# 26

# Alkaloids



Alkaloid-containing plants constitute an extremely varied group both taxonomically and chemically, a basic nitrogen being the only unifying factor for the various classes. For this reason, questions of the physiological role of alkaloids in the plant, their importance in taxonomy, and biogenesis are often most satisfactorily discussed at the level of a particular class of alkaloid. A similar situation pertains with the therapeutic and pharmacological activities of alkaloids. As most alkaloids are extremely toxic, plants containing them do not feature strongly in herbal medicine but they have always been important in the allopathic system where dosage is strictly controlled and in homoeopathy where the dose-rate is so low as to be harmless.

## INTRODUCTION

A precise definition of the term 'alkaloid' (alkali-like) is somewhat difficult because there is no clear-cut boundary between alkaloids and naturally occurring complex amines. Typical alkaloids are derived from plant sources, they are basic, they contain one or more nitrogen atoms (usually in a heterocyclic ring) and they usually have a marked physiological action on man or other animals. The name 'proto-alkaloid' or 'amino-alkaloid' is sometimes applied to compounds such as hordenine, ephedrine and colchicine which lack one or more of the properties of typical alkaloids. Other alkaloids, not conforming with the general definition, are those synthetic compounds not found in plants but very closely related to the natural alkaloids (e.g. homatropine). In practice, those substances present in plants and giving the standard qualitative tests outlined below are termed alkaloids, and frequently in plant surveys this evidence alone is used to classify a particular plant as 'alkaloid-containing'.

### HISTORY

The first isolations of alkaloids in the nineteenth century followed the reintroduction into medicine of a number of alkaloid-containing drugs and were coincidental with the advent of the percolation process for the extraction of drugs. The French apothecary Derosne probably isolated the alkaloid afterwards known as narcotine in 1803 and the Hanoverian apothecary Sertürner further investigated opium and isolated morphine (1806, 1816). Isolation of other alkaloids, particularly by Pelletier and Caventou, rapidly followed; strychnine (1817), emetine (1817), brucine (1819), piperine (1819), caffeine (1819), quinine (1820), colchicine (1820) and coniine (1826). Coniine was the first alkaloid to have its structure established (Schiff, 1870) and to be synthesized (Ladenburg, 1889), but for others, such as colchicine, it was well over a century before the structures were finally elucidated. Modern methods and instrumentation have greatly facilitated these investigations, and it is interesting to note that the yields of 'minor' alkaloids, too small for further investigation, isolated by chemists during the first quarter of the last century would now be sufficient, several thousand times over, for a complete structure analysis. In the second half of the twentieth century alkaloids featured strongly in the search for plant drugs with anticancer activity. A notable success was the introduction of *Catharanthus* alkaloids and paclitaxel into medicine and there is much current interest in other alkaloids having anticancer properties as well as those exhibiting antiaging and antiviral possibilities.

### DISTRIBUTION

Some 150 years of alkaloid chemistry had resulted by the mid-1940s in the isolation of about 800 alkaloids; the new technology of the next 50 years increased this figure to the order of 10 000.

True alkaloids are of rare occurrence in lower plants. In the fungi the lysergic acid derivatives and the sulphur-containing alkaloids, e.g. the gliotoxins, are the best known. Among the pteridophytes and gymnosperms the lycopodium, ephedra and *Taxus* alkaloids have medicinal interest. Alkaloid distribution in the angiosperms is uneven. The dicotyledon orders Salicales, Fagales, Cucurbitales and Oleales at present appear to be alkaloid-free. Alkaloids are commonly found in the orders Centrospermae (Chenopodiaceae), Magnoliales (Lauraceae, Magnoliaceae), Ranunculales (Berberidaceae, Menispermaceae, Ranunculaceae), Papaverales (Papaveraceae, Fumariaceae), Rosales (Leguminosae, subfamily Papilionaceae), Rutales (Rutaceae), Gentiales (Apocynaceae, Loganiaceae, Rubiaceae), Tubiflorae (Boraginaceae, Convolvulaceae, Solanaceae) and Campanulales (Campanulaceae, sub-family Lobelioideae; Compositae, subfamily Senecioneae).

Hegnauer, who has made an intensive study of alkaloid distribution, while recognizing the undoubted potential chemotaxonomic significance of this group, is cautious about its use without due regard to all the other characters of the plant. Nevertheless it continues to be a popular area of research.

Nearly 300 alkaloids belonging to more than 24 classes are known to occur in the skins of amphibians along with other toxins. They include the potent neurotoxic alkaloids of frogs of the genus *Phyllobates*, which are among some of the most poisonous substances known. Other reptilian alkaloids are strongly antimicrobial. Alkaloids derived from mammals include ones of indole and isoquinoline classes a few are found in both plants and animals.

## PROPERTIES

Most alkaloids are well-defined crystalline substances which unite with acids to form salts. In the plant they may exist in the free state, as salts or as *N*-oxides (see below). In addition to the elements carbon, hydrogen and nitrogen, most alkaloids contain oxygen. A few, such as coniine from hemlock and nicotine from tobacco, are oxygen-free and are liquids. Although coloured alkaloids are relatively rare, they are not unknown; berberine, for example, is yellow and the salts of sanguinarine are copper-red.

A knowledge of the solubility of alkaloids and their salts is of considerable pharmaceutical importance. Not only are alkaloidal substances often administered in solution, but also the differences in solubility between alkaloids and their salts provide methods for the isolation of alkaloids from the plant and their separation from the non-alkaloidal substances also present. While the solubilities of different alkaloids and salts show considerable variation, as might be expected from their extremely varied structure, it is true to say that the free bases are frequently sparingly soluble in water but soluble in water but soluble in organic solvents; with salts the reverse is often the case, these being usually soluble in water but sparingly soluble in organic solvents. For example, strychnine hydrochloride is much more soluble in water than is strychnine base. It will soon be realized that there are many exceptions to the above generalizations, caffeine (base) being readily extracted from tea with water and colchicine being soluble in either acid, neutral or alkaline water. Again, some alkaloidal salts are sparingly soluble—for example, quinine sulphate is only soluble to the extent of 1 part in 1000 parts of water, although 1 part quinine hydrochloride is soluble in less than 1 part of water.

## STRUCTURE AND CLASSIFICATION

Alkaloids show great variety in their botanical and biochemical origin, in chemical structure and in pharmacological action. Consequently, many different systems of classification are possible. In the arrangement of the well-known drugs which follow later in the chapter, the phytochemical arrangement introduced in the eleventh edition of this book and based on the origin of the alkaloids in relation to the common amino acids has been used. For practical purposes it is useful, therefore, to maintain the well-established classifications based on chemical structures, Fig. 26.1, and Table 26.1 closely follows that used by a number of authors. There are two broad divisions:

I. Non-heterocyclic or atypical alkaloids, sometimes called 'proto-alkaloids' or biological amines.
II. Heterocyclic or typical alkaloids, divided into 14 groups according to their ring structure.

### The nitrogen of alkaloids

Alkaloids, taken in their broadest sense, may have a nitrogen atom which is primary (mescaline), secondary (ephedrine), tertiary (atropine) or quaternary (one of the N atoms of tubocurarine), and this factor affects the derivatives of the alkaloid which can be prepared and the isolation

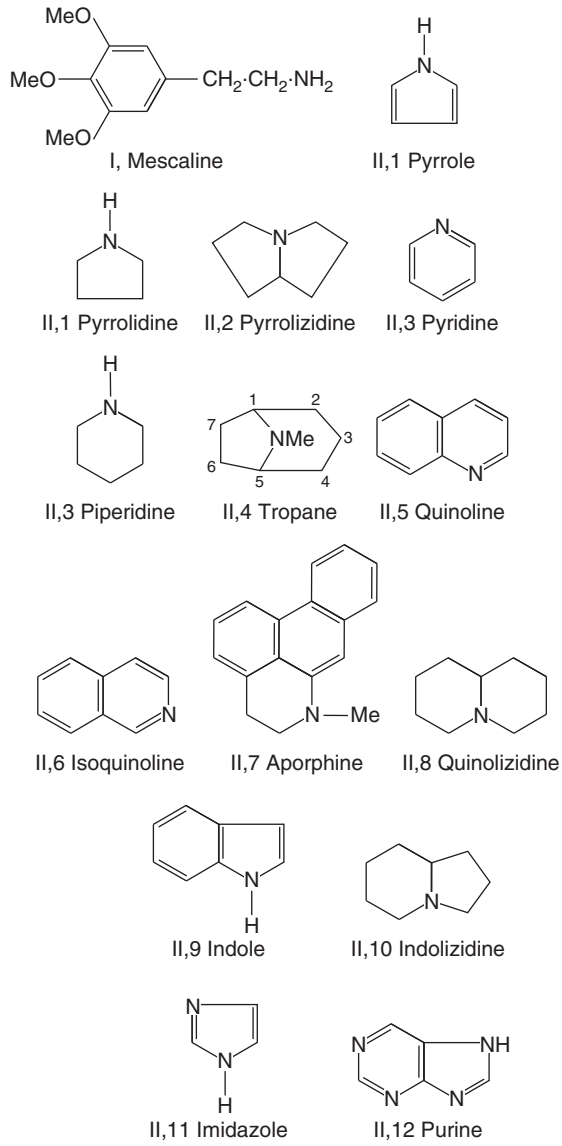



**Fig. 26.1**
Skeletal structures of alkaloids found in medicinal plants. (Numbers refer to location in Table 26.1).

**Table 26.1 Types of alkaloid and their occurrence.**

| | |
|---|---|
| **I. Non-heterocyclic alkaloids** | |
| Hordenine or *N*-methyltyramine | In germinating barley, *Hordeum distochon* |
| Mescaline, related to tryptamine (see formula) | *Lophophora williamsii* (Cactaceae) |
| Ephedrine | *Ephedra* spp. (Ephedraceae) |
| Colchicine (tropolone nucleus with nitrogen in side-chain) | *Colchicum* spp. and related genera (Liliaceae) |
| Erythromycin (an antibiotic) | *Streptomyces erythreus* (Bacteriophyta, Actinomycetales) |
| Jurubin (steroid with 3-amino group) | *Solanum paniculatum* (Solanaceae) |
| Pachysandrine A (steroid with *N*-containing C-17 side-chain) | *Pachysandra terminalis* (Buxaceae) |
| Taxol (a modified diterpene pseudo alkaloid) | *Taxus brevifolia* (Taxaceae) |
| **II. Heterocyclic alkaloids** | |
| 1. *Pyrrole and pyrrolidine* | |
| Hygrines | *Coca* spp. (Erythroxylaceae); often associated with tropane alkaloids of the Solanaceae |
| Stachydrine | *Stachys tuberifera* (Labiatae), soya bean and other Leguminosae |
| 2. *Pyrrolizidine* | |
| Symphitine, echimidine | *Symphytum* spp. |
| Senecionine, seneciphylline, etc. | *Senecio* spp. |
| 3. *Pyridine and piperidine* | |
| Trigonelline | Fenugreek (Leguminosae), strophanthus (Apocynaceae), coffee (Rubiaceae) |
| Coniine | *Conium maculatum* (Umbelliferae) |
| Arecoline | *Areca catechu* (Palmae) |
| Lobeline | *Lobelia* spp. (Lobeliaceae) |
| Pelletierine | *Punica granatum*, the pomegranate (Punicaceae) |
| Nicotine (pyridine + pyrrolidine) | *Nicotiana tabacum* and other spp. (Solanaceae) |
| Anabasine | *Nicotiana glauca; Anabasis aphylla* (Chenopodiaceae) |
| Piperine | *Piper* spp. (Piperaceae) |
| Ricinine | *Ricinus communis* (Euphorbiaceae) |
| 4. *Tropane* (piperidine/*N*-methyl-pyrrolidine) | |
| Hyoscyamine, atropine, hyoscine, meteloidine, etc. | Species of *Atropa, Datura, Hyoscyamus, Duboisia, Mandragora* and *Scopolia* (Solanaceae) |
| Calystegines | *Convolvulus* spp., *Ipomoea polpha* (Convolvulaceae), some solanaceous spp., *Morus* spp. (Moraceae) |
| *Cocaine* | *Coca* spp. (Erythroxylaceae) |
| Pesudo-pelletierine | *Punica granatum* (Punicaceae) |
| 5. *Quinoline* | |
| Quinine, quinidine, cinchonine, cinchonidine | *Cinchona* spp. (Rubiaceae), *Remijia* spp. (Rubiaceae) |
| Cusparine | *Angostura or cusparia bark, Galipea officinalis* (Rutaceae) |
| 6. *Isoquinoline* | |
| Papaverine, narceine, narcotine | *Papaver somniferum* (Papaveraceae) |
| Corydaline | *Corydalis* and *Dicentra* spp. (Fumariaceae) |
| Hydrastine, berberine | Numerous genera of the Berberidaceae, Ranunculaceae and Papaveraceae |
| Emetine, cephaeline | *Cephaelis* spp. (Rubiaceae) |
| Tubocurarine | Curare obtained from plants of Menispermaceae |
| Morphine, codeine | *Papaver somniferum* (Papaveraceae) |
| Erythraline | *Erythrina* spp. (Leguminosae) |
| Galanthamine | *Leucojum aestivum* (Amaryllidaceae) |
| 7. *Aporphine* (reduced isoquinoline/naphthalene) | |
| Boldine | *Peumus boldus* (Monimiaceae) |
| 8. *Quinolizidine* | |
| Sparteine, cytisine, lupanine, laburnine | Sometimes called 'the lupin alkaloids'. Occur particularly in the Leguminosae, subfamily Papilionaceae, e.g. broom. *Cytisus scoparius;* dyer's broom, *Genista tinctoria; Laburnum* and *Lupinus* spp. |
| 9. *Indole or benzopyrrole* | |
| Ergometrine, ergotamine | *Claviceps* spp. (Hypocreaceae) |
| Lysergic acid amide, clavine alkaloids | *Rivea corymbosa, Ipomoea violacea* (Convolvulaceae) |
| Physostigmine | *Physostigma venenosum* (Leguminosae) |
| Ajmaline, serpentine, reserpine | *Rauwolfia* spp. (Apocynaceae) |
| Yohimbine, aspidospermine | *Aspidosperma* spp. (Apocynaceae) |
| Vinblastine, vincristine | *Catharanthus roseus* (Apocynaceae) |
| Calabash curare alkaloids | *Strychnos* spp. (Loganiaceae) |
| Strychnine, brucine | *Strychnos* spp. (Loganiaceae) |

(*Continued*)

**Table 26.1 Types of alkaloid and their occurrence. (Cont'd)**

| | |
|---|---|
| 10. *Indolizidine* | |
| Castanospermine | *Castanospermum australe* (Leguminosae), *Alexa* spp. (Leguminosae) |
| Swainsonine | *Swainsona* spp. (Leguminosae), Loco plants (Leguminosae) |
| 11. *Imidazole or glyoxaline* | |
| Pilocarpine | *Pilocarpus* spp. (Rutaceae) |
| 12. *Purine* (pyrimidine/imidazole) | |
| Caffeine | Tea (Ternstroemiaceae), coffee (Rubiaceae), maté (Aquifoliaceae), guarana (Sapindaceae), cola nuts (Sterculiaceae) |
| Theobromine | Cocoa (Sterculiaceae) |
| 13. *Steroidal* (some combined as glycosides) | |
| Solanidine (glycoside = solanine) | Shoots of potato (Solanaceae), etc. |
| Veratrum alkamine esters and their glycosides | *Veratrum* spp. and *Schoenocaulon* spp. (Liliaceae) |
| Conessine | *Holarrhena antidysenterica* (Apocynaceae) |
| Funtumine | *Funtumia elastica* (Apocynaceae) |
| 14. *Terpenoid* | |
| Aconitine, atisine, lyctonine, etc. | *Aconitum* and *Delphinium* spp. (Ranunculaceae) |

procedures. In the plant, alkaloids may exist in the free state, as salts or as amine or alkaloid *N*-oxides.

**Alkaloid *N*-oxides.** *N*-oxidation products of alkaloids, particularly the *N*-oxides of tertiary alkaloids, are well-known laboratory products, easily prepared from the original base. As early as the 1920s quite extensive pharmacological and toxicological comparisons had been made of common alkaloids such as morphine, strychnine and hyoscyamine and their corresponding *N*-oxides. Some enthusiasm for the clinical use of *N*-oxides was engendered by their purported delayed-release properties, low toxicities and low addictive properties compared with the corresponding tertiary alkaloids.

Although the formation of *N*-oxides and other *N*-oxidation products of alkaloids in animal systems is well-known, forming part of the wider scheme for the metabolism of amines, the occurrence of such compounds in plants has, until relatively recently, received little attention. This was possibly due to a belief that such compounds represented artefacts arising during the extraction and work-up of tertiary alkaloids. Secondly, because of the high polarity, and water-solubility of alkaloid *N*-oxides, they were discarded by the normal alkaloid extraction procedures.

One group of alkaloids known to occur extensively as the natural *N*-oxides comprises the quinolizidines of the Boraginaceae, Compositae and Papilionaceae; these are alkaloids, including those of *Senecio* spp., which cause extensive liver damage in animals using plants containing them as fodder. A number of *N*-oxide alkaloids of the indole series have been isolated from plant materials, and among those of pharmaceutical significance are the simple hallucinogenic indole derivatives of *Amanita* spp., reserpine, strychnine, and some *Mitragyna* alkaloids. Fresh *Atropa, Datura, Hyoscyamus, Scopolia* and *Mandragora* each contain the two isomeric *N*-oxides of hyoscyamine.

One of the two possible *N*-oxides of hyoscine has been isolated from species of the first four genera above. Morphine and codeine *N*-oxides are natural constituents of the opium poppy latex, and *Nicotiana* spp. contain two isomeric nicotine *N*-oxides based on the pyrrolidine nitrogen. Some *N*-oxides—for example, aspergillic acid and iodinin (1,6-dihydroxyphenazine dioxide)—isolated from microorganisms, possess antibacterial activity.

As with the tertiary alkaloids themselves, there is little evidence to suggest what role the *N*-oxides may play in the plant's metabolism. Ontogenetic studies of hyoscyamine *N*-oxide production in belladonna indicate a dynamic role for the *N*-oxide with a maximum build-up in the developing fruits. Oxidation–reduction involving *N*-oxides and tertiary bases is a probability. It has been suggested that *N*-oxides may be involved in demethylations and their participation in the biosynthesis of benzylisoquinoline alkaloids has also been proposed. The solubility properties of *N*-oxides could influence the transport of alkaloids both throughout the plant and also within the cell itself.

## Tests for alkaloids

Most alkaloids are precipitated from neutral or slightly acid solution by Mayer's reagent (potassiomercuric iodide solution), by Wagner's reagent (solution of iodine in potassium iodide), by solution of tannic acid, by Hager's reagent (a saturated solution of picric acid), or by Dragendorff's reagent (solution of potassium bismuth iodide). These precipitates may be amorphous or crystalline and are of various colours: cream (Mayer's), yellow (Hager's), reddish-brown (Wagner's and Dragendorff's). Caffeine and some other alkaloids do not give these precipitates (see below). Care must be taken in the application of these alkaloidal tests, as the reagents also give precipitates with proteins. During the extraction of alkaloids from the plant and subsequent evaporation, some proteins will not be extracted and others will be made insoluble (denatured) by the evaporation process and may be filtered out. If the original extract has been concentrated to low bulk and the alkaloids extracted from an alkaline solution by means of an organic solvent, and then transferred into dilute acid (e.g. tartaric), the latter solution should be protein-free and ready to test for alkaloids.

As mentioned above, caffeine, a purine derivative, does not precipitate like most alkaloids. It is usually detected by mixing with a very small amount of potassium chlorate and a drop of hydrochloric acid, evaporating to dryness and exposing the residue to ammonia vapour. A purple colour is produced with caffeine and other purine derivatives. This is known as the murexide test. Caffeine easily sublimes and may be extracted from tea by heating the broken leaves in a crucible covered with a piece of glass. Colour tests are sometimes useful—for example, the yellow colour given by colchicine with mineral acids or the bluish-violet to red colour given by indole alkaloids when treated with sulphuric acid and *p*-dimethylaminobenzaldehyde. Other examples will be given under individual drugs.

For the identification of drugs containing known alkaloids, pharmacopoeias commonly employ TLC separations using reference compounds to establish the presence of individual alkaloids. In this respect, some of the alkaloid reagents quoted above are useful for detection of the separated bases.

## EXTRACTION OF ALKALOIDS

Extraction methods vary with the scale and purpose of the operation, and with the raw material. For many research purposes chromatography gives both speedy and accurate results. However, if an appreciable quantity of alkaloid is required, one of the following general methods will usually serve.

**Process A.** The powdered material is moistened with water and mixed with lime which combines with acids, tannins and other phenolic substances and sets free the alkaloids (if they exist in the plant as salts). Extraction is then carried out with organic solvents such as ether or petroleum spirit. The concentrated organic liquid is then shaken with aqueous acid and allowed to separate. Alkaloid salts are now in the aqueous liquid, while many impurities remain behind in the organic liquid.

**Process B.** The powdered material is extracted with water or aqueous alcohol containing dilute acid. Pigments and other unwanted materials are removed by shaking with chloroform or other organic solvents. The free alkaloids are then precipitated by the addition of excess sodium bicarbonate or ammonia and separated by filtration or by extraction with organic solvents.

Large-scale extractions based on the above principles are sometimes done in the field and the crude mixtures of alkaloids afterwards sent to a factory for separation and purification. This has been done for both cinchona and coca alkaloids in South America and Indonesia, the crude alkaloids then being sent to Europe, USA or Japan for purification. The separation and final purification of a mixture of alkaloids may sometimes be done by fractional precipitation or by fractional crystallization of salts such as oxalates, tartrates or picrates. Chromatographic methods are particularly suitable if the mixture is a complex one and if small quantities of alkaloids will suffice. Supercritical fluid extraction (Chapter 17), although not yet applied to many alkaloids, will probably become of increasing importance for these compounds.

Volatile liquid alkaloids such as nicotine and coniine are most conveniently isolated by distillation. An aqueous extract is made alkaline with caustic soda or sodium carbonate and the alkaloid distilled off in steam. Nicotine is an important insecticide, and large quantities of it are prepared from those parts of the tobacco plant which cannot be used for tobacco manufacture.

### Cell, tissue and organ culture

The production of alkaloids using cell, tissue and organ cultures has now been extensively investigated for its commercial potential, as a means of obtaining new alkaloids and for elucidating biosynthetic pathways. These aspects are considered in Chapter 13 and under individual drugs.

## FUNCTIONS OF ALKALOIDS IN PLANTS

The characteristic nature of alkaloids and their often very marked pharmacological effects when administered to animals naturally led scientists to speculate on their biological role in the plants in which they occurred. In spite of many suggestions over the years, however, little convincing evidence for their function has been forthcoming. The following points are noteworthy.

1. Being of such diverse nature, alkaloids as a group could not be expected to have a common role (if any) in the plant, except possibly in situations requiring a non-specific basic compound. In this respect the increase in putrescine in barley seedlings when grown in a medium deficient in potassium is of interest.
2. Alkaloids often occur in plants in association with characteristic acids—for example, the tropane alkaloids of the Solanaceae and Erythroxylaceae are esters, the cinchona alkaloids occur with quinic and cinchotannic acids, opium alkaloids are associated with meconic acid. In some cases the alkaloids could provide either a means of storing or transporting in soluble form the particular acids. In the case of solanaceous plants it has been shown that tropane esters formed in the roots are translocated to the aerial parts, where hydrolysis of the alkaloid and breakdown of the liberated acid occurs.
3. As the majority of alkaloids are biosynthesized from readily available units by a series of ubiquitous reactions, their presence in the plant may be purely chance, depending on the enzymes present and the availability of precursors. Being apparently harmless to the plant, they are not eliminated through necessity by natural selection.
4. By the use of suitable grafts, plants which normally accumulate alkaloids in the aerial parts (e.g. *Nicotiana, Datura*) are produced free of alkaloids. The lack of alkaloid in the scion appears in no way to impair its development, which suggests the non-essential nature of the alkaloid.
5. Plants which do not normally contain alkaloids appear usually to suffer no adverse reaction when administered alkaloids (colchicine is an exception). Some 'foreign' alkaloids may be metabolized.
6. Current research constantly demonstrates not only that alkaloids participate in plant metabolism over the long term, but also that daily variation in alkaloid content (qualitative and quantitative) is very common in some species. This implies that even if the presence of alkaloids is not vital to the plant, they do participate in metabolic sequences and are not solely the waste, endproducts of metabolism.
7. Pertinent to the above, it has been suggested (R. A. Larson and K. M. Marley, *Phytochemistry*, 1984, **23**, 2351; R. A. Larson, *Phytochemistry*, 1988, **27**, 969) that alkaloids may have a role in the defence of the plant against singlet oxygen ($^1O_2$), which is damaging to all living organisms and is produced in plant tissues in the presence of light. Of 15 alkaloids tested, most showed a good ability to quench singlet oxygen, with brucine and strychnine being especially efficient. Circumstantial evidence quoted is the turnover of poppy alkaloids on a diurnal basis and the formation of oxidized serpentine at the expense of the reduced ajmaline when *Catharanthus roseus* tissue cultures are exposed to light. Further, to concur with the above hypothesis, one would expect plants inhabiting regions with a high ultraviolet light intensity to accumulate more alkaloids, and confirmatory examples quoted are berberine in *Berberis* and tomatidine in *Lycopersicum*. To these could be added quinine in *Cinchona*.

### Further reading

Cordell GA (ed) 1991 The alkaloids. Chemistry and pharmacology, Vols 41–. Academic Press, London. *Vol. 53 appeared in 2000. This is a continuation of the original Manske and Holmes series started in 1950; followed by A Brossi as editor, Vols 23–41*

Roberts MR, Wink M (eds) 1998 Alkaloids. Biochemistry, ecology and medical applications. Plenum, New York

Southon IW Buckingham J 1989 Dictionary of Alkaloids. Chapman and Hall, London

## ORNITHINE-DERIVED ALKALOIDS

As indicated in Fig. 26.2, the amino acid ornithine, its decarboxylation product, putrescine, and proline constitute the basic unit of the tropane, ecgonine, nicotine (pyrrolidine ring), necine and stachydrine groups of alkaloids. Biogenetically ornithine is linked to arginine (Fig. 18.15); putrescine can also be formed from arginine without the involvement of ornithine and this has led to problems in the understanding of the

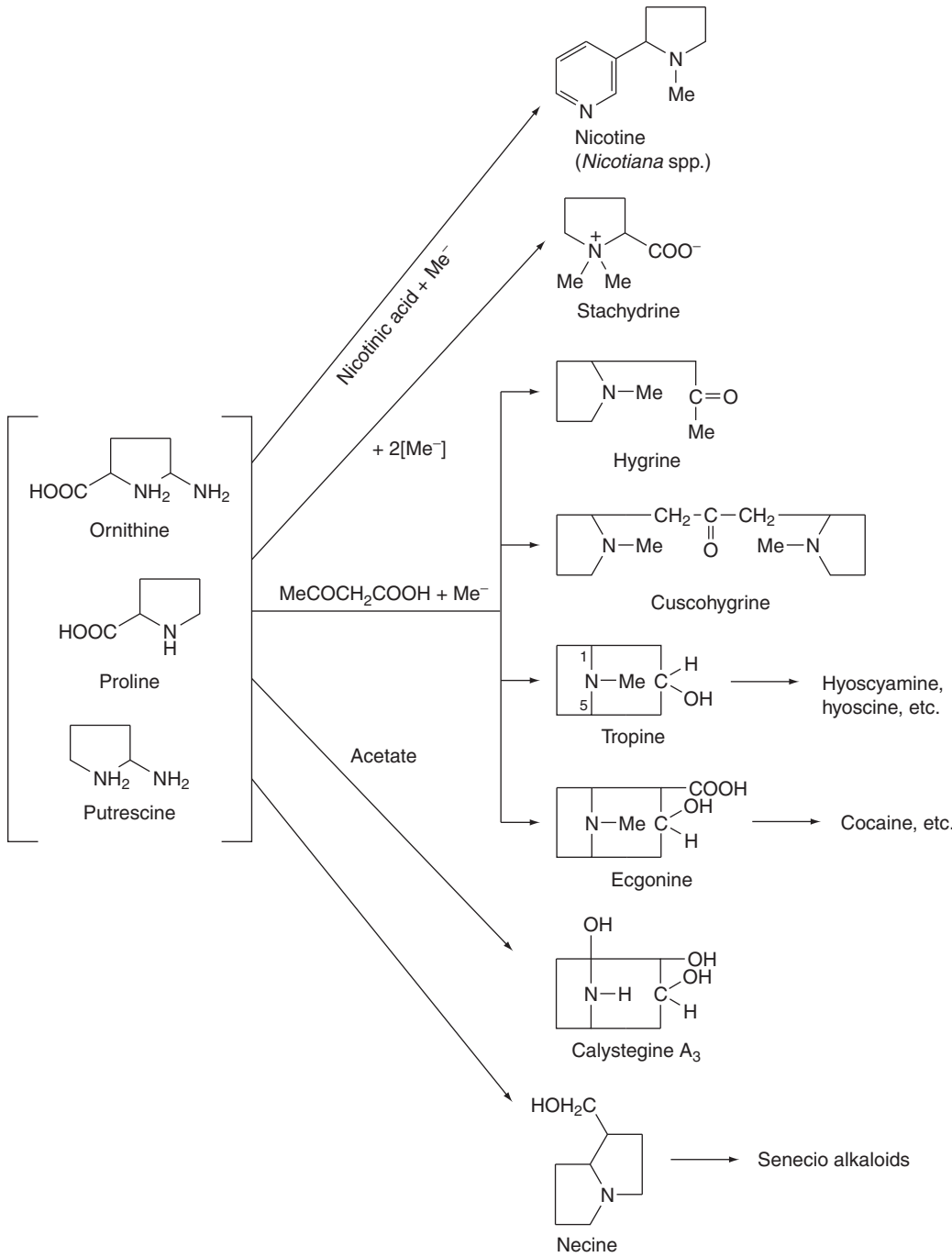
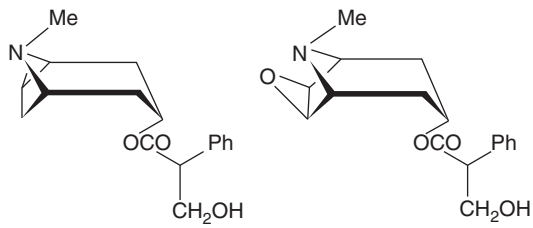



**Fig. 26.2**
Some ornithine-derived alkaloids.

stereospecific incorporation, or otherwise, of precursors into particular alkaloids, see below. Pharmaceutically, the tropane group is important.

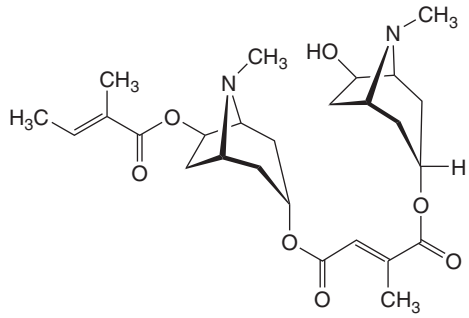
## TROPANE ALKALOIDS

The principal alkaloids of medicinal interest in this group are (–)-hyoscyamine; its more stable racemate atropine, and hyoscine (scopolamine). The compounds are esters and are hydrolysed by heating at 60°C with baryta water; atropine yields tropic acid and tropine; hyoscine gives tropic acid and oscine (scopine is actually formed by enzymatic hydrolysis but the chemical treatment converts it to the more stable geometric isomer, oscine).




These three specific alkaloids are confined to the Solanaceae, in which some 40 different ester bases of the tropane type have now been discovered; they constitute an interesting chemotaxonomic study

within the family. Examples of tropanol esters are given in Table 26.2. Dimeric and trimeric tropanol ester alkaloids involving the dicarboxylic acids mesaconic and itaconic acids are found in *Schizanthus*. For isolations from *S. porrigens* see O. Muñoz and S. Cortés, *Pharm. Biol*., 1998, **36**, 162, and from *S. hookeri* see M. Jordan *et al.*, *Phytochemistry*, 2006, **67**, 570. Other tropane bases occur in the Erythroxylaceae (see cocaine in coca leaves), Convolvulaceae, Dioscoreaceae, Rhizophoraceae, Cruciferae and Euphorbiaceae.



Schizanthine Z— a tropandiol mesaconic acid diester

Altogether over 200 tropane alkaloids have now been recorded. Semisynthetic derivatives, e.g. hyoscine butylbromide (Buscopan), are of medicinal importance.

## BIOGENESIS OF TROPANE ALKALOIDS

As the characteristic alkaloids of the group are esters of hydroxytropanes and various acids (tropic, tiglic, etc.) there are, for each alkaloid, two distinct biogenetic moieties which warrant consideration. Most studies in this connection have utilized various species of *Datura* because, for a number of reasons, they are one of the most convenient of the Solanaceae with which to work. However, with the advent of isolated root culture techniques the study of alkaloid formation in other genera has become more evident and Japanese workers in particular have employed species of *Hyoscyamus* and *Duboisia* with considerable success.

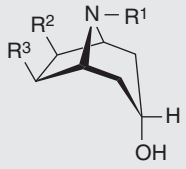
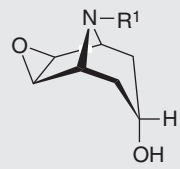
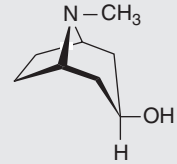
**Tropane moiety.** The available evidence suggests that the formation of the tropane ring system is generally similar for all Solanaceae studied but there are still apparent variations between species, particularly in the stereospecific incorporation of some precursors.

Early work with isotopes indicated that ornithine and acetate were precursors of the tropane nucleus; later, the incorporation of ornithine was shown to be stereospecific. Hygrine can also serve as a precursor of the tropane ring but is not now considered to lie on the principal pathway. The *N*-methyl group of the tropane system can be supplied by methionine and can be incorporated at a very early stage of biosynthesis, as demonstrated by the intact incorporation of *N*-methylornithine into hyoscine and hyoscyamine of *Datura metel* and *D. stramonium*. Early involvement of the *N*-methyl group was reinforced by the isolation in 1981 of naturally occurring δ-*N*-methylornithine from belladonna plants. Also supporting the stereospecificity of the ornithine incorporation was the work of McGaw and Woolley (*Phytochemistry*, 1982, **21**, 2653) which showed that for *D. meteloides* the C-2 of hygrine was specifically incorporated into the C-3 of the tropine moiety of the isolated alkaloid. Putrescine (the symmetrical diamine formed by the decarboxylation of ornithine) and its *N*-methyl derivative also serve as precursors, which, taken in conjunction with the stereospecific incorporation of ornithine, makes it difficult to construct a single pathway for tropane ring formation. A scheme for the biogenesis of the tropane moiety, consistent with the above findings, is shown in Fig. 26.3.

Studies on the enzyme putrescine *N*-methyltransferase in cultured roots of *Hyoscyamus albus* support the role of this enzyme as the first committed enzyme specific to the biosynthesis of tropane alkaloids. (N. Hibi *et al*., *Plant Physiol*., 1992, **100**, 826.)

It will be observed from Fig. 26.3 that the reduction of tropinone yields both tropine (3α-hydroxytropane) and pseudotropine (3β-hydroxytropane). These reductions are brought about by two independent tropinone reductases (EC 1.1.1.236), often referred to as TR-I and TR-II, which accept NADPH as coenzyme. After considerable research involving principally *D. stramonium* root cultures both enzymes were separately purified and characterized. Furthermore, cDNA clones coding for the two separate enzymes TR-I and TR-II have been isolated and shown to involve polypeptides containing 272

**Table 26.2 Examples of ester components of tropane alkaloids of the Solanaceae.**

| | |
|---|---|
| Genera of pharmaceutical interest | *Atropa, Acnistus, Scopolia, Physochlaina, Przewalskia, Hyoscyamus, Physalis, Mandragora, Datura, Solandra, Duboisia, Anthocercis* |
| Tropanol components of esters |  $R^1$ = H or $CH_3$<br>$R^2$ = H or OH<br>$R^3$ = H or OH<br>(Tropine: $R^1 = CH_3$, $R^2 = R^3 = H$) |
| |  Scopine: $R^1 = CH_3$<br>Norscopine: $R^1$ = H<br>(Esterified with tropic or atropic acid only) |
| |  φ-Tropine<br>(Esterified with tiglic acid only) |
| Esterifying acids | Acetic, propionic, isobutyric, isovaleric, 2-methylbutyric, tiglic, nonanoic, tropic, atropic, 2-hydroxy- 3-phenylpropionic, 2,3-dihydroxy-2-phenylpropanoic, *p*-methoxyphenylacetic, anisic |

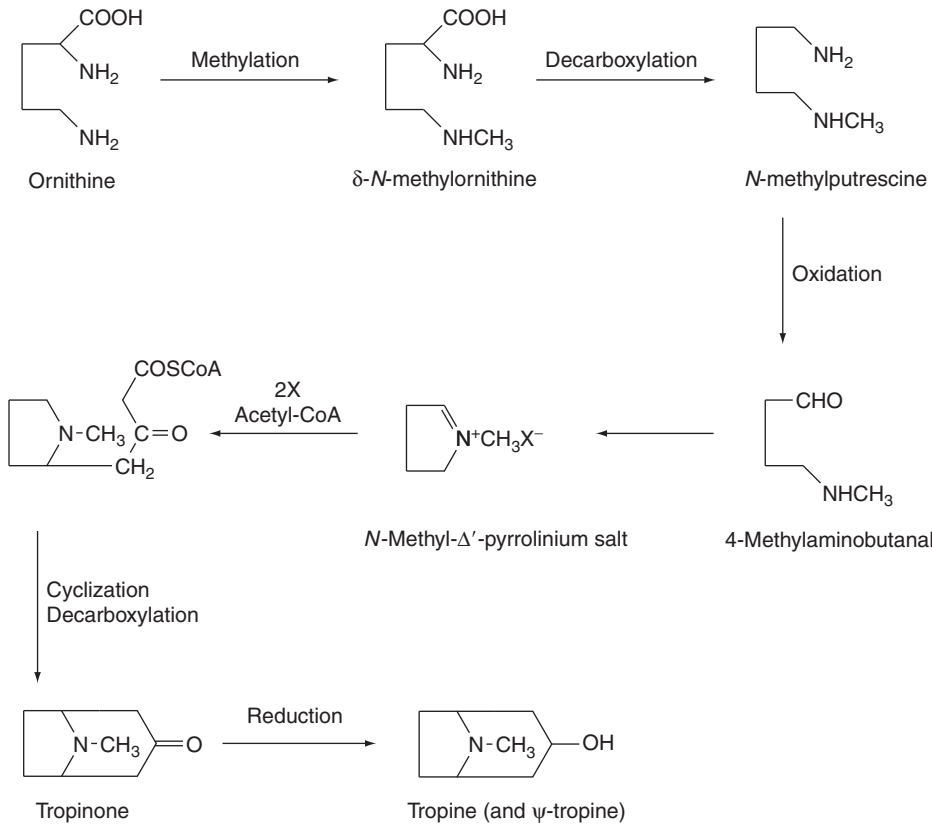



**Fig. 26.3**
Possible biogenetic routes for tropine and pseudotropine (see text for additional comments).

and 260 amino acids respectively. These clones were expressed in *Escherichia coli* and the same reductive specificity demonstrated as for the natural TRs isolated from plant material.

As indicated in Table 26.2 for solanaceous alkaloids, hydroxyls and ester groups are also common at C-6 and C-7 ($R^2$ and $R^3$) of the tropane ring system. Current evidence suggests that hydroxylation of these carbons probably occurs after the C-3 hydroxyl has been esterified.

**Esterification.** The next stage in the biosynthesis of hyoscyamine, the esterification of tropine and tropic acid, has been demonstrated by feeding experiments and isolated enzymes. It was some 40 years ago that Kaçzkowski first recorded the presence of a hyoscyamine esterase in *D. stramonium*; later, Robins *et al*. (*FEBS Lett*., 1991, **292**, 293) demonstrated the involvement of two acetyl-CoA-dependent acyltransferases in the respective formation of 3α- and 3β-acetoxytropanes in *D. stramonium*-transformed root cultures.

**Acid moiety.** The tropic acid fragment of hyoscine and hyoscyamine is derived from phenylalanine, as is the α-hydroxy-β-phenylpropionic acid (phenyllactic acid) of the tropane alkaloid littorine. The specific incorporations obtained with phenylalanine are given in Fig. 26.4. The sequence involved in the rearrangement of the side-chain in the conversion of phenylalanine to tropic acid has been the subject of longstanding debate. Ansarin and Woolley (*Phytochemistry*, 1994, **35**, 935), by feeding phenyl [1,3$^{13}C_2$]lactic acid to *D. stramonium* and examining the $^{13}$C-NMR spectra of the subsequently isolated hyoscine and hyoscyamine, have substantiated the hypothesis that tropic acid is formed by an intramolecular rearrangement of phenyllactate. Furthermore, it has been demonstrated that hyoscyamine is biosynthesized from littorine by a

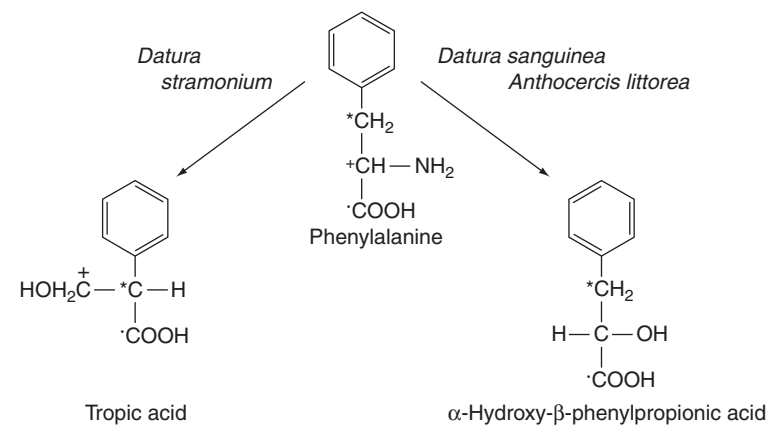



**Fig. 26.4**
Demonstrated incorporations of phenylalanine into the tropic acid and α-hydroxy-β-phenylpropionic acid (phenyllactic acid) moieties of hyoscyamine and littorine, respectively.

process involving the intramolecular rearrangement of the phenyllactate moiety of the alkaloid. In concordance with this, transformed roots of *Datura stramonium* will convert exogenously added littorine to hyoscyamine (35% metabolism recorded) but, in contrast, exogenously added hyoscyamine is not metabolized to littorine.

Isoleucine serves as a precursor of the tigloyl and 2-methylbutanoyl moieties of various mono- and di-esters of the hydroxytropanes.

**Biogenesis of hyoscine (scopolamine).** Work initiated by Romeike in 1962 showed that hyoscine appeared to be formed in the leaves of *D. ferox* from hyoscyamine via 6-hydroxyhyoscyamine and 6,7-dehydrohyoscyamine. The former intermediate has been well substantiated, as indicated below, and occurs in quantity in some other genera (*Scopolia*, *Physochlaina*, *Przewalskia*) but the latter, although incorporated into hyoscine when fed as a precursor to *D. ferox*, has never been isolated from normal plants. Some 25 years later Hashimoto's group, using *Hyoscyamus niger* cultured roots, isolated and partially purified the enzyme responsible for the conversion of hyoscyamine to 6-hydroxyhyoscyamine. They used this enzyme to prepare [6-$^{18}$O]-hydroxyhyoscyamine from hyoscyamine and showed that when the labelled compound, fed to *Duboisia myoporoides*, was converted to hyoscine the $^{18}$O was retained, thus eliminating 6,7-dehydrohyoscyamine from the pathway (Fig. 26.5). For this reaction to proceed the epoxidase enzyme requires 2-oxo-glutarate, ferrous ions and ascorbate as cofactors, together with molecular oxygen.

The elucidation of the above pathway, which has spanned many years, aptly illustrates the value of biotechnology and enzymology in contributing to the resolution of some uncertainties resulting from traditional labelled-precursor experiments.

**Ontogenesis.** In some plants of the Solanaceae (e.g. belladonna and scopolia) hyoscyamine is the dominant alkaloid throughout the life cycle of the plant. In *D. stramonium* hyoscyamine is the principal alkaloid at the time of flowering and after, whereas young plants contain principally hyoscine; in many other species of *Datura* (e.g. *D. ferox*) hyoscine is the principal alkaloid of the leaves at all times. The relative proportions of hyoscine and hyoscyamine in a particular species not only vary with age of the plant, but also are susceptible to other factors, including day length, light intensity, general climatic conditions, chemical sprays, hormones, debudding and chemical races. Isolated organ cultures of belladonna, stramonium and hyoscyamus indicate that the root is the principal site of alkaloid synthesis; however, secondary modifications of the alkaloids may occur in the aerial parts, for example, the epoxidation of hyoscyamine to give hyoscine, and the formation of meteloidine from the corresponding 3,6-ditigloyl ester.

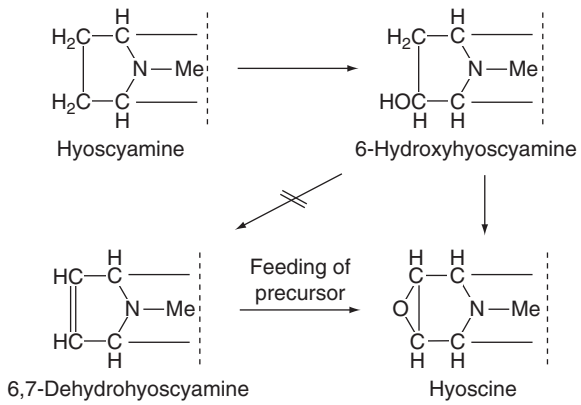



**Fig. 26.5**
Route for the formation of hyoscine from hyoscyamine (partial formulae).

## Further reading

Griffin WJ, Lin GD 2000 Chemotaxonomy and geographical distribution of tropane alkaloids. Phytochemistry 53: 623–637

Lounasmaa M, Tamminen T 1993 Tropane alkaloids. In: Cordell GA (ed) The alkaloids. Chemistry and pharmacology, Vol 44. Academic Press, London. *A review with 484 references listing all known tropane alkaloids*

Robins RJ, Walton NJ 1993 The biosynthesis of tropane alkaloids. In: The alkaloids. Chemistry and pharmacology. *A review with 191 references*

## STRAMONIUM LEAF

Stramonium Leaf *BP*/*EP* (*Thornapple Leaves; Jimson* or *Jamestown Weed*) consists of the dried leaves or dried leaves and flowering tops of *Datura stramonium* L. and its varieties (Solanaceae). The drug is required to contain not less than 0.25% of alkaloids calculated as hyoscyamine. The plant is widespread in both the Old and New Worlds. British supplies are derived mainly from the Continent (Germany, France, Hungary, etc.).

