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Bioactive Alkaloids: Structure and Biology

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Ecological Roles of Alkaloids

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1.1
Introduction: Defense Strategies in Plants

Plants are autotrophic organisms and serve as both a major and the ultimate source of food for animals and microorganisms. Plants cannot run away or fight back when attacked by a herbivore, nor do they have an immune system to protect them against pathogenic bacteria, fungi, viruses, or parasites. Plants struggle for life, as do other organisms, and have evolved several strategies against herbivorous animals, parasites, microorganisms, and viruses. Plants also compete with neighboring plants for space, light, water, and nutrients [1–8].

Apparently plants have evolved both physical and chemical defense measures, similar to the situation of sessile or slow moving animals. Among physical defense strategies we find [8]

- formation of indigestible cell walls containing cellulose, lignin, or callose;
- presence of a hydrophobic cuticle as a penetration barrier for microbes and against desiccation;
- formation of a thick bark in roots and stems against water loss, microbes, and herbivores;
- development of spines, thorns, hooks, trichomes, and glandular and stinging hairs (often filled with noxious chemicals) against herbivores;
- formation of laticifers and resin ducts (filled with gluey and noxious fluids);
- a high capacity for regeneration so that parts that have been browsed or damaged by infection can be readily replaced (so-called open growth).

Secondly, plants are masters of chemical defense, with a fascinating ability to produce a high diversity of chemical defense compounds, also known as secondary metabolites or allelochemicals [1–17]. Chemical defense involves macromolecular compounds, such as diverse defense proteins (including chitinase [against fungal cell

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walls], β-1,3-glucanases [against bacteria], peroxidase, and phenolase, lectins, protease inhibitors, toxalbumins, and other animal-toxic peptides), polysaccharides, and polyterpenes. More diverse and more prominent are low molecular weight secondary metabolites, of which more than 100 000 have been identified in plants (Figure 1.1).

Among the secondary metabolites that are produced by plants, alkaloids figure as a very prominent class of defense compounds. Over 21 000 alkaloids have been identified, which thus constitute the largest group among the nitrogen-containing secondary metabolites (besides 700 nonprotein amino acids, 100 amines, 60 cyanogenic glycosides, 100 glucosinolates, and 150 alkylamides) [2,3,18,19]. However, the class of secondary metabolites without nitrogen is even larger, with more than 25 000 terpenoids, 7000 phenolics and polyphenols, 1500 polyacetylenes, fatty acids, waxes, and 200 carbohydrates.

1.2 Ecological Roles of Alkaloids

Alkaloids are widely distributed in the plant kingdom, especially among angiosperms (more than 20 % of all species produce alkaloids). Alkaloids are less common but present in gymnosperms, club mosses (*Lycopodium*), horsetails (*Equisetum*), mosses, and algae [1–5,17]. Alkaloids also occur in bacteria (often termed antibiotics), fungi, many marine animals (sponges, slugs, worms, bryozoa), arthropods, amphibians (toads, frogs, salamanders), and also in a few birds, and mammals [1–5,13,17,20].

Alkaloids are apparently important for the well-being of the organism that produces them (Figures 1.1–1.3). One of the main functions is that of chemical defense against herbivores or predators [2,3,8,18]. Some alkaloids are antibacterial, antifungal, and antiviral; and these properties may extend to toxicity towards animals. Alkaloids can also be used by plants as herbicides against competing plants [1,3,8,18]. The importance of alkaloids can be demonstrated in lupins which – as wild plants – produce quinolizidine alkaloids (''bitter lupins''), that are strong neurotoxins (Table 1.1) [21,22]. Since lupin seeds are rich in protein, farmers were interested in using the seeds for animal nutrition. This was only possible after the alkaloids (seed content 2–6 %) had been eliminated. Plant breeders created so-called sweet lupins with alkaloid levels below 0.02 %. If bitter and sweet lupins are grown together in the field it is possible to study the importance of alkaloids for defense. For example, Figure 1.3 shows that rabbits strongly discriminate between sweet and bitter lupins and prefer the former. This is also true for insects, as aphids and mining flies always favor sweet lupins. In the wild, sweet lupins would not survive because of the lack of an appropriate chemical defense [8,21].

Secondary metabolites are not only mono- but usually multifunctional. In many cases, even a single alkaloid can exhibit more than one biological function. During evolution, the constitution of alkaloids (that are costly to produce) has been modulated so that they usually contain more than one active functional group, allowing them to interact with several molecular targets and usually more than one group of enemies [3,18,19,21–24]. Many plants employ secondary metabolites (rarely alka-

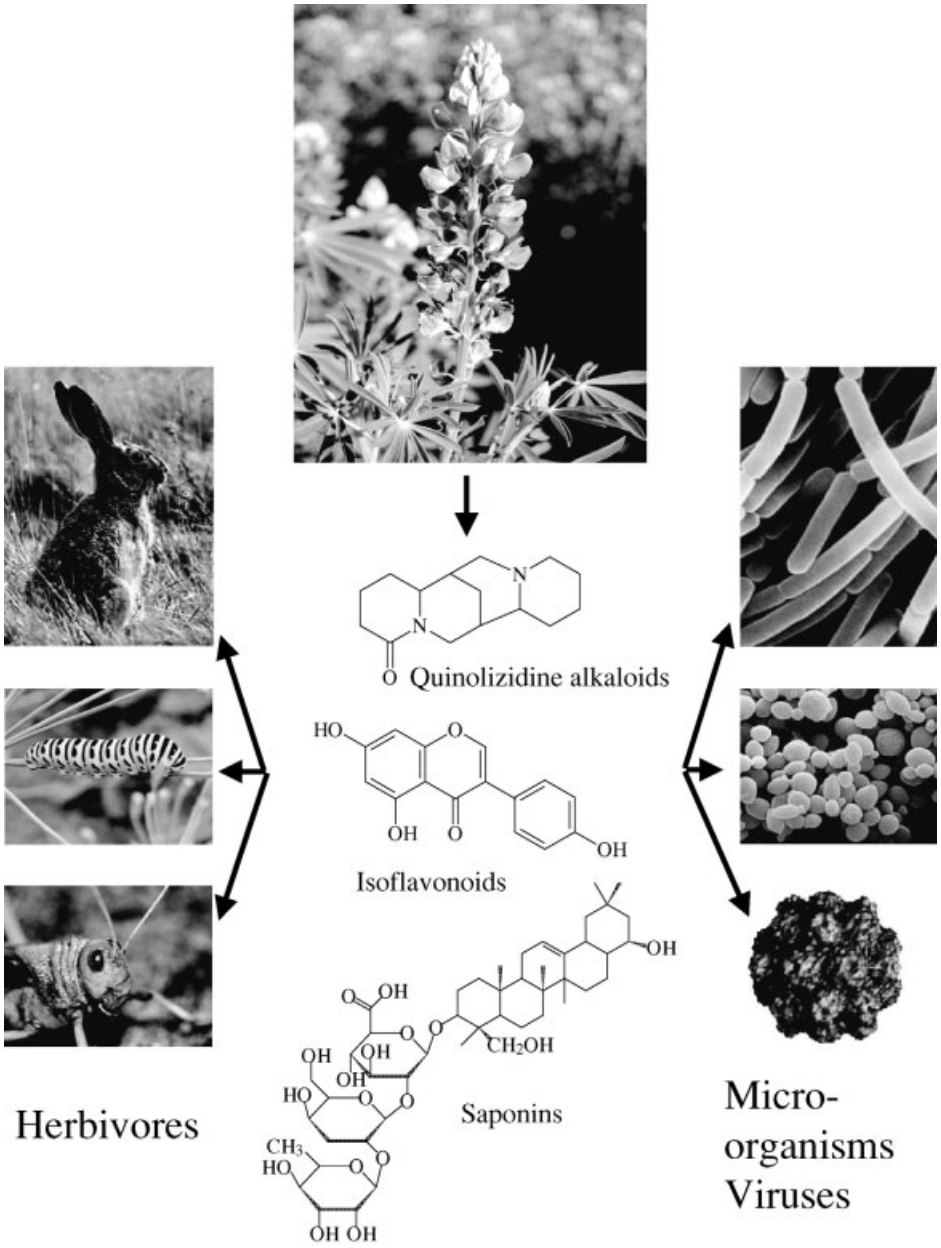


Fig. 1.1 Relationships between plants, their secondary metabolites, and potential enemies (herbivores, microorganisms, and viruses). Example: Lupins produce quinolizidine alkaloids, isoflavonoids, and saponins as main defense compounds.

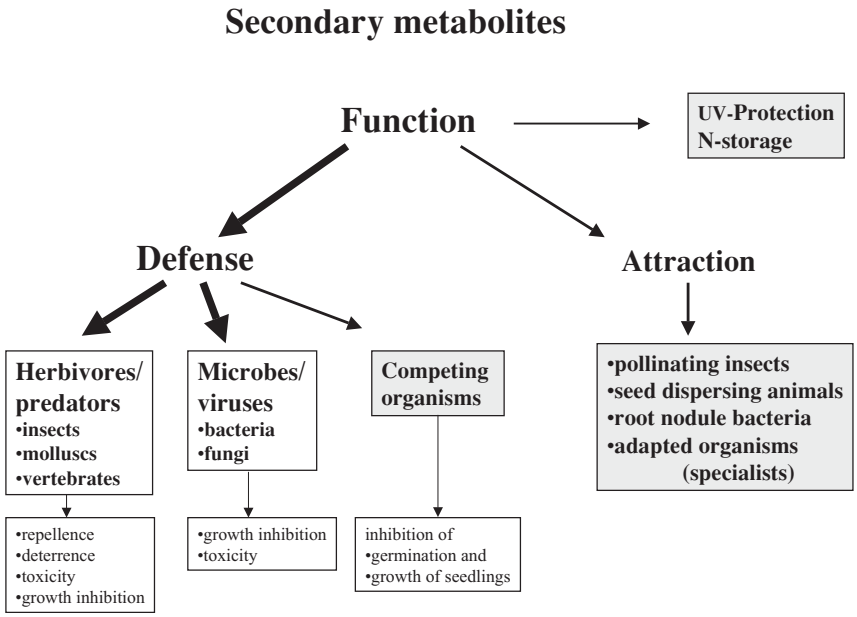


Fig. 1.2 Overview of the ecological functions of secondary metabolites.

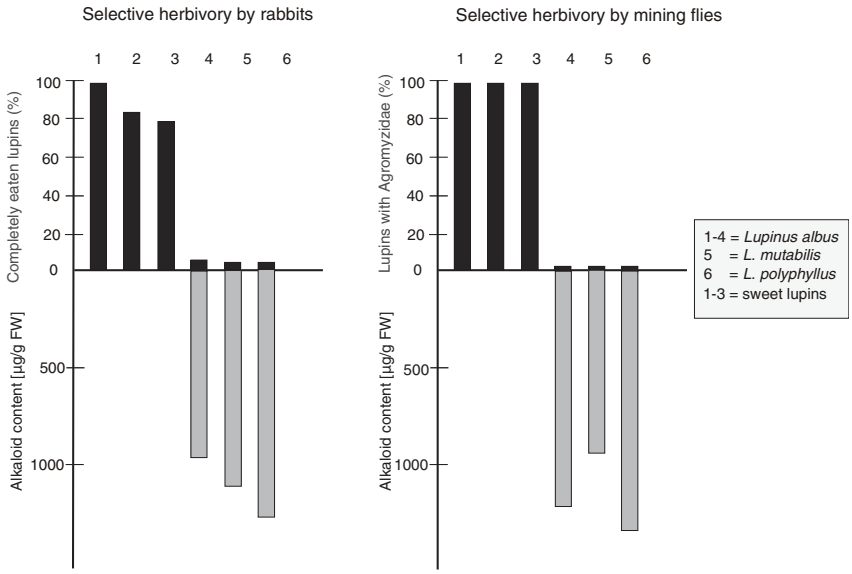


Fig. 1.3 Importance of quinolizidine alkaloids for lupins against herbivores.In this experiment, lupins with or without alkaloids were grown in the field. When rabbits got into the field, they preferentially consumed the sweet, alkaloid-free lupins. Also larvae of mining flies preferred sweet lupins.

Tab. 1.1 Molecular targets of alkaloids in neuronal signal transduction [2,3,19].

Target	Selected alkaloids
Neuroreceptor	
Muscarinic acetylcholine receptor	Hyoscyamine, scopolamine, and other tropane alkaloids (AA); acetylheliosupine and some other pyrrolizidine alkaloids; arecoline (A); berbamine, berberine, and other isoquinoline alkaloids; dicentrine and other aporphine alkaloids; strychnine, brucine; cryptolepine (AA); sparteine and other quinolizidine alkaloids (A); pilocarpine (A); emetine; himbacine and other piperidine alkaloids (A); imperialine (AA); muscarine (A)
Nicotinic acetylcholine receptors	Nicotine and related pyridine alkaloids (A); Ammodendrine (A); anabasine (A); arborine (AA); boldine and other aporphine alkaloids (AA); berberine and related protoberberine alkaloids; C-toxiferine (AA); coniine and related piperidine alkaloids (A); cytisine, lupanine, and other quinolizidine alkaloids (A); tubocurarine (AA); codeine (A); erysodine and related Erythrina alkaloids (AA); histrionicotoxin (AA); lobeline (A); methyllycaconitine (AA); pseudopelletierine (A)
Adrenergic receptors	Acetylheliosupine and related pyrrolizidine alkaloids; ajmalicine, reserpine (AA); arecoline; berbamine, berberine, laudanosine, and other isoquinoline alkaloids (AA); boldine, glaucine, and other aporphine alkaloids (AA); cinchonidine and other quinoline alkaloids; corynanthine, yohimbine, and other indole alkaloids (AA); emetine; ephedrine; ergometrine, ergotamine, and related ergot alkaloids (A/AA); ephedrine and related phenylethylamines (A); higenamine (A); *N*-methyldopamine, octopamine (A)
Dopamine receptor	Agroclavine, ergocornine, and related ergot alkaloids (A); bulbocapnine and related aporphine alkaloids (AA); anisocycline, stylopine, and related protoberberine alkaloids; salsolinol and related isoquinolines (A); tyramine and derivatives (A)
GABA receptor	Bicuculline (AA), cryptopine, hydrastine, corlumine, and related isoquinoline alkaloids (AA); securinine; harmaline and related β-carboline alkaloids (A); muscimol (A); securinine (AA)
Glycine receptor	Corymine, strychnine, and related indole alkaloids (AA)
Glutamate receptor	Histrionicotoxin and related piperidines (AA); ibogaine and related indole alkaloids (AA); nuciferine and related aporphine alkaloids (AA)
Serotonine receptor	Akuaminine and related indole alkaloids (A); annonaine, boldine, liriodenine and related aporphine alkaloids (AA); berberine and related protoberberine alkaloids; ergotamine, ergometrine, and related ergot alkaloids (AA); psilocin, psilocybine (A); bufotenine, *N,N*-dimethyltryptamine, and related indoles (A); harmaline and related β-carboline alkaloids (A); kokusagine and related furoquinoline alkaloids (AA); mescaline (A); ibogaine and other monoterpene indole alkaloids (A); gramine; *N,N*-dimethyltryptamine and derivates (AA)
Adenosine receptor	Caffeine, theobromine, and other purine alkaloids (AA)
Opiate receptor	Morphine and related morphinan alkaloids (A); akuammine, mitragynine (A), ibogaine and related indole alkaloids

(*continued*)

Tab. 1.1 (*Continued*)

Target	Selected alkaloids
Acetylcholine esterase	Galanthamine (AA); physostigmine and related indole alkaloids (AA); berberine and related protoberberine alkaloids (AA); vasicinol and related quinazolines (AA); huperzine (AA); harmaline and related β-carboline alkaloids (AA); demissine and related steroidal alkaloids (AA)
Monoamine oxidase	Harmaline and related β-carbolines (AA); carnegine, salsolidine, *O*-methylcorypalline, and related isoquinolines (AA); *N,N*-dimethyltryptamine and related indoles (AA);
Neurotransmitter uptake (transporter)	Ephedrine and related phenylalkyl amines (AA); reserpine, ibogaine, and related indole alkaloids (AA); cocaine (AA); annonaine and related aporphine alkaloids (AA); arecaidine (AA); norharman and related β-carboline alkaloids (AA); salsolinol and related isoquinolines (AA)
Na^+, K^+ channels	Aconitine and related diterpene alkaloids (A); veratridine, zygadenine, and related steroidal alkaloids (A); ajmaline, vincamine, ervatamine, and other indole alkaloids (AA); dicentrine and other aporphine alkaloids (AA); gonyautoxin (AA); paspalitrem and related indoles (AA); phalloidin (AA); quinidine and related quinoline alkaloids (AA); sparteine and related quinolizidine alkaloids (AA); saxitoxin (AA); strychnine (AA); tetrodotoxin (AA)
Ca^{2+} channels	Ryanodine (A); tetrandrine, berbamine, antioquine, and related bis-isoquinoline alkaloids (AA); boldine, glaucine, liriodenine, and other aporphine alkaloids (AA); caffeine and related purine alkaloids (A/AA); cocaine (AA); corlumidine, mitragynine, and other indole alkaloids (A/AA); bisnordehydrotoxiferine (AA)
Adenylate cyclase	Ergometrine and related ergot alkaloids (AA); nuciferine and related aporphine alkaloids (AA)
cAMP phosphodiesterase	Caffeine and related purine alkaloids (AA); papaverine (AA); chelerythrine, sanguinarine, and related benzophenanthridine alkaloids (AA); colchicines (AA); infractine and related indole alkaloids (AA)
Protein kinase A (PKA)	Ellipticine and related indole alkaloids (AA)
Protein kinase C (PKC)	Cepheranthine and related bis-isoquinoline alkaloids (AA); michellamine B and related isoquinoline alkaloids (AA); chelerythrine and related benzophenanthridine alkaloids (AA); ellipticine and related indole alkaloids (AA)
Phospholipase (PLA_2)	Aristolochic acid and related aporphine alkaloids (AA); berbamine and related bis-isoquinoline alkaloids (AA)

A = agonist; AA = antagonist.

loids, mostly colored phenolics and fragrant terpenoids) to attract pollinating and seed-dispersing animals; the compounds involved are usually both attractant and feeding deterrents. Attracted animals are rewarded by nectar or fleshy fruit tissues but should leave seeds or flowers undamaged. Hence, a multifunctional or pleiotropic effect is a common theme in alkaloids and other secondary metabolites.

An alkaloid never occurs alone; alkaloids are usually present as a mixture of a few major and several minor alkaloids of a particular biosynthetic unit, which differ in functional groups. Furthermore, an alkaloid-producing plant often concomitantly accumulates mixtures of other secondary metabolites, mostly those without nitrogen, such as terpenoids and polyphenols, allowing them to interfere with even more targets in animals or microorganisms. When considering the total benefits to a plant from secondary metabolites or the pharmacological activities of a drug, the potential additive or even synergistic effect of the different groups of secondary metabolites should be taken into account [10,25].

The multiple functions that alkaloids can exhibit concomitantly include a few physiological tasks: sometimes, alkaloids also serve as toxic nitrogen storage and nitrogen transport molecules [3,8]. Plants that produce few and large seeds, nearly always invest in toxic defense compounds (often alkaloids) that are stored together with proteins, carbohydrates, or lipids [8]. Since nitrogen is a limiting factor for plant growth, nitrogen apparently is a valuable asset for plants. In many species that store nitrogen in proteins and/or secondary metabolites in seeds or tubers, a remobilization has been observed after germination or regrowth in spring [2]. In plants that shed their leaves, alkaloids are usually exported to storage organs prior to leaf fall [2]. Alkaloids are definitely not waste products as had previously been assumed.

Aromatic and phenolic compounds can mediate UV-protecting activities, which might be favorable for plants living in UV-rich environments, such as high altitudes [1]. Alkaloids (such as isoquinoline, quinoline, and indole alkaloids) that derive from aromatic amino acids, such as phenylalanