Secondary Metabolites of Plants

5

Secondary metabolites are substances manufactured by plants that make them competitive in their own environment. These small molecules exert a wide range of effects on the plant itself and on other living organisms. They induce flowering, fruit set and abscission, maintain perennial growth or signal deciduous behaviour. They act as antimicrobials and perform the role of attractants or, conversely, as repellents. Over 50,000 secondary metabolites have been discovered in the plant kingdom. Medicinal herbs and many modern medicines rely on secondary plant metabolites for their actions.

The search for new secondary products in plants with the hope of discovering new products or, even better, new approaches for the treatment of disease is an on-going process involving academic and pharmaceutical institutions. At one time, another reason was the hope that understanding the distribution of natural products would assist in the classification of plants (Lawler 1986a, b). This secondary reason is no longer important today because plant classification is being increasingly approached by comparing DNA sequences.

Phytoalexins

In their natural environment, orchids are naturally exposed to many micro-organisms, and in response to such microbial challenge they produce phytoalexins, which are low-molecular-weight compounds that confer resistance against such organisms (Letcher and Nhamo 1975; Stoessl and Arditti 1984). The main phytoalexins of orchids are 9,10-dihydrophenanthrenes. Orchinol was the first phytoalexin to be isolated, from *Orchis militaris* infected with *Rhizoctonia repens* (Boller et al. 1957). Loroglossol, an isomer of orchinol, was next isolated from *Loroglossum hircinum* infected with *Rhizoctonia versicolor* (Hardegger et al. 1963), followed by hircinol. More than 40 dihydrophenanthrene phytoalexins have been isolated from orchids, and many, including the three original phytoalexins, have been synthesised (Stoessl and Arditti 1984). Feeding experiments using radioactive L-phenylalanineas as a precursor demonstrated that the biosynthetic sequence for production of 9,10-dihydrophenanthrenes starts with L-phenylalanine and passes through *m*-coumaric acid, dihydro-*m*-coumaric acid, and 3,3′,5-trihydroxybibenzyl (Fritzememeier and Kindl 1983). From their biosynthesis, dihydrophenanthrenes can be classified as stilbenoids because they are derivatives of dihydrostilbenes or bibenzyls (Reinecke and Kindl 1994). Resveratrol is the best publicised stilbenoid. Present on the skin of grapes and playing a role in warding off attack by fungi and bacteria on the fruit, it is alleged to have many beneficial effects on plants and animals

E.S. Teoh, *Medicinal Orchids of Asia*, DOI 10.1007/978-3-319-24274-3_5

and even cytotoxic activities, but its global benefit is controversial and has never been replicated in humans.

Ordinarily, phytoalexins are present only in minute amounts in healthy orchids. When attacked by pathogenic fungi, the orchid responds by an intense activation of genes encoding phytoalexin enzymes, but this response is transient (Reinecke and Kindl 1994a, 1994b). Phytoalexin concentrations decline markedly when symbiosis is established between the orchid and the mycorrhiza. Nevertheless, upon destruction of the mycorrhiza, phytoalexin production increases in proportion to the amount of fungal material present (Gehlert and Kindl 1991). In young, sterile plants of *Phalaenopsis*, bibenzyls and their oxidative products, the 9,10-dihydrophenanthrene phytoalexins, are not present. Following infection with fungi, such as *Botrytis cinerea* and *Rhizoctonia* spp., there is a greater than 100-fold increase in bibenzyl synthase activity. This is the key enzyme for the formation of phytoalexins (Reinecke and Kindl 1994b). Since mycoheterotrophic plants are unable to photosynthesise, they are totally dependent on their mycorrhiza for carbon supplies, and therefore they need to be able to defend themselves against microbes and herbivores (Roy et al. 2013). Over 50 chemical substances have been isolated from *Gastrodia elata*, and it would not be surprising if similarly large numbers of phytochemicals are also found in other highly successful, large, mycoheterotrohic orchids (e.g. various species of *Gastrodia, Galeola, Cephalantera, Corallorhiza*, and *Cymbidium micorhizon*).

Orchinol and loroglossol inhibit spore germination of *Phytophora infestans* at 0.000006 M concentration and disrupt vegetative growth of newly germinated *Monilinia fructicola* (Ward et al. 1975). Phytoalexins are bacteristatic and fungistatic, while being neither bactericidal nor fungicidal. In this respect, it is interesting to note that, in Nepal, pertaining to skin lesions, native medicine only makes use of orchids for minor conditions like wounds (employing *Coelogyne, Dactylorhiza, Gymadenia, Rhynchostylis* and *Vanda*), pimples (*Dendrobium*), boils (*Coelogyne, Cymbidium, Dendrobium, Pholidota* and *Vanda*) or as a demulcent (*Dactylorhiza*); orchids are not used for sores or carbuncles (Manandhar and Manandhar 2002).

