Plant Alkaloids

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Based in part on the previous version of this Encyclopedia of Life Sciences (ELS) article Plant Alkaloids by Jack G Woolley.

Advanced article

Article Contents

Online posting date: 15th March 2010

Alkaloids are traditionally defined as basic (alkali-like), nitrogen-containing organic constituents that occur mainly in plants. The nitrogen in the alkaloid molecule is derived from amino acid metabolism. Since the amino acid skeleton is often largely retained in the alkaloid structure, alkaloids originating from the same amino acid show similar structural features and can be classified according to their biosynthetic origin. The biosynthesis of alkaloids often follows complex pathways and includes stereospecific steps. Alkaloids often have pronounced bioactivities and are therefore thought to play an important role in the interaction of plants with their environment. Alkaloids and extracts of alkaloid-containing plants have been used throughout human history as remedies, poisons and psychoactive drugs.

Introduction

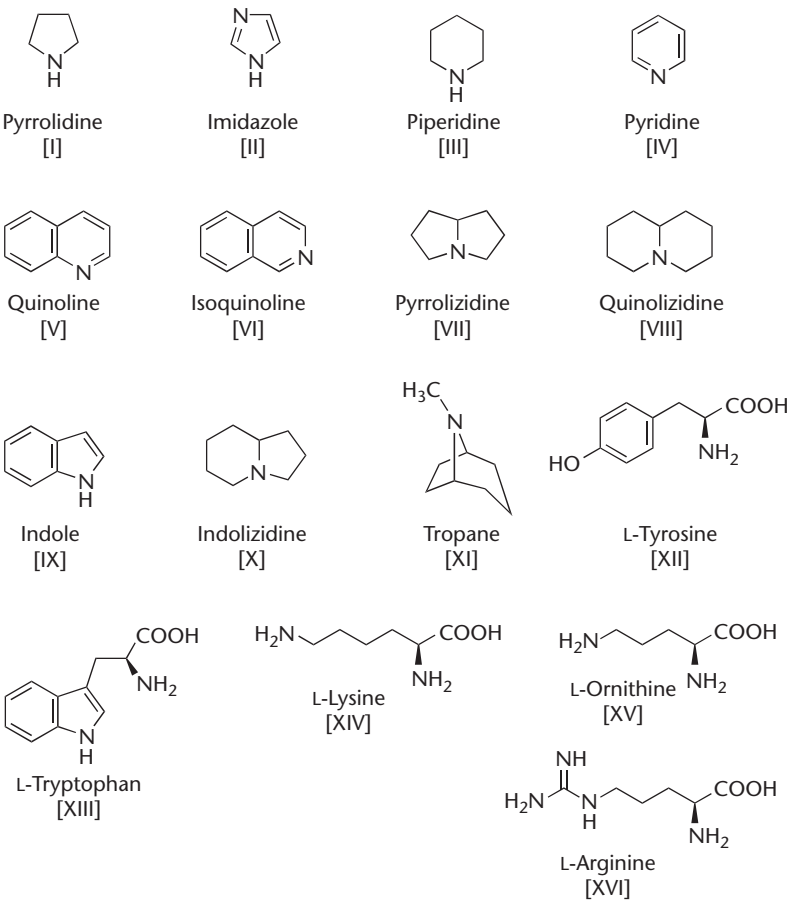
Alkaloids are organic nitrogenous bases predominantly found in plants, often with heterocyclically bound nitrogen, which is derived from amino acid metabolism. Owing to structural variations, however, their basicity varies greatly, and even neutral alkaloids have been described. Pseudoalkaloids are compounds that are not derived from amino acids, but acquire the nitrogen by transamination of a terpenoid, steroid, polyketide or shikimate-derived core. Examples are the steroid alkaloids and the diterpene alkaloid paclitaxel (Taxol®). The term protoalkaloid is used for nonheterocyclic amines that arise from amino acids, for example, ephedrine and mescaline [**XXXII**]. Many alkaloids are poisonous (e.g. strychnine), others are addictive (e.g. cocaine) and some are used clinically (e.g. morphine [**XXVII**]). More than 12 000 alkaloids are now known, the first discovered being narcotine (syn. noscapine) [**XXX**], isolated from opium by Derosne in 1803. Alkaloids occur as salts of organic acids, for example, acetic acid, oxalic acid, tartaric acid, in the cell sap of plants. They can be extracted from the cell with acidified water or alcohol, or alternatively they are soluble in organic solvents (e.g. chloroform) when the plant extract is rendered alkaline. Many alkaloids give an orange precipitate with Dragendorff's reagent (potassium tetraiodobismutate) and a white precipitate with Mayer's reagent (potassium tetraiodomercurate). These nonspecific detection reactions are based on the formation of insoluble salts between a large cation (alkaloid) and a large anion (reagent). Alkaloids are normally classified according to the heterocyclic ring system they possess (**Scheme 1**), but some authors prefer a classification based on their biosynthetic origins from amino acids. For example, tyrosine [**XII**] is the precursor of the benzyltetrahydroisoquinoline and Amaryllidaceae alkaloids, while indole and quinoline alkaloids are produced from tryptophan [**XIII**]. Lysine [**XIV**] gives rise to piperidine, quinolizidine and indolizidine alkaloids, and the biosynthesis of tropane and pyrrolidine alkaloids starts from ornithine [**XV**] or arginine [**XVI**]. **See also**: Cocaine and Amphetamines; Glycosides: Naturally Occurring; Opiates; Phenylpropanoid Metabolism; Shikimate Pathway: Aromatic Amino Acids and Beyond

ELS subject area: Plant Science

How to cite:
Fester, Karin (March 2010) Plant Alkaloids. In: Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester.
DOI: 10.1002/9780470015902.a0001914.pub2

Occurrence in the Plant Kingdom

Approximately 20–30% of all plants produce alkaloids. Alkaloids are common in the angiosperms (mono- and dicotyledons), but rare in nonflowering plants, although there are exceptions, for example, paclitaxel from yew (*Taxus*, a gymnosperm), lycopodine [**XVII**] from clubmoss (*Lycopodium*) and palustrine [**XVIII**] from horsetail (*Equisetum*) (**Figure 1**). Several classes of alkaloids are of limited occurrence and can be used as chemotaxonomic markers, for example, the terpene indol alkaloids that are most abundant in the families Apocynaceae, Loganiaceae,



Scheme 1 Classification of alkaloids.

