: it is able to detect down to 10^{-13} g (one tenth of a picogram!) of the analyte.

However, the full power of GC was achieved when mass spectrometry detectors were made available at an affordable price. This technique allows the measurement of the molecular weight of a compound and, once a molecular ion has been identified, it is possible to measure this ion accurately to ascertain the exact number of hydrogens, carbons, oxygens and other atoms that may be present in the molecule. This will give the molecular formula. A number of ionization techniques are available in MS, of which **electron impact** is widely used. This technique gives good fragmentation of the molecule and is useful for structure elucidation purposes as the fragments can be assigned to functional groups present in the compound. The disadvantage of this technique is that molecular ions are sometimes absent. Softer techniques such as **chemical ionization** (CI), **electrospray ionization** (ESI) and **fast**

atom bombardment (FAB) mass spectrometry ionize the molecule with less energy; consequently, molecular ions are generally present, but with less fragmentation information for structure elucidation purposes.

GC-FID-MS or GC-ECD-MS is a highly sensitive and powerful instrumental technique when coupled to an electronic library capable of searching for compounds with the same MS ionization pattern. Modern software enables the MS spectra of eluting peaks to be compared with spectra stored electronically, thereby enabling early identification of known compounds or, usefully, the comparison of novel compounds with a similar MS spectrum, which may indicate structural similarity. It is also possible to increase the size of these electronic libraries and improve the searching power of the technique.

The strength and limitation of GC is that only relatively volatile compounds are able to be separated and detected. It is therefore the method of choice for essential oils, that by definition are mixtures of volatile metabolites from plant materials. The analysis of essential oils exploiting the information contained in electronic libraries allows – in most cases – for the fast resolution and identification of typically 80–99% of the components, which is unmatched by any other instrumental technique. Fig. 7.8 shows the GC chromatogram of an essential oil.

Essential oils are made up by molecules usually below 300 $g \cdot mol^{-1}$. Heavier natural compounds may need chemical modification to improve their volatility. For example, pentacyclic triterpenes with molecular masses exceeding 400 $g \cdot mol^{-1}$ can be separated by GC if their hydroxyl and carboxylic acid groups are derivatized with trimethylchlorosilane (TMCS) in pyridine (Jemmali et al 2016).

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

The final instrumental technique discussed in this section is high-performance liquid chromatography (HPLC). This method is widely used for the analysis and isolation of bioactive natural products. The analytical sensitivity of the technique, particularly when coupled with UV/VIS detection, enables the acquisition of spectra of eluting peaks from 190 nm to 800 nm. The flow-rates of this system are typically 0.5–2.0 ml/min and sample loading in the analytical mode allows the detection and separation of sub-microgram amounts of material.

HPLC pumps solvents at high pressures (typically up to 4,000 psi; up to 15,000 psi in modern ultra-HPLC machines) through stainless steel columns densely packed with the stationary phase, thus maximizing its interaction with the solutes. These HPLC systems are computer-driven and not only run samples, but may be programmed to process data and print out chromatograms and spectra automatically. A scheme of an HPLC instrument is shown in Fig. 7.9.

It operates with virtually all types of stationary phases. Standard sorbents such as normal phase (silica) and reverse-phase (C_{18} and C_8) and more 'exotic' stationary phases such as phenyl, cyano, C_4, and chiral phases provide a vast array of adsorption/partition chromatography methods tailored to virtually every type of phytochemical. Other separation techniques such as ion exchangers for the isolation of highly polar compounds and gel size-exclusion for the separation of proteins can be easily implemented. In addition, some HPLC columns employ radial compressed column technology providing a long column life, few void volumes, homogeneous column packing with little solvent channelling and excellent 'band flow' of components as they flow through the column.

This versatility of stationary phases has made HPLC a highly popular method for separation. However, the most widely used stationary phase is still C_{18} (reverse-phase) chromatography, generally employing water/acetonitrile or water/methanol mixtures as the mobile phase. Despite the presence of water, it can be used for the majority of natural products that are soluble in organic solvents. These mobile phases may be run in **isocratic elution mode**, in which a constant composition (e.g. 70% acetonitrile in water) is maintained for a set period of time, or in **gradient elution mode**, in which the concentration of a particular solvent is increased over a period of time, starting, for example, with 100% water and increasing to 100% acetonitrile over 30 minutes. The latter is achieved with the aid of computer-controlled pumping systems accurately mixing solvents over time, which results in superb control of elution power.

Detection in analytical HPLC generally utilizes a UV/VIS detector recording one wavelength only. Alternatively, the photo-diode array (PDA) detector is able to monitor and record all wavelengths at the same time. This is the most useful detector in phytochemistry as crude extracts contain phytochemicals with very diverse UV/VIS behaviour.

Despite the analytical power packed in a HPLC-UV/VIS-PDA system, phytochemical analyses pose such a challenge in terms of polarities and UV spectra that even in the best of the conditions not all the components of an extract may be fully resolved. Fig. 7.10 shows a typical HPLC-UV/VIS chromatogram (at 254.4 nm) of a plant extract together with the UV/VIS spectra of each major peak. Not all peaks represent a pure compound, but are made of a group of compounds with very similar

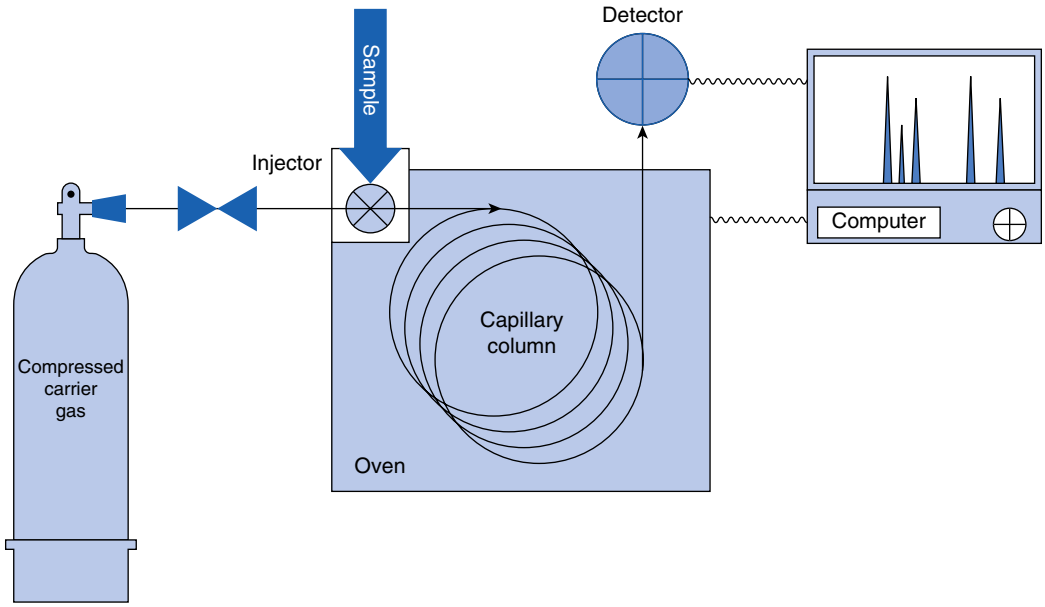


Fig. 7.7 Scheme of a gas chromatograph. © Jose M Prieto Garcia.

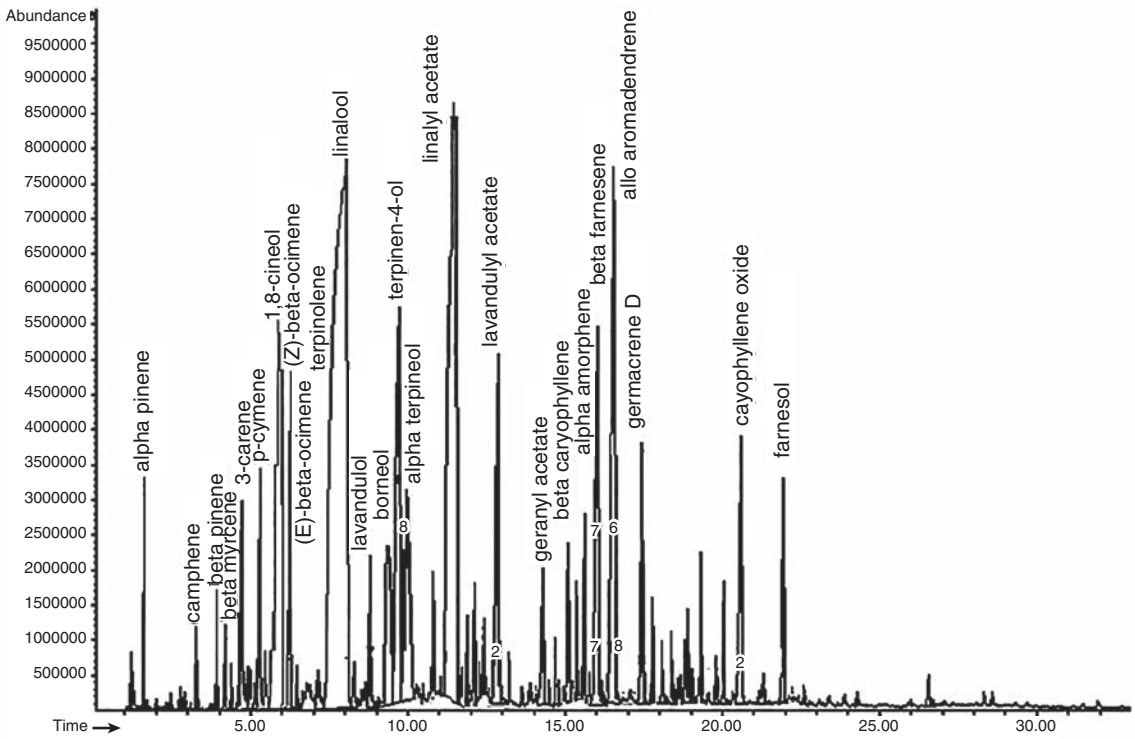


Fig. 7.8 Typical GC–MS chromatogram of *Lavandula angustifolia* essential oil showing the separation of chemical components.

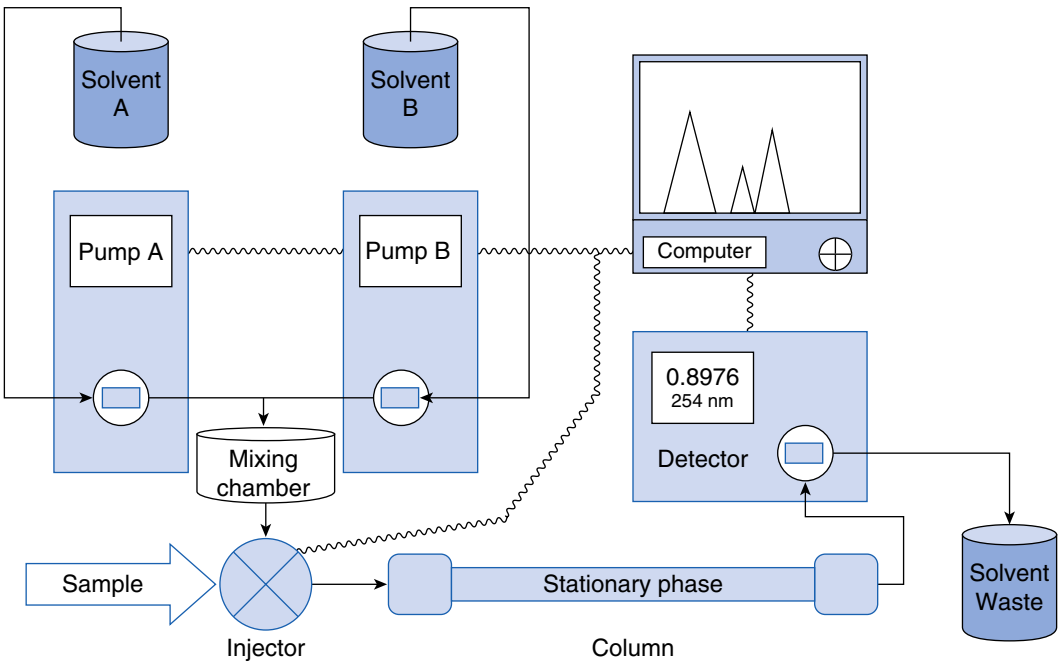


Fig. 7.9 Scheme of HPLC equipment. © Jose M Prieto Garcia.

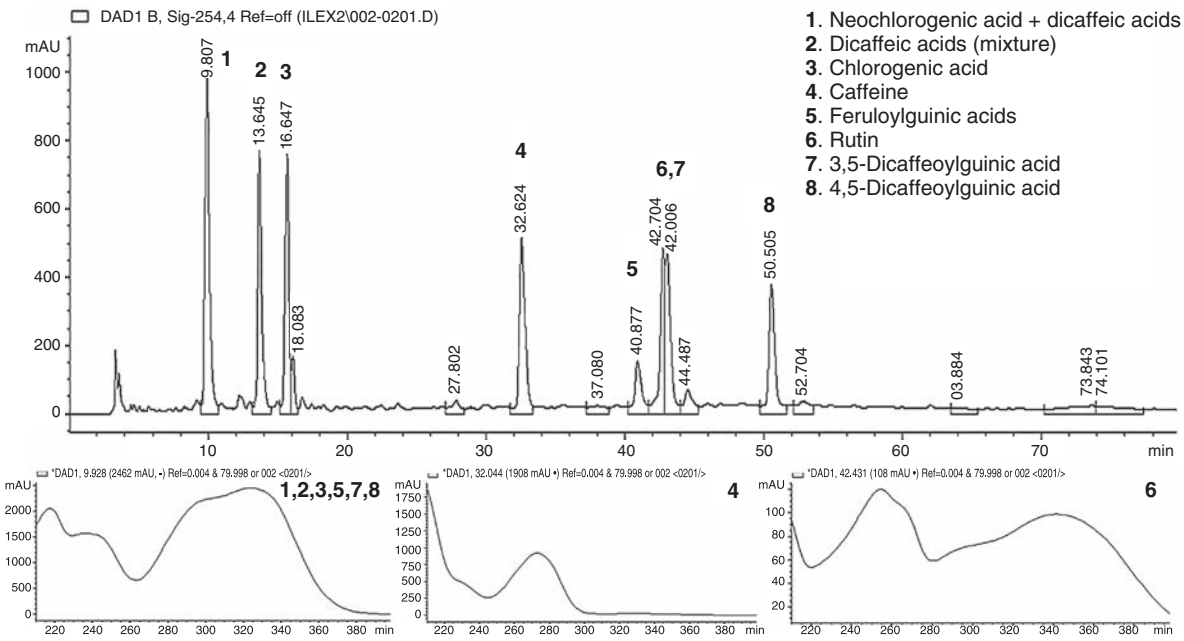


Fig. 7.10 Typical HPLC-UV-VIS-PDA chromatogram of a plant extract (in this case at 254.4 nm, up) and the UV spectra of every major peak (down). © Jose M Prieto Garcia.

polarities eluting together (such as 1, 2 and 5). Other peaks in the chromatogram are single compounds well resolved from each other. Their identification must be carried out by matching both the retention time (t_R) and UV/VIS spectra with those of pure standards under the same elution conditions. Alternatively, co-injection of the sample and reference substance should result in a perfect overlapping of the two peaks.

Compounds with poor UV characteristics cannot be detected by PDA UV/VIS detection. This is especially true in the analysis of natural products such as terpenoids or polyketides, which may have no unsaturation or **chromophores** that give rise to a characteristic UV signature. In these cases, a **refractometry index** (RI) detector has been the classical alternative. However, they have two major limitations: they are 100–1000 times less sensitive than UV detectors and must be used with isocratic conditions only. To overcome these limitations **evaporative light scattering** (ELS) detectors were developed. Three processes occur within the ELS detector: first the eluate is nebulized, second the mobile phase is evaporated and third the scattered light by the analyte particles is detected. This detector works better with volatile mobile phases and its response is both related to the quantity of the eluate (mass not the concentration) as well as the molecular mass of the molecules. The ELS detector is a perfect choice for the analysis of high molecular weight, UV/VIS transparent compounds such as polysaccharides.

Fluorescence can be also used to improve the detection of analytes. The operation of fluorescence detectors is similar to the UV/VIS detector but with the excitation light axial to the detector cell. Its sensitivity is up to 1000 times higher than UV/VIS. Unfortunately, not all molecules are endowed with this property and in some cases a fluorescent derivatization reagent is added pre- or post-column to form a fluorescent derivative of the natural product of interest.

Without doubt, one of the most important additions to the detection power in HPLC systems is mass spectrometry (MS). As with GC-MS, HPLC-MS becomes a powerful technique when coupled to an electronic library capable of searching for compounds with a known UV/VIS spectrum and MS ionization pattern. Actually, HPLC-MS is now considered as the golden standard in terms of versatility and sensitivity for the analysis of all types of natural products.

FINGERPRINTING OF NATURAL PRODUCTS

Instrumental analytical techniques were originally designed to detect the presence of one or a few compounds (qualitative analysis) and/or their precise quantity (quantitative analyses) within a complex sample. These goals require optimal eluting conditions achieving well-resolved peaks that must be matched with t_R and spectral characteristics of the pure standards.

The application of powerful instrumental techniques such as GC-MS and HPLC-MS for metabolomics studies – defined as the analysis of all metabolites present in an organism – has been applied to the study (**fingerprinting)** of complex natural products. This type of analysis aims to record the overall profile of metabolites without necessarily identifying them one by one. Theoretically, a fingerprint of the extract of a natural product (e.g. a herbal drug) facilitates comparison with similar materials. The degree of similarity may be surmised by qualitative comparison of chromatograms and UV/VIS-MS spectra stored in an electronic library. This is currently requested by many journals to ensure the reproducibility of experimental data obtained with non-commercial natural products such as plants collected in the wild. This untargeted approach exploits the gradient elution mode to elute as many compounds of different polarity as possible within a reasonable time but without trying to achieve perfect resolution. Therefore, it is not accepted for the quality control of herbal medicines for which reproducible quantitative methods of certain well resolved quality or stability markers must be met. The fingerprint needs to disclose absolutely all analytical parameters, including the processing and extraction protocols of the materials, the brand of the equipment, the brand and batch of the columns and solvents, as well as every detail of the elution program and settings of each detector. Failure to do this results in an irreproducible set of data of no intra- or interlaboratory use.

By applying multivariate analysis and chemometrics to the data gathered from fingerprint analyses, the analyst can check consistency across batches of the same herbal product as well as rule out adulterations, which otherwise would have been unobserved by focusing on one phytochemical marker only. Fig. 7.11 shows the overlapped fingerprint analysis of 19 samples of *Ginkgo biloba* L. The chemometric analysis (scatter plot) reveals that three samples (1, 2 and 4) stand out from the rest because peak no. 8 is too predominant (as seen in the single chromatograms on the right). These products were adulterated by addition of the inexpensive flavonoid rutin, thus artificially increasing their total flavonoid content (Xie et al 2006).

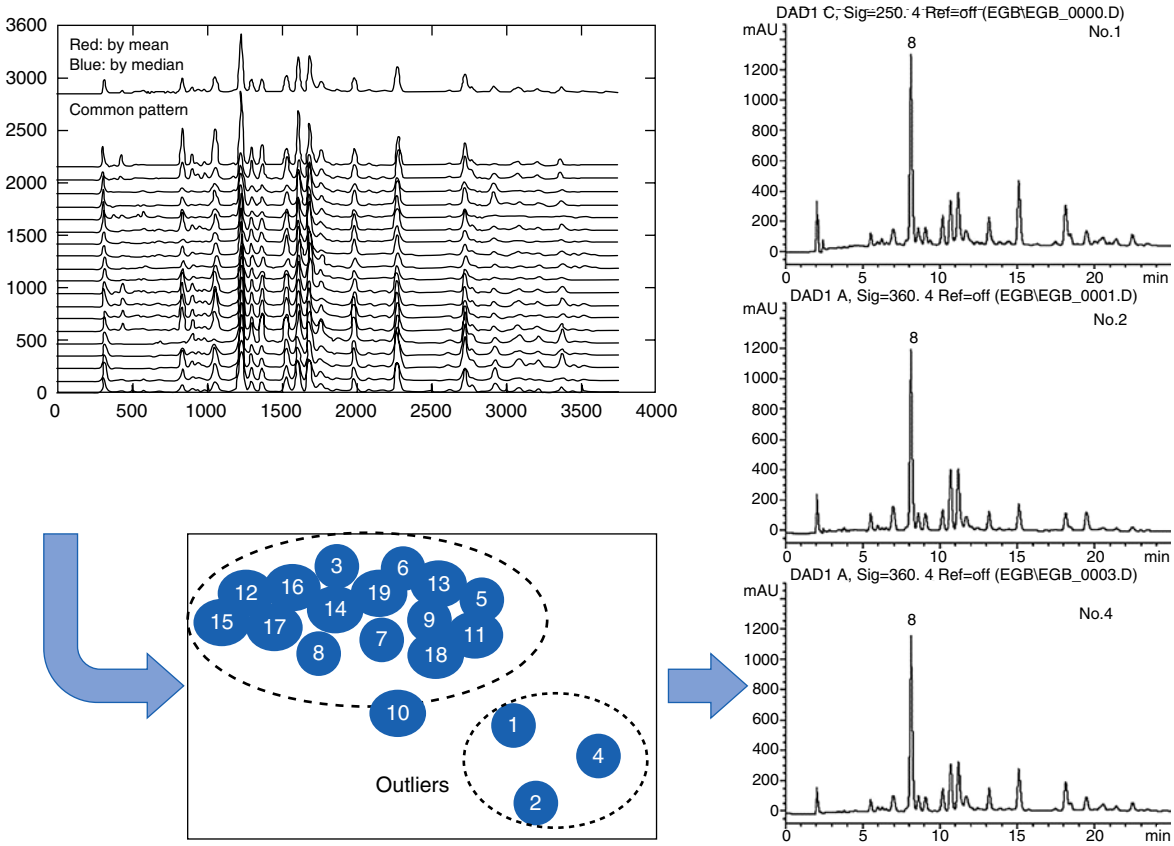


Fig. 7.11a–c HPLC fingerprints of 19 commercial samples of *Ginkgo biloba* L. extracts analysed by computer-aided-similarity-evaluation software helps to pinpoint adulterated products. Adapted from: Xie P, Chen S, Liang Y-z, Wang X, Tian R, Upton R 2006 Chromatographic fingerprint analysis—a rational approach for quality assessment of traditional Chinese herbal medicine. Journal of Chromatography A 1112(1–2):171–180. http://dx.doi.org/10.1016/j.chroma.2005.12.091

CURRENT TRENDS IN NATURAL PRODUCTS CHEMICAL ANALYSIS

Phytochemical analyses benefit from the continuous developments in the wider field of analytical chemistry. Consequently, improvements in experimental design, the creation of new types of separation or detection tools and the application of computation and statistical analyses to the results (chemometrics) are constantly applied to the analyses of natural products, thus providing both new insights and more convenient technical approaches. For example, with the advent of higher field NMR machines (>800 Hz), their use as online detectors is starting to become possible. For a glimpse at the latest development in the natural products analyses the reader is encouraged to read the excellent themed collection 'Modern Methods in Plant Natural Products' published in Natural Products Reports (see Further reading).

The next frontier is to make separation an unnecessary analytical step. This may be achieved by direct analyses of the natural substances by purely spectroscopic techniques such as mass spectroscopy (MS), near-infrared spectroscopy (NIR) or nuclear magnetic resonance (NMR). These approaches vastly reduce processing time, thus avoiding the generation of artefacts, but require high sensitivity and computational power. Solubilized extracts may be directly infused into MS detectors to obtain fingerprints (Politi et al 2009). The use of special methods in NMR such as diffusion-ordered spectroscopy allows for the identification of expired Valerian-Hops commercial tinctures without the need of any processing or deuterated solvents (Prieto et al 2016). ^{1}H NMR spectroscopy coupled with multivariate analysis software (NMR-omics) applied to the chemistry of important commodities of the herbal market such as *Curcuma longa* L. can effectively correlate

some variations in product composition for selected producers and identify strengths and weaknesses of some types of value chains (Booker et al 2014). Finally, NIR has become a promising technique for the direct characterization of the final medicinal product regardless of whether it contains synthetic (Said et al 2011) or natural active adulterant ingredients (Peerapattana et al 2013) and is becoming so powerful that tablets can be analysed without even taking them out of their blister packets, thus allowing for continuous online quality control of the whole manufacturing process.

References

Booker, A., Frommenwiler, D., Johnston, D., Umealajekwu, C., Reich, E., Heinrich, M., 2014. Chemical variability along the value chains of turmeric (*Curcuma longa*): a comparison of nuclear magnetic resonance spectroscopy and high performance thin layer chromatography. J. Ethnopharmacol. 152, 292–301.

Ćetković, G.S., Dilas, S.M., Čanadanović-Brunet, J.M., Tumbas, V.T., 2003. Thin-layer chromatography analysis and scavenging activity of marigold (*Calendula officinalis* L.) extracts. APTEFF 34, 1–148.

Jemmali, Z., Chartier, A., Dufresne, C., Elfakir, C., 2016. Optimization of the derivatization protocol of pentacylic triterpenes prior to their gas chromatography-mass spectrometry analysis in plant extracts. Talanta 147, 35–43.

Peerapattana, J., Otsuka, K., Otsuka, M., 2013. Application of NIR spectroscopy for the quality control of mangosteen pericarp powder: quantitative analysis of alpha-mangostin in mangosteen pericarp powder and capsule. J. Nat. Med. 67, 452–459.

Politi, M., Zloh, M., Pintado, M.E., Castro, P.M.L., Heinrich, M., Prieto, J.M., 2009. Direct metabolic fingerprinting of commercial herbal tinctures by nuclear magnetic resonance spectroscopy and mass spectrometry. Phytochem. Anal. 20, 328–334.

Prieto, J.M., Mellinas-Gomez, M., Zloh, M., 2016. Application of diffusion-edited and solvent suppression ^{1}H-NMR to the direct analysis of markers in valerian-hop liquid herbal products. Phytochem. Anal. 27, 100–106.

Said, M.M., Gibbons, S., Moffat, A.C., Zloh, M., 2011. Near-infrared spectroscopy (NIRS) and chemometric analysis of Malaysian and UK paracetamol tablets: a spectral database study. Int. J. Pharm. 415, 102–109.

Xie, P., Chen, S., Liang, Y-z, Wang, X., Tian, R., Upton, R., 2006. Chromatographic fingerprint analysis—a rational approach for quality assessment of traditional Chinese herbal medicine. J. Chromatogr. A 1112, 171–180.

Further reading

Fried, B., Sherma, J., 1999. Thin-Layer Chromatography, Revised and Expanded. Dekker, New York.

Satyanshu, K., 2016. Analytical techniques for natural product research. CABI, Wallingford

Various authors, 2013. Modern methods in plant natural products – a themed issue. Nat. Prod. Rep. 30, 477–524..

Chapter 8

Isolation and structure elucidation of natural products

In this chapter we deal, in detail, with the fractionation of **biomass** (plant or microbe), and the isolation and characterization processes of its constituents. Most of these **preparative** techniques are based on the same chromatographic and spectroscopic **analytical** techniques described in Chapter 7, the only difference being the quantity of sample processed. We will use *Thymus vulgaris*, an example of a natural product endowed with antibiotic principles, to illustrate the initial process in the selection of extraction methods and screening in different formats (high- and low-throughput screening), the isolation of the active components (bioassay-guided isolation) and the structure elucidation of the active principles. In this way we intend to give you an insight into the drug discovery processes leading to a drug lead. The final drug will need further chemical semisynthesis informed by preclinical pharmacological and toxicological assays prior to being subjected to clinical trials. If successful, it may be marketed as a medicine after a typical timeframe of 10–20 years.

NATURAL PRODUCTS IN DRUG DISCOVERY

A survey of any pharmacopoeia will show that natural products have a key role as biologically active agents; in fact, it has been estimated that 20–25% of all medicines are derived from such sources. In this definition, the medicinal agent may be a natural product isolated straight from the producing organism (e.g. the β-lactamase inhibitor **clavulanic acid** isolated from the bacterium *Streptomyces clavuligerus*), a natural product that has undergone a minor chemical modification (semisynthetic) (e.g. **aspirin**, derived from **salicylic acid**, which occurs as esters and glycosides in *Salix* spp.), or a compound that was totally synthesized based on a particular natural product possessing biological activity (e.g. **pethidine**, which was based on **morphine** from the opium poppy, *Papaver somniferum*). It is sometimes difficult to see how the fully synthetic compound was modelled on the natural product (Fig. 8.1).

Natural products are historically the core of medicines and they are still a major source of **drug leads**, which is a term used to describe compounds that may be developed into medicines. A particular example of a natural product that is currently one of the best-selling drugs is **paclitaxel**, marketed as **Taxol** (Fig. 8.2). This drug was developed by BristolMyers Squibb and marketed for the treatment of ovarian and mammary cancers, and became available for use in the USA in 1993. The compound was initially isolated from the bark of the Pacific yew tree, *Taxus brevifolia*, and demonstrates the best possible qualities of a natural product, being highly functional and chiral. Additionally, paclitaxel occurs in the bark with a wide range of structurally related compounds (taxane diterpenes); this is a further important and valuable quality of natural products when they are considered as a source in the search for biologically active drug leads. Paclitaxel has many functional groups and chiral centres (11) and these qualities give rise to its distinct shape and fascinating biological activity. It is important not to be overwhelmed by such a complex molecule, but to look at the functional groups that make up the total structure of the compound. Even a natural product that is as structurally complex as paclitaxel can be broken down into the simple chemical features of functional groups and chiral centres.

Natural drugs have long captured the imagination of the general public who have the impression that

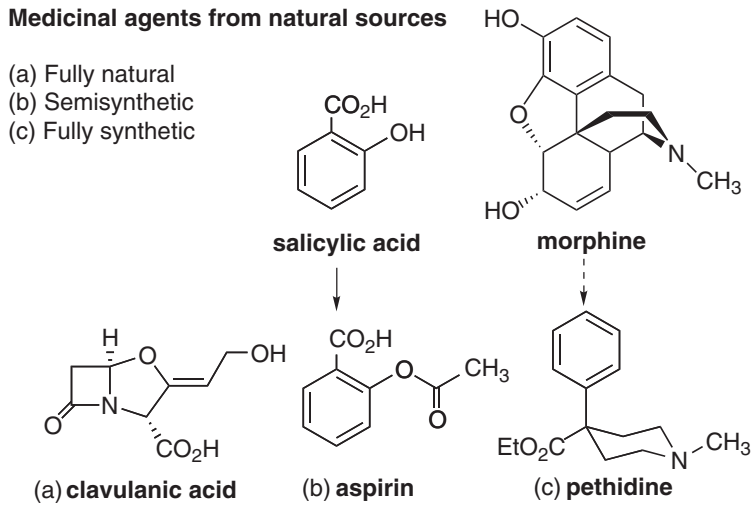


Fig. 8.1

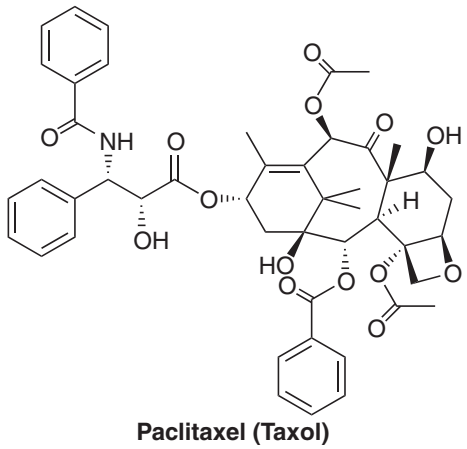


Fig. 8.2

natural products are safe and non-toxic, but, as we will see, some of the most potent poisons are derived from nature. This view of natural medicines is in part based on the romantic notion of **bioprospecting** for new **drug leads** from areas with an exceptionally high level of **biodiversity** and beauty, such as the Amazonian rainforest. Although public interest in natural drugs is high, investment in the discovery of natural drugs from industry sources has been highly cyclical, due to the development of alternative ways of finding new drug leads (e.g. **combinatorial chemistry**). An additional perceived benefit of compounds derived from nature is that they are 'eco-friendly' and that they may be produced as a renewable resource by growing the plants or by fermenting the micro-organisms that produce them. This approach has both advantages and disadvantages over the synthetic production of biologically active agents, but synthetic chemistry cannot yet readily mimic the ability of organisms to produce such structurally complex and diverse natural product molecules.

There are a number of approaches that can be used to discover new drug leads from nature, and all of the following have been used by large and small pharmaceutical companies in an attempt to harness the biological potential of natural products.

In the **ethnobotanical** approach, knowledge of the use of a particular plant by an indigenous people is used to direct a search for a drug lead. In this case, observation of a particular usage of a plant, usually made by a highly trained observer (ethnobotanist), allows the collection of that plant and its subsequent testing for biological activity. Examples of such uses include arrow poisons made from trees by South American Indians as a way of hunting animals for food. One of these agents, curare (from the liana *Chondrodendron tomentosum*) acts as a muscle relaxant, which kills by paralysing the muscles required to breathe. The active component in curare is **tubocurarine**. This ethnobotanical observation of curare used as an arrow poison led to the development of a muscle relaxant used in surgery known as atracurium. The use of ethnobotanical information is greatly underexploited and, as many of the more remote regions of the planet become more readily accessible, without trained personnel to interview and retrieve this information, it is highly likely that much valuable local medicinal knowledge will be lost.

In the **chemotaxonomic** approach, knowledge that a particular group of plants contains a certain class of natural product may be used to predict that taxonomically related plants may contain structurally similar compounds. This approach is highly useful when the chemistry and biological activity of a compound are well described and compounds with similar chemical structure are needed for further biological testing. A good example of this is the plant family Solanaceae, which is a rich source of alkaloids of the tropane type. The knowledge that deadly nightshade (*Atropa belladonna*) produces hyoscyamine (a smooth muscle relaxant) would enable one to predict that the thorn apple (*Datura stramonium*) would contain structurally related compounds, and this is certainly the case, with hyoscine being the major constituent of this solanaceous plant (see Fig. 6.63).

Using the **random** approach, plants are collected regardless of any existing previous knowledge of their chemistry or biological activity. This approach relies on the availability of plants that are abundant in a certain area (and may previously have been extensively studied). Plants that are rare or only exist in a specific habitat (e.g. alpine or parasitic plants) may be neglected and access to chemical diversity lost. This approach is purely serendipitous in that there is a chance that random plant selection will give access to extracts (and therefore compounds) with biological activity (**bioactivity**).

The **information-driven** approach utilizes a combination of ethnobotanical, chemotaxonomic and random approaches together with a database that contains all of the relevant information concerning a particular plant species. The database is used to prioritize which plants should be extracted and screened for bioactivity. This approach is favoured by large organizations (particularly pharmaceutical companies) interested in screening thousands (in some cases hundreds of thousands) of samples for bioactivity as it may reduce costs by a process known as **dereplication** – the process of avoiding the repeated discovery of common or known drugs. This is most important where millions of dollars are spent in the natural product drug lead discovery process.

An example of when a database would be of use in the information-driven approach is in the discovery of antitumour agents. Should bioactivity be demonstrated upon screening extracts from the English yew (*Taxus baccata*), then chemotaxonomic knowledge could be entered into the database indicating that this species is related to *Taxus brevifolia* and consequently may produce related chemical constituents and should be prioritized accordingly. This is certainly the case as *Taxus brevifolia* produces the antitumour drug paclitaxel.

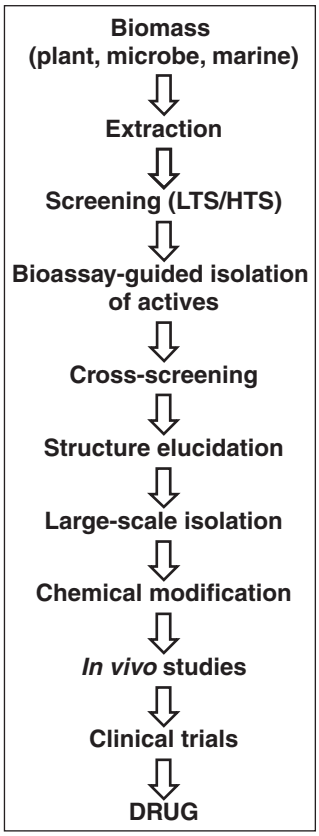


Fig. 8.3

The discovery of drugs from nature is complex and is depicted schematically in Fig. 8.3. The **biomass** (plant, microbe, marine organism) is collected, dried and extracted into a suitable organic solvent to give an **extract**, which is then screened in a **bioassay** to assess its biological activity (bioactivity). Screening or assessment of biological activity is generally divided into two formats depending on the number of extracts to be assessed. In **low-throughput screening** (LTS), small numbers of extracts (a single extract up to hundreds of extracts) are dispensed into a format that is compatible with the bioassay (e.g. a 96-well microtitre plate, sample tubes). This approach is used widely in academic laboratories where only a relatively low number of extracts are assessed. In **high-throughput screening** (HTS), thousands of extracts are dispensed into a format (usually microtitre plates with hundreds of wells, e.g. 384 wells per plate) and screened in the bioassay. This approach is favoured by the pharmaceutical industry, which may have hundreds of thousands

of samples (both natural and synthetic) for biological evaluation. This large-scale approach means that decisions can be made rapidly about the status of an extract, which has an impact on the cost of the discovery process.

Active extracts are **fractionated** using **bioassay-guided isolation**, in which **chromatographic techniques** are used to separate the extract into its individual components; the biological activity is checked at all stages until a pure active compound is obtained. The natural product isolated will be designated as a **lead compound** and will be assessed for biological activity in a bank of other assays. This process is known as **cross-screening** and will give information on how **selective** the compound is – i.e. is it active in all the assays or does it exhibit specificity for one particular assay? This is an important consideration as one of the criteria for the selection of a compound for further development is **specificity**. Whilst biological evaluation is on-going, **structure elucidation** will be necessary to determine the three-dimensional structure of the active molecule. This will enable a search to be done to establish whether the compound is novel, what chemical class it belongs to and whether that type of compound has previously been reported to possess biological activity in the bioassay of interest or other bioassays.

Once novelty and potent biological activity have been established, large amounts of the lead compound are isolated and the decision is made as to whether the compound can be synthesized *de novo* or whether **chemical modification** needs to be made to enhance the biological activity. The lead compound will undergo extensive ***in vivo*** **studies** to establish activity, toxicity and efficacy; these studies are sometimes known as **preclinical studies**. Only once all of these steps have been completed will a drug lead finally enter **clinical trials**, which is the most extensive evaluation stage of a drug candidate during which many drug leads fail through toxicity or lack of efficacy in humans. Successful completion of these trials usually results in a product licence, which means that the compound is now a **drug**.

Given the complexity of the process described above, it is not surprising that many natural product drug leads fail to make their way onto the market. Some estimates state that only 1 in 10,000 drug leads may actually make their way to the market. The process is also very lengthy and it may take 12–15 years from the collection of the original biomass to the granting of a licence for a new natural product drug. Additionally, the process is very costly but the rewards are enormous. For example, although high costs could occur for the development of Taxol (US$300 million), these can be readily recovered, with initial sales in excess of US$1 billion per annum. In 2009 the best-selling brand name drug was Lipitor (Atorvastatin), which made $13.3 billion; this highlights the potential value of the drug discovery process.

The expense, complexity and time of the natural drug lead process have militated against natural products in the past, but the fact remains that natural products are a tried and tested source and there are many examples of natural drugs. The most important strengths of natural products are their complex chemistry and structural diversity.

BIOASSAY-GUIDED ISOLATION

Bioassay-guided isolation is the physical process used to isolate biologically active chemicals from a natural source. Many of the chemicals described in Chapter 6 are from plant sources, but microbes are also an exceptionally valuable source of chemical diversity, in particular the filamentous bacteria (the Actinomycetes) of which the antibiotic-producing genus *Streptomyces* is the most widely studied for bioactive compounds. The fungi are also important and microbiologists spend time working in **biota**-rich environments such as the Amazon basin collecting, typing (identifying) and culturing samples for shipment back to the laboratory to be screened for bioactivity. As with plants, this process can be highly complicated, particularly in the identification of fungi, of which there may be potentially millions of new species waiting to be described in remote locations. This exercise is extremely worthwhile, as it is highly likely that new species will contain new chemistry that may have interesting bioactivity when fully screened. This will be particularly relevant for the **Basidiomycetes**, a large group of fruiting fungi that produce a mushroom cap (basidium) and are sometimes difficult to grow in solution fermentation.

ISOLATION AND INITIAL FRACTIONATION METHODS

Once an extract has been generated by a suitable extraction protocol (see Chapter 7) and activity is demonstrated in a bioassay (e.g. an antibacterial test), the next step is to fractionate the extract using a separation method so that a purified biologically active component can be isolated.

Possibly the simplest separation method is partitioning, which is widely used as an initial extract purification and 'clean up' step. Partitioning uses two immiscible solvents to which the extract is added; this can be sequential by using immiscible organic solvents of increasing polarity. Typically, this may take place in two steps: (1) water/light petroleum ether (hexane) to generate a non-polar fraction in the organic layer; (2) water/dichloromethane or water/chloroform or water/ethyl acetate to give a medium-polar fraction in the organic layer. The remaining aqueous layer will contain polar water-soluble natural products. This is a soft separation method and relies on the solubility of natural products and not a physical interaction with another medium (e.g. adsorption on silica gel in thin-layer chromatography [TLC]; see Chapter 7). Partitioning may give rise to excellent separations, particularly with compounds that differ greatly in solubility; for example, monoterpenes are easily separated from phenolics such as tannins.

GEL CHROMATOGRAPHY

Assuming that the extract is still active, the next step is chromatography. A procedure that is widely used as an initial clean-up is **gel chromatography**, also known as size **exclusion chromatography**. This technique employs a cross-linked dextran (sugar polymer) that, when added to a suitable solvent (e.g. chloroform or ethyl acetate), swells to form a gel matrix. The gel contains pores of a finite size that allow small molecules (< 500 Da) to be retained in the matrix; larger molecules (> 500 Da) are excluded and move quickly through the gel. This gel is loaded into a column and the extract is added to the top of the column. Large molecules are the first to elute, followed by molecules of a smaller size. This is an excellent method for separating out chlorophylls, fatty acids, glycerides and other large molecules that may interfere with the biological assay. Different sorts of gels are available, which may be used in organic solvents (e.g. LH-20) or aqueous preparations such as salts and buffers (e.g. G-25). Therefore, both non-polar and polar natural products can be fractionated using this technique. Additionally, not only are compounds fractionated according to size, but also a small amount of adsorption chromatography occurs, as the dextran from which the gel is made contains hydroxyl groups that interact with natural products, facilitating some separation according to polarity.

This is a non-destructive 'soft' method with a high recovery (compounds are rarely strongly adsorbed) and a high quantity of extract (hundreds of milligrams to grams) may be separated. A further benefit of this technique is that many different gels are available with a variety of pore sizes that can be used to separate compounds from 500 to 250,000 Da. This is the method of choice for large molecules, in particular proteins, polypeptides, carbohydrates, tannins and glycosides, especially saponin and triterpene glycosides.

ION-EXCHANGE CHROMATOGRAPHY

The separation of small polar compounds, in particular ionic natural products, is often problematic. It is possible to separate these metabolites from larger molecules (using gels), but they are generally very strongly adsorbed with normal-phase sorbents such as silica or alumina, and, even with the use of polar solvents and modifiers (e.g. acid and base), efficient separations may not be achievable. Additionally, these compounds are not retained on reverse-phase sorbents such as C_{18} or C_8. These natural products possess functional groups, such as CO_2H, -OH, $-NH_2$, that contribute to the polarity of the molecule, and this may be used to develop a separation method using **ion-exchange chromatography**.

This technique is limited to natural products that can carry charge on their functional groups. The **sorbent** or **stationary phase** has charged groups and mobile counter ions, which may exchange with ions of the functional groups present in the natural product as the mobile phase moves through the sorbent. Separation is achieved by differences in affinity between ionic components (polar natural products) and the stationary phase. These ion-exchange sorbents or resins are divided into two groups: **cation** exchangers, which have acidic groups (CO_2H, $-SO_3H$) and are able to exchange their protons with cations of natural products, and **anion** exchangers, which have basic groups ($-N^+R_3$) that are incorporated into the resin and can exchange their anions with anions from the natural product. These ion-exchange resins may be used in open-column chromatography or in closed columns in applications such as high-performance liquid chromatography (HPLC).

2,5-Dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP) from *Lonchocarpus sericeus* (Fabaceae) is a nematocidal polyhydroxylated alkaloid (PHA), and also inhibits insect α- and β-glucosidases. Compounds of this type are bases and form cations in acidic solutions. When added to a cation exchanger [e.g. Amberlite CG-120, which has a sulphonic acid bound to the resin that can exchange its proton (cation)], the DMDP

cations are retained (bound) by the cation exchanger and protons are displaced. If the cation exchanger is then eluted with a solution containing a stronger cation such as NH_4^+ (e.g. from 0.2 M NH_4OH), then the DMDP cation is desorbed from the exchanger and is unbound and mobile. This affinity can be used to separate such alkaloids from acidic (anionic) or neutral components that would not be retained by the cation exchanger and may be washed from the resin by water.

Plant extracts that contain DMDP are used as a nematocide against infected crops (bananas) in Costa Rica and are licensed by the National Institute of Biodiversity. This is an example of a renewable resource as the extracts may be prepared from the seeds of the plant and DMDP is ecologically friendly as it is biodegradable.

FLASH CHROMATOGRAPHY

Flash chromatography may be used for quick efficient separations. This employs pre-packed solvent-resistant plastic cartridges (Fig. 8.4), which contain the sorbent (silica, alumina, C_{18}, HP-20, or ion exchange resin). These cartridges are introduced into a **radial compression module** (the metal cylinder in Fig. 8.4), which pressurizes the cartridge and sorbent radially. This results in a very homogeneous packed material (sorbent), reduces the possibility of solvent channeling when the system is run and minimizes void spaces on the column head.

Using this technique, milligrams to tens of grams can be separated. The bioactive extract can be dissolved in solvent and loaded onto the column directly; solvent is then pumped through the column and fractions are collected, resulting in a rapid separation of extract components. This is a rapid method; 10 g of extract can be fractionated into 12 fractions of increasing polarity in 30 min using a step gradient solvent system. There are a number of benefits to this, particularly that speed minimizes contact with reactive sorbents (e.g. silica) and that hazardous sorbents such as silica, which when free may cause silicosis, are contained in the cartridges. Additionally, the cartridges may be re-used, reducing the cost of the bioassay-guided process. The high flow-rates employed by this technique (20–250 ml/min) retain 'band-like' movement of the components through the column, resulting in a high resolution. Compounds eluting from the column may be detected by TLC (of fractions) or the eluant may be passed through a UV detector so that compounds that absorb UV light can be detected as they elute from the column. Some laboratories run several of these flash columns simultaneously, resulting in a high number of fractionated extracts having sufficient mass for further purification of the active components.

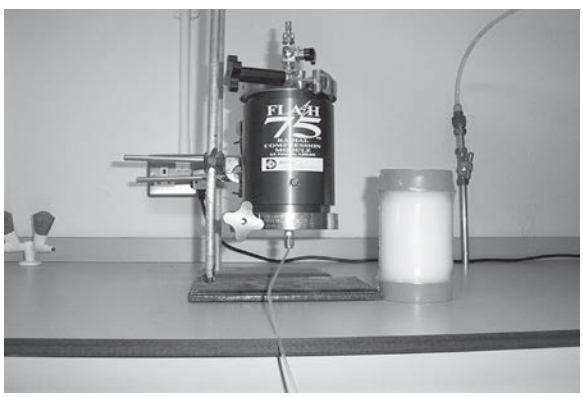


Fig. 8.4 Biotage™ flash chromatograph. Fast separations are achieved with good resolution.

PREPARATIVE THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography (TLC) is one of the most widely used and easiest methods for purifying a small number (2–4) of components, typically following a flash separation. This method employs glass or aluminium plates that are pre-coated with sorbent (e.g. silica gel) of varying thickness dependent on the amount of material to be loaded onto the plates. The coating of preparative plates may be 1–2 mm thick, that is up to 10 times thicker than analytical plates. The compound mixture is loaded at 1–2 cm from the bottom edge of the plate as either a spot or a continuous band. The plate is then lowered into a tank containing a predetermined solvent that will migrate up the plate and separate the compound mixture according to the polarity of the components.

Preparative scale TLC loadings of 1–100 mg can readily produce enough purified material for biological assays and structure elucidation. It is rapid and cheap and has been the method of choice for separating lipophilic compounds. Preparative plates are available from suppliers as pre-coated plates of 1–2 mm thickness in silica, alumina or C_{18}. However, home-made plates offer greater flexibility by allowing the incorporation of modifying agents into the sorbents (e.g. silver nitrate for separation of olefinic compounds – known as argentation TLC), use of other sorbents (ion exchange, polyamide, cellulose) and the addition of indicators and binders.

The scale-up from analytical to preparative mode is crucial, as an increase in the sample load may drastically change the separation of the components. Normally, the method developed on the analytical scale must be modified, generally with a reduction of solvent system polarity. Preparative TLC is mostly used as a simple, final clean-up procedure to separate 2–4 compounds. The sample is dissolved in a small volume of solvent and applied as a thin line 2 cm from the bottom of the plate and dried. The plate is then eluted in a suitable solvent and UV-active compounds are visualized at 254 or 366 nm. Natural products that are not UV-active will need development using a suitable spray reagent such as vanillin-sulphuric acid, Dragendorff's reagent, phosphomolybdic acid or antimony trichloride. In this case, an edge of the plate is sprayed with the reagent (taking care that the rest of the plate is covered) and separated compounds are visualized as coloured bands. The bands containing pure natural product are scraped off the plate and the natural product is desorbed from the sorbent. This desorption may be carried out by placing the compound-rich sorbent into a sintered glass funnel and washing with a suitable solvent followed by collection and concentration of the filtrate. The purified 'band' should then be assessed for purity by analytical TLC. Disadvantages of preparative TLC are poor loadings and speeds that compare unfavorably to flash chromatography.

PREPARATIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

The scaling up of HPLC technology allows for the injection and separation of milligram to gram quantities of samples into their components. As with TLC, an analytical HPLC method is developed for scale-up to preparative HPLC; this mainly implies an increase of flow-rates up to 50–300 ml/min. A large sample loading of tens of milligrams to grams of material can be achieved and rapid isolation can be facilitated by the use of intelligent fraction collectors that can 'peak collect' compounds as they elute from the column by receiving input from the UV-VIS, ELS or MS detector. The major disadvantage of this technique, however, is its expense, as analytical instrumentation may cost upwards of £20,000, and preparative HPLC may be £30,000 onwards depending on their capacity and the type of detector. Consumables for this technique are also expensive, especially preparative columns (£2,000), which may have a short life and at high solvent flow-rates there is a high cost for purchase and disposal of high-purity solvents.

ISOLATION STRATEGY

How do the isolation methods described above fit together? Fig. 8.5 gives a general isolation protocol starting with selection of biomass (e.g. plant or microbe), which is then extracted using a Soxhlet apparatus, cold or hot percolation or supercritical fluid extraction. Hydrophilic extracts will then typically undergo ion-exchange chromatography with bioassay of generated fractions. A further ion-exchange method of bioactive fractions would yield pure compounds, which could then be submitted for structure elucidation. Lipophilic extracts could initially be partitioned to generate a further hydrophilic fraction which could be dealt with by ion-exchange chromatography as described above.

The lipophilic portion may then be either subjected to gel chromatography to remove or separate large

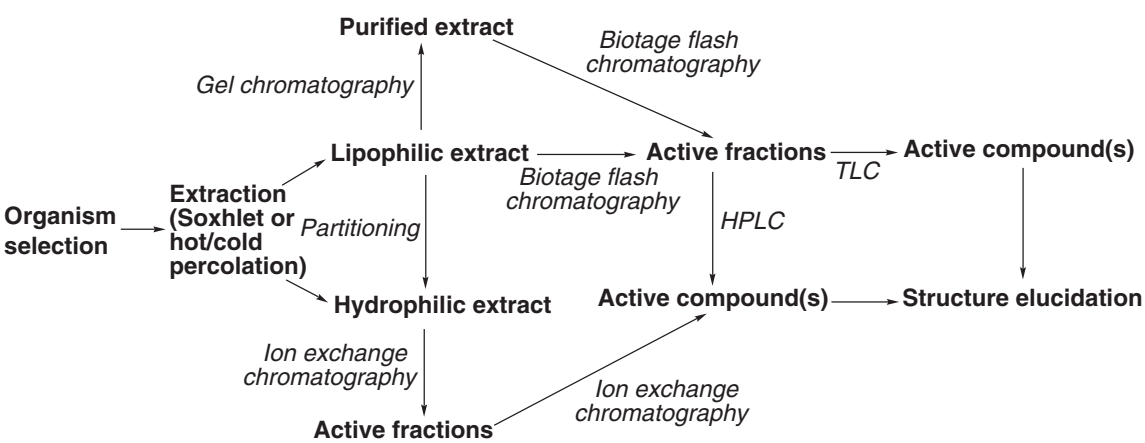


Fig. 8.5 General isolation strategy for purification of bioactive natural products.

components (e.g. chlorophylls, fatty acids or glycerides), or this step skipped, and then flash chromatography used to generate a series of fractions that would undergo bioassay. Active fractions can then be further purified by using either HPLC or TLC to give pure compounds, for submission to structure elucidation. This is only a general outline for isolation of bioactive natural products; there is no tailored protocol and the physicochemical properties of natural products differ enormously, sometimes making the isolation of chemicals from nature a highly difficult and intellectually challenging problem. In very rare cases, organisms produce very simple extracts (2–4 components), which may be readily separated into pure compounds, but in the majority of cases extracts are highly complex and the active component may be present at a very low concentration or even be unstable, further contributing to the difficulty of the bioassay-guided isolation process. It is also possible that activity may even diminish during the separation process; this could be due to a synergistic effect occurring through several components working in concert in the bioassay. There is currently much interest in this area, and it is possible that the value and efficacy of certain herbal medicines (which contain tens to hundreds, or even thousands, of natural products) are due to several active components; thus the bioassay-guided isolation approach may not be appropriate for the study of these agents. The facts remain, however, that many useful pharmaceutical entities were developed using this approach and, because of the sheer number of organisms still to be investigated, it is certain that bioassay-guided isolation will yield many new medicines in the future.

STRUCTURE ELUCIDATION

Ideally, bioassay-guided methods should afford a pure natural product of at least 5 mg in weight. Current structure elucidation techniques are available that can determine the structures of compounds on micrograms of material, although larger quantities are of benefit for further biological assays and will save time and money in the pharmaceutical industry setting, so that valuable resources do not need to be used in the acquisition of more biomass and a potentially lengthy bioassay-guided isolation process.

Structure elucidation of natural products generally employs the classical spectroscopic techniques of **mass spectrometry** (MS) and **nuclear magnetic resonance** (NMR) **spectroscopy**. The first steps, however, should be the recording of infrared (IR) and ultraviolet-visible (UV/Vis) spectra to determine the presence of certain functional groups and conjugation in the molecule. Rather than a theoretical approach to this subject, we will use a bioactive natural product to highlight the strengths of these techniques.

In a project to isolate and characterize antibiotics from plants, *Thymus vulgaris* (thyme), a member of the mint family (Lamiaceae), was extracted with hexane and ethyl acetate. Each of these extracts was highly active in a bioassay to discover compounds with activity against meticillin-resistant *Staphylococcus aureus*. Extracts were analysed by TLC and bulked due to similarity. Flash chromatography, followed by preparative HPLC, led to the isolation of the pure active natural product, compound X, which was a pale yellow volatile oil with a pungent aroma. The UV spectrum showed a maximum at 277 nm, indicative of the presence of an aromatic ring. The IR spectrum showed absorptions attributable to aromatic and aliphatic C-H groups and a broad peak at 3600 nm indicative of an hydroxyl functional group.

Compound X was submitted to FAB-MS; the spectrum is shown in Fig. 8.6. The scale on the *x*-axis is the mass (*m*) to charge (*z*) ratio (*m/z*). As compound X readily forms single ions, *m/z* is in effect *m/1* and therefore directly related to the weight of fragments and, in the case of the molecular ion, the molecular weight of the compound.

A molecular ion (M^+) is seen at *m/z* 150. This is supported by additional peaks where the molecule picks up a hydrogen ion at *m/z* 151 $[M+H]^+$ and loses a hydrogen ion at *m/z* 149 $[M–H]^+$. The spectrum was run using FAB ionization and little fragmentation is evident. There are some useful fragments, however, in particular at *m/z* 135, which is 15 mass units less than the molecular ion and almost certainly corresponds to $[M–Me]^+$, indicating that this molecule contains a methyl group (15 mass units), which is readily lost in the mass spectrometer.

Accurate mass measurement of the molecular ion at *m/z* 150 gave a figure of 150.104700. If a computer program is used to calculate the number of carbon, hydrogen and oxygen atoms that would be required to give this weight, a formula of $C_{10}H_{14}O$ is produced. The theoretical mass of this formula is 150.104465, which is very close to the measured accurate mass. The theoretical mass takes into account the accurate masses of carbon, hydrogen and oxygen, and the nearest 'fit' to the measured mass gives the $C_{10}H_{14}O$ formula. Interestingly, compound X has 10 carbon atoms and, as it is a volatile oil, it is likely to be a member of the monoterpene group of natural products.

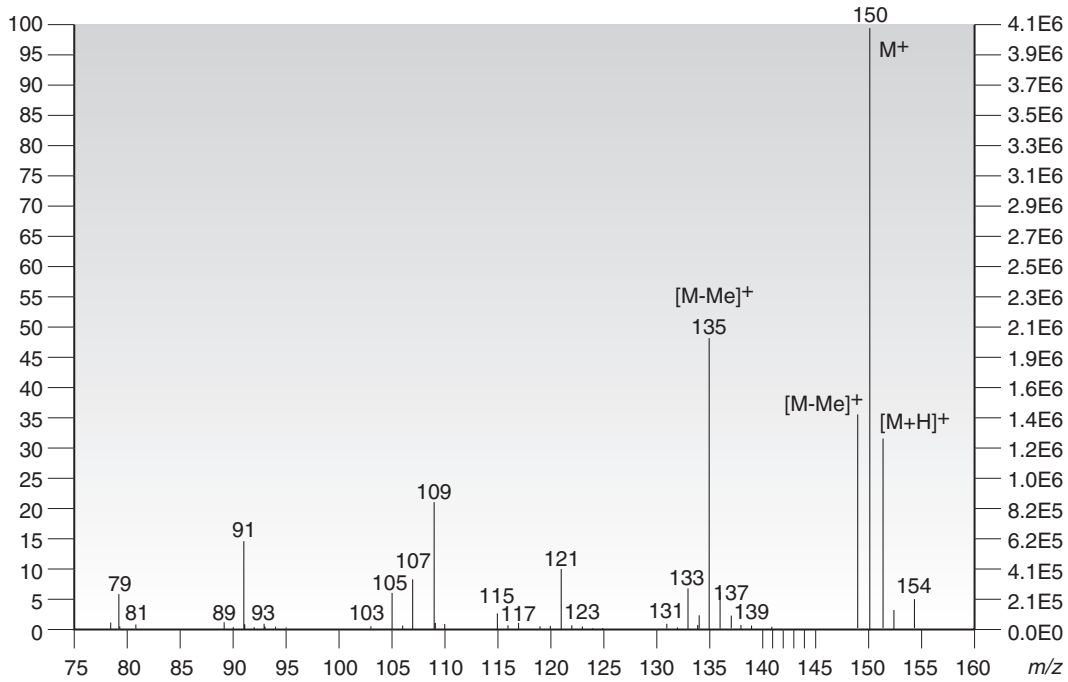


Fig. 8.6 Fast atom bombardment mass spectrum (FAB-MS) of compound X.

At this stage it would be possible to perform a database search on this molecular formula and the producing organism (*Thymus vulgaris*) from sources such as SciFinder™ (**Chemical Abstracts)** or the **Dictionary of Natural Products**, although there are many natural products with this formula and therefore further structure elucidation is required.

NMR SPECTROSCOPY

^{1}H NMR spectroscopy

The next step in this process is the recording of an ^{1}H NMR spectrum (Fig. 8.7). This will indicate the number of hydrogen atoms associated with a particular group (**integration**) and how **shielded** or **deshielded** that group is. Shielding and deshielding occur due to the presence of groups that are either electron withdrawing (deshielding) or electron donating (shielding).

Inspection of the ^{1}H NMR spectrum of compound X recorded in the solvent deuteron-chloroform $CDCl_3$, which has a peak at 7.27 ppm (**Z** in the spectrum), shows three deshielded peaks (**A, B** and **C**), each integrating for one proton (the figures under the *x*-axis are the integration and indicate how many protons are associated with each peak). These protons occur in the aromatic region (6.00–8.00 ppm) and have a particular coupling pattern. Additional signals include a broad peak (**D**), indicating that it is exchangeable and possibly an hydroxyl group, a multiplet at 2.84 ppm (**E**) integrating for one proton and a singlet at 2.23 ppm (peak **F**) integrating for three protons, which is due to a methyl group. The last peak in the spectrum (**G**) is a doublet (two lines) integrating for six protons. This is due to two methyl groups occurring in the same position of the spectrum as they are equivalent. This equivalence occurs because they are in the same 'environment'. This signal appears as a doublet because these methyls are coupled to one proton (multiplicity = $n + 1$, where n is the number of nearest neighbouring protons), and the most likely candidate for this single proton is the multiplet at 2.84 ppm. This proton is a complex multiplet because it couples to all six of the protons of the two coincident methyl groups. This coupling system indicates that these two groups form an isopropyl group [$(CH_3)_2$CH-]. The one-proton multiplet at 2.84 ppm (peak **E**) and methyl group at 2.23 ppm (**F**) are slightly deshielded (higher ppm) with respect to the methyl groups at 1.23 ppm (**G**), indicating that they are attached to a group that causes electron withdrawal (possibly an aromatic ring, which is inferred by the presence of the aromatic protons).

Expansion of the aromatic region 6.6–7.1 ppm (Fig. 8.8) shows the coupling pattern of the aromatic ring.

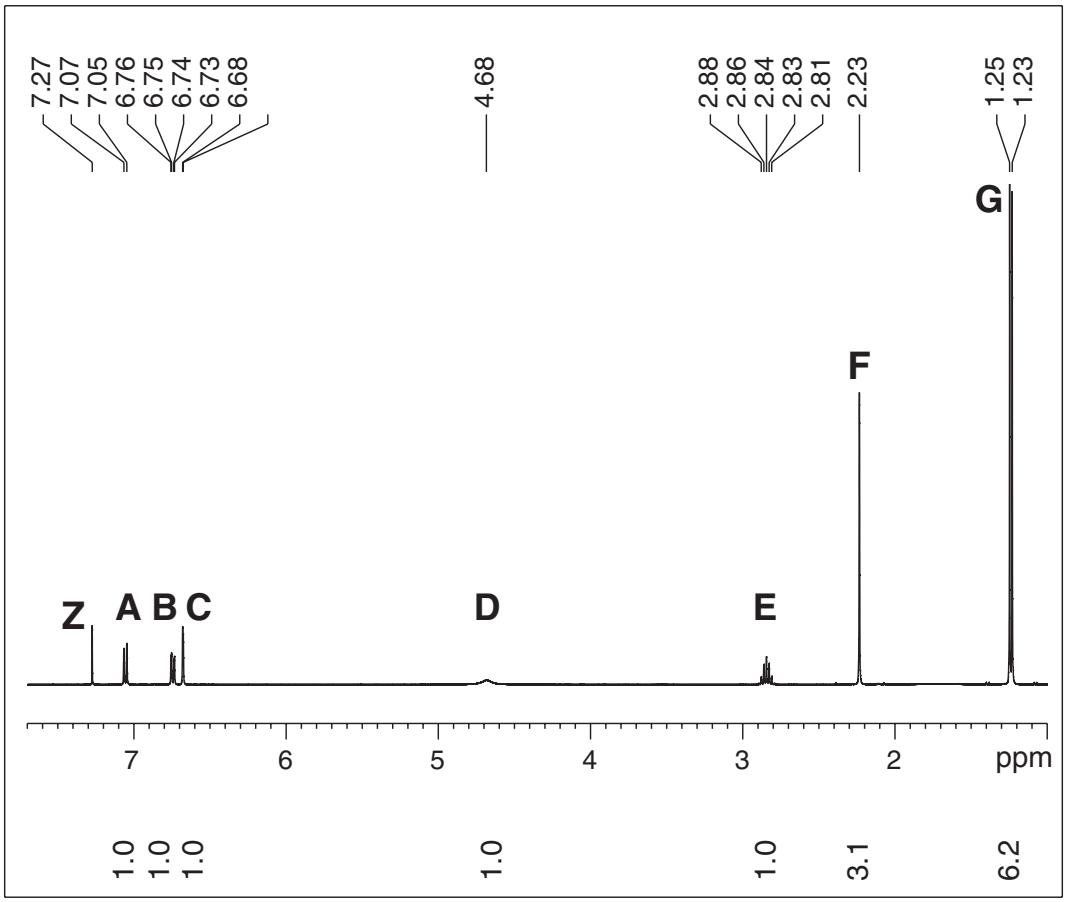


Fig. 8.7 ^{1}H NMR spectrum of compound X recorded in $CDCl_3$.

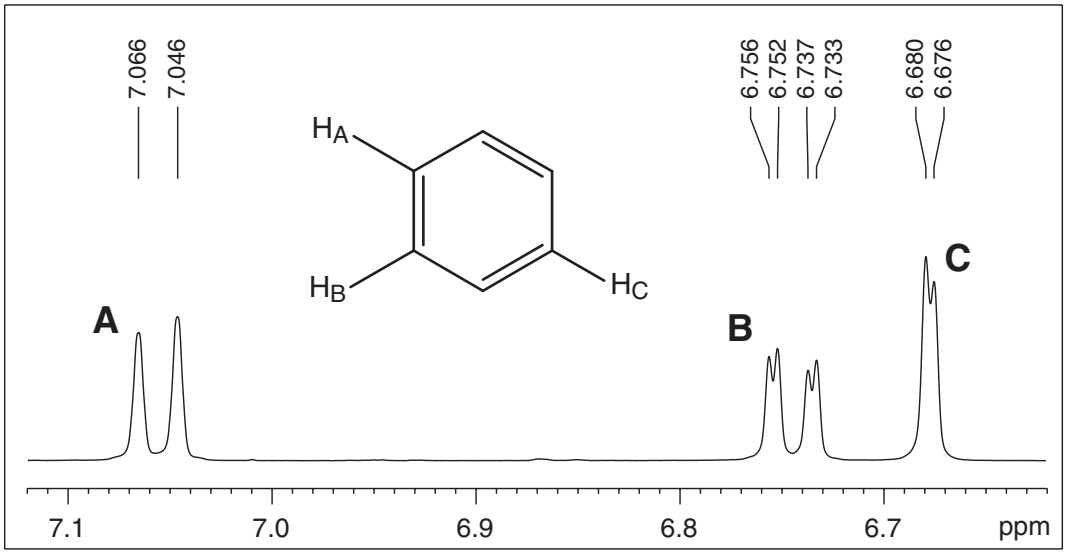


Fig. 8.8 An expansion of the aromatic region of the ^{1}H NMR spectrum of compound X.

Inspection of this area allows measurement of coupling constants, referred to as *J* values. Taking peak **A**, which is a doublet (two lines), as an example, this is done by subtracting the lower ppm value for this peak from the higher ppm value and multiplying the difference by the field strength at which this experiment was measured (400 MHz in this case). This gives: (7.066 – 7.046 ppm) × 400 = 8 Hz

The size of this coupling constant indicates that peak A is coupled to another proton that is *ortho* to itself (*ortho* coupling constants are of the order of 6–9 Hz). Peak **B** is a double doublet (and has four lines) with two couplings (8 and 1.6 Hz), indicating that this is the proton that is *ortho* to peak **A** (it has the same coupling constant of 8 Hz) and the smaller coupling constant (1.6 Hz) is indicative of a *meta* coupling to another proton (meta coupling constants are typically 1–2 Hz). Peak **C** at 6.67 ppm is a doublet (1.6 Hz) that has the same coupling constant as one of those for peak **B**, indicating that this is the proton that is *meta* to **B**. This part of the spectrum therefore tells us that we have an aromatic ring with three protons attached to it in the 1, 2 and 4 positions (Fig. 8.7).

Taking all of the fragments from the ^{1}H spectrum into consideration, there are three aromatic protons, a broad exchangeable peak, a multiplet, a methyl singlet and a six-proton doublet corresponding to two coincident methyl groups. This total of 14 protons is identical to the number found in the molecular formula using MS.

^{13}C NMR spectroscopy

The proton spectrum has revealed much about the number of protons present and their chemical environments – i.e. whether they are shielded or deshielded by electron-donating or electron-withdrawing groups, respectively. The next step is the acquisition of a ^{13}C NMR spectrum, which will give further information regarding the environment of the different groups and the number of carbons present. Two ^{13}C NMR spectra of compound X are shown in Fig. 8.9.

The top spectrum is the **broadband decoupled** spectrum that shows all of the carbons present; the carbons appear as singlets due to proton decoupling. The top spectrum of compound X was recorded in $CDCl_3$,

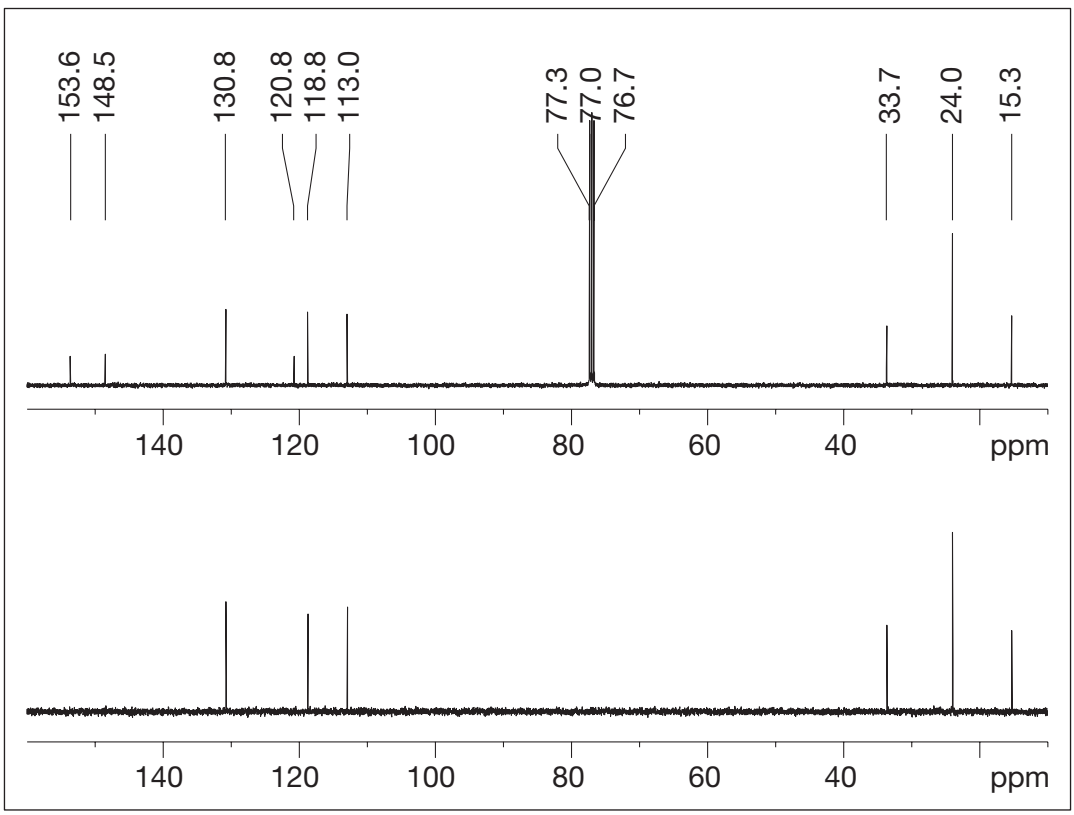


Fig. 8.9 Broadband decoupled ^{13}C spectrum (top) and DEPT-135 ^{13}C spectrum (bottom) of compound X.

which occurs as three lines at 77.0 ppm. There are only nine carbons evident, which might be confusing as we know from the mass spectrum that compound X should contain 10 carbons. However, as the ^{1}H spectrum has two coincident methyl groups, it is possible that there are two carbons associated with the peak at 24 ppm. If both methyl groups were in the same environment (as they are in an isopropyl group), then they would occur at the same position in the spectrum. As with the proton spectrum, the carbon signals occur over a large range, which is again determined by whether the carbons are deshielded (high ppm value) or shielded (low ppm value). The lower ^{13}C spectrum has been produced by a special experiment called **DEPT-135**, which lacks the solvents signals, but, more importantly, it only shows carbons that have protons attached to them (CH, CH_2 and CH_3). As compound X does not have any CH_2 groups (there are no groups in the ^{1}H spectrum that integrate for two protons), only CH and CH_3 carbons are shown. This is useful as it allows quaternary carbons (carbons with no protons attached) to be identified, and it can be seen that there are three additional aromatic quaternaries in the top spectrum, at 153.6, 148.5 and 120.8 ppm. The range for aromatic carbons is 110–160 ppm. The carbon at 153.6 is highly deshielded and it is possible that this carbon is attached to an oxygen atom (from the OH group in compound X). The three peaks at 130.8, 118.8 and 113.0 are all carbons bearing one proton (CH or methine carbons); these correspond to proton peaks **A, B** and **C** in the ^{1}H spectrum (Fig. 8.7). The remaining peaks at 33.7, 24.0 and 15.3 are carbons associated with the multiplet (peak **E**), two coincident methyl groups (peak **G**) and a methyl singlet (peak **F**).

Homonuclear correlation spectroscopy

The next technique that can aid in the structure determination of compound X is **COrrelation SpectroscopY** (COSY), which reveals couplings between protons that are close (two, three or four bonds distant from each other). It is referred to as a **homonuclear** (same nuclei, both of which are ^{1}H) two-dimensional technique because the data are displayed in a matrix format with two one-dimensional experiments (^{1}H spectra) displayed on the *x*- and *y*-axes (Fig. 8.10). A diagonal series of peaks correspond to the ^{1}H spectrum signals. Peaks that are away from the diagonal (referred to as **cross-peaks**) indicate coupling between signals.

For example, inspection of Fig. 8.10 shows a cross-peak between the signal at 1.23 ppm (coincident methyl groups, **G**) and the multiplet signal at 2.84 ppm (group **E**), confirming that they are coupled to each other (implied by the couplings in the ^{1}H spectrum) and that together **E** and **G** are an isopropyl group. Additionally, group **F** (a methyl singlet at 2.23 ppm) shows a coupling to proton **A**, indicating that the methyl group is *ortho* to this proton (Fig. 8.10).

Inspection of an expansion of the aromatic region (Fig. 8.11) provides further support for the coupling pattern already suggested by the ^{1}H spectrum. H_A has an *ortho* coupling to H_B and, in addition to coupling to H_A, H_B has a *meta* coupling to H_C and appears as a double doublet (four lines). This coupling pattern is indicative of a 1,2,4-protonated aromatic ring and confirms the data from the ^{1}H spectrum.

The related technique of **Nuclear Overhauser Effect SpectroscopY** (NOESY) is also useful as it shows through space correlations and through bond coupling between protons. Once the through bond correlations are determined by a COSY spectrum, the through space correlations can be seen. This allows the measurement of how close one proton is to another, which can be very useful in assigning the stereochemistry of a natural product.

Heteronuclear correlation spectroscopy

COSY spectra are referred to as homonuclear spectra as they are acquired by detecting only one type of nucleus (^{1}H), but it is also possible to detect the interactions between two different nuclei such as ^{1}H and ^{13}C. This is known as **heteronuclear correlation spectroscopy**, of which two types will be discussed here: **Heteronuclear Single Quantum Coherence** (HSQC) and **Heteronuclear MultiBond Coherence** (HMBC).

HSQC shows which protons are attached to which carbons. Fig. 8.12 shows an HSQC spectrum for compound X from which clear correlations can be seen for protons **A–C** and **E–G** with the carbons to which they are attached. Proton **D** is a proton attached to oxygen (an hydroxyl group), so there is no carbon to correlate to (and therefore no signal). The aromatic protons all correlate to carbons at higher ppm; the aliphatic protons correlate to lower ppm carbons.

HMBC shows correlations between protons and the carbon atoms that are two and three bonds distant; these couplings are referred to as ^{2}J and ^{3}J, respectively. The experiment is set to show correlations that occur where the coupling constant between protons and carbons is of the order of 7 Hz. Two bond correlations are not always present in the spectrum as the coupling constant for ^{2}J correlations may be less or

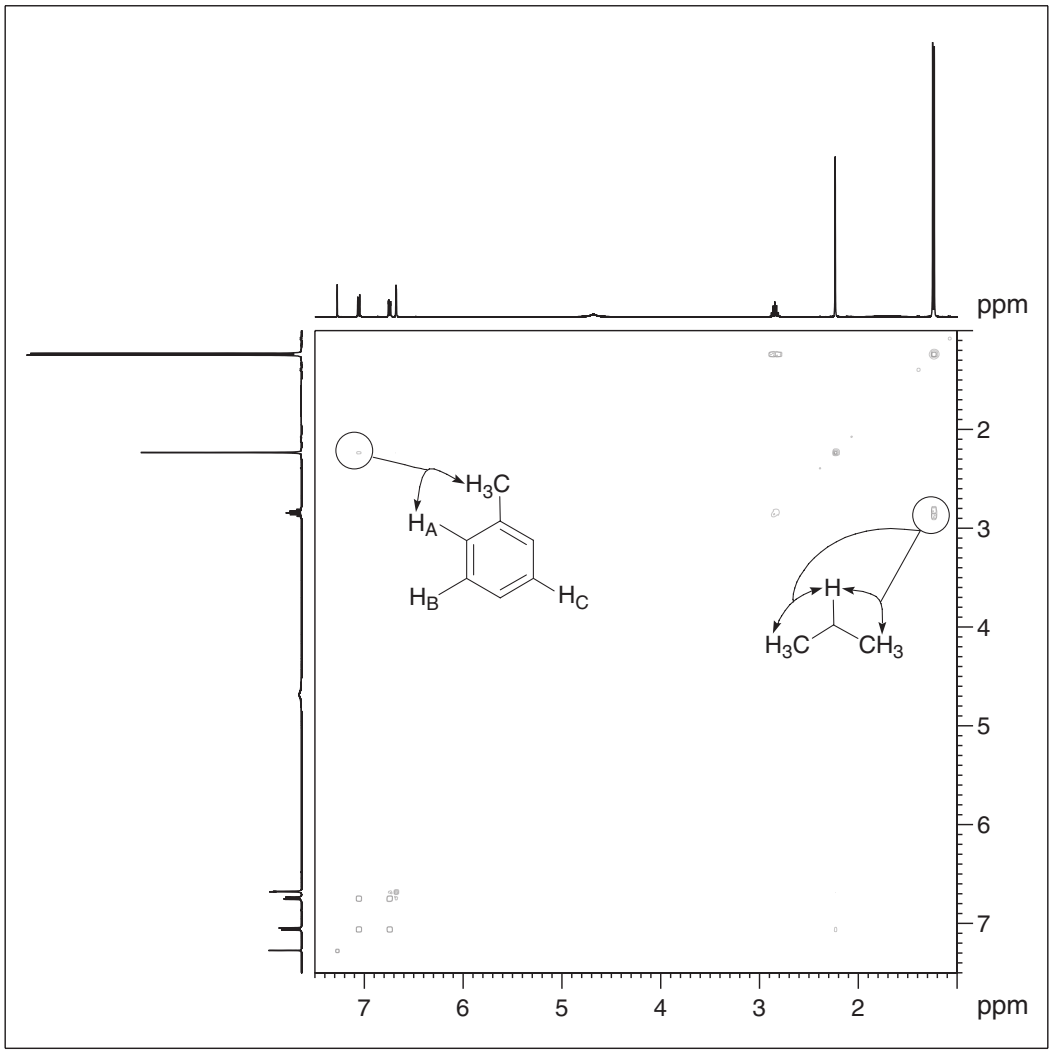


Fig. 8.10 ^{1}H-^{1}H correlation spectroscopy (COSY) spectrum of compound X.

greater than 7 Hz. Fig. 8.13 shows the HMBC spectrum for compound X.

HMBC spectra are highly informative and allow partial structure fragments to be constructed that can enable the full structure elucidation of natural products. The correlations for each proton group of compound X are given in Fig. 8.14.

It is already known from the HSQC spectrum which protons are attached to which carbons and the HMBC spectrum allows the final structure of compound X to be pieced together. For peak **A** (1 H aromatic doublet proton at 7.05 ppm) there are three correlations, one to the carbon associated with peak **F** and two to quaternary carbons.

Peak **B** (1 H aromatic double doublet proton at 6.74 ppm) correlates to the carbon to which peak **E** is attached; this fixes the isopropyl group next to peak **B** on the aromatic ring (peak **E** is part of the isopropyl system with peak **G**). Further correlations for peak **B** include couplings to carbons that are directly attached to peak **C** and to a quaternary carbon.

Peak **C** (1 H aromatic doublet proton at 6.68 ppm) also couples to the carbon bearing the proton associated with peak **E**; this confirms the position of the isopropyl side-chain between protons **B** and **C**. Proton **C** also couples to the same quaternary carbon as **B** and to the carbon attached to proton **B**. There is also a small coupling to the most downfield carbon at 153.6 ppm.

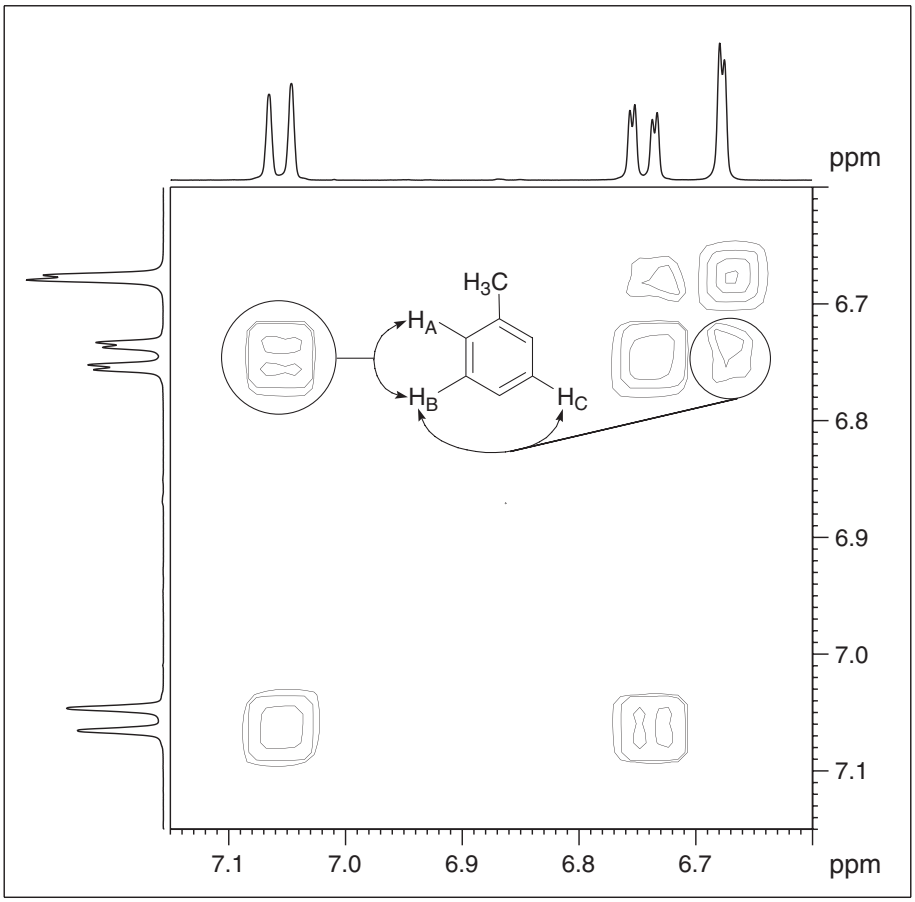


Fig. 8.11 An expansion of the aromatic region of the COSY spectrum of compound X.

Peak **D** (hydroxyl group, 4.68 ppm) is broad, and long-range correlations to carbons are absent. Peak **E** (1 H multiplet proton at 2.84 ppm) shows correlations to the carbons attached to peak **G** (these are the coincident methyl groups), to both carbons attached to peaks **B** and **C** and to a quaternary carbon, which is the carbon to which the isopropyl group is directly attached.

For peak **F** (3 H singlet protons at 2.23 ppm) there are two correlations that appear equidistant at 15.3 ppm in the carbon domain and are an artifact of the HMBC spectrum (they are in fact the unsuppressed direct correlation between the protons of peak **F** and the carbon to which they are directly attached; compare with the HSQC spectrum in Fig. 8.12). There are three couplings for peak **F**: to the carbon attached to proton A, and to two quaternary carbons, one of which is the most downfield carbon (153.6 ppm).

Finally, peak **G** (6 H doublet at 1.23 ppm) shows a correlation to the neighbouring methyl carbon of the isopropyl group (and an unsuppressed one-bond signal equidistant about the peak **G** signal), a correlation to the carbon directly attached to peak **E** and to an aromatic quaternary carbon.

Fig. 8.14 shows all of the correlations for each peak. The position of the hydroxyl group has yet to be assigned, but, as there is only one position available on the aromatic ring, the hydroxyl must be placed *ortho* to the methyl group. This is supported by the fact that the carbon to which this hydroxyl group is attached is the most downfield aromatic quaternary carbon (153.6 ppm), and heteroatoms such as oxygen are known to deshield carbon nuclei (compare the ppm value of this quaternary carbon with other quaternary aromatic carbons in compound X).

A database search indicates that compound X is **carvacrol** (see Fig. 7.14), which is a common component of volatile oils, especially those from plants belonging to the mint family (Lamiaceae) of which *Thymus vulgaris* is a member.

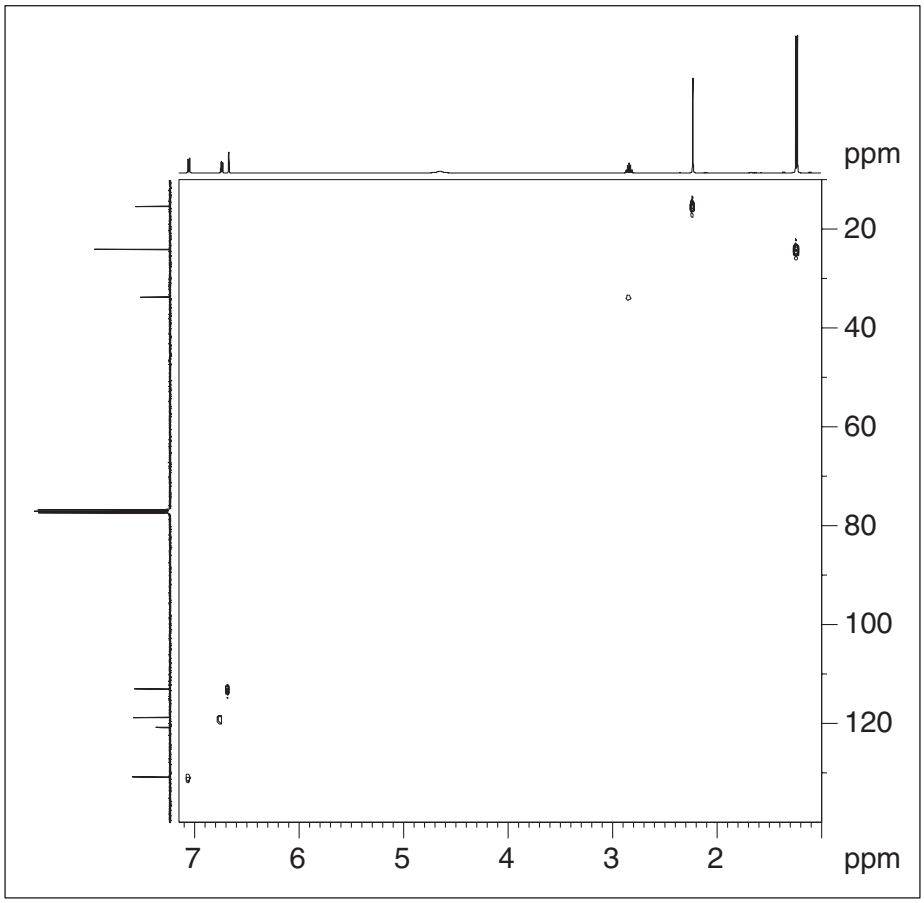


Fig. 8.12 HSQC spectrum for compound X showing correlations between protons and the carbon atoms to which they are directly attached.

X-RAY STRUCTURAL ANALYSIS

HMBC spectra are a very powerful way of determining the fragments and full structure of natural products and, although carvacrol is a simple example, the technique can be extended to highly complex natural products such as cardiac glycosides and polyketides. Together with simple spectroscopic techniques such as UV-visible and IR spectroscopy, NMR and MS are now the most widely used methods to determine structure.

Possibly the most comprehensive way to determine the three-dimensional structure of a molecule is to use X-ray structural analysis. This technique requires the compound to be in the form of a crystal of suitable quality, which is then placed in the path of an X-ray source. The atoms of the crystal diffract the X-rays in a pattern that is characteristic of the arrangement and type of atoms present. The pattern can then be interpreted by computer programs to give a three-dimensional structure of the compound. Unfortunately, natural products do not always readily form crystalline substances (for example carvacrol is an oil); moreover, the amounts of material produced by some organisms are small and can make the production of crystals challenging. However, X-ray structural analysis can provide information on the stereochemistry of a molecule and, if crystals are readily available, the structure can be solved in a matter of hours.

THE INDUSTRIAL APPROACH TO NATURAL PRODUCT DRUG LEAD DISCOVERY

Industry is currently putting much emphasis on discovering drugs from synthetic rather than natural sources, which, given the rich history that natural products have in the development of new drugs, is foolish. Nevertheless, the companies involved in natural product drug discovery use a highly organized

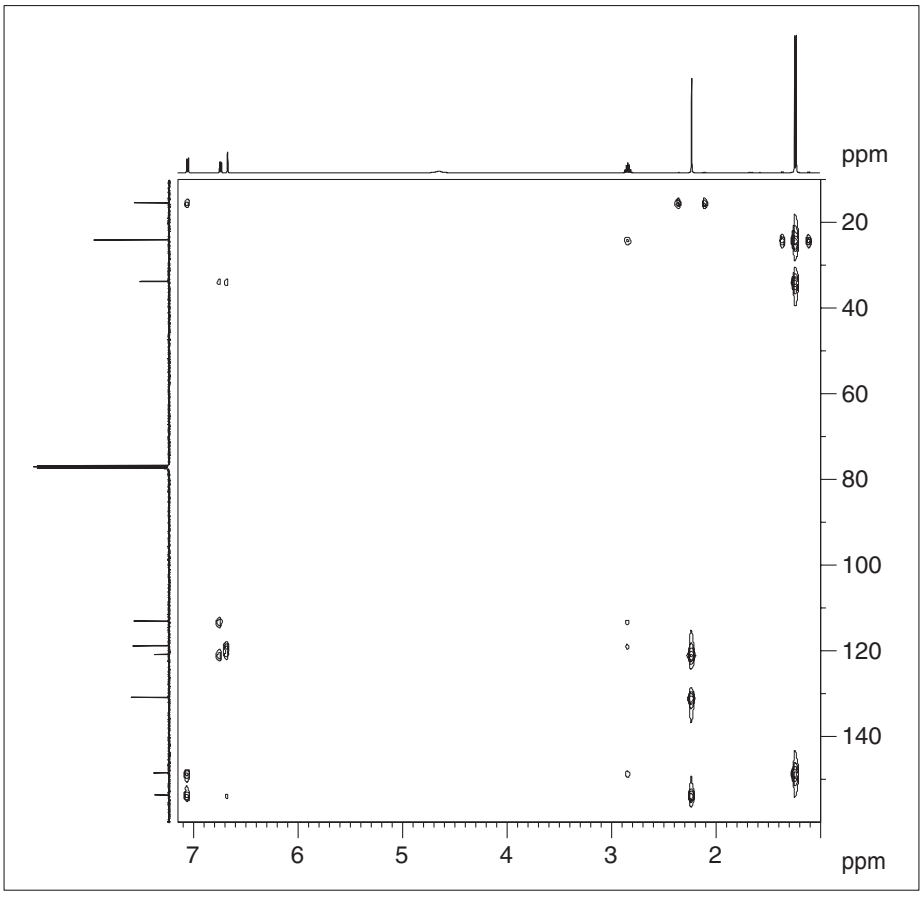


Fig. 8.13 HMBC spectrum for compound X showing correlations between protons that are two and three bonds distant from carbon atoms.

approach to reduce the time taken to find a biologically active compound and put it into drug development.

CHOICE OF BIOLOGICAL TARGET

The first decision a company will make is to choose the target – i.e. for what disease state is a drug being sought. Clearly, this decision is market-driven, with many large companies preferring to look for anticancer or antiarthritis drugs, which have enormous potential markets, rather than antimalarials or antitubercular drugs, for which there is an enormous need, but among populations that are less able to pay for these drugs. Some companies have an historical association with a particular medicinal area. It is also true that the decision to work on a particular assay for a disease state is fashion-driven. For example, several companies may be working on the same target that has recently been described in the literature as being particularly important in the development of a disease state.

ASSAY SELECTION AND DEVELOPMENT

The selection of an assay is the next step. Clearly, the assay should show a good correlation with, and reflect, the particular disease state. For example, to select a target such as the inhibition of a certain enzyme is of limited use, if it later transpires that the enzyme is not key to the proliferation of the disease. The decision to look at just a cellular or a molecular assay is important; ideally, both cellular and molecular mechanisms should be investigated using several assays. The relevance to disease state, the ease and speed of performing the assay, a good response, sensitivity and insensitivity to common natural products, and the ability to perform the assay in a high-throughput screening (HTS) format are all issues that will impact on the selection of a

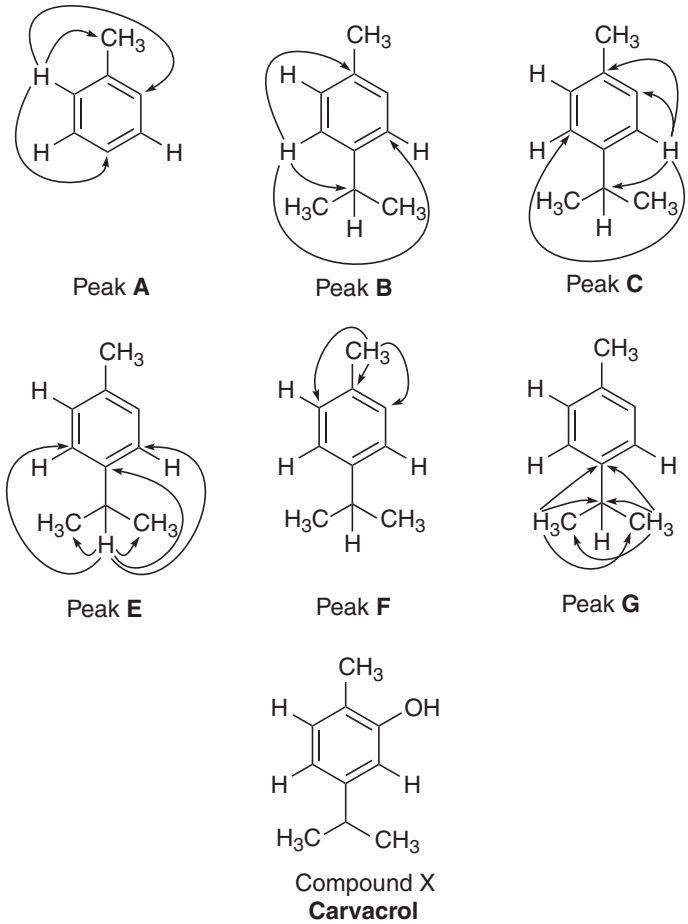


Fig. 8.14 HSQC and HMBC correlations confirming the structure and identity of Compound X.

biological assay. Development of this assay (creation, evaluation and validation for HTS) may take many months and in some cases years. As this process can be very expensive, the need for speed of assay development is vital.

PROGRAMME STRUCTURE

Assays are part of the remit of a specific programme. Examples include **anti-infectives** (assays for antibacterial, antifungal and antiviral agents), **immune-inflammation** (assays for disease states such as arthritis, eczema, asthma and psoriasis) and **anticancer programmes** (assays for cytotoxic agents and resistance reversing agents). These programmes are managed by a programme head who is responsible for several **project teams** working on various assays within the programme to discover new drug leads. The project teams are a truly multidisciplinary group with expertise in all aspects of the drug discovery process and include a **project leader** who runs the team, reports to the programme head and manages the logistics of the team, such as budgets and timelines for implementation of all aspects of the project. Many project teams use a **microbiologist** who is a specialist in the collection and taxonomy of organisms (sometimes working in the field). If organisms have already been collected and stored, the microbiologist will be involved in the fermentation of microbes for screening and in the optimization of fermentation for scale-up once a biologically active extract has been identified.

Some projects use the expertise of a **field botanist**, who is often based in a university botanic garden and has expertise in the identification and collection of plant species. The botanist will collect and dry samples and send them to the company for extraction and assay. Collaboration with such an expert can be extremely valuable as it will allow access to diverse

biological samples from biota-rich tropical sources (e.g. Central America), or access to plants that are known to come from taxa that are adept at producing bioactive molecules.

An essential member of the team is the **biochemist**, who will assay extracts prepared by the microbiologist or **chemist**. This is a vital step in which thousands (sometimes tens of thousands) of extracts are assayed (screened for biological activity) in an HTS format. The biochemist then retests active extracts to ascertain if there is any cross-screening data on an extract – i.e. is it active in other assays? This is important, as some active compounds may be non-specifically active or show adverse activity (toxicity). Cross-screening can enable a decision to be made on the selection of an extract for further evaluation. The project chemist is responsible for the extraction of biomass (plants/microbes), the isolation of pure bioactive natural products and elucidation of their structure using spectroscopic techniques. A **molecular pharmacologist** carries out detailed mechanistic studies of pure active compounds and performs *in vitro* and *in vivo* preclinical evaluation.

DEREPLICATION

After a screening process (the assay), a series of extracts will be active, but it is particularly important to establish that no replicates (i.e. extracts with the same chemistry) are present or that no compounds are present that are already known to be active in the assay. This is done by the process of **dereplication**, which is a strategy to avoid known compounds or common molecules that interfere with assays (e.g. tannins which may bind in a non-specific manner to many proteins). This may be important if the assay uses a protein (e.g. an enzyme). To give a further example, if plant extracts are being screened for cytotoxic activity, it would be unhelpful to re-isolate taxol. The main reason for dereplication is cost; because projects and programmes are expensive to run and it can take weeks (sometimes months) to isolate active components, it is important that they are novel and are not known to be active in an assay. Novelty is an important factor, and the technology must be protected by patent so that the drug can be exploited commercially.

It is possible to avoid some known metabolites by accurate literature searching of the organisms under study. This is best achieved by on-line searching of databases such as **Chemical Abstracts**, **Dictionary of Natural Products** and **NAPRALERT,** which offer a quick method to find chemical information on a species. There is no perfect database (they are all incomplete by their nature), but possibly the most comprehensive survey of natural chemistry can be found in Chemical Abstracts, which may be accessed online through a fee-paying service, although it is wise to use as many databases as possible.

The databases can lead to primary literature information on molecular weight, IR, UV/Vis and NMR data, which can be used to recognize common metabolites at an early stage.

Most dereplication methods use physical methods on plant/microbial extracts before the assay, possibly the most comprehensive of which is automated HPLC, which can give retention times and UV data on eluting peaks. Combined techniques are more important. For example, **HPLC-MS** can provide more information such as retention times of peaks, their UV spectra (with photo-diode array) and molecular weight (and fragment) information, which is acquired as peaks elute from the UV detector into the mass spectrometer. All of these data can be built into a spectral library to allow searching of peaks with characteristic retention times, UV and mass spectra with those already acquired. This process is very powerful and enables recognition of known peaks to be made rapidly, thereby reducing costs in the discovery process.

For compounds that are lipophilic and volatile, a combination of **gas chromatography and mass spectrometry (GC-MS**) can be used to separate components to give retention times and eluting peaks that can be fed into a mass spectrometer to acquire characteristic molecular weight and fragmentation information. This technique can also be applied to polar water-soluble components such as the calystegines and other polyhydroxylated alkaloids if they are first derivatized with a suitable agent (e.g. trimethylsilyl chloride) to increase their volatility. Other more exotic dereplication processes for polar compounds include **capillary electrophoresis–mass spectrometry (CE-MS**), which can be used for compounds that are positively or negatively charged. In capillary electrophoresis, extracts are separated in a capillary filled with buffer, which has a potential difference (voltage) applied to it. Compounds of varying charge can be separated by this technique and, when coupled to a UV detector and mass spectrometer, the technique has great utility.

NATURAL PRODUCT LIBRARIES

Traditionally, drug-lead discovery has used HTS of extracts in microtitre plates in high number to discover active extracts. Extracts are then produced in large amounts (scale-up) and compounds are isolated

using the bioassay-guided route. This process is highly productive and there are many examples of drugs that have been discovered in this way. There are, however, a number of drawbacks with this process; it is expensive, time-consuming and may not lead to a drug candidate if there are problems with acquiring large amounts of the natural product or if the active component is a well-known compound with an uninteresting broad spectrum of activities. It is also true that this approach has come under threat from other competing methods of drug lead discovery, in particular combinatorial chemistry libraries in which high numbers of compounds are synthesized and dispensed into microtitre plates and screened for bioactivity.

Natural product chemistry has had to evolve to cope with this approach, and several companies now market **natural product libraries**. These libraries are banks of microtitre plates with pure natural products in individual wells at a known concentration and, in effect, the chemistry has already been done. Organisms are selected on the basis of unknown chemistry or chemotaxonomic information, extracted with solvents, and teams of chemists isolate hundreds of compounds from the extracts. This process can be highly automated, with flash chromatography and preparative HPLC being run in parallel to separate extracts into fractions and fractions into pure compounds. There are varying levels of structure elucidation information available on pure compounds (i.e. full NMR, MS, IR, UV); this is determined by the customer, who will be the company who buys (or leases) the library from the library producer.

There are a number of benefits to this procedure over conventional HTS and synthetic libraries:

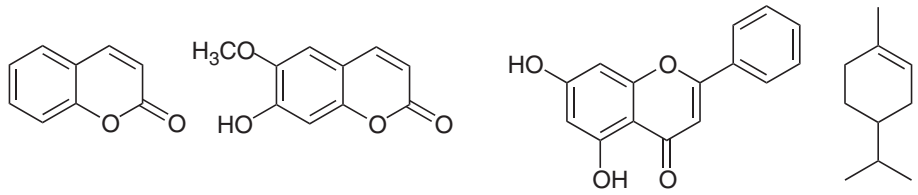
- The isolation chemistry in HTS has always been the slow bottleneck. The process is faster in the library generation, which enables decisions about active natural products to be made rapidly.
- All compounds have a **defined concentration** in the microtitre plate and so the potency of the compound in the assay is known immediately; this is not true with extract screening. Most active extracts may contain one major component with poor activity or, even worse, the least-active extract may have a low concentration of a very potent compound, which may be deprioritized.
- Screening a pure compound will give a better assay response, as single components have a cleaner interaction with the biological target and there are no interfering compounds present in the extract (e.g. tannins, which may mask the presence or absence of an active component).
- The dereplication process is more successful with pure compounds and a large amount of data can be acquired.
- Identification of the active compound may be total before screening and this can give useful information on any structure–activity relationships with other screened compounds.
- The whole process is now much quicker; the limiting step is now assay technology, which is itself very rapid.
- Large amounts of pure compounds can be isolated (5–10 mg), so retesting and further assays can be performed quickly without delay in further isolation.
- Most importantly, the **cost** is less than HTS as the time for discovery of a natural lead is reduced.

Several companies have adopted this approach, and market curated natural product libraries. These companies sell (or licence) the libraries for drug and agrochemical discovery to larger companies. For compounds that are of further interest to the customer, the same companies may then scale-up the extraction and purification of the compound at their facilities.

ALTERNATIVE APPROACHES IN NATURAL PRODUCT DRUG LEAD DISCOVERY

Many fungi and bacteria do not produce natural products when fermented and it is possible that they require an external stimulus from another organism to do so (e.g. other microbes or molecules secreted into their environment by another organism). This presence of unproductive organisms results in a loss of access to chemical diversity. Additionally, only 5% of microbes may be culturable and fermentable and there is, therefore, great potential to discover new therapeutic agents if ways are discovered to tap into the genetic, and, therefore, chemical, capability of these organisms.

Some companies utilize the procedure of combinatorial biosynthesis, in which DNA is taken from uncultivable organisms or from soil to build a library of characterized DNA fragments. It is then possible to insert this DNA into a host such as *Escherichia coli* or a yeast, which are readily fermentable organisms. The importance of this technique is based on the assumption that the host may use this DNA in the biosynthesis of new natural products. The host is then fermented and screened for bioactivity; this process may also be used to generate compounds for natural product



Analogues **Different classes**

Fig. 8.15

library production. This is an innovative approach and may lead to new classes of natural products and allow full exploitation of microbial chemistry. Unfortunately, it may need much research to exploit this opportunity, especially given that many kilobases of DNA are required to produce even simple natural products. Additionally, the host may not utilize the foreign DNA, or it is possible that the DNA inserted may not code for proteins that make natural products.

WHY NATURAL PRODUCTS AS DRUGS?

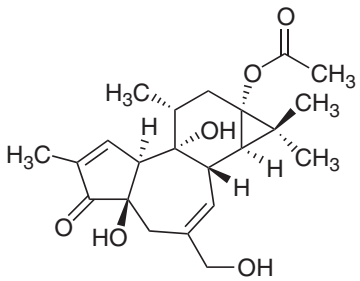
In the previous sections we briefly looked at some of the classes of compounds produced by nature and how they can be isolated and their structures elucidated; but why are natural products such an important source of drugs? There are many examples of drugs from nature and this is possibly a result of biological and chemical diversity.