**Plant.** *D. Stramonium* is a bushy annual attaining a height of about 1.5 m and having a whitish root and numerous rootlets. The erect aerial stem shows dichasial branching with leaf adnation. The stem and branches are round, smooth and green. The flowers are solitary, axillary and short-stalked. They have a sweet scent. Each has a tubular, five-toothed calyx about 4.5 cm long, a white, funnel-shaped corolla about 8 cm long, five stamens and a bicarpellary ovary. The plant flowers in the summer and early autumn. The fruit is originally bilocular but as it matures a false septum arises, except near the apex, so that the mature fruit is almost completely four-celled. The ripe fruit is a thorny capsule about 3–4 cm long. Stramonium seeds (see Fig. 41.6) are dark brown or blackish in colour, reniform in outline and about 3 mm long. The testa is reticulated and finely pitted. A coiled embryo is embedded in an oily endosperm.

*D. stramonium* var. *tatula* closely resembles the above; its stems are reddish and the leaves have purplish veins, as also have the lavender-coloured corollas. Varieties of both the above forms occur with spineless capsules.

**History.** Stramonium was grown in England by Gerarde towards the end of the sixteenth century from seeds obtained from Constantinople. The generic name, *Datura*, is derived from the name of the poison, *dhât*, which is prepared from Indian species and was used by the Thugs.

**Macroscopical characters.** Fresh stramonium leaves or herbarium specimens should first be examined, since the commercial leaves are much shrunken and twisted, and their shape can only be ascertained by careful manipulation after soaking them in water.

The dried leaves are greyish-green in colour, thin, brittle, twisted and often broken. Whole leaves are 8–25 cm long and 7–15 cm wide; they are shortly petiolate, ovate or triangular-ovate in shape, are acuminate at the apex and have a sinuate-dentate margin. They are distinguished from the leaves of the Indian species, *D. innoxia*, *D. metel* and *D. fastuosa*, by the margin, which possesses teeth dividing the sinuses, and by the lateral veins which run into the marginal teeth.

The commercial drug contains occasional flowers and young capsules, which have been described above. The stems are often flattened, longitudinally wrinkled, somewhat hairy and vary in colour from light olive brown (*D. stramonium*) to purplish-brown (var. *tatula*). Stramonium has a slight but unpleasant odour, and a bitter taste.

**Microscopical characters.** A transverse section of a leaf (Fig. 26.6) shows that it has a bifacial structure. Both surfaces are covered with a

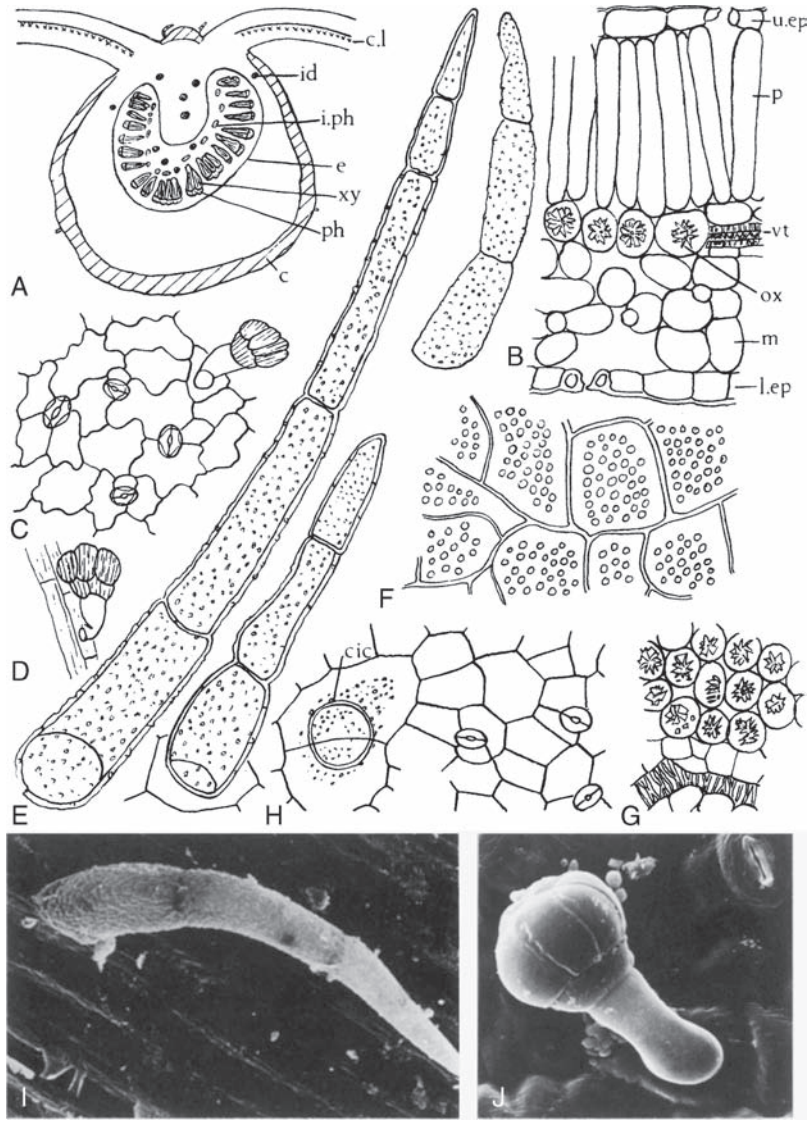



**Fig. 26.6**
*Datura stramonium* leaf. A, Transverse sections of midrib (×15); B, transverse section portion of lamina; C, lower epidermis with stomata and glandular trichome; D, glandular trichome over vein; E, clothing trichomes (all ×200); F, arrangement of calcium oxalate crystals in crystal layer, surface view (×50); G, calcium oxalate crystals in cells; H, upper epidermis showing cicatrix and stomata (G and H, ×200). I, J, Scanning electron micrographs of (I) clothing trichome and (J) glandular trichome. c, Collenchyma; cic, cicatrix; c.l, crystal layer; e, endodermis; id, idioblast containing micro-crystals; i.ph, intraxylary phloem; l.ep, lower epidermis with stoma; m, mesophyll; ox, calcium oxalate crystal; p, palisade layer; ph, phloem; u.ep, upper epidermis with stoma; vt, veinlet; xy, xylem. (Photographs: L. Seed and R. Worsley.)

smooth cuticle and possess both stomata and hairs. Cluster crystals of calcium oxalate are abundant in the mesophyll (Fig. 26.6F, G), and microsphenoidal and prismatic crystals are also found. The stomata are of the anisocytic and anomocytic types. The epidermal cells have wavy walls, particularly those of the lower epidermis. The uniseriate clothing hairs are three- to five-celled, slightly curved, and have thin, warty walls (Fig. 26.6E). The basal cell is usually more than 50 µm long (distinction from *D. metel*). Small glandular hairs with a one- or two-celled pedicel and others with a two-celled pedicel and an oval head of two to seven cells are also found. If portions of the leaf are cleared with chloral hydrate solution, the abundance of the cluster crystals of calcium oxalate and their distribution with regard to the veins may be noted.

The midrib shows a bicollateral structure and characteristic subepidermal masses of collenchyma on both surfaces. The xylem forms a strongly curved arc. Sclerenchyma is absent.

Stems are present, but few of these should exceed 5 mm diameter. They possess epidermal hairs up to 800 µm long and have perimedullary phloem. The stem parenchyma contains calcium oxalate similar to that found in the leaf.

**Constituents.** Stramonium usually contains 0.2–0.45% of alkaloids, the chief of which are hyoscyamine and hyoscine, but a little atropine may be formed from the hyoscyamine by racemization. At the time of collection these alkaloids are usually present in the proportion of about two parts of hyoscyamine to one part of hyoscine, but in young plants hyoscine is the predominant alkaloid. The TLC test for identity given in the *BP*/*EP* enables other *Datura* species containing different proportions of alkaloids to be detected. The larger stems contain little alkaloid and the official drug should contain not more than 3% stem with a diameter exceeding 5 mm. Stramonium seeds contain about 0.2% of

mydriatic alkaloids and about 15–30% of fixed oil. The roots contain, in addition to hyoscine and hyoscyamine, ditigloyl esters of 3,6-dihydroxytropane and 3,6,7-trihydroxytropane, respectively, and a higher proportion of alkamines than the aerial portions. For a recent report on the distribution of alkaloids in different organs and in three varieties of *D. stramonium*, see S. Berkov *et al*., *Fitoterapia*, 2006, **77**, 179. *D. stramonium* cell and root cultures have been considerably utilized in biogenetic studies.

*Prepared Stramonium BP/EP* is the finely powdered drug adjusted to an alkaloid content of 0.23–0.27%.

**Allied species.** All *Datura* species examined to date contain those alkaloids found in stramonium, but frequently hyoscine, rather than hyoscyamine, is the principal alkaloid.

Commercial 'datura leaf' consists of the dried leaves and flowering tops of *D. innoxia* and *D. metel*; it is obtained principally from India. Like those of stramonium, the dried leaves are curled and twisted, but are usually somewhat browner in colour, with entire margins and with differences in venation and trichomes. The leaves contain about 0.5% of alkaloids. Variations in hyoscine and atropine contents in different organs of *D. metel* during development have been studied (S. Afsharypuor *et al*., *Planta Med*., 1995, **61**, 383). Over 30 alkaloids have been characterized from *D. innoxia* by capillary GLC–mass spectrometry. For studies on the anatomy of the leaf of *D. metel*, see V. C. Anozie, *Int. J. Crude Drug Res*., 1986, **24**, 206; and for the isolation of 3α-anisoyloxytropane see S. Siddiqui *et al*., *J. Nat. Prod*., 1986, **49**, 511. 'Datura seeds' are derived from *D. metel* and possibly other species. Each seed is light brown in colour and ear-shaped. They are larger and more flattened than stramonium seeds but resemble the latter in internal structure. The alkaloid content, hyoscine with traces of hyoscyamine and atropine, is about 0.2%. *D. ferox*, a species having very large spines on its capsules, contains as its major alkaloids hyoscine and meteloidine.

The 'tree-daturas' constitute Section Brugmansia of the genus; these arboraceous, perennial species are indigenous to South America and are widely cultivated as ornamentals. They produce large, white or coloured trumpet-shaped flowers and pendant unarmed fruits. Some species constitute a potential source of hyoscine (W. C. Evans, *Pharm. J*., 1990, **244**, 651) and *D. sanguinea*, in particular, has proved a most interesting plant with respect to its wide range of tropane alkaloids and has been cultivated commercially in Ecuador. It yields about 0.8% hyoscine. Plantations have an economically useful life of about 10 years. Chemical races of *D. sanguinea* are evident, particularly one producing relatively large amounts of 6β-acetoxy-3α-tigloyloxytropane. Various tree datura hybrids developed at Nottingham University, UK, have been used by a number of workers for alkaloid studies involving hairy root and root cultures; as an example see P. Nussbaumer *et al*., *Plant Cell Rep*., 1998, **17**, 405.

The South American Indians have long cultivated various races of these plants for medicinal and psychotropic use (for a comparison of native assessment of their potency with alkaloid content, see Bristol *et al*., *Lloydia*, 1969, **32**, 123).

Withanolides (q.v.) have also been recorded in a number of species of the genus; these include various hydroxywithanolides.

**Adulteration.** Adulterants cited are the leaves of species of *Xanthium* (Compositae), *Carthamus* (Compositae) and *Chenopodium* (Chenopodiaceae), which are, however, easily distinguished from the genuine drug.

**Uses.** Atropine has a stimulant action on the central nervous system and depresses the nerve endings to the secretory glands and plain muscle. Hyoscine lacks the central stimulant action of atropine; its sedative properties enable it to be used in the control of motion sickness. Hyoscine hydrobromide is employed in preoperative medication, usually with papaveretum, some 30–60 min before the induction of anaesthesia. Atropine and hyoscine are used to a large extent in ophthalmic practice to dilate the pupil of the eye.

## HYOSCYAMUS LEAF

Hyoscyamus Leaf (*Henbane*) *BP/EP* 2001 consists of the dried leaves or the dried leaves and flowering tops of *Hyoscyamus niger* (Solanaceae). It is required to contain not less than 0.05% of total alkaloids calculated as hyoscyamine. The pharmacopoeial description refers to petiolate as well as sessile leaves, the first-year biennial leaves being thus admitted. Henbane is no longer cultivated commercially in Britain and supplies are imported from central Europe. The plant is also cultivated in the USA.

**Plant.** Henbane is a biennial (var. α-*biennis*) or annual (var. β-*annua*) plant. It is found wild, chiefly near old buildings, both in the UK and in the rest of Europe, and is widely cultivated. Before examining commercial henbane leaves it is advisable to study growing plants or herbarium specimens. The differences tabulated in Table 26.3 should be noted.

*Henbane flowers* have the formula K(5), C(5), A5, G(2). The hairy, five-lobed calyx is persistent. The fruit is a small, two-celled pyxis (see Fig. 41.6B), which contains numerous seeds.

*Henbane seeds* are dark grey in colour, somewhat reniform in shape and about 1.5 mm long. They have a minutely reticulated testa and an internal structure closely resembling that of stramonium seeds. Henbane seeds contain about 0.06–0.10% of alkaloids (hyoscyamine with a little hyoscine and atropine) together with calystegines (nortropane alkaloids). A number of non-alkaloidal components include various lignanamides (C.-Y. Ma *et al*., *J. Nat. Prod.*, 2002, **65**, 206).

**History.** Henbane, probably the Continental *H. albus*, was known to Dioskurides and was used by the ancients. Henbane was used in

**Table 26.3 Comparison of commercial varieties of hyoscyamus.**

| First-year biennial | Second-year biennial | Annual |
|---|---|---|
| Stem very short | Stem branched and up to 1.5 m high | Stem simple and about 0.5 m high |
| Leaves in a rosette near the ground. Ovate-lanceolate and petiolate, up to 30 cm long, the lamina being up to 25 cm long. Hairy | Leaves sessile, ovate-oblong to triangular-ovate. 10–20 cm long. Margin deeply dentate or pinnatifid. Very hairy, especially in the neighbourhood of the midrib and veins | Leaves sessile. Smaller than those of the biennial plant, with a less incised margin and fewer hairs |
| Does not normally flower in the first year | Flowers May or June. Corolla yellowish with deep purple veins | Flowers July or August. Corolla paler in colour and less deeply veined |

England during the Middle Ages. After a period of disuse in the eighteenth century the drug was restored to the *London Pharmacopoeia* of 1809, largely owing to the work of Störck.

**Collection and preparation.** Biennial henbane was the variety traditionally grown in England, but much of the current drug is now of the annual variety or is derived from the allied species *H. albus*. The germination of henbane seeds is slow and often erratic and may often be assisted by special treatments (e.g. concentrated sulphuric acid, gibberellic acid or splitting of the testa). The plant may be attacked by the potato beetle, and spraying with derris or pyrethrum may be necessary.

The annual plant usually flowers in July or August and the biennial in May or June. The leaves should be dried rapidly, preferably by artificial heat at a temperature of about 40–50°C.

**Macroscopical characters.** Commercial henbane consists of the leaves and flowering tops described above. The leaves are more or less broken but are characterized by their greyish-green colour, very broad midrib and great hairiness. If not perfectly dry, they are clammy to the touch, owing to the secretion produced by the glandular hairs. The stems are mostly less than 5 mm diameter and are also very hairy. The flowers are compressed or broken but their yellowish corollas with purple veins are often seen in the drug. Henbane has a characteristic, heavy odour and a bitter, slightly acrid taste.

**Microscopical characters.** A transverse section of a henbane leaf shows a bifacial structure (Fig. 26.7A). Both surfaces have a smooth cuticle, epidermal cells with wavy walls, stomata of both anisocytic and anomocytic types, and a large number of hairs, which are particularly abundant on the midrib and veins. The hairs are up to 500 μm long; some are uniseriate and two to six cells long, while others have a uniseriate stalk and a large, ovoid, glandular head, the cuticle of which is often raised by the secretion (Fig. 26.7E). Similar hairs are found on the stems. The spongy mesophyll contains calcium oxalate, mainly in the form of single and twin prisms, but clusters and microsphenoidal crystals are also present (Fig. 26.7B,D). The broad midrib contains a vascular bundle, distinctly broader than that of stramonium, showing the usual bicollateral arrangement, which is also to be seen in the stems. The mesophyll of the midrib is made up of two thin zones of collenchyma immediately within the epidermi and a ground mass of colourless parenchyma showing large, intercellular air spaces and containing prisms or, occasionally, microsphenoidal crystals of calcium oxalate.

The calyx possesses trichomes and stomata, as in the leaf. The corolla is glabrous on the inner surface but exhibits trichomes on the outer surface, particularly over the veins (Fig. 26.7G). Those cells of the corolla which contain bluish anthocyanins turn red with chloral hydrate solution. Numerous pollen grains are present, about 50 μm diameter, tricolpate with three wide pores and an irregularly, finely pitted exine (Fig. 26.7F). The testa of the seeds has an epidermis with lignified and wavy anticlinal walls, and sclereids are present in the pericarp.

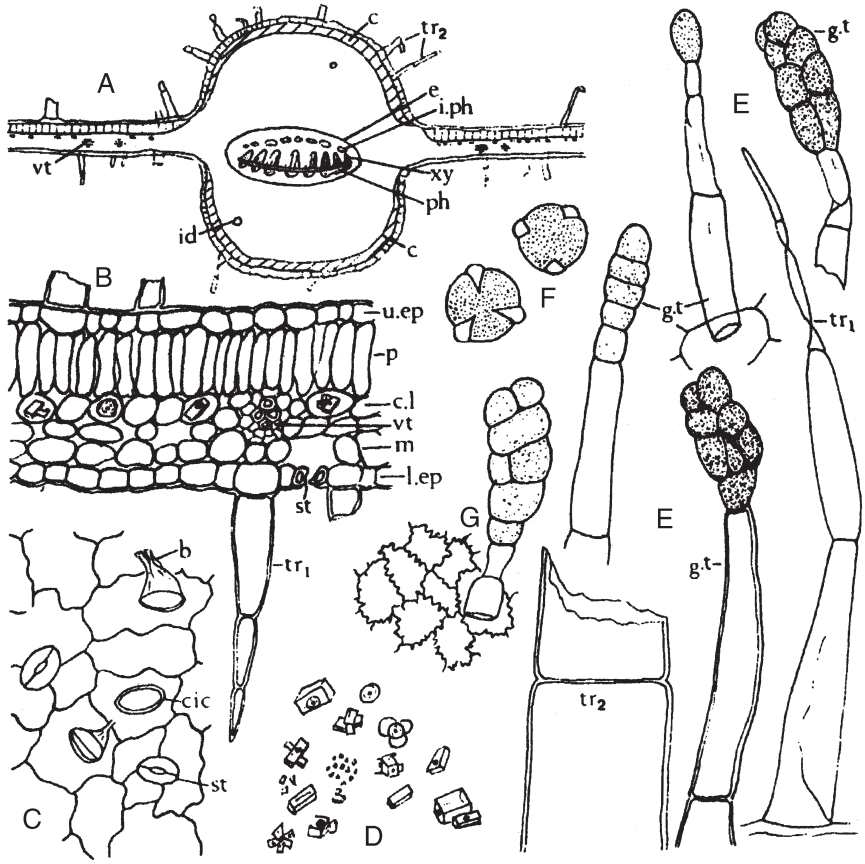



**Fig. 26.7**

*Hyoscyamus niger*. A, Transverse section of midrib of leaf (×40); B, transverse section of portion of leaf lamina; C, portion of leaf upper epidermis, surface view; D, calcium oxalate crystals; E, trichomes; F, pollen grains; G, portion of epidermis of corolla with attached glandular trichome (all ×200). b, Base of trichome; c, collenchyma; cic, cicatrix; c.l, crystal layer; e, endodermis; g.t, glandular trichome or portion of; id, idioblast; i.ph, intraxylary phloem; l.ep, lower epidermis; m, mesophyll; p, palisade layer; ph, phloem; st, stoma; $tr_1$, $tr_2$, whole and broken clothing trichomes, respectively; u.ep, upper epidermis; vt, veinlet; xy, xylem.

**Constituents.** Henbane leaves contain about 0.045–0.14% of alkaloids and yield about 8–12% of acid-insoluble ash (*BP*/*EP* 2001 not more than 12%). Hyoscyamine and hyoscine are the principal alkaloids. The petiole appears to contain more alkaloid than the lamina or stem.

*Prepared Hyoscyamus BP/EP* 2001 is the drug in fine powder adjusted to contain 0.05–0.07% of total alkaloids. It has a 'loss on drying' requirement of not more than 5.0%.

**Allied drugs.** *Hyoscyamus albus* is grown on the Continent, particularly in France, and in the Indian subcontinent. It has petiolate stem-leaves and the flowers have pale yellow, non-veined corollas. Unlike *H. niger*, the fruits are barely swollen at the base. Quantitatively and qualitatively its alkaloids appear similar to those of *H. niger*. It has been used in biogenetic studies (q.v.) and the hairy roots (transformed with *Agrobacterium rhizogenes*) have been analysed for 7β- and 6β-hydroxyhyoscyamine, littorine, hyoscine and hyoscyamine (M. Sauerwein and K. Shimomura, *Phytochemistry*, 1991, **30**, 3277). In traditional medicine of the Tuscan archipelago the seeds are pressed into the cavities of decayed teeth to obtain pain relief (R. E. Uncini Manganelli and P. E. Tomei, *J. Ethnopharmacology*, 1999, **65**, 181).

*Hyoscyamus muticus* is indigenous to India and Upper Egypt; it has been introduced into Algiers. For further details, see below.

*Indian henbane*. Under this name considerable quantities of drug were imported into Britain during World War II. Although *H. niger* is grown in India and Pakistan, much of the drug came from a closely related plant, *H. reticulatus*. This contains hyoscine and hyoscyamine and microscopically it is almost identical to *H. niger*.

*Hyoscyamus aureus* and *H. pusillus* are two species which produced hyoscine as the principal alkaloid.

**Uses.** Henbane resembles belladonna and stramonium in action but is somewhat weaker. The higher relative proportion of hyoscine in the alkaloid mixture makes it less likely to give rise to cerebral excitement than does belladonna. It is often used to relieve spasm of the urinary tract and with strong purgatives to prevent griping.

**Hyoscyamus for Homoeopathic Preparations.** This is described in the *BP*/*EP* Vol. III and consists of the whole, fresh, flowering plant of *Hyoscyamus niger* L. There is a limit for foreign matter (max. 5%) and a *minimal* loss on drying at 100–105°C for 2 h of 50%. An assay is described for the Mother Tincture.

### Egyptian henbane

Egyptian henbane consists of the dried leaves and flowering tops of *Hyoscyamus muticus* (Solanaceae). The plant is a perennial about 30–60 cm in height. It is indigenous to desert regions in Egypt, Arabia, Iran, Baluchistan, Sind, western Punjab, and has been introduced into Algiers and is cultivated in southern California. In Egypt it is collected from wild plants by Arab shepherds.

**Macroscopical characters.** The drug consists of leaves, stems, flowers and fruits. The leaves are usually matted and form a lower proportion of the drug than in the case of European henbane. The leaves are pubescent, pale green to yellowish, rhomboidal or broadly elliptical and up to about 15 cm long. Midrib broad, venation pinnate, margin entire or with about five large teeth on each side. Petiole almost absent or up to 9 cm long. The stems are greyish-yellow, striated, slightly hairy and hollow. The flowers are shortly stalked, with large hairy bracts, a tubular five-toothed calyx and a yellowish-brown corolla which in the dry drug may show deep purple patches. The fruit is a cylindrical pyxidium surrounded by a persistent calyx and containing numerous yellowish-grey to brown seeds. Odour, slightly foetid; taste, bitter and acrid.

**Microscopical characters.** Egyptian henbane is easily distinguished from *H. niger* by the numerous branched and unbranched glandular trichomes, which have a one- to four-celled stalk and unicellular heads. Additional characters are the striated cuticle, the prisms of oxalate 45–110 μm, twin prisms and occasional clusters and microsphenoids.

**Constituents.** Ahmed and Fahmy found about 1.7% of alkaloids in the leaves, 0.5% in the stems and 2.0% in the flowers. The chief alkaloid is hyoscyamine for the isolation of which (as atropine) the plant is principally used. The alkaloidal mixture of plants grown in Afghanistan had the following composition: hyoscyamine 75%, apoatropine 15%, hyoscine 5%, with smaller quantities of noratropine and norhyoscine. A number of non-alkaloidal ketones, an acid and sitosterol have been characterized from plants raised in Lucknow, India. The formation of alkaloids in suspension cell cultures has been widely investigated with variable results; with callus cultures the addition of phenylalanine to the medium produced maximum alkaloid production (3.97%) whereas isoleucine gave the greatest growth (M. K. El-Bahr *et al*., *Fitoterapia*, 1997, **68**, 423).

## BELLADONNA LEAF

Belladonna Leaf *BP*/*EP* (*Belladonna Herb*) consists of the dried leaves and, occasionally fruit-bearing flowering tops of *Atropa belladonna* L. (Solanaceae); it contains not less than 0.30% of total alkaloids calculated as hyoscyamine. Traditionally the *BP* drug consisted of all the aerial parts (Belladonna Herb) but under the European requirements there is a limit (3%) of stem with a diameter exceeding 5 mm. The *USP*, which requires 0.35% alkaloid, also admits *A. acuminata* (see below) in the Belladonna Leaf monograph.

*A. belladonna* is cultivated in Europe and the USA.

**Plant.** The deadly nightshade, *A. belladonna*, is a perennial herb which attains a height of about 1.5 m. Owing to adnation, the leaves on the upper branches are in pairs, a large leaf and a smaller one.

The flowers appear about the beginning of June. They are solitary, shortly stalked, drooping and about 2.5 cm long. The corolla is campanulate, five-lobed and of a dull purplish colour. The five-lobed calyx is persistent, remaining attached to the purplish-black berry. The latter is bilocular, contains numerous seeds and is about the size of a cherry (see Fig. 41.6C). In the USA the plant is often known as the 'Poison Black Cherry', while the German name is 'Tollkirschen' (i.e. Mad Cherry). A yellow variety of the plant lacks the anthocyanin pigmentation; the leaves and stems are a yellowish-green and the flowers and berries yellow.

**History.** Belladonna was probably known to the ancients but it is not clearly recorded until the beginning of the sixteenth century. The leaves were introduced into the *London Pharmacopoeia* of 1809, but the root was not used in Britain until a liniment prepared from it was introduced by Squire in 1860.

**Cultivation, collection and preparation.** Belladonna is grown from seed. The leaves are said to be richest in alkaloid at the end of June or in July, and a sunny position is said to give more active leaves than a shady one. Plants about 3 years old are sufficiently large to give a good yield of leaves and, if the roots are being collected, it would seem to be best to replant about every third year (see also 'Belladonna Root'). Two or more crops of leaves may be collected annually. Leaves left in an imperfectly dry state deteriorate and give off ammonia. They should

therefore be dried immediately after collection and be carefully stored. Leaves of a good colour may be obtained by drying in thin layers starting with a moderate heat which is gradually increased to about 60°C and then gradually decreased. Sometimes the leaves are badly attacked by insects and the roots by a fungus.

**Macroscopical characters.** The drug consists of leaves and the smaller stems, the latter seldom exceeding 5 mm diameter, together with flowers and fruits as described above. If the drug is little broken, the arrangement of the leaves in unequal pairs may be seen. The leaves are dull green or yellowish-green in colour, the upper side being somewhat darker than the lower. Each has a petiole about 0.5–4 cm long and a broadly ovate, slightly decurrent lamina about 5–25 cm long and 2.5–12 cm wide. The margin is entire and the apex acuminate. A few flowers and fruits may be found. If the leaves are broken, the most useful diagnostic characters are the venation and roughness of the surface. The latter is due to the presence of calcium oxalate in certain of the mesophyll cells which causes minute points on the surface of the leaf as the other cells contract more on drying.

**Microscopical characters.** A transverse section of the leaf of *A. belladonna* is shown in Fig. 26.8A. It has a bifacial structure. The epidermal cells have wavy walls and a striated cuticle (Fig. 26.8E). Stomata of the characteristic anisocytic type and also some of the anomocytic type are present on both surfaces but are most common on the lower. Hairs are most numerous on young leaves. Some of the hairs are uniseriate, two- to four-celled clothing hairs; others resemble these but have a unicellular glandular head; while a third kind has a short pedicel

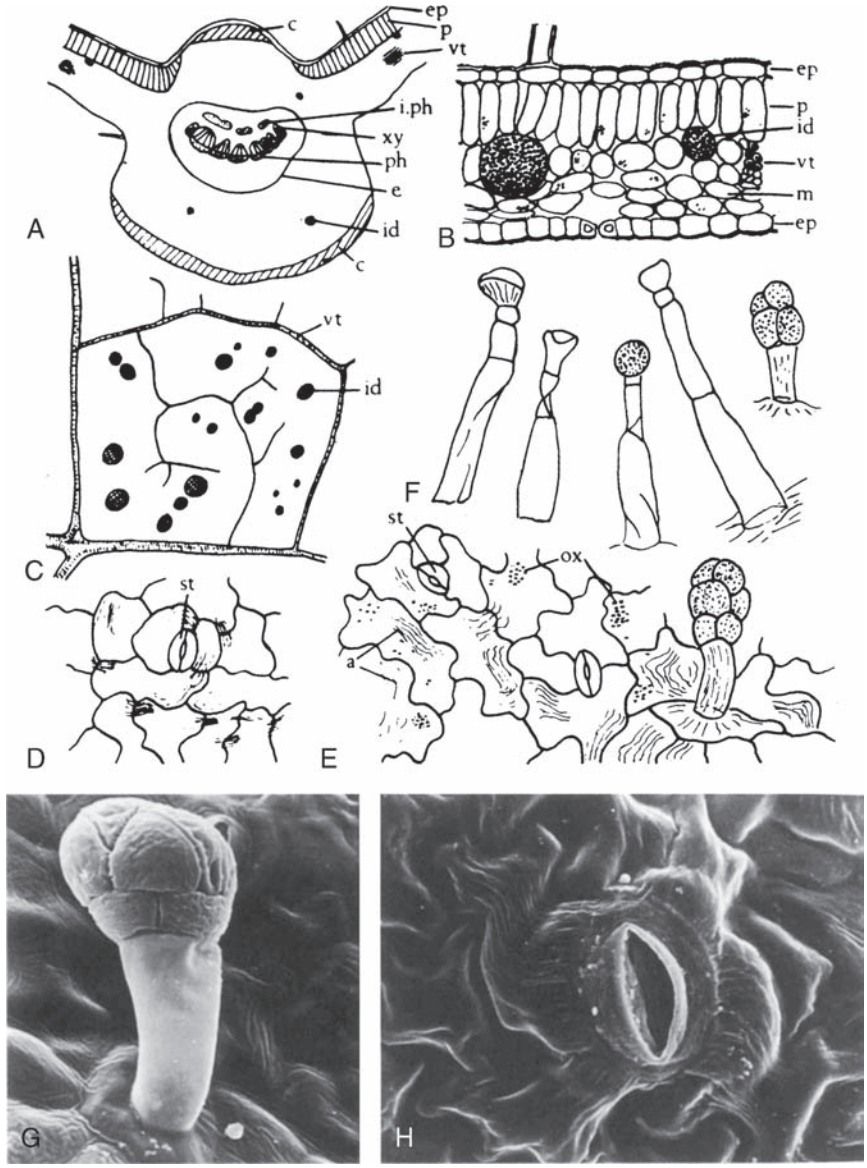



**Fig. 26.8**
*Atropa belladonna* leaf. A, Transverse section of midrib (×40); B, transverse section of portion of lamina (×200); C, distribution of idioblasts, surface view of leaf cleared in chloral (×50); D, upper epidermis; E, lower epidermis; F, trichomes (all ×200); G and H, Scanning electron micrographs (G) of glandular trichome and epidermal cells with striated cuticle and (H) stoma and striated cuticle. a, Striations of cuticle; c, collenchyma; e, endodermis; ep, epidermis; id, idioblast containing crystals of calcium oxalate; i.ph, intraxylary phloem; m, mesophyll; ox, calcium oxalate crystals; p, palisade layer; ph, phloem; st, stoma; vt, veinlet; xy, xylem. (Photographs, L. Seed and R. Worsley.)

and a multicellular glandular head (Fig. 26.8F). Certain of the cells of the spongy mesophyll are filled with microsphenoidal ('sandy') crystals of calcium oxalate (Fig. 26.8B, C). The midrib is convex above and shows the usual bicollateral vascular bundle. A zone of collenchyma underlies both epidermi in the region of the midrib.

**Constituents.** The drug from *A. belladonna* contains 0.3–0.60% of alkaloids, the chief of which is hyoscyamine. Small quantities of volatile bases, such as pyridine and *N*-methylpyrroline, are present, and if not removed during the assay of the drug by heating, increase the titration and appear in the result as hyoscyamine. The leaves also contain a fluorescent substance, β-methylaesculetin (scopoletin), and calcium oxalate. They yield about 14% of ash and not more than 4% of acid-insoluble ash.

*Prepared Belladonna Herb* is the finely powdered drug adjusted to contain 0.28–0.32% of total alkaloids. Note the 'loss on drying' requirement.

**Allied drugs.** *Indian belladonna* from *A. acuminata* Royle ex Lindley differs from that derived from *A. belladonna* in that its flowers are yellowish-brown and its leaves brownish-green, oblong-elliptical and tapering towards both base and apex. It grows wild in the Himalayan regions of northern India (1800–3400 m) and is cultivated in the Kashmir valley.

*Atropa baetica* Willk. is a species native to southern Spain and northern Morocco; it produces yellow flowers and black berries and is regarded as an endangered species. R. Zárate *et al*. have described a rapid *in vitro* propagation method for the plant, and from hairy root cultures have isolated tigloylpseudotropine—alkaloid not found in the mature plant (*Plant Cell Rep*., 1999, **18**, 418).

**Adulterants.** Of the numerous recorded adulterants of belladonna leaves, those of *Phytolacca decandra* (Phytolaccaceae) and *Ailanthus glandulosa* (Simaroubaceae) are perhaps the most important. In *Phytolacca* the lamina is denser and less decurrent than in belladonna; the epidermal cells have straight walls, the stomata are of the anomocytic type and some of the mesophyll cells contain bundles of needle-shaped crystals of calcium oxalate. *Ailanthus* leaves are triangular-ovate, have straight-walled epidermal cells showing a strongly striated cuticle, cluster crystals of calcium oxalate, and on both surfaces white, unicellular clothing hairs which are lignified (Fig. 42.3F).

**Uses.** Belladonna leaves are mainly used for internal preparations which are used as sedatives and to check secretion. Preparations of the root are mainly used externally.

### Belladonna root

Belladonna root consists of the dried roots or rootstock and roots of *Atropa belladonna* (Solanaceae).

**Collection and preparation.** Much of the *A. belladonna* drug is of small size and poor quality. The first-year roots are not profitable to collect from the commercial point of view, although they contain a high proportion of alkaloids. The autumn of the third year would seem to be a suitable time for collection. The roots are dug up, washed, sliced and dried.

**Macroscopical characters.** *Atropa belladonna* rapidly develops a large branching root. The aerial stems die back each year and new ones arise independently from the large crown. Dried roots of 3-year-old plants are about 3 cm diameter and roots over 4 cm diameter are exceptional. Most commercial drug is about half this thickness.

The drug is usually cut into short lengths, which are sometimes split longitudinally. The outer surface is a pale greyish-brown. The root breaks with a short fracture and then shows a whitish or, if overheated during drying, brownish interior. A yellowish-green colour in the region of the cambium is often seen.

A transverse section of the bark is non-fibrous and the wood does not show a radiate appearance. The wood consists of scattered groups of vessels, tracheids and fibres which are most abundant near the cambium; there is a central mass of primary xylem (Fig. 41.8G). The extensive parenchyma of bark and wood contains sandy crystals of calcium oxalate and abundant simple and compound starch grains.

The structure gradually changes as the roots pass into rhizome, the wood becoming denser and exhibiting a distinctly radiate structure; the rhizome also shows a distinct pith and internal phloem. The aerial stems found on the upper surface of the crown are hollow.

**Constituents.** *Atropa belladonna* root contains about 0.4–0.8% of alkaloids calculated as hyoscyamine.

Samples of belladonna root examined by Kuhn and Schäfer showed 0.3–1.0% of alkaloids, of which 82.8–97.3% was hyoscyamine, 2.7–15.2% atropine, and 0.0–2.6% scopolamine. Capillary GLC–mass spectrometry data revealed the presence of hygrine, hygroline, cuscohygrine, tropinone, tropine, pseudotropine and nine tropanol esters (F. Oprach *et al*., *Planta Med*., 1986, 513). Other constituents previously reported include belladonnine together with β-methylaesculetin, calcium oxalate and starch.

A pseudotropine-forming, tropinone reductase (see biogenesis of tropane alkaloids), not entirely similar in chemical and catalytic properties to other samples of the enzyme previously described, has been isolated from transformed belladonna root cultures. The pseudotropine could have implications for the formation of calystegines (q.v). Littorine has been detected in both non-transformed and hairy root-cultures (F. Nakanishi *et al*., *Plant Cell Rep*., 1998, **18**, 249).

**Allied drug.** *Indian belladonna root* from *Atropa acuminata* (see under 'Belladonna Leaf') consists of brownish-grey roots, stolons, rootstock and stem bases. It has been described in detail by Melville. The roots are cylindrical, longitudinally wrinkled, occasionally branched, and 0.5–3 cm diameter. Young roots resemble those of *A. belladonna* but older ones show concentric zonation of the secondary xylem. The rootstock is 3–9 cm diameter at the top and bears the bases of 4–12 aerial stems. The rootstock, stem bases and stolons all possess a pith which becomes hollow in the stem bases. The constituents are similar to those of European belladonna.

**Adulterant.** The root of *Phytolacca decandra* (Phytolaccaceae) is sometimes sliced and mixed with samples of belladonna. It bears little resemblance to belladonna root, but a casual and inexperienced observer might perhaps mistake it for pieces of an old belladonna crown. The transverse section shows a number of concentric cambia, each producing a ring of wood bundles. The parenchyma contains abundant acicular crystals of calcium oxalate.

## DUBOISIA LEAVES

Three species of *Duboisia* are indigenous to Australia and two of these, *D. myoporoides* and *D. leichhardtii*, have for over 55 years been a major world source of tropane alkaloids, particularly hyoscine. The third species, *D. hopwoodii*, contains principally nicotine and related alkaloids and was used by the Australian aborigines for the preparation of 'pituri' by mixing powdered leaves with an alkaline wood ash to form a quid which was held in the cheek pouch.

*D. myoporoides*, discovered by Robert Brown, naturalist to the Flinders expedition of 1802, occurs along the east coast of Australia, where the rainfall exceeds a monthly mean of 5 cm for 11 months of the year and where frosts rarely occur. *D. leichhardtii* was described by Mueller in 1877 and is named after the explorer Ludwig Leichhardt, who originally collected the plant; it occurs naturally in a limited area of south-east Queensland known locally as the South West Burnett. *D. hopwoodii* is of wide distribution in Western and Central Australia.

Of the two tropane alkaloid-containing species *D. myoporoides* is the larger and more densely leaved; both, however, are bushy trees and have the advantage that in one year repeated harvests can often be taken from the same plants. For collection, the small branches are removed, tied in bundles and stood in sheds to dry; the leaves are then easily removed by beating.

In addition to hyoscine and hyoscyamine, minor alkaloids occur in variable amounts and include norhyoscyamine, 6β- hydroxyhyoscyamine, valeroidine, tigloidine, poroidine, isoporoidine, valtropine, 3α-tigloyloxytropane, 3α-acetoxytropane, 3α-nonanoyloxytropane, butropine and apohyoscine. Two discopine esters were identified in 1980 in *D. leichhardtii* and the greenhouse leaves have yielded calystegines $B_1$, $B_2$, $B_4$, $C_1$, and $C_2$ (A. Kato *et al*., *Phytochemistry*, 1997, **45**, 425). Other constituents include the triterpenoids ursolic acid and betulonic acid and a number of recently reported aliphatic constituents.

A number of chemical races occur, particularly in *D. myoporoides*, and include the well-established 'northern' and 'southern' races which differ in their relative contents of hyoscine and hyoscyamine, and a race which contains nicotine and anabasine as principal bases.

For a number of years, growers have also been cultivating a hybrid of the two species, the origin of which is doubtful, but which Griffin considers may derive from the experimental work of the CSIRO carried out in the early 1950s. Established plantations of the hybrid exhibit no morphological differences and propagation is carried out vegetatively. In a series of experiments on the hybrid, Griffin and Luanratana (*J. Nat. Prod*., 1980, **43**, 552; 1982, **45**, 270) have shown that the total alkaloid content of the leaves does not vary throughout the year but there is a decrease in hyoscine content from January to June (summer to autumn) and a gradual increase from June to September; the reverse is true for hyoscyamine. Repeated sprayings of plants with cytokinin solution (which also has a beneficial effect on plant growth), in the form of a sea-weed extract, prevented the hyoscine decline. Such treatment of plants could possibly enhance the hyoscine yield from all-year harvesting. There is evidence (Y. Kitamura *et al*., *Phytochemistry*, 1996, **42**, 1331) that in the plant the tropic acid moiety of atropine may be recycled.

Addition of putrescine and spermidine to the culture medium of *D. myoporoides* root cultures has been shown to increase the hyoscine content.

Most of the Australian crop (some 1200 tonnes) is exported to West Germany, Switzerland and Japan for processing. Plantations have also been established in Ecuador.

### Scopolia

All species of *Scopolia* investigated appear to contain tropane alkaloids similar to those found in belladonna (q.v.). Although little used in western Europe, these plants constitute a useful source of hyoscyamine and galenicals in regions where the plant is available locally. *Scopolia carniolica* is a central and eastern European species somewhat smaller than belladonna. In shape the leaves resemble those of belladonna, although they are more lanceolate and translucent. The cuticle is striated but less markedly so than in belladonna, sandy crystals are less numerous, hairs are rare or absent, and stomata are present on the lower surface only. The fruit, a pyxis, may often be found in the drug. The rhizomes (*BPC* 1934), which are nearly black in colour and bear numerous depressed stem scars, are used similarly to belladonna root. In addition to hyoscyamine and hyoscine, other alkaloids reported in this species are cuscohygrine, 3α-tigloyloxytropane, pseudotropine and tropine. *S. caucasia*, *S. lurida* and *S. tangutica* all appear to be suitable as sources of hyoscyamine; the last two also contain 6-hydroxyhyoscyamine and an alkaloid named daturamine (anisodine) which is a 'hydroxyhyoscine'. Both these alkaloids are produced commercially in China. The dried rhizomes of *S. japonica* ('Japanese Belladonna Root') were official in the *Japanese Pharmacopoeia* 1961; the isolation of steroidal glycosides (scopolosides) has been reported from this species (S. Okamura *et al*., *Chem. Pharm. Bull*., 1992, **40**, 2981).

*Przewalskia tangutica* is a related tropane alkaloid-containing plant and is used in Tibetan traditional medicine. The roots have a high content of hyoscyamine with total alkaloids amounting to 1.7–3.8%; 6β-hydroxyhyoscyamine and small amounts of hyoscine are also present.

### Mandrake

The true mandrake, *Mandragora officinarum*, is one of several Mediterranean species. It was well known to Dioskurides (see R. T. Gunther's English edition of *The Greek Herbal of Dioscorides*, 1934, Oxford, UK: OUP). B. P. Jackson, in an investigation of the botanical source of the drug, found that the species *M. autumnalis* is also involved. The leaves and roots were official in France (1818–1883) and in Spain. The roots occur in fusiform or two-branched pieces and their microscopical structure and distinction from belladonna root has been described by Berry and Jackson (*Planta Med*., 1976, **30**, 281). The plant is surrounded with much folklore and superstition and even the collection of the root was formerly accompanied by special rites. The drug, like belladonna, has long been known to contain atropine and the fluorescent substance scopoletin. Recent investigations have established the presence of several other solanaceous alkaloids.

For a review of the isolated constituents of *Mandragora* spp., including alkaloids, volatile compounds, lipids and related compounds, coumarins and pigments (78 refs), see L. O. Hanus *et al*., *Phytochemistry*, 2005, **66**, 2408.

## COCA LEAF AND COCAINE

Coca leaves are derived from two cultivated shrubs of the Erythroxylaceae, namely *Erythroxylum coca* Lam. and *E. novogranatense* (Morris) Hieron. Each comprises two subspecies, as indicated below.

**History.** Coca leaves have been used in South America as a masticatory from very early times. They were formerly reserved for the sole use of the native chiefs and Incas. Coca was introduced into Europe about 1688 and cocaine was isolated in 1860. By employing the alkaloid in ophthalmic surgery in 1884 Carl Koller was the first to introduce it into clinical practice so heralding the era of modern anaesthetics. For reviews covering both the historical and other aspects of coca see the special issue of *The Journal of Ethnopharmacology* (1981), Vol. 3: *Coca and Cocaine*.

**Cultivation and collection.** These differ depending on geographical source. For Andean coca, plants are raised from seed and cultivated at an altitude of 500–2000 m. Pruning limits the height to about 2 m and traditionally three harvests are collected annually, the first from the pruned twigs, the second in June and the third in November. The leaves are artificially or sun dried and packed into bags. On the other hand, in the Amazon, plants are raised from cuttings, often in jungle clearings and interplanted between other staple crops.

**Varieties and characters:**

1. *Huanco* or *Bolivian coca* consists of the leaves of *E. coca* var. Lam; it is produced as described above on the eastern slopes of the Andes in Bolivia and Peru. The leaves are shortly petiolate, oval, 2.5–7.5 cm long and 1.5–4 cm wide. The lamina is greenish brown to brown and glabrous; margin entire. The midrib is prominent on the lower surface, bears a ridge on its upper surface, and projects slightly beyond the lamina as an apiculus. The latter is often broken in the commercial drug but the leaves are otherwise fairly entire. The lower surface shows two, very characteristic, curved lines, one on either side of the midrib. Odour, characteristic; taste, at first bitter and slightly aromatic, the alkaloids afterwards causing numbness of the tongue and lips.
2. The subspecies *E. coca* var. *ipadu* Plowman is an Amazonian coca cultivated sparingly in the western area of the Amazon basin. The leaves are broadly elliptic and rounded at the base; on the lower surface the characteristic 'parallel lines' of *E. coca* var. *coca* are often indistinct or lacking and the cocaine content is consistently lower.
3. *Columbian coca* comprises the leaves of *E. novogranatense* var. *novogranatense*; it has been cultivated throughout the mountains of present Columbia and Venezuela since pre-Columbian times. It thrives at lower altitudes and in hotter drier climates than does *E. coca*; this variety was widely planted in the Old World tropics, especially in the former British colonies, as an ornamental plant and minor source of cocaine.
4. *Truxillo, Trujillo* or *Peruvian coca*, derived from *E. novogranatense* var. *truxillense*, is the well-known commercial variety of the drug. It is well adapted to the desert conditions of N. Peru and was cultivated for 'coca chewing' and the manufacture of coca-based soft drinks (originally containing cocaine but now legally devoid of the alkaloid). Leaves of a Truxillo-type coca were formerly exported from Indonesia for the manufacture of cocaine (*Javanese coca* leaves). The leaves are pale green in colour, are more papery in texture than the Huanuco and are usually broken. Lamina about 1.6–5 cm long; lines on the lower surface usually indistinct. Flowers of a species of *Inga* (Leguminosae, subfamily Mimosoideae) are sometimes added to the leaves.

**Microscopical characters.** A transverse section of a coca leaf shows upper epidermis, palisade parenchyma containing prisms of calcium oxalate, spongy parenchyma and a very characteristic lower papillose epidermis with numerous stomata. The midrib is partly surrounded by an arc of pericyclic fibres, above and below which is a considerable amount of collenchyma. A surface preparation of the lower epidermis shows the papillae as well-marked circles, and numerous stomata (see Fig. 42.2J), each with four subsidiary cells, two of which have their long axes parallel to the pore.

**DNA analysis.** For a report on the differentiation of the cocaine-producing species and varieties of *Erythroxylum* using AFLP DNA analysis, see E. L. Johnson *et al*., *Phytochemistry*, 2003, **64**, 187; 132 *Erythroxylum* samples were examined.

**Constituents.** Coca leaves contain about 0.7–1.5% of total alkaloids, of which cocaine, cinnamylcocaine and α-truxilline are the most important (Fig. 26.9). They occur in different proportions in different commercial varieties. Javanese leaves are usually richest in total alkaloids, of which the chief is cinnamylcocaine, while the Bolivian and Peruvian leaves contain less total alkaloid but a higher proportion of cocaine. Other substances isolated from various varieties of the leaves are hygrine, hygroline, cuscohygrine, dihydrocuscohygrine, tropacocaine (3β-benzoyloxytropane), crystalline glycosides and cocatannic acid. 1-Hydroxytropacocaine (free hydroxyl situated at a bridgehead carbon) has been isolated as a major alkaloid of greenhouse-cultivated *E. novogranatense* var. *novogranatense*; much lower amounts were detected in var. *truxillense* and in field cultivated coca from Colombia and Bolivia (J. M. Moore *et al*., *Phytochemistry*, 1994, **36**, 357).

The leaves also contain essential oil and as early as 1894 Van Romburgh identified methyl salicylate as a component; this was confirmed (13.6%) in a recent study, together with *N*-methylpyrrole (3.7%) and possibly *N*,*N*-dimethylbenzylamine (0.5%) and two dihydrobenzaldehydes (38.9%). The grassy odour of the leaves is explained to a large extent by the presence in the oil of *trans*-2-hexenal (10.4%) and *cis*-3-hexen-1-ol (16.1%); no mono- or sesquiterpenes were detected (M. Novák *et al*., *Planta Med*., 1987, **53**, 113).

Although it had been generally assumed that ecgonine, the basic moiety of the cocaines, was ornithine-derived (Fig. 26.2), the practical demonstration of the incorporation of the usual precursors proved difficult. Then Leete (*J. Am. Chem. Soc*., 1982, **104**, 1403) obtained a significant level of radioactivity in cocaine isolated from *Erythroxylum coca*, the leaves of which were painted with an aqueous solution of DL-[5-$^{14}$C]ornithine HCl. The pathway to ecgonine appears to be similar to that for tropine except that the carboxyl is retained and the different stereospecificities need to be accommodated. The benzoyl moiety of cocaine is derived from phenylalanine. For work on the incorporation of labelled 1-methyl-$\Delta^1$-pyrrolinium chloride into cuscohygrine, indicating the alkaloid to be a mixture of its *meso* and optically active diastereomers, see E. Leete *et al*., *Phytochemistry*, 1988, **27**, 401.

**Manufacture of cocaine.** The crude alkaloids may be extracted with dilute sulphuric acid or by treatment with lime and petroleum or other organic solvents. Non-alkaloidal matter is roughly separated by

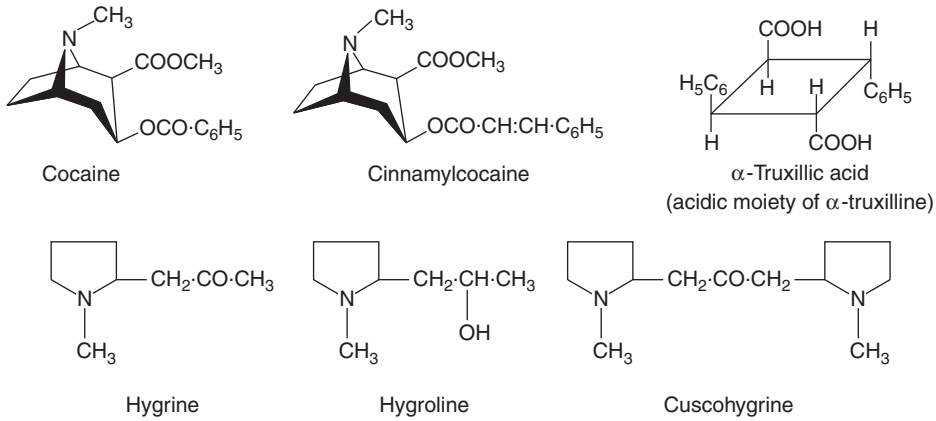



**Fig. 26.9**
Constituents of coca.

transferring the alkaloids from one solvent to another. The crude alkaloids are obtained in solid form either as free bases by precipitation with alkali, or as hydrochlorides by concentrating an acidified solution.

Pure cocaine is prepared from the leaves, the crude bases or the crude hydrochlorides. The process depends on the fact that cocaine, cinnamylcocaine and α-truxilline are closely related derivatives of ecgonine (Fig. 26.2), which is produced by hydrolysing them with boiling dilute hydrochloric acid.

Cocaine → ecgonine + methyl alcohol + benzoic acid
Cinnamylcocaine → ecgonine + methyl alcohol + cinnamic acid
α-Truxilline (1 mol) → ecgonine (2 mols) + methyl alcohol (2 mols) + α-truxillic acid (1 mol)

The ecgonine hydrochloride is purified and converted into the free base. This is benzoylated by interaction with benzoic anhydride and the benzoylecgonine purified. The benzoylecgonine is methylated with methyl iodide and sodium methoxide in methyl alcohol solution, to give methylbenzoylecgonine or cocaine. The latter is converted into the hydrochloride and purified by recrystallization.

Much illicit cocaine is extracted locally in South America and despite the unsophisticated methods employed a high degree of purity can be attained.

In view of the importance of quantitatively determining cocaine and its metabolite, benzoylecgonine, in body fluids, etc., many assays are available for these alkaloids.

**Allied species.** There are over 200 species of *Erythroxylum* found throughout the tropical and pantropical regions of the world. Few of the non-cocaine-producing species have been systematically examined but the majority of those that have contain a range of tropane alkaloids (W. C. Evans, *J. Ethnopharmacol*., 1981, **3**, 265 and for Pt. 12 of a further series of papers see P. Christen *et al*., *Phytochemistry*, 1995, **38**, 1053). Subsequently, other workers have isolated a number of the same alkaloids from other species, together with the characterization of new tropane alkaloids, some named pervilleines and others catuabines. Nortropanols (calystegins, see below) are present in some species. Trimethoxybenzoic acid and trimethoxycinnamic acid commonly occur as esterifying acids. D. Bieri *et al.* (*J. Ethnopharmacology*, 2006, **103**, 439) have studied the cocaine distribution in 51 species of *Erythroxylum* from S. America, 28 of which had received no previous phytochemical examination; cocaine was reported for the first time in 14 species, with *E. laetevirens* having the highest cocaine content of the wild species. It is of interest to note that in this study, the time between collection and analysis of the samples varied from 20 to 25 years.

**Uses.** Cocaine and its salts were the earliest of the modern local anaesthetics but, because of their toxic and addictive properties, their use is now almost entirely confined to ophthalmic, ear, nose and throat surgery.

### Calystegines

These relatively new alkaloids are trihydroxy-, tetrahydroxy- or pentahydroxy derivatives of nortropane. They were originally isolated from the roots of the bindweed *Calystegia sepium* and given the names calystegine $A_3$ (a 1,2,3-trihydroxynortropane) (Fig. 26.2) and calystegine $B_2$ (a 1,2,3,4-tetrahydroxynortropane). By 1998 the structures of nine such alkaloids had been elucidated including the 3-*O*-β-D-glucopyranoside of calystegine $B_1$. In chemotaxonomic studies of the convolvulaceae involving GC-MS analyses, T. Schimming *et al.* (*Phytochemistry*, 1998, **49**, 1989; 2005, **66**, 469) record the occurrence of 11 calystegines and the calystegine patterns in 135 species.

Calystegines have also been reported in the Solanaceae including belladonna and hyoscyamus root cultures. R. J. Nash *et al*., (*Phytochemistry*, 1993, **34**, 1281) found these compounds to be present in the tubers and leaves of potato plants and that these alkaloids can be isolated from certain moths and butterflies, the larvae of which feed on the plant. Other sources are the Brassicaceae, Erythroxylaceae (see 'Coca' above) and the Moraceae.

The formation of calystegines in root cultures of *Calystegium sepium* involves tropinone and pseudotropine as metabolic intermediates (Y. Scholl *et al*., *Phytochemistry*, 2003, **62**, 325).

Pharmaceutically, interest lies in the calystegines because they are potent inhibitors of glycosidases (R. J. Molyneux *et al*., *Arch. Biochem. Biophysics*, 1993, **304**, 81) making them possible candidates for the development of antiviral, anticancer and antidiabetic drugs (cf. the trihydroxyindolizidine alkaloids castanospermine and swainsonine and the tetrahydroxypyrrolizidine alkaloid australine).

### Further reading

Biastoff S, Dräger B 2007 Calystegines. The Alkaloids 64: 49–102.
*A review with 347 refs covering structures, chemical properties, occurrence, biosynthesis, chemical syntheses, activities*

### Tobacco alkaloids

The principal alkaloids of the genus *Nicotiana* have a pyridine moiety associated with either a pyrrolidine ring (ornithine-derived) or a piperidine ring (lysine-derived). The former group is represented by nicotine (Fig. 26.2) and the latter by anabasine (Fig 26.11).

Although, with the exception indicated below, no drugs are derived from these alkaloids, they have been extensively studied in relation to tobacco manufacture and smoking, and as insecticides (see Chapter 40). Consequently, much is known of their plant biochemistry and genetics of formation.

A pharmaceutical introduction is that of nicotine chewing-gum, nasal spray or patch, intended to help smokers who want to give up smoking but who experience great difficulty in so doing because of their nicotine dependence.

## PYRROLIZIDINE ALKALOIDS

Although these alkaloids have at present no great medicinal significance they are important in that they constitute the poisonous hepatotoxic constituents of plants of the genus *Senecio* (Compositae), well-known for their toxicity to livestock. Some of the alkaloids also show carcinogenic and mutagenic properties and have caused concern in that they occur in small quantities in some herbal products such as comfrey (Boraginaceae) and coltsfoot (Compositae). These alkaloids are known to have an ecological role in some species of butterfly affording protection to some and converting to female flight arrestants in others. In the first demonstration of its kind, the presence of alkaloids on leaf surfaces has been indicated in eight different samples of *Senecio jacoboea* (K. Vrieling and S. Derridj, *Phytochemistry*, 2003, **64**, 1223). Indicine *N*-oxide has antitumour properties (q.v.). Australine, recently characterized from the seeds of the leguminous tree *Castanospermum australe*, is a tetrahydroxypyrrolizidine alkaloid. It was obtained by use of repeated preparative centrifugal TLC. Like the polyhydroxyindolizidine alkaloids it exhibits glycosidase inhibitory activity (for further details see R. J. Molyneux *et al*., *J. Nat. Prod*., 1988, **51**, 1198). The alkaloids frequently occur as esters, being linked with characteristic mono- or dibasic acids called the necic acids. They are biosynthesized from ornithine via a symmetrical intermediate and labelling experiments have shown the involvement of putrescine and homospermidine. Two molecules of putrescine are required to form one of homospermidine. This pathway

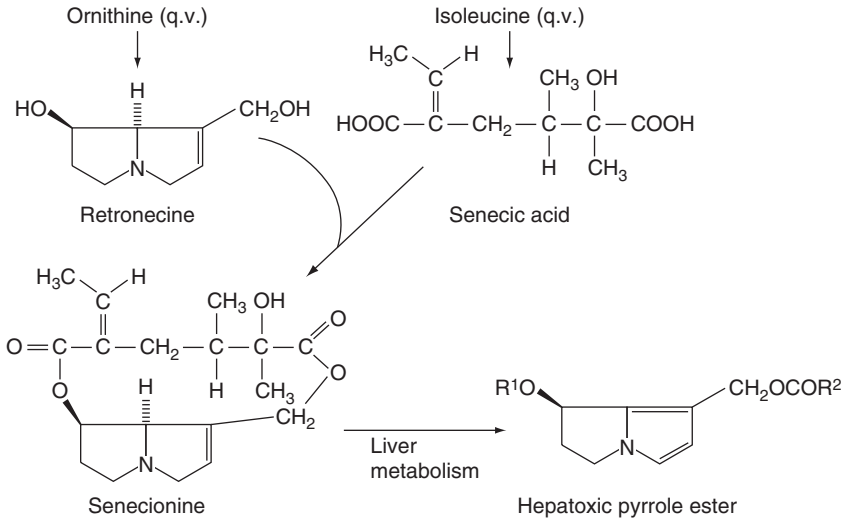



**Fig. 26.10**
Formation and liver metabolism of pyrrolizidine-ester alkaloids.

has been supported by the isolation, partial purification and characterization of the $NAD^+$-dependent enzyme homospermidine synthase, the first pathway-specific enzyme in pyrrolizidine alkaloid biosynthesis (F. Böttcher *et al*., *Phytochemistry*, 1993, **32**, 679). The components of senecionine are illustrated in Fig. 26.10. The hepatotoxic properties are believed to arise by breakdown of the alkaloids in the liver to strongly alkylating pyrrole esters.

For reports on pyrrolizidine alkaloids see: T. Hartmann and L. Witte (1995), *Alkaloids, Chemical and Biological Perspectives* (ed. S. W. Pelletier), Vol. 9, New York: Wiley, p. 155. A general review: K. Ndjoko *et al.*, *Planta Med*., 1999, **65**, 562. Determination in *Senecio* species.

## LYSINE-DERIVED ALKALOIDS

As the next homologue to ornithine, lysine and its associated compounds give rise to a number of alkaloids, some of which are analogous to the ornithine group (see Fig. 26.11). The lycopodium alkaloids are also derived from lysine. Although in some cases, such as the quinolizidine lupin alkaloids, lysine is incorporated via a symmetrical precursor, e.g. cadaverine, in the majority of examples (anabasine, sedamine, *N*-methylpelletierine) the incorporation is asymmetric. In general, for the simple α-substituted piperidines, the C-2 of lysine becomes the point of attachment of the α side-chain.

The wide distribution of these bases throughout the plant kingdom is illustrated by the drugs which follow.

### LOBELIA

Lobelia *BHP*; *BP* 1988 (*Lobelia Herb*, *Indian Tobacco*) consists of the dried aerial parts of *Lobelia inflata* (Campanulaceae), an annual herb indigenous to the eastern USA and Canada. It is cultivated in the USA and Holland.

**History.** Lobelia has long been used by the North American Indians. It was recommended for use in asthma by Cutler in 1813 and was introduced to the English medical profession by Reece in 1829.

**Cultivation and collection.** Lobelia is grown from seed which is sown either in the autumn or in March and April. The plant produces an aerial stem about 50 cm high. It bears alternate leaves 3–8 cm long and pale blue, bilabiate flowers. The inferior ovary develops into an inflated capsule. The plants are cut in August or September, when they bear numerous capsules. After drying, the drug is exported in bales or compressed packets. The seeds are sometimes separated by thrashing.

**Macroscopical characters.** Up to about 60% (*BP*, 1988, upper limit) of the drug consists of stems. These are green or purplish, winged and very hairy in the upper part but becoming more rounded and channelled and less hairy below. The pale green leaves are usually more or less broken and are covered with bristly hair. Entire leaves are ovate to ovate-lanceolate in shape. The margin is irregularly serrate-dentate and the teeth bear water-pores. The flowers are rarely seen in the drug. The fruits are 5–8 mm long, ribbed and crowned by the calyx teeth. Each is bilocular and contains numerous oval-oblong, brown reticulated seeds about 0.5–0.7 mm long. The drug has a slightly irritating odour and an acrid taste.

**Microscopical characters.** The epidermis of the stem is composed of rectangular cells, covered with a striated cuticle and with anticlinal walls clearly pitted, giving a characteristic beaded appearance. The epidermis bears stomata with the pore parallel to the stem axis and large, conical, warty-walled, unicellular hairs up to 600 µm long (Fig. 42.3). The cortex is composed of rounded, thin-walled, chlorophyll-containing parenchyma except in the wings, where the cells are collenchymatous. The endodermis is well-differentiated, the cells clearly showing the Casparian strip. A pericycle composed of small groups of fibres is distinguishable in the lower part of the stem. The phloem is composed of small groups of delicate sieve-tube tissue enclosing the anastomosing latex vessels, readily seen after staining with iodine. The xylem is composed of elongated, thick-walled xylem fibres and spiral and scalariform vessels. The pith is composed of pitted lignified parenchyma.

With the leaf the upper epidermis is composed of straightwalled, papillose cells with the anticlinal walls showing the beaded appearance (Fig. 42.2). The lower epidermal cells have wavy walls, and numerous stomata, without special subsidiary cells, are present. Unicellular covering hairs, like those present on the stem, are borne on both epidermal surfaces. The mesophyll is differentiated into a single-layered palisade tissue and a spongy mesophyll. The mesophyll cells contain small fat crystals. The palisade tissue is interrupted in the midrib and groups of collenchyma occur above and below the midrib bundle. The phloem contains the characteristic latex vessels. Numerous water-pores occur on the upper surface of the marginal teeth.

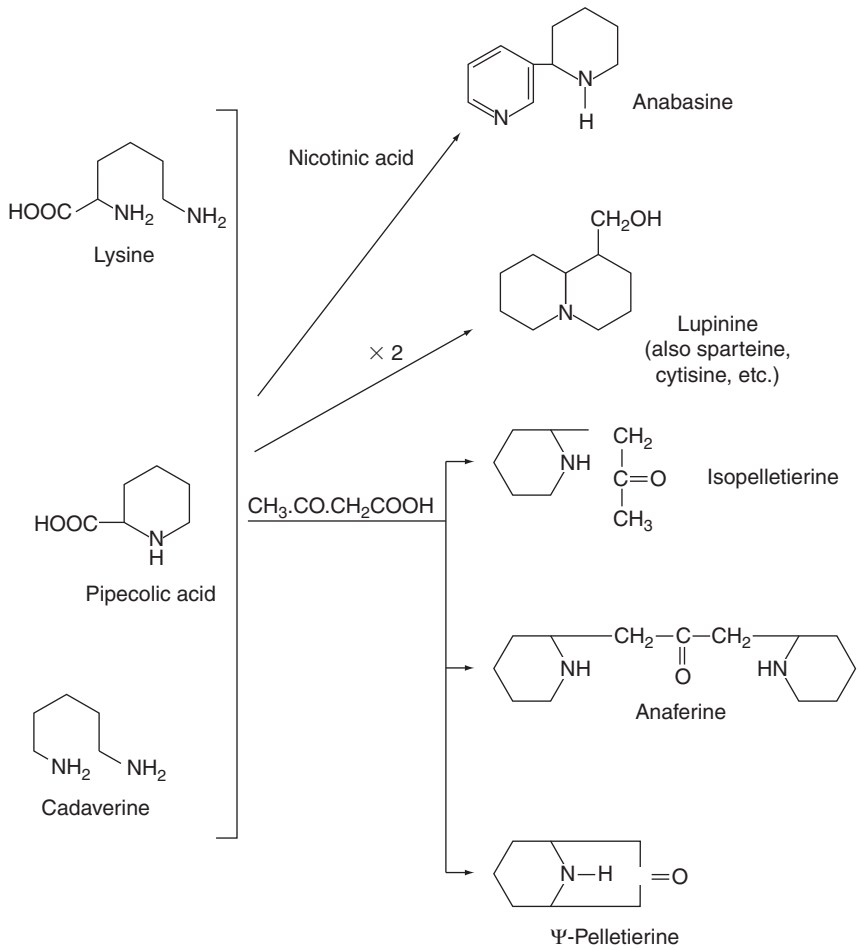



**Fig. 26.11**
Lysine as a precursor of alkaloids.

The surface of the seed is characteristically reticulate. The pollen grains are roughly spherical, 20–30 μm diameter, and show three pores.

**Constituents.** Lobelia contains about 0.24–0.4% of alkaloids (*BP* 1988, not less than 0.25% as determined by a standard Stas-Otto procedure) the most important of which is lobeline. This and many related alkaloids including lobelidine, lobelanine, lobelanidine and isolobelanine, have a piperidine nucleus (Fig. 26.12). Others are piperideines (i.e. the ring system is unsaturated).

By analogy with the biosynthesis of coniine (Fig. 26.38), the above alkaloids could be formed from a polyketo acid involving two benzoyl units and acetate. However, the demonstration that phenylalanine can be incorporated by the plant into the lobelia alkaloid lobinaline and into the related sedamine (*Sedum acre*, Crassulaceae), and the incorporation of lysine into the piperidine ring of lobeline, does not support this hypothesis. A number of Brazilian species contain similar constituents.

Root cultures of *L. inflata* transformed by *Agrobacterium rhizogenes* have been shown to produce lobeline at the same concentration as normal pot-grown plants (H. Yonemitsu *et al*., *Plant Cell Rep.* 1990, **9**, 307).

**Uses.** Lobelia is used in spasmodic asthma and chronic bronchitis; it is included in some antismoking preparations. In its pharmacological action, lobeline resembles nicotine in having both central and peripheral effects. In toxic doses the drug has a paralytic effect and its continuous use should be avoided.

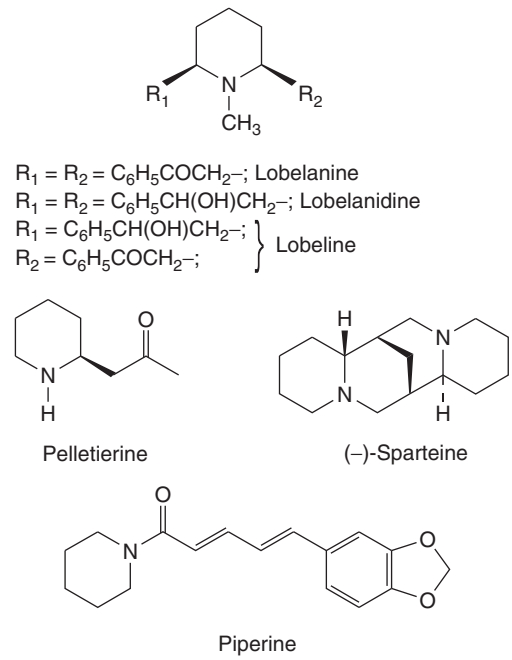



**Fig. 26.12**
Alkaloids of lobelia, pomegranate, broom and pepper.

## Indian lobelia

This drug, which is official in India, consists of the dried aerial parts of *L. nicotianaefolia*, a biennial or perennial herb found in many parts of India at altitudes of 700–2200 m. The leaves and stems are larger than those of the American lobelia and in the form of powder the drug may be distinguished by the trichomes and palisade ratio. Indian lobelia contains not less than 0.8% alkaloids calculated as lobeline.

## Pomegranate

The pomegranate, *Punica granatum* L. Punicaceae, is cultivated thoughout subtropical and tropical regions of the world; some 800 000 metric tons are produced annually, principally for dietary purposes. However, the barks, fruit-rind, flowers and seeds all find medicinal use.

Both stem and root barks are used and occur in curved or channelled pieces about 5–10 cm long and 1–3 cm wide. The outer surface of the stem bark shows longitudinal corky furrows, a few shallow depressions and the bark apothecia of lichens, while that of the root bark shows depressions where the outer layers have exfoliated. The barks are smooth and yellowish on their inner surfaces and break with a short granular fracture. They contain about 0.5–0.9% of volatile liquid alkaloids, the chief of which are pelletierine and pseudopelletierine, together with about 22% of tannin.

Pelletierine tannate, a mixture of the tannates of the alkaloids, was included in the *BP* 1948 and was used as an anthelminthic with a specific action on tapeworms.

The dried pericarp of the fruit occurs in thin, curved pieces about 1.5 mm thick, some of which bear the remains of the woody calyx or a scar left by the stalk. The outer surface is brownish-yellow or reddish and the inner surfaces bear impressions left by the seeds. A high tannin content (28%) affords its use as an astringent in the treatment of diarrhoea.

The seeds have been studied for their bioactive constituents with new compounds recently reported. Major components are monoacylglycerols, glycerides, sterols, proteins, pectins and sugars (M. Yusuph and J. Mann, *Phytochemistry*, 1997, **44**, 1391; R.-F. Wang *et al*., *J. Nat. Prod.*, 2004, **67**, 2096). The validity of a seed extract for use in the treatment of diarrhoea, as practised in traditional Indian and Bangladesh medicine, has been experimentally verified (A. K. Das *et al*., *J. Ethnopharm*., 1999, **68**, 205).

The flowers are used in Chinese medicine; a new polyphenol and six known compounds including maslinic acid (see 'Olive Leaf') have been reported (R. Wang *et al*., *Fitoterapia*, 2006, **77**, 534).

For a study on the antioxidant, antimalarial and antimicrobial tannin-rich fractions, ellagitannins and phenolic acids of pomegranate, see M. K. Reddy *et al*., *Planta Medica*, 2007, **73**, 461.

N. P. Seeram *et al.* (see 'Further reading') have listed 122 compounds isolated from various organs of the pomegranate; they are classed as ellagitannins and gallotannins, ellagic acid derivatives, catechin and procyanidins, anthocyanins and anthocyanidins, flavonols, organic acids, simple galloyl derivatives, fatty acids and triglycerides, sterols and terpenoids, alkaloids and other compounds.

For studies on the biosynthesis of *N*-methylpelletierine involving the feeding of $^{13}$C-labelled acetoacetate and acetate see Hemscheidt and Spenser (*J. Am. Chem. Soc*., 1990, **112**, 6360). Their results constitute evidence in support of the classical biogenetic concepts regarding the incorporation of acetate into the alkaloid (compare findings for the incorporation of acetate into the tropane skeleton).

The role of pomegranate juices and extracts as nutraceuticals is discussed in Chapter 32: The plant nutraceuticals.

## Further reading

Seeram NP *et al* (eds), Hardman R (series ed) 2006 Medicinal and aromatic plants industrial profiles, Vol 43. Pomegranates – ancient roots to modern medicine. CRC, Taylor and Francis Group, Boca Raton, FL

## Broom

The broom, *Cytisus scoparius* (Leguminosae), is a perennial shrub about 1–2 m high. The lower part is woody but the long, straight branches are green and glabrous. The upper parts of the stem bear five prominent, longitudinal ridges. The lower leaves are stalked and consist of three obovate leaflets, but the upper leaves are sessile and usually reduced to a single leaflet.

The flowers are typical of the subfamily Papilionaceae. The fruit is a black, hairy pod about 3–5 cm long.

Broom tops are described in the *BHP* 1988 and *BPC* 1949. An aqueous decoction or other extract was used as a mild diuretic. Their chief constituents are quinolizidine alkaloids, including the volatile liquid alkaloid sparteine (Fig. 26.12), a yellow isoflavone scoparin, and flavonoids. The drug has diuretic and cathartic actions but is now little used.

## Pepper

Black pepper (*Piper Nigrum*) consists of the dried, unripe fruits of *Piper nigrum* (Piperaceae), a perennial climbing plant cultivated in the Malay Archipelago, southern India, South America and the West Indies. Large quantities are obtained from Indonesia, Sarawak and Brazil. The structure of the pepper market is extremely complex and highly speculative, with dealers often selling one shipment of pepper several times over on behalf of various principals.

Pepper was known to Theophrastus and other ancient writers. It was the most important spice used in the Middle Ages and was imported into England about AD 1000. The high cost of pepper and other Eastern spices was a big inducement to the Portuguese to find a sea route to India; competition for the spice trade has played a large part in the colonial expansion of European nations.

Pepper is essentially a crop of the wet tropics and is propagated from cuttings (Sarawak) or runners (India). However, it is subject to various diseases which are transmitted by vegetative propagation so that shoot-culture techniques designed for the mass culture of selected disease-free vines have been investigated (V. J. Philip *et al*., *Plant Cell Reps*., 1992, **12**, 41).

**Production.** The vines are grown on poles or trees. The inflorescence is a spike of about 20–30 sessile flowers, which develop into sessile fruits (see earlier editions for diagrams). The latter are picked when the lower fruits of the spike turn red. They are then removed from the axis and dried, either in the open air or by artificial heat. The fire-dried spice is most esteemed but the ground spice is usually a blend of different varieties.

White pepper, which is largely used in the East, is also obtained from *P. nigrum*, but the fruits are allowed to become more completely ripe. After storing them for some days or soaking them in water, the outer part of the pericarp is removed by rubbing and washing and the fruits are dried.

Black pepper fruits are almost globular and 3.5–6 mm diameter. The surface is dark brown or greyish-black and strongly reticulated. The apex shows the remains of the sessile stigmas and a basal scar indicates the point of attachment to the axis. Pepper has an aromatic odour and pungent taste.

Bacterial and fungal contamination of the stored peppers can be reduced by washing and redrying with subsequent maintenance of the moisture content at less than 11%.

In white pepper, owing to the removal of the outer part of the pericarp, the vascular bundles, about 16 in number, run on the outside of the fruit from base to apex.

**Constituents.** Pepper contains 1–2.5% of volatile oil, 5–9% of the crystalline alkaloids piperine and piperettine, and a resin. The aroma of the spice is due to the volatile oil, which consists largely of terpenes, while the pungency is ascribed to piperine and the resin. Piperine, first isolated in 1819, is also found in the long pepper (1–2%) and in Ashanti pepper, the fruits of *Piper guineense*. Analyses for the volatile oil are given as β-caryophyllene (21.59–27.70%), limonene (21.06–22.17%), sabinene (8.5–17.6%), β-pinene (9.16–11.08%), α-pinene (5.07–6.18%), myrcene (2.2–2.3%), *p*-cymeme (0.0–0.18%) and oxygenated constituents (3.39–5.68%); 40 compounds were identified in oil from Sao Tome e Principe (A. P. Martíns *et al*., *Phytochemistry*, 1998, **49**, 2019).

Pepper was once employed in the treatment of gonorrhoea and chronic bronchitis. Large quantities are used as a condiment.

*Long pepper* is the dried unripe fruit of *Piper retrofractum* (*P. officinarum*) and *P. longum*, grown in Indonesia, India and the Philippines. The spice consists of whole spikes of small fruits forming a structure about 4 cm long and 6 mm in diameter. Individual fruits show a similar structure to black pepper. For a report on the component alkamides and other constituents see B. Das *et al*., *Planta Med*., 1996, **62**, 582. Guineesine, a recently isolated alkaloid, is an acyl-CoA:cholesterol acyltransferase inhibitor and has been proposed as an attractive target for the prevention and treatment of hypercholesterolemia and atherosclerosis (S. W. Lee *et al*., *Planta Medica*, 2004, **70**, 678).

*Cubebs* or tailed pepper are the dried, full-grown fruits of *Piper cubeba*, a native of Indonesia, Borneo and Sumatra. The fruits are collected while green and dried in the sun. They were used in Europe as a spice as early as the eleventh century. The spikes of cubebs bear more fruits than those of pepper and become falsely stalked as they mature, owing to an abnormal development of the base of the pericarp. The upper part of the cubeb fruit is globular, 3–6 mm diameter and covered with a greyish-brown, reticulated pericarp, which is prolonged at the base into a straight stalk. Cubebs yield 10–18% of volatile oil containing terpenes and sesquiterpenes, a crystalline inodorous substance (−)-cubebin (formula Table 21.7) and a number of other lignans, a white amorphous substance cubebic acid (1%), and amorphous resin (3%).

The above gives only a token appreciation of the genus as a whole—there are some 700 species of *Piper* globally of which only about 12% have been studied phytochemically. Nevertheless the literature is extensive and in a review (341 refs) covering the secondary metabolites (V. S. Parmar *et al*., see 'Further reading') nearly 600 constituents are listed. Classes of metabolites considered are alkaloids/amides, propenylphenols, lignans, neolignans, terpenes, steroids, kawa pyrones, piperolides, chalcones, dihydrochalcones, flavones, flavanones and miscellaneous compounds.

### Further reading

Parmar VS, Jain SC, Bisht KS *et al* 1997 Phytochemistry of the genus *Piper*. Phytochemistry 46(4): 597–673

Ravindran PA (ed), Hardman R (series ed) 2000 Medicinal and aromatic plants—industrial profiles, Vol 13. Black pepper, *Piper nigrum*. Harwood Academic, Amsterdam

### Lycopodium

Lycopodium consists of the spores of the clubmoss, *Lycopodium clavatum* (Lycopodiaceae, Phylum Pteridophyta). Most of the commercial drug is collected in Poland and E. Europe, but Indian and Pakistani supplies are obtained from the Himalayas. The sporangial spikes are cut and dried, and the spores are separated by shaking and then freed from vegetable debris by sieving through four sieves. The drug is exported in sacks, which are usually enclosed in matting.

Lycopodium is a light, yellow, extremely mobile powder without odour or taste. It floats on water without being wetted. The spores are 25–40 μm diameter and have the shape of a three-sided pyramid with a convex base. The surface is covered with polygonal-shaped reticulations which form a projecting ridge at the edge of the spore. Viewed from the apex of the pyramid, the edges of the flat sides form a distinct, triradiate marking. On crushing, yellowish drops of oil exude.

Lycopodium consists about 50% of fixed oil, which consists mainly of the glycerides of lycopodiumoleic acid. The drug also contains about 3% of sugars, phytosterin and alkaloids of the annotine type, which are characteristic of the genus, together with traces of nicotine. The lycopodium alkaloid lycopodine, first reported in 1881, is, like pelletierine, derived from lysine and acetate (for the role of the latter in its biosynthesis in the intact plant see T. Hemscheidt and I. D. Spenser, *J. Amer. Chem. Soc*., 1993, **115**, 2052). For a review of the lycopodium alkaloids see Ayer (*Nat. Prod. Rep*., 1991, **8**, 455).

Adulteration with the pollen of *Pinus* species, *Corylus avellana*, *Typha latifolia*, etc., or with roasted and coloured starches, dextrin, sulphur or inorganic salts, can readily be detected by means of the microscope.

Lycopodium was once used to a limited extent in dusting powders and medicated snuffs, and as a dusting powder for pills. It is employed in quantitative microscopy (see Chapter 43).

### Further reading

Kobayashi J, Morita H 2005 The lycopodium alkaloids (*a review with 154 refs*). The Alkaloids 61, 1–57

## PHENYLALANINE-, TYROSINE- AND DIHYDROXYPHENYLALANINE-DERIVED ALKALOIDS

The title compounds and their corresponding decarboxylation products are the precursors of a large number of alkaloids which include simple protoalkaloids, the benzylisoquinolines, phthalideisoquinolines, aporphines and proaporphines, protoberberines, protopines, naphthaphenanthridines, the *Erythrina*, Amaryllidaceae and ipecacuanha alkaloids and the morphine and rhoeadine-type alkaloids. A group of compounds, first recognized in 1965, is that comprising the phenethylisoquinoline alkaloids; it is from these that colchicine arises. Some of the pharmaceutically more important groups are illustrated in Fig. 26.13.

In addition to the reading matter quoted at the end of the first section of this chapter, see also the following reviews: Isoquinoline alkaloids, H. Guinaudeau and J. Brunetonin *Methods in Plant Biochemistry* (ed. P. G. Waterman) 1993, Vol 8. London: Academic, p. 373; bisbenzylisoquinoline alkaloids, P. L. Schiff, *J. Nat. Prod*., 1983, **46**, 1; 1987, **50**, 529; 1991, **54**, 645; dimeric aporphinoid alkaloids, H. Guinaudeau *et al*., *J. Nat. Prod*., 1988, **51**, 1025; 1994, **57**, 1025.

## PROTOALKALOIDS

Those alkaloid-like amines which do not have the nitrogen as part of a heterocyclic ring system are often termed protoalkaloids. They are not restricted to any particular class of alkaloids and are often classified according to the amino acids from which they are derived. Some are quaternary bases as, for example, the protoberberine alkaloids which constitute a large group with diverse structures and a wide distribution

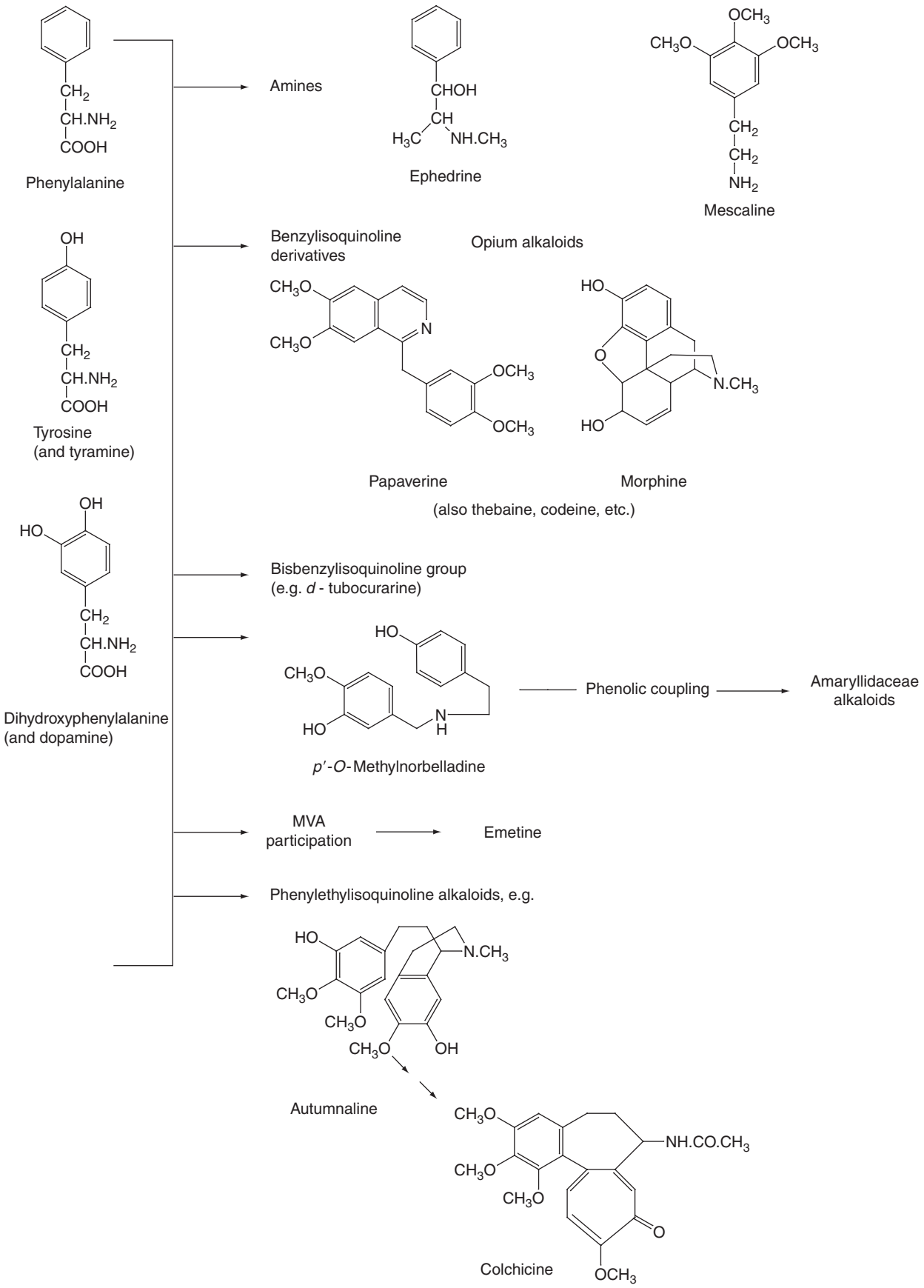



**Fig. 26.13**
Some bases derived from phenylalanine, tyrosine and dihydroxyphenylalanine.

in nature. Although not prominent in common herbal drugs the alkaloids are being studied for their antibacterial, antimalarial (Chapter 28) and potential genotoxic properties. For reviews with many references see E. V. L. Da-Cunha *et al*., *The Alkaloids*, 2005, **62**, 1–75; L. Grycova *et al*., *Phytochemistry*, 2007, **68**, 150.

26

## Ephedra

Various species of *Ephedra* (*Ma-huang*) (Ephedraceae) are used as a source of the alkaloids ephedrine and pseudoephedrine, which may also be prepared by synthesis. Among these are the Chinese species *Ephedra sinica* and *E. equisetina* and the Indian and Pakistani *E. gerardiana*, *E. intermedia* and *E. major*.

Although ma-huang was known to the Chinese over 5000 years ago and ephedrine was isolated in 1887, it only came into extensive use during the last century.

**Collection.** The drug is collected in the autumn, this being important, as the amount of alkaloid present shows considerable variation at different seasons. The drug is imported in bales. It consists of slender, more or less broken aerial stems which are woody and usually branch only at the base.

**Characters.** The stems of the ephedras bear numerous, fine, longitudinal ridges. The leaves are small, connate at the base, and usually in whorls of two (less commonly, whorls of three or four) and decussate.

1. *Ephedra sinica* Stapf. The stems are about 30 cm long, ashy greyish-green in colour, and slightly rough. The diameter at the lowest green node is about 1 or 2 mm, while the internodes are about 3–6 cm long. The leaves, which are about 4 mm long, have a subulate, recurved apex; the lamina is whitish and the base reddish-brown. In the transverse section the young stem 9 shows 6–10 bundles. The pith is largely unlignified.
2. *E. equisetina* Bunge. The stems are very woody and much branched, 25–200 cm in length and ashy yellow-green in colour. The internodes are shorter than *E. sinica*, being about 1–2.5 cm long. The apex of the leaves is shorter than the cup and not recurved. The leaves are of a brownish-purple colour, the lower ones tending to go black. Transverse sections show that the number of bundles in the stem is not constant (usually about 10). The pith is lignified.
3. *E. distachya*. The stems are slightly woody and branching takes place from the upper and lower parts of the main stem. Stems about 37 cm long, rough and greenish-yellow in colour. Internodes 2.5–6 cm long. Leaf apex short but acute and often fissured at the base. There are no cortical or perimedullary fibres, but the pith is lignified. *Ephedra*, when dry, has little odour. The taste is slightly bitter.

**Constituents.** The ephedras contain about 0.5–2.0% of alkaloids. Of the total, ephedrine (and its isomers) forms from 30 to 90%, according to the species. Pseudoephedrine is also present (see Fig. 26.14). The roots also contain a number of macrocyclic alkaloids (ephedradines) and feruloylhistamine which have hypotensive properties.

**Biosynthesis of ephedrine and related alkaloids.** These alkaloids are formed by a union of a $C_6$–$C_1$ unit and a $C_2$ unit. For many years it has been known that phenylalanine is the originator of the $C_6$–$C_1$ moiety, being converted first to benzaldehyde or benzoic acid. GrueSørensen and Spenser (*J. Am. Chem. Soc*., 1993, **115**, 2052 and references quoted therein) using $^{13}C$- and $^{2}H$-labelled precursors in feeding experiments with *E. gerardiana* have shown that benzoic acid combines with the intact $CH_3CO$ group of pyruvic acid to form ephedrine and related alkaloids with 1-phenylpropan-1,2-dione and (*S*)-(−)-2-amino-1-phenylpropan-1-one (cathinone) serving as intermediates. The route is illustrated in Fig. 26.14. 1-Phenylpropan-1,2-dione and cathinone are known constituents of *Catha edulis* (see below) but the previous non-isolation of cathinone from ephedra is attributed to its high efficiency of incorporation into the nor-alkaloids.

**Uses.** Ephedrine is used for the relief of asthma and hay fever. Its action is more prolonged than that of adrenaline, and it has the further advantage that it need not be given by injection but may be administered by mouth. In oriental medicine the ephedras are also used as anti-inflammatory drugs and this action is ascribed to an oxazolidone related to ephedrine. In contrast to the herb, which has a sudorific action, the root has been used clinically in China for its antisudorific effect.

## Khat or Abyssinian tea

This consists of the fresh leaves of *Catha edulis* Forsk. (Celastraceae). The plant is cultivated in Abyssinia, in parts of east and southern Africa,

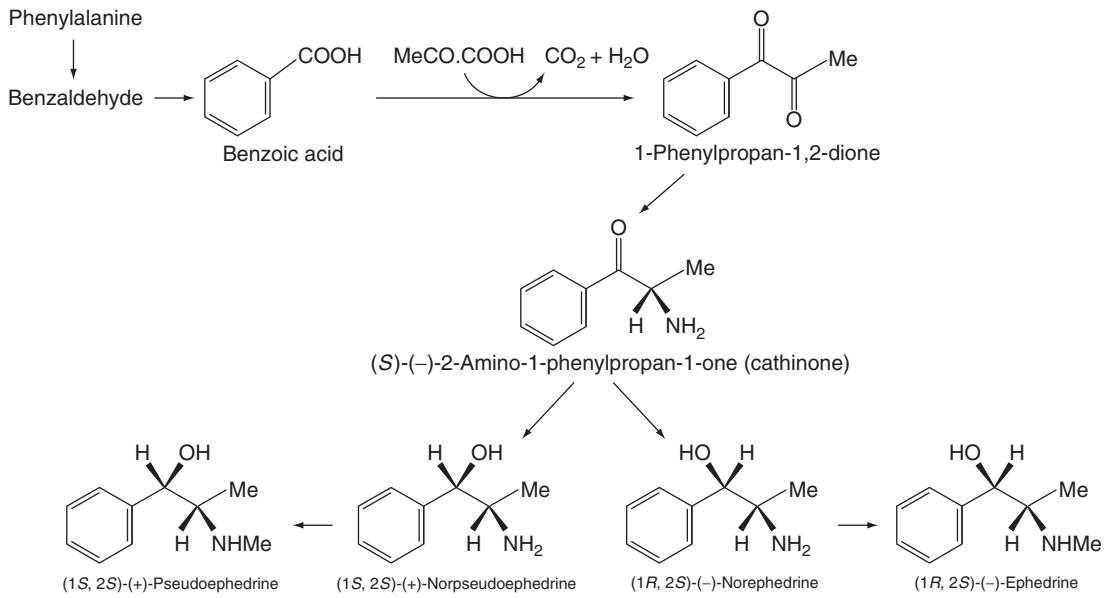



**Fig. 26.14**
Biogenesis of ephedrine and related alkaloids.

and in southern Arabia. There appear to be a number of different varieties of the plant varying in 'khatamine' content between 0.1% (Yemen, Madagascar) and 0.5% (Kenya). It is widely employed in African and Arab countries, particularly in the Yemen, for chewing, and its misuse has been surveyed by the WHO. Its traditional use is similar to that of coca in that the fresh leaves, when chewed, have a stimulatory effect with the alleviation of depression and of the sensations of hunger and fatigue. The drug constitutes a problem in the West.

On a dry weight basis the leaves contain about 1.0% of (+)-norpseudoephedrine, and for many years this was thought to be the principle responsible for the stimulant effect of the drug. In 1975 another phenylpropane, (−)-α-aminopropiophenone (cathinone), was isolated at UN laboratories and is considered the principal CNS stimulant of the fresh plant. (−)-Cathinone has pharmacological properties analogous to those of (+)-amphetamine, possessing a similar potency and the same mechanism of action. Many other components (alkaloids, sesquiterpenes, triterpenes, flavonoids, numerous acids as esters), including an essential oil containing about 40 components, have been characterized. Tissue cultures of the plant have been shown to produce quinone-methide triterpenes; these compounds do not appear to be present in the normal plant although they have been reported in several other members of the Celestraceae. Crombie and Whiting have reviewed (64 refs) the alkaloids of khat (*Alkaloids*, 1990, **39**, 139).

## BENZYLISOQUINOLINE DERIVATIVES

A number of important drugs come within this heading. Phytochemically the group is often subdivided.

### Opium poppy

The opium poppy, *Papaver somniferum* L., is an annual herb about 50–150 cm in height. The stem and leaves are glaucous. The latter are about 10 cm in length, entire, sessile and amplexicaul. The margin is dentate but varies somewhat in the different varieties. The flowers, which are borne on a slightly hairy peduncle, are solitary, nodding in the bud, and have caducous sepals. They have the floral formula K2, C2 + 2, $A_{\infty}$, G(∞). The unilocular ovary contains numerous ovules attached to parietal placentas. It bears at its apex a flat disc formed by the union of the radiating stigmas. The capsule opens by means of small valves, which are equal in number to the carpels and situated immediately below the stellate stigma.

In addition to numerous garden hybrids, the following varieties are recognized:

*P. somniferum* var. *glabrum* Boiss., cultivated in Turkey; flowers purplish but sometimes white; capsule subglobular; stigmata, 10–12; seeds, white to dark violet.

*P. somniferum* var. *album* D.C., cultivated in India; flowers and seeds white; capsules more or less egg-shaped, 4–8 cm diameter, no pores under the stigma.

*P. somniferum* var. *nigrum* D.C., cultivated in Europe for the seeds, which are slate-coloured and are known as 'maw seeds' (probably a corruption of *Mohnsamen*). The leaves and calyx are glabrous, the flowers violet and the capsules somewhat smaller and more globular than those of the var. *album*.

*P. somniferum* var. *setigerum* D.C., a truly wild form found in southern Europe. The peduncles and leaves are covered with bristly hairs. The leaf lobes are sharply pointed and each terminates in a bristle.

Poppy capsules contain, when ripe, 0.18–0.28% of morphine. Poppy seeds contain only very small quantities of narcotine, papaverine and thebaine in addition to morphine and codeine, all detectable by GC/MS. It has been pointed out that the detection of the former three bases in urine samples may be used to differentiate between poppy seed consumption and the illegal use of morphine or heroin (B. D. Paul *et al*., *Planta Med*., 1996, **62**, 544). Importantly the seeds also contain 50–60% of a drying oil which is used by artists and also for cooking.

*Papaver* breeding has received some attention; a morphine-rich strain suitable for mechanical harvesting and a low-morphine variety for seed production have been described. Other strains with little alkaloid, and ones with a higher proportion of codeine, have also been produced.

The red or corn poppy, *Papaver rhoeas*, was formerly used in pharmacy. The fresh scarlet petals were particularly used as a colouring matter in the form of a syrup and are described in more detail in Chapter 33. They contain the anthocyanidin glucoside mecocyanin, an isomer of the cyanin found in red rose petals. A number of alkaloids are produced (e.g. rhoeadine of the benzyltetrahydroisoquinoline type); they have no morphine-like activity.

## OPIUM

Opium (*Raw Opium*) is the latex obtained by incision from the unripe capsules of *Papaver somniferum* (Papaveraceae) and dried partly by spontaneous evaporation and partly by artificial heat. It is worked into irregularly shaped masses and is known in commerce as Indian opium. Indian opium is specifically stated because this is a legally available source of the drug. However, a number of countries e.g. Turkey, former USSR and Yugoslavia and Australia (Tasmania) grow considerable quantities of the opium poppy for alkaloid extraction and seed production. For strategic purposes, a relatively small crop is raised in southern England. Much illegal opium is produced in S.E. Asia.

The *BP*/*EP* monograph for Raw Opium states that it is intended only as a starting material for the manufacture of galenical preparations (e.g. Tincture of Opium) and is not dispensed as such. It should contain not less than 10% of morphine and not less than 2.0% of codeine. The thebaine content is limited to 3%. The alkaloidal assays are performed by liquid chromatography on the drug dried at 100–105°C.

*Prepared Opium BP*/*EP* is raw opium powdered and dried at a temperature not exceeding 70°C. It contains 9.8–10.2% morphine and a minimum 1.0% of codeine; thebaine is limited to 3.0%. The powder may be adjusted to strength by the addition of raw opium or a suitable excipient, which must be recorded on the label.

**History.** Opium was well known to the ancients. Dioskurides, about AD 77, distinguishes between the latex of the capsules, *opos*, and an extract of the whole plant, *mekonion*. The use of opium spread from Asia Minor to Persia, where opium eating became popular, and from there to India and China. However, it was not until the second half of the eighteenth century that opium smoking began to be extensively practised in China and the Far East.

Asia Minor has from very early times been an important source of opium production. In Macedonia cultivation was started as recently as 1865. Persian opium was imported into England from about 1870 to 1955. Opium was cultivated in India during the Middle Ages, and the monopoly of the Mogul Government was taken over first by the East India Company and then by the British Government. Formerly, Indian opiums, being prepared mainly for smoking, were little esteemed for pharmaceutical purposes. However, that now imported is of good quality and constitutes the principal British source for the manufacture of alkaloids.

**Production.** The plant cultivated in India under licence is *P. somniferum* var. *album*. Sowing takes place in November and collection from April to June. The incisions are made in the afternoon with an

instrument known as a 'nushtur'. This bears narrow iron spikes which are drawn down the capsule to produce several longitudinal cuts. The incision must not penetrate into the interior of the capsule or latex will be lost. The latex, which is at first white, rapidly coagulates and turns brown. Early in the morning of the day following the making of the incisions the partly dried latex is scraped off with a trowel-like 'see-tooar'. Each capsule is cut several times at intervals of 2 or 3 days. After collection the latex is placed in a tilted vessel so that the dark fluid (pussewah) which is not required may drain off. By exposure to air the opium acquires a suitable consistency for packing.

Indian opium is exported in 5 kg blocks, packed 12 to a lightweight wooden case to facilitate air transport. Each block is wrapped in grease-proof paper, tied with tape and placed in a polythene bag. The drug has a soft consistency and so arrives as rounded, somewhat flattened, cakes. It contains about 9–12% of morphine. Being difficult to dry and powder because of its plastic nature, Indian opium is less suitable than some other types for the preparation of powdered opium.

Former varieties of legal opium were obtained from Turkey (Turkish Government Monoply Opium) and the former Yugoslavia, and opium is also produced in former Kirghiz SSR and China under government control for national use. Iran and Egypt, former producers, no longer cultivate the plant. Such opiums were very characteristic in appearance and packaging, and illustrations of some different varieties together with tools for production will be found in the 10th edition (1972) of this book. Turkish government opium was until relatively recently much used and consisted of cubical stamped blocks each weighing 2 kg; it was much easier to powder than the Indian drug.

In addition to the above countries, opium has been produced, often only on an experimental scale, in most European countries, the USA, the East Indies and parts of Africa. Experiments have shown that a hot climate is not essential—opium of excellent quality has been produced in Scotland and Norway.

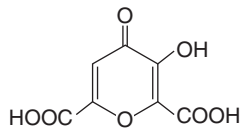
**Microscopy.** Opium examined under the microscope shows agglomerated latex granules in irregular masses. Other smaller amounts of characteristic material which arise as a result of the preparation process are best seen by examining the residue left after water-extraction of the opium. These particles include occasional spherical pollen grains, fragments of vessels and portions of the epicarp of the capsule the latter showing in surface view polygonal thick-walled cells with a stellate lumen. Pointed trichomes and a few starch grains may be present.

**Constituents.** Opium contains some 30 alkaloids, which are largely combined with the organic acid meconic acid; the drug also contains sugars, salts (e.g. sulphates), albuminous substances, colouring matters and water. Six principal alkaloids are listed in Table 26.4.

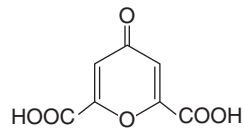
The first group (e.g. morphine) consists of alkaloids which have a phenanthrene nucleus whereas those of the papaverine group have a benzylisoquinoline structure. Some of the less important opium alkaloids (e.g. protopine and hydrocotarnine) are of different structural types. The morphine molecule has both a phenolic and an alcoholic hydroxyl group, and when acetylated forms diacetyl morphine or heroin. Codeine is an ether of morphine (methylmorphine), and other morphine ethers which are used medicinally are ethylmorphine and pholcodine.

New alkaloids continue to be isolated from *P. somniferum*—a number were recognized during investigations on the biogenesis of morphine and Repasi *et al*. (*Planta Med*., 1993, **59**, 477) obtained 5′-*O*-demethylnarcotine during the purification of narcotine from poppy straw; it was also detected in a sample of Indian opium.

*Meconic acid*, a dibasic acid, is easily detected either in the free state or as a meconate by the formation of a deep red colour on the addition of a solution of ferric chloride. As it is invariably found in opium, its presence has long been used to indicate opium. However, research has shown that some species of *Papaver* which produce no morphine but other morphinanes may also contain this acid; it may serve as a chemotaxonomic marker for the Papaveraceae. It is notable that the related acid, chelidonic acid, is found in some other members of the Papaveraceae such as in the root of Greater Celandine (q.v).



Meconic acid



Chelidonic acid

### Papaveretum BP

Papaveretum BP is a mixture of the hydrochlorides of opium alkaloids containing 80.0–88.4% anhydrous morphine HCl, 8.3–9.2% papaverine HCl and 6.6–7.4% codeine HCl. It is assayed by liquid chromatography. Well-known preparations of papaveretum are the trade products Omnopon and Nepenthe which are used mainly for premedication and as analgesics during and after operations. Formerly, Papaveretum (*BPC*, 1973) also contained the opium alkaloid noscapine but this has now been removed from the preparation on account of its genotoxicity. Noscapine has also been a constituent of many cough mixtures and these have now been withdrawn by the manufacturers.

## BIOGENESIS OF THE OPIUM AND RELATED ALKALOIDS

The step-by-step elucidation of the biogenetic pathway of the opium alkaloids constitutes a brilliant chapter in the history of phytochemical research. The principal features of the biosynthesis of thebaine, codeine and morphine as now envisaged are given in Fig. 26.15; a further consideration of the initial stages of this scheme follows later.

In 1910 Winterstein and Trier suggested that there was a structural relationship between the benzylisoquinoline alkaloids and dihydroxyphenylalanine. This was extended by Robinson's observation that morphine could be derived from these alkaloids by rotation of the

**Table 26.4 Historic isolations of principal opium alkaloids.**

| Alkaloid | Formula | Discoverer | Date | Properties |
|---|---|---|---|---|
| Morphine | $C_{17}H_{19}O_3N$ | Sertürner | 1816 | Strong bases, which are alkaline to litmus and highly toxic |
| Codeine | $C_{18}H_{21}O_3N$ | Robiquet | 1832 | |
| Thebaine | $C_{19}H_{21}O_3N$ | Thiboumèry | 1835 | |
| Noscapine | $C_{22}H_{23}O_7N$ | Derosne | 1803 | Feeble bases, which are slightly toxic |
| Narceine | $C_{23}H_{22}O_8N$ | Pelletier | 1832 | |
| Papaverine | $C_{22}H_{21}O_4N$ | Merck | 1848 | |

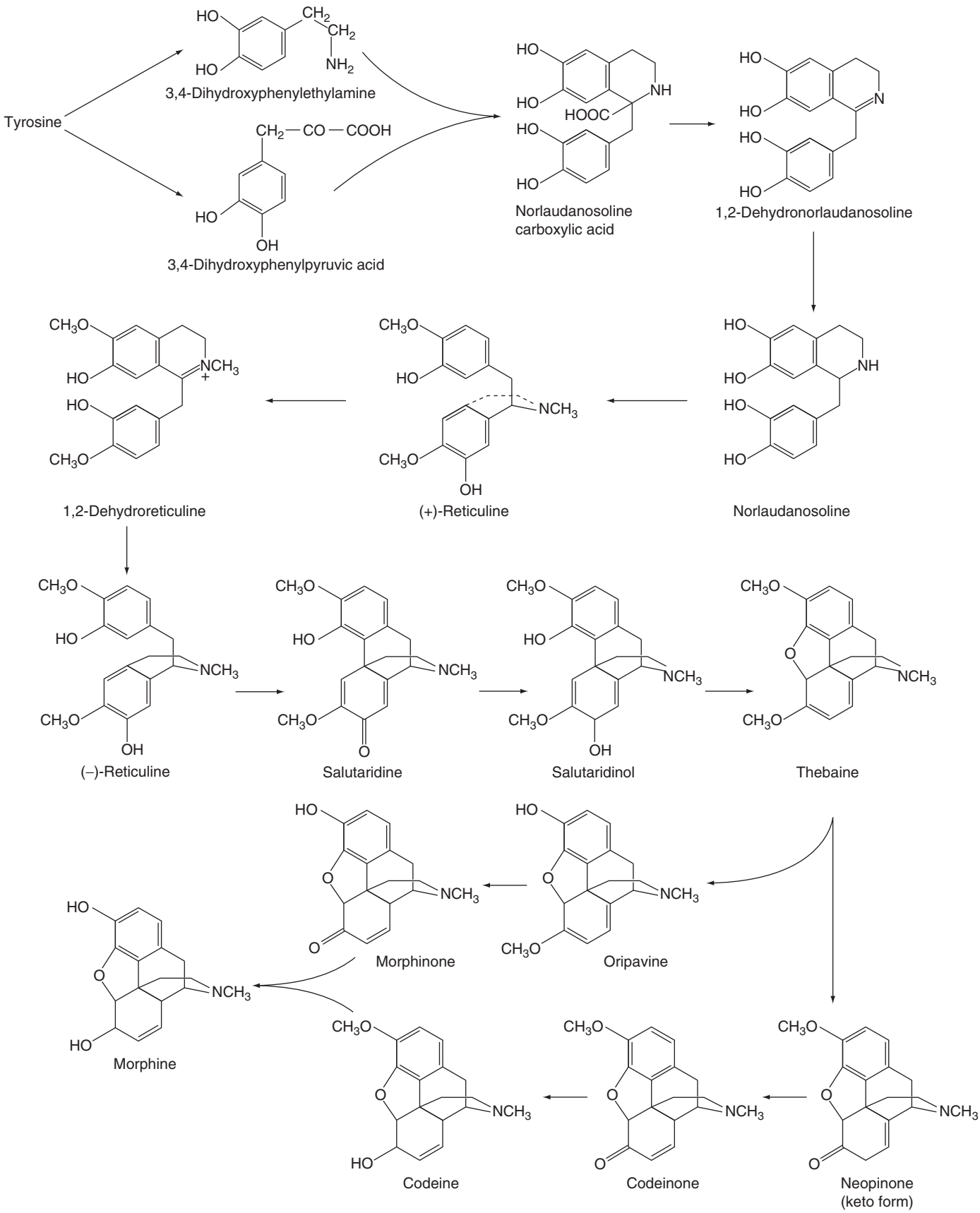



**Fig. 26.15**
Biogenesis of thebaine, codeine and morphine; see also Fig. 26.16.

tetrahydroisoquinoline residue followed by oxidative ring closure. The validity of such schemes remained untested until the advent of radiochemical techniques, when in 1958–60 experiments with labelled tyrosine, administered to poppy capsules, demonstrated that two molecules of precursor were incorporated into the morphine molecule, in full accord with Robinson's theory. Further, the intermediate stage was confirmed by the demonstration that norlaudanosoline acted as a more efficient precursor for morphine than did tyrosine and yielded a product labelled as required by the theory. By the cultivation of poppy plants in $^{14}CO_2$, and by injection of labelled alkaloids into the plant, it was shown that the first major alkaloid formed is thebaine; this is irreversibly converted to codeine and then to morphine.

Many details of the above outline pathway have now been filled in. Theory required that in the oxidative coupling of norlaudanosoline, the hydroxyl groups not involved in the reaction be protected. A base of the type required, in which two of the hydroxyls were methylated, had previously been isolated from another plant (*Annona reticulata*, Annonaceae, order Magnoliales); this was reticuline. Labelled reticuline and norreticuline both proved to be very efficient precursors of morphine in the poppy, surpassing norlaudanosoline in this respect. Subsequently in 1964 reticuline was found to be a normal, minor component of *P. somniferum*; this is another instance of the isolation of a natural product from a plant after its presence has been suggested on phytochemical grounds. It also illustrates the point that what may be a principal alkaloid in one plant (reticuline in *Annona*) is, in another, a transient metabolite, which is essential to some metabolic pathway but which does not accumulate. In support of the theory, when tetrahydropapaverine (all hydroxyl groups methylated) was fed to the opium poppy, negligible incorporation into the alkaloids was obtained. The stages in the conversion of reticuline to thebaine and of thebaine to codeine were demonstrated by the feeding of appropriate labelled intermediates, alkaloids which have since been isolated as minor components of the opium alkaloid mixture.

One sequence of the pathway shown in Fig. 26.15, which has been difficult to establish unequivocally involves the initial stages leading to the formation of (+)-reticuline. Now norcoclaurine (also called higenamine, and a constituent of *Annona squamosa*, q.v.) is a favoured trihydroxylated precursor. In 1987 the (*R*)-isomer was shown to be specifically incorporated into thebaine when applied as a labelled precursor to *P. somniferum* seedlings. Also with cell cultures and plants of *Berberis*, *Peumus*, *Eschscholtzia* and *Argemone* spp. it was specifically incorporated into protoberberine, aporphine and benzophenanthridine alkaloids (see R. Stadler *et al*., *Phytochemistry*, 1989, **28**, 1083). The experiments indicated that dopamine and *p*-hydroxyphenylacetaldehyde (both derived from tyrosine) condense to give norcoclaurine, thus explaining the observed lack of incorporation of DOPA and dopamine into the benzylic portion of reticuline-derived alkaloids. The origin of (+)-reticuline by this route is shown in Fig. 26.16.

A second pathway for the terminal steps in the biosynthesis of morphine has been demonstrated (E. Brochmann-Hanssen, *Planta Med*., 1984, **50**, 343) by using two strains of the opium poppy—a Tasmanian strain known to contain the alkaloid oripavine and the Indra strain. Both species converted labelled oripavine to morphine, and morphinone was also isolated with good incorporation of radioactivity, albeit in small quantity owing to its unstable nature. Codeine and thebaine were not radioactive, demonstrating that the demethylation of the phenolic ether of thebaine is not reversible. This alternative final stage would therefore appear to be thebaine → oripavine → morphinone → morphine (Fig. 26.15).

Two alkaloids which arise as branches of the principal biogenetic pathway are neopine, the presence of which in opium is explained as a reduction product of neopinone, and papaverine, which arises by methylation of norreticuline (Fig. 26.17) followed by dehydrogenation. The presence of some of the other minor alkaloids of opium can be explained by various methylations and dehydrogenations of laudanosoline, reticuline and their nor-derivatives. Various oxidative couplings of reticuline account for other minor alkaloids (e.g. corytuberine and isoboldine).

**Role of reticuline in alkaloid biosynthesis.** The alkaloids involved in Fig. 26.15 are derived from (−)-reticuline, and the enzymic racemization of reticuline, which is essential for the biosynthesis of the principal opium alkaloids, is very substrate specific. Thus the *N*-ethyl, 6-ethoxy and 4′-ethoxy analogues of reticuline are completely resistant to racemization. (+)-Reticuline also gives rise to a

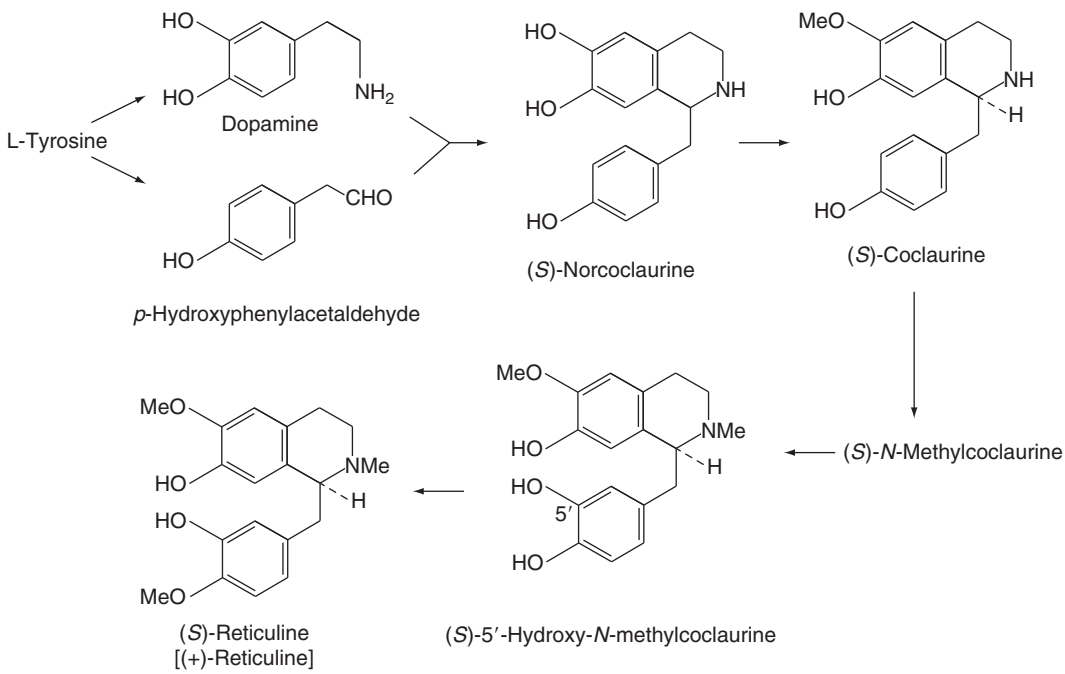



**Fig. 26.16**
A revised biogenetic route from L-tyrosine to (*S*)-reticuline.

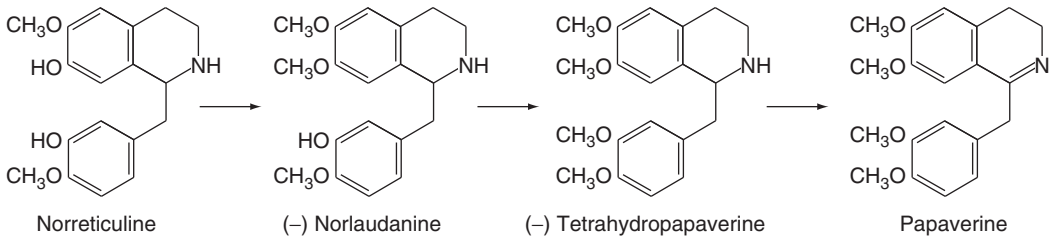



**Fig. 26.17**
A major pathway in the biogenesis of papaverine.

number of bases: narcotine (noscopine) and narceine of opium; canadine, berberine and hydrastine of *Hydrastis* (Berberidaceae); and sinomenine (the enantiomer of the opium alkaloid salutaridine, Fig. 26.15, of *Sinomenium acutum*, Menispermaceae). With the exception of sinomenine, these alkaloids are termed 'berberine bridged' alkaloids and they arise from norlaudanosoline and a one-carbon unit, which is derived from the *N*-methyl group of (+)-reticuline. The methylenedioxy group of these alkaloids is formed by oxidative cyclization of an *o*-methoxyphenol function. A scheme indicating the origin of some of these alkaloids is given in Fig. 26.18. For berberine, 13 enzymes are involved in its biosynthesis from two molecules of tyrosine. The enzyme associated with the final reaction, the formation of the methylenedioxy bridge, has now been detected in microsomal preparations from different Ranunculaceae and Berberidaceae cell cultures (M. Rueffer and M. H. Zenk, *Phytochemistry*, 1994, **36**, 1219).

**Enzyme studies.** As indicated in Chapter 13 cell cultures of *P. somniferum* do not produce morphine-type alkaloids but accumulate large amounts of sanguinarine and other alkaloids. However, some of the enzymes of the morphinan pathway are present in such cell cultures as shown by the reduction of codeinone to codeine by immobilized cells of *P. somniferum* and by the isolation from cell cultures of the enzyme which reduces salutaridine to (7*S*)-salutaridinol. This enzyme has been fully characterized (R. Gerardy and M. H. Zenk, *Phytochemistry*, 1993, **34**, 125) as has 1,2-dehydroreticuline reductase isolated from poppy seedlings. The same group has shown (R. Wilhelm and M. H. Zenk, *Phytochemistry*, 1997, **46**, 701) that the enzyme necessary for the enol cleavage in the conversion of thebaine to neopinone (Fig. 26.15) is present in cell cultures, as evidenced by the formation of a new alkaloid, thebainone, as a result of feeding labelled thebaine to low-thebaine producing *P. somniferum* cultures. For work on genes encoding (*S*)-*N*-methylcoclaurine-5′-hydroxylase and codeinone reductase see F.-C. Huang and T. M. Kutchan, *Phytochemistry*, 2000, **53**, 555.

**Tests for opium alkaloids.** Tests for morphine and other alkaloids are given in the pharmacopoeias. The solubility of morphine in sodium hydroxide solution is explained by its phenolic nature. Conversely, codeine is precipitated by sodium hydroxide.

**Storage.** Opium requires careful storage if the morphine content is to be maintained. In the past, Indian opium appears to have suffered more morphine loss during preparation and storage than the other varieties, but when it is dried at 100°C and stored out of contact with air, the loss of morphine is small. Abraham and Rae (1926) ascribe the loss

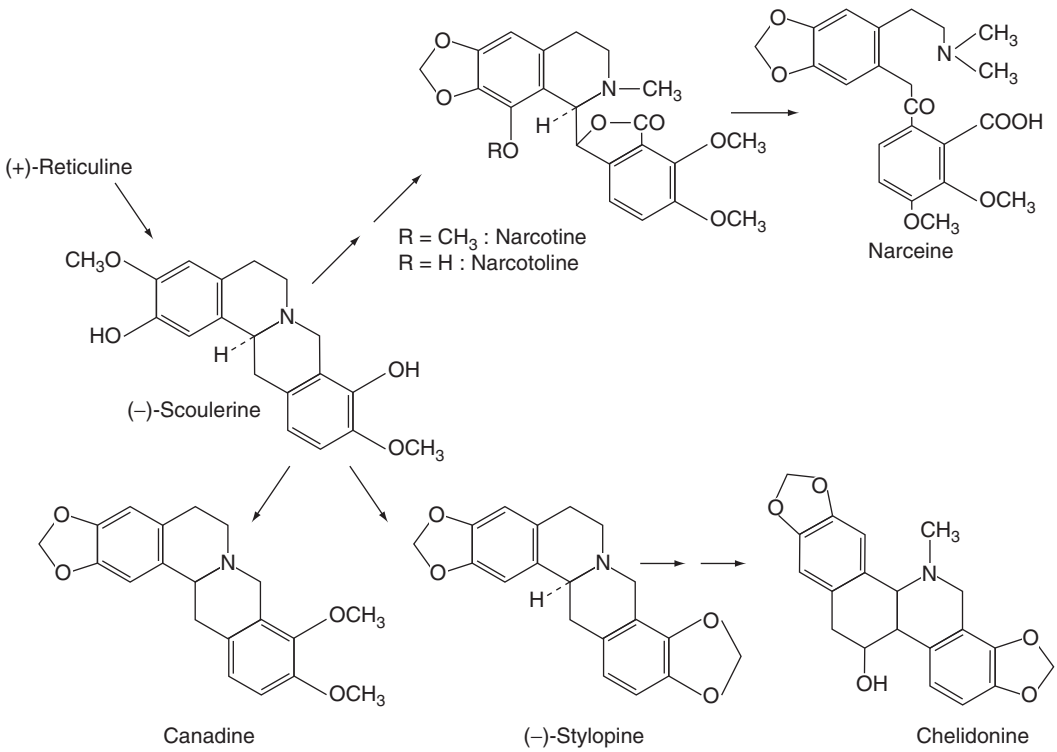



**Fig. 26.18**
Some alkaloids derived from (+)-reticuline.

of morphine to a peroxidase which they called opiase. More recently a phenoloxidase which acts on morphine has been isolated from poppy capsules.

**Adulteration.** Opium has been adulterated with sugary fruits, gum, powdered poppy capsules and other substances too numerous to mention. As far as legitimate commerce is concerned, such adulteration is now pointless because the product is analysed and the price paid is governed by the content of morphine and other alkaloids.

**Uses.** The alkaloids present in opium in greatest proportion decrease in narcotic properties in the order morphine, codeine, noscopine. Opium and morphine are widely used to relieve pain and are particularly valuable as hypnotics, as, unlike many other hypnotics, they act mainly on the sensory nerve cells of the cerebrum. Codeine is a milder sedative than morphine and is useful for allaying coughing. Both morphine and codeine decrease metabolism, and the latter, particularly before the introduction of insulin, was used for the treatment of diabetes. Opium, while closely resembling morphine, exerts its action more slowly and is therefore preferable in many cases (e.g. in the treatment of diarrhoea). Opium is also used as a diaphoretic. The habitual use of codeine may, in some individuals, produce constipation.

**Manufacture of opium alkaloids.** The majority of legal opium is used for the isolation of its constituent alkaloids, and in Britain some 90% of the morphine produced is converted into other bases such as codeine, ethylmorphine and pholcodine. In recent years attempts have been made to reduce the illicit traffic in opium either by banning the cultivation of the opium poppy or by cultivation under strict licences. However, two methods by which the opium stage is eliminated are by extraction of the whole poppy capsule and by the use of other species (e.g. *P. bracteatum*) which do not contain morphine.

**Extraction of poppy capsules and straw.** The feasibility of extracting poppy straw has long been known and utilized in Europe and recently there has been a world-wide trend towards the extraction of the dried poppy capsules (e.g. in Hungary, former USSR, Tasmania). In a study of poppy capsule drying and storage under commercial conditions in Tasmania it has been shown that kiln-drying of immature capsules (42 days old) at various temperatures (40–100°C) resulted in a loss of morphine content of up to about 11% without effect on codeine and thebaine. However, this morphine loss was significantly less than with the field-dried material. To avoid deterioration of the dried product the moisture content should not exceed 16%. Processes have also been developed in France and in the UK for the harvesting and processing of the green capsule, one technical difficulty being the separation of the seed from the fruit at this stage of development.

**Use of morphine-free species of *Papaver*.** The increasing abuse of opiates has stimulated the search for raw materials other than *Papaver somniferum* which would meet the requirements of the pharmaceutical industry. Thus, plants containing non-addictive thebaine as principal alkaloid could be used for the manufacture of codeine, naloxone (a narcotic antagonist prescribed for babies of heroin addicts) and etorphine (a 'Bentley' compound used for sedating large wild animals).

In this respect attention focused on three closely related perennial species of *Papaver* of the section *Oxytona* Bernh. of the family, namely, *P. bracteatum*, *P. orientale* and *P. pseudo-orientale*. These are indigenous to the mountainous districts of Iran, eastern Turkey and the Transcaucasian former USSR. Confusion concerning the identity of the characteristic alkaloids for each species had arisen from various factors, including incorrect identification, variations of chromosome number within a species, the existence of chemical races and geographical influences.

### *Papaver bracteatum*

Thebaine is the predominant alkaloid of this species and in a UN-backed programme of large-scale cultivation trials were organized in various countries. High yielding strains were introduced, for example, Ayra II, a race obtained from west Iran in 1974 which gives 3.5% thebaine in the dried capsules. Problems associated with the development were the insufficiency of seed of high-yielding strains and the poorer crops obtained when the plants were removed from their normal environment. However, political decisions also jeopardized the continuation of the programme.

*P. bracteatum* produces some 27 alkaloids belonging to 10 of the 14 alkaloid groups described for *Papaver*. The biogenesis of thebaine follows the same pathway as in *P. somniferum*. Feeding experiments with labelled intermediates have shown that the plant is capable of converting codeinone to codeine but cannot perform either of the demethylations leading to codeine or directly to morphine. Thebaine does not appear to be entirely an end-product and undergoes further metabolism to unknown products (for a report, see H. G. Theuns *et al*., *Phytochemistry*, 1985, **24**, 581). A more recently described new alkaloid is salutaridine-*N*-oxide (G. Sariyar *et al*., *Planta Med*., 1992, **58**, 368).

### *Papaver orientale*

This species is commonly cultivated as the ornamental poppy and a number of alkaloid chemotypes have been described. Generally, oripavine (formed by demethylation of the aromatic ring of thebaine, Fig. 26.15) is the principal alkaloid, but Phillipson *et al*. (*Planta Med*., 1981, **43**, 261) described one chemotype with mecambridine (a berberine type alkaloid) as principal constituent.

### *Papaver pseudo-orientale*

The plant ($2n = 42$) is intermediate in many of its characters between those of the above two species and may have arisen as an allohexaploid from them. Phillipson (above reference) divided 16 Turkish samples in three cytological and alkaloid groups. Thirteen samples contained isothebaine, mecambridine and orientalidine as major alkaloids, two contained principally salutaridine and thebaine, and one sample possessed salutaridine as the major component. (For other alkaloids and biogenetic transformations see G. Sźariya *et al*., *Phytochemistry*, 1986, **25**, 2403.)

(For a comprehensive review of the morphine alkaloids covering general chemistry, biogenesis, occurrence and structure elucidation (391 refs) see C. Sźantay *et al*., *The Alkaloids*, 1994, **45**, 128.)

## BOLDO LEAVES

Boldo leaves *BP*/*EP* are derived from *Peumus boldus* (Monimiaceae); they contain aporphine-type alkaloids, chiefly boldine. The drug has antihepatoxic activity and is described in Chapter 29.

## GOLDENSEAL ROOT

Goldenseal root *BP*/*EP*, *BHP* 1990 consists of the dried rhizome and roots of *Hydrastis canadensis* (Berberidaceae), a small perennial plant indigenous to the woods of eastern Canada and the eastern USA.

The wild plants have been exterminated in many districts and the species now has CITES listing (q.v.) for the USA and Canada. Most of the commercial drug is now obtained from cultivated plants grown in America and in Europe. The use of hydrastis, both as a drug and as a dye, was learned by the early European settlers from the Cherokee Indians.

**Characters.** The drug consists of almost cylindrical rhizomes about 1–5 cm long and 2–10 mm diameter. The rhizomes grow more or less obliquely and bear numerous, short branches, which terminate in cup-shaped scars and bear encircling cataphyllary leaves. Similar scale leaves are found on the rhizome, the outer surface of which is yellowish-brown or greyish-brown. The roots, which originate on the ventral and lateral surfaces, are long and wiry, and in the commercial drug are often broken at a distance of a centimetre or so from the rhizome. The drug breaks with a short, waxy fracture. It has a slight but distinctive odour and bitter taste.

A transverse section of the rhizome shows a fairly thick, yellow or yellowish-brown bark; 12–20 radially elongated, bright yellow wood bundles, separated by wide medullary rays; a large pith.

**Constituents.** Hydrastis contains the alkaloids hydrastine, berberine, canadine and other minor ones. Commercial samples yield 1.5–4% of hydrastine and 0.5–6.0% of berberine. The latter, as a constituent of an extract of the root, is responsible for activity against multiple drug resistant *Mycobacterium tuberculosis* (see E. J. Gentry *et al*., *J. Nat. Prod*., 1998, **61**, 1187)

The *BP*/*EP* requires a minimum content of 2–5% (dried drug) for hydrastine and a minimum of 3.0% for berberine. These are determined by liquid chromatography with absorbance measurements at 235 nm using a reference solution of the above alkaloids for comparison. These alkaloids are also identified in the TLC test for the drug using visualization with UV light at 365 nm.

**Uses.** The use of hydrastis to check uterine haemorrhage, as a bitter stomachic and locally in the treatment of catarrhal conditions of the genito-urinary tract is largely based on empirical observations. Hydrastine hydrochloride and hydrastinine hydrochloride have been used in various forms to control uterine haemorrhage.

## FUMITORY

Fumitory *BP*/*EP*, *BHP* consists of the dried aerial parts of *Fumaria officinalis* L., family Fumariaceae, collected when in flower. It is an annual herb common as a weed and on roadsides throughout most of Europe; it has spread world-wide. The leaves are greyish-green, stalked, each pinnately divided several times giving flattened lanceolate, often toothed segments. The flowers are arranged in racemes with pink, red-tipped petals forming a tubular corolla. Fruits are somewhat flattened achenes, 2.0–2.5 mm, with rough surfaces. Microscopical features of the olive-green powder include leaf epidermi with anomocytic stomata and spherical pollen grains, about 35–40 µm in diameter, with pitted exine and six large pores; also in abundance are features of the stems, flowers and fruits.

**Constituents.** A range of isoquinoline-type alkaloids includes protoberberines, spirobenzylisoquinolines, benzophenanthridines and indenobenzazepines. Protopine (Fig. 26.19) is the principal alkaloid, the *BP*/*EP* requiring a minimum total alkaloid content of 0.40% calculated as this alkaloid. A titrimetric assay is employed, sonication being used for dissolving the plant extract produced in the alkaloid purification procedure.

Other constituents include flavonoids, principally glycosides of quercetin, and acids, including chlorogenic and caffeic acids.

**Uses.** Fumitory is employed for its choleretic action, see Chapter 29.

## GREATER CELANDINE

Greater celandine *BP*/*EP* consists of the dried whole or cut aerial parts of *Chelidonium majus* L. family Papaveraceae, collected parts of *Chelidonium majus* L. family Papaveraceae, collected during the flowering period. There is a minimum requirement of 0.6% for total alkaloids expressed as chelidonine.

This perennial herb is widespread throughout Europe, northern Asia and the north-eastern USA, growing along banks, in hedgerows, against walls and in waste areas. The hollow, ribbed stems are up to about 90 cm in height, branched and leafy. When fresh, the stems and leaves exude a milky sap, which turns to orange–red

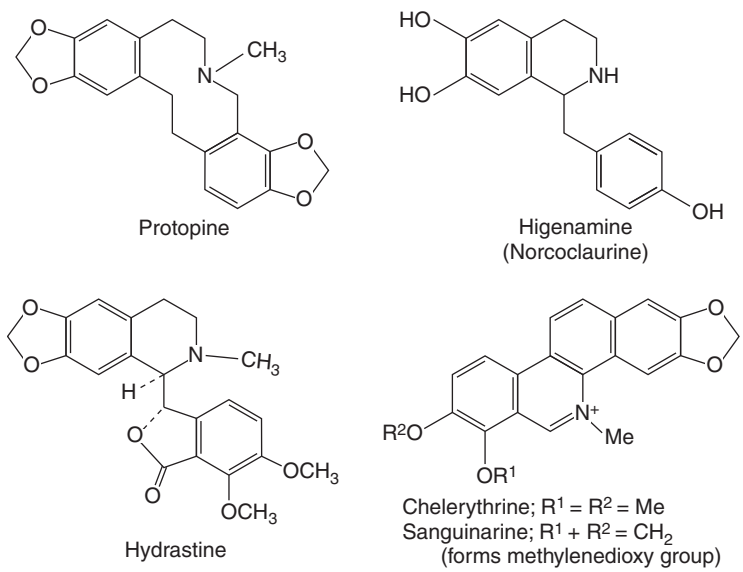



**Fig. 26.19**
Selected alkaloids of drugs described in the accompanying text.

when exposed to the air and is a skin irritant. The leaves are almost pinnate and divided into five to seven ovate to oblong leaflets, the terminal ones often three-lobed; margins are crenately toothed, the upper surfaces glabrous and dark green and the lower somewhat glaucous. The bright yellow flowers, 2–2.5 cm in diameter each have two greenish-yellow sepals and four petals. Stamens are yellow and numerous. Fruit is a capsule 3–5 cm in length, containing black seeds having a white appendage; mature fruits are rarely to be found in the drug.

Microscopic features include: anomocytic stomata on the lower leaflet surfaces, uniseriate possibly fragmented covering trichomes, vascular tissues, associated latex canals with brown contents, corolla fragments with oil droplets, spherical three-pored pollen grains up to 40 μm in diameter.

**Constituents.** Greater celandine contains up to 4% of alkaloids including α- and β-allocryptopine, berberine (Fig. 28.1), chelerythrine (Fig. 26.19), chelidonine (Fig. 26.18), chelirubine, choline, coptisine, hydroxychelidonine, hydroxysanguinarine, protopine (Fig. 26.19), sanguinarine (Fig. 26.19), sparteine, (Fig. 26.12) and others.

The *BP*/*EP* TLC test for identity produces a number of unidentified separated components; the assay for total alkaloids is performed on an extract of the drug treated in acid solution with the *BP* chromotropic, sodium salt reagent and absorbance measurements at 570 nm.

**Uses.** The drug has many traditional uses based on its reputed anodyne, antispasmodic, caustic, diaphoretic, diuretic, hydragogue, narcotic and purgative properties, Some of these activities have support from pharmacological tests involving particular alkaloids. *C. majus* is also used in homoeopathic practice and in Chinese medicine.

### *Annona squamosa* (Annonaceae)

Various parts of this plant have featured in the folk medicine of Africa, India and the Far East for the treatment of a number of conditions including heart disorders. The cardioactive effect has been attributed to the alkaloid higenamine (Fig. 26.19) an important precursor of a number of other isoquinolines.

### Bloodroot

Bloodroot consists of the dried rhizomes and roots of *Sanguinaria canadensis* (Papaveraceae), a perennial herb widely distributed in the woods of North America. The drug consists of dark brown, more or less cylindrical pieces of rhizome, 2–7 cm long and 5–15 mm diameter. Some of the pieces are branched and some show numerous wiry roots. The latter, however, are usually broken off in the commercial drug. The rhizome breaks with a short fracture and, if not overheated during drying, shows numerous red dots (secretion cells) distributed throughout the starch-containing parenchyma of the bark and large pith. If dried at too high a temperature, the secretion escapes from its containing cells and the whole section assumes a deep red or brownish-red colour. A ring of small, yellow, fibrovascular bundles lies about 1 mm from the outside. Odour, slight; taste, acrid and bitter. Bloodroot contains the benzophenanthridine alkaloids sanguinarine, chelerythrine (Fig. 26.19), allocryptopine, protopine and dihydrosanguilutine. Sanguinarine and chelerythrine, although themselves colourless, form red and yellow salts, respectively. The drug also contains red resin and starch.

In a report by Rho *et al*. (*Appl. Microbiol. Biotechnol*., 1992, **36**, 611) *S. canadensis* cultured cells produced sanguinarine (about 80% of the total alkaloid) together with chelirubine and chelerythrine. In contrast, in the normal rhizome sanguinarine and sanguirubine together account for some 70% of the total alkaloids. (For elicitor studies see G. B. Mahady and C. W. W. Beecher, *Phytochemistry*, 1994, **37**, 415.)

Bloodroot is used mainly in the USA, where it is an ingredient of Compound White Pine Syrup. Sanguinarine, like colchicine, causes the doubling of the chromosomes in cells.

### Calumba root

Calumba is the dried, sliced root of *Jateorhiza palmata* (*J. columba*) (Menispermaceae), a dioecious climbing plant indigenous to the forests of Mozambique and Madagascar (Malagasy Republic) and other east African countries. It is exported to Europe from Tanzania and the name derives from the fact that it was at one time exported from Colombo (Sri Lanka).

**Collection.** Attempts to cultivate the drug in various areas do not appear to have been successful and collection is from the wild. The plant possesses a somewhat slender rhizome from which numerous large fusiform roots arise. The older reports state that these are dug up during dry weather (March), the rhizomes are rejected and the roots cut into transverse or oblique slices and dried in the shade. The imported 'natural calumba' is frequently washed, brushed and graded, the product being known as 'washed calumba'.