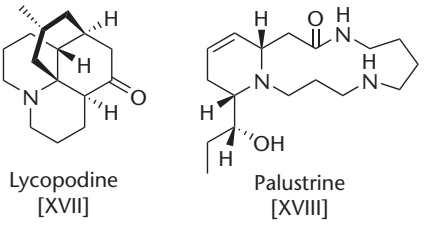


Figure 1 Alkaloids from nonflowering plants.

Rubiaceae and Gelsemiaceae. Benzyltetrahydroisoquinoline alkaloids, in contrast, occur frequently in early angiosperms of the subclass Magnoliids and in the families Ranunculaceae and Papaveraceae.

The distribution of alkaloids in the plant kingdom is displayed in **Table 1**. Owing to the large number and diversity of alkaloids in plants, only selected species and alkaloids are listed. **See also**: Evolution of Secondary Plant Metabolism

Occurrence of alkaloids in animals and fungi

Many authors consider alkaloids to be exclusively plant products, but similar compounds are found in fungi and animals, for example, insects and frogs. Beetles of the Coccinellidae (ladybirds) synthesize alkaloids as defence against predators, for example, coccinelline [**XIX**] from the 7-spot ladybird (*Coccinella septempunctata*) (**Figure 2**). Over 800 alkaloids have been isolated from amphibian skins (Daly *et al*., 2005). Of particular interest are the extremely toxic skin secretions from 'poison dart' frogs of South America (e.g. batrachotoxin from *Dendrobates* and *Phyllobates*). Simply wiping the dart across the back of the frog is said to be sufficient to produce a poison dart. *Epipedobates tricolor* (Ecuadoran tree frog) is of much interest since epibatidine [**XX**] (**Figure 2**) is *c*. 200 times more potent than morphine [**XXVII**] as an analgesic. In most cases, the Amphibians themselves do not biosynthesize the alkaloids, but rather take them up from dietary sources like ants and mites (Daly *et al*., 2005). Recently, it became evident that even mammals can synthesize low amounts of the alkaloid morphine [**XXVII**] (Poeaknapo *et al*., 2004; Böttcher *et al*., 2005).

Toxic ergot alkaloids are produced by fungal parasites (*Claviceps* species) or endophytes (e.g. *Epichloë* and *Neotyphodium* species) of grasses. *Claviceps purpurea*, the producer of rye ergot, is causative agent of ergotism, a disease especially prevalent in the Middle Ages that is characterized by gangrenes of extremities or neurological

Table 1 Distribution of alkaloids in the plant kingdom

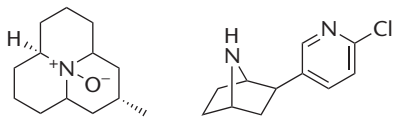
Family	Alkaloid	Plant genus[a]	Medical use[b]
Amaryllidaceae	Lycorine	*Amaryllis* (b)	Antiviral
	Galanthamine	*Galanthus* (b), *Narcissus* (b)	Alzheimer disease
Ancistrocladaceae	Michellamine B	*Ancistrocladus* (l)	Anti-HIV
Apiaceae	Coniine	*Conium* (fr)	Neurotoxic
Apocynaceae	Alstonine	*Alstonia* (bk)	Antipsychotic
	Ajmalicine	*Rauvolfia* (r, rh)	Antihypertensive
	Ajmaline [**XXII**]	*Rauvolfia* (r, rh)	Antiarrhythmic
	Aspidospermine	*Aspidosperma* (bk)	Respiratory stimulant
	Conessine	*Holarrhena* (bk)	Antidysenteric
	Ellipticine	*Ochrosia* (bk)	Anticancer
	Reserpine [**XXI**]	*Rauwolfia* (r, rh)	Antihypertensive
	Vinblastine [**XXIII**], Vincristine [**XXIV**]	*Catharanthus* (l)	Anticancer
	Yohimbine	*Yohimbe* (bk)	Aphrodisiac
Arecaceae	Arecoline	*Areca* (s)	Anthelmintic, stimulant (chewed seeds)
Aristolochiaceae	Aristolochic acid	*Aristolochia* (rh)	Tumour-inducing
Asteraceae	Senecionine [**XLVIII**]	*Senecio*	Hepatotoxic, carcinogenic
Berberidaceae	Berberine [**XXXI**]	*Berberis* (bk)	Antibacterial
	Berberine [**XXXI**], Oxyacanthine	*Mahonia* (bk)	Antibacterial, antipsoriatic
Boraginaceae	Indicine *N*-oxide	*Heliotropium* (l)	Anticancer, hepatotoxic
Cactaceae	Mescaline [**XXXII**]	*Lophophora* (l)	Hallucinogen
Celastraceae	Cathine, Cathinone	*Catha* (l)	CNS stimulant
Chenopodiaceae	Anabasine	*Anabasis* (l)	Insecticidal
Colchicaceae	Colchicine	*Colchicum* (c)	Antigout, induces polyploidy
Convolvulaceae	Calystegines [**XLIV**]	*Calystegia* (r)	Antihyperglykaemic
Dioncophyllaceae	Dioncophylline C	*Triphyophyllum* (l)	Antimalarial
Ephedraceae	Ephedrine	*Ephedra* (hb)	CNS stimulant
Equisetaceae	Palustrine [**XVIII**]	*Equisetum* (hb)	Toxic
Erythroxylaceae	Cocaine	*Coca* (l)	Local anaesthetic, CNS stimulant
Fabaceae	Castanospermine [**L**]	*Castanospermum* (l)	Antiviral, 'locoism' (stock)
	Anagyrine	*Anagyris* (hb)	Teratogenic
	Cytisine	*Laburnum*	Very toxic
	Monocrotaline [**XLVII**]	*Crotalaria* (l)	Hepatotoxic, tumour-inducing
	Physostigmine	*Physostigma* (s)	Glaucoma
	Sparteine	*Cytisus* (hb)	Antiarrhythmic
	Swainsonine [**XLIX**]	*Swainsona*	'Locoism' (stock)
Fumariaceae	Chelerythrine	*Dicentra*	Antimicrobial
Melanthiaceae	Cevadine	*Schoenocaulon* (s)	Insecticidal
	Rubijervine	*Veratrum* (r)	Antihypertensive
Loganiaceae	Strychnine	*Strychnos* (s)	Very poisonous
Lycopodiaceae	Lycopodine [**XVII**]	*Lycopodium* (l)	
Menispermaceae	Tubocurarine	*Chondrodendron* (bk)	Neuromuscular blocking agent, muscle relaxant
Moraceae	Calystegines [**XLIV**]	*Morus* (l)	Antiviral, antihyperglykaemic
Nyssaceae	Camptothecin [**XXVI**]	*Camptotheca*	Anticancer
Orchidaceae	Dendrobine	*Dendrobium* (hb)	
Papaveraceae	Codeine [**XXVIII**]	*Papaver* (lt)	Analgesic, cough suppresssant