In screening for new bioactive compounds, it is important to access as wide a range of diverse biological specimens as possible because it is thought that this high range of biological diversity mirrors or gives rise to a high degree of chemical diversity (i.e. a wide array of structurally unrelated molecules). Ideally, a drug discovery programme should have access to as many different species as possible from groups such as plants, fungi, filamentous bacteria, corals, sea animals and amphibia. The tropics hold a vast repository of numbers of such species, and there is, therefore, enormous potential to discover new bioactive entities in this region of the world. Using Costa Rica as an example, the Instituto Nacional de Biodiversidad (INBio) has been conducting a national inventory of plants, insects, microbes and animals, and has so far assessed almost 500,000 species. This incredible genetic resource could potentially generate millions of natural products to be assessed for biological activity. Individual species are also adept at producing not only different classes of natural product (e.g. flavonoids and monoterpenes simultaneously), but also analogues of the same natural product class (Fig. 8.15).

It is this richness in chemistry, coupled with the structural complexity of certain types of natural product (e.g. paclitaxel), that makes natural products such a valuable commodity for the discovery of new drugs. It has been estimated that 25% of all prescription medicines owe their origin to a natural source and that, in the field of anticancer drugs, almost 60% of agents are either natural products or are derived from a natural product source. Additionally, many natural products are produced by the organism as a chemical defence (e.g. as an antimicrobial or as an antifeedant substance) against another organism; thus, in many cases, *there is already an inherent biological activity associated with natural products*.

Large pharmaceutical companies have been scaling down their interest in natural products, preferring to access their chemical diversity through synthetically produced libraries of compounds. Unfortunately, many of these libraries lack the true chemical diversity, chirality, structural complexity and inherent biological activity of natural products. It is therefore a matter of time before tried and tested natural product sources once again become widely used in drug discovery.

The **Convention on Biological Diversity** is a treaty between 182 countries that recognizes the authority that countries/states have over their genetic resources (see also Chapter 5). The treaty recognizes that access to biodiversity, be it plant, microbial or animal, is governed by the sovereign authority of that particular state. This means that it is not possible to acquire biological specimens from an area without 'prior informed consent' on 'mutually agreed terms' and that, should any commercial benefit arise from collection of biota (e.g. in the discovery of a new drug), then 'equitable sharing of benefits' should occur. This is an exceptionally important concept for profit sharing and one that can be highlighted by the example of **prostratin** (Fig. 8.16) from *Homolanthus nutans* (Euphorbiaceae).



Prostratin

Fig. 8.16

Paul Cox, an ethnobotanist working in Samoa, became intrigued by the local use of the inner bark of *Homolanthus nutans* to treat yellow fever, which is a clinical manifestation of the viral disease hepatitis. He collected samples of these species and sent them for testing at the National Cancer Institute (NCI) for assessment of antiviral activity in anti-AIDS assays. The active component, **prostratin**, was isolated and the structure determined as a diterpene related to the phorbol ester group of natural products. Interest waned in this compound as the phorbol esters are known to be strongly tumour-promoting. However, Paul Cox was not deterred by this because of the local use of the plant and he urged the NCI to assess prostratin for tumour promotion. Interestingly, prostratin does not promote tumour growth. On the contrary, recent research shows that prostratin represses tumorigenesis in *K-Ras* mutant pancreatic cancer cells. It appears to prolong the life of HIV-infected cells and stops infection of healthy cells by HIV. Whilst prostratin is still in development, the authorities of the village from which the discovery originated have negotiated an agreement signed by the prime minister of Samoa. The AIDS Research Alliance who are developing prostratin will ensure that 20% of commercial profits that come from prostratin will go back to Samoa. In this settlement, revenues will go back to the village and to the families of the traditional healers who gave the original information on the use of *H. nutans*. This example highlights the fact that it is not only important that financial recompense is made to the originators of ethnobotanical research, but also that this traditional knowledge has value that must be preserved.

Further reading

Balick, M.J., Cox, P.A., 1996. Plants, people and culture: the science of ethnobotany. Scientific American Library, New York.

Crews, P., Rodriguez, J., Jaspars, M., 2009. Organic structure analysis, second ed. Oxford University Press, Oxford.

Hostettmann, K., Marston, A., Hostettmann, M., 2010. Preparative chromatography techniques: applications in natural product isolation, second revised ed. Springer-Verlag, Berlin.

Sarker, S.D., Latif, Z., Gray, A.I., 2010. Natural products isolation. Methods in biotechnology, second ed. Humana Press, Totowa.

Wang, M.-T., Holderfield, M., Galeas, J., et al., 2015. K-ras promotes tumorigenicity through suppression of non-canonical Wnt signaling. Cell 163 (5), 1237–1251.

Williams, D.H., Fleming, I., 2007. Spectroscopic methods in organic chemistry, sixth ed. McGraw-Hill, London.

Chapter 9

Anticancer natural products

Plants have been the basis of sophisticated medical systems for thousands of years, particularly in China and India, and they have an essential role in health care. The World Health Organization has estimated that 80% of the Earth's inhabitants rely on traditional medicines for primary health care, and plant products are highly important in the remaining 20% of the population (Farnsworth et al 1985). During the period 1959–1980 approximately 25% of US prescription medicines contained plant extracts, plant constituents or agents that were derived from natural sources. Over 119 chemicals from 90 plant species are important drugs in many countries and three-quarters of these were derived from studying the chemistry of traditional medicines (Farnsworth et al 1985).

Natural products have made an enormous impact on the discovery of compounds that kill cancer cells; in fact, possibly 60% of all cancer drugs that are used clinically are either natural products or owe their origin to a natural source. The most comprehensive study conducted on cytotoxic agents from nature has been carried out by the **National Cancer Institute (NCI)**, a US government agency that has invested in the identification of a number of anticancer drugs.

THE NATIONAL CANCER INSTITUTE

The **National Cancer Institute (NCI)** was established in the USA in 1937 'to provide for, foster and aid in coordinating research related to cancer'. This organization has been involved in many of the great discoveries in fundamental research of cancer and particularly in the characterization of cytotoxic natural products. During the 1950s it was realized that there was a need for screening of natural extracts in a discovery programme and the **Cancer Chemotherapy National Service Center** was set up (Cragg et al 1993). The objectives of this organization were to procure compounds and to screen and evaluate them in a preclinical and, finally, full clinical setting. This service developed into the modern organization known as the **Developmental Therapeutics Program (DTP)**.

Apart from endeavours to find anticancer agents from natural and synthetic sources, the DTP has also initiated a programme to discover and preclinically develop anti-AIDS agents. Being a US government-funded agency, the NCI accepts and screens compounds from many sources, including academics in universities and pharmaceutical companies. This philanthropic activity increases the chances of finding lead compounds with activity against the many types of cancer and against HIV. Much of the early NCI screening focused on natural products produced by fermentation of microbes, such as filamentous bacteria, and, prior to 1960, only a small number of plants (1500) were investigated.

Early successes in the exploitation of plants as a source of anticancer agents included the characterization of podophyllotoxin-type lignans from *Podophyllum peltatum*, and their semi-synthetic derivatives, and vincristine and vinblastine from *Catharanthus roseus* (Fig. 9.1). These discoveries drove further interest in plants at the NCI, and collections of plant material were expanded to include 60 countries. This collection strategy was quite extensive and, between 1960 and 1982, 114,000 extracts from 35,000 plants species were screened against a number of tumour types (Cragg et al 1993).

Methods to assess antitumour activity are being continually improved and, after 1975, the P388 mouse leukaemia cell line was used to assay for the

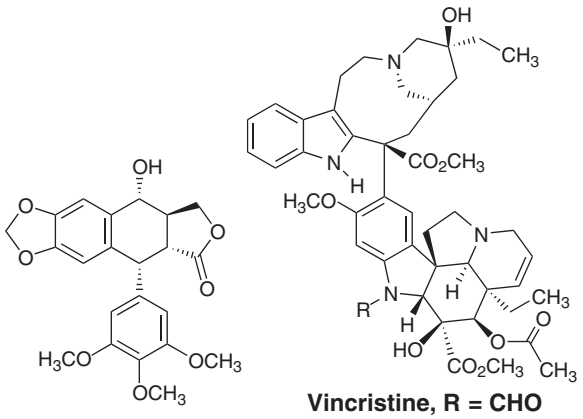


Fig. 9.1 Podophyllotoxin, vincristine and vinblastine.

presence of compounds that are cytotoxic. Following bioassay-guided isolation of the active agents, a secondary screen known as the human xenograft mouse model was used to assess agents with significant activity. In this model, human tumour cells are introduced beneath the skin of mice lacking an immune response. The cells rapidly grow to form a tumour; compounds may then be administered to the mouse and any reduction in tumour size measured. This mouse model is still used today and cells from different tumour types may be introduced. Any compounds with broad-spectrum activity were prioritized for preclinical development and eventually clinical trials.

Bioassay-guided fractionation of active extracts led to the characterization of a large number of agents from many different natural product classes. Arguably the most significant discovery from the NCI screening programme was **taxol**, from *Taxus brevifolia* (Taxaceae) (Fig. 9.2). The compound had been previously isolated in 1971 by Wall and co-workers and its activity against a melanoma cell line and in the human xenograft model led to its selection for preclinical development in 1977. Initially, there were problems in acquiring large amounts of the compound, but solutions to these problems and the report of its unique mode of action by promotion of tubulin polymerization and stabilization of microtubules against depolymerization increased interest. Taxol (now known as paclitaxel; see below) has excellent activity against ovarian and breast cancers and was approved in the USA by the FDA in 1993.

Another agent of note, again from the group of Wall, is **camptothecin** from the Chinese ornamental tree, *Camptotheca acuminata* (Nyssaceae) (Fig. 9.2).

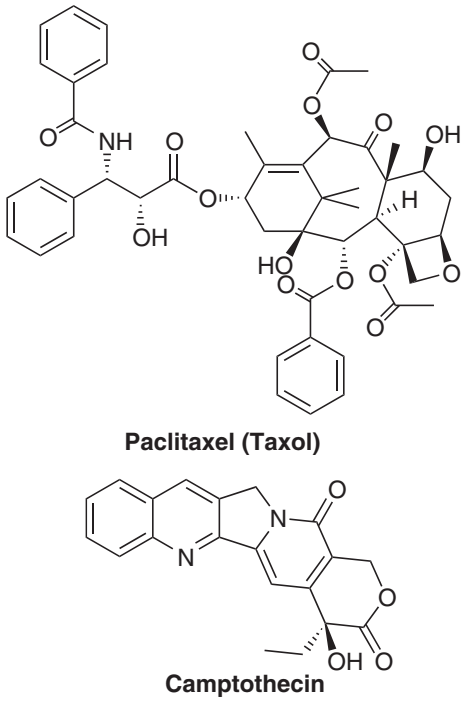


Fig. 9.2 Paclitaxel and camptothecin.

Chinese clinical trials were conducted on this agent, which showed promise against a number of different cancer types, including gastric, liver, head, neck and bladder cancers. However, US clinical trials of the sodium salt were terminated due to a limited number of responses. There has been much work on this class of natural product, with two companies (SmithKline Beecham and Daiichi) having developed and released two products: **topotecan** and **irinotecan**.

Between 1985 and 1990 the NCI developed a new *in vitro* screen that uses 60 human cell lines from nine cancer types representing leukaemia, lung, colon, central nervous system (CNS), melanoma, ovarian, renal, prostate and breast cancers. Synthetic or natural products are made up at concentrations ranging from 10^{-4} M to 10^{-8} M and then tested against the 60 different tumour cell lines. The data that are acquired from this test system are evaluated to decide if further investigation is warranted (Alley et al 1988, Grever et al 1992).

The results from the screening are reported in a graph format that calculates the potency of the compound against all 60 cell lines in terms of three indicators:

- GI_{50}: the concentration at which growth is inhibited by 50%.

- TGI: the concentration at which cell growth is totally inhibited.
- LC_{50}: the concentration at which 50% of the cells are killed.

The importance of these three factors is that a fingerprint of a compound's anticancer activity is generated that can be correlated, using a mathematical model, with test results for known anticancer drugs (Paull et al 1989). This can even give an idea of whether a compound works by a similar mechanism of action as a known anticancer agent such as vincristine. Promising compounds with interesting selective profiles of activity (e.g. activity against one particular cell line) are then retested to confirm the reproducibility of these results. In 1995 a three-cell line prescreen was introduced to increase the throughput of compounds whilst not reducing the number of compounds with activity in the 60-cell line assay. These cell lines were from lung, CNS and breast tumour types.

A new acquisition programme was initiated in 1986 with contracts for the cultivation and extraction of fungi and bacteria and the collection of marine organisms and terrestrial plants (Cragg et al 1997). Marine organisms were collected from many diverse locations, including the Caribbean, Australasia, Central and South Pacific, Indian Ocean and Polynesia. Plant material was collected in over 25 countries in the tropical and subtropical regions by specialist organizations with botanical expertise such as the Missouri Botanical Garden, the University of Chicago and the New York Botanical Garden.

Collaborations with the NCI rely on working closely with qualified organizations (e.g. universities, research institutes, botanical gardens) in the source country that has access to the biological diversity. Botanists and biologists from source countries collaborate in collections, which are invaluable to the NCI, and in many cases training is provided and workshops are organized for local personnel so that there is a transfer of expertise in certain areas. The source countries send biomass back to the National Products Repository in Frederick, Maryland. In the past, invitations have been made to local scientists of source countries to visit the NCI for collaborative research in natural products (Cragg et al 1997). This fosters openness and true collaboration between the NCI and source country.

For biomass collection, dried plant material (usually in the dry weight range of 0.3–1.0 kg) and frozen marine organism samples (1 kg) are shipped to Frederick and stored at −20°C, lessening the possibility of sample degradation. Biomass is extracted with methanol/dichloromethane (1:1) and then water to give organic and aqueous extracts that are given NCI numbers and stored at −20°C until required for screening (Cragg et al 1997). The extracts are then tested in the *in vitro* human cancer cell line screen and active extracts undergo bioassay-guided fractionation to isolate pure natural products. Structure elucidation is carried out using NMR and mass spectrometry. Compounds demonstrating significant antitumour activity are selected for secondary testing in *in vivo* systems and those with good activity advance to preclinical and, finally, clinical development. During this process there is the opportunity to license compounds out to companies for development; this is the preferred route due to the large costs of conducting the development process.

Importantly, agreements ensure that any royalties that may arise are shared with the country of origin and, as previously mentioned with the Convention on Biodiversity, it is a legal requirement that the collection of organisms is only possible with the 'prior informed consent' of the authorities of the source country (see Chapter 5).

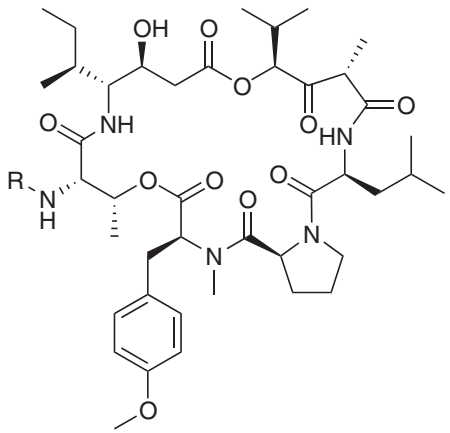
The popularization of cell culture methods in the late 1990s and first decade of the 21st century facilitated the work of academic groups all over the world. As a result, a huge number of plants have been screened for their cytotoxic activities, thus contributing to the efforts of governmental agencies. Unfortunately, there is a tendency to use incorrect terminology when reporting on the activities of natural drugs in cancer research and as a result we have hundreds of scientific papers on false 'anticancer' natural products. It is important to bear in mind that when a drug is active at the *in vitro* level only, the correct adjective is **cytotoxic** or **antiproliferative**. When the drug is also active in *in vivo* animal models then the drug has **antitumour** properties. Only when the drug proves to be of therapeutic value in clinical trials (i.e. on human patients) can we talk about an **anticancer** effect.

A full review of antitumour agents is beyond the scope of this text but we will briefly cover the three main sources of anticancer agents (marine sources, plants and microbes), giving selected examples of agents and their sources. The reader is urged to consult the excellent reviews by Cragg and co-workers on this subject (Cragg and Newman 1999, 2000, Cragg et al 1993, 1997, Newman and Cragg 2016).

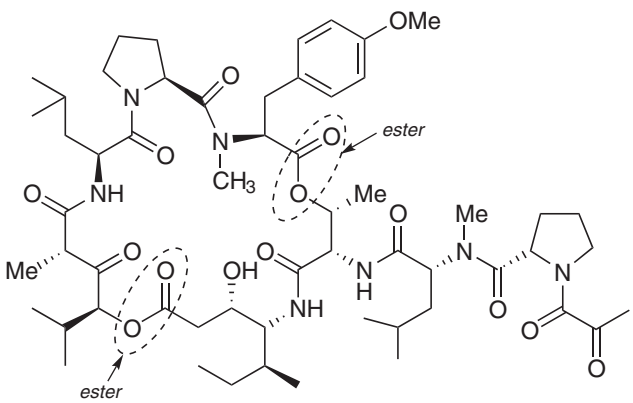
MARINE ANTICANCER NATURAL PRODUCTS

Marine organisms have no history of medicinal use but, because the oceans cover 70% of the Earth's surface, there is a vast reserve for the discovery of new natural product drugs. This huge environment is home to a fantastic range of diverse organisms and, of the 28 major animal classes, 26 exist in aquatic areas and eight are exclusively aquatic (Cragg et al 1997). Collection of biomass is usually carried out by divers and in some cases submersibles can give access to organisms that occur in deeper sites, although this is a highly expensive procedure.

The discipline of marine natural product chemistry is comparatively new compared to phytochemistry, with relatively small numbers of natural products having been reported. At present there is one marine natural product that is used as an anticancer drug clinically (Ecteinascidin-743, Yondelis®), and a number are in clinical trials and their potent activities highlight the future importance of this source. A compound in development worth noting is **bryostatin-1**, which is a novel macrocyclic lactone derived from the marine bryozoan, *Bugula neritina*. This compound modulates protein kinase C activity and phase I studies have demonstrated activity against several tumour types. Bryostatin-1 has subsequently been investigated extensively in phase II clinical trials as a single agent and data suggest that it may have potential in combination with other cytotoxic agents. **Didemnin B** (Fig. 9.3) (*Tridemnum solidum*) was the first marine natural product to enter clinical trials and has recently been shown to induce apoptosis (programmed cell death) in a wide range of cell lines. **Aplidine** (Fig. 9.3) is a marine **depsipeptide** obtained from the **tunicate** *Aplidium albicans*, originally found in the Mediterranean. Tunicates, which are also known as **sea squirts**,



R = Lac-Pro-*N*-Me-L-Leu; Didemnin B



Aplidine

Fig. 9.3 Didemnin B and aplidine.

are organisms that attach themselves to submerged objects and feed by removing microscopic organisms from the water that is drawn through them. Depsipeptides are cyclic peptides that also possess a cyclic ester functional group. Aplidine blocks the cell division cycle in human tumour cell lines and prevents the onset of DNA synthesis. Like didemnin B, it has also been shown to be an inducer of apoptosis in many cell models. Additionally, using the xenograft assay, grafted into athymic mice, aplidine demonstrated considerable activity against colon, bladder, lung, prostate and stomach tumours, and against melanoma and lymphoma tumours. This agent is currently in clinical trials. Aplidine demonstrates some of the very best features of a natural product, being highly chiral and exhibiting great functionality. Both of these features contribute to the high cytotoxicity of this molecule, which in nature is probably produced by *Aplidium* species as a chemical defence mechanism.

One of the most promising groups of agents comes from the sea hare (*Dolabella auricularia*), a herbivorous mollusc from the Indian Ocean that produces cytotoxic linear peptides. An interesting member of this class is **dolastatin-10** (Fig. 9.4), an inhibitor of microtubule assembly, currently in clinical trials.

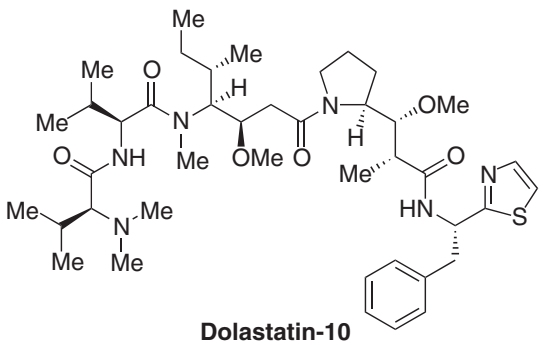
It is possible to synthesize these compounds; their peptide chemistry is highly amenable to the production of many analogues of the dolastatin class of which a number have been synthesized to date.

Perhaps the most interesting antitumour marine compounds and the first anticancer drug to come from this marvelous source is **ecteinascidin-743** (= trabectidin**),** now marketed as Yondelis® (Fig. 9.5), from *Ecteinascidia turbinata*, which is a tunicate found in the Caribbean and Mediterranean. Ecteinascidin-743 has very potent activity against a broad spectrum of tumour types in animal models; it binds to the minor groove of the DNA double helix and inhibits cell proliferation, leading to apoptosis of cancer cells. This binding to the minor groove of DNA allows an alkylation reaction to occur between a guanine residue of the DNA and an electron-deficient carbon on the molecule (* in Fig. 9.5).

It was shown that ecteinascidin-743–DNA adducts are recognized by the nucleotide excision repair system, which is inherent to each cell and is present to protect the cell from accumulation of mutations and DNA damage. When this repair system encounters the adduct, rather than repairing the cell, apoptosis occurs. Of particular interest is that ecteinascidin-743 cytotoxicity only occurs during active transcription of genes; this has obvious potential in cancer cells, which rely on increased transcription and translation. Ecteinascidin-743 also inhibits the induction of the gene *MDR1*, which encodes a membrane pump responsible for multidrug resistance, and this can drastically affect the potency of antitumour agents. These characteristics make ecteinascidin-743 unique and this compound was approved as an anticancer drug in combination with doxorubicin, particularly for the treatment of relapsed ovarian cancer, in the European Union in 2009.

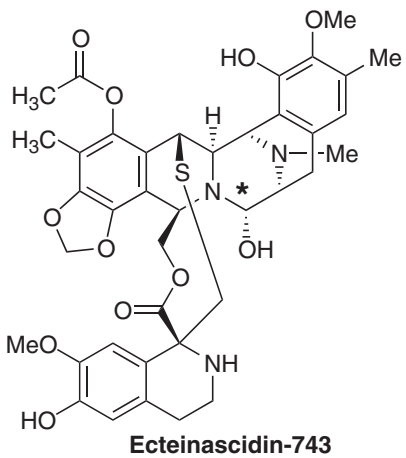
PLANT ANTICANCER AGENTS

Plants have been an excellent source of anticancer agents and there is much anecdotal evidence for their use against tumours in traditional systems of medicine, particularly in the Chinese, Ayurvedic, Jamu and African systems. The track record of plant



Dolastatin-10

Fig. 9.4 Dolastin-10.



Ecteinascidin-743

Fig. 9.5 Ecteinascidin 743.

anticancer agents is excellent and over the last 40 years some major chemotherapy agents derived from this source have been released onto the market: **alkaloids** from *Vinca* species (**vinblastine, vincristine, vindesine** and **vinorelbine**), the **camptothecin**-derived cytotoxics (**topotecan** and **irinotecan**) and more recently **homoharringtonine** from *Cephalotaxus* trees; **lignans** based on the **podophyllotoxin class** (**etoposide** and **teniposide)**; **terpenes** like **betulin**, the **taxanes** (**paclitaxel** and **taxotere)** and **ingenol mebutate**; and the **macrolide maytansine** from *Gymnosporia* spp. Plants are, therefore, a superb source of cytotoxic agents and, given the enormous number still to be investigated, there is great potential to discover new anticancer drugs. It is also true that advances in genome biology and target selection will ensure that plants will continue to provide new anticancer leads. There is even a case to reinvestigate plant extracts that have already been evaluated against old assays to assess them in new assays based on targets recently described by elucidation of the genome.

THE ALKALOIDS

Alkaloids have always been a great source of bioactive natural products due to the mandatory presence of an amine functional group. This feature confers the molecules with a versatile chemical behaviour depending on the pH and mimics the chemistry of endogenous amines acting as physiological messengers. Many alkaloids are endowed with an exquisite selectivity for their targets, allowing them to exert their effects at very low concentrations. In actuality they have been used as poisons since ancient times. In addition, they are easily extracted and purified with simple partition and precipitation methods so they were isolated in high purity very early in the history of drug discovery.

Camptotheca acuminata Decne. (Nyssaceae)

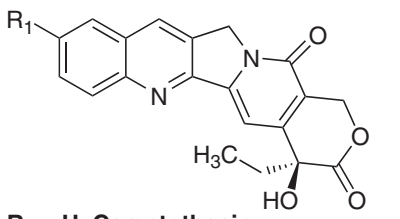
In 1957, a small number of ethanolic plant extracts (1,000) were screened at the Cancer Chemotherapy National Center (USA) and extracts of *Camptotheca acuminata* were shown to possess high activity. The late Dr Monroe Wall, who will be remembered as a leading expert in plant antitumour agents and a wonderful enthusiast for natural product research, established a natural product research group at the Research Triangle Institute that conducted some research funded by the NCI. Dr Wall, who was later joined by Dr Mansukh Wani, became interested in the constituents of *Camptotheca*, particularly due to the high activity of these extracts. Wood and bark (20 kg) were collected for extraction and the extracts were shown to be active against a mouse leukaemia life prolongation assay in which it was unusual to find activity (Wall and Wani 1995). During the 1960s, the bioassay-guided isolation process was slow and it could take as long as 3 months to get bioassay results from the mouse life-prolongation assay back to the chemists working on the extracts (Wall and Wani 1995).

When compared with cellular assays today, in which it may only take a few days to decide if extracts and fractions are active, it is worth noting how diligent, persistent and thorough these researchers were. Extraction of the plant material was carried out first using a non-polar solvent (hot heptane) to remove very non-polar natural products. The plant material was then extracted with ethanol and this extract was treated with chloroform and aqueous ethanol to give two phases, the lipophilic chloroform phase being highly active. Additional extraction of the aqueous phase with chloroform gave a further active chloroform extract. High activity was associated with all the chloroform extracts of the ethanolic portion, indicating that the active natural product had a degree of lipophilicity. Column chromatography was initially attempted on these active extracts using alumina as the sorbent, but what was not known at the time was that the active component binds very tightly to alumina and could not be eluted from the column. Extensive partitioning of the extract was tried in separating funnels using the quaternary system (four solvents) chloroform/carbon tetrachloride/methanol/water and each of the fractions were assayed in the mouse life-prolongation assay and *in vitro* cellular cytotoxicity assay. This partitioning system gave excellent separation and active fractions were combined to yield a yellow precipitate, which was subjected to chromatography on silica to which the active agent did not irreversibly bind. Further crystallization of active fractions led to the isolation of the active agent, **camptothecin** (Fig. 9.6). The structure elucidation of this natural product was carried out by conversion to its chloroacetate, which was then further converted to the iodoacetate salt, which gave crystals of sufficient quality for X-ray structural analysis.

At the time, camptothecin had a unique structure, possessing an α-hydroxylactone, and being a highly unsaturated alkaloid (nitrogen-containing) natural product of the quinoline alkaloid group. When treated with sodium hydroxide, the lactone ring opened with

the formation of a sodium salt that was much more water-soluble than camptothecin. When this salt was acidified, the lactone was again regenerated and the water solubility decreased.

Camptothecin was shown to be extremely active in the life-prolongation assay of mice treated with leukaemia cells and in solid tumour inhibition. These activities encouraged the NCI to initiate clinical trials with the water-soluble sodium salt. Trials were conducted in a small number of patients (18) and there were five partial responses against gastrointestinal tumours of short duration (Gottlieb and Luce 1972). There were, however, toxic side effects. A phase II study was conducted but unfortunately there were only two responses to the treatment (Moertel et al 1972). The sodium salt was also studied in a much larger clinical trial in China using 1000 patients with better results against head, neck, gastric, intestinal and bladder carcinomas. These results were more promising than those of the US trial, possibly due to the fact that the US patients had already been treated with other cytotoxic drugs and their tumours may have become multidrug-resistant. It is also now known that the lactone (which is absent in the lactone ring-opened sodium salt) of the compound is important for activity.



R_1 = H, Camptothecin
R_1 = OH, 10-Hydroxycamptothecin

Fig. 9.6 Camptothecin.

Wall and Wani continued to isolate and evaluate *Camptotheca* metabolites and found that one such product in particular, **10-hydroxycamptothecin** (Fig. 9.6), was more active than camptothecin, and this may have stimulated a number of companies to prepare further water-soluble analogues of 10-hydroxycamptothecin.

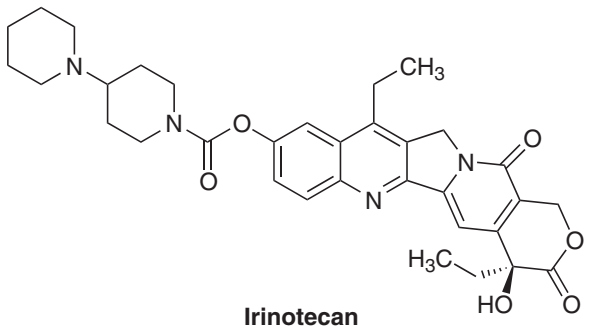
Interest in this natural product and its analogues was low until 1985 when it was discovered that camptothecin works by inhibition of topoisomerase I, which is an enzyme involved in many important cellular processes by interacting with DNA. This finding fuelled further interest in this class of compound as the mechanism by which it functioned as an inhibitor of tumour growth was understood. Much work was conducted by pharmaceutical companies on this template and two products became available as a result of this research, **irinotecan** and **topotecan** (Fig. 9.7). The structural importance of the parent natural product, 10-hydroxycamptothecin, as a template can be seen in both of these products.

Irinotecan was approved in 1994 in the USA. It is less toxic than camptothecin and is used to treat metastatic colorectal cancer; it is also effective against lung cancer and leukaemias. Irinotecan has much greater water solubility than camptothecin. It is a prodrug, being metabolized *in vivo* by hydrolysis of the carbamate ester to give the phenolic topoisomerase I inhibitor, which is 1000 times more potent than the parent compound.

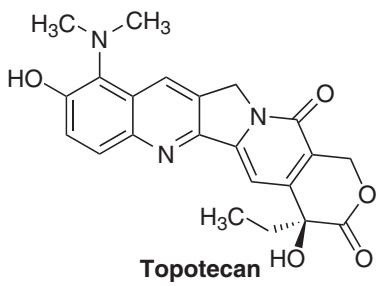
Topotecan (Fig. 9.7) was approved for use in the USA in 1996. It is active against ovarian cancer.

Catharanthus roseus (L.) G. Don (Apocynaceae)

The Madagascar periwinkle (*Catharanthus roseus*, syn. *Vinca rosea*) was originally native to Madagascar. It has been widely cultivated for hundreds of years and can now be found growing wild in most countries, including the UK. The natural wild plants are a pale pink with

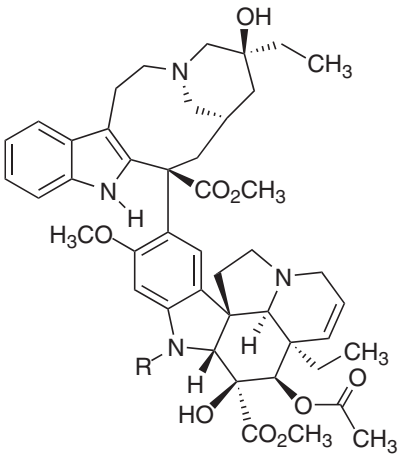


Irinotecan



Topotecan

Fig. 9.7 Irinotecan and topotecan.



Vincristine, R = CHO
Vinblastine, R = CH_3

Fig. 9.8 Vincristine and vinblastine.

a purple eye in the centre but many colours have been developed by horticulturalists, ranging from white to pink and purple. The plant has had a long history of treating a wide assortment of diseases and was used as a folk remedy for diabetes in Europe for centuries. In China, the plant has been used for its astringent and diuretic properties and as a cough remedy, and in the Caribbean it is used to treat eye infections and for diabetes. Historically, the periwinkle has had a reputation as a magical plant: Europeans thought it could ward off evil spirits, and the French referred to it as 'the violet of the sorcerers'.

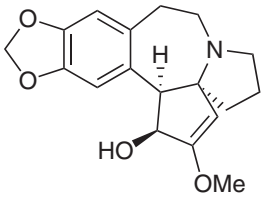
Jamaicans have traditionally used a tea from *C. roseus* to treat diabetes, although it has not been possible to find a basis for this use. Over 150 alkaloids have been characterized in the plant, a number of which are **indole alkaloids** and include **dimeric** or **bis-indole alkaloids**. These components are broadly referred to as **vinca alkaloids** after the name of a synonym for this plant, *Vinca rosea*.

The discovery of the vinca alkaloids from the Madagascan periwinkle is a classic example of serendipitous drug discovery and, although extracts of the species had a reputation for being useful in the treatment of diabetes, a screening programme at the pharmaceutical company Eli Lilly revealed that extracts inhibited the growth of certain types of cancer cells. Bioassay-guided isolation of extracts of the plant led to discovery of the characterization of the active alkaloidal compounds **vincristine** and **vinblastine** (Fig. 9.8).

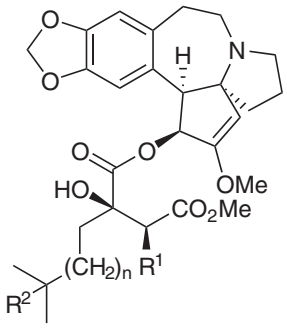
These natural products are structurally highly complex and are dimeric indole alkaloids that can be synthesized but are too expensive to produce in this way. The natural yield of the drugs in the plant is exceptionally low (0.0002% for vincristine), which makes them very expensive antitumour agents. They exert their anticancer effects by inhibiting mitosis by binding to tubulin, thus preventing the cell from making the spindles it needs to be able to move its chromosomes around as it divides. Vinblastine is marketed as Velbe® by Eli Lilly and is useful for treating Hodgkin's disease, lymphomas, advanced testicular cancer, advanced breast cancer and Kaposi's sarcoma. This drug has a number of side effects, including hair loss, nausea, lowered blood cell counts, constipation and mouth sores. Vincristine is marketed as Oncovin® by Eli Lilly and is used to treat acute leukaemia, Hodgkin's disease and other lymphomas. Semi-synthetic vinca alkaloids of note include **vindesine** (marketed under the name Eldisine®), used to treat leukaemia and lung cancers, and **vinorelbine** (marketed as Navelbine® by GlaxoSmithKline), which is used as a treatment for ovarian cancer. Vinorelbine has a wider range of antitumour activity than the other vinca alkaloids and is used, in combination with cisplatin, in treating patients with non-small-cell lung cancers.

Cephalotaxus fortunei Hook. (Taxaceae)

In the 1970s, the People's Republic of China embarked on an ethnopharmacology-driven drug discovery process informed by the millenary knowledge of its traditional medicine. One of its most remarkable achievements was the isolation of the antimalarial artemisinin (see Fig. 24.8). Some 40 years after, in 2012, another natural molecule discovered thanks to this scientific endeavor finally made its way to the clinical armamentarium: **homoharringtonine** (= omacetaxine), a naturally occurring alkaloid in *Cephalotaxus* trees (Fig. 9.9). It is commercialized in the form of a mepesuccinate ester called Ceflatonin® for the treatment of chronic myeloid leukaemia (CML) in cases resistant to other therapies. The development of homoharringtonine has been possibly the longest in the history of anticancer research due to several factors: difficult production and unreliable source supply, toxicity profile of the original dose schedules, and the initial success of other synthetic drugs in CML. It also represents a rare case of collaboration between China and the USA in a time of global military confrontation: a '*Cephalotaxus* Research Coordination Group' was established by the Chinese Academy of Medical Sciences – mainly dedicated to



Cephalotaxine



Harringtonine: R^1 = H, R^2 = OH, n = 1
Isoharringtonine: R^1 = OH, R^2 = H, n = 1
Homoharringtonine: R^1 = H, R^2 = OH, n = 2
Deoxyharringtonine: R^1 = H, R^2 = H, n = 1

Fig. 9.9 Cephalotaxin, harringtonine, homoharringtonine, isoharringtonine and deoxyharringtonine.

running clinical trials – and staff of the U.S. Department of Agriculture – taking on the isolation and characterization of the active principles from entire *Cephalotaxus* trees cultivated in Maryland.

Cephalotaxus is a genus comprising a few species of evergreen coniferous trees with leaves similar to the yew. They grow in humid valleys or in forests of Japan, India and China. Crude extracts from Chinese *Cephalotaxus* trees showed promising antitumour activity in early screenings. Further research revealed that alkaloids from any part of the trees (bark, roots, leaves, dried stems) had similar activity. Extraction of the alkaloids from *Cephalotaxus* typically involved percolation of the biomass in 95% EtOH for 12–24 hours for three consecutive times. The combined alcoholic extracts were evaporated under reduced pressure (below 40°C) and the concentrate was then diluted with a 5% tartaric acid solution. The acidic solution could then be extracted repeatedly with $CHCl_3$ to separate the non-polar impurities (terpenes, fats, pigments, etc.) and then made basic (pH 9) by the addition of ammonia. Repeated extraction of the basic solution with $CHCl_3$ and evaporation of the combined extracts yielded crude alkaloids that represented 0.08% of the original mass. Note that this process is a variation of the alkaloid extraction strategy described in p. 108 of Chapter 7.

One of the alkaloids, **cephalotaxine** (Fig. 9.9) was crystallized by slow evaporation of an ether solution. However, it was biologically inactive, indicating that the antitumour activity was due to one or more of the remaining alkaloids. All attempts to crystallize them (from methanol, ether, benzene, petroleum ether, or mixtures of these solvents) failed but fortunately each gave a well resolved single spot on TLC. After preparative TLC, ^{1}H NMR analyses in a 100-MHz apparatus yielded very clean NMR spectra revealing that all were very similar to cephalotaxine. Only an X-ray crystallographic study of the methiodide salt could establish the correct structure for cephalotaxine. Interestingly, both the crystal and the amorphous material precipitated from the crude alkaloid extract were optically inactive contrarily to the amorphous, pure compound isolated by preparative TLC. This suggested that cephalotaxine may occur naturally as a partial racemate.

After these efforts, the structures of the remaining alkaloids were elucidated as the cephalotaxine esters **harringtonine**, **homoharringtonine**, **isoharringtonine**, and **deoxyharringtonine** (Fig. 9.9). Harringtonine and homoharringtonine were the most promising and entered a series of initial clinical trials from which homoharringtonine emerged as a better overall drug lead. However, further clinical studies were hampered by the large quantities of *Cephalotaxus* trees required to maintain a steady supply of the drug. These species are rare in China and cannot be grown easily. Because homoharringtonine is present in low concentrations but cephalotaxine is ubiquitous and abundant in all parts of *Cephalotaxus* species, methods of semisynthesis by addition of acyl moieties to the cephalotaxine ring were developed to ensure a sustainable supply of this anticancer drug. Semisynthetic homoharringtonine is known as omacetaxine and is structurally identical to its natural counterpart.

Homoharringtonine and its analogues are inhibitors of protein synthesis, although direct effects at the DNA level cannot be ruled out. They affect the initial stage of the elongation phase of translation by preventing substrate binding to the acceptor site on the 60S ribosome subunit, therefore blocking aminoacyl–tRNA binding and peptide bond formation. Interestingly, they do not interfere with the protein synthesis when this has already started, and *in vivo*

studies show the recovery of protein synthesis within 24 hours of injection. This implies that continuous drug exposure is required to achieve a maximal antitumour effect thus leading to unacceptable adverse effects, which include cardiotoxicity and hyperglycemia. To reduce patient exposure to homoharringtonine, *in vitro* combination studies with other cytotoxic agents were carried out, revealing a significant synergistic effect with cytarabine. This association underwent further clinical trials in the U.S. and showed a better therapeutic outcome *in vivo*, characterized by a dramatic increase in the cure rates (up to 40%) with less adverse effects. Furthermore, the reduced doses could be administered by subcutaneous injections, making self-administration by patients possible. By the time homoharringtonine was ready for approval as an anticancer drug, a synthetic tyrosine kinase inhibitor (imatinib, Gleevec©) took the clinical world by storm and temporarily outclassed all other drugs. It took further clinical experience to appreciate that homoharringtonine has a therapeutic edge in a subset of patients suffering CML and T315I mutations. The failure of several tyrosine kinase inhibitors to increase survival in these cases finally prompted FDA approval of semisynthetic homoharringtonine (omacetaxine) in CML for the narrow indication of 'CML in chronic or accelerated phases post-failure of two or more tyrosine kinase inhibitors'.

THE TERPENES

Terpenes made their entry in the anticancer therapy arena later than other natural products due to difficulties in their isolation and characterization. These properties are inherent to their chemistry: they do not precipitate or form crystals easily, they have very low polarity making separation of the very complex mixtures of naturally occurring terpenes a challenge until the development of adsorption chromatography, and the arrangements of their aliphatic carbons could not be readily discerned by NMR methods until the advent of bidimensional techniques. However, they are endowed with extraordinary bioactivities. Triterpenes and phytosteroids are obviously good candidates because their structures mimic those of many physiologically relevant animal metabolites such as hormones. This is less obvious for the diterpene class; however, after the discovery of the taxanes and ingenol esters this class is turning out to be the next frontier in natural drug discovery and has extraordinary promise for therapeutic approaches in the future.

Taxus brevifolia Nutt. (Taxaceae)

In the early 1960s a number of plants were collected from the USA and assessed for antitumour activity under an NCI-supported programme. One of these specimens was *Taxus brevifolia*. This plant, commonly known as the Pacific yew, is a member of the Taxaceae family and is related to the English yew (*Taxus baccata*), which is common in churchyards in the UK. The Pacific yew is a slow-growing tree common to the western coast of the USA. Active extracts were tested at the Research Triangle Institute in 1964 (Wall and Wani 1995).

The extraction of the plant material was conducted using a partitioning protocol similar to that employed in the isolation of camptothecin. The isolation of the active component employed a highly extensive partitioning procedure utilizing many steps between aqueous and organic solvents. The biological activity of the fractions was monitored at all stages and 0.5 g of the active compound was isolated. This was an exceptionally low yield (0.004%) from 12 kg of plant material and the name **taxol** was assigned to the active natural product. Taxol was shown to be active against solid tumours and highly active against leukaemia models in the mouse life-prolongation assay. The compound was also active against a melanoma cell line. As with camptothecin, structure elucidation was not a trivial process (it can still be difficult today!) and there were only a handful of techniques that could be used to determine the structure, including UV, IR, elemental analysis (determination of C, H and N composition) and mass spectrometry; there were not many NMR techniques available in the early 1960s, which made structure elucidation an extremely difficult task. The structure elucidation of this compound is an example of a masterful piece of natural product research and the reader is urged to consult the superb review by Wall and Wani (1995) and the book by Goodman and Walsh (2001).

^{1}H NMR spectra suggested that taxol had a number of ester groups attached to it and was possibly a diterpene natural product of the taxane group. Elemental analysis and mass spectrometry gave a molecular formula of $C_{47}H_{51}NO_{14}$ for taxol, but it was not possible to obtain crystals of the compound of sufficient quality for X-ray structural analysis. At this stage chemical degradation techniques were used to help elucidate the structure. Base-catalysed methanolysis yielded compounds that could be crystallized and submitted for X-ray structural analysis for structure elucidation.

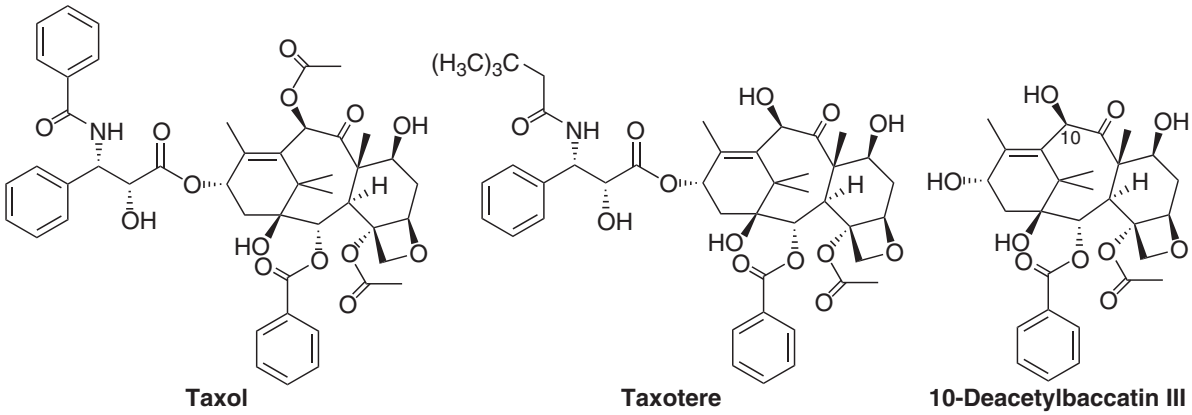


Fig. 9.10 Taxol, taxotere and 10-deacetylbaccatin III.

Elegant work, which included the use of further chemical degradation plus ^{1}H NMR and high-resolution mass spectrometry, was conducted to piece these fragments together to give the final structure of taxol (Fig. 9.10) (Wall and Wani 1995).

This was the first report of a taxane diterpene with cytotoxic activity. Taxol is a highly functional molecule possessing esters, epoxides, hydroxyls, amide, ketone groups and unsaturation. It has a large number of chiral centres (11) and is very difficult to synthesize; this was achieved in 1994 after 26 steps and is obviously not a feasible option for production on a large scale.

For the antitumour activity it is essential that the ester group at position C_{13} is present as the hydrolysed product is inactive. Work on taxol at RTI ended in 1971. Although Dr Wall and co-workers tried to get the NCI to assess taxol further, it was thought that the concentration in the plant was too low, the extraction and isolation were too difficult and the tree supply too limited. There were two developments that re-ignited interest in this compound, the first being its activity in a melanoma cell line and the discovery of its unique mode of action. Initial work suggested that taxol had similar activity to other agents that were 'spindle poisons', such as vincristine and colchicine. The work of Susan Horowitz's group demonstrated that, whilst taxol inhibited mitosis, it actually stabilized microtubules and inhibited their depolymerization back to tubulin, which is the exact opposite of other agents that bind to soluble tubulin, and inhibit the polymerization of tubulin into microtubules.

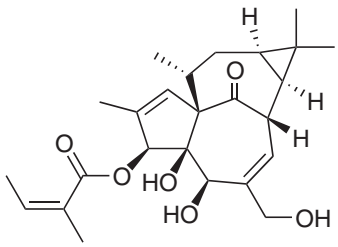
The fact that taxol worked by a new mechanism and that it was a highly unusual and novel structure encouraged further research into this agent, resulting in clinical trials and the development of analogues such as taxotere (Fig. 9.10).

The supply issues concerning taxol were overcome with its semi-synthesis by the conversion of metabolites present in larger amounts (e.g. **10-deacetylbaccatin III**; Fig. 9. 10) in the needles of the related English yew (*Taxus baccata* L.). As the needles are a renewable resource, there is no need to destroy trees by the removal of bark. Taxol is now commercially produced by plant cell culture by large-scale fermentation of the plant cells.

Taxol, now renamed **paclitaxel**, was approved in 1993 and marketed under the trade name **Taxol®** by Bristol Myers Squibb for ovarian cancer and the secondary treatment for breast and non-small-cell lung cancers. Docetaxel is marketed as Taxotere by Rhone-Poulenc Rorer and, like Taxol, prevents the mitotic spindle from being broken down by stabilizing microtubule bundles. Docetaxel, approved for use in the USA in 1995, is slightly more water-soluble than Taxol and is also administered by the intravenous route. It is used for the treatment of breast and ovarian cancers.

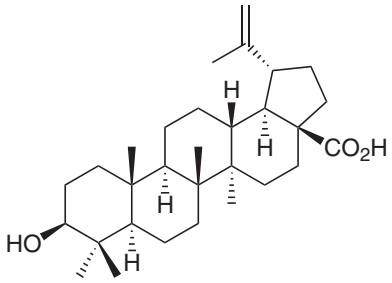
Euphorbia peplus L. (Euphorbiaceae)

The spurges (a group of *Euphorbia* species) have been known since ancient times for their topical pro-inflammatory activity and drastic laxative effects when taken internally. Their characteristic milky sap is loaded with diterpenes. Careful application onto warts removes them very effectively, but it is not devoid of local adverse effects, usually acute painful oedema. Chronic application of naturally occurring diterpenes in spurges,



Ingenol mebutate

Fig. 9.11 Ingenol mebutate.



Betulinic acid

Fig. 9.12 Betulinic acid.

notably phorbol esters, is known to both induce cancerous changes on normal cells and differentiation in cancer cells. These activities and the extraordinary diversity of diterpene skeletons in the plant kingdom provide the opportunity to find structures with valuable cancer prevention effects as illustrated by the approval in 2012 of the diterpene **ingenol mebutate** (Fig. 9.11) (commercialized as Picato®) for the topical treatment of actinic keratosis.

The development of this chemopreventative drug has its origins in the use of *Euphorbia peplus* L. in Australian folk medicine for the treatment of actinic keratoses and skin cancer. Actinic keratosis is a precancerous condition that, if untreated, usually leads to a melanoma.

The diterpene ingenol was first isolated in 1968 by Opferkuch and Hecker. These authors were searching for the '*Euphorbia* factors' (skin irritant and tumor promoting principles) present in *Euphorbia ingens* E. Mey. However, they also isolated some 'non-irritant' esters of ingenane-type polyfunctional diterpene alcohols devoid of 'reasonable tumorigenic activity in mouse skin'. Actually, they were 90% less irritant than PMA (phorbol 12-myristate 13-acetate), a naturally occurring protein kinase C activator diterpene in *Croton* spp., which is classified as a Category 2 carcinogenic chemical hazard. These authors could elucidate the structure through X-ray crystallography in 1970 and described it as endowed with a unique macrocyclic core. Pharmacological work carried out during the 1990s shown that ingenol is endowed with both antitumor and *in vitro* anti-HIV activities. Mechanistic studies of its effects on actinic keratosis unveiled a dual mechanism of action including rapid necrosis of the affected skin cells and a specific neutrophil-mediated, antibody-dependent cellular cytotoxicity. Destruction of actinic keratosis lesions is accomplished in 2 or 3 days only and the subsequent immune-mediated response prevents the development of any residual dysplastic epidermal cells.

Direct isolation of ingenol mebutate from *E. peplus* is relatively inefficient (ca. 1 mg of ingenol mebutate per kg of plant) but still commercially viable so it was chosen as the herbal drug for pharmaceutical production. Partial or total synthesis has been extensively explored as a means to obtain both the active principle and other derivatives with improved therapeutic profile. A total synthesis of ingenol was reported in 2004 by Nickel and co-workers. A more recent and viable semisynthetic approach starting from the comparatively simple and inexpensive chiral monoterpene (+)-3-carene was reported by Baran' s group in 2014.

The first post-marketing studies on the safety and tolerability of Picato® in the treatment of actinic keratosis seem to be very favourable: reported adverse effects are mild to moderate in intensity (i.e. erythema, flaking/scaling and crusting) and resolve quickly. It looks like ingenol mebutate will be a drug of choice for the treatment of actinic keratosis in the years to come.

Betula pubescens Ehrh. (Betulaceae)

Betulinic acid (Fig. 9.12) is a pentacyclic triterpene that occurs in several plants. It can be chemically derived from **betulin** (Fig. 9.12), a natural product found in abundance in the outer bark of white birch trees (formerly known as *Betula alba* L.).

There is currently interest in betulinic acid as it selectively kills human melanoma cells, leaving healthy cells alive. The incidence of melanoma has been increasing at a higher rate than any other type of cancer and, therefore, the need for new anticancer agents that selectively target melanoma is great. The effectiveness of betulinic acid against melanoma cancer cells has also been evaluated in a xenograft assay with athymic (nude) mice. When mice were injected with melanoma cells, the tumour size was observed for 40 days following injections of betulinic acid. Betulinic

acid effectively inhibited tumour growth in the mice, with little drug toxicity and side effects such as weight loss. This natural product has been shown to be an inducer of apoptosis (programmed cell death) in cancer cells with a high degree of specificity for melanoma cells. This unique specificity is unusual when compared to other cytotoxic agents such as camptothecin or paclitaxel, which exhibit a far broader spectrum of activity.

This natural product appears to have limited toxicity, is inexpensive and abundantly available from the bark of white birch trees in the form of betulin (the hydroxyl derivative), which can be readily converted to betulinic acid through a simple oxidation reaction. Both **betulin** and **betulinic acid** are currently undergoing preclinical development by a large network of clinical trial groups with the support of the U.S. National Cancer Institute. So far, a good level of evidence has been reached in the investigational treatment for actinic keratosis. In a prospective, randomized pilot study of 45 patients, the twice-daily application of betulin-based oleogel for 3 months achieved 64% complete clearing. In the future, these triterpenes may be used to complement **ingenol mebutate-**based therapies for the prevention of melanoma, although the commercialization of these ubiquitous and relatively accessible natural molecules may be hampered by difficulties in intellectual property protection.

THE LIGNANS

Lignans are polymers of phenylpropene units providing a wide array of different arrangements featuring an interesting mix of chemical functionality. Overall their chemical behaviour is somewhere between the classic shikimate polar metabolites and the terpenes. Their intermediate polarity and the difficulty in precipitating or crystallizing posed an inherent challenge to their separation until adsorption chromatography arrived. However, their structural characterization was relatively easy with classic non-spectroscopic methods. This, together with very homogeneous and powerful pharmacological effects, facilitated their clinical use as crude extracts well before isolation was a viable option.

Podophyllum peltatum L. (Berberidaceae)

Podophyllum peltatum has a number of common names, including the mayapple, Devil's apple and American mandrake, and is a perennial plant found in the woodlands of Canada and eastern USA.

The plants reach 10–45 cm in height and have long, thin rhizomes that are the underground stem from which the roots grow. The rhizomes are known to be poisonous and are the most important part of the plant, containing high concentrations of **podophyllotoxin** (see Fig. 9.1) and α- and β-**peltatin**, all of which are cytotoxic.

The closely related Asian species, *P. emodi* (syn. *P. hexandrum*), which is known as Indian podophyllum, contains these active lignan ingredients at a lower concentration. Podophyllotoxin and related lignans are also found in the rhizomes of another species *P. pleianthum*, which in Japan and China is used to make a preparation to treat snakebites and genital tumours.

The rhizomes of *P. peltatum* have a long history as a medicine among native North American tribes (Penobscot Indians of Maine). They are gathered in autumn, dried and ground to a powder, and the material is eaten or drunk as an infusion of the powder as a laxative or to get rid of intestinal worms. The powder was also used as a poultice to treat warts and skin growths. Currently, extracts of the plant are used in topical medications for genital warts and some skin cancers. However, the mayapple rhizome powder has a strong purgative action and the compounds in it are too toxic for self-medication.

Ethanolic extracts of the rhizomes are known as **podophyllin**, which is included in many pharmacopoeias for the topical treatment of warts and *condylomata acuminata*, which are benign tumours. Podophyllin resin is highly irritant and unpleasant and cannot be used systemically. The main natural product is the podophyllotoxin lignan class (see Chapter 6) of which podophyllotoxin was the first to be isolated in 1880 and the structure proposed in 1932. There are many components in podophyllin; the most important from the antitumour perspective are the lignans. **Podofilox®** is a purified form of podophyllin that acts as a cell poison against cells undergoing mitosis, and, although this extract is not a systemic chemotherapeutic agent, it is used topically in creams as a treatment for genital warts.

Chemists working at the Sandoz pharmaceutical company developed the hypothesis that podophyllin resin may contain anticancer lignan glycosides that are more water-soluble and less toxic than podophyllotoxin, and several of these natural products were isolated. The compounds did, indeed, possess greater water solubility but unfortunately had less antitumour activity. Much work was done on these glycosides to make them resistant to enzymic hydrolysis, maintain their water solubility and improve their cellular uptake.

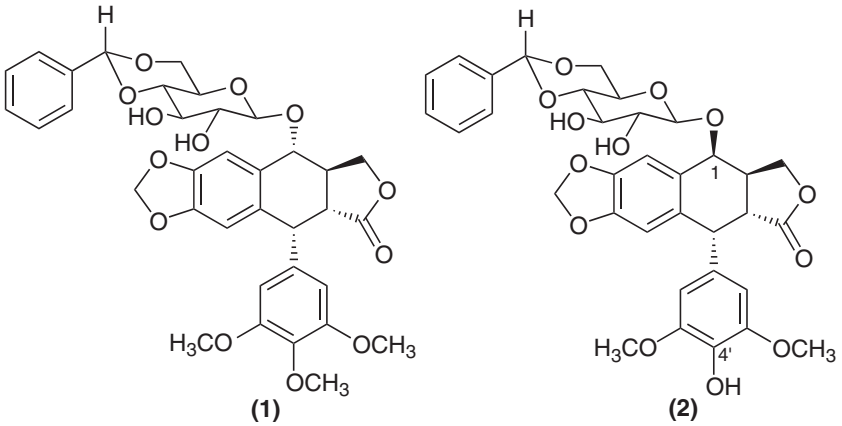


Fig. 9.13 Structures of podophyllotoxin benzylidene glucoside (1) and 4′-demethylepipodophyllotoxin benzylidene glucoside (2).

This was a difficult task as, paradoxically, improving the water solubility decreased the cellular uptake (and therefore activity).

By a serendipitous event, a crude fraction of podophyllin was treated with benzaldehyde, resulting in a mixture of products that were mainly benzyl derivatives of lignan glycosides (Stähelin and von Wartburg 1991). The crude reaction mixture was highly cytotoxic and active in the mouse life-prolongation assay against a leukaemia cell line. Some of the components of this mixture were isolated but were not as active as the whole mixture. Condensation of benzaldehyde with the reaction mixture generated a series of acetals that were not hydrolysed by glucosidase enzymes and were less water-soluble. Additionally, the crude mixture worked by a different mechanism from the purified products by stopping the tumour cells from undergoing mitosis. This preparation was marketed for cancer treatment under the name of Proresid. Work on Proresid with bioassay-guided isolation against a mouse life-prolongation assay with a leukaemia cell line indicated that there was a highly active agent present. As with paclitaxel and camptothecin, in the 1960s it was very difficult to isolate and identify minor components and the process took several years to complete. The most abundant component of Proresid is **podophyllotoxin benzylidene glucoside** (**1**) (Fig. 9.13); a minor component is **4′-demethylepipodophyllotoxin benzylidene glucoside** (**2**), which was found to be the most active agent (Fig. 9.13).

There was much synthetic work conducted to produce analogues that retain the same structural features of compound (**2**), which is epimeric at position

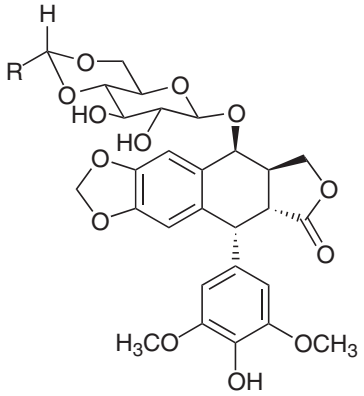


Fig. 9.14 Etoposide and teniposide.

1 and lacking a methyl at position 4′ with respect to compound (**1**). The two most important analogues synthesized so far are **etoposide** and **teniposide** (Fig. 9.14), which have much more potency than the parent compound.

Etoposide is marketed as Vepesid for small-cell lung cancer, testicular cancer and lymphomas; **teniposide** is also used in the treatment of brain tumours. Podophyllotoxin binds to tubulin and is a member of the 'spindle poison' group of agents and functions by preventing microtubule formation. Etoposide and teniposide work via a different mechanism by inhibiting the enzyme topoisomerase II preventing DNA synthesis and replication. The difference

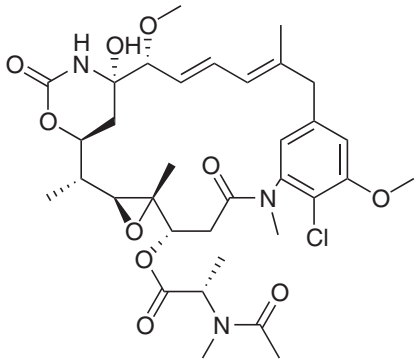


Fig. 9.15 Maytansine.

in mechanism is attributable to the small adjustment in structure with etoposide and teniposide being 4′-demethyl compounds and having different stereochemistry at position C_1.

THE MACROLIDES

Macrolides are traditionally a class of microbial secondary metabolites contributing to the defence against other organisms. Many have powerful antibiotic and cytotoxic properties towards other microbes and mammal cells, respectively. Historically, a number of macrolides have been found from plants and until recently were considered 'exotic' plant polyketides. In fact, recent scientific efforts are slowly unveiling that these 'plant-derived' natural products are actually biosynthesized by endophytic microorganisms. Despite this recent evidence, we present here maytansine as a plant anticancer product on the grounds of a fundamental pharmaceutical reason: 'plant macrolides' cannot be directly obtained from fermentation just yet, but from the whole 'plant' biomass only.

Gymnosporia buchananii Loes. (Celastraceae)

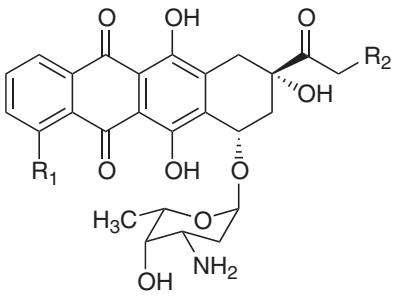
The macrolide **maytansine** (Fig. 9.15), is yet another recent success of the extensive screening of natural products fostered by the Cancer Chemotherapy National Service Center during the 1950s–1970s. The crude extract of fruits, roots and the wood of stems from Ethiopian and Kenyan *Gymnosporia buchananii* (at the time known as *Maytenus ovatus* L.), showed significant inhibitory activity *in vitro* against human carcinoma of the nasopharynx (KB cell line) and *in vivo* against five animal tumour models.

The isolation of the active principle was carried out in 1971 by Morris Kupchan and co-workers at the Department of Chemistry of the University of Virginia with a grant from the NCI. Kilogram quantities of biomass provided by the U.S. Department of Agriculture were extracted in alcohol and after removal of the solvent, partitioned in the biphasic system ethyl acetate–water. The organic layer retained the activity and pilot experiments revealed that treatment with acetic anhydride-pyridine facilitated the separation without affecting the activity. The bioguided fractionation of the acetylated extract made extensive use of column chromatography on silica gel and a final purification step with preparative TLC afforded one active principle (yield: 1 mg/kg of plant). NMR analysis complemented with X-ray crystallography of the 3-bromopropyl derivative revealed the structure and absolute configuration of a novel ansamycin macrolide with significant *in vivo* tumour inhibitory activity.

The new compound was named **maytansine** (Fig. 9.15) and showed significant antileukaemic activity against P-388 lymphocytic leukaemia over a 50–100-fold dosage range at the microgram per kilogram level, and cytotoxicity (ED_{50}) against KB cell cultures at 10^{-4} to 10^{-5} μg/ml. Morris Kupchan and co-workers already noted in their original communication that maytansine was structurally close to the rifamycins, a group of antibiotics synthesized by the bacterium *Amycolatopsis rifamycinica*. In fact, recent evidence shows that what was thought to be a 'plant-derived' natural product is actually a biosynthetic product of associated endophytic microorganisms.

Early clinical trials were initiated by the NCI in 1975 in patients with advanced disease refractory to conventional therapy. Despite the challenging cohort and a dose-limiting toxicity in the range of 1–2 mg/m^2 the positive response of a few patients warranted phase II clinical trials. By 1984 the compound was evaluated as a single agent in over 35 tumour types in more than 800 patients with only one complete and 20 partial responses. Based on these poor results, clinical research with maytansine was discontinued. The potency of this drug was too high to be ignored and preclinical research continued. During the 1980s it was established that maytansine exerted its cytotoxic activity by interacting with tubulin in the vinca domain, similarly to vinca alkaloids and the dolastatins. In the 1990s it was clear that a targeted delivery of maytansine was the only viable strategy to harness its anticancer power. In Japan, Takeda Chemical Industries set on conjugating it with a monoclonal antibody reactive to both

the drug and the human transferrin receptor (TfR). In the US, Immuno-Gen Inc. took a different approach and developed a thiol-containing maytansine derivative named DM1 which can be conveniently conjugated with many different tumour-specific monoclonal antibodies. DM1 has since been used by major pharmaceutical industries to target specific markers. In 2013 the maytansine–herceptin linked monoclonal antibody Kadcyla® was approved to treat HER2-positive breast cancer that has spread to other parts of the body (metastatic breast cancer) after prior treatment with Herceptin® and a taxane. Other conjugates are in clinical development and, if they prove successful, maytansine may become the cytotoxic component of many chemotherapy protocols in the near future.



R_1 = OCH_3, R_2 = H, Daunorubicin
R_1 = OCH_3, R_2 = OH, Doxorubicin
R_1 = H, R_2 = H, Idarubicin

Fig. 9.16 Daunorubicin, doxorubicin and idarubicin.

MICROBIAL ANTICANCER AGENTS

Microbial sources of chemical diversity are probably the most important for the pharmaceutical industry, with collection of microbes (*Actinomycetes* and fungi), culturing and fermentation leading to a greater ease of access to extracts with no need for resupply of biomass from distant sources. There are, however, issues concerning refermentation, and in some instances extracts produced by fermentation of an organism may vary. An advantage of microbes as a source of chemistry is that cultures of the microbe can be stored at −135°C indefinitely, but there is still need for material collection agreements with the source country.

Like plants, microbes are an enormous source of bioactive natural products; the anticancer metabolites are a good example, with many important therapeutic groups such as the **anthracycline, bleomycin** and **actinomycin** classes. These agents have been used for quite some time and highlight the need for further investigation of microbes as a source, and there are still many species that need to be cultured, extracted and screened, particularly amongst the fungi. Microbes are also present in many harsh environments; this may give rise to unique chemistry and thus these organisms possibly have great untapped potential.

THE ANTHRACYCLINES

This group is a large complex family of antibiotics and many were investigated before a useful antitumour agent was isolated. They are structurally and biosynthetically related to the tetracyclines (Chapter 6), being derived from the polyketide pathway. One of the first agents to be described in this class was **daunorubicin** from *Streptomyces peucetius* and *Streptomyces caeruleorubidis* (Fig. 9.16).

This agent is active against leukaemias. One of the most widely used related antitumour agents is **doxorubicin** (Adriamycin) from *Streptomyces peucetius* var. *caesius*, discovered in the late 1960s, which is highly active against a broad spectrum of both solid and liquid tumours. A number of semi-synthetic analogues have been produced, of which **idarubicin** has enhanced antitumour potency and is less cardiotoxic than doxorubicin. These compounds possess a tetracyclic linear ring system (based on **anthracene**, hence the term **anthracycline antitumour antibiotics**) to which an amino sugar is attached. These agents bind to DNA and inhibit DNA and RNA synthesis. The main antitumour action of this group is by inhibition of topoisomerase II.

THE BLEOMYCINS

This group consists of a closely related mixture of glycopeptide antibiotics from the filamentous bacterium *Streptomyces verticillus*. They were discovered in 1966 by screening of culture filtrates against tumours. The bleomycin mixture is partly resolved (purified) prior to formulation for clinical use under the name blenoxane, which consists of a mixture of **bleomycin A_2** (55–70%) and **bleomycin B_2** (30%) (Fig. 9.17). These natural products occur as blue copper chelates.

The different analogues are distinct from each other only in the terminal amine functional group and a

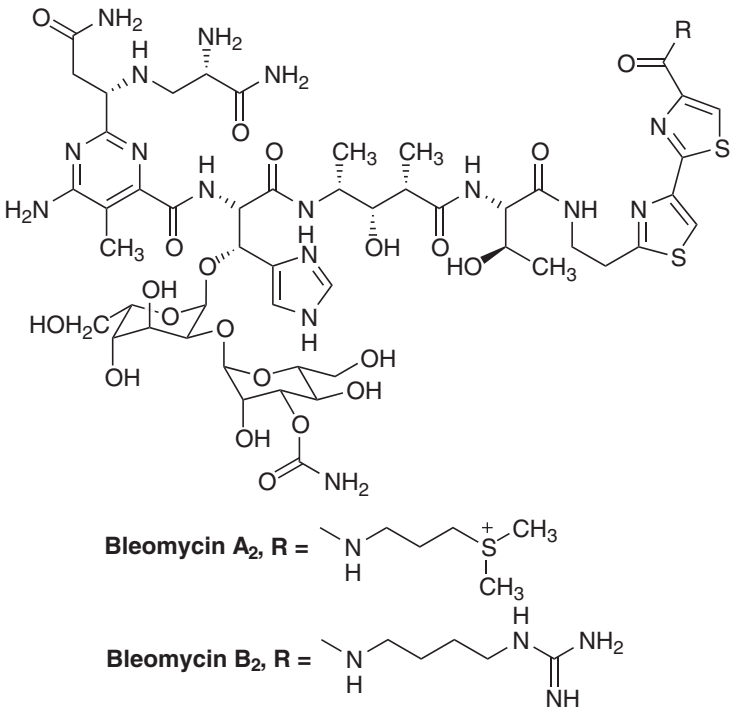


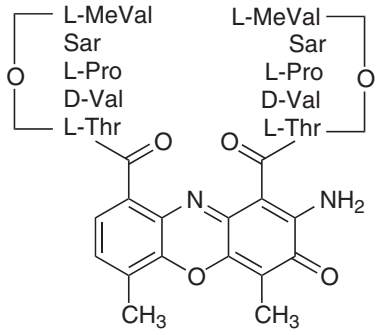
Fig. 9.17 Bleomycin A2 and bleomycin B2.

number of unusual functional groups are present in the bleomycin skeleton, including amino acids, pyrimidine rings and sugars. The bleomycins are DNA-cleaving drugs and cause single- and double-strand breaks in DNA. The dithiazole groups are essential for activity and are believed to be important in the binding of the bleomycins to DNA. This class of drugs has use in the treatment of lymphomas, head and neck tumours and testicular cancer with very little bone-marrow toxicity.

THE ACTINOMYCINS

Actinomycin D (**dactinomycin**) is an antibiotic from *Streptomyces parvullus* first isolated in 1940. It is an antimicrobial compound that is toxic and has limited use as an antitumour agent. Structurally, it consists of a planar phenoxazinone dicarboxylic acid attached to two identical pentapeptides (Fig. 9.18). A number of analogues of different peptide composition are known.

The planar group of this agent intercalates with double-stranded DNA, inhibits topoisomerase II and RNA synthesis, and can also cause single-strand DNA breaks. The principal use of this group is in paediatric tumours, including kidney (Wilms) tumours, but the use is limited due to unpleasant side effects.



Actinomycin D

Fig. 9.18 Actinomycin D.

FURTHER STRATEGIES FOR THE DISCOVERY OF ANTICANCER AGENTS

There are a number of potential strategies that can be applied to attempts to discover new natural product cytotoxic agents. The most obvious is to obtain biomass in previously unexplored environments, for example the collection of marine organisms in places such as the Persian Gulf, the Red Sea and deep-water collections in the Pacific. Such collections can only work with close

collaboration with organizations based in the countries controlling these areas. In the marine environment, there are still many areas that could harbour extensive chemical resources, such as deep sea vents, which occur along ocean ridges of the East Pacific and the Galapagos Rift. In these areas on the sea bed, superheated water seeps up from areas of geothermal activity and the environments around these vents are rich in both microscopic and macroscopic life. These organisms are subject to extremes of pressure and heat that may have profound effects on their natural product chemistry capability.

In terms of terrestrial plants, there is still much work to be done; of the 250,000 higher plant species, only a fraction (10–15%) have been systematically investigated for chemistry and biological activity. If the correct agreements can be put in place, there are still vast tracts of rainforest that are virtually untapped as sources for chemical diversity.

Terrestrial animals, such as insects, of which there are millions of species, are to all intents and purposes practically uninvestigated for biological activity, and organizations such as the Costa Rican Instituto Nacional de Biodiversidad (INBio) have been carrying out a national inventory of species, including insects. What is needed is investment in this resource, with pharmaceutical companies developing collaborations and screening extracts from these institutes.

Further investment in research is needed to extend searches into many of the world's ecosystems, some of which may well disappear with changes in climate.

Recent advances indicate that a number of what were thought to be 'plant-derived' natural products such as **taxol** or **maytansine**, or 'animal-derived' such as **ecteinascidin 743**, are in fact produced either totally or partially by endophytic microbes, frequently fungi. Whether this is due to horizontal gene transfer from the fungi to the plant or vice versa is difficult to say at this moment. Therefore, fermentation of either plant cells or microbes as a source of some natural anticancer drugs holds the promise of a more sustainable supply to an ever-expanding market. For example, the yield of Taxol (the best-selling cancer drug ever manufactured, with annual sales of the drug reaching $1.6 billion) from natural plant sources cannot be further improved. This prompted a number of patents on alternative production methods focusing on biotechnology approaches. One such patent filed by Novopharm Limited back in 1996 already describes the isolation of Taxol-producing microorganisms from the leaves, branches, twigs and bark of the ornamental yew shrub *Taxus hicksii*, which may be used in fermentation processes to produce a biomass containing taxol in relatively large quantities. Other companies such as Phyton Biotech (now part of the pharmaceutical giant Bristol-Myers Squibb), developed services to offer plant-derived natural actives utilizing a proprietary Plant Cell Fermentation (PCF®) platform technology, from a catalogue of cryopreserved plant cells covering 450 species. They can scale up the fermentation of *Taxus* cells in bioreactors and are *de facto* the only Paclitaxel supplier in the world that controls the entire upstream and downstream production process internally under strict GMP conditions.

However, very often access to microbially derived chemical diversity is limited to the supply of certain types of organisms (e.g. readily culturable *Actinomycetes*). It has been estimated that less than 1% of bacterial species and less than 5% of fungal species are currently known; thus millions of species remain undiscovered and enormous chemical diversity remains to be tapped. Moreover, research into the development of new culturing techniques will in the future give access to this chemistry. Companies such as Hypha Discovery Ltd (UK) are specialized not only in fermentation of rare exotic fungi, but also in the stimulation of dormant metabolic pathways enabling these microorganisms to synthesize metabolites, which in normal conditions would not be formed. This is possibly the reason why fungal metabolites are under-represented in anticancer drug research. This strategy will certainly provide chemical libraries with an array of potentially unique molecules.

Investment is, therefore, needed in natural product resources. At present, pharmaceutical companies are scaling down their interest in natural product drug discovery, preferring either to farm out this process to smaller companies or to investigate synthetic compounds that can be produced in-house in their thousands by combinatorial chemistry techniques. Unfortunately, the synthetic chemistry that produces these combinatorial libraries is in most cases simple, introducing limited functional group chemistry and, most importantly, little stereochemistry into the synthesized compounds. Natural products, on the other hand, are in many cases highly functional and chiral; these facets have evolved as the producing organism has evolved. In many cases it can also be demonstrated that these natural products confer an advantage on the organism, for example as antibiotic substances (e.g. the tetracyclines and macrolides).

What is more important, and what many large pharmaceutical companies seem determined to ignore, is that natural products are a tried, tested and proven route to new drugs, and that the fascination with new discovery technology is only of any value if it produces new therapeutic agents.

References

Alley, M.C., Scudiero, D.A., Monks, A., et al., 1988. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res. 48, 589–601.

Cragg, G.M., Boyd, M.R., Cardellina II, J.H., et al., 1993. Role of plants in the National Cancer Institute Drug Discovery and Development Program. In: Kinghorn, A.D., Balandrin, M.F. (Eds.), Human medicinal agents from plants. American Chemical Society, Washington, DC.

Cragg, G.M., Newman, D.J., 1999. Discovery and development of antineoplastic agents from natural sources. Cancer Invest. 17, 153–163.

Cragg, G.M., Newman, D.J., 2000. Antineoplastic agents from natural sources: achievements and future directions. Expert Opin. Investig. Drugs 9, 2783–2797.

Cragg, G.M., Newman, D.J., Weiss, R.B., 1997. Coral reefs, forests and thermal vents: the world wide exploration of nature for novel antitumour agents. Semin. Oncol. 24, 156–163.

Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D., Guo, Z., 1985. Medicinal plants in therapy. Bull. World Health Organ. 63, 965–981.

Goodman, J., Walsh, V., 2001. The story of taxol. Cambridge University Press, Cambridge.

Gottlieb, J.A., Luce, J.K., 1972. Treatment of malignant melanoma with camptothecin (NSC-100880). Cancer Chemother. Rep. 56, 103–105.

Grever, M.R., Schepartz, S.A., Chabner, B.A., 1992. The National Cancer Institute: cancer drug discovery and development program. Semin. Oncol. 19, 622–638.

Moertel, C.G., Schutt, A.J., Reitemeier, R.J., et al., 1972. Phase II study of camptothecin (NSC-100880) in the treatment of advanced gastrointestinal cancer. Cancer Chemother. Rep. 56, 95–101.

Newman, D.J., Cragg, G.M., 2016. Natural products as sources of new drugs from 1981 to 2014. J. Nat. Prod. 79, 629–661.

Paull, K.D., Shoemaker, R.H., Hodes, L., et al., 1989. Display and analysis of patterns of differential activity of drugs against human tumor cell lines: development of mean graph and COMPARE algorithm. J. Nat. Cancer Instit. 81, 1088–1092.

Stähelin, H.F., von Wartburg, A., 1991. The chemical and biological route from podophyllotoxin glucoside to etoposide. Ninth Cain Memorial Award Lecture. Cancer Res. 51, 5–15.

Wall, M.E., Wani, M.C., 1995. Camptothecin and taxol: discovery to clinic. Thirteenth Bruce F Cain Memorial Award Lecture. Cancer Res. 55, 753–760.

Further reading

Ali-Seyed, M., Jantan, I., Vijayaraghavan, K., 2016. Betulinic acid: recent advances in chemical modifications, effective delivery, and molecular mechanisms of a promising anticancer therapy. Chem. Bio. Drug Des. 87, 517–536.

Beale, J.M., Block, J., 2010. Wilson and Gisvold's textbook of organic medicinal and pharmaceutical chemistry, twelfth ed. Lippincott-Raven, Philadelphia.

Cassady, J.M., Chan, K.K., Floss, H.G., Leistner, E., 2004. Recent developments in the maytansinoid antitumor agents. Chem. Pharm. Bull. 52, 1–26.

Cragg, G.M., Grothaus, P.G., Newman, D.J., 2009. Impact of natural products on developing new anti-cancer agents. Chem. Rev. 109, 3012–3043.

Ernst, M., Grace, O.M., Saslis-Lagoudakis, C.H., Nilsson, N., Simonsen, H.T., Rønsted, N., 2015. Global medicinal uses of *Euphorbia* L. (Euphorbiaceae). J. Ethnopharmacol. 176, 90–101.

Kantarjian, H.M., Talpaz, M., Santini, V., Murgo, A., Cheson, B., O'Brien, S.M., 2001. Homoharringtonine: history, current research, and future direction. Cancer 92, 1591–1605.

Newman, D.J., Cragg, G.M., 2009. Microbial antitumor drugs: natural products of microbial origin as anticancer agents. Curr. Opin. Investig. Drugs 10, 1280–1296.

Newman, D.J., Cragg, G.M., 2007. Natural products as sources of new drugs over the last 2years. J. Nat. Prod. 70, 461–477.

Pan, L., Chai, H., Kinghorn, A.D., 2010. The continuing search for antitumor agents from higher plants. Phytochemistry Lett. 3, 1–8.

Pratt, W.B., Ruddon, R.W., 1994. The anticancer drugs, second ed. Oxford University Press, Oxford.

Ritter, J.M., Flower, R.J., Henderson, G., Rang, H.P., 2015. Rang & Dale's Pharmacology, eighth ed. Elsevier, London.

Rosen, R.H., Gupta, A.K., Tyring, S.K., 2011. Dual mechanism of action of ingenol mebutate gel for topical treatment of actinic keratoses: rapid lesion necrosis followed by lesion-specific immune response. J. Am. Acad. Dermatol. 66, 486–493.

Section 4

Medicines and nutraceuticals/botanicals derived from plant extracts

SECTION CONTENTS

Chapter 10

Production, quality control and standardization of herbal materials

PRODUCTION

High-quality herbal products can only be made with good plant starting material, and in 2015/2016 the discovery of toxic pyrrolizidine alkaloids in St John's wort products, due to contamination with weeds such as *Senecio*, illustrates this very well. Traceability is becoming increasingly important in assuring quality and starts with cultivation under Good Agricultural Practice (GAP). Wild-collected plants are still widely used but present extra challenges of authentication and/or contamination with other species in addition to concerns about sustainability and conservation. Some medicinal plants are endangered species and others are extremely expensive, such as wild ginseng and saffron, and thus even more liable to adulteration or falsification. The lack of regulation of these products means that some are of very poor quality indeed, and have caused the deaths of an unknown number of people.

Quality assurance starts with agricultural production of the plant or collection from the wild, continues with the processing and manufacture of the herbal product through the packaging and sale, and ends with post-marketing surveillance and eventual pharmacovigilance, as shown in Fig. 10.1. More information can be found in the World Health Organization (WHO) publications 'Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants' (WHO 2003); 'Guidelines on Good Manufacturing Practices (GMP) for Herbal Medicines' (WHO 2007a); 'Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems' (WHO 2004); 'Quality Control Methods for Herbal Materials' (WHO 2011) and 'Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues' (WHO 2007b).

Plant-derived medicines include traditionally used medicinal plants, which may be sold loose or in teabags to make infusions. These are sometimes referred to as *botanical crude drugs* (or 'crude drugs') and are usually unprocessed, although in traditional Chinese medicine (TCM) many different processing methods can be used. Plant extracts are also widely used and include 'instant teas', tinctures and dry extracts. These may be *standardized extracts,* where the major *active* constituents are known and can be controlled, and have well-established chemical and pharmacological profiles. *Non-standardized extracts* are less clearly characterized regarding their active components and their pharmacological effects. *Pure compounds*, or *highly purified natural products* isolated from botanical drugs are not considered to be herbal medicines and their quality assurance is the same as for any single-entity chemical substance.

As defined in the European Pharmacopoeia (PhEur 2016) and the British Pharmacopoeia (BP 2016):

Herbal drugs are mainly whole, fragmented or cut, plants, parts of plants, algae, fungi, and lichens in an unprocessed state, usually in the dried form but occasionally fresh (referred to as 'crude drugs'). They are precisely defined by the botanical scientific name according to the binominal system (genus, species, variety and author).

Herbal drug preparations are materials made from the herbal drug, for example essential oils and extracts. They are obtained from cultivated or wild plants [unless endangered and Convention on International Trade in Endangered Species (CITES) controlled] using suitable collection, cultivation, harvesting, drying, fragmentation and

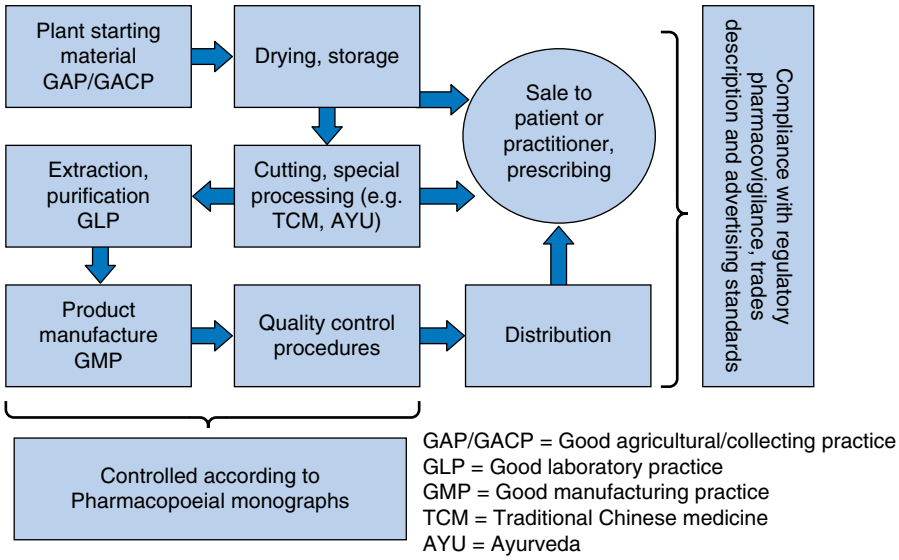


Fig. 10.1 Regulatory processes involved in monitoring the quality of herbal products.

storage conditions to guarantee the quality. They are, as far as possible, free from impurities such as soil, dust, dirt and other contaminants such as fungal, insect and other animal contaminations. In traditional Chinese medicine (TCM), processing is commonplace and requires separate monographs.

Herbal drugs are usually gathered during the flowering period (aerial parts, leaves, flowers), during spring (bark) and at the end of the vegetative season (root and rootstock). However, there are exceptions; for example, cloves, the unopened flower buds of *Syzygium aromaticum* (L.) Merr. & L.M.Perry. The time of harvesting affects the concentrations of the active constituents and is an important part of the quality assurance process.

HOW CONTAMINATION ARISES

There are many forms of adulteration, both deliberate (e.g. substituting a cheaper plant species) and accidental (misidentification of species). Contamination of the starting material will lead to contamination of the final product, and other factors may be involved, such as poor storage conditions that allow spoilage due to fungal and bacterial growth, as summarized in Box 10.1.

In addition to the factors relating to the raw material listed above, production methods including extraction and further processing influence the composition of the final product, shown as an overview in Fig. 10.2.

MEASURING QUALITY DURING PRODUCTION: THE ROLE OF THE PHARMACOPOEIA

Monographs providing quality standards, usually from an official source such as a pharmacopoeia, are available and are legally enforceable. They provide the means for an independent judgement as to the overall quality and apply throughout the shelf-life of a product. Inclusion in a pharmacopoeia does not indicate that a substance is either safe or effective for the treatment of any disease; it gives guidance only on quality.

However, if the plant has been grown according to GACP, checking its botanical identity should be straightforward, and if a crop has been grown organically, and is certified as such, it is not necessary to carry out pesticide residue testing at the stage of production, unless there is cause to suspect contamination. This illustrates an important point: herbal material sold as pharmacopoeial quality does not have to be subjected to every test routinely as part of its quality assurance; however, it must conform to those standards and is expected to pass the tests if carried out. Pharmacopoeial methods are accepted by regulatory authorities, and if different procedures are used to assure quality, these must be validated.

The EU, UK, US, China, India, Japan and other countries publish official standards for herbal drugs and there are others, such as the American Herbal Pharmacopoeial monographs, which are not official but are very comprehensive and well-referenced.

BOX 10.1 How contamination of herbal materials arises

At the growing and collecting stage:

- Growing under poor environmental conditions (soil, light, etc.)
- Harvesting at the wrong time of the plant growth cycle
- Poor agricultural or collecting practice, e.g. contamination with toxic weeds (e.g. *Senecio* and *Aristolochia* species)
- Contamination with human pathogens such as *Escherichia coli* and *Salmonella* spp. from sewage
- Misidentification of species, e.g. *Belladonna* leaf mistaken for *Arctium lappa*
- *Foreign organic matter*: inclusion of other plant species or non-medicinal parts (e.g. stalks and twigs in fruit and flower material)
- *Foreign inorganic matter*, such as excess soil and gravel in roots

At the production stage:

- Insufficient drying, leading to spoilage and toxic fungal metabolites, e.g. aflatoxins, or degradation of the active constituent to more toxic compounds (e.g. coumarins converted to the anticoagulant dicoumarol in *Melilotus*)
- Poor storage, allowing contamination with insects and animal parts such as mice and rat faeces and hairs
- Deliberate falsification, e.g. cheap liquorice substituted for expensive ginseng
- Use of 'exhausted' plant material (previously extracted and re-dried)
- Use of inferior raw material for producing extracts
- Introduction of isolated chemical constituents to boost content of 'actives', such as synthetic terpenes to an essential oil
- Photo-decomposition, e.g. essential oils, cardiac and anthraquinone glycosides
- Atmospheric oxidation: resinification of essential oils, rancidification of fatty oils

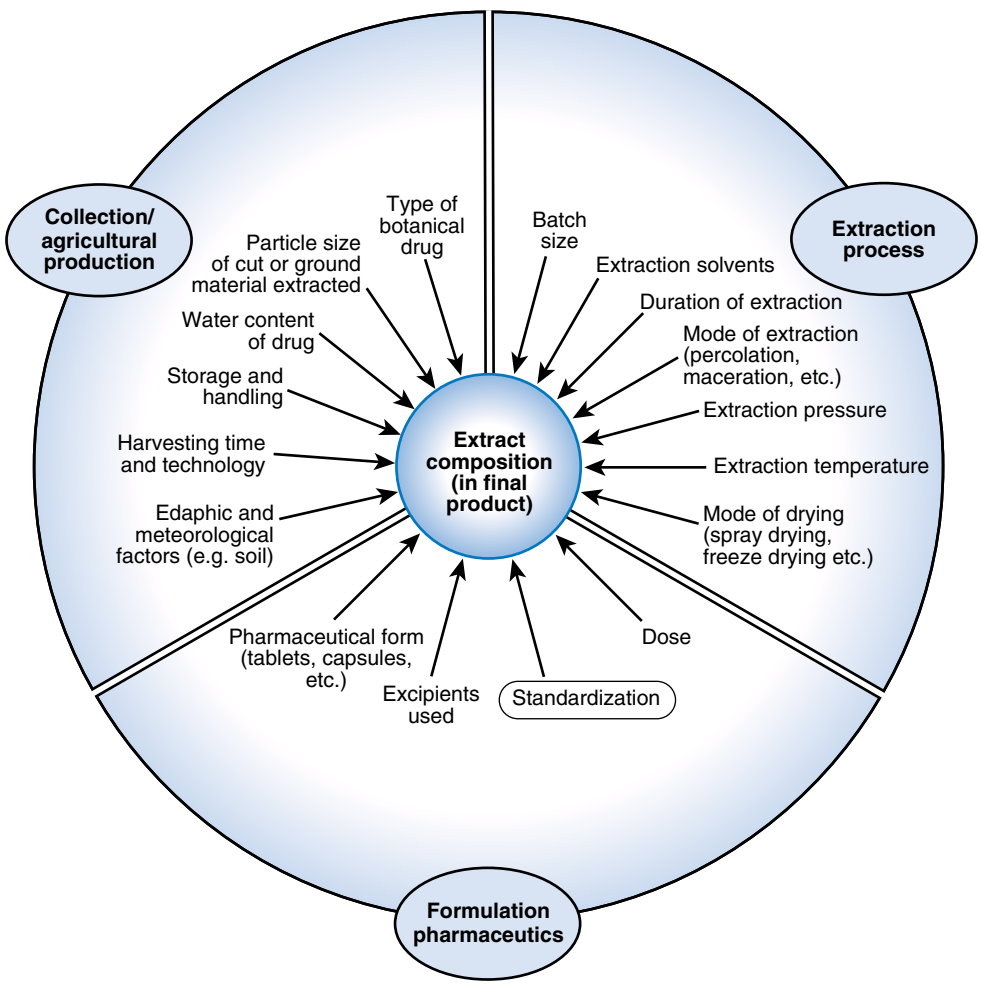


Fig. 10.2 The extraction process and factors that influence the composition and quality of an extract and the final product.

BOX 10.2 Methods used in pharmacopoeial monographs

- Macroscopical and microscopical descriptions, and chemical/chromatographic tests for identification
- DNA tests for identification of species in some cases (under development)
- Tests for absence of any related species
- Microbial test to assure microbial quality
- Tests for inorganic impurities and non-specific purity tests, including extractives, ash values and tests for heavy metals where appropriate
- Moisture content test, such as *Loss on drying* or *Water*
- Wherever possible, a method for assaying the active constituent(s) or suitable marker constituent(s)

Monographs usually follow a format similar to that shown in Box 10.2, and the following summary discusses the tests in the order in which they are covered in a typical herbal monograph.

Identification

Botanical identification. A pharmacopoeial monograph provides standards for botanical identification of herbal material, for the macroscopic appearance (i.e. that visible to the unaided eye or with a hand lens) and also its microscopic characteristics. Most botanical drugs are supplied in a cut form, and may be difficult to identify, and in such cases, microscopy can help to confirm identity. It is also useful for powdered samples, although most powders are much more easily degraded and commercial trade tends to be in cut and dried samples or extracts. Microscopy is often overlooked as an analytic tool; however, with some very basic equipment and a small amount of training it is possible to gain a great deal of information quickly and easily. It is also very useful for picking out micro-contamination such as sand, fungal hyphae, insect parts and animal hairs, as these may be too small to see with the unaided eye but are very obvious when viewed under the microscope.

Microscopy is also useful for distinguishing which part of a plant the powder is made from. It may be difficult to distinguish a leaf from a whole herb powder by just looking at it, but the botanical material can be identified because of the presence of large amounts of stem (which are limited in leaf drugs) as well as elements of the flower and seed. Pollen grains are useful for identifying medicinal plants but may also be present in good leaf samples because pollen is so ubiquitous.

Figures 10.3 to 10.5 show examples of microscopical characteristics of the major plant organs and tissues that can be used to identify medicinal plants. Their abundance or absence indicates the part of plant, and the characteristic appearance of plant cell types, discussed next, is the basis for identifying the species. Pictures and photographs of macroscopical and microscopical features of many herbal drugs are now included in pharmacopoeial monographs, and their online versions, and should be consulted for further information and examples.

Plant cell types as diagnostic features. Certain plant cell types are found in all plant tissues, for example parenchyma, the 'basic' cell type and also xylem (water-conducting tissue), but their abundance can indicate the tissue type: for example, bark is composed of mainly cork and phloem, with its associated parenchyma and fibres, but very little xylem tissue, because it has been removed from the outer surface of a tree or root, whereas heartwood contains almost only xylem elements, together with its associated parenchyma and fibres. Other cell types occur only in particular organs, for example epidermis, trichomes (hairs, both covering and glandular) and stomata in plant parts, such as the leaf, which are in contact with the atmosphere. Cells inclusions, such as starch, are highly diagnostic of plant storage organs, such as roots and rhizomes, as well as seeds and fruits, which often have a high content of oils and proteins.

Some examples of characteristics that can be seen in powdered botanical drugs are illustrated in Figs 10.3, 10.4 and 10.5, but this is not a comprehensive list, and there is insufficient space here to cover microscopic techniques and the diagnostic characteristics of individual herbal drugs. Further detailed information can be obtained from most pharmacopoeias, the American Herbal Pharmacopoeia (AHP) 'Microscopic Characterization of Botanical Medicines' (Upton et al 2011) and the classic 'Atlas of Microscopy of Medicinal Plants, Culinary Herbs and Spices' by Jackson and Snowden, republished in 2005, which is available online. These publications include guidelines for slide preparation and clearing and staining techniques, as well as contain illustrations of powdered botanical drugs. Photographs can also be viewed in the AHP and on the websites of many pharmacopoeias.

The overview below of leaf characteristics, which are crucial for identifying herbaceous plant material; lignified tissues, which are important diagnostic features

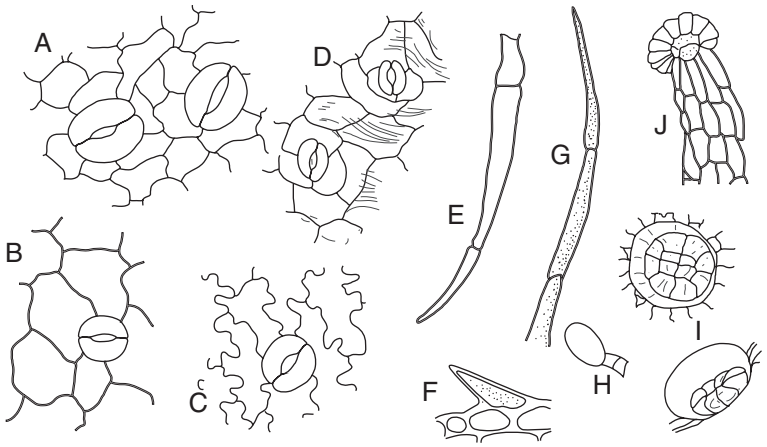


Fig. 10.3 Leaf epidermal tissues in powdered botanical drugs (varying magnifications). (A) Leaf epidermis with anomocytic stomata (i.e. no particular arrangement); (B) epidermis with thickened ('beaded') walls; (C) wavy-walled epidermis; (D) strait-walled epidermis with anisocytic stomata (i.e. one much smaller adjacent cell) and cuticular; (E) multicellular covering trichome with smooth walls; (F) unicellular conical trichome; (G) multicellular covering trichome with thick, warty walls; (H) small glandular trichome with unicellular head; (I) glandular trichome characteristic of the mint family, surface and side view; (J) unusual glandular trichome, unique to cannabis.

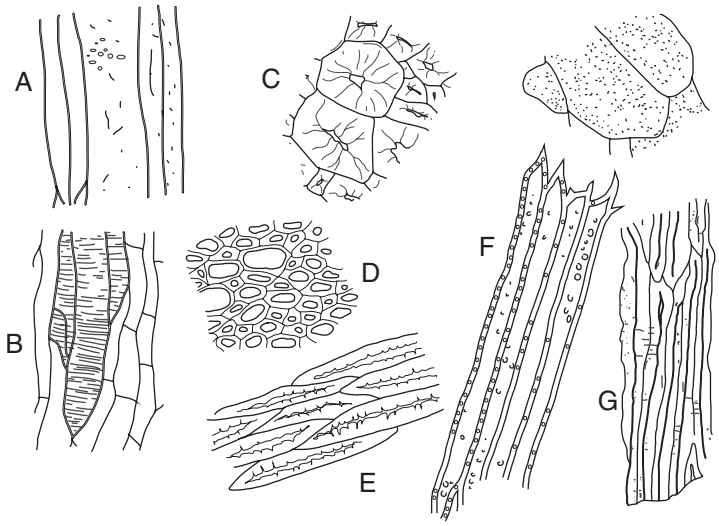


Fig. 10.4 Lignified tissue in powdered botanical drugs (varying magnifications). (A) Xylem vessels, bordered pitted; (B) xylem vessels, reticulately thickened; (C) thick-walled stone cells; (D) isodiametric stone cells from seed testa; (E) thick-walled sclereids; (F) thin-walled fibres; (G) thick-walled fibres.

in most herbal drugs and especially barks, roots, seeds and other woody materials; and some cell inclusions shows the application of microscopy to identification.

Leaf characteristics. Fragments of the epidermis indicate the presence of leaf, stem, fruit or flower parts. The epidermis is a waterproof layer of cells and contains pores known as stomata that open and close to allow passage of air and water, which are usually more abundant on the lower surface of the leaf. The shape of the cell walls and arrangement of the cells and also the stomata are characteristic of plant families and sometimes individual species. Some epidermises have a striated waxy cuticle that can be seen as faint lines on the cell surface and some have thickened cell

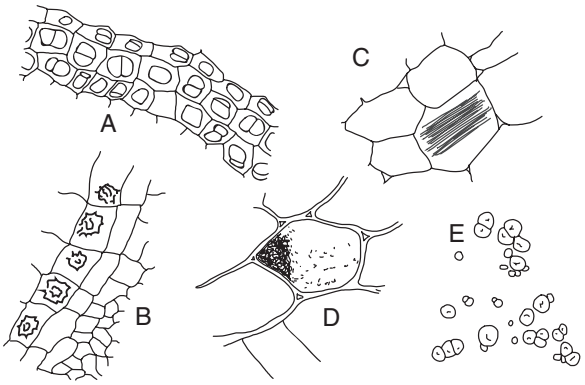


Fig. 10.5 Cell inclusions in powdered herbs (varying magnifications). (A) Calcium oxalate prisms arrangement in a crystal sheath; (B) calcium oxalate cluster crystals; (C) calcium oxalate needle crystals; (D) calcium oxalate microsphenoids or crystal sand; (E) starch grains, simple and compound.

walls, giving a beaded appearance. Trichomes (hairs) may be present and are useful characteristics for identifying a plant family, genus and even species. There are two types, covering and glandular trichomes, but within these types there are a wide variety of shapes and sizes, from unicellular to highly organized multicellular structures, and they may have thin or thick walls and a warty appearance.

Some examples of microscopic leaf features that can be seen in powdered herbal leaf drugs are shown in Fig. 10.3. Other plant parts are present in herbs (whole plant or aerial parts), which contain more fragments of stem and also flower and fruiting parts.

Lignified tissue. Lignified (woody) cells are present in older plants and organs that need strength to support the plant, as with stems and tree trunks, and to confer protection, as in seeds and fruits. Lignified cells include fibres that are long, narrow, tapered, thick-walled cells found in groups and adjacent to specialized cells, such as phloem and xylem, to provide strength with flexibility. In a few cases (notably ginger rhizome) the fibres are not lignified, but this is unusual. Xylem (water-conducting) elements are also usually lignified, especially in older tissues, and parenchyma cells may also become lignified through age and where extra supporting strength is required. Sclereids are thick-walled cells and if the walls are very thick indeed, these are called stone cells. They are mainly isodiametric, but sometimes with projections; for example, giving a stellate appearance. They usually occur in groups and confer hardness in a tissue without the long fracture associated with fibres (in other words, they snap rather than bend) and are very common in barks and seeds, and the 'stones' of soft fruit. As lignin is waterproof, they have pores or gaps in the thickening to allow passage of water and air to other parts of the organ, and these patterns of thickening may also help in identification of plant material. Lignin can be identified by staining, as explained by Upton et al (2011) and Jackson and Snowden (2005). Some of the most common types of lignified tissue, from different medicinal plants showing their different types of thickening, are illustrated in Fig. 10.4.

Cell inclusions. Substances such as starch, calcium oxalate, protein, inulin, silica, calcium carbonate and oils and fats are stored in cells and can be used as diagnostic tools. Calcium oxalate crystals occur in various shapes and sizes: tiny particles known as microsphenoids or crystal sand; cluster or rosette crystals; needle crystals or raphides; and prism crystals, which are often found alongside fibres as a crystal sheath. They can be viewed under polarized light to confirm their identity (Fig. 10.5).

Starch is extremely common in storage organs of plants, and occurs in simple and compound granules. It can be stained with iodine solution (it goes dark blue) or viewed under polarized light, where it shows an interesting light on dark cross pattern. Starch must be viewed before the slide is cleared to look for other cell features.

Chemical identification. The main supporting technique for identification is by chemical analysis, most commonly fingerprinting using thin-layer chromatographic (TLC) methods. High-performance TLC, i.e. HPTLC, is now considered the gold standard for fingerprinting techniques (Reich 2007). Although it is not absolutely necessary to use specialist equipment to carry out HPTLC, if standardization of all steps is applied strictly, it is certainly more efficient to do so. HPTLC is covered elsewhere in this book and explains the techniques described in the pharmacopoeia in detail. Monographs give precise details as to how to conduct the analysis using validated methods and contain descriptions and illustrations of the expected results.

Tests. Related species commonly found as adulterants, which are sometimes highly toxic, may be included with the authentic drug or a herbal preparation. For example, adulteration of star anise, *Illicium verum* Hook.f., with Japanese star anise fruit, *Illicium anisatum* L., has caused deaths in infants who were given an infusion to soothe colic. Microscopical examination of the powdered fruit only shows one difference between these species, in the shape of the stone cells, and a chemical test is needed in addition, to show the absence of anisatin, the neurotoxin present in *Illicium anisatum.*

Foreign matter (see Box 10.1), is specified in the BP and PhEur as not more than 2%, unless otherwise specified. It is measured simply by weighing 100 g to 500 g of the herb, spreading it out in a thin layer, examining with the unaided eye or a lens (6 ×), separating out foreign matter, weighing it and calculating its percentage.

Foreign inorganic matter measures the amount of dust, sand or soil present on the herb, and is mainly applied to underground organs such as roots, rhizomes and tubers. It is measured as an *ash value*, calculated by incinerating a sample of the herbal drug. However, crude drugs contain inorganic substances (calcium salts especially) naturally, so to differentiate these, the *total ash* can be dissolved in weak acid, leaving the *acid insoluble ash* as a measure of the soil and sand content.

Water content, if too high, may result in spoilage by bacteria and moulds and enzymatic degradation. It is usually measured as '*loss on drying*' or (less often) by chemical methods. For high volatile oil-containing plants (>1% oil): distillation methods are used instead, because the drying process would also remove some of the essential oil and give an inaccurate result.

There are other tests that can be performed on specific herbal drugs; for example, the *extractable matter* (which shows if herbal material has been previously extracted), the *swelling index* (for mucilage-containing botanical drugs and bulk laxatives), and the *bitterness value*.

TESTS FOR BIOLOGICAL CONTAMINATION OF HERBAL MATERIAL

Microbial examination

Some level of microbial burden is unavoidable, and limits are set for total aerobic microbial count (TAMC) and the total combined yeasts/moulds count (TYMC). Contamination with human pathogens (mainly from sewage), such as *Escherichia coli* and *Salmonella* spp., is tested for specifically: *E. coli* levels are limited and *Salmonella* must be absent.

Microbial toxins and mycotoxins

Fungal infection (usually from incomplete drying) can produce carcinogenic, mutagenic, teratogenic and hepatotoxic compounds, such as the aflatoxins and ochratoxin A, produced by *Aspergillus* species. Tests for these are included in some herbal drugs, such as liquorice.

Specific compounds

Certain well-known toxins, such as the aristolochic acids and the pyrrolizidine alkaloids, are tested for if there is any likelihood of them being present due to contamination with weeds or as part of a traditional formula.

TESTS FOR CHEMICAL CONTAMINATION OF HERBAL MATERIALS

Heavy metals

Contamination (with lead, arsenic, mercury, cadmium, etc.) may arise from environmental pollution. Some metals may be accumulated in certain plants, and are then even further concentrated during processing. Lead and arsenic have also been found in imported herbal products, where they have been included as part of a traditional formula.

Unless otherwise stated, the limits are: cadmium, 1.0 ppm; lead, 5.0 ppm; mercury, 0.1 ppm.

Pesticide residues

The most important are the persistent chlorinated and organophosphate insecticides. Many of these are banned globally, but poor agricultural practices and lack of traceability may lead to occurrences.