Phytoalexins are also produced by a large number of plants consumed by humans, but generally they are in such small amounts that they would not cause problems unless the vegetable in question is consumed excessively. Garden peas contain pisatin and green bean phaseolin, both of which will lyse bovine red blood cells, the former at a concentration of 200 ppm (parts per million), the latter at 17.5 ppm. Carrots contain myristicin which is insecticidal and in humans produces cerebral excitation. However, a 70-kg man would need to consume 5 kg of carrots to experience hallucinations. Damaged sweet potato is toxic to cattle and humans due to elevated levels of ipomeamarone which damages the liver and lungs. Blighted white potato is known to have caused poisoning and deaths in humans due to the presence of two glycoalkaloids, alpha-sloanine and alpha-chaconine. Good agricultural practice reduces the amount of phytoalexins in agricultural crops and is also important from the consumer acceptance standpoint (Surak 1978).

Hydrocarbons

These are the simplest compounds. They contain only hydrogen and carbon. They occur as straight chains (aliphatic hydrocarbons) or with ring forms, and form the basic skeleton of more complex molecules. A carbon atom is capable of binding to four hydrogen atoms, and when fewer hydrogen atoms are present relative to the carbon, the hydrocarbon is said to be unsaturated. Such compounds carry double or triple bonds. Marsh gas, methane (CH4), is a saturated hydrocarbon, and the four bonds of carbon are all attached to hydrogen. The waxy coat on leaves and fruits contain many saturated hydrocarbons which are insoluble in water. They prevent water sticking on the surface of leaves and fruit. Olive oil also contains a number of saturated hydrocarbons.

Another gas, ethylene ($H_2C{=}CH_2$), and an example of an unsaturated hydrocarbon, is a plant hormone. It is released by apples and by

fading flowers of *Papilionanthe* and their hybrids. Ethylene causes ripening of fruit, abscission of leaves and fading of adjacent flowers, especially in an enclosed space which prevents dissipation of the gas.

When a hydroxyl group (−OH), hydrogen and oxygen, is attached to a hydrocarbon, the latter becomes an alcohol, for drinking, or ethanol, C_2H_5OH.

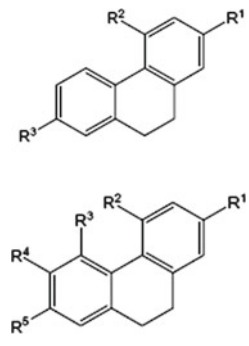
Terpenes (Terpenoids and Steroids)

Terpenes are important plant metabolites. They include substances like floral fragrances, which serve as insect attractants, pine oil, growth inhibitors, the two plant hormones, gibberelic acid and abscisic acid, and some which are insecticidal. The 30,000 terpenes that have been identified share one common characteristic: they all possess repeating five-carbon isoprene units (a five-carbon ring, Fig. 5.1).

The number of five-carbon isoprene units determines their classification into:

1. Hemiterpenes (single isoprene unit)
2. Monoterpenes (two isoprene units)
3. Sesquiterpenes (three isoprene units)
4. Diterpenes (four isoprene units)
5. Sesterterpenes (five isoprene units)
6. Triterpenes (six isoprene units)
7. Carotenoids (eight isoprene units).

Although their structures were first elucidated in the nineteenth century, terpene-based essential oils, found in frankincense, for instance, have Biblical usage. Monoterpenes such as linalool are major components of the scent produced by orchids (Kaiser 1993). The modern antimalarial, artemesinin, a sequiterpene, comes from the Chinese medicinal plant *Quinhao* (*Artemesia annua*) which had been in use as a fever medicine for over two millennia. It was mentioned in the *52 Remedies* recovered from the Mawangdui Tomb dating from the Han Dynasty (206 BC–221) located in Henan Province (Harper 1998). It has the empiric formula C15H22O5 and is chemically known as 3*R*,5a*S*,6-*R*,8a*S*,9*R*,12*S*,12a*R*)-Octahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyranol[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one. Artemesinin is effective against the dangerous chloroquine-resistant falciparium malaria which sometimes involves the brain (Anonymous 1979). Another life-saving terpene is placitaxol (a diterpene with a very complex molecular structure) effective against ovarian, breast, colon, non-small cell lung cancer and malignant melanoma. It has the empiric formula $C_{47}H_{51}NO_{14}$ and is known as 5beta,20-epoxy-1,2alpha,4,7beta,10beta,13alpha-hexhydroxytax-11-en-9-one 4,10 diacetate 2-benzoate 13-ester with (2R,3S)-*N*-benzoyl-3-phenylisoserine (Evangelista 1995).



R1 = OMe, R2= OMe, R3 = OH

Orchinol

R1 = OMe, R2 = OMe, R3 = OH, R4 = H, R5 = H

Loroglossal

R1 = OH, R2 = OMe, R3 = OH, R4 = H, R5 = H

Hirsinol

Fig. 5.1 Orchinol is the first phytoalexin to be isolated, from *Orchis militaris* infected with *Rhizoctonia repens*, followed by loroglossal (Hardegger et al. 1963) and hircinol (Fisch et al. 1973). Analogues of orchinol like coelonin and lusianthridin occur in other orchid species

Terpenoids (diterpenoids, sesquiterpenoids, triterpenoids) and lignoids also possess antiviral activities, and at least 22 have been shown to inhibit corona-viruses, including the dangerous SARS-Corona Virus which created such havoc in the Far East in 2007. Betulinic acid and savinin are competitive inhibitors of a protease (an enzyme which breaks down proteins) produced by the SARS-CoV 3CL virus. Terpenes in orchids are therefore a topic of great interest to researchers.