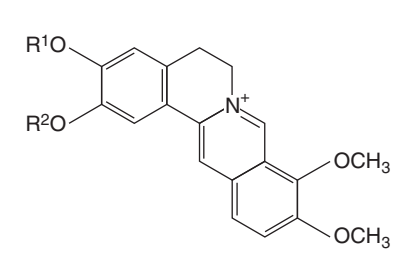
**Macroscopical characters.** Calumba occurs in circular or oblique slices. These are usually 2–8 cm diameter and 3–12 mm thick.

The cork is thin, greyish-brown or reddish-brown in colour and longitudinally wrinkled. Within it lies a broad, greenish-yellow zone which extends to the cambium and contains in its outer part isolated sclerenchymatous cells within which are dark-grey, sinuous strands of sieve tissue. The greyish wood, which is separated from the bark by a dark cambium line, shows numerous narrow, radiating lines of yellow vessels separated by abundant parenchyma. The vessels are close together in the region near the cambium and again in the extreme centre of the root, but they are less numerous in the intermediate zone, which therefore shrinks considerably and becomes depressed on drying. Some pieces show two or more concentric zones of wood. The fracture is short and starchy; odour, slight and somewhat musty; taste, bitter.

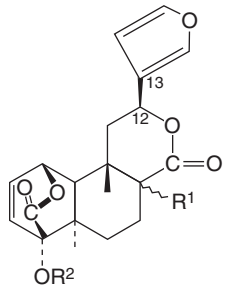
Calumba frequently contains occasional slices of *calumba rhizome*. These average about 2–3 cm diameter. The structure is markedly radiate and, owing to its greater woodiness in that region, is not depressed in the centre.

**Microscopical characters.** The drug is characterized by the sclereids which have unevenly thickened, yellow walls and contain a number of prisms of calcium oxalate, by abundant parenchymatous cells containing starch grains, each grain measuring about 20–85 μm long and having an eccentric, very distinct radiate or cleft hilum, and by the yellow reticulate vessels. The walls of both the sclerenchymatous cells and vessels on treatment with 66% v/v sulphuric acid change colour from yellow to green.

**Constituents.** Calumba contains about 2–3% of isoquinoline alkaloids, palmatine, jatrorrhizine and columbamine. Bisjatrorrhizine is a quaternary dimeric alkaloid formed by *ortho*-oxidative coupling of the phenolic group of jatrorrhizine. Other constituents are the non-alkaloidal furanoditerpenes columbin, isocolumbin, palmarin, chasmanthin, jateorin and isojateorin. Some of these occur as glucosides and have been named palmatosides A to G.




Palmatine: $R^1 = R^2 = CH_3$
Jatrorrhizine: $R^1 = H$, $R^2 = CH_3$
olumbamine: $R^1 = CH_3$, $R^2 = H$




Columbin: $R^1$ = ◀ H, $R^2 = H$
Isocolumbin: $R^1$ = — H, $R^2 = H$
Columbinyl glucoside: $R^1$ = ◀ H, $R^2$ = glycosyl

Other diterpenes are similar isomers differing in the positions of the epoxide ring and in the stereochemistry of the C-12–C-13 bond.

**Uses.** Calumba is used as a bitter tonic and, as it contains no tannin, may be prescribed with iron salts. In the *BHP* it is specifically indicated for anorexia and flatulent dyspepsia.

### Serpentary

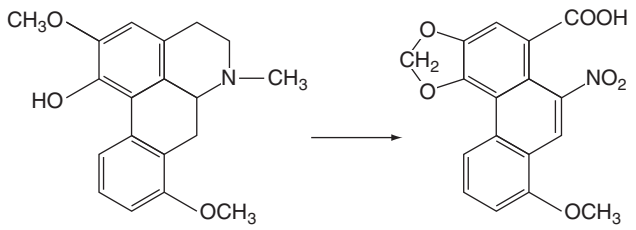
Serpentary consists of the dried rhizome and roots of *Aristolochia reticulata* (Aristolochiaceae). This is known in commerce as Texan or Red River snake-root and is collected in the woods of Texas, Louisiana, Arkansas and Oklahoma.

**Macroscopical characters.** The drug has a yellowish colour when fresh, becoming brown on keeping. It consists of small rhizomes bearing the remains of subaerial stems and numerous wiry roots. The rhizomes are about 1–2 cm long and 2–3 mm diameter, while the roots are about 10 cm long and 0.2–1.2 mm diameter. The drug contains up to 10% of subaerial stems. Odour, camphoraceous; taste, camphoraceous and bitter.

A transverse section of the rhizomes shows a starchy, eccentric pith (nearer the upper surface of the rhizome than the lower), wedge-shaped, yellowish vascular bundles separated by wide medullary rays, and a narrow bark.

**Allied drugs.** *Virginian snake-root*, from *Aristolochia serpentaria*, was formerly official but its regular importation has now ceased. It closely resembles the Texan drug, but has smaller rhizome and more wiry roots. *Indian aristolochia* or Indian birthwort consists of the roots and rhizome of *Aristolochia indica*; it contains aristolochic acid together with other phenanthrene derivatives, an *N*-glycoside and steroids. *A. heterophylla* is used in China, and *A. constricta*, recently investigated (L. Pastrelli *et al*., *J. Nat. Prod*., 1997, **60**, 1065), is widely employed in folk medicine in S. America. *A. clematis* (birthwort) is European and has been used both internally and externally. It contains similar constituents to other species.

**Constituents.** Many species of *Aristolochia* including *A. reticulata* contain aristolochic acid and the tumour-inhibiting properties of this compound are of interest. However, in experimental animals it can cause tumour formation and has been associated with cases of renal failure. Aristolochic acid is not alkaloidal, belonging to a small group of naturally occurring nitro-compounds, but is included here because of its direct derivation from isothebaine derivatives in the plant.




Isothebaine isomer — Aristolochic acid

(For a review on the structure of the aristolochic acids and their corresponding lactams (aristolactams) see D. B. Mix *et al*., *J. Nat. Prod*., 1982, **45**, 657 and for the isolation of aristolochic acids I–IV and aristolactams I–III from *A. auricularis* see P. J. Houghton and M. Ogutveren, *Phytochemistry* 1991, **30**, 253.)

**Uses and dangers.** Snakeroot has been traditionally employed as an aromatic bitter but other *Aristolochia* spp. have also been employed in Chinese herbal medicines for various treatments. As from 28 July 1999 an emergency ban on the import, sale and supply of medicinal products containing *Aristolochia* spp. came into force for the UK. This arose in part from two UK cases of end-stage renal failure in patients using Chinese medicines containing *Aristolochia* and from many cases of renal failure in Belgium when *Aristolochia* was substituted for *Stephania* in a herbal preparation. Apparently in Chinese herbal preparations it is liable to be substituted for other innocuous components such as *Stephania*, *Akebia* or *Clematis* (MCA/CSM, UK, *Current Problems in Pharmacovigilance*, 1996, **22**, 10; see also the report, *Pharm. J*., 2000, **265**, 10).

## CURARE

The term ‘curare’ is a generic one applied to various South American arrow poisons. These extracts are made from a number of different plants, particularly members of the Menispermaceae (e.g. *Chondrodendron*) and the Loganiaceae. From the former, tubocurarine is obtained and the (+)-hydrochloride of this is included in the *BP*/*EP* as a muscle relaxant.

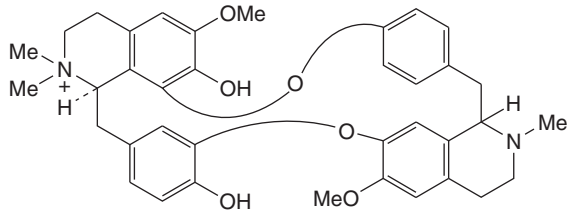
Curares from the upper Amazon (Brazil and Peru) seem to be mainly menispermaceous in origin. The original genus *Chondrodendron* has been divided into two by Barneby and Krukoff (*Mem. N. Y. Bot. Gdn*, 1971, **22**, 1); *Chondrodendron* itself with three species (*Ch. tomentosum*, *Ch. platyphyllum*, *Ch. microphyllum*) and *Curarea* with four species (*Cu. toxicofera*, *Cu. candicans*, *Cu. tecunarum* and *Cu. cuartecasasii*).

The principal ones used in curare are *Ch. tomentosum* (the only plant known to contain tubocurarine), and the first three of the *Curarea* species. Several other genera of Menispermaceae are known to have been, or to be, ingredients of curares including species of *Sciadotenia*, *Abuta*, *Telitoxicum* and *Cissampelos*, but little is known about the muscle-relaxant activity, if any, of their alkaloids.

The curares from Guyana, Venezuela and Colombia owe much of their activity to species of *Strychnos* (Loganiaceae); around 20 species are known to have been incorporated into curares and these include *S. toxifera*, *S. jobertiana*, *S. peckii* and *S. guianensis*.

(For reviews covering the history, uses, botany and chemistry of curare see N. G. Bisset, *J. Ethnopharmacology*, 1992, **36**, 1; *Alkaloids*, *Chem. Biol. Perspect*., 1992, **8**, 1.)

**History.** There is a close botanical relationship between Pareira Brava Radix of the seventeenth- and eighteenth-century pharmacopoeias and the menispermaceous plants yielding curare. In both cases the botanical source of the drugs has long been in doubt. It is now known that Pereira Brava is obtained from two species of *Chondrodendron*, namely, *C. microphyllum*, which contains (+)-bebeerine, and *C. platyphyllum*, which contains (−)-bebeerine. A similar case is the so-called *C. tomentosum*, which sometimes yields



(+)-Tubocurarine

(+)-tubocurarine and sometimes the less active (−)-tubocurarine. This led King (1948) to write: 'It seems very probable therefore that two species are involved under the name *C. tomentosum*'.

Boehm (1895) distinguished three kinds of curare differentiated first by their containers, and second by their different chemical characteristics.

1. *Tube-curare*, packed in bamboo tubes. This came from Brazil and Peru, was mainly menispermaceous in origin and contained the alkaloid which Boehm (1895) isolated as amorphous 'tubocurarine' and which King (1935) first prepared crystalline as tubocurarine chloride.
2. *Calabash-curare* is packed in gourds and comes mainly from Guiana, Venezuela and Columbia. It was formerly the type of curare most commonly found in commerce. Chemical investigations of Wieland *et al*. (1937–41), King (1949) and Karrer (1946–54) show that *Strychnos* species furnish important constituents.
3. *Pot-curare*, which is no longer a commercial article, was packed in earthenware pots. These varied in size and were glazed, unglazed or ornamented with paint. King's examination of one small pot led him to the conclusion that it was menispermaceous in origin and contained no *Strychnos* spp. However, Bauer (1969, 1981) showed in an extensive investigation of museum samples of curare that pot curares were usually of mixed Loganiaceae/Menispermaceae origin.

**Curare in tins.** Much curare has been imported in tins containing about 1 kg of a viscous dark-brown or blackish extract. It has little odour but a very bitter taste.

This drug is similar to the specimen examined by King (1948), who received it from Asher Kates y Cia S.A. of Lima, Peru, together with the leaves of the plant from which it was prepared. These leaves were indistinguishable from those of *C. tomentosum* and its menispermaceous nature, and similarity to the old tube-curare, was confirmed by the isolation of (+)-tubocurarine chloride and four other alkaloids.

**Constituents.** (1) *Menispermaceous* tube-curare and the form now imported in tins contain tubocurarine. From the tin-curare King (1948) isolated (+)-tubocurarine chloride and four non-quaternary bases (isochondrodendrine dimethyl ether, (−)-curine (bebeerine), (+)-chondrocurine and (+)-isochondrodendrine). (2) *Loganiaceous* calabash-curare derives its activity largely from *Strychnos* species, particularly *S. toxifera.* King (1949) has shown that the bark of this species contains 12 crystalline quaternary alkaloids, the toxiferines I–XII, of which two had previously been isolated by Wieland *et al*. (1937–41). These latter authors examined calabash-curare, probably from Venezuela, and isolated several alkaloids known as C-curarines (C signifies calabash). Toxiferines I and II have been found in calabash-curare.

More recently calabash-curares and *Strychnos* spp. have been examined by Karrer and his colleagues with the isolation of a large number of new alkaloids. (+)-Tubocurarine is a bisbenzylisoquinoline alkaloid and as such is derived from dopamine, whereas the Loganiaceous curares are indolic and contain $C_{40}$ compounds of the dimeric strychnine type.

**Uses.** Curare is now little used except as a source of alkaloids. Tubocurarine chloride, official in the *BP/EP*, is used to secure muscular relaxation in surgical operations and in certain neurological conditions.

## TETRAHYDROISOQUINOLINE MONOTERPENOID ALKALOIDS AND GLYCOSIDES

These alkaloids and alkaloid-glycosides derive from the condensation of dopamine with secologanin (a $C_{10}$ monoterpene) to give two series of compounds. The best-known examples of their limited occurrence are in species of *Cephaëlis* (Rubiaceae) and *Alangium* (Alangiaceae). The former gives ipecacuanha root, and the root, bark, fruits and leaves of *A. lamarckii* are used in Ayurvedic medicine for the treatment of a number of conditions.

### IPECACUANHA

Ipecacuanha (*Ipecacuanha Root*) of the *BP* is the dried root or rhizome and root of *Cephaëlis ipecacuanha* (Brotero) A. Richard (Rubiaceae), known in commerce as Matto Grosso Ipecac. or of *Cephaëlis acuminata* Karsten, known in commerce as Costa Rica Ipecac. A mixture of both species is also permissible. It should contain a minimum of 2% of ether-soluble alkaloids.

*C. ipecacuanha* is a shrub 20–40 cm high found over a large area in Brazil, particularly in the moist and shady forests of Matto Grosso and Minãs Geraes; plantations have been established in the Matto Grosso area. It is cultivated to some extent in Malaya, Burma and the Darjeeling Hills of West Bengal. *C. acuminata* is exported from Colombia, Nicaragua and Costa Rica; Costa Rica is at present the principal source of the drug. However India is now in full production of Costa Rican type root which is of high quality (in excess of 3.5% total alkaloid) and extremely competitive in price; extracts of the Indian root are now being produced and exported.

**History.** What appears to have been ipecacuanha was mentioned, under the name of *Igpecaya*, by a Portuguese friar around 1600. It was introduced into Europe in 1672.

**Collection and preparation.** In the Matto Grosso district of Brazil the drug is collected from wild plants. The collector, using a pointed stick, levers the plant from the ground and, having removed most of the roots, replaces it in the ground, where it usually lives to produce further crops. The roots are dried in the sun or by fires and transported down river to ports such as Rio de Janeiro, Bahia and Pernambuco from which they are exported in bales. Other South American ipecacuanhas are collected in a similar way. The supply of S. American ipecacuanha has been erratic for many years as a result of habitat destruction, overcollection and the uprooting of plants. The high price of the drug has stimulated both cultivation in other areas (see above) and the promotion of cell and root cultures for alkaloid production (see below).

**Macroscopical characters.** The underground portion consists of thin, horizontal rhizomes from the lower surface of which roots are given off. Some of the latter remain thin, while others develop an abnormally thick bark and become annulated.

The Matto Grosso drug occurs in tortuous pieces up to 15 cm long and 6 mm diameter, but it is usually smaller. The colour of the outer surface varies from a deep brick-red to a very dark brown, the colour being very largely dependent on the type of soil in which the plant has been grown. Most of the roots are more or less annulated externally, and some have a portion of the rhizome attached (Fig. 26.20A), while separate portions of rhizome and non-annulated roots are also found. Generally, the drug of present-day commerce is less markedly annulated than was formerly the case, a fact that points to earlier collection. The ridges are rounded and completely encircle the root; here and there the bark has completely separated from the wood.

The root breaks with a short fracture and shows a thick greyish bark and a small, dense wood, but no pith. The rhizomes, on the other hand, have a much thinner bark and a definite pith (Fig. 26.20C, D). The drug has little odour, but is irritating and sternutatory when in fine powder, and has a bitter taste.

The Costa Rica drug (Fig. 26.20B) is exported from Cartagena and Savanilla. The main differences between the Rio and Cartagena drugs are listed in Table 26.5.

*Ipecacuanha stems*, although containing the same alkaloids as the roots, usually contain them in smaller proportion. An excessive amount of stem must, therefore, be regarded as an adulteration.

**Microscopical characters.** A transverse section of the root (Fig. 26.20C) shows a thin, brown cork, the cells of which contain brown, granular material. Within this is a wide secondary cortex (phelloderm), the cells of which are parenchymatous and contain starch, usually in compound grains with from two to eight components, or raphides of calcium oxalate. The individual starch grains are muller-shaped and up to 15 or 20 µm diameter. The phloem is entirely parenchymatous, containing no sclerenchymatous cells or fibres. The compact central mass of xylem is composed of small tracheidal vessels, tracheids, substitute

**Table 26.5 Comparison of ipecacuanha (*Cephaëlis*) roots.**

| | *C. ipecacuanha* | *C. acuminata* |
|---|---|---|
| Usual diameter | 1–4 mm | 4–6.5 mm |
| Colour | Brick-red to brown | Greyish-brown |
| Annulations | Very crowded | Less crowded and less projecting |
| Starch | Individual grains up to 15 µm | Individual grains up to 20 µm |

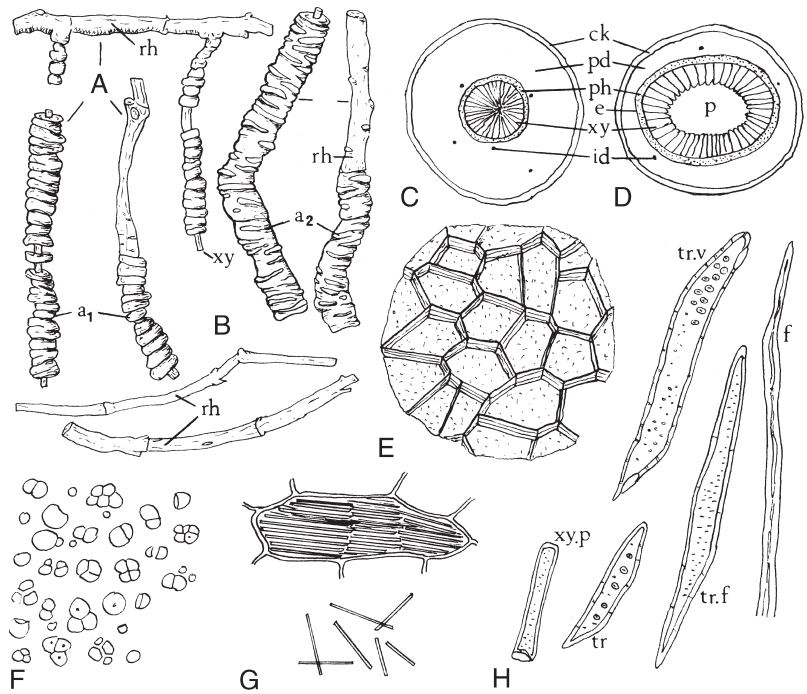



**Fig. 26.20**
Ipecacuanha. A, *Cephaëlis ipecacuanha* roots with rhizome; B, *C. acuminata* roots with rhizome (both ×1); C, transverse section of root; D, transverse section of rhizome (both ×4); E, cork cells in surface view; F, starch granules (mounted in cold lactophenol); G, idioblast containing calcium oxalate crystals; H, elements from Schultze maceration of wood (all ×200). $a_1$, Complete annulation of *C. ipecacuanha*; $a_2$, incomplete annulation of *C. acuminata*; ck, cork; e, endodermis; f, fibrous cell; id, idioblast containing calcium oxalate; p, pith; pd, phelloderm; ph, phloem; rh, rhizome; tr.v, tracheid vessel; xy, xylem; xy.p, xylem parenchyma.

fibres, xylem fibres and xylem parenchyma. Starch is present in the xylem parenchyma and substitute fibres contain starch (Fig. 26.20E–H).

The transverse section of ipecacuanha rhizome (Fig. 26.20D) shows the presence of a ring of xylem and a large pith. The pericycle contains characteristic sclerenchymatous cells. Spiral vessels occur in the protoxylem. The pith is composed of pitted parenchyma which shows some lignification.

**Adulterants.** At one time other 'ipecacuanhas' were regularly imported, the name being applied in South America to a number of different roots which were reputed to have emetic properties. Most of these, briefly described in the 10th edition, are very easily distinguished from the genuine drug and are now rarely imported.

**Constituents.** Ipecacuanha contains the alkaloids emetine (Pelletier and Magendie, 1817), cephaëline (Paul and Cownley, 1894), psychotrine, psychotrine methylether and emetamine. These are isoquinoline derivatives of a group only known with certainty to occur in the families Alangiaceae, Icacinaceae and Rubiaceae. However, emetine-type alkaloids are not necessarily a characteristic of the genus *Cephaëlis* as a whole as Solis *et al*. (*Phytochemistry*, 1993, **33**, 1117) recorded a new *indole* alkaloid and four other known indole alkaloids from the aerial parts of *C. dichroa* from Western Panama.

In a review (411 refs) of the ipecacuanha and related bases (T. Fujii and M. Ohba, *The Alkaloids*, 1998, **51**, 271) 39 new alkaloids from ipecacuanha and *Alangium* are recorded for the 14 years to 1997. For further new alkaloids of *A. longiflorum*, see for example N. Sakurai *et al*., *Phytochemistry*, 2006, **67**, 894.

Other constituents of the official drug are monoterpenoid isoquinoline glucosides including ipecoside (Fig. 26.22), alangiside and, reported in a series of papers (1989–), a number of other novel glycosides closely related to the known ipecacuanha alkaloids (A. Itoh *et al*., *Phytochemistry*, 2002, **59**, 91 and the refs cited therein). The iridoid glucosides sweroside and 7-dehydrologanin together with starch and calcium oxalate are also found in the root. Ipecacuanhin and ipecacuanhic acid, originally designated glycosidal tannins, are now thought to have been impure mixtures of ipecoside and sucrose.

As may be seen from Fig. 26.21, the principal alkaloids are closely related to one another; emetine and psychotrine methylether are non-phenolic, whereas cephaëline and psychotrine are phenolic.

Thus, emetine, which is the alkaloid usually required in medicine, may be prepared by methylating the cephaëline originally present in the drug. These alkaloids may be regarded as being formed in the plant from two phenylethylamine units and a $C_9$ terpenoid precursor. The latter is provided in the plant by secologanin and is incorporated via desacetylisoipecoside into the emetine alkaloids (Fig. 26.22). The glucosidic compound ipecoside is formed from the epimer desacetylipecoside (cf. Fig. 26.22, which illustrates the involvement of secologanin in the formation of some indole alkaloids; in this case, however, only the α-epimer, strictosodine, is formed, but it can serve as a precursor for alkaloids with β-configuration). The isolation by Itoh *et al*., (*Chem. Pharm. Bull*., 1994, **17**, 1460) of tetrahydroisoquinoline-monoterpenoid glucosides, from *Alangium lamarckii* fruits, with the same stereo-configuration as desacetylisoipecosides supported the role of the latter as an intermediate in the formation of emetine-type alkaloids. Furthermore cell-free extracts of *A. lamarckii* have been shown to contain two enzyme activities promoting the condensation of dopamine and secologanin to give the (*S*)- and (*R*)-products respectively (W. De-Eknamkul *et al*., *Phytochemistry*, 1997, **45**, 477).

Various varieties of ipecacuanha contain different proportions of the principal alkaloids. Thus, the Rio drug, which is now difficult to obtain commercially and is the most esteemed, contains 2–2.4% alkaloids, of which 60–75% is emetine. Those varieties derived from *C. acuminata* yield 2–3.5% alkaloids, of which emetine may constitute 30–50%.

**Cell and root cultures.** Production of the ipecacuanha alkaloids by artificial culture would be commercially highly desirable and some research in this area has been reported. The composition of the culture medium and the nature of the added hormones greatly influences alkaloid production but generally root cultures appear to be more satisfactory than callus or suspension cultures. Increased growth of roots is obtained by the induction of hairy roots. In contrast to whole roots, cell cultures produce more cephaëline than emetine, and immobilized cell systems give higher amounts of cephaëline compared with static cell cultures. (For original research papers see K. Yoshimatsu *et al*., *Phytochemistry*, 1991, **30**, 507; S. Jha *et al*., *ibid*., **30**, 3999; C. Veeresham *et al*., *ibid*., 1994, **35**, 947.) The roots of *C. ipecacuanha* transformed with *Agrobacterium rhizogenes* grow well in a gamboge medium yielding 112 mg/l of cephaeline and 14 mg/l of emetine after eight weeks of culture (K. Yoshimatsu *et al.*, *Planta Med.*, 2003, **69**, 1018).

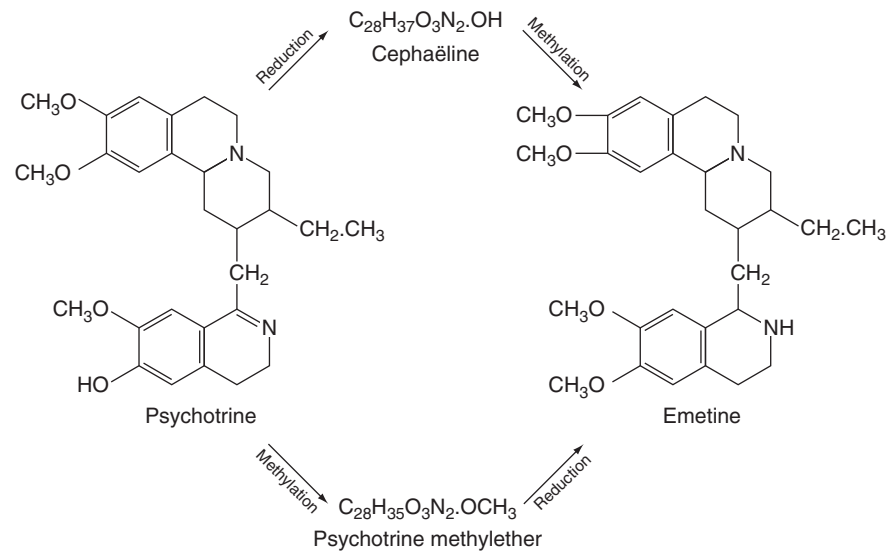



**Fig. 26.21**
Relationship between the principal alkaloids of ipecacuanha.

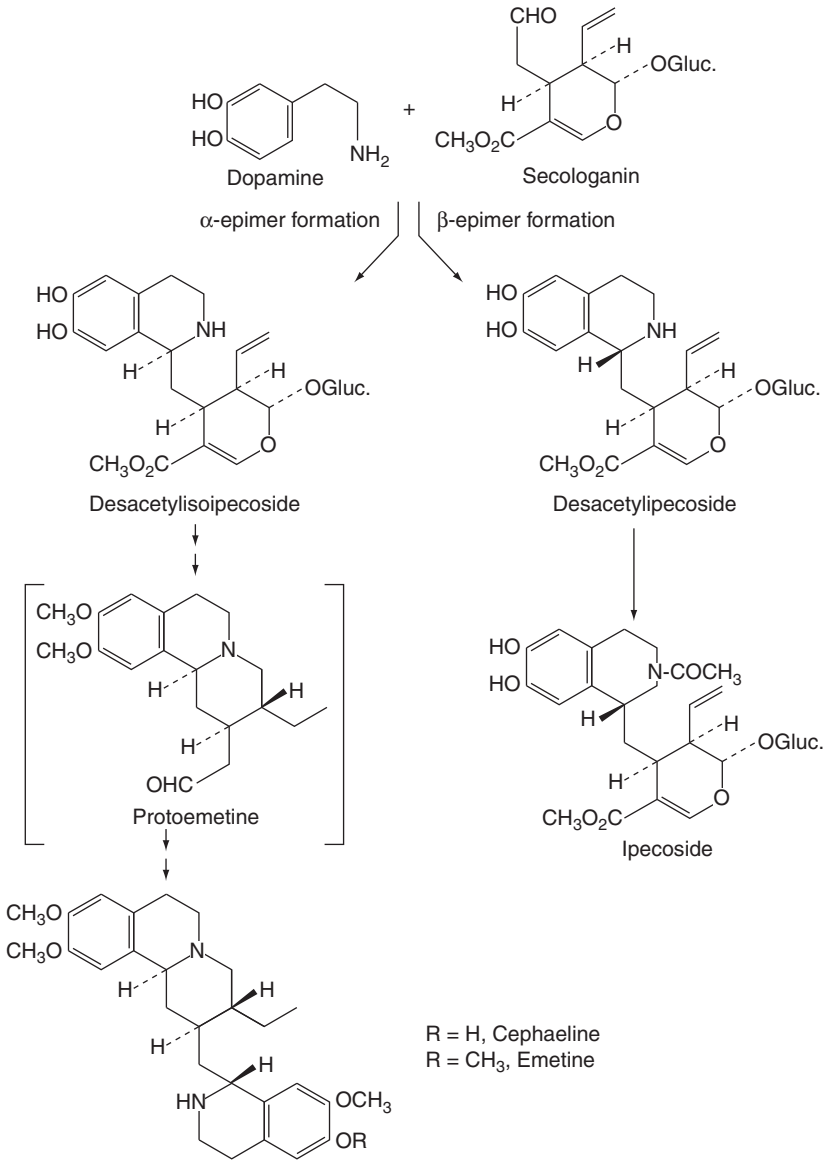



**Fig. 26.22**
Biosynthetic sequence for the biosynthesis of cephaëline, emetine and the alkaloidal glucoside ipecoside.

A simple one-step method for the production of ipecacuanha plants from root cultures has been described (K. Yoshimatsu and K. Shimomura, *Plant Cell Rep*., 1994, **14**, 98).

**Test for emetine.** Mix 0.5 g of the powdered drug with 20 ml of hydrochloric acid and 5 ml of water; filter, and to 2 ml of the filtrate add 0.01 g of potassium chlorate; if emetine is present, a yellow colour appears, which, on standing for about 1 h, gradually changes to red.

*Prepared Ipecacuanha* is the drug reduced to a fine powder and adjusted to contain 1.90–2.10% of total alkaloids. It is assayed (*BP/EP*) by extraction of the alkaloids followed by back-titration of the standarized acid solution with sodium hydroxide.

**Uses.** Ipecacuanha is used as an expectorant and emetic and in the treatment of amoebic dysentery (Chapter 28). Emetine has a more expectorant and less emetic action than cephaëline, a fact that accounts for the preference shown for the Brazilian drug. In the treatment of amoebic dysentery emetine hydrochloride is frequently given by injection, and emetine and bismuth iodide by mouth. Psychotrine and its *O*-methyl ether are selective inhibitors of human immunodeficiency virus and their study could lead to the development of therapeutically useful agents (G. J. Tan *et al*., *J. Biol. Chem*., 1991, **266**, 23529).

## Cocillana

Cocillana *BP* 2001 (Grape Bark, Guapi Bark) is the dried bark of *Guarea rusbyi* (Meliaceae) and other closely related species. The trees are native to the South American Andes and the bark is collected in Bolivia and Haiti.

**Macroscopical characters.** The commercial bark which has a slight aromatic odour, occurs in fairly large flattish or curved pieces, up to 60 cm long and 5–20 mm thick. Externally, the cork may be quite extensive and fissured, but the outer layers are missing in some areas and covered by lichen patches in others. The inner surface shows longitudinal striations and is lighter in colour than the grey-brown or orange brown outer tissues.

**Microscopical characters.** The lignified cork cells occur in bands alternating with layers of lignified parenchyma and sclereids. In the phloem the narrow medullary rays, some cells of which are sclerenchymatous, run between numerous fibre groups, each containing a

prism sheath. Many cells contain pigmented contents and a little starch is present. For illustrations showing the macroscopical and microscopical features of the drug, see previous editions.

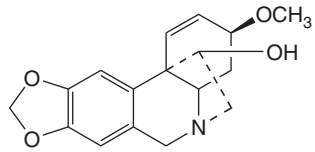
**Constituents.** There appears to be no recent work which delineates the active constituents of the drug and it is placed in this position because of its association with ipecacuanha. Arising from work carried out at the end of the nineteenth century, the bark is described as containing 2.3% resins, 2.5% fixed oil, tannin, a small quantity of alkaloid and possibly a glycoside. A study carried out in 1966, which duplicated the earlier methods of extraction and fractionation, gave different results and added credence to the widespread belief that present commercial supplies may not be identical with the earlier ones.

**Uses.** A liquid extract, with other ingredients, is used in the form of a linctus as an expectorant giving an alternative to ipecacuanha in the treatment of coughs.

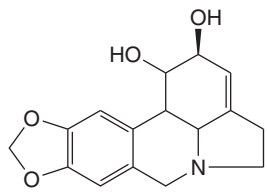
## AMARYLLIDACEAE ALKALOIDS

The bulbs of this family are well-known for their toxic properties, at least one fatality in the UK being recorded in 1999 as a result of mistaken consumption of daffodil bulbs for onions.

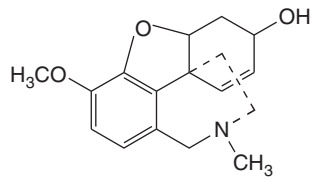
The alkaloids are derived from one molecule of phenylalanine and one of tyramine and biochemically fall into three series depending on the type of oxidative phenolic coupling undergone by the precursor *p′-O*-methylnorbelladine (formula Fig. 26.13). These series are represented by the alkaloids haemanthamine (*p*,*p′*coupling, lycorine (*o*,*p′* coupling) and galanthamine (*o′*,*p* coupling) (Fig. 26.23), all three types often co-occurring in one species. Some 20 genera of the family produce these alkaloids but in the past they have been of minor significance in Western medicine. However *Narcissus tazetta* var. *chinensis*, the bulbs of which contain lycorine, pseudolycorine and tazettine, is described in Chinese traditional and herbal remedy texts.




Haemanthamine




Lycorine




Galanthamine

**Fig. 26.23**
Structural types of Amaryllidaceae alkaloids.

O. Hoshino has reviewed (203 refs) the Amaryllidaceae alkaloids considering ten types plus miscellaneous alkaloids (*The Alkaloids*, 1998, **51**, 323).

Currently there is much interest in galanthamine which has emerged as a useful drug for the treatment of Alzheimer's disease.

### Galanthamine

This base was first isolated from the Caucasian snowdrop *Galanthus woronowi* by Russian workers in 1952 and subsequently by other scientists from the common snowdrop *G. nivalis*. It was rapidly shown to be a metabolite of many species of the family (J. J. Willaman and H.-L. Li, *J. Nat. Prod*., 1970, **33**, 17) from which *Leucojum aestivum* has emerged as a commercial source with the consequence that wild plants are, like many other medicinal plants, becoming an endangered species.

A. Poulev *et al*. (*Planta Medica*, 1993, **59**, 442) have used ELISA techniques (Ch. 16) for the quantitative determination of galanthamine in plant materials and obtained yields of from 0.1 to 2.0% for the dried bulbs of *L. aestivum* with *Phaedranassa negistrophylla* from Peru giving 7.4%. Various *Narcissus* cultivars have been examined for the alkaloid and it has been shown that planting depth, planting density, bulb size and flower bud removal did not affect the galanthamine content (R. M. Moraes-Cerdeira *et al*., *Planta Medica*, 1997, **63**, 472). *N. confusus*, endemic to Spain, is reported to have the highest galanthamine content of the genus reaching in the emerging bulb a maximum concentration of up to 2.5%, dry weight (S. Lopez *et al.*, *Planta Medica*, 2003, **69**, 1166).

There is a considerable body of literature on the pharmacology of galanthamine (see review by A. L. Harvey, *Pharmac. Ther*., 1995, **68**, 113) and readers will note a possible confusion in nomenclature in a number of the more recent publications by use of the designation 'galantamine', a name also employed by the manufacturer to describe the new product mentioned below.

Galanthamine is an acetylcholinesterase inhibitor and as such has been used extensively since the late 1950s in Eastern Europe and the former Soviet Union in anaesthesia as a curare reversal agent. Secondly, the alkaloid also acts centrally by penetrating the blood–brain barrier to serve as a modulator of nicotinic cholinergic receptors, thus augmenting central cholinergic neurotransmission. It is the latter which has invoked investigation into its use in the palliative treatment of Alzheimer's disease.

Clinical trials with the isolated alkaloid involving patients presenting with mild to moderate symptoms of Alzheimer's disease have given positive findings for the improvement of memory and intellectual functioning. The drug, as Reminyl®, was granted European marketing approval in July 2000.

### Further reading

Hanks GR (ed), Hardman R (series ed) 2002 Medicinal and aromatic plants – industrial profiles, Vol 21. Narcissus and daffodil – the genus *Narcissus*. CRC Press, Taylor and Francis Group. Baca Raton, FL

Heinrich M, Teoh HL 2004 Galanthamine from snowdrop – the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. J Ethnopharmacology 92: 147–162. *Review with about 80 references*

## PHENETHYLISOQUINOLINE ALKALOIDS

This group of alkaloids was first recognized in 1966 during investigations on the biosynthetic origin of colchicine, the principal alkaloid of the autumn crocus. They represent analogues of the benzyltetrahydroisoquinoline alkaloids and are found in a number of genera of the Liliaceae.

Colchicine-type alkaloids are present in many species of *Colchicum* (e.g. *C. luteum* and *C. speciosum*). Also, the genera *Androcymbium*, *Bulbocodium*, *Camptorrhiza*, *Dipidax*, *Gloriosa*, *Iphigenia*, *Littonia*, *Merendera*, *Ornithoglossum* and *Sandersonia* possess similar constituents.

## Colchicum seed and corm; Colchicine

Colchicum seed and corm are derived from the autumn crocus or meadow saffron, *Colchicum autumnale* (Liliaceae). The plant, whose life cycle is described below, is found in Britain and in many other parts of Europe. Commercial supplies come from Poland, Czechoslovakia, former Yugoslavia and The Netherlands. *Colchicum luteum* is used in Indian medicine.

**History.** Drugs believed to have been derived from species of *Colchicum* have long been known under the names of 'colchicum', 'hermodactyl', 'surinjan' and 'ephemeron', and some have been identified as *C. autumnale*. Dioskurides was aware of the poisonous nature of a *Colchicum* which may or may not have been the species now used in medicine. The genus derives its name from Colchis on the Black Sea, one of the places where this plant is found. The drug was recommended in Arabian writings for use in gout, but it was little employed in either classical or mediaeval times, owing to the wholesome fear inspired by its poisonous properties. Colchicum corm appeared in the *London Pharmacopoeias* of 1618, 1627, 1632 and 1639. It was then deleted but reappeared in the edition of 1788. The uncertain action of the corm led Dr W. H. Williams, of Ipswich, to introduce the use of the seeds about 1820, and these were admitted to the *Pharmacopoeia* of 1824. Colchicine was isolated by Pelletier and Caventou in 1820.

**Life cycle.** The corm consists of an enlarged underground stem bearing foliage leaves, sheathing leaves and fibrous roots. If the plants are examined in the latter part of the summer, it will be found that a new corm is developing in the axil of a scale leaf near the base of the old corm, the new plant occupying an infolding in the side of the parent corm. In September the parent corm bears the remains of recently withered leaves and is very much larger than the daughter corm. For medicinal purposes the corm would have been collected shortly after the withering of the leaves ('early summer') and before the enlargement of its axial bud. The corms are surrounded by a dark, membranous coat. The young corm develops fibrous roots at its base, and in August or September two to six flowers emerge from it, but its foliage leaves do not appear above ground until the following spring. The flowers are 10–12 cm long. Each has six stamens and a perianth consisting of six lilac or pale-purple segments which fuse into an exceptionally long perianth tube, at the base of which lies the superior ovary. More than half the length of the flower is below ground, and the fruit lies protected throughout the winter by the surrounding corm and earth. The fruit is a three-lobed, three-celled, septicidal capsule, which is carried above ground in the spring by the expanding leaves. The fully grown leaves are radical, linear-lanceolate and about 12 cm long. During these changes the daughter corm grows at the expense of the parent, which now gradually perishes. Before doing so, however, it may produce in its second spring one or more small corms by means of which the number of plants may be increased.

**Characters of seed.** The seeds are collected when ripe, usually in July or August, and dried. They are ovoid or globular in shape and 2–3 mm in diameter. They are extremely hard and have a reddish-brown, minutely pitted testa. During drying the seeds darken in colour and become covered with a sugary exudation. The seed, as in most Liliaceae, develops from an amphitropous ovule. From a slight projection at the hilum there extends for about one-quarter of the circumference a well-marked strophiole. The small embryo lies embedded in horny endosperm.

Microscopical examination shows that the testa consists of somewhat thick-walled reddish-brown parenchyma; that the endosperm cells have pitted walls and contain fixed oil and aleurone grains up to 5 mm in diameter; and that the strophiole contains starch.

Colchicum seeds contain 0.6–1.2% of colchicine, a number of other colchicine-type alkaloids, a resin, fixed oil and reducing sugars.

**Characters of corm.** The corms are collected about July, cut into transverse slices and dried at a temperature not exceeding 65°C. The outer membranes are rejected. The whole corms are 2–3 cm diameter, but the dried drug consists of somewhat reniform, transverse slices and occasional more ovate longitudinal slices, about 2–5 mm thick. The epidermal surface is cinnamon-brown and slightly wrinkled. The interior is white and starchy and, if carefully smoothed, shows scattered fibrovascular bundles. The drug breaks with a short mealy fracture. The odour is much less marked than in the fresh drug. Taste, bitter.

Microscopical examination shows numerous starch grains contained in parenchyma, some simple but the majority consisting of two to seven components. Individual grains are from 6 to 30 µm diameter, and show a triangular or star-shaped hilum. Their shape varies from spherical or ovoid to polygonal. Vessels with a spiral or annular thickening and portions of brownish epidermis with very occasional circular stomata may also be seen.

On treating the drug with 60–70% sulphuric acid or with concentrated hydrochloric acid, a yellow colour, due to colchicine, is produced. The corms contain up to about 0.6% colchicine, other related alkaloids and starch.

## Colchicine

This is an amorphous, yellowish-white alkaloid, which darkens on exposure to light and gives a yellow coloration with strong mineral acids. Colchicine readily dissolves in water, alcohol or chloroform but is only slightly soluble in ether or petroleum spirit. It is a weak base and may be extracted from either acid or alkaline solution by means of chloroform. Colchicine *BP/EP* is assayed by non-aqueous titration.

The rather unusual chemical structure of colchicine meant that its probable biogenetic origin from simpler molecules could not be easily predicted. Examples of the occurrence of the tropolone ring (ring C) are rare in higher plants, although it features in mould metabolism; also, the position of the nitrogen atom is unusual. Owing mainly to the work of Battersby, Leete and their coworkers involving tracer studies on *C. autumnale* and *C. byzantium*, the principal pathway for the biogenesis of colchicine has now been established.

Ring A and carbons 5, 6 and 7 are derived from phenylalanine; the tropolone moiety arises from tyrosine by ring cleavage followed by closure to give a seven-membered ring. In contrast to mould metabolism, acetate does not contribute directly to the tropolone ring but is merely effective in supplying the *N*-acetyl group. An intermediate formed early in the pathway as the result of union of the two amino acids is a 1-phenylethylisoquinoline derivative. This is a member of a class of alkaloids first reported in 1966, the first two representatives being androcymbine and the dimer melanthioidine, alkaloids of *Androcymbium melanthioides*, a close relative of colchicum. Demecolcine, also a constituent of *Colchicum* spp., is a more immediate precursor of colchicine. The sequential formation of these compounds is indicated in Fig. 26.24. (For a study on the early stages of colchicine biosynthesis leading to the formation of phenethylisoquinoline intermediates see R. B. Herbert *et al*., *Tetrahedron*, 1990, **46**, 7119 and for more recent refinements to the biosynthetic sequence see A. Nasreen *et al*., *Phytochemistry*, 1997, **46**, 107).

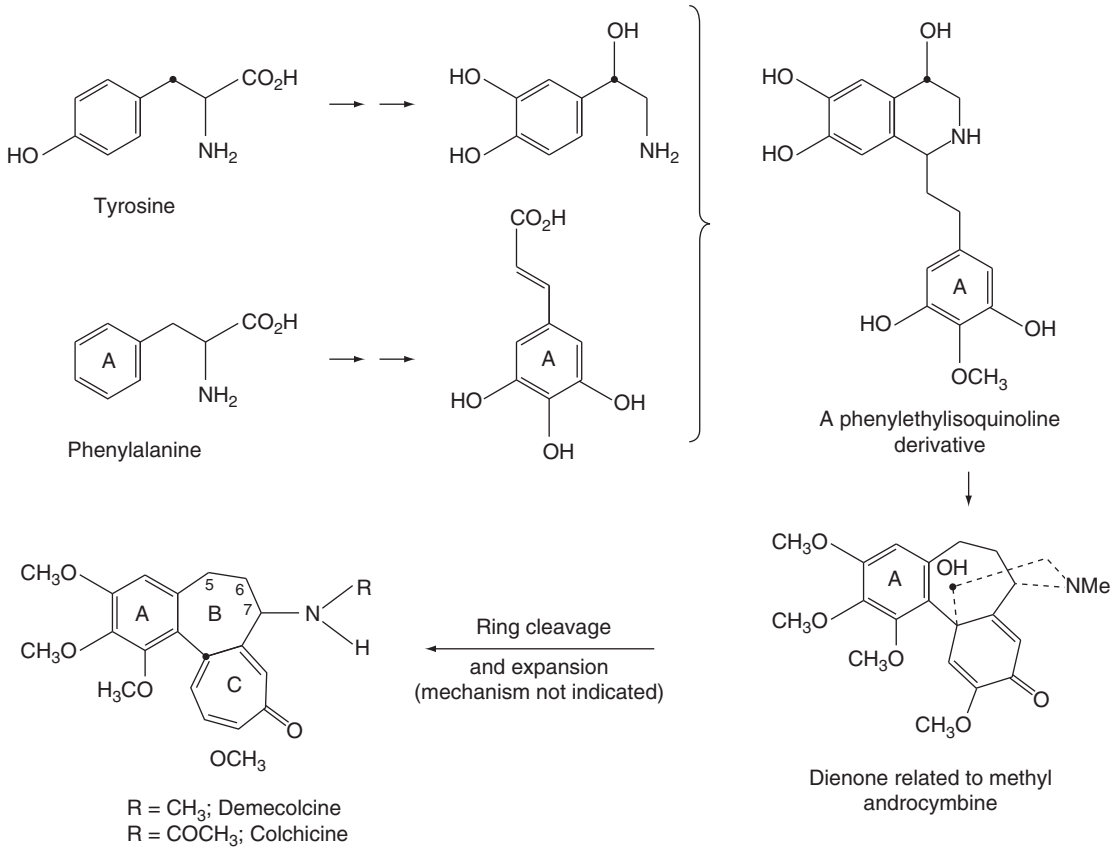



**Fig. 26.24**
Steps in the biogenesis of colchicine.

The richest sources of colchicine are the corms and seeds, but the difficulty of obtaining adequate supplies of these has led Šantavý and coworkers (*Planta Med*., 1979, **36**, 119; 1981, **43**, 153) to investigate the possibility of using leaves and flowers for extraction purposes. In colchicine content the flowers compare with the seeds. The leaves contain only one-fifteenth the alkaloid content of the seeds but, compared with the corms, they contain half the amount of 2-demethyldemecolcine. The latter alkaloid can be chemically converted to demecolcine. On slow drying of the leaves, the proportion of 2- and 3-demethylated derivatives of colchicine increases; these are not glycosidic breakdown products but arise from unknown compounds as a result of enzymatic liberation. Suspension and callus cultures of *C. autumnale* have been shown to produce colchicine.

**Uses.** Colchicum preparations are used to relieve gout, but must be employed with caution. Colchicine is frequently prescribed in tablet form and transdermal preparations containing colchicine are the subject of a Japanese patent (1991). The alkaloid is also used in biological experiments to produce polyploidy or multiplication of the chromosomes in a cell nucleus (see Chapter 14).

### Further reading

Lettello C 2000 The pharmacology and therapeutic aspects of colchicine. Alkaloids 53: 287–352

## TRYPTOPHAN-DERIVED ALKALOIDS

With a few minor exceptions, tryptophan and its decarboxylation product, tryptamine, give rise to the large class of indole alkaloids. These bases usually contain two nitrogen atoms; one is the indolic nitrogen and the second is generally two carbons removed from the β-position of the indole ring. Of the several alkaloid groups within the indole class, two may be produced, depending on the type of condensation occurring between tryptamine and an aldehyde or ketoacid. A Mannich reaction involving the α-carbon atom of the indole nucleus affords a β-carboline derivative; reaction involving the β-position gives rise to an indolenine (Fig. 26.25).

A number of simple tryptamine derivatives and β-carbolines have psychomimetic properties; for a review of their phytochemistry, chemotaxonomy and pharmacology, see Allen and Holmstedt (*Phytochemistry*, 1980, **19**, 1573).

Some examples of alkaloids of pharmaceutical interest, derived from tryptamine, are given in Fig. 26.26. It will be noted that the more complex indole alkaloids contain a non-tryptophan-derived portion of the molecule and this is supplied by mevalonic acid, which in the case of the ergot alkaloids is a $C_5$-isopentenyl unit and with the alkaloids of the Apocynaceae, Loganiaceae, Rubiaceae etc., a $C_{10}$-geraniol (monoterpenoid) contribution. Some 2000 monoterpenoid alkaloids are known and Fig. 26.27 illustrates how a number of alkaloid types within this group can arise.

A key intermediate in the biogenesis of the monoterpene indole alkaloids is 3α(*S*)-strictosidine; it was first isolated in 1968 by G. N. Smith from *Rhazya stricta* and until 1997 its structure was based on compounds of known stereochemistry, then, direct instrumental measurements furnished its first detailed stereochemical analysis (Á. Patthy Lukats, *J. Nat. Prod*., 1997, **60**, 69). It is formed by the enzymatic condensation of tryptamine and secologanin (Fig. 26.28). The enzyme responsible for this important reaction, strictosidine synthase, has been isolated and characterized from

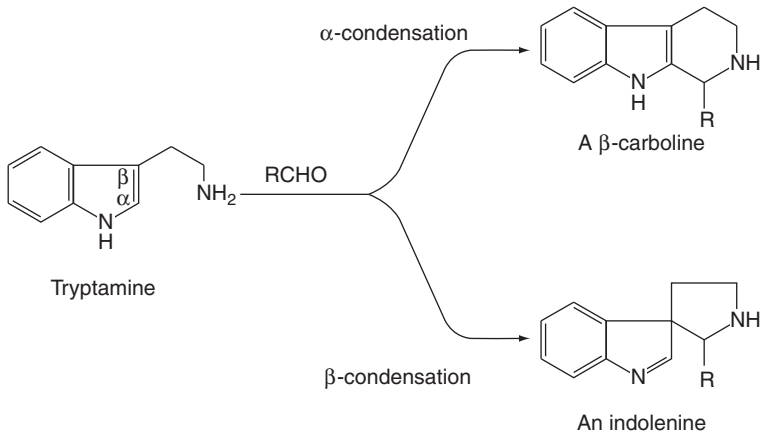



**Fig. 26.25**
Formation of the indole alkaloids.

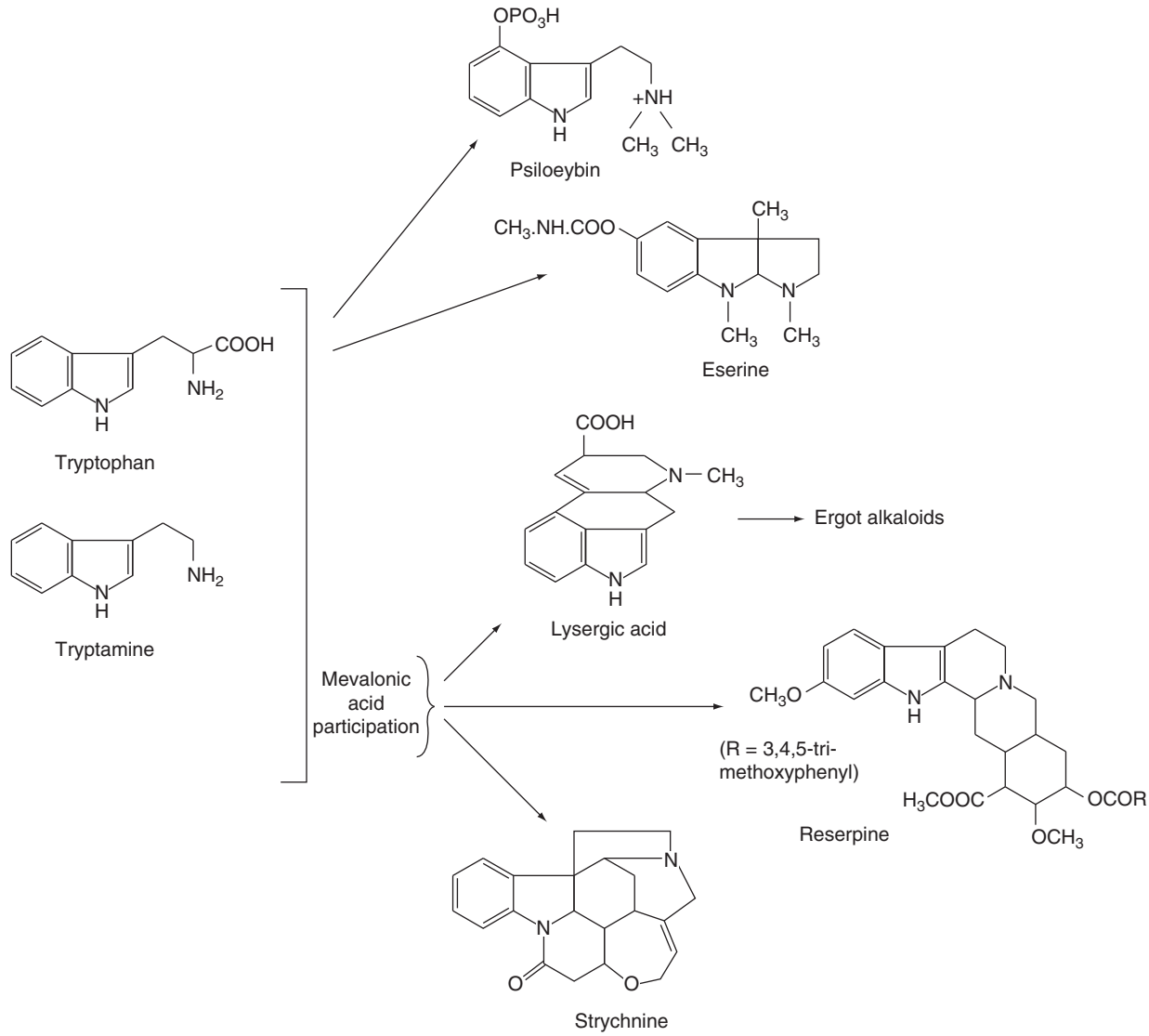



**Fig. 26.26**
Tryptophan and tryptamine as precursors of indole alkaloids.

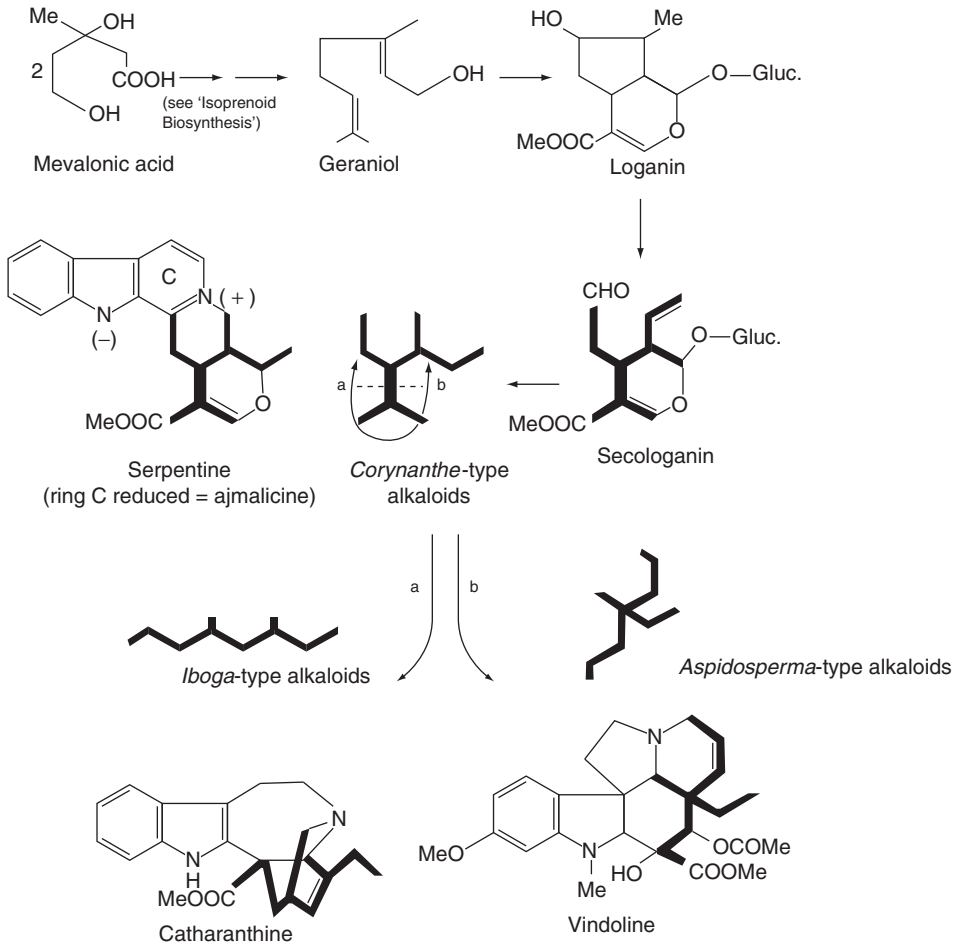



**Fig. 26.27**
Incorporation of mevalonic acid into some indole alkaloids.

cell cultures of a number of species including *Rauwolfia serpentina*, *Cinchona robusta* and *Catharanthus roseus* and a number of isoforms have been described. The *R. serpentina* gene relating to this enzyme has been cloned and heterologously expressed in microorganisms including *Escherichia coli* and *Saccharomyces cerevisiae* (baker's yeast). This example represented the first cloning of cDNA for an enzyme of alkaloid biosynthesis. The gene is a single polypeptide $M_r$ about 34 000, possessing a 5.3% carbohydrate content. An investigation of 10 spp. of *Rauwolfia* using a polymerase chain reaction comparison showed the gene to be highly conserved, which was unexpected considering the geographical range of the species and the fact that it would be conventionally considered as an unimportant gene of secondary metabolism.

Strictosidine glucosidase was reported in 1996 from a suspension cell culture of *Tabernaemontana divaricata* (T. J. C. Luijendijk *et al*., *Phytochemistry*, 1996, **41**, 1451).

Prior to 1977, 3β(*R*)-vincoside (Fig. 26.28), the epimer of 3α(*S*)-strictosidine was accepted as the naturally occurring precursor of the monoterpenoid indole alkaloids and elaborate biomimetric models had to be conceived to accommodate the necessary inversion of configuration at C-3 to give the natural alkaloids. Quinine is also derived from tryptophan but this is not immediately obvious by inspection of its formula; its biogenesis is outlined under 'Cinchona'. The antileukaemic alkaloids of *Catharanthus roseus*, vinblastine and vincristine are dimeric alkaloids of this group (see Chapter 27) and their biogenesis, production in artificial culture and enzymic aspects remain a most active area of research.

## ERGOT AND ERGOT ALKALOIDS

Ergot (*Ergot of Rye*) is the dried sclerotium of a fungus, *Claviceps purpurea* Tulasne (Clavicipitaceae), arising in the ovary of the rye, *Secale cereale*. Controlled field cultivation on rye is the main source of the crude drug. The most important producers are Czechoslovakia, Hungary, Switzerland and former Yugoslavia. With modern farming the supply of 'natural' ergot is decreasing and fields of rye are devoted to its cultivation. Different selected strains of *C. purpurea* are used for the production of the alkaloids ergotamine, ergocristine, or ergocornine and ergokryptine. Commercially, ergot of rye is becoming less important and by 1994 UK dealers were trading mainly in ergot of wheat.

**History.** There is considerable doubt as to whether ergot and ergotism were known to the ancients, and it is impossible to say whether the 'ignis sacer' of the Romans referred to ergotism. The outbreaks of 'ignis St Antonii', or St Antony's fire, which occurred during the Middle Ages, do, however, appear to have been of ergot origin. Outbreaks of ergotism occurred in Germany in 1581, 1587 and 1596 and at intervals in Europe until recent years. Ergotism was never common in England, probably owing to the fact that rye is little grown, and the only serious outbreak recorded, which took place in 1762, was caused by wheat.

World-wide, sporadic reports of ergot poisoning still appear in the literature and in 1992 an analysis of rye flour sold in Canada showed that low-level contamination by the fungus still exists; of 128 samples tested 118 proved positive for ergot alkaloids at concentrations of 70–414 ng $g^{-1}$ whereas with wheat flour the incidence and levels were much lower.

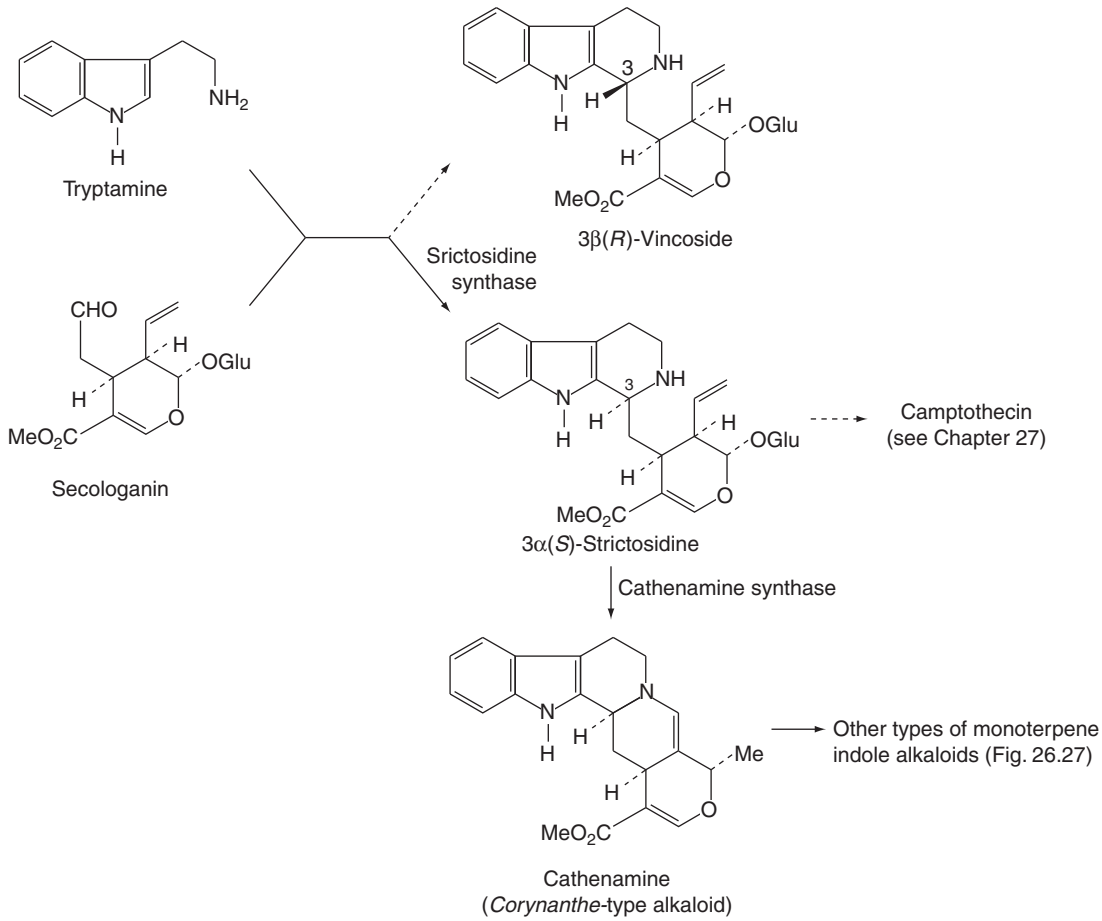



**Fig. 26.28**
Formation and metabolism of strictosidine.

The obstetric use of ergot was known in the sixteenth century, but the drug was not widely employed until the nineteenth century. It was first introduced into the *London Pharmacopoeia* of 1836. The fungoid origin of ergot was recognized by Münchausen in 1764, while the life history of the fungus was worked out and the name *Claviceps purpurea* given to it by Tulasne in 1853.

**Life history and collection.** The fungus *C. purpurea* and other species such as *C. microcephala* Wallr., *C. nigricans* Tul. and *C. paspali* produce ergots on many members of the Gramineae (including the genera *Triticum*, *Avena*, *Festuca*, *Poa*, *Lolium*, *Molinia* and *Nardus*) and Cyperaceae (including the genera *Scirpus* and *Ampelodesma*). Many of these ergots appear to be extremely toxic and to produce typical ergotism.

For the life-cycle and illustrations of the fungus, see earlier editions.

**Macroscopical characters.** The drug consists almost entirely of sclerotia, the amount of other organic matter being generally limited to not more than 1%. Each sclerotium is about 1.0–4 cm long and 2–7 mm broad; fusiform in shape and usually slightly curved. The outer surface, which is of a dark, violet-black colour, is often longitudinally furrowed and may bear small transverse cracks. Ergot breaks with a short fracture and shows within the thin, dark outer layer a whitish or pinkish-white central zone of pseudoparenchyma in which darker lines radiating from the centre may be visible. Ergot has a characteristic odour and an unpleasant taste.

Powdered ergot when treated with sodium hydroxide solution develops a strong odour of trimethylamine. In filtered ultraviolet light it has a strong reddish colour by means of which its presence in flour may be detected.

**Microscopical characters.** Ergot shows an outer zone of purplish-brown, rectangular cells, which are often more or less obliterated. The pseudoparenchyma consists of oval or rounded cells containing fixed oil and protein, and possessing highly refractive walls which give a reaction for chitin. Cellulose and lignin are absent.

**Constituents.** The ergot alkaloids (ergolines) can be divided into two classes: (1) the clavine-type alkaloids, which are derivatives of 6,8-dimethylergoline and have been extensively studied in cultures of the mycelium of the ergot fungus; and (2) the lysergic acid derivatives, which are peptide alkaloids. It is the latter class that contains the pharmacologically active alkaloids that characterize the ergot sclerotium (ergot). Each active alkaloid occurs with an inactive isomer involving isolysergic acid; the inactive isomers are not formed initially in the sclerotium but tend to accumulate as a result of unsuitable processing and poor or long storage. These alkaloids have been studied over many years and were not easy to characterize. Thus 'ergotoxine', which since its isolation in 1906 (by Barger and Carr and independently by Kraft) had been accepted as a pure substance, and in the form of ergotoxine ethanosulphonate was formerly used as a standard, was shown to be a mixture of the three alkaloids ergocristine, ergocornine and ergocryptine.

Six pairs of alkaloids predominate in the sclerotium and fall into either the water-soluble ergometrine (or ergonovine) group or the water-insoluble ergotamine and 'ergotoxine' groups. Table 26.6 gives the more

**Table 26.6 Alkaloids of ergot.**

| | Alkaloid | Formula | Discovered |
|---|---|---|---|
| I. Ergometrine group | Ergometrine<br>Ergometrinine | $C_{19}H_{22}O_2N_3$ | Dudley and Moir (1935) |
| II. Ergotamine group | Ergotamine<br>Ergotaminine | $C_{33}H_{35}O_5N_5$ | Spiro and Stoll (1920) |
| | Ergosine<br>Ergosinine | $C_{30}H_{37}O_5N_5$ | Smith and Timmis (1937) |
| III. Ergotoxine group | Ergocristine<br>Ergocristinine | $C_{35}H_{39}O_5N_5$ | Stoll and Burckhardt (1937) |
| | Ergocryptine<br>Ergocryptinine | $C_{32}H_{41}O_5N_5$ | Stoll and Hoffmann (1938, 1943) |
| | Ergocornine<br>Ergocorninine | $C_{31}H_{39}O_5N_5$ | Stoll and Hoffmann (1938, 1943) |

physiologically active member of each pair first. Alkaloids of groups II and III are polypeptides in which lysergic acid or isolysergic acid is linked to other amino acids. In the ergometrine alkaloids lysergic acid or its isomer is linked to an amino alcohol. Ergometrine was synthesized by Stoll and Hofmann in 1943. Other, new, peptide alkaloids have been isolated from submerged cultures of *C. purpurea* and from the field-growing fungus (L. Cvak *et al*., *Phytochemistry*, 1996, **42**, 231; 1997, **44**, 365).

Among the less important constituents of ergot may be mentioned histamine, tyramine and other amines and amino acids; acetylcholine; colouring matters; sterols (ergosterol and fungisterol); and about 30% fat. The cell walls are chitinous.

*Variation in alkaloid constituents*. Not only are chemical races very evident in *C. purpurea* with respect to alkaloid production but also the host plant is not without influence. Thus a new commercial strain of ergot adapted from a wild grass (*Anthraxon lancifolius*) to rye gave sclerotia containing 0.5% total alkaloids involving ergometrine (33%), ergotamine (17.6%), ergocornine (18.7%) and ergocryptine (22.7%). However, sclerotia produced on the grass as a result of natural infection did not contain ergometrine (K. K. Janardhanan *et al*., *Planta Med*., 1982, **44**, 166). The application of specific amino acids to maturing sclerotia can also be used to influence the type of alkaloids produced (a technique also used with saprophytic cultures).

For recent studies and references on the investigation of the alkaloid gene cluster in *C. purpurea* see T. Haarmann *et al*., *Phytochemistry*, 2005, **66**, 1312.

**Alkaloid production in artificial cultures.** The artificial culture of the ergot fungus has received considerable attention, and, obviously, large-scale submerged fermentation with selected strains to give alkaloids of choice has commercial possibilities. Abe's initial work in Japan showed that submerged cultures did not produce the typical alkaloids associated with the sclerotium but, rather, a series of new non-peptide bases (clavines) which unfortunately possessed no significant pharmacological action. Attempts were made by many workers to influence alkaloid production by modification of the culture medium and the fungus strain. As a result of successful experiments in 1960, the commercial manufacture of simple lysergic acid derivatives by fermentative growth of a strain of *Claviceps paspali* became feasible. The alkaloids produced are converted to lysergic acid which is used for the part-synthesis of ergometrine and related alkaloids. Other strains are now available which produce the peptide alkaloids in culture; not only can different chemical races of the fungus be used to produce specific groups of alkaloids but synthesis can also be directed by the addition of certain amino acids or their analogues to the fermentation liquid. In this way new unnatural alkaloids can be produced.

Flieger *et al*. (*J. Nat. Prod*., 1989, **52**, 1003) found that with submerged cultures in the postproduction stage both the alkaloid concentration and the composition of the alkaloid mixtures underwent dramatic changes including the production of two new alkaloids, 8-hydroxyergine and 8-hydroxyerginine. (For papers pertaining to the isolation of new and unnatural alkaloids from submerged cultures of *C. purpurea* see N. C. Perellino *et al*., *J. Nat. Prod*., 1992, **55**, 424; 1993, **56**, 489.)

**Biosynthesis.** The majority of biosynthetic studies were at first directed to the clavine alkaloids, which could be easily produced in cultures but, until recently, their biological relationship to the lysergic acid derivatives remained obscure. The ergoline nucleus is derived from tryptophan and mevalonate, and current work has involved elucidating the biosynthetic relationship between the various clavine alkaloids, determining which of these intermediates is the true natural precursor of lysergic acid, and studying the initial hydrogen elimination from the C-4 of mevalonate to yield the stereo-configuration of chanoclavine-I. Possible biosynthetic routes for lysergic acid involving two isomeric intermediates are given in Fig. 26.29. A major problem has been not so much in discovering which reactions the fungus can effect when supplied with a given substrate as which route is actually involved in its normal metabolism. As a result of work with cell-free systems, Abe assigned a primary role to 4-isopentenyltryptophan and lysergene rather than to the dimethylallyl compound, agroclavine or chanoclavine. Later work by Gröger *et al*. (*Planta Med*., 1980, **40**, 109) appeared to favour a scheme involving the latter compounds and this has now been generally substantiated with the intermediates involved with the ring closure between dimethylallyltryptophan and chanoclavine having been investigated (A. P. Kozikowski *et al*., *J. Amer. Chem. Soc*., 1993, **115**, 2482).

Work on the origin of the nitrogen of the peptide portions of the ergot alkaloids indicates that appropriate amino acids are specifically incorporated. Abe's scheme is shown in Fig. 26.30 and he obtained intact incorporation of the units shown, in certain strains, but workers in Germany were unable to confirm these results. As has been mentioned earlier, unnatural amino acids can also be incorporated into the alkaloids.

**Varieties.** Commercial ergot varies considerably in activity from batch to batch, and the differences cannot be fully explained by differences in storage; further, it is often found that inferior-looking ergot is highly active. Such variations are apparently due to the fact that there are a number of different chemical races of *C. purpurea* and in cultivating ergot by modern methods it is obviously important to prepare the spore-cultures used for infecting the rye from a race of the fungus known to develop ergots having a high content of the required

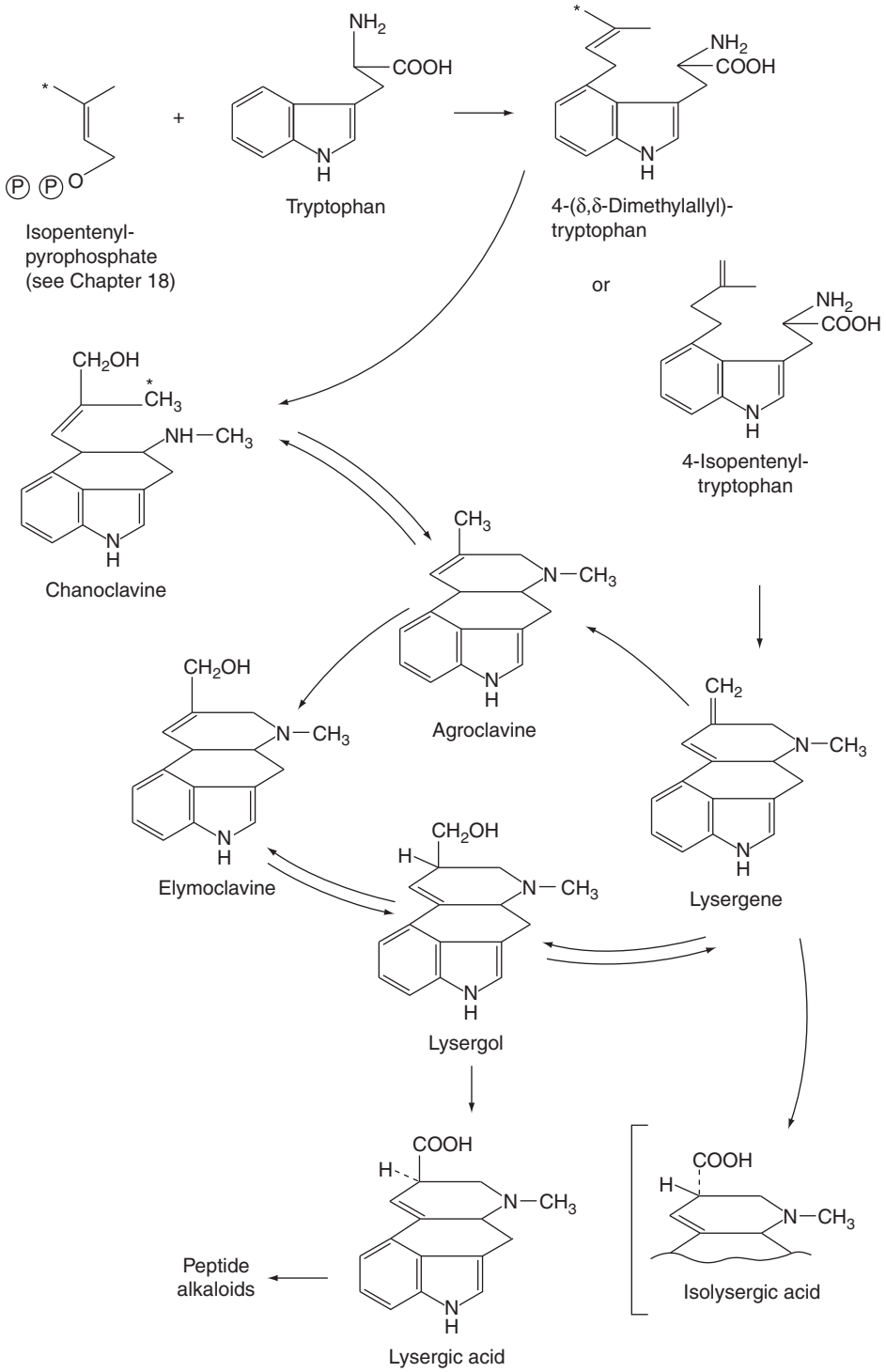



**Fig. 26.29**
Possible biogenesis of lysergic acid.

alkaloids. Cultivated ergot may contain up to 0.5% of total alkaloids, and 0.15% is a minimum commercial value. In addition, there may be a minimal requirement for water-soluble alkaloids. The alkaloids can be determined by colorimetry, as they give a blue colour with a solution of *p*-dimethylaminobenzaldehyde.

**Substitutes.** *Ergot of wheat* is now imported into Britain and has been used medicinally in France. The sclerotia are shorter and thicker than those of rye. Instances of ergot on barley and rye in Britain, and on wheat and rye in the USA and Canada have been reported.

*Ergot of oats* has been used medicinally in Algiers. The sclerotia are black in colour, 10–12 mm long and 3–4 mm diameter.

*Ergot of diss*, which is produced on the Algerian reed *Ampelodesma tenax*, has appeared in commerce and is said to be highly active. The sclerotia may attain as much as 9 cm in length and are spirally twisted.

**Storage.** Ergot is particularly liable to attack by insects, moulds and bacteria. After collection it should be thoroughly dried, kept entire, and stored in a cool, dry place. If powdered and not immediately defatted, the activity decreases, but if defatted and carefully stored in an air-tight

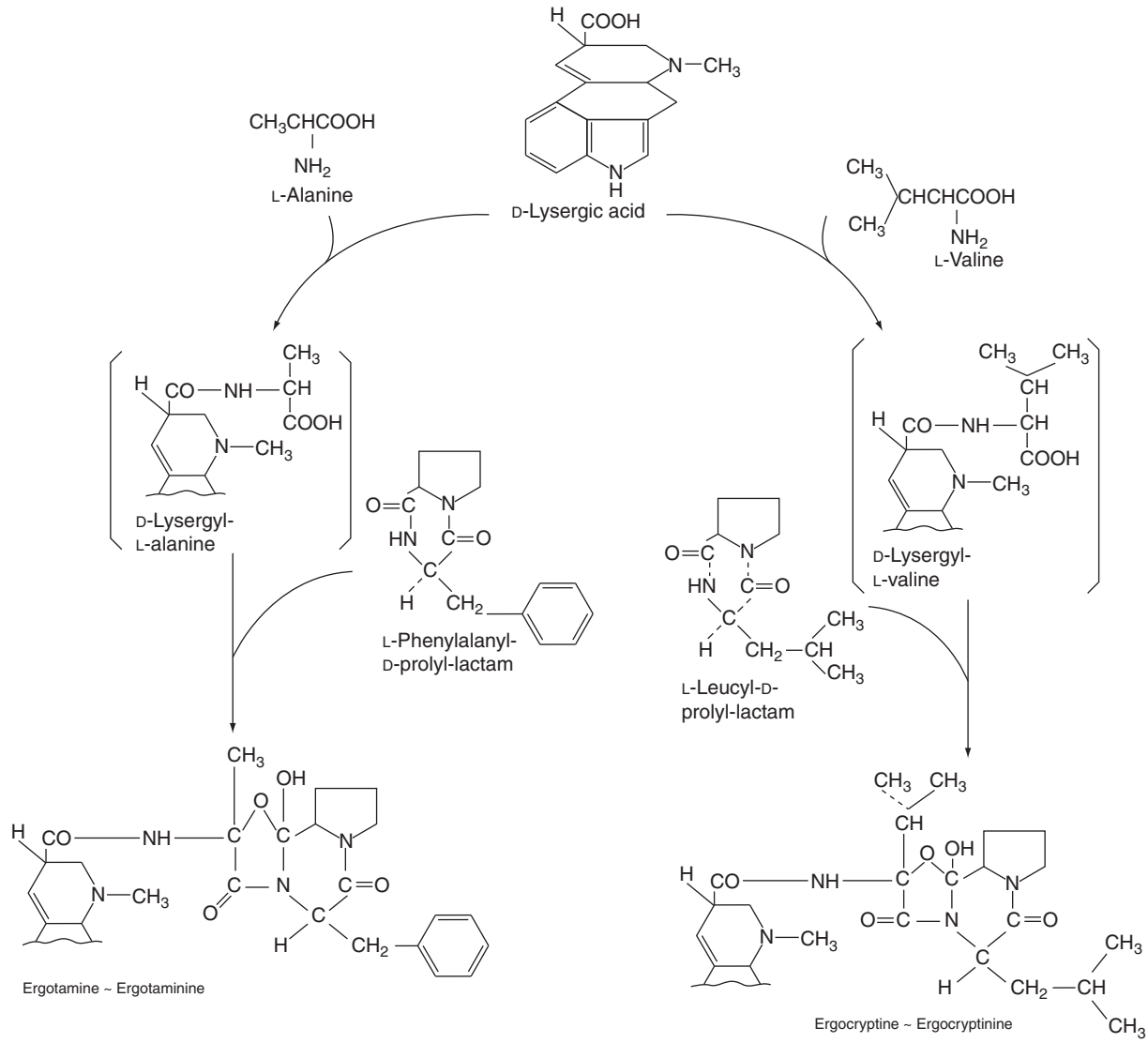



**Fig. 26.30**
Biogenesis of ergot peptide alkaloids (after Abe).

container, it will remain active for a long period. However, as indicated above, under certain conditions, loss of activity arises by the conversion of the pharmacologically important alkaloids to inactive isomers. Any sample of ergot which shows worm holes or a considerable amount of insect debris will almost certainly deteriorate further on storage.

**Uses.** Although whole ergot preparations were traditionally used in labour to assist delivery and to reduce post-partum haemorrhage, ergot itself has been largely replaced in the pharmacopoeias by the isolated alkaloids. Only ergometrine produces an oxytocic (literally 'quick delivery') effect, ergotoxine and ergotamine having quite a different action. Ergometrine is soluble in water or in dilute alcohol. It is often known, particularly in the USA, as ergonovine. Ergotamine and the semisynthetic dihydroergotamine salts are employed as specific analgesics for the treatment of migraine. Lysergic acid diethylamide (LSD-25), prepared by partial synthesis from lysergic acid, is a potent specific psychotomimetic.

## Calabar bean and physostigmine

Calabar beans (*Ordeal beans*) are the dried ripe seeds of *Physostigma venenosum* (Leguminosae), a perennial woody climber found on the banks of streams in West Africa. The plant bears typical papilionaceous flowers, and legumes about 15 cm long, each containing two or three seeds.

**History.** The seeds were formerly used by the west African tribes as an 'ordeal poison'. They were first known in England in 1840. The myotic effect of the drug was noted in 1862 by Fraser, and physostigmine was isolated in 1864 by Jobst and Hesse.

**Characters.** Calabar beans have a somewhat flattened, reniform shape. They are 15–30 mm long, 10–15 mm wide and up to 15 mm thick. The seeds are extremely hard. The dark brown testa is smooth, except in the neighbourhood of the grooved hilum, which runs the whole length of the convex side and round one end, where it is somewhat wrinkled. On either side of the groove is a well-marked ridge and in the groove itself are the greyish, papery remains of the funiculus. A transverse section shows a large central cavity and two, very hard, concavo-convex cotyledons.

**Constituents.** The seeds contain the alkaloids physostigmine or eserine,eseramine, isophysostigmine, physovenine, geneserine, *N*-8-norphysostigmine, calabatine and calabacine. The structure of geneserine,

long regarded as an *N*-oxide, has been revised to include the oxygen in a ring system. The chief alkaloid, physostigmine, is present to the extent of about 0.15%. It is derived from tryptophan (see Fig. 26.26). On exposure to air it oxidizes into a red compound, rubreserine, and should therefore be protected from air and light. Both physostigmine salicylate and sulphate are included in the *BP*/*EP*. The former of the two is more stable and non-deliquescent. For both salts there is a colorimetic test for the elimination of eseridine and a non-aqueous titration assay.

**Uses.** Physostigmine salicylate is used for contracting the pupil of the eye, often to combat the effect of mydriatics. It has also been investigated as an intravenous injection for reversing the effects of a number of sedatives. With Alzheimer's disease it has shown some evidence of inducing a slight improvement in intellectual and cognitive performance (*Pharm. J.*, 1992, **249**, 376) but galanthamine (q.v.) may prove superior. Physovenine has the same order of activity but that of eseramine is much lower.

## Nux vomica

Nux vomica consists of the dried, ripe seeds of *Strychnos nux-vomica* (Loganiaceae), a tree 10–13 m high with a distribution including Ceylon, India, East Bengal, Burma, Thailand, Laos, Cambodia and S Vietnam. The drug is mainly collected in India and exported from Mumbai (Bombay), Madras, Cochin, Cocanada and Calcutta.

**History.** Nux vomica was known in Europe in the sixteenth century and was sold in England in the time of Parkinson (1640), mainly for poisoning animals. Strychnine was isolated in 1817 and brucine in 1819.

**Collection and preparation.** The fruit is a berry about the size of a small orange. When ripe it has a rather hard orange-yellow epicarp and a white, pulpy, interior in which 1–5 seeds are embedded. The seeds are washed free from pulp and dried. They are exported in small sacks, known as 'pockets', holding about 18–25 kg.

**Macroscopical characters.** Nux vomica seeds are extremely hard and should be boiled in water for at least an hour in order to soften them sufficiently for dissection. The seeds are greenish-grey, disc-shaped, 10–30 mm diameter and 4–6 mm thick. Most of the seeds are nearly flat and regular in shape, but a few are irregularly bent and somewhat oval in outline. The edge is rounded or acute. The testa is covered with silky, closely appressed, radiating hairs. In the centre of one of the flattened sides is a distinct hilum, and a small prominence on the circumference marks the position of the micropyle, which is joined to the hilum by a radial ridge. To examine further, a boiled seed should be cut transversely and another one opened like an oyster by inserting the blade of a small knife or scalpel at a point on the circumference opposite the micropyle. The small embryo with two cordate cotyledons and a cylindrical radicle, the latter directed towards the micropyle, will be seen embedded in a grey, horny endosperm (Fig. 26.31). In the centre of the seed is a slit-like cavity. The seeds are odourless when dry; but if soaked in water and left for a day or two, they develop a very unpleasant odour. They have a very bitter taste.

**Microscopical characters.** A radial section shows a very thin testa consisting of collapsed parenchyma and an epidermal layer of very characteristic lignified hairs (Fig. 41.7M). The latter have a very large, thick-walled base with slit-like pits. Surface irregularities in the bases of the hairs cause them to interlock with one another. The upper portions of the hairs are set at almost a right angle to the bases and all radiate out towards the margin of the seed, giving the testa its characteristic silky appearance. On the ridge connecting hilum and micropyle, however, the hairs are irregularly arranged. The upper part of the wall

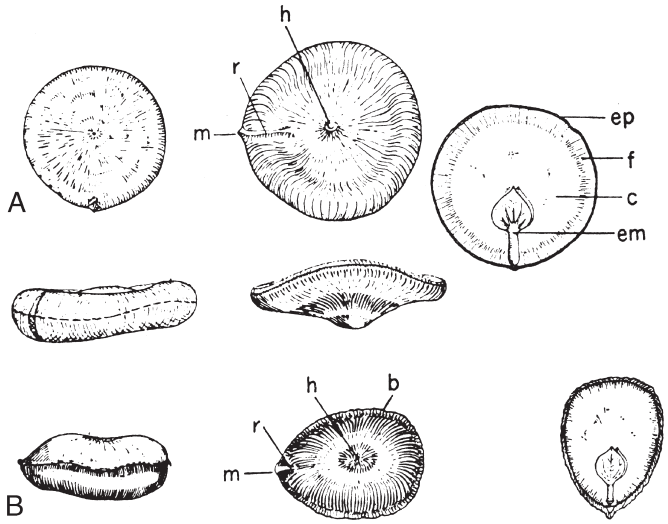



**Fig. 26.31**
A, *Strychnos nux-vomica* seed; B, *S. nux-blanda* seed. Surface and lateral views of entire seed and inner surface of horizontally split seeds. All ×0.8. m, Micropyle; r, ridge; h, hilum; b, lateral ridge; ep, epidermis; f, area of fusion of two endosperm halves; c, area of central cavity; em, embryo. (Drawn by Dr. T. D. Turner. For further details, see *J. Pharm. Pharmacol.*, 1963, **15**, 594.)

of the hair is composed of about 10 longitudinal ridge-like thickenings united by a thin wall so that the lignified ribs readily separate from one another on powdering. The lumen is circular in the upper part, but in the base has branches corresponding with the oblique pits in the wall. Fragments of testa, removed from a soaked seed, may be disintegrated by treatment with 50% nitric acid and a little potassium chlorate; the hairs can then be separated.

The endosperm consists of large, thick-walled cells, which are non-lignified and yield galactose and mannose on hydrolysis. When mounted in solution of iodine, they show well-marked protoplasmic threads (plasmodesma) passing through the walls (see Fig. 42.1G) and an oily plasma containing a few aleurone grains and the alkaloids strychnine and brucine. Strychnine is most abundant in the inner part of the endosperm and brucine in the outer layers. The presence of strychnine is shown by mounting a section in a solution of ammonium vanadate in sulphuric acid, when a violet colour is produced; of brucine, by mounting in nitric acid, when a crimson colour is observed.

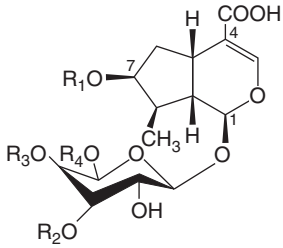
The length of lignified ribs of the hairs per milligram of nux vomica seed has been used for the determination of the content of seeds in veterinary medicines, see 'Quantitative Microscopy'.

**Constituents.** Nux vomica usually contains about 1.8–5.3% of the indole alkaloids strychnine and brucine. Strychnine (formula Fig. 26.26) is physiologically much more active than brucine and the seeds are therefore assayed for strychnine and not for total alkaloids. They usually contain about 1.23% of strychnine and about 1.55% of brucine. Minor related alkaloids include α-colubrine, β-colubrine, icajine, 3-methoxyicajine, protostrychnine, vomicine, novacine, *N*-oxystrychnine, pseudostrychnine and isostrychnine.

For a review (188 refs) of recent studies concerning the synthesis of strychnine, see M. Shibasaki and T. Ohshima, *The Alkaloids*, 2006, **64**, 103.

Iridoids of the seeds include the glycoside loganin (Fig. 26.27), loganic acid and 7-*O*-acetyl loganic acid together with three new iridoids, 6′-*O*-acetyl loganic acid, 4′-*O*-acetyl loganic acid and 3′-*O*-acetyl loganic acid (X. Zhang *et al.*, *Phytochemistry*, 2003, **64**, 1341).

The seeds also contain chlorogenic acid (see Fig. 19.5) and about 3% of fixed oil.




Loganic acid: $R_1 = R_2 = R_3 = R_4 = H$
Acetyl loganic acids: acetyl groups occur singly on $R_1$, $R_2$, $R_3$, or $R_4$ (see text)

Loganin, although present only in small amounts in the seed, occurs to the extent of about 5% in the fruit pulp together with secologanin; these compounds are intermediates in the biogenesis of the strychnine-type alkaloids (Fig. 26.27).

Seasonal variations in alkaloid content of *S. nux-vomica* have been studied (K. H. C. Baser and N. G. Bisset, *Phytochemistry*, 1982, **21**, 1423).

**Uses.** The action of the whole drug closely resembles that of strychnine. The alkaloid was formerly used as a circulatory stimulant in such cases as surgical shock, but its use is now more limited to that of a respiratory stimulant in certain cases of poisoning. Like other bitters, strychnine improves the appetite and digestion, but it has been considerably misused as a 'general tonic'. Nux vomica is used in Chinese medicine for much the same purposes as in Western medicine and the seeds are usually processed to reduce their toxicity. Heat-treatment of the seeds reduces the normal levels of the principal alkaloids and the amounts of isostrychnine, isobrucine, strychnine *N*-oxide and brucine *N*-oxide are increased (B.-C. Cai *et al*., *Chem. Pharm. Bull*., 1990, **38**, 1295).