ASSAY

An assay is included whenever possible, and only in unusual circumstances (such as where the chemical composition of the plant is incompletely known or a validated method is proving difficult to develop) is this not the case. Work usually continues on incomplete monographs and an assay is added as soon as possible. The assay is usually HPLC-based. In some older monographs it may be colorimetric. It is intended to measure at least one of the active substances, if known! The relevant phytochemical techniques involved are discussed in the analysis section [see Ch 7].

MONOGRAPHS FOR TRADITIONAL HERBAL MEDICINES

The increase in global use of Traditional Herbal Medicinal Products (THMP), now classed as medicines under EU Directive 2004/24/EC, has led to a surge in efforts to define their quality through the collaboration of analysts from all over the world, particularly

China and India. The same standards are required for THMPs as for any other herbal drug, but the monograph may also cover aspects such as traditional processing methods, which may require the addition of processing aids, for example, honey, vinegar, wine, milk and salt, and may also involve heat treatment by stir-baking or roasting. See Zhao et (2010) for more information.

EXTRACTS

Herbal products often use extracts rather than raw material, to provide a more concentrated form of the active constituent(s). Extracts are prepared from the herbal starting material that complies with the monograph for that herb, and are also subjected to similar quality-control analysis, although their identification does not involve botanical characters of course. In addition, tests for residues are included for extracts that have been produced using organic solvents.

The method of extraction, for example infusion in hot water (herbal tea or tisane), decoction (boiling in water), percolation (repeated extraction with hot solvent), maceration (soaking), and pressing of fresh plant material (for expressed juice, oils or fats) depends on the type of raw material and the finished herbal product required. Leaf drugs are more suitable for infusion, whilst hard and woody drugs such as barks and roots may require decoction or percolation.

DRUG–SOLVENT RATIO (DSR) AND DRUG–EXTRACT RATIO (DER)

Simple parameters can be stated for an extract by defining it in relation to the amount of starting material, the type of solvent(s) used and the mode of extraction. These are rarely used nowadays, as better analytical methods are available, but may occasionally be applied to crude extracts. If included in a monograph, an acceptable range for both may be specified.

The *drug–solvent ratio (DSR)* represents the amount of plant material used to produce a measured amount of extract. It is calculated simply by dividing the amount of herbal material by the amount of solvent used: e.g. a DSR of '1:4 (ethanol 70%) by maceration' means that the herbal drug was macerated with a fourfold amount of 70% ethanol.

The *drug–extract ratio (DER*) is more useful: it gives information on *the amount of extract obtained from the herbal drug.* Therefore, a DER of 4:1 (maceration, 70% ethanol) means that 4 units (e.g. kg) of the herbal drug have yielded 1 unit of dried extract. The DER varies considerably depending on the herbal drug and the solvents used. If chamomile flowers, for example, are extracted in water, the DER is in the range 6–8:1 (meaning that 6–8 kg botanical drug produce 1 kg extract); however, if turmeric is extracted with 96% ethanol, the DER typically is in the range of 20–50:1. In other words, in the first case a large amount of extract can be obtained from a botanical drug (12–18%), in the second case only a very small amount (2–5%) can be obtained.

TYPES OF EXTRACT

As explained under 'production', different kinds of extract can be prepared, which exert varying degrees of control over the concentration of the active ingredients:

Standardized extracts are adjusted to a defined content of one or more constituents with known therapeutic activity. This is achieved by adjustment with inert excipients or by blending batches of the extract.

Quantified extracts are adjusted to one or more active markers, controlled within a limited, specified range. Adjustments are made by blending batches of the extract.

Other extracts are not adjusted to a particular content of constituents. For control purposes, one or more constituents are used as analytical markers. The minimum content for these analytical markers is given in an individual monograph.

Standardized herbal extracts

Standardization is 'the establishment of reproducible quality by comparing a product with established reference substances and by defining minimum and sometimes maximum concentrations of one or more compounds or groups of compounds'. It is important for the safe use of potent medicinal plants, and for conducting clinical studies, to define more precisely the composition of the herbal drug under investigation. It also enables a dose regimen to be defined so patients can purchase herbal products and self-medicate. There are monographs for standardized extracts for *Senna, Digitalis, Belladonna, Ipecacuanha* and others in the BP and PhEur, reflecting their well-known chemical composition and potent effects.

Standardization may be related to one compound or a range:

Defined single content: for example, for *Ipecacuanha liquid extract, standardized*, the content of total alkaloids is stated as 1.80–2.20%, calculated as emetine. The acceptable tolerance is usually within the range ± 5% to ± 10% taking into account the nature of the extract and the method of assay.

Defined range of content: for *Frangula bark dry extract, standardized*, the content of assayed constituents is stated as 15.0–30.0% – much broader than for *Ipecacuanha*. However, it is intended that an extract will consistently be produced within the defined range, taking into account an acceptable tolerance.

Special extracts

Production of a standardized extract can include combining batches to give a consistent product, and/or removing unwanted constituents to give a more concentrated product. It does not include the addition of isolated substances, of herbal or other origin, or anything not normally found in the plant, unless as part of a finished herbal product and stated on the label.

Quantified extracts. Some extracts are assayed, but the contents are not adjusted, and their content must be within the values given in the definition section of an individual monograph. If a plant is grown and processed under controlled conditions, then the resulting extracts will have a certain amount of batch-to-batch uniformity.

Refined extracts. Extracts may be processed to remove unwanted constituents. One example is *Ginkgo*, which is sometimes refined to limit the levels of ginkgolic acids (considered to be allergenic) to less than 5 ppm. A refined extract is usually further characterized as a *Refined and standardized extract*, as for Bilberry, or a *Refined and quantified extract*, as for *Ginkgo*, shown below. Any suitable method of production can be used, as long as it gives the defined levels of constituents required, as illustrated in Box 10.3, which shows the limits required for Refined and Quantified *Ginkgo* Dry Extract.

BOX 10.3 Limits of content in refined and quantified ginkgo dry extract

Definition

Refined and quantified dry extract produced from *Ginkgo leaf*.

Content

- Flavonoids, expressed as flavone glycosides: 22.0–27.0% (dried extract)
- Bilobalide: 2.6–3.2% (dried extract)
- Ginkgolides A, B and C: 2.8–3.4% (dried extract)
- Ginkgolic acids: maximum 5 ppm (dried extract).

Production

The extract is produced from the herbal drug by an appropriate procedure using organic solvents and their mixtures with water, physical separation steps as well as other suitable processes.

NEW METHODS FOR QUALITY ASSURING HERBAL PRODUCTS

Herbal medicines are so complex that no current methods are ideal for assuring their quality, but given the importance of correct identification of the plant species, which cannot be done in the same way as for single-entity drugs, improving authentication is a good place to start. Botanical identification, unlike other pharmacopoeial tests, is unique to herbal medicines.

DNA BARCODING METHODS FOR QUALITY TESTING

With advances in molecular genetics, reliable methods of identifying medicinal plants are now available using their DNA profile. The use of DNA-based methods is controversial, and their appropriate use is not always understood. A case in 2015 where the US Attorney General published a report on herbal products suggesting that 'in a large number of the tested products, there was no detectable plant DNA whatsoever' was widely criticized on scientific grounds, including the fact that extracts do not contain DNA! (see Tyler 2015). Nevertheless, there are some distinct advantages to using these methods. The first routine methods, which used to clearly distinguish the pharmaceutically used species from other ones was published in 2017 in the BP: *Ocimum tenuiflorum* L. or tulsi.

Advantages of DNA-based methods

- DNA profiles are highly accurate and diagnostic, if used in context.
- DNA-based methods are ideal for routine screening, once the method is set up.
- They are highly sensitive in discriminating between closely related species.

Limitations of DNA-based methods

- DNA profiles provide only information about the botanical identity of the plant, not the quality of the herbal material, which is affected by environmental factors explained earlier and summarized in Fig. 10.2.
- DNA analysis does not give information on the part of plant included.
- DNA-based methods cannot be used in extracts from which the plant material has been removed.
- Processing methods may destroy the DNA.
- DNA will not pick up contamination with any plant that is not actively being looked for.
- It is very complicated to use these techniques on multi-herbal formulae.
- There is a high risk of contamination of the working materials with environmental, user or different sample DNA.

The BP method uses short regions of DNA with species-specific sequences as barcodes for recognition. In the BP, selected barcode sequences are the basis for any molecular identification technique, and where a DNA-based method is specified, the identified region and its sequence will be published as part of the monograph. DNA barcoding of plant material is achieved in multiple stages involving extraction, polymerase chain reactions (PCRs) and Sanger sequencing. General guidance on how to conduct DNA-based identification methods for herbal drugs is also included in the new Appendix to the BP 2016, but other methods can be used if validated.

CONCLUSIONS

Quality assurance of herbal products can only be guaranteed by taking care throughout the entire process, from growing the medicinal plant to its eventual administration to a patient. Pharmacopoeial standards and guidelines for good practice are there to assist at all stages, for the benefit of the manufacturer, who has a responsibility to ensure that the product is safe and of good quality, and also the consumer, who needs that assurance too. Monographs are produced by the secretariat of the relevant pharmacopoeial commission, supported by dedicated analytical laboratories and in collaboration with groups of independent experts.

Many herbal producers, especially those making THR products, already carry out additional tests that are not mandatory in the pharmacopoeial monograph. They also assist in the work of the pharmacopoeia by seconding staff as members of expert groups, sharing analytical methods, taking part in collaborative trials and testing new methods, usually at no charge. This may benefit the company in that its own methods are included, but it does not confer a monopoly since other methods can be used as long as they are also validated. For the small producer, using a BP or PhEur method means they have easy access to validated methods.

Finally, it should be noted that if an official monograph is available for any medicinal plant or herbal preparation, compliance with such a monograph is usually essential for inclusion in a registered product.

References

Council of Europe, 2017. European Pharmacopoeia. Coucil of Europe, Strasbourg. Available online at http://online.edqm.eu/EN/entry.htm.

Jackson, B.P., Snowden, D., 2005. Atlas of Microscopy of Medicinal Plants Culinary Herbs and Spices. CBS Publishers & Distributors, New Delhi.

Medicines and Healthcare Products Regulatory Agency (MHRA), 2017. British Pharmacopoeia. MHRA, London. Available online at https://www.pharmacopoeia.com.

Reich, E., 2007. High-performance thin-layer chromatography for the analysis of medicinal plants. Springer-Verlag, Berlin.

Tyler, S., 2015. The supplement saga: a review of the New York Attorney General's herbal supplement investigation. HerbalGram 106, 44–55. Available online at www.herbalgram.org.

Upton, R., Graff, R., Jolliffe, G., Länger, R., Williamson, E.M. (Eds.), 2011. American Herbal Pharmacopoeia: Microscopic Characterization of Botanical Medicines. CRC Press, Boca Raton.

WHO, 2003. Guidelines on good agricultural and collection practices (GACP) for medicinal plants. WHO, Geneva. Available online at http://apps.who.int/medicinedocs/en/d/Js4928e/#Js4928e.

WHO, 2004. Guidelines on safety monitoring of herbal medicines in pharmacovigilance systems. WHO, Geneva. Available online at http://apps.who.int/medicinedocs/en/m/abstract/Js7148e/.

WHO, 2007a. Guidelines on good manufacturing practices (GMP) for herbal medicines. WHO, Geneva. Available online at http://apps.who.int/medicinedocs/en/m/abstract/Js14215e/.

WHO, 2007b. Guidelines for assessing quality of herbal medicines with reference to contaminants and residues. WHO, Geneva. Available online at http://apps.who.int/medicinedocs/en/m/abstract/Js14878e/.

WHO, 2011. Quality control methods for herbal materials. WHO, Geneva. Available online at http://apps.who.int/medicinedocs/en/m/abstract/Jh1791e/.

Zhao, Z., Liang, Z., Chan, K., et al., 2010. A unique issue in the standardization of Chinese materia medica: processing. Planta Med. 76 (17), 1975–1986.

Further reading

Wagner, H., Bauer, R., Melchart, D., Xiao, P.-G., Staudinger, A., 2011. Chromatographic Fingerprint Analysis of Herbal Medicines. Thin-layer and High Performance Liquid Chromatography of Chinese Drugs, second ed. Springer Verlag, Berlin.

Chapter 11

The complex chemistry and pharmacology of herbal medicines

THE NATURE OF HERBAL AND PHYTOMEDICINES

Herbal medicines, as complex mixtures of compounds (even if consisting of only one herbal ingredient), have particular attributes that single compounds do not. There is also a continuum between products that are mainly (health) foods, composed of benign and innocuous compounds, and those that are potent (herbal) medicines, and contain highly toxic moieties. Generally, highly toxic drugs must be used as single chemical entities since the dose needs to be very precise, but if the active principles are generally safe, the natural mixture found in a plant extract may have benefits conferred by interactions between the components. There may be toxic ingredients present that do not contribute to the therapeutic benefits, as discussed in Chapter 12, and these botanical drugs should be avoided in clinical practice.

Phytomedicines often take a while to produce a measurable improvement and appear to have a cumulative effect; for this reason, long-term therapy is routine. This is not a unique property of natural products but is also found in conventional medicines (e.g. the antidepressants, where several weeks of treatment may be necessary before a clinical improvement is seen). The use of drug combinations is also not confined to herbal products; for example, cancer chemotherapy and the treatment of HIV and hypertension routinely use drug combinations. In addition, although many phytomedicines may have been characterized phytochemically, their mechanism of action is still unknown, which makes isolation of one constituent impossible.

PROPERTIES OF MIXTURES (WHOLE AND REFINED EXTRACTS)

Herbal practitioners have always held the tenet that the effects of combinations of ingredients, such as those found in herbal medicines, contribute to their efficacy, but until recently there has been little clinical evidence to demonstrate that this is the case. Indirect evidence may be useful where it appears that the dose of an 'active' constituent, calculated on the basis of a single compound, is too low to have an effect, even though the clinical and pharmacological evidence has shown that the preparation is in fact effective. This is the case with willow bark, discussed later. In general, synergistic and other interactions within herbal mixtures are considered to be positive, enabling lower doses of potent compounds to be used and reducing the incidence and severity of side effects. The properties of mixtures described below apply to all types of combinations between two or more drugs, whether of herbal or synthetic origin. They also apply to drug interactions, which are also a form of synergistic, antagonistic or additive effect, and the mechanisms of interaction are the same. These are discussed in Chapter 19 Herbal medicine interactions.

SYNERGISTIC, MULTI-FACTORIAL AND POLYVALENT EFFECTS

Synergy and other forms of interaction between the constituents of herbal extracts are expected, and widely cited but poorly documented. Interactions may result in enhancement of a therapeutic effect, reduction of toxicity or preservation of stability. Synergy is

a specific type of interaction, and needs to be proven experimentally; however, although it may take place, it may not actually be the most important type of interaction occurring in herbal mixtures. Not only may two or more components of a mixture interact with each other, single constituents may interact with different pharmacological targets. Thus, the various interaction mechanisms involved in the action of phytomedicines are now mainly referred to as **multi-factorial effects** (also known as **polyvalent action**) and may include the following:

- **Several compounds affecting a single target, either directly or indirectly:** this may include synergy, the metabolism of one active altered by the presence of others in the extract, or the bioavailability of a component changed by the presence of another. These may include pharmacokinetic and pharmacodynamic interactions.
- **A single compound affecting multiple targets:** this is not an interaction between components of a mixture of course, but it helps to explain why a particular herbal medicine (or any drug, for that matter) can be used for different purposes.
- **Multiple compounds affecting multiple related targets:** these are the result of a number of constituents acting in different ways; and interactions could certainly be taking place. There may be cases where the effect of one compound cancels out the effect of another by antagonism and this would not be known unless fractionation of an extract before testing had been carried out.

More than one of these mechanisms may be taking place at the same time, and the overall effect is therefore the result of a complex interaction between different components of a mixture and different targets, which may all be relevant in the treatment of a particular condition. The use of phytomedicines has been described as the 'herbal shotgun' approach, as opposed to the 'silver bullet' method of conventional medicine. Systems biology methods are now being used to investigate the relationships between genes, proteins and transcription factors, and the single-drug single-target view is giving way to a more complex understanding of networks within an organism and the complexity of the therapeutic intervention.

Mechanisms of interaction are the same as those involved in herb–drug interactions (Chapter 19), and include *pharmacodynamic processes*, where the effects of one drug are altered by the presence of another at the site of action, and *pharmacokinetic* interactions, where one drug affects the absorption, distribution, metabolism or excretion of another.

An example of both is provided by Ayurveda, where an ancient combination formula known as 'Trikatu' contains black pepper (*Piper longum*). Pepper contains the alkaloid piperine, which has many useful pharmacological activities (anti-inflammatory, anti-allergic, digestive) that add to the desired effects of the other ingredients in the formula. These could be considered to be pharmacodynamic interactions. However, piperine is also known to increase the bioavailability of a number of drugs by enhancing absorption. Piperine modulates the multidrug transporter P-glycoprotein and influences the efflux of other compounds, both herbal and synthetic drugs, from cells (Najar et al 2010). Piperine, along with many other natural compounds, also inhibits both constitutive and inducible cytochrome P450 (CYP-450) drug-metabolizing enzymes.

MEASURING SYNERGY

The general understanding of synergy is that it is an effect seen by a combination of substances that is greater than would have been expected from a consideration of individual contributions. It does, however, depend on the method used to prove it. Such methods have been discussed extensively (Williamson 2001, Wagner and Ulrich-Merzenich 2009, Zhou et al 2016).

Currently, no unified methodology is available to facilitate the understanding of mechanisms of synergy, although two main approaches have been developed: Loewe additivity, when the drugs have similar modes of action on the same target or pathway, and Bliss independence, which is expected to hold true drugs that elicit their responses independently. In recent years, many other methods based on these two models have been developed and adapted for the study of synergistic effects of multi-component preparations. The combination index and isobole methods (derived from Loewe additivity) are mainly used, and systems biology analysis can be used for multi-target synergy. The strengths and weaknesses of these approaches are summarized below (see also Zhou et al 2016).

The combination index (CI)

This is a quantitative determination of synergistic effects: a CI < 1 indicates synergy, a CI = 1 indicates an additive effect and a CI > 1 indicates antagonism. It is the most conclusive and practical method for demonstrating synergy and has no limitations regarding

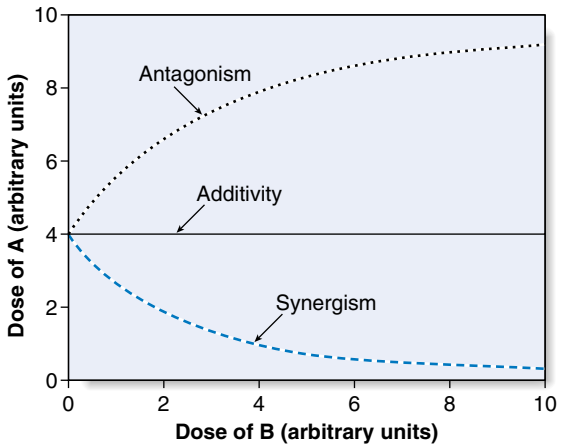


Fig. 11.1 Example of an isobole showing synergism, additivity and antagonism.

the number of ingredients in the tested combination. However, it must be possible to determine the dose-responses of individual constituents and also the combination.

The isobole method

An old and established method, this is independent of the mechanism of action of the agents. An isobole is an 'iso-effect' curve, in which a combination of ingredients (d_a,d_b) is represented by a point on a graph, the axes of which are the dose axes of the individual agents (D_a and D_b). If there is no interaction, the isobole (the line joining the points representing the combination to those on the dose axes representing the individual doses with the same effect as the combination) will be a straight line. If synergy is present, the dose of the combination needed to produce the same effect will be less than that for the individual components and the curve will be 'concave up'. The opposite applies for antagonism, which produces a 'concave down' isobole, as shown in Fig. 11.1. It is necessary to determine the dose-response of the individual constituents and the combination and is most suited to two-component mixtures.

Systems biology

This is a method of computational and mathematical modelling based on experimental data, used for predicting and understanding networks of components and protein/gene targets. It is useful for studying the synergy of multiple components, prodrugs, and novel targets, and for investigating mechanisms of action of combinations and identifying key active components. It is being adopted particularly in studying interactions in TCM multi-herb formulations. However, it requires large data sets including chemical, genetic, pharmacological data and information on potential targets.

MULTIPLE PHARMACOLOGICAL EFFECTS IN A SINGLE PLANT

Ispaghula, *Plantago ovata* Forssk.

Ispaghula, or psyllium husk, is (paradoxically) effective in both constipation and diarrhoea. The laxative effect is achieved principally through its fibre content, but the reason why it is more effective in chronic constipation than other types of fibre may be due to the fact that the seed also contains constituents with gut-stimulatory properties, mediated partly through cholinergic activation, which is likely to enhance the laxative effect. Interestingly, it also contains gut-inhibitory constituents, which could provide a scientific explanation for the traditional use of ispaghula in diarrhoea. In addition to gut-stimulatory and -inhibitory constituents, ispaghula also contains anti-amoebic constituents explaining its traditional use in amoebic dysentery, thus demonstrating multiple effects, some supporting and some opposing a particular activity, in one medicinal plant (Gilani and Rahman 2005).

Ispaghula has also been shown to improve glycaemic control in patients who were being treated for type-2 diabetes, but without lowering glucose levels in non-diabetic patients (Gibb et al 2015), again illustrating differential effects in the same herbal product.

Liquorice, *Glycyrrhiza glabra* L.

In traditional Chinese medicine (TCM), liquorice (*Glycyrrhiza glabra*) is added to many formulae as a synergistic agent, to enhance activity and detoxify, and provides a number of instances of synergism not only between its own constituents but also with other herbal preparations. For example, blood levels of glycyrrhizin are lower, due to reduced absorption, if it is taken as part of an extract or mixture rather than as an isolated compound.

Whole extracts of liquorice inhibit angiogenesis, granuloma formation and fluid exudation in inflammation, as does isoliquiritin, whereas glycyrrhizin and glycyrrhetinic acid tend to promote angiogenesis

(reviewed in Williamson 2001). The whole plant extract, and isolated glabridin, inhibit the release of PGE_2 and TXB_2, cyclo-oxygenase (COX) products and LTB_4 (a LOX product) and isoliquiritigenin inhibits COX products. Glycyrrhizin attenuates LPS-induced acute lung injury by inhibiting cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase expression. Liquorice has many other beneficial effects, and is still the subject of intensive research (reviewed by Hosseinzadeh and Nassiri-Asl 2015) and it is likely that even more mechanisms will be discovered. These examples illustrate the complexity of the effects involved in even a single herbal extract, and it may be that this is found much more commonly than was previously thought.

CLINICAL EXAMPLES OF SYNERGY AND POLYVALENT ACTION

Ginkgo, *Ginkgo biloba* L.

The ginkgolides are known to be platelet-activating factor (PAF) antagonists, a mechanism of anti-inflammatory activity, and a synergistic interaction between ginkgolides A and B has been shown using an *in vitro* platelet aggregation test. A positive interaction was shown by an isobole curve using a 50% mixture of the two. Furthermore, the presence of the other ginkgolides and the ginkgoflavones also had an effect on the overall activity: a mixture of ginkgolides A, B and C, at a dose of 100–240 mg, generated a PAF-antagonizing effect in humans that was equivalent to a dose of 120 mg of a standardized ginkgo extract containing only 6–7 mg of ginkgolides, together with bilobalide and flavonol glycosides (reviewed by Williamson 2001, Wagner and Ulrich-Merzenich 2009). However, the ginkgo flavones are also anti-inflammatory, the combination being considered additive and possibly synergistic in effect as well as increasing blood circulation to the brain, and a total ginkgo extract acts as an antioxidant activity in brain preparations. Clinical studies have shown ginkgo to be effective in improving cognitive function; with the preparation tested being a total extract, suggesting polyvalent as well as synergistic activity.

Ginkgo, taken in combination with other herbal preparations also shows synergistic-like interactions: a double-blind, cross-over trial using 20 young, healthy volunteers tested ginseng (*Panax ginseng*) with ginkgo extract and found it to be more effective in improving cognitive function than either alone. Cognitive performance was assessed in three studies, using 'serial threes' or 'serial sevens', which are arithmetic tests involving subtracting from a random number. Although single treatments showed improvements, the combination produced a significant and sustained improvement, especially in the 'serial sevens' test (Scholey and Kennedy 2002).

Cannabis, *Cannabis sativa* L.

Cannabis has potential as a therapeutic agent in chronic conditions such as rheumatoid arthritis, HIV infection and multiple sclerosis. Documented reports of interactions within the botanical drug and its preparations include the fact that levels of tetrahydrocannabinol (THC) in the brain can be elevated by cannabidiol, and it is known that THC taken alone can induce anxiety, which can be attenuated by the presence of cannabidiol in the herb. There is additional evidence to show that the effect of the botanical preparations is both qualitatively and quantitatively different to that of isolated THC (Wilkinson et al 2003). The plant extract is a better antispastic agent than THC alone, as measured in an immunogenic model of multiple sclerosis (Fig. 11.2). The graph shows that the extract acts more rapidly than isolated THC. The rest of the extract has no effect in this system, suggesting that in this case, the effect of THC is enhanced by the presence of other compounds in the extract, but there is no additive effect.

Willow bark, *Salix alba* L.

Standardized extracts of willow bark for the treatment of inflammatory diseases such as osteoarthritis have confirmed efficacy, and a consideration of their concentrations in plant extracts, and the doses used clinically, suggest the involvement of some form of synergy. Firstly, the gastrointestinal side effects commonly encountered with non-steroidal anti-inflammatory drugs, including aspirin, were not seen at the doses used, although it is usually assumed that willow bark is effective due to its salicin (and therefore salicylic acid) content. Furthermore, when the amount of salicin in the study preparation was taken into account, the dose used (equivalent to 240 mg of salicin daily) was insufficient to explain the activity. Investigations were then carried out to see if another mechanism might be operating. The effect on COX-1 (a cyclooxygenase isoenzyme) was examined and no involvement found

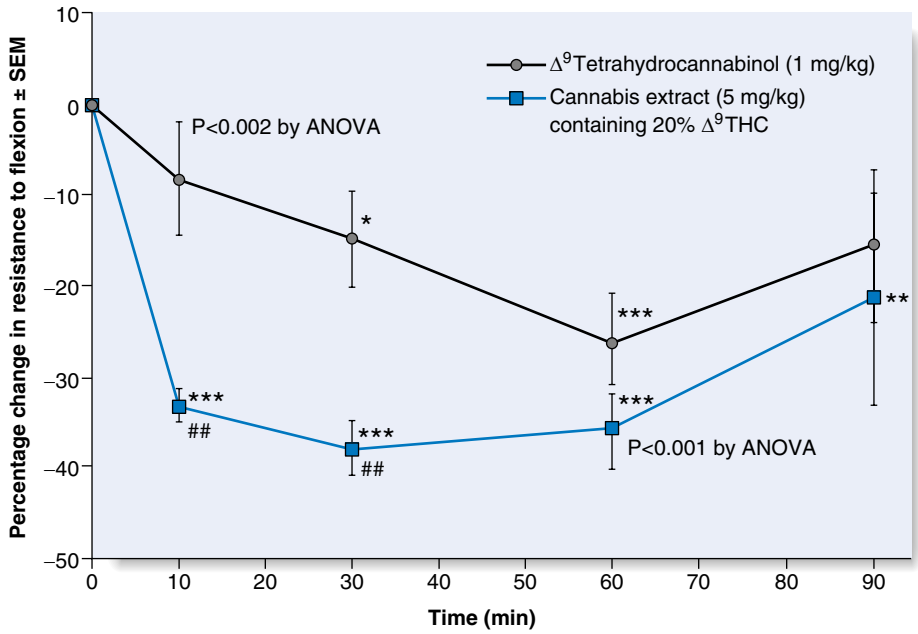


Fig. 11.2 The antispasticity effects of isolated Δ^9-tetrahydrocannabinol (Δ^9-THC) compared with that of cannabis extract containing a matched dose of Δ^9-THC, in a mouse models of multiple sclerosis.

(despite the fact that COX-1 is inhibited by aspirin). COX-1 is responsible for many of the side effects, especially those on the digestive system. However, COX-2 and lipoxygenase, which are also involved in pain and inflammation, were both inhibited. This shows that phytomedicines do not necessarily work in the same way as isolated constituents, and indicated that interactions were taking place in the willow bark extract.

IMPLICATIONS OF SYNERGY

Bioassay-led isolation of actives

Scientists often investigate and extract medicinal plants with a view to finding the chemical compounds responsible for the effects, but this may lead to inconclusive results. If a combination of substances is needed for the effect, then the bioassay-led method of investigation, narrowing activity down first to a fraction and eventually to a compound, is doomed to failure, and this has led to the suggestion that some widely used medicinal plants are devoid of activity. Evidence of herbal interactions was provided by a clinically successful formulation of Chinese herbs used to treat eczema, but when investigated phytochemically and pharmacologically, the activity was lost during the fractionation procedure and was only present with the mixture (reviewed by Williamson 2001). If activity appears to be lost during purification, interactions should be suspected and a search for synergy could be instigated.

ESTABLISHING THE ACTIVE PHARMACEUTICAL INGREDIENT (API) OF A PRODUCT

There are many examples of multifactorial effects in a single plant due to several constituents acting at multiple targets, and once the optimum composition of a product has been determined by pre-clinical and clinical studies, this specific extract should be considered the 'active pharmaceutical ingredient' (API). For a manufacturer, this provides an opportunity for patent protection. It also means that all extracts are not necessarily equivalent in therapeutic effect, or bioavailability.

OTHER REASONS FOR NOT ISOLATING INDIVIDUAL CONSTITUENTS

The most important reason for not isolating an individual component is the presence of multifactorial

or polyvalent effects in a mixture, and the enhanced therapeutic benefit that such effects are expected to produce. However, the following should also be taken into account:

- **Unstable constituents:** Sometimes the presence of all of the components isolated from the plant material, which may include antioxidants for example, may 'protect' the actives from decomposition. Examples of botanical drugs in which this is thought to take place include valerian (*Valeriana* spp.), garlic (*Allium sativum* L.), ginger (*Zingiber officinale* Roscoe) and hops (*Humulus lupulus* L.).
- **Unknown active constituents:** For some herbs, even those that are widely used, the actives may not have been completely identified. This is in fact very common, and as can be seen from the example of liquorice discussed earlier, even for herbs with a very long history, new effects for old compounds are continually being discovered. Other examples include chasteberry (*Vitex agnus-castus* L.), passion flower (*Passiflora edulis* Sims) and hawthorn (*Crataegus* spp.).
- **A range of actives identified:** It is unusual for a plant to contain only one active constituent. Even for cannabis, where there is only one significant psychoactive ingredient, tetrahydrocannabinol, other constituents of the plant may enhance its activity, as shown in Fig. 11.2. Other examples include *Echinacea*, devil's claw (*Harpagophytum procumbens*), artichoke (*Cynara scolymus*) and St John's wort (*Hypericum perforatum*).

References

Gilani, A.H., Rahman, A.U., 2005. Trends in ethnopharmacology. J. Ethnopharmacol. 100 (1–2), 43–49.

Hosseinzadeh, H., Nassiri-Asl, M., 2015. Pharmacological effects of glycyrrhiza spp. and its bioactive constituents: update and review. Phytother. Res. 29 (12), 1868–1886.

Najar, I.A., Sachin, B.S., Sharma, S.C., Satti, N.K., Suri, K.A., Johri, R.K., 2010. Modulation of P-glycoprotein ATPase activity by some phytoconstituents. Phytother. Res. 24 (3), 454–458.

Scholey, A.B., Kennedy, D.O., 2002. Acute, dose-dependent cognitive effects of *Ginkgo biloba*, *Panax ginseng* and their combination in healthy young volunteers: differential interactions with cognitive demand. Hum. Psychopharmacol. 17 (1), 35–44.

Wagner, H., Ulrich-Merzenich, G., 2009. Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine 16 (2–3), 97–110.

Wilkinson, J.D., Whalley, B.J., Baker, D., et al., 2003. Medicinal cannabis: is Δ-9 THC responsible for all its effects? J. Pharm. Pharmacol. 55 (2), 1687–1694.

Williamson, E.M., 2001. Synergy and other interactions in phytomedicines. Phytomedicine 8 (5), 401–409.

Zhou, X., Seto, S.W., Chang, D., et al., 2016. Synergistic effects of Chinese herbal medicine: a comprehensive review of methodology and current research. Front. Pharmacol. 7, 201.

Further reading

Gibb, R.D., McRorie Jr., J.W., Russell, D.A., Hasselblad, V., D'Alessio, D.A., 2015. Psyllium fiber improves glycemic control proportional to loss of glycemic control: a meta-analysis of data in euglycemic subjects, patients at risk of type 2 diabetes mellitus, and patients being treated for type 2 diabetes mellitus. Am. J. Clin. Nutr. 102 (6), 1604–1614.

Chapter 12

Toxicity of herbal constituents

CLASSES OF TOXIC COMPOUNDS

Toxic plant species are often used in medicine and provide some of our most important drugs as isolated compounds (natural products), but there are others whose toxicity far outweighs any therapeutic benefit they may possess. These are not suitable for use as herbal medicines and many are banned, but in some traditional systems of medicine they are still used, and may also occur as contaminants in other commercial plant batches. Traditional use is not always a reliable indication of safety, since toxicity, which results only from chronic use, or manifests after a long interval between taking the medicine and the onset of a reaction, may make the connection difficult. New integrated approaches are being advocated to examine more subtle forms of herbal toxicity (e.g. Williamson et al 2014). An added complication is that patients often do not consider herbs to be 'drugs', so an association may not be made with an adverse reaction. Even though a particular botanical drug has been used for hundreds of years there may remain cause for vigilance, and research continues to look at mechanisms of the action and metabolism of the herbal constituents, which may help to predict toxicity problems, as shown in the examples of *Aristolochia* constituents and the pyrrolizidine alkaloids.

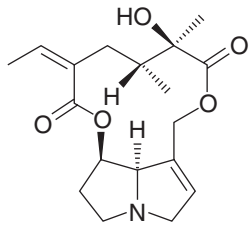
Many toxic effects of herbal medicines are due to the poor quality of the product. For example, herbal medicines may be adulterated with toxic herbs, either accidentally or deliberately, as discussed in Chapter 11, and also with synthetic medicines or contamination with heavy metals. Some toxic compounds, including the pyrrolizidine alkaloids and aristolochic acids, are present as both contaminants and as ingredients of traditional formulas, and still pose potential serious risks. Allergic reactions can be elicited by any drug, and idiosyncratic responses are not restricted to plant medicines, although some plant families, especially the daisy family (Asteraceae), are notorious for causing allergic reactions.

PYRROLIZIDINE ALKALOIDS

These are found mainly in the plant families Boraginaceae, Asteraceae, Leguminosae. Herbs affected include comfrey (*Symphytum* spp.), butterbur (*Petasites hybridus*), and coltsfoot (*Tussilago farfara*). Pyrrolizidine alkaloids that are unsaturated at the 1,2-position (e.g. senecionine; Fig. 12.1) cause veno-occlusive disease as well as being hepato-carcinogenic, and their effects are cumulative. The European Medicines Agency (EMA) has concluded (December 2015) that there is no level of PA ingestion that is without risk, and exposure should be reduced to as low as reasonably practicable (EMA 2016). PAs have recently been found in herbal THR products such as St John's wort, which are due to contamination with weeds. This happens during the collecting process, but it has also been shown that interspecific transfer of PAs can occur when, for example, *Jacobaea vulgaris* Gaertn. (syn.: *Senecio jacobaea* L.) is used as a mulch for growing chamomile or parsley (Nowak et al 2016). It shows the importance of good agricultural and collecting practice, and traceability of the plant material.

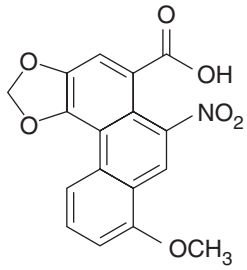
ARISTOLOCHIC ACIDS

Most species of birthwort (*Aristolochia*, known as snakeroot) and related genera including *Asarum*, all from the family Aristolochiaceae, contain aristolochic acids



Senecionine

Fig. 12.1

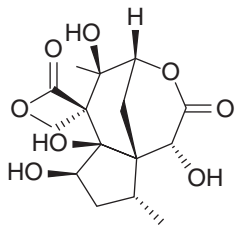


Aristolochic acid

Fig. 12.2

(Fig. 12.2) and aristolactams. Aristolochic acid nephropathy (AAN), which causes kidney failure and urothelial malignancies, was reported in >100 patients from the same Belgian clinic, after the intake of slimming pills containing the Chinese herb, *Aristolochia fangchi*. In the first report (1993), the plant was identified as *Stephania tetrandra* – or 'hang fang ji', but *Aristolochia fangchi* – 'guang fang ji' – had been used instead. Whether this was accidental or deliberate (substitution by herbs with similar properties is allowed in traditional Chinese medicine [TCM]) is not clear, but all plants in the genus *Aristolochia* contain aristolochic acids (AA) and are banned in Europe and the US. A recent study suggests a much wider spectrum of toxicity in related compounds found in related species, such as *Asarum*, and these may need to be revisited (Michl et al 2016).

Contamination of foods with *Aristolochia* is a major concern in some parts of the world: for example, AA-nephropathy has been reported in China, Bangladesh and in central Europe where it is known as Balkan endemic nephropathy. On the other hand, in many regions of the world species from the genus are used locally as traditional medicines, especially in the treatment of gastrointestinal complaints, snake bites, poisoning and for gynaecological conditions including the treatment of sexually transmitted diseases (STDs) such as syphilis and gonorrhoea (Heinrich et al 2009), and these practices need to be addressed.



Anisatin

Fig. 12.3

MONOTERPENES, SESQUITERPENES AND PHENYLPROPANOIDS

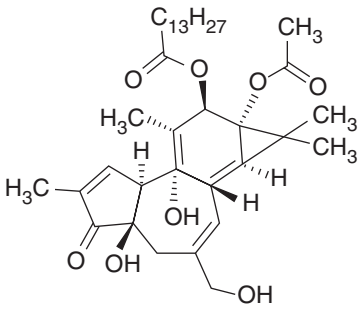
Most mono- and sesquiterpenes found in essential oils are fairly safe, apart from causing irritation when used undiluted and allergies in susceptible individuals. However, some have been shown to be carcinogenic, for example safrole (from *Sassafras* bark), and β-asarone (from *Acorus calamus*). However, they do not appear to give cause for concern when present in minute amounts in other oils. Methysticin, from nutmeg, is toxic in large doses, and has been postulated as being a metabolic precursor of the psychoactive drug MDMA (methylene dioxymethamphetamine). Thujone, which is present in wormwood (*Artemisia absinthium*) and in the liqueur absinthe, is also toxic and hallucinogenic in large doses.

Camphor is present in many essential oils and plant balsams. It inhibits nicotinic receptors, and is also an agonist at several TRVP (transient receptor potential cation channel subfamily V (TrpV) or capsaicin receptor) channels. Its CNS-stimulatory effects may cause nausea, vomiting, headache, dizziness, tremor, convulsions and delirium, and in severe overdose, death. It is absorbed very easily through the skin.

SESQUITERPENE LACTONES

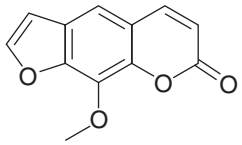
These compounds are present in many Asteraceae plants, and are often responsible for the biological activity of the herb. Some are cytotoxic and some highly allergenic, which can cause problems if misidentification occurs, for example with mayweed (*Anthemis cotula* L.) instead of chamomile (*Anthemis nobilis* L. = Chamaemelum nobile (L.) All. or *Matricaria chamomilla* L.). Anthecotulide is such an allergen, and is present in several species of the Asteraceae, the daisy family (Compositae).

Anisatin (Fig. 12.3) is a neurotoxin occurring in the fruits of shikimi fruit *Illicium anisatum* L.; (see Chapter 12), which may occur as adulterants of star anise, *Illicium verum* Hook.f. (See Wang et al (2011) for review). Symptoms of anisatin poisoning include diarrhoea, vomiting,



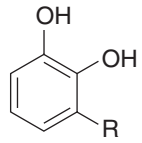
Tetradecanoyl phorbol acetate

Fig. 12.4



Xanthotoxin

Fig. 12.5



Urushiols

Fig. 12.6

stomach pain, nervous system excitation, seizures, loss of consciousness and respiratory paralysis.

DITERPENE ESTERS

The phorbol, daphnane and ingenol esters found in the Euphorbiaceae and Thymeliaceae are highly pro-inflammatory and are known to activate protein kinase C, as well as having tumour-promoting (co-carcinogenic) activity. The most important is tetradecanoyl phorbol acetate (phorbol myristate acetate; Fig. 12.4), which is a tumour promoter and an important biochemical research tool. Some of these plants were formerly used as drastic purgatives (e.g. croton oil, from *Croton tiglium* L., Euphorbiaceae) but should now be avoided in herbal products.

PLANT LECTINS AND AGGLUTININS

Castor beans, which are used to produce castor oil for use in medicines and cosmetics, contain a highly toxic lectin, ricin, which is denatured during manufacture of the oil, but the oil, and the seed cake remaining (which is used as animal feed), should not be used without heat processing. Pokeweed (*Phytolacca americana* L.), which is sometimes used as an anti-inflammatory herb, contains phytoagglutinins called pokeweed mitogens. These have been known to cause gastrointestinal upset when taken in the fresh herb, but as they are heat-labile they may denature on processing. They are also used as biochemical tools in immunology research, for example in blood grouping, erythrocyte polyagglutination, and lymphocyte subpopulation studies.

FURANOCOUMARINS

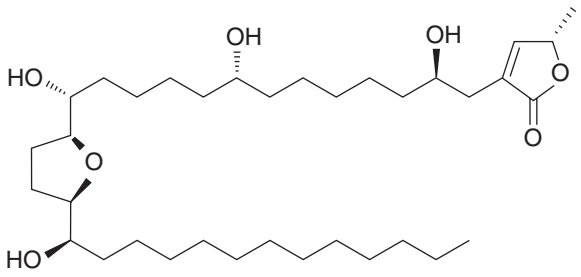
Some furanocoumarins, e.g. imperatorin, psoralen and xanthotoxin (Fig. 12.5), which are found in giant hogweed (*Heracleum mantegazzianum*) and other umbelliferous plants and citrus peels, are phototoxic and produce photodermatitis on contact. Psoralen plus UV-A radiation (PUVA therapy) is occasionally used in the treatment of psoriasis, in specialist hospital clinics.

URUSHIOL DERIVATIVES

The urushiols (Fig. 12.6), anacardic acids and ginkgolic acids are phenolic compounds with a long side-chain. The uroshiols are found in poison ivy (*Toxicodendron radicans*) and poison oak (*T. quercifolium*) and cause severe contact dermatitis. The anacardic acids, which are found in the liquor surrounding the cashew nut (*Anacardium occidentale* L.), are less toxic. The ginkgolic acids are reputed to cause allergic reactions; however, they are present in *Ginkgo biloba* fruit rather than the leaf, which is the medicinal part.

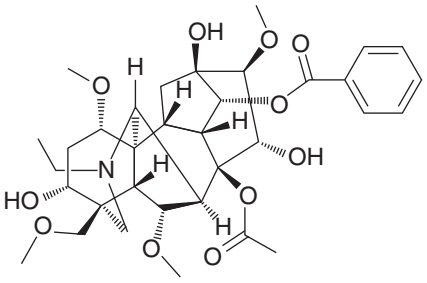
ANNONACINS

Soursop (also known as graviola) is used as an unproven cancer treatment and, although consumption of the fruit and juice is widespread and considered safe, there are neurotoxic acetogenins, the annonacins (Fig. 12.7), which are also the anticancer compounds, in the leaf and bark. In countries such as Guadeloupe, where the leaf and bark are consumed as herbal medicines (and not only for cancer), there is a significantly higher incidence of atypical Parkinson's disease (PD) than elsewhere. The annonacins cause atypical PD in animal models by destroying dopaminergic neurons and this treatment cannot be recommended (Champy et al 2009).



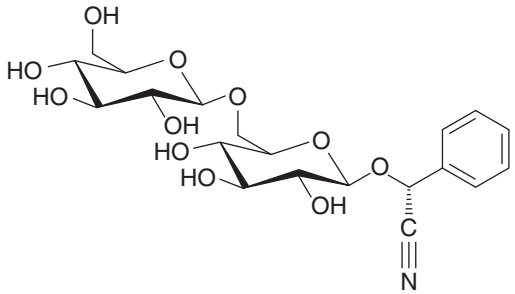
Annonacin 1

Fig. 12.7



Aconitine

Fig. 12.9



Amygdalin

Fig. 12.8

CYANOGENETIC GLYCOSIDES

Cyanogenetic glycosides, such as amygdalin (Fig. 12.8), are present in many foods and herbs, including nuts such as almonds. They are so-called because they release hydrogen cyanide when crushed, via the action of various enzymes, and also generate flavour compounds such as benzaldehyde. Their presence in small amounts in fruits and nuts is not a concern. However, herbal remedies containing high levels of cyanogenetic glycosides or laetrile (synthesized from amygdalin by hydrolysis) are promoted as alternative cancer treatments. In some cases, simply ingesting crushed apricot kernels (*Prunus armeniaca*) is recommended, and this practice has resulted in documented hospital admissions.

PROCESSING AS A MEANS OF DETOXIFICATION

Chemical and physical methods are used in traditional medicine systems to detoxify herbal materials, but they are not infallible and reports of intoxication still occur. These have been more fully discussed in the context of TCM by Liu et al (2014), but the species most often involved in reports of toxicity is aconite (*Aconitum* spp.), reviewed briefly below.

ACONITINE

The tubers and roots of *Aconitum* (Ranunculaceae) have been used medicinally for centuries in TCM for the treatment of syncope, rheumatic fever, painful joints, gastroenteritis, diarrhoea, oedema, bronchial asthma, tumours and others. It contains aconitine (Fig. 12.9) and related diterpene alkaloids, which can be denatured by special processing. The improper use of *Aconitum* in India, Japan, and especially China still results in deaths (Chan 2015). In China, only the processed (i.e. detoxified) tubers and roots of *Aconitum* are allowed to be administered orally or adopted as raw materials for pharmaceutical manufacturing. More than 70 techniques are applied for processing *Aconitum* roots to lower toxic alkaloid levels to below a certain threshold, a principle that is not accepted in Europe.

References

Champy, P., Guérineau, V., Laprévote, O., 2009. MALDI-TOF MS profiling of Annonaceous acetogenins in *Annona muricata* products for human consumption. Molecules 14, 5235–5246.

Chan, T.Y.K., 2015. Incidence and causes of aconitum alkaloid poisoning in Hong Kong from 1989 to 2010. Phytother. Res. 29 (8), 1107–1111.

EMA, 2016. Public statement on contamination of herbal medicinal products/traditional herbal medicinal products1 with pyrrolizidine alkaloids. Available online at http://www.ema.europa.eu/docs/en_GB/document_library/Public_statement/2016/06/WC500208195.pdf.

Heinrich, M., Chan, J., Wanke, S., et al., 2009. Local Uses of Aristolochia Species and Content of Nephrotoxic Aristolochic Acid 1 and 2–A Global Assessment Based on Bibliographic Sources. J Ethnopharmacol. 125 (1), 108–144.

Liu, X., Wang, Q., Song, G., et al., 2014. The classification and application of toxic Chinese Materia Medica. Phytother. Res. 28 (3), 334–347.

Michl, J., Kite, G.C., Wanke, S., et al., 2016. LC-MS- and (1)H NMR-based metabolomic analysis and in vitro toxicological assessment of 43 aristolochia species. J. Nat. Prod. 22;79 (1), 30–37.

Nowak, M., Wittke, C., Ledere, I., et al., 2016. Interspecific transfer of pyrrolizidine alkaloids: an unconsidered source of contaminations of phytopharmaceuticals and plant derived commodities. Food. Chem. 213, 163–168.

Wang, G.W., Hu, W.T., Huang, B.K., et al., 2011. *Illicium verum*: a review on its botany, traditional use, chemistry and pharmacology. J. Ethnopharmacol. 136 (1), 10–20.

Williamson, E.M., Chan, K., Xu, Q., et al., 2014. Evaluating the safety of herbal medicines: integrated toxicological approaches. The Art and Science of Traditional Medicine 2, S47–S49.

Further reading

Michl, J., Jennings, H.M., Kite, G.C., et al., 2013. Is aristolochic acid nephropathy a widespread problem in developing countries? A case study of *Aristolochia indica* L. in Bangladesh using an ethnobotanical-phytochemical approach. J. Ethnopharmacol. 149 (1), 235–244.

Section 5

Medicinal plants in traditional medicine systems, complementary and integrative medicine

SECTION CONTENTS

The importance of medicinal plants in modern pharmacy and medicine was discussed in Chapter 1. This section provides an overview of medicinal plants used in traditional medical systems (Chapter 13), discusses several important traditional medicine systems involving the use of medicinal plants (Chapters 14–17), and briefly considers several 'complementary therapies', such as homeopathy and aromatherapy, that involve the use of substances sourced from plants (Chapter 18). Also, interactions between herbal and conventional medicines are discussed in Chapter 19.

Chapter 13

Overview of medicinal plants in healthcare systems

Both developed and developing countries have their own traditional systems of medicine and, in many countries, these traditional medical systems remain an important part of healthcare. In some cases, their practice and delivery have evolved to meet today's needs: in China, for example, traditional Chinese medicine is practiced alongside Western medicine in hospital settings and is taught in schools of pharmacy and medicine, as well as other contexts. The practice of these traditional medical systems is not limited to the country or region in which they first originated: global migration has meant that many of these healthcare approaches also have migrated and are practiced in new regions and accessed by individuals from other cultures.

DEFINITIONS

The World Health Organization (WHO) defines 'traditional medicine' (TM) as 'the sum total of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness' (WHO 2013). An important feature is that the traditional knowledge and practices are handed down from generation to generation orally, or in writing.

Traditional medicine includes diverse health practices and approaches, incorporating use of traditional medicines (i.e. the preparations used in traditional medical systems), spiritual therapies, manual techniques and exercises applied singularly or in combination. Many traditional medicines are based on plant material, i.e. they are herbal medicines. In some systems, traditional medicines may contain natural organic or inorganic active ingredients that are not of plant origin (e.g. insect, animal and mineral materials).

In some countries, traditional medicine systems are grouped with complementary/alternative medicine (CM or CAM), because, usually (but not always), they are organized, practiced and accessed outside of the conventional mainstream medical healthcare framework. Thus, the WHO and other organizations often refer to 'traditional and complementary medicine (T&CM).

Increasingly, the CAM sector is being referred to as 'integrative' or 'integrated' medicine/health. It has been defined by the Academic Consortium for Integrative Medicine & Health (a North America-based organization whose members include esteemed academic medical centres and other institutions) as: 'Integrative medicine and health reaffirms the importance of the relationship between practitioner and patient, focuses on the whole person, is informed by evidence, and makes use of all appropriate therapeutic and lifestyle approaches, healthcare professionals and disciplines to achieve optimal health and healing' (ACIMH 2016).

WORLD HEALTH ORGANIZATION AND TRADITIONAL MEDICINE

The WHO first published a Traditional Medicine (TM) strategy in 2002; the sector was also covered in the WHO's Medicines Strategy documents of 2004–2007 and 2008–2013, reflecting its importance in the overall context of medicines and health. The new WHO TM strategy (2014–2023) recognizes the continued and global use and practice of TM, its economic and cultural importance, and advances in regulation and

standards. The strategy's goals are 'to support WHO member states in:

- harnessing the potential contribution of TM to health, wellness and people-centred health care;
- promoting the safe and effective use of TM by regulating, researching and integrating TM products, practitioners and practice into health systems, where appropriate' (WHO 2013).

The WHO's activities with respect to TM include supporting 20 WHO Collaborating Centres for Traditional Medicine worldwide, and convening the WHO's Expert Advisory Panel on Traditional Medicine. It has also published numerous monographs and guidelines relating to TM, including, for example, on good agricultural and collection practices for medicinal plants, safety monitoring of herbal medicines in pharmacovigilance systems, and development of consumer information for the use of traditional medicines (WHO 2003, 2004a, 2004b). Further information about the WHO's activities in this sector can be found on the webpage (http://www.who.int/topics/traditional_medicine/en/).

EXTENT OF USE

Almost 18,000 plant species are recorded as used in medicines (RBG Kew 2016), and a substantial proportion of this relates to use in traditional medicine; the materia medica of traditional Chinese medicine, for example, uses over 3000 plant species. Not surprisingly, trade in raw materials used in traditional medicine is vast. The demand for medicinal plants was estimated as being US$14 billion in 2006 (Booker et al 2012). Unsustainable and/or illegal trade in endangered plant (and animal) species for medicinal purposes has contributed to the extinction risk for these species (RBG Kew 2016, CITES 2016).

The prevalence of use of, and expenditure on, 'true' traditional medicine is difficult to estimate since it involves several points of access (e.g. self-treatment, consultations with TM practitioners) and because studies often categorize traditional medicines and traditional medicine practice together with other complementary medicines or natural health products, and complementary health approaches.

In developing countries in particular, a substantial proportion of the population (up to 80% in parts of Africa) relies on the use of traditional medicines and traditional healers as their only, or main, source of healthcare (WHO 2013). The extent to which individuals consult different types of traditional or complementary medicine practitioners varies across different countries (WHO 2012), and the cultural and ethnic 'mix' of the population is likely to have some bearing on this. For example, in England in 2005, a relatively small proportion (around 1%) of a nationally representative sample had consulted a TCM practitioner in the previous 12 months (Hunt et al 2010); by contrast, in China, in 2009, there were over 900 million TCM 'visits' (this figure may include multiple visits by the same individual) (WHO 2013).

Market research data from several sources indicate that sales of manufactured herbal and other complementary medicines are substantial (Mintel 2007, Smith et al 2015); for example, in the USA, sales of botanical (herbal) products were estimated to be worth more than US$6.4 billion in 2014 (Smith et al 2015). Nationally representative studies from several developed countries also indicate a high prevalence of use of complementary medicines for health maintenance as well as prevention and treatment of chronic diseases (Barnes et al 2008, Eisenberg et al 1998, MacLennan et al 2006, Morgan et al 2012).

LEGAL STATUS

In recent years, many countries have introduced regulatory frameworks for manufactured herbal and, in some cases, other types of complementary medicines and natural health products. Typically, these are simplified regulatory systems for 'low-risk' products used for minor, self-limiting conditions suitable for self-treatment; they usually require manufacturers to meet pharmaceutical quality standards for their products, whereas evidence of traditional use, or other 'lower' levels of evidence, is accepted with respect to efficacy, and safety assessment is limited to literature review or use of ingredients on a permitted list. In some countries, such as Australia and Canada, many plants used in manufactured traditional medicines have been included on 'permitted substances lists'. By contrast, the implementation of the Traditional Herbal Medicinal Products Directive in the European Union effectively meant that manufactured TCM products (with only one or two exceptions) could not meet the traditional use requirements in the UK and can be no longer legally sold. These regulatory frameworks typically apply to manufactured products sold in retail outlets and are not intended to control herbal medicines administered or supplied by traditional medicine practitioners. The regulatory frameworks for

herbal medicines and other natural health products in selected countries are summarized in Chapter 1.

The regulation of traditional and complementary medicine practitioners varies along a spectrum. In some countries, certain types of practitioners are educated through university programmes, have a high level of professional organization, and are subject to statutory professional regulation (WHO 2013). In other instances, the extent and quality of education and training can be highly variable, there can be little or no professional organization, and neither self- nor statutory regulation.

The regulation of herbal medicinal preparations that can be supplied or administered by traditional medicine practitioners is a separate, but related, issue. Again, the approach to this differs across countries. In some developed countries (e.g. the UK and New Zealand), current regulations allow herbalists or 'natural-health' practitioners (not defined) to make herbal remedies and other 'individualized' natural-health treatments for specific patients; in some other instances, the prescribing and supply of certain herbal preparations is restricted to specific categories of registered traditional medicine practitioners. In many countries, certain herbal medicines are controlled substances and can only be prescribed by a medically qualified practitioner (i.e. doctor) and sold or supplied by a pharmacist.

EFFICACY AND EFFECTIVENESS OF TRADITIONAL MEDICINE PRACTICES AND TRADITIONAL MEDICINES

Obtaining scientific evidence to support or refute the theoretical basis of the different traditional medicine systems is virtually impossible because they are based on abstract philosophical frameworks, and it is not possible to test the existence or otherwise of the different concepts to which they refer. In the countries from which these traditional medical systems originate, there is strong confidence and cultural acceptance of them as a healthcare option; outside these countries, general uncertainty about their practice and effectiveness is more common (Teng et al 2006).

In contrast, scientific evidence relating to the efficacy of traditional medicines, including individualized traditional treatments (which reflects how they are often used in practice), can be obtained through assessing their effects in randomized, controlled, clinical trials. Many studies with traditional Chinese medicines, Ayurvedic medicines and other traditional medicines have been carried out, but these studies have not always met standards for methodological quality. The Cochrane Library, compiled by the Cochrane Collaboration (an international organization dedicated to the creation and maintenance of systematic reviews of clinical trials of healthcare interventions), contains around 100 systematic reviews of clinical trials of Chinese herbal medicines in different medical conditions. These provide some limited evidence of efficacy for the preparations tested; typically, these reviews conclude that most of the available trials are of low methodological quality and that further, rigorous, studies are required.

SAFETY AND PHARMACOVIGILANCE OF TRADITIONAL MEDICINE PRACTICES AND TRADITIONAL MEDICINES

The safety of traditional medicines and traditional medicine practices has not been subject to the comprehensive scientific scrutiny that is applied to assessing the safety of conventional pharmaceutical medicines. A history of safe use in traditional medicine can provide some limited degree of assurance about (lack of) acute toxicity, but cannot provide reliable information on many other aspects of safety, such as the effects of long-term use, latent adverse reactions, use in contemporary medical conditions (e.g. HIV infection), and concurrent use with conventional medicines (Ernst et al 1998). Preparing certain traditional medicines in particular ways (e.g. 'cooking' or boiling certain herbs used in TCM) is used to reduce toxicity (Teng et al 2006), but these practices need to be underpinned by scientific evidence, and universally adopted if demonstrated effective.

While the public health risk of using traditional medicines needs to be kept in perspective, serious adverse reactions associated with the use of certain herbal preparations have been reported (Barnes 2012). In some cases, these are due to the toxicity of constituents of the plant material used; in other instances, the adverse reaction may have occurred due to failures in quality, such as contamination of the plant material with toxic weeds or other plant species, micro-organisms, or pesticides. A particular area of concern is the potential for drug interactions to occur between plant medicines and conventional pharmaceutical medicines taken concurrently. This topic is considered of such importance in pharmacy, medicine and herbal medicine that it is considered in depth in this section (see Chapter 19 Herbal medicines interactions).

Capturing these cases of adverse reactions associated with traditional medicines has many challenges. Many of the countries in which traditional-medicine use is most prevalent do not have well-established and well-resourced pharmacovigilance (drug safety monitoring) systems; even where systems do exist, these systems are unlikely to attract reports from traditional-medicine practitioners, as these individuals may be unaware of, or uncertain about, such systems, their purpose, and how to use them. Further, the reporting forms that are used to collect data on suspected adverse drug reactions have been designed for conventional medicines, and are not easily applied to collecting data on suspected adverse reactions associated with traditional-medicine preparations (Barnes 2003). For example, some traditional medicines contain up to 15 or more different ingredients of plant or other origin; the preparation and its component ingredients can be named ambiguously, or even unidentified.

Even in countries with well-established pharmacovigilance systems, reports of suspected adverse reactions associated with traditional medicines reported by traditional-medicine practitioners are scarce. This is, in part, because these schemes are not usually promoted to this group of health practitioners, and as there may be a reluctance to report due to fear of the consequences for their own practice and on the continued availability of traditional medicines implicated in reports (Barnes 2003). In some countries, such as the UK, responsible practitioner organizations have developed their own adverse reaction-reporting schemes, which usually have a link to the national pharmacovigilance system, to which their members are invited to submit reports (Broughton 2011). Although the reporting forms used in such schemes are designed specifically for herbal medicine practitioners, the schemes are likely to be subject to the same limitations described above. Many pharmacovigilance systems now provide for direct reporting of suspected adverse reactions by patients; these initiatives may contribute to the identification of safety concerns associated with traditional (and complementary) medicines, including where they are used in self-treatment.

There is international recognition of the need to develop safety monitoring for herbal and other traditional medicines: the WHO has produced guidelines on this, including designing a reporting form that is intended to cater more satisfactorily for the complex nature of many herbal medicines (WHO 2004), and the Uppsala Monitoring Centre (the WHO Collaborating Centre for International Drug Monitoring) initiated a programme to stimulate reporting for herbal medicines (WHO UMC).

References

Academic Consortium for Integrative Medicine & Health, 2016. Definition of integrative medicine and health. Available online at https://www.imconsortium.org/about/about-us.cfm.

Barnes, J., 2003. Pharmacovigilance of herbal medicines. A UK perspective. Drug. Saf. 26 (12), 829–851.

Barnes, P.M., Bloom, B., Nahin, L., 2008. Complementary and alternative medicine use among adults and children: United States, 2007. Natl. Health Stat. Rep. 12, 1–23.

Barnes, J., 2012. Adverse drug reactions and pharmacovigilance of herbal medicines. In: Talbot, J., Aronson, J., Stephens' Detection and Evaluation of Adverse Drug Reactions, sixth ed. Chichester: Wiley, pp. 645–683.

Booker, A., Johnston, D., Heinrich, M., 2012. Value chains of herbal medicines – research needs and key challenges in the context of ethnopharmacology. J. Ethnopharmacol. 140 (3), 624–633.

Broughton, A., 2011. An overview of Yellow Card reporting by NIMH practitioners. J. Herb. Med. 1, 15–29.

Convention on International Trade in Endangered Species of Wild Fauna and Flora, 2016. Available online at https://cites.org/eng/disc/what.php.

Eisenberg, D.M., Davis, R.B., Ettner, S.L., et al., 1998. Trends in alternative medicine use in the United States, 1990–1997: results of a follow-up national survey. JAMA 280 (18), 1569–1575.

Ernst, E., De Smet, P.A., Shaw, D., Murray, V., 1998. Traditional remedies and the "test of time". Eur. J. Clin. Pharmacol. 54 (2), 99–100.

Hunt, K.J., Coelho, H.F., Wider, B., et al., 2010. Complementary and alternative medicine use in England: results from a national survey. Int. J. Clin. Pract. 64 (11), 1496–1502.

MacLennan, A.H., Myers, S.P., Taylor, A.W., 2006. The continuing use of complementary and alternative medicine in South Australia: costs and beliefs in 2004. Med. J. Aust. 184 (1), 27–31.

Mintel, 2007. Complementary medicines. Market Intelligence. Mintel International Group, London.

Morgan, T.K., Williamson, M., Pirotta, M., Stewart, K., Myers, S.P., Barnes, J., 2012. A national census of medicines use: a 24-hour snapshot of Australians aged 50 years and older. Med. J. Aust. 196 (1), 50–53.

RBG Kew, 2016. The state of the world's plants report – 2016. Royal Botanic Gardens, Kew, London.

Smith, T., Lynch, M.E., Johnson, J., Kawa, K., Bauman, H., Blumenthal, M., 2015. Herbal dietary supplement sales in US increase 6.8% in 2014. HerbalGram 107, 52–59.

Teng, L., Shaw, D., Barnes, J., 2006. Traditional Chinese herbal medicine. Pharm. J. 276, 361–363.

WHO, 2003. WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants. WHO, Geneva. Available online at http://apps.who.int/medicinedocs/en/d/Js4928e/.

WHO, 2004a. WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems. WHO, Geneva. Available online at http://apps.who.int/medicinedocs/en/m/abstract/Js7148e/.

WHO, 2004b. WHO Guidelines on Developing Consumer Information on Proper Use of Traditional, Complementary and Alternative Medicine. WHO, Geneva. Available online at http://apps.who.int/medicinedocs/en/d/Js5525e/.

WHO, 2012. World Health Organization Regional Office for the Western Pacific. The Regional Strategy for Traditional Medicine in the Western Pacific (2011–2020). WHO, Geneva.

WHO, 2013. WHO traditional medicine strategy: 2014–2023. WHO, Geneva. Available online at http://apps.who.int/iris/bitstream/10665/92455/1/9789241506090_.

WHO, 2016. WHO Uppsala Monitoring Centre. Monitoring of herbal medicines. WHO, Geneva. Available online at http://www.who-umc.org/DynPage.aspx?id=105337&mn1=7347&mn2=7252&mn3=7322&mn4=7492.

Chapter 14

Characteristics of traditional medicine systems using herbal substances

All modern medicine is derived originally from ancient herbal traditions. These have evolved to produce the conventional medicine known in the West, which uses both synthetic drugs and isolated natural compounds. Plant extracts are now rarely used by physicians or in hospitals, although herbal remedies are popular with the public. Improvements in the formulation of herbal products have resulted in a new generation of phytomedicines that are more potent than before and also chemically standardized. There is also a resurgence of interest in the older traditional medicine systems; this is due partly to dissatisfaction with conventional treatments and partly to the constantly growing interest in all things natural, environmentally friendly and biodegradable. These older types of traditional medicine are philosophically based, and are holistic in that they treat the patient as a whole rather than as the 'owner' of a disease or malfunctioning organ. The Oriental systems of traditional medicine have much in common with traditional medical herbalism as it was, and still is, practised in Europe and America. Whether or not pharmacists, doctors and other healthcare professionals accept the validity of these older medical systems, it is necessary for them to know about their basic principles for two main reasons. First, to be in a position to advise patients who may wish to consult a traditional-medicine practitioner and, second, because traditional use of natural substances is a common starting point in the ongoing search for new drugs.

It is necessary to consider the cultural environment in which traditional remedies are being used to make our expectations more reasonable, put these treatments in the context of Western thinking and widen the criteria of selection of remedies for our own use within modern medicine.

The following chapters in this section consider several different types of traditional medicine:

- 'Western' traditional herbal medicine.
- 'Oriental' or 'Asian' systems of traditional medicine, e.g. traditional Chinese medicine (TCM), Kampo, Ayurveda.
- Latin and South American, African and Oceanian traditional medical systems.

TCM and Ayurveda, for example, are popular and highly sophisticated and have evolved through the ages whilst keeping their philosophy intact. Others are examples of mainly oral traditions, which is typical of many types of indigenous medicine. All these systems use herbs as well as other forms of treatment. Discussion here, however, is restricted to herbs, since they have pharmacological activities and are, therefore, within the remit of this book. First, it is useful to identify some aspects of the general approach of traditional medical systems to health, disease and treatment that affect perceptions of them.

In the context of the systems discussed in this chapter, diseases can be considered as minor or self-limiting disorders, or chronic or serious disorders.

MINOR OR SELF-LIMITING DISORDER

Such ailments include aches and pains, diarrhoea, wounds or injuries, for which a common remedy will usually be offered; in addition, the facilitation of childbirth will also be considered. The traditional remedy used would usually be an indigenous plant or herb, or something that is obtainable from a local market, and would be well known within the community.

CHRONIC OR SERIOUS DISORDERS

These may be fatal, life-threatening or debilitating conditions, or those that cannot be diagnosed by indigenous healers; in some traditional medical systems (e.g. traditional African medicine) they are considered to have a 'supernatural' component. Examples include forms of cancer, and some genetic or metabolic diseases where obvious lesions may not be seen. Plant remedies will certainly be used, but they may be used as part of a ritual, and treatment will often also involve practices such as divination to find out which gods or ancestors have been offended and what sacrifices may be necessary to appease the supernatural entity.

DOSE

In traditional medicine, this usually means a lack of specific dose. Typically, a calabash (a dried hollow shell of a gourd, used as a bowl), seashell or a tumbler of a decoction may be taken from time to time. Traditional medicine is more concerned with *how* to take the remedy rather than *how much*. This aspect of traditional medicine is very important, because it means that highly potent plants are rarely part of a traditional pharmacopoeia – pure compounds, accurate balances and volume measurements are not part of such cultures – and some plants that we now find useful were considered dangerous. For example, the foxglove, a source of the cardiac glycoside digoxin, has no historical documentation as a herbal medicine, and it was not until the 1800s that Withering used it for cardiac dropsy (congestive heart failure). Due to the narrow therapeutic index of the drug, it was necessary to develop a standardized preparation, which was powdered leaf, assayed biologically, and compressed into a tablet, before the drug gained widespread acceptance.

CORRELATION OF TRADITIONAL USE WITH SCIENTIFIC EVIDENCE

Clearly, the rationale behind the use of traditional remedies for minor ailments is more logical to Western thought, and scientists would therefore look, for example, for antipyretic or analgesic compounds in a herb known as a cure for fever, or for haemostatic substances in a plant used to staunch bleeding. It may even be possible to speculate, with some knowledge of chemotaxonomy, on the types of compounds that are present (e.g. tannins in a haemostatic plant, salicylates or sesquiterpene lactones in an antipyretic plant). However, if ritual is more often involved in the use of a particular plant, it is almost impossible to surmise what compounds may be present; this approach is therefore much less useful. The plant may be a treasure trove of useful compounds, but the traditional use will not provide any information about it.

Thus, there may be a correlation between traditional usage and pharmacological action, such as the isolation of antipyretic principles from a 'fever' remedy, but, even so, the results may turn out to be different to our expectations. A classic example is 'fever bark' (*Cinchona*), which was traditionally used in South America for fever, but in many tropical countries 'fever' really means 'malaria'. Quinine is actually antipyretic to some extent, but it has a much more relevant property, which is to kill the malaria parasite *Plasmodium*. Therefore, extracts of plants based on traditional usage should be tested not only for the activity expected, but also a battery of tests, since some important modern drugs have been developed from plants used for a different purpose entirely. For example:

- In the Caribbean the periwinkle *Catharanthus* (*Vinca*) *rosea* (L.) G.Don (originally from Madagascar) was used traditionally for treating diabetes, but on further investigation it yielded the powerful anticancer alkaloids vincristine and vinblastine.
- *Fagara xanthoxyloides* (Lam.) Zepern. & Timler, which is used in Nigeria as a chewing stick for cleaning the teeth, has been found to be not only antimicrobial, but also to have antisickling activity, and this finding has led to further work into this painful and chronic genetic condition.

Despite the reservations mentioned, traditional medicines have yielded many useful modern drugs, although not as many as poisonous species. Although toxic plants are only occasionally used as medicines, they are well known to the healers and may also be used for nefarious purposes, witchcraft or as 'ordeal' poisons, as, for example, Calabar bean (*Physostigma venenosum* Balf.), which is the source of the anticholinesterase physostigmine (eserine).

Chapter 15

Western herbal medicine

HISTORY

In the UK, Western herbal medicine, or medical herbalism, traces its historical traditions partly to Galen's (a Greek physician of the 2nd century AD) model of 'bodily humours' (blood, black bile, yellow bile, phlegm), their 'temperaments' (e.g. hot, cold, damp) and the belief that illness resulted from an imbalance in these humours. Herbs were used to correct the imbalance and were often described as, for example, 'heating' or 'cooling'; a 'cooling' herb, such as peppermint, would be used to treat a 'hot' condition, such as fever. A significant evolution in the development of medical herbalism occurred with the publication of 'Culpeper's Herbal' in 1652, one of the first comprehensive compilations of medical and pharmaceutical knowledge on plant medicines. Western herbalism has also drawn on other traditions, such as the use of herbs in North America after Samuel Thomson, although Thomson was himself influenced by herbalism in Europe. (For a review of the historical development of Western medical herbalism, see Tobyn et al 2010.)

MODERN HERBALISM

Today, medical herbalism, practised by medical herbalists, continues to draw on traditional knowledge, but, increasingly, this is interpreted and applied in a modern context. For example, herbalists use current knowledge of the causes and consequences of disease as well as some of the diagnostic tools, such as blood pressure measurement, used in conventional medicine. Also, there is an increasing emphasis, particularly among professionally organized medical herbalists, on using evidence from modern randomized controlled clinical trials to support the traditional use of herbal preparations. In essence, herbal medicine in the UK and other countries in which 'Western herbal medicine' has developed (e.g. Australia, New Zealand) covers a wide spectrum of practice: there are traditional herbalists who refer mainly to the older traditions and philosophy, those whose view is aligned more closely with a 'modern' rational or scientific phytotherapeutic approach, and those whose practice is somewhere between the two (Nissen and Evans 2012).

The following are important aspects of modern herbalism as practiced by herbalists:

- A patient's psychological and emotional wellbeing, as well as physical health, is considered, resulting in the claim that a holistic therapy is offered.
- Herbalists select herbs on an individual basis for each patient (in line with the holistic approach), thus it is likely that even patients with the same physical symptoms will receive different combinations of herbs.
- Herbalists also aim to identify the underlying cause (e.g. stress) of a patient's illness and to consider this in the treatment plan.
- Herbs are used to stimulate the body's healing capacity, to 'strengthen' bodily systems and to 'correct' disturbed body functions rather than to treat presenting symptoms directly.
- Herbs may be used, for example, with the aim of 'eliminating toxins' or 'stimulating' the circulation. The intention is to provide long-term relief from the particular condition.

Importantly, different chemical constituents of a medicinal plant are seen as acting together in some

(undefined) way that has beneficial effects. For example, the constituents may have additive effects, or interact to produce an effect greater than the total contribution of each individual constituent (known as 'synergy'), or the effects of one constituent reduce the likelihood of adverse effects due to another constituent. Thus, herbalists believe it is important to use the plant material in a more 'whole' form, as opposed to isolating and extracting a specific chemical constituent that has been shown to have the relevant pharmacological activity (as would be done in the development of a conventional medicine where the original source of the compound was plant material). Similarly, it is also believed that some combinations of different herbs interact in a beneficial way. There is some experimental (but little clinical) evidence that such interactions occur, although it cannot be assumed that this is the case for all herbs or for all combinations of herbs. Synergy is discussed in detail in Chapter 11.

CONDITIONS TREATED

Medical herbalists treat a wide range of acute (e.g. infections) and, more usually, chronic conditions, and all patient groups from pregnant women, breast-feeding mothers and children, to older patients. Herbalists treat patients with: allergies; autoimmune conditions, such as Crohn's disease, lupus, multiple sclerosis and rheumatoid arthritis; infections; exhaustion, fatigue, and fatigue syndromes, such as myalgic encephalitis (ME); issues and conditions relating to fertility, pregnancy, childbirth and breast-feeding; digestion and nutrition; skin disorders, including acne, eczema, dermatitis; heart and circulatory issues; musculoskeletal problems; emotional and mental health conditions, including anxiety and depression; and many other issues, such as menstrual problems, migraine, and sleep disorders.

The herbalists' approach to treating illness is usually multi-faceted and may involve, for example, depending on the particular condition treated and the individual patient, the use of herbs to relieve symptoms, reduce inflammation, strengthen the immune system, aid 'detoxification' or hormonal balance, and to support the body and specific organs. Typically, herbalists would explain that this multi-layered approach to healing and the 'gentle' nature of the action of herbs, means that a response to treatment can take longer than with conventional medicines.

Table 15.1 Examples of herbal prescriptions

PLANT	PLANT PART
Menopausal symptoms	
Actaea racemosa L. (syn.: *Cimicifuga racemosa* (L.) Nutt., black cohosh)	Roots, rhizome
Leonorus cardiaca L. (motherwort)	Aerial parts
Hypericum perforatum L. (St John's wort)	Aerial parts
Alchemilla vulgaris aggr.* L. (Lady's mantle)	Aerial parts
Stress	
Passiflora incarnata L. (passion flower)	Aerial parts
Valeriana officinalis L. (valerian)	Root
Verbena officinalis L. (vervain)	Aerial parts
Leonorus cardiaca L. (motherwort)	Aerial parts

*'aggr.' – aggregate refers to a group of closely related species generally with very similar morphology

HERBALISTS' PRESCRIPTIONS

A first consultation with an herbalist may last for an hour or more, during which the herbalist will take a full case history, including the detailed history of the illness, discuss diet and lifestyle, employ or arrange for diagnostic tests and examinations (e.g. examine the skin, measure blood pressure) depending on the nature of the presenting illness and, at the first appointment, take a full family medical history. The practitioner would then develop a treatment plan, comprising treatment with herbal medicines, as well as dietary advice and recommendations for dietary supplements where appropriate (Denham et al 2011).

Generally, a combination of several different herbs (usually four to six) is used in the treatment of a particular patient (Barnes and Ernst 1998). Some examples of such combinations are given in Table 15.1, although there are no 'typical' prescriptions for specific conditions; as stated above, even patients with the same condition are likely to receive different prescriptions. Sometimes, a single herb may be given, for example, *Vitex agnus-castus* L. (chasteberry) for premenstrual syndrome and dysmenorrhoea. Each patient's treatment is reviewed regularly and is likely to be changed depending on whether or not there has been a response.

Herbalists usually prescribe herbal medicines as tinctures, although sometimes more concentrated formulations (fluid extracts) are used. Where a prescription requires several herbs, tinctures and fluid extracts are blended into a mixture. Some herbalists

Table 15.2 Comparison of herbalism and rational phytotherapy

HERBALISM	RATIONAL PHYTOTHERAPY
Assumes that synergy or additive effects occur between herbal constituents or between herbs	Seeks evidence that synergy or additive effects occur between herbal constituents or between herbs
Holistic (individualistic) prescribing of herbs	Not holistic; uses symptom- or condition-based prescribing
Preparations mainly formulated as tinctures	Preparations mainly formulated as tablets and capsules
Mainly uses combinations of herbs	Single-herb products used mainly
Some opposition towards tight standardization of preparations	Aims at using standardized extracts of plants or plant parts
Not scientifically evaluated	Science-based approach

will prepare their own stock material, others purchase it from specialist suppliers and most dispense their own herbal prescriptions. Other oral formulations (herbal teas, tablets, capsules) and topical preparations (creams, lotions, ointments) of herbs may also be prescribed. The acts of prescribing and dispensing would both be undertaken by the herbalist: the herbal-medicine practitioner would write the patient's prescription and make entries in their patient records, and then prepare and dispense the herbal preparation(s) themselves, sometimes with recommendations to the patient to purchase certain nutritional supplements.

TRADITIONAL MEDICAL HERBALISM AND THE 'RATIONAL' OR SCIENTIFIC PHYTOTHERAPEUTIC APPROACH

Rational phytotherapy is the practice of science- or evidence-based herbal medicine. It is the use of well-characterized, standardized botanical medicines, selected on the basis of their known phytochemistry and clinical pharmacology as appropriate evidence-based therapeutic agents for the prevention and treatment of specific health and medical conditions (Schulz et al 1998). Its approach is thus the same as that of conventional medicine, although the interventions are botanical drugs. The approach originates from Germany, where the herbal medicines industry is organized and regulated much like the conventional pharmaceuticals industry.

There are some similarities and important differences between traditional medical herbalism and a rational or scientific phytotherapeutic approach that it is important to understand. Clearly, both traditional medical herbalism and a rational or scientific phytotherapeutic approach involve the use of herbal medicines in the prevention and treatment of health conditions, and both use material doses of those herbal preparations. (N.B. herbal medicine is sometimes confused with homeopathy, which uses highly dilute preparations, not all of plant origin, and has a very different philosophical approach.)

However, importantly, the herbalist's treatment approach (outlined earlier) has not been evaluated scientifically, whereas 'rational phytotherapy' is very much a science-based approach to the use of herbal medicines as therapeutic agents. While many of the same medicinal plants are used in each of the two approaches, the formulations of those herbs are usually very different. For example, St John's wort (*Hypericum perforatum* L.) is used in both traditional medical herbalism and rational phytotherapy. However, traditional medical herbalists are likely to use a tincture of *H. perforatum* herb that is not standardized on its content of any particular constituent, whereas in rational phytotherapy, the preparations used are likely to be well-characterized extracts of the *H. perforatum* herb (leaves and tops) standardized on hypericin and/or hyperforin content and formulated as tablets. A comparison of traditional medical herbalism and rational phytotherapy is provided in Table 15.2.

The terminology applied to the two approaches is often similar. Herbalism is sometimes also referred to as phytotherapy, and both herbalism and rational phytotherapy are sometimes described as 'herbal medicine'. Likewise, preparations used in either approach may be referred to as 'herbal medicines' or 'phytomedicines' or 'phytotherapies'.

EVIDENCE OF EFFICACY AND SAFETY

There is a significant body of clinical evidence on the potential benefits and potential risks associated with the use of specific herbal medicines (for the

most important species see Part B). The vast majority of this information relates to the use of specific herbal medicines formulated as phytomedicines and used with the same approach as that for conventional pharmaceuticals to treat or prevent disease. There has been very little investigation of the efficacy and safety of herbal medicines and combinations of herbal medicines used by traditional medical herbalists. Furthermore, while the herbalist's treatment approach is well-illustrated in case studies (e.g. Owen 2011), robust scientific evaluation of the efficacy and safety of herbalism as a treatment approach is very limited (Denham et al 2011).

In most countries, medical herbalists are not recognized as state-registered health professionals, and there is no legal requirement for medical herbalists to have completed specific education and training programmes. That said, to become a member of most professional organizations of medical herbalists, it is necessary to have successfully completed a training programme (usually around three years in duration) recognized by the professional organization, and to comply with the organization's professional code of ethics and standards. In several countries, including the UK, there have been applications or proposals to regulate herbal-medicine practitioners, but this outcome has not been achieved to date.

References

Barnes, J., Ernst, E., 1998. Traditional herbalists' prescriptions for common clinical conditions: a survey of members of the National Institute of Medical Herbalists. Phytother. Res. 12, 369–371.

Denham, A., Green, J., Hawkey, S., 2011. What's in the bottle? Prescriptions formulated by medical herbalists in a clinical trial of treatment during the menopause. J. Herb. Med. 1, 95–101.

Nissen, N., Evans, S., 2012. Exploring the practice and use of Western herbal medicine: perspectives from the social science literature. J. Herb. Med. 2 (1), 6–15.

Owen, N., 2011. An herbal therapeutic approach to food intolerance and immune dysfunction: an illustrative case history. J. Herb. Med. 1 (2), 53–63.

Schulz, V., Hänsel, R., Tyler, V.E., 1998. Rational phytotherapy: a physicians' guide to herbal medicine, second ed. Springer, Heidelberg.

Tobyn, G., Denham, A., Whitelegg, M., 2010. Illustrated by Marije Rowling. The Western herbal tradition: 2000 years of medicinal plant knowledge. Churchill Livingstone/Elsevier, Edinburgh.

Further reading

Eldin, S., Dunford, A., 1999. Herbal medicine in primary care. Butterworth-Heinemann, Oxford.

Mills, S.Y., Bone, K., 2000. Principles and practice of phytotherapy. Churchill Livingstone, Edinburgh.

Chapter 16

'Oriental' and 'Asian' forms of traditional medicine

The more systematic forms of traditional medicine arose in the East, particularly in India and China, and from there they spread to other parts of the world where they influenced Greek, Arab and other highly sophisticated cultures. Ayurveda originated in India, and traditional Chinese medicine (TCM) in China, but in both cases they have evolved as they were incorporated into local customs; for example, **Jamu** arose in Indonesia and **Kampo** in Japan. These medical systems were (and still are) complicated and structured, and had a philosophical and religious aspect far removed from the primitive magic, superstition, divination and sacrifice that are characteristic of African, South American, pre-Islamic Arabian and other cultures, where medical knowledge was an oral (rather than written) tradition. **Tibb-I-Islam** is Islamic medicine and, therefore, practised by Muslims throughout Asia and the rest of the world, but it is more of a cultural way of looking at health (as dictated by the Koran) than a type of medicine, although of equal impact.

TRADITIONAL CHINESE MEDICINE

The study of TCM is a mixture of myth and fact, stretching back well over 5000 years. At that time, none of the knowledge was written down, apart from primitive inscriptions of prayers for the sick on pieces of tortoise carapace and animal bones, so a mixture of superstition, symbolism and fact was passed down by word of mouth for centuries. TCM still contains very many remedies, which were selected by their symbolic significance rather than proven effects; however, this does not necessarily mean that they are all 'quack' remedies! There may be some value in medicines such as tiger bone, bear gall, turtle shell, dried centipede, bat dung and so on, but these substances have not been comprehensively researched in robust studies; also, of substantial importance is that at least some of these are endangered or threatened with extinction and as such are CITES-listed species. The herbs, however, are well researched and are becoming increasingly popular for several reasons, including the popular interest in 'natural health products' and, among some people, a dissatisfaction with Western medicine. Again, Chinese medicine is philosophically based, and as an holistic therapy (one that considers all aspects of a person's illness, including the physical, emotional, and spiritual) the concept of balance and harmony is supremely important. The relevant records and documents were discussed earlier (Chapter 2), but additional historical milestones will be included here to show the evolution of TCM into what it is today.

THE DEVELOPMENT OF TCM

Shen Nong, the legendary Chinese Emperor, is credited with the discovery of herbal medicine in around 2800 BCE, and he is also reputed to have defined the opposing yet complementary principles eventually known as **yin** and **yang**. **Confucius** (551–479 BC) is celebrated as China's greatest sage. He established a code of rules and ethics based on the premise that there is an order and harmony to the universe resulting from a delicate balance of yin and yang forces. Humans should cultivate the five virtues of benevolence, justice, propriety, wisdom and sincerity, in order to exert their own life force in this cycle. **Lao Zi** extended the Confucian doctrine and taught that man can only achieve personal harmony by bowing to the

inevitable. His followers invented a spiritual destination in the mythical 'Island of the Eastern Sea', where a herb with the power to bestow immortality was thought to grow. The way (or path) within this natural order of things is called the **Tao** and the religion is now called **Taoism**. Along this path, the two basic expressions of **yin** (negative and passive) and **yang** (positive and active) are interlocked. This philosophy was refined and extended by **Dong Zhongshu** to include the inner reaches of man himself, i.e. man as the universe on a small scale, containing within himself the locked cycle of yin and yang. It, therefore, follows that Taoist principles apply to the well-being of man, and extend to diet and medicine as factors in ensuring a balance between physical and mental health.

Food and medicine became inter-related. Herbal medicine was initially the domain of shamans and mountain recluses, who believed that the mountain mists contained high concentrations of **qi**, the vital essence of life. They practised the 'way of long life', which involved a herbal diet and medicine combined with martial arts – a link that continues today. The principles of TCM became consolidated and a search for the 'elixir of life' began to obsess the Chinese aristocracy. In TCM, drugs to rejuvenate and increase longevity are still prized.

The **Han dynasty** (206 BC to 220 AD) saw remedies (including veterinary) recorded in handy booklets or **Gansu**, which were strips of bamboo or wood bound together. All herbal medicines were collected together in the *Shen Nong Ben Cao Jing* (the pharmacopoeia of Shen Nong) and classified into three categories:

- **Upper**: drugs that nurture life.
- **Middle**: drugs that provide vitality.
- **Lower**: 'poisons' used for serious disease.

The most noted physician of these times was **Zhang Zhogjing**, who divided diseases into six types: three 'yin' and three 'yang'. His prescriptions aimed to correct any imbalances of these forces. He also contributed to acupuncture by drawing a map of meridians along which the body's vital energy (qi) is said to flow. During this period, the theory of the circulation of the blood was described, and anaesthetics were used, based mainly on *Datura*. By the end of the Han dynasty all the elements we regard as vital to TCM were in place, and refinement went on throughout the Tang, Song and Ming dynasties.

During the **Ming dynasty**, **Li Shizhen** (1518–1593 CE) produced the classic herbal encyclopaedia *Ben Cao Gang Mu*. It took 27 years to compile and consists of 52 volumes containing 1892 medicines. It was translated into Japanese, Korean, English, French, German and other languages, and is said to mark the beginning of a cultural exchange between Chinese and Western medicine.

In the **20th century**, TCM came under attack from Western influences. Missionary doctors translated Western medical journals into Chinese, and Chinese doctors who had studied abroad turned against traditional herbal medicine. However, after the 1949 communist revolution, the government of the new People's Republic of China reinstated TCM and set up new medical colleges, intended to break China's dependence on the West. The 3rd (or Cultural) Revolution of 1966–1976 brought culture to a standstill, and 'barefoot doctors' with no more than 6 months of training were sent to rural areas to replace the denounced 'intellectual' Westerners. Today, both systems coexist and even Western-style medical schools teach students TCM and acupuncture.

CONCEPTS IN TCM

Qi, the essential life force

Qi (or **chi**) permeates everything. It is transferable. For example, digestion extracts qi from food and drink and transfers it to the body; and breathing extracts qi from the air and transfers it to the lungs. These two forms of qi 'meet' in the blood and form 'human qi', which circulates through the body. It is the quality, quantity and balance of qi that determines your state of health and lifespan. Obviously, therefore, food and air affect health, so diet and breathing exercises are very important. These aspects of treatment will be considered first, before herbs are introduced. It is considered that the original vital energy, **yuan qi**, is gradually dissipated throughout life, so it is important to conserve it using diet, kung fu, breathing exercise and herbal medicine.

Yin and yang

These have already been mentioned as central to Taoism, and the theory of yin and yang still permeates all aspects of Chinese thought. Attributes of both are:

- **Yin**: negative/passive/dark/female/water.
- **Yang**: positive/active/bright/male/fire.

Yin is considered to be the stronger: fire is extinguished by water, and water is 'indestructible'. So yin

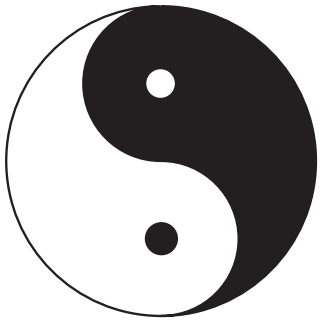


Fig. 16.1 The yin-yang symbol.

is always mentioned before yang; however, they are always in balance. Consider the well-known symbol (Fig. 16.1): where yin becomes weak, yang is strong and vice versa. Both contain the seed of each other: their opposites within themselves.

The five elements

The earth is divided into wood, fire, earth, metal and water. They dominate everything on earth, and each is associated with a vital organ of the body:

- Heart: fire.
- Liver: wood.
- Spleen: earth.
- Lungs: metal.
- Kidneys: water.

The vital organs

These do not correspond to our organs exactly. Exact anatomy was not considered important since it was the relationship between the organs, the five elements, qi and yin and yang that mattered. Also, until the 20th century, cutting up a human body (dead or alive) was considered a grave insult to the ancestors. An example of the relationship between them all and its treatment is that of a person with a red complexion (fire colour) and who laughs a lot (fire sound) may have an over-fired heart; in this case, herbs to sedate the heart will be given.

The organs are also considered to be yin or yang and are paired. Coupled organs are connected by meridians, or energy channels, through which qi flows. Meridians are not associated with the nervous system and cannot be seen physically. They are stimulated with herbs and by acupuncture and will have a direct effect on a particular organ as well as a toning effect on the system.

Causes of disease

Bacteria, viruses and chemicals are not considered to be causes. If an organ is weak, it may be attacked, and, therefore, the weakness is the cause and must be rectified. It may be the result of external forces and internal emotional factors. The external 'cosmological' forces are called the **six excesses:**

- Wind.
- Cold.
- Summer heat.
- Dampness.
- Dryness.
- Fire.

Most people, if healthy, are not affected by the six excesses but, if the body is deficient in qi or weather conditions are abnormal (i.e. not what is expected), then this may cause problems.

The seven emotions

These are considered to be the major internal causes of disease. Excessive emotional activity causes a severe yin/yang imbalance, blockage of qi in the meridians and impairment of vital organ function. This leads to damage of the organs and allows disease to enter from outside, or a minor weakness from inside to develop. The seven emotions are:

- Joy.
- Anger.
- Anxiety.
- Concentration.
- Grief.
- Fear.
- Fright.

Once physical damage has occurred, by whatever cause, it will need more than emotional factors to cure it and herbs will be used. There are a few other causes, which are not emotional or external excesses. These are the exceptions not the rule, and include epidemics, insect and animal bites, worm infestation and hereditary diseases.

Diagnosis

Various methods are used:

- **Examination of the tongue**: a very important aspect.

Table 16.1 Treatment of disease in traditional Chinese medicine according to the nature of disease and the remedy

TYPE OF DISEASE	EXAMPLE OF DISEASE	NATURE OF DISEASE	NATURE OF REMEDY	EXAMPLE OF REMEDY	DESIRED EFFECT
Cold	Nausea, vomiting	Yin	Yang	*Zingiber officinale* Roscoe	Warming
Hot	Malaria, fever	Yang	Yin	*Artemisia annua* L.	Cooling
Empty	Fatigue, diabetes	Yin, yang, qi deficiency	Tonic	*Panax ginseng* C.A. Mey.	Nourishing
Full	Congestion in chest	Yang	Yin	*Scutellaria baicalensis* Georgi	Cooling
Internal	Weak pulse	Yin	Yang	*Aconitum carmichaelii* Debeaux	Warming
External	Psoriasis	Yang	Yin	*Arctium lappa* L.	Cooling

- **Pulse diagnosis**: more than one pulse will be taken, depending on the pressure exerted.
- **Palpation of internal organs**: carried out to determine consistency and tone.
- **Massage**: used to detect temperature and knotted muscles or nerves.
- **Interviewing**: vital – questions are asked about sleep patterns, tastes in food and drink, stool and urine quality, fever, perspiration and sexual activity.

Treatment

The purpose is to rectify harmony, restore qi and the yin/yang balance. For example, 'cold' diseases, such as cold in the lungs, coughs, vomiting and nausea are considered to be a deficiency of yang and treatment would be with a warming herb such as ginger (see examples in Table 16.1). A list of common herbs and their indications is given in Table 16.2. Once the prescription has been formulated, the patient may be given a crude herb mixture with written instructions on how to prepare it at home, perhaps as an infusion or tea. Pastes and pills are prepared by the herbalist and may take several days to complete. Slow-release preparations are made using beeswax pills; tonic wines, fermented dough (with herbs in) and external poultices are also common.

KAMPO

Kampo (also known as 'kanpo') is Japanese herbal medicine and is part of Japanese traditional medicine, along with acupuncture and acupressure (shiatsu), which has been practised for around 1500 years (Yu et al 2006). Kampo originally derives from ancient traditional Chinese herbal medicine, merged with indigenous Japanese practices and culture; it can be considered a simplified form of TCM. It has retained some similarities with TCM, such as holistic diagnostic patterns of disease and, originally, individualized prescriptions, but it has also continued to evolve through practice in Japan.

As with other forms of traditional medicine, kampo recognizes the relationship between the human body and its environment, and views the occurrence of disease as resulting from imbalance in the patient's normal state or equilibrium, and the aim of treatment is to restore balance or equilibrium. In kampo, treatment is based on a patient's 'Sho', which is the patient's symptoms at a given moment. In modern kampo, the Sho (kampo diagnosis) is often named in terms of a specific treatment formula; thus, the pathological condition is related to the prescription for its treatment. Often, the same formula can be used to treat several different conditions, and there are many different formulae that are indicated for a particular condition (Borchers et al 2000). Many ingredients and their respective formulae are used for their immunomodulatory properties.

A kampo formula typically comprises five to nine herbs; most formulae are included in the older text *Shang Han Za Bing Lun* and described in modern books. An example of a well-known kampo medicine is 'Shosaiko-to' (or shosaiko-to). This formula is used for the treatment of acute fever, pneumonia, bronchitis, influenza, chronic gastrointestinal disorders, chronic hepatitis and other liver diseases, and comprises seven crude herbal drugs: bupleurum (*Bupleurum falcatum* L.), root;

Table 16.2 Some important herbs in traditional Chinese medicine and their uses

BOTANICAL NAME/ENGLISH NAME	CHINESE NAME	NATURE	MEDICAL USE
Aconitum carmichaelii Debeaux			
Aconitum	Chuan wu tou	Very pungent and hot, yang	Heart tonic, diarrhoea, analgesic
Angelica sinensis (Oliv.) Diels			
Chinese angelica	Dang gui	Sweet, pungent, warm, yang	Menstrual disorders, analgesic
Arctium lappa L.			
Great burdock	Niu bang zi	Pungent, bitter, cold, yin	Sore throat, pneumonia, psoriasis
Artemisia annua L.			
Sweet wormwood	Qing hao	Bitter, cold, yin	Malaria, fever
Cinnamomum cassia (L.) J.Presl			
Chinese cinnamon	Rou gui	Pungent, sweet, very hot, yang	Diarrhoea, tonic, dysmenorrhoea
Coix lacryma-jobi L.			
Job's tears	Yi yi ren	Sweet, plain, slightly cold, yin	Dysentery, painful joints, diuretic
Cyperus rotundus L.			
Nut grass	Xiang fu	Pungent, sweet, neutral	Liver disorders, amenorrhoea, sedative
Ephedra sinica Stapf			
Ephedra	Ma huang	Pungent, slightly bitter, warm, yang	Bronchial asthma, hayfever
Glycyrrhiza uralensis Fisch. ex DC.			
Chinese liquorice	Gan cao	Sweet, neutral	Asthma, bronchitis, ulcers, steroid activity
Lonicera japonica Thunb.			
Japanese honeysuckle	Jin yin hua	Sweet, cold, yin	Fever, throat infections, ulcers
Paeonia lactiflora Pall.			
Chinese white peony	Bai shao yao	Bitter, slightly cold, yin	Fever, haemostatic anti-inflammatory
Panax ginseng C.A.Mey.			
Ginseng	Ren shen	Sweet, neutral	Tonic, aphrodisiac, appetite stimulant
Perilla frutescens (L.) Britton			
Beefsteak plant	Zi su	Pungent, warm, yang	Allergic reactions, fever
Rheum palmatum L.			
Rhubarb	Da huang	Bitter, cold, yin	Constipation, burns, diarrhoea, jaundice
Salvia miltiorrhiza Bunge			
Salvia	Dan shen	Bitter, cold, yin	Menstrual disorders, chest pain, blood clots
Schisandra chinensis (Turcz.) Baill.			
Schisandra	Wu wei zi	Sour, warm, yang	Diarrhoea, thirst, asthma, coughs
Scutellaria baicalensis Georgi			
Baical skullcap	Huang qin	Bitter, cold, yin	Dysentery, jaundice

Table 16.2 Some important herbs in traditional Chinese medicine and their uses—cont'd

BOTANICAL NAME/ENGLISH NAME	CHINESE NAME	NATURE	MEDICAL USE
Styphnolobium japonicum (L.) Schott (syn.: *Sophora japonica* L.)			
Pagoda tree	Huai hua	Bitter, slightly cold, yin	Blood disorders, clots, reduces cholesterol
Terminalia chebula Retz.			
Myrobalan	He zi	Bitter, sour, neutral	Chronic diarrhoea, dysentery, haemostatic
Tribulus terrestris L.			
Caltrops	Ci ji li	Sweet, warm, yang	Liver and kidney tonic, lumbago, tinnitus
Zingiber officinale Roscoe			
Ginger	Gan jiang	Pungent, sweet, very hot, yang	Nausea, vomiting, colds, diarrhoea
Ziziphus jujuba Mill.			
Chinese jujube	Suan zao ren	Sweet, sour, neutral	Liver and heart tonic

Different parts of the plant, whether fresh or dried, and the type of preparation will affect the nature and medical uses of a herb, so the above are only examples. Many of the plants are the same as those used in Ayurvedic medicine, sometimes for different purposes but mainly for the same indications.

pinellia [*Pinellia ternata* (Thunb.) Makino], tuber; scutellaria (*Scutellaria baicalensis* Georgi.), root; jujube (*Zizyphus jujuba* Mill.), fruit; ginseng (*Panax ginseng* C.A. Mey.), root; licorice (*Glycyrrhiza uralensis* Fisch. ex DC., *G. glabra* L.), root; ginger (*Zingiber officinale* Roscoe), root.

Today, kampo is widely practised in Japan and is fully integrated into the healthcare system (Yu et al 2006). Kampo is formally recognized by the Japanese government, and this has also influenced practice. Kampo medicine can only be practised by conventional medical doctors in Japan; there is no specific training or license required for them to do this, although many medical universities now include kampo in their medical programmes. Some kampo medicines are included in Japanese clinical practice guidelines, and approved formulae are covered by national health insurance.

Modern kampo uses around 200 recognized formulae, and most access to kampo is through use of manufactured products containing these formulae. This can sometimes be restrictive if there is not a formula to suit the patient's condition. Some kampo prescriptions may have the same name as some TCM formulae, but the ingredients could be different. The Japanese Pharmacopoeia XIV included over 100 crude herbal medicines, animal and mineral products used in kampo medicine. The number of medicinal plants and other ingredients used in kampo medicine is relatively limited; the main differences lie in the precise combinations of crude ingredients that are used in different formulae, and the quantity and proportion of each ingredient used (Borchers et al 2000).

There are over 350 randomized controlled trials of kampo medicines in Japan, although these studies have not always used kampo diagnosis (Motoo et al 2014) and few high-quality studies are available (Motoo et al 2011). Adverse drug reactions, including allergic reactions, gastrointestinal effects, fever, headache and haematuria, have been reported following the use of kampo medicines (Ikegami et al 2004). Also, since kampo medicines are chemically complex mixtures, there is also the potential for drug interactions to occur where kampo medicines and conventional Western medicines are taken concurrently. As with other traditional medicines, the efficacy and safety of Kampo require further investigation.

AYURVEDA

Ayurveda is considered to be the most ancient of all medical disciplines. It is a system of sacred Hindu medicine, originating in India, and as well as being an oral tradition, is also fairly well documented. Over 5000 years ago, the great seers (or 'rishis') organized the 'fundamentals of life' into what became known as Ayurveda, and this has evolved and adapted over the years whilst still retaining the philosophical basis on which it was founded. It now accommodates modern

science, especially in relation to the testing of medicines, and research and adaptation are actively encouraged. Like other forms of holistic medicine, Ayurveda considers the patient as an individual and 'normality' as what is appropriate for that particular person. The patient is viewed as unique, and is, therefore, subject to unique imbalances. This is in contrast to Western medicine, where populations are generalized and 'normal' means what is applicable to the majority. Another important difference is that Eastern thought greatly values subjectivity, and even considers it to be a vital addition to objectivity, which is the goal in Western medicine.

There are only a few Ayurvedic practitioners ('vaid') in the West at present, but popularity is growing rapidly and Ayurvedic medicines are now being exported from India to many other countries. It is worthy of study for this reason, not only because it is the most ancient system of medicine still in use today, but also because it has influenced so many other types. Many ethnic populations from India and Pakistan continue to use their own traditional remedies while living in Europe, Australia or the USA, and it is important that healthcare professionals should have some knowledge of the background and remedies that they are using.

Philosophically, Ayurveda has similarities with traditional Chinese medicine, in that the concept of humanity as a microcosm within the macrocosm of the universe is accepted. There is a life force, which can be nourished, protected, and of course dissipated, as well as opposing forces or 'humors' whose balance is vital to health. In TCM there are two (yin and yang), and in Ayurveda there are three (the tridosha). There are five elements in both, but they are slightly different and will be outlined in the appropriate section. Many remedies are common to both systems, although the philosophical rationale for their application may be a little different.

CONCEPTS IN AYURVEDA

Prana, the life energy

Prana is the vital energy, activating both body and mind. Nutrient prana from the air gives energy to the vital prana in the brain, via respiration, and is thus the equivalent of qi in Chinese medicine. In the body it is seated in the head, and governs emotions, memory, thought and other functions of the mind. Prana kindles the bodily fire (**agni**) and governs the functioning of the heart, entering the bloodstream from where it controls the vital organs or **dhatus**.

Bhutas, the five elements

The ether (space), air, fire, water and earth are considered to be the basic elements, which are manifestations of cosmic energy. They are related to the five senses (hearing, touch, vision, taste and smell) and from them to resultant actions. As an example, ether is related to hearing, since sound is transmitted through it, and from there to the ear, the associated sense organ, leading to speech, from the organs of action, which are the tongue and vocal cords. Likewise, fire is associated with the eyes as sense organs, leading to an action such as walking, by an organ of action, such as the feet.

Tridosha: vata, pitta and kapha – the three humors

Ether, air, fire, water and earth (the five basic elements) are manifest in the human body as three basic principles or humors known as the '**tridosha**', which is unique to Ayurveda. The three humors, known as **vata, pitta** and **kapha** (individually called **doshas**), govern all biological, psychological and physiopathological functions of the body and mind. The primary requirement for the diagnosis and treatment of disease is to understand the relationship between these. Some similarities can be drawn with the ancient Greek system of medicine in which the humoral theory considers the body to consist of four fluids (phlegm, blood, yellow bile and black bile) and disease is thought to occur when these fluids are out of balance. Similarly, when the tridosha works in harmony and functions in a balanced manner, the result is health and a feeling of well-being in the individual. However, in cases of imbalance and disharmony, the result is illness or disease. The tridosha affects the creation, maintenance and destruction of bodily tissues and the elimination of toxins (**ama**) from the body. It is also responsible for psychological phenomena, including basic human emotions such as fear, anger and greed, and more complicated sentiments such as understanding, compassion and love, and as such is the foundation of the psychosomatic nature of man.

The tridosha (vata, pitta and kapha) has recently been redefined as an equilibrium, balance and coordination between the three vital body systems: the central nervous system (CNS) corresponding to vata, the endocrine system to pitta and the immune axis to kapha, operating with both positive and negative feedback. To try to correlate this ancient philosophy with

modern science is difficult, but some analogies can be drawn. The tridosha can be considered to govern all metabolic activities: catabolism (vata), metabolism (pitta) and anabolism (kapha). When vata is out of balance, the metabolism will be disturbed, resulting in excess catabolism, which is the breakdown or deterioration process in the body; excess would, therefore, induce emaciation. When anabolism is greater than catabolism (excess kapha), there is an increased rate of growth and repair of organs and tissues. Excess pitta disturbs metabolism generally. The tridosha can be described further:

- **Vata**, affiliated to air or ether (space), is a principle of movement. It can be characterized as the energy controlling biological movement and is thus associated with the CNS, and governs functions such as breathing, blinking, all forms of movement, the heartbeat and nervous impulses.
- **Pitta** is affiliated to fire and water, and governs bodily heat and energy. It, therefore, controls body temperature, is involved in metabolism, digestion, excretion and the manufacture of blood and endocrine secretions, and is also involved with intelligence and understanding.
- **Kapha** is associated with water and earth. It is responsible for physical structure, biological strength, regulatory functions, including that of immunity, the production of mucus, synovial fluid and joint lubrication and assists with wound healing, vigour and memory retention.