Triterpenes and Steroids

Tetracyclic triterpenes (compounds) and steroids have similar structures, but are biosynthesised through different pathways. The plant steroids contain three six-membered and one five-membered rings. Such steroids exert profound physiological effects on animals. Some are employed as an oestrogen substitute in menopausal women. Cardiac glycosides consisting of a sugar molecule bound to a steroid, such as digitalis (digitoxin) from foxglove (*Digitalis purpurea,* not an orchid), are used to treat cardiac insufficiency. Steroidal saponins are important precursors for the manufacture of steroid drugs ranging from anti-inflammatory agents to sex hormones such as androgens, oestrogens, progestogens and oral contraceptives. Triterpene saponins have antitussive (cough preventing), expectorant, analgesic, anti-inflammatory and cytotoxic effects. Liquorice, which is used in the treatment of coughs, is one example. The ginsenocides from ginseng are another. All saponins are surfactants, and when mixed with water and shaken, they form a foamy solution. Many saponins are haemolytic (they rupture red blood cells). They are toxic to cold-blooded animals like fish (de Padua et al. 1999).

Stilbenoids and Bibenzyls

Bibenzyl is a hydrocarbon whose basic structure consists of two benzene rings attached to ethane. They occur commonly in plants. Bibenzyls in orchids are synthesised from dihydro-*p*-coumaric acid and acetate or malonate (Fritzememeier and Kindl 1983; Friederich et al. 1998). Gigantol and batatasin III are the two commonest bibenzyls occurring in orchids (Chen et al. 2008) which have cytotoxic activity. Gigantol (from *Dendrobium draconis*) inhibits migration of non-small cell lung cancer in vitro (Charoenrungruang et al. 2014). Erianin, a bibenzyl which occurs naturally in *Dendrobium chrysotoxum*, is often employed as an antipyretic and analgesic in traditional Chinese medicine (Su et al. 2011). Erianin possesses antiangiogenic properties (Gong et al. 2004a, b); furthermore, it induces apoptosis in human leukaemia HL-60 cells and hepatocarcinoma (HCC) Huh7 cells, in vitro (Li et al. 2001; Su et al. 2011). Should it be applicable as an antitumour agent, that would make the drug more valuable. Erianin was successfully synthesised in 2008 (Zou et al. 2008).

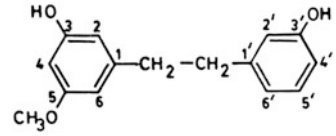


Fig. 5.2 Batatasin III, a common bibenzyl in orchids

Tamoxifen and diethylstilbenoids are examples of synthetic stilbenoids which have been used to treat hormone-dependent breast cancer, and clomiphene is a synthetic stilbenoid that is used for ovulation induction Fig. 5.2.

Phenanthrenes

Phenanthrene, C14H10, is an angular polynuclear hydrocarbon which is related to certain alkaloids like morphine, and figure in the structure of steroids. It is postulated that they are formed through the oxidative coupling of the aromatic rings in stilbene or diterpenoid precursors. Many phenanthrenes occur in higher plants, particularly orchids, in such medicinal genera as *Bletilla, Bulbophyllum, Dendrobium, Coelogyne, Cymbidium, Eria* and *Flickingeria.*

Table 5.1 Properties of phenanthrenes present in medicinal orchid species

Pharmacological action	Orchid species
Anti-allergic	*Gymadenia conopsea*
Anti-inflammatory	*Dendrobium moniliforme*
Antimicrobial	*Bletilla striata* *Cypripedium macranthos*
Anti-oxidant	*Pholidota yunnanensis*
Antithrombotic	*Dendrobium loddigesii*
	Dendrobium xantholeucum (syn. *Ephemerantha lonchophylla*)
Cytotoxic	*Bulbophyllum kwangtungense*
	Cremastra appendiculata
	Dendrobium catenatum
	Dendrobium nobile
	Dendrobium chrysanthum
	Dendrobium thrysiflorum

There is on-going interest in natural phenanthrenes because some of them have been shown to be cytotoxic against specific human cancer cell lines, while other possess anti-allergic, antimicrobial, anti-inflammatory, anti-oxidant, antiplatelet (antithrombotic) and spasmolytic properties (Kovacs et al. 2008). Examples of such laboratory-demonstrated pharmacological actions found in various species are shown in Table 5.1.

Antitumour effects are probably the most important property of phenanthrenes to be investigated. Monomeric phenanthrenes, generally the commonest, in *Cremastra appendiculata* were ineffective in all tested cancer cell lines, whereas its biphenanthrenes and triphenanthrene displayed antitumour activity (Xue et al. 2006; Kovacs et al. 2008). Denbinobin, a phenanthroquinone, and lusianthridin, a dihydroxymethoxy phenanthrene from *Dendrobium nobile*, exhibit cytotoxic effects in vitro and in vivo, with denbinobin being more potent. A free phenolic hydroxyl group appears to be essential for the inhibitory activity (Lee et al. 1995; Kovacs et al. 2008).