**Allied drugs.** The genus *Strychnos* continues to attract considerable attention. (For extensive reviews on the taxonomy, chemistry and ethnobotany of the American, African and Asian species see N. G. Bisset *et al.*, *Lloydia*, **33**, 201; **34**, 1; **35**, 95, 193; **36**, 179; **37**, 62; **39**, 263; also Bisset's review on alkaloids of the Loganiaceae in *Indole and Biogenetically Related Alkaloids*, 1980 (eds J. D. Phillipson and M. K. Zenk) p. 27, London: Academic Press; and J. Quetin-Leclercq *et al.*, *J. Ethnopharm*., 1990, **28**, 1, review with *c.* 150 refs.)

*Ignatius beans* are the seeds of *Strychnos ignatii*, a plant occurring in the Philippines, Vietnam and elsewhere. The fruits are larger than those of nux vomica and may contain as many as 30 seeds. These are about 25 mm long, dark grey in colour and irregularly ovoid in shape. The structure closely resembles that of nux vomica, but the testa, which bears irregularly arranged greyish hairs, is easily rubbed off and is almost entirely absent in the commercial drug. The seeds contain about 2.5–3.0% of total alkaloids, of which about 46–62% is strychnine. They are mainly used for the preparation of strychnine and brucine. The seeds of *S. ignatii* from Java (*S. tieute*) contain 1.5% strychnine and no brucine and from Hainen (*S. hainanensis*) mainly brucine with little strychnine.

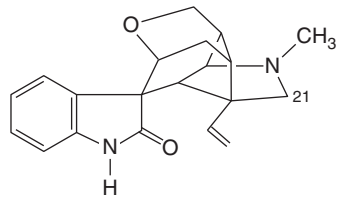
In addition to the seeds, other parts of the plants of *Strychnos* spp. may contain alkaloids including strychnine (B. De Datta and N. G. Bisset, *Planta Med*., 1990, **56**, 133; G. Massiot *et al.*, *Phytochemistry*, 1992, **31**, 2873).

*S. potatorum*, from India, and *S. nux-blanda*, from Burma, have been substituted for nux vomica; although they contain no strychnine or brucine, seeds of the former have been reported to contain the alkaloid diaboline and its acetyl derivative, triterpenes and sterols. They are best distinguished by means of the ammonium vanadate reagent. The seeds of *S. potatorum* are used in India for clearing water, whence the specific name. They will also flocculate heavy metal contaminants in water and are capable of mopping up radioactive isotopes from nuclear waste. The protein responsible for this property has now been isolated (*Pharm. J*., 1994, **252**, 238). The tannins present in the seeds are suggested as the possible active constituents associated with the folklore treatment of chronic diarrhoea (S. Biswas *et al.*, *Fitoterapia*, 2002, **73**, 43).

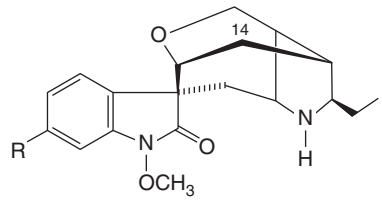
## Gelsemium

Gelsemium consists of the dried rhizomes and roots of the American yellow jasmine, *Gelsemium sempervirens* (*G. nitidum*) family Loganiaceae, indigenous to southern USA. It is a climbing plant and produces scented yellow flowers; it should not be confused with the yellow-flowering jasmine (*Jasminum nudiflorum*, family Oleaceae) cultivated as an ornamental in Europe.

The drug occurs in cylindrical pieces 3–20 cm long and 3–30 mm diameter. The outer cork cells of the rhizome are reddish-brown and the inner ones yellowish. As growth takes place, the outer cork cells crack and the inner cork shows itself as a yellowish-brown reticulation. The roots are somewhat smaller than the rhizome and have a uniform yellowish-brown cork. Gelsemium breaks with an irregular splintery fracture. It has a slightly aromatic odour and a bitter taste. A transverse section of the rhizome shows a thick cork, a cortex containing groups of sclerenchyma, a dense wood, internal as well as external phloem and a small pith. The roots, on the other hand, have no sclerenchyma in the cortex and no pith.




Gelsemine




Gelsedine: R = H
Gelsemicine: R = $OCH_3$

Gelsemium contains extremely toxic alkaloids of unique skeletal type. Gelsemine is the principal alkaloid and is the one most studied although it is not as toxic as gelsemicine. Other oxindole bases characterized are sempervirine, 1-methoxy- and 21-oxo-gelsemine, 14-hydroxygelsemicine, gelsedine and 14-hydroxy-gelsedine. Three new alkaloids of the gelsidine type, together with an iridoid, have been reported by M. Kitajima *et al.*, *Chem. Pharm. Bull.*, 2003, **51**, 1211.

In a review (129 refs) H. Takayama and S. Sakai list 45 alkaloids derived from *G. elegans*, *G. sempervirens* and *G. rankinii*; they are classified into five groups according to structure (*The Alkaloids*, 1997, **49**, 1).

Scopoletin is responsible for the blue fluorescence of the broken drug in ultraviolet light. Iridoids and glucoiridoids have been isolated from the aerial parts.

Gelsemium is used (*BHP*, 1983) in the treatment of trigeminal neuralgia and migraine, but its use requires great care, as dangerous side-effects may develop. It has been studied for its anticancer properties.

*G. elegans* is used in Oriental folk medicine for much the same purposes as *G. nitidum*. For information on new and known alkaloids of the leaves and stems of this species see Y.-K. Xu *et al*., *J. Nat. Prod.*, 2006, **69**, 1347.

## Rauwolfia (Rauvolfia)

Rauwolfia consists of the dried rhizome and roots of *Rauwolfia serpentina* (*Rauvolfia serpentina*), Apocynaceae, a small shrub found in India, Pakistan, Burma, Thailand and Java. The geographical source appears to influence the alkaloidal content, and manufacturers tend to prefer drug obtained from India or Pakistan. Reserpine, the most important constituent, is contained in many other species of *Rauwolfia* (see 'African Rauwolfia' below); it is included in the *BP*/*EP*.

**History.** Although used in India from time immemorial, it was not until 1942 that favourable reports were published of the use of the drug in powdered form. Since then research workers have studied the pharmacognosy, chemistry, pharmacology and clinical uses of many species of *Rauwolfia* and of the alkaloids obtained from them.

**Collection and preparation.** The drug is collected mainly from wild plants, but cultivation of the drug will probably increase as wild plants become more scarce; in parts of India collectors are required to leave some root from each plant in the ground for future growth. Nevertheless, and coupled with the low seed viability, the plant is regarded as an endangered species in India. Consequently, the potential for the regeneration of plants from cell cultures and the possible utilization of nodal culture has received some attention (see C. M. Ruyter *et al*., *Planta Med*., 1991, **57**, 328; N. Sharma and K. P. S. Chandel, *Plant Cell Rep*., 1992, **11**, 200).

As other species of *Rauwolfia* are found in India, care is needed to identify the correct plant. When first imported, many commercial samples were found to be adulterated; this was due in many cases to lack of knowledge, and substitution of, or adulteration with, other species has become much rarer in recent years. After collection the drug is cut transversely into convenient-sized pieces and dried.

**Macroscopical characters.** The first detailed description of the drug was made by Wallis and Rohatgi in 1949. It usually occurs in cylindrical or slightly tapering, tortuous pieces about 2–10 cm long and 5–22 mm in diameter (Fig. 26.32A). The roots are rarely branched and rootlets, 0.5–1 mm in diameter, are rare. Pieces of rhizome closely resemble the root but may be identified by a small central pith; they occasionally have attached to them small pieces of aerial stem.

The outer surface is greyish-yellow, light brown or brown with slight wrinkles (young pieces) or longitudinal ridges (older pieces); occasional circular scars of rootlets. In this species the bark exfoliates readily, particularly in the older pieces, and may leave patches of exposed wood. The drug breaks readily with a short fracture. The smoothed transverse surface shows a narrow, yellowish-brown bark and a dense pale yellow wood, which occupies about three-quarters of the diameter. Both bark and wood contain abundant starch. Some commercial samples show mould. The recently dried drug has a slight odour which seems to decrease with age. Taste, bitter.

**Microscopical characters.** The cork is stratified into about two to eight zones (Fig. 26.32E), which consist of smaller and radially narrower suberized but unlignified cells alternating with larger radially broader cells which are lignified. In many pieces much of the cork is exfoliated, and for section cutting it may be best to select pieces with little exfoliation, separate these from the wood and cut sections of the bark and wood separately. Most of the cells of the secondary cortex are parenchymatous and contain starch; isolated latex cells may occur in this region, particularly in the Dehra Dun variety. The phloem is narrow and consists mainly of parenchyma with scattered sieve tissue. Sclerenchyma is absent (distinction from many other species such as *R. tetraphylla* (*R. canescens*), *R. micrantha*, *R. densiflora*, *R. perakensis* and *R. vomitoria*; see Fig. 26.32 C, D). Most of the parenchymatous cells of the bark contain starch grains, and others prisms or conglomerate crystals of calcium oxalate.

The xylem is entirely lignified and usually shows three to six annual rings. The medullary rays, which are one to five cells wide, contain starch and alternate with the rays of the secondary xylem, which consist of vessels, fibres and xylem parenchyma. Compared with many other species of *Rauwolfia*, the vessels of *R. serpentina* are small (up to 57 μm) and are less numerous than in most of the likely adulterants. The starch grains are larger in the wood than in the bark and measure from 5–**8** to **12**–20 μm.

**Constituents.** Rauwolfia contains at least 30 alkaloids, which total some 0.7–2.4%. Other substances present include phytosterols, fatty acids, unsaturated alcohols and sugars.

In 1931 Siddiqui and Siddiqui isolated ajmaline (rauwolfine), ajmalinine, ajmalicine, serpentine and serpentinine. The chief therapeutically important alkaloids are reserpine (isolated in 1952; formula Fig. 26.26) and rescinnamine (isolated in 1954). These are esters derived from methyl reserpate and trimethoxybenzoic acid in the case of reserpine and trimethoxycinnamic acid in the case of rescinnamine. New alkaloids continue to be isolated; recently, five anhydronium bases (e.g. 3,4,5,6-tetradehydroyohimbine) for the first time (O. Wachsmuth and R. Matusch, *Phytochemistry*, 2002, **61**, 705) and five new indole alkaloids together with a new iridoid glycoside, 7-epiloganin, a new sucrose derivative and 20 known compounds (A. Itoh *et al.*, *J. Nat. Prod.*, 2005, **68**, 848).

**Cell and root cultures.** *R. serpentina* cell suspension cultures have proved an important tool in the elucidation of monoterpenoid indole alkaloid biogenesis and in this connection the significance, and isolation, of strictosidine synthase has been considered. By the use of cell cultures Stöckigt in 1988 was able to clarify a 10-step biosynthetic pathway from strictosidine to the typical rauwolfia alkaloid, ajmaline. His group has also shown that the principal alkaloid of cell suspension cultures of *R. serpentina* is raucaffricine occurring in amounts of up to 1–6 g $l^{-1}$ in the nutrient medium, representing 2.3% of the dried cells, a value some 67 times higher than found for the roots. Ajmalicine, an antiarrhythmic drug, is also produced to the extent of 0.6% (cell dry wt.) together with over 30 different monoterpenoid alkaloids in trace amounts including five glucoalkaloids. Addition of high levels of ajmaline to the cell culture medium promoted the formation of a new group of alkaloids, the raumaclines, not found in the roots (for further details see S. Endress *et al*., *Phytochemistry*, 1993, **32**, 725 and references cited therein).

Hairy root cultures of *R. serpentina*, produced by *Agrobacterium rhizogenes* transformation, synthesized ajmaline (0.045% dry wt.) and serpentine (0.007% dry wt.) (B. D. Benjamin *et al*., *Phytochemistry*, 1994, **35**, 381); minor alkaloids have also been recorded (H. Falkenhagen *et al*., *Can. J. Chem*., 1993, **71**, 2201). *R. verticillata* hairy roots have been shown to produce reserpine and aimaline. Working on hairy root cultures, Stockigt's group has reported on the isolation of three new monoterpenoid indole alkaloids of the sarpagine group along with 16 known compounds together with the first *natural* occurrence (cf. cell cultures above), of the rare raumacline type alkaloids (Y. Sheludko *et al.*, *J. Nat. Prod.*, 2002, **65**, 1006; *Planta Medica* 2002, **68**, 435).

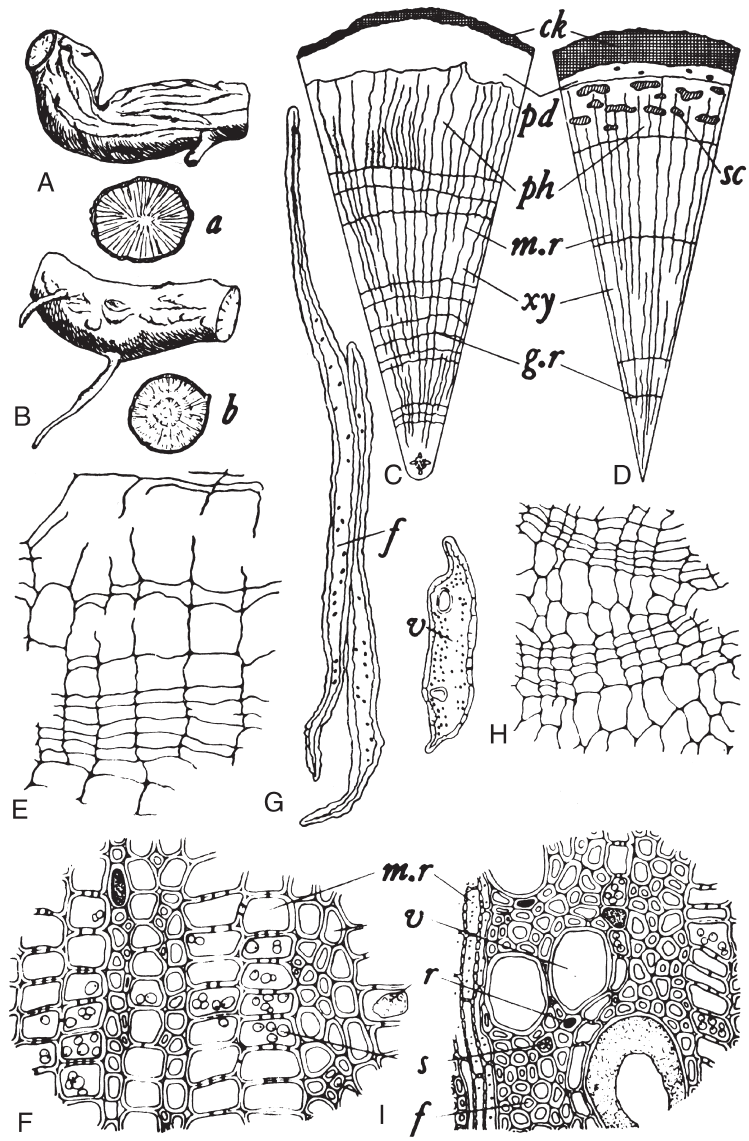



**Fig. 26.32**
*Rauwolfia serpentina* and *R. vomitoria* roots. A, Root of *R. serpentina* (×1); a, transverse section (TS) of same (×1); B, root of *R. vomitoria*, ×1; b, TS of same (×1); C, diagrammatic TS of *R. serpentina* root (×15); D, diagrammatic TS of *R. vomitoria* root (×15); E, TS of cork of *R. serpentina*; F, TS of the secondary wood of *R. serpentina*; G, fibres and vessel of *R. serpentina*, isolated by maceration; H, TS of cork of *R. vomitoria*; I, TS of the secondary wood of *R. vomitoria*; E, F, G, H and I, (all ×150). ck, cork; f, fibre; g.r, growth ring; m.r, medullary ray; pd, phelloderm; ph, phloem; r, resinous material; s, starch; sc, group of sclereids; v, wood vessel; xy, xylem (J. D. Kulkarni, partly after T. E. Wallis and S. Rohatgi (*R. serpentina*) and W. C. Evans (*R. vomitoria*))

Two monoterpenoid indole alkaloids and four β-carbolines have been isolated from cultured hybrid cells of *Rauwolfia serpentina* and *Rhazya stricta*, not all of the compounds being found in the parent plants (N. Aimi *et al*., *Chem. Pharm. Bull*., 1996, **44**, 1637).

**Standardization.** An assay for total alkaloids is not a true measure of therapeutic activity, since only some of the alkaloids have the desired pharmacological action. The *BPC* 1988 and *USP/NF* 1995 determine the reserpine-like alkaloids by utilizing the colour reaction between an acid solution of reserpine (and rescinnamine) and sodium nitrite solution.

**Allied drugs.** The 110 *Rauwolfia* species classified by Pichon in 1947 were reduced by Woodson in 1957 to 86. Some of these occur in more than one geographic area but their approximate geographic areas are as follows: Central and South America 34, Africa 20, Far East 24, India and Burma 7, Hawaii, New Guinea and New Caledonia 6. A large number of these species have been examined for reserpine and related alkaloids.

In the identification of the roots of species of *Rauwolfia* useful characters to be seen in transverse sections are: cork (whether stratified or lignified); cortex and phloem (presence or absence of sclereids or fibres); wood (relative number, distribution and size of vessels). As the species vary from herbs to large trees, the roots vary considerably in size. Some samples of drug contain aerial stems, which usually contain less reserpine than the roots and have unlignified pericyclic fibres.

*R. tetraphylla* (*R. canescens*, *R. hirsuta*) is a species of wide distribution—tropical South America, the Caribbean, India, Australia

(Queensland). The root was at one time occasionally substituted for *R. serpentina* and could be recognized by its non-stratified cork, and sclereid groups in the phloem. It has served as a commercial source of reserpine and the alkaloid deserpidine, possibly particularly important as *R. serpentina* is now classed as an endangered species. Micropropagation protocols have been described for *in vitro* mass multiplication of the plant (D. Sarma *et al*., *Planta Medica*, 1999, **65**, 277).

*R. nitida* is a West Indian species from the root-bark of which 33 indole alkaloids have been isolated.

**Uses.** Rauwolfia preparations and reserpine are used in the management of essential hypertension and in certain neuropsychiatric disorders. Ajmaline, which has pharmacological properties similar to those of quinidine, is marketed in Japan for the treatment of cardiac arrhythmias.

An estimated 3500 kg of ajmalicine is isolated annually from either *Rauwolfia* or *Catharanthus* spp. by pharmaceutical industries for the treatment of circulatory diseases.

Conflicting reports on the possible involvement of the rauwolfia alkaloids in breast cancer engendered a natural hesitation in their use. A report in *the Lancet* (1976) suggested that the alkaloids do not initiate the carcinogenic process but that they promote breast cancer from previously initiated cells.

## African Rauwolfia (African Rauvolfia)

African rauwolfia consists of the dried roots of *Rauwolfia vomitoria* Afz. The plant is a bush or tree widely distributed in tropical Africa from the west coast to Mozambique. It is the most important African rauwolfia for the commercial preparation of reserpine.

**Collection and preparation.** As the tree may attain a height of 10 m, the roots are larger than those of *R. serpentina*. Before drying they are cut transversely, but are rarely sliced longitudinally. Roots up to 5 cm or more in diameter are sometimes found but the commercial drug usually consists of much smaller pieces. Occasional shipments have been made consisting of the bark only.

**Macroscopical characters.** The first detailed description of the drug was published by Evans in 1956. It occurs in cylindrical or flattened pieces, usually 0.15–1.5 cm diameter and up to 30 cm long. The roots taper slightly and are occasionally branched. The outer surface is greyish-brown, longitudinally furrowed or rubbed smooth, since the outer cork easily flakes off. Pieces do not break easily, but the fracture is short in the bark and splintery in the wood. The smoothed transverse surface shows a narrow brown bark and a buff or yellowish, finely radiate wood. Odourless; taste, bitter. Pieces of rootstock with attached stem-bases are sometimes found in the drug.

**Microscopical characters.** The drug is easily distinguished from *R. serpentina* by the groups of sclereids in the bark arranged in up to five discontinuous bands and by the large vessels of the wood which are up to 180 βm in diameter (Fig. 26.32 D and I).

**Constituents.** African rauwolfia contains reserpine and rescinnamine and alkaloids of the same type such as reserpoxidine and seredine. Many other alkaloids such as ajmaline, alstonine and yohimbine are also present. Court's group (see *Planta Med*., 1982, **45**, 105) isolated 42 indole alkaloids from the stem-bark and identified 39. The major alkaloids were heteroyohimbines (especially reserpiline) and $N_a$-demethyldihydroindoles. The interrelationship of these alkaloids with those in the root of the plant is discussed in the same paper. New indole alkaloids from *R. vomitoria* extracts have continued to be reported.

**Allied drugs.** Other African rauwolfias containing reserpine are *R. caffra* (*R. natalensis*), *R. mombasiana*, *R. oreogiton*, *R. obscura*, *R. cumminsii*, *R. volkensii* and *R. rosea*. Court and coworkers have made a systematic study of the microscopy and chemistry of African species (for a report see W. E. Court, *Planta Med*., 1983, **48**, 228). The roots of *R. caffra* closely resemble those of *R. vomitoria* but the cork has not the same tendency to flake off. In sections the main difference is that in *R. vomitoria* there are alternating lignified and unlignified cork cells, while in *R. caffra* all the cork cells are lignified. *R. caffra* contains the alkaloid raucaffricine—one of relatively few examples of monoterpenoid indole glucoalkaloids within the group. *R. mombasiana* differs from *R. vomitoria* in the structure of the wood of the root; *R. rosea*, *R. volkensii* and *R. obscura* lack sclereid development.

## *Alstonia* barks

Several *Alstonia* species (Apocynaceae) have been used in the past as antimalarials, and the barks of the Indian *Alstonia scholaris* and the Australian *A. constricta* were included in the 1914 edition of the *British Pharmacopoeia*. At least 11 species are known to contain alkaloids, such as alstonine, alstoniline, cillastonine and echitamine. Interest in them was again awakened by the isolation in 1955 of reserpine in moderate yield from the root-bark of *A. constricta*; reserpine has since been isolated from *A. venenata*. For a report on the isolation of a new indole alkaloid and a new glycosidic indole alkaloid from the trunk bark of Indonesian *Alstonia scholaris*, see A. A. Salim *et al.*, *J. Nat. Prod.*, 2004, **67**, 1591. Descriptions of *Alstonia* barks will be found in the older editions of reference books (e.g. the 22nd edition of the *USD*, p. 1227).

## Yohimbe bark

Yohimbe bark is derived from *Pausinystalia yohimbe* (Rubiaceae), a tree growing in the Cameroon Republic. It occurs in flat or slightly quilled pieces up to 75 cm long and 2 cm thick. The grey-brown cork has furrows and cracks and patches of lichen. The inner surface is reddish-brown and striated. Taste, bitter. It contains the indole alkaloid yohimbine, which is structurally related to reserpine.

The bark is well-recognized for its aphrodisiac property and yohimbine is effective in the symptomatic treatment of erectile dysfunction, producing fewer side-effects than invasive treatments (M. H. Pittler, *Fortschritte der Medizin*, 1998, **116**, 32).

## *Aspidosperma* barks

The large genus *Aspidosperma* (Apocynaceae) contains many alkaloid-containing South American trees. The alkaloids are of various indole types formed by a number of different biogenetic pathways. Among the many investigated are yohimbine, which is structurally related to reserpine, and aspidospermine, which has the same general structure as vindoline (see Fig. 26.27). The *Aspidosperma* barks are therefore potential sources of alkaloids, because the trees are large and the barks would be commercially available cheaply and in almost unlimited quantities.

## Catharanthus roseus.

For an account of this important plant, see Chapter 27.

## *Mitragyna* leaves

The genus *Mitragyna* (Rubiaceae) occurs in West and East Africa, India and S.E. Asia. More than 30 different alkaloids have been characterized, and the majority of these are indole or oxindole structures with an open or closed ring E; they exist in various isomeric forms. One alkaloid, mitragynine, isolated from *Mitragyna speciosa* has analgesic and antitussive properties similar to those of codeine.

Shellard and coworkers published extensively on the genus during the 1960s and 1970s (for more recent work on *M. speciosa* from the same Department see P. J. Houghton *et al*., *Phytochemistry*, 1991, **30**, 347). The mis-use of mitragyna as an hallucinogen is considered in Chapter 39.

### *Uncaria* species

In addition to *Uncaria gambir*, the source of catechu (q.v.), the genus is notable for its alkaloids, which resemble those of *Mitragyna*. Uncaria hooks, the dried climbing hooks and stems of *U. sinensis,* have sedative and antispasmodic properties. They are used in Chinese medicine for the relief of headaches and dizziness caused by hypertension and for the treatment of convulsions in children. The drug contains indole alkaloids, e.g. rhyncophylline and indole alkaloid glycosides which exhibit a long-lasting hypotensive effect (K. Endo *et al*., *Planta Med*., 1983, **49**, 188; S. Kawazoe *et al*., *ibid*., 1991, **57**, 47).

*Uncaria rhynchophylla*, a species also used in Chinese medicine, is reported to contain various alkaloids including rhynchophylline, corynoxeine, corynantheine and among others, hirsutine which exhibit antihypertensive, neuroprotective and vasodilator effects; (+)-catechin and (−)-epicatechin have been isolated for the first time from this species (W.-C. Hou *et al.*, *J. Ethnopharmacology* 2005, **100**, 216). Other research suggests this species to be an effective anxiolytic agent acting via the serotonergic nervous system (K. W. Jung *et al.*, *J. Ethnopharmacology*, 2006, **108**, 193).

*U. tomentosa*, one of two species found in S. America, features in the traditional medicine of Peru. It produces similar hooks to *U. sinensis* being known locally by the Spanish as 'una degato' (tomcat's claw).

In a number of reports on this species, M. Kitajima *et al.*, have recorded a new glucoindole akaloid, 3,4-dehydro-5-carboxystrictosodine, various triterpenes including nor-triterpene glycosides and cincholic acid glycosides (see *Chem. Pharm. Bull.*, 2004, **52**, 1258 and references cited therein). The plant has a potential immunostimulant action and has been examined for its pharmacological and toxicological properties (K. Keplinger *et al.*, *J. Ethnopharmacology*, 1999, **64**, 23; I. Lemaire *et al.*, *J. Ethnopharmacology*, **64**, 109). It is being used traditionally to treat a large number of conditions, including cancer (R. Pilarski *et al.*, *J. Ethnopharmacology*, 2006, **104**, 18; L. De Martino *et al*., *J. Ethnopharmacology*, 2006, **107**, 91; G. Gonçalves *et al.*, *Phytochemistry*, 2005, **66**, 89).

### Further reading

Heitzman ME, Neto CC, Winiarz E *et al* 2005 Ethnobotany, phytochemistry pharmacology of *Uncaria* (Rubiaceae). Phytochemistry 66(1): 5–29

### *Vinca major* and *V. minor*

The greater periwinkle (*Vinca major*) together with the lesser periwinkle (*V. minor*) are the only members of the essentially tropical and subtropical family Apocynaceae found wild in the British Isles. The former is listed in the *BHP* (1983) for the treatment of menorrhagia and topically as an application for haemorrhoids but is now seldom used. The following indole alkaloids have been characterized: reserpine, majdine, akuammicine, strictosodine (Fig. 26.34), pseudoakuammigine, akuammine and possibly 10-hydroxycathofoline. New alkaloids continue to be reported e.g. Atta-ur-Rahman *et al*., *Phytochemistry*, 1995, **38**, 1057. More than 50 alkaloids have been isolated from the leaves of *V. minor*, a few of them quaternary (for a recent report see D. Uhrin *et al*., *J. Nat. Prod*., 1989, **52**, 637). Vincamine, first isolated from *V. minor* in 1953, is available as a vasodilatory drug.

## CINCHONA

Cinchona bark consists of various species, races and hybrids of *Cinchona* (Rubiaceae), large trees indigenous to Colombia, Ecuador, Peru and Bolivia. The *BP*/*EP* recognizes the whole or cut, dried bark of *Cinchona pubescens* Vahl (*C. succirubra* Pavon), *C. calisaya* (Weddell), of *C. ledgeriana* (Moens ex Trimen) or its varieties or hybrids, containing not less than 6.5% of total alkaloids, 30–60% of which consists of quinine-type alkaloids. The former importance of cinchona bark and its alkaloids in the treatment of malaria has been lessened by the introduction of synthetic drugs, but it remains of great economic importance, and salts of quinine and quinidine are included in most pharmacopoeias.

Collection from wild trees was soon replaced by cultivation, and most research was undertaken by the Dutch in Java and the British in India to obtain hybrids which are rich in alkaloids. While Indonesia and India remain important producers of cinchona, a high percentage of the total crop is now grown on plantations in Tanzania, Kenya, Guatemala and Bolivia.

**History.** The natives of South America do not appear to have been acquainted with the medicinal properties of cinchona bark, the bitter taste of which inspired them with fear. Although Peru was discovered in 1513, the bark was first used for the cure of fevers about 1630. The name 'Cinchona' is said to be derived from a Countess of Chinchon, wife of a viceroy of Peru who it was long believed was cured in 1638 from a fever by the use of the bark. According to recent study of the Count's diary, it appears that the Countess never suffered from malaria or other fever during her stay in Peru, and although the Count himself did so, there is no record of his having been treated with cinchona bark. The remedy, which became known as 'Pulvo de la Condesa', acquired a considerable reputation and was known in Spain in 1639. The further distribution of the bark was largely due to the Jesuit priests, and the drug became known as Jesuit's Powder or Peruvian Powder. It first appeared in the *London Pharmacopoeia* in 1677 under the name of 'Cortex Peruanus'.

The bark was originally obtained by felling the wild trees, which were exterminated in many districts. Ruiz (1792) and Royle (1839) suggested the cultivation of cinchonas in other parts of the world. Weddell germinated seeds in Paris in 1848, and the plants were introduced into Algiers in the following year but without much success. A further attempt by the Dutch was made in 1854, seeds and plants being obtained from Peru by Hasskarl and introduced into Java. An English expedition under Markham in 1860 led to the introduction of *C. succirubra* (the most hardy species), *C. calisaya*, and *C. micrantha* into India. Seeds of *C. ledgeriana* were obtained in Bolivia by Charles Ledger in 1865 and were bought by the Dutch for their Javanese plantations. A fascinating book covering Ledger's exploits is *The Life of Charles Ledger* (*1818–1905*) by G. Grammicia, Macmillan Press, London, 1988. World War II and subsequent fighting in Malaya and Vietnam increased the demand for cinchona and stimulated cultivation in Africa and Central and South America.

**Cultivation, collection and preparation.** The production of cinchona bark is a highly specialized section of tropical agriculture. An acid soil, rainfall and altitude are all important factors in cinchona production. Selection of high-yielding strains is of paramount importance, and grafting techniques with *C. succirubra* as stock may be employed. Seedlings need careful treatment and propagation to avoid disease attack, etc. Since the mid-1970s a disease of the cinchona tree, known as stripe canker, has posed a threat to the plantations of Central Africa. The disease, also known in Central America, is caused by the phytopathogenic fungus *Phytophthora cinnamomi*, which causes sunken necrotic stripes in the bark and kills thousands of trees a year.

**General characters**

1. *Stem-bark*. The commercial 'druggist's' quills are up to 30 cm long and usually 2–6 mm thick. Bark for manufacturing purposes is frequently in small curved pieces. The outer surface frequently bears moss or lichen. The cork may or may not be longitudinally wrinkled, and usually bears longitudinal and transverse cracks, which vary in frequency and distinctness in the different varieties. The inner surface is striated and varies in colour from yellowish-brown to deep reddish-brown. The fracture is short in the outer part but somewhat fibrous in the inner part. Odour, slight; taste, bitter and astringent.
2. *Root-bark*. Root-bark occurs in channelled, often twisted pieces about 2–7 cm long. Both surfaces are of similar colour, the outer, however, being somewhat scaly, while the inner surface is striated.

**Special characters.** In view of the number of hybrids which are cultivated, the distinction of the various commercial cinchona barks is a matter of some difficulty. In Table 26.7 the notes on four important species have been made as concise as possible to facilitate comparison.

**Microscopical characters.** Cinchona barks have the general microscopical structure shown in Fig. 26.33. The cork is composed of several layers of thin-walled cork cells, arranged in regular radial rows and appearing polygonal in surface view. Their cell contents are dark reddish in colour. Within the cork cambium is a phelloderm of several layers of regular cells with dark walls. The cortex is composed of tangentially elongated, thin-walled cells containing amorphous reddish-brown matter or small starch grains 6–10 μm diameter. Scattered in the cortex are idioblasts containing microcrystals of calcium oxalate and secretion cells. The phloem consists of narrow sieve-tubes showing transverse sieve plates, phloem parenchyma resembling that of the cortex and large characteristic spindle-shaped phloem fibres with thick conspicuously striated walls traversed by funnel-shaped pits. The phloem fibres occur isolated or in irregular radial rows. The distribution and size of the phloem fibres differ in the various species

**Table 26.7 Comparison of *Cinchona* species.**

| *C. succirubra* (Fig. 26.33) | *C. calisaya* | *C. ledgeriana* | *C. officinalis* |
|---|---|---|---|
| Frequently 20–40 mm diameter, and 2–6 mm thick | Diameter 12–25 mm and 2–5 mm thick | Similar to *C. calisaya* | Up to 12 mm diameter, and 1 mm thick |
| Well-marked longitudinal wrinkles, relatively few transverse cracks. Some pieces, but by no means all, show reddish warts | Broad longitudinal fissures; transverse cracks about 6–12 mm apart | Similar to *C. calisaya*, but cracks more numerous and less deep. Some pieces show longitudinal wrinkles and reddish warts | Transverse cracks, very numerous, often less than 6 mm apart |
| Powder reddish-brown | Powder cinnamon-brown | Powder cinnamon-brown | Powder yellowish |

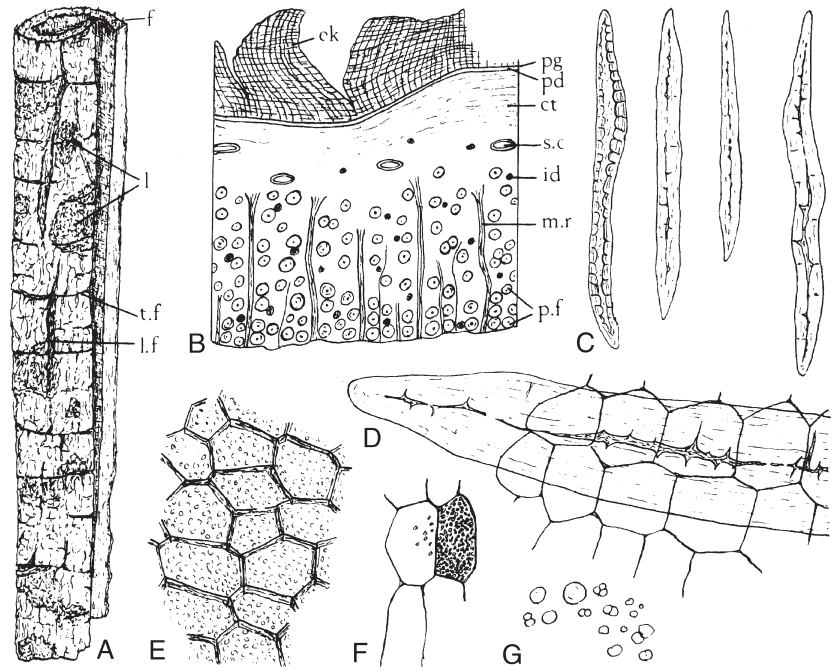



**Fig. 26.33**
Cinchona bark. A, specimen of *Cinchona succirubra* (× 0.5); B, transverse section of bark (×25); C, isolated phloem fibres (×50); D, portion of phloem fibre with surrounding parenchyma; E, cork cells in surface view; F, idioblast with calcium oxalate; G, starch (all × 200). ck, Cork; ct, cortex; f, fibres protruding from fracture; id, idioblast; l, lichen patches; l.f, longitudinal fissure; m.r, medullary ray; pd, phelloderm; pg, phellogen; p.f, phloem fibres; s.c, secretory cell; t.f, transverse fissure.

(those of *C. succirubra* are 350–**600**–**700**–1400 μm long and 30–**40**–**70**–100 μm diameter; for other species see 10th edition). The medullary rays are two or three cells wide, the cells being thin-walled and somewhat radially elongated.

**Constituents.** Cinchona bark contains quinoline alkaloids (see Fig. 26.34). The principal alkaloids are the stereoisomers quinine and quinidine and their respective 6′-demethoxy derivatives, cinchonidine and cinchonine. The quinine series has the configuration 8*S*, 9*R* and the quinidine 8*R*, 9*S* (Fig. 26.34); other alkaloids of lesser importance have been isolated. Some of these (e.g. quinicine and cinchonicine) are amorphous. The amount of alkaloids present and the ratios between them vary considerably in the different species and hybrids, also according to the environment of the tree and the age and method of collection of the bark.

The alkaloids appear to be present in the parenchymatous tissues of the bark in combination with quinic acid and cinchotannic acid. Quinic acid (see Fig. 19.5) is present to the extent of 5–8%. Cinchotannic acid is a phlobatannin and a considerable amount of its decomposition product, 'cinchona red', is also found in the bark. Other constituents are quinovin (up to 2%), which is a glycoside yielding on hydrolysis quinovaic acid and quinovose (isorhodeose).

Anthraquinones, which as a group of compounds are associated with the family Rubiaceae (see Table 21.3), are not normally found in quantity in the bark of cinchona as indicated by the isolation of norsolorinic acid, a tetrahydroxyanthraquinone, in 0.0008% yield from the bark of *C. ledgeriana*. However, they are produced in cell cultures of the plant and by infection of the bark with *Phytophthora cinnamomi*. The latter case may be associated with a phytoalexin defence mechanism; in infected material, alkaloid production is lowered. In connection with the production of anthraquinones in cell cultures, enzymes associated with the later stages of glycoside formation have been isolated. This work involved glucosidases in *C. succirubra* cell cultures and the

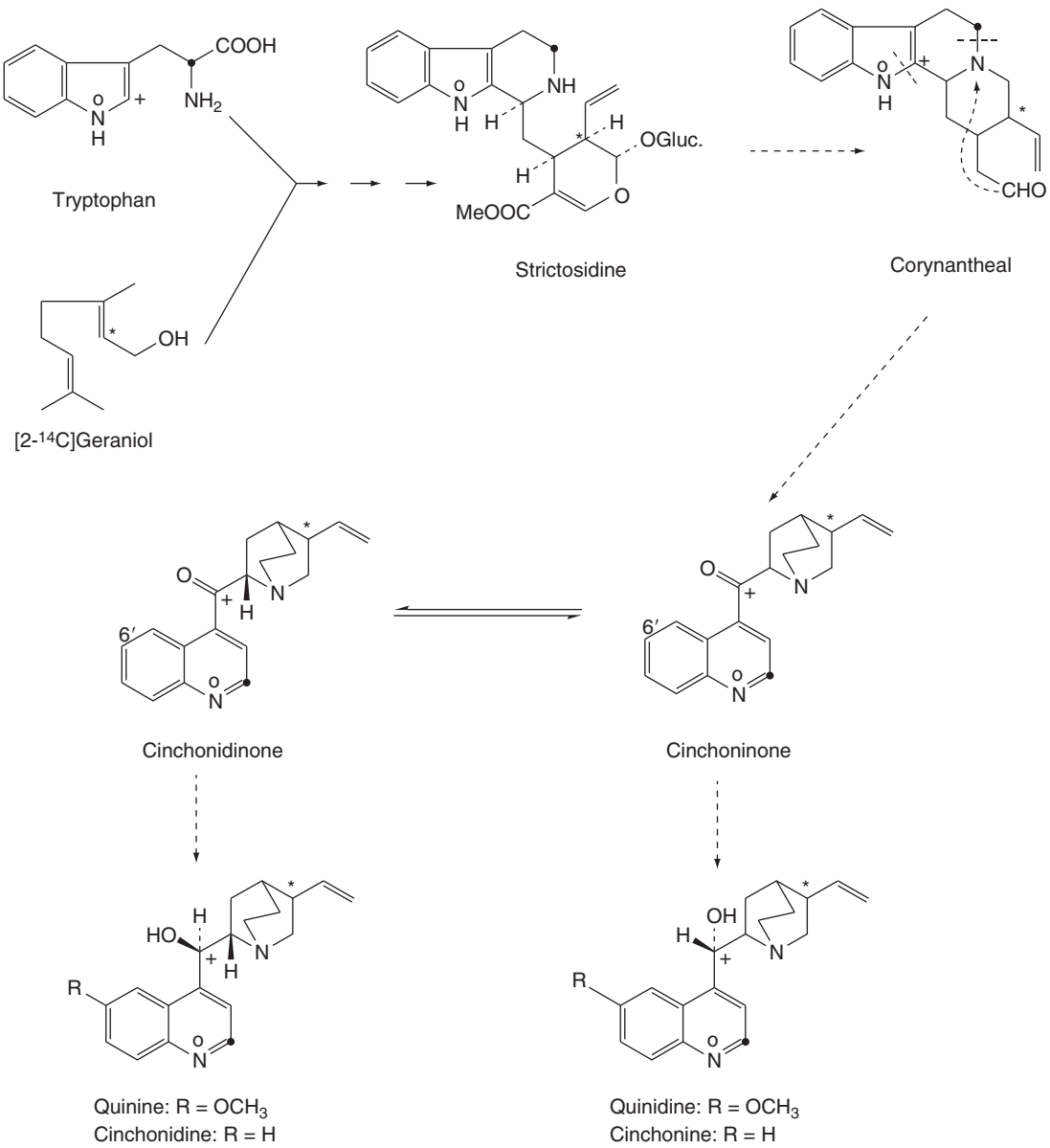



**Fig. 26.34**
Outline of possible biogenetic pathway for *Cinchona* alkaloids. Marked atoms illustrate the structural changes.

isolation of five distinct glucosyltransferases (EC 2,4,1,-) which catalyse the transfer of the glucosyl moiety from UDP-glucose to the hydroxyl groups of those anthraquinones found in cinchona cultures (e.g. emodin, anthrapurpurin, quinizarin, etc.).

**Biosynthesis of alkaloids.** Grafting experiments and organ culture suggest that the alkaloids are formed principally in the aerial parts of the plant. Although the quinoline alkaloids have structures which by inspection might suggest anthranilic acid as a biological precursor, they are, in fact, as originally suggested by Janot *et al*. in 1950, derived from indolic precursors. This has been demonstrated by the specific incorporation of tryptophan (indole moiety), loganin and geraniol (terpenoid moiety) into the quinine of *Cinchona* spp. The pathway, largely established by Battersby's and Leete's groups, involves alkaloids of the serpentine type as illustrated in Fig. 26.34.

The proposed biosynthetic route has been supported and elaborated by enzyme studies. The important role of strictosidine synthase in the initial stages of the biogenesis of some tryptophan-derived alkaloids and its isolation from *C. robusta* was mentioned at the beginning of this section. The enzyme tryptophan decarboxylase (EC 4.1.1.28), which provides tryptamine, is also involved in these early reactions. An enzyme (cinchoninone: NADPH oxidoreductase) associated with the pathway has been isolated from cells of a suspension culture of *Cinchona ledgeriana*; it catalyses the reduction of cinchoninone to an unequal mixture of cinchonine and cinchonidine. The enzyme can be resolved (by ion exchange) into two isoenzymic forms both of which have an absolute requirement for NADPH and catalysed reversible reactions. Isoenzyme I acts specifically on cinchoninone in the forward direction of the pathway and on cinchonidine and cinchonine in the reverse direction. Isoenzyme II has a broad specificity acting on all the quinoline alkaloids of cinchona tested.

**Allied drugs.** The barks of certain species of *Remijia* (Rubiaceae) contain alkaloids. That of *R. pedunculata* is quoted (*USP*) as a source of quinidine. It also contains cupreine, an alkaloid which responds to the thalleioquin test and by methylation forms quinine. False cuprea bark (*R. purdiena*) contains no quinine but an alkaloid cusconidine and small proportions of cinchonine and cinchonamine.

**Cinchona leaf alkaloids.** Alkaloids of the indole type (e.g. cinchophylline) are generally considered to typify the leaves, so that it is of interest that Phillipson *et al*. (*J. Pharm. Pharmacol*., 1981, **33**, 15P) isolated quinine from leaves of *C. succirubra* grown in Thailand. Thirteen alkaloids have been separated by HPLC.

**Uses.** Galenicals of cinchona have long been used as bitter tonics and stomachics. On account of the astringent action, a decoction and acid infusion are sometimes used as gargles. Before World War II, quinine was the drug of choice for the treatment of malaria but became largely superseded by the advent of synthetic antimalarials developed during that period. It has, however, remained of importance in Third World countries and has re-emerged as suitable for the treatment of *Plasmodium falciparum* infections (falciparum malaria) in the many areas where the organism is now resistant to chloroquine and other antimalarials.

Quinidine is employed for the prophylaxis of cardiac arrhythmias and for the treatment of atrial fibrillation; it also has antimalarial properties and like quinine is effective against chloroquine-resistant organisms.

## MISCELLANEOUS ALKALOIDS

There are a number of relatively small groups of alkaloids, some of whose biosynthetic relationships to particular amino acids have not been firmly established or whose formation does not involve direct amino acid participation.



## INDOLIZIDINE ALKALOIDS

Only a small number of indolizidine alkaloids are currently known but they have recently become of pharmaceutical interest through the discovery of the tetrahydroxy alkaloids castanospermine and 6-epi-castanospermine, which are possible lead compounds in the search for anti-AIDS drugs (see Chapter 30). Also, like the above, swainsonine, the toxic constituent of locoweeds and Australian *Swainsona* spp., is a powerful glycosidase inhibitor; this alkaloid is a trihydroxy-indolizidine. Both alkaloids are biosynthesized from lysine via pipecolic acid.

(For a review of the simple indolizidine alkaloids (154 refs) see J. Takahata and T. Momose, *Alkaloids*, 1993, **44**, 189.)

## IMIDAZOLE ALKALOIDS

The most important pharmaceutical examples of this group are the *Pilocarpus* alkaloids, pilocarpine finding use as an ophthalmic cholinergic drug. Possible biosynthetic routes to pilocarpine (see formula under 'Jaborandi Leaf and Pilocarpine', below) could involve either of the amino acids histidine or threonine.

### JABORANDI LEAF AND PILOCARPINE

The name 'jaborandi' is now applied to the leaflets of various species of *Pilocarpus* (Rutaceae), a genus of trees and shrubs well represented in South America and found to a lesser extent in the West Indies and Central America. The principal jaborandi now imported, Maranham jaborandi, is that derived from the Brazilian plant *Pilocarpus microphyllus*.

The state of Maranhão accounts for about 90% of the Brazilian leaf. Traditionally the crop is collected from wild plants but over the years production has fallen due to non-sustainability of the supply and attempts to cultivate the plant commercially have been undertaken. As with a number of medicinal plants, transforming a wild species into a cultivable crop is not necessarily easy and a balanced collection between wild and cultivated plants may be desirable.

A comprehensive account relating to jaborandi production is given by C. U. B. Pinheiro (*Economic Botany*, 1997, **51**, 49).

Jaborandi was formerly official but is now used mainly as a source of the medicinally important alkaloid pilocarpine.

#### Characters of principal sources

1. *Maranham jaborandi*. The plant *P. microphyllus*, which produces the Maranham drug, bears imparipinnate compound leaves with about seven leaflets (Fig. 26.35). The leaflets are attached to a somewhat winged rachis which is almost glabrous (*Swartzia* is hairy). The drug consists of separated leaflets, a certain amount of rachis and an occasional fruit. The leaflets are 2–5 cm long, 1–3 cm broad, and emarginate at the apex. The terminal leaflets are oval, symmetrical and have a petiolule 5–15 mm long, with a winged margin which passes imperceptibly into the lamina. The remaining leaflets are

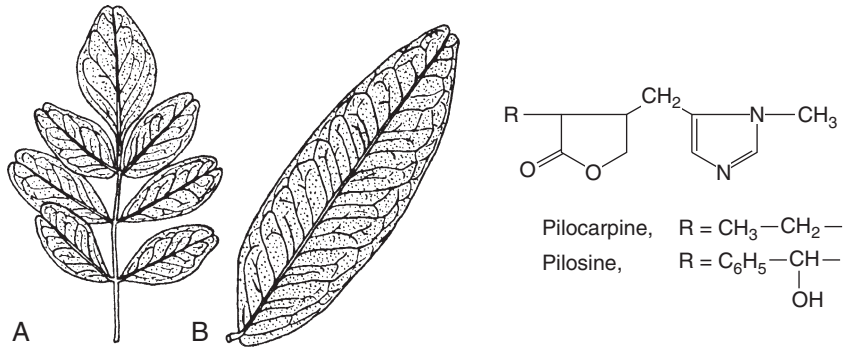



**Fig. 26.35**
Jaborandi. A, Leaf of *Pilocarpus microphyllus*; B, leaflet of *P. jaborandi* (×0.5).

obovate, asymmetrical and sessile. Leaflets of the left and right sides of the leaf may be distinguished from one another by the fact that the broader side of each leaflet lies away from the rachis. The veins are pinnate and anastomose near the margin. The drug is greyish-green to greenish-brown and brittle in texture. Numerous small oil cells may be seen by transmitted light. Odour, when crushed, slightly aromatic; taste, bitterish and aromatic with induction of salivation.

2. *Pernambuco jaborandi*. Pernambuco jaborandi consists of the leaflets of *Pilocarpus jaborandi* Holmes, which are obtained from a compound imparipinnate leaf with 1–9 leaflets. Leaflets 4–12 cm long and 2–4 cm broad (Fig. 26.35), petiolules short; apex emarginate; base usually asymmetric; margin entire and slightly revolute. Upper surface glabrous and greyish to brownish-green; lower surface yellowish- or greenish-brown and slightly pubescent. Midvein not prominent on the upper surface but very prominent on the lower surface (in the midveins of the Maranham leaflets the reverse is the case). A transverse section of the midveins is often useful for distinguishing between the various species of *Pilocarpus*—for example, the Pernambuco variety shows a complete ring of pericyclic sclerenchyma, the Maranham a broken ring.

   A compound, 1-phenyl-5-vinyl-5,9-dimethyl decane has been obtained from the foliar epicuticular wax of *P. jaborandi* and by TLC it can be used to distinguish this species from others of *Pilocarpus* (G. Negri *et al*., *Phytochemistry*, 1998, **49**, 127).
3. *Paraguay jaborandi*. Paraguay jaborandi, derived from *P. pennatifolius* Lemaire. Greyish-green; papery in texture; usually equal at base; veins not prominent on the upper surface and the anastomoses not marked. Pericyclic sclerenchyma more broken than in Maranham or Pernambuco. The above three varieties have a single palisade layer, a point which distinguishes them from the Guadeloupe and Aracati varieties.
4. *Ceara jaborandi*. Ceara jaborandi is derived from *P. trachylophus* Holmes and is exported from the Brazilian provinces of Ceara and Maranhão. Leaflets are smaller than those of *P. jaborandi*; oblong or elliptical; coriaceous; both surfaces bearing short curved hairs, which are particularly abundant on the lower surface.

**Constituents.** Maranham leaves contain about 0.7–0.8% of the alkaloids, pilocarpine, isopilocarpine, pilosine and isopilosine and about 0.5% of volatile oil. An examination of the volatile oil composition of a number of species indicated a total of 22 components occurring throughout the samples. These included monoterpenes (e.g. limonene, sabiene, α-pinene) sesquiterpenes (e.g. caryophyllene) but not in *P. jaborandi*, and 2-undecanone or 2-tridecanone.

Pilocarpine, the lactone of pilocarpic acid, contains a glyoxaline nucleus and with heat or alkalis is converted into its isomer isopilocarpine. Isopilocarpine occurs in small quantity in the leaf but more is formed during the extraction process. The dried leaves soon lose their activity on storage.

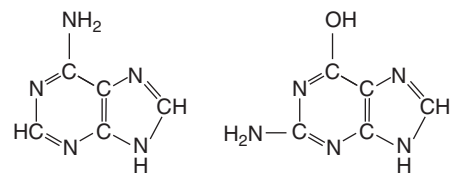
**Uses.** Salts of pilocarpine (e.g. Pilocarpine Hydrochloride *BP*/*EP* and Nitrate *BP*/*EP*) are used in ophthalmic practice, as they cause contraction of the pupil of the eye, their action being antagonistic to that of atropine. In early glaucoma treatment they serve to increase the irrigation of the eye and relieve pressure. A study in the USA involving 207 patients suffering from dry mouth resulting from radiation treatment for head or neck cancer indicated that oral pilocarpine can possibly offer relief (*Pharm. J*., 1993, **251**, 215). In 1994 its use was approved for this purpose by the US Food and Drug Administration.

## PURINE ALKALOIDS

The purine nucleotides, together with the pyrimidine nucleotides, constitute vital structural units of the nucleic acids; they also function as coenzymes (Chapter 18) and as portions of complex substrate molecules. Adenine and guanine are the purines most commonly involved in these roles, but xanthine and hypoxanthine feature in their biosynthesis.

'Purine alkaloids' constitute secondary metabolites and are derivatives of xanthine; three well-known examples are caffeine (1,3,7-trimethylxanthine), theophylline (1,3-dimethylaxanthine) and theobromine (3,7-dimethylxanthine).

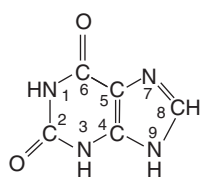
Beverages such as tea and coffee owe their stimulant properties to these substances. Caffeine stimulates the central nervous system and has a weak diuretic action, whereas theobromine acts in the reverse way. Theophylline has generally similar properties to the above, with a shorter, though more powerful diuretic action than caffeine; it relaxes involuntary muscles more effectively than either caffeine or theobromine. The three alkaloids are official in the *EP* and *BP*.



Adenine



Guanine



Xanthine

**Biogenesis.** The ring formation of the purine alkaloids appears to follow the classical scheme for the biosynthesis of purine nucleotides with $C_1$-moieties arising from such compounds as formates and formaldehyde. Methylamine is also effectively incorporated into the ring system; studies by Suzuki *et al*. indicated that methylamine is oxidized to formaldehyde and then metabolized as a $C_1$ compound. For caffeine, the purine bases such as hypoxanthine, adenine and guanine, and the nucleosides can also be incorporated by the plant into the molecule.

In both tea and coffee plants and in suspension cultures of *Coffea arabica* it has been clearly demonstrated that theobromine is methylated to caffeine. In 1979, as a result of work involving *N*-methyltransferases, Roberts and Waller suggested the pathway 7-methylxanthosine → 7-methylxanthine (heteroxanthin) → 3,7-dimethylxanthine (theobromine) → 1,3,7-trimethylxanthine (caffeine) which has been substantiated by later work. *S*-Adenosylmethionine is utilized as a donor of the methyl groups. Attempts to isolate the individual *N*-methyltransferase enzymes do not yet appear to have been successful due partly to their extreme lability. However progress has been made on their biochemical characterization and their time course during leaf development of *Coffea arabica* (S. S. Mösli Waldhauser *et al*., *Phytochemistry*, 1997, **44**, 853). The origin of the caffeine molecule is shown in Fig. 26.36.

(For a review on this aspect see T. Suzuki *et al*., *Phytochemistry*, 1992, **31**, 2575.)

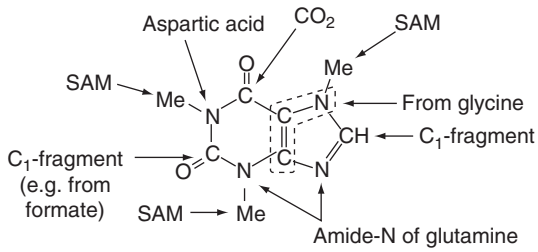



**Fig. 26.36**
Biogenetic origin of the caffeine molecule (SAM = *S*-adenosylmethionine).

## COLA

Commercial cola (*Kola seeds*, *bissy* or *gooroo* nuts) consists of the dried cotyledons of the seeds of various species of *Cola* (Sterculiaceae), trees found in West Africa, the West Indies, Brazil and Java. The colour of the fresh seeds varies, those of *C. acuminata* being white or crimson, *C. astrophora* red, *C. alba* white and *C. vera (C. nitida*) (which is possibly a hybrid of the two latter species) either red or white. The *BP*/*EP* specifies *C. nitida* and its varieties as well as *C. acuminata*, containing not less than 1.5% caffeine. The dried cotyledons are usually of a dull, reddish-brown colour and more or less broken. They are usually graded as 'halves' and 'quarters'. The whole seeds are 2–5 cm long, and in the seeds usually imported there are two cotyledons. A microscopical examination of the powder shows portions of thick-walled, reddish polygonal cells of the cotyledons containing concentrially striated starch granules, reniform to ovoid in shape and 5–25 μm in size. Odourless; taste, slightly astringent.

Cola seeds contain caffeine (1–2.5%) and a little theobromine, which appear to be partly in the free state and partly combined. kola also contains about 5–10% of tannoids (the 'kolatin' of earlier workers), particularly catechol and epicatechol. During preparation, oxidation and polymerization of these produces the insoluble phlobaphene 'kola-red'. It has been suggested (C. Maillard *et al.*, *Planta Med*., 1985, 515) that the differences in the stimulatory action between fresh and dried seeds may be due to the formation of a caffeine–catechin complex in the latter.

The pharmacopoeia uses a TLC test for identity using caffeine and theobromine as reference compounds. Liquid chromatography is used for the official assay with absorption measurements at 272 nm.

## COCOA SEED

Cocoa seeds (*Cocoa Beans*) are obtained from *Theobroma cacao* (Sterculiaceae), a tree usually 4–6 m high. Cocoa is produced in South America (Ecuador, Colombia, Brazil, Venezuela and Guiana), Central America, the West Indies, West Africa (Ivory Coast, Nigeria and Ghana), Ceylon and Java.

**History.** Cocoa has long been used in Mexico and was known to Columbus and Cortez. Cocoa butter was prepared as early as 1695.

**Collection and preparation.** Cocoa fruits are 15–25 cm long and are borne on the trunk as well as on the branches. Cocoa plantations are very vulnerable to pest attack and recently modern pheromone technology has been used to control the cocoa pod borer, also known as the cocoa moth (*Conopomorpha cramerella*), the most serious pest of the crop in S.E. Asia. Collection continues throughout the year, but the largest quantities are obtained in the spring and autumn. The fruits have a thick, coriaceous rind and whitish pulp in which 40–50 seeds are embedded. In different countries the seeds are prepared in different ways, but the following may be taken as typical: the fruits are opened and the seeds, embedded in the whole pulp or roughly separated from it, are allowed to ferment. Fermentation occurs in tubs, boxes or cavities in the earth; the process lasts 3–9 days, and the temperature is not allowed to rise above 60°C. In Jamaica fermentation is allowed to proceed for 3 days at a temperature of 30–43°C. During this process a liquid drains from the seeds, which change in colour from white or red to purple, and also acquire a different odour and taste. After fermentation the seeds may or may not be washed. They are then roasted at 100–140°C, when they lose water and acetic acid and acquire their characteristic odour and taste. Roasting facilitates removal of the testa. The seeds are cooled as rapidly as possible and the testa removed by a 'nibbling' machine. The nibs or kernels are separated from the husk by winnowing. Sometimes the seeds are simply dried in the sun but these are not as highly regarded owing to their astringent and bitter taste.

*Plain* or *bitter chocolate* is a mixture of ground cocoa nibs with sucrose, cocoa butter and flavouring. Milk chocolate contains in addition milk powder.

**Macroscopical characters.** Cocoa seeds are flattened ovoid in shape, 2–3 cm long and 1.5 cm wide. The thin testa is easily removed from prepared cocoa beans, but is difficult to remove from those that have not been fermented and roasted. The embryo is surrounded by a thin membrane of endosperm. The cotyledons form the greater part of the kernel and are planoconvex and irregularly folded. Each shows on its plane face three large furrows, which account for the readiness with which the kernel breaks into angular fragments. Both testa and kernel are of a reddish-brown colour, which varies, however, in different commercial varieties and depends on the formation of 'cacao-red' during processing.

**Constituents.** Cocoa kernels contain 0.9–3.0% of theobromine and the husks contain 0.19–2.98% of this alkaloid. The seeds also contain 0.05–0.36% caffeine, cocoa fat or butter (nibs 45–53%, husk 4–8%). During the fermentation and roasting, much of the theobromine originally present in the kernel passes into the husk. The constituents other than fat and theobromine are extremely complex and have been intensely studied in recent years. The fresh seeds contain about 5–10% of water-soluble polyphenols (epicatechol, leucoanthocyanins and anthocyanins) which are largely decomposed during processing, forming the coloured complex formerly known as 'cocoa-red'. Condensed tannins are also present, and some 84 different volatile compounds, including glucosinolates, are responsible for the aroma (see M. S. Gill *et al.*, *Phytochemistry*, 1984, **23**, 1937).

*Theobromine* is produced on the commercial scale from cocoa husks. The process consists of decocting the husks with water, filtering, precipitating 'tannin' with lead acetate, filtering, removing excess of lead and evaporating to dryness. Theobromine is extracted from the residue by means of alcohol and purified by recrystallization from water.

Theobromine is 3,7-dimethylxanthine (see p. 000), the lower homologue of caffeine (trimethylxanthine). It is isomeric with theophylline (1,3-dimethylxanthine), which occurs in small quantities in tea. Theobromine crystallizes in white rhombic needles. It gives the murexide reaction (see p. 000), and may be distinguished from caffeine by the fact that it is precipitated from a dilute nitric acid solution by silver nitrate. Theobromine sublimes at 220°C, caffeine at 178–180°C.

Callus and suspension cultures of cocoa both produce caffeine, theobromine and theophylline and are considered useful for studying secondary metabolism *in vitro*.

**Uses.** Cocoa has nutritive, stimulant and diuretic properties. Theobromine is used as a diuretic. It has less action on the central nervous system than caffeine but is more diuretic. With its isomer, theophylline, the diuretic effect is even more marked. Oil of theobroma (q.v.) is used in pharmacy chiefly as a suppository base.

**Allied products.** *Guarana* (*Pasta Guarana* or *Brazilian cocoa*) is a dried paste prepared mainly from the seeds of *Paullinia cupana* (Sapindaceae). The seeds are collected from wild or cultivated plants in the upper Amazon basin by members of the Guaranis tribe. The kernels are roughly separated from the shell, broken and made into a paste with water, starch and other substances being frequently added. The paste is then made into suitable shapes and dried in the sun or over fires.

The drug usually occurs in cylindrical rolls 10–30 cm long and 2.5–4 cm diameter. Portions of broken seed project from the dark chocolate-brown outer surface. When broken, similar fragments project from the fractured surface. The drug has no marked odour but an astringent bitter taste.

Guarana contains 2.5–7.0% of caffeine, other xanthine derivatives, tannins about 12% ('guarana red') and other constituents resembling, as far as is known, those of cola and cocoa. Guarana resembles tea and coffee in its action and the powder grated from the masses is used in South America with water to make a drink. In the West it is now a popular remedy for combating fatigue, for slimming, and for the treatment of diarrhoea. The fat content of the drug is stated to effect a slow but steady release of the alkaloids (for a short article on guarana see P. Houghton, *Pharm. J.*, 1995, **254**, 435).

*Coffee* consists of the seeds of *Coffea arabica* and other species of *Coffea* (Rubiaceae). It contains caffeine (1–2%), tannin and chlorogenic (caffeotannic) acid (see Fig. 19.5), fat, sugars and pentosans.

Prepared coffee is the kernel of the dried ripe seeds of various species, including *C. arabica* (Arabica coffee), *C. liberica* and *C. canephora* (Robusta coffee) (Rubiaceae), deprived of most of the seed coat and roasted. The kernels are dark brown, hard and brittle, elliptical or planoconvex and about 1.0 cm long. Coffee has a characteristic odour and taste. A decoction is used as a flavouring agent in Caffeine Iodide Elixir BPC (1979). Prepared coffee contains about 1–2% of caffeine, probably combined with chlorogenic acid and potassium. Other constituents include nicotinic acid, fixed oil and carbohydrates caramelized during roasting.

*C. arabica*, both as whole plants and as cell suspension cultures, has been considerably employed to study purine alkaloid variations and biosynthesis (q.v.).

*Tea* consists of the prepared leaves of *Camellia sinensis (Thea sinensis)* (Theaceae), a shrub cultivated in India, Sri Lanka, East Africa, Mauritius, China and Japan. The leaves contain thease, an enzymic mixture containing an oxidase, which partly converts the phlobatannin into phlobaphene. This oxidase may be destroyed by steaming for 30 s. Tea contains 1–5% of caffeine and 10–24% of tannin; also small quantities of theobromine, theophylline and volatile oil. The alkaloid content of the leaves is very much dependent on age and season.

*C. sinensis* is known locally as the Chinese tea plant, the flower buds and seeds of which contain acylated oleane-type triterpenes (theasaponins) with antiallergic activities (M. Yashikawa *et al.*, *Chem. Pharm. Bull.*, 2007, **55**, 57; **55**, 598). The flower buds of *C. japonica* yield noroleane and oleane-type triterpenoids having gastroprotective and platelet aggregation activities (M. Yashikawa *et al.*, *Chem. Pharm. Bull.*, 2007, **55**, 606).

Callus and root suspension cultures of *C. sinensis* have been shown to accumulate caffeine and theobromine (A. Shervington *et al.*, *Phytochemistry*, 1998, **47**, 1535).

The possible beneficial effects of drinking black or green tea have received considerable coverage in the medical and national press. An infusion of tea contains in addition to caffeine a mixture of polyphenols including epigallocatechin-3-gallate possessing strong antioxidant and free-radical scavenging properties. Possible beneficial effects are: inhibition of angiogenesis, a process involving the growth of blood vessels necessary for tumour growth and metastasis; the treatment of genetic haemochromatosis by the inhibition of absorption of iron by tannates and other ligands; treatment of blindness caused by diabetes (an angiogenic related condition); and a lowering of the risk of ischemic heart disease in older men (a finding not substantiated with tea with milk added) see M. G. L. Hertog *et al*., *Amer. J. Clin. Nutr*., 1997, **65**, 1489; J. P. Kaltwasser, E. Werner, K. Schalk *et al*., *Gut*, 1998, **43**, 649; Y. Cao and R. Cao, *Nature*, 1999, **398**, 381.

## Maté leaf

Maté (*Yerba maté; Paraguay tea*) consists of the dried and cured leaves of *Ilex paraguensis* (Aquifoliaceae) and other species of *Ilex*, small trees or shrubs indigenous to the region where Argentina, Paraguay and Brazil meet. The drug is obtained partly from wild plants (e.g. in Brazil) and partly from cultivated ones (in Argentina).

The branches are cut when the fruits are ripe and 'toasted' for a moment over a fire until they show blisters. The leaves are then separated and spread on a platform over a small wood fire for about 24–36 h. They are then reduced to a coarse powder and put into sacks (formerly into hide serons), in which the leaf should be allowed to mature for at least a year. Rapid drying in ovens gives an inferior product.

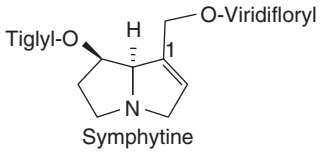
The whole leaves, seldom seen in commerce, are shortly petiolate, ovate or oblong-lanceolate, 5–15 cm long, and dark green to yellowish-green. They have a crenate-serrate margin and a coriaceous texture. The commercial drug consists of fragments of leaf with a variable

amount of 'stalk'. It has a characteristic odour and a somewhat bitterish taste.

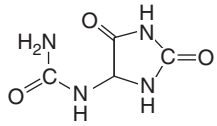
Maté contains about 0.2–2% of caffeine, about 10–16% of chlorogenic acid (caffeotannic acid) and a little volatile oil. It is said to be very rich in vitamins. Maté tea is very widely used in South America with some consumption in Europe and America. (For studies on maté drinking in S. America, see A. Vázquez and P. Moyna, *J. Ethnopharm.*, 1986, **18**, 267.)

### Comfrey

The roots and aerial parts of *Symphytum officinale* (comfrey), family Boraginaceae, have long been important drugs in herbal medicine for the treatment of pulmonary and gastric conditions and various rheumatic complaints. In addition to mucilage and tannin these contain allantoin (0.6–0.8% in the roots) which can be regarded as a breakdown product of uric acid. Allantoin stimulates tissue regeneration and therefore the drug has been used for external injuries and gastric ulcers. A new saponin involving oleanic acid glycosylated at C-3 with arabinose-glucose-glucose has been reported (V. U. Ahmad *et al.*, *J. Nat. Prod.*, 1993, **56**, 329). (For a report on the isolation of other bidesmosidic triterpenoidal saponins see F. V. Mohammed *et al.*, *Planta Med.*, 1995, **61**, 94.) The relatively recent discovery of a range of pyrrolizidine alkaloids in comfrey and in Russian comfrey (*S.* × *uplandicum*) has cast doubt on the desirability of using the drug for internal medication (see p. 000). N.-C. Kim et al. (*J. Nat. Prod.*, 2001, **64**, 251) have described the separation of three such alkaloids by counter-current chromatography, each having 1,2-unsaturation of the pyrrolizidine nucleus and ester functions on two side-chains (variously angelic, tiglic, viridifloric or echimidinic acids), e.g. symphytine q.v. Only the root is included in the *BHP* 1996 and is listed as a vulnerary.



Symphytine



Allantoin

## REDUCED PYRIDINE ALKALOIDS

In addition to the lysine-derived alkaloids (see Fig. 26.11), there are a number of other alkaloids having a reduced pyridine moiety. They include coniine (from hemlock), arecoline (see 'Areca Nut') and ricinine, the alkaloid of the castor seed (q.v.). Ricinine has been shown to be derived from nicotinic acid or other participants of the pyridine nucleotide cycle; hence, glycerol and succinic acid proved to be good precursors but quinolinic acid was not detected as an intermediate, as originally expected in the pathway shown in Fig. 26.37.

### Hemlock fruit

(*Conii Fructus*). The drug consists of the dried unripe fruits of *Conium maculatum* (Umbelliferae), the spotted hemlock, a poisonous biennial plant indigenous to Europe.

Hemlock was the plant used by the Greeks for preparing a draught by means of which criminals were put to death. It was employed in Anglo-Saxon medicine and was in considerable use until about 80 years ago. Although now rarely employed, it merits attention as one of the commonest of our indigenous poisonous plants and on account of the fact that coniine was the first alkaloid to be synthesized (Ladenburg, 1886).

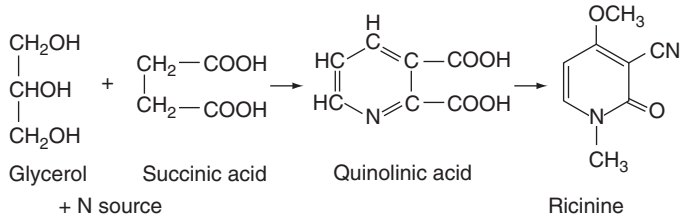



**Fig. 26.37**
Biosynthesis of ricinine.

The fruit is a broadly ovate, somewhat laterally compressed cremocarp about 3 mm long. It bears a small stylopod and the remains of the stigmas. Each mericarp has five prominent, primary ridges, the width of which is constantly altering so as to give them a beaded appearance. The transverse section differs from that of most umbelliferous fruits in not showing conspicuous vittae, although numerous very small ones are actually present. The endosperm is deeply grooved and is surrounded by well-marked, alkaloid-containing layers.

When hemlock is treated with solution of potassium hydroxide, it develops a strong, mouse-like odour owing to liberation of the alkaloid coniine. The latter is volatile and may be steam-distilled. It is present to the extent of 1–2.5% together with *N*-methyl coniine, conhydrine, pseudoconhydrine, conhydrinone and γ-coniceine. Roberts reported (*Phytochemistry*, 1981, **20**, 447) South African *Conium* to contain a high volatile oil composition, the main component being myrcene. The alkaloids were similar to those of European plants but consisted, in addition, of *N*-methyl pseudoconhydrine.

Daily fluctuations in the proportions of these alkaloids in the living plant have been reported. Unlike a large number of alkaloids, coniine does not appear to be biosynthesized in the plant directly from an amino acid, but from four molecules of acetic acid with the participation of ammonia or some other nitrogen source. Leete's experiments (*J. Am. Chem. Soc.*, 1972, **94**, 5472) involving the isolation of [1′-$^{14}$C]-coniine and γ-[1′-$^{14}$C]-coniceine after feeding hemlock plants with 5-oxo[6-$^{14}$C]octanol and 5-oxo[6-$^{14}$C] octanoic acid are consistent with Fig. 26.38 for the biogenesis of coniine and related alkaloids from acetate.

The origin of the nitrogen may be indicated by Roberts' work, in which an enzyme, mol. wt 56 200, catalysing a transamination between 5-ketooctanal and L-alanine to give γ-coniceine and pyruvic acid, has been isolated.

### Further reading

Reynolds T 2005 Hemlock alkaloids from Socrates to poison aloes. *Phytochemistry* 66(12): 1399–1406

### Areca nut

Areca nuts (*betel nut*) are the seeds of *Areca catechu* (Palmae), a feather-palm 15–17 m high, which is cultivated in tropical India, Sri Lanka, Malaysia, south China, the East Indies, the Philippine Islands and part of East Africa (including Zanzibar and Tanzania). Large quantities are exported from Madras, Singapore, Penang and Sri Lanka.

**History.** Areca was known in China under the name *pinlang* (probably a corruption of the Malay name for the tree *pinang*) from at least

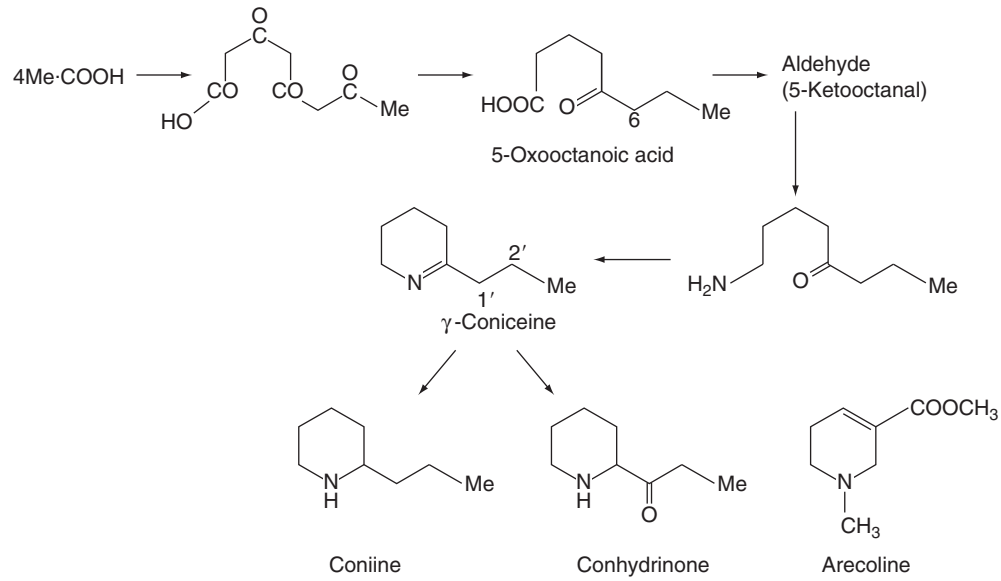



**Fig. 26.38**
Biogenesis of hemlock alkaloids.
Formula of arecoline.

100 BC. Immense quantities have been consumed in the East from very early times in the form of a masticatory known as betel, which consists of a mixture of areca nuts, the leaves of *Piper betle*, and lime. The value of areca as a taenicide was also known in the East.

**Collection and preparation.** The fruits, of which about 100 are annually borne on each tree, are detached by means of bamboo poles and the seeds extracted. The latter, before exportation, are usually boiled in water containing lime, and dried.

**Characters.** The areca nut is about 2.5 cm long and rounded conical in shape. Patches of a silvery coat, the inner layer of the pericarp, occasionally adhere to the testa. The deep brown testa is marked with a network of depressed, fawn-coloured lines. The seed is very hard, has a faint odour when broken and an astringent, somewhat acrid taste. Sections of the seed show dark-brown, wavy lines (folds of testa and perisperm) extending into the lighter-coloured interior (ruminate endosperm). At the flattened end of the seed is a small embryo.

**Constituents.** Areca contains alkaloids which are reduced pyridine derivatives. Of these, arecoline (methyl ester of arecaine) (Fig. 26.38), arecaine (*N*-methylguvacine) and guvacine (tetrahydronicotinic acid) may be mentioned. Only arecoline, which is present to the extent of 0.1–0.5%, is medicinally important. Ether extraction yields about 14% of fat, consisting mainly of the glycerides of lauric, myristic and oleic acids; subsequent extraction with alcohol yields about 15% of amorphous red tannin matter (areca red) of phlobaphene nature.

## TERPENOID ALKALOIDS

Included in the terpenoid alkaloids are monoterpenes (e.g. skytanthine), sesquiterpenes (e.g. patchoulipyridine) and diterpenes (e.g. the alkaloids of *Aconitum*, *Delphinium* and *Taxus* spp.). Various *Taxus* spp. are considered elsewhere and aconite, which has some medicinal interest, is described below. Valerian root, which contains monoterpene alkaloids of the skytanthine type, is grouped with the iridoids in Chapter 24. (For reviews of the literature covering diterpenoid alkaloids for the period 1985–92 (308 refs in all) see N. S. Yunusov, *Nat. Prod. Rep*., 1991, **8**, 499; 1993, **10**, 471; also F. P. Wang and X. T. Liang, *Alkaloids*, 1992, **42**, 151 (196 refs) and Atta-ur-Rahman and M. I. Choudhary, *Nat. Prod. Rep*., 1995, **12**, 361).

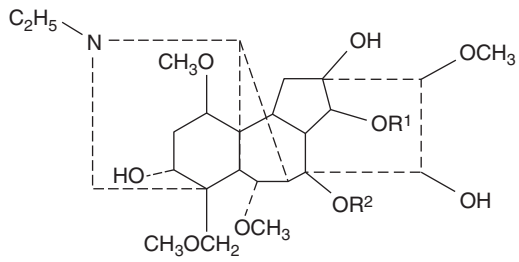
### Aconite root

Aconite (*Wolfsbane Root*) consists of the dried roots of *Aconitum napellus* (Ranunculaceae), collected from wild or cultivated plants. *A. napellus* is a polymorphic aggregate extending from Western Europe to the Himalayas. Cultivated forms have deeper coloured flowers, and darker green and less narrowly divided leaves than the wild plants; the former are in considerable demand in Europe as cut flowers and to meet this demand a rapid micropropagation method using floating membrane rafts and shoot tips has been developed (A. A. Watad *et al.*, *Plant Cell Rep*., 1995, **14**, 345). The greater part of the commercial drug is derived from wild plants grown in central and southern Europe, particularly Spain.

**Macroscopical characters.** Aconite differs in appearance according to the season of collection. The aconite formerly cultivated in England was harvested in the autumn and consisted of both parent and daughter roots. Both are obconical in shape, dark-brown in colour, 4–10 cm long and 1–3 cm diameter at the crown. Most Continental aconite is collected from plants at the flowering stage and therefore consists mainly of parent roots. The parent roots bear the remains of aerial stems and are more shrivelled than the daughter roots, which bear large, apical buds. Rootlets may be present but these are usually broken off. The odour is usually slight but samples vary in this respect. Taste at first slightly sweet, followed by tingling and numbness (taste with care; long chewing may be painful).

Transverse sections cut about one-third of the length from the crown show a stellate cambium with five to eight angles. The amount of lignified tissues is small, the greater part of the root consisting of starch-containing parenchyma of the pith and secondary phloem.

**Constituents.** Aconite contains terpene ester alkaloids, of which the most important is aconitine.




| | $R^1$ | $R^2$ |
|---|---|---|
| Aconine | H | H |
| Benzoylaconine | $CO{\cdot}C_6H_5$ | H |
| Aconitine | $CO{\cdot}C_6H_5$ | $CO{\cdot}CH_3$ |

Aconite also contains other alkaloids such as mesaconitine, hypaconitine, neopelline, napelline and neoline. Hikino *et al. (J. Nat. Prod*., 1984, **47**, 190) isolated eight alkaloids from roots of Swiss origin, five being new to the species.

From ssp. *vulgare* Arlandini *et al*., (*J. Nat. Prod*., 1987, **50**, 937) isolated *N*-deethylaconitine, and Chen *et al*., (*J. Nat. Prod*., 1999, **62**, 701) obtained twelve diterpenoid alkaloids, characterized by NMR and MS, from the herb and flowers.

The percentage of total alkaloid in the drug is about 0.3–1.2%. About 30% of the total is ether-soluble aconitine. In view of the different groups of alkaloids reported by workers over the years, and the large variation in aconitine contents of roots, it seems that in all probability there is considerable chemical variation between varieties of *A. napellus*. Aconite also contains aconitic acid (q.v.) and abundant starch.

Other *Aconitum* species may contain aconitine or similar alkaloids of very varied toxicity and the hydrolysis products of those given in Table 26.8 may be compared with aconitine. According to Zhu *et al. (Phytochemistry*, 1993, **32**, 767) more than 96 spp. of *Aconitum* have been studied chemically, resulting in reports regarding over 250 $C_{19}$-diterpene alkaloids and a number of $C_{20}$-diterpene alkaloids. For a recent report on the alkaloids of the aerial parts of *A. variegatum* from the Carpathians and Pyrenees, see J. G. Diaz *et al.*, *Phytochemistry*, 2005, **66**, 837.

The employment of *Aconitum* spp. as arrow poisons in China, India and other parts of Asia has been reviewed by Bisset in a series of publications (*J. Ethnopharmacology*, 1981, **4**, 247; 1984, **12**, 1; 1989, **25**, 1; 1991, **32**, 71).

### Japanese aconite

Japanese aconite was formerly an article of European commerce. The roots are shorter and plumper than the European drug, and dark grey or brownish in colour. *Aconitum japonicum* possesses cardiotonic properties and the principal alkaloid associated with this activity is higenamine [(±)- demethylcoclaurine], formula Fig. 26.19, which is active at about the same dosage levels as the *Digitalis* glycosides. The only other cardioactive alkaloid obtained is coryneine chloride (dopamine methochloride) from *A. carmichaelii*. The reported yield of both alkaloids was small. These species are important in Oriental medicine and have clinical usage.

### Chinese aconites

*A. carmichaelii*, *A. kusnezofii* and *A. brachypodum* are three species employed in Chinese medicine. Traditionally, as with other very poisonous drugs, such as nux vomica, the toxicity is reduced by processing—in this case by soaking or boiling in water which causes some hydrolysis of the alkaloids. However this treatment may not always be properly controlled and as reported in the *Lancet* (1992), in Hong Kong 17 Chinese were poisoned, two fatally, as a result of consuming a herbal preparation involving the above species.

For the characterization of trans-2,2′,4,4′-tetramethyl-6,6′-dinitroazobenzene from the traditional Chinese medicinal plant, *A. sungpanense* see X. Wang *et al.*, *Fitoterapia*, 2004, **75**, 789.

### Indian aconites

The *Indian Pharmacopoeia* includes the dried root of *A. chasmanthum*. This it describes as being 2.5–4.5 cm long. It contains indaconitine. Several other aconites have been imported from India and Pakistan, including roots from *A. deinorrhizum* and *A. balfourii*, with smaller quantities of *A. spicatum* and *A. laciniatum*. In 1970 Mehra and Purie considered that some six species were being collectively exported under the commercial name of *A. ferox*. Samples often consist of daughter roots about 15 cm long and 4 cm diameter at the crown. The surface is dark brown and coarsely wrinkled. The drug is very hard and horny, the starch being usually gelantinized by excessive heating. (For the isolation of alkaloids from *A. ferox* see J. B. Hanuman and A. Katz, *J. Nat. Prod*., 1993, **56**, 801.)

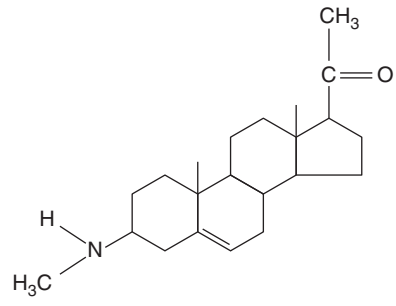
**Uses.** Aconite is a very potent and quick-acting poison which is now rarely used internally in the UK, except in homeopathic doses. The drug was included in the *BPC* (1973) and was formerly used for the preparation of an antineuralgic liniment.

## STEROIDAL ALKALOIDS

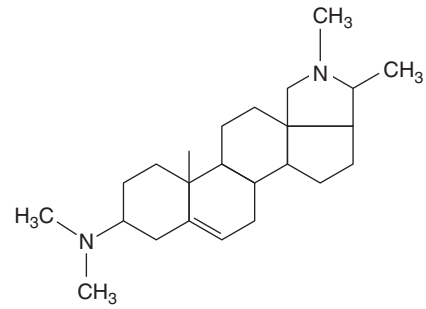
Steroidal alkaloids arise by the inclusion of a basic nitrogen at some point in the steroid molecule. Those of the $C_{27}$ group include the *Solanum* alkaloids mentioned in Chapter 23 in relation to their potential as steroid precursors, and the *Veratrum* alkaloids, considered in more detail below, which have a similar structure. A second, $C_{21}$ group, of which many examples are found in the Apocynaceae (*Holarrhena* and *Funtumia*) and in the Buxaceae, probably arise from pregnenolone by amination at either C-3 or C-20 (see formulae of examples overleaf). Conessine is a common alkamine of the group and represents a desirable starting material for the synthesis of some hormones (e.g. aldosterone). Whereas

**Table 26.8 Hydrolysis products of *Aconitum* alkaloids.**

| Alkaloid | Base | Acids |
|---|---|---|
| Hypaconitine | Hypaconine | Acetic and benzoic acids |
| Jesaconitine | Aconine | Acetic acid and anisic acid (*p*-methoxybenzoic acid) |
| Pseudaconitine | Pseudaconine | Acetic acid and veratric acid (Fig. 19.5) |
| Lycaconitine | Lycoctonine | Lycoctoninic acid (*N*-succinyl anthranilic acid) |




Holaphylline




Conessine

holaphylline has little toxicity, the quaternary diamine malouetine, which is found in the same family, is a potent curare-type poison.

For reviews of steroidal alkaloids see R. Shakirov *et al*., *Nat. Prod. Rep*., 1990, **7**, 557; Atta-ur-Rahman and M. I. Choudhary, *ibid*., 1995, **12**, 361. References pertaining to the investigation and traditional uses of neotropical S. American steroidal akaloids may be found in J. Nino *et al.*, *Pharm. Biol.*, 2006, **44**, 14.

## Veratrums

American veratrum (Green Hellebore), *Veratrum viride* (Liliaceae), and European veratrum (White Hellebore), *V. album*, are very similar perennial herbs, whose rhizomes and roots are almost indistinguishable either macroscopically or microscopically. Some alkaloidal constituents are common to both species. The American drug is collected in the eastern parts of Canada and the USA and white hellebore in central and southern Europe.

**History.** The North American Indians were aware of the therapeutic activity of American hellebore and it was employed by the early European settlers. Its use spread to England about 1862. In Europe the closely allied drug obtained from *V. album* had long been used. Until about 1950 veratrums, except as insecticides, were being little used. Since then they have been the subject of much research and are now employed in the treatment of hypertension.

**Collection and preparation.** The rhizome is dug up in the autumn, often sliced longitudinally into halves or quarters to facilitate drying, and sometimes deprived of many of the roots.

**Macroscopical characters.** The rhizome, if entire, is more or less conical and 3–8 cm long and 2–3.5 cm wide; externally brownish-grey. The roots, if present, are numerous and almost completely cover the rhizome. Entire roots are up to 8 cm long and 4 mm diameter, light brown to light orange, and usually much wrinkled (for transverse section, see Fig. 41.8H). Commercial American veratrum is more frequently sliced than is the drug from *V. album*, and more of the roots remain attached to the rhizome. Odourless, but sternutatory; taste, bitter and acrid.

**Microscopical characters.** The various species of *Veratrum* resemble one another very closely in microscopical structure. The rhizomes of *V. viride* and *V. album* are virtually identical microscopically but minor differences occur in the roots. Microscopical distinction of the powders is nevertheless difficult.

**Allied drugs.** Youngken (1952) reported on the following substitutes for *V. viride*, which have been offered commercially: *V. album*, *V. eschscholtzii*, *V. woodii*, *V. californicum* and what is believed to be a variety of *V. viride* from Montana. In addition to these, *V. fimbriatum* has been the subject of chemical investigation.

**Constituents.** Numerous steroidal alkaloids are present in both *V. album* and *V. viride*; over 100 have been recorded in the former and new alkaloids of both groups (see below) continue to be isolated (Atta-ur-Rahman *et al.*, *J. Nat. Prod*., 1992, **55**, 565; K. A. El Sayed *et al*., *Int. J. Pharmacognosy*, 1996, **34**, 111). *V. nigrum* L. var. *ussuriense* is used for the preparation of the Chinese drug 'Li-lu', together with other species (W. Zhao *et al*., *Chem. Pharm. Bull.*, 1991, **39**, 549). Both drugs have long been used as insecticides, but their more recent importance results from those alkaloids that have hypotensive properties. Alkaloids present in some other species, e.g. *V. californicum*, can cause serious damage to livestock grazing in locations where the plant occurs as they have teratogenic properties (see 'Teratogens of Higher Plants', Chapter 39).

There are two distinct chemical groups of veratrum steroidal alkaloids and these are now referred to as the jerveratrum and ceveratrum groups.

*Jerveratrum alkaloids* contain only 1–3 oxygen atoms and occur in the plant as free alkamines and also combined, as glucosides, with one molecule of D-glucose. Examples are pseudojervine derived from jervine and veratrosine derived from veratramine.

*Ceveratrum alkaloids* are highly hyroxylated compounds with 7–9 oxygen atoms. They usually occur in the plant esterified with two or more various acids (acetic, α-methylbutyric, α-methyl-α-hydroxybutyric, α-methyl-α,β-dihydroxybutyric), but are also found unconjugated. It is these ester alkaloids that are responsible for the hypotensive activity of veratrum; examples are the esters of germine, protoverine and veracevine.

**Uses.** American veratrum is used for the preparation of Veriloid, a mixture of the hypotensive alkaloids. European veratrum is used for the preparation of the protoveratrines. Both drugs, and the closely related cevadilla seeds (*Schoenocaulon officinale*), are used as insecticides.

## Cevadilla seeds

Cevadilla seeds, which contain alkaloids similar to those of veratrum, are considered under 'Pesticides', Chapter 40.

## Kurchi or holarrhena bark

The stem-bark of *Holarrhena pubescens* (*H. antidysenterica*) (Apocynaceae), has long been valued for its antidysenteric properties. The plant is a small tree found in many parts of India and up to about 1250 m in the Himalayas; Than reports that the Burmese material is also satisfactory. The drug should be obtained from trees about 8–12 years old, which yield a stem bark about 6–12 mm in thickness.

The pieces are recurved. The outer surface shows deep cracks and is buff to brownish in colour. Fracture, brittle and splintery. Odour, none; taste, bitter.

Kurchi contains numerous steroidal-type alkaloids (1.8–4.5%) including conessine, norconessine, isoconessine and kurchine. Bhutani *et al. (Phytochemistry*, 1988, **27**, 925; 1990, **29**, 969) isolated six new steroidal alkaloids named regholarrhenines A.-F, and P. J. Houghton and M. L. Dias Diogo (*Int. J. Pharmacognosy*, 1996, **34**, 305) have reported on two bark samples from Malawi showing levels of conessine comparable with those of Nepalese material.

The bark, official in India, is required to contain not less than 2% of alkaloids and Kurchin Bismuth Iodide, a preparation much used for amoebic dysentery, 23–27% of total alkaloids. Conessine hydrobromide is manufactured from the seeds of *H. antidysenterica* and the polymorphous W. African species *H. floribunda* has been cultivated for the same purpose. For the isolation of a new steroidal alkaloid from the seeds of holarrhena and for other references associated with the drug see A. Kumar and M. Ali, *Fitoterapia*, 2000, **71**, 101.

The root-bark also contains conessine and other steroidal alkaloids.

New isolated flavonoids of the leaves are recorded by P. Tuntiwachwuttikul *et al.*, *Fitoterapia*, 2007, **78**, 271.

Callus and cell suspension cultures of *H. antidysenterica* produce principally conessine and the addition of cholesterol to the nutrient medium has been shown to enhance alkaloid production.