Prakruti, the human constitution

Humans can also be divided into personality types, and the constitution of an individual (prakruti) is determined by the state of the parental tridosha at conception (unlike astrology, which depends on time of birth). Most people are not completely one type or another, but can be described as vatapitta or pittakapha, for example. People of vata constitution are generally physically under-developed with cold, rough, dry and cracked skin. People with pitta constitution are of medium height with moderate muscle development. Kapha people have well-developed bodies. Table 16.3 can be used to indicate constitution in a superficial way, but it is not a tool for self-diagnosis!

As well as the vata, pitta and kapha type of personalities, three attributes provide the basis for distinctions in human temperament, individual differences, and psychological and moral dispositions. These basic attributes are satva, rajas and tamas. In brief, **satva** expresses essence, understanding, purity, clarity, compassion and love; **rajas** describes movement, aggressiveness and extroversion; and **tamas** manifests in ignorance, inertia, heaviness and dullness. In

Table 16.3 Determining the human constitution according to the tridosha

ASPECT OF CONSTITUTION	VATA CHARACTER	PITTA CHARACTER	KAPHA CHARACTER
Bodyweight	Low	Moderate	Overweight
Skin	Dry	Soft	Thick
	Rough	Oily	Oily
	Cool	Warm	Cool
Eyes	Small	Sharp	Large
	Dark	Green	Blue
	Dull	Grey	
Hair	Dry	Oily	Oily
	Dark	Fair	Thick
	Curly		Dark or fair
Appetite	Poor	Good	Steady
	Variable	Excessive	
Thirst	Variable	Excessive	Scanty
Mind	Restless	Aggressive	Calm
	Active	Intelligent	Slow
Emotional temperament	Insecure	Irritable	Calm
	Unpredictable	Aggressive	Greedy
Speech	Fast	Penetrating	Slow
Physical activity	Very active	Moderate	Lethargic
Sleep	Interrupted	Little sound	Heavy
			Long

To find the Ayurvedic constitution, just tick the most relevant description, and count up the ticks in each column to determine the dominant type. It may not just show one pure type, but, for example, vata-pitta or pitta-kapha. Then the best diet for a particular constitution can be found. Foods that aggravate a particular dosha should not be taken in excess by a person of that type; for example, a vata person should not take excessive amounts of lamb, cabbage, potatoes or dried fruits. However, eggs, rice, cooked vegetables and sweet fruits would be beneficial to someone of vata constitution. It can also be used to decide which type of food to eat in different seasons. For example, in summer, pitta predominates, and those foods that aggravate pitta should be avoided; but winter is the season of kapha, so seafood, melon and cows' milk products are not recommended then. Autumn is the season of vata, and spring is kapha-pitta.

Ayurveda, a state of health exists when the digestive fire (**agni**) is in a balanced condition and the bodily humors (vata-pitta-kapha) are in equilibrium. The three waste products (**mala**), which are urine, faeces and sweat, should be produced at usual levels, the senses functioning normally, and the body, mind and consciousness working in harmony. When the balance of any of these systems is disturbed, the disease process begins.

Agni, the digestive fire

Agni governs metabolism and is essentially pitta in nature. An imbalance in the tridosha will impair agni and, therefore, affect metabolism. Food will not be digested or absorbed properly, and toxins will be produced in the intestines, and may find their way into the circulation. These toxins are known as ama and are the root cause of disease. Overactive ama is also detrimental in that over combustion of nutrients may occur, leading to vata disorders and emaciation.

Malas, the three waste products

These are, as may be expected, the faeces, urine and sweat, and production and elimination of these are vital to health. Their appearance and properties can give many indications of the state of the tridosha and, therefore, health. As an example, the colour of urine depends on the diet, and, if the patient has a fever or jaundice (pitta disorders), it may be darker. Substances such as coffee and tea, which stimulate urination, also aggravate pitta and render the urine dark yellow.

Dhatus, the seven tissues

The human body consists of seven basic tissues or organs (constructing elements) or dhatus. When there is a disorder in the balance of the tridosha, the dhatus are directly affected. Health can be maintained by taking steps to keep vata-pitta-kapha in balance through a proper diet, exercise and rejuvenation programme. The dhatus do not correspond to our definition of anatomy, but are more a tissue type than an individual organ.

Gunas, the attributes

Ayurveda encompasses a subtle concept of attributes or qualities called gunas. Caraka, the great Ayurvedic physician, theorized that organic and inorganic substances, as well as thought and action, all have definite attributes. These attributes contain potential energy while their associated actions express kinetic energy. Vata, pitta and kapha each have their own attributes, and substances having similar attributes will tend to aggravate the related bodily humor. The concepts governing the pharmacology, therapeutics and food preparation in Ayurveda are based on the attributes' actions on and reactions to one another. Through the understanding of these attributes, the balance of the tridosha may be maintained. The diseases and disorders ascribed to vata, pitta and kapha are treated with the aid of medicines that are characteristic of the opposite attribute, to try to correct the deficiency or excess. Vata disorders are corrected with the aid of sweet (**madhur**), sour (**amla**) or saline and warm (**lavana**) medicines. The excitement or 'aggravation' of pitta is controlled by sweet (madhur), bitter (**katu**) or astringent and cooling (**kashaya**) herbs. Kapha disorders are corrected with pungent (**tikta**), bitter (katu) or astringent and dry (kashaya) herbs. The use of herbs in correcting any imbalance is of extreme importance and is essential for the proper functioning of the organism. There is often little distinction between foods and medicines, and controlling the diet is an integral part of Ayurvedic treatment. Foods are also described according to their properties, such as their taste (**rasa**) and physical and chemical properties (**guna**); these affect the tridosha (Table 16.4).

APPLICATION OF AYURVEDA

Diagnosis

Taking the case history involves astrological considerations, as well as a thorough medical examination where the appearance of the tongue, properties of the urine, sweat and sputum will also be examined. **Karma**, the good and bad effects across reincarnations, is also taken into account.

Treatment

This may involve diets, bloodletting, fasting, skin applications and enemas, which are used to cleanse the system. There is a programme consisting of five types of detoxification, known as **panchkarma**. Drugs may then be given to bring the dhatus into balance again. These include herbal treatments as well as minerals, and there are thousands in use. In addition, yogic breathing and other techniques are used. All of the abovementioned will have their properties

Table 16.4 Effect of different foods on the tridosha

DOSHA	VATA		PITTA		KAPHA	
FOOD TYPE	AGGRAVATES	BALANCES	AGGRAVATES	BALANCES	AGGRAVATES	BALANCES
Meat	Lamb, pork, venison	Beef, eggs, turkey (white meat), chicken	Beef, lamb, pork, egg yolk	Chicken, turkey, egg white	Beef, lamb, pork, seafood	Chicken, turkey (dark meat), rabbit, eggs
Cereals	Rye, barley	Oats (cooked), rice, wheat	Barley, oats (cooked), brown rice	White rice, wheat, barley, oats (cooked)	Oats (cooked), rice, wheat	Barley, rye, corn
Vegetables	Raw veg, cauliflower, sprouts, cabbage, aubergine, lettuce, mushrooms, onion (raw), peas, potatoes	Cooked veg, carrots, garlic, green beans, cucumber, avocado, courgettes	Carrots, aubergine, garlic, onion, spinach, tomatoes, hot peppers	Broccoli, sprouts, lettuce, peas, cauliflower, mushrooms, courgettes	Cucumber, tomatoes, courgettes	Cauliflower, sprouts, cabbage, carrots, aubergine, lettuce, mushrooms, onions, peas, potatoes
Fruit	Dried fruit, apples, pears, water melon	Sweet fruits, apricots, peaches, bananas, cherries, grapes, citrus	Sour fruits, peaches, bananas, grapes, lemons, oranges, pineapple	Sweet fruits, apples, melon, coconut, raisins, prunes	Bananas, coconut, grapefruit, grapes, lemon, orange, melon, pineapple	Apples, apricots, peaches, pears, cherries, raisins, prunes
Dairy	All OK	All OK	Buttermilk, cheese, yogurt,	Butter, milk	None	Goats milk
Oils	All OK	All OK	Corn, sesame, almond	Sunflower, soya, olive	None	None
Condiments	All OK	All OK	Most	Coriander, fennel, turmeric	All	Salt

described: rasa, guna and their karma, as well as which doshas they affect. Some of the most popular herbs of Ayurveda are shown in Table 16.5.

In modern Indian herbal medicine the Ayurvedic properties are described together with the conventional pharmacological and phytochemical data. Drugs are prepared as tinctures, pills, powders and some formulae unique to Ayurveda (Table 16.6). Ayurveda is very metaphysical – too much so for many Westerners to grasp – and practitioners view it as a way of life as opposed to a career.

Rasayana

Rasayana are remedies considered to have diverse action and, therefore, affect many systems of the body, leading to a positive effect on health – panaceas in other words. The most important are *Asparagus racemosus* Willd. (**shatavari**), *Phyllanthus emblica* L. (**amla**), also known as *Emblica officinalis Gaertn.*, *Piper longum* (**pimpli**), *Terminalia chebula* Retz. (**haritaki**), *Tinospora cordifolia* (Willd.) Hook.f. & Thomson (**guduchi**) and *Withania somnifera* (L.) Dunal (**ashwagandha**). They are included in many recipes and are used to strengthen the tissues of the body. In general, modern research has found them to have antioxidant, immunomodulating and various other activities.

Acceptance of Ayurveda in Europe and other regions of the world is growing, but often its main role is in uses associated with 'wellness' and spa treatments, resulting in a dilution of traditional Ayurvedic concepts.

UNANI

Unani (also spelt, yunani) medicine refers to the traditional medicine system of what was Persia and Arabia. It may also be referred to as Unani Tibb, Al-Tibb-Al-Arabi, Al-Tibb-Al-Unani, or Al-Tibb-Al-Aa'Shaab and other names, and as Arabian or Islamic medicine. Its origin is in the teachings of the Greek physicians Hippocrates and Galen and, later, by the Persian philosopher, scientist and writer, Avicenna; Unani has been

Table 16.5 Some important herbs of Ayurveda and their uses

ENGLISH NAME	AYURVEDIC NAME	EFFECT ON DOSHA	MEDICAL USE
Acorus calamus L.			
Sweet flag	Vacha	Pacifies vata and kapha	Nerve stimulant, digestive
Adhatoda vasica Nees (now classed as *Justicia adhatoda* L.)			
Malabar nut	Vasaka	Pacifies pitta and kapha	Respiratory disorders, fevers
Aegle marmelos (L.) Corrêa			
Bengal quince	Bael, bel	Promotes pitta	Antidysenteric, digestive, tonic
Andrographis paniculata (Burm.f.) Nees			
(Common) andrographis	Kalmegh	Pacifies kapha and pitta	Liver protectant, jaundice
Green chiretta			
Eclipta prostrata (L.) L. (syn.: *Eclipta alba* (L.) Hassk.)			
Trailing eclipta	Bhringarajah	Pacifies kapha and pitta	Skin and hair disorders
Embelia ribes Burm.f.			
Embelia	Viranga	Pacifies kapha and vata	Vermifuge, contraceptive
Nigella sativa L.			
Black cumin	Kalonji	Pacifies vata and kapha	Digestive, antiseptic
Ocimum tenuiflorum L. (syn: *O. sanctum* L.)			
Holy basil	Tulsi	Pacifies kapha and vata	Expectorant, febrifuge, immunomodulatory
Phyllanthus emblica			
Indian gooseberry	Amla	Balances tridosha	Improves memory and intelligence, tonic
Phyllanthus niruri L.			
Stone breaker	Bhumyamlaki	Pacifies kapha and pitta	Diabetes, jaundice, liver protectant
Picrorrhiza kurroa Royle ex Benth.			
Kutki, yellow gentian	Katurohini	Pacifies kapha and pitta	Hepatoprotective, immunomodulator
Piper nigrum L.			
Black pepper	Kalmirch	Pacifies vata and pitta	Digestive, respiratory disorders
Swertia chirayita (Roxb.) H.Karst.			
Chiretta	Chirayita	Balances tridosha	Appetite stimulant, liver disorders
Terminalia arjuna (Roxb. ex DC.) Wight & Arn.			
Arjun myrobalan	Arjuna	Pacifies pitta and kapha	Heart tonic, angina, hypertension
Terminalia chebula Retz.			
Black myrobalan	Haritaki	Balances tridosha	Digestive, blood tonic, antiasthmatic
Tribulus terrestris L.			
Caltrops	Gokhru	Pacifies vata and pitta	Digestive, diuretic, aphrodisiac
Withania somnifera (L.) Dunal			
Winter cherry	Ashwagandha	Pacifies kapha and vata	Analgesic, sedative, rejuvenator

influenced by other traditions, particularly Ayurveda and traditional Chinese medicine.

Today, Unani medicine is practised in India, Pakistan and Middle Eastern countries. In India, Unani medicine is recognized by the government, and is taught to students in medical colleges to undergraduate (five years) and postgraduate levels. Medical practitioners of Unani medicine are known as 'hakims'.

Table 16.6 Methods of preparing Ayurvedic medicines

FORMULATION	METHOD OF PRODUCTION
Juice (swaras)	Cold-pressed plant juice
Powder (churna)	Shade-dried, powdered plant material
Cold infusion (sita kasaya)	Herb/water 1:6, macerated overnight and filtered
Hot infusion (phanta)	Herb/water 1:4, steeped for a few minutes and filtered
Decoction (kathva)	Herb/water 1:4 (or 1:8, 1:16 then reduced to 1:4), boiled
Poultice (kalka)	Plant material pulped
Milk extract (ksira paka)	Plant boiled in milk and filtered
Tinctures (arava, arista)	Plant fermented, macerated or boiled in alcohol
Pills or tablets (vati, gutika)	Soft or dry extracts made into pills or tablets
Sublimates (kupipakva rasayana)	Medicine prepared by sublimation
Calcined preparations (bhasma)	Plant or metal is converted into ash
Powdered gem (pisti)	Gemstone triturated with plant juice
Scale preparations (parpati)	Molten metal poured on leaf to form a scale
Medicated oils or ghee (sneha)	Plant heated in oil or ghee
Medicated linctus/jam (avaleha)	Plant extract in syrup

Unani takes an holistic view of health, believing the body and mind to be interconnected, and an individualistic approach to treatment. Unani focuses on the underlying cause of illness rather than only its symptoms, and part of the approach to treatment is to effect lifestyle changes to improve health and quality of life.

Unani still refers to the galencial model of four bodily 'humours' ('akhlat') – phlegm ('balgham'), blood ('dam'), yellow bile ('safra') and black bile ('sauda') – that was important in early Western traditional medical herbalism. Each person is believed to have a unique humoral constitution, which represents the healthy state of the individual (Rafatullah and Asqasoumi 2008). The mix of these humours also determines the person's temperament ('mizaj'), depending on the dominant humour: phlegm (phlegmatic, 'balghami'); blood (sanguine, 'damvi'); yellow bile (choleric, 'safravi'); black bile (melancholic, 'saudavi'); individuals can also be assigned a combination of temperaments, depending on their compostion of humours (Javed et al 2009). The humours are also linked to the four elements, which is similar to the concept of the bhutas or elements in Ayurveda. Unani medicine links phlegm with water, blood with air, yellow bile with fire, and black bile with earth; the elements are believed to be present in these body fluids and if their equilibrium is disturbed, this leads to illness.

The body is believed to have the power to maintain the healthy state, but if that power fails or weakens, this leads to disturbances in the humours. It is believed that this leads to pathological changes and to the manifestation of clinical symptoms. Management of the condition requires diagnosing the disease, eliminating the cause, and normalizing the humours, tissues and organs. The approach to treatment may comprise one or more of the following: pharmacotherapy with Unani drugs; use of modalities, such as cupping, massage, aromatherapy, and leeching; dietary modification and surgery.

The Unani pharmacopoeia is vast and comprises over 2000 substances from herbal, mineral and animal sources. Unani medicines can be single-ingredient preparations, or, more usually, multi-ingredient preparations comprising several ingredients. In some preparations, powdered toxic metals, including mercury and arsenic, are used in the belief that this improves the effectiveness of the remedies; however, the inclusion of these ingredients can result in toxic effects. Access to treatment can be through a Unani practitioner; manufactured, formulated Unani medicinal products can readily be purchased online.

JAMU

Jamu is the traditional medical system practised in Indonesia and other parts of south-east Asia, including Malaysia and Singapore. Like other traditional medicine systems, jamu considers physical symptoms and illnesses to indicate imbalance, and the aim of treatment is to correct this. Jamu is also the name given to the traditional medicines prepared from plants that are used in the practice of jamu. Jamu preparations are viewed as essential supplements to maintain health, and as being both preventive and curative; as such, they are usually taken daily, and for long periods of time.

Traditionally, medicinal plant parts were taken fresh, or dried, as an infusion, typically prepared

in the home; typically, a mixture of several dried or powdered herbs would be used. In some respects, preparation of these home jamu remedies evolved into 'jamu gendong', whereby 'jamu women' sell home-made jamu on the streets from a pack carried on their back. These jamu gendong are usually freshly prepared liquid formulations of plant medicines, and are taken in quantities of 200–300 ml daily as a health tonic, and for the treatment of minor ailments (Limyati and Juniar 1998).

Today, the production of jamu is a major industry, particularly in Indonesia, and preparations are available in numerous pharmaceutical forms, including solid dose forms such as tablets and pills, powders and tonics for internal use, and creams, ointments and so forth for external use (Tuschinsky 1995). 'Jamu-jamu majun' are medicines formulated as pills for enhancing male vitality. Traditional 'majun' are large soft pills prepared from cooked, powdered herbal ingredients kneaded with honey and beef fat and then shaped into the soft pills; these are increasingly also available formulated as capsules.

Safety problems resulting from poor-quality jamu products have been reported. There are case reports of serious adverse reactions, including agranulocytosis, necrotizing fasciitis, Stephens-Johnson syndrome and toxic epidermal necrosis resulting in death, associated with the use of jamu medicines adulterated with pharmaceutical agents, including phenylbutazone and similar drugs (Doshi et al 2009, Giam et al 1986, Paul et al 2005). Aflatoxins have been detected in commercial jamu traditional herbal products (Ali et al 2005), and substantial microbial contamination of jamu gendong raw materials and finished products has been reported (Limyati and Juniar 1998).

References – TCM and Kampo

Borchers, A.T., Sakai, S., Henderson, G.L., 2000. Shosaiko-to and other Kampo (Japanese herbal) medicines: a review of their immunomodulatory activities. J. Ethnopharmacol. 73 (1–2), 1–13.

Ikegami, F., Fujii, Y., Satoh, T., 2004. Toxicological considerations of Kampo medicines in clinical use. Toxicology 198 (1–3), 221–228.

Motoo, Y., Seki, T., Tsutani, K., 2011. Traditional Japanese Medicine, Kampo: its history and current status. Chin. J. Integr. Med. 17 (2), 85–87.

Motoo, Y., Arai, I., Tsutani, K., 2014. Use of Kampo diagnosis in randomized controlled trials of Kampo products in Japan: a systematic review. PLoS One 9 (8) e104422–e104422.

Yu, F., Takahashi, T., Moriya, J., et al., 2006. Traditional Chinese Medicine and Kampo: a review from the distant past for the future. J. Int. Med. Res. 34, 231–239.

Further reading – Ayurveda

Govindarajan, R., Vijayakumar, M., Pushpangadan, P., 2005. Antioxidant approach to disease management and the role of Rasayana herbs of Ayurveda. J. Ethnopharmacol. 99, 165–178.

Lad, V., 1990. Ayurveda the science of self-healing. Lotus Press, Wisconsin.

Mukherjee, P.K., Mukherjee, D., Venkatesh, M., Rai, S., Heinrich, M., 2009. The sacred lotus *(Nelumbo nucifera)* – phytochemical and therapeutic profile. J. Pharm. Pharmacol. 61, 407–422.

Sairam, T.V., 2000. Home remedies, vols. I–III. Penguin, India.

Williamson, E.M. (Ed.), 2002. Major herbs of Ayurveda. Churchill Livingstone, Edinburgh.

References – Unani

Javed, G., Anwar, M., Siddiqui, M.A., 2009. Perception of psychiatric disorders in the Unani system of medicine – a review. Eur. J. Integr. Med. 1 (3), 149–154.

Rafatullah, S., Alqasoumi, S., 2008. Unani medicine: an integral part of health care system in Indian subcontinent. Eur. J. Integr. Med. 1 (Suppl. 1), S39–S40.

References – Jamu

Ali, N., Hashim, N.H., Saad, B., Safan, K., Nakajima, M., Yoshizawa, T., 2005. Evaluation of a method to determine the natural occurrence of aflatoxins in commercial traditional herbal medicines from Malaysia and Indonesia. Food. Chem. Toxicol. 43 (12), 1763–1772.

Doshi, H.K., Thambiah, J., Chan, C.L., Nga, M.E., Tambyah, P.A., 2009. Necrotising fasciitis caused by adulterated traditional Asian medicine: a case report. J. Orthop. Surg. 17 (2), 223–226.

Giam, Y.C., Tham, S.N., Tan, T., Lim, A., 1986. Drug eruptions from phenylbutazone in Jamu. Ann. Acad. Med. Singapore. 15 (1), 118–121.

Limyati, D.A., Juniar, B.L., 1998. Jamu Gendong, a kind of traditional medicine in Indonesia: the microbial contamination of its raw materials and endproduct. J. Ethnopharmacol. 63 (3), 201–208.

Paul, J., Duncan, J.R., Sharp, P., et al., 2005. Agranulocytosis and citrobacter infection associated with jamu, a herbal remedy containing phenylbutazone. Clin. Infect. Dis. 40 (12), 1859–1860.

Tuschinsky, C., 1995. Balancing hot and cold – balancing power and weakness: social and cultural aspects of Malay jamu in Singapore. Soc. Sci. Med. 41 (11), 1587–1595.

Chapter 17

African, American and Oceanian traditional medicine using herbal preparations

TRADITIONAL AFRICAN MEDICAL SYSTEMS

Traditional African medicine (TAM) is a mainly oral tradition with few written records. Many of the features of traditional African medical systems (TAMS) are similar to those of cultures in other parts of the world, and, like them, many practitioners were illiterate. The term 'medical system' is used to describe the complexity of medical practices in a society. The cultural diversity in Africa has resulted in a variety of different TAMS, although these often share important elements. TAM assumes the existence of supernatural forces in the cause of disease to a greater extent than most other systems and employs magic and divination. These and some of the more exotic practices, which include the use of animal parts, especially in the religion vodun (voodoo) in West Africa, undermine its credibility. However, these aspects are an important part of TAM so it is necessary to understand the cultural context in which the herb is used, which may not reflect its true pharmacological activity, to accurately evaluate any contribution to medicine. The African flora remains a huge resource for the discovery of new bioactive compounds: from an estimated biodiversity of ~45,000 plant species, only 5,000 have documented medicinal use.

CONCEPTS IN TRADITIONAL AFRICAN MEDICAL SYSTEMS

The causes of disease, as they are defined culturally, are essential for an understanding of TAMS. In African thought, all living things are connected to each other and to the gods and ancestral spirits. If harmony exists between all of these then good health is enjoyed, but, if not, misfortune or ill-health will result. Forces can be directed at humanity by displeased gods, ancestors and also by witches, resulting in disharmony, which must be resolved before good health can be restored. Treatment may also involve much more than medicine; practices such as divination and incantation may be carried out to help with diagnosis, and sacrifices may be needed to placate the supernatural entity. The traditional healer is likely also to be a religious leader, since health and spirituality are closely intertwined in TAMS.

Apart from physical examination, the diagnosis may also involve several other forms of diagnosis and treatment:

- **Confessions** may be extracted. These are thought to be both healing and prophylactic. In the case of a child, the mother may need to examine her previous behaviour, since the sins of the mother can be visited on the child (this compares with ideas in Christianity and other religions).
- **Divination** may be required, and may involve throwing objects and interpreting the pattern in which they fall. This is a consistent feature of many cultures and still persists in Europe in such forms as the 'reading of tea leaves'.

Serious illness is considered to be due to supernatural causes much more than minor aches and pains are, and treatment will correspondingly be more concerned with ritual, incantation and sacrifice than it would for minor disorders. Herbal medicines will be part of the ritual and less likely to lead to expected or anticipated pharmacological or therapeutic activity. The treatment of disease is based on mind–body dualism and is, therefore, holistic; it is concerned with

the whole lifestyle of the patient. This spiritual emphasis is important and can be rationalized with respect to modern life to some extent. If **stress** is caused by a breakdown in relationships with neighbours, family or work colleagues, or to immoral behaviour and the resulting guilt, or sins due to disobedience of religious laws, lowered immune resistance and ill-health may ensue. We now recognize the importance of psychosomatic factors and the **placebo** effect demonstrates this very well. Although we are at present looking at TAMS mainly as a source of new drugs, it may well be that the practices of making amends, sorting out your life and living according to a reasonable moral code has as many lessons for modern medicine as testing medicinal plants for pharmacological activity!

In TAMS, medicinal plants are used in two ways, only one of which corresponds to the Western perception of drug therapy. When we talk of research into traditional medicines, we are really talking about the scientific analysis of medicinal plants. Some of the most commonly used African ones are listed in Table 17.1. The traditional healer will, however, use medicinal plants not only for their pharmacological properties, but also for their power to restore health as supernatural agents. This is based on two important assumptions:

- Plants are living and it is thought that all living things generate a vital force, which can be harnessed.
- The release of the force may need special rituals and preparations such as incantations to be effective. This belief is rather similar to the concept held by some people nowadays that uncooked vegetables, particularly things such as sprouting beans, somehow contain a 'life-force' that is beneficial to health.

The method of application is essential for understanding TAMS. In conventional medicine, some form of absorption of the drug must take place. It can be orally, rectally, parenterally, topically or by inhalation. In TAMS, this is not necessarily important (although it will happen in many instances) as ingredients can be encapsulated and worn as an amulet, necklace or around the wrist or ankle. They may not even come into contact with the patient at all; perhaps being placed above the door, or under a mat or pillow. These

Table 17.1 Examples of common and widely used African medicinal plants and the main conditions for which they are used

SPECIES	WELL KNOWN INDIGENOUS USES
Aframomum alboviolaceum (Ridl.) K.Schum. (syn.: *Aframomum latifolium* K.Schum.; Zingiberaceae)	Pain, inflammation, lumbago
Carica papaya L (Caricaceae)	Contraceptive
Diospyros mespiliformis Hochst. ex A.DC. (Ebenaceae)	Dysentery, diarrhoea
Euphorbia drupifera Thonn. (syn.: *Elaeophorbia drupifera* (Thonn.) Stapf; Euphorbiaceae)	Worms
Ficus obliqua G.Forst. (syn.: *Ficus elegans* Miq.; Moraceae)	Dysentery, diarrhoea
Harpagophytum procumbens (Burch.) DC. ex Meisn.[a] (Pedaliaceae)	Pain, inflammation, lumbago
Hunteria umbellata (K.Schum.) Hallier f. (syn.: *Picralima umbellata* (K.Schum.) Stapf; Apocynaceae)	Worms
Piper guineense Schumach. & Thonn. (Piperaceae)	Oedema
Plumbago zeylanica L. (Plumbaginaceae)	Skin diseases
Prunus africana (Hook.f.) Kalkman[a] (Rosaceae)	Male urinary problems
Pycnanthus angolensis (Welw.) Warb. (syn.: *Pycnanthus kombo* Baill.) Warb.; Myristicaceae)	Pain, inflammation, lumbago
Rauvolfia vomitoria Afzel. (Apocynaceae), *Holarrhena floribunda* (G.Don) T.Durand & Schinz (syn.: *Holarrhena wulfsbergii* Stapf; Apocynaceae)	Mental illness (especially schizophrenia)
Ricinus communis L. (Euphorbiaceae)	
Senna alata (L.) Roxb. (syn.: *Cassia alata* L.; Caesalpiniaceae)	Skin diseases
Xylopia aethiopica (Dunal) A.Rich. (Annonaceae)	Oedema

[a]Developed into phytomedicines used in Europe and North America.

practices are to ward off the evil spirits, which may be causing the disease.

In some cases, the choice of a species has been made on a basis that has no bioscientific rationale; for example, a plant that bears many fruits may be used to treat infertility. This is rather like the ancient 'Doctrine of Signatures', where the plant was thought to display features indicating to the healer what it should be used for. It is a recurrent theme in the history of medicine. For example, walnuts were thought to be good for the brain because they resemble the cerebellum, and in Chinese medicine the use of animal parts is thought to endow the person consuming them with the properties of the animal. Tigers' bones are taken to imbue strength and courage, and the horn of the rhinoceros to increase sexual vigour. These observations show that plants used in these ways may *not* necessarily lead us to new drugs, except as a random result.

Nevertheless, effective strategies for using TAM herbal knowledge are available, for example from studies of antimalarial plants used by Nigerian healers. An important consideration is the division between those plant species used as medicines and those employed for more nefarious purposes. Highly poisonous species are rarely used for healing, because it is not possible to accurately control the dose. Paradoxically, these have more often led to the development of modern drugs than the relatively innocuous species. For example, *Physostigma venenosum* Balf., the Calabar bean, which yields the alkaloid physostigmine (eserine) and its derivatives neostigmine (used for myasthenia gravis) and rivastigmine (used for Alzheimer's disease), has no traditional medical use. It was an ordeal poison in Nigeria, administered to those accused of witchcraft, where death indicated guilt. Other African species yielding important drugs include the Madagascar periwinkle, *Catharanthus roseus* (L.) G. Don, which contains vincristine and vinblastine (used for leukaemias and Hodgkin's lymphoma) and the Cape bushwillow, *Combretum caffrum* (Eckl. & Zeyh.) Kuntze, which yields the combretastatins that are currently under investigation as antiangiogenic agents.

Popular herbal medicines from African species include Devil's claw, from the root of *Harpagophytum* species, Umckaloabo, from the root of *Pelargonium* species, yohimbe, from the bark of *Pausinystalia johimbe* (K.Schum.) Pierre ex Beille (syn: *Corynanthe johimbe* K.Schum.), and the *Hoodia* 'cactus'. Other pantropical and temperate species that are used in other parts of the world, such as the various types of ginger (*Zingiber* species), turmeric (*Curcuma* species), basil (*Ocimum* species) and wormwoods (*Artemisia* species), are also widely used throughout Africa.

LOCAL AND TRADITIONAL MEDICINES IN THE AMERICAS

Until Europe's invasion and 'discovery' of the New World in 1492, American medicine was truly traditional and developed through small local networks, relying on the local resources in the hugely diverse ecozones of the continent. After the Spanish and Portuguese conquest, interest in plants known to American indigenous groups produced manifold contributions to world medicine and science. Famously, in 1552, Martín de la Cruz wrote the Little Book of the Medicinal Herbs of the Indians (*Libellus de Medicinalibus Indorum Herbis)*, which, since the 16th century, has given rise to a wide range of studies on the local and traditional use of medicinal plants (see Chapter 2, p. 17) (Ortiz de Montellano, 1975). Over the following centuries American plants yielded important natural products and many species were seen as potential panaceas, a trend that continues today with regular reports about acclaimed (rainforest) superfruits and other miracle drugs. Since the Convention of Rio de Janeiro (1992) and subsequent treaties and agreements, such as the Nagoya protocol (2010), the nations in most American countries are now keen to ascertain their rights to equitable benefit sharing (see Secretariat of the Convention on Biological Diversity, 2011); however, so far, there is no good example of a successful product development.

The long and highly dominant colonial rule resulted in the Americas' medicines being the product of a syncretic development between local and non-American traditions, most importantly from Europe and Africa. Today, a huge diversity of medicines is used. Between around 1978 [initiated by the World Health Organization (WHO)] and the end of the 20th century some countries focused on the use of medicinal plants with the dual aim of scientific validation and therapeutic applicability. In the early 21st century, such research seemed to slow down, but since around 2010 many countries have started new initiatives to study and use medicinal plants available locally (Bye and Linares 2015, Cañigueral and Sanz-Bisset 2015, Heinrich et al 2014). In Canada and the USA, such medicine is often no longer practised by indigenous groups and the historical sources documenting medicinal plant use and traditional medicine have now become a source for community representatives interested in reinvigorating such traditions. By contrast, in Cuba, for example, the state has actively promoted the development and use of herbal medicines and some commercial preparations are used widely.

Today, in the American nations, a wide range of traditional practices is important, including several

influenced by African cultures, those maintaining a strong indigenous element and, more recent practices influenced, for example, by traditional Asian medicine systems. Importantly, a key focus has been to foster autochthonous developments and intercultural health programmes that support the local use of traditional medicines. In some countries, their inclusion into national health systems has become a political priority, but has received only limited financial support. In many countries, they remain an alternative to mainstream medicine. Such an alternative is, in fact, not an 'alternative', but more often the only accessible form of healthcare available and/or affordable for a population. Especially in many Central American, some Caribbean and South American countries, healthcare at all levels remains limited and poorly supported, making it accessible only for wealthy individuals.

Uniquely, many American psychoactive plants have become globally famous. Starting in the mid-20th century, neuropharmacology has benefited from the study of hallucinogenic plants and mushrooms. In some regions of rural México, important species, such as Mexican morning glory (*Turbina corymbosa* (L.) Raf.), and mushrooms, such as *Psilocybe* spp. (Agaricales), have been used for centuries. Some were described as early as the 16th century in codices (see above). Phytochemical investigations of the Amazonian Ayahuasca brews have contributed to a better understanding of the psychoactive effects of tryptamine-rich admixtures. The role of *Banisteriopsis caapi* (Spruce ex Griseb.) Morton as an important monoamine oxidase (MAO) inhibitor (*in vitro*), preventing the breakdown of active constituents, such as the psychoactive dimethyltryptamine (DMT), found, for example, in *Psychotria viridis* Ruiz & Pav., has been studied in detail and demonstrates the comprehensive medical understanding of Amazonian practitioners of traditional medicines.

The role of herbal medicines, and natural products derived from them, in America has been crucial and today's wide usage and their spread to global markets is testament both of the biocultural history and diversity of the continent, and of the continued need for plant-based healthcare in many countries and regions of the Americas.

AUSTRALIAN ABORIGINAL TRADITIONAL MEDICINE

Traditional Aboriginal medicine is the traditional medicine system of Australian Aborigines, the indigenous people of Australia. Like many other indigenous systems of medicine, it is an oral tradition (i.e. the traditional knowledge is handed down by word of mouth and, for example, through song and dance); unfortunately, much of the Aboriginal traditional medical use knowledge has been lost (Stack 1989). An Aboriginal pharmacopoeia has been published, which brings together traditional knowledge from the Northern Territory (Barr et al 1988).

As with some other indigenous systems of medicine, Aborigines attributed serious illness and death to malevolent spirits or sorcery. Spiritual 'doctors' of great wisdom, power and standing would be summoned to determine the cause of death or illness and, through performing sacred rites, to cure the illness; these esteemed healers were also believed to hold the power to inflict illness or a 'death curse' as punishment (Byard 1988).

With respect to minor ailments, due to the nomadic nature of their existence, initially all Aborigines used plants and other substances (such as animal fats and oils, earth, mud, clay and sand) as treatments essential for their survival. Blood-letting or cutting (to allow pain to 'escape'), massage, wearing of amulets, chants and ceremonies also played a role. Most medicinal plants were prepared as ointments or pastes composed of crushed plant parts mixed with animal fats or oils, and applied externally; infusions of leaves or bark were also applied to the body, or sometimes taken internally. Some plants were burnt over a fire and the vapour inhaled.

Aboriginal medicine is still practised today by Aboriginal tribes in central and northern Australia; its practice varies widely in other regions (Oliver 2013). The practice has evolved, and modalities such as blood-letting are unlikely to be used; however, the belief that serious illness results from bad spirits and sorcery remains.

Medicinal plants ('bush medicines') remain an important component for treating minor ailments, such as coughs and colds, boils and other skin disorders, bites and stings, burns and other wounds. The plants most commonly used are those that are easily accessible and require minimal preparation. The traditional remedies selected, indications for use and methods of preparation differ across different Aboriginal tribes and areas. In some tribes, only certain individuals are permitted to reveal the location of the native plants and describe their uses, although all members of the tribe would have the knowledge (Stack 1989). In some instances, special rituals, including singing of special songs, are performed

during collection of plants, and this is believed to be important for the medicinal value of the remedies. Some important traditional medicines are listed in Table 17.2. Some native plants used by indigenous Australians, such as the volatile oils (tea tree oil) from the Australian tea tree *Melaleuca alternifolia* (Maiden & Betche) Cheel and eucalyptus oil from the eucalyptus tree (*Eucalyptus* spp.), have an important place in the Australian community, being used for antimicrobial activity and respiratory conditions, respectively (Barnes et al 2016).

Improving Aboriginal health in Australia is among government priorities for future policy development and investment, including in medical and health research relating to indigenous medicine. The National Aboriginal and Torres Strait Islander Health Plan 2013–2023 of the Australian Government (Commonwealth of Australia, 2013) notes 'the significance of culture to wellbeing, and therefore good health, is also demonstrated by using traditional knowledge and the practices of traditional healers, which are adapted by many people for complementary use with western science in an integrated health care system'. At present, there are some examples of state-funded access to ngangkiri (traditional indigenous healers), and of use of traditional healers and traditional medicine both alone and in combination with Western healthcare (Oliver 2013).

In Australia there is substantial interest in exploring the potential medicinal value of traditional Aboriginal

Table 17.2 Some important medicinal plants of Australian Aboriginal medicine and selected uses

SCIENTIFIC NAME; FAMILY	COMMON NAMES	PREPARATION AND ADMINISTRATION	TRADITIONAL MEDICAL USES
Acacia melanoxylon R.Br. Fabaceae, s.l.	Blackwood	Bark, infusion used externally	Painful joints
Dodonaea polyandra Merr. & L.M.Perry; Sapindaceae	Uncha	Leaves, applied to tooth or cavity	Toothache
Duboisia hopwoodii (F.Muell.) F.Muell.; Solanaceae		Leaves, prepared as pituri;† chewed	Intoxicant, euphoric, narcotic
Eremophila alternifolia R.Br.; Scrophulariaceae	Narrow leaf fuchsia bush, native honeysuckle	Leaves, infusion used internally and externally	As decongestant, expectorant, analgesic, colds, influenza, fever, headache, septic wounds
Eremophila longifolia (R.Br.) F.Muell.; Scrophulariaceae	Fuchsia bush, weeping emu bush		Coughs, colds
Eremophila freelingii F. Muell.; Scrophulariaceae	Rock fuchsia bush	Leaves, decoction applied as wash	Aches, pains, including headache
Eucalyptus spp.; Myrtaceae	Gum tree	Leaves, infusion Bark, poultice applied externally	 Pain relief
Ficus opposita Miq.; Moraceae		Leaves, infusion applied to skin	Scabies
Ipomoea pes-caprae (L.) R. Br.; Convolvulaceae	Beach convolvulus	Leaves, heated and applied to wound Juice	Analgesia, skin infections, green ant bites, insect stings, scabies Diuretic, laxative
Phylloxylon xylophylloides (Baker) "Du Puy, Labat & Schrire" (syn.: *Exocarpus cupressiformis* Baker)' Fabaceae s.st.	Cherry ballart, native cherry	Sap, externally	Snake bites
Solanum laciniatum Aiton, *Solanum aviculare* G. Forst.; Solanaceae	Kangaroo apple	Fruit; poultice	Joint swellings

†Small dried fragments of plant material mixed with alkaline wood ash.
From: Barr et al 1988, Byard 1988, Stack 1989.

plant medicines through a combined approach comprising understanding the traditional knowledge relating to use of plants by Aborigines with rigorous scientific investigation with the ultimate aim of identifying potential new therapeutic compounds (Locher et al 2013, Simpson et al 2013).

RONGOĀ MĀORI

The traditional medical system of the native people (known as Māori) of Aotearoa New Zealand is Rongoā Māori. Like many other indigenous systems of medicine, it is an oral tradition (i.e. the traditional knowledge is handed down by word of mouth), and pays particular attention to spiritual aspects of a person's health.

Historically, Māori believed that some illnesses were caused by supernatural forces, and the tohunga (Māori traditional healers) were believed to be the earthly conduit of the spirits that controlled all aspects of life. As with other traditional systems, illness was believed to result from some sort of disharmony or imbalance with nature: the role of tohunga was to determine the nature of this discord, and to use plant, physical (e.g. massage) and spiritual treatments to correct it (Anon, 2008; Jones).

The arrival of European settlers to New Zealand in the 1800s brought new diseases and other challenges for Māori that had a devastating effect on the population's health. Also, due to the emergence of 'quack' practitioners, the Tohunga Suppression Act 1907 was passed (along with a Quackery Prevention Act in 1908 in response to 'quack' doctors). The Tohunga Suppression Act was passed with the support of the (few) Māori members of parliament, and was rarely enforced. However, it caused the practice of Rongoā Māori to become secretive and discussed only among Māori communities; the traditional knowledge relating to rongoā continued to thrive and develop during this time (Anon, 2008). The Act was repealed in 1962.

Today, Rongoā Māori involves the use of traditional herbal medicines (known as rongoā rākau), massage (mirimiri) and prayer (karakia) and is an holistic system of healthcare incorporating physical, social, cultural, emotional, family (whanau) and spiritual aspects of health; each aspect is important to ensure full recovery of the patient.

The use of native plant medicines is an essential component of Rongoā Māori; these plants are typically collected from the wild by tohunga, often from specific locations only, and have specific methods of collection and preparation in accordance with customs (tikanga), and to reduce potential toxicity. The remedies are usually crude water or ethanol extracts of crushed, fresh or dried plant material, sometimes boiled, and taken orally as an infusion. With some plants, the leaves or shoots are chewed, or these and other plant parts are made into poultices or compresses for topical use. Māori traditional medicines are used to treat a range of conditions, often minor ailments, including wounds and other skin conditions, gastrointestinal disorders, aches and pains, and upper respiratory tract infections. Māori may also access rongoā to help with chronic medical conditions, such as diabetes and mental health issues, in part because of a lack of response to conventional medicine, or because they feel that rongoā better addresses all their needs as a patient. Some important medicinal plants of Rongoā Māori and selected uses are listed in Table 17.3. Māori may use rongoā concurrently with conventional medicine.

Tohunga still have a substantial role in the practice of Rongoā Māori. Practitioners do not usually advertise their services, and accessing a tohunga would usually be through local knowledge, or a marae (Māori meeting place); some work in a clinic offering Rongoā Māori services. The practice of Rongoā Māori and use of medicinal plants vary between practitioners and in different regions, and treatment of patients and conditions is also individualized (Anon, 2008). There is no formal training programme for tohunga: practitioners learn through the oral transmission of knowledge by elders and/or through an 'apprenticeship' with a practising tohunga.

In line with the WHO's strategic goals with respect to traditional medicine (see Chapter 13), the New Zealand Ministry of Health has introduced several initiatives, including the publication of standards for traditional Māori healing (Ministry of Health 1999) and a Rongoā development plan for how Rongoā Māori (Ministry of Health 2006) will be made available and supported within the health and disability sector in New Zealand.

Information on the traditional uses, chemical constituents and other scientific information on New Zealand's medicinal plants has been accumulated (Ngā Tipu Whakaoranga – Māori Plant Use Database; Brooker et al 1987), though, in general, there has been little scientific study of the clinical pharmacology of Māori plant medicines.

Table 17.3 Some important medicinal plants of Rongoā Māori and selected uses

BOTANICAL NAME; FAMILY	MĀORI NAME(S); OTHER COMMON NAMES	PREPARATION AND ADMINISTRATION	TRADITIONAL MEDICAL USES
Kunzea spp., Myrtaceae	Kānuka;† white tea tree, white mānuka	Bark; decoction	Diarrhoea, dysentery,
Kunzea ericoides (A.Rich.) Joy Thomps. (syn.: *Leptospermum ericoides* A.Rich.) and *L. scoparium* J.R.Forst. & G.Forst.; Myrtaceae	Mānuka, kahikatoa; red tea tree	Bark; decoction Gum; topical Seedpods; decoction Seedpods; chewed	Mouthwash, diarrhoea, dysentery, back pain Burns Dysentery Diarrhoea
Macropiper excelsum Miq.; Piperaceae	Kawakawa, kawa; Māori pepper tree	Leaves, pulped, applied as poultice Leaves; decoction Leaves, root; chewed	Toothache, boils, Boils, other skin disorders, bruises, wounds, stomach pains, gonorrhoea Toothache, dysentery
Phormium tenax J.R.Forst. & G.Forst.; Hemerocallidaceae	Harakeke, korari; flax	Gum; topical	Burns, scalds, wounds
Pomaderris kumeraho A.Cunn. ex Fenzl; Rhamnaceae	Kūmarahou, pāpapa; gumdigger's soap, poverty weed, golden tainui	Leaves; boiled Rhizome; poultice Root; decoction Root juice; lotion	Asthma, bronchitis, coughs, colds, mild laxative, tuberculosis Boils Constipation, wounds ringworm

†The name kānuka is in common use today, but in early records, the name mānuka was used, thus records for mānuka are also relevant here.
From: Ngā Tipu Whakaoranga – Māori Plant Use Database.

Further reading TAMS

Kahumba, J., Rasamiravaka, T., Okusa, P.N., et al., 2015. Traditional African medicine: from ancestral knowledge to a modern integrated future? Science 350 (6262), S61–S63.

Neuwinger, H.D., 2000. African traditional medicine. Medpharm, Stuttgart.

Okpako, D.T., 1999. Traditional African medicine: theory and pharmacology explored. Trends. Pharm. Sci. 20, 482–484.

Sofowara, A. (Ed.), 1979. African medicinal plants: Proceedings of a conference. University of Life Press, Nigeria.

Thomas, O.O., 1989. Perspectives on ethno-phytotherapy of Yoruba medicinal herbs and preparations. Fitoterapia 60, 49–60.

References – the Americas

Bye, R., Linares, E., 2015. Perspectives on ethnopharmacology in Mexico. In: Heinrich, M., Jaeger, A.K. (Eds.), Ethnopharmacology. ULLA Book series, Wiley, Chichester, pp. 393–404.

Cañigueral, S., Sanz-Biset, J., 201). Ethnopharmacology in Central and South America. In: Heinrich, M., Jaeger, A.K. (Eds.), Ethnopharmacology. ULLA Book series, Wiley, Chichester, pp. 379–391.

Heinrich, Leonti, M., Frei Haller, B., 2014. A perspective on natural products research and ethnopharmacology in México. The eagle and the serpent on the prickly pear cactus. J. Nat. Prod. 77, 678–689.

Ortiz de Montellano, B.R., 1975. Empirical Aztec medicine. Science 188 (4185), 215–220.

Secretariat of the Convention on Biological Diversity, 2011. Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity. Montreal (Quebec), Secretariat of the Convention on Biological Diversity.

References – Australian Aboriginal traditional medicine

Barnes, J., McLachlan, A., Sherwin, C., Enioutina, E.Y., 2016. Herbal medicines: challenges in the modern world. Part 1. Australia and New Zealand. Expert. Rev. Clin. Pharmacol. 9 (7), 905–915.

Barr, A., 1988. Traditional bush medicines: an Aboriginal pharmacopoeia. Greenhouse Publications, Richmond, Vic.

Byard, R., 1988. Traditional medicine of aboriginal Australia. CMAJ 139 (8), 792–794.

Commonwealth of Australia, 2013. National Aboriginal and Torres Strait Islander Health Plan, 2013–2023. Available at http://www.health.gov.au/NATSIHP [last accessed August 4, 2016].

Locher, C., Semple, S.J., Simpson, B.S., 2013. Traditional Australian Aboriginal medicinal plants: an untapped resource for novel therapeutic compounds? Future Med. Chem. 5 (7), 733–736.

Oliver, S.J., 2013. The role of traditional medicine practice in primary health care within Aboriginal Australia: a review of the literature. J. Ethnobiol. Ethnomed. 9, 46.

Simpson, B.S., Claudie, D.J., Smith, N.M., McKinnon, R.A., Semple, S.J., 2013. Learning from both sides: Experiences and opportunities in the investigation of Australian aboriginal medicinal plants. J. Pharm. Pharm. Sci. 16 (2), 259–271.

Stack, E.M., 1989. Aboriginal pharmacopoeia. The Third Eric Johnston Lecture. Northern Territory Library Service, Darwin. Available online at https://www.nt.gov.au/__data/assets/pdf_file/0008/241865/occpaper10.pdf [last accessed August 5, 2016].

References – Rongoā Māori

Anon, 2008. Demystifying Rongoā Māori: Traditional Māori Healing. Best. Pract. J. 32 (13), 32–36. Available online at http://www.bpac.org.nz/magazine/2008/may/docs/bpj13_rongoa_pages_32-36_pf.pdf.

Brooker, S.G., Cambie, R.C., Cooper, R.C., 1987. New Zealand medicinal plants. Heinemann, Auckland.

Jones R Rongoā – medicinal use of plants – Understanding rongoā. Te Ara – the Encyclopedia of New Zealand, URL: http://www.TeAra.govt.nz/en/rongoa-medicinal-use-of-plants/.

Ministry of Health Manatu Hauora, 1999. Standards for Traditional Māori Healing, June 1999. Ministry of Health, Wellington, New Zealand.

Ministry of Health, 2006. Taonga Tuku Iho – Treasures of our Heritage: Rongoā Development Plan. Ministry of Health, Wellington, New Zealand.

Ngā Tipu Whakaoranga – Māori Plant Use Database. Available online at http://maoriplantuse.landcareresearch.co.nz.

Chapter 18

Complementary/alternative or 'integrative' therapies involving use of plant substances

This chapter discusses several so-called 'complementary/alternative' or 'integrative' therapies (as they are increasingly known, see Chapter 13, p. 185), that include in their approach the use of substances derived from plants. Collectively, complementary therapies, also known as complementary and alternative medicine (CAM), are a highly diverse group of approaches to health care and are based on philosophies towards health and illness that are fundamentally different from the approach of conventional, scientific medicine (biomedicine) and pharmacy (Box 18.1). CAM includes therapies such as acupuncture, chiropractic, osteopathy, hypnotherapy, massage in some contexts, reflexology and many others, including several others, such as homeopathy, aromatherapy, and anthroposophical medicine, which involve the use of plant-derived substances (Box 18.2). Traditional medical systems, such as traditional Chinese medicine, Ayurveda, and others, are also sometimes considered as part of CAM; these systems are discussed in Chapters 15 to 17 in this section.

HOMEOPATHY

HISTORY

Homeopathy was developed around 200 years ago by Samuel Hahnemann, a German physician and apothecary. His development of the principles of this controversial approach to treatment needs to be considered against the background of medical practice at the time, when the use of leeches, bloodletting, strong purgatives and emetics, and preparations containing toxic heavy metals, such as arsenic and mercury, was widespread. It is reported that Hahnemann was dissatisfied with these harsh therapeutic strategies and that this led him to give up the practice of medicine. During this period, he was stimulated to experiment with cinchona bark (which was used to treat malaria) and found that, while taking high doses of the substance, he experienced symptoms that were similar to those of malaria. Hahnemann then used this approach (which he called a 'proving') with healthy volunteers who were given many other substances in order to build up a 'symptom picture' for each substance. On the basis of his findings from these experiments, Hahnemann outlined three basic principles of (classical) homeopathy:

1. A substance which, used in large doses, causes a symptom(s) in a healthy person can be used to treat that symptom(s) in a person who is ill. For example, Coffea, a remedy prepared from the coffee bean (a constituent, caffeine, is a central nervous system stimulant) would be used to treat insomnia. This is the so-called 'like cures like' concept (in Latin, *similia similibus curentur*).
2. The minimal dose of the substance should be used in order to prevent toxicity. Initially, Hahnemann used high doses of substances, but this often led to toxic effects. Subsequently, substances were diluted in a stepwise manner and subjected to vigorous shaking ('succussion') at each step. This process is called potentization. It is claimed that the more dilute the remedy, the more potent it is. This completely opposes current scientific knowledge.
3. Only a single remedy or substance should be used in a patient at any one time.

MODERN HOMEOPATHY

Despite the controversies, homeopathy has spread widely and is a very popular form of healthcare in

BOX 18.1 Complementary and alternative medicines (CAM)

Several of these therapies involve the administration (internally or externally) of plant-derived preparations, such as essential oils. In homeopathy, other substances are also used (e.g. minerals) and they are administered in a highly diluted form ('potentiation'), an approach that as such differentiates homeopathy from biomedicine. Several of these approaches, such as **homeopathy**, are described as 'an holistic' or complete systems of healing in that they proffer a philosophy for health and illness, together with a distinct approach to the diagnosis and treatment of a wide range of complaints and disorders. In addition to the therapies listed above, CAM includes acupuncture, chiropractic, massage, osteopathy, reflexology and other therapies.

It should be noted that individuals with a scientific interest in herbal medicines do not consider the rational use of herbal preparations (i.e. science-based phytotherapy) to be part of CAM (see Chapter 15, Western herbal medicine).

BOX 18.2 Core characteristics of some important forms of complementary and alternative medicine which make use of medicinal plants

Homeopathy also focuses on understanding a patient's psychological, emotional and physical health, but treatment with specially prepared highly diluted ('potentiated') material is used. Thus its philosophical basis and therapeutic approaches are completely different from approaches where biologically active preparations are used.

Anthroposophical medicine also focuses on an holistic understanding of illness in terms of how the four 'bodies' and the functional systems interact with each other. Diagnosis involves conventional tools, the patient's life story and social context, and even bodily expressions. It uses an integrated therapeutic programme including diet, therapeutic movement (eurythmy), artistic therapies and massage, and anthroposophic medicines.

Aromatherapy is the therapeutic use of essential oils generally distilled from plants and used for therapeutic purposes generally or in order to increase a person's wellbeing.

Flower remedies of various types are obtained using a very simple extraction procedure used on the flowers of a range of common plant species and they are widely available for self-treatment.

many European and Asian communities. Hahnemann's principles of homeopathy still form the basis of modern homeopathic practice, with the exception of the single remedy rule, which is ignored by many homeopaths in favour of multiple prescribing. Today, around 1200 homeopathic remedies are commonly used. For many of these, homeopaths rely on Hahnemann's provings and, therefore, guidance on which symptoms the remedies can be used to treat. Modern-day provings involving healthy volunteers are sometimes undertaken, and several have involved rigorous study design (randomized, double-blind, placebo-controlled). However, Hahnemann did not use rigorous study design, although he did specify certain criteria; for example, subjects were not permitted to have coffee during the course of a proving.

In addition to the key principles of homeopathy outlined above, homeopaths also claim the following:

- Illness results from the body's inability to cope with challenging factors such as poor diet and adverse environmental conditions.
- The signs and symptoms of disease represent the body's attempt to restore order.
- Homeopathic remedies work by stimulating the body's own healing activity (the 'vital force') rather than by acting directly on the disease process.
- The 'vital force' is expressed differently in each individual, so treatment must be chosen on an individual basis and thus needs to be holistic.

In choosing a remedy for a particular patient, a homeopath will consider the patient's physical, mental and emotional symptoms, as well as personal characteristics, likes and dislikes. This information is then used to select the homeopathic remedy with a 'symptom picture' that most closely matches that of the patient. Computerized repertories (databases of homeopathic remedy symptom pictures) are now available that facilitate this process.

HOMEOPATHIC REMEDIES

Homeopathic remedies and herbal medicines are often confused and/or deemed to be similar. Even though they are not a part of phytotherapy, they are covered here, since very often such preparations are derived from plant extracts.

Table 18.1 Examples of homeopathic remedies originating from plant material

COMMON NAME OF THE REMEDY	PLANT SOURCE	COMMON PLANT NAME(S)	PLANT PART
Aconite	*Aconitum napellus* L.	Monkshood	Whole plant
Arnica	*Arnica montana* L.	Arnica, leopard's bane	Dried flowers
Allium cepa	*Allium cepa* L.	Red onion	Whole fresh plant
Belladonna	*Atropa belladonna* L.	Deadly nightshade	Whole fresh plant
Bryonia	*Bryonia alba* L.	White bryony	Root
Euphrasia	*Euphrasia officinalis* L.	Eyebright	Whole plant
Hydrastis	*Hydrastis canadensis* L.	Goldenseal	Fresh root
Rhus tox	*Toxicodendron pubescens* Mill. (syn.: *Rhus toxicodendron* L.)	Poison ivy	Fresh leaves
Staphisagria	*Delphinium staphisagria* L.	Stavesacre	Seeds
Stramonium	*Datura stramonium* L.	Thorn apple, Jimson weed	Fresh plant

The fundamental differences between the two types of preparation are:

- Homeopathic remedies are (mostly) highly dilute, whereas herbal medicines are used at material strengths. However, since homeopathic preparations are first extracted from, for example, plant material and then diluted, there is a borderline group including 'mother tinctures' and lower potencies (i.e. less diluted) that still may contain biomedically relevant amounts of active ingredients.
- Many homeopathic remedies (around 65%) originate from plants, whereas by definition all herbal medicines originate from plants (for examples of plant-based homeopathic preparations see Table 18.1).

Many of the species used for preparing homeopathic remedies have a history of medicinal use as herbal drugs; others are poisonous if used undiluted. Other types of material used in the preparation of homeopathic remedies include animal, insect, biological, drug/chemical and mineral.

The starting point for the production of most homeopathic remedies is a mother tincture, usually an alcohol/water extract of crude plant material, such as dried arnica flowers. The mother tincture is then diluted according to either the decimal (dilution steps of 1 in 10; denoted by D or X) or centesimal (dilution steps of 1 in 100; denoted by C or cH) scale to form homeopathic remedies or potencies. For example, on the decimal dilution scale, a 1X (or D1) remedy is prepared by taking one part mother tincture and adding it to nine parts diluent (dilute alcohol) and succussing the resulting 1 in 10 dilution. A 2X remedy is prepared by taking one part 1X remedy and adding it to nine parts diluent and succussing the resulting dilution, which is now a dilution of 1 in 100, and so on. The centesimal scale uses the same procedure except that each step involves adding one part mother tincture to 99 parts diluent, so that the first step produces a 1 in 100 dilution (1C or 1cH), the second step a 1 in 10,000 dilution (2C) and so on. The centesimal scale goes as far as M (1 in 10^{2000} dilution, i.e. 2000 centesimal dilution steps) and 10M (1 in $10^{20,000}$) dilutions. These potencies are usually prepared robotically. There are also LM potencies, which involve serial dilutions of 1 in 50,000 at each step.

Potencies at the lower end of the decimal (i.e. 1X, 2X, 3X to around 6X) and centesimal scales (usually up to 3C) still contain reasonable quantities of starting material and, depending on the nature of the starting material, may elicit pharmacological or toxicological effects. For this reason, some homeopathic remedies at these lower dilutions are classified as prescription-only medicines (POM) in the UK. Some examples of plant-derived homeopathic remedies and the potencies below which they are POM include:

- Aconite (*Aconitum napellus* L., monkshood), 3C or 6X
- Belladonna (*Atropa bella-donna* L., deadly nightshade), 2C or 3X
- Croton (*Croton tiglium* L.), 3C or 6X

- Hyoscyamus (*Hyoscyamus niger* L., henbane), 2C or 3X
- Nux vomica (*Strychnos nux-vomica* L.), 3C or 6X.

Potencies of 24X and 12C and above are diluted beyond Avogadro's number; thus it is highly unlikely that even a single molecule of the original starting material is present.

Quality control needs to be carried out on the source materials, and the manufacturing process for homeopathic preparations needs to adhere to the principles of good manufacturing practice to ensure that contamination does not occur.

POTENTIZATION

According to Hahnemann's second principle (but in opposition to modern pharmacological principles), the more dilute the preparation, the more potent it is. So, for example, a 6X remedy is claimed to be 'stronger' (more potent) than a 2X remedy, and a 12C remedy more potent than a 6C remedy. Also, although 2X and 1C preparations are the same concentration, a 2X is considered to be more potent because it has undergone two steps involving succussion, whereas 1C preparations have undergone only one succussion step.

Homeopaths have put forward several arguments in an attempt to explain how highly dilute homeopathic remedies could work. One of the most well-known of these is the 'memory of water' theory. Proponents of this theory claim that the process of succussion somehow alters the solvent molecules such that they become rearranged to form 'imprints' of molecules of the original starting material. Research exploring this theory has drawn on the disciplines of physics and biology, and has involved investigation of the physicochemical principles of homeopathic remedies. It has been reported for some homeopathic remedies that their physicochemical principles are different to those of the relevant solvent alone. However, these preliminary findings have had a negligible impact on the wider scientific community.

EVIDENCE OF EFFICACY

Homeopathic treatment has been investigated in over 100 clinical trials, and the results of these studies have been subject to systematic review and meta-analysis. A meta-analysis of data from 89 placebo-controlled trials of homeopathy indicated that the effects of homeopathy are not completely due to placebo. Restricting the analysis to high-quality trials only reduced, but did not eliminate, the effect found. However, there was insufficient evidence to demonstrate that homeopathy is clearly efficacious in any single clinical condition (Linde et al 1997).

Many trials, particularly those with negative results for homeopathy, have been criticized by proponents of homeopathy because all participants received the same homeopathic treatment rather than individualized treatment. So, another meta-analysis considered all placebo-controlled trials (n= 19) of 'individualized' homeopathy (i.e. where patients are prescribed the remedy most appropriate for their particular symptoms and personal characteristics; Linde and Melchart 1998). The study found that individualized homeopathy was significantly more effective than placebo, but, when the methodologically best trials only were considered, no effect over that of placebo was seen for homeopathy. A more recent meta-analysis of trials of individualized homeopathy reported cautiously positive results for homeopathy, compared with placebo (Mathie et al 2014).

Further work has provided strong evidence that, in homeopathy, clinical trials of better methodological quality tend to yield less positive results. There have been several high-quality trials published since Linde et al's original meta-analysis that report negative results, and it seems likely that the original meta-analysis 'at least overestimated the effects of homeopathic treatments' (Linde et al 1999).

There are now over 300 controlled clinical trials that have investigated the efficacy of homeopathic interventions, including, increasingly, studies that have assessed individualized homeopathy, as well as over 30 systematic reviews of trials of homeopathic interventions in specific health conditions. Around one half of these systematic reviews report positive, or cautiously positive, effects for the homeopathic interventions tested, compared with placebo. However, stronger conclusions cannot be drawn due to the low quality of the original controlled trials (Mathie 2015). A review of evidence relating to the effectiveness of homeopathy and undertaken by the National Health and Medical Research Council of Australia concluded '*there are no health conditions for which there is reliable evidence that homeopathy is effective*' (NHMRC 2015).

SAFETY

It is, perhaps, thought unlikely that highly dilute homeopathic remedies can lead to adverse drug reactions. However, isolated cases of adverse reactions, including serious cases, associated with the use of

homeopathic remedies, have been reported (Barnes 1998a, Syrigou et al 2014). There are also isolated reports of adulterated homeopathic remedies. Pooled data from placebo-controlled clinical trials involving homeopathic remedies indicate that adverse effects occur more frequently with homeopathy than with placebo, but that adverse effects are mild and transient, and the types (e.g. headaches, tiredness, skin reactions, dizziness and diarrhoea) are similar for both homeopathy and placebo (Dantas and Rampes 2000).

The potential for toxicity with homeopathic remedies at low dilutions is an important consideration, since such preparations can still contain reasonable quantities of starting material. Several strategies for evaluating the safety of raw materials used in homeopathic medicinal products have been proposed, including the lowest human recommended dose, threshold of toxicological concern, and the 'first safe dilution' (Buchholzer et al 2014, WHO 2009).

Several countries regulate homeopathic preparations. In the UK and European Union, the regulatory framework is the European directive 92/73/EEC, which became law in the UK in 1994 and provides regulations for homeopathic medicinal products. The directive required member states to set up a simplified registration procedure based on quality and safety, but not efficacy, for homeopathic medicinal products that met certain criteria, including:

- Oral or external use only
- Minimum dilution of 4X
- No claims for therapeutic efficacy.

ANTHROPOSOPHICAL MEDICINE

HISTORY

Anthroposophical medicine is a philosophical vision of health and disease based on the work of Austrian philosopher and esotericist Rudolf Steiner (1861–1925). Steiner's work explored how human beings and the natural world could be described, not only in physical terms, but also in terms of soul and spirit. He called this philosophy 'anthroposophy'. Its relevance in medicine, education and agriculture became an increasing part of Steiner's work, and resulted in what is now known as anthroposophic medicine.

Steiner believed that consciousness could not be defined in physical terms, as in conventional medicine, and explored how human's soul and spiritual nature relate to the health and function of the body. Nevertheless, he aimed anthroposophic medicine to be an extension, not an alternative, to conventional medicine. Steiner viewed each person as having four 'bodies' or 'forces':

- A physical body
- An etheric body, or life force
- An astral body, or conscious awareness
- A spiritual body, or self-awareness or ego.

And he considered the human-being to be made of three functional systems:

- The 'sense-nervous' system (the head and spinal column), focusing on 'cooling' and 'hardening' processes (e.g. the development of arthritis).
- The 'reproductive-metabolic' system, which includes parts of the body that are in constant motion (e.g. the limbs and digestive system) and which focuses on warming and softening processes (e.g. fevers).
- The 'rhythmic' system (the heart, lungs and circulation), which balances the other two systems. Steiner believed that health is maintained by harmonious interaction of the three systems, and that cacophonous (inharmonious) interactions between the systems result in illness.

MODERN ANTHROPOSOPHIC MEDICINE

Anthroposophic medicine today is still based on Steiner's philosophy. Practitioners of anthroposophy aim to understand illness holistically in terms of how the four 'bodies' and the functional systems interact with each other. Diagnosis involves not only several conventional tools, such as history-taking, physical examination and laboratory investigations, but also the patient's life story, social context and even body shape, movements, social behaviour and modes of artistic expression. Anthroposophic practitioners may use a range of therapies, including diet, therapeutic movement (eurythmy), artistic therapies and massage, as well as anthroposophic medicines, in an integrated therapeutic programme.

Anthroposophic medicine is particularly well-developed in Austria, Germany, Switzerland and The Netherlands, where there are hospitals specializing in anthroposophic medicine, as well as many general practitioners who practise an anthroposophical approach. In the UK, there are only a few medically qualified practitioners who practise anthroposophic medicine.

CONDITIONS TREATED

Several hospitals in Germany specializing in anthroposophic medicine provide a range of treatments that are also provided by general hospitals. Anthroposophic medicine is used as a therapeutic approach, under medical supervision, for several serious conditions, including supportive treatment in cancer. There is also a wide range of over-the-counter (OTC) medicines (both general sales list and pharmacy only) used for the symptomatic relief of conditions suitable for OTC treatment, such as indigestion, constipation, coughs, colds, sore throat, catarrh, sleeplessness, muscular pain and certain skin conditions.

ANTHROPOSOPHIC MEDICINES

Steiner believed that the sizes of different parts of plants, such as flowers, leaves and roots, are disproportionate in plants with medicinal properties. Usually, the disproportionately sized part would be used therapeutically. For example, nettle (*Urtica dioica*) produces an abundance of green leaves, whereas the flowers and fruit are insignificant in terms of size. Therefore, from an anthroposophic perspective, nettle leaves are deemed to have medicinal properties. However, sometimes, the whole plant, or a part of the plant other than the disproportionately sized part would be used therapeutically. In addition, in anthroposophic medicine, it is believed that the specific part of a medicinal plant relates to one of the three different 'systems' of the body (see above): roots relate to the 'sense-nervous system', flowers and fruit relate to the 'reproductive-metabolic system' and leaves act on the 'rhythmic system'. Continuing to use the nettle as an example, nettle leaves are used to stimulate the assimilation of iron (e.g. in anaemia), which is important in blood circulation.

Anthroposophic medicines are derived mainly from plants and minerals, such as calcium, iron and copper. Many products are combinations of herbal ingredients, and some products contain both herbal and mineral ingredients. Herbal and mineral ingredients are usually described by their Latin binomial name together with the plant part for herbs. For example:

- *Aconitum napellus* L., planta total (= aconite, whole plant)
- *Natrium carbonicum* (= sodium carbonate).

Ingredients of anthroposophical medicines are sometimes 'potentized' using the X or D potency series (steps of 1 in 10 dilution) rather than the C potency series (steps of 1 in 100 dilution) (see Homeopathy above). Thus, an ingredient with a potency of 1X (or D1) has a concentration of 1 in 10 or 10%, a 2X potency has undergone two steps of 1 in 10 dilution so is 1 in 100 or 1%. As with homeopathic remedies, at each dilution stage for an anthroposophical ingredient, the liquid is rhythmically succussed, which is claimed to 'release' the therapeutic properties of the substance. In anthroposophical medicines, ingredients are usually used at potencies below 6X (or D6). These are low dilutions, so reasonable quantities of plant constituents will be present. Thus, anthroposophical medicines containing plant-derived ingredients at dilutions below 6X can, from a pharmaceutical perspective, be considered to be herbal medicines.

Another group of products derived from the anthroposophical approach is mistletoe (*Viscum album* L.) preparations. Mistletoe is a semi-parasite, extracting water and mineral salts from host trees. The preparations contain a specially processed fermented aqueous mistletoe extract growing on a range of host trees, such as apple (*Malus pumila* Mill., syn: *M. domestica* Borkh.), pine (*Pinus* spp.), or oak (*Quercus* spp.). The three types are also available formulated with low concentrations (10^{-8} g per 100 mg fresh plant extract) of certain metal salts, such as those of copper and mercury. A lectin-standardized extract, also prepared according to the anthroposophic approach, is available, although this formulation does not include metal salts. Lectin-standardized mistletoe extracts, which are distinct from anthroposophical mistletoe preparations, are also available, particularly in Germany. Mistletoe products prepared from different host trees are prescribed for patients with different types of cancer. Treatment is usually given by subcutaneous injection, although the intravenous injection route is sometimes used, and oral formulations are also available.

In the preparation of anthroposophical medicines, particular attention is paid to the source and methods of farming used in growing plant raw materials. Plant materials are grown according to the principles of biodynamic farming, which is similar to organic farming. Pharmaceutical manufacturing companies exist that are dedicated to the production of anthroposophical medicines.

AROMATHERAPY

HISTORY

Aromatic plants and their extracts have been used in cosmetics and perfumes and for religious purposes for

thousands of years, although the link with the therapeutic use of essential oils is weak. One of the foundations of aromatherapy is attributed to Rene-Maurice Gattefosse, a French perfumer chemist, who first used the term aromatherapy in 1928. Gattefosse burnt his hand while working in a laboratory and found that lavender oil helped the burn to heal quickly with little scarring. Jean Valnet developed Gattefosse's ideas of the benefits of essential oils in wound healing, and used essential oils more widely in specific medical disorders. Marguerite Maury popularized the ancient uses of essential oils for health, beauty and wellbeing and so played a role in the modern renaissance of aromatherapy.

MODERN AROMATHERAPY

Aromatherapy is the therapeutic use of essential oils. These are obtained from plant material (e.g. roots, leaves, flowers, seeds) usually by distillation, although physical expression (using compression and pressure) is the method used to obtain some essential oils, mainly those from the skin of citrus fruits. Some of the key aspects of the use of essential oils in aromatherapy are described below:

- Aromatherapists believe that essential oils can be used not only for the treatment and prevention of disease, but also for positive effect on mood, emotion and wellbeing.
- Aromatherapy is claimed to be an holistic therapy: an essential oil, or a combination of essential oils, is selected to suit each client's symptoms, personality and emotional state, and treatment may change at subsequent visits.
- Essential oils are described both with reference to reputed pharmacological properties (e.g. antibacterial, anti-inflammatory) and to concepts not recognized in conventional medicine (e.g. 'balancing', 'energizing'). There is often little agreement among aromatherapists on the 'properties' of specific essential oils.
- Aromatherapists claim that the constituents of essential oils, or combinations of oils, work synergistically to improve efficacy or to reduce the occurrence of adverse effects (described as 'quenching') associated with particular (e.g. irritant) constituents (Barnes 1998b).

CONDITIONS TREATED

Aromatherapy is widely used as an approach to relieving stress, and many essential oils are claimed to be 'relaxing'. Many aromatherapists also claim that essential oils can be used in the treatment of a wide range of conditions. Often, many different properties and indications are listed for each essential oil, and conditions range from those that are relatively minor to those considered serious. For example, indications for peppermint leaf oil (*Mentha* × *piperita* L.) listed by one text include flatulence, ringworm, skin rashes, cystitis, indigestion, nausea, gastritis and sciatica, as well as migraine, hepatitis, jaundice, cirrhosis, bronchial asthma and impotence (Price and Price 1995). Many users self-administer essential oils either as a beauty treatment, as an aid to relaxation, or to treat specific ailments, many of which may not be suitable for self-treatment. Aromatherapy is also used in a variety of conventional healthcare settings, such as in palliative care, intensive care units, mental health units and in specialized units caring for patients with HIV/AIDS, physical disabilities and severe learning disabilities.

On a first appointment and before treating a client, an aromatherapist will take a case history, including gathering details of the client's medical history, lifestyle, diet and moods/emotions. Information gathered during the consultation is used to select essential oils thought to be appropriate for the individual concerned. The most common method used by aromatherapists for the application of essential oils is by massage, where drops of (usually) two to three essential oils are diluted in a vegetable carrier (or base) oil, such as grapeseed oil, jojoba oil, wheatgerm oil, sweet almond oil or sesame oil. The resulting 'blend' is then applied either during a full-body massage or localized massage. Other methods of applying essential oils used by aromatherapists or in self-treatment include the following:

- Addition of essential oils to baths and footbaths (water should be agitated vigorously to aid dispersion)
- Inhalations
- Compresses
- Use in aromatherapy equipment (e.g. burners and vaporizers).

Some practitioners advocate the oral administration of essential oils, described as 'aromatology'. However, essential oils should never be taken internally without medical supervision. Some aromatherapists also suggest that essential oils can be administered vaginally (e.g. via tampons or a douche) or rectally, but administration by these routes may cause mucosal membrane irritation and is not recommended.

ESSENTIAL OILS

Typically, an essential oil contains around 100 or more chemical constituents, mostly present at concentrations below 1%, although some constituents are present at much lower concentrations. Some essential oils contain one or two major constituents, and the therapeutic and toxicological properties of the oil can largely be attributed to those constituent(s). However, other constituents present at low concentrations can be important. The composition of an essential oil will vary according to the plant's environment and growing conditions, the plant part used and on methods of harvesting, extraction and storage. The major constituents of an essential oil can also vary in different chemotypes of the same species of plant. The constituents of essential oils are largely volatile compounds that are sensitive to the effects of light, heat, air and moisture and should, therefore, be stored in a cool place in tightly closed, darkened bottles. Even when stored correctly, the composition of essential oils can change during storage, so qualitative and quantitative analyses relate only to the composition of the oil at the time of testing. There is also the possibility of adulteration and contamination occurring during processing. Gross adulteration can be detected using established analytical techniques such as gas chromatography–mass spectrometry (GC-MS).

Essential oils should be referred to by the Latin binomial name of the plant species from which a particular oil is derived. The plant part used should be specified and, sometimes, further specification is necessary to define the chemotype of a particular plant; for example, *Thymus vulgaris* L. CT thymol describes a chemotype of a species of thyme that has thymol as a major chemical constituent (Clarke 2002).

EFFICACY AND SAFETY

Essential oils are believed to act both by exerting pharmacological effects following absorption into the circulation and via the effects of their odour on the olfactory system. There is evidence that essential oils are absorbed into the circulation after topical application (i.e. massage) and after inhalation, although amounts entering the circulation are likely to be very small (Vickers 1996).

Certain essential oils have been shown to have pharmacological effects in animal models and in *in vitro* studies, but there is little good-quality clinical research investigating the effects of essential oils and aromatherapy as practised by aromatherapists. Most of the clinical trials that have been conducted do not show that massage with essential oils is significantly better than massage with carrier oil alone (Barnes 1998b). A systematic review of eleven randomized clinical trials assessing the effects of inhaled essential oils (mostly lavender) on sleep reported cautiously positive findings (Lillehei and Halcon 2014). Similar conclusions were drawn following a 'systematic review' of all levels of evidence (e.g. including case reports) of inhaled peppermint oil or ginger oil for nausea and vomiting (Lua and Zakaria 2012). There is evidence that tea tree oil applied topically is effective in the treatment of certain skin infections, but these studies have not tested aromatherapy as practised by aromatherapists.

Data regarding the safety of essential oils as used in aromatherapy are limited. Few adverse effects associated with aromatherapy treatment have been reported; most reports relate to cases of contact dermatitis in patients or aromatherapists. Minor transient adverse effects, such as drowsiness, headache and nausea, can occur after aromatherapy treatment. The increasing use of essential oils during pregnancy and labour is of concern. Because of uncertainties about the safety of essential oils during these periods, general advice is that the use of essential oils should be avoided during pregnancy, particularly during the first trimester. The use of certain essential oils should also be avoided by patients with epilepsy.

FLOWER REMEDY THERAPY

Bach (pronounced 'batch') flower remedies are probably the most well-known of this type of preparation, although there are many other types of flower remedies (also known as flower essences). Different types are usually derived from native plants of the particular region or country, such as Australian bush flower essences, rain forest essences (Brazil), Alaskan flower essences.

HISTORY

Bach flower remedies were developed by Dr Edward Bach (1886–1936), a physician and homeopath. Bach believed that negative states of mind caused physical illness, and his approach to maintaining health was focused on the patient's psychological state. His theory was that by treating patients' emotional and mental responses to their illness, physical symptoms would then be relieved. He identified 38 negative psychological states (e.g.

jealousy, hopelessness, guilt, indecision) and sought natural remedies that could be used to 'correct' these negative states of mind. It is claimed that to do this Bach visited the countryside, concentrated on these specific emotional states and was intuitively drawn towards particular wild flowers that he believed could relieve them.

MODERN FLOWER REMEDY THERAPY

Flower remedies of various types are widely available for self-selection and self-treatment. In addition, some individuals undertake training to become a flower-remedy practitioner; this includes some healthcare professionals, such as some general practitioners, who use flower remedies alongside their day-to-day conventional medical practice.

FLOWER REMEDIES

Bach developed 38 flower remedies, 37 of which are based on single wild flowers and tree blossoms, and one (rock water), which is made from natural spring water. He intended each remedy to be used for a specific emotional or mental state. Some examples are:

- Gentian (*Gentianella amarella* (L.) Börner) for despondency
- Holly (*Ilex aquifolium* L.) for jealousy
- Impatiens (*Impatiens glandulifera* Royle) for impatience
- Pine (*Pinus sylvestris* L.) for guilt
- Rock rose (*Helianthemum nummularium* [L.] Mill.) for terror.

Bach also developed a preparation termed Rescue Remedy, which is a combination of five of other remedies: impatiens (*Impatiens glandulifera* Royle), star of Bethlehem (*Ornithogalum umbellatum* L.), cherry plum (*Prunus cerasifera* Ehrh.), rock rose (*Helianthemum nummularium* [L.] Mill.) and clematis (*Clematis vitalba* L.). Bach recommended this preparation be used in difficult and demanding situations, such as shock, terror, bereavement.

Bach flower remedies are prepared from mother tinctures, which are themselves made from plant material and natural spring water using either an infusion ('sun') method or a 'boiling' method (Kayne 2002). The infusion method is used to prepare mother tinctures for 20 of the Bach remedies: flower heads from the appropriate plant are added to a glass vessel containing natural spring water and are left to stand in direct sunlight for several hours, after which the flowers are discarded and the infused spring water retained. The boiling method involves the addition of plant material to natural spring water, which is then boiled for 30 minutes, cooled and strained. With both methods, the resulting solution is diluted with an equivalent volume of alcohol (brandy) to make the mother tincture. Flower remedies are then prepared by adding two drops of the appropriate mother tincture to 30 ml of grape alcohol. It is claimed that the resulting solution is equivalent to a 1 in 100,000 dilution. This is the same dilution as a 5X potency in homeopathy, but preparation of flower remedies does not involve serial dilution and succussion. Thus, in material terms, flower remedies and 5X potencies can be considered equal, although from a homeopathic perspective they are not.

Flower remedies are usually taken orally (2–4 drops added to a cold drink and sipped), although, in some cases, drops are placed directly under the tongue and even on the wrist or temples. Rescue Remedy is also available as a cream for external use.

EFFICACY AND SAFETY

Although there are many anecdotal reports of the benefits of flower remedies, there is only limited experimental and clinical research into their reputed effects (Barnes 1998c). A systematic review of randomized clinical trials identified seven studies; all six placebo-controlled trials did not find evidence of benefit with flower remedies (Ernst 2010).

Thus, although this form of self-treatment is very popular, it remains highly controversial.

Flower remedies are widely claimed to be completely free from adverse effects. Adverse effects are unlikely to occur, given that the preparations contain only highly dilute material. However, as flower remedies contain alcohol, they may be unsuitable for some individuals. Excessive use of a flower remedy could be of concern if an individual was relying on self-treatment with flower remedies for conditions such as anxiety or depression, for which medical treatment and other professional support may be required.

GENERAL CONCLUSION

The therapies discussed above are part of European and other medical traditions and while evidence for their clinical usefulness is very limited they remain a popular healthcare choice and one that often involves the use of plant-derived medicines. Consequently, these products are – in pharmaceutical terms – as important as other medications.

References

Barnes, J., 1998a. Homeopathy. Pharm. J. 260, 492–497.

Barnes, J., 1998b. Aromatherapy. Pharm. J. 260, 862–867.

Barnes, J., 1998c. Complementary medicine. Other therapies. Pharm. J. 261, 490–493.

Buchholzer, M.-L., Werner, C., Knoess, W., 2014. Current concepts on integrative safety assessment of active substances of botanical, mineral or chemical origin in homeopathic medicinal products within the European regulatory framework. Regul. Toxicol. Pharma. 68 (2), 193–200.

Clarke, S., 2002. Essential chemistry for safe aromatherapy. Churchill Livingstone, Edinburgh.

Dantas, F., Rampes, H., 2000. Do homeopathic medicines provoke adverse effects? A systematic review. Br. Homeopathy J. 89 (Suppl. 1), S35–S38.

Ernst, E., 2010. Bach flower remedies: a systematic review of randomised clinical trials. Swiss. Med. Wkly. 140, w13079.

Lillehei, A.S., Halcon, L.L., 2014. A systematic review of the effect of inhaled essential oils on sleep. J. Altern. Complement. Med. 20 (6), 441–451.

Linde, K., Clausius, N., Ramirez, G., et al., 1997. Are the clinical effects of homeopathy placebo effects? A meta-analysis of placebo-controlled trials. Lancet 350 (9081), 834–843.

Linde, K., Melchart, D., 1998. Randomized controlled trials of individualized homeopathy: a state-of-the-art review. J. Altern. Complement. Med. 4 (4), 371–388.

Linde, K., Scholz, M., Ramirez, G., et al., 1999. Impact of study quality on outcome in placebo-controlled trials of homeopathy. J. Clin. Epidemiol. 52 (7), 631–636.

Lua, P.L., Zakaria, N.S., 2012. A brief review of current scientific evidence involving aromatherapy use for nausea and vomiting. J. Altern. Complement. Med. 18 (6), 534–540.

Mathie, R.T., 2015. Controlled clinical studies of homeopathy. Homeopathy 104 (4), 328–332.

Mathie, R.T., Lloyd, S.M., Legg, L.A., 2014. Randomised placebo-controlled trials of individualised homeopathic treatment: systematic review and meta-analysis. Syst. Rev. 3, 142.

National Health and Medical Research Council, Australian Government, 2015. NHMRC Statement: statement on homeopathy. March 2015. Available online at https://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/cam02_nhmrc_statement_homeopathy.pdf.

Price, S., Price, L., 1995. Aromatherapy for health professionals. Churchill Livingstone, Edinburgh.

Syrigou, E., Gkiozos, I., Dannos, I., Grapsa, D., Tsimpoukis, S., Syrigos, K., 2014. Giant tuberculin reaction associated with the homeopathic drug tuberculinum: a case report. Clin. Infect. Dis. 58 (7), e119–e121.

Vickers, A., 1996. Massage and aromatherapy. A guide for health professionals. Chapman & Hall, London.

World Health Organization, 2009. Safety issues in the preparation of homeopathic medicines. Key technical issues of quality impacting on the safety of homeopathic medicines. WHO, Geneva.

Further reading

Astin, J.A., 1998. Why patients use alternative medicine. Results of a national study. JAMA 279, 1548–1553.

Department of Health, 2001. Government response to the House of Lords Select Committee on Science and Technology's report on complementary and alternative medicine. The Stationery Office, London.

Fulder, S., 1996. The handbook of alternative and complementary medicine, third ed. Oxford University Press, Oxford.

House of Lords Select Committee on Science and Technology, 2000. Session 1999–2000, 6th report. Complementary and alternative medicine. The Stationery Office, London.

Kayne, S., 1997. Homeopathic pharmacy. An introduction and handbook. Churchill Livingstone, Edinburgh.

Kayne, S.B., 2002. Complementary therapies for pharmacists. Pharmaceutical Press, London.

Tisserand, R., Balacs, T., 1995. Essential oil safety. A guide for health professionals. Churchill Livingstone, Edinburgh.

Chapter 19

Herbal medicine interactions

INTRODUCTION

Herbal medicinal products (HMPs), including nutritional supplements, are widely used to maintain health, prevent disease, and for self-medication of chronic and refractory diseases. They are often taken alongside conventional medicines, often referred to as 'drugs' in this context. This can lead to interaction between the herbal medicine and the drug, causing changes in blood levels that could, in the most serious cases, lead to toxicity due to increased drug plasma levels or even treatment failure due to decreased levels.

It is also possible for herb–drug interactions to enhance treatment by improving drug bioavailability, but harmful herb–drug interactions are understandably a higher priority in terms of public health. Some of the most important are shown in Table 19.1, but these are current examples and databases that are continually updated should always be consulted. Notably, the anticoagulant warfarin is the most common drug involved, and the most common herb is St John's wort. The concomitant use of St John's wort (*Hypericum perforatum* L.) with immunosuppressive (e.g. ciclosporin), antiretroviral (e.g. indinavir, nevirapine), cardiac (e.g. digoxin) or antineoplastic (e.g. irinotecan, imatinib) drugs may result in reduced plasma concentration of the drug and reduced efficacy by various mechanisms (Russo et al 2014).

Ginkgo biloba L. extract, used for improving cognitive functioning, has been reported to cause spontaneous bleeding and may produce additive effects with anticoagulants and antiplatelet agents. Ginseng, widely used for its physical and mental stimulatory effects, is generally well tolerated, but has been implicated as a cause of decreased response to warfarin. Many reports are of individual clinical cases and are poorly recorded (Izzo et al 2016), but even well-documented reports may not be able to establish a cause-and-effect relationship.

Herbal medicines may also interact with each other, and many herb combinations, selected to increase efficacy and attenuate toxicity, are composed according to traditional principles governing appropriate combinations. These are outside the scope of this chapter.

Under-reporting of cases of suspected adverse effects associated with herbal medicines is a known problem (see Safety and pharmacovigilance of traditional medicine practices and traditional medicines, Chapter 13, p.187), and physicians often also fail to ask their patients about their use of herbal medicines and other supplements. This means that there are no overall reliable statistics for the incidence of clinical herbal–drug interactions.

Herbal products may also be taken to enhance the effect of conventional drugs and reduce side effects, showing the potential for beneficial herb–drug interactions. *Schisandra sphenanthera* Rehder & E.H. Wilson is used in liver transplant patients taking the immune suppressant tacrolimus: the combination increases the oral bioavailability of tacrolimus, reduces some of its side effects and improves liver function (Wei et al 2013). A herbal medicine, PHY 906, based on an ancient Chinese formula, is used in cancer chemotherapy to improve outcomes and reduce side effects (Saif et al 2014). This type of combination therapy should only be undertaken in specialist centres with expertise in integrative therapy.

MECHANISMS OF HERBAL MEDICINE INTERACTIONS

Interactions can be synergistic or additive, where the effect is increased, or antagonistic where it is decreased, or new and unexpected, and may occur via

Table 19.1 Some examples of documented interactions* for herbs that may be taken as self-medication

HERBAL DRUG	PRESCRIBED DRUG	RESULT OF INTERACTION
Cranberry *Vaccinium macrocarpon* Aiton, V. oxycoccos L. fruit	Warfarin Tacrolimus	Increased plasma levels of warfarin Decreased plasma levels of tacrolimus
Don quai *Angelica sinensis* (Oliv.) Diels root	Warfarin Paracetamol Saquinavir	Increased anticoagulant effect Changes in paracetamol pharmacokinetics Decreased saquinavir blood concentration
Ginkgo *Ginkgo biloba* L. herb	Omeprazole Tolbutamide Tanilolol	Decreased omeprazole blood concentration Decreased tolbutamide blood concentration Increased tanilolol blood concentration
Ginseng, Asian *Panax ginseng* C.A. Mey. root	Phenelzine	Sleeplessness, tremor and headaches
Ginseng, American *Panax quinquefolius* L.	Warfarin	Reduced warfarin concentration/ anticoagulation
Hibiscus (Roselle), *Hibiscus sabdariffa* L.	Chloroquine Paracetamol	Reduced blood concentration of chloroquine Changes in paracetamol pharmacokinetics
Magnolia vine *Schisandra chinensis* (Turcz.) Baill., *S. sphenanthera* Rehder & E.H. Wilson fruit	Tanilolol	Increased tanilolol blood concentration
St John's wort *Hypericum perforatum* L.	Alprazolam, amitriptyline, bupropion, ciclosporin, digoxin, gliclazide, imatinib, indinavir, irinotecan, midazolam, nevirapine, nifedipine, omeprazole, verapamil, warfarin, zolpidem, etc. Oral contraceptive Paroxetine, venlafaxine	Decreased blood concentrations. With ciclosporin, changes in pharmacokinetics were associated with rejection episodes in transplant patients Reduced efficacy; breakthrough bleeding Serotonin syndrome

*Taken from well-documented case reports; multiple case reports and case series, pharmacokinetic trials in patients or healthy volunteers and clinical trials.

pharmacodynamic or *pharmacokinetic* mechanisms. An overview of these processes is shown in Fig. 19.1.

PHARMACODYNAMIC INTERACTIONS

Pharmacodynamic (PD) interactions involve receptor binding, post-receptor effects, systemic or organ effects and chemical interactions. They can sometimes be predicted on the basis of their pharmacology and chemistry.

PHARMACOKINETIC INTERACTIONS

Pharmacokinetic (PK) interactions occur when drug **A**bsorption, **D**istribution, **M**etabolism or **E**xcretion (ADME) are altered by another drug; this is the most common type of mechanism currently reported for HMPs.

P-GLYCOPROTEIN

P-glycoprotein 1 (P-gp) is a multidrug-resistance protein that transports many important drugs, regulating their bioavailability. In the intestinal epithelium it pumps drugs back into the lumen, in the liver it excretes them into bile ducts, in the kidney it excretes them into the urine, and in the blood–brain barrier and blood–testis barrier it pumps them back into the capillaries. *Increased* intestinal expression of P-gp reduces absorption of drugs so plasma levels may be too low, whereas *decreased* P-gp expression can lead to toxic levels of the drug. Some cancer cells express large amounts of P-gp, making them multi-drug resistant. P-gp is inhibited by some natural compounds (e.g. quercetin and curcumin), which may provide a means of delaying resistance or improving drug bioavailability.

CYTOCHROME ENZYME ACTIVITY

Cytochrome (CYP) 450 enzymes are important in Phase 1 metabolism. Six of the known CYPs are responsible for the metabolism of 90% of drugs; the two most significant are CYP3A4 and CYP2D6:

CYP enzyme induction: if enzyme activity is increased, the metabolism of another substrate drug will

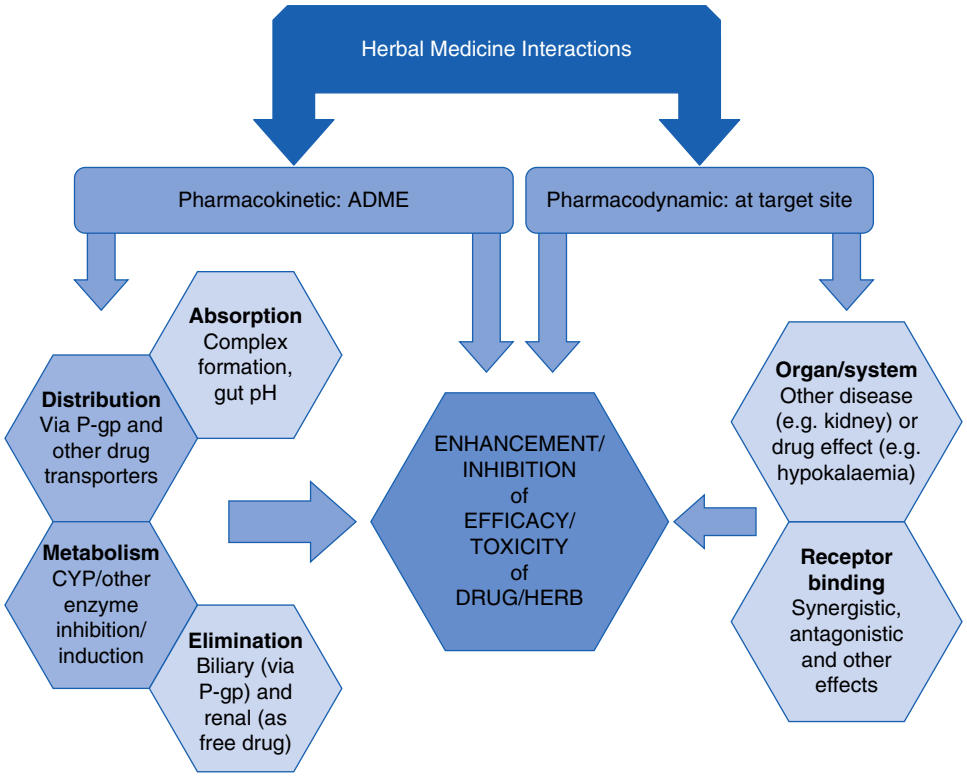


Fig. 19.1 Herbal–drug interactions: an overview of processes involved.

also be increased, leading to reduced plasma drug levels and even treatment failure (unless the substrate drug is a prodrug, in which case, the increased metabolism will lead to increased concentrations of the active metabolite).

CYP enzyme inhibition: if enzyme activity is inhibited, the metabolism of another substrate drug will be decreased, leading to increased plasma levels and possible toxicity.

Most herbal remedies involved in drug interactions have been shown to up- or down-regulate CYP450s and/or P-gp, and, more recently, the role of other drug transporters, including the organic anion and cation transporters (OATs and OCTs), has been described. Nuclear receptors (NRs), such as the pregnane X receptor, are activated by some herbs, but have not been well investigated.

PHARMACOGENOMICS AND PHARMACOGENETICS

Pharmacogenomics (genetic variations in individuals and populations) and *pharmacogenetics* (single drug–gene interactions) influence drug response. Taking genetic factors into account when prescribing treatment is now termed 'personalized medicine'. In traditional and complementary medicine, individualized medicine is not a new approach at all, with aspects of a patient's physical and psychological make-up (rather than a genetic profile) considered in addition to disease symptoms.

The phenomenon of pharmacogenetics was first described by Pythagoras (ca 510 BCE), when he linked fava bean consumption with haemolytic anaemia ('favism') in some individuals, which was later found to be due to a deficiency of the enzyme glucose 6-phosphodiesterase. A patient's response to drugs can also be broadly categorized according to their speed of metabolism, ranging from '*ultra-rapid*' to '*poor*' metabolizers in addition to describing individual gene variations.

EVALUATING HERBAL MEDICINE INTERACTIONS

Protocols and standards used for the evaluation of interactions of herbal medicines are similar to those for other drug interactions and are specifically discussed in the WHO document 'Guidelines on safety

Table 19.2 Cytochrome and P-glycoprotein activity of five popular herbs

HERB	CYTOCHROME ENZYME INVOLVEMENT	P-GLYCOPROTEIN EFFECTS	CLINICAL INTERACTION REPORTS AND POTENTIAL FOR INTERACTION
Echinacea *Echinacea* spp.	No significant inhibition of CYP2D6, 1A2, but weak induction of 3AA4	Induction via PXR activation	No clinical reports; many *in vivo* studies show equivocal effects, suggesting weak interaction potential
Ginkgo *Ginkgo biloba* L.	Inhibits CYP1A2, 2C9, 2E1 (extract); induces CYP 3A4 (human *in vitro*)	Inhibition	Yes, but causality mainly not established as used by vulnerable patient groups (elderly) liable to CV events. May augment antiplatelet activity
Ginseng *Panax ginseng* C.A. Mey.	Inhibits CYP2C9, 2C19, 2D6, 3A4 (extract). Different profile for individual components	Inhibition	Yes, but causality not clear; constituents have opposing actions. Interaction potential weak but may augment stimulant activity
St John's wort *Hypericum perforatum* L.	Potent inducer of CYP3A4, 2C9, 2C19, 2E1 (extracts, hyperforin). Activation of pregnane receptor X (hyperforin)	Strongly induces	Many recorded events supported by clinical and mechanistic evidence. High potential for interaction by several mechanisms
Valerian *Valeriana officinalis* L.	No effect on CYP1A2, 2E1; induces CYP2D6, CYP3A4 *in vitro* but not *in vivo*	Weak inhibitor (also UGT1A1 and 2B7)	Review suggests no pharmacokinetic interaction, but may add to sedative effects

UGT, UDP-glucuronosyltransferase

monitoring of herbal medicines in pharmacovigilance systems (WHO 2004). The quality of herbal medicines is rarely as high as that of licensed medicines and herbal medicines are subject to wide variations in composition. Herbal medicines also have the potential for long and unsupervised consumption, which may be important with respect to the potential for drug interactions to occur.

Individual herbs may interact by more than one mechanism: for example, in conjunction with other antidepressants, St John's wort may lead to a serotonergic syndrome by pharmacodynamic mechanisms, whereas taken with ciclosporin it reduces blood levels via pharmacokinetic processes. It induces several CYP enzymes as well as P-glycoprotein, and interacts with the pregnane X receptor, making it susceptible to several different mechanisms of interaction. Most other herbal medicines do not pose the same danger, but St John's wort provides an example of how experimental results can reflect patient risk. The most important mechanisms have been well reviewed by Butterweck and Nahrstedt (2012), Cho and Yoon (2015) and Gurley et al (2012). In most traditional and complementary medicine systems, multiple herbs are more commonly used than single herbs, which introduces further complexity, and the combination must be treated as the active ingredient.

Table 19.2 illustrates the mechanistic activity profiles of five popular herbs that are commonly taken as self-medication, and reports of their clinical interaction. The herbs are important for different reasons: *Ginkgo biloba* L. and *Panax ginseng* C.A. Mey. are used in vulnerable patients such as the elderly; *Valeriana officinalis* L. (valerian) is an ingredient in many herbal sleeping and relaxation products; *Echinacea* species are taken by children (and others) for upper respiratory conditions; and *Hypericum perforatum* L. (St John's wort) is the herb with the greatest documented potential for interactions. Of the list, *H. perforatum* L. is the only herb with consistent pre-clinical results that would predict interactions.

PATIENT SUSCEPTIBILITY TO HERB–DRUG INTERACTIONS

Herbal medicines are widely used by older adults and children, and both groups have different rates of metabolism. Pregnant and lactating women are usually prescribed only essential drugs (e.g. for epilepsy), but their use of herbal medicines is rarely monitored and some herbs are used specifically at these times. These include raspberry leaf tea (*Rubus idaeus* L.) during pregnancy and fenugreek (*Trigonella foenum-graecum* L.) and fennel (*Foeniculum vulgare* Mill.) during breastfeeding. Although these have not raised serious issues, as yet, their complete safety has not been proven. Older patients, in addition to having a generally slower metabolism, may take herbal medicines for degenerative conditions and are also more likely to be taking multiple medications. Patients with certain diseases,

such as cancer, are also more likely to use complementary therapies of all types, including herbal medicine and nutritional supplements (Alsanad et al 2014).

ISSUES FOR PHARMACISTS AND OTHER HEALTHCARE PROFESSIONALS

Western-trained health professionals have often received little or no training in the appropriate use of herbal medicines, which predisposes them to advise patients not to take them. However, if the patient has been taking a combination without perceiving any harm, they may decide that the doctor has a lack of expertise in this area and not inform them of their HP use. Pharmacists are ideally placed to advise patients on herb–drug interactions if they have information available, because they are trained to understand the issues involved and are easily approached by patients, especially if they are also dispensing their prescribed medicines. Some of the specific issues that pharmacists need to consider are listed in Box 19.1.

BOX 19.1 Specific issues for pharmacists to consider

- Is the patient in a vulnerable patient group: e.g. elderly, pregnant?
- Do any of the patient's prescribed medicines have a narrow therapeutic index?
- Are any of their medicines known to be liable, or have mechanisms that predispose, to drug interactions: e.g. warfarin, protease inhibitors, immune system suppressants?
- Is the herbal medicine known to be liable to interaction: e.g. St John's wort?
- Will the herbal product be taken over a long period of time?
- Is the herbal product of good quality/licensed/registered?

CONCLUSIONS

The use of herbal and nutritional supplements is increasing and the practice of integrated medicine is becoming more acceptable. This means that pharmacists and other health professionals may be asked by patients about the advisability of taking certain herbal medicines or food supplements with their prescribed medicines. It is not possible to list all important potential herb–drug interactions here, so an up-to-date reference should always be consulted. Whenever possible, registered herbal products should be recommended as these are certified as good quality and also include patient information leaflets that give more details about combinations to avoid.

References

Alsanad, S.M., Howard, R.L., Williamson, E.M., 2014. Cancer patients at risk of herb–food–supplement interactions. Phytother. Res. 12, 1749–1755.

Butterweck, V., Nahrstedt, A., 2012. What is the best strategy for preclinical testing of botanicals? A critical perspective. Planta. Med. 78 (8), 747–754.

Cho, H.J., Yoon, I.S., 2015. Pharmacokinetic interactions of herbs with cytochrome p450 and p-glycoprotein. Evid. Based Complement. Altern. Med. 736431.

Gurley, B.J., Fifer, E.K., Gardner, Z., 2012. Pharmacokinetic herbal drug interactions (part 2): drug interactions involving popular botanical dietary supplements and their clinical relevance. Planta Med. 78 (13), 1490–1514.

Izzo, A.A., Kim, S.H., Radhakrishnan, R., Williamson, E.M., 2016. A critical approach to evaluating clinical efficacy, adverse events and drug interactions of herbal remedies. Phytother. Res. 30 (5), 691–700.

Liu, S.H., Cheng, Y.C., 2012. Old formula, new Rx: the journey of PHY906 as cancer adjuvant therapy. J. Ethnopharmacol. 140 (3), 614–623.

Russo, E., Scicchitano, F., Whalley, B.J., et al., 2014. *Hypericum perforatum:* pharmacokinetic, mechanism of action, tolerability, and clinical drug–drug interactions. Phytother. Res. 28, 643–655.

Saif, M.W., Li, J., Lamb, L., et al., 2014. First-in-human phase II trial of the botanical formulation PHY906 with capecitabine as second-line therapy in patients with advanced pancreatic cancer. Cancer Chemother. Pharmacol. 73 (2), 373–380.

Wei, H., Tao, X., Di, P., et al., 2013. Effects of traditional Chinese medicine Wuzhi capsule on pharmacokinetics of tacrolimus in rats. Drug Metab. Dispos. 41, 1398–1403.

World Health Organization, 2004. WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems. WHO, Geneva. Available online at http://apps.who.int/medicinedocs/en/m/abstract/Js7148e/.

PART B

Important natural products and phytomedicines used in pharmacy and medicine

This part is devoted to plant-derived medicines, arranged in therapeutic categories in a manner analogous to that of the British National Formulary (BNF), although the classification differs in some ways. For example, there is no section on immunological products and vaccines, or anaesthesia, since plant medicines are not used for these purposes and, therefore, are not represented in these categories; natural anticancer drugs are covered in Chapter 9. A chapter on miscellaneous supportive therapies for stress, ageing, cancer and debility (chapter 32) is also included. These preparations are often of Oriental or Asian origin, and are becoming important throughout the rest of the world; examples include ginseng, ashwagandha, reishi, schisandra and green tea.

This part (chapters 20 to 32) is not a prescribing guide, herbal compendium or pharmacopoeia, but a summary of the most important drugs obtained from plant sources and their medicinal uses. Entries are not necessarily consistent in length, or the amount or nature of the information included: more emphasis is given to the most important herbal drugs, or those not covered extensively elsewhere. Inclusion in Part B is not a recommendation or endorsement, but an acknowledgement that these herbal drugs are in use and that information on them is needed. For many, sound evidence for clinical effects or effectiveness exists, but this is covered elsewhere (Edwards et al 2015). Both pure compounds and herbal medicines are included; isolated natural products are used mainly in conventional medicine and are treated in the same way as any other drug; examples given include morphine, codeine, digoxin, pilocarpine, atropine and colchicine. Many plant drugs are used as extracts, either in crude form, or modified and standardized in some way, and these are normally described as herbal products or phytomedicines. The preparation of such extracts has been discussed in Chapter 9.

Many of these herbal drugs have now been incorporated into the European / British Pharmacopoeia (Eur. Ph. / BP) and, if so, they are marked with the symbol (**EP**) and the official Latin drug name is included. In these cases the Eur. Ph. should be consulted for definitions and analytical and quality control procedures. The information in each monograph has been taken from reviews, primary references and reputable textbooks. The textbooks are standard, well-referenced works that give more details of the botanical drugs described. Some (e.g. Edwards et al. 2015; Pharmaceutical Press Editorial 2016; Williamson et al 2016) provide detailed information about the constituents, evidence and literature citations of a smaller number of herbs, others give a briefer overview of a much larger number (e.g. Williamson 2003), whereas others approach the subject from a prescribing point of view (Bone & Mills 2013, Schulz et al 2004). Pharmacognosy reference books (Evans 2009, Hänsel and Sticher 2014), Ayurvedic reference books (Williamson 2002), Chinese herbal medicine books (Tang & Eisenbrand 1992) and specialist literature on essential oils (e.g. Tisserand & Balacs 1995) have also been used.