(*Continued*)

Table 1 Continued

Family	Alkaloid	Plant genus	Medical use
	Morphine [**XXVII**]		Analgesic, narcotic
	Narcotine [**XXX**]		Cough suppresssant
	Papaverine [**XXIX**]		Spasmolytic
	Thebaine		
Peganaceae	Harmaline	*Peganum* (s)	Stimulant, hallucinogen
Ranunculaceae	Aconitine	*Aconitum* (r)	Rheumatism, neuralgia (topical)
	Ajaconine	*Delphinium* (hb)	
Rubiaceae	Caffeine	*Coffea* (s)	Stimulant
	Emetine [**XXXIII**]	*Psychotria* (r, rh)	Emetic, expectorant
	Quinine [**XXV**]	*Cinchona* (bk)	Antimalarial
	Quinidine		Antiarrhythmic
Rutaceae	Acronycine	*Acronychia* (bk)	Anticancer
	Canthine-6-one	*Zanthoxylum* (bk)	Antifungal
	Pilocarpine	*Pilocarpus* (l)	Glaucoma, miotic
Solanaceae	Capsaicin	*Capsicum* (fr)	Hot taste
	Scopolamine [**XLII**], Hyoscyamine [**XL**]	*Atropa* (hb), *Datura* (hb), *Duboisia* (hb), *Hyoscyamus* (hb), *Mandragora* (r)	Scopolamine: motion sickness Atropine (racemic hyoscyamine): Preoperative treatment, mydriatic, antidote against organophosphorous insecticides
	Solanine	*Solanum* (tb)	Toxic
	Nicotine	*Nicotiana* (l)	Insecticidal, smoking
Sterculiaceae	Theobromine	*Theobroma* (s)	
Taxaceae	Paclitaxel, Baccatin III	*Taxus* (bk, l)	Anticancer
Theaceae	Caffeine	*Camellia* (l)	Stimulant

[a]Part of plant used: (l), leaves; (s), seeds; (hb), herbs; (bk), bark; (rh), rhizome; (tb), tuber (green); (fr), fruits; (lt), latex; (r), roots; (c), corm and (b), bulb.
[b]Biological activity relates to the alkaloids that are listed as representative examples of the families, but in most cases also to the plant itself.



Coccinelline [XIX] Epibatidine [XX]