Phenanthrenes from orchids are classified into three main groups: monophenanthrenes, diphenenthrenes and triphenenthrenes. There are 210 compounds in the first group, the monophenenthrenes, of which almost half are only hydroxyl- and/or methoxy-substituted. Almost all the remainder are 8,10-dihydro- or dehydro derivatives (Kovacs et al. 2008). Glycosides are rare, but three were discovered in *Bletilla striata* (Yamaki et al. 1993) and one in *Dendrobium chrysanthum* (Ye et al. 2003). A unique monophenanthrene with a spirolactone ring was also isolated from *Bletilla striata*; it was named blespirol (Yamaki et al. 1993). An additional monophenanthrene with a spironolactone ring was isolated from *Dendrobium chrysanthum* and named dendrochrysanene (Yang et al. 2006). Phenanthraquinones form another group of monomeric phenanthrenes and have been isolated from *Spiranthes sinensis* and *Cremastra appendiculata* (Tezuka et al. 1990; Xue et al. 2006). Bibenzyl derivatives of phenanthrenes were discovered in *Pleione bulbocodioides* (Bai et al. 1996) and *Pholidota yunnanensis* (Guo et al. 2006).

Diphenthrenes are less common. They have been isolated from *Agrostophyllum callosum* and *A. khasiyanum* (Majumder and Sabzabadi 1988), *Bletilla striata* (Honda and Yamaki 1989, 2000; Bai et al. 1991), *B. formosana* (Lin et al. 2005), *Bulbophyllum reptens* (Majumder et al. 1999), *B. maculosum* (*Cirrhopetalum maculosum*) (Majumder et al. 1990) *B. vaginatum* (Leong and Harrison 2004), *Cremastra appendiculata* (Xue et al. 2005), *Dendrobium plicatile* (Honda and Yamaki 2000), *D. thyrsiflorum* (Zhang et al. 2005), *Eria flava* (Majumder and Banerjee 1988), *Eulophia nuda* (Tuchinda et al. 1988) *Gymadenia conopsea* (Matsuda et al. 2004), *Pleione bulbodioides* (Bai et al. 1996) and *Pholidota yunnanensis* (Guo et al. 2006). The single orchidaceous triphenenthrene was isolated from the tubers of *Cremastra appendiculata* (Xue et al. 2006). Their phytochemistry and pharmacology have been well reviewed by Kovacs et al. (2008).

Some of the bioactive compounds may originate in the endophytic fungi associated with the orchid. Ten endophytic fungi from *Dendrobium devonianum* and 11 from *D. thyrsiflorum* exhibited antimicrobial activity against at least

one species of bacteria or fungus among the six pathogenic microbes that were tested (*Escherichia coli, Bacillus subtilis, Streptococcus aureus, Candida albicans, Cryptococcus neoformans* and *Aspergillus fumigatus*). Antibacterial activity of *Epicoccum nigrum* from *D. thyrsiflorum* was stronger than ampicillin. *Fusarium* from the two *Dendrobium* species was effective against both bacteria and fungus (Xing et al. 2011). These findings suggest that tribal usage of orchids to treat infection may be based on experience of beneficial effects.

Alkaloids

The term alkaloid is used as a name for plant-derived compounds, containing one or more nitrogen atoms, usually in a heterocyclic ring (an amine functional group), and which have a marked effect on animals, including humans. They are optically active. Like proteins, they are derived from amino acids, but they differ in being alkaline. The term has an Arabic origin. Soda ash is known as *al qali* in Arabic. Alkaloids are bitter to taste. Among their functions, they are thought to play a role in germination and in protecting plants from predators, in particular herbivores and microbes. They are present in around 20 % of higher plants. Sometimes, they are also present in animals, for instance in the skin of some species of frogs.

Many alkaloids act on the nervous system. Poppy was employed in the Middle East over 3000 years ago, and coffee drinking originated in Ethiopia. Poppy is narcotic, caffeine and nicotine are stimulants, while cocaine is an anaesthetic, and scopolamine induces "twilight sleep." Codeine, which is more commonly employed by doctors to suppress severe coughing, is also present in the latex of the poppy capsule, and structurally very similar to morphine. Codeine is now a controlled drug. Aminophylline is a bronchodilator, while papaverine is a vasodilator which had a role in treating erectile dysfunction before the discovery of Viagra. Reserpine which lowers blood pressure is an ancient Indian remedy derived from *Rauwolfia serpentina,* now totally replaced by a wide range of more potent and reliable antihypertensives. Many alkaloid stimulants (e.g. morphine, cocaine, nicotine) are addictive. Improperly applied, some stimulants and sedatives are deadly. Strychnine is used as a rat poison. In 339 BC, the Greek philosopher, Socrates, was killed by being forced to drink hemlock which contains the alkaloid, coniine.

Taxol which has a diterpenoid core possesses an alkaloid side chain. It is an indispensable component in the chemotherapeutic cocktail employed in the treatment of ovarian and breast cancer. Vincristine and vinblastine are two alkaloids derived from the periwinkle, *Catharanthus roseus* (not an orchid), and are also cytotoxic agents but their use is limited to late-stage cancers because of their high toxicity. Camptothecin, a quinoline alkaloid obtained from the Chinese 'tree of joy' (*Camptotheca accuminata*), is used for treating advanced ovarian cancer that is resistant to taxol. Many synthetic compounds are derived from natural plant materials, and some of these are safer to use although they retain some toxicity along with the beneficial properties. Codeine derived from morphine is one of these. Sometimes, the derivative is more potent and far more dangerous, like heroine, which is also derived from the hydrolysis of morphine.

A shortage of quinine and several medicinal alkaloids during World War II precipitated by the interruption of supplies provided the impetus for governments, the pharmaceutical industry and scientists to undertake extensive screening of plants for alkaloids during the 1950s and 1960s (Lawler 1986a, b).

Alkaloids being so important in the pharmaceutical industry, it is not surprising that they were among the first secondary metabolites to be studied in orchids (Suzuki et al. 1932; Chen and Chen 1935; Yamamura and Hirata 1964; Inubushi et al. 1964; Luning 1964, 1967, 1974, 1975, 1980; Nishikawa and Hirata 1967, 1968; Brandange and Granelli 1973; Slaytor 1977; Lawler 1984), but many species that were screened did not contain appreciable amounts of the such metabolites. In 1974, Luning reported that 2044 species of orchids from 281 genera had been screened for alkaloids. Over half (numbering 30, or 53.6 %) of the 56 medicinal orchid

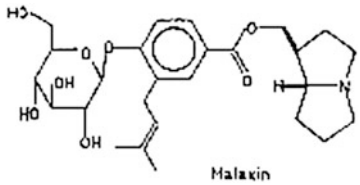


Fig. 5.3 Malaxin is a useful anti-malarial alkaloid

genera from Asia that were screened contained species that tested positive for alkaloids, albeit not all their species were medicinal. Only 14.6 % of all orchid species tested gave a positive test for alkaloids (i.e. present in amounts of 0.1 % or more). Genera that contained the largest number of alkaloid-positive species were *Liparis* Fig. 5.3 (with 28 species), *Dendrobium* (24), *Phalaenopsis* (19), *Malaxis* (18) and *Bulbophyllum* (9) (Table 5.2). Alkaloid-rich species were found in only four genera, *LIparis, Malaxis, Oberonia* and *Bulbophyllum*, when 314 orchid species in Bougainville, Papua New Guinea, were screened for alkaloids (Lawler and Slaytor 1969). (It should be noted that *Liparis* and *Malaxis* species are related. In the recent taxonomic revision, many species have been reassigned to different genera.) There were no appreciable amounts of alkaloids in 29 genera that had medicinal species (Table 5.3). However, single species of plants are not homogenous in their chemical content and individual plants of species that tested negative in past studies may actually contain undiscovered alkaloids. For instance, 8 out of 10 Himalayan *Coelogyne* species (*Coelogyne cristata, Coelogyne elata, Coelogyne flavida, Coelogyne nitida, Coelogyne ovalis* and *Coelogyne virescens* (=*Coelogyne brachyptera* Rchb. f) tested negative for alkaloids when they were screened by Luning (1964), but ten (different) species of *Coelogyne* from Bougainville, Papua New Guinea, were found to contain small amounts of alkaloids (Lawler and Slaytor 1969). Most of these alkaloid-rich genera occur in India and Southeast Asia. Only 5–10 % of their species have been screened, so there is much opportunity for good work to be done.

Table 5.2 Alkaloid-positive medicinal orchid genera from Asia

Genus	Number positive	% positive	Number tested
Anoectochilus	1		11
Arachnis	1	50	2
Bulbophyllum	9	6.5	138
Calanthe	2	7.7	26
Coelogyne	2	7.7	26
Corymborkis	1	25	4
Cymbidium	2	5.4	37
Cyrtochis	1	16.7	6
Dendrobium	24	8.3	384
Eria	14	18.2	77
Eulophia	2	15.4	13
Gastrochilus	1	50	2
Gastrodia	1	50	2
Goodyera	1	9.1	11
Habenaria	2	16.7	12
Liparis	28	41.8	67
Malaxis	18	36.7	49
Malleola	1	100	1
Nervilia	4	33	12
Oberonia	5	17.2	29
Paphiopedilum	1	4.3	23
Phalaenopsis	19	50	38
Plocoglottis	2	28.6	7
Renanthera	1	20	5
Cleisostoma (as *Sarcanthus*)	2	25	8
Vanda	3	13.6	22
Zeuxine	1		205
Total	149	14.6	1015

Reference: Luning 1974

Note: *Doritis* and *Kingiella* are now in *Phalaenopsis*. *Cirrhopetalum* is in *Bulbophyllum*. *Sarcanthus* are *Cleisostoma*. *Eria* species are not assigned contemporary nomenclature because of insufficient data in the original.