Standard references consulted for the preparation of monographs

British National Formulary. British Medical Association and the Royal Pharmaceutical Society London (published biannually).

Bone, K., Mills, S., 2000. Principles and Practice of Phytotherapy, second ed. Elsevier, Edinburgh.

Edwards, S., da Costa-Rocha, I., Williamson, E.M., Heinrich, M., 2015. Phytopharmacy – an evidence-based guide to herbal medicines. Wiley, Chichester.

Evans, W.C., 2009. Trease and Evans's pharmacognosy, sixteen ed. WB Saunders, London.

Hänsel, R., Sticher, O., 2014. Pharmakognosie – Phytopharmazie, 10. Wissenschaftliche Verlagsgesellschaft, Stuttgart. Aufl.

Pharmaceutical Press Editorial, 2016. Herbal medicines (online edition). Pharmaceutical Press, London.

Schulz, V., Hänsel, R., Blumenthal, M., Tyler, V., 2004. Rational phytotherapy. Springer-Verlag, Berlin.

Tang, W., Eisenbrand, G., 1992. Chinese drugs of plant origin. Springer-Verlag, Berlin.

Tisserand, R., Balacs, T., 1995. Essential oil safety. Churchill Livingstone, Edinburgh.

Williamson, E. (Ed.), 2002. Major herbs of Ayurveda. Churchill Livingstone, Edinburgh.

Williamson, E., 2003. Potter's herbal Cyclopedia CW Daniels. Saffron Walden.

Williamson, E.M., Driver, S., Baxter, K., 2016. Stockley's Herb-Drug Interactions (online edition). Pharmaceutical Press, UK.

Chapter 20

The gastrointestinal and biliary system

Gastrointestinal (GI) and liver disorders account for minor, everyday complaints as well as major health problems. Dietary measures can improve symptoms that are caused, for example, by poor eating habits, but, if these are not successful, phytotherapy is also useful. In fact, natural products are still the most commonly used remedies in cases of constipation, diarrhoea and flatulence. Plants and their derivatives also offer useful treatment alternatives for other problems such as irritable bowel syndrome, motion sickness and dyspepsia. In the case of some liver diseases, phytotherapy provides the only effective remedies currently available.

DIARRHOEA

Diarrhoea of sudden onset and short duration is very common, especially in children. It normally requires no detailed investigation or treatment, as long as the loss of electrolytes is kept under control. However, chronic, serious cases of diarrhoea caused by more virulent pathogens are still a major health threat to the population of poor tropical and subtropical areas. The World Health Organization (WHO) has estimated that approximately 5 million deaths are due to diarrhoea annually (2.5 million in children under 5 years).

The first-line treatment is oral rehydration therapy using sugar–salt solutions, often with added starch, and the use of gruel rich in polysaccharides (e.g. rice or barley 'water') is an effective measure. The polysaccharides of rice (*Oryza sativa*) grains are hydrolysed in the GI tract; the resulting sugars are absorbed because the co-transport of sugar and Na^+ from the GI lumen into the cells and mucosa is unaffected. Rice suspensions thus actively shift the balance of Na^+ towards the mucosal side, enhance the absorption of water and provide the body with energy, and the efficacy of rice starch has been demonstrated in several clinical studies. The treatment of diarrhoea in adults, particularly for travellers, may also include opiates or their derivatives, to reduce gastrointestinal motility. Many classical anti-diarrhoeal preparations contain opium extracts, or the isolated alkaloids morphine and codeine (e.g. kaolin and morphine mixture, codeine phosphate tablets), although these are controlled by law in some countries. Opioid derivatives such as loperamide, which have limited systemic absorption and, therefore, fewer central nervous system side effects, have superseded these agents to some extent but the natural substances are still used and are highly effective. Dietary fibre, including that found in bulk-forming laxatives (quantum vis [q.v.]) can also be used to treat diarrhoea; in this case, the fibre is taken with only a small amount of water. There are other plant drugs that act in varying ways (for review see Palumbo 2006).

STARCH (AMYLUM) EP

Starch is used for rehydration purposes and may be derived from rice (*Oryza sativa* L.), maize (*Zea mays* L.) or potato (*Solanum tuberosum* L.). Giving starch-based foods (like gruels) has been shown to be therapeutically beneficial and is a first-line treatment in minor self-limiting cases. These are also used as excipients for tablet production. Starch particles give a very characteristic microscopic picture, which can be used to differentiate the various types, but chemical analysis is rarely carried out.

TANNIN-CONTAINING DRUGS

Tannins are astringent, polymeric polyphenols, and are found widely in plant drugs. The most important medicinal plants used in the treatment of diarrhoea

include Greater Burnet – *Sanguisorba officinalis* L., Black Catechu – *Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb. [better known under its synonym: *Acacia catechu* (L.f.) Willd.], Oak bark – *Quercus robur* L., Tormentil – *Potentilla erecta* (L.) Raeusch., and even (black) tea and coffee (Palumbo 2006). Tannin-containing drugs are generally safe, but care should be taken with concurrent administration of other drugs since tannins are not compatible with alkalis or alkaloids, and form complexes with proteins and amino acids.

Constipation is often due to an inappropriate diet and lack of physical activity, for example while being confined to bed during illness, or the result of taking other medication (especially opioids). It is characterized by reduced and difficult bowel movements, and is said to be present when the frequency of bowel movements is less than once in 2 or 3 days. Although the causes are not usually serious in nature, continuous irregularity in bowel movements should be investigated in case there is a risk of malignant disease. The subjective symptoms (straining heavily, hard stools, painful defecation and a feeling of insufficient evacuation) make it one of the most commonly reported health problems. Constipation is often associated with other forms of discomfort such as abdominal cramps, dyspepsia, bloating and flatulence. Alternating diarrhoea and constipation is a symptom of irritable bowel syndrome.

CONSTIPATION

Various types of plant-derived laxative are used: stimulant laxatives (purgatives), which act directly on the mucosa of the GI tract; bulk-forming laxatives, which act mainly via physicochemical effects within the bowel lumen; and osmotic laxatives, which act by drawing water into the gut and thus softening the stool. Osmotic laxatives may be mineral in origin, for example magnesium salts, or derived from natural products such as milk sugars.

Patients generally require rapid relief from constipation, and the immediate effect of stimulant and saline purgatives is very well known. Although there is no problem using them occasionally, or on a short-term basis (less than 2 weeks), or prior to medical intervention such as X-ray (Roentgen) diagnostics, long-term use should be discouraged. The exception is for patients taking opioids for pain management, who may need to use stimulant laxatives routinely. The most important adverse effect of the long-term use of the stimulant laxatives and saline purgatives is electrolyte loss. Hypokalaemia, pathologically reduced levels of potassium (K^+), may even worsen constipation and cause damage to the renal tubules. The risk of hypokalaemia is increased with administration of some diuretics and hypokalaemia exacerbates the toxicity of the cardiac glycosides (e.g. digoxin), which are often prescribed for elderly patients. Hyperaldosteronism, an excess of aldosterone production, which leads to sodium (Na^+) retention, and again to potassium loss and hypertension, is also a risk. In general, the use of bulk-forming or osmotic laxatives is preferred, unless there are pressing reasons for using a stimulant laxative.

BULK-FORMING LAXATIVES

These are bulking agents with a high percentage of fibre and are often rich in polysaccharides, which swell in the GI tract. They influence the composition of food material in the GI tract, especially via the colonic bacteria, which are thus provided with nutrients for proliferation. This in turn influences the composition of the GI flora and the metabolism of the food in the tract (including an increase in gas, or flatus). Fibre-rich food is part of a healthy diet, but processed food and modern lifestyles have generally reduced fibre intake. Bulk-forming laxatives are generally not digested or absorbed in the GI tract, but pass through it largely unchanged.

Bulking agents can be distinguished from swelling agents in that bulking material contains large amounts of fibre, whereas swelling material is generally composed of plant material (seeds) with a dense cover of polysaccharides on the outside. Both types of medicinal drugs may swell to a certain degree by the uptake of water, but swelling agents in the strict sense include only medicinal plants that form mucilage or gel. The **swelling factor** (which compares the volume of drug prior to and after soaking it in water) is an indicator of the amount of polysaccharides present in the drug and is generally used as a marker for the quality of bulk-forming laxatives. The European Pharmacopoeia requires a minimal value of the swelling factor for each agent, and the swelling factors of the phytomedicines detailed below are shown in Table 20.1. Preparations of bulk-forming laxatives are always taken with plenty of water. They can, paradoxically, be used to treat diarrhoea if given with very little fluid; they then absorb the fluid from the lumen and increase the consistency of the stool.

Linseed (flax), *Linum usitatissimum* L. (Lini semen) EP

Linseeds are the ripe, dried seeds of flax (*L. usitatissimum*, Linaceae), a plant grown for its fibre (used in the

clothing industry) and for the seed oil, which is used in paints and varnishes, and to make oil cloth ('linoleum'). Flax, with its characteristic blue flowers, is an annual, and has long been under human cultivation. The dark brown (less often yellowish-white) seeds are oblong or ovate with a characteristically pointed end. They are tasteless but slowly produce a mucilaginous feel when placed in the mouth. The outer layer of the seed (testa) is rich in polysaccharides, while the inner part of the seed, which contains the endosperm and the cotyledons, is rich in fatty oil. If the seeds are taken whole, the inner layer of the testa is only partially digested in the GI tract and they will be excreted in the entire form, and the fatty acids will not be released. The swelling factor should at least be 4 (entire seeds) or 4.5 (powdered drug). Linseed also possesses cholesterol-lowering properties, and contains phytoestrogenic lignans.

Plantago species

Ispaghula, *Plantago ovata* Forssk. (syn.: *Plantago ispaghula* Roxb. – Plantaginis ovatae semen) EP. The various types of psyllium yield useful and commonly used emollients and bulk laxatives that help in maintaining a regular bowel movement.

The dark brown, glossy seeds from *Plantago ovata* (Indian fleawort, blond psyllium, Plantaginaceae) are useful in the treatment of chronic constipation. In cases of simple and chronic constipation and among the elderly, the use of ispaghula husk is reported to be effective. Ispaghula should not be taken within at least half to one hour of any other medication, as it may delay their absorption (EMA 2013b).

The seeds are broadly elliptical in shape, up to 3.5 mm long, and are practically tasteless, becoming mucilaginous when chewed. They can help to maintain or achieve a regular bowel movement and are also useful in irritable bowel syndrome. The swelling factor should be > 9 for the entire seeds and > 40 for the seed husk, which is the most widely used part. The usage is similar to that of psyllium (fleawort, below).

Psyllium, *Plantago indica* L. (syn.: *Plantago psyllium* L. and *P. arenaria* Waldst. & Kit.) and *Plantago afra* L. (Psyllii semen) EP. The brown, shiny, elliptical to ovate seeds (2–3 mm long) are obtained from two species of the plantain family (Plantaginaceae). *Plantago indica* (fleawort, black or dark psyllium, plantain) is used in a similar way and the seeds are narrower and somewhat smaller than ispaghula seeds. An essential characteristic of high-quality material is a high swelling factor.

Wheat bran, *Triticum aestivum* L. Bran is less useful as a laxative (except when taken as a natural part of the diet, e.g. in breakfast cereal), since it contains phytic acid, which in high concentrations can complex with and, therefore, reduce the bioavailability of vitamins and minerals taken at the same time. However, in some patients, wheat bran (the husk from the grains of *Triticum aestivum*) is more effective than other swelling agents, and preparations containing it are available for prescribing. These are taken in water.

Table 20.1 Swelling factors of various bulk-forming laxatives

COMMON NAME	BOTANICAL SOURCE	SWELLING FACTOR (EUR. PH.)	NOTES
Ispaghula seed	*Plantago ovata*	≥ 9 (seed) ≥ 40 (testa of the seed)	
Psyllium seed	*Plantago indicum* and *P. afra*	≥ 10	
Linseed	*Linum usitatissimum*	≥ 4 (entire seed) ≥ 4.5 (ground seed)	Also rich in fatty acids (in endosperm of seed)
Wheat bran	*Triticum aestivum*	–	Rich in fibre

OSMOTIC LAXATIVES

Osmotic laxatives, such as lactulose or lactose, which are dimeric sugars derived from milk, are a useful and widely used approach to the treatment of long-term constipation. Lactose is split in the GI tract into glucose and galactose, and galactose is not generally resorbed well. Consequently, the bacteria of the colon metabolize this sugar. The resulting acids, including lactic acid and acetic acid, have an osmotic effect, and the bacteria in the colon multiply more rapidly. This results in softening and an increase in the amount of faeces, with a subsequent increase in GI peristalsis.

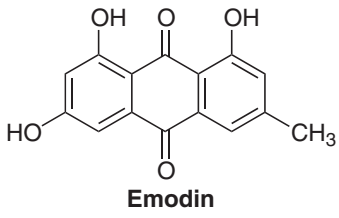
STIMULANT LAXATIVES

Stimulant laxatives are derived from a variety of unrelated plant species, which only have in common the fact that they contain similar chemical constituents. These are anthraquinones such as emodin (Fig. 20.1) and aloe-emodin, and related anthrones and anthranols.

Anthraquinones are commonly found as glycosides in the living plant. Several groups are distinguished, based on the degree of oxidation of the nucleus and whether one or two units make up the core of the molecule. The anthrones are less oxygenated than the anthraquinones and the dianthrones are formed from two anthrone units (Fig. 20.2). Studies using dianthrone glycosides such as sennosides A and B suggest that most of these compounds pass through the upper GI tract without any change; however, they are subsequently metabolized to rhein anthrone in the colon and caecum by the natural flora (mainly bacteria) of the GI tract. Anthranoid drugs act directly on the intestinal mucosa, influencing several pharmacological targets, and the laxative effect is due to increased peristalsis of the colon, reducing transit time and, consequently, the re-absorption of water from the colon. Additionally, the stimulation of active chloride secretion results in an inversion of normal physiological conditions and a subsequent increased excretion of water. Overall, this results in an increase of the faecal volume with an increase in the GI pressure. These actions are based on the well-understood effects of chemically defined constituents; consequently, phytomedicines containing them are usually standardized to specified anthranoid content (see Chapter 10, p. 169). While anthranoid-containing extracts are clearly clinically effective, in palliative care patients, who used laxatives to counteract the effects of opioid analgesics, Miles et al (2006) found no significant clinical differences in effectiveness between lactulose and senna, or lactulose with senna, compared to magnesium hydroxide and liquid paraffin. Another Cochrane review concluded that in the management of childhood constipation, senna compared with lactulose was identical in terms of passing stools (Gordon et al 2012).

Safety concerns related to anthranoid drugs

The monomeric aglycones (especially emodin and aloe-emodin) have been shown to have genotoxic and mutagenic effects using bacterial and *in vitro* systems such as the Ames test, and in mammalian cell lines. The long-term use of anthranoids may result in a (reversible) blackening of the colon (*Pseudomelanosis coli*), which is due to the incorporation of metabolites of the anthranoids and is thought to be associated with an increased risk of colon carcinoma. In practice, few toxic effects have been described, apart from those involving electrolyte loss described above. More immediate effects of anthranoid-containing drugs are colic and griping pains due to increased spastic contractions of the smooth musculature of the GI tract. Aloes and senna leaves are particularly prone to producing these. A synthetic derivative, danthrone (also known as dantron; not to be confused with the naturally occurring dianthrone), has been developed and, although effective, it is used only in palliative care due to its carcinogenic potential.

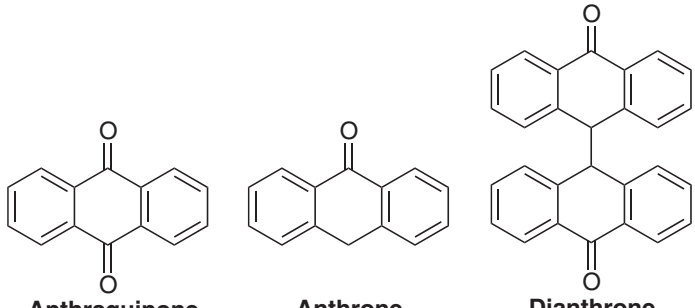


Emodin

Fig. 20.1

Frangula, *Frangula alnus* Mill., (syn.: *Rhamnus frangula* L. – Frangulae cortex); buckthorn, *R. cathartica* L. (Rhamni cathartici cortex) and cascara, *Frangula purshiana* Cooper (syn.: *R. purshiana* DC. – Rhamni purshiani cortex) EP

The barks of several species of Rhamnaceae are used for their strong purgative effects. *F. alnus* (glossy buckthorn, frangula) has a milder action than *R. cathartica*



Anthraquinone **Anthrone** **Dianthrone**

Fig. 20.2

(European buckthorn) and the berries are used in veterinary medicine. (The fruit also yields a dye, the colour of which depends partly on the ripeness.) The bark of *F. purshiana* (American buckthorn, known in commerce as Cascara sagrada) is the other main species used medicinally.

F. alnus is a densely foliated, thornless bush or tree, reaching a height of 1–7 m, common in damp environments such as bogs and along streams in North and Central Europe, as well as northern Asia. The cut bark is grey-brown with numerous visible grey-white lenticels. The leaves are broadly elliptical to obovate, about 3.5–5 cm long. The black, pea-sized berries develop from small greenish-white flowers.

Buckthorn (*R. cathartica*) is a thorny shrub with toothed leaves and a reddish brown bark; the berries are black and globular.

Cascara (*F. purshiana*) is native to the Pacific coast of North America but grows widely elsewhere. It is found in commerce in quilled pieces, often with epiphytes (lichen and moss) attached.

Constituents.

F. alnus: Glucofrangulin A (Fig. 20.3) and B, which are diglucosides differing only in the **type of sugar at C6.**

***R. cathartica*:** Emodin, aloe-emodin, chrysophanol and rhein glycosides, frangula-emodin, rhamnicoside, alaterin and physcion.

***F. purshiana*:** Cascarosides A (Fig. 20.3), B, C, D, E and F (which are stereoisomers of aloin and derivatives), with minor glycosides including barbaloin, frangulin, chrysaloin, palmidin A, B and C and the free aglycones.

The anthrone and dianthrone glycosides, which are present in the fresh bark of these species, have emetic effects and may result in colic. In order to oxidize these compounds to anthraquinones with fewer undesirable side effects, the drug has either to be kept for a year or it is 'aged artificially' by heating it for several hours to 80–100°C.

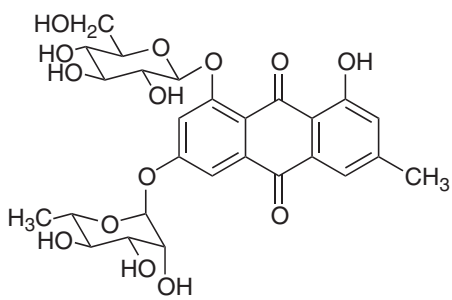
Senna, *Cassia senna* L. (Senna) (Sennae fructus, Sennae folium) EP

The genus *Cassia* (Caesalpiniaceae) is very large, with about 550 species, mostly occurring in warm temperate and tropical climates. The species are not native to Europe and were an important drug of early trading; the name 'senna' is of Arabic origin and was recorded as early as the 12th century. Cassia senna L. is today named Senna alexandrina Mill. and yields the drugs senna leaves and senna fruit. The species was previously split into two species based on their origin: Alexandrian senna (*Cassia senna* L. also known as *C. acutifolia* L.) and Tinnevelly senna (*C. angustifolia* Vahl). The common names were derived from their original trade sources and are only applied to the fruits (pods). The second origin is considered to be the milder in activity. Both the leaves and the fruits have typical microscopic characteristics, including the highly diagnostic, single-celled warty trichomes and the crystal sheath of calcium oxalate prisms around the fibres, but it is possible to distinguish the two species microscopically.

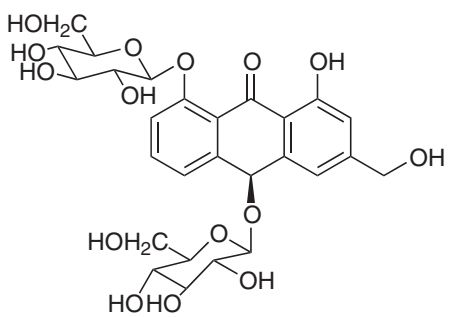
Constituents.

***Leaf*.** Sennosides A and B (Fig. 20.4), which are based on the aglycones sennidin A and sennidin B; sennosides C and D, which are glycosides of heterodianthrones of aloe-emodin and rhein; palmidin A, rhein anthrone and aloe-emodin glycosides and some free anthraquinones. *C. senna* usually contains greater amounts of the sennosides.

***Fruit*.** Sennosides A and B and a related glycoside sennoside A1. The sennosides, which are dianthrones, differ in their stereochemistry at C_{10}

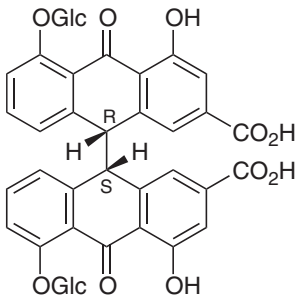


Glucofrangulin A



Cascaroside A

Fig. 20.3



Sennoside B

Fig. 20.4

and C_{10}, as well as in their substitution pattern. *C. senna* usually contains greater amounts of the sennosides.

The structure of sennoside B is given in Fig. 2.4. The Eur. Ph. standard is for a glycoside content of not less than 2.5% for the leaf, 3.5% for *C. senna* fruit and 2.2% for *C. angustifolia* fruit, calculated as sennoside B. Other secondary metabolites such as flavonoids, tannins and bitter compounds are also present but not defined in the standard. The way in which the plant material is dried has a strong influence on the amount of glycosides remaining and accordingly on the quality of the product (see above).

The other main botanical anthranoid drugs are aloes *Aloe vera* (L.) Burm.f., (syn.: *A. barbadensis* Mill.; and other species) and rhubarb (*Rheum rhaponticum* L. and others). These are used to a lesser extent nowadays.

Often combinations of different types of laxatives are used like whole linseed (*L. usitatissimum*) combined with senna leaf (*S. alexandrina*) and frangula bark (*F. alnus*) and while there is a long tradition of use of such products, this is largely based on empirical evidence. Very little research is being conducted on these botanical drugs and preparations.

INFLAMMATORY GI CONDITIONS: GASTRITIS AND ULCERS

Inflammation of the gastric mucosa, or gastritis, is an acute inflammatory infiltration of the superficial gastric mucosa, predominantly by neutrophils. It is generally treated with antacids (magnesium and aluminium salts) and emollients (alginate, mucilages), but other phytomedicines are still occasionally used (e.g. chamomile and liquorice). These agents, especially liquorice, were used to treat gastritis and ulceration until superseded by the synthetic H_2-receptor-blocking agents (cimetidine, ranitidine, etc.) and proton pump inhibitors (omeprazole, lansoprazole). Now that infection with *Helicobacter pylori* is known to be a causal factor in ulceration, antibiotic therapy is the first-line treatment of choice. Most pharmaceuticals for mild gastric inflammation contain a mixture of an emollient, to line and soothe the mucosa (e.g. an agar suspension), an antacid and possibly a carminative such as peppermint or anise oil (see section on dyspepsia).

Alginate EP

Alginate, or alginic acid, is an anionic polysaccharide distributed widely in the cell walls of brown algae including *Laminaria*, and *Ascophyllum nodosum*. Raw or dried seaweed is washed with acid to remove cross-linking ions that cause the alginate to be insoluble. It is then dissolved in alkali, typically sodium hydroxide, to produce a viscous solution of alginate. The solution is filtered to remove the cell wall debris and leave a clear alginate solution. Alginate binds with water to form a viscous gum and acts as a protective coating over the walls of the stomach and oesophagus.

Chamomile *Matricaria chamomilla* L. (Matricariae flos) EP

German (or Hungarian) chamomile flowers are derived from *M. chamomilla* L. (syn. *Chamomilla recutita* (L.) Rauschert, *M. recutita* L, Asteraceae, the daisy family). They have a pleasant aromatic odour. The flower heads have a diameter of approximately 10 mm and are composed of many minute flowers (called florets), which are either tongue-shaped ('ligulate florets', found at the margin) or tubular ('disk' florets, found in the disk-like centre). True chamomile has a hollow receptacle (the part of the stalk where the flower parts are attached) and is devoid of the small leaf-like structures (stipules) that are common with the non-medicinal members of this genus. Chamomile is grown on a large scale, especially in Eastern Europe, Spain, Turkey, Egypt and Argentina, and has been known as a medicinal plant for several thousands of years. It is used internally for spasmodic and inflammatory illnesses of the GI tract.

Constituents. The flower heads are rich in essential oil. Two types of essential oil are recognized: one rich in bisabolol (levomenol) (Fig. 20.5) and the other in bisabolol oxides. Both contain other terpenoid compounds, including guaianolides, such as matricin, which are only found in the crude drug. The characteristic, dark blue azulenes (e.g. chamazulene; Fig. 20.5) are produced during steam distillation and only found in the essential oil. Flavonoids (up to 6%), especially apigenin and apigenin-7*O*-glycoside, caffeic acid derivatives and spiro ethers are also present. The components of the essential oil levomenol (α-bisabolol), its oxides, chamazulene,

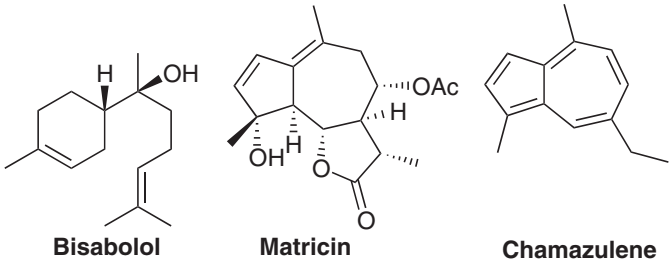


Fig. 20.5

some unusual spiro ethers and the flavonoids (especially apigenin) are all essential for the pharmacological effects of the drug. The minimum amount of essential oil required by the Eur. Ph. is 0.4%.

Therapeutic uses and available evidence. Anti-inflammatory, spasmolytic, antibacterial and antifungal effects are well established both pharmacologically and clinically (see McKay and Blumberg 2006a) and its use is based on empirical evidence. Chamomile is generally considered to be safe, although allergic reactions may occur as with all plants of the Asteraceae. Aside from its medical use, chamomile is also used as a food in the form of an infusion due to its mild digestive properties.

Note. The flower heads of *Chamaemelum nobile* (L.) All. (syn. *Anthemis nobilis* L., English chamomile, Roman chamomile) have a pharmacological profile similar to that of *Matricaria recutita*, and are included in the Eur. Ph. However, there is much less scientific and clinical evidence to support their use than for *M. recutita*.

Liquorice, *Glycyrrhiza glabra* L. (Liquiritiae radix) EP

Liquorice (licorice) root is derived from the inner part of the root and underground stem (rhizome) of *G. glabra* (Fabaceae, the bean family). The peeled drug is of much higher quality than the root with the bark, and is produced in several south-eastern European countries, Turkey, China and Russia. It has a very characteristic taste and smell, and is used in confectionery. The sweet taste also makes the identification of the drug relatively easy and so adulteration is uncommon. Microscopic identification is possible and uses characteristic crystals of oxalate, especially in the form of a sheath of parenchyma surrounding the phloem fibres, as well as the structure of the parenchyma.

Liquorice is used to relieve gastric inflammation, specifically in the case of peptic ulcers and duodenal ulcers, but its use as a GI remedy is controversial because of its mineralocorticoid action. Due to the small size of the clinical studies using liquorice extract in GI and ulcerative conditions, no meaningful conclusions

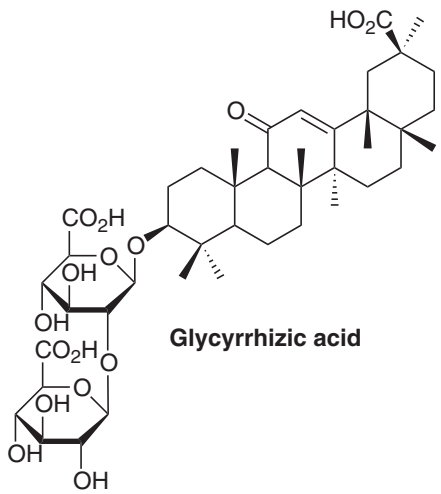


Fig. 20.6

can be drawn (EMA 2013a). Beneficial effects in terms of the relief of the symptoms of functional dyspepsia were shown in a small clinical study (Raveendra et al. 2012).

The dose should not exceed 200–600 mg of glycyrrhizin daily and the duration of treatment should be at most 4–6 weeks. More potent synthetic pharmaceuticals are now available, and it is now rarely used for this purpose. Liquorice and its preparations are contraindicated in cholestatic liver disorders, liver cirrhosis, hypertension, hypokalaemia, severe renal failure and pregnancy. With excessive use, liquorice-containing confectionery may result in similar undesired side effects. Liquorice is also used in respiratory complaints as an expectorant, mucolytic and antitussive agent.

Constituents. The most important bioactive secondary metabolite is glycyrrhizic acid (also known as glycyrrhizin; Fig. 20.6), a water-soluble pentacyclic triterpene saponin that gives the drug its characteristic sweet taste (it is about 50 times sweeter than sucrose). The genin (glycyrrhetinic acid or glycyrrhetin), on the other hand, is not sweet but very bitter. Liquorice also contains numerous flavonoids (chalcones and isoflavonoids), coumarins and polysaccharides, which contribute to the activity.

INFLAMMATORY GI CONDITIONS: INFLAMMATORY BOWEL DISEASE (IBD)

This condition, which includes ulcerative colitis and Crohn disease is characterized by the intestines becoming inflamed, but its aetiology is poorly understood. Irritable bowel syndrome (IBS) is characterized by pain in the left iliac fossa, diarrhoea and/or constipation. Symptoms are usually relieved to some extent by defecation or the passage of wind, and can be treated successfully by the use of bulk laxatives with or without antispasmodic (carminative) drugs. Clearly, inflammatory cytokines such as TNF-α are crucial mediators. Ulcerative colitis is limited to the colon and Crohn disease may involve any part of the entire gastrointestinal tract. IBD increases the risk of developing colon cancer. The standard treatment includes anti-inflammatory medicines, immunosuppressants, and TNF blockers with the generally poor treatment outcomes, the high cost and adverse effects associated limiting their therapeutic usefulness. Some of the tropane alkaloids have been used. Atropine has been replaced by hyoscine, in the form of the *N*-butyl bromide, which, as a quaternary ion, is poorly absorbed from the GI tract and, therefore, has fewer anti-muscarinic side effects.

No established phytotherapeutic products are available and a sound evidence base with well-designed randomized clinical trials with large cohorts will be needed. Natural remedies include peppermint oil and other essential oil carminatives, and in the case of ulcerative colitis some data exist, for example, for turmeric (*Curcuma longa*, see below, Ali et al 2012) and ispaghula, *Plantago ovata* (see above). In the case of Crohn's disease the traditional Chinese medicines thunder duke vine (*Tripterygium wilfordii* Hook.f., Celastraceae), and *Artemisia absinthium* (wormwood, see below) are examples for which some evidence exists (Ng et al 2013). Artichoke extract is also useful in irritable bowel syndrome (Walker et al 2001; see under dyspepsia and biliousness). It is also known that patients self-prescribe marihuana (*Cannabis sativa* L.), but there is no evidence providing a clear basis for or against its use (Ravikoff Allegretti et al 2013).

DYSPEPSIA AND BILIOUSNESS

Dyspepsia and 'biliousness' are closely associated with eating habits and are very common complaints. Patients describe the symptoms as nausea, pain and cramps, distension, heartburn and the 'inability to digest food', often experienced after rich meals. The condition is treated either with cholagogues or with bitter stimulants. A cholagogue is an agent that stimulates bile production in the liver, or promotes emptying of the gallbladder and bile ducts. Although clinical evidence is largely lacking, plant-based cholagogues are frequently prescribed by family doctors in Germany based on observational evidence and a long tradition of use, but they should not be used in cases of bile duct obstruction or cholestatic jaundice.

Liver disease is not treated as such by conventional medicine, but herbal medicine has a number of clinically proven treatments that help to protect the liver from damage and reverse some of the indicators of liver malfunction. The most important of these is silymarin, but other medicinal plants are widely used for liver disease, although mainly with much less clinical evidence in support.

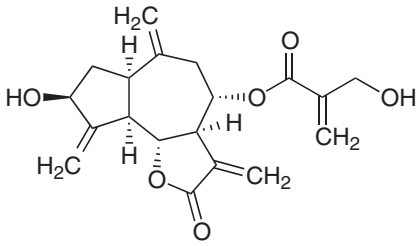
Phytomedicines used as bitter stimulants, such as gentian and wormwood, act directly on the mucosa of the upper part of the GI tract and especially of the bitter receptors on the tongue, stimulating the secretion of saliva and gastric juices and influencing the secretion of gastrin. An aperitif containing 'bitters', taken about half an hour before eating, stimulates gastric and biliary secretion; however, it is not known whether these effects are restricted to patients with a reduced secretory reflex, or whether an increase also occurs in healthy people.

Artichoke, *Cynara cardunculus* L. (syn.: *Cynara scolymus* L. – Cynarae folium) EP

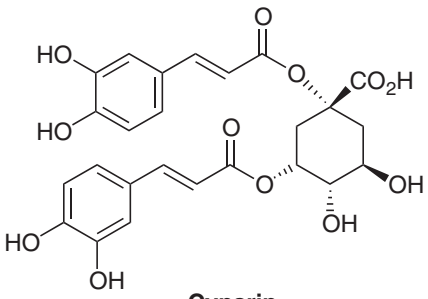
This well-known member of the Asteraceae yields the globe artichoke (a food common in French cuisine), which is the large flower head of the plant. The medicinal part is the leaf, which is used to treat indigestion and dyspepsia, and to lower cholesterol levels.

Constituents. The leaf contains the bitter sesquiterpene lactone cynaropicrin, several flavonoids and derivatives of caffeoylquinic acid, including cynarin (Fig. 20.7).

Therapeutic uses and available evidence. In the case of hypercholesterolaemia and based on a Cochrane review including only randomized clinical trials (262 participants), there is some evidence for a cholesterol-lowering effect of single-herb artichoke leaf extract, although the effect is modest (Wider et al 2013). Antihepatotoxic effects, cholagogue activity and a reduction of cholesterol and triglyceride levels have been reported, and are now known to be due to inhibition of cholesterol biosynthesis. A cohort study conducted for 60 days in outpatients with a clinical diagnosis of functional dyspepsia showed that a commercial artichoke product significantly reduced the symptom severity associated with dyspepsia along with a modest decrease in total choles-



Cynaropicrin



Cynarin

Fig. 20.7

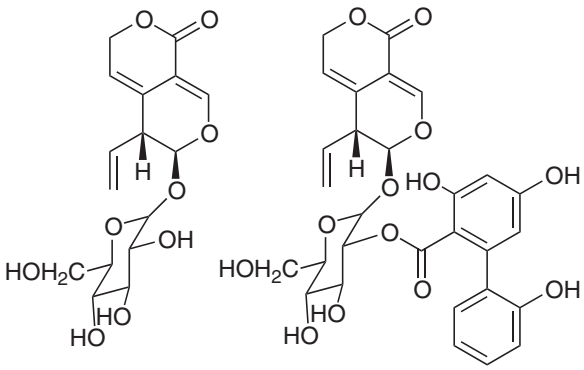
terol, LDL cholesterol and triglyceride levels (Sannia 2010). Clinical studies have shown that artichoke leaf extract can improve parameters such as fat intolerance, bloating, flatulence, constipation, abdominal pain and vomiting, and increase bile flow, at a daily mean dose of 1500 mg. For further details, see Bundy et al (2008) and Wider et al (2013). Artichoke extract is also useful in irritable bowel syndrome (Walker et al 2001).

Gentian, *Gentiana lutea* L. (Gentianae radix) EP

The yellow gentian (*G. lutea*, Gentianaceae) is, after ethanol, the most important ingredient of the Alpine beverage *Enzianschnaps*, used as a digestive stimulant, taken after a large meal. Most medicinal products are made using the rapidly dried and non-fermented drug.

The species is rare (but locally abundant) and distributed in the alpine regions of Europe and western Asia. It is a perennial herb up to 1.4 m high with showy yellow flowers. Because of the high risk of overexploitation (for use as an ornamental and as a medicine) the species is now protected throughout most of its range and attempts are being made to cultivate it. Gentian root in commerce consists of the dried rhizomes and roots of the species. The rhizome is cylindrical and may have a diameter of up to 4 cm, with long roots attached.

Constituents. The compounds responsible for the highly bitter taste are monoterpenoid compounds (Fig. 20.8) such as gentiopicroside – a seco-iridoid with a bitter value of 12,000 – and amarogentin – with a bitter value of 58,000,000, which is only present in minute amounts. The normally white inner part of the rootstock turns yellow during fermentation, due to the formation of xanthones, including gentisin. Chemical analysis is carried out following the method of the Eur. Ph., but the 'bitter value' test is also useful. This is a simple and useful measure for establishing the quality of bitter-tasting (botanical) drugs. It is the inverse concentration of the dilution of an extract (or a pure compound), which can still be detected as being bitter to testers with normal



Gentiopicroside Amarogentin

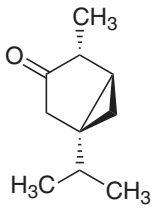
Fig. 20.8

bitter taste receptors. In the case of gentian, it should at least be 10,000 (i.e. an extract that has been diluted 10,000 times should still leave a bitter taste).

Therapeutic uses and available evidence. Gentian is indicated for poor appetite, flatulence and bloating, although clinical trial evidence is lacking. Extracts stimulate gastric secretion in cultured rat gastric mucosal cells and gentiopicroside has been shown to suppress chemically and immunologically induced liver damage in mice.

Wormwood, *Artemisia absinthium* L. (Absinthii herba) EP

Wormwood is a bitter stimulant derived from the aerial parts of *Artemisia absinthium* (Asteraceae) and is popularly used as a tea. It is a commonly cultivated garden plant. The liqueur was a popular stimulant in many European countries during the latter part of the 19th century and early part of the 20th century, and gave rise to the condition known as absinthism, a form of mental disorder, reputed to affect the artist Van Gogh. The plant is still commonly grown in Mediterranean gardens. The leaves and young stems are densely covered with characteristic greyish-white hairs, which give the species its typical appearance.



Thujone

Fig. 20.9

A large number of related species are also used as a food (estragon – *A. dracunculus* L., Obolskiy et al 2011) or medicine (*A. annua* L. – the source of artemisinin, used to treat malaria and to isolate artemisinin, see p. 291)

Constituents. The essential oil contains β-thujone (Fig. 20.9) as the major component, as well as thujyl alcohol, azulenes, bisabolene and others. Sesquiterpene lactones are also present, especially absinthin, anabsinthin, artemetin, artabsinolides A, B, C and D, artemolin and others, and flavonoids. During the process of distillation, the intensively blue chamazulene is formed, which together with the other constituents gives the oil of absinth its characteristic green-blue colour. The sesquiterpene lactone absinthin is responsible for the intensive bitter taste, which according to the Eur. Ph. should be at least 10,000 (Deutsches Arzneibuch 15,000) for the crude extract (see 'Gentian', p. 243 for explanation of bitter value). β-Thujone, a monoterpene, is also partly responsible for the bitterness. The essential oil content should be at least 0.2% and the bitter sesquiterpenoids 0.15–0.4%, according to the Eur. Ph. where the methods are described.

Therapeutic uses and available evidence. It is commonly used as a bitter tonic, a choleretic and also as an anthelmintic. Although its use in the form of a tea is considered safe, the essential oil, and the liqueur 'absinthe' distilled from this plant, are harmful in large doses due to the thujone content, and are not now used except where the thujone has been removed. Most of the evidence is empirical and clinical evidence is limited.

Toxicological risks. Thujone, a major component of the essential oil, is neurotoxic and hallucinogenic in large doses and can produce epileptic fits and long-lasting psychiatric disturbances. These are considered to be a problem only with the distilled ethanolic beverage (absinthe). Thujone is found in the essential oil of many unrelated species, including sage (*Salvia officinalis*) and thuja (*Thuja occidentalis*), and is still used medicinally with few ill-effects. There is also some dispute as to whether 'absinthism' is anything more than plain alcoholism, since thujone levels are not always high enough to be considered as causing such severe damage.

NAUSEA AND VOMITING

'Travel sickness' or 'motion sickness' is particularly common in children and is caused by the repetitive stimulation of the labyrinth of the ear. It is most common when travelling by sea, but also happens in cars, aeroplanes and when horse-riding. Vomiting, nausea, dizziness, sweating and vertigo may occur. Prophylactic treatment includes the use of antihistamines (mainly phenothiazines) and cinnarizine, and natural compounds such as the antimuscarinic alkaloid hyoscine, found in the Solanaceae (nightshade family). Morning sickness of pregnancy is also common but few (if any) synthetic drugs are licensed for such a use because of fears of toxicity to the unborn child. Ginger can be a useful anti-emetic for this condition, as well as for travel sickness.

Ginger, *Zingiber officinale* Roscoe (Zingiberis rhizoma) EP

Ginger (Zingiberaceae) is one of the most commonly used culinary spices in the world and has a variety of medicinal uses. The odour and taste are very characteristic, aromatic and pungent. Ginger is cultivated in moist, warm tropical climates throughout south and south-eastern Asia, China, Nigeria and Jamaica. The rhizome is the part used and is available commercially either peeled or unpeeled. African dried ginger is usually unpeeled, and the fresh rhizome, which is widely available for culinary purposes, is always unpeeled. The medicinal use of ginger in Europe has an ancient history and can be traced back to Greek and Roman times. The plant has also been mentioned in Ayurvedic and other religious scriptures dating back to 2000 BCE, where it was recognized as an aid to digestion and for cases of rheumatism and inflammation.

Constituents. The rhizome contains 1–3% essential oil, the major constituents of which are zingiberene and β-bisabolene. The pungent taste is produced by a mixture of phenolic compounds with carbon side-chains consisting of seven or more carbon atoms, referred to as gingerols, gingerdiols, gingerdiones, dihydrogingerdiones and shogaols (Fig. 20.10). The shogaols are produced by dehydration and degradation of the gingerols and are formed during drying and extraction. The shogaols are twice as pungent as the gingerols, which accounts for the fact that dried ginger is more pungent than fresh ginger.

Therapeutic uses and available evidence. Modern uses of ginger are diverse and include as a carminative, anti-emetic, spasmolytic, antiflatulent, antitussive, hepatoprotective, antiplatelet aggregation and hypolipi-

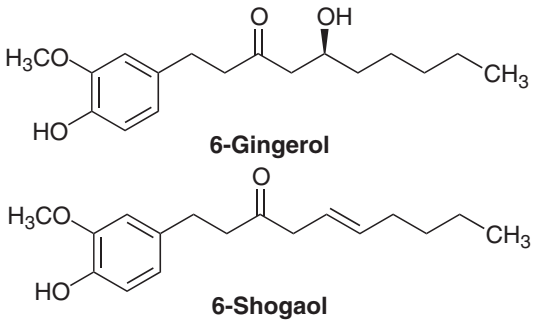


Fig. 20.10

daemic. Some of these actions are substantiated by pharmacological *in vivo* or *in vitro* evidence. Of particular importance is the use in preventing the symptoms of motion sickness and postoperative nausea, as well as vertigo and morning sickness in pregnancy, and there is some clinical evidence for the efficacy of ginger in these conditions (Matthews et al 2010). Ginger consumption has also been reported to have a beneficial effect in alleviating the pain and frequency of migraine headaches, and studies on the action in rheumatic conditions have shown a moderately beneficial effect. Anti-ulcer activity has been described in animals and attributed to the volatile oil, especially the 6-gingesulfonic acid content, and hepatoprotective effects have been noted in cultured hepatocytes, with the gingerols being more potent than the homologous shogaols found in dried ginger. Both groups of compounds are antioxidants and possess free radical scavenging activity. Ginger is well known to produce a warming effect when ingested, and the pungent principles stimulate thermogenic receptors. In addition, zingerone induces catecholamine secretion from the adrenal medulla (for review, see Ali et al 2008). In Oriental medicine, ginger is so highly regarded that it forms an ingredient of about half of all multi-item prescriptions. A distinction is made between the indications for the fresh rhizome (vomiting, coughs, abdominal distension and pyrexia) and the dried or processed rhizome (abdominal pain, lumbago and diarrhoea). This is probably justifiable since the constituents are present in different proportions in the different preparations. Ginger, both in the fresh and dried form, is generally regarded as safe.

Hyoscine (scopolamine) EP

The alkaloid hyoscine (Fig. 20.11) is usually isolated from *Datura* or *Scopolia* spp., although, as the name suggests, it was originally found in *Hyoscyamus niger*. It is a popular remedy for motion sickness, given at an oral dose of 400 µg or, more recently, as a transdermal

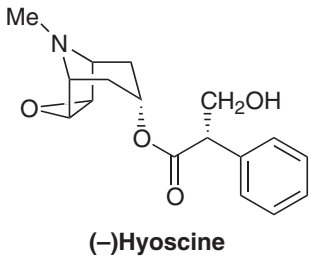


Fig. 20.11

patch containing 2 mg of the alkaloid, which is delivered through the skin over 24 h. Hyoscine is also used as a premedication, usually in combination with an opiate, to relax the patient and dry up bronchial secretions prior to administration of halothane anaesthetics.

BLOATING AND FLATULENCE

Flatulence, which is the passage of excessive wind from the body, is a condition for which phytotherapy offers useful therapeutic approaches. Carminatives are usually taken with food; they produce a warm sensation when ingested and promote postprandial elimination of gas. Plant-based carminatives are usually rich in essential oil, such as the fruits ('seeds') of species of the Apiaceae (celery family) and some members of the Lamiaceae (mint family). Many condiments such as cumin and caraway have carminative effects and are used as spices because of their taste *and* their pharmacological effect. The clinical validity of carminatives is based on long historical observation and is well established. The effect of many of these botanical drugs is due to their spasmolytic action, for which some *in vitro* evidence exists (Ford et al 2008), but the precise mechanism of action is unclear. It seems likely that not only is the essential oil responsible for the effect, but that the other components (e.g. the flavonoids) also contribute.

MINT LEAVES AND OILS: *MENTHA* SPECIES

Members of the mint family are widely used for their digestive effects and flavouring qualities. They contain similar compounds, but in differing proportions, which results in subtle differences in their taste and properties.

Peppermint, *Mentha × piperita* L. (Menthae piperitae folium) EP

Peppermint is a hybrid of *Mentha aquatica* L. and *M. spicata* L., which originated spontaneously and has been

known for over 2500 years; the first records are from old Egyptian graves (2600–3200 BCE). Peppermint has a very characteristic, strongly aromatic and penetrating smell and taste. All species of the genus *Mentha* (the mints) have quadrangular (square) stems and decussate, elongated, dentate leaves with a pointed apex, and pinkish-blue flowers up to 5 mm long, and microscopical characteristics include glandular hairs, which are typical of the Lamiaceae. Both the leaves, in the form of a tea, and the oil are used for digestive problems.

Constituents. Peppermint leaf is rich in essential oil (0.5–4%), the main components being (–)-menthol, menthone, menthylacetate and menthofuran. The plant also contains the non-volatile polyphenolics rosmarinic acid and derivatives, flavonoids and triterpenes.

Peppermint oil is derived from the fresh plant by steam distillation and contains approximately 50% (–)-menthol (see Fig. 22.4).

Therapeutic uses and available evidence. Overall, the evidence is mostly empirical and based on many years of practice. Peppermint is often taken in the form of a tea, which provides a refreshing beverage as well as a mild digestive soothing effect (see McKay and Blumberg 2006b). The oil can be given well-diluted with water or as an emulsion (2%, v/v, dispersed in a suitable vehicle) for treating colic and GI cramps in both adults and children, and in the form of enteric-coated capsules for IBS, where it is released directly into the intestine and bowel. The antispasmodic effect of peppermint oil has been well established using a series of *in vitro* models, the effect being marked by a decline in the number and amplitude of spontaneous contractions, and due at least in part to Ca^{2+}-antagonistic effects. Peppermint oil has also been shown clinically to enhance gastric emptying. Peppermint chloroform-water (or emulsion) must be used with great care. A tragic incident with such an extemporaneous preparation caused the death of a young baby, although the toxicity was due to the excipient rather than to the peppermint oil (by adding concentrated chloroform water rather than the diluted form, two pharmacists inadvertently produced a lethal medication).

Japanese mint, *Mentha arvensis* L

Japanese mint is rich in menthol (about 80% of the total volatile oil, see below) and is employed as a cheaper substitute for peppermint or for extracting menthol.

Spearmint, *Mentha* × *spicata* L. (syn. *M. crispa*, *M. spicata* subsp. *crispa*)

Spearmint gives toothpaste, mouthwash and chewing gum their typical taste and smell. It is an important flavouring agent, but is of limited pharmaceutical importance. Material from this species is easily identifiable from the taste and odour.

UMBELLIFEROUS FRUITS

The fruits (not 'seeds' as they are commonly known) of several members of the celery family (Apiaceae or Umbelliferae) are used as carminatives as they are rich in essential oil and have an antispasmodic effect. Many of these species are also important as spices. Flower heads of these species are umbels of white or pinkish flowers, which produce the characteristic schizocarp (double) fruits, in which two mericarps are united to form an easily separated fruit on a carpophor (a stalk which means 'carrier of fruit').

Caraway, *Carum carvi* L. (Carvi fructus) EP

Caraway is the fruit of a mountain herb common in many regions of Europe and Asia.

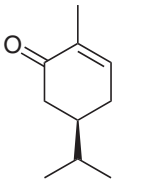
Constituents. The essential oil (3–7%) consists mainly of (+)-carvone and (+)-limonene, accounting for 45–65% and 30–40%, respectively, of the total oil. Carvone (Fig. 20.12) is considered to be the main component responsible for the spasmolytic action.

Therapeutic uses and available evidence. The fruits are used in cases of dyspepsia, minor GI cramps and flatulence. Little modern clinical evidence is available but caraway has long been used in products such as infant gripe water. The aqueous extract, and even more so the essential oil, acts as a spasmolytic and has antimicrobial activity.

Fennel, *Foeniculum vulgare* Mill. (Foeniculi fructus) EP

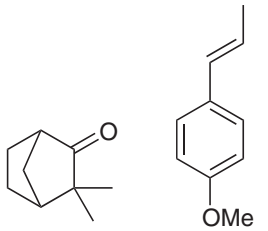
The common fennel is a perennial herb yielding fruit and oil that are used for stomach and abdominal discomfort, as well as a spice in sweets and liqueurs. Other varieties yield the commonly used vegetable fennel. Two pharmaceutically important varieties are distinguished: *Foeniculum vulgare* var. *dulce* (sweet fennel), which is richer in anethole and has a sweet and aromatic taste, and *F. vulgare* var. *vulgare* (bitter fennel), which is rich in fenchone, resulting in a bitter taste. The two varieties are nearly impossible to distinguish microscopically; consequently, taste and smell differentiation, as well as thin-layer chromatography (TLC) analysis, are essential for differentiating the two.

Constituents. All of the aerial parts of fennel are rich in essential oil, with bitter fennel fruit containing 2–6%, mostly *trans*-anethole (> 60% of the oil)



(–)-Carvone

Fig. 20.12



Fenchone Anethole

Fig. 20.13

and fenchone (> 15%) (Fig. 20.13), and with not more than 10% estragole. Sweet fennel contains 1.5–3% essential oil, composed of *trans*-anethole (80–90%) but with very little fenchone (< 1%) and less than 5% estragole. Fatty oil and protein are also found in fennel fruit.

Therapeutic uses and available evidence. Fennel is used empirically as a carminative, for indigestion and colic in children, and clinical data for fennel preparations show encouraging results. It is considered to be a very safe drug and is widely used as a health-food supplement as well as a spice. However, some safety concerns were raised based on the content of the potential carcinogenic compound found in the essential oil – estragole (methylchavicol; ca 5–10% of the total essential oil), but no clinical reports for toxicity for fennel have been recorded. Also, the clinical relevance of some studies using pure estragole at high doses has been disputed. Fennel oil (the distilled essential oil of both varieties of fennel) is used for the same indications and has been shown to be bacteriostatic.

Other fruit drugs used for these indications are anise (Anisi fructus) from *Pimpinella anisum* L., star anise (Anisi stellata fructus) from *Illicium verum* Hook. F. and coriander (Coriandri fructus) from *Coriandrum sativum* L. These agents are all in the Eur. Ph.

The oils of all of these fruits are the subject of monographs in the Eur. Ph. where quantification of compounds is achieved after separation by gas chromatography. Differentiation between the two types of fennel is possible using the TLC method described in the Eur. Ph. where bitter fennel shows an additional yellow zone after spraying with sulphuric acid.

LIVER DISEASE

Liver damage, cirrhosis and poisoning should only be treated under medical supervision. There is, however, a useful phytomedicine derived from the milk thistle, *Silybum marianum* (L.) Gaertn. (Asteraceae), in the form of an extract known as silymarin. Other medicinal plants as shown below, are widely used for liver disease, although with less clinical evidence in support. Medicinal plants used for 'biliousness' (see section on Dyspepsia and Biliousness) are also used in mild liver disease.

Andrographis, *Andrographis paniculata* (Burm.f.) Nees (Andrographis herba) EP

Andrographis is widely used in many Asian systems of medicine to treat jaundice and liver disorders. There are few clinical studies available to support these uses, although numerous *in vitro* experiments have shown it has liver-protective effects against a variety of hepatotoxins. 14-Deoxyandrographolide, a constituent, desensitizes hepatocytes to TNF-alpha-induced signalling of apoptosis (Roy et al 2010, Chua et al. 2014). It appears to be well-tolerated but caution should be exercised when given in conjunction with anti-thrombotic drugs. In the West, it is more often used as an immune stimulant (see Chapter 22, pp. 269–270, Respiratory System, for more detail, including constituents).

Berberis species and other berberine-containing drugs

Berberine (Fig. 20.14) is contained in *Berberis* species, for example *B. vulgaris* L., *B. aristata* DC, in Blood root, *Sanguinaria canadensis* L., Goldenseal, *Hydrastis canadensis* L., Gold Thread, *Coptis chinensis* Franch, and Greater Celandine, *Chelidonium majus* L.

Berberine has antibacterial and amoebicidal properties and is used either in the form of the pure compound or as a component of plant extracts, to treat dysentery and liver disease (Imanshahidi and Hosseinzadeh 2008). Care should be taken when given together with anticancer drugs and with ciclosporin, since theoretical drug interactions have been described for these combinations, and berberine is known to be a substrate of P-glycoprotein and to affect expression of cytochrome P(CYP) 450 enzymes 3A4 and others.

Milk Thistle, *Silybum marianum* (L.) Gaertn. (Silybi Marianae Fructus) EP

The seeds of the milk thistle, *Silybum marianum* (Asteraceae), yield a flavolignan fraction known as silymarin.

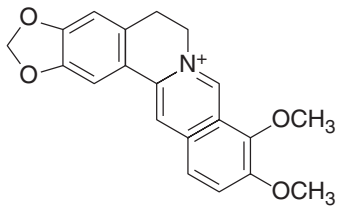
Constituents. The active constituents of the extract silymarin are flavolignans, mainly silybin (= silibinin), with isosilybin, dihydrosilybin, silydianin, silychristin and others.

Therapeutic uses and available evidence. In many parts of Europe, silymarin is used extensively for liver disease and jaundice. It has been shown to exert an antihepatotoxic effect in animals against a variety of poisons, particularly those of the death cap mushroom *Amanita phalloides*. This fungus contains some of the most potent liver toxins known (the amatoxins and the phallotoxins), both of which cause fatal haemorrhagic necrosis of the liver. Silymarin has been used at doses of 420 mg daily to treat patients with chronic hepatitis and cirrhosis; it is also partially active against hepatitis B virus, is hypolipidaemic and lowers fat deposits in the liver in animals. This extract can be used not only for serious liver disease, but also for general biliousness and other digestive disorders (see Saller et al 2008). The long-term administration of silymarin (in open studies) significantly increased survival time of patients with alcohol-induced liver cirrhosis and anti-inflammatory effects are a key mechanistic basis for these uses. An inhibitory effect of silymarin on the development of metastases has also been reported (both in liver and other cancers), offering opportunities for its use in adjuvant therapy (Féher and Lengyel 2012) even though there is practically no clinical evidence.

Schisandra, *Schisandra chinensis* (Turcz.) Baill. (Schisandrae chinensis fructus) EP

Schisandra (Schizandra) berries are the fruit of the Magnolia vine, and are very important in traditional Chinese medicine where they are used to treat liver disorders and many other conditions of general debility. Related species are also used.

Constituents. The active constituents are dibenzocyclooctene lignans, known as schisandrins (schizandrins) and gomisins. The nomenclature is confused and, for example, 'schisandrin' is sometimes referred to in the literature as 'schisandrol A', and 'gomisin A' as 'schisandrol B'.



Berberine

Fig. 20.14

Therapeutic uses and available evidence. A great deal of research has been carried out on the pharmacology of this plant (see review by Panossian and Wikman 2008), and liver protectant effects have been observed in animals, but clinical studies are lacking

Turmeric, *Curcuma longa* L. (Curcumae domesticae rhizoma) EP

Turmeric is used in Asian medicine to treat liver disorders as well as inflammatory conditions. For details regarding the drug and its constituents, see Chapter 27, p. 311 (Musculoskeletal system). Related species include Javanese turmeric (*Curcuma xanthorrhiza* Roxb., Eur. Ph.), which is mostly used for dyspepsia and other gastrointestinal problems. Turmeric and the curcuminoids are hepatoprotective against liver damage induced by various toxins, including paracetamol (acetaminophen), aflatoxin and cyclophosphamide; they protect against stomach ulcers in rats, and have antispasmodic effects. Turmeric is also hypoglycaemic in animals, and hypocholesterolaemic effects have been observed both in animal and human clinical studies, although clinical studies for liver disease are lacking. In addition, turmeric is antibacterial and antiprotozoal *in vitro*. The underlying mechanism seems to be linked to the modulation of numerous signalling cascades, these complex and, as it seems, sometimes non-specific effects pose a particular challenge in its further development as an herbal medical product of a botanical supplement. Turmeric is well tolerated but the bioavailability is poor and daily doses of at least 2 g are normally used (Epstein et al 2010, Gupta et al 2013, Rivera-Espinoza and Muriel 2009).

References

Ali, B.H., Blunden, G., Tanira, M.O., Nemmar, A., 2008. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. Food Chem. Toxicol. 46, 409–420.

Ali, T., Shakir, F., Morton, J., 2012. Curcumin and inflammatory bowel disease: biological mechanisms and clinical implication. Digestion 85 (4), 249–255.

Bundy, R., Walker, A.F., Middleton, R.W., Wallis, C., Simpson, H.C., 2008. Artichoke leaf extract *(Cynara scolymus)* reduces plasma cholesterol in otherwise healthy hypercholesterolemic adults: a randomized, double blind placebo controlled trial. Phytomedicine 15, 668–675.

Chua, L.S., 2014. Review on liver inflammation and antiinflammatory activity of Andrographis paniculata for hepatoprotection. Phytother. Res. 28 (11), 1589–1598.

EMA, 2013a. Assessment report on *Glycyrrhiza glabra* L. and/or *Glycyrrhiza inflata* Bat. and/or *Glycyrrhiza uralensis* Fisch., radix. Eur. Med. Agency. Available online at: http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_HMPC_assessment_report/2012/08/WC500131285.pdf.

EMA, 2013b. Community Herbal monograph on *Plantago ovata* Forssk., Seminis tegumentum. Eur. Med. Agency. Available online at: http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_Community_herbal_monograph/2013/07/WC500146508.pdf.

Epstein, J., Sanderson, I.R., Macdonald, T.T., 2010. Curcumin as a therapeutic agent: the evidence from in vitro, animal and human studies. Br. J. Nutr. 103, 1545–1557.

Gupta, S.C., Patchva, S., Aggarwal, B.B., 2013. Therapeutic roles of curcumin: lessons learned from clinical trials. AAPS Journal 15 (1), 195–218.

Féher, J., Lengyel, G., 2012. Silymarin in the prevention and treatment of liver diseases and primary liver cancer. Current Pharmaceutical Biotechnology 13 (1), 210–217.

Ford, A.C., Talley, N.J., Spiegel, B.M., et al., 2008. Effect of fibre, antispasmodics, and peppermint oil in the treatment of irritable bowel syndrome: systematic review and meta-analysis. BMJ 337, a2313.

Gordon, M., Naidoo, K., Akobeng, A.K., Thomas, A.G., 2012. Osmotic and stimulant laxatives for the management of childhood constipation. Cochrane. Database. Syst. Rev. 7, CD009118.

Imanshahidi, M., Hosseinzadeh, H., 2008. Pharmacological and therapeutic effects of Berberis vulgaris and its active constituent, berberine. Phytother. Res. 22, 999–1012.

Matthews, A., Dowswell, T., Haas, D.M., Doyle, M., O'Mathúna, D.P., 2010. Interventions for nausea and vomiting in early pregnancy. Cochrane Database Syst. Rev. 8, CD007575.

McKay, D.L., Blumberg, J.B., 2006a. A review of the bioactivity and potential health benefits of chamomile tea (*Matricaria recutita* L.). Phytother. Res. 20, 519–530.

McKay, D.L., Blumberg, J.B., 2006b. A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). Phytother. Res. 20, 619–633.

Miles, C.L., Fellowes, D., Goodman, M.L., Wilkinson, S., 2006. Laxatives for the management of constipation in palliative care patients. Cochrane Database Syst. Rev. (1), CD003448.

Ng, S.C., Lam, Y.T., Tsoi, K.K.F., et al., 2013. Systematic review: the efficacy of herbal therapy in inflammatory bowel disease. Aliment. Pharmacol. Ther. 38 (8), 854–863.

Obolskiy, D., Pischel, I., Feistel, B., Glotov, N., Heinrich, M., 2011. *Artemisia dracunculus* L. (tarragon): a critical review of its traditional use, chemical composition, pharmacology and safety. J. Agric. Food. Chem. 59, 11367–11384.

Palumbo, E.A., 2006. Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. Phytother. Res. 20, 717–724.

Panossian, A., Wikman, G., 2008. Pharmacology of *Schisandra chinensis* Bail.: an overview of Russian research and uses in medicine. J. Ethnopharmacol. 118, 183–212.

Raveendra, K.R., Jayachandra Srinivasa, V., et al., 2012. An extract of *Glycyrrhiza glabra* (gutgard) alleviates symptoms of functional dyspepsia: a randomized, double-blind, placebo-controlled study. Evid. Based Complement. Altern. Med. 2012, 216970.

Ravikoff Allegretti, J., Courtwright, A., Lucci, M., Korzenik, J.R., Levine, J., 2013. Marijuana use patterns among patients with inflammatory bowel disease. Inflamm. Bowel Dis. 19 (13), 2809–2814.

Rivera-Espinoza, Y., Muriel, P., 2009. Pharmacological actions of curcumin in liver diseases or damage. Liver Int. 29, 1457–1466.

Roy, D.N., Mandal, S., Sen, G., Mukhopadhyay, S., Biswas, T., 2010. 14-Deoxyandrographolide desensitizes hepatocytes to tumour necrosis factor-alpha-induced apoptosis through calcium-dependent tumour necrosis factor receptor superfamily member 1A release via the NO/cGMP pathway. Br. J. Pharmacol. 160, 1823–1843.

Saller, R., Brignoli, R., Melzer, J., Meier, R., 2008. An updated systematic review with meta-analysis for the clinical evidence of silymarin. Forsch. Komplement. Med. 15, 9–20.

Sannia, A., 2010. Phytotherapy with a mixture of dry extracts with hepato-protective effects containing artichoke leaves in the management of functional dyspepsia symptoms. Minerva Gastroenterol. Dietol. 56 (2), 93–99.

Walker, A.F., Middleton, R.W., Petrowicz, O., 2001. Artichoke leaf extract reduces symptoms of irritable bowel syndrome in a post-marketing surveillance study. Phytother. Res. 15, 58.

Wider, B., Pittler, M.H., Thompson-Coon, J., Ernst, E., 2013. Artichoke leaf extract for treating hypercholesterolaemia. Cochrane Database Syst. Rev. 3, CD003335.

Chapter 21

The cardiovascular system

Cardiovascular (CV) disorders are responsible for many years of ill health and multiple deaths around the Western world, and are mostly a consequence of lifestyle and diet as well as being linked to some extent to genetic predisposition. Serious conditions such as heart failure may be treated only under the guidance of a qualified physician, but some minor forms of CV disease respond well to changes in diet and taking more exercise, as well as phytotherapy. Cardiology has benefited greatly from the introduction of some newer semi-synthetic drugs based on natural products, including aspirin, an antiplatelet agent derived from salicin, and warfarin, an anticoagulant derived from dicoumarol. Others have been developed using a natural product as a template. For example, verapamil, a calcium channel antagonist used to treat hypertension and angina, is based on the opium alkaloid papaverine; nifedipine, a calcium channel antagonist, and amiodarone, an anti-arrhythmic, were both developed from khellin, the active constituent of khella [*Visnaga daucoides* Gaertn., syn.: *Ammi visnaga* (L.) Lam.]. Cocaine has cardioactive as well as central nervous system effects, and was the starting material for the development of the anti-arrhythmics procaine and lignocaine, which are more effective and without the unwanted stimulant activity (Hollmann 1992).

Cardiovascular conditions discussed here include heart problems, venous insufficiency, thrombosis and atherosclerosis. Other conditions such as hypertension and cardiac arrhythmias are rarely treated with phytomedicines, although there are some natural products used in their treatment that will be mentioned briefly. Synthetic diuretics are widely used as antihypertensives, but phytomedicines are not suitable for this purpose as they are not sufficiently potent to reduce blood pressure. Instead, they are often incorporated into remedies for urinary tract complaints (see Chapter 24) or to reduce bloating (mild water retention, for example pre-menstrual).

HEART FAILURE AND ARRHYTHMIAS

These conditions can be treated with cardiac glycosides (cardenolides and bufadienolides) such as digoxin, isolated from the foxglove. Lily of the valley (*Convallaria majalis* L.) contains convallotoxin (a mixture of cardenolides), and squill [*Drimia maritima* (L.) Stearn] contains the bufadienolides scillaren A and proscillaridin, but these are rarely used now. Ouabain, isolated from *Strophanthus* spp., has been used in emergency cases. The cardiac glycosides have a positive inotropic effect, meaning that they increase the force of the contractions of the heart. They are emetic and toxic in large doses and have a cumulative effect and are therefore unsuitable for use in the form of herbal extracts. Single isolated compounds for which the pharmacokinetics can be monitored are used instead.

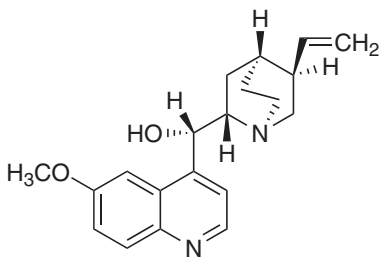
There are, however, other herbal drugs that have beneficial effects upon the heart, the most important of which are hawthorn (*Crataegus* spp.) and motherwort (*Leonurus cardiaca* L.). Hawthorn has anti-arrhythmic activity, and will be discussed briefly below. There is not enough evidence available at present to justify the inclusion of motherwort. In general, however, arrhythmias are treated with isolated compounds, most of which are synthetic, although quinidine (an alkaloid from *Cinchona* spp.; Fig. 21.1) is still used occasionally. Ajmaline, from *Rauvolfia* spp., is used as an antiarrhythmic in some parts of the world; sparteine, from broom [*Cytisus scoparius* (L.) Link], was formerly employed. Both of these compounds are alkaloids.

FOXGLOVE (DIGITALIS) LEAF, *DIGITALIS PURPUREA* L. (DIGITALIS PURPUREAE FOLIUM) EP

The very common purple foxglove (*Digitalis purpurea*) and its close relative the woolly foxglove (*D. lanata* Ehrh., Plantaginaceae), yield cardiac glycosides. They are indigenous to Europe and cultivated elsewhere. They do not have a long history of herbal use because of their toxicity, although in 1870, based on reports from a local herbalist in Shropshire, the famous surgeon William Withering described their use for 'dropsy' (an old term for congestive heart failure), and this was the first time an effective treatment for this condition had been found. The leaves are the source of the drug and are usually gathered in the second year of growth.

Constituents

Both species contain cardenolides, which are glycosides of the steroidal aglycones digitoxigenin, gitoxigenin and gitaloxigenin. There are very many cardiac glycosides, but the most important is digoxin (Fig. 21.2) and to a much lesser extent digitoxin, and the purpurea glycosides A and B. *Digitalis lanata* contains higher concentrations of glycosides, including digoxin and lanatosides, and is the main source of digoxin for the pharmaceutical industry.



Quinidine

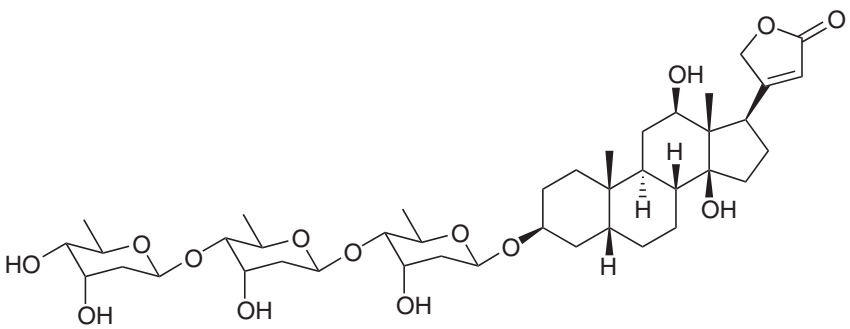
Fig. 21.1

Therapeutic uses and available evidence

Digoxin increases the force of myocardial contractility and reduces conductivity within the atrioventricular node. It is used primarily in the treatment of supraventricular tachycardia and heart failure and is given as a once-daily dosage in the range 62.5–250 micrograms. Digitalis glycosides increase the force of the contractions of the heart without increasing the oxygen consumption, and slow the heart rate when atrial fibrillation is present. Due to their cumulative effect, the glycosides can easily give rise to toxic symptoms, such as nausea, vomiting and anorexia, especially in the elderly, thus blood levels should be monitored.

HAWTHORN, *CRATAEGUS* SPP. (CRATAEGI FOLIUM CUM FLORE, CRATAEGI FRUCTUS) EP

Hawthorn (sometimes known as mayflower or whitethorn) is a common plant found in hedgerows and gardens throughout Europe and elsewhere. The flowers, leaves and berries of at least three species and their hybrids are used: mainly *C. laevigata* (Poir.) DC., *C monogyna* Jacq., (Rosaceae) as well as *C. rhipidophylla* Gand. (syn.: *C. oxyacantha* L.). These are hairless, thorny, deciduous shrubs with 3–5 lobed leaves, bearing white, dense clusters of flowers, followed by deep red fruits containing one seed (in *C. monogyna*) or two seeds (in *C. laevigata*). The flowers appear in early summer and the berries or 'haws' in early autumn. Jams and wines are often made from the fruit.



Digoxin

Fig. 21.2

Constituents

The main constituents of the leaf are flavonoids, including vitexin, vitexin-4-rhamnoside, quercetin and quercetin-3-galactoside, hyperoside, rutin, vicentin, orientin; the fruit contains flavonoids, procyanidins, catechins and epicatechin dimers, as well as phenolic acids such as chlorogenic and caffeic acids. Amines such as phenethylamine and its methoxy derivative, as well as dopamine, acetylcholine and tyramine, have also been isolated (Edwards et al 2012). It is thought that a mixture of active constituents may be necessary for the therapeutic effect. The drug prepared from hawthorn is often standardized to contain 4–30 mg of flavonoids, calculated as hyperoside, or 30–160 mg of procyanidins, calculated as epicatechin. In the Eur. Ph., the leaf and flower, and also the berries, are identified using a thin-layer chromatography (TLC) method with the hyperoside, rutin and chlorogenic acid as reference standards. The assays are different, however: whereas the leaf and flower use the flavonoid content, the berries are assayed for anthocyanin content.

Therapeutic uses and available evidence

Hawthorn is used as a cardiac tonic, hypotensive, coronary and peripheral vasodilator, anti-atherosclerotic and antiarrhythmic. Animal studies have shown beneficial effects on coronary blood flow, blood pressure and heart rate, as well as improved circulation to the extremities (Anonymous 2010). Hawthorn extract inhibits myocardial Na^+, K^+-ATPase and exerts a positive inotropic effect and relaxes the coronary artery. It blocks the repolarizing potassium current in the ventricular muscle and so prolongs the refractory period, thus exerting an anti-arrhythmic effect. It seems to protect heart muscle by regulating Akt and HIF-1 signaling pathways (Jayachandran et al 2010), and reducing oxidative stress (Bernatoniene et al 2009, Swaminathan et al 2010).

There are as yet few clinical trials of the drug, although a double-blind pilot study indicated a promising role for hawthorn extract in mild essential hypertension (Walker et al 2002). A large consistent positive outcome from clinical trials using hawthorn extracts for cardiovascular disease has been reported but there are inconsistencies in terms of the clinical criteria used (sample size, preparation, dosage, among others) (Koch and Malek 2011). Tolerance to exercise was not improved in patients with class II congestive heart failure (Zick et al 2009). It seems likely that hawthorn can be used as an adjunct or sole treatment only in milder cases of heart disease. The usual recommended dose of standardized extract (see Constituents, above) is 160–900 mg daily. Few side effects have been observed, and both patients and physicians rated the tolerance of the drug as good, although nausea and headache have been reported infrequently.

VENOUS INSUFFICIENCY AND CIRCULATORY DISORDERS

Improvements in circulatory disorders arise from a number of different pharmacological effects, particularly those involving anti-inflammatory and antioxidant activity. Plant drugs with these actions are important in the treatment of haemorrhoids, varicose veins, impaired visual acuity and even in memory enhancement, when blood flow to the brain may be affected. They usually contain saponins with anti-inflammatory activity, or anthocyanidins and other antioxidants. The most important are bilberry, butcher's broom, horse chestnut, ginkgo and garlic.

BILBERRY, *VACCINIUM MYRTILLUS* L. (MYRTILLI FRUCTUS) EP

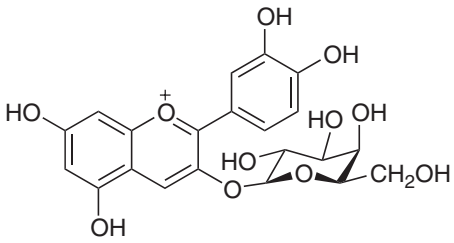
The bilberry, also known as the huckleberry or blueberry (*Vaccinium myrtillus*, Ericaceae), grows on acid soil in hilly and mountainous regions of Europe, Asia and North America. It is cultivated extensively for its delicious fruit, which ripen from July to September. The soft blue-black berries, about 0.5–1 cm in diameter, have a persistent calyx ring at the apex and contain numerous small oval seeds. Both the ripe fruit and the leaves are used medicinally. Some other members of the genus are used for similar indications.

Constituents

The fruit contains anthocyanosides (Fig. 21.3), mainly galactosides and glucosides of cyanidin, delphidin and malvidin, together with vitamin C and volatile flavour components, such as *trans*-2-hexenal and ethyl-2- and -3-methylbutyrates. Unlike other *Vaccinium* spp., bilberry does not contain arbutin or other hydroquinone derivatives. The anthocyanins can be estimated using their absorption at 528 nm, as described in the Eur. Ph.

Therapeutic uses and available evidence

The berries were traditionally used as an antidiabetic, and an astringent and antiseptic for diarrhoea.



Cyanidin-3-*O*-galactoside

Fig. 21.3

However, they are now more important as agents to improve blood circulation in conditions such as haemorrhoids, and especially vision disorders such as retinopathy caused by diabetes or hypertension, as well as for other forms of venous insufficiency. The anthocyanosides are mainly responsible for these effects, due to their antioxidant and free radical scavenging properties, particularly in the case of the ophthalmic and vascular systems. They also have a spasmolytic action on the gut. The anthocyanoside fraction has antiplatelet effects and inhibits some proteolytic enzymes. Extracts are also anti-inflammatory, antiulcer and anti-atherosclerotic and reduce fluid retention; many of these effects have been supported by clinical studies (e.g. Christie et al 2001), although the quality of the trials for improving vision has been criticized due to their methodology (Canter and Ernst 2004). The usual daily dose of a standardized anthocyanoside extract of bilberry is 480 mg, taken in divided doses. Few side effects have been observed, as would be expected of a widely consumed food substance.

In rats fed a high-fat and high-cholesterol diet blueberry extracts improved vascular reactivity and lowered blood pressure (Rodriguez-Mateos et al 2013).

BUTCHER'S BROOM, *RUSCUS ACULEATUS* L. (RUSCI RHIZOMA) EP

Butcher's broom (*Ruscus aculeatus*, Asparagaceae) is an evergreen shrub native to Europe, and is found in dry woods and among rocks. It is often cultivated for its tough, spiky twigs and 'leaves', which can be preserved and used for decoration. The true leaves are reduced to small scales and the stems flattened at the ends into oval 'cladodes' that resemble leaves, each bearing a small white flower in the centre, followed by a round scarlet berry, and ending in a sharp spine. The rhizome or whole plant can be used.

Constituents

Actives are saponin glycosides, including ruscine and ruscoside, aculeosides A and B, which are based on ruscogenin (1β-hydroxydiosgenin) and neoruscogenin.

Therapeutic uses and available evidence

Butcher's broom has anti-inflammatory effects and is used mainly for venous insufficiency, especially varicose veins and haemorrhoids. The ruscogenin derivatives have been shown to reduce vascular permeability and improve symptoms of retinopathy and lipid profiles of diabetic patients. The reduction of thrombin-induced hyperpermeability of endothelial cells *in vitro* was linked to several of the steroidal saponins, most importantly deglucoruscin, ruscin, and esculin (Barbič et al 2013).

The extract is either taken internally as a decoction or, more often, applied topically in the form of an ointment (or a suppository in the case of haemorrhoids). The saponins inhibit elastase activity *in vitro* and for this reason extracts are widely used in cosmetic preparations (Redman 2000). When applied topically, few side effects have been observed, apart from occasional irritation.

GINKGO, *GINKGO BILOBA* L. (GINKGO FOLIUM) EP

See Chapter 23 for more detail about the plant. *Ginkgo biloba* (Ginkgoaceae) can also be used in cases of peripheral arterial occlusive disease and other circulatory disorders. Although probably less potent than some synthetic drugs, it has the advantage of being well tolerated. Ginkgo improves blood circulation and can alleviate some of the symptoms of tinnitus, intermittent claudication and altitude sickness. Ginkgo extracts have complex effects on isolated blood vessels. The ginkgolides are specific platelet-activating factor (PAF) antagonists and inhibit effects produced by PAF, including platelet aggregation and cerebral ischaemia. The usual dose is 120–160 mg of extract daily.

HORSE CHESTNUT, *AESCULUS HIPPOCASTANUM* L. (HIPPOCASTANI SEMEN) EP

Aesculus hippocastaum (syn.: *Hippocastanum vulgare* Gaertn., Sapindaceae) is native to western Asia, but is now cultivated and naturalized in most temperate regions. It is a large tree, bearing large sticky leaf buds,

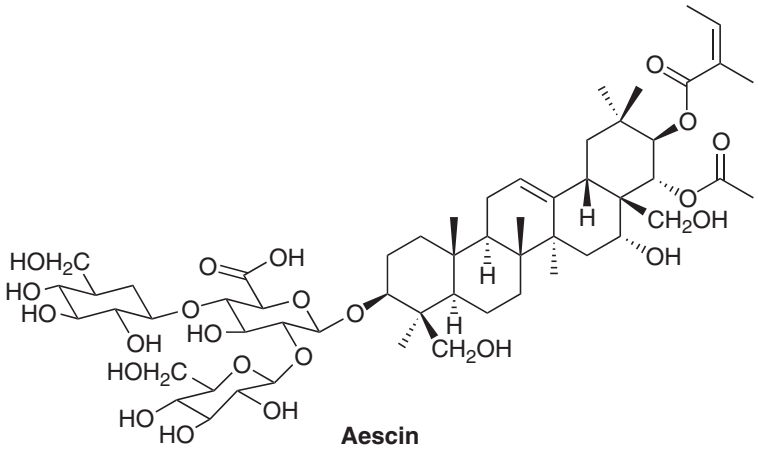


Fig. 21.4

which open in early spring. The leaves are composed of 5–7 large oval leaflets; the flowers have a candle-like appearance and are white or pink in colour. The bark is thick, rough, grey or brown on the external surface and pinkish-brown and finely striated on the inside. The fruits are spiny capsules, each with two to four compartments containing the well-known large shiny brown seeds or 'conkers'. The seeds, and occasionally the bark, are used.

Constituents

Both seeds and bark contain a complex mixture of saponins based on protoescigenin and barringtogenol-C, which is known as 'aescin' (or 'escin'), although this term refers more properly to the isomeric compound aescin (Fig. 21.4). More than 30 saponins have been identified, including α- and β-escin, together with escins Ia, Ib, IIa, IIb, IIIa, etc. Sterols and other triterpenes such as friedelin, taraxerol and spinasterol are present, as well as coumarins (e.g. esculin = aesculin) and fraxin, flavonoids and anthocyanidins.

Therapeutic uses and available evidence

Extracts of horse chestnut, or, more usually, extracts standardized to the aescin content, are used particularly for conditions involving chronic venous insufficiency (CVI), bruising and sports injuries. They can be taken internally or applied topically. In a Cochrane review Pittler and Ernst (2012) showed that oral horse chestnut seed extracts mono-preparation (standardized to 'aescin') and compared with placebo and reference treatment was safe and efficacious for treating patients with CVI-related signs and symptoms [leg pain, pruritus (itching), oedema (swelling) as well as leg volume and leg circumference at ankle and calf]. It is also recommended for preventing thrombosis during long flights and was found to be beneficial in the treatment of cerebral oedema following road accidents. Venotonic effects, and an improvement in capillary resistance, have also been noted in healthy volunteers (Suter et al 2006). Aescin has been shown to reduce oedema, decrease capillary permeability and increase venous tone, and horse chestnut extract to contract both veins and arteries *in vitro*, with veins being the more sensitive. The extract also significantly reduced ADP-induced human platelet aggregation, and these effects appear to be at least partly mediated through 5-HT(2A) receptors (Felixsson et al 2010). Horse chestnut extract also antagonizes some of the effects of bradykinin and produces an increase in plasma levels of adrenocorticotrophin, corticosterone and glucose in animals. Escin is widely used in cosmetics. The usual dose is 600 mg of extract daily, which corresponds to about 100 mg of escin. Extracts are well tolerated at therapeutic doses, but higher amounts can cause gastrointestinal upset with internal use, and occasional irritation with external application.

RED ROOT SAGE, *SALVIA MILTIORRHIZA* BUNGE, LAMIACEAE (SALVIAE MILTIORRHIZAE RADIX)

The root and rhizome of *Salvia miltiorrhiza* Bunge, also known as Danshen, are widely used in traditional Chinese medicine to treat many types of cardiovascular diseases, including ischaemic and circulatory disorders such as angina, after stroke, and atherosclerosis. The root has a characteristic bright red colour, which is due to the active constituents, the tanshinones.

Constituents

The main active constituents are diterpene quinones, known as tanshinones (one of the transliterations of *danshen* is *tan-shen*, hence the quinones were called tanshinones). Tanshinone I, tanshinone II, and cryptotanshinone are the major constituents, although nearly 40 variants of the basic tanshinone structures have been found in the roots. The total tanshinone content of the roots is about 1%, with tanshinone I and II and cryptotanshinone being the major components.

Therapeutic uses and available evidence

In recent years preclinical research on the species has flourished, but no clear therapeutic recommendations can be made. Animal studies have shown many relevant effects, such as protecting heart muscle from ischaemia and improving microcirculation. The isolated tanshinones have been shown to reduce fever and inflammation, inhibit platelet aggregation, dilate the blood vessels, and aid urinary excretion of toxins. *Salvia miltiorrhiza* has a mild vasodilatory effect, but does not increase cardiac output. Clinical studies carried out in China have shown benefit to patients with heart and circulatory diseases, including ischaemic stroke and acute myocardial infarction, but many of these do not fulfil the criteria required for acceptance in the West, and the efficacy has therefore not been accepted there (Wu et al 2007, 2008, Yu et al 2009). In general, *Salvia miltiorrhiza* seems to be safe and well-tolerated at the usual dose of 2–6 g/day for the dried root, or equivalent in the form of an extract, but there is potential for drug interactions, especially with other cardiovascular drugs (Williamson et al 2009).

RED VINE LEAF, *VITIS VINIFERA* L. (VITIS VINIFERAE FOLIUM)

Certain varieties of grape vine (*Vitis vinifera* L., Vitaceae, a plant that needs no description) produce red leaves that are used in the treatment of CVI and, in particular, varicose veins. Unusually, however, the definition of the botanical drug not only covers the species and a certain plant part but also the use of a specific prominently coloured variety.

Constituents

Grape leaves contain a wide range of polyphenols including quercetin-3-*O*-beta-D-glucuronide and isoquercetrin (the main flavonoids), anthocyanins, oligomeric proanthocyanidins, catechin, epicatechin monomers and dimers, gallic acid and astilbine. The phytoalexin *trans*-resveratrol, another polyphenolic substance belonging to the stilbene group, can also be found in grape vine, along with organic acids, mainly malic and oxalic acid. The dried leaves of red vine should contain at least 4% of total polyphenols and 0.2% of anthocyanins.

Therapeutic uses and available evidence

Positive effects in terms of improving the symptoms of CVI (tired, heavy and swollen legs or pain and tension in the legs) and reducing leg oedema compared to placebo were shown for ethanolic and aqueous wine leaf extracts (EMA 2010). Red vine leaf extracts are also used to improve the microcirculation and aid wound healing (Wollina et al 2006). *In vitro* studies indicate that they have antioxidant and anti-inflammatory properties, and that they inhibit platelet aggregation and hyaluronidase, and reduce oedema, possibly by reducing capillary permeability. Preclinical *in vivo* experiments demonstrated anti-inflammatory and capillary wall thickening effects.

Red vine leaf extract can be applied topically and taken internally, and is well-tolerated, although minor gastrointestinal effects have been reported. Commercial products are usually standardized to 90% polyphenols and 5% astilbine, with a daily dose of 360 mg red vine leaf extract being the recommended internal dose.

ANTIPLATELET AND ANTI-ATHEROSCLEROTIC DRUGS

Thrombosis and atherosclerosis are the result of a sedentary lifestyle and high sugar and fat consumption. Their incidence is rising and the age at which patients show signs of these conditions is becoming younger. These conditions are closely related in that atherosclerosis predisposes to thrombus formation, and can result in peripheral arterial disease, myocardial infarction and stroke. As well as improving diet and taking regular exercise, preventative drugs can be taken, many of which are natural products of some kind. Antiplatelet drugs are used prophylactically to decrease platelet aggregation and inhibit thrombosis. The standard antiplatelet drug is aspirin, which is used in doses that are lower (75–300 mg daily) than for pain relief (300 mg to 1 g, up to four times daily).

Many items in our diets also have antiplatelet effects, for example, the flavonoids and anthocyanidins,

as well as garlic, which is used as a food supplement for this and other purposes (e.g. as an antimicrobial). Garlic also has anti-atherosclerotic effects and is part of the 'Mediterranean diet' or the 'French paradox', in which there is a generally lower incidence of heart disease despite a cuisine rich in cream and butter. Other elements of this diet include olive oil, which contains monounsaturated fatty acids, which also lower blood cholesterol levels, and red wine, which contains anthocyanidins (see bilberry, above). Ginkgo has antiplatelet activity and is used to improve peripheral blood circulation; this has a beneficial effect on memory and cognitive processes and will be discussed in more detail in Chapter 23. Generally, these medicines are very safe, but care should be taken if they are used in conjunction with anticoagulants or prior to surgery. High-fibre phytomedicines, also used as bulk laxatives, such as ispaghula and psyllium husk, lower plasma lipid levels and can be used as adjuncts to a low-fat diet. Oat bran (*Avena sativa* L.) and guar gum (*Cyamopsis tetragonolobus* (L.) Taub.; see also Chapter 25) lower cholesterol levels; these are thought to act by binding to cholesterol.

GARLIC, *ALLIUM SATIVUM* L. (ALLII SATIVI BULBUS) EP

The garlic bulb (*Allium sativum*, Amaryllidaceae) is composed of a number of small bulbs or 'cloves', covered with papery, creamy-white bracts. Garlic is cultivated worldwide and is used in many forms of cooking. The drug in commerce is the powder prepared from the cut, dried or freeze-dried bulb.

Constituents

Garlic contains a large number of sulphur compounds, which are responsible for the flavour and odour of garlic, as well as the medicinal effects. The main compound in the fresh plant is alliin, which on crushing undergoes enzymatic hydrolysis by alliinase to produce allicin (*S*-allyl-2-propenthiosulphinate; Fig. 21.5). This in turn forms a wide range of compounds, such as allylmethyltrisulphide, diallyldisulphide, ajoene and others, many of which are volatile.

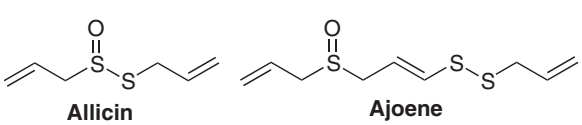


Fig. 21.5

Sulphur-containing peptides such as glutamyl-*S*-methylcysteine, glutamyl-*S*-methylcysteine sulphoxide and others are also present.

Therapeutic uses and available evidence

Different types of garlic preparations are available, such as quantified, allicin-rich extracts, aged garlic extracts (particularly in the Far East) and capsules containing the oil (older products). All have different compositions, but it is recognized that the sulphur-containing compounds must be present for the therapeutic effect. Deodorized products, except for those containing the precursor allicin, are ineffective. A wide range of clinical data has been reported, but the evidence remains mixed. In hypercholesterolemia the prolonged use (longer than 2 months) was effective in reducing total serum cholesterol (TC) and low-density lipoprotein (LDL-c) in a meta-analysis for patients with high cholesterol, reducing the risk of coronary problems and with minor side effects (Ried et al 2013). A Cochrane review on the use of garlic as monotherapy in patients diagnosed with hypertension found some blood pressure-lowering effect but insufficient evidence to support its use in lowering the risk of cardiovascular morbidity and mortality (Stabler et al 2012). In a systematic review no statistically significant effect on reducing some of the risk factors associated with atherosclerosis, such as peripheral arterial occlusive disease, was found (Jepson et al 2013), nor is there conclusive evidence for the prevention of pre-eclampsia and its complications in pregnant women and their babies (Meher and Duley 2010).

A large body of data exists using *in vivo* and *in vitro* pharmacological approaches. Hypolipidaemic activity has been observed in animals with garlic extract; this has been attributed to *S*-allylcysteine, which is regarded as important in this activity. *S*-Allylcysteine inhibits NF-κB synthesis and low-density lipoprotein (LDL) oxidation, which are both implicated in atherosclerosis. Allicin is also antioxidant, and garlic extracts protect endothelial cells from oxidized LDL damage. It is known that ajoene (see Fig. 21.5) is a potent antithrombotic agent, as well as 2-vinyl-4*H*-1,3-dithiin to a lesser extent. Cardiovascular benefits are supported by the antithrombotic activity, which has been shown in several studies, and an antiplatelet effect demonstrated by aged garlic extracts in humans.

Other health benefits attributed to garlic are antibacterial, antiviral and antifungal effects, and, more importantly, chemopreventative activity against carcinogenesis in various experimental models.

Diallylsulphide is thought to inhibit carcinogen activation via cytochrome P450-mediated oxidative metabolism, and epidemiological evidence suggests that a diet rich in garlic reduces the incidence of cancer. Hepatoprotection against paracetamol (acetaminophen)-induced liver damage has been described and attributed to similar mechanisms. The evidence for the health benefits of taking garlic is generally good despite the poor quality of some trials (Aviello et al 2009). The usual dose of garlic products is equivalent to 600–900 mg of garlic powder daily.

Garlic has few side effects, but due to the antiplatelet effects, care should be taken if given in combination with other cardiovascular drugs. Interaction with antiplatelet drugs, warfarin and related drugs increasing the risk of bleeding needs to be kept in mind for garlic supplements.

References

Anonymous, 2010. Alternative Medicine Review (Crataegus) 15 (2), 164–167.

Aviello, G., Abenavoli, L., Borrelli, F., et al., 2009. Garlic: empiricism or science? Natural Product Communications 4 (12), 1785–1796.

Barbič, M., Willer, E.A., Rothenhöfer, M., Heilmann, J., Fürst, R., Jürgenliemk, G., 2013. Spirostanol saponins and esculin from Rusci rhizoma reduce the thrombin-induced hyperpermeability of endothelial cells. Phytochemistry 90, 106–113.

Bernatoniene, J., Trumbeckaite, S., Majiene, D., et al., 2009. The effect of crataegus fruit extract and some of its flavonoids on mitochondrial oxidative phosphorylation in the heart. Phytotherapy Research 23 (12), 1701–1707.

Canter, P.H., Ernst, E., 2004. Anthocyanosides of vaccinium myrtillus (bilberry) for night vision – a systematic review of placebo-controlled trials. Survey of Ophthalmology 49 (1), 38–50.

Christie, S., Walker, A.F., Lewith, G.T., 2001. Flavonoids – a new direction for the treatment of fluid retention? Phytotherapy Research 15 (6), 467–475.

Edwards, J.E., Brown, P.N., Talent, N., Dickinson, T.A., Shipley, P.R., 2012. A review of the chemistry of the genus Crataegus. Phytochemistry 79, 5–26.

EMA, 2010. Assessment report on Vitis vinifera L., folium. European Medicines Agency. Available online at http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_HMPC_assessment_report/2011/01/WC500100390.pdf.

Felixsson, E., Persson, I.A., Eriksson, A.C., Persson, K., 2010. Horse chestnut extract contracts bovine vessels and affects human platelet aggregation through 5-HT(2A) receptors: an in vitro study. Phytotherapy Research 24 (9), 1297–1301.

Hollmann, A., 1992. Plants in Cardiology. BMJ Books, London.

Jayachandran, K.S., Khan, M., Selvendiran, K., Devaraj, S.N., Kuppusamy, P., 2010. Crataegus oxycantha extract attenuates apoptotic incidence in myocardial ischemia-reperfusion injury by regulating Akt and HIF-1 signaling pathways. Journal of Cardiovascular Pharmacology 56 (5), 526–531.

Jepson, R.G., Kleijnen, J., Leng, G.C., 2013. Garlic for peripheral arterial occlusive disease. Cochrane Database of Systematic Reviews 4, CD000095.

Koch, E., Malek, F.A., 2011. Standardized extracts from hawthorn leaves and flowers in the treatment of cardiovascular disorders – preclinical and clinical studies. Planta Medica 77 (11), 1123–1128.

Meher, S., Duley, L., 2010. Garlic for preventing pre-eclampsia and its complications. Cochrane Database of Systematic Reviews 3, CD006065.

Pittler, M.H., Ernst, E., 2012. Horse chestnut seed extract for chronic venous insufficiency. Cochrane Database of Systematic Reviews 11, CD003230.

Redman, D.A., 2000. Ruscus aculeatus (butcher's broom) as a potential treatment for orthostatic hypotension, with a case report. Journal of Alternative and Complementary Medicine 6 (6), 539.

Ried, K., Toben, C., Fakler, P., 2013. Effect of garlic on serum lipids: an updated meta-analysis. Nutrition Reviews 71 (5), 282–299.

Rodriguez-Mateos, A., Ishisaka, A., Mawatari, K., Vidal-Diez, A., Spencer, J.P., Terao, J., 2013. Blueberry intervention improves vascular reactivity and lowers blood pressure in high-fat-, high-cholesterol-fed rats. British Journal of Nutrition 109 (10), 1746–1754.

Stabler, S.N., Tejani, A.M., Huynh, F., Fowkes, C., 2012. Garlic for the prevention of cardiovascular morbidity and mortality in hypertensive patients. Cochrane Database Systematc Reviews 8, CD007653.

Suter, A., Bommer, S., Rechner, J., 2006. Treatment of patients with venous insufficiency with fresh plant horse chestnut seed extract: a review of 5 clinical studies. Advance in Therapy 23 (1), 179–190.

Swaminathan, J.K., Khan, M., Mohan, I.K., et al., 2010. Cardioprotective properties of Crataegus oxycantha extract against ischemia-reperfusion injury. Phytomedicine 17 (10), 744–752.

Walker, A., Marakis, G., Morris, A.P., Robinson, P.A., 2002. Promising

hypotensive effect of hawthorn extract: a randomized double-blind pilot study of mild, essential hypertension. Phytotherapy Research 16, 48–54.

Williamson, E.M., Driver, S., Baxter, K., 2009. Stockley's Herb–Drug Interactions. Pharmaceutical Press, UK.

Wollina, U., Abdel-Naser, M.B., Mani, R., 2006. A review of the microcirculation in skin in patients with chronic venous insufficiency: the problem and the evidence available for therapeutic options. International Journal of Lower Extremity Wounds 5 (3), 169–180.

Wu, B., Liu, M., Zhang, S., 2007. Dan Shen agents for acute ischaemic stroke. Cochrane Database Systematic Review 2, CD004295.

Wu, T., Ni, J., Wu, J., 2008. Danshen (Chinese medicinal herb) preparations for acute myocardial infarction. Cochrane Database Systematic Review 2, CD004465.

Yu, S., Zhong, B., Zheng, M., Xiao, F., Dong, Z., Zhang, H., 2009. The quality of randomized controlled trials on DanShen in the treatment of ischemic vascular disease. Journal of Alternative and Complementary Medicine 15 (5), 557–565.

Zick, S.M., Vautaw, B.M., Gillespie, B., Aaronson, K.D., 2009. Hawthorn Extract Randomized Blinded Chronic Heart Failure (HERB CHF) trial. European Journal of Heart Failure 11 (10), 990–999.

Chapter 22

The respiratory system

Minor common disorders of the respiratory system can generally be treated successfully using phytotherapeutic preparations and it may be helpful as a supportive measure in more serious diseases, such as bronchitis, emphysema and pneumonia. For severe infections, antibiotic therapy may be needed and, although most antibiotics are natural products, their study is a separate issue and will not be dealt with here.

Upper respiratory tract infection or the common cold is one of the leading reasons for visiting a healthcare professional and only some treatments are considered to be therapeutically beneficial (Fashner et al 2012). For colds and flu-like virus infections, decongestants (e.g. menthol and eucalyptus), broncholytics and expectorants (including ipecacuanha, thyme and senega), demulcents (e.g. mallow), antibacterials and antivirals (e.g. linden and elder flowers, pelargonium) and immune system modulators (e.g. echinacea, andrographis) are popular and effective. Traditionally, garlic and echinacea have been used for allergic and infective rhinitis. In children, a review only identified honey (buckwheat) and *Pelargonium sidoides* (geranium) extracts as potentially beneficial therapies using natural product-derived preparations, for adults *Andrographis paniculata, Echinacea purpurea* and *Pelargonium sidoides* (geranium) extracts were considered to be of potential therapeutic benefit (Fashner et al 2012) and it is now generally recommended not to use antibiotics as a first-line treatment.

Asthma is becoming more prevalent for reasons as yet unknown, but is best treated aggressively with inhaled steroids and bronchodilators. Many bronchodilators are either of natural origin (e.g. theophylline and ephedrine) or have been developed from natural products. Although isolated ephedrine and pseudoephedrine are theoretically contraindicated in asthma because they can precipitate an attack, ephedra herb has a long history of use without apparent ill-effects; this is attributed to other constituents in the whole extract. A range of herbal extracts are used, generally as an adjuvant therapy. Also, omega-3 (n–3) polyunsaturated fatty acids (n–3 PUFAs) have well documented anti-inflammatory properties, but for conditions such as asthma, the evidence is limited. Overall, therapeutic benefits of most treatments are limited (Fashner et al 2012)

Antimuscarinic drugs (e.g. atropine), which have bronchodilator effects and also dry up secretions, have largely been superseded by derivatives such as ipratropium. An important compound, sodium cromoglycate, is an anti-allergic drug developed from khellin, which stabilizes mast cells and is used in the form of an inhaler to treat asthma. Leukotriene antagonists are used for asthma therapy and, although no plant products are yet in use, there are several natural products (e.g. quercetin) with this property and they may become available in the future. Cough suppressants are very popular and available over the counter (OTC), although there is no clinical evidence for their effectiveness (Smith et al 2014). The most important antitussives are codeine and other opiate derivatives obtained from the opium poppy.

Butterbur, *Petasites hybdridus* (L.) G.Gaertn., B.Mey. and Scherb., has often been recommended for allergic, respiratory problems and there is good scientific evidence to support its use for the prevention of allergic rhinitis (see below), but the drug cannot be recommended due to safety concerns. This is due to the presence of unsaturated pyrrolizidine alkaloids (PAs) in the species, which are known to be hepatotoxic. Even though commercial preparations are on the market that have the PAs removed, the use of such preparations

cannot be recommended. Similarly, ephedra and its constituents should not be used as a food supplement or for self-medication.

BRONCHODILATORS AND DECONGESTANTS

SYSTEMIC DRUGS

Ephedra, *Ephedra* spp. (Ephedrae Herba) EP

Ephedra, also known as Ma Huang (*Ephedra sinica* Stapf and other species of the Ephedraceae) is an ancient Chinese medicine, which is now used worldwide. It was the original source of ephedrine, a useful decongestant and bronchodilator. Traditionally, it is used to treat asthma and nasal congestion, in the form of nasal drops. Pseudoephedrine is now used more widely for respiratory congestion as it has fewer central nervous system (CNS) stimulatory properties. The plant has slender green stems, which are jointed in branches of about 20 tufts about 15 cm long, and terminate in a sharp, recurved point. These are the medicinally used part. The leaves are reduced to sheaths surrounding the stems.

Constituents. Alkaloids, up to about 3%, but widely varying; the major alkaloid is (–)-ephedrine (Fig. 22.1), together with many others. These include (+)-pseudoephedrine, norephedrine, norpseudoephedrine, ephedroxane, *N*-methylephedrine, maokonine, transtorine and the ephedradines A–D. Other components are catechin derivatives, and diterpenes, including ephedrannin A and mahuannin A, have been isolated from other species of *Ephedra*.

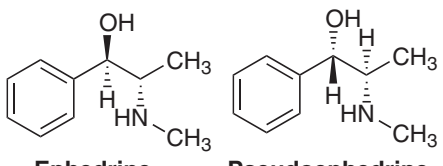
Therapeutic uses and available evidence. *Ephedra* has been used since ancient times in China for asthma and hay fever, as a bronchodilator, sympathomimetic and CNS and cardiac stimulant. Herbalists also use it to treat enuresis, allergies, narcolepsy and other disorders, and anti-inflammatory activity has been observed in extracts. Ephedrine is used in the form of elixirs and nasal drops, and has an additional use as a heart rate accelerator in the treatment of some types of bradycardia. Pseudoephedrine, the D-isomer of ephedrine produced by chemical synthesis, is usually the compound of choice for isolated alkaloid preparations. Ephedra herb, ephedrine and pseudoephedrine are all the subject of European Pharmacopoeia (Eur. Ph.) monographs. Ephedra herb is used as an anti-allergic agent; this is supported by evidence that it induces immunoglobulin A in Peyer's patches and blocks complement activation by both the classical and alternative pathways.

Ephedra and preparations containing ephedra alkaloids are of significant safety concern (see next paragraph) and should not be used as food supplements (EFSA 2013) or in self-treatment.

Toxicological risks. The botanical drug has been abused as a slimming aid, and as an ergogenic aid in sports and athletics, but this is dangerous (Fleming 2008). For example, hypertension and other cardiovascular events, and a case of exacerbation of hepatitis, have been noted with high doses. The absorption of ephedrine and pseudoephedrine is slower after ingestion of the botanical drug than for isolated alkaloid preparations, and the other constituents, the ephedradins, mahuannins and maokonine, are mildly hypotensive; but both the drug and the isolated alkaloids should be avoided by hypertensive patients as well as in cases of thyrotoxicosis, narrow-angle glaucoma and urinary retention. Therapeutic doses of the drug are calculated to deliver up to 30 mg of the alkaloids, calculated as ephedrine. There is a lack of sufficient toxicological data for hazard characterization (EFSA 2013).

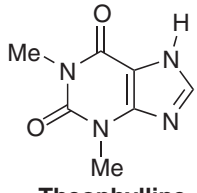
Theophylline EP

Although a natural xanthine, theophylline (Fig. 22.2), which is found in cocoa (*Theobroma cacao* L.), coffee (*Coffea* spp.) and tea (*Camellia sinensis* (L.) Kuntze), is almost invariably used as the isolated compound. It is indicated in reversible airways obstruction, particularly in acute asthma. Because of the narrow margin between the therapeutic and the toxic dose, and the fact



Ephedrine **Pseudoephedrine**

Fig. 22.1



Theophylline

Fig. 22.2

that the half-life is highly variable between patients, especially smokers and in heart failure or with concurrent administration of other drugs, care must be taken. The usual dose is 125–250 mg in adults, three times daily, and half of that in children.

Side effects include tachycardia and palpitations, nausea and other gastrointestinal upsets. These can be reduced using sustained-release preparations, and this is the usual form of theophylline products.

INHALATIONS

Essential-oil-containing drugs are often used with aromatic compounds (especially camphor) as chest rubs, steam inhalations or nasal sprays, for their decongestant properties. They are particularly useful for infants, children, asthmatics and pregnant women for whom systemic decongestants may not be appropriate. They may also be used orally, in pastilles, lozenges, or 'cough sweets'. Oils distilled from the aerial parts of members of the pine family [e.g. the common Pumilio (Alpine) pine (*Pinus mugo* Turra), the European larch (*Larix decidua* Mill.) and the fir tree (*Abies* spp.)] and the Australian Myrtaceae (e.g. eucalyptus and tea-tree oil) are used frequently. These oils can also be used in steam baths.

Camphor EP

Camphor (Fig. 22.3), a pure natural product, is derived from the Asian camphor tree (*Cinnamomum camphora* (L.) J.Presl, Lauraceae). It is often combined with the essential-oil-containing drugs as an aromatic stimulant and decongestant.

Camphor has antiseptic, secretolytic and decongestant effects. Small doses were formerly taken internally for colds, diarrhoea and other complaints, but it is now used only externally.

Toxicological risks. Camphor has been in use for many years; however, in 2011 'camphorated oil' was taken off the market since, in large quantities, camphor may be absorbed through the skin causing systemic toxicity. Overdose causes vomiting, convulsions and palpitations, and can be fatal. However, when used externally in therapeutic doses it is generally well tolerated.

Eucalyptus oil, *Eucalyptus* spp. (Eucalypti aetheroleum) EP

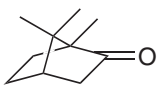
The blue gum tree, *Eucalyptus globulus* Labill., and other species (Myrtaceae) yield a highly characteristic oil that is widely used as a decongestant and solvent. The leaves are scimitar-shaped, 10–15 cm long and about 3 cm wide, short-stalked and rounded at the base, with numerous transparent oil glands.

Constituents. The oil contains 1,8-cineole (eucalyptol; see Fig. 22.4) as the major component, with terpineol, α-pinene, *p*-cymene and small amounts of ledol, aromadendrene and viridoflorol, aldehydes, ketones and alcohols.

Therapeutic uses and available evidence. The oil is antiseptic, antispasmodic, expectorant, stimulant and insect repellent. It is a traditional Australian Aboriginal remedy for coughs, colds and bronchitis. It may be taken internally in small doses (0.05–0.2 ml), as an ingredient of cough mixtures, sweets and pastilles, or as an inhalation; it is applied externally in the form of a liniment, ointment or 'vapour rub'. The leaf extract and oil have well-defined antiseptic effects against a variety of bacteria and yeasts. The oil is also insect-repellent and larvicidal, and is used in pharmaceutical products for these properties as well as for its antiseptic and flavouring properties in dentifrices and cosmetics. It is widely used in Menthol and Eucalyptus Inhalation BP for steam inhalation as a decongestant. Eucalyptus oil is irritant and, although safe as an inhalation, caution should be exercised when taken internally as fatalities have been reported (Sadlon and Lamson, 2010).

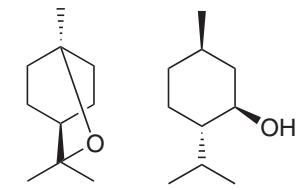
Menthol EP

Menthol is a monoterpene (Fig. 22.4) extracted from mint oils, *Mentha* spp. (especially *M. arvensis* L.) or it can be made synthetically. Whole peppermint oil is used in herbal combinations to treat colds and



Camphor

Fig. 22.3



1,8-Cineole (–)-Menthol

Fig. 22.4

influenza (as well as for colic, etc.; see Chapter 20, pp. 245–246), but isolated menthol is an effective decongestant used in nasal sprays and inhalers. Menthol can be irritant and toxic in overdose, but is generally well tolerated in normal usage.

ANTI-ALLERGICS

Most antihistamines are synthetic in origin and, although many flavonoids have anti-allergic properties, they are nowhere near as potent as, for example, cetirizine, desloratidine, fexofenadine or chlorpheniramine. An extract of butterbur (see below) was found to be equivalent in activity to cetirizine. However, its use is not recommended due to toxicity concerns (see below). Smooth muscle relaxant drugs have been used widely in asthma, and one of these, khellin (used particularly in the Mediterranean region, and isolated from *Ammi visnaga*), was investigated as a lead compound for development. One derivative, sodium cromoglycate, was discovered to have anti-allergic effects (see below).

Butterbur, *Petasites hybdridus* (L.) G.Gaertn., B.Mey. and Scherb

Petasites hybridus (syn. *P. vulgaris, Tussilago petasites*, Compositae) is a downy perennial, common in damp places throughout Europe, with very large heart-shaped leaves and lilac-pink brush-like flowers that occur in early spring before the leaves appear. The root and herb are used.

Constituents. Butterbur contains sesquiterpene lactones (eremophinolides), including a series of petasins and isopetasins, neopetasin, petasalbin, furanopetasin, petasinolides A and B, and flavonoids including isoquercetin glycosides. All parts of the plant contain unsaturated, toxic pyrrolizidine alkaloids (PAs - senecionine, integerrimine, senkirkine, petasitine and neopetasitine), usually with higher concentrations in the root.

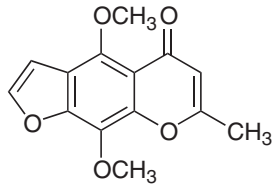
Therapeutic uses and available evidence. Butterbur is traditionally used as a remedy for asthma, colds, headaches and urinary tract disorders. It is used as an antihistamine for seasonal allergic rhinitis, and a randomized, double-blind comparative study using 125 patients over 2 weeks of treatment showed that butterbur extract is as potent as cetirizine. The anti-inflammatory activity is due mainly to the petasin content. Extracts inhibit leukotriene synthesis and are spasmolytic, and reduce allergic airway inflammation and AHR by inhibiting the production of the Th2 cytokines IL-4 and IL-5, and RANTES (Brattström et al 2010), thus supporting its use in asthma. Use as prophylactic treatment for migraine has also been suggested but further evidence of efficacy is needed (Agosti et al 2006). The usual dose is an extract equivalent to 5–7 g of herb or root. In general, the drug cannot be recommended due to safety concerns linked to the PAs, which are known to be hepatotoxic (NTP 2009). Maximum intake of the alkaloids should be less than 1 μg daily for fewer than 6 weeks per year. Even though commercial preparations are on the market that have the PAs removed, the use of such preparations cannot be recommended.

Khella, *Visnaga daucoides* Gaertn. [syn.: *Ammi visnaga* (L.) Lam.]

Also known as the 'toothpick plant', as the woody pedicels can be used for this purpose, khella (Apiaceae) is an herbaceous annual reaching 1.5 m in height, with divided filiform leaves and typically umbelliferous flowers. The botanical drug is derived from the fruits, which are very small, broadly ovoid and usually found as separate greyish-brown merocarps. The drug has a long history of use in the Middle East, especially Egypt, as an antispasmodic in renal colic, for asthma and as a coronary vasodilator for angina.

Constituents. Key active principles are furanocoumarins, the most important being khellin (Fig. 22.5), together with visnagin, visnadin and khellol glucoside.

Therapeutic uses and available evidence. Khellin, visnadin and visnagin are vasodilators, with calcium-channel-blocking and spasmolytic activity. Khellin was the starting material for the development of several important semi-synthetic derivatives such as sodium cromoglycate, which is widely used as a prophylactic treatment for asthma, hay fever and other allergic conditions, often in the form of an inhaler or eyedrops. It was also the basis for the development of nifedipine (a calcium channel antagonist and vasodilator) used in heart disease, and amiodarone, a cardiac antiarrhythmic.



Khellin

Fig. 22.5

EXPECTORANTS AND MUCOLYTICS

The purpose of these drugs is to reduce the viscosity of mucus in the respiratory tract to enable expectoration of phlegm in cases of chest and throat infection. Frequently, essential oils are used with expectorant aromatic compounds such as camphor. Many expectorants are included in cough mixtures and, although efficacy is difficult to demonstrate, these products are very popular with patients in the absence of other treatments. All of the expectorants are used for coughs and colds, bronchitis and sinusitis, usually in conjunction with other decongestants, demulcents, analgesics and, occasionally, antibiotics. Some of these drugs contain essential oils and salicylates (e.g. poplar buds, thyme), and may also include the decongestants mentioned above (eucalyptus, menthol); others contain saponins (e.g. senega, ivy).

Balm of Gilead (poplar buds), *Populus* spp.

Poplar buds (from various *Populus* spp., including *P.* × *candicans* Aiton (syn.: *P.* x *gileadensis* Rouleau), *P. balsamifera* L. and *P. nigra* L., Salicaceae) are collected in the spring before they open. The species are cultivated in Europe (*P.* × *candicans* and *P. nigra*) and North America (*P.* × *candicans* and *P. balsamifera*). The buds of all species are similar, being about 2 cm long and 0.5 cm wide, with narrow, brown, overlapping scales; the inner scales are sticky and resinous. The bark of these species is also used.

Constituents. All species contain the phenolic glycosides salicin (salicyl alcohol glucoside), populin (benzoyl salicin) and a volatile oil containing α-caryophyllene, with cineole, bisabolene and farnesene. Flavonoids (pinocembrin and pinobanksin) and, in *P. nigra* at least, lignans, based on isolariciresinol, have been isolated.

Therapeutic uses and available evidence. Balm of Gilead is an expectorant, stimulant, antipyretic and analgesic. It is a common ingredient of herbal cough mixtures, and also ointments used for rheumatic and other muscular pains. The phenolic glycosides (e.g. salicin) and the volatile oil constituents have antiseptic and expectorant activity. Little evidence is available for efficacy, but the drug has a long history of traditional use. The bark of poplar species is used in a similar way to willow bark, as an antirheumatic.

Balm of Gilead is generally non-toxic, except for patients who are allergic to salicylates. If excessive amounts of these drugs are taken, adverse effects such as stomach upset and tinnitus are possible, due to the salicylate content.

Thyme and wild thyme, *Thymus vulgaris* L. and *Thymus serpyllum* L. (Thymi herba and Serpylli herba) EP

Thymus vulgaris (known as garden or common thyme) and wild thyme (*T. serpyllum*, mother of thyme or serpyllum, Lamiaceae) are indigenous to Europe, especially the Mediterranean region, and are cultivated extensively. They are small, bushy shrubs with small, elliptical, greenish-grey, shortly stalked leaves. Those of thyme are up to about 6 mm long and 0.5–2 mm broad, with entire recurved margins. The leaves of wild thyme are a little broader and the margins are not recurved; it has leaves with long trichomes at the base. Microscopically, the two botanical drugs are similar; both having the characteristic Lamiaceous glandular trichomes; the rather subtle differences are described in the Eur. Ph. Both have a characteristic odour of thymol and are used as culinary herbs.

Constituents. The active principle is the volatile oil, which has the major constituent thymol, with lesser amounts of carvacrol, 1,8-cineole, borneol, thymol methyl ether and α-pinene. However, the flavonoids (apigenin, luteolin, thymonin, etc.) and the polyphenolic acids (labiatic, rosmarinic and caffeic) are expected to contribute to the anti-inflammatory and antimicrobial effects.

Therapeutic uses and available evidence. Thyme, and oil of thyme, are carminative, antiseptic, antitussive, expectorant and spasmolytic, and, as such, are used for coughs, bronchitis, sinusitis, whooping cough and similar respiratory complaints. Most of the activity is thought to be due to the thymol, which is expectorant and highly antiseptic. Thymol and carvacrol are spasmolytic and the flavonoid fraction has a potent effect on the smooth muscle of guinea pig trachea and ileum. Thymol (see Fig. 30.1) is a popular ingredient of mouthwashes and dentifrices because of its antiseptic and deodorant properties. The oil may be taken internally in small doses of up to 0.3 ml, unless for use in a mouthwash, which is not intended to be swallowed in significant amounts.

Thymol is irritant, and toxic in overdose, and should be used with care.

Senega, *Polygala senega* L. (Polygalae radix) EP

Senega (snake root, rattlesnake root, *Polygala senega* L., Polygalaceae) is native to the USA. In Chinese

medicine, senega may also refer to *P. tenuifolia* Willd.; both species are used for similar purposes. The root is light yellowish-grey with a knotty crown, from which slender stems arise, bearing the remains of rudimentary leaves and buds at the base.

Constituents. The active constituents are triterpenoid saponins, the mixture generally known as 'senegin'. These are based on the aglycones presenegenin, senegenin, hydroxysenegin, polygalacic acid and senegnic acid, including the E- and Z-senegins II, III and IV, E- and Z-senegasaponins a, b and c, and others.

Therapeutic uses and available evidence. Senega is used primarily for chronic bronchitis, catarrh, asthma and croup. The saponins are the active constituents, as with other mucolytic plant drugs. Senega is usually taken orally as an infusion. The saponins also have immunopotentiating activity to protein and viral antigens, and exhibit less toxicity than quillaia saponins. They are anti-inflammatory and antiseptic. Senega extracts, the senegasaponins and the senegins are hypoglycaemic in rodents, the senegasaponins are inhibitors of alcohol absorption, and the senegins also have anticancer and anti-angiogenic effects *in vitro* (Arai et al 2011). The dose is usually equivalent to 0.5–1 g of the powdered root.

The saponins are irritant and haemolytic, but taken orally do not appear to pose many problems. Nausea and vomiting are the most common side effects and, in view of the other pharmacological actions, care should be taken with senega when given in high doses or to sensitive individuals.

Ivy, *Hedera helix* L. (Hederae folium) EP

Ivy is a saponin-containing expectorant. It is a common European plant, found also in northern and eastern Asia and introduced into America. *Hedera helix* L. (Araliaceae) has dark green leathery leaves, shiny, with 3–4 triangular lobes. The berries are small, purplish-black and globular, with the calyx ring visible at the apex. Both leaves and berries may be used as part of phytotherapeutic preparations. The berries are somewhat toxic if consumed.

Constituents. The actives are saponins based on oleanolic acid, bayogenin and hederagenin, including the hederosaponins (or hederacosides) B, C and D, and α- and β-hederin, the polyyne falcarinol, and also flavonoids.

Therapeutic uses and available evidence. Ivy extracts are used in preparations for bronchitis and catarrh, as an expectorant. The saponins and sapogenins are the main active ingredients; they are expectorant and antifungal. Few clinical studies have been carried out, and further work is needed (Holzinger and Chenot 2011). One study showed that after 7 days of therapy with dried ivy leaf extract, 95% of patients showed improvement or healing of their symptoms, and it was safe and well-tolerated: the overall incidence of adverse events was 2.1%, mainly gastrointestinal disorders (Fazio et al 2009).

Both the saponin and the flavonoid fractions have spasmolytic effects. Overall, there is some evidence specifically linked to a special extract (Lang et al 2015), which is the best-investigated ivy-based preparation.

A specific mode of action relevant for respiratory conditions of the saponins has been postulated: hederacoside C (which is converted into α-hederin by esterases) as well as its aglucone hederagenin, acts on G protein-linked β_2-adrenergic receptors of epithelial lung cells, resulting ultimately in an indirect β_2-sympathomimetic effect (Hegener 2004). Ivy extracts are often used in cosmetic preparations to treat cellulite, with some success. Ivy saponins are being widely investigated for their antileishmanial, molluscicidal, antimutagenic, antithrombin and anticlastogenic effects. The usual therapeutic dose as an expectorant is 0.3 g of crude drug, or equivalent.

Like all saponin-containing drugs, ivy can be irritant and allergenic. These effects are also due at least in part to the falcarinol content.

Tolu balsam, *Myroxylon balsamum* (L.) Harms (Balsamum tolutanum) EP

The resin, which is collected from incisions in the bark and sapwood of *Myroxylon balsamum* (Fabaceae), is a light brown, fragrant, balsamic resin, softening when warm and becoming brittle when cold. It has a pleasant, sweetish, aromatic, vanilla-like odour.

Constituents. The main constituents of the balsam are cinnamic and benzoic acids, their esters such as benzyl benzoate and cinnamyl cinnamate, and esters with resin alcohols, including coniferyl and hydroconiferyl benzoates.

Therapeutic uses and available evidence. Balsam of tolu is expectorant, stimulant and antiseptic. It is used in cough mixtures and pastilles, and as a lozenge base. Although there is no modern clinical evidence, many balsams are used for similar purposes and it is generally agreed to have a useful therapeutic role as expectorants, antiseptics and demulcents. Balsam of tolu is an ingredient in Friar's balsam, which is used

as a steam inhalation and also as a protectant in skin formulations. The antimicrobial activity is due to the benzyl benzoate and benzyl cinnamate content.

Tolu balsam, like many other balsam resins, can cause allergic reactions.

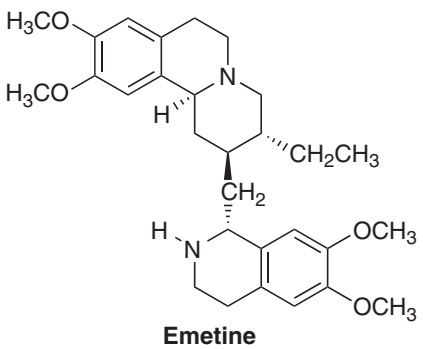
Ipecacuanha, *Carapichea ipecacuanha* (Brot.) L.Andersson (Ipecacuanhae radix) EP

'Ipecac' is obtained from the root and rhizome of *Carapichea ipecacuanha* (better known under its synonyms *Cephaelis ipecacuanha* A.Rich and also known as *Psychotria ipecacuanha* Stokes as well as *C. acuminata* Karsten; Rubiaceae). Rio, Matto Grosso and Brazilian ipecac were described as *C. ipecacuanha* and Cartagena, Nicaragua or Panama ipecac, *C. acuminata*. Thus, while they were previously described as two species they are now considered to be one. *C. ipecacuanha* root is slender, twisted and reddish brown, up to about 4 mm in diameter, with a characteristic ringed appearance. *C. acuminata* is larger, with fewer annulations. The root can be identified microscopically by the characteristic tracheids and bordered pitted xylem vessels, and the needle crystals of calcium oxalate (see Eur. Ph.). It is native to tropical central and south America and cultivated in southern Asia.

Constituents. Both species contain isoquinoline alkaloids as the active principles, usually about 2–3%. The most important are emetine (Fig. 22.6) and cephaeline, with psychotrine and some others.

Therapeutic uses and available evidence. Ipecac extract is an ingredient of many cough preparations, both elixirs and pastilles, because of its expectorant activity. It is also well known as an emetic and has been employed to induce vomiting in cases of drug overdose, particularly in children. This use is, however, highly controversial (Quang and Woolf 2000). The alkaloids are amoebicidal, but the emetic activity means that they are rarely used for this purpose. There is little clinical evidence for the use of ipecac as an expectorant but it has a long history of traditional use. Ipecacuanha Liquid Extract BP is given at a dose of 0.25–1 ml.

Ipecac causes vomiting in large doses and the alkaloids are cytotoxic.



Emetine

Fig. 22.6

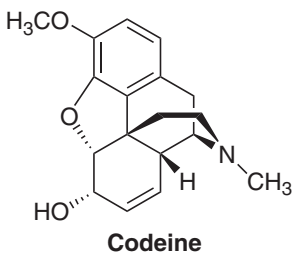
COUGH SUPPRESSANTS

Cough is a reflex action and a symptom of other diseases such as asthma and colds due to 'nasal drip'. Cough suppressants may be useful in some instances, but efficacy is not fully proven and if expectoration is required, for example to avoid sputum retention, they should not be used. They are not recommended for small children who are highly susceptible to respiratory depression caused by opiates. Codeine and semisynthetic opiates such as dextromethorphan are the most common antitussives; in serious disease, such as in lung cancer, stronger opiates such as methadone may be used.

Codeine EP

Although found in opium (*Papaver somniferum* L.), codeine (Fig. 22.7) is usually used as the isolated alkaloid, in the form of a salt (usually phosphate) formulated as a linctus, at a dose of 5–10 mg 4-hourly, to treat cough. The dose for treating diarrhoea and pain is much higher (up to 240 mg daily in divided doses). Only two clinical trials are recorded and codeine appeared to be no more effective than placebo in reducing cough symptoms (Smith et al 2014)

Codeine is sedating and constipating. In large doses it may cause respiratory depression and should not be used where there is hepatic or renal impairment. It is also liable to abuse and is available only on prescription in many countries.



Codeine

Fig. 22.7

GENERAL PHYTOMEDICINES USED IN COLDS AND INFLUENZA – DEMULCENTS AND EMOLIENTS

Some of these medicinal plants have antiviral and anti-inflammatory activity, some are demulcents or stimulate the immune system, and many have several of these properties. They are often used in combination with other ingredients as herbal teas for the supportive or symptomatic therapy of respiratory disease.

Many herbal teas, made particularly from flowers and leaves, are used to obtain symptomatic relief from colds and influenza. Some are diaphoretic (induce sweating), some are anti-inflammatory and analgesic, others are mucilagenous and soothing, and many have some antiviral activity due to the polyphenolic constituents. They are used as a general supportive measure and are usually pleasant to take. As well as the plants discussed here, other botanical drugs rich in mucilage are also used for respiratory conditions, for example the lichen 'Icelandic Moss' from *Cetraria islandica* (L.) Ach. (Parmeliaceae).

Coltsfoot, *Tussilago farfara* L. (Tussilago folium) EP

Coltsfoot (Asteraceae) is a common wild plant in Britain and Europe, growing in damp places. The flowers appear in early spring before the leaves. The leaves are hoof-shaped, with angular teeth on the margins, green above and coated with matted, long white hairs on the lower surface. The flowers are bright yellow, with a characteristic scaly pedicel. Both the leaves and flowers are used medicinally.

Constituents. The main constituent is a mucilage composed of acidic polysaccharides, together with flavonoids, triterpenes and sterols. Pyrrolizidine alkaloids, including senkirkine, tussilagine and isotussilagine, may be present in variable amounts, usually very minor (about 0.015%) or absent, depending on source.

Therapeutic uses and available evidence. Coltsfoot is used for pulmonary complaints, irritating or spasmodic coughs, whooping cough, bronchitis, laryngitis and asthma. The polysaccharides are anti-inflammatory and immunostimulating, as well as demulcent, and the flavonoids also have anti-inflammatory and antispasmodic action.

The pyrrolizidine alkaloids are known to cause hepatotoxicity in rats fed daily on high doses, but not on daily low-dose regimens, and appear not to cause damage to human chromosomes *in vitro*. However, coltsfoot is not suitable for self-medication nor should it be recommended.

Elderflower and Elderberry (fruit), *Sambucus nigra* L. (Sambuci flos, Sambuci fructus) EP

Sambucus nigra (Adoxaceae or Sambucaceae), the black or European elder (berry), is a common European hedge tree or shrub. The flowers appear in May as small, creamy-white, flat-topped umbel-like clusters and are followed by small, shiny, purplish-black berries. Most parts of the plant are used, but most commonly the flowers and berries, which are also used to make refreshing drinks and country-style wines. The berries should not be eaten raw as they contain lectins, which can cause gastrointestinal disturbances, but which are destroyed by heat. Related species are toxic (e.g. Danewort, *S. ebulus* L.).

Constituents. Triterpenes including ursolic and oleanolic acid derivatives, flavonoids (rutin, quercetin, nicotoflorin, hyperoside), and phenolic acids such as chlorogenic acid are the main actives. The flowers contain an essential oil.

Therapeutic uses and available evidence. Elder flowers are used as an infusion or herbal tea, and a mixture with peppermint is a traditional remedy for colds and influenza. They induce perspiration, which is thought to be beneficial in such cases. *In vitro* it shows activity against several strains of influenza virus, and a clinical study has also demonstrated a reduction in the duration of flu symptoms for the berries (see Vlachojannis et al 2010 for review). The effect was attributed to an increase in inflammatory cytokine production as well as a direct antiviral action. The usual dose is about 3 g of flowers infused with 150 ml of hot water, but is not critical. Elder flowers are non-toxic and no side effects have been reported. Both the berries and the flowers are used to make cordials, which are taken medicinally for their reputed antioxidant and antiviral properties (Mikulic-Petkovsek 2015).

Linden flowers, *Tilia* spp. (Tiliae flos) EP

Linden flowers (although called 'lime flowers' they are not related to lime fruit) are from *Tilia rubra* (Weston) DC. (better known under its synonym *Tilia platyphylla* Scop.), *T. cordata* Mill. and their hybrids (Tiliaceae). They are ornamental trees native to Europe. The pedicel bears three to six yellowish-white, five-petalled, fragrant flowers on stalks half-joined to an oblong bract.

Constituents. The flowers contain volatile oil (linalool, germacrene, geraniol, 1,8-cineole, 2-phenyl ethanol and others), flavonoids (hesperidin, quercetin,

astralagin, tiliroside), a mucilage of arabinose, galactose and rhamnose polysaccharides, polyphenolics such as chlorogenic and caffeic acids, and GABA (γ-aminobenzoic acid).

Therapeutic uses and available evidence. Linden flowers are used for feverish colds, catarrh, coughs and influenza. They are used as herbal teas to induce diaphoresis (perspiration) like elder and at a similar dose (see above). The polysaccharides are soothing and adhere to epithelial tissue, producing a demulcent effect. The other main use of the flowers is for nervous disorders; the extract is thought to act as an agonist for the peripheral benzodiazepine receptor. There is evidence that components of the aqueous extract of the flowers bind to GABA receptors in rat brain (an effect not due entirely to the GABA content of the extract) and mild sedative effects were confirmed using the elevated maze anxiety test in mice (Anesini 1999). Linden flowers are non-toxic and no side effects have been reported.

Mallow flower and leaves, *Malva sylvestris* L. (Malvae flos and Malvae folium) EP

The common mallow (*Malva sylvestris* L., Malvaceae) is a wild plant indigenous to southern Europe but naturalized worldwide. The leaves are downy, with 5–7 lobes, and prominent veins on the under surface. The flowers are mauve, with darker veins; both are used for their mucilage content.

Constituents. The main constituents are mucilages, sulphated flavonol glycosides such as gossypin-3-sulphate, hypolaetinglucoside-3′-sulphate and others, and anthocyanins (malvin, the diglucoside of malvidin, and delphinidin).

Therapeutic uses and available evidence. Mallow is a demulcent and pectoral. An infusion is used for colds and coughs, and the mucilage from the leaves is anti-inflammatory with anticomplement activity. Little clinical evidence is available but there is a long tradition of historical use. No adverse effects are known.

Marshmallow leaf and root, *Althea officinalis* L. (Althaeae folium, Althaeae radix) EP

Both the leaves and the rootstock of the marshmallow (Malvaceae) are used as a demulcent, expectorant and emollient. The plant is a downy perennial reaching up to 2 m in height with leaves broadly ovate or cordate, 10–20 cm long and about 10 cm wide, with three to seven rounded lobes, palmate veins and a crenate margin. The flowers are pink, five-petalled, and up to 3 cm in diameter. The root as it appears in commerce is dried, fibrous, cream-white when peeled, deeply furrowed longitudinally and with some root scars. It is largely tasteless.

Constituents. Both rootstock and leaves are rich in mucilage, consisting of a number of polysaccharides (composed of L-rhamnose, D-galactose, D-galacturonic acid and D-glucuronic acid) and others. It also contains common flavonoids, especially derivatives of kaempferol and quercetin.

Therapeutic uses and available evidence. Both the leaves and root are used internally for coughs and bronchial complaints. Extracts of both are used occasionally for gastric and urinary inflammation in general, and for cystitis. They may be applied externally as a soothing poultice and vulnerary. The mucilages have proven biological activity, including the stimulation of phagocytosis *in vitro.* Antimicrobial and anti-inflammatory activities have also been documented. Several of the polysaccharides isolated from the roots have been found to have antitussive activity. The most common use of extracts of marshmallow root is in the making of confectionery.

GENERAL PHYTOMEDICINES USED IN COLDS AND INFLUENZA – GENERAL ANTI-INFECTIVE AGENTS

A range of medicinal plants are used as antimicrobial agents and, in the context of respiratory conditions, one stands out as being of particular relevance.

Pelargonium, *Pelargonium sidoides* DC. and *P. reniforme* (Andrews) Curtis (Pelargonii radix) EP

Pelargonium is obtained from two southern African species, *Pelargonium sidoides* and *P. reniforme* (Geraniaceae) where the tubers, stems and root have been used for centuries to treat a range of infectious conditions. Umckaloabo is a fusion of two Zulu terms ("Umkuhlune" = Coughing and fever, fused with "Uhlabo" = chest pains; A. Viljoen, pers. comm. 2016) and this term refers to a medicine used traditionally in South Africa as a treatment for respiratory tract infections. This material is derived from the roots of either *Pelargonium sidoides* or *P. reniforme* (Geraniaceae). A decoction of the roots is used to treat chest infections and this material is the subject of a book by Charles Stevens ('Stevens Cure'), a 19th century army officer credited with introducing it to the United Kingdom.

Constituents. The main active components are hydrolysable tannins, (+)-catechin, gallic acid and methyl

gallate, including a unique series of O-galloyl-C-glucosylflavones. Flavonoids including myricetin and quercetin-3-O-beta-d-glucoside, coumarins including scopoletin, umckalin, 5,6,7-trimethoxycoumarin and 6,8-dihydroxy-5,7-dimethoxycoumarin, are present in both species. A series of benzopyranones has been isolated from *P. sidiodes*. Pelargoniins (a type of ellagitannin) and a diterpene, reniformin, have been found in *P. reniforme*.

Therapeutic uses and available evidence. In Germany, a standardized extract of *Pelargonium sidoides* (EPs® 7630, also known as Umckaloabo®) is registered by the Federal Institute for Drugs and Medical Devices (BfArM) for the indication 'acute bronchitis'. Based on a systematic review (Timmer et al 2013) assessing the efficacy of *P. sidoides* preparations in acute respiratory infections in randomized controlled trials showed that it may be effective in alleviating symptoms of acute rhinosinusitis and the common cold in adults, as well as in relieving symptoms in acute bronchitis in adults and children, and sinusitis in adults. Reliable data on treatment for other acute respiratory infections are lacking (Timmer et al 2013) and the authors raised concerns about the quality of the studies under review. The extract EPs® 7630 has multiple effects that are beneficial in respiratory infections, and include antiviral, antibacterial, immunomodulatory and cytoprotective effects. It also increases the frequency of ciliary beats, thus helping to remove pathogens for the upper respiratory tract, and inhibits the interaction between bacteria and host cells. Extracts of *Pelargonium* species have been shown to inhibit the adherence of bacteria to cells of the mucous membrane and there is some published chemistry and biology on methoxylated coumarins from *P. sidoides* which have weak antibacterial activity (Kayser and Kolodziej 1997).

EPs® 7630 interferes with the replication of different respiratory viruses including seasonal influenza A virus strains, respiratory syncytial virus, human coronavirus, parainfluenza virus and coxsackie virus (Michaelis et al 2011).

This extract is also given to athletes to help strengthen their immune systems, which can be compromised by extreme exercise, and to protect against colds. A study in athletes submitted to intense physical activity found that *P. sidioides* increased the production of secretory immunoglobulin A in saliva, and decreased levels of both interleukin-15 and interleukin-6 in serum, suggesting a strong modulating influence on the immune response associated with the upper airway mucosa (Luna et al 2011).

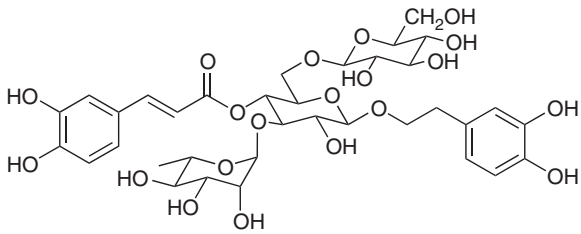
IMMUNOSTIMULANTS

Immune stimulation is usually measured using parameters such as an increase in numbers of circulating immune cells, or enhanced phagocytosis after inoculation with a pathogen. It is notoriously difficult to substantiate claims for the prevention of disease, since very large clinical studies are needed for statistical validity, and these are difficult and expensive to perform. However, echinacea is taken widely and the use of an Oriental medicinal plant, astragalus, is increasing in the West for the same indications.

Echinacea, *Echinacea pallida* (Nutt.) Nutt., *E. purpurea* (L.) Moench and *E. angustifolia* DC (Echinaceae herba, radix) EP

Members of the genus *Echinacea* (Asteraceae) are widely distributed in North America and have a long tradition of use, both by the American Indians and the settlers, who developed the first commercial preparations during the 19th century. Both aerial parts and secondary roots are used. The indigenous people used *E. pallida* in particular for a variety of illnesses, such as pain, inflammatory skin conditions and toothache. The three botanical species are used as immunostimulants in the preparation of phytomedicines to 'prevent colds and other respiratory infections'. The complex situation regarding species, quality of products made from them and method of production makes an assessment of the clinical efficacy very difficult. Echinacea is often combined with garlic, for the treatment of colds and allergic rhinitis.

Constituents. Numerous compounds have been identified, but the most pharmacologically relevant ones are not known. All species contain similar types



Echinacoside

Fig. 22.8

of compounds, although not necessarily the same individual ones. The most important are the caffeic acid derivatives, including echinacoside (Fig. 22.8) (*E. pallida* root), cichoric acid (*E. purpurea* aerial parts) and others, and the alkylamides (found throughout the plant in all three species), which are a complex mixture of unsaturated fatty acid derivatives. Some have a diene or diyne structure (with two unsaturated and two triple unsaturated groups) or a tetraene structure (with four unsaturated groups) linked via an esteramide to a (2)-methylpropane or (2)-methylbutane residue.

Therapeutic uses and available evidence. Echinacea preparations are available both as traditional herbal medicinal products used to relieve the symptoms of the common cold and influenza-type infections, and as preparations with a well-established use. There is some evidence in the treatment and prevention of respiratory infections, but more limited evidence for slow-healing wounds using topical applications. Clinical evidence for use as an immunostimulant is available for some of the chemically characterized extracts. Overall a series of meta-analyses showed that Echinacea preparations *seem to be* efficacious both therapeutically (reducing symptoms and duration) and in terms of prophylaxis against the common cold (Shah et al 2007, Woelkart et al 2008). A large (755 healthy subjects) randomized, double-blind, placebo-controlled trial assessed the safety and efficacy of specific *Echinacea purpurea* extract products over a period of 4 months. Participants were given the herbal preparation (standardized to contain 5 mg/100 g of dodecatetraenoic acid isobutylamide; 0.9 ml 3 times a day corresponding to 2400 mg of extract) for illness prevention or placebo. The study concluded that this herbal preparation was safe and, compared to placebo, effective. Consequently the authors state that it can be recommended as a prophylactic treatment (Jawad et al 2012). However, *Echinacea* preparations tested in clinical trials differ greatly. There is better evidence that preparations based on the aerial parts of *E. purpurea* might be effective for the early treatment of colds in adults but the results are not fully consistent. A mechanism of action has been postulated by Chicca et al (2009), suggesting that the alkylamides dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (A1) and dodeca-2*E*, 4*E*-dienoic acid isobutylamide (A2) bind to the cannabinoid-2-(CB2) receptor and are the main anti-inflammatory and immune-modulatory principles, acting in synergy. In addition, alkylamides potently inhibit LPS-induced inflammation in human whole blood and exert modulatory effects on cytokine expression, but these effects are not exclusively related to CB2 binding.

Echinacea appears to be safe, although allergic reactions have been reported. The risk of interactions seems to be very limited (Modarai et al 2007).

Astragalus, *Astragalus mongholicus* Bunge (Astragali radix) EP

Astragalus mongholicus Bunge [syn: *Astragalus membranaceus* (Fisch.) Bge., Fabaceae] is an herbaceous perennial native to north-eastern China, central Mongolia and Siberia. The drug is known in Chinese medicine as Huang qi. The use of *Astragalus* root, as a general tonic, dates back to the legendary Chinese emperor Shen-Nong. The root consists of a long cylindrical tap root, which is internally yellowish in colour, but rootlets should be absent.

Constituents. Triterpenoid saponins, the astragalosides I–VIII, and their acetyl derivatives, agroastragalosides I–IV, astramembranins I and II and others; isoflavones including formononetin and kumatakenin, and polysaccharides known as astrogaloglucans.

Therapeutic uses and available evidence. Overall, there is anecdotal, but little clinical evidence that astragalus alone or in combination may help in the treatment of the common cold or for impaired immunity. A number of clinical studies, supported by data from over 1000 patients in China, confirm the use of astragalus as an immunostimulant for use in colds and upper respiratory infections. It is also used prophylactically. In China it is used as an adjunctive in the treatment of cancer, and appears to potentiate the effects of interferon, but the evidence is limited (Auyeung et al 2016). Antioxidant, hepatoprotective and antiviral activity and enhancement of cardiovascular function have been reported (Anon 2003). Many animal studies have been carried out, but specific data on toxicity are sparse. Anti-inflammatory properties via reducing the release of inflammatory mediators and modulating the MAPK signalling pathway, resulting in inactivation of NF-κB, have been shown (Lai et al 2013). In general, astragalus is well tolerated but should probably be avoided in autoimmune diseases.

Andrographis, *Andrographis paniculata* (Burm.f.) Wall. (Andrographis paniculatae herba)

Andrographis paniculata (Acanthaceae), also known as 'green chiretta', is an erect annual herb found in north-eastern India and many other parts of Asia. It is

extremely bitter in taste and has been referred to as the 'king of bitters'. It is an important medicinal plant in Ayurveda where it is known as kalmegh.

Constituents. The main actives are diterpenes, known as andrographolides, and consist of andrographolide and its many analogues, including neoandrographolide, isoandrographolide, 14-deoxyandrographolide, 14-deoxy-14,15-dehydroandrographolide, 3,19-isopropylideneandrographolide and 14-acetylandrographolide and many others. Flavonoids and polyphenols such as 7-O-methylwogonin, apigenin, onysilin and 3,4-dicaffeoylquinic acid are also present. An alkaloid, andrographine, and a series of sesquiterpene lactones, paniculides A, B, and C, are also present in the root (Sareer et al 2014).

Therapeutic uses and available evidence. The most recent systematic reviews for the use of *A. paniculata* in upper respiratory tract infection (URTI) (Akbar 2011) included one study in children and were mostly conducted using a standardized commercial preparation of *A. paniculata* extract in a fixed combination with *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. (Kan Jang). It was considered to be effective in reducing the severity and duration of the symptoms in URTI, both in children and adults but further research is warranted. Andrographis is most commonly used as an immune stimulant, but is also reputed to possess antihepatotoxic, antimicrobial, antithrombogenic, anti-inflammatory and anticancer properties (Anon. 2003). Andrographolide has been shown to have immunostimulatory activity, shown by an increase in proliferation of lymphocytes and production of interleukin-2, and the anti-inflammatory activity has been demonstrated by an inhibition of NF-κB, nitric oxide, PGE2, IL-1β, IL-6, LTB4, TXB2 and histamine (Bao et al 2009, Chandrasekaran et al 2010). *In vitro* the activation and proliferation of immune-competent cells as well as the production of key cytokines and immune activation markers was inhibited by andrographolide and Kan Jang (a standardized fixed combination of *A. paniculata* extract SHA-10 and *E. senticosus* extract SHE-3) (Panossian et al 2002).

In one Chilean study, andrographis herb had a significant drying effect on the nasal secretions of cold sufferers who took 1200 mg of the extract daily for 5 days (Cáceres et al 1999). A systematic review of the literature has suggested the herb alone (or in combination with *Eleutherococcus*) may be an appropriate treatment for uncomplicated acute upper respiratory tract infection (Poolsup et al 2004).

References

Agbabiaka, T.B., Guo, R., Ernst, E., 2008. *Pelargonium sidoides* for acute bronchitis: a systematic review and meta-analysis. Phytomedicine 15, 378–385.

Agosti, R., Duke, R.K., Chrubasik, J.E., Chribasik, S., 2006. Effectiveness of *Petasites hybridus* preparations in the prophylaxis of migraine: a systematic review. Phytomedicine 13, 743–746.

Akbar, S., 2011. *Andrographis paniculata*: a review of pharmacological activities and clinical effects. Alternative Medicine Reviews 16 (1), 66–77.

Anesini, C., Werner, S., Borda, E., 1999. Effect of *Tilia cordata* Mill. flowers on lymphocyte proliferation. Participation of peripheral type benzodiazepine binding sites. Fitoterapia 70, 361–367.

Anon, 2003. *Astragalus membranaceus.* Monograph. Alternative Medicine Reviews 8, 72–77. Available online at www.thorne.com.

Arai, M., Hayashi, A., Sobou, M., et al., 2011. Anti-angiogenic effect of triterpenoidal saponins from *Polygala senega*. J. Nat. Med 65, 149–156.

Auyeung, Kathy K., Han Quan-Bin., Ko Joshua K., 2016. Astragalus membranaceus: A review of its protection against inflammation and gastrointestinal cancers. Am. J. Chin. Med. 44,1–22

Aydin, A.A., Valentin, Z., Harun, P., Th, Letzel, 2013. The medical plant butterbur (*Petasites*): analytical and physiological (re)view. Journal of Pharmaceutical and Biomedical Analysis 75, 220–229.

Bao, Z., Guan, S., Cheng, C., et al., 2009. A novel antiinflammatory role for andrographolide in asthma via inhibition of the nuclear factor-kappaB pathway. American Journal of Respiratory and Critical Care Medicine 179, 657–665.

Brattström, A., Schapowal, A., Maillet, I., Schnyder, B., Ryffel, B., Moser, R., 2010. Petasites extract Ze 339 (PET) inhibits allergen-induced Th2 responses, airway inflammation and airway hyperreactivity in mice. Phytotherapy Research 24, 680–685.

Cáceres, D.D., Hancke, J.L., Burgos, R.A., Sandberg, F., Wikman, G.K., 1999. Use of visual analogue scale measurements (VAS) to assess the effectiveness of standardized *Andrographis paniculata* extract SHA-10 in reducing the symptoms of common cold. A randomized double blind-placebo study. Phytomedicine 6, 217–223.

Chandrasekaran, C.V., Gupta, A., Agarwal, A., 2010. Effect of an extract of *Andrographis paniculata* leaves on inflammatory and allergic mediators in vitro. Journal of Ethnopharmacology 129, 203–207.

Chicca, A., Raduner, S., Pellati, F., et al., 2009. Synergistic immunomopharmacological effects of N-alkylamides in *Echinacea purpurea* herbal extracts. International Immunopharmacology 9, 850–858.

EFSA (EFSA Panel on Food Additives and Nutrient Sources), 2013. Scientific opinion on safety evaluation of *Ephedra* species in food. EFSA Journal 11 (11), 3467. http://dx.doi.org/10.2903/j.efsa.2013.3467.

Fashner, J., Ericson, K., Werner, S., 2012. Treatment of the common cold in children and adults. American Family Physician 86, 153–160.

Fazio, S., Pouso, J., Dolinsky, D., et al., 2009. Tolerance, safety and efficacy of *Hedera helix* extract in inflammatory bronchial diseases under clinical practice conditions: a prospective, open, multicentre postmarketing study in 9657 patients. Phytomedicine 16, 17–24.

Fleming, R.M., 2008. Safety of ephedra and related anorexic medications. Expert Opinion on Drug Safety 7, 749–759.

Hegener, O., Prenner, L., Runkel, F., Baader, S.L., Kappler, J., Häberlein, H., 2004. Dynamics of beta2-adrenergic receptor-ligand complexes on living cells. Biochemistry 43, 6190–6199.

Holzinger, F., Chenot, J.F., 2011. Systematic review of clinical trials assessing the effectiveness of ivy leaf (*Hedera helix*) for acute upper respiratory tract infections. Evidence Based Complementary and Alternative Medicine 382789.

Jawad, M., Schoop, R., Suter, A., Klein, P., Eccles, R., 2012. Safety and efficacy profile of *Echinacea purpurea* to prevent common cold episodes: a randomized, double-blind, placebo-controlled trial. Evidence Based Complementary and Alternative Medicine 841315.

Kayser, O., Kolodziej, H., 1997. Antibacterial activity of extracts and constituents of *Pelargonium sidoides* and *Pelargonium reniforme*. Planta Medica 63, 508–510.

Lai, P.K., Chan, J.Y., Wu, S., et al., 2013. Anti-inflammatory activities of an active fraction isolated from the root of *Astragalus membranaceus* in RAW 264.7 macrophages. Phytotherapy Research 28 (3), 395–404.

Lang, C., Röttger-Lüer, P., Staiger, C., 2015. A valuable option for the treatment of respiratory diseases: review on the clinical evidence of the ivy leaves dry extract EA 575®. Planta Medica 81, 968–974.

Luna Jr., L.A., Bachi, A.L., et al., 2011. Immune responses induced by *Pelargonium sidoides* extract in serum and nasal mucosa of athletes after exhaustive exercise: modulation of secretory IgA, IL-6 and IL-15. Phytomedicine 18, 303–308.

Mikulic-Petkovsek, M., Samoticha, J., Eler, K., Stampar, F., Veberic, R., 2015. Traditional elderflower beverages: a rich source of phenolic compounds with high antioxidant activity. Journal of Agricultural and Food Chemistry 63, 1477–1487.

Michaelis, M., Doerr, H.W., Cinatl Jr., J., 2011. Investigation of the influence of EPs(W) 7630, a herbal drug preparation from *Pelargonium sidoides*, on replication of a broad panel of respiratory viruses. Phytomedicine 18, 384–386.

Modarai, M., Gertsch, J., Suter, A., Heinrich, M., Kortenkamp, A., 2007. Cytochrome P450 inhibitory action of Echinacea preparations differs widely and co-varies with alkylamide content. Journal of Pharmacy and Pharmacology 59, 567–573.

NTP, 2009. Chemical Information Review Document for Butterbur (Petasites hybridus, ext.) [CAS No. 90082-63-6]. National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health. U.S. Department of Health and Human Services Research Triangle Park, NC. Available online at http://ntp.niehs.nih.gov/.

Panossian, A., Davtyan, T., Gukassyan, N., et al., 2002. Effect of andrographolide and Kan Jang – fixed combination of extract SHA-10 and extract SHE-3 – on proliferation of human lymphocytes, production of cytokines and immune activation markers in the whole blood cells culture. Phytomedicine 9 (7), 598–605.

Poolsup, N., Suthisisang, C., Prathanturarug, S., Asawamekin, A., Chanchareon, U., 2004. *Andrographis paniculata* in the symptomatic treatment of uncomplicated upper respiratory tract infection: systematic review of randomized controlled trials. Journal of Clinical Pharmacy and Therapeutics 29, 37–45.

Quang, L., Woolf, A.D., 2000. Past, present, and future role of ipecac syrup. Current Opinion in Pediatrics 12, 153.

Sareer, O., Ahmad, S., Umar, S., 2014. *Andrographis paniculata:* a critical appraisal of extraction, isolation and quantification of andrographolide and other active constituents. Natural Product Research 28, 2081–2101.

Sadlon, A.E., Lamson, D.W., 2010. Immune-modifying and antimicrobial effects of Eucalyptus oil and simple inhalation devices. Alternative Medicine Reviews 15 (1), 33–47.

Shah, S.A., Sander, S., White, C.M., Rinaldi, M., Coleman, C.I., 2007. Evaluation of echinacea for the prevention and treatment of the common cold: a meta-analysis. The Lancet. Infectious Diseases 7, 473–480.

Smith, S.M., Schroeder, K., Fahey, T., 2014. Over-the-counter (OTC) medications for acute cough in children and adults in community settings. Cochrane Database of Systematic Reviews11, CD001831. http://dx.doi.org/10.1002/14651858.CD001831.pub5.

Timmer, A., Günther, J., Motschall, E., Rücker, G., Antes, G., Kern, W.V., 2013. *Pelargonium sidoides* extract for treating acute respiratory tract infections. Cochrane Database Systematic Reviews 10, CD006323.

Vlachojannis, J.E., Cameron, M., Chrubasik, S., 2010. A systematic review on the sambuci fructus effect and efficacy profiles. Phytotherapy Research 24 (1), 1–8.

Woelkart, K., Linde, K., Bauer, R., 2008. Echinacea for preventing and treating the common cold. Planta Medica 74, 633–637.

Chapter 23

The central nervous system

Drugs acting on the central nervous system (CNS) include the centrally acting (mainly opioid) analgesics, anti-epileptics and anti-Parkinson agents, as well as those used for psychiatric disorders. Drugs of plant origin are important in all these areas, although not usually for self-medication. They are also of historical interest; for example, the antipsychotic drug reserpine, isolated from *Rauvolfia* species, revolutionized the treatment of schizophrenia and enabled many patients to avoid hospitalization before the introduction of the phenothiazines (such as chlorpromazine) and the newer atypical antipsychotics (olanzapine and risperidone). Unfortunately, reserpine depletes neurotransmitter concentrations in the brain (it is used as a pharmacological tool in neuroscience for this reason) and so can cause severe depression. There are no other currently useful antipsychotics obtained from plants. Similarly, the useful anti-epileptics are synthetic, with the possible exception of the cannabinoids, from *Cannabis sativa* L., which are currently under investigation; clinical studies, however, are limited, and efficacy and safety have not been established (Gloss and Vickrey 2014).

Phytotherapy can provide useful support for milder psychiatric conditions. The prevalence of mental health problems, particularly depression and anxiety, in the general population is around one in six people, and around 40% of people with mental health problems will have symptoms of both anxiety and depression. Depression is more common in women than men: around one-half of women and one-quarter of men will be affected by depression at some time. However, other than in mild cases, these disorders are not suitable for self-treatment, and medical supervision is necessary. Sleep disturbances, such as insomnia and early morning awakening, are characteristic of depression and anxiety, although they can also occur independently of mental health problems. Around one-third of adults experience insomnia, and most do not seek treatment from a physician. Phytotherapy may help to re-establish a regular pattern of sleep. Valerian (Fig. 23.1), for example, has been advocated as a means of alleviating the symptoms of benzodiazepine withdrawal.

Migraine is a common disorder, and can be debilitating. Opioid analgesics are used, and the synthetic 5-HT_1 (5-hydroxytryptamine) antagonists (sumatriptan, rizatriptan, zolmitriptan and others) are highly effective in the treatment of acute migraine, but are not used for migraine prophylaxis. Ergotamine is a potent drug used as a last resort in attacks of migraine. Feverfew is sometimes used to prevent attacks.

In dementia and Alzheimer's disease, some natural compounds have played a role in symptomatic treatment. Galantamine (from the snowdrop, *Galanthus nivalis* L.) and derivatives of physostigmine (e.g. rivastigmine) are used clinically as cholinesterase inhibitors. Some plant extracts, such as sage and rosemary, have similar, but milder, effects and are being investigated for memory improvement. *Ginkgo biloba* L. has cognition-enhancing properties and is used in mild forms of dementia.

HYPNOTICS AND SEDATIVES

Whether a substance produces a sedative (having a calming effect, reducing agitation, and permitting sleep) or hypnotic (producing sleep) effect generally relates to the dose at which it is used. Plant products used as sedatives and/or hypnotics are not as potent as synthetic drugs, but, as with synthetic hypnotics, these herbal medicines are generally intended for short-term use.

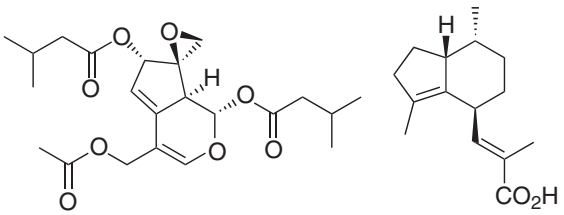


Fig. 23.1

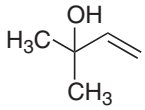


Fig. 23.2

HOPS, *HUMULUS LUPULUS* L. (HUMULI LUPULI STROBULI) EP

Humulus lupulus L. (Cannabaceae), often referred to by its common name of hops, has been used traditionally for insomnia, neuralgia and excitability. It is cultivated in several European countries, including England, France and Germany. The part of the plant used pharmaceutically is the female flower heads (known as 'strobiles'). These are composed of overlapping bracts, which enclose the ovary. Hops have a characteristic odour.

Constituents

The main active constituents of hops are the bitter principles found in the oleo-resin. These include the α-acid humulone and the β-acid lupulone, and their degradation products, such as 2-methyl-3-buten-2-ol (Fig. 23.2). Other constituents include flavonoids, chalcones, tannins and volatile oils.

Therapeutic uses and available evidence

Modern pharmaceutical indications for hops include sleep disturbances and restlessness. Sedative and hypnotic activities have been documented *in vivo* (mice) for extract of hops, and for the bitter acid degradation product 2-methyl-3-buten-2-ol. Clinical studies provide some evidence of the hypnotic effects of hops given in combination with the herbal sedative-hypnotic valerian (Salter and Brownie 2010, Zanoli and Zavatti 2008). Antibacterial and antifungal activities have been documented *in vitro* for certain constituents of hops.

Preclinical studies indicate that extracts of hops may influence the effects of some medicines, including paracetamol and diazepam (Williamson et al 2016). Hops are non-toxic, as their use in beer would suggest. Cheers!

LEMON BALM, *MELISSA OFFICINALIS* L. (MELISSAE FOLIUM) EP

Melissa officinalis L. (syn. 'melissa', 'balm' and 'sweet balm', Lamiaceae) has been used traditionally for its sedative effects, as well as for gastrointestinal disorders. The dried leaves are the parts used pharmaceutically. This herbal medicine is described in Chapter 24.

Constituents

The volatile oil of melissa contains numerous constituents, mainly monoterpenes, particularly aldehydes (e.g. citronellal, geranial and neral) and sesquiterpenes (e.g. β-caryophyllene). Flavonoids, including quercetin, apigenin and kaempferol, and polyphenols (e.g. hydroxycinnamic acid derivatives) are also present in the herb. Melissa is listed in the Eur. Ph., which states that the drug contains not less than 4.0% of total hydroxycinnamic derivatives, expressed as rosmarinic acid, calculated with reference to the dried drug.

Therapeutic uses and available evidence

Sedative and antispasmodic effects have been documented for melissa extracts using *in vivo* studies (mice, rats). It is used for nervous or sleeping disorders and functional gastrointestinal complaints. There has been limited clinical investigation of the sedative effects of melissa alone in individuals with sleeping disorders (Cases et al 2011). Other clinical trials have explored the effects of melissa in combination with other herbal sedatives (e.g. valerian and hops) and provide some evidence to support the sedative and hypnotic effects of such preparations (Pharmaceutical Press Editorial 2016).

Clinical trials have reported that melissa leaf extract improved cognitive performance in patients with dementia, but that melissa oil used in aromatherapy treatment did not reduce agitation in institutionalized patients with dementia (Burns et al 2011, Perry and Howes 2011). The pharmacological activities of lemon balm provide some supporting evidence for these effects (Perry and Howes 2011).

Preclinical studies have reported antithyroid and cholinergic activity for extracts of balm; the clinical relevance of these effects is not known. Dried lemon balm is usually taken internally in the form of a herbal tea, at a dose of 2–4 g three times a day. Melissa extracts are also applied topically in cases of *herpes simplex labialis* resulting from HSV-1 infection (see Anti-infectives).

Lemon balm is regarded as non-toxic, although it should not be taken excessively due to its reputed antithyroid activity.

KAVA, *PIPER METHYSTICUM* G.FORST. (PIPERIS METHYSTICI RHIZOME)

Piper methysticum G.Forst (Piperaceae), also known as *Macropiper methysticum* (G.Forst.) Miq., and the common names kava-kava or kawa, has been used in the Pacific Islands, notably Fiji, for hundreds of years. It is a small shrub with heart-shaped leaves and thick, woody roots and rhizomes, which are ground or chewed to release the actives. These are then fermented to make the ceremonial drink kava, which induces a relaxed sociable state, and is given to visiting dignitaries. Kava is used medicinally for its tranquillizing properties and numerous other disparate complaints. Due to concerns about the hepatotoxic effects associated with kava use, in 2003, in the UK, kava was prohibited in unlicensed medicines and, in the EU, all licensed kava products underwent market withdrawal; several other countries internationally took regulatory action. In 2014, a German Federal court ruled that there was no justification for withdrawal of kava marketing authorizations and that the benefit–risk balance for kava was favourable, meaning that kava preparations could be marketed again in Germany (Schmidt 2014). The German medicines regulatory authority has appealed against this decision.

Constituents

The main constituents of kava are the kavalactones (also known as kavapyrones), including kavain, dihydrokavain, methysticin, yangonin and desmethoxyyangonin.

Therapeutic uses and available evidence

Preclinical studies using kava extracts or isolated kavalactones indicate that these substances potentiate $GABA_A$ receptor activity and have effects on certain neurotransmitters (Sarris et al 2011).

The efficacy of kava extracts in relieving anxiety is supported by data from randomized, controlled trials (Sarris et al 2011), including in patients with both anxiety and depression (Sarris et al 2009). Overall, studies indicate reductions in anxiety after 4–12 weeks of treatment with kava extracts at dosages equivalent to 60–240 mg of kavalactones daily.

Kava extracts are generally well tolerated when used at recommended doses for limited periods. However, case reports of liver toxicity associated with the use of kava preparations have been reported since the year 2000 in several different countries. The liver effects described in these reports range from abnormal liver function test results and jaundice to irreversible liver failure requiring liver transplant, and death. Assessment of the causal role of kava in cases of hepatotoxicity is complicated by several factors, including use of alcohol and other concomitant drugs associated with liver toxicity. The hepatotoxic effect is idiosyncratic, and the exact cause remains unknown. There are conflicting views as to whether or not the use of poor-quality kava raw material is involved, and whether there are toxic constituents or metabolites; there may also be a pharmacogenomic component to the toxicity (Sarris et al 2011, Zhang et al 2011).

PASSION FLOWER, *PASSIFLORA INCARNATA* L. (PASSIFLORAE HERBA) EP

Passion flower (*Passiflora incarnata* L., Passifloraceae) is also known by the common names passion vine, maypop and others. The plant is a climbing vine, native to South America, but now also grown widely, including in the USA and India. The dried leafy aerial parts, which normally include the flowers and fruits, are used pharmaceutically. The flower shows a distinctive shape of a cross, and was given the name passion (which refers to Christian connotations rather than romantic). There are numerous curling tendrils and the leaves are three-lobed. The edible passion fruit is from *P. edulis* Sims.

Constituents

The active constituents have not yet been clearly established, and various compounds likely contribute to the clinical effects; the flavonoids, particularly chrysin and related compounds (schaftoside, isoschaftoside, orientin, homoorientin, vitexin, isovitexin, kaempferol, luteolin, quercetin, rutin, saponaretin and saponarin), are thought to be important (Miroddi et al 2013). Alkaloids of the harman type are present in low concentrations

(the presence of harmine, harmaline, harmol and harmalol has been disputed) as well as β-carbolines. *P. edulis* contains similar types of compounds and cycloartane triterpenoids, such as the cyclopassifloic acids and cyclopassiflosides. Passion flower is included in the Eur. Ph. The drug should contain not less than 1.5% of total flavonoids, expressed as vitexin, assayed by a colorimetric method.

Therapeutic uses and available evidence

The historical medicinal uses of passion flower include treatment of insomnia, hysteria, nervous tachycardia and neuralgia. Modern pharmaceutical uses include nervous restlessness and insomnia due to nervous tension. In the UK, marketed products authorized under the Traditional Herbal Medicinal Products Directive (THMPD) containing passion flower are indicated for the temporary relief of symptoms associated with stress, such as mild anxiety, based on traditional use only.

Preclinical studies have described several pharmacological activities, including sedative and anxiolytic activity, for passion flower extracts and certain constituents (Miroddi et al 2013). Modulation of the GABA system is known to be involved in the anxiolytic effects. Sedative effects are attributed at least in part to the flavonoid, particularly chrysin, content.

There are few clinical studies of passiflora, and systematic reviews have reported that there was insufficient research to draw definitive conclusions regarding the effects of passion flower extracts in anxiety (Miroddi et al 2013, Miyasaka et al 2007). Generally, passiflora is well tolerated with few side effects; however, there are isolated case reports of nausea and tachycardia, and vasculitis.

VALERIAN, *VALERIANA OFFICINALIS* L. (VALERIANAE RADIX) EP

Valeriana officinalis L.(Valerianaceae), commonly known as valerian, all-heal, and by many other vernacular (common) names, is among the most well documented of all medicinal plants, particularly in northern Europe. It is an herbaceous plant, reaching about 1 m in height, and is cultivated in many European countries, as well as in Japan and North America. Valerian has a long history of traditional use. Historically, it was used in the treatment of conditions involving nervous excitability, such as hysterical states and hypochondriasis, as well as in insomnia. The parts used pharmaceutically are the root, rhizomes and stolons, which are yellowish-grey to pale greyish-brown. The rhizomes may be up to 50 mm long and 30 mm in diameter, whereas the roots may be around 100 mm long and 1–3 mm in diameter. Valerian root has a characteristic smell, which is usually described as unpleasant.

Constituents

The main components of valerian are the volatile oil and the iridoid valepotriate constituents. The volatile oil contains monoterpenes and sesquiterpenes, such as β-bisabolene, caryophyllene, valeranone, valerianol, valerenol, valerenal, valerenic acid and derivatives (see Fig. 23.1). The valepotriate compounds include valtrate, didrovaltrate and isovaltrate. The valepotriates readily decompose on storage and processing to form mainly baldrinal and homobaldrinal, which are also unstable. Valerian also contains alkaloids, including valerianine and valerine, and amino acids such as arginine, γ-aminobutyric acid (GABA), glutamine and tyrosine.

Valerian root is listed in the Eur. Ph., which requires that it contains not less than 5 ml/kg volatile oil for the whole drug, and not less than 3 ml/kg for the cut drug, calculated with reference to the dried drug. It should also contain not less than 0.17% of sesquiterpenic acids, expressed as valerenic acid.

Therapeutic uses and available evidence

In Europe, valerian and its various preparations (tablets, tinctures) have been approved for the temporary relief of symptoms of mild anxiety and to aid sleep, generally based on traditional use evidence. The sedative effects of valerian root are well documented. *In vivo* studies (in mice) have demonstrated CNS-depressant activity for the volatile oil, the valepotriates and the valepotriate degradation products. The sedative effects of valerian root are thought to be due to the activities of these different components, particularly valerenal and valerenic acid (constituents of the volatile oil), and the valepotriate compounds. Therefore, the profile of these constituents, and their concentrations, in a specific valerian preparation will determine its activity. Biochemical studies have indicated that certain components of valerian, particularly valerenic acid, may lead to increased concentrations of the inhibitory neurotransmitter GABA in the brain by inhibiting its catabolism, inhibiting uptake and/or by inducing GABA release. Increased GABA concentrations are associated with decreased CNS activity, which may, at least partly, explain valerian's sedative activity. It is not clear whether valerian root extracts have effects on the binding of benzodiazepines to receptors.

Modern medicinal uses for valerian root preparations are for insomnia, stress and anxiety. Clinical trials have tested the effects of valerian preparations on subjective (e.g. sleep quality) and objective (e.g. sleep structure) sleep parameters, and on measures of stress. Some, but not all, of these studies provide evidence to support the traditional uses of valerian (Pharmaceutical Press Editorial 2016). Several preparations contain valerian root in combination with other herbs reputed to have hypnotic and/or sedative effects, such as hops (*Humulus lupulus* L.) and Melissa (*Melissa officinalis* L.) (see Salter and Brownie 2010 for review).

It is recommended that valerian preparations should not be taken for up to 2 hours before driving a car or operating machinery; also, the effect of valerian preparations may be enhanced by alcohol consumption. There are isolated reports of hepatotoxicity associated with valerian-containing products, although causality has not been established. Valerian root extracts may cause gastrointestinal symptoms, such as nausea and abdominal cramps.

ANTIDEPRESSANTS

SAFFRON, *CROCUS SATIVUS* L. (STIGMA CROCI)

Crocus sativus L. (Iridaceae), also known as saffron and in some countries as 'red gold', is a perennial herb indigenous to south-western Asia and southern Europe and is cultivated in several other countries, including China and India. The flower produces drooping red stigmas, which, when dried, are the part used medicinally. Saffron has a range of traditional uses, including as an emmenagogue, and for treatment of abdominal pain and fever. Clinical research has explored the effects of saffron in depression, Alzheimer's disease, and other conditions. Saffron has a culinary use as a spice, and is also used in fragrances and as a colouring agent (Woolven and Snider 2016).

Saffron is considered to be the most expensive spice in the world. Its high cost is due to the extensive manual labour required for its collection: around 150,000 flowers are individually picked to produce 1 kg of the spice (Soffritti et al 2016). The high price means it is a common target for adulteration; reported adulterants include other plant materials, animal substances, chalk, synthetic dyes and others. Other plant materials that have been reported as adulterants in saffron include: petals from safflower (*Carthamus tinctorius* L.) and calendula (*Calendula officinalis* L.); powdered rhizomes from curcuma (*Curcuma longa* L.); dye extracted from buddleia flowers (*Buddleja officinalis* Maxim.) (Soffritti et al 2016). 'Old' saffron material is also sometimes used as a 'bulking agent' (Nenadis et al 2016). Safflower (and, sometimes, curcuma) are sometimes mislabeled as being 'saffron'.

Constituents

The major constituents are carotenoids, including crocins (e.g. crocin, a glucoside) and crocetin, safranal (a monoterpene), and picrocrocins (e.g. picrocrocin, a monoterpene glucoside); the vitamins riboflavin and thiamine are also present (Christodoulou et al 2015).

Therapeutic uses and available evidence

The important active constituents are considered to be the crocins, crocetin, and safranal. Several pharmacological activities, including anti-inflammatory, immunomodulatory, anti-oxidant and anti-platelet effects, have been described for saffron and/or its constituents in preclinical studies (Boskabady and Farkhondeh 2016, Lopresti and Drummond 2014). While these activities have been postulated to contribute to the antidepressant effect of saffron (reported in clinical studies), there is limited evidence to support this. There is some evidence from preclinical studies for the antidepressant, e.g. serotonergic, activity and anxiolytic effects of saffron or its constituents (Christodoulou et al 2015).

In the clinical setting, a systematic review of six randomized, double-blind trials involving patients diagnosed with major depressive disorder found that *C. sativus* stigma or petal was more effective than placebo and as effective as certain conventional antidepressant medicines in relieving symptoms of depression when administered at doses of 30 mg/day for up to 8 weeks (Lopresti and Drummond 2014). Larger trials are required to confirm these results. Types of adverse events reported for saffron in these clinical trials were similar to those reported for placebo and standard antidepressants, though actual numbers of events differed.

The clinical relevance of the effects of saffron on platelet activity and blood coagulation reported in preclinical studies is unclear. Until further information is available, the use of saffron by patients taking antiplatelet or anticoagulant medicines should be avoided. Although saffron is used in foods, further investigation of its safety profile, including that of its important constituents, when used as a medicine is required (Alavizadeh and Hosseinzadeh 2014). Saffron is toxic at daily doses of 5 g or higher.

ST JOHN'S WORT, *HYPERICUM PERFORATUM* L. (HYPERICI HERBA) EP

The common name St John's wort is used for several hundred different species of the genus *Hypericum*. In developed countries, it is usually used to refer to herbal medicinal products containing *Hypericum perforatum* L., though other *Hypericum* species are used medicinally in other parts of the world.

St John's wort (Hypericaceae) has a history of medicinal use, particularly as a 'nerve tonic' and in the treatment of nervous disorders. It is commonly used to treat mild and moderate forms of depression and is registered in the UK for the treatment of 'slightly low mood and mild anxiety'.

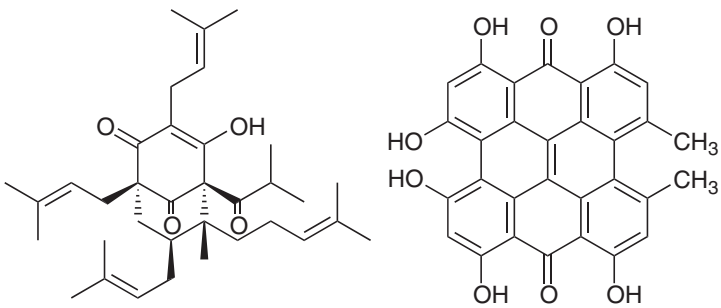
It is an herbaceous perennial plant native to Europe and Asia. The name St John's wort may have arisen as the flowers bloom in late June around St John's day (24 June). Herbal products containing St John's wort have been among the top-selling herbal preparations in developed countries in recent years. The dried herb (consisting mainly of the flowering tops, including leaves, unopened buds and flowers) is the part used pharmaceutically.

Constituents

St John's wort contains a series of naphthodianthrones, which include hypericin and pseudohypericin, and the prenylated phloroglucinols, such as hyperforin and adhyperforin. Initially, hypericin was considered to be the antidepressant constituent of St John's wort, although evidence has now emerged that hyperforin (Fig. 23.3) is also a major constituent required for antidepressant activity (Barnes et al 2001). Further research is necessary to determine which other constituents contribute to the antidepressant effect. The Eur. Ph. states that the drug should contain not less than 0.08% of total hypericins, expressed as hypericin, calculated with reference to the dried drug. Most products containing standardized extracts of St John's wort are still standardized on hypericin content, as hyperforin is fairly unstable. St John's wort also contains other biologically active constituents, such as flavonoids. The leaves and flowers also contain an essential oil, of which the major components are β-caryophyllene, caryophyllene oxide spathulenol, tetradecanol, viridiflorol, α- and β-pinene, and α- and β-selinene. In 2016, the Medicines and Healthcare products Regulatory Agency (MHRA) recalled several batches of St John's wort products as they were found to contain higher than acceptable concentrations of toxic pyrrolizidine alkaloids; the compounds do not occur naturally in *Hypericum perforatum* and their presence in these batches is thought to be due to the collection of pyrrolizidine-alkaloid-containing weeds along with St John's wort, during the harvesting process (MHRA 2016).

Therapeutic uses and available evidence

The precise mechanism of action for the antidepressant effect of St John's wort is unclear. Results of biochemical and pharmacological studies have suggested that St John's wort extracts inhibit synaptosomal uptake of the neurotransmitters, serotonin (5-hydroxytryptamine, 5-HT), dopamine and noradrenaline (norepinephrine) and GABA. Studies involving small numbers of healthy male volunteers have indicated that St John's wort extracts may have dopaminergic activity and effects on cortisol, which may influence concentrations of certain neurotransmitters. Previous *in vitro* studies suggested that St John's wort inhibited monoamine oxidase, although other studies failed to confirm this. Experimental studies involving animal models of depression provide supporting evidence for the antidepressant



Hyperforin **Hypericin**

Fig. 23.3

effects of St John's wort (Barnes et al 2001). Evidence from randomized controlled trials indicates that preparations of St John's wort extracts are more effective than placebo, and as effective as certain conventional antidepressants, in the treatment of major depression (Linde et al 2008, Nahrstedt and Butterweck 2010). Generally, a few weeks' treatment is required before marked improvement is seen. St John's wort is not recommended for self-treatment of depression.

Standardized extracts of St John's wort are generally well tolerated when used at recommended doses for up to 12 weeks. Adverse effects reported are usually mild, and include gastrointestinal symptoms, dizziness, confusion and tiredness and, rarely, photosensitivity (due to the hypericin content). Data from clinical trials of St John's wort indicate that it has a more favourable short-term safety profile than that of some conventional antidepressants (Linde et al 2008). However, constituents of St John's wort preparations induce the activity of certain cytochrome P450 drug-metabolizing enzymes in the liver, and P-glycoprotein (a drug-transport protein), and can have important interactions with some prescribed medicines. These include anticonvulsants, ciclosporin, digoxin, HIV protease inhibitors, oral contraceptives, selective serotonin reuptake inhibitors, tacrolimus, theophylline, triptans and warfarin. Patients taking these medicines with St John's wort should seek medical advice: in most cases, St John's wort treatment should be stopped, and dose adjustment of the prescribed medicines concerned may be necessary. Women taking St John's wort concurrently with an oral contraceptive should use additional contraceptive measures. Many other medicines are metabolized by the cytochrome P450 (CYP) enzymes induced by St John's wort, and a reduction in drug plasma concentrations is possible with concurrent use. St John's wort should not be used during pregnancy and lactation.

STIMULANTS

CNS stimulants are now rarely employed therapeutically, with the exception of caffeine, although they were important in the treatment of barbiturate poisoning (e.g. picrotoxin) or as a tonic (strychnine). Cola nut extract is used in many herbal tonics and, of course, in the ubiquitous soft drink of the same name. Guarana is an ingredient of some 'energy' drinks and 'healthy' nutritional products. Both cola nut and guarana contain caffeine as the active constituent. Cocaine is more useful medicinally as a local anaesthetic, but its use as a recreational drug is an increasing problem throughout the world.

CAFFEINE EP

Caffeine is a methylxanthine derivative found in tea, coffee and cocoa (Fig. 23.4). It is a mild stimulant, and is added to many analgesic preparations to enhance activity; there is some evidence from clinical trials to support this, though the mechanism is not well-understood (Derry et al 2014). High doses of caffeine may lead to gastric irritation, insomnia, a feeling of anxiety, diuresis, muscle tremors and tachycardia, among other symptoms, and can induce withdrawal syndrome following prolonged use.

COLA NUT, *COLA* SPP. (COLAE SEMEN) EP

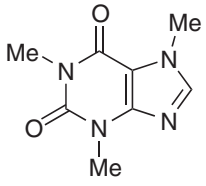
Cola, or kola nut [*Cola nitida* (Vent.) Schott & Endl., *Cola acuminata* (P.Beauv.) Schott & Endl., Sterculiaceae], is native to West Africa and extensively cultivated in the tropics, particularly Nigeria, Brazil and Indonesia. The seed is found in commerce as the dried, fleshy cotyledons, without the testa. They are red-brown in colour, convex on one side and flattened on the other, up to 5 cm long and about 2.5 cm in diameter. The cotyledons of C. *acuminata* are generally smaller and divided into four or six segments.

Cola contains the xanthine derivative caffeine (see Fig. 23.4), with traces of theobromine and theophylline. Tannins and phenolics, including catechin, epicatechin, kolatin, kolatein, kolanin, and amines, including dimethylamine, methylamine, ethylamine and isopentylamine, are also present, together with thiamine and other B vitamins.

Caffeine is a mild stimulant and has diuretic properties; cola extracts are also astringent and antidiarrhoeal due to the tannin content. Cola extracts are an ingredient of many tonics for depression and tiredness, and to stimulate the appetite. Cola extract is safe, apart from any effects due to high doses of caffeine (see above).

GUARANA, *PAULLINIA CUPANA* KUNTH

Guarana (Sapindaceae) is a vine indigenous to the Amazonian rain forest. The seeds are ground to a



Caffeine

Fig. 23.4

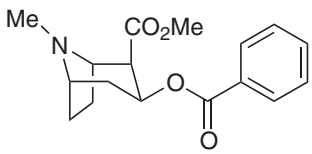
paste and used in cereal bars, or extracted and made into a stimulant drink. The effects are similar to those of cola (see above). The main active constituent is caffeine, which was formerly known as guaranine, and other methylxanthines; it also contains tannins. The stimulant properties of guarana are well-documented in preclinical studies and, to a lesser extent, clinical trials (Schimpl et al 2013).

COCAINE EP

Cocaine (Fig. 23.5) is a tropane alkaloid extracted from the leaf of coca [*Erythroxylum coca* Lam. and *E. novogranatense* (D.Morris) Hieron, Erythroxylaceae]. These are shrubs growing at high altitudes in the South-American Andes. The leaf is still chewed by the local people (along with lime to assist buccal absorption), in order to alleviate symptoms of altitude sickness and fatigue. Cocaine is rarely used medicinally, except as a local anaesthetic in eye surgery, but is now a major illicit drug responsible for many health problems and associated crime. The supply and use of cocaine is strictly regulated in most countries.

ANALGESICS

Two types of analgesics are usually recognized: those that act via the CNS (the opioids), which will be discussed briefly here, and the non-opiate and non-steroidal anti-inflammatory drugs, such as aspirin, which will be covered in Chapter 27. It is very common for the two types to be used in combination, for example, aspirin with codeine. The opioid analgesics and their derivatives have never been surpassed as painkillers in efficacy or patient acceptability despite their disadvantages. They are obtained from the opium poppy (*Papaver somniferum* L.) and the most important are still the alkaloids morphine and codeine. Numerous derivatives, such as oxycodone, dihydrocodeine, fentanyl, buprenorphine and etorphine, have been developed that have different therapeutic and pharmacokinetic profiles, or can be administered via a different route (buccal tablets such as those containing buprenorphine, or transdermal patches, such as with fentanyl). The pharmacology of the opiates is covered in depth in many textbooks and these should be referred to for further information.



Cocaine

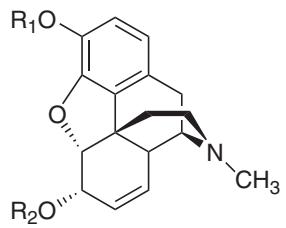
Fig. 23.5

OPIUM, *PAPAVER SOMNIFERUM* L. (OPII CRUDUM; OPII PULVATUS NORMATUS) EP

The opium poppy (*Papaver somniferum* L., Papaveraceae) is an annual that is native to Asia, but is cultivated widely for food (the seed and seed oil), for medicinal purposes and as a garden ornamental. It has been used since time immemorial as a painkiller, sedative, cough suppressant and antidiarrhoeal, and features in ancient medical texts, myths and histories. The flowers vary in colour from white to reddish purple, but are usually pale lilac with a purple base spot. The capsules are subspherical, depressed at the top with the radiating stigma in the centre, below which are the valves through which the seeds are dispersed. The seeds are small, greyish and kidney-shaped. The latex, which exudes from the unripe capsule when scored, dries to form a blackish tarry resin, which is known as opium. For pharmaceutical use it can be treated to form 'prepared opium', but opium or the whole dried capsule (known as 'poppy straw') are now used commercially to extract the alkaloids. The supply and use of these products are strictly regulated in most countries. Poppy seeds are used in cooking.

Constituents

Alkaloids represent about 10% of the dried latex. The major alkaloid is morphine (Fig. 23.6), with codeine and thebaine and lesser amounts of very many others including narceine, narcotine, papaverine, salutaridine, oripavine and sanguinarine.



Morphine, $R_1 = R_2 = H$
Codeine, $R_1 = CH_3$, $R_2 = H$

Fig. 23.6

Therapeutic uses and available evidence

Opium has potent narcotic and analgesic properties. The total alkaloidal extract is known as 'papaveretum' and is used for pre-operative analgesia (now with the narcotine removed due to its reported genotoxicity). Morphine is a very potent analgesic, used for severe pain in the short term (e.g. kidney stone), or for terminal illness, and is the starting material for the production of diamorphine (heroin). Codeine is less potent than morphine, although it is a very useful analgesic for moderate to severe pain, for example for migraine, dental and gynaecological pain. All the opioid analgesics have side effects, which include nausea, constipation and drowsiness; they cause respiratory depression and have a potential for dependence, which varies according to their capacity to induce euphoria. A withdrawal syndrome is common after illicit use (especially with heroin); in recent years, prescription-opioid dependence has emerged as a complex problem (Brady et al 2016).

MIGRAINE

The aetiology of migraine is not fully known and various drugs are used in its treatment. The analgesics mentioned above (particularly codeine) can be used to relieve an attack, although their capacity to induce nausea can cause problems, and aspirin can cause stomach discomfort. The newer synthetic drugs sumatriptan, naratriptan and others are highly effective in acute attacks, and β-blockers and pizotifen taken regularly are used to prevent recurrences. If all else fails, ergotamine can be used in limited doses for acute attacks. Butterbur and feverfew are herbal medicines that have been investigated for migraine prophylaxis.

ERGOTAMINE

Ergotamine is an alkaloid extracted from ergot (*Claviceps purpurea*), a parasitic fungus that grows on cereals, usually rye. It can be used to treat severe migraine that cannot be controlled with other drugs, but it can cause severe adverse reactions and there are restrictions as to the maximum daily and weekly doses. It is not suitable for children.

FEVERFEW, *TANACETUM PARTHENIUM* (L.) SCH. BIP. (TANACETI PARTHENII HERBA) EP

Feverfew [syn. *Chrysanthemum parthenium* (L.) Bernh., Asteraceae] is a perennial herb reaching 60 cm, with a downy erect stem. It has been a common garden plant for many centuries and was found in peasants' gardens throughout Europe. It is still a popular medicinal plant in many parts of the world, to treat rheumatism and menstrual problems. The aerial parts are used. The leaves are yellowish-green, alternate, stalked, ovate and pinnately divided with an entire or crenate margin. The flowers, which appear in June to August, are up to about 2 cm in diameter and arranged in corymbs of up to 30 heads, with white ray florets and yellow disc florets and downy involucral bracts.

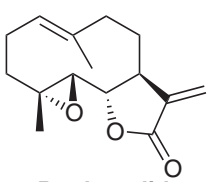
Constituents

The sesquiterpene lactones are essential for the biological activity, the major one being parthenolide (Fig. 23.7), with numerous others reported from the species (e.g. santamarine). It also contains small amounts of essential oil (0.02–0.07%), with α-pinene and derivatives, camphor and others.

Therapeutic uses and available evidence

The main use of feverfew today is as a prophylactic and treatment for migraine. The fresh leaves may be eaten, usually with other foods to disguise the nauseous taste, or a standardized extract taken daily to prevent migraine attacks. Although some clinical studies have shown efficacy, others have not, and further work is needed to identify which extracts may be effective (Wider et al 2015).

Feverfew extracts inhibit secretion of serotonin from platelet granules and proteins from polymorphonuclear leukocytes (PMNs). Since serotonin is implicated in the aetiology of migraine and PMN secretion is increased in rheumatoid arthritis, these findings substantiate the use of feverfew in these conditions. Parthenolide is considered to be the main active constituent, and is a potent inhibitor of NF-κB. The sesquiterpene lactones as a class have effects on several other targets, including the inhibition of prostaglandin production and arachidonic acid release. This explains the antiplatelet and antifebrile actions to some extent, but



Parthenolide

Fig. 23.7

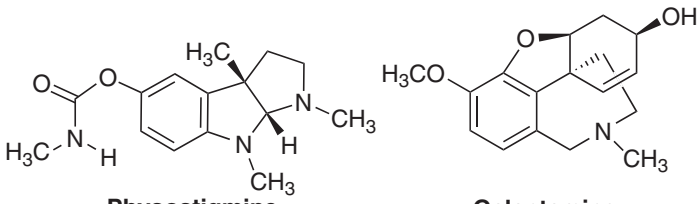


Fig. 23.8

feverfew extract with the parthenolide removed also has anti-inflammatory activity (Sur et al 2009). Feverfew may produce side effects such as dermatitis, and soreness or ulceration of the mouth. Contact dermatitis has been described in workers handling material from this species, caused by exposure to the allergenic sesquiterpene lactones.

BUTTERBUR, *PETASITES HYBRIDUS* (L.) G.GAERTN., B.MEY. & SCHREB., PETASIDIS RHIZOME

Background information on butterbur (Asteraceae) and evidence relating to its use in seasonal allergic rhinitis are discussed in Chapter 22. Butterbur rhizome has also been investigated clinically for its effects in migraine prophylaxis.

Therapeutic uses and available evidence

Butterbur and some of its constituents have been shown to have anti-inflammatory effects in preclinical studies, and this is the basis for their use in migraine prophylaxis. Clinical investigation, however, is limited to a few small studies. A review of two higher-quality studies, both involving the same butterbur rhizome extract standardized for 15% petasins, found that use of 150 mg of the extract daily for 3–4 months was associated with fewer migraine attacks (Agosti et al 2006).

However, use of butterbur in migraine prophylaxis cannot be recommended due to safety concerns. All parts of the plant contain unsaturated pyrrolizidine alkaloids (PAs) (e.g. senecionine, integerrimine, senkirkine) and these compounds are known to be hepatotoxic. Some marketed commercial preparations have steps in their manufacturing process to remove the PAs, but the risks of harm from exposure to even very low concentrations mean that even the use of these preparations cannot be recommended (see Chapter 22, Butterbur).

DRUGS USED FOR COGNITIVE ENHANCEMENT AND IN DEMENTIA

There are few effective treatments for improving memory, especially in dementia. Acetylcholinesterase-inhibiting drugs are available to treat Alzheimer's disease with varying degrees of success. Rivastigmine is a reversible, non-competitive inhibitor of acetylcholinesterase. It is a semi-synthetic derivative of physostigmine, an alkaloid found in the Calabar bean (*Physostigma venenosum* Balf.); a highly poisonous plant indigenous to West Africa. Galantamine (= galanthamine), an alkaloid extracted from the snowdrop (*Galanthus nivalis* L.), was introduced around 2001 (Fig. 23.8, Heinrich and Teoh 2004). These drugs appear to slow down progression of the disease for a period of time, but do not cure it, and have side effects making them unacceptable to many patients.

GINKGO, *GINKGO BILOBA* L. (GINKGO FOLIUM) EP

Ginkgo, the maidenhair tree (Ginkgoaceae), is an ancient 'fossil' tree indigenous to China and Japan and cultivated elsewhere. It is very hardy and is reputed to be the only species to have survived a nuclear explosion. The leaves are glabrous and bi-lobed, each lobe being triangular with fan-like, prominent, radiate veins. The leaves are used medicinally and the fruits are eaten.

Constituents

Ginkgo contains two major classes of actives, both of which contribute to the activity: ginkgolides A, B and C, and bilobalide, which are diterpene lactones; and the biflavone glycosides, such as ginkgetin, isoginkgetin and bilobetin (Fig. 23.9). Ginkgolic acids are present in the fruit, but normally only in very minor amounts in the leaf.

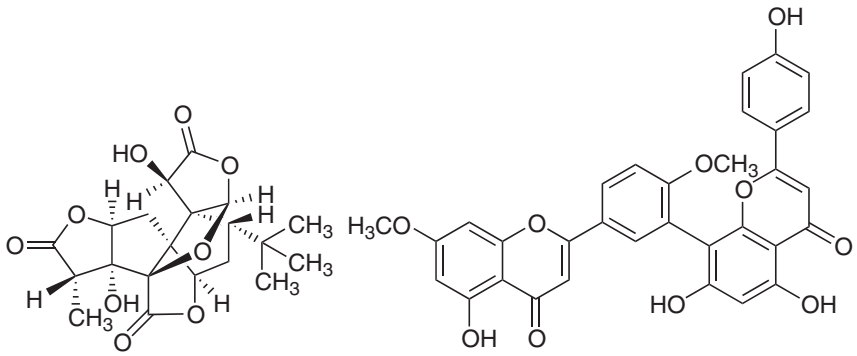


Fig. 23.9

Therapeutic uses and available evidence

Ginkgo has many different uses and has been investigated clinically for its effects in a range of conditions including tinnitus, peripheral arterial occlusive disease, chronic venous insufficiency, acute ischaemic stroke, visual disorders and antidepressant-related sexual dysfunction. It is perhaps best known for its use in cognitive impairment, including reducing or preventing memory deterioration, due to ageing and milder forms of dementia, including the early stages of Alzheimer's disease. It is thought to enhance cognitive processes by improving blood circulation to the brain and through its anti-inflammatory and antioxidant effects. The effects of ginkgo on the CNS are not yet well defined, but include effects on neurotransmitter uptake, neurotransmitter receptor changes during ageing, cerebral ischaemia and neuronal injury; inhibition of nitric oxide may also be involved.

More than 35 clinical trials have assessed the effects of ginkgo extracts (most commonly the standardized *Ginkgo biloba* L. leaf extract EGb-761) in patients with cognitive impairment, including Alzheimer's disease and other forms of dementia, although not all of these trials were of high methodological quality. A Cochrane systematic review concluded that there was no reliable and convincing evidence that ginkgo extracts are efficacious in improving symptoms of cognitive impairment and dementia (Birks and Grimley Evans, 2009). Another review, limited to higher-quality studies, found that ginkgo was more effective than placebo in patients with dementia, but that the clinical importance of this was unclear (Weinmann et al, 2010). There are conflicting results as to whether ginkgo has beneficial effects on cognitive function in cognitively intact individuals (Pharmaceutical Press Editorial 2016).

The usual dose of ginkgo (standardized) extracts is 120–240 mg daily in 2 to 3 divided doses.

Ginkgo has been reported to cause dermatitis and gastrointestinal disturbances in large doses, although rarely. Allergic reactions in sensitive individuals are more likely to be due to ingestion of the fruits due to the ginkgolic acids, which usually are absent from leaf extracts and ginkgo products, or present only in very small amounts. There are isolated reports of bleeding associated with use of ginkgo either alone and when used concurrently with antiplatelet and anticoagulant agents, and non-steroidal anti-inflammatory drugs (Williamson et al 2016).

References

Agosti, R., Duke, R.K., Chrubasik, J.E., Chrubasik, S., 2006. Effectiveness of *Petasites hybridus* preparations in the prophylaxis of migraine: a systematic review. Phytomedicine: International Journal of Phytotherapy and Phytopharmacology 13, 743–746.

Alavizadeh, S.H., Hosseinzadeh, H., 2014. Bioactivity assessment and toxicity of crocin: A comprehensive review. Food and Chemical Toxicology 64, 65–80.

Barnes, J., Anderson, L.A., Phillipson, J.D., 2001. St John's wort (*Hypericum perforatum* L.): a review of its chemistry, pharmacology and clinical properties. Journal of Pharmacy and Pharmacology 53, 583–600.

Birks, J., Grimley Evans, J., 2009. *Ginkgo biloba* for cognitive impairment and dementia. Cochrane Database of Systematic Reviews 1,

CD003120. http://dx.doi.org/10.1002/14651858.CD003120.pub3.

Boskabady, M.H., Farkhondeh, T., 2016. Antiinflammatory, antioxidant, and immunomodulatory effects of *Crocus sativus* L. and its main constituents. Phytotherapy Research 30 (7), 1072–1094.

Brady, K.T., McCauley, J.L., Back, S.E., 2016. Prescription opioid misuse, abuse, and treatment in the United States: an update. American Journal of Psychiatry 173 (1), 18–26.

Burns, A., Perry, E., Holmes, C., et al., 2011. A double-blind placebo-controlled randomized trial of *Melissa officinalis* oil and donepezil for the treatment of agitation in Alzheimer's disease. Dementia and Geriatric Cognitive Disorders 31 (2), 158–164.

Cases, J., Ibarra, A., Feuillère, N., Roller, M., Sukkar, S.G., 2011. Pilot trial of *Melissa officinal*is L. leaf extract in the treatment of volunteers suffering from mild-to-moderate anxiety disorders and sleep disturbances. Mediterranean Journal of Nutrition and Metabolism 4 (3), 211–218.

Christodoulou, E., Kadoglou, N.P., Kostomitsopoulos, N., Valsami, G., 2015. Saffron: a natural product with potential pharmaceutical applications. Journal of Pharmacy and Pharmacology 67 (12), 1634–1649.

Derry, C.J., Derry, S., Moore, R.A., 2014. Caffeine as an analgesic adjuvant for acute pain in adults. Cochrane Database of Systematic Reviews 12, CD009281. http://dx.doi.org/10.1002/14651858.CD009281.pub3.

Gloss, D., Vickrey, B., 2014. Cannabinoids for epilepsy. Cochrane Database of Systematic Reviews 3, CD009270. http://dx.doi.org/10.1002/14651858.CD009270.pub3.

Heinrich, M., Lee Teoh, H.L., 2004. Galanthamine from snowdrop – the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. Journal of Ethnopharmacology 92, 147–162.

Linde, K., Berner, M.M., Kriston, L., 2008. St John's wort for major depression. Cochrane Database of Systematic Reviews 4, CD000448. http://dx.doi.org/10.1002/14651858.CD000448.pub3.

Lopresti, A.L., Drummond, P.D., 2014. Saffron (*Crocus sativus*) for depression: a systematic review of clinical studies and examination of underlying antidepressant mechanisms of action. Human Psychopharmacology 29 (6), 517–527.

Medicines and Healthcare products Regulatory Agency 2016 Press release, February 2016. Precautionary recall – six batches of St John's wort tablets 8. Available online at https://www.gov.uk/government/news/precautionary-recall-six-batches-of-st-johns-wort-tablets.

Miroddi, M., Calapai, G., Navarra, M., Minciullo PL., Gangemi, S., 2013. *Passiflora incarnata* L.: ethnopharmacology, clinical application, safety and evaluation of clinical trials. [Review] Journal of Ethnopharmacology 150 (3), 791–804.

Miyasaka, L.S., Atallah, Á.N., Soares, B., 2007. Passiflora for anxiety disorder. Cochrane Database of Systematic Reviews 1, CD004518. http://dx.doi.org/10.1002/14651858.CD004518.pub2.

Nahrstedt, A., Butterweck, V., 2010. Lessons learned from herbal medicinal products: the example of St. John's wort (perpendicular). Journal of Natural Products 73, 1015–1021.

Nenadis, N., Heenan, S., Tsimidou, M.Z., Van Ruth, S., 2016. Applicability of PTR-MS in the quality control of saffron. Food Chemistry 196, 961–967.

Perry, E., Howes, M.-J., 2011. Medicinal plants and dementia therapy: herbal hopes for brain aging? CNS Neuroscience and Therapeutics 17 (6), 683–698.

Pharmaceutical Press Editorial, 2016. Herbal medicines. Pharmaceutical Press, London.

Salter, S., Brownie, S., 2010. Treating primary insomnia – the efficacy of valerian and hops. Australian Family Physician 39, 433–437.

Sarris, J., Kavanagh, D.J., Byrne, G., Bone, K.M., Adams, J., Deed, G., 2009. The Kava Anxiety Depression Spectrum Study (KADSS): a randomized, placebo-controlled crossover trial using an aqueous extract of *Piper methysticum*. Psychopharmacology (Berl) 205, 399–407.

Sarris, J., LaPorte, E., Schweitzer, I., 2011. Kava: a comprehensive review of efficacy, safety, and psychopharmacology. [Review] Australian and New Zealand Journal of Psychiatry 45 (1), 27–35.

Schimpl, F.C., Ferreira da Silva, J., Francisco de Carvalho, J., Mazzafera, G.P., 2013. Guarana: revisiting a highly caffeinated plant from the Amazon. Journal of Ethnopharmacology 150 (1), 14–31.

Schmidt, M., 2014. German court ruling reverses kava ban; German regulatory authority appeals decision. HerbalGram 11 (7).

Soffritti, G., Busconi, M., Sanchez, R.A., et al., 2016. Genetic and epigenetic approaches for the possible detection of adulteration and auto-adulteration in saffron (*Crocus sativus* L.) spice. Molecules 21 (3), 343. http://dx.doi.org/10.3390/molecules21030343.

Sur, R., Martin, K., Liebel, F., Lyte, P., Shapiro, S., Southall, M., 2009. Anti-inflammatory activity of parthenolide-depleted Feverfew *(Tanacetum parthenium)*. Inflammopharmacology 17, 42–49.

Weinmann, S., Roll, S., Schwarzbach, C., Vauth, C., Willich, S.N., 2010. Effects of *Ginkgo biloba* in dementia: systematic review and meta-analysis. BMC Geriatrics 10, 14.

Wider, B., Pittler, M.H., Ernst, E., 2015. Feverfew for preventing migraine.

Cochrane Database of Systematic Reviews 4, CD002286. http://dx.doi.org/10.1002/14651858.CD002286.pub3.

Williamson, E.M., Driver, S., Baxter, K., Preston, C.L., 2016. Stockley's herbal medicines interactions. Pharmaceutical Press, London.

Woolven, L., Snider, T., 2016. Saffron: the salubrious spice. HerbalGram 110, 61–71.

Zanoli, P., Zavatti, M., 2008. Pharmacognostic and pharmacological profile of *Humulus lupulus* L. Journal of Ethnopharmacology 116, 383–396.

Zhang, L.Y., Rowe, A., Ramzan, I., 2011. Does inflammation play a role in kava hepatotoxicity? Phytotherapy Research 25 (4), 629–630.

Chapter 24

Infectious diseases

Preparations from traditional medicines form the basis of many anti-infective medicines used to cure topical and systemic infections caused by microbes, in particular bacteria, and there is an enormous body of primary literature concerning modern and historical uses of plants to treat a wide range of infections. The plant may be prepared as a poultice of the crude drug or an extract to be applied topically to improve wound healing. These preparations may prevent microbial growth and some cases also promote wound repair by stimulating cellular growth. Natural products produced by plants also have antiprotozoal and insecticidal activity, and many, especially those containing essential oils, are active against all of these. Intestinal worms can be treated with medicinal plants such as wormseed and wormwood (*Artemisia* spp.), and tannin-containing herbs, but the most effective and least toxic anthelmintic drugs at present are synthetic, so these will not be covered here.

Therefore, plants are a valuable source of antimicrobial natural products and the most commonly occurring are essential oil components such as the phenolic monoterpene **thymol** (Fig. 24.1) from thyme (*Thymus vulgaris* L., Lamiaceae; see Chapter 22).

Thymol has a range of antibacterial and antifungal properties (Marchese et al 2016a) and is likely to be produced by the plant to protect itself from plant pathogenic microbes and insects present in its environment. This is an example of an **intrinsic antimicrobial** natural product that the plant produces as a normal part of its chemistry that can be used medicinally. Plants also have the ability to produce antimicrobial natural products when they are under attack from microbes, herbivores and insects. These compounds are very quickly synthesized by the plant and are called **phytoalexins**, which display antimicrobial properties to a wide range of bacteria and fungi. Examples of this phenomenon include the potato, which when inoculated with a fungus synthesizes the antimicrobial coumarin **scopoletin** (Fig. 24.2; see also Chapter 6) and 3,5-dihydroxy-bisbenzyl, which is produced by a species of yam [*Dioscorea cayennensis* subsp. *rotundata* (Poir.) J.Miège, Dioscoreaceae].

Berberine (Fig. 24.3) is a protoberberine benzylisoquinoline alkaloid found in many widely used medicinal plant species, such as *Coptis chinensis* Franch. (Ranunculaceae), and species of *Berberis*, *Mahonia*, *Hydrastis* and *Phellodrendron*. It has antidiabetic and other effects in addition to its antibacterial and antiprotozoal effects (Kumar et al 2015). Berberine has potent antibacterial effects against, for example, *Staphylococcus aureus*, but it is readily extruded by multi-drug resistance (MDR) pumps, leading to resistance. However, several *Berberis* species also synthesize an inhibitor of the NorA MDR pump, 5′-methoxyhydnocarpin (5′-MHC), which has no antimicrobial activity alone but strongly potentiates the action of berberine by inhibiting MDR-dependent efflux from *S. aureus* cells. The level of accumulation of berberine in bacterial cells is increased strongly in the presence of 5′-MHC, indicating that it effectively disables

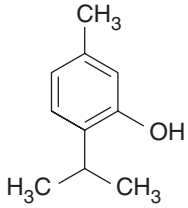
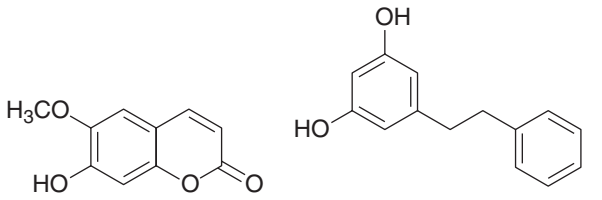
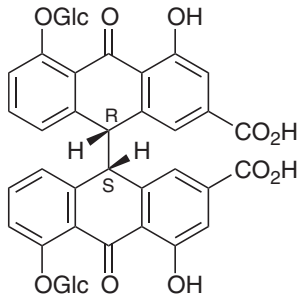


Fig. 24.1 Thymol



Scopoletin **3,5-dihydroxy-bisbenzyl from *Dioscorea rotundata***

Fig. 24.2 Scopetin



Sennoside B

Fig. 24.3 Berberine

the resistance mechanism (Stermitz et al 2000). This example also illustrates the use of plant compounds as resistance-modifying agents, as discussed in Chapter 6, Natural Product Chemistry.

Plant antibacterials are very different in their chemistry and mechanisms of antimicrobial action to existing antibiotics (Appendino et al 2010, Gibbons 2004, 2008), which are often derived from fungi, such as the beta-lactams (e.g. penicillins from *Penicillium* species), or different classes of bacteria, such as the tetracyclines and macrolides (the erythromycins, from actinobacteria such as *Streptomyces*). Plant-derived antibacterials function through an as yet poorly understood mechanisms of action: for example, thymol has been shown to cause perturbation of the lipid fraction of the bacterial plasma membrane, resulting in the leakage of intracellular materials, and it also inhibited biofilm formation and inactivated mature biofilms of *Listeria monocytogenes* (Marchese et al 2016a). Ginger constituents interfere with quorum sensing (Kumar et al 2014), and liquorice flavonoid components inhibited protein synthesis, DNA gyrase and dihydrofolate reductase of *Helicobacter pylori in vitro* (Asha et al 2013). Cranberry extracts (see below) exhibit anti-adhesion properties, preventing bacteria binding to the uroepithelium cells. Berberine inhibits the synthesis of proteins associated with the growth and cleavage of bacteria (Kumar et al 2015).

This variety of mechanisms makes plant-derived antibacterials valuable where bacterial resistance to conventional antibiotics (beta-lactams, macrolides, tetracyclines) has arisen, offering opportunities for treating MRSA (meticillin-resistant *Staphylococcus aureus*), which has become practically impossible to treat, and infections such as tuberculosis where the causative organism, *Mycobacterium tuberculosis*, is often resistant to existing antibiotics. New agents that function by a different mechanism are thus urgently needed, and plants offer great potential in this area (see Abreu et al [2012]).

BROAD-SPECTRUM ANTIMICROBIAL AGENTS

For details on Umckaloabo [*Pelargonium sidoides* DC., Geraniaceae, and *P. reniforme* (Andrews) Curtis (Pelargonii radix)] see Chapter 22 (The respiratory system).

LEMON BALM, *MELISSA OFFICINALIS* L. (MELISSAE FOLIUM, LAMIACEAE) EP

This aromatic species has long use (Shakeri et al 2016) as an antimicrobial and carminative and mild sedative (for the latter see Chapter 23, The central nervous system).

There are a number of topical formulations that are marketed for *Herpes simplex* virus skin lesions and there are clinical data and some *in vitro* studies confirming these using extracts of *Melissa officinalis*. It is generally well-tolerated, although it has been suggested that long-term use may interfere with thyroid function.

Constituents

Both the polyphenolics and the essential oil are thought to be responsible for the antimicrobial effects.

Phenolics include protocatechuic acid, caffeic acid, rosmarinic acid and tannins, in significant amounts, together with flavonoids such as cynaroside, cosmosiin, isoquercitrin and others. The volatile oil consists mainly of α- and β-citral (= neral and geranial), with caryophyllene oxide, linalool, citronellal, nerol, geraniol, germacrene-D, traces of eugenyl acetate, *cis*- and *trans*-β-ocimene, copaene and others.

Therapeutic uses and available evidence

Lemon balm is antimicrobial, carminative and sedative (Shakeri et al 2016). Hot-water extracts have antiviral properties, mainly due to the polyphenolic acids. Topical formulations are used for *herpes simplex* virus skin lesions, the antiviral activity having been confirmed *in vitro* and also in clinical trials. Aqueous extracts also inhibit division of tumour cells, and tannin-free extracts inhibit protein biosynthesis. It is used as an ingredient of herbal teas, often with other medicinal plants, for nervous disorders and insomnia (see Chapter 23). The activity is at least in part due to the presence of rosmarinic acid and related compounds. Lemon balm is well tolerated, although it should not be taken internally in high doses over a long period because of its reputed antithyroid activity.

GARLIC, *ALLIUM SATIVUM* L. (ALLII SATIVI BULBUS) EP

Garlic, and other *Allium* sp. (Amaryllidaceae), have a very long history in anti-infective preparations used topically and systemically. The many other acclaimed therapeutic benefits of garlic, including its antihypertensive, antithrombotic, fibrinolytic, anticancer, antidiabetic and lipid-lowering properties, are not covered here, but some are covered in Chapter 21 (The cardiovascular system).

There any many *in vitro* studies showing the effects of the extracts and oils of the bulbs of various *Allium* species with activity against various bacteria, fungi and viruses. Members of the genus have a long and rich usage as culinary herbs with onions, garlic, shallots and chives all having antimicrobial properties.

Constituents

The antimicrobial constituents are the sulphur compounds, which include allicin (Fig. 24.4), which degrades naturally to form allylmethyl trisulphide, diallyl disulphide, diallyl trisulphide, diallyl tetrasulphide, allylpropyl disulphide, and glycosides such as

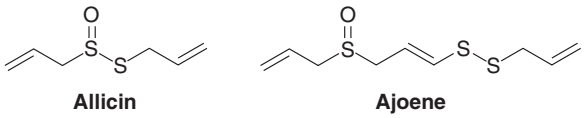


Fig. 24.4 Allicin and ajoene.

sativoside B1 (Casella et al 2013). Monoterpenoids (citral, geraniol, linalool and α- and β-phellandrene) and flavonoids based on kaempferol and quercetin are also present. It is possible that the subterranean bulbs of these species produce these sulphur natural products as a protection against microbes in their environment.

Therapeutic uses and available evidence

Garlic extracts have been shown to have antibiotic, expectorant and anti-thrombotic properties and many garlic preparations are marketed for their anti-blood-clotting properties to give some protection against atherosclerosis. Preparations from common garlic have also found much use in the treatment for respiratory tract infections, such as common cold, flu and bronchitis, and the allyl sulphides such as allicin and ajoene are strongly antimicrobial having activity against *Staphylococcus aureus*, *Streptococcus* species and even some Gram-negative bacteria such as *Helicobacter pylori*, the major bacterial causative agent of stomach ulcers. The chemical mechanisms underlying the antimicrobial activity of allicin are poorly understood, but it is known to alter the free amino acid l-cysteine through formation of allyl-disulphides (Marchese et al 2016b).

Garlic preparations are generally very well tolerated with low toxicity (other than what some consider an offensive smell!), although it has been suggested that these materials may have the ability to interfere with anti-platelet drugs.

TEA TREE AND TEA TREE OIL, *MELALEUCA ALTERNIFOLIA* (MAIDEN ET BETCHE) CHEEL (MELALEUCAE ATHEROLEUM) EP

The oil from the leaves and stems of this tree has a long traditional usage amongst indigenous peoples of North Australia and New South Wales. This tree grows with other species of Myrtaceae, such as *Eucalyptus*, which are also used topically as antimicrobial agents. Over the last 30 years there has been an explosion in the usage of tea tree oil products in Europe and the United States and one cannot go into a pharmacy without seeing dozens of preparations including soaps, shampoos, creams, lotions and gels containing this oil, which has a distinctive 'dry'

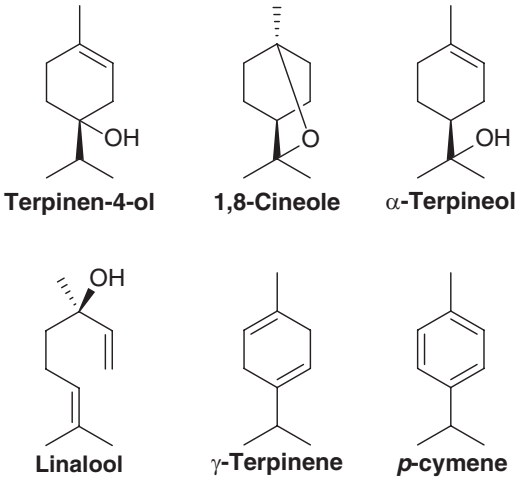


Fig. 24.5 Terpine-4-ol etc

aroma. The leaves and twigs undergo distillation to produce the oil, which is pale yellow to colourless. Traditionally the oil is used topically as an antimicrobial for skin infections, to reduce bruising and for insect bites.

Constituents

Tea tree oil is a highly complex mixture of monoterpenes and the major component is terpinen-4-ol (Fig. 24.5), which may be present at concentrations as high as 30%.

Some varieties are rich in 1,8-cineole, which is present in *Eucalyptus* oil, but the best-quality tea tree oils are low in 1,8-cineole and high in terpinen-4-ol. Other monoterpenes present include γ- and α-terpineol, α- and β-pinene, α-terpineol, limonene and cymene, and the sesquiterpenes cubebol, epicubebol, cubenol, epicubanol and δ-cadinene. The composition of the oil may also depend on the method of distillation.

Therapeutic uses and available evidence

Tea tree oil is now used worldwide in the form of skin creams for pimples and acne, pessaries for vaginal thrush, as an inhalation for respiratory disorders and in pastilles for sore throats. It is also popular as a lotion for the treatment of lice and scabies infestations and for dandruff and other hair and scalp disorders. The oil has broad-spectrum antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and various pathogenic fungi and yeasts including *Candida albicans*, and also against the protozoa *Leishmania major* and *Trypanosoma brucei*. There have also been studies conducted using preparations containing the oil to reduce the spread of meticillin-resistant *Staphylococcus aureus* (MRSA) in hospital units (Warnke et al 2009) and there has been much research into the use of this oil as an antiseptic. Importantly, there is no evidence for the induction of bacterial resistance after repeated low-dose application (habituation) of tea tree oil (Thomsen et al 2013). In a study on oral microorganisms, Karbach et al (2015) demonstrated the antimicrobial effects of tea tree oil, with lemon grass [*Cymbopogon citratus* (DC.) Stapf] being a more potent antibacterial agent.

The most active constituents include terpinen-4-ol, γ-terpinene, α-terpineol and linalool with minimum inhibitory concentrations in the range of 0.125–0.25% v/v (Carson et al 2006, Cox et al 2001). These compounds demonstrate broad-spectrum activity towards Gram-negative bacteria. Clinical trials have supported many of the uses of tea tree oil, including for *herpes labialis*, although most of the studies are rather small (see Carson et al 2006). Undiluted essential oils can cause skin irritation and tea tree oil should be used with care. It should only be taken internally in small doses.

The related manuka tree (*Leptospermum scoparium* J.R.Forst. et G.Forst.) is sometimes referred to as 'New Zealand tea tree', and is used for similar purposes. Honey made from this plant (Manuka honey) is widely promoted as an antibacterial agent.

BEARBERRY, *ARCTOSTAPHYLOS UVA-URSI* (L.) SPRENG. (UVAE URSI FOLIUM) EP

The leaves of the shrub *Arctostaphylos uva-ursi* (Ericaceae), widely known as Uva-Ursi or bearberry, are used to treat cystitis and urethritis, although the efficacy is not supported by evidence from randomized controlled trials.

Constituents

Hydroquinone derivatives are characteristic, notably the glycoside **arbutin,** which is hydrolyzed *in vivo* by the enzyme β-glucosidase to give the diphenol, **hydroquinone** (Fig. 24.6) with antiseptic properties. Other constituents include terpenoids such as α- and β-amyrin, flavonoids and tannins.

Therapeutic uses and available evidence

Hydroquinone is a potent phenolic antiseptic that is very active against many bacteria, in particular those

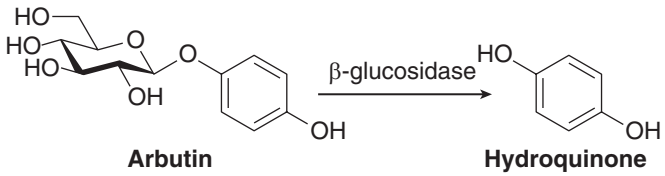


Fig. 24.6 Hydrolyzation of arbutin to hydroquinone

that are liable to cause urinary tract infections such as *Escherichia coli* and *Pseudomonas aeruginosa*. Activity has also been demonstrated against bacterial species such as *Bacillus subtilis* and *Staphylococcus aureus*. Uva-ursi is also mildly diuretic and antilithuric (Butterweck and Khan 2009). Uva ursi preparations require that the urine is alkaline for it to have antiseptic properties and, as such, acidic foods including cranberry juice (see below) should be avoided during treatment. Hydroquinone is a very reactive and biologically active compound and is cytotoxic and mutagenic. High doses and prolonged usage of bearberry products should be avoided and it should not be used during pregnancy or by anyone who has a kidney infection.

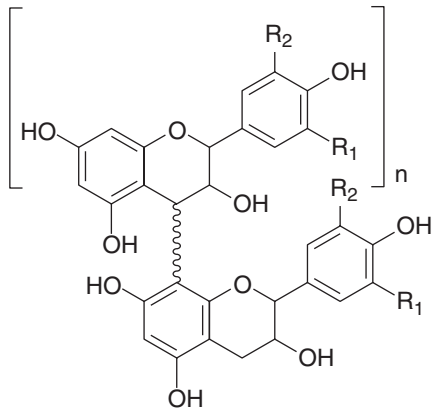


Fig. 24.7 Proanthocyanidins

CRANBERRY JUICE, *VACCINIUM MACROCARPON* AITON

This is one of the most popular of the plant-derived products with preparations generally being taken to treat urinary tract infections in the form of the juice of the berries of *V. macrocarpon* and related species (Ericaceae) or a freeze-dried extract, which is then re-suspended in water. In North America the plant was used traditionally for the same purpose.

Constituents

The chemistry of this plant is still not well understood because it contains many complex **flavonoid polymers**, in particular the **proanthocyanidins** that are believed to be important for the antibacterial activity of this species (Fig. 24.7).

These proanthocyanidins are exceptionally complex and vary in the number of flavonoid units in the polymer, the way in which each of the units is connected, and the functional groups present on each unit (R_1 and R_2 groups in Fig. 24.7 may be OH or OMe for example). This can give rise to a highly complex mixture. These compounds are polar and soluble in water, ethanol and methanol, which can make their analysis by conventional methods such as HPLC and HPLC-MS difficult.

Therapeutic uses and available evidence

A large number of clinical trials in a range of urinary tract problems has been reported and the best evidence for cranberry's effectiveness seems to be in clinical trials assessing the *prevention* of recurrent urinary tract infections in generally healthy women, although recent studies have not provided supporting evidence (Jepson et al 2012). In complicated urinary tract infections or as a treatment for urinary tract infections (Liska et al 2016) there seem to be differences in the response of different groups of patients.

In vitro experiments with cranberry juice and proanthocyanidins have shown that they have the ability to affect the binding of *Escherichia coli*, which is a major causative agent of urinary tract infections, to uroepithelial cells inhibiting adherence of this bacterium allowing its clearance. Cranberry juice is also thought to act by increasing levels of hippuric acid

(a metabolite of benzoic acid) and therefore acidity of the urine.

Cranberry juice has a high calorific content, and patients with diabetes who wish to use cranberry juice should use sugar-free preparations. Reports that cranberry juice interacts with warfarin are controversial, and may depend on a patient's pharmacogenetics, but it would be prudent for all patients on anticoagulant therapy of all types to avoid taking cranberry products regularly. Cranberries have also been used for unrelated disorders such as kidney stones, and are frequently used in foods.

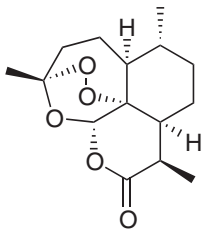
ANTIPROTOZOAL AGENTS

The classical antiprotozoal drug, used to treat malaria, is quinine, from *Cinchona* bark. It is still occasionally used to treat the disease, but more importantly it is the template for the production of newer semi-synthetics such as chloroquine and mefloquine, and others now under development. Most of these are also used for malaria prophylaxis.

SWEET WORMWOOD (SYN. QINGHAOU), *ARTEMISIA ANNUA* L.

The most recent antimalarial to be introduced clinically is artemisinin (from some types of sweet or annual wormwood, *Artemisia annua* L., Asteraceae) or the more stable derivative, artemether, which is on the WHO list of essential medicines and used for multiple drug-resistant strains of *Plasmodium falciparum* malaria. Another important derivative is the water-soluble artesunate, which can therefore be given in injections. The discovery of artemisinin marks a milestone in natural product research and in 2015 the achievements of Prof Youyou Tu (China) were rewarded with the Nobel Prize in Medicine/Physiology (Tu 2016).

Sweet or annual wormwood (*Artemisia annua* L.), is a native of temperate parts of Asia, particularly China. It is a prostrate or erect annual with woody stems, pinnately divided leaves and small yellow flowers arranged in panicles. It has a characteristic sweet, aromatic, odour. The plant has been used for thousands of years in China for fevers and disorders of the liver. It is highly effective for the treatment of malaria, especially against resistant strains of *Plasmodium berghei* and *P. falciparum*, and this is now the major use of the plant.



Artemisinin

Fig. 24.8 Artemisinin, artesunate, etc.

Constituents

The herb contains sesquiterpene lactones, the most important of which is artemisinin (qinghaosu; Fig. 24.8), as well as the arteannuins A–O, artemisitine, artemisinic acid, hydroarteannuin, and others. There is also a volatile oil containing artemisia ketone, cadinene and others, and flavonoids including artemetin.

Therapeutic uses and available evidence

Artemisinin is one of the most rapidly acting antiplasmodial compounds known. Several more stable and effective derivatives, such as artemether, arteether and artesunate (Fig. 24.8), have been developed and are being used clinically for both the prophylaxis and treatment of malaria (see Cui and Su 2009). The species appears to be fairly non-toxic, although cytotoxicity *in vitro* and teratogenic effects have been observed in mice. There is evidence that the whole extract of the aerial parts may be superior to isolated artemisinin, since the flavonoids present in the leaves have been linked to suppression of CYP450 enzymes responsible for altering the absorption and metabolism of artemisinin in the body, and also to a beneficial immunomodulatory activity in subjects afflicted with parasitic and chronic diseases (Ferreira et al 2010).

A huge number of clinical, biochemical, pharmacological and other studies is available on these substances and on many other derivatives. For example, based on a Cochrane analysis of eight trials, involving a total of 1664 adults and 5765 children, Sinclair et al (2012) showed that parenteral artesunate is clearly superior to quinine for the treatment of severe malaria in both adults and children and in different regions of the world.

The most important protozoal diseases are endemic in the tropics (e.g. bacillary dysentery), and many (e.g. *Leishmania*) involve a non-human vector,

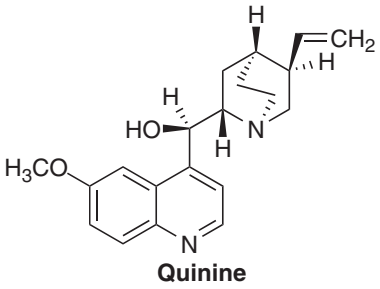


Fig. 24.9 Quinine

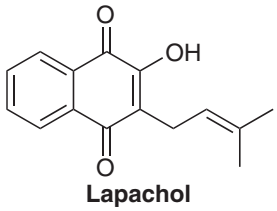


Fig. 24.10 Lapachol

which may be an insect, larva or snail. Control of these diseases therefore includes the use of pesticides to destroy the vector, improvement in hygiene and water supplies, as well as targeting the parasite. For many developing countries, the use of plant-based antiprotozoals, mosquito-repellents and pesticides represents the best chance of some sort of disease control.

CINCHONA, *CINCHONA* SPP. (CINCHONAE CORTEX) EP

Trees of the genus *Cinchona* (Rubiaceae) are used as a source of quinine (Fig. 24.9). Red cinchona, 'cinchona rubra', is *C. pubescens* Vahl [=*C. succirubra* Pav. ex Klotzsch; yellow cinchona, 'cinchona flava', is *C. calisaya* Wedd. Other species and hybrids of the genus *Cinchona* are also used. It has been called Peruvian bark, from the country of origin, and also Jesuit's bark, since it was originally introduced into Europe by Jesuit missionaries. It is native to mountainous regions of tropical America, and cultivated in South-East Asia and parts of Africa. The bark is found in commerce as quills or flat pieces. The external surface is brownish-grey, usually fissured, and lichens and mosses may be seen as greyish-white or greenish patches.

Constituents

The actives are fluorescent quinoline alkaloids, the major being quinine (Fig. 24.9), with quinidine, cinchonine, cinchonidine, epi- and hydro- derivatives of these, quinamine and others. The total alkaloid content of the bark should be not less than 6.5%, with 30–60% being of the quinine type. Identification is by thin-layer chromatography (TLC). Their discovery is one of the classical examples of research in the 19th century (see Chapter 2, p. 24).

Therapeutic uses and available evidence

Quinine was primarily used as an antimalarial before the advent of semi-synthetics, which have improved efficacy, especially against resistant strains, different pharmacokinetic profiles and reduced toxicity. The bark was formerly used as a febrifuge, tonic, orexigenic, spasmolytic and astringent, but it is only used now for the extraction of the alkaloids, quinine and its isomer quinidine. Both quinine and quinidine have antimalarial activity, although quinine is more widely used. Both are cardiac anti-arrhythmic agents (see Chapter 21), which limits their usefulness as antimalarials, and quinidine is still used clinically for this purpose. Quinine salts are used for the prevention of night cramps (the dose for this purpose is 200–300 mg of quinine sulphate or bisulphate) and in low doses is an ingredient of some analgesic and cold and flu remedies. Chronic overdosage can result in the condition known as cinchonism, which is characterized by headache, abdominal pain, rashes and visual disturbances. Cinchona and quinine should not be taken in large doses during pregnancy except for treating malaria.

LAPACHO (TAHEEBO, PAU D'ARCO), *TABEBUIA* SPP.

Lapacho is obtained from the bark of several tropical tree species of the genus *Handroanthus* (Bignoniaceae), including *H. impetiginous* (Mart. ex DC.) Mattos. (syn.: *Tabebuia avellanedae* Lorentz ex Griseb.), *H. serratifolius* (Vahl) S.O.Grose (syn.: *T. serratifolia* (Vahl) G.Nicholson) and *T. rosea* (Bertol.) Bertero ex A.DC. and others indigenous to South America. Lapacho is used traditionally for infectious diseases, including protozoal, bacterial, fungal and viral infections, to enhance immune function and for treating various cancers. Lapachol (Fig. 24.10) is antiprotozoal against *Leishmania*, *Trypanosoma* and *Schistosoma* spp., as well as being anti-inflammatory.

Constituents

The active constituents are naphthoquinones, the most important being lapachol (Fig. 24.10), with deoxylapachol, α- and β-lapachone and others. It also contains anthraquinones, benzoic acid and benzaldehyde derivatives.

Therapeutic uses and available evidence

Antimicrobial effects are documented against *Candida, Brucella* and *Staphylococcus* spp., and for several viruses. Lapacho is also becoming popular as an unlicensed anticancer treatment; antitumour activity has been shown *in vitro* and *in vivo* and a few uncontrolled trials have been carried out. The evidence available is so far inconclusive and this botanical drug should not be recommended for the treatment of cancer. Semi-synthetic derivatives of lapachol are being prepared in order to increase activity and reduce toxicity. Lapachol is cytotoxic in large doses, and inhibits pregnancy in mice; however, there is little evidence of toxicity for the herbal drug when used in normal doses. For a review, see Gómez Castellanos et al (2009).

References

Abreu, A.C., McBain, A.J., Simões, M., 2012. Plants as sources of new antimicrobials and resistance-modifying agents. Natural Product Reports 29 (9), 1007–1021.

Appendino, G., Fontana, G., Pollastro, F., 2010. Natural products drug discovery. In: Mander, L., Lui, H.W. (Eds.), Comprehensive Natural Products II Chemistry and Biology. vol. 3. Elsevier, Oxford, pp. 205–236.

Asha, M.K., Debraj, D., Prashanth, D., et al., 2013. In vitro anti-*Helicobacter pylori* activity of a flavonoid rich extract of *Glycyrrhiza glabra* and its probable mechanisms of action. Journal of Ethnopharmacology 145 (2), 581–586.

Butterweck, V., Khan, S.R., 2009. Herbal medicines in the management of urolithiasis: alternative or complementary? Planta Medica 75 (10), 1095–1103.

Carson, C., Hammer, K.A., Riley, T.V., 2006. *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. Clinical Microbiology Reviews 19 (1), 50–62.

Casella, S., Leonardi, M., Melai, B., Fratini, F., Pistelli, L., 2013. The role of diallyl sulfides and dipropyl sulfides in the in vitro antimicrobial activity of the essential oil of garlic, *Allium sativum* L., and leek, *Allium porrum* L. Phytotherapy Research 27 (3), 380–383.

Cox, S.D., Mann, C.M., Markham, J.L., 2001. Interactions between components of the essential oil of *Melaleuca alternifolia*. Journal of Applied Microbiology 91, 492–497.

Cui, L., Su, X.Z., 2009. Discovery, mechanisms of action and combination therapy of artemisinin. Expert Review of Anti-infective Therapy 7 (8), 999–1013.

Ferreira, J.F., Luthria, D.L., Sasaki, T., Heyerick, A., 2010. Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. Molecules 15 (5), 3135–3170.

Gibbons, S., 2004. Anti-staphylococcal plant natural products. Natural Product Reports 21, 263–277.

Gibbons, S., 2008. Phytochemicals for bacterial resistance – strengths, weaknesses and opportunities. Planta Medica 74, 594–602.

Gómez Castellanos, J.R., Prieto, J.M., Heinrich, M., 2009. Red Lapacho (*Tabebuia impetiginosa*) – a global ethnopharmacological commodity? Journal of Ethnopharmacology 121 (1), 1–13.

Jepson, R.G., Williams, G., Craig, J.C., 2012. Cranberries for preventing urinary tract infections. Cochrane Database of Systematic Reviews. Issue 10. Art. No.: CD001321. DOI: 10.1002/14651858.CD001321.pub5.

Karbach, J., Ebenezer, S., Warnke, P.H., Behrens, E., Al-Nawas, B., 2015. Antimicrobial effect of Australian antibacterial essential oils as alternative to common antiseptic solutions against clinically relevant oral pathogens. Clinical Laboratory 61 (1–2), 61–68.

Kumar, A., Ekavali, Chopra K., Mukherjee, M., Pottabathini, R., Dhull, D.K., 2015. Current knowledge and pharmacological profile of berberine: an update. European Journal of Pharmacology 761, 288–297.

Kumar, N.V., Murthy, P.S., Manjunatha, J.R., Bettadaiah, B.K., 2014. Synthesis and quorum sensing inhibitory activity of key phenolic compounds of ginger and their derivatives. Food Chemistry 159, 451–457.

Liska, D.J., Kern, H.J., Maki, K.C., 2016. Cranberries and urinary tract infections: how can the same evidence lead to conflicting advice? Advances in Nutrition 7 (3), 498–506.

Marchese, A., Orhan, I.E., Daglia, M., et al., 2016a. Antibacterial and antifungal activities of thymol: a brief review of the literature. Food Chemistry 210, 402–414.

Marchese, A., Barbieri, R., Sanchez-Silva, A., et al., 2016b. Antifungal and antibacterial activities of allicin: a review. Trends in Food Science and Technology 52, 49–56.

Shakeri, A., Sahebkar, A., Javadi, B., 2016. *Melissa officinalis* L. – A review of its traditional uses, phytochemistry and pharmacology. Journal of Ethnopharmacology 188, 204–228.

Sinclair, D., Donegan, S., Isba, R., Lalloo, D.G., 2012. Artesunate versus quinine for treating severe malaria. Cochrane Database of Systematic Reviews 6 CD005967.

Stermitz, F.R., Lorenz, P., Tawara, J.N., Zenewicz, L.A., Lewis, K., 2000. Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 5′-methoxyhydnocarpin, a multidrug pump inhibitor. Proceedings of the National Academy of Science (USA) 97 (4), 1433–1437.

Thomsen, N.A., Hammer, K.A., Riley, T.V., Van Belkum, A., Carson, C.F., 2013. 2013 Effect of habituation to tea tree (*Melaleuca alternifolia*) oil on the subsequent susceptibility of *Staphylococcus* spp. to antimicrobials, triclosan, tea tree oil, terpinen-4-ol and carvacrol. International Journal of Antimicrobial Agents 41, 343–351.

Tu, Y., 2016. Artemisinin—A Gift from Traditional Chinese Medicine to the World (Nobel Lecture). Angewandte Chemie – International Edition 55 (35), 10210–10226.

Warnke, P.H., Becker, S.T., Podschun, R., et al., 2009. The battle against multi-resistant strains: renaissance of antimicrobial essential oils as a promising force to fight hospital-acquired infections. Journal of Cranio-maxillofacial Surgery 37 392–387.

Chapter 25

The endocrine system

Phytomedicines are often used in the treatment of hormonal disorders, although they are not a substitute for hormone replacement, whether for insulin in diabetes, or natural female and male sex hormones. Nor do they have a place in the management of thyroid deficiency, which should be treated with thyroxine. In diabetic patients, many foods and herbs can help to reduce blood glucose concentrations and may assist in controlling hyperglycaemia in milder cases of non-insulin-dependent diabetes. Phytomedicines are much less potent in effect than the sex hormones, but this can be an advantage, as, for example, with the phytoestrogens. There are several conditions for which phytomedicines may offer at least some symptomatic relief, for example, premenstrual syndrome, which affects around 20–30% of women for up to 2 weeks before the start of menstruation, and the menopause, which affects all women, usually around the age of 51 years. Certain phytomedicines also have beneficial effects in benign prostatic hyperplasia, which is very common in men over 50 years of age and is characterized by a swollen prostate gland, causing frequent or difficult urination.

HYPOGLYCAEMIC AND ANTIDIABETIC HERBS

Many plants and foods lower blood glucose concentrations by a variety of mechanisms. Although type 1 diabetes (insulin-dependent diabetes mellitus) must be controlled by injections of insulin, type 2 diabetes (non-insulin-dependent diabetes mellitus) responds well to improvements in diet and use of hypoglycaemic drugs. Phytomedicines have a part to play and are very popular in Asia, where a wide range of herbs is used. Complex carbohydrate preparations, such as guar gum, act by inhibiting glucose absorption from the gut and hence preventing the surge in blood glucose that can occur immediately after a meal. This is a feature of high-fibre diets, which are also recommended in diabetes; such diets also have the advantage of regulating blood cholesterol concentrations.

FENUGREEK, *TRIGONELLA FOENUM-GRAECUM* L. (TRIGONELLAE FOENUGRAECI SEMEN) EP

Fenugreek (also known as goat's horn and numerous other common names) is obtained from the dried, ripe seeds of *Trigonella foenum-graecum* L. (Fabaceae), an aromatic annual plant that grows up to 60 cm high. It is indigenous to China, India, Indonesia and the Mediterranean region and cultivated worldwide. The seeds are oblong-rhomboidal in shape, and can be up to 5 mm long; they are yellowish-brown to reddish-brown in colour, and taste slightly bitter. Fenugreek has an important culinary use, particularly in the Indian subcontinent.

Constituents

The seeds contain pyridine-type alkaloids, including trigonelline and choline, saponins [including the steroidal sapogenins diosgenin, yamogenin, tiogenin and others, fenugreekine (a sapogenin-peptide ester), and the furostanol glycosides trigofoenosides A–G], sterols, including β-sitosterol, and flavonoids, including orientin, isoorientin, and isovitexin (Barnes et al 2007). There is a high mucilage content; a small quantity of essential oil is also present. It is not clear which compounds are responsible for the pharmacological

activities, although the steroidal saponins may be important, and the mucilaginous fibre likely accounts for some of the traditional uses.

Therapeutic uses and available evidence

Fenugreek has a long history of traditional use for a range of conditions, including as an appetite stimulant and aphrodisiac. Modern uses are also broad, and include use as an antidiabetic, and galactogogue.

Hypoglycaemic and hypocholesterolaemic effects have been described for fenugreek extracts in animal models of diabetes and hypercholesterolaemia (Bahmani et al 2016). Hypoglycaemic activity has been documented for trigonelline, and steroidal saponins isolated from fenugreek have cholesterol-lowering effects in chemically induced diabetes in preclinical studies.

The hypoglycaemic and hypocholesterolaemic effects of fenugreek are supported by some clinical evidence, although many of the available studies are of low methodological quality. A systematic review of 12 randomized controlled trials involving patients with diabetes or pre-diabetes found that fasting and postprandial blood glucose concentrations, glycosylated haemoglobin and total cholesterol concentrations were reduced with fenugreek preparations (Gong et al 2016).

There is very limited evidence of effectiveness for fenugreek, from one randomized controlled trial, in improving pain scores in women with dysmenorrhea (Pattanittum et al 2016). There is no definitive evidence to support the use of fenugreek as a galactogogue and this cannot be recommended.

Fenugreek may cause gastrointestinal disturbances, including diarrhoea, dyspepsia, abdominal pain and flatulence; dizziness may also occur. There are isolated reports of allergic reactions (including facial oedema) to the seeds; cross-reactivity may occur in individuals with peanut or chickpea allergy (Ouzir et al 2016). Other toxicological effects, including antifertility and teratogenic effects, have been described in animal studies; the seeds should not be used during pregnancy and lactation.

GUAR GUM, *CYAMOPSIS TETRAGONOLOBA* (L.) TAUB. (CYAMOPSIDIS SEMEN) EP

Guar gum (also known as cluster bean) is obtained from the ground endosperms of the seeds of *Cyamopsis tetragonoloba* (L.) Taub. (Leguminosae), a species indigenous to Africa and parts of Asia, producing a yellowish-white flour that can be used to supplement the diet and even be made into food items such as pasta. The flour is a source of fibre and used as an adjunct in the treatment of diabetes.

Constituents

These are polysaccharides composed of straight and branched chains of D-galactose and D-mannose polymers.

Therapeutic uses and available evidence

The flour reduces pre- and postprandial glucose concentrations and is usually given with meals, and may also be of use in lowering blood lipid levels (Butt et al 2007). Like other bulk fibre preparations, it has other clinical effects such as the alleviation of diarrhoea, and it has been advocated as a slimming aid, and this seems to be justified. It is also used as a thickening and suspending agent in foods, and as a tablet binder (Mudgil et al 2014). The usual dose of the powdered gum is 5 g given with each meal. Few adverse effects have been noted, but patients sometimes consider guar to be rather unpalatable when made into foods.

GYMNEMA, *GYMNEMA SYLVESTRE* (RETZ.) R.BR. EX SM.

Gymnema sylvestre (Retz.) R.Br. ex Sm. (Asclepidaceae) grows wild in India, Sri Lanka and tropical Africa. It is a large woody climber with small yellow flowers. The leaves, which are ovate and hairy on both surfaces, have a slightly bitter taste, and if chewed this is followed by a remarkable temporary loss of sensitivity to the taste of sugar and other sweeteners. This unusual property has no relation to the hypoglycaemic effects, although may have originally been the rationale for its traditional use.

Constituents

The leaves and root contain saponin glycosides known collectively as ‘gymnemic acid’, which consists of a mixture of gymnemic acids, gymnemasins, gymnasides, gymnemosides and their aglycones (Di Fabio et al 2015). Gurmarin, a polypeptide, is responsible for the desensitization of the palate to sweet tastes.

Therapeutic uses and available evidence

The herb is a traditional treatment for diabetes in India and is widely used. The antihyperglycaemic

properties are due to the gymnemic acids and other saponins. In preclinical studies involving rodent models of hyperglycaemia and/or hyperlipidaemia, various different extracts of gymnema (e.g. methanolic, ethanolic, aqueous) reduced blood glucose concentrations, increased plasma insulin concentrations, and/or lowered serum total cholesterol or triglycerides (Pothuraju et al 2014). Studies using a specific standardized extract of gymnema [OSA(R); 1 g/day, orally for 60 days] found significant increases in circulating insulin and C-peptide, which were associated with a reduction in fasting and post-prandial blood glucose. *In vitro* experiments using isolated human islets of Langerhans demonstrated direct stimulatory effects of the same extract on insulin secretion from human β-cells, consistent with an *in vivo* mode of action through enhancing insulin secretion (Al-Romaiyan et al 2010).

Definitive clinical evidence to support the effects of gymnema in diabetes, however, is lacking and the herb cannot be recommended. A systematic review of clinical studies described some evidence in type 1 and type 2 diabetes, including reductions in blood glucose concentrations (Ulbricht et al 2011). However, this came from poor-quality studies involving small numbers of patients and cannot be considered conclusive.

In a small randomized, double-blind, placebo-controlled trial involving patients with metabolic syndrome treated with gymnema capsules for 90 days, gymnema prevented a decrease in insulin sensitivity and compensatory hyperinsulinemia (Martinez-Abundis et al 2015).

A recent randomized, double-blind, placebo-controlled trial of a multi-ingredient product containing gymnema leaf extract as well as ivy gourd fruit extract, fenugreek, chromium picolinate, and biotin, in patients with newly diagnosed hyperglycaemia reported a significant reduction in mean glycosylated haemoglobin (HbA1c) concentrations in treated participants, although the contribution of gymnema to this outcome is not clear (Thacker et al 2016).

The usual dose is up to 4 g of leaf daily. Gymnemic acids are well tolerated, but care should be taken when used in conjunction with other antidiabetic agents.

KARELA, *MOMORDICA CHARANTIA* L.

The bitter gourd or bitter melon, karela (*Momordica charantia* L., Cucurbitaceae), is grown throughout India, China, Africa and parts of America. It is a slender, climbing shrub with kidney-shaped, lobed leaves. The fruit resembles a cucumber with numerous ridges or warts and soft spines. It has an intensely bitter taste. Both the leaves and fruit are used medicinally.

The plant is widely used in the treatment of diabetes. The fruit is eaten as a vegetable; the leaf may be made into a type of 'bush tea', called 'cerassie'.

Constituents

The species contains triterpene (cucurbitane-type) glycosides called momordicosides A–L and the goyaglycosides A–H, as well as momordicin, momordicinin and cucurbitanes I, II and III and goyasaponins I, II and III. Proteins and lectins present include α-, β- and γ-momorcharins and momordins a and b.

Therapeutic uses and available evidence

Both the fruit and the leaf have hypoglycaemic effects. There is a substantial body of evidence demonstrating hypoglycaemic effects for karela extracts in preclinical studies (Grover and Yadav 2004, Habicht et al 2014). However, clinical research is far more limited: hypoglycaemic effects have been seen in some (but not all) small studies involving patients with diabetes (Habicht et al 2014). Also, a recent Cochrane review found no statistically significant difference in glycaemic control for momordica preparations, compared with placebo, and concluded that there is insufficient evidence to recommend momordica for type 2 diabetes mellitus. More research is required to address issues of standardization and quality control of preparations (Ooi et al 2012).

Karela has also been used to treat asthma, skin infections and hypertension (Grover and Yadav 2004). Contraceptive and teratogenic effects have been described in animals, so karela should be avoided by pregnant women until further information is available; cooking the vegetable may destroy many of the toxins.

PHYTOESTROGENS

Many plants contain oestrogenic substances (phytoestrogens), and pharmacological and epidemiological evidence suggests that they act as mild oestrogens or, in certain circumstances, as anti-oestrogens (by binding to oestrogen receptors and preventing occupation by natural oestrogens). They generally have beneficial effects, including chemopreventive activity. As well as the herbs mentioned

below, many pulses (which are legumes) contain phytoestrogens, as do linseed and hops. The main chemical types of phytoestrogen are the isoflavones, coumestans and lignans, and some species of palm even contain similar hormones (e.g. estriol) to those found in the human body. The common occurrence of these substances has implications for men as well as for women, in that the incidence of benign prostatic hyperplasia in men, and menopausal symptoms in women, is lower in societies consuming significant amounts of foods containing these substances in their normal diet. A case-control study in the UK found no significant associations between phytoestrogen intake and breast cancer risk, although colorectal cancer risk was inversely associated with enterolignan intake in women, but not in men (Ward and Kuhnle 2010). Soya phytoestrogen intake may even have a beneficial effect on tumour recurrence (Roberts 2010). As the majority of studies have not involved women with breast cancer and are of short duration, it would be wise for patients with hormone-dependent cancers to avoid taking phytomedicines known to affect hormone concentrations.

RED CLOVER, *TRIFOLIUM PRATENSE* L.

Red (or pink) clover (Fabaceae) is widely distributed throughout Europe, naturalized in North America and found in many other parts of the world. The flower heads are ovoid, red or pinkish purple, about 2–3 cm in diameter, composed of numerous individual, keeled flowers. The leaflets are trefoil, often with a whitish crescent in the centre. Both the leaves and isolated isoflavones are used medicinally.

Constituents

The major actives are phytoestrogens of two types: the isoflavones genistein (Fig. 25.1), afrormosin, biochanin A, daidzein, formononetin, pratensein, calyconin, pseudobaptigenin, orobol, irilone and trifoside, and their glycoside conjugates; and the coumestans coumestrol (see Fig. 25.1) and medicagol.

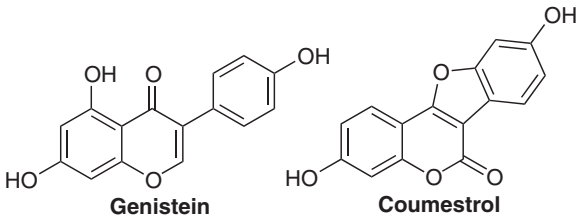


Fig. 25.1

Therapeutic uses and available evidence

Red clover was traditionally used for skin complaints such as psoriasis and eczema, and as an expectorant in coughs and bronchial conditions. However, it has recently been used more as a source of the isoflavones, for a natural method of hormone replacement therapy (for review, see Sabudak and Guler 2009). The isoflavones are oestrogenic in animals, and biochanin A inhibits metabolic activation of the carcinogen benzo(a)pyrene in a mammalian cell culture, suggesting chemopreventive properties. Red clover extracts also inhibit cytochrome P450 3A4 *in vitro*, which supports such a use.

Clinical evidence for use of red clover by menopausal women is limited. A systematic review and meta-analysis (conducted according to Cochrane guidelines) of randomized trials of red clover extracts for treating hot flushes in menopausal women found a significant effect for red clover following 3–4 months' treatment; however, there was no significant effect for 12 months' treatment (Gartoulla and Han 2014). Also, a Cochrane systematic review concluded there was no definitive evidence that phytoestrogen 'supplements', including red clover preparations, reduce the frequency or severity of hot flushes and night sweats in peri- or post-menopausal women; there was some indication that preparations containing high concentrations of the isoflavone genistein might reduce the daily number of hot flushes (Lethaby et al 2013). A new randomized, double-blind, placebo-controlled trial published since these systematic reviews found that red clover dried leaf extract 80 mg daily for 12 weeks reduced the severity of menopausal symptoms in post-menopausal women (Shakeri et al 2015).

Red clover is generally considered safe, although there has not been a comprehensive investigation of its safety profile. A systematic review of soy, red clover and isoflavones for menopausal symptoms in women with breast cancer concluded that better evidence confirming safety is required before use of higher doses (> 100 mg daily) of isoflavones can be recommended in women with breast cancer (Fritz et al 2013).

SOYA, *GLYCINE MAX* (L.) MERR.

Soya (Fabaceae) is a low-growing, typically leguminous crop plant, producing white or yellow beans. It is an important item in the diet of people from many countries and is used in many ways. For example, 'soya milk' is used as a substitute for animal milk in allergic

people (especially babies) and by some vegetarians, and can be made into yoghurt. The protein is used as a meat substitute and to make tofu, and is fermented into condiments such as 'soy sauce'. The bean sprouts are eaten raw in salads and used in stir-fry dishes; the flour can be made into bread and cakes.

Constituents

Soya contains phytoestrogens of two chemical types: isoflavones including genistein, daidzein and their derivatives, ononin, isoformononetin and others, and coumestans such as coumestrol (especially in the sprouts). There is a fixed oil composed mainly of linoleic and linolenic acids, and phytosterols including β-sitosterol and stigmasterol.

Therapeutic uses and available evidence

Preparations containing the isolated isoflavones are used medicinally. The available epidemiological evidence suggests that a diet high in soya can reduce menopausal symptoms and prostate enlargement, although clinical trials have not yet proved these benefits. A Cochrane systematic review concluded there was no definitive evidence that phytoestrogen 'supplements', including dietary soy and soy extracts, reduce the frequency or severity of hot flushes and night sweats in peri- or post-menopausal women; these was some indication that preparations containing high concentrations of the isoflavone genistein might reduce the daily number of hot flushes (Lethaby et al 2013).

The isoflavones and coumestans are oestrogenic, and are now being used as a 'natural' form of hormone replacement therapy, although the evidence for their effectiveness is not conclusive. Dietary inclusion of whole soya foods appears to produce a reduction in some clinical risk factors for osteoporosis; also, soy isoflavone supplements moderately decreased the bone resorption marker deoxypyridinoline, but did not affect the bone formation markers alkaline phosphatase and serum osteocalcin in menopausal women, although the effects varied between studies (Taku et al 2010).

Cardiovascular disease and lipid profiles in menopausal women have been reported to be improved by consumption of whole soya foods, although results have been conflicting. There is still much work to be done on the clinical effects of the isoflavones in soya, but at present it appears that they are beneficial with few adverse effects (for a review, see Messina 2010). Opposing advice has been given regarding the safety of dietary phytoestrogen use for women with previous breast cancer; as mentioned above, the majority of studies have not been conducted in women with breast cancer, and many studies are of short duration. A systematic review of soy, red clover and isoflavones for menopausal symptoms in women with breast cancer concluded that better evidence confirming safety is required before use of higher doses (> 100 mg daily) of isoflavones can be recommended in women with breast cancer (Fritz et al 2013). For a review of the molecular mechanisms of genistein and its beneficial and harmful effects in different types of cancer, see Russo et al (2016).

Soya is considered to be non-toxic.

HORMONAL IMBALANCE IN WOMEN

Although the phytoestrogens can be considered to affect hormone activity, there are other herbal medicines that are considered to have the capacity to regulate hormone levels without necessarily being oestrogenic. Their mechanism of action is generally not known.

BLACK COHOSH, *ACTAEA RACEMOSA* L. (CIMICIFUGAE RACEMOSAE RHIZOME) EP

Black cohosh (syn. *Cimicifuga racemosa* (L.) Nutt., Ranunculaceae) is also known as 'squawroot', because of its traditional use for female complaints. In North America, where the species originates, it was also used to treat snakebite, hence another synonym, 'black snakeroot'. It has also been used for a variety of disparate disorders, including rheumatism, sciatica, chorea and tinnitus. The parts used medicinally are the rhizomes and roots, which are dark brown in colour. There are substantial issues with the quality of black cohosh raw material in the supply chain: adulteration with other *Actaea* species in particular is a significant problem (Foster 2013).

Constituents

The active components of black cohosh are considered to be the triterpene glycosides, such as actein (Fig. 25.2),

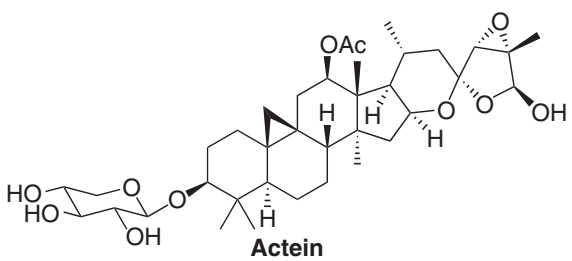


Fig. 25.2

27-deoxyactein and several cimicifugosides; the flavonoids may contribute to the activity.

Therapeutic uses and available evidence

Hormonal and anti-inflammatory effects have been described for black cohosh. Reductions in serum luteinizing hormone concentrations have been documented for methanolic and lipophilic extracts in preclinical studies, but there are conflicting data on the oestrogenic activity of the herb (Barnes et al 2007).

Evidence from well-designed, randomized, controlled trials to support the effects of black cohosh preparations in humans does not consistently demonstrate significant beneficial effects. A Cochrane systematic review of 16 randomized controlled trials of black cohosh preparations in peri- or post-menopausal women did not find any significant differences between black cohosh and placebo in the frequency of hot flushes or menopausal symptom scores (Leach and Moore 2012). Further research is warranted, although a recent study, published since the Cochrane systematic review, also reported no significant differences between black cohosh and placebo on menopausal symptoms in peri- or post-menopausal women (Tanmahasamut et al 2015).

Evidence from epidemiological studies and clinical trials indicates that black cohosh use is not associated with an increased risk of breast cancer (Fritz et al 2014), although many studies in this area are of short duration (Roberts 2010).

Black cohosh has been associated with hepatotoxic reactions, ranging from abnormal liver function test values to severe hepatitis requiring liver transplantation (EMEA 2006). Regulatory action was taken in several countries, usually requiring label warnings to be added to products containing black cohosh root/rhizome regarding the association between use of such products and liver adverse reactions. There is a view that poor-quality black cohosh material in the supply chain is an alternative explanation for at least some of the cases observed (Teschke et al 2011). New cases continue to be reported, which do not adequately describe the black cohosh product used (Kim et al 2015).

Other adverse effects may include skin reactions, gastrointestinal disturbances, and lowering of blood pressure with high doses. Black cohosh should be avoided in pregnancy and lactation because of insufficient data.

CHASTEBERRY, *VITEX AGNUS-CASTUS* L. (AGNI CASTI FRUCTUS) EP

Vitex agnus-castus L. (Lamiaceae) is also known by the common names 'chasteberry' and 'chaste tree', but is often referred to simply as 'agnus castus'. It has a history of traditional use for menstrual problems, including premenstrual symptoms and dysmenorrhoea, and also for menopausal complaints. It was considered historically to reduce the libido, especially in men, hence the names 'chasteberry', 'agnus castus' (which means 'chaste lamb') and 'monk's pepper'. *Vitex agnus-castus* is native to the Mediterranean. It is a shrub or small tree, which grows to 1–6 m in height. The fruit (berries) is reddish-black and around 2–4 mm in diameter, and is the part used pharmaceutically.

Constituents

The pharmacologically active components of agnus castus have not yet been clearly established, although the diterpene constituents (e.g. rotundifuran) are likely to be important. Flavonoids, mainly vitexin, casticin, and others, such as kaempferol and quercetagetin, iridoids are also present.

Therapeutic uses and available evidence

Extracts of agnus castus and isolated diterpene constituents display dopamine receptor-binding activity *in vitro.* For example, dopaminergic activity and inhibition of prolactin secretion have been demonstrated *in vitro* for rotundifuran. Dopaminergic activity is associated with inhibition of prolactin synthesis and release.

Modern pharmaceutical uses of agnus castus include menstrual cycle disorders, premenstrual syndrome (PMS) and cyclical mastalgia (breast pain).

There is some evidence from randomized controlled trials to support the effects of proprietary preparations containing agnus castus in relieving breast pain in women with mastalgia (Barnes et al 2007). A systematic review of randomized, controlled trials of *Vitex agnus-castus* extracts (mostly, but not all, proprietary preparations) found that vitex preparations were more effective than controls in treating premenstrual syndrome (van Die et al 2013). Newer randomized, controlled trials have also reported beneficial effects in PMS (Mirghafourvand et al 2016, Schellenberg et al 2012), including a dose-dependent effect for a proprietary preparation of agnus castus in PMS, with a daily dose of 20 mg of the ethanolic extract of the fruit being required for beneficial effects (Schellenberg et al 2012).

There was also some evidence of benefits with agnus castus preparations in premenstrual dysphoric disorder, and in lowering prolactin concentrations in hyperprolactinaemia; some of these studies included

women experiencing mastalgia (van Die et al 2013). Other studies have reported that agnus castus does not markedly affect prolactin concentrations in women with normal basal prolactin concentrations.

Evidence from rigorous randomized controlled trials is lacking for agnus castus in the alleviation of menopausal symptoms, but emerging pharmacological evidence suggests this area warrants investigation (van Die et al 2009).

Agnus castus is generally considered to be safe, but comprehensive investigation of its safety profile is lacking. Agnus castus is not recommended during pregnancy (Dugoua et al 2008), and should not be used during lactation as it may suppress milk production; its effects upon neonates are not known.

BENIGN PROSTATIC HYPERPLASIA

Benign prostatic hyperplasia (BPH) is so common in older men that it can almost be considered a normal part of ageing. The symptoms are increased frequency, and difficulty, of micturition (urination). BPH requires a medical diagnosis to eliminate the possibility of prostate cancer, and so self-treatment is only suitable after consultation with a medical practitioner. Severe cases are treated with surgery, which is not always entirely successful, or drug treatment with either specific α-adrenergic-blocking agents or testosterone 5α-reductase inhibitors. α-Blockers relax smooth muscle in BPH and improve urinary flow, but have side effects including drowsiness, syncope and dry mouth. The enzyme 5α-reductase catalyses the conversion of testosterone to the more potent androgen, dihydrotestosterone, and if this is prevented, a reduction in prostate size and consequent improvement in urine flow ensues. Phytotherapy is thought to be as successful (or almost) as synthetic drug treatment, but complete reversal of the enlargement of the prostate is not possible.

NETTLE, *URTICA DIOICA* L. (URTICAE HERBA, RADIX) EP

Urtica dioica L. (Urticaceae) is commonly known as stinging nettle or simply urtica. Traditionally, it has been used to treat a disparate range of conditions, including uterine haemorrhage, epistaxis, cutaneous eruptions, and nervous and infantile eczema, which have little relation to its modern pharmaceutical uses. It has also been used as supportive therapy in rheumatic ailments. The plant grows to around 60–120 cm in height and has serrated leaves with stinging hairs and bristles. The herb and the roots are the parts used pharmaceutically.

Constituents

The phytochemistry of nettle is well documented, although it is not clear precisely which constituents are responsible for the various activities. Lignans present in the root, including pinoresinol, secoisolariciresinol, dehydrodiconiferyl alcohol and neo-olivil, may be important in inhibiting the interaction between sex hormone-binding globulin and 5α-testosterone. Other constituents include lectins, the mixture known as UDA (*Urtica dioica* agglutinin), composed of at least six isolectins, and triterpenes such as oleanolic and ursolic acid derivatives. The leaf contains flavonoids, mainly isorhamnetin, kaempferol and quercetin glycosides, and glycoprotein, with indoles such as histamine and serotonin, betaine, acetylcholine, caffeic, chlorogenic and caffeoylmalic acids.

Therapeutic uses and available evidence

Modern uses of nettle extracts are focused mainly on symptom relief in BPH, adjuvant treatment (i.e. in addition to non-steroidal anti-inflammatory drugs) in arthritis and rheumatism and, more recently, diabetes.

Evidence from *in vitro* and *in vivo* (mice) studies measuring sex hormone-binding globulin to human prostate membranes, and by inhibition of proliferation of human prostatic epithelial and stromal cells, suggests that nettle root extracts have beneficial effects on BPH tissue. Several compounds from the roots are reported to be aromatase inhibitors. Nettle leaf extracts also inhibit the pro-inflammatory transcription factor NF-κB, partially inhibit cyclo-oxygenase and lipoxygenase, and inhibit tumour necrosis factor and interleukin-1β secretion stimulated by lipopolysaccharide. Numerous preclinical studies have explored the effects of nettle leaf extracts on blood glucose and insulin concentrations, triglyceride profiles and other parameters in animal models of diabetes.

There is evidence from some clinical trials to support the use of nettle root extracts for the relief of symptoms associated with BPH (Chrubasik et al 2007). Clinical investigation of nettle extracts in diabetes in humans is limited. In a randomized, double-blind, placebo-controlled trial involving patients with type 2 diabetes, nettle leaf extract (1500 mg daily for 3 months) lowered fasting and post-prandial blood glucose and glycosylated haemoglobin concentrations (Kianbakht et al 2013).

Nettle preparations are generally considered safe, although a comprehensive investigation of safety is lacking. Few adverse events are reported in clinical trials of nettle preparations. The stinging sensation and dermatitis resulting from contact with nettle plants is, of course, well known, and is thought to be due to both chemical and mechanical irritation (Cummings and Olsen 2011).

PYGEUM BARK, *PRUNUS AFRICANA* (HOOK.F.) KALKMAN. (PRUNI AFRICANI CORTEX) EP

The African prune (also known as *Pygeum africanum*, Hook.f., Rosaceae) is a tropical evergreen tree indigenous to central and southern Africa. The whole or fragmented dried bark of the stems and branches is the part used pharmaceutically. The use of pygeum bark is becoming more widespread throughout Europe and the USA, and the tree is in danger of becoming scarce due to over-harvesting of the bark (Bodeker et al 2014, Cunningham et al 2016).

Constituents

The bark contains sterols and pentacyclic triterpenes, including abietic, oleanolic, ursolic and crataegolic acids, *N*-butylbenzene-sulphonamide, and esters of ferulic acid.

Therapeutic uses and available evidence

Traditionally used for micturition problems and now for BPH, pygeum extract has antiproliferative and apoptotic effects on proliferative prostate fibroblasts and myofibroblasts, but not on smooth muscle cells (Quiles et al 2010). The compound *N*-butylbenzene-sulphonamide, isolated from *P. africanum*, is a specific androgen receptor antagonist that inhibits both endogenous prostate serum antigen expression and growth of human prostate cancer cells, but does not interact with oestrogen receptors (Papaioannou et al 2010). Other *Prunus* species have recently been investigated in preclinical studies for their effects in testosterone-induced BPH (Jena et al 2016).

A Cochrane systematic review of clinical trials of pygeum concluded the extract may be a useful treatment option for men with lower urinary tract symptoms consistent with BPH. However, due to methodological short-comings of the studies further work is needed before conclusions on efficacy can be made (Pagano et al 2014, Wilt et al 2002).

Acute and chronic toxicity and mutagenicity tests have shown no adverse effects, and the extract appears to be well-tolerated in men when administered over long periods. Pygeum is often used in combination with nettle.

SAW PALMETTO, *SERENOA REPENS* (W.BARTRAM) SMALL (SABALIS SERRULATAE FRUCTUS) EP

Serenoa repens (W.Bartram) Small (Arecaceae) is also known as *S. serrulata* (Michx.) Hook.f. ex B.D.Jacks. and *Sabal serrulata* (Michx.) Schult.f., as well as the common name 'saw palmetto'. Saw palmetto is a small 'fan palm' that produces berries with a diameter of 1–2 cm. The fruit (berries) is the part used pharmaceutically. It has been used traditionally for cystitis and sex hormone disorders, including prostatic enlargement. Most commercial saw palmetto comes from the USA.

Constituents

The phytochemistry of saw palmetto is fairly well known, although precisely which components are responsible for the pharmacological effects has yet to be established. Constituents likely to be important include: the fatty acids capric, caprylic, lauric, oleic, myristoleic, palmitic, linoleic and linolenic acids; the monoacyl glycerides 1-monolaurin and 1-monomyristicin; and phytosterols, such as β-sitosterol, campesterol, stigmasterol, lupeol and cycloartenol (Fig. 25.3). Long-chain alcohols (farnesol, phytol and polyprenolic alcohols) and flavonoids are present, as well as immunostimulant, high-molecular-weight polysaccharides containing galactose, arabinose, mannose, rhamnose and glucuronic acid. As with other herbal medicines, there are substantial variations in the pharmaceutical

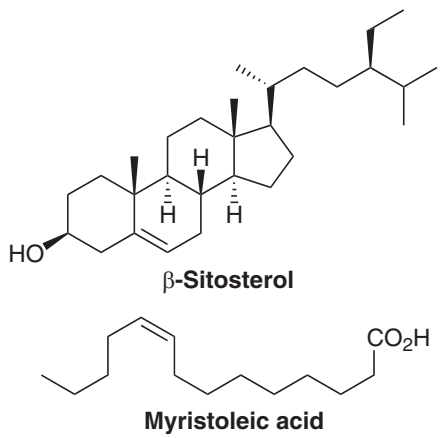


Fig. 25.3

quality of saw palmetto products; novel approaches to pharmaceutical analysis of such products provide opportunities for more robust assessment of the chemical complexity of herbal medicines (Booker et al 2014).

Therapeutic uses and available evidence

The main use of saw palmetto is in the treatment of symptoms of BPH. The use of saw palmetto in this condition is supported by evidence from preclinical studies. Liposterolic and ethanolic extracts of saw palmetto inhibit 5α-reductase (the enzyme that catalyses the conversion of testosterone to 5α-dihydrotestosterone in the prostate) *in vitro*; other studies have described beneficial effects in animal models of BPH (Barnes et al 2007). Spasmolytic activity, which may also contribute to improvements in BPH, has also been documented *in vivo* (rats) for an ethanolic extract of saw palmetto. *In vitro* growth arrest of prostate cancer LNCaP, DU145, and PC3 cells (Yang 2007) and *in vivo* oestrogenic and anti-inflammatory activities have been reported for extracts of saw palmetto, and may be due to the high content of β-sitosterol. The anti-inflammatory effects may also be due to the presence of the polysaccharides.

The preclinical evidence, however, is no longer supported by results of clinical studies. A Cochrane systematic review of 32 randomized controlled trials, involving over 5000 men, of saw palmetto extracts concluded that single- or multi-ingredient preparations of saw palmetto did not have beneficial effects on urinary flow measures, or prostate size, in men with lower urinary tract symptoms consistent with BPH (Tacklind et al 2012).

In the Cochrane systematic review, the few adverse events reported were mild and did not occur any more frequently than with placebo or conventional medicines (Tacklind et al 2012). Post-marketing surveillance studies also suggest that saw palmetto extracts are well tolerated. Saw palmetto is, however, sometimes used in untested indications, such as hirsutism and androgenic alopecia, and in children; there are isolated case reports of adverse effects occurring in this context (Morabito et al 2015).

References

Al-Romaiyan, A., Liu, B., Asare-Anane, H., et al., 2010. A novel *Gymnema sylvestre* extract stimulates insulin secretion from human islets in vivo and in vitro. Phytotherapy Research 24, 1370–1376.

Bahmani, M., Shirzad, H., Mirhosseini, M., Mesripour, A., Rafieian-Kopaei, M., 2016. A review on ethnobotanical and therapeutic uses of fenugreek (*Trigonella foenum-graceum* L). Journal of Evidence-Based Complementary and Alternative Medicine 21 (1), 53–62.

Barnes, J., Anderson, L.A., Phillipson, J.D., 2007. Herbal medicines, third ed. Pharmaceutical Press, London.

Bodeker, G., van't Klooster, C., Weisbord, E., 2014. *Prunus africana* (Hook.f.) Kalkman: the overexploitation of a medicinal plant species and its legal context. Journal of Alternative and Complementary Medicine 20 (11), 810–822.

Booker, A., Suter, A., Krnjic, A., et al., 2014. A phytochemical comparison of saw palmetto products using gas chromatography and (1) H nuclear magnetic resonance spectroscopy metabolomic profiling. Journal of Pharmacy and Pharmacology 66 (6), 811–822.

Butt, M.S., Shahzadi, N., Sharif, M.K., Nasir, M., 2007. Guar gum: a miracle therapy for hypercholesterolemia, hyperglycemia and obesity. CRC Critical Reviews in Food Science and Nutrition 47, 389–396.

Chrubasik, J.E., Roufogalis, B.D., Wagner, H., Chrubasik, S., 2007. A comprehensive review on the stinging nettle effect and efficacy profiles. Part II: urticae radix. Phytomedicine 14, 568–579.

Cummings, A.J., Olsen, M., 2011. Mechanism of action of stinging nettles. Wilderness and Environmental Medicine 22 (2), 136–139.

Cunningham, A., Anoncho, V.F., Sunderland, T., 2016. Power, policy and the *Prunus africana* bark trade, 1972–2015. Journal of Ethnopharmacology 178, 323–333.

Di Fabio, G., Romanucci, V., Di Marino, C., Pisanti, A., Zarrelli, A., 2015. *Gymnema sylvestre* R. Br., an Indian medicinal herb: traditional uses, chemical composition, and biological activity. Current Pharmaceutical Biotechnology 16 (6), 506–516.

Dugoua, J.J., Seely, D., Perri, D., Koren, G., Mills, E., 2008. Safety and efficacy of chastetree *(Vitex agnus-castus)* during pregnancy and lactation. Canadian Journal of Clinical Pharmacology 15 (1), e74–e79.

EMEA Public Statement on herbal medicinal products containing *Cimicifugae racemosae* rhizome (black cohosh, root) – serious hepatic reactions, July 2006. European Medicines Agency 18. Doc. Ref: EMEA/269259/2006. Available online at http://www.ema.europa.eu/docs/en_GB/document_library/Public_statement/2009/12/WC500016766.pdf.

Foster, S., 2013. Exploring the peripatetic maze of black cohosh adulteration. HerbalGram 98, 32–51. Available online at http://cms.herbalgram.org/herbalgram/issue98/hg98feat-blackcohosh.html.

Fritz, H., Seely, D., Flower, G., 2013. Soy, red clover, and isoflavones and breast cancer: a systematic review. PLoS ONE 8 (11), e81968.

Fritz, H., Seely, D., McGowan, J., et al., 2014. Black cohosh and breast cancer: A systematic review. Integrative Cancer Therapies 13 (1), 12–29.

Gartoulla, P., Han, M.M., 2014. Red clover extract for alleviating hot flushes in postmenopausal women: a meta-analysis. Maturitas 79 (1), 58–64.

Gong, J., Fang, K., Dong, H., Wang, D., Hu, M., Lu, F., 2016. Effect of fenugreek on hyperglycaemia and hyperlipidemia in diabetes and prediabetes: a meta-analysis. Journal of Ethnopharmacology 194, 260.

Grover, J.K., Yadav, S.P., 2004. Pharmacological actions and potential uses of *Momordica charantia*: a review. Journal of Ethnopharmacology 93, 123–132.

Habicht, S.D., Ludwig, C., Yang, R.Y., Krawinkel, M.B., 2014. *Momordica charantia* and type 2 diabetes: From in vitro to human studies. Current Diabetes Reviews 10 (1), 48–60.

Jena, A.K., Vasisht, K., Sharma, N., Kaur, R., Dhingra, M.S., Karan, M., 2016. Amelioration of testosterone induced benign prostatic hyperplasia by *Prunus* species. Journal of Ethnopharmacology 190, 33–45.

Kianbakht, S., Khalighi-Sigaroodi, F., Dabaghian, F.H., 2013. Improved glycemic control in patients with advanced type 2 diabetes mellitus taking urtica dioica leaf extract: a randomized double-blind placebo-controlled clinical trial. Clinical Laboratory 59 (9–10), 1071–1076.

Kim, H., Fisher, C., Laudenslager, M., Goodman, Z., Thapar, M., 2015. A case of sub acute liver failure associated with black cohosh use. JSM Gastroenterol Hepatol 3 (2), 1045.

Leach, M.J., Moore, V., 2012. Black cohosh (*Cimicifuga* spp.) for menopausal symptoms. Cochrane Database of Systematic Reviews 9, CD007244. http://dx.doi.org/10.1002/14651858.CD007244.pub2.

Lethaby, A., Marjoribanks, J., Kronenberg, F., Roberts, H., Eden, J., Brown, J., 2013. Phytoestrogens for menopausal vasomotor symptoms. Cochrane Database of Systematic Reviews 12, CD001395. http://dx.doi.org/10.1002/14651858.CD001395.pub4.

Martinez-Abundis, E., Zuniga, L.Y., Gonzalez-Ortiz, M., 2015. Effect of *Gymnema sylvestre* administration on metabolic syndrome, insulin sensitivity and insulin secretion. Diabetologia 58 (1 Suppl. 1), S398.

Mirghafourvand, M., Mohammad-Alizadeh-Charandabi, S., Ahmadpour, P., Javadzadeh, Y., 2016. Effects of vitex agnus and flaxseed on cyclic mastalgia: A randomized controlled trial. Complementary Therapies in Medicine 24, 90–95.

Messina, M., 2010. A brief historical overview of the past two decades of soy and isoflavone research. Journal of Nutrition 140, 1350S–1354S.

Morabito, P., Miroddi, M., Giovinazzo, S., Spina, E., Calapai, G., 2015. Serenoa repens as an endocrine disruptor in a 10-year-old young girl: a new case report. Pharmacology 96 (1–2), 41–43.

Mudgil, D., Barak, S., Khatkar, B.S., 2014. Guar gum: processing, properties and food applications – a review. Journal of Food Science and Technology-Mysore 51 (3), 409–418.

Ooi, C.P., Yassin, Z., Hamid, T.A., 2012. *Momordica charantia* for type 2 diabetes mellitus. Cochrane Database of Systematic Reviews 8, CD007845. http://dx.doi.org/10.1002/14651858.CD007845.pub3.

Ouzir, M., El Bairi, K., Amzazi, S., 2016. Toxicological properties of fenugreek *(Trigonella foenum graecum)*. Food and Chemical Toxicology 96, 145–154.

Pagano, E., Laudato, M., Griffo, M., Capasso, R., 2014. Phytotherapy of benign prostatic hyperplasia. A minireview. Phytotherapy Research 28 (7), 949–955.

Papaioannou, M., Schleich, S., Roell, D., et al., 2010. NBBS isolated from *Pygeum africanum* bark exhibits androgen antagonistic activity, inhibits AR nuclear translocation and prostate cancer cell growth. Investigational New Drugs 28, 729–743.

Pattanittum, P., Kunyanone, N., Brown, J., et al., 2016. Dietary supplements for dysmenorrhoea. Cochrane Database of Systematic Reviews 3, CD002124. http://dx.doi.org/10.1002/14651858.CD002124.pub2.

Pothuraju, R., Sharma, R.K., Chagalamarri, J., Jangra, S., Kumar Kavadi, P., 2014. A systematic review of *Gymnema sylvestre* in obesity and diabetes management. Journal of the Science of Food and Agriculture 94 (5), 834–840.

Quiles, M.T., Arbós, M.A., Fraga, A., de Torres, I.M., Reventós, J., Morote, J., 2010. Antiproliferative and apoptotic effects of the herbal agent *Pygeum africanum* on cultured prostate stromal cells from patients with benign prostatic hyperplasia (BPH). Prostate 70, 1044–1053.

Roberts, H., 2010. Safety of herbal medicinal products in women with breast cancer. Maturitas 66, 363–369.

Russo, M., Russo, G.L., Daglia, M., et al., 2016. Understanding genistein in cancer: The "good" and the "bad" effects: A review. Food Chemistry 196, 589–600.

Sabudak, T., Guler, N., 2009. *Trifolium* L. – a review on its phytochemical and pharmacological profile. Phytotherapy Research 23, 439–446.

Schellenberg, R., Zimmermann, C., Drewe, J., Hoexter, G., Zahner, C., 2012. Dose-dependent efficacy of the Vitex agnus castus extract Ze 440 in patients suffering from premenstrual syndrome. Phytomedicine 19 (14), 1325–1331.

Shakeri, F., Taavoni, S., Goushegir, A., Haghani, H., 2015. Effectiveness of red clover in alleviating menopausal symptoms: a 12-week randomized, controlled trial. Climacteric 18 (4), 568–573.

Tacklind, J., MacDonald, R., Rutks, I., Stanke, J.U., Wilt, T.J., 2012. Serenoa repens for benign prostatic hyperplasia. Cochrane Database of Systematic Reviews 12, CD001423. http://dx.doi.org/10.1002/14651858.CD001423.pub3.

Taku, K., Melby, M.K., Kurzer, M.S., Mizuno, S., Watanabe, S., Ishimi, Y., 2010. Effects of soy isoflavone supplements on bone turnover markers in menopausal women: systematic review and meta-analysis of randomized controlled trials. Bone 47, 413–423.

Tanmahasamut, P., Vichinsartvichai, P., Rattanachaiyanont, M., Techatraisak, K., Dangrat, C., Sardod, P., 2015. *Cimicifuga racemosa* extract for relieving menopausal symptoms: a randomized controlled trial. Climacteric 18 (1), 79–85.

Teschke, R., Schwarzenboeck, A., Schmidt-Taenzer, W., Wolff, A., Hennermann, K.H., 2011. Herb induced liver injury presumably caused by black cohosh: a survey of initially purported cases and herbal quality specifications. Annals of Hepatology 10 (3), 249–259.

Thacker, H., Bantwal, G., Jain, S., 2016. Evaluation series on safety and efficacy of nutritional supplements in newly diagnosed hyperglycemia: A placebo-controlled, randomized study. North American Journal of Medical Sciences 8 (2), 106–113.

Ulbricht, C., Abrams, T.R., Basch, E., 2011. An evidence-based systematic review of gymnema (*Gymnema sylvestre* R. Br.) by the natural standard research collaboration. Journal of Dietary Supplements 8 (3), 311–330.

van Die, M.D., Burger, H.G., Teede, H.J., Bone, K.M., 2009. *Vitex agnus-castus* (chaste-tree/berry) in the treatment of menopause-related complaints. Journal of Alternative and Complementary Medicine 15, 853–862.

van Die, M.D., Burger, H.G., Teede, H.J., Bone, K.M., 2013. *Vitex agnus-castus* extracts for female reproductive disorders: a systematic review of clinical trials. Planta Medica 79 (7), 562–575.

Ward, H.A., Kuhnle, G.G.C., 2010. Phytoestrogen consumption and association with breast, prostate and colorectal cancer in EPIC Norfolk. Archives of Biochemistry and Biophysics 501, 170–175.

Wilt, T., Ishani, A., Mac Donald, R., Rutks, I., Stark, G., 2002. *Pygeum africanum* for benign prostatic hyperplasia. Cochrane Database Systematic Reviews 1, CD001044.

Yang, Y., Ikezoe, T., Zheng, Z., et al., 2007. Saw palmetto induces growth arrest and apoptosis of androgen-dependent prostate cancer LNCaP cells via inactivation of STAT 3 and androgen receptor signaling. International Journal of Oncology 31, 593–600.

Chapter 26

The reproductive tract

Drugs used in hormonal disorders have been covered in Chapter 25. Other drugs that are used in obstetrics, including substances used in childbirth, and genitourinary disorders and erectile dysfunction in men, will be mentioned briefly here.

PREGNANCY AND CHILDBIRTH

Taking (any) medicine during pregnancy is generally not advisable as safety to the mother and foetus cannot be guaranteed. Raspberry leaf is included here because it has a widespread folklore use in facilitating childbirth, and it is often recommended that it be taken during pregnancy for this purpose, despite a lack of evidence as to its efficacy.

ERGOMETRINE EP

Ergometrine (Fig. 26.1) is an alkaloid extracted from ergot (*Claviceps purpurea* Tul.), a parasitic fungus growing on cereals, usually rye. It is sometimes used to manage the third stage of labour (in conjunction with oxytocin), and to control postpartum haemorrhage if the placenta has not been completely expelled. It must be used only under the care of a midwife or obstetrician.

RASPBERRY LEAF, *RUBUS IDAEUS* L. (RUBI IDAEI FOLIUM)

Raspberry leaf (*Rubus idaeus*, Rosaceae) 'tea' has been used for centuries to facilitate childbirth, and it is usually recommended that it be drunk freely before and during confinement for maximum benefit. The raspberry shrub is well known and will not be described. It is cultivated in many temperate countries for the fruit.

Constituents

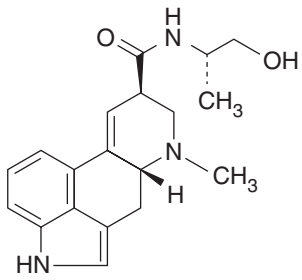
The leaves contain tannins and flavonoids, mainly glycosides of kaempferol and quercetin, including rutin, uncharacterized polypeptides (Edwards et al 2015).

Therapeutic uses and available evidence

A retrospective observational study on 108 mothers in Australia indicated that a shortening of labour and reduction in medical intervention occurred, with no untoward effects apart from a single case of diarrhoea and anecdotal reports of strong Braxton Hicks contractions. However, a larger, randomized placebo-controlled trial of 192 women by the same authors did not confirm such benefits, although no adverse effects for either mother or baby were noted (Simpson et al 2001). Uterine relaxant effects have been demonstrated in animals (Rojas-Vera et al 2002), and raspberry leaf appears to affect only the pregnant uterus from both rats and humans, with no activity on the non-pregnant uterus. Recent reviews have concluded that in the absence of good clinical data, raspberry leaf cannot be recommended in pregnancy (Holst et al 2009), although it has shown no evidence of causing harm (Smeriglio et al 2014). The potential for drug interactions is not high, and few adverse events have been described (Edwards et al 2015).

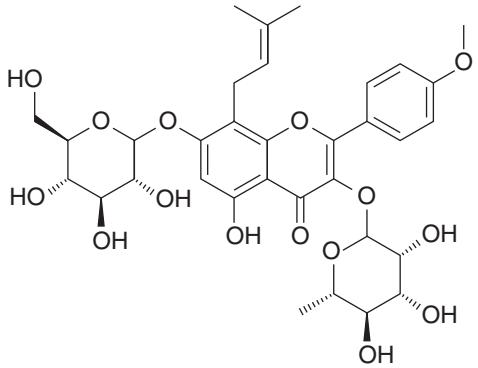
MALE SEXUAL DYSFUNCTION (IMPOTENCE)

Male impotence (failure to produce a satisfactory or sustainable erection) may result from psychogenic, vascular, neurogenic or endocrine abnormalities (such



Ergometrine

Fig. 26.1



Icariin

Fig. 26.2

as diabetes), or drug treatment (e.g. with antihypertensives and antidepressants). It is conventionally treated with sildenafil and similar drugs or intracavernosal injections of alprostadil (prostaglandin E1).

There are several herbal products available that claim to treat this distressing disorder. The most common are probably damiana, epimedium and yohimbe, a traditional aphrodisiac, and there are other botanical mixtures usually sold under the description 'Herbal Viagra'. There is no good clinical evidence of efficacy for any of these, although epimedium has some pharmacological actions in common with those of sildenafil (Viagra®).

DAMIANA

Damiana, *Turnera diffusa* Willd ex Schult. (Passifloraceae) grows in tropical and subtropical areas of Central and South America. The leaves are used as a tonic and to treat male sexual dysfunction, and in some areas are smoked to produce relaxation. They have been included in 'legal highs', but there is little clinical evidence available for any of these uses.

Constituents

Damiana contains traces of cyanogenetic glycosides and the hydroquinone glycoside arbutin, together with an essential oil composed of pinenes, thymol and others (see Edwards et al 2015).

Therapeutic uses and available evidence

No clinical evidence is available for the use of damiana in humans, but a study in rats has found that the extract can enhance recovery in sexually exhausted rats, via the nitric oxide pathway (Estrada-Reyes et al 2013).

HORNY GOAT WEED

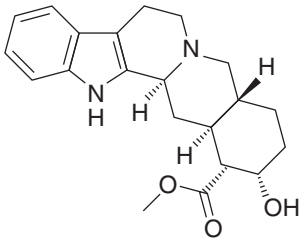
Epimedium brevicornu Maxim (Berberidaceae), *E. sagittatum* (Siebold and Zucch.) Maxim and related species (Epimedii herba) are widely used in traditional Chinese medicine (TCM) for a variety of disorders, but are becoming increasingly used to treat impotence. Its properties were apparently discovered by the Chinese, who noticed that when goats had eaten it, they were eager to mate, and for this reason they called the herb 'yin yang huo', or 'licentious goat plant'. Epimediums are sprawling perennial herbs, with cordate leaves and white, cream, pink, yellow or lavender flowers. Although native to Asia and the Mediterranean region, they are widely cultivated. Epimedium has been used for the treatment of erectile dysfunction in TCM for many years. It is also used to ease menopausal symptoms in women and to treat and prevent osteoporosis. The isoflavones possess oestrogenic properties and this may contribute to their clinical effects, in addition to those outlined below.

Constituents

The main constituents are prenylated flavonoids, the most important being icariin (Fig. 26.2) and its analogues and metabolites epimedins A, B, and C and the saggitatosides, together with other flavonoids.

Therapeutic uses and available evidence

Erectile dysfunction. Icariin has phosphodiesterase type 5 inhibiting effects (the mechanism of action of sildenafil) and may also have neurotrophic effects, and a small double-blind clinical trial found that a daily dose



Yohimbine

Fig. 26.3

of an epimedium-containing herbal product enhanced sexual satisfaction (reviewed by Edwards et al 2015).

A study of the effects of icariin administered daily to cavernous nerve-injured rats found that the ratio of intracavernous pressure to arterial pressure was significantly higher compared with control (and also single-dose icariin-treated) animals. The penile tissue of rats treated with icariin showed greater positivity for neuronal nitric oxide synthase and calponin, and cultured pelvic ganglia treated with icariin had significantly greater neurite length (Shindel et al 2010).

Bone health. Clinical studies on *Epimedium* extract and isolated icariin suggest that on bone anabolism it may exert its osteogenic effects through the induction of bone morphogenetic protein-2 and NO synthesis, subsequently regulating gene expression and contributing to the induction of osteoblast proliferation and differentiation (Zhang et al 2013).

YOHIMBE, *PAUSINYSTALIA JOHIMBE* (K. SCHUM.) PIERRE (NOW CLASSED AS *CORYNANTHE JOHIMBE* K.SCHUM)

Yohimbe bark (Rubiaceae) occurs as flat or slightly quilled pieces, often covered with lichen. It is a very popular supplement used to treating sexual dysfunction, but has been linked to reports of toxicity, including priapism, hypertension and even heart attack, and is on the USFDA list of dangerous supplements. There are many contra-indications and potential for drug interactions, so its use cannot be recommended (reviewed by Edwards et al 2015).

Constituents

The bioactive constituents are indole alkaloids, the major one being yohimbine, together with coryantheine, α- and β-yohimbane, and pseudoyohimbine.

Therapeutic uses and available evidence

Yohimbine (Fig. 26.3) is an α-2 adrenergic blocker and coryantheine is an α-1 adrenergic blocker, both of which may contribute to activity, so yohimbe bark may be more effective than yohimbine alone, if the alkaloids are present in sufficient concentrations. Priapism has been reported as a result of taking the bark extract as well as from yohimbine.

References

Edwards, S.E., Da Costs Rocha, I., Williamson, E.M., Heinrich, M., 2015. Phytopharmacy: an evidence-based guide to herbal medicinal products. Wiley-Blackwell, Chichester, UK.

Estrada-Reyes, Carro-Juarez M., Martinez-Mota, L., 2013. Pro-sexual effects of *Turnera diffusa* Willd. (Turneraceae) in male rats involve the nitric oxide pathway. Journal of Ethnopharmacology 146 (1), 164–172.

Holst, L., Wright, D., Nordeng, H., Haavik, S., 2009. Raspberry leaf – should it be recommended to pregnant women? Complementary Therapies in Clinical Practice 15 (4), 204–208.

Rojas-Vera, J., Patel, A.V., Dacke, C.G., 2002. Relaxant activity of raspberry (*Rubus idaeus*) leaf extract in guinea-pig ileum in vitro. Phytotherapy Research 16 (7), 665–668.

Shindel, A.W., Xin, Z.C., Lin, G., et al., 2010. Erectogenic and neurotrophic effects of icariin, a purified extract of horny goat weed (*Epimedium* spp.) *in vitro* and *in vivo*. The Journal of Sexual Medicine (4 Pt 1), 1518–1528.

Simpson, M., Parsons, M., Greenwood, J., Wade, K., 2001. Raspberry leaf in pregnancy: its safety and efficacy in labor. J Midwifery Womens Health 46 (2): 51–9.

Smeriglio, A., Tomaino, A., Trombetta, D., 2014. Herbal products in pregnancy: Experimental studies and clinical reports. Phytotherapy Research 28 (8), 1107–1116.

Zhang, X., Liu, T., Huang, Y., Wisjmeijer, D., Liu, Y., 2013. Icariin: does it have an osteogenic potential for bone tissue engineering. Phytotherapy Research 28 (4), 498–509.

Chapter 27

The musculoskeletal system

Short-lived and self-limiting inflammatory disorders are not normally treated with phytomedicines, but over recent decades the use of some botanical preparations for chronic inflammatory conditions has become increasingly widespread. The use of analgesic and anti-inflammatory drugs such as paracetamol, aspirin and ibuprofen is common for such conditions, but the side effects of these drugs can limit their acceptability. Non-steroidal anti-inflammatory drugs (NSAIDs) act mainly via inhibition of cyclo-oxygenase (COX) enzymes, also known as prostaglandin synthases (PGS). At present three are known, COX-1, COX-2 and COX-3 (a splice variant of COX-1, sometimes referred to as COX-1b). Inhibition of COX-1 (e.g. with aspirin, ibuprofen and diclofenac) reduces levels of the gastroprotective prostaglandins, leading to inflammation of the gastrointestinal lining and even ulceration and bleeding. COX-2, however, is only induced in response to pro-inflammatory cytokines, and is not found in normal tissue (unlike COX-1). It is associated particularly with oedema and the nociceptive and pyretic effects of inflammation. Treatment with inhibitors of COX-2 does not produce such severe gastrointestinal side effects, but there are concerns about their cardiovascular safety. Other targets for treating inflammatory diseases include 5-lipoxygenase (LOX), NF-κB (which is activated in rheumatoid arthritis and other chronic inflammatory conditions), and certain cytokines that inhibit the activity of tumour necrosis factor-α (TNFα). Chronic expression of NO is also associated with various inflammatory conditions, including arthritis.

DRUGS USED IN ARTHRITIS, RHEUMATISM AND MUSCLE PAIN

The classic NSAID, aspirin, was originally developed as a result of studies on salicin, obtained from willow bark (see below and – for historical aspects – Chapter 2). Although it was thought at first that the effects of salicin were due only to the hydrolyzed product salicylic acid, it is now known that plant anti-inflammatory agents tend to have fewer gastrointestinal side effects than salicylates in general. There are also several combination herbal products on the market, for which little clinical data are available, but which are very popular and seem to produce few side effects.

BROMELAIN (ANANASE)

Bromelain is a mixture of proteolytic enzymes extracted from the fruit and stem of the pineapple [*Ananas comosus* (L.) Merr.] and other species of bromeliads (Bromeliaceae). The active constituents are protease-inhibiting enzymes, having molecular weights between 5000 and 6000.

Bromelain has been proposed for the treatment of atherosclerosis, osteoarthritis, dysmenorrhoea, scleroderma, infection and sports injuries.

It is anti-inflammatory in animal studies and is used clinically to treat bruising, arthritis, joint stiffness and pain, and to improve healing postoperatively, including after dental procedures. It is considered to be an effective alternative to NSAIDs, as shown by a number of clinical trials. Some evidence exists for the efficacy of bromelain in treating postoperative oedema and pain after tooth extraction (Inchingolo et al 2010). Bromelain, given once daily in acute tendon injury at a dosage of 7 mg/kg for 14 days, promoted healing by stimulating tenocyte proliferation in rats (Aiyegbusi et al 2011). While bromelain is often acclaimed to be useful in the treatment and prevention of osteoarthritis, the current evidence does not support this use even though *in vitro* and *in vivo* preclinical data provide

some evidence (Gonzalez-Sarrias et al 2013). Bromelain is generally well tolerated, but side effects include minor gastrointestinal upsets (Mncwangi et al 2012).

DEVIL'S CLAW, *HARPAGOPHYTUM PROCUMBENS* (BURCH.) DC. EX MEISSNER (HARPAGOPHYTI RADIX) EP

Devil's claw (Pedaliaceae) has fairly recently been developed into a successful and relatively well-characterized medicine. The name arises from the claw-like appearance of the fruit. The secondary storage roots are collected in the savannahs of southern Africa (mainly the Kalahari Desert) and, while still fresh, they are cut into small pieces and dried. The main exporters are South Africa and Namibia. Devil's claw was used traditionally as a tonic for 'illnesses of the blood', fever, kidney and bladder problems, during pregnancy and as an obstetric remedy for induction or acceleration of labour, as well as for expelling the retained placenta (Heinrich and Booker 2015). *H. zeyheri* Decne. is also used as a source of the drug.

Constituents

The most important actives are considered to be the bitter iridoids, harpagide and harpagoside (Fig. 27.1), with 8-*O*-*p*-coumaroylharpagide, procumbide, 6′-*O*-*p*-coumaroylprocumbide, pagide and procumboside; the triterpenoids oleanolic and ursolic acids; β-sitosterol; and a glycoside harproside. Other compounds present include phenylethyl glycosides, such as verbascoside and isoacteoside, polyphenolic acids (caffeic, cinnamic, and chlorogenic acids), and flavonoids, such as luteolin and kaempferol. According to the European Pharmacopoeia (Eur. Ph.) the drug must contain ≥ 1.2% harpagide and harpagoside, expressed as harpagoside.

Therapeutic uses and available evidence

In Europe, a tea (made from a dose of about 1.5 g/day of the powdered drug) has been used for the treatment of dyspeptic disorders such as indigestion and lack of appetite. This effect is due to the presence of bitter glycosides, the iridoids, which are present in large amounts.

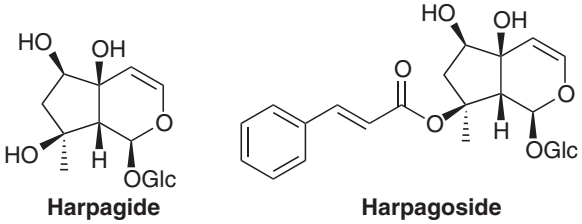


Fig. 27.1

Most pharmacological and clinical research has been conducted using standardized extracts for the treatment of rheumatic conditions and lower back pain. Several clinical studies, including some placebo-controlled double-blind trials, demonstrate the superiority of these extracts to placebo in patients with osteoarthritis, non-radicular back pain and other forms of chronic and acute pain. Other studies show their therapeutic equivalence to conventional forms of treatment. In 2016 a Cochrane analysis covering a range of herbal medicines concluded that even though *H. procumbens* seems to reduce pain more than placebo, evidence was overall of moderate quality (Gagnier et al 2016). Devil's claw is generally well tolerated and appears to be a suitable alternative to NSAIDs, which often have gastrointestinal side effects (for a review, see Barnes 2009, Cameron et al 2009).

The mechanism of action is not fully known: fractions of the extract containing the highest concentration of harpagoside inhibited COX-1 and COX-2 activity and greatly inhibited NO production, whereas in contrast, the fraction containing mainly the other iridoids increased COX-2 and did not alter NO and COX-1 activities. A fraction containing mainly cinnamic acid was able to reduce only NO production (Anauate et al 2010). An extract of *Harpagophytum procumbens* showed a significant anti-inflammatory effect in the rat adjuvant-induced chronic arthritis model, and harpagoside dose-dependently suppressed the lipopolysaccharide (LPS)-induced production of inflammatory cytokines (IL-1β, IL-6, and TNF-α) in mouse macrophage cells (Inaba et al 2010). These demonstrate that harpagoside is probably the main active constituent responsible for the effect of devil's claw, but that other components from the crude extract can antagonize or increase the synthesis of inflammatory mediators. In summary, both the pharmacological mechanism and the compounds responsible for this activity have to be investigated further, and by *in vivo* methods. There are implications for the production methods for preparing devil's claw extracts. A great difference in the composition of various commercial products and consequently their anti-inflammatory activity was demonstrated, which led to the conclusion that the harpagoside content is not a reliable method of predicting the therapeutic efficacy (Ouitas and Heard 2010). Extracts of devil's claw are generally well tolerated but should not be used for patients with gastric or duodenal ulceration.

The aqueous extract possesses spasmogenic, uterotonic action on rat uterine muscles (Mahomed and Ojewole 2009), leading credence to the folkloric obstetric uses, but suggesting that it should be avoided in pregnant women. Side effects include minor gastrointestinal upsets (Mncwangi et al., 2012).

ROSEHIP, *ROSA CANINA* L. (ROSAE PSEUDOFRUCTUS; ALSO KNOWN AS ROSAE FRUCTUS OR ROSAE PSEUDOFRUCTUS CUM FRUCTIBUS)

The fruits of the wild or dog rose, *Rosa canina* (Rosaceae), are known as rosehips, and are botanically 'pseudofruits', composed of achenes enclosed in a fleshy receptacle or hypanthium. The trichomes found inside rose hips are irritant and are often removed before powdering the fruit. There are several types of rosehip preparation available: rosehip and seed (the ripe pseudofruits, including the seed); rosehip (the ripe seed receptacle, freed from seed and attached trichomes); and rosehip seed (the ripe, dried seed). The whole pseudofruit, i.e. rosehip with seed, is most commonly used and widely investigated.

Constituents

Anti-inflammatory constituents isolated from rosehip extracts include the triterpene acids, oleanolic, betulinic and ursolic acids; oleic, linoleic and alpha-linolenic acids, and a series of galactolipids, which are thought to be a major contributor to the effects (Chrubasik et al 2008b).

Therapeutic uses and available evidence

Traditionally, rose hips were used as a source of vitamin C and were made into syrups for that purpose, but modern use is now focused on their anti-inflammatory effects (for review, see Chrubasik et al 2008a). In a pilot surveillance study that included 152 patients with acute exacerbations of chronic pain, mainly of the lower back and knee, patients were recommended rose hip and seed powder at a dose providing up to 3 mg of galactolipid/day for up to 54 weeks. Multivariate analysis suggested an appreciable overall improvement, irrespective of type of pain, and this was reflected for most of the individual measures. There were no serious adverse events (Chrubasik et al 2008b). In a double-blind placebo-controlled trial of 89 patients with rheumatoid arthritis, treatment with encapsulated rose-hip powder 5 g daily for 6 months suggested that patients with rheumatoid arthritis may benefit from additional treatment with rose hip (Willich et al 2010). A study comparing powdered rose hip with and without the seeds found that extracts derived from rose hip without fruits were more effective in assays carried out for inhibition of COX-1, COX-2 and 5-LOX-mediated leukotriene B(4) formation, as well as for antioxidant capacity (Wenzig et al 2008). Extracts of rosehips have displayed potent anti-inflammatory and antinociceptive activities in several *in vivo* experimental models (Deliorman Orhan et al 2007), but the mechanism of action and the active constituents are still not fully known. Bioassay-guided fractionation of rosehip powder yielded the triterpene acids, oleanolic acid and ursolic acid, as inhibitors of lipopolysaccharide-induced interleukin-6 release (Saaby et al 2011), but these are ubiquitous compounds and may only play a part in the overall activity.

TURMERIC, *CURCUMA DOMESTICA* VAL. (CURCUMAE DOMESTICAE RHIZOMA) EP

The rhizomes of turmeric (syn. *C. longa* L., Zingiberaceae) are imported as a ready-prepared and ground, dark yellow powder with a characteristic taste and odour. The distinctive colour and presence of starch grains (as both simple and compound grains) and cork make the microscopic identification of the drug relatively straightforward. Turmeric is used in religious ceremonies by Hindus and Buddhists. It is important in the preparation of curry powders and is increasingly being used as a colouring agent because of the increased use of natural ingredients in foods. A related species is Javanese turmeric (*Curcuma xanthorrhiza* Roxb., Curcumae xanthorrhizae rhizoma; Eur. Ph.), which is mostly used for dyspepsia and other gastrointestinal problems.

Constituents

Three classes of compounds are particularly important: the curcuminoids – the mixture known as curcumin (Fig. 27.2) – consisting of several phenolic diarylheptanoids including curcumin, monodemethoxycurcumin and bisdemethoxycurcumin; an essential oil (about 3–5%), containing about 60% sesquiterpene ketones

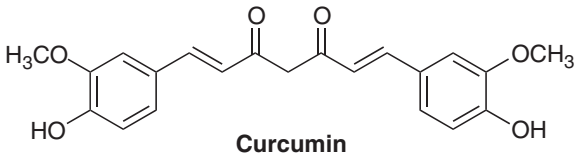


Fig. 27.2

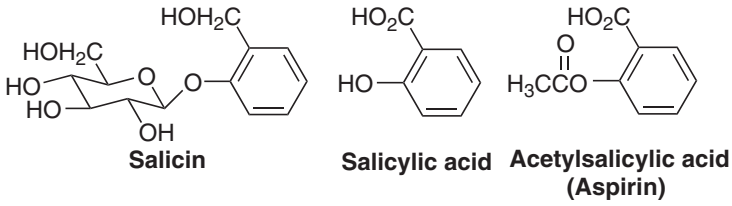


Fig. 27.3

(turmerones), including arturmerone, α-atlantone, zingberene, with borneol, α-phellandrene, eugenol and others; and polysaccharides such as glycans, the ukonans A–D.

Therapeutic uses and available evidence

Turmeric is becoming increasingly popular in the West as an anti-inflammatory and antihepatotoxic agent (see also Chapter 20, p. 248, Gastrointestinal and biliary system). It is also widely used in Ayurveda and Chinese medicine as an anti-inflammatory, digestive aid, blood purifier, antiseptic and general tonic. It is given internally and also applied externally to wounds and insect bites. Most of the actions are attributable to the curcuminoids, although some of the essential oil components are also anti-inflammatory. The efficacy of curcumin and its regulation of multiple targets, as well as its safety for human use, mean that turmeric has received considerable interest as a potential therapeutic agent for the prevention and/or treatment of various malignant diseases, arthritis, allergies, Alzheimer's disease and many other inflammatory illnesses (for a review, see Zhou et al 2011). Anti-inflammatory properties have been documented in numerous pharmacological models, and the use of turmeric seems promising, despite the limited number of clinical studies and poor bioavailability (for a review, see Henrotin et al 2010). Curcumin has been studied as an anticancer drug and inhibits iNOS (inducible nitric oxide synthase) in both *in vitro* and *in vivo* mouse models via a mechanism involving the pro-inflammatory transcription factor NF-κB. It has also been shown to inhibit the activation of another transcription factor (AP-1), indicating that curcumin may be a non-specific inhibitor of NF-κB. Reports also indicate cyclo-oxygenase inhibition and free radical scavenging ability as potential targets (for a review, see Epstein et al 2010). Immunostimulant activity, due to the polysaccharide fraction, has been shown, and anti-asthmatic effects have been noted, together with antimutagenic and anticarcinogenic effects. It is the subject of much current research but clinical evidence is urgently needed. Turmeric is well tolerated.

WILLOW BARK, *SALIX* SPP. (SALICIS CORTEX) EP

Salix spp., including *S. purpurea* L., *S.* x *fragilis* L., S. *daphnoides* Vill. and S. *alba* L. (Salicaceae), are the source of the drug 'willow bark'. They are trees and shrubs common in alpine ecosystems, flooded areas and along the margins of streams. Willow bark is a European phytomedicine with a long tradition of use for chronic forms of pain, rheumatoid diseases, fever and headache. As is well known, one of its main compounds, salicin, served as a lead molecule for the development of aspirin (acetylsalicylic acid).

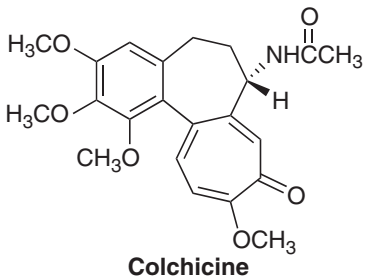
Constituents

Phenolic glycosides, including salicin (Fig. 27.3), phenolic acids, tannins (mainly dimeric and polymeric procyanidins) and flavonoids are the most prominent groups of compounds. The most commonly used willow bark dry extract has a salicin content of 15–18%.

Very few pharmacological studies of individual compounds from willow bark (and their metabolites) have been conducted. The extract, however, exerts effects on several pro-inflammatory targets, including both isoforms of cyclo-oxygenase and willow bark water extract STW 33-1 has been shown to produce a significant inhibition of TNFα and NF-κB in activated monocytes (Bonaterra et al 2010).

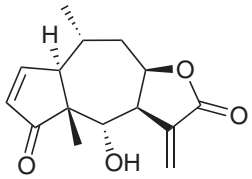
Therapeutic uses and available evidence

Willow bark has been studied clinically. The effectiveness of an extract of willow bark (which is licensed as a medicine in Germany) has been shown to be superior to placebo for osteoarthritis and lower back pain, and with fewer side effects than, for example, aspirin (for a review, see Vlachlojannis et al 2009). However, further more stringent clinical and mechanistic studies are needed. In very high doses, the side effects of salicylates may be encountered, although these are rarely seen at therapeutic levels of the extract. In general, the effective dose contains lower amounts of salicylate



Colchicine

Fig. 27.4



Helenalin

Fig. 27.5

than would be expected by calculation, and a form of synergy is thought to be operating within the extract.

GOUT

Gout is a very painful, localized inflammation of the joints (particularly those of the thumb and big toe) caused by hyperuricaemia and the consequent formation of needle-like crystals of uric acid in the joint. For prevention, the xanthine oxidase inhibitor allopurinol is the drug of choice, but an alternative is sulfinpyrazone, which increases excretion of uric acid. Prophylactic treatment should never be initiated during an acute attack as it may prolong it. Acute gout is normally treated with indometacin or other NSAIDs (but not aspirin), but, if inappropriate, colchicine can be used.

COLCHICINE EP

Colchine (Fig. 27.4) is a pure alkaloid extracted from the corms and flowers of *Colchicum autumnale* L., the autumn crocus or meadow saffron (Colchicaceae, formerly Liliaceae). The plant grows from bulbs in meadows throughout Europe and North Africa, typically appearing during the autumn, with the fruit developing over winter and dispersing prior to the first mowing of the meadows. The leaves and the fruit appear during spring. The plant extract is not used because colchicine is highly toxic and the dose must be rigorously controlled.

Colchicine is used in the acute phase of gout, particularly when NSAIDs are either ineffective or contraindicated (for a review, see Schlesinger et al 2009). Colchicine is occasionally also used as prophylaxis for Mediterranean familial fever. It is an important tool for biochemical research, as an inhibitor of the separation of the chromosomes during mitosis (e.g. used in breeding experiments to produce polyploid organisms). Colchicine causes gastrointestinal upsets such as nausea, vomiting, abdominal pain and diarrhoea. The dose is 1 mg initially, followed by increments of 500 µg every 2–3 hours until relief is obtained, to a maximum of 6 mg. The course should not be repeated within 3 days.

TOPICAL ANTI-INFLAMMATORY AGENTS

Most topical antirheumatics are rubifacients, which act by counter-irritation. They are used for localized pain or when systemic drugs are not appropriate. Many contain salicylates, and capsaicin is used for severe pain (e.g. with shingles). They should not be used in children, pregnant or breastfeeding women or with occlusive dressings. Arnica is also widely employed, despite little clinical evidence to support its use.

ARNICA, *ARNICA MONTANA* L. (ARNICAE FLOS) EP

Arnica (Asteraceae) is widely used in many European countries, including the UK. The flower heads are the part used, and, as *A. montana* is protected, other species are being investigated as substitutes. Extracts and tinctures are applied topically, for bruising, sprains, swellings and inflammation, usually in the form of a cream or gel.

Constituents

Arnica species are rich in sesquiterpene lactones of the pseudoguianolide type. The most abundant sesquiterpene lactone in *A. montana* is helenalin (Fig. 27.5), with 11α,13-dihydrohelenalin. Flavonoids, including quercetin and kaempferol derivatives, some coumarins and an essential oil are the other groups of natural products found typically in the flower heads of arnica.

Therapeutic uses and available evidence

Extracts of arnica and the pure sesquiterpene lactones with an exocyclic methylene group (e.g. helenalin) have been shown to exert anti-inflammatory effects *in vivo* in animal models, although few clinical studies have been carried out. A randomized, double-blind study in 204 patients with active osteoarthritis of the hands, carried out to compare ibuprofen gel (5%) with arnica gel (50 g tincture/100 g, drug extract ratio 1:20), found that there were no differences in pain relief and hand function after 21 days' treatment between the two groups. Adverse events were reported by five patients (4.8%) on arnica, slightly lower than the ibuprofen group (Widrig et al 2007).

However, in a trial with 53 subjects who were carrying out eccentric calf exercises arnica increased leg pain 24 hours after exercise (Adkison et al 2010). However this effect did not extend to the 48-hour measurement, and it is not clear how this model relates to most of the clinical situations in which arnica is used. There was no difference in muscle tenderness or ankle range of motion.

Helenalin is well known for its *in vitro* effects on several transcription factors, including NF-κB and NF-AT. Arnica preparations also suppress matrix metalloproteinase-1 (MMP1) and MMP13 mRNA levels in articular chondrocytes at low concentrations, possibly due to inhibition of DNA binding of the transcription factors AP-1 and NF-κB (Jäger et al 2009). The cytotoxicity of the sesquiterpene lactones is well documented, and allergic reactions may occur. Arnica is used externally, except in homeopathic preparations, but the sesquiterpene lactones have been shown to be absorbed through the skin (Tekko et al 2006).

CAPSAICIN

Capsaicin is the pungent oleo-resin of the fruit of the chilli pepper (*Capsicum frutescens* L., and some varieties of *C. annuum* L., Solanaceae), also known as capsicum, cayenne, or hot chilli. Green and red (or bell) peppers and paprika are produced by milder varieties. The plant is indigenous to tropical America and Africa, but is widely cultivated.

Constituents

Capsaicin itself is 8-methyl-*N*-vanillyl-non-6-enamide; other capsaicinoids such as dihydrocapsaicin, nordihydrocapsaicin, and homodihydrocapsaicin are present in the natural product. These are esters of vanillyl amine with C_8–C_{13} fatty acids.

Therapeutic uses and available evidence

Capsaicin acts on vanilloid receptors, causing inflammation, but it also desensitizes sensory nerve endings to pain stimulation by depleting the neuropeptide substance P from local C-type nerve fibres. It is used as a local analgesic in the treatment of postherpetic neuralgia, diabetic neuropathy, osteoarthritis and for pruritus (Papoiu and Yosipovitch 2010). In the management of intractable neuropathic pain, it may provide a degree of pain relief to some patients (Derry et al 2009). Capsaicin has long been used in cough and cold remedies, and findings that the vanilloid 1 (TRPV1) receptor is a sensor of airway irritation and initiator of the cough reflex (Geppetti et al 2010) may provide a rationale for that usage.

For external use, capsaicin is normally formulated as a cream containing 0.025%, 0.075% or 0.75%. Capsaicin can produce severe irritation. It causes burning on initial application and should not be applied near the eyes, mucous membranes or to broken skin. It should be avoided in children and pregnant or breastfeeding women.

WINTERGREEN OIL, *GAULTHERIA PROCUMBENS* L., *BETULA LENTA* L.

Wintergreen oil is now most often obtained from *Betula lenta* (Betulaceae) rather than *Gaultheria procumbens* (Ericaceae), although both have similar compositions. It has a characteristic odour of methyl salicylate.

Constituents

The oil contains methyl salicylate (about 98%), which is produced by enzymatic hydrolysis of phenolic glycosides during maceration and steam distillation.

Therapeutic uses and available evidence

Methyl salicylate is anti-inflammatory and antirheumatic. Oil of wintergreen is used mainly in the form of an ointment or liniment for rheumatism, sprains, sciatica, neuralgia and all kinds of muscular pain. Methyl salicylate can cause irritation. It should not be applied near the eyes, mucous membranes or to broken skin, and should be avoided in children and pregnant or breastfeeding women.

NOCTURNAL LEG CRAMPS

Night cramps are common in elderly people, and particularly in patients with liver disease such as cirrhosis.

QUININE EP

Quinine is isolated from the bark of *Cinchona* spp. Quinine salts can be effective in reducing their incidence, but should be avoided for routine use in the management of muscle cramps because of their potential for cardiac toxicity. However, in select patients they can be considered once potential side effects are taken into account. It has been recommended that quinine should be used more in patients with cirrhosis, subject to further investigations (Corbani et al 2008). Quinine salts are used in doses of 200–300 mg at bedtime, in ambulatory patients. For further information on quinine, including the structure, see Chapter 24 (pp. 291–292).

OSTEOPOROSIS AND BONE HEALTH

Osteoporosis is a common disorder affecting mainly elderly people and especially women, where it is exacerbated by the steep decline in oestrogen occurring after the menopause. Most conventional treatments rely either upon oestrogen replacement, or selective oestrogen receptor modulation (SERM) in women, or the use of drugs that prevent the breakdown of bone tissue, such as the bisphosphonates. A good diet is important for maintaining bone health and several studies have shown that consumption of fruit and vegetables can enhance bone growth (reviewed by Putnam et al 2007). Although there are few herbal treatments for osteoporosis, TCM offers some examples of potentially useful treatments, namely *Epimedium* aerial parts (see Chapter 26, p. 307, 'The reproductive tract') and kudzu, *Pueraria lobata* (Willd.) Ohwi. Epimedium (horny goat weed) contains the osteogenic and phytoestrogenic prenylated flavonoid icariin. Kudzu root, from *Pueraria* species, is used for many diseases in China and one of the most important is the treatment of osteoporosis.

KUDZU

The kudzu vines, [*Pueraria montana* var. *lobata* (Willd.) Sanjappa & Pradeep [syn *P. lobata* (Willd.) Ohwi], *P. montana* var. *chinensis* (Ohwi) Sanjappa & Pradeep (syn *P. thomsonii* Benth) and *P. candolle*i var. *mirifica* (Airy Shaw & Suvat.) Niyomdham] are today found in many warmer and human regions around the globe and are invasive species, especially in the southern states of America and on some Oceanian islands. In addition to its other activities, kudzu root has recently been advocated as a treatment for alcoholism, with some clinical evidence to support this use, as well as for some cerebro-vascular and neurological degenerative conditions. The roots of these species are phytoestrogenic and also can be used as hormone-replacement aids.

Constituents

The root contains isoflavones including puerarin, daidzen, genistein and their derivatives (reviewed by Zhang et al 2013, Wong et al 2011).

Therapeutic uses and available evidence

The isoflavones are phytoestrogenic, and exert some of their beneficial properties via this mechanism. Puerarin is the major active constituent and is thought to be responsible for most of the clinical effects. Several animal models have shown osteogenic effects for puerarin, mediated via various complementary mechanisms (reviewed by Zhou et al 2014), but clinical studies are lacking in osteoporosis.

References

Adkison, J.D., Bauer, D.W., Chang, T., 2010. The effect of topical arnica on muscle pain. Annals of Pharmacotherapy 44, 1579–1584.

Aiyegbusi, A.I., Duru, F.I., Anunobi, C.C., Noronha, C.C., Okanlawon, A.O., 2011. Bromelain in the early phase of healing in acute crush Achilles tendon injury. Phytotherapy Research 25, 49–52.

Anauate, M.C., Torres, L.M., de Mello, S.B., 2010. Effect of isolated fractions of *Harpagophytum procumbens* D.C. (devil's claw) on COX-1, COX-2 activity and nitric oxide production on whole-blood assay. Phytotherapy Research 24 (9), 1365–1369.

Barnes, J., 2009. Devil's claw *(Harpagophytum procumbens)*. Journal of Primary Health Care 1, 238–239.

Bonaterra, G.A., Heinrich, E.U., Kelber, O., Weiser, Metz J., Kinscherf, R., 2010. Anti-inflammatory effects of the willow bark extract STW 33-I (Proaktiv(W)) in LPS-activated human monocytes and differentiated macrophages. Phytomedicine 17, 1106–1113.

Cameron, M., Gagnier, J.J., Little.V, Parsons TJ., Blümle, A., Chrubasik, S., 2009. Evidence of effectiveness of herbal medicinal products in the treatment of arthritis. Part I: osteoarthritis. Phytotherapy Research 23, 1497–1515.

Chrubasik, C., Roufogalis, B.D., Müller-Ladner, U., Chrubasik, S., 2008a. A systematic review on the *Rosa canina* effect and efficacy profiles. Phytotherapy Research 22, 725–733.

Chrubasik, C., Wiesner, L., Black, A., Müller-Ladner, U., Chrubasik, S.,

2008b. A one-year survey on the use of a powder from *Rosa canina* lito in acute exacerbations of chronic pain. Phytotherapy Research 22, 1141–1148.

Corbani, A., Manousou, P., Calvaruso, V., Xirouchakis, I., Burroughs, A.K., 2008. Muscle cramps in cirrhosis: the therapeutic value of quinine. Is it underused? Digestive and Liver Disease 40, 794–799.

Deliorman Orhan, D., HarteviogÆlu, A., Küpeli, E., Yesilada, E., 2007. In vivo anti-inflammatory and antinociceptive activity of the crude extract and fractions from *Rosa canina* L. fruits. Journal of Ethnopharmacology 112, 394–400.

Derry, S., Lloyd, R., Moore, R.A., McQuay, H.J., 2009. Topical capsaicin for chronic neuropathic pain in adults. Cochrane Database Systematic Reviews 4, CD007393.

Epstein, J., Sanderson, I.R., Macdonald, T.T., 2010. Curcumin as a therapeutic agent: the evidence from in vitro, animal and human studies. British Journal of Nutrition 103, 1545–1557.

Gagnier, J.J., Oltean, H., Tulder, M.W., van Berman, B.M., Bombardier, C., Robbins, B., 2016. Herbal medicine for low back pain a Cochrane review. Spine 41, 116–133.

Geppetti, P., Patacchini, R., Nassini, R., Materazzi, S., 2010. Cough: the emerging role of the TRPA1 channel. Lung 188, S63–S68.

Gonzalez-Sarrias, A., Larrosa, M., Garcia-Conesa, M.T., Tomás-Barberán, F.A., Espín, J.C., 2013. Nutraceuticals for older people: facts, fictions and gaps in knowledge. Maturitas 75, 313–334.

Heinrich, M., Booker, A., 2015. Can there be an ethnopharmacology of inflammation? In: Heinrich, M., Jaeger, A.K. (Eds.), Ethnopharmacology. Wiley, Chichester, pp. 159–167.

Henrotin, Y., Clutterbuck, A.L., Allaway, D., et al., 2010. Biological actions of curcumin on articular chondrocytes. Osteoarthritis Cartilage 18, 141–149.

Inaba, K., Murata, K., Naruto, S., Matsuda, H., 2010. Inhibitory effects of devil's claw (secondary root of *Harpagophytum procumbens*) extract and harpagoside on cytokine production in mouse macrophages. Journal of Natural Medicine 64, 219–222.

Inchingolo, F., Tatullo, M., Marrelli, M., et al., 2010. Clinical trial with bromelain in third molar exodontia. European Review for Medical and Pharmacological Science 14, 771–774.

Jäger, C., Hrenn, A., Zwingmann, J., Suter, A., Merfort, I., 2009. Phytomedicines prepared from Arnica flowers inhibit the transcription factors AP-1 and NF-kappaB and modulate the activity of MMP1 and MMP13 in human and bovine chondrocytes. Planta Medica 75, 1319–1325.

Mahomed, I.M., Ojewole, J.A., 2009. Uterotonic effect of *Harpagophytum procumbens* DC (Pedaliaceae) secondary root aqueous extract on rat isolated uterine horns. Journal of Smooth Muscle Research 45, 231–239.

Mncwangi, N., Chen, W., Vermaak, I., Viljoen, A.M., Gericke, N., 2012. Devil's claw—a review of the ethnobotany, phytochemistry and biological activity of *Harpagophytum procumbens*. Journal of Ethnopharmacology 143, 755–771.

Ouitas, N.A., Heard, C., 2010. Estimation of the relative anti-inflammatory efficacies of six commercial preparations of *Harpagophytum procumbens* (devil's claw). Phytotherapy Research 24, 333–338.

Papoiu, A.D., Yosipovitch, G., 2010. Topical capsaicin. The fire of a `hot' medicine is reignited. Expert Opinion on Pharmacotherapy 11, 1359–1371.

Putnam, S.E., Scutt, A.M., Bicknell, K., Priestley, C.M., Williamson, E.M., 2007. Natural products as treatments for metabolic bone disorders and for maintenance of bone health. Phytotherapy Research 21 (2), 99–112.

Saaby, L., Jäger, A.K., Moesby, L., Hansen, E.W., Christensen, S.B., 2011. Isolation of immunomodulatory triterpene acids from a standardized rose hip powder (*Rosa canina* L.). Phytotherapy Research 25, 195–201.

Schlesinger, N., Dalbeth, N., Perez-Ruiz, F., 2009. Gout – what are the treatment options? Expert Opinion on Pharmacotherapy 10, 1319–1328.

Tekko, I.A., Bonner, M.C., Bowen, R.D., Williams, A.C., 2006. Permeation of bioactive constituents from *Arnica montana* preparations through human skin in-vitro. The Journal of Pharmacy and Pharmacology 58, 1167–1176.

Vlachlojannis, J.E., Cameron, M., Chrubasik, S., 2009. A systematic review on the effectiveness of willow bark for musculoskeletal pain. Phytotherapy Research 23, 897–900.

Wenzig, E.M., Widowitz, U., Kunert, O., et al., 2008. Phytochemical composition and in vitro pharmacological activity of two rose hip (*Rosa canina* L.) preparations. Phytomedicine 15, 826–835.

Widrig, R., Suter, A., Saller, R., Melzer, J., 2007. Choosing between NSAID and arnica for topical treatment of hand osteoarthritis in a randomised, double-blind study. Rheumatology International 27, 585–591.

Willich, S.N., Rossnagel, K., Roll, S., et al., 2010. Rose hip herbal remedy in patients with rheumatoid arthritis – a randomised controlled trial. Phytomedicine 17, 87–93.

Wong, K.H., Li, G.Q., Li, K.M., Razmovski-Naumovskia, V., Chan, K., 2011. Kudzu root: traditional uses and potential medicinal benefits in diabetes and cardiovascular diseases. Journal of Ethnopharmacology 134, 584–607.

Zhang, Z., Lam, T.M., Zuo, Z., 2013. Radix Puerariae: an overview of its chemistry, pharmacology, pharmacokinetics and clinical use. Journal of Clinical Pharmacology 53 (8), 787–811.

Zhou, H., Beevers, C.S., Huang, S., 2011. The targets of curcumin. Current Drug Targets 12, 332–347.

Zhou, Y.X., Zhang, H., Peng, C., 2014. Puerarin: a review of its pharmacological effects. Phytotherapy Research 28 (7), 961–975.

Chapter 28

The skin

Inflammatory and infectious skin diseases have a high prevalence in both developing and developed countries. Anti-infective preparations have been covered separately in Chapter 24. It is in the areas of dry and itchy skin, inflammation and wound healing that medicinal plants have an important place. Cosmetic products used widely are only included if preparations with well-defined medical indications are available.

DRY AND ITCHY SKIN CONDITIONS, AND ECZEMA

Dry and scaly skin conditions are very common and can arise from many causes. Diagnosis should be carried out initially by a medical practitioner in order to exclude infection, infestation or other serious disorders. Emollient preparations, such as oil-based preparations based on peanut (arachis) oil, or oat extracts, are usually the first line of treatment. Plant extracts are often incorporated into these preparations and can be very useful.

ARACHIS OIL (ARACHIDIS OLEUM RAFFINATUM) EP

Arachis oil (also known as groundnut or peanut oil) is expressed from *Arachis hypogaea* L. (Fabaceae). It is a fixed oil consisting mainly of glycerides of oleic and linoleic acids. It is an ingredient of emollient creams and bath oils. Peanuts are dangerously allergenic to some individuals, and the oil should be avoided in these patients as a precaution.

OATS, *AVENA SATIVA* L.

Oats (*Avena sativa*, Poaceae) are a widely distributed cereal crop. The seeds, with the husks removed, are crushed to form a coarse powder, which is creamy white in colour.

Constituents

Oats contain proteins (prolamines known as avenin, avenalin and gliadin), polyphenols known as avenanthramides, starch and soluble polysaccharides (mainly β-glucans and arabinogalactans), saponin glycosides including avenacosides A and B, and soyasaponin I. The fatty oil is composed of phytosterols including cholesterol, β-sitosterol and Δ^5-avenasterol, avenoleic, oleic, ricinoleic and linoleic acids and vitamin E.

Therapeutic uses and available evidence

Oats are externally emollient, and a colloidal fraction is used in bath preparations for eczema, and itchy or dry skin, often with success, especially if used regularly over a long period. Cells treated with avenanthramides showed a significant inhibition of TNF-alpha- induced NF-κB activity and subsequent reduction of interleukin-8 (IL-8) release. Topical application of avenanthramides mitigated inflammation in murine models of contact hypersensitivity and neurogenic inflammation and reduced pruritogen-induced scratching in a murine itch model. Avenanthramides are thus potent anti-inflammatory agents that appear to mediate the anti-irritant effects of oats (Sur et al 2008). The many clinical properties of colloidal

oatmeal derive from its variety of chemical constituents: the starches and beta-glucans are responsible for the protective and water-holding functions of oat and the presence of phenolics confers antioxidant and anti-inflammatory activity. Some of the oat phenols are also strong ultraviolet absorbers and the cleansing activity of oat is mostly due to saponins (Kurtz and Wallo 2007). Together, these make colloidal oatmeal a cleanser, moisturizer, buffer, as well as a soothing and protective anti-inflammatory agent (for a review, see Cerio et al 2010).

Oat tinctures are also taken internally for their reputed sedative activity, but this has not yet been proven. Ingestion of oats lowers cholesterol levels; an effect attributed to the saponins and polysaccharides.

INFLAMMATORY SKIN CONDITIONS

Allergic reactions, psoriasis, burns, bruising and general inflammation of skin are common. Severe cases are treated with corticosteroids as well as emollient preparations, ideally under medical supervision. However, minor disorders respond well to phytotherapy, with soothing and anti-inflammatory herbal products, as outlined below.

ALOE VERA, *ALOE VERA* (L.) BURM.F. [SYN.: *ALOE BARBADENSIS* MILL.]

The name 'aloe vera' is usually applied to the gel obtained from the centre of the fleshy leaves of various species of aloe, to differentiate from the anthraquinone-rich exudate or 'aloes', which is used as a purgative. The botanical nomenclature of the genus *Aloe* (Asphodeliaceae) is complex and changing. The succulent, non-fibrous leaves are about 30–40 cm long, up to 5 cm in diameter and occur in a terminal, sessile rosette. It is common practice in the tropics to use the gel and the heated leaves for burns and other inflammatory skin conditions. Aloe vera is added to shampoo, skin creams, 'after sun' preparations (and even washing powder), but normally in concentrations too low to have any therapeutic effect.

Constituents

An ill-defined extract of the fleshy leaves, obtained by cutting open and scraping out the gel, is used in the preparation of externally used products, especially cosmetics. It contains polysaccharides consisting mainly of glucomannans, glycoproteins such as the aloctins, enzymes such as carboxypeptidases and variable amounts of anthraquinone glycosides.

Therapeutic uses and available evidence

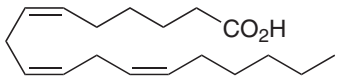
Aloe vera gel is used mainly in the form of the pure gel, applied as a lotion. It may be stabilized and a preservative added for this purpose. For dermatological preparations, there is some evidence for antibacterial, anti-inflammatory, emollient and moisturizing effects. The polysaccharides are important as soothing and immunostimulating agents. Some of the glycoproteins have similar effects, while the anthraquinone derivatives are antibacterial. Enzymes extracted from aloe vera gel have been shown to be analgesic and inhibit thermal damage and vascular permeability in mice. The fresh leaf pulp is antioxidant and induces carcinogen-metabolizing phase I enzymes. Taken internally, the gel has been reported to be effective in the treatment of stomach and aphthous (mouth) ulcers. Although few good clinical studies are available, aloe vera gel seems to be helpful in the treatment of burns and to aid wound healing (for a review, see Maenthaisong et al 2007). Aloe vera cream was found to be at least as effective as 0.1% triamcinolone acetonide in reducing the clinical symptoms of psoriasis in patients (Choonhakarn et al 2010) as well as in mice (Dhanabal et al 2012). In UV-induced erythema, aloe vera gel (97.5%) displayed some anti-inflammatory effects superior to those of 1% hydrocortisone in placebo gel (Reuter et al 2008), supporting its use as an after-sun treatment and post-radiotherapy emollient. The sterol hexadecanoic acid has antifungal effects (Bawankar et al 2013).

EVENING PRIMROSE OIL, *OENOTHERA BIENNIS* L. (OENOTHERA BIENNIS OLEUM) AND OTHER SPP.

Oenothera spp. (Onagraceae) are common ornamentals that came into use in Western phytomedicine as a result of indigenous uses by North American Indians. Today, the seed oil is used in the treatment of atopic eczema. The plant is a medium or tall hairy perennial with alternate, lanceolate leaves and relatively large yellow, four-petalled flowers, developing into long elongated capsules containing the seeds from which the oil is extracted.

Constituents

The seed oil contains about 70% *cis*-linoleic acid and about 9% *cis*-γ-linolenic acid (Fig. 28.1).



γ-Linolenic acid

Fig. 28.1

Therapeutic uses and available evidence

The fatty oil has been extensively investigated, and its therapeutic benefits ascribed mainly to the γ-linolenic acid content. It is taken internally as well as applied externally. Supplementation with omega-6 essential fatty acids (omega-6 EFAs) is of potential interest in the treatment of atopic dermatitis since patients with atopic dermatitis have been reported to have imbalances in EFA levels. EFAs play a vital role in skin structure and physiology and deficiency replicates the symptoms of atopic dermatitis.

To date, most studies of EFA supplementation in atopic dermatitis have produced conflicting results. A Cochrane review assessed 19 studies using evening primrose oil orally in the treatment of eczema and found that it failed to significantly improve overall eczema symptoms compared to placebo (Bamford et al 2013). An earlier meta-analysis found that evening primrose oil has a simultaneous beneficial effect on itching, crusting, oedema and redness that becomes apparent between 4 and 8 weeks after treatment is initiated. This effect is said to be reduced in association with increasing frequency of using potent steroids (Morse and Clough 2006). The main indications for which clinical evidence exists are: atopic eczema, especially in infants, mastalgia, rheumatoid arthritis and premenstrual syndrome, although the evidence of efficacy is equivocal. Evening primrose oil is usually taken in conjunction with vitamin E to prevent oxidation.

Note: The seed oil of *Borago officinalis* L. (borage, Boraginaceae), also known as Star Flower oil, is used in a similar way to evening primrose oil, but contains two to three times more γ-linolenic acid. It seems to provide some benefit to patients with atopic eczema (Foster et al 2010).

MARIGOLD, *CALENDULA OFFICINALIS* L. (CALENDULAE FLOS) EP

Calendula officinalis (Scotch or 'pot') marigold is one of the best known medicinal plants of Europe and has a long tradition of pharmaceutical use. Its origin is unclear and it has been cultivated for many centuries. Consequently, many varieties exist and its usage as an ornamental has increased the botanical variability of the species. The flower heads are relatively large, with a diameter of up to 5 cm, and yellow-orange. Some varieties have both ligulate (tongue-shaped) and radiate florets, others have only the ligulate type. The European Pharmacopoeia (Eur. Ph.) requires that only flower heads exclusively containing ligulate florets should be used.

Constituents

Marigold flowerheads contain saponins based on oleanolic acid, including calendasaponins A, B, C and D, and triterpene pentacyclic alcohols such as faradol, arnidiol, erythrodiol, calenduladiol, heliantriols A1, B0, B1 and B2, taraxasterol, lupeol and ursatriol. It also contains flavonoids, including hyperoside and rutin; sesquiterpene and ionone glycosides such as officinosides A, B, C and D, loliolide and arvoside A; a volatile oil and polysaccharides PS-I, -II and -III; and chlorogenic acid.

Therapeutic uses and available evidence

Pharmaceutical uses include inflammatory skin conditions such as topical application for wound healing and after radiotherapy. The flower heads and extracts from them are well known for their anti-inflammatory properties, which are mainly due to the lipophilic triterpene alcohols, notably the esters of faradiol. These were demonstrated using *in vivo* models such as phorbol ester-induced mouse ear oedema or croton oil-induced irritation.

Marigold extract prevented ultraviolet B (UVB) irradiation-induced growth-stimulating hormone (GSH) depletion in the skin of hairless mice after oral administration and increased gelatinase activity, which may be beneficial for skin healing and pro-collagen synthesis (Fonseca et al 2010). Both oral and topical applications of *Calendula* flower extract improved healing of excision wounds in rats and reduced the time needed for re-epithelization (Preethi and Kuttan 2009). Extracts stimulated proliferation and migration of fibroblasts at low concentrations (Fronza et al 2009), again supporting its use in wound healing, while the essential oil exerts (*in vitro*) antibacterial and antifungal effects. Immunostimulant effects have been reported for polysaccharide fractions. Few clinical studies are available to further validate these pharmacological data, although some preliminary studies indicate efficacy.

WITCH HAZEL, *HAMAMELIS VIRGINIANA* L. (HAMAMELIS FOLIUM, AND HAMAMELIS FOLIUM ET CORTEX) EP

Witch hazel (*Hamamelis virginiana* L., Hamamelidaceae) is indigenous to North America and Canada. The leaves are broadly oval, up to 15 cm long, 7 cm broad, the margin dentate or crenate, the apex acute and the base asymmetrically cordate. The distilled extract, known simply as 'witch hazel', is used as an astringent in skin and eye inflammation.

Constituents

The leaves and bark contain tannins, composed mainly of gallotannins with some condensed catechins and proanthocyanins. These include 'hamamelitannin', which is a mixture of related tannins, including galloylhamameloses and flavonoids.

Therapeutic uses and available evidence

Witch hazel is widely used for the treatment of haemorrhoids, bruises, skin irritation, spots and blemishes and redness of the eye. Hamamelitannin inhibits TNF-mediated endothelial cell death without altering TNF-induced upregulation of endothelial adhesiveness (Habtemariam 2002), which may explain the antihaemorrhagic use. The proanthocyanidins, gallotannins and gallates are highly active as free radical scavengers. Witch hazel phenolics protected red blood cells from free radical-induced haemolysis and were mildly cytotoxic to 3T3 fibroblasts and HaCat keratinocytes; they also inhibited the proliferation of tumoral SK-Mel 28 melanoma cells at lower concentrations than grape (*Vitis vinifera* L.) and pine (*Pinus* spp.) procyanidins (Touriño et al 2008) and the effects on fibroblast cells against hydrogen peroxide-induced damage have been linked to the reported therapeutic benefits (Thring et al 2011).

Antiviral activity against *herpes* viruses has been shown, and several clinical studies have demonstrated the efficacy of topically applied witch hazel in inflammatory conditions, including UV-irradiated burning and atopic dermatitis (Hughes-Formella et al 2002). An observational study in children (age 27 days to 11 years) with minor skin injuries, diaper dermatitis, or localized inflammation of skin found hamamelis ointment to be as effective as dexpanthenol, and concluded that hamamelis ointment is an effective and safe treatment for certain skin disorders in children up to the age of 11 years (Wolff and Kieser 2007). However, a review of the literature of hamamelis water in women suffering episiotomy pain following childbirth found it to confer no advantage over ice packs (East et al 2007). Witch hazel is used in after-shave lotions and in cosmetic preparations. The evidence for its use is mostly based on empirical evidence and very little systematic data providing a clinical evidence base are available.

Rarely allergic contact dermatitis has been reported (EMA/HMPC 2009).

WOUND HEALING

CENTELLA ASIATICA (L.) URB. (CENTELLAE HERBA) EP

Centella asiatica (Apiaceae), also known as gotu kola, Indian Pennywort, Brahmi and Manduukaparani, is an important medicinal plant throughout the world. The leaves of this small plant are kidney-shaped (reniform), long-stalked with rounded apices. The pinkish to red flowers are borne in small rounded umbels. It is a native of tropical and subtropical Asia and generally grows along streams, ditches and in low wet areas. As a consequence, this makes the species prone to exposure to sewerage, so there is a risk of high levels of bacterial and other contaminations (including heavy metals). In Sri Lanka and other countries it is an element of the local cuisine, used as a vegetable or in salads.

Constituents

The triterpenes are generally considered to be the major active metabolites. They are mainly pentacyclic triterpenic acids of the ursane or oleanane types, and their glycosides, and include asiatic acid, the asiaticosides, madecassic acid, madecassoside, brahmoside, brahmic acid, brahminoside, thankuniside, isothankuniside, centelloside, madasiatic acid, and the centellasaponins. There are some differences depending on the geographical origin of the plant. Flavonoids based on kaempferol and quercetin are present and there is a small amount of essential oil, with α-humulene, β-caryophyllene and bicyclogermacrene as the main constituents.

Therapeutic uses and available evidence

A wide range of pharmacological uses has been reported, including as a general tonic (see also Chapter 32; Bylka et al 2014). Most importantly, it is being used in

skin conditions, including wound healing, inflammation, psoriasis, keloid and prevention of stretch marks during pregnancy. Creams containing extracts are applied topically, but products derived from the plant are also taken internally for mental disorders, to improve memory, for atherosclerosis and to improve venous insufficiency and microangiopathy (for a review, see Brinkhaus et al 2000).

The current scientific and especially clinical data are too limited to determine the therapeutic benefits of *C. asiatica* and its preparations in wound healing (Bylka et al 2014). Despite the lack of clinical studies, pharmacological studies support the use of Centella in skin conditions. Extracts have been shown to significantly increase the wound breaking strength in a rat incision wound model, improving the rate of epithelization and wound contraction (Shetty et al 2006). Different constituents affect the different phases of wound repair: the triterpenes stimulated extracellular matrix accumulation in rat experimental wounds (Coldren et al 2003), whereas asiatic acid stimulated collagen synthesis, and madecassoside increased collagen secretion (Lee et al 2006). Centella has been suggested as a topical anti-psoriatic agent, and extracts have been shown to inhibit keratinocyte replication. The effect was thought to be due to the two triterpenoid glycosides madecassoside and asiaticoside (Sampson et al 2001).

INSECTICIDAL AGENTS

Control of insects is a huge problem due to their potential for transmitting disease, spoiling foods and devastating crops, and causing skin infestations in animals and humans. Agricultural methods and eradicating insect disease vectors – such as the *Anopheles* mosquito which transmits malaria, and *Aedes* species which carry the Zika and Dengue fever viruses - are obviously different to those involved in treating humans, although similar insecticidal compounds are used in many applications if they meet safety requirements.

Before the advent of synthetic pesticides, all insecticides were derived from natural products, mainly terpenoids such as essential oils (e.g. tea tree, see above, lavender, citronella), which are still widely used as insect repellants, pyrethrum, and quassia. Delphinium and veratrum alkaloids were used formerly but they are highly toxic and can be absorbed through the skin. Pyrethrum is extremely important as an insecticidal product, both in the natural form and as the starting material for the semisynthetic pyrethroids, and is used for infestations of lice and scabies.

HEADLICE

The most common application of herbal insecticidal agents in medicine is probably treating infestations with head lice, *Pediculus humanus capitis* (Anoplura: Pediculidae), a common problem throughout the world. This is a common problem in most countries, irrespective of wealth or status of patients, and particularly amongst schoolchildren where they are easily passed from head to head. Adult head lice are actually easy to kill, they do not survive long away from the body and can be suffocated with occlusive treatments such as dimethicone and mineral oil. The eggs (nits) are the main problem; they have a waterproof covering and most insecticides only act once the louse has hatched. This means that either multiple treatments or persistent insecticides must be used; however, the use of persistent compounds facilitates the development of resistance: as concentrations fall, lice can survive more easily and acquire resistance. This has occurred with permethrin (see below). Body lice are more robust and can survive for much longer periods away from the body; they also transmit disease whereas head lice are considered to be mainly a social problem. A review comparing the use of synthetic, semi-synthetic, natural and mechanical agents for head lice infestation is available from Burgess (2011), but the following natural medicines are also used.

PYRETHRUM (INSECT) FLOWERS, *CHRYSANTHEMUM* SPP.

Chrysanthemum cinerariaefolium (Trev.) Vis., *C. coccineum* Willd. and *C. marshallii* Aschers (Asteraceae) are all known as insect flowers. Dalmation insect flowers are *C. cinerariifolium* [formerly *Pyrethrum cinerarii folium* Trev. or *Tanacetum cinerariifolium* (Trev.) Sch. Bip.]; *C. coccineum* and *C. marshallii* are known as Persian and Causasian insect flowers, respectively. They are indigenous to the Balkans but widely cultivated elsewhere. The unopened flower heads are about 7 cm in diameter, with creamy-white ligulate and yellow tubular florets. There are 2-3 rows of lanceolate greenish-yellow, hairy bracts and a flat receptacle without paleae.

Constituents

All species contain pyrethrins, which are esters of chrysanthemic and pyrethric acids and are the actives.

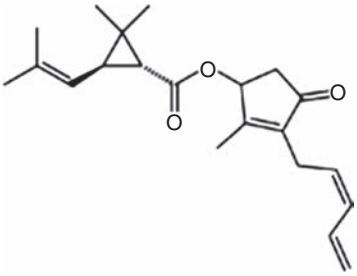


Fig. 28.2 Pyrethrin I

They are known as pyrethrins I (Fig. 28.2) and II, cinerins I and II and jasmolins I and II.

Therapeutic uses and available evidence

The natural pyrethrins are used to treat lice and scabies infestations and to kill other types of insect (houseflies etc.) which are not necessarily causes of skin infestation. Pyrethrin I (Fig. 28.2) is the most potent of the naturally occurring compounds, although all have a knock-down effect on insects. The natural products have been used to develop semi-synthetic derivatives such as permethrin, phenothrin, tetramethrin, cypermethrin and decamethrin, which can be more potent and offer more chemical stability. All of these have been shown to have clinical efficacy, but the semi-synthetic compounds are more likely to lead to resistance arising because of their persistence. Pyrethrum is mostly considered to be harmless to humans and animals, and may be used as a spray, lotion or powder, or fumigant. Pyrethroids are much less toxic to humans than synthetic insecticides, but care should be taken as they can cause irritation or allergic reactions.

QUASSIA WOOD, *PICRASMA EXCELSA* (SW.) PLANCH AND *QUASSIA AMARA* L.

Picrasma excelsa and *Quassia amara* (Simaroubaceae), known as quassia or bitter wood, and Japanese quassia (*P. ailanthoides* Planch) occur in commerce as logs, chips or shredded. They have a wide folk-lore use as insecticides but extracts must be used immediately after preparation, as they are unstable, which limits their usefulness.

Constituents

The wood contains quassinoids such as quassin, isoquassin (= picrasmin) and others, depending on the species. *P. excelsa* and *Q. amara* also contain carboline alkaloids and *P. ailanthoides* contains a series of picrasidine alkaloids.

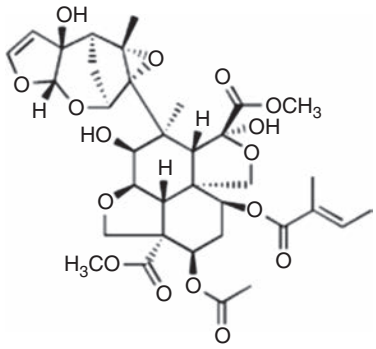


Fig. 28.3 Azadirachin

Therapeutic uses and available evidence

Quassia is an insecticide, anthelmintic, febrifuge and antimalarial, although efficacy in malaria is unproven. It has been used as a fresh infusion to treat head lice, although little evidence for efficacy is available. The quassinoids are insecticidal, cytotoxic and amoebicidal both *in vitro* and *in vivo*. Quassia is used to flavour bitter alcoholic and soft drinks and to stimulate the appetite. It is non-toxic when applied externally, and safe in small doses when ingested.

NEEM, *AZADIRACHTA INDICA* A. JUSS. (MELIACEAE)

The Neem tree is a vitally important medicinal plant in Asia, where it is used to treat a wide spectrum of diseases, especially skin conditions. There is a strong tradition in India of neem use as a personal insecticide and to control infestations in crops. All parts of the plant are used, and lotions and shampoos containing seed oil and extracts are marketed as headlice treatments. These are very popular, and although their efficacy has not been conclusively proven in clinical trials, there is some evidence to show their usefulness (reviewed by Edwards et al 2015).

Constituents

Neem contains limonoids such as the azadiractin (Fig. 28.3) and nimbin derivatives as the active constituents, together with flavonoids, tannins and coumarins,

Therapeutic uses and available evidence

Neem seed extracts have shown activity against headlice and their eggs (e.g. Abdel-Ghaffar et al 2012), and when used topically they are considered safe. Neem

extracts are also used internally in some traditional medicine systems, but there are concerns about their genotoxicity, cytotoxicity and abortifacient properties.

ESSENTIAL OILS

Many essential oils and their components have pediculicidal (and other insecticidal) effects. Tea tree, and its component terpenen-4-ol, are particularly effective. Some mono- and sesquiterpenes are more potent at killing lice than eggs, and vice versa, so a mixture (as is found naturally in an essential oil) may be more clinically effective than an isolated terpene (reviewed by Williamson 2007). Essential oils also have the advantage of penetrating the egg covering and killing the developing louse. Despite the long history of use and the wide application in this area, there are few good clinical trials showing efficacy. Many essential oils are known allergens and they are also readily absorbed through the skin, but their use in moderate concentrations does not seem to pose any great risk.

References

Abdel-Ghaffar, F., Al-Quraishy, S., Al-Rasheid, K.A., Mehlhorn, H., 2012. Efficacy of a single treatment of head lice with a neem seed extract: an *in vivo* and *in vitro* study on nits and motile stages. Parasitol Res. 110 (1), 277–280.

Bamford, J.T., Ray S., Musekiwa A., van Gool C., Humphreys R., Ernst E., 2013. Oral evening primrose oil and borage oil for eczema. Cochrane Database Syst Rev. 2013 Apr 30;(4):CD004416. doi: 10.1002/14651858.CD004416.pub2.

Bawankar, R., Deepti, V.C., Singh, P., Subashkumar, R., Vivekanandhan, G., Babu, S., 2013. Evaluation of bioactive potential of an *Aloe vera* sterol extract. Phytotherapy Research 27 (6), 864–868.

Brinkhaus, B., Lindner, M., Schuppan, D., Hahn, E.G., 2000. Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella asiatica*. Phytomedicine 7 (5), 427–448.

Burgess, I.F., 2011. Head lice. BMJ Clin Evid. May 16, 2011 pii: 1703.

Bylka, W., Znajdek-Awizeń, P., Studzińska-Sroka, E., Dańczak-Pazdrowska, A., Brzezińska, M., 2014. *Centella asiatica in* dermatology: An overview. Phytotherapy Research 28 (8), 1117–1124.

Cerio, R., Dohil, M., Jeanine, D., Magina, S., Mahé, E., Stratigos, A.J., 2010. Mechanism of action and clinical benefits of colloidal oatmeal for dermatologic practice. Journal of Drugs Dermatology 9 (9), 1116–1120.

Choonhakarn, C., Busaracome, P., Sripanidkulchai, B., Sarakarn, P., 2010. A prospective, randomized clinical trial comparing topical aloe vera with 0.1% triamcinolone acetonide in mild to moderate plaque psoriasis. Journal of the European Academy of Dermatology and Venereology 24 (2), 168–172.

Coldren, C.D., Hashim, P., Ali, J.M., Oh, S.K., Sinskey, A.J., Rha, C., 2003. Gene expression changes in the human fibroblast induced by *Centella asiatica* triterpenoids. Planta Medica 69 (8), 725–732.

Dhanabal, S.P., Dwarampudi, L.P., Muruganantham, N., Vadivelan, R., 2012. Evaluation of the antipsoriatic activity of *Aloe vera* leaf extract using a mouse tail model of psoriasis. Phytotherapy Research 26 (4), 617–619.

East, C.E., Begg, L., Henshall, N.E., Marchant, P., Wallace, K., 2007. Local cooling for relieving pain from perineal trauma sustained during childbirth. Cochrane Database Systematic Reviews 4 CD006304.

Edwards, S.E., Da Costs Rocha, I., Williamson, E.M., Heinrich, M., 2015. Phytopharmacy: an evidence-based guide to herbal medicinal products. Wiley-Blackwell, Chichester, UK.

EMA/HMPC, 2009. Assessment report on *Hamamelis virginiana* L., cortex, *Hamamelis virginiana* L., folium, *Hamamelis virginiana* L., folium et cortex aut ramunculus destillatum. EMA/HMPC/114585/2008. Available online at http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_HMPC_assessment_report/2010/04/WC500089242.pdf.

Fonseca, Y.M., Catini, C.D., Vicentini, F.T., Nomizo, A., Gerlach, R.F., Fonseca, M.J., 2010. Protective effect of *Calendula officinalis* extract against UVB-induced oxidative stress in skin: evaluation of reduced glutathione levels and matrix metalloproteinase secretion. Journal of Ethnopharmacology 127 (3), 596–601.

Foster, R.H., Hardy, G., Alany, R.G., 2010. Borage oil in the treatment of atopic dermatitis. Nutrition 26 (7–8), 708–718.

Fronza, M., Heinzmann, B., Hamburger, M., Laufer, S., Merfort, I., 2009. Determination of the wound healing effect of *Calendula* extracts using the scratch assay with 3T3 fibroblasts. Journal of Ethnopharmacology 126 (3), 463–467.

Habtemariam, S., 2002. Hamamelitannin from *Hamamelis virginiana* inhibits the tumour necrosis factor-alpha (TNF)-induced endothelial cell death in vitro. Toxicon 40 (1), 83–88.

Hughes-Formella, B.J., Filbry, A., Gassmueller, J., Rippke, F., 2002. Anti-inflammatory efficacy of topical preparations with 10% hamamelis distillate in a UV erythema test. Skin Pharmacology and Applied Skin Physiology 15 (2), 125–132.

Kurtz, E.S., Wallo, W., 2007. Colloidal oatmeal: history, chemistry and clinical properties. Journal of Drugs in Dermatology 6 (2), 167–170.

Lee, J., Jung, E., Lim, J., et al., 2006. Asiaticoside induces human collagen I synthesis through TGFbeta receptor I kinase (TbetaRI kinase)-independent Smad signaling. Planta Medica 72 (4), 324–328.

Maenthaisong, R., Chaiyakunapruk, N., Niruntraporn, S., Kongkaew, C., 2007. The efficacy of aloe vera used for burn wound healing: a systematic review. Burns 33 (6), 713–718.

Morse, N.L., Clough, P.M., 2006. A meta-analysis of randomized, placebo-controlled clinical trials of Efamol evening primrose oil in atopic eczema. Where do we go from here in light of more recent discoveries? Current Pharmaceutical Biotechnology 7 (6), 503–524.

Preethi, K.C., Kuttan, R., 2009. Wound healing activity of flower extract of *Calendula officinalis*. Journal of Basic Clinical Physiology and Pharmacology 20 (1), 73–79.

Reuter, J., Jocher, A., Stump, J., Grossjohann, B., Franke, G., Schempp, C.M., 2008. Investigation of the anti-inflammatory potential of *Aloe vera* gel (97.5%) in the ultraviolet erythema test. Skin Pharmacology and Physiology 21 (2), 106–110.

Sampson, J.H., Raman, A., Karlsen, G., Navsaria, H., Leigh, I.M., 2001. In vitro keratinocyte antiproliferant effect of *Centella asiatica* extract and triterpenoid saponins. Phytomedicine 8 (3), 230–235.

Shetty, B.S., Udupa, S.L., Udupa, A.L., Somayaji, S.N., 2006. Effect of *Centella asiatica* L (Umbelliferae) on normal and dexamethasone-suppressed wound healing in Wistar Albino rats. International Journal of Lower Extremity Wounds 5 (3), 137–143.

Sur, R., Nigam, A., Grote, D., Liebel, F., Southall, M.D., 2008. Avenanthramides, polyphenols from oats, exhibit anti-inflammatory and anti-itch activity. Archives of Dermatological Research 300 (10), 569–574.

Thring, T.S., Hili, P., Naughton, D.P., 2011. Antioxidant and potential anti-inflammatory activity of extracts and formulations of white tea, rose, and witch hazel on primary human dermal fibroblast cells. Journal of Inflammation. 8, 27.

Touriño, S., Lizárraga, D., Carreras, A., et al., 2008. Highly galloylated tannin fractions from witch hazel *(Hamamelis virginiana)* bark: electron transfer capacity, in vitro antioxidant activity, and effects on skin-related cells. Chemical Research in Toxicology 21 (3), 696–704.

Williamson, E.M., 2007. The medicinal use of essential oils and their components for treating lice and mite infestations. Nat. Prod. Comm. 2 (12), 1303–1310.

Wolff, H.H., Kieser, M., 2007. Hamamelis in children with skin disorders and skin injuries: results of an observational study. European Journal of Paediatrics 166 (9), 943–948.

Chapter 29

The eye

Diagnosis of functional disorders of the eye (glaucoma, etc.) always needs to be carried out by a medical practitioner. The usual precautions when using extracts in the eye (e.g. sterility of eye drops, absence of irritating solutions, etc.) must also be taken. Simple eye lotions containing mildly astringent and soothing plant products are very popular, especially those containing distilled witch hazel and eyebright herb extracts. However, ophthalmology clearly is not an area where phytotherapy is of wider importance.

INFLAMMATION OF THE EYE

Inflammation may be a result of an allergic reaction, infection or irritation due to dust or particles. Simple irritation of the eye can be treated with an eye lotion or drops, usually containing either extract of witch hazel (see Chapter 28, p. 321), or the herb eyebright.

EYEBRIGHT, *EUPHRASIA OFFICINALIS* L. AND *EUPHRASIA OFFICINALIS* SUBSP. *ROSTKOVIANA* (HAYNE) F.TOWNS. (EUPHRASIAE HERBA)

Euphrasia spp. (Scrophulariaceae) have a long history of use in eye disorders, as the name would suggest. Both *E. officinalis* and its subspecies *rostkoviana* (syn.: *Euphrasia rostkoviana* Hayne) are used medicinally as Euphrasiae herba. The species is found in meadows and grassy vegetation throughout Europe and temperate Asia. The leaves are opposite near the base and alternate above, about 1 cm long, lanceolate, with four or five teeth on each side; the axillary flowers are two-lipped, small and white, often tinged with purple or with a yellow spot.

Constituents

Eyebright contains iridoid glycosides, such as aucubin, geniposide, catalpol, luproside, eurostoside, euphroside, veronicoside, verproside and others, lignans including coniferyl glucosides and eukovoside, and tannins and polyphenolic acids, including gallic, caffeic and ferulic acids.

Therapeutic uses and available evidence

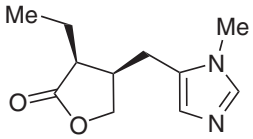
Extract of *Euphrasia* is a traditional remedy for disorders of the eye, such as conjunctivitis. Practically no clinical studies have been carried out, but single-dose eye drops containing extracts of the herb were evaluated in a clinical prospective cohort trial for conjunctivitis, and efficacy and tolerability were deemed 'good to very good' by both patients and physicians (Stoss et al 2000). Overall, the evidence for beneficial effects is very limited (Wszelaki and Melzig 2011), even though there are some *Euphrasia* preparations available on the market to treat tired eyes (often registered as a medical device) and the plant is widely used as a local and traditional medicine.

DISTILLED WITCH HAZEL, AQUA HAMAMELIDIS

Distilled witch hazel is prepared by macerating the dormant and partially dried twigs of *Hamamelis virginiana* L. (Hamamelidaceae). It is often used in eye drops and eye lotions to soothe the eye and clear redness (for further details, see witch hazel, p. 321, Chapter 28, The Skin).

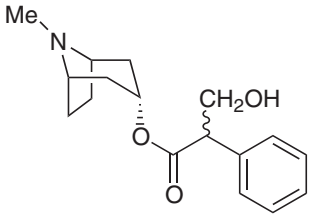
GLAUCOMA

Glaucoma is always treated under medical supervision. It is associated with increased intraocular pressure and



Pilocarpine

Fig. 29.1



Atropine

Fig. 29.2

can cause blindness if not treated. Most of the drugs used are synthetic sympathomimetics, such as dipivefrine and brimonidine, β-blockers, such as timolol, or prostaglandin analogues, but there is a useful plant-derived miotic (a substance causing constriction of the pupil), the alkaloid, pilocarpine, which is widely used. It reduces intraocular pressure by opening the drainage channels in the trabecular meshwork, which may be affected by a spasm or contraction of the ciliary muscle.

PILOCARPINE EP

Pilocarpine (Fig. 29.1) is an alkaloid obtained from Jaborandi leaf (*Pilocarpus microphyllus* Stapf ex Wardleworth and other species of *Pilocarpus*, Rutaceae). Pilocarpine is a sympathomimetic agent, causing salivation and tachycardia and other effects if taken systemically. Its main use is in ophthalmicpreparations as a miotic, in open-angle glaucoma and to contract the pupil after the use of atropine (BNF, 2017). It is a prescription-only medicine in most countries.

ANTERIOR UVEITIS

Anterior uveitis is an inflammatory disorder of the anterior segment. It is treated (under medical supervision) with atropine or its derivatives, homatropine and tropicamide.

ATROPINE EP

Atropine (Fig. 29.2) is a tropane alkaloid extracted from deadly nightshade (*Atropa belladonna* L., Solanaceae). It is occasionally used as eye drops (0.5%) or ointment (1%) to open the iris for examination or surgical procedures, and to treat anterior uveitis (see BNF, and Chapter 4, p. 48, and Chapter 6, p. 103).

References

British National Formulary, May 2017. London: BMJ Group and Pharmaceutical Press, 2017.

Stoss, M., Michels, C., Peter, E., Beutke, R., Gorter, R.W., 2000. Prospective cohort trial of *Euphrasia* single-dose eye drops in conjunctivitis. Journal of Alternative and Complementary Medicine 6 (6), 499.

Wszelaki, N., Melzig, M.F., 2011. Eyebright – *Euphrasia officinalis* L. [Augentrost – *Euphrasia officinalis* L.] Zeitschrift fur Phytotherapie 32 (1), 40–44.

Chapter 30

Ear, nose and oropharynx

Infections of the ear, nose and throat are treated under medical supervision with antibiotics, but a number of soothing and antiseptic preparations from plant sources are available for use. Decongestants have already been discussed in Chapter 22, pp. 260–262.

THE EAR

Infections of the ear are treated with either topical or systemic antibiotics. However, the removal of wax from the ear is achieved with the aid of softening agents such as almond, arachis or olive oil (BNF), sometimes followed by ear syringing. For details of arachis oil, see Chapter 28, p. 318.

ALMOND OIL, *PRUNUS DULCIS* (MILL.) D.A.WEBB (SYN.: *PRUNUS AMYGDALUS* BATSCH – OLEUM AMYGDALI) EP

Almond oil is obtained from the seed of *Prunus dulcis* = (Rosaceae). It is a fixed oil (i.e. a non-volatile oil of animal or plant origin), also known as sweet almond oil, and consists of triglycerides, mainly triolein and trioleolinolein, together with fatty acids, including palmitic, lauric, myristic and oleic acids.

OLIVE OIL, *OLEA EUROPAEA* L. (OLEUM OLIVAE) EP

Olive oil is expressed from the fruits of *Olea europea* (Oleaceae). Virgin (or cold-expressed) olive oil has a greenish tinge and is used as a food; refined oil is yellowish. Both have a characteristic odour. Olive oil is a fixed oil containing glycerides of oleic acid (about 70–80%), with smaller amounts of linoleic, palmitic and stearic acid glycerides.

THE OROPHARNYX

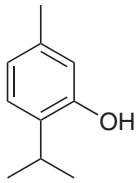
Simple oral and throat irritation can be treated with an anti-inflammatory and antiseptic mouthwash, including the thymol type associated with a visit to the dentist. Many essential oils are used as oral antiseptics, deodorizers and anti-inflammatory agents, including mint, clove, eucalyptus and lemon oils, as well as menthol and thymol. These can be incorporated into artificial saliva products, used for relieving dry mouth, which are composed of either animal mucins or hydroxymethoxycellulose derivatives.

THYMOL EP

Thymol (see Fig. 30.1) was originally extracted from thyme (*Thymus* spp.) and is present in many oils, including ajowan, but is now more easily synthesized chemically. It is antiseptic, deodorizing and anti-inflammatory and is widely used in dental products (e.g. compound thymol glycerin). Thymol causes irritation in high concentrations when applied externally, and should not be swallowed in significant amounts. Normal doses associated with the herb do not normally cause problems.

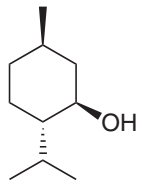
PEPPERMINT OIL, *MENTHA* × *PIPERITA* L. (MENTHAE PIPERITAE AETHEROLEUM) EP

Peppermint oil is antiseptic, deodorizing and anti-inflammatory and is widely used in skin and dental products. Other species of mint, such as spearmint,



Thymol

Fig. 30.1



(–)-Menthol

Fig. 30.2

are also used for the same purpose (for details, see Chapter 20, pp. 245–246).

Constituents

The major constituents are menthol (30–55%) and menthone (14–32%), with isomenthone (2–10%), menthofuran (1–9%), menthyl acetate (3–5%), 1,8-cineole (6–14%), limonene (1–5%), pulegone (not more than 4.0%) and carvone (not more than 1.0%). Menthol (see Fig. 30.2) can cause irritation in high concentrations.

SAGE, *SALVIA OFFICINALIS* L. (SALVIAE FOLIUM) EP

Salvia officinalis (garden or red sage, Lamiaceae) is indigenous to Europe, especially the Mediterranean region, and cultivated extensively. Spanish sage is *S. officinalis* subspp. *lavandulifolia* (Vahl) Gams; Greek sage is *Salvia fruticosa* Mill. (better known under its synonym *S. triloba* L. f.). The leaves are stalked, 3–5 cm long and 1–2.5 cm broad, oblong or lanceolate and rounded at the base and at the apex. Sage has a strong, characteristic, odour. It is widely used as a culinary herb.

The genus *Salvia* is one of the largest of the family Lamiaceae and many of its species, especially those rich in essential oil, have pharmaceutical uses. This common garden plant (garden sage) and culinary shrub has conspicuous blue flowers and relatively large leaves (3–5 cm long, 1–2.5 cm broad), which are oblong or lanceolate, rounded at the base and at the apex, and crenulate at the margin. The young leaves especially are covered with a white layer of fine hairs. The leaves have a characteristic uneven upper surface and prominent lower venation. The taste and odour are characteristic, pungent and aromatic. Sage is a popular culinary herb. *S. triloba* L.f. is also rich in essential oil and has similar topical uses as *S. officinalis*.

Constituents

The leaves are rich in essential oil with α- and β-thujone as the major components (normally about 50%), with cineole, borneol and others. It also contains rosmarinic acid. Diterpenes and flavonoids are two other important classes of natural products prominent in this species. There are differences in the composition of the essential oil depending on the origin of the plant material.

Spanish sage does not contain thujone; Greek sage contains only small amounts. Diterpene bitters picrosalvin (carnosol), carnosolic acid, abietane derivatives called royleanones, and flavonoids such as salvigenin, genkwanin, luteolin and derivatives are present, together with the polyphenolic acids salvianolic, rosmarinic and caffeic acids.

Therapeutic uses and available evidence

The use of sage leaf, in the form of a tea used as a gargle, is traditional for soothing inflammation of the mucous membranes of the mouth and throat including pharyngitis, tonsillitis, sore gums, mouth ulcers and other similar disorders.

Rosmarinic acid is well known for its antiviral and anti-inflammatory effects, sage essential oil is antibacterial and antifungal, and an aqueous extract of sage leaf has been shown to have analgesic and anti-inflammatory effects in rats, supporting this use of sage. In the doses used for mouthwashes it is generally considered to be safe.

A throat spray containing echinacea and sage was compared to a chlorhexidine and lidocaine spray in a multicenter, randomized, double-blind, double-placebo controlled trial involving 154 patients with acute sore throats. They used two puffs every 2 hours, up to 10 times daily until they were symptom-free, for a maximum of 5 days. The main outcome measure was the comparison of response rates during the first 3 days, a response being defined as a decrease of at least 50% of the total symptoms compared to baseline. The echinacea/sage treatment showed similar efficacy to the chlorhexidine/lidocaine spray during the first 3 days, i.e. 63.8% in the echinacea/sage group and 57.8%

in the chlorhexidine/lidocaine group. For all assessments of efficacy by the physician and patient, no difference between the two treatments was seen, and both were very well tolerated (Schapowal et al 2009).

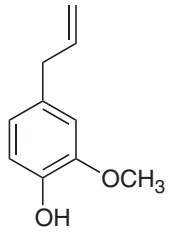
Sage has a reputation for enhancing memory, and there is some clinical trial evidence (Scholey et al 2008, Miroddi et al 2015), and the reported anticholinesterase activity as well as some clinical data support this use.

CLOVE, *SYZYGIUM AROMATICUM* (L.) MERR. & L.M. PERRY (CARYOPHYLLI FLOS) EP

Cloves are obtained from the flower buds of *Syzygium aromaticum* (syn. *Eugenia caryophyllata* Thunb., Myrtaceae), which are collected prior to opening. The buds are brown, about 1–1.5 cm long, with a very characteristic shape, the lower portion consisting of the calyx tube enclosing in its upper half the immature flower. Taste and odour are highly characteristic. On pressing a clove with the fingernail, oil should be exuded. Cloves are used as a culinary spice.

Constituents

The buds are very rich in essential oil (15–20%), consisting mainly of eugenol (Fig. 30.3; Kamatou et al 2012), usually 85–90%, and numerous minor constituents, including acetyl eugenol, α- and β-caryophyllene, methyl salicylate. Tannins such as eugeniin, casuarictin, tellimagrandin I, and flavonoids, are found in the plant material but not in the oil.



Eugenol

Fig. 30.3

Therapeutic uses and available evidence

Clove oil is used for the symptomatic relief of toothache and is a constituent of many dental preparations. The oil is useful in the treatment of inflammation of the mucous membranes of the mouth and throat. It has antiseptic, antispasmodic, antihistaminic and anthelmintic properties, many of which are due to the eugenol content. Eugenol inhibits prostaglandin synthesis and the metabolism of arachidonic acid by human polymorphonuclear leukocytes, inhibits smooth muscle activity *in vitro* and is anti-inflammatory (for review, see Chaieb et al 2007). Clove extracts are used in cosmetics and perfumery.

References

British National Formulary. Published biannually, BMJ Group and the Pharmaceutical Press, London. Available online at: http://bnf.org/bnf/index.htm.

Chaieb, K., Hajlaoui, H., Zmantar, T., et al., 2007. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzygium aromaticum* L. Myrtaceae): a short review. Phytotherapy Research 21, 501–506.

Kamatou, G.P., Vermaak, I., Viljoen, A.M., 2012. Eugenol-from the remote maluku islands to the international market place: a review of a remarkable and versatile molecule. Molecules 17, 6953–6981.

Miroddi, M., Navarra, M., Quattropani, M.C., Calapai, F., Gangemi, S., Calapai, G., 2015. Systematic review of clinical trials assessing pharmacological properties of *Salvia* species on memory, cognitive impairment and Alzheimer's disease. CNS Neuroscience and Therapeutics 20, 485–495.

Schapowal, Berger, D., Klein, P., Suter, A., 2009. Echinacea/sage or chlorhexidine/lidocaine for treating acute sore throats: a randomized double-blind trial. European Journal of Medical Research 14, 406–412.

Scholey, A.B., Tildesley, N.T., Ballard, C.G., et al., 2008. An extract of *Salvia* (sage) with anticholinesterase properties improves memory and attention in healthy older volunteers. Psychopharmacology (Berl) 198, 127–139.

Chapter 31

Weight loss supplements

It has been estimated that up to 15% of adults have taken products intended to increase weight loss. This may be by reducing calorie intake, for example by suppressing the appetite or inhibiting fat and/or carbohydrate absorption, as with fibre supplementation or by 'speeding up' metabolism, inducing thermogenesis and increasing lipolysis. There is a lack of good clinical evidence as to the efficacy of these approaches. Many of these herbal and nutritional supplements are also taken for the purpose of improving athletic performance and increasing stamina, and several may be taken at the same time.

Some of the most popular weight loss supplements contain caffeine, which acts as a stimulant and appetite suppressant. These include **guarana** (see Chapter 23), **green tea** (see Chapter 32) and **green coffee bean** extracts. **Hoodia** is an appetite suppressant, and **bitter orange peel**, **chilli peppers**, **garcinia** and **kelp** are considered to increase metabolism. Many other supplements are advertised as beneficial during weight loss regimes but few are clinically proven, and some, such as the stimulant **ephedra** (see Chapter 22), are responsible for many cases of poisoning.

BITTER ORANGE, *CITRUS* X *AURANTIUM* L.

Bitter orange peel extract and its constituent *p*-synephrine are widely used in weight loss products.

CONSTITUENTS

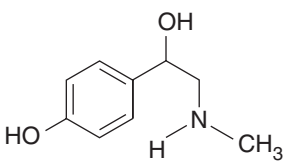
Bitter orange peel contains *p*-synephrine (Fig. 31.1) as the main active compound involved in weight-loss claims. There are also citrus flavonoids such as naringenin and essential oil components.

THERAPEUTIC USES AND AVAILABLE EVIDENCE

There are few clinical studies showing efficacy for bitter orange extracts or *p*-synephrine, but animal studies have shown thermogenic effects. Concerns have been expressed about the use of products containing *C. aurantium* extract; however, there is some confusion between *p*-synephrine and *m*-synephrine (phenylephrine), a stimulant used as a nasal decongestant. Based on current knowledge, the use of bitter orange extracts and *p*-synephrine appears to be safe at doses recommended (Stohs et al 2011), and a clinical study assessing the cardiovascular effects of bitter orange extract (49 mg p-synephrine) in 18 healthy subjects found no significant changes in heart rates, systolic blood pressure or blood chemistry at any time point in either the control or treated groups. No adverse effects were reported (Shara et al 2016).

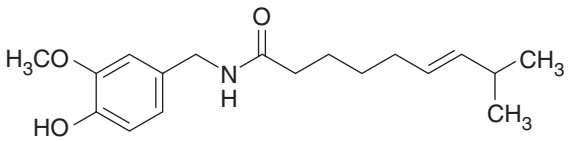
CHILLI PEPPERS, *CAPSICUM FRUTESCENS* L. AND OTHERS

Chilli peppers are eaten everywhere and used widely in medicine. See Chapter 27 for details.



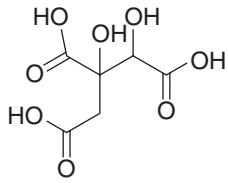
***p*-Synephrine**

Fig 31.1



Capsaicin

Fig 31.2



Hydroxycitric acid

Fig 31.3

CONSTITUENTS

Pungent substances, capsaicinoids, based on capsaicin (Fig. 31.2). Chillies also contain non-pungent analogues called capsinoids, and many other compounds (reviewed by Edwards et al 2015).

THERAPEUTIC USES AND AVAILABLE EVIDENCE

Capsaicinoids are thermogenic and human studies have shown them to increase satiety and fullness, preventing overeating (Janssens et al 2014). The capsinoids are also of interest for weight loss and appear to be safe in humans (Saito and Yonishiro 2013).

GREEN COFFEE BEAN, *COFFEA ARABICA* L. AND OTHER VARIETIES (RUBIACEAE)

Extracts of unripe coffee beans are a fairly recent addition to the range of weight-loss products.

CONSTITUENTS

Green coffee beans contain caffeine, and high concentrations of chlorogenic acid derivatives.

THERAPEUTIC USES AND AVAILABLE EVIDENCE

A systematic review (Onakpoya et al 2011) has concluded that the results from published trials are promising, showing a significant but moderate difference in body weight compared with placebo, but the studies are all of poor methodological quality.

HOODIA, *HOODIA GORDONII* (MASSON) SWEET EX DECNE. AND RELATED SPECIES (APOCYNACEAE)

The stem of the Hoodia plant is traditionally used as an appetite suppressant by bushmen in the Kalahari desert. It is becoming endangered due to overharvesting and there is thought to be considerable illegal trade and adulteration of Hoodia products (reviewed by Edwards et al 2015).

CONSTITUENTS

Steroidal (pregnane) glycosides, based on hoodigogenin and calogenin. A mixture of glycosides, known as P57, is the main appetite suppressant component.

THERAPEUTIC USES AND AVAILABLE EVIDENCE

Several human clinical studies have confirmed appetite suppression of a P57-enriched extract of Hoodia (reviewed by Edwards et al 2015), although side effects such as nausea, flatulence, and increases in blood pressure and heart rate are considered to be associated with consumption of the high doses required to achieve a clinical effect (Smith et al 2014). A study of a *Hoodia parviflora* extract showed efficacy with only mild transient gastro-intestinal side effects (Landor et al 2015).

"GARCINIA CAMBOGIA", *GARCINIA GUMMI-GUTTA* (L.) ROXB. [FORMERLY *G. CAMBOGIA* (GAERTN.) DESR.] (CLUSIACEAE)

The extract of the rind of the fruit of *Garcinia cambogia* and its major constituent hydroxycitric acid are widely used in weight loss products. The fruit is also used as a flavouring in food.

CONSTITUENTS

Plant acids, mainly hydroxycitric acid (Fig. 31.3), and also ascorbic acid.

THERAPEUTIC USES AND AVAILABLE EVIDENCE

The evidence is conflicting: one review (Semwal et al 2015) suggests that *Garcinia* extracts exhibit anti-obesity activity by regulating serotonin levels related to satiety, increasing fat oxidation and decreasing *de novo* lipogenesis. However, a systematic review of clinical studies concludes most studies have been conducted on small samples over a short term, and that none has shown effects persisting beyond 12 weeks of intervention (Márquez et al 2012). Liver toxicity has been reported and also a possible interaction with serotonin re-uptake inhibiting drugs (see Semwal et al 2015).

KELP, BLADDERWRACK *FUCUS VESICULOSUS* L., *F. SERRATUS* L., *ASCOPHYLLUM NODOSUM* (L.) LE JOLIS (FUCACEAE)

The thallus (whole plant) of these seaweeds is used to aid weight loss and also as a source of iodine, to support thyroid function, which may also help weight loss.

CONSTITUENTS

Polysaccharides, the fucoidans, alginic acid and laminarin, with minerals, mainly iodine (reviewed by Edwards et al 2015).

THERAPEUTIC USES AND AVAILABLE EVIDENCE

There is little clinical evidence for the use of kelp for weight loss, although it does alter glucose homeostasis in humans (reviewed by Edwards et al 2015). A study in mice has shown anti-obesity effects for fucoidan, with reduced triglyceride, cholesterol and low-density lipoprotein levels (Kim et al 2014). High doses should be avoided due to the iodine content, which is very variable.

References

Edwards, S.E., Da Costs Rocha, I., Williamson, E.M., Heinrich, M., 2015. Phytopharmacy: an evidence-based guide to herbal medicinal products. Wiley-Blackwell, Chichester, UK.

Janssens, P.L., Hursel, R., Westerterp-Plantenga, M.S., 2014. Capsaicin increases sensation of fullness in energy balance, and decreases desire to eat after dinner in negative energy balance. Appetite 77, 44–49.

Kim, M.J., Jeon, J., Lee, J.S., 2014. Fucoidan prevents high-fat diet-induced obesity in animals by suppression of fat accumulation. Phytotherapy Research 28 (1), 137–143.

Landor, M., Benami, A., Segev, N., Loberant, B., 2015. Efficacy and acceptance of a commercial *Hoodia parviflora* product for support of appetite and weight control in a consumer trial. Journal of Medicinal Food 18 (2), 250–258.

Márquez, F., Babio, N., Bulló, M., Salas-Salvadó, J., 2012. Evaluation of the safety and efficacy of hydroxycitric acid or *Garcinia cambogia* extracts in humans. Critical Reviews in Food Science and Nutrition 52 (7), 585–594.

Onakpoya, I., Terry, R., Ernst, E., 2011. The use of green coffee extract as a weight loss supplement: a systematic review and meta-analysis of randomised clinical trials. Gastroenterology Research Practice 382852.

Saito, M., Yonishiro, T., 2013. Capsinoids and related food ingredients activating brown fat thermogenesis and reducing body fat in humans. Current Opinion in Lipidology 24 (1), 71–77.

Semwal, R.B., Semwal, D.K., Vermaak, I., Viljoen, A., 2015. A comprehensive scientific overview of *Garcinia cambogia*. Fitoterapia 102, 134–148.

Shara, M., Stohs, S.J., Mukattash, T.L., 2016. Cardiovascular safety of oral p-synephrine (bitter orange) in healthy subjects: a randomized placebo-controlled cross-over clinical trial. Phytotherapy Research 30 (5), 842–847.

Smith, C., Krygsman, A., 2014. Hoodia gordonii: to eat, or not to eat'. Journal of Ethnopharmacology 155, 987–991.

Stohs, S.J., Preuss, H.G., Shara, M., 2011. The safety of *Citrus aurantium* (bitter orange) and its primary protoalkaloid *p*-synephrine. Phytotherapy Research 25 (10), 1421–1428.

Chapter 32

Miscellaneous supportive therapies for stress, ageing, cancer and debility

Although their effects are very difficult to evaluate, preventative medicines are extremely popular with patients and are an essential element of most types of Oriental medicine. They have been collected together here as they are generally used for a multitude of disorders, and to prevent degenerative conditions, including ageing and some forms of cancer. Most of the herbs mentioned here originated in Asia and China. In traditional Chinese medicine they are used to treat 'empty' diseases, to restore 'qi' energy and tonify the organs, having a balancing effect on yin and yang rather than affecting only one. They are thought to strengthen the immune system, improve memory and alertness, enhance sexual performance, promote healing and stimulate the appetite. In the West, the most important Chinese herbs are ginseng, ginkgo, astragalus, shizandra, reishi mushroom, baical scullcap and tea. In Ayurveda, some rejuvenating and tonic herbs are called 'rasayanas' and are considered to have a beneficial effect, balancing the tridosha. In Asian medicine, ashwagandha and *Centella asiatica* are very widely used. Many of these herbs contain saponins or steroidal compounds of some kind and it has been suggested that they act in a similar way to corticosteroids or enhance the effect of naturally occurring steroid hormones in the body. This type of drug is known as an adaptogen, and is considered to be a substance that helps the body to deal with, or adapt to, stress or other adverse conditions.

While very contentious, this group is a fast-growing area of phytotherapy, but use is often based on very limited scientific evidence.

CANCER CHEMOPREVENTION

During the 1960s and 1970s, work done at the University of Minnesota by LW Wattenberg showed that various compounds, especially from fruits and vegetables (indoles and isothiocyanates), could inhibit chemically induced tumours in laboratory animals. Termed 'chemoprophylaxis of carcinogenesis', this was of obvious benefit to maintenance of human health and had enormous dietary significance. After intensive studies using retinoids (vitamin A-related natural products), the term 'cancer chemoprevention' was first used.

Cancer chemoprevention can be defined as 'the prevention of cancer in human populations by ingestion of chemical agents that prevent carcinogenesis'. It is also important to differentiate between cancer prevention (e.g. cessation of cigarette smoking) and cancer chemotherapy (the use of cytotoxic drugs after cancer diagnosis). Cancer chemoprevention has now developed into a well-defined discipline. Several recent epidemiological studies have demonstrated that dietary factors may reduce the incidence of cancers. In one study, involving 250,000 people, an inverse correlation was found between the incidence of lung cancer among people who smoke and consume carotene-rich foods. As well as carotenoids, there was a similar finding for vitamin C and oesophageal and stomach cancers; selenium and various cancers; and vitamin E and lung cancer. Epidemiological studies may help to find leads for chemopreventive agents, which can then be tested in laboratory experiments. Almost 600 'chemopreventive' agents are known and they are usually classified as: inhibitors of carcinogen formation (ascorbic acid, tocopherols, phenols), inhibitors of initiation (phenols, flavones) and inhibitors of post-initiation events (β-carotene, retinoids, terpenes). Many are items of food or beverages (e.g. tea) and are sometimes called 'functional foods' or 'nutraceuticals'. Those that are used *purely* as foods

are not covered here, but some others can be found in the chapters for which they are most useful (e.g. ginger, Chapter 20; garlic, Chapter 21).

Chemopreventive agents may even work in synergy, with several components contributing to the overall effect, which may be the case with plant drugs. This approach has great promise, with both natural products and synthetics being potentially useful. Dietary campaigns by government bodies, the American Cancer Society and others recommend that 5–7 servings of vegetables be consumed daily to function as a source of cancer chemopreventive agents. However, it is not reasonable to assume that chemopreventive agents will safeguard humans from known carcinogenic risks such as smoking. As knowledge of these agents increases they will play an increasing role in cancer prevention. Chemoprevention will not be covered further, apart from tea, as it is a vast subject in its own right. However, there are some useful recent reviews that discuss compounds, mechanisms and future directions available, such as Shu et al (2010), Gullett et al (2010) and Cerella et al (2010). Some data on the chemopreventive effects of garlic, anthocyanins (bilberry) and others can be found in the monographs of these plants.

CANCER SUPPORT

Many cancer patients use other holistic therapies to help their recovery, and dietary measures are often advocated. These usually involve increasing vegetable and whole grain, and reducing or omitting meat intake. Herbal and nutritional supplements are also popular, as an attempt to improve general health before or during cancer treatment, to enhance well-being and counteract the effects of chemo- or radiotherapy. This has led to concerns of clinical interactions with prescribed drugs, but a systematic review of cancer patients in the UK has shown that the most commonly used supplements are fairly innocuous (for example, green tea and garlic) and do not pose a real risk (Alsanad et al 2014). The dangers of co-administration of St John's wort with many drugs are now recognized and patients are made aware of them. In China, traditional Chinese medicine (TCM) is routinely used as a supporting or adjunctive therapy in cancer patients, with linzhi mushroom and *Ganoderma lucidum* being particularly widely used. Beneficial interactions between herbs and drugs may be involved and are expected, but this kind of treatment should be only practised by clinical specialists, usually in integrated TCM hospitals.

TONICS, STIMULANTS, ADAPTOGENS AND SUPPORTIVE THERAPIES

AÇAI BERRY, *EUTERPE OLERACEA* MART. (EUTERPE FRUCTUS)

The fruits of the palm *Euterpe oleracea* Mart. (açai, Arecaceae) have been acclaimed as having a wide range of health-promoting and therapeutic benefits due to their reportedly high levels of antioxidants. Açai has a history of use as a medicinal plant and as a staple food in many parts of Brazil. Traditionally, it has been used to treat fevers, skin complications, digestive disorders and parasitic infections. In recent years, açai berry has been advertised widely, for example, via the internet.

Constituents

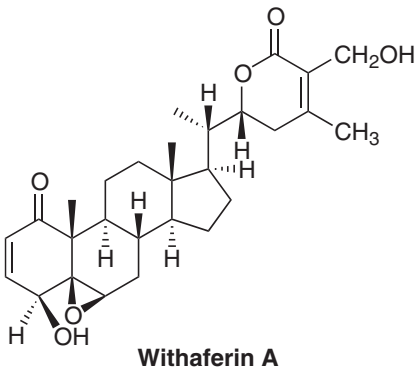
Açai has a relatively high content of polyphenols. Most notable are anthocyanins and flavonoids, as well as fatty acids. Small amounts of lignans have also been reported.

Therapeutic uses and available evidence

This botanical drug has become very popular despite a lack of definitive scientific evidence (Heinrich et al 2011). The high content of polyphenols has been linked with several activities, including antioxidant, anti-inflammatory, antiproliferative and cardioprotective properties, following preclinical studies (de Moura and Resende 2016, Yamaguchi et al 2015). Clinical data are mostly limited to small studies involving healthy volunteers (Ulbricht et al 2012); one small study has explored the anti-inflammatory effects of an açai beverage in patients with metabolic syndrome.

ASHWAGANDHA, *WITHANIA SOMNIFERA* (L.) DUNAL

Ashwagandha, also known as winter cherry (Solanaceae), is a woody shrub native to the Middle East, Africa and parts of Asia, growing in stony and semi-arid regions; it is cultivated widely. The leaves are elliptical with an acute apex and the flowers campanulate and greenish yellow, developing into red berries enclosed in a papery membrane. The dried root is used medicinally. Ashwagandha has been used in Ayurvedic medicine for over 4000 years, as an adaptogen, sedative and tonic for debility. It is used to enhance fertility in both men and women, and as an aphrodisiac.



Withaferin A

Fig. 32.1

The name 'ashwagandha' comes from the Sanskrit *ashva* (meaning 'horse') and *gandha* (meaning 'smell'), and refers to the odour of the root. It is also widely used for inflammation, colds, asthma and many other disorders.

Constituents

The root contains steroidal lactones (the withanolides A–Y), withaferin A (Fig. 32.1), withasomniferols A–C and others, phytosterols (such as the sitoindosides) and the alkaloids anahygrine, cuscohygrine, ashwagandhine, ashwagandhinine, withasomnine, withaninine, somniferine and others.

Therapeutic uses and available evidence

Extracts are antioxidant, immunomodulatory and sedative but, despite their wide usage, much of the clinical knowledge is still anecdotal. Many of the pharmacological effects have been substantiated in animal studies: for example, the adaptogenic and antistress activity was comparable to that of ginseng, in mice and rats, and immunomodulatory activity has been confirmed. The extract is also reported to be anxiolytic, acting via the GABA-ergic system (Bhattarai et al 2010). Numerous other actions have been documented in preclinical studies, including antimicrobial, anti-inflammatory, antitumour, neuroprotective, cardioprotective, and antidiabetic activities (for reviews, see Kulkarni and Dhir 2008, Dar et al 2015). The usual dose of powdered root is 3–6 g daily.

Conclusive evidence from well-designed clinical trials to support the pharmacological effects of ashwagandha is limited. A systematic review of five trials of different preparations and regimens of *W. somnifera* in patients with anxiety or stress found positive results in three studies, although due to the low methodological quality of the trials, further research is needed to confirm these findings (Pratte et al 2014). Few adverse events occurred in patients who received ashwagandha, but clinical trials have the power only to detect acute, very common adverse reactions, and comprehensive investigation of the safety profile of ashwagandha is required. High doses can cause gastrointestinal irritation.

CENTELLA, *CENTELLA ASIATICA* (L.) URB. (CENTELLAE HERBA)

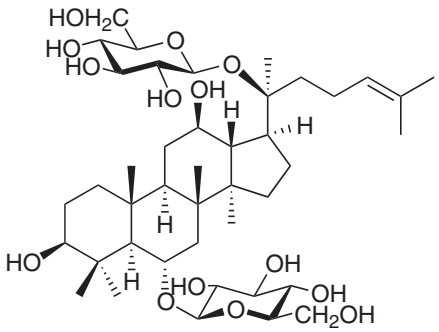
The herb has already been described in Chapter 28 (The skin). In addition to the wound-healing effects, the plant is considered a 'rasayana' in Ayurvedic medicine; it enhances the immune system and is considered to have a rejuvenating, neurological 'tonic' with a mild sedative effect. The immunomodulating effects of the herb have been shown *in vitro* and *in vivo* in mice. Studies in rats have shown that asiatic acid has some benefits on memory and learning (Nasir et al 2011), and that the extract can protect against certain types of neurodegeneration (Haleagrahara and Ponnusamy 2010), but in general, evidence for this use is lacking. Other reported effects for the herb include anti-ulcer activity and spasmolytic effects (for reviews, see Brinkhaus et al 2000, James and Dubery 2009, Chandrika and Kumara 2015).

Small randomized, double-blind, placebo-controlled trials involving healthy volunteers have reported positive effects for centella on the acoustic startle response (Bradwejn et al 2000) and, in older participants, on age-related cognitive decline, mood, health-related quality of life, and lower-extremity strength (Wattanathorn et al 2008, Mato et al 2011).

Centella is taken orally, often as an infusion, and also applied topically. The powdered leaf is taken at an internal dose of 0.5–1 g daily, or the equivalent in the form of an extract.

GINSENG, *PANAX GINSENG* C.A. MEY. *(PANAX RADIX)*, *ELEUTHEROCOCCUS SENTICOSUS* (RUPR. & MAXIM.) MAXIM. AND RELATED 'GINSENG' SPECIES

Ginseng root in commerce is obtained from *Panax ginseng* C.A.Mey. (Korean or Chinese ginseng, Araliaceae) and other species. American ginseng is from *P. quinquefolius* L., but Siberian ginseng, *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim., comes from a different but related genus.



Ginsenoside Rg_1

Fig. 32.2

P. ginseng is native to China but cultivated widely elsewhere. The root is spindle-shaped, ringed, and divided into two or three equal branches. Red Korean ginseng (from *P. ginseng*) is about 8 years old; it is matured and roasted, and is the most highly regarded form. Adulteration and substandard material is common in the trade and there is 'an acute need for proper authentication' (Foster 2016). Adulteration with other species of the same genus, or with unrelated species (including sawdust), or the admixing of other plant parts of *P. ginseng* are just some of the many examples of adulteration. Sadly, there seems to be no limit to the ingenuity of adulterating a botanical drug like *P. ginseng* (root) and this clearly impacts both on the patients' experience with the use of products and the results of bioscientific research on the species.

Constituents

All types contain saponin glycosides (the ginsenosides Ra, Rb, Rg_1, Rg_2, Rs, etc.; Fig. 32.2). The ginsenosides are sometimes referred to as the panaxosides, but this nomenclature uses the suffixes A–F, which do not correspond to those of the ginsenosides.

In Siberian ginseng (*Eleutherococcus*), the saponins (eleutherosides A–F) are chemically different, but have similar properties. Glycans (panaxans) also occur in *P. ginseng*. The actual composition of ginseng extracts depends upon the species and method of preparation.

Therapeutic uses and available evidence

Ginseng is taken as a tonic for debility, insomnia, natural and premature ageing; to increase alertness and improve sexual inadequacy; for diabetes; and as an adaptogen to relieve stress and improve stamina and concentration. It has been suggested that these effects are due to changes in cholinergic activity and also neuro-protection, as well as through antioxidant activity (Wang et al 2007). The adaptogenic effect may be due to the elevation of serum concentrations of corticosteroids and the reduction of catecholamines, which results in homeostasis. Ginsenoside Rb_1 acts as a central nervous system sedative and Rg_1 has antifatigue and stimulant properties. In animals, an extract increases the capacity of skeletal muscle to oxidize free fatty acids in preference to glucose to produce cellular energy, which would support the anti-fatigue activity seen in conventional exhaustion tests. Ginseng also has a traditional use in diabetes, and the glycans (panaxans A–E) are hypoglycaemic in mice. Other documented effects include immunomodulatory activity, potentiation of analgesia and anticancer effects (by ginsenosides R_{s3} and R_{s4}). For reviews of the pharmacological effects of ginseng see: *Panax ginseng* (Ru et al 2015); *Eleutherococcus senticosus* (Huang et al 2011).

Trials of ginseng for improving 'quality of life', including mental health parameters, show beneficial effects for up to 8 weeks of treatment. With respect to effects on cognition, a Cochrane systematic review of clinical trials found only studies involving healthy volunteers, and concluded that higher-quality studies are needed, involving patients with cognitive disorders, including dementia (Geng et al 2010). A systematic review that included two trials involving patients with Alzheimer's disease reached similar conclusions (Lee et al 2009); a comprehensive systematic review across all health conditions reported some positive results for *P. ginseng* with respect to immunomodulatory effects, although confirmatory studies are still required (Shergis et al 2013). A recent randomized, double-blind, placebo-controlled trial reported beneficial effects for a bespoke extract of *P. ginseng* roots in patients with idiopathic chronic fatigue treated for four weeks (Kim et al 2013); another controlled study found no effects for single-dose ginseng on driving performance, but attributed this to the small sample size (LaSala et al 2015).

The dose of ginseng used is very variable but, in general, for a short course in the young and healthy, 0.5–1 g daily is recommended for up to 20 days; for long-term treatment in the sick or elderly, 0.4–0.8 g daily is more usual. Ginseng is taken widely, and side effects are relatively well documented; they include oestrogenic effects, hypertension and irritability. A 'ginseng abuse' syndrome has been described (Paik and Lee 2015).

LINGZHI OR REISHI MUSHROOM, *GANODERMA* SPP.

Ganoderma lucidum (Curtis Fr.) P. Karst., *G. japonicum* (Fr. Lloyd) and other species of mushroom (Polyporaceae) grow on tree stumps (mainly conifers) in China, Japan and North America. This mushroom is now cultivated for commerce. In China, the fungus is known as lingzhi, and in Japan, as reishi. The fruiting body takes several forms, including a rare, branched or 'antler' type, in addition to the more usual mushroom shape. The colour varies from red, through orange and brown, to black, with the red and antlered varieties being more highly prized. The cap is circular, kidney- or fan-shaped, leathery with a smooth or rippled upper surface, and an under surface that shows the spore tubes. Lingzhi is a very important Chinese medicine; it has been immortalized in Oriental paintings and was used frequently by Taoist monks.

Constituents

The mature fruiting body of the fungus contains a series of triterpenes, mainly lanostanes such as the ganoderic acids A–Z, ganoderals A and B, ganoderiols A and B, epoxyganoderiols A–C, ganolucidic acids A–E, lucidones A–C, and lucidenic acids A–M. Polysaccharides, mainly glucans and arabinoxyoglucans, and peptidoglycans (known as ganoderans A–C) are also present (for a review, see Xia et al 2014).

Therapeutic uses and available evidence

Lingzhi is used as an adaptogen and general tonic, in the hope of prolonging life, retarding ageing and generally improving wellbeing and mental faculties. The most common indication is to enhance the immune system, and various animal and clinical studies support this. More recently, reishi has been applied as an adjunctive treatment to chemotherapy and radiation in cancer patients, to support immune resistance. The active principles are considered to be the triterpenes and polysaccharides. Extracts inhibit angiotensin-converting enzyme and produce hypotensive effects; cholesterol-lowering effects have been seen in animals. It is also a sedative, liver protectant and cholesterol-lowering agent (for reviews, see Sanodiya et al 2009, Bishop et al 2015).

Clinical evidence to support the use of ganoderma preparations is limited. A Cochrane systematic review of trials of *G. lucidum* in Chinese patients with cancer found limited evidence of improved outcomes when reishi was included with certain cancer chemotherapy regimens; however, this finding comes from only a small number of studies that had methodological limitations (Jin et al 2016). Another Cochrane review, also based on trials with methodological issues, found no benefit for *G. lucidum* on cardiovascular risk factors in patients with type 2 diabetes mellitus (Klupp et al 2015).

The dose of the dried fruiting body of the fungus is 6–12 g daily, or the equivalent in extract. Lingzhi is well tolerated, although transient side effects of gastrointestinal disturbance and rashes in sensitive individuals have been reported. Animal studies have shown no toxic effects after long-term high doses, but as with other immune modulators it should probably be avoided in auto-immune disease.

ROSENROOT, *RHODIOLA ROSEA* L. (RHODIOLAE ROSEAE RHIZOMA)

Also known as *Sedum roseum* (L.) Scop., Golden root, Aaron's rod, *Rhodiola rosea* (Crassulaceae) grows in cold regions of the world, including much of the Arctic, mountainous regions of Central Asia and Europe, and the Rocky Mountains. It is a dioecious perennial reaching 5 to 35 cm in height.

Constituents

The main active constituents of the root and rhizome are monoterpene alcohols and their glycosides, such as salidroside (previously known as rhodioloside or rhodosin), rhodioniside, rhodiolin, rosin, rosavin, rosarin and rosiridin (for a summary, see Barnes et al 2007). These compounds are thought to be responsible for the adaptogenic properties of rhodiola. Other phenolic constituents such as p-tyrosol, gallic acid, caffeic acid, chlorogenic acid and flavonoids (catechins and proanthocyanidins) are present. Geraniol and other essential oil constituents, such as geranyl formate, geranyl acetate, benzyl alcohol and phenylethyl alcohol, give the root its rose-like odour.

Therapeutic uses and available evidence

Rhodiola has a long history of use in traditional medicine, particularly in traditional Chinese medicine; it is used as a 'brain tonic' and stimulant, to reduce fatigue and improve stamina, and to prevent stress. Rhodiola rhizome preparations are used in a contemporary context for their adaptogenic effects, including neuroprotective, cardioprotective,

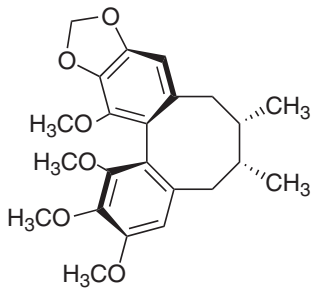
antifatigue, antidepressive, anxiolytic and CNS-stimulating activities. There is a substantial literature based on preclinical studies that provides evidence for these activities (Panossian et al 2010). Unregulated products are often of poor quality and an additional problem relates to the use of a different species in Chinese medicine. According to the Chinese Pharmacopoeia 2010 only *R. crenulata* can be used medicinally, and, consequently, this species also enters the trade. Of course, this problem is linked both to poor pharmacognostic authentication and to a lack of knowledge along the value chains of the botanical drugs (Booker et al 2016).

Mechanisms of action that may contribute to the reported effects include interactions with the HPA system (reducing cortisol levels) and defence mechanism proteins (some heat shock proteins) (Panossian and Wikman 2009).

Due to the low quality of clinical trials, evidence for *Rhodiola* preparations is usually inconclusive. A systematic review of clinical trials assessing the effects of preparations of *Rhodiola* in physical and mental fatigue identified 10 randomized controlled trials, but concluded there was insufficient evidence to assess efficacy due to the low methodological quality of the studies (Ishaque et al 2012); another systematic review drew cautiously positive conclusions (Hung et al 2011). A similar outcome was reported in a systematic review of trials of *Rhodiola* in patients with ischaemic heart disease (Yu et al 2014). Few adverse events associated with *Rhodiola* have been reported in clinical trials, but comprehensive investigation of the safety profile of *Rhodiola* is lacking.

SCHISANDRA, *SCHISANDRA CHINENSIS* (TURCZ.) BAILL. (SCHISANDRAE CHINENSIS FRUCTUS)

The magnolia vine (*Schisandra*, Schisandraceae) is also known as gomishi in Japan and as Wu-wei-Zi in China. It is a monoecious liana, native to Northern China, Korea, Japan and eastern Russia, usually found climbing round tree trunks. The leaves are elliptical and the flowers are cream with a pleasant odour. The fresh berries are scarlet, small and ovoid, hanging in clusters and are the main botanical drug used. When dry they are wrinkled, dark reddish brown, containing a sticky pulp and a yellow kidney-shaped seed. *Schisandra sphenanthera* Rehder & E.H.Wilson is also widely used for similar purposes.



Schisandrin B

Fig. 32.3

Constituents

The active constituents are lignans, including schizandrin A (= deoxyschisandrin or wuweizu A), schizandrin B (= wuweizu B or γ-schizandrin B; Fig. 32.3), schizandrol A (= schizandrin), schizandrol B (= gomisin A), schisandrin C, schisantherin A (= gomisin C), schisantherin B (= gomisin B), gomisins H, K, L, M, N, schizanhenol, wuweizu C, schisantherin C and others.

Therapeutic uses and available evidence

Schisandra has been used in China since ancient times to prolong life and increase energy ('qi') and act as a general and sexual tonic, especially for men. It is also used to reduce sweating, detoxify the liver, enhance kidney function and suppress cough in lung disease. Many pharmacological studies support the use of schisandra; for example, the adaptogenic and antifatigue properties have been tested in racehorses in which a beneficial effect on physical recovery and a general improvement in performance were observed. Schizanhenol and schizandrin B protect against peroxidative damage associated with ageing and ischaemia in the rat brain. Hepatoprotective effects have been documented in animal and cell culture studies. Schizandrin B, schisandrin C and gomisin A reduced liver enzyme concentrations and prevented histological damage in experimental models of liver injury, inhibited lipid peroxidation and stimulated glycogen synthesis in the liver. Antioxidant and free radical scavenging effects have also been described *in vivo* and *in vitro*. Deoxyschisandrin, gomisin A, B and C increase liver cytochrome P450 enzymes, which supports the detoxifying and anticancer properties attributed to the plant. Antitumour-promoting and anti-inflammatory properties have also been shown in skin and the lignans are known to be platelet activating factor antagonists, and to have several cardiovascular effects in the

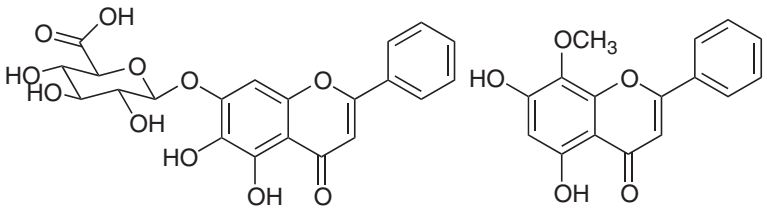


Fig. 32.4

preclinical settings [for reviews, see Chun et al (2014), Panossian and Wikman (2008, 2009)].

Clinical evidence to support the uses of schisandra is limited. Many studies are available in the older literature and describe studies conducted in Russia, although few of these trials meet today's standards (for a review, see Panossian and Wikman 2008). In a recent small randomized controlled trial involving obese Korean women, ingestion of schisandra fruit was associated with some changes in composition of the gut microbiota, but did not lead to any significant changes in obesity-related outcomes (Song et al 2015). Another placebo-controlled trial, involving individuals with raised alanine aminotransferase (ALT) or aspartate aminotransferase (AST) concentrations, found improvements in these indicators of liver function, but no change in bilirubin, following administration of tablets containing schisandrin B, sesamin (a lignan from sesame seed) and vitamin E for five months (Chiu et al 2013). Daily doses of powdered berry are usually 1.5–6 g, or sometimes higher.

Few toxicity studies have been carried out and comprehensive investigation of the safety profile of schisandra is required. Schisandra is reputed to increase gastric acidity and may cause allergy in susceptible individuals. It should be avoided during pregnancy (possible uterine stimulation) and epilepsy.

SKULLCAP, *SCUTELLARIA BAICALENSIS* GEORGI (SCUTELLARIA BAICALENSIS RADIX)

Scutellaria baicalensis (Huan qin, Lamiaceae) is sometimes known as baical skullcap to differentiate it from American skullcap (*S. laterifolia* L.). It grows in northern China, Siberia and Manchuria (a large region in northeast Asia). The leaves are opposite, lanceolate and sessile with an acute apex. The flowers are blue, with a helmet-shaped upper lip (hence the name). The root is the part used medicinally.

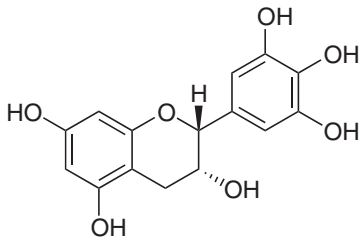
Constituents

The root contains flavonoids, including baicalin, baicalein, wogonin, chrysin, oroxylin A, skullcapflavones I and II, and others (Fig. 32.4).

Therapeutic uses and available evidence

Skullcap is used for a wide variety of ailments, particularly fevers, infections, jaundice, thirst and nosebleeds, and as an antidote and sedative. Baicalin is anti-inflammatory and anti-allergic; it inhibits the formation of lipoxygenase products and, to a lesser extent, cyclo-oxygenase products in leukocytes. It also inhibits the generation of inflammatory cytokines and is synergistic with β-lactam antibiotics against meticillin-resistant *Staphylococcus aureus in vitro*. Extracts of *S. baicalensis* inhibit lipid peroxidation in rat liver. Wogonin also suppresses production of hepatitis B virus surface antigen. The flavones interact with the benzodiazepine-binding site of the $GABA_A$ receptor, with wogonin and baicalein being the most potent; this supports the sedative use of the herb. Baicalein is antigenotoxic *in vitro* and inhibits adhesion molecule expression induced by thrombin and cell proliferation of several types of cells. Wogonin inhibits nitric oxide production in activated C6 rat glial cells, acting via NF-κB inhibition and thus suppressing cell death. It also reduces skin inflammation in mice (induced by phorbol ester expression of COX-2) and inhibits monocyte chemotactic protein-1 gene expression in human endothelial cells. Antioxidant and antibiotic activities have also been reported for extracts. These activities all support the anti-inflammatory and other uses of skullcap (for reviews, see Li et al 2011, Li-Weber 2009, Wang et al 2007).

Despite the extensive traditional use of skullcap, clinical evidence from well-designed randomized controlled trials to support the uses of skullcap is lacking. A recent trial of single doses (100 to 2800 mg) of baicalein administered to healthy volunteers found this



(–)-Epigallocatechin

Fig. 32.5

constituent to be well tolerated when taken in this context (Li et al 2014). Comprehensive investigation of the safety profile of baical skullcap is required.

The daily dose of skullcap root is usually 5–8 g. Baical skullcap is usually well tolerated, but comprehensive investigation of its safety profile is required.

TEA, *CAMELLIA SINENSIS* (L.) KUNTZE (GREEN TEA LEAVES: THEAE VIRIDIS FOLIUM; BLACK TEA LEAVES: THEAE NIGRAE FOLIUM)

Tea (*Camellia sinensis* (L.) Kuntze, Theaceae) is cultivated in China, India, Sri Lanka, Kenya, Indonesia and elsewhere. Green tea is produced in China and Japan; it is not processed and thus differs from black tea, which is fermented and produced in India, Sri Lanka and Kenya. Oolong tea is partially fermented. The leaf buds and very young leaves are used to prepare the beverage and extracts for medicinal use.

Constituents

Tea contains caffeine, and much smaller amounts of other xanthines such as theophylline and theobromine. The polyphenols are the antioxidant constituents [in green tea these are mainly (–)-epigallocatechin; Fig. 32.5], together with theogallin, trigalloyl glucose. In black tea, they have been oxidized to form the 'tea pigments', the theaflavins, thearubigens and theaflavic acids.

Therapeutic uses and available evidence

Tea is a stimulant, diuretic, astringent and antioxidant. Green tea is used medicinally more frequently than black tea. The stimulant and diuretic properties are due to the caffeine content, and the astringency and antioxidant effects to the polyphenols (Wang et al 2007). Tea is also antimicrobial and anticariogenic, and is reputed to help weight loss. Tea is useful in diarrhoea and, in China, is used for many types of dysentery.

The polyphenols in green tea have cancer chemopreventive properties due to their antioxidant capacity. Anti-inflammatory and antitumour effects have been described, and attributed to inhibition of the transcription factor NF-κB.

Black tea consumption has been associated with a lower risk of death from ischaemic heart disease and has been shown to reverse endothelial dysfunction in coronary heart disease. Habitual consumption of green tea is generally associated with a lower incidence of cancer (for a review, see Lambert and Elias 2010) and black tea is now known to have similar health benefits, which are ascribed to the tea pigments (for a review, see Kumar et al 2010). However, recent Cochrane systematic reviews of randomized trials and observational studies concluded that there was insufficient and conflicting evidence for green tea in cancer prevention (Boehm et al 2009), and that green tea had no significant effect on achieving or maintaining weight loss in overweight or obese adults (Jurgens et al 2012). Consumption of green or black tea was associated with a reduced risk of cardiovascular disease, but this evidence was not definitive due to methodological limitations of the studies (Hartley et al 2013). This body of work did indicate though that consumption of three to five cups of tea daily was not associated with safety concerns.

Tea is drunk in nearly every country in the world for its refreshing, and mildly stimulating effects. There is no recommended dose for tea, and consumption varies widely. Tea as a beverage is non-toxic in the usual amounts ingested, although it can cause gastrointestinal upsets and nervous irritability, due to the caffeine content. However, there is now some concern about the safety of concentrated preparations or excessive consumption of green tea. Cases of hepatotoxicity have been associated with consumption of high doses of green-tea-containing dietary supplements (10–29 mg/kg/d p.o.). In most cases, patients presented with elevated serum alanine aminotransferase (ALT) and bilirubin levels, and in some cases liver biopsies were performed, and periportal and portal inflammation was observed. All cases resolved following cessation of supplement consumption, and re-injury was observed in some studies when the subject began reusing the same preparations, suggesting a causative effect of the green tea (for a review, see Mazzanti et al 2015).

'SUPERFOODS'

Superfoods is a contemporary term that is used to describe foods that are believed to be, or promoted as being, beneficial to health (Anon 2013) and this is often driven by marketing strategies. Many health claims have been made in association with these foods, including lowering blood pressure, lowering cholesterol, improving exercise performance, strengthening immunity, preventing dementia, and preventing cancer.

Foods that have in recent years been hailed as superfoods include those that contain high concentrations of antioxidant compounds, such as vitamins A, C and E, and omega-3 fatty acids. Many of the so-called superfoods are fruits or vegetables, or otherwise originate from plants; some are animal products or animal by-products (e.g. salmon, eggs, yoghurt). Table 32.1 lists some popular 'superfoods' and their important constituents. Rather than looking to a few 'superfoods' for their health benefits, it is probably more appropriate to adhere to a 'superdiet' that is balanced and healthy, and rich in fruits, vegetables and wholegrain foods.

Although superfoods are promoted as being beneficial to health, most of the evidence relating to health claims associated with their consumption is inconclusive. Also, there is evidence that some may be harmful. For example, a Cochrane systematic review of antioxidant supplements used for prevention of a range of medical conditions found no evidence of benefit and some evidence that certain supplements were associated with a greater risk of mortality (Bjelakovic et al 2012). It is also important to consider that research with superfoods often uses extracts, rather than the food in its natural state. The European Food Safety Authority has issued guidance on the types of studies required to substantiate health claims relating to antioxidants (EFSA 2011).

Table 32.1 Examples of 'superfoods' from plants, and their important constituents

FOOD	IMPORTANT CONSTITUENTS
Beetroot	Iron, magnesium; folate; antioxidants (betacyanin)
Blueberries and other coloured berries, such as raspberries and blackberries	Blueberries: anthocyanins; vitamins C and K; fibre
Broccoli and other cruciferous vegetables, including Brussels sprouts, cauliflower, cabbage, or kale	Broccoli: vitamins A, C, K; folate; fibre; sulforaphane and isothiocyanates
Beans and other low-fat legumes	Folate, potassium, magnesium, iron, protein, fibre
Chocolate (dark)	Cocoa: iron, magnesium, manganese, phosphorous, zinc; antioxidants (catechins, procyanidins)
Oatmeal	Fibre
Olive oil	Unsaturated fats
Pomegranate	Vitamins A, C, E; iron
Quinoa	Essential amino acids; vitamins; minerals; protein
Spinach	Iron (but is poorly absorbed); lutein, zeaxanthin
Tomatoes	Lycopene
Walnuts	Omega-3 fatty acids; fats (help absorb vitamins A, D, E and K)

References

Alsanad, S.M., Howard, R.L., Williamson, E.M., 2014. Cancer patients at risk of herb-food-supplement interactions. Phytotherapy Research 12, 1749–1755.

Anonymous, 2013. Twelve "superfoods" you should be eating. Harvard Women's Health Watch 20 (7) 1,7.

Barnes, J., Anderson, L.A., Phillipson, J.D., 2007. Rhodiola. In: Herbal Medicines. Pharmaceutical Press, London, pp. 500–505.

Bjelakovic, G., Nikolova, D., Gluud, L.L., Simonetti, R.G., Gluud, C., 2012. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. Cochrane Database of Systematic Reviews 3, CD007176.

Bhattarai, J.P., Ah Park, S., Han, S.K., 2010. The methanolic extract of *Withania somnifera* acts on GABAA receptors in gonadotropin releasing hormone (GnRH) neurons in mice. Phytotherapy Research 24, 1147–1150.

Bishop, K.S., Kao, C.H., Xu, Y., Glucina, M.P., Paterson, R.R., Ferguson, L.R., 2015. From 2000 years of *Ganoderma lucidum* to recent developments in nutraceuticals. Phytochemistry 114, 56–65.

Boehm, K., Borrelli, F., Ernst, E., et al., 2009. Green tea (*Camellia sinensis*) for the prevention of cancer. Cochrane Database of Systematic Reviews 3, CD005004.

Booker, A., Zhai, L.X., Gkouva, C.h., Li, S.Y., Heinrich, M., 2016. From traditional resource to global commodities: a comparison of *Rhodiola* species using NMR spectroscopy—metabolomics and HPTLC. Frontiers in Pharmacology 7, 254.

Bradwejn, J., Zhou, Y., Koszycki, D., Shlik, J., 2000. A double-blind, placebo-controlled study on the effects of gotu kola *(Centella asiatica)* on acoustic startle response in healthy subjects. Journal of Clinical Psychopharmacology 20 (6), 680–684.

Brinkhaus, B., Lindner, M., Schuppan, D., Hahn, E.G., 2000. Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella asiatica*. Phytomedicine 7 (5), 427–448.

Cerella, C., Sobolewski, C., Dicato, M., Diederich, M., 2010. Targeting COX-2 expression by natural compounds: a promising alternative strategy to synthetic COX-2 inhibitors for cancer chemoprevention and therapy. Biochemistry and Pharmacology 80, 1801–1815.

Chandrika, U.G., Prasad Kumara, P.A., 2015. Gotu kola *(Centella asiatica)*: nutritional properties and plausible health benefits. Advances in Food & Nutrition Research 76, 125–157.

Chiu, H.F., Chen, T.Y., Tzeng, Y.T., Wang, C.K., 2013. Improvement of liver function in humans using a mixture of schisandra fruit extract and sesamin. Phytotherapy Research 27 (3), 368–373.

Chun, J.N., Cho, M., So, I., Jeon, J.H., 2014. The protective effects of *Schisandra chinensis* fruit extract and its lignans against cardiovascular disease: A review of the molecular mechanisms. Fitoterapia 97, 224–233.

Dar, N.J., Hamid, A., Ahmad, M., 2015. Pharmacologic overview of *Withania somnifera*, the Indian Ginseng. Cellular & Molecular Life Sciences 72 (23), 4445–4460.

de Moura, R.S., Resende, A.C., 2016. Cardiovascular and metabolic effects of Acai, an Amazon plant. Journal of Cardiovascular Pharmacology 68 (1), 19–26.

EFSA, 2011. Panel on Dietetic Products, Nutrition and Allergies (NDA); Guidance on the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health. EFSA Journal 9 (12), 2474.

Foster, S., 2016. Towards an understanding of ginseng adulteration. Herbalgram 111, 36–57.

Geng, J., Dong, J., Ni, H., et al., 2010. Ginseng for cognition. Cochrane Database Systematic Reviews 12, CD007769.

Gullett, N.P., Ruhul Amin, A.R., Bayraktar, S., et al., 2010. Cancer prevention with natural compounds. Seminars in Oncology 37, 258–281.

Haleagrahara, N., Ponnusamy, K., 2010. Neuroprotective effect of *Centella asiatica* extract (CAE) on experimentally induced parkinsonism in aged Sprague-Dawley rats. The Journal of Toxicological Sciences 35, 41–47.

Hartley, L., Flowers, N., Holmes, J., et al., 2013. Green and black tea for the primary prevention of cardiovascular disease. Cochrane Database of Systematic Reviews 6, CD009934.

Heinrich, M., Dhanji, T., Casselman, I., 2011. Açai (*Euterpe oleracea* Mart.) – a phytochemical and pharmacological assessment of the species' health claims. Phytochemistry Letters 4 (1), 10–21.

Huang, L., Zhao, H., Huang, B., Zheng, C., Peng, W., Qin, L., 2011. *Acanthopanax senticosus:* review of botany, chemistry and pharmacology. Pharmazie 66 (2), 83–97.

Hung, S.K., Perry, R., Ernst, E., 2011. The effectiveness and efficacy of *Rhodiola rosea* L.: a systematic review of randomized clinical trials. Phytomedicine 18 (4), 235–244.

Ishaque, S., Shamseer, L., Bukutu, C., Vohra, S., 2012. Rhodiola rosea for physical and mental fatigue: a systematic review. BMC Complementary and Alternative Medicine 12, 70. Available online at http://www.biomedcentral.com/1472-6882/12/70.

James, J.T., Dubery, I.A., 2009. Pentacyclic triterpenoids from the medicinal herb, *Centella asiatica* (L.) Urban. Molecules, a Journal of Synthetic Chemistry and Natural Product Chemistry 14 (10), 3922–3941.

Jin, X., Ruiz Beguerie, J., Sze, D.M.Y., Chan, G.C.F., 2016. *Ganoderma lucidum* (Reishi mushroom) for cancer treatment. Cochrane Database of Systematic Reviews 4, CD007731.

Jurgens, T.M., Whelan, A.M., Killian, L., Doucette, S., Kirk, S., Foy, E., 2012. Green tea for weight loss and weight maintenance in overweight or obese adults. Cochrane Database of Systematic Reviews 12, CD008650.

Kim, H.G., Cho, J.H., Yoo, S.R., et al., 2013. Antifatigue effects of *Panax ginseng* C.A. Meyer: a randomised, double-blind, placebo-controlled trial. PLoS ONE [Electronic Resource] 8 (4), e61271.

Klupp, N.L., Chang, D., Hawke, F., 2015. *Ganoderma lucidum* mushroom for the treatment of cardiovascular risk factors. Cochrane Database of Systematic Reviews 2, CD007259.

Kulkarni, S.K., Dhir, A., 2008. *Withania somnifera:* an Indian ginseng. Progress in Neuro-psychopharmacology and Biological Psychiatry 32, 1093–1105.

Kumar, G., Pillare, S.P., Maru, G.B., 2010. Black tea polyphenols-mediated in vivo cellular responses during carcinogenesis. Mini Reviews in Medicinal Chemistry 10, 492–505.

Lambert, D., Elias, R.J., 2010. The antioxidant and pro-oxidant activities of green tea polyphenols: a role in cancer prevention. Archives of Biochemstry and Biophysics 501, 65–72.

LaSala, G.S., McKeever, R.G., Patel, U., Okaneku, J., Vearrier, D., Greenberg, M.I., 2015. Effect of single-dose *Ginkgo biloba* and *Panax ginseng* on driving performance. Clinical Toxicology 53 (2), 108–112.

Lee, M.S., Yang, E.J., Kim, J.I., Ernst, E., 2009. Ginseng for cognitive function in Alzheimer's disease: a systematic review. Journal of Alzheimer's Disease 18 (2), 339–344.

Li, C., Lin, G., Zuo, Z., 2011. Pharmacological effects and pharmacokinetics properties of *Radix scutellariae* and its bioactive flavones. Biopharmaceutics & Drug Disposition 32 (8), 427–445.

Li, M., Shi, A., Pang, H., 2014. Safety, tolerability, and pharmacokinetics of a single ascending dose of baicalein chewable tablets in healthy subjects. Journal of Ethnopharmacology 156, 210–215.

Li-Weber, M., 2009. New therapeutic aspects of flavones: the anticancer properties of Scutellaria and its main active constituents Wogonin, Baicalein and Baicalin. Cancer Treatment Reviews 35, 57–68.

Mato, L., Wattanathorn, J., Muchimapura, S., et al., 2011. 2011 *Centella asiatica* improves physical performance and health-related quality of life in healthy elderly volunteer. Evidence-based Complementary and Alternative Medicine 579467.

Mazzanti, G., Di Sotto, A., Vitalone, A., 2015. Hepatotoxicity of green tea: an update. Archives of Toxicology 89 (8), 1175–1191.

Nasir, M.N., Habsah, M., Zamzuri, I., Rammes, G., Hasnan, J., Abdullah, J., 2011. Effects of asiatic acid on passive and active avoidance task in male Spraque-Dawley rats. Journal of Ethnopharmacology 134, 203–209.

Paik, D.J., Lee, C.H., 2015. Review of cases of patient risk associated with ginseng abuse and misuse. Journal of Ginseng Research 39 (2), 89–93.

Panossian, A., Wikman, G., 2008. Pharmacology of *Schisandra chinensis* Bail.: An overview of Russian research and uses in medicine. Journal of Ethnopharmacology 118, 183–212.

Panossian, A., Wikman, G., 2009. Evidence-based efficacy of adaptogens in fatigue, and molecular mechanisms related to their stress-protective activity. Current Clinical Pharmacology 4, 198–219.

Panossian, A., Wikman, G., Sarris, J., 2010. Rosenroot (*Rhodiola rosea*): traditional use, chemical composition, pharmacology and clinical efficacy. Phytomedicine 17, 481–493.

Pratte, M.A., Nanavati, K.B., Young, V., Morley, C.P., 2014. An alternative treatment for anxiety: a systematic review of human trial results reported for the Ayurvedic herb ashwagandha *(Withania somnifera)*. Journal of Alternative and Complementary Medicine 20 (12), 901–908.

Ru, W., Wang, D., Xu, Y., 2015. Chemical constituents and bioactivities of *Panax ginseng* (C. A. Mey.). Drug Discoveries & Therapeutics 9 (1), 23–32.

Sanodiya, B.S., Thakur, G.S., Baghel, R.K., Prasad, G.B., Bisen, P.S., 2009. *Ganoderma lucidum*: a potent pharmacological macrofungus. Current Pharmacology and Biotechnology 10, 717–742.

Shergis, J.L., Zhang, A.L., Zhou, W., Xue, C.C., 2013. *Panax ginseng* in randomised controlled trials: a systematic review. Phytotherapy Research 27 (7), 949–965.

Shu, L., Cheung, K.L., Khor, T.O., Chen, C., Kong, A.N., 2010. Phytochemicals: cancer chemoprevention and suppression of tumor onset and metastasis. Cancer Metastasis Reviews 29, 483–502.

Song, M.Y., Wang, J.H., Eom, T., Kim, H., 2015. *Schisandra chinensis* fruit modulates the gut microbiota composition in association with metabolic markers in obese women: a randomized, double-blind placebo-controlled study. Nutrition Research 35 (8), 655–663.

Ulbricht, C., Brigham, A., Burke, D., 2012. An evidence-based systematic review of acai *(Euterpe oleracea)* by the natural standard research collaboration. Journal of Dietary Supplements 9 (2), 128–147.

Wang, C.Z., Mehendale, S.R., Yuan, C.S., 2007. Commonly used antioxidant botanicals: active constituents and their potential role in cardiovascular illness. American Journal of Chinese Medicine 35, 543–558.

Wattanathorn, J., Mator, L., Muchimapura, S., 2008. Positive modulation of cognition and mood in the healthy elderly volunteer following the administration of *Centella asiatica*. Journal of Ethnopharmacology 116 (2), 325–332.

Xia, Q., Zhang, H., Sun, X., 2014. A comprehensive review of the structure elucidation and biological activity of triterpenoids from *Ganoderma* spp. Molecules 19 (11), 17478–17535.

Yamaguchi, K.K., Pereira, L.F., Lamarao, C.V., Lima, E.S., da Veiga-Junior, V.F., 2015. Amazon acai: chemistry and biological activities: a review. Food Chemistry 179, 137–151.

Yu, L., Qin, Y., Wang, Q., et al., 2014. The efficacy and safety of Chinese herbal medicine, Rhodiola formulation in treating ischemic heart disease: a systematic review and meta-analysis of randomized controlled trials. Complementary Therapies in Medicine 22 (4), 814–825.

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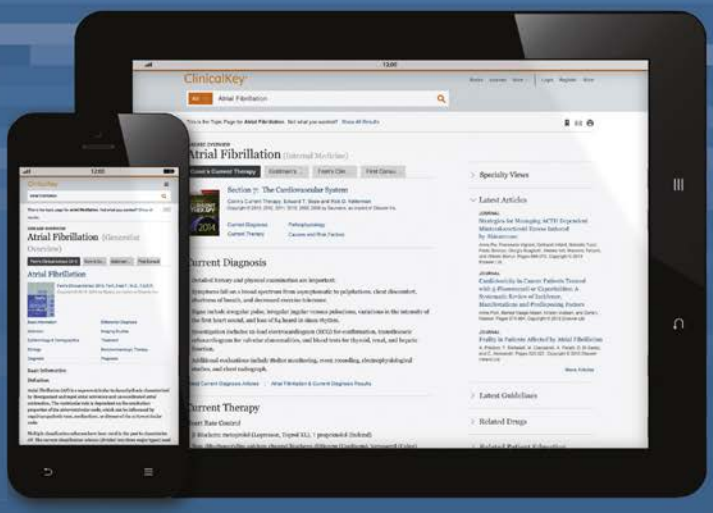

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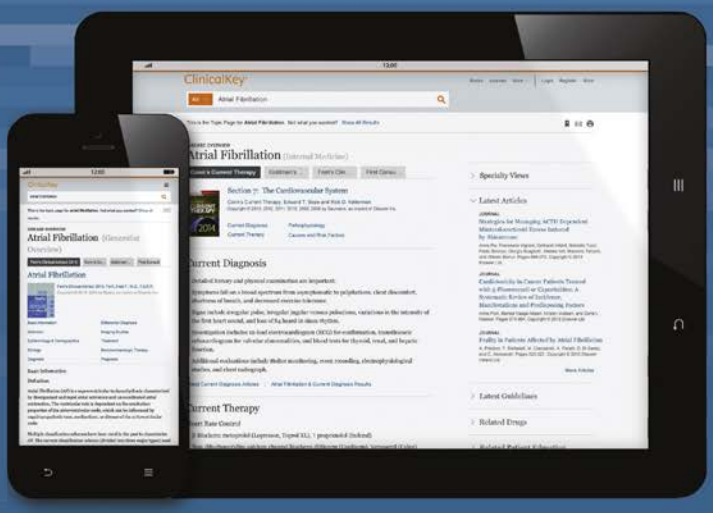

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