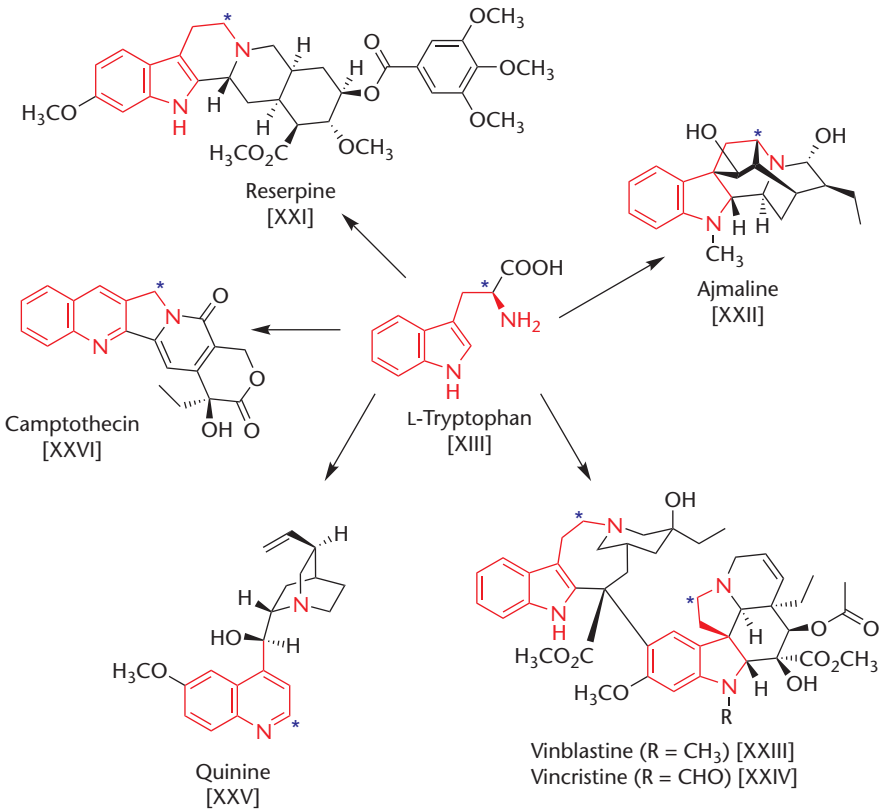
Figure 2 Two examples for alkaloids that have been isolated from animals: coccinelline [**XIX**] from the seven-spot ladybird (*Coccinella septempunctata*) and epibatidine from the Ecuadoran tree frog *Epipedobates tricolor* [**XX**].

symptoms like convulsions and hallucinations. Some ergot alkaloids are still in medical use today, for example, ergometrine as vasoconstrictor after childbirth. Several genera of the Agaricales (gilled mushrooms) produce hallucinogenic alkaloids, for example, muscimol from *Amanita* species and psilocybin from *Psilocybe* species. *Psilocybe mexicana* was used by the Aztecs in religious ceremonies. **See also**: Fungal Metabolites

Biosynthetic Pathways

Alkaloids are produced by secondary metabolism of primary metabolites, usually amino acids. These pathways are long, intricate, stereochemically precise and energy consuming, and are assumed to confer a selective advantage on the organism concerned. Normally these routes are explored using isotopically labelled precursors that are introduced into the plant, as shown in **Scheme 2** and **Scheme 3**. **See also**: Plant Secondary Metabolism; Secondary Metabolites: Deterring Herbivores

In many instances enzymes mediating single steps in the pathways have been isolated and characterized and in a growing number of cases the gene encoding the enzyme has been isolated and cloned. A candidate gene can be recognized because of similarity between its predicted protein product and enzymes already known to be involved in alkaloid synthesis. Expression of the gene in a heterologous host, for example, the bacterium *Escherichia coli* or the

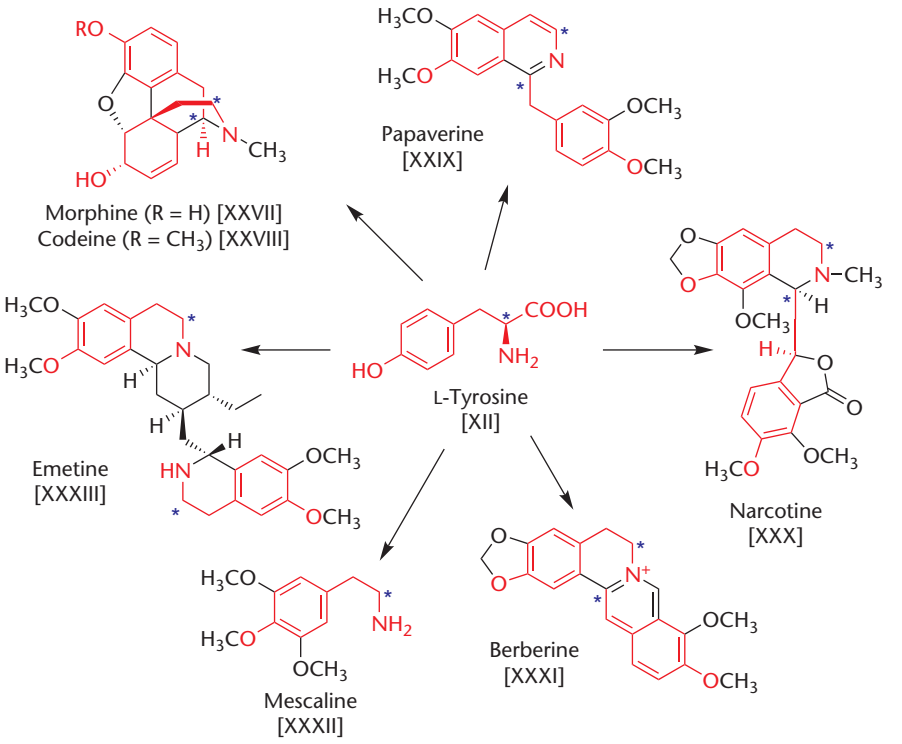


Scheme 2 Some alkaloids are derived from the amino acid L-tryptophan [**XIII**]. Labelled (*) tryptophan was employed in feeding experiments to determine the biosynthetic origin of the alkaloids. Although the indole ring of tryptophan is retained in reserpine [**XXI**] and ajmaline [**XXII**] from *Rauvolfia serpentina* as well as in vinblastine [**XXIII**] and vincristine [**XXIV**] from *Catharanthus roseus*, it is rearranged to a quinoline system for the biosynthesis of quinine [**XXV**] (*Cinchona succirubra*) and camptothecin [**XXVI**] (*Camptotheca acuminata*). The atoms derived from the precursor tryptophan are labelled red in the alkaloids.

yeast *Saccharomyces cerevisiae*, followed by tests of substrate specificity can be used to establish its precise role in the pathway under study. An interesting example for a pathway under investigation is the biosynthesis of tropane alkaloids. The *PMT* (putrescine *N*-methyltransferase) gene, the *TRI* and *TRII* (tropinone reductases I and II) genes involved in the biosynthesis of hyoscyamine [**XL**] and calystegines [**XLIV**] and the *H6H* (hyoscyamine 6-hydroxylase) gene involved in scopolamine (syn. hyoscine) [**XLII**] formation have been isolated from *Atropa* and *Hyoscyamus* (Biastoff and Dräger, 2007; Oksman-Caldentey, 2007). The H6H enzyme is unusual in that it catalyses two steps, a hydroxylation and an epoxydation, as illustrated in **Scheme 4**. This enzyme may become important for the biotechnological production of scopolamine, since there is a much higher demand for scopolamine than for hyoscyamine. Several genes of the tropane alkaloid biosynthetic pathway are still not known, and it will require novel approaches utilizing transcriptomics and metabolomics to isolate these genes. These techniques are based on the high-throughput analysis of transcripts and metabolites of plants grown under different conditions or in different stages of development. If the expression of an unknown gene correlates with that of a known gene or a metabolite of the pathway under study, it is likely to be involved in the same pathway. **See also**: Functional Genomics in Plants

Sequestration by Insects from Plants

Moths of the Arctiidae and Ctenuchidae are well known to sequester pyrrolizidine alkaloids, but many insects of the Coleoptera, Hemiptera, Lepidoptera and Orthoptera do the same as a means of protection against predators. Aphids ingest pyrrolizidines from the sap of plants and ladybirds feeding on the aphids are thereby able to accumulate these alkaloids. In plants and adapted insects, pyrrolizidines are usually stored as nontoxic, water-soluble *N*-oxides. These *N*-oxides are reduced in the gut of nonadapted herbivoures or predators to toxic and lipophilic-free alkaloid bases. Adapted herbivoures that use pyrrolizidines for their defence have developed mechanisms to either suppress the reduction or reconvert the free bases to polar *N*-oxides or glycosides (Hartmann and Ober, 2000). Some moths and butterflies convert sequestered pyrrolizidine alkaloids, for example, heliotrine [**XLV**], into the male courtship pheromone hydroxydanaidal [**XLVI**]



Scheme 3 The biosynthetic origin of some alkaloids derived from the amino acid L-tyrosine [**XII**]. The precursor tyrosine is labelled with isotopic carbon (*) and during biosynthesis the alkaloidal metabolites are specifically labelled at the sites shown (*). The atoms derived from tyrosine are marked red in the depicted alkaloids. The respective feeding experiments were carried out with *Papaver somniferum* (morphine [**XXVII**], codeine [**XXVIII**], papaverine [**XXIX**] and narcotine [**XXX**]), *Hydrastis canadensis* (berberine [**XXXI**]), *Lophophora williamsii* (mescaline [**XXXII**]) and *Psychotria ipecacuanha* (emetine [**XXXIII**]).

(**Figure 3**), which signals the alkaloid load of the male to the female and increases its success during courtship and mating. In many butterfly species, the male transfers sequestered plant pyrrolizidine alkaloids as a nuptial gift to the female during copulation. Eggs thus acquire the alkaloids from both parents and are subsequently laid on a pyrrolizidine alkaloid containing plant. On hatching the larvae sequester pyrrolizidines from eating the leaves of the host. The types of alkaloid sequestered by adults of a particular insect species depend on the host plant of its larval form. For example, the cinnabar moth (*Tyria jacobaea*) feeds on ragwort (*Senecio jacobaea*) and accumulates senecionine [**XLVIII**] and other bases. The death's-head hawk moth (*Acherontia atropus*) sequesters calystegines [**XLIV**] from potato plant leaves (Nash *et al*., 1993). **See also**: Ecology of Invertebrate Nutrition; Predator-induced Polyphenism

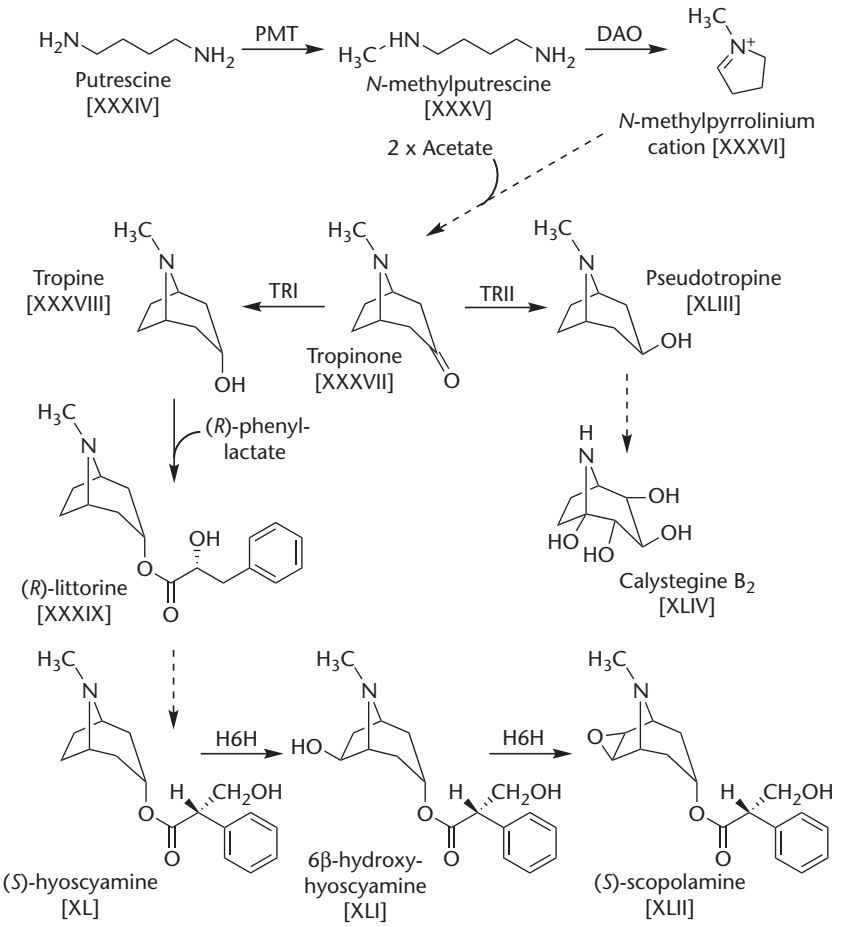
Physiological Properties

The poisonous and therapeutic effects of plants have been known since time immemorial, but the active constituents have been studied for only approximately 200 years. After the discovery of the first alkaloid, narcotine [**XXX**], other alkaloids were rapidly discovered: morphine [**XXVII**] (opium), strychnine (*Strychnos nux-vomica* seeds), emetine [**XXXIII**] (ipecacuanha root), quinine [**XXV**] (*Cinchona* bark) and coniine (*Conium maculatum*).

Alkaloids are used medicinally in their own right, for example, the anticholinergic hyoscyamine [**XL**] and the anticancer compound vincristine [**XXIV**] and they have often provided lead compounds for the development of synthetic drugs. For example, cocaine was the first local anaesthetic, quinine the first antimalarial and tubocurarine the first neuromuscular blocking agent. Modern synthetic analogues are the local anaesthetics benzocaine and lidocaine, the antimalarial drugs chloroquine and mefloquine, and muscle relaxants like atracurium and pancuronium, respectively. Recent discoveries of alkaloids with important pharmacological activities include the anticancer compounds camptothecin [**XXVI**] from the Chinese tree *Camptotheca acuminata* and paclitaxel from the Pacific Yew (Oberlies and Kroll, 2004). Alkaloids frequently have powerful physiological effects; some of these are listed in **Table 2**. **See also**: Acetylcholine; Cocaine and Amphetamines; Hallucinogenic Drugs; History of Anticancer Drugs; History of Drug Discovery; Opiates; Taxol

Alkaloids and Stock Poisoning

Alkaloids of the pyrrolizidine [**VII**] and indolizidine [**X**] types cause serious toxicity (and death) in livestock, mainly



Scheme 4 Biosynthesis of the tropane alkaloids (*S*)-hyoscyamine [**XL**] and (*S*)-scopolamine [**XLII**] which are confined to the Solanaceae family, and of the more abundantly occurring calystegine *nor*tropanes [**XLIV**]. Some enzymes involved in this biosynthetic pathway are putrescine methyltransferase (PMT), diamine oxidase (DAO), tropinone reductases I and II (TRI and TRII), and hyoscyamine 6-hydroxylase (H6H). Dashed lines indicate reactions that remain to be elucidated and may require more than one enzyme.

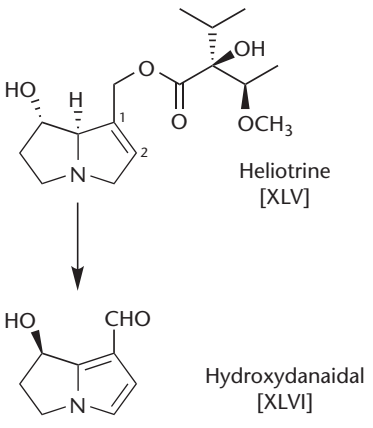


Figure 3 Some alkaloids are sequestered and metabolized by insects. The pyrrolizidine alkaloid heliotrine [**XLV**] can be converted by the Asian arctiid moth *Creatonotos transiens* to the pheromone hydroxydanaidal [**XLVI**] (Schulz *et al.*, 1993).

horses, cattle and sheep that graze on plants containing them. Pyrrolizidines, especially those bases with a C1–C2 double bond, for example, heliotrine [**XLV**] from *Heliotropium* species, cause chronic liver damage and malignant tumours, and monocrotaline [**XLVII**] occurring in the genus *Crotalaria* damages the lungs as well. Indolizidines, for example, swainsonine [**XLIX**] (*Swainsona canescens*) and castanospermine [**L**] (*Castanospermum australe*), are powerful glycosidase inhibitors that cause 'locoism', leading to histologically visible damage to neurons in the brain and ultimately death (**Figure 4**). **See also**: Plant Defences Against Herbivore and Insect Attack; Secondary Metabolites: Deterring Herbivores

Less well known are the recently described calystegines (*nor*tropanes), for example, calystegine B_2 [**XLIV**], which are, like the indolizidines, sugar-mimic glycosidase inhibitors. These alkaloids are widely distributed and occur in edible fruits of the Solanaceae, Moraceae, Convolvulaceae and edible cabbage species. Since glycosidase

Table 2 Some biological actions of alkaloids

Biological activity	Alkaloid
Cholinergic neurotransmission	
Acetylcholine receptors (allosteric modulator)	Galanthamine
Muscarinic acetylcholine receptors (activation)	Hyoscyamine [**XL**], scopolamine [**XLII**], arecoline, pilocarpine
Nicotinic acetylcholine receptors antagonists	Lobeline, nicotine (in low doses activator)
Nicotinic acetylcholine receptor antagonist at motor end plate	Tubocurarine
Acetylcholinesterase inhibitors	Galanthamine, physostigmine
Catecholamine neurotransmission	
α-Adrenoceptor agonists	Yohimbine
α-Adrenoceptor antagonists	Ajmalicine (raubasine)
Catecholamine release	Cathine, cathinone, ephedrine
Catecholamine depletion of synapses	Reserpine [**XXI**]
Other neurotransmission	
Adenosine receptor antagonists (low doses)	Caffeine, theobromine
Monoamine reuptake inhibitor	Cocaine
Monoamine oxidase A inhibitor (reversible)	Harmaline
μ Receptor agonist	Morphine [**XXVII**], codeine [**XXVIII**]
Serotonine 5-HT_3 receptor agonist	Emetine [**XXXIII**]
Serotonine 5-HT_{2A} receptor agonist	Mescaline [**XXXII**]
Antidysenteric	Conessine
Antimalarial	Quinine [**XXV**], dioncophylline C
Antimicrobial	Berberine [**XXXI**], chelerythrine
DNA damage or intercalation	Acronycine, ellipticine, indicine-*N*-oxide
Glycosidase inhibitors	Calystegines [**XLIV**], swainsonine [**XLIX**]
Inhibition of HIV reverse transcriptase and HIV-mediated cellular fusion and syncytium formation	Michellamine B
Inhibition of tubulin polymerization	Colchicine, vinblastine [**XXIII**], vincristine [**XXIV**]
Insecticidal	Cevadine
Na^+ channel blockade	Ajmaline [**XXII**], quinidine, sparteine
Phosphodiesterase inhibitor	Papaverine [**XXIX**]
Stabilization of DNA/topoisomerase I complex	Camptothecin [**XXVI**]
Stabilization of microtubules	Paclitaxel

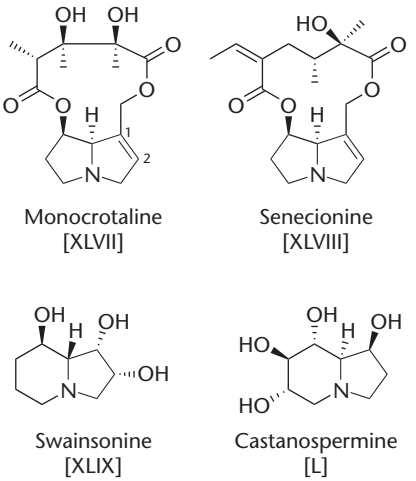


Figure 4 Some alkaloids that cause poisoning of livestock.

inhibitors may interfere with enzymes involved in sugar, polysaccharide and glycoprotein degradation, concern has been expressed as to their possible toxicity to humans and livestock. Calystegine-containing plants like *Solanum kwebense* and *Solanum dimidiatum* are known to cause neurological disorders in cattle. Isolated calystegines, however, have not shown any toxic effects under experimental conditions, and it remains to be proven whether they can be absorbed from the intestine despite their hydrophilicity (Biastoff and Dräger, 2007).

Site of Synthesis in Plants

Establishing the site of synthesis is difficult since the site of storage is not necessarily the site of synthesis. Grafting experiments with members of the Solanaceae (nightshade) family showed that nicotine and tropane alkaloids are

Table 3 Alkaloids from plants that have been grown in tissue culture conditions

		Culture type[a]					
Alkaloid	Plant	r	hr	sh	c	sc	References
Ajmalicine	*Catharanthus roseus*	+	+	+	+	+	Ciau-Uitz *et al*. (1994);
Catharanthine			+	+	+	+	Miura *et al*. (1988);
Serpentine		+	+	+	+	+	Ramani and
Vindoline				+		+	Jayabaskaran (2008) and
Vinblastine [**XXIII**]				+			van Der Heijden *et al*. (2004)
Berberine [**XXI**]	*Coptis japonica*				+	+	Verpoorte *et al*. (1991)
	Berberis wilsonia					+	
	Thalictrum minus				+	+	
Calystegines [**XLIV**]	*Calystegia sepium*	+					Biastoff and Dräger (2007)
	Hyoscyamus niger	+					
	Solanum lycopersicon	+					
Camptothecin [**XXVI**]	*Camptotheca acuminata*		+		+	+	Sirikantaramas *et al*. (2007)
	Ophiorrhiza pumila		+				
Emetine [**XXXIII**]	*Psychotria ipecacuanha*	+	+	+	+		Verpoorte *et al*. (1991) and Yoshimatsu *et al*. (2003)
Galanthamine	*Leucojum aestivum*			+	+		Berkov *et al*. (2009)
	Narcissus confusus			+	+		
Hyoscyamine[b] [**XL**] and scopolamine [**XLII**]	*Atropa belladonna*	+	+		+	(+)	Verpoorte *et al*. (1991)
	Datura innoxia	+	+		+	(+)	
	Datura metel	+			+	(+)	
	Datura stramonium	+	+		+	(+)	
	Duboisia leichhardtii	+	+		+		
	Duboisia myopyroides	+	+		+		
	Hyoscyamus muticus	+	+		+	(+)	
	Hyoscyamus niger	+	+		+	(+)	
Nicotine	*Nicotiana rustica*	+	+		+	(+)	Verpoorte *et al*. (1991)
	Nicotiana tabacum	+	+		+	+	
Paclitaxel and baccatin III	*Taxus chinensis*				+	+	Bringi *et al*. (1995)
	Taxus media					+	Yukimune *et al*. (1996)
Quinine [**XXV**] and quinidine	*Cinchona ledgeriana*	+	+	+	+	(+)	Anderson *et al*. (1982); Chung and Staba (1987); Mulder-Krieger *et al*. (1982b); Payne *et al*. (1987) and Robins *et al*. (1986)
	Cinchona pubescens				+	(+)	Mulder-Krieger *et al*. (1982a) and Robins *et al*. (1987)
Reserpine [**XXI**]	*Rauvolfia serpentina*		+	+	+	(+)	Verpoorte *et al*. (1991) and Madhusudanan *et al*. (2008)
Ajmaline [**XXII**]	*Rauvolfia serpentina*		+		+	+	Stöckigt (1995)
Vincamine	*Vinca minor*			+		+	Tanaka *et al*. (1995) and Verpoorte *et al*. (1991)

[a]r, root; hr, hairy root; sh, shoot; c, callus and sc, suspension cultures. +, alkaloid production and (+), only trace amounts of alkaloids detected or instable production.
[b]Since (*S*)-hyoscyamine easily undergoes racemization, atropine, the mixture of the (*S*)- and (*R*)-enantiomers, is determined in most cases rather than the enantiopure alkaloid.

produced in the stock (root) and transported into the scion (shoot). Root cultures of Solanaceous plants (*Datura*, *Atropa*, *Hyoscyamus*, *Duboisia*, *Nicotiana*, etc.) all produce alkaloids in the absence of the aerial parts. In contrast, the shoot is the site of synthesis in *Ephedra* (ephedrine), *Conium maculatum* (coniine) and lupin (lupanine). At the subcellular level, alkaloids frequently accumulate in the vacuole, where they are trapped in their nondiffusable protonated form due to the acidic vacuolar milieu.

Since the genes encoding many alkaloid biosynthetic enzymes are now known, it is possible to identify tissues and cells in which the genes are expressed by *in situ* hybridization using labelled DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) probes. In addition, it is possible to determine the localization of the corresponding enzymes by immunohistochemistry using antibodies raised against the recombinant proteins. The biosynthesis of the cytotoxic alkaloid vincristine [**XXIV**] in Madagascar periwinkle (*Catharanthus roseus*) was shown to involve multiple cell types. Early biosynthetic genes are expressed in phloem parenchyma of young aerial parts, whereas some of the genes for downstream reactions are expressed in epidermal cells. Late biosynthetic steps are localized to idioblast (highly differentiated cells with specialized functions) and laticifers (ducts containing milky sap). This suggests that the biosynthesis of vincristine requires intercellular shuttling of biosynthetic intermediates and is under developmental control (De Luca and St Pierre, 2000; Kutchan, 2005). **See also**: Immunofluorescence; *In Situ* Hybridization; Plant Anatomy; Plant Cell: Overview; Plant Vacuoles

Production in Cell Culture

Many useful plants grow in inaccessible parts of the world or where access is limited by political uncertainties. Some are in short supply and do not lend themselves to farming, or are perhaps slow growing (e.g. the Pacific Yew, *Taxus brevifolia*, the source of paclitaxel). Sometimes the desired alkaloid is present only in trace quantities (e.g. vinblastine [**XXIII**] and vincristine [**XXIV**] from *Catharanthus roseus*). Consequently, there has been an enormous effort to grow medicinal plants in flasks (or fermenters) where alkaloid production may be optimized, carried out without seasonal variations and when required. Under the influence of plant hormones (IAA, indoleacetic acid, or the synthetic weed-killer 2,4-D), plants dedifferentiate into identical single-cell clumps (callus). Callus can be broken down into a suspension culture when the culture fluid is agitated and this kind of homogeneous culture is ideal for large-scale production. Unfortunately, callus and suspension cultures often do not produce the same quantity of alkaloid found in the parent plant, and after many transfers their ability to synthesize the alkaloid might be lost. Moreover, the alkaloid profile may differ from the intact plant. Some alkaloids, for example, vincristine [**XXIV**], even cannot be produced in dedifferentiated callus and suspension cultures, because their biosynthesis takes place in various cell types that require differentiation. Therefore, differentiated tissue cultures like root or shoot cultures can prove more suitable for alkaloid production and biosynthetic studies. **See also**: Plant Cell Culture; Plant Cell Differentiation; Plant Growth Factors and Receptors

Hairy root cultures are much used in research into the biosynthesis of alkaloids. They are produced by exposing sterilized plant parts to strains of the soil microorganism *Agrobacterium rhizogenes*, which is able to transfer part of its own DNA (T-DNA) from a plasmid (a circular loop of double-stranded DNA) to the plant's nuclear genome. The T-DNA carries genes encoding the biosynthesis of auxins (plant hormones controlling cell division and differentiation) and for the production of opines, unusual nutritional amino acids required by the microorganism. As a consequence the invaded tissue, when rid of *Agrobacteria* using antibiotics, continues to produce opines and generates hairy roots (even from leaf tissue) due to increased auxin production or sensitivity to auxins (Sevón and Oksman-Caldentey, 2002). These hairy roots usually grow faster than normal roots and show a stable and high production of secondary metabolites, provided the respective compounds are also produced in the root of the untransformed plant. By inserting genes encoding enzymes or regulators of alkaloid production into their plasmids, *Agrobacteria* can also be used to engineer hairy roots with increased alkaloid content. For example, by introducing the *H6H* gene of *Hyoscyamus muticus* into *Atropa belladonna*, hairy roots with increased levels of scopolamine [**XLII**] were generated (Hashimoto *et al*., 1993). Some plants that have been grown in culture are listed in **Table 3**. **See also**: *Agrobacterium tumefaciens*-mediated Transformation of Plant Cells; Plant Transformation

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