Orchid alkaloids commonly fall into two main classes: alkaloids of the pyrrolizidine type and (2) alkaloids of the dendrobine type (Fig. 5.4). *Dendrobium* is the genus richest in alkaloids, but their most important alkaloids are pyrrolizidine compounds, not the dendrobine type (Hausen 1984). Bibenzyl alkaloids have been identified in many orchid species. A picrotoxinin-type alkaloid has recently been isolated from the *Dendrobium, D*. Snowflake "Red Star" (Morita et al. 2000).

Dendrobine, the first alkaloid discovered in *Chin Shih Hu* (*Dendrobium nobile*) was isolated

Table 5.3 Alkaloid-negative medicinal orchid genera from Asia

Genera	Number tested negative
Arundina	1
Bletilla	1
Bronheadia	2
Cephalanthera	1
Cremastra	1
Cypripedium	4
Dactylorhiza	3
Epipactis	3
Geodorum	3
Grammatophyllum	5
Gymnadenia	1
Hetaeria	5
Luisia	8
Neottia	1
Nephelaphyllum	3
Orchis	4
Ornithochilus	2
Pelantheria	2
Phaius	13
Pholidota	14
Platanthera	1
Pleione	4
Polystachya	15
Rhynchostylis	2
Robiquetia	3
Satyrium	2
Spathoglottis	8
Spiranthes	7
Vanilla	5
Total	124
Total number of species in alkaloid-positive genera	1015
Total number of species in medicinal genera reported	1139

Reference: Luning (1974)

Note: *Doritis* and *Kingiella* are now in *Phalaenopsis*. *Cirrhopetalum* is in *Bulbophyllum*. *Eria* species are not assigned contemporary nomenclature because of insufficient data in the original

by Suzuki, Keimatsu and Ito in Japan in 1932, and pharmacological action was reported by Chen and Chen in 1935. It is the major alkaloid in *D. nobile*, and was subsequently found to be also present in *D. linawianum* (Suzuki et al. 1932, 1934). Another 14 alkaloids related to dendrobine are present in *Dendrobium* species. These include nobiline or nobilonine (Yamamura and Hirata 1964; Onaka et al. 1965), dendramine (6-hydroxydendromine), dendrine, dendroxine, 4-hydroxydendroxine and 6-hydroxydendoxine in *D. nobile* (Inubushi and Nakano 1965; Inubushi et al. 1966; Okamoto et al. 1966a, b); 2-hydroxydendrobine in *D. finlayanum* (Granelli et al. 1970); 6-hydroxynobilonine in *D. hildebrandii* (Elander and Leander 1971); and the isopentenyl derivatives of dendroxine and 6-hydroxydendroxine in *D. hildebrandii* and *D. friedricksianum* (Hedman et al. 1971).

More than 30 alkaloids have now been isolated from the genus *Dendrobium*. Although *Dendrobium* is the genus richest in alkaloids, only 8.33 % of the 384 species tested were found to have an alkaloid content which amounted to 0.1 % or greater (Luning 1974). A more recent tally discovered alkaloids to be present in appreciable amounts in 42 species of *Dendrobium*, particularly those of the northern clade, among which are species included within *shihu* (such as *D. nobile, D. liniawanium, D. findlayanum, D. moniliforme, D. hildebrandii, D. friedricksianum, D. wardianum, D. crepidatum, D. aphyllum, D. chrysanthum, D. lohohense, D. primulum, D. parishii* and *D. anosmum*) (Zhang et al. 2003; Liu et al. 2007).

Malaxin is the first of the pyrrolizidine-based alkaloids to be elucidated. Present in *Malaxis* and *Liparis*, malaxin was first isolated from *M. congesta* by Luning and Leander in 1967 (Luning 1974) and subsequently discovered in *L. bicallosa* and *L. hachijoensis* (Nishikawa and Hirata 1968; Nishikawa et al. 1967). Malaxin is dihydroartemesinin, an ester of laburnine (an alkaloid present in *Trudelia cristata, Vanda hindsii* and *V. helvola*) and malaxinic acid. Following its synthesis in 1969 (Tanino et al. 1969), it is now employed in Korea and several African countries for the treatment of uncomplicated falciparium malaria (Jackson et al. 2006; Anonymous, undated, http://www.act.watch.info.). Treatment failures with artemisine-based therapies have been reported, but these may be due to suboptimal dosage caused by poor pharmaceutical practice (Green et al. 2001; Jackson et al. 2006). Fakes have also been reported.

More complex pyrrolizidine alkaloids are present in several genera of monopodial orchids like *Vanda, Vandopsis Phalaenopsis,* and *Doritis*

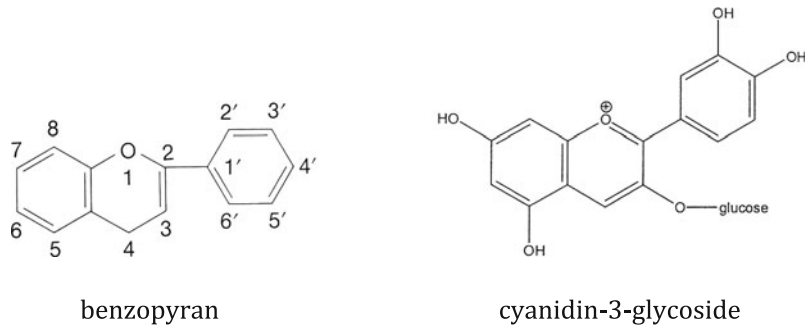


Fig. 5.4 Structure of benzopyran and cyanidin-3-glycoside

(Slaytor 1977). Shihunine isolated from *Dendrobium lohohense* is an early example of a phthalide-pyrrolidine alkaloid (Inubushi et al. 1964).

Phenols

Probably the largest group of secondary metabolites, phenols range from simple compounds with a single aromatic ring to complex compounds which are polymers like tannins and lignins. They include coumarins, quinones, napthoquinones and anthraquinones, all flavonoids which give odour, scent and colour to plants. Some of the compounds have physiological effects on animals. Vanillin is widely used to flavour food. It is a simple phenol. The ubiquitous salicylic acid is a precursor of aspirin.

Denbinobin, a 1,4-phenanthrenequinone first isolated from *Ephermerantha lonchophylla* (*Flickingeria xantholeuca*), and subsequently found to be present in *Dendrobium nobile* and *D. candidum* (Lin et al. 2001; Li et al. 2010; Yang et al. 2011), has been found to inhibit HIV-1 replication through an NF-kappaB-dependent pathway (Sanchez-Duffhunes et al. 2008). In vitro studies also show that denbinobine causes apoptosis of numerous human cancer cell lines (leukaemia; breast, lung, colorectal and stomach cancers) (Huang et al. 2005; Kuo et al. 2008, 2009; Chen et al. 2008; Sanchez-Duffhues et al. 2009; Chen et al. 2011; Song et al. 2012). Additionally, it may suppress tumour growth by blocking angiogenesis (Tsai et al. 2011) and prevent invasion or spread of breast and stomach cancers (Chen et al. 2011; Song et al. 2012). By causing selective apoptosis in hepatic stellate cells but not in normal hepatic cells, denbinobin exerts an antifibrotic effect on the liver and may thus be a useful starting point for developing compounds to protect the liver against cirrhosis (Yang et al. 2011). Denbinobin has been synthesised (Kraus and Zhang 2002; Wang et al. 2005), and so more studies and clinical testing should be forthcoming. This is probably the most promising phenanthrene or phenol that has been isolated from orchids.

Flavonoids

Flavonoids, phenols and tannins are aromatics. By that it is meant that their chemical structure contains a cyclic carbon (aromatic) ring instead of being merely straight or branched chains. The double bonds of the benzene ring effectively absorb ultraviolet radiation, and modifications to the ring move the absorbance towards longer wavelengths. Through their absorbance of various wavelengths of visible light, flavonoids give rise to the various colour pigments in plants (Lee 2007).

Flavonoids are constituted by a large family of compounds, estimated as exceeding 10,000. Their structural diversity results from various modification reactions, an important example being O-methylation regulated by a wide range of *O*-methyl transferases. The biological activities of a flavonoid and its O-methylated derivative are dissimilar (Kim et al. 2010). Flavonoids are commonly recommended

because of their antioxidant activity. Quercetin, the most abundant dietary flavonoid (it is also present in *Dendrobium catenatum*), is a potent antioxidant with anti-allergic and anti-inflammatory properties. Some flavanoids are antibacterial, antiviral (against the common cold sore virus), anti-allergic, anti-inflammatory, antiplatelet and antineoplastic (Liu 2011).

Anthocyanins, which impart yellow, red, mauve, pink, magenta and purple colouring to flowers, play an important role in insect mimicry. Colour in many orchids is often decided by two genes. In *Spathoglottis plicata*, the presence of both the dominant gene for pink colour (P) and the dominant gene for pale pink (T, which results in a flowers with a tinge of colour) results in flowers of deep purple. The presence of a single dominant gene results in either pink or tinge, and two recessive genes produce white (Storey 1950, 1958).

The parent compound of anthocyanins is 2-phenylbenzopyran. Few studies have been conducted on orchid anthocyanins. The bulk of these have been focused on orchids from the New World, and comments on anthocyanin pigments in Asian orchids are sometimes speculative. Hybrids of the *Vanda-Aranda-Renanthera* group of cultivated orchids contain a single anthocyanin which is cyanidin-based and only present in the flowers. Cyanidin-3-glycoside was chemically identified in flowers of *Cymbidium finlaysonianum*, *Grammatophyllum speciosum*, and *Pogonia japonica*, and cyanidin-3-rutinoside in the *Dendrobium* hybrid, *Dendrobium* Caesar (Arditti and Fish 1977).

Flavonoids have a wide range of pharmacological activities that include anti-oxidant, anti-microbial, anti-inflammatory, antimutagenic, antitumour, antidiabetic vaso-relaxant, immunomodulatory and both oestrogenic and anti-oestrogenic activities (Lin et al. 2014). Anti-oxidant activity is exhibited by floral anthocyanins extracted from a hybrid between *Papillionanthe teres* and *P. hookeriana* (*Vanda* Miss Joaquim) (Junka et al. (2012). Coumarin class compounds which exhibit anticoagulant or antiplatelet activities are phenylpropanoids (with three carbon side chains attached to a phenol).

Podophyllotoxin is a lignan used to treat warts. Etoposide and related anticancer drugs are derived from podophyllotoxin. Unfortunately, these derivatives are extremely toxic, and ordinarily they would only be employed as a last resort.

Among the flavonoids are phyto-oestrogens: quercetin which possesses anti-oxidant activity, and genistein and galangin which show some antibacterial activity. The potency of these compounds is weak and much work needs to be done to enhance their therapeutic value. Nevertheless, in their present state, they may have a role in tribal medicine.

Bulbophyllum odoratissimum is employed to treat respiratory infections and injuries in China, and this usage may have some justification because the orchid contains chrysin, a flavanoid with anti-inflammatory and pain-relieving properties. Chrysin suppresses lipopolysaccharide- induced cyclooxygenase-2-expression (COX2 expression) through the inhibition of nuclear factor for IL-6 (NF-IL6) DNA-binding activity (Woo et al. 2005). In the health supplement trade, chrysin is promoted as an aromatase inhibitor on the basis of in vitro testing; and from this it is inferred that it may encourage muscle development and possibly enhance libido. However, in vivo studies found that orally-administered chrysin did not alter steroid levels in humans nor in experimental animals (Saarinen et al. 2001). In nature, the commonest source of chrysin is the blue passion flower, *Passiflora caerulea*.

The other flavonoid present in *Bulbophyllum odoratissimum* is pinobanksin, subsequently also isolated from sunflower honey. It exhibits anti-oxidant activity against low density lipoproteins (LDL) (Oridrias et al. 1997). Oxidation of LDL is thought to contribute to atherosclerosis. When vitamin E was found to possess anti-oxidant activity against LDL, many studies for atherosclerosis prevention included prophylactic vitamin E supplementation. The intervention studies failed to show any benefit (Upston et al. 1999), the reason being that, under different conditions, vitamin E can be either pro- or anti-oxidant (Thomas et al. 1997).

Flavonoids are abundant in the plant kingdom. Orchids being relatively rare and smaller plants

would seldom be a choice to supply a source for their isolation.

Polysaccharides

Bioactive polysaccharides or carbohydrates with beta 1–3, 1–4 or 1–6 branch-chains from herbs are widely promoted in TCM and Kanpo medicine as tonics and anticancer agents. They are principally derived from fungi but some are also present in other herbs, such as aloe, cinnamon, gingers, ginseng and *lallang*. Polysaccharides in orchids are attracting scientific attention in China and Japan, the work still being restricted to the classic traditional herbs like *shihu* and *baiji* (Diao et al. 2008; Hua et al. 2004; Hsieh et al. 2008; Luo et al. 2008, 2010; Sun et al. 2005; Tagaki et al. 1983; Wang et al. 2006, 2010; Wu et al. 2010; Yamaki et al. 1989; Zhao et al. 2007). They exhibit immuno-modulatory activity in vitro. Other actions include an antimicrobial action against *Streptococcus mutans*, induction of cell differentiation, inhibition of angiogenesis and an antimetastatic effect. Polysaccharides vary greatly in their efficacy; their greater complexity in the branch chains and higher molecular weight are directly related to higher bioactivity.

They are usually administered in conjunction with conventional chemotherapy and radiotherapy. Lack of standardisation and a paucity of acceptable controlled trial data restrict their acceptance as adjunctive therapy.

The Orchids

The secondary metabolites of many orchids have been studied. They are discussed in the concluding 'OVERVIEW' of the various orchid genera which have been used as medicinal orchids in Asia. These compounds include alkaloids, terpenes, stilbenoids, bibenzyls, phenanthrenes, coumarins and flavonoids. Polysaccharides of orchids with medicinal properties are being intensively studied in China.

Genetic transformation is currently being studied as a tool to improve orchids of horticultural value (Sanjaya and Chan 2007). When the process is mastered, it could be employed to improve medicinal orchids or to extend their range of pharmaceutically important compounds.

Comment

To qualify for testing in a clinical situation, a compound must be effective at extremely low dosage (indicated by IC50), be non-lethal or with a lethal dose (LD50) much below 1 % of the minimum effective dose, and possess few serious side effects or none at all. Animal experiments are essential before human trials. The compound's structure must be known and, preferably, synthesis of it achieved. How it acts is explained at the molecular level. Exceptions may be made for anticancer agents; many of them elicit serious side effects which have to be carefully monitored. New compounds should always be introduced via clinical trial studies and their efficacy proven beyond doubt before they are approved for clinical use. There should be a system for voluntary notification of side effects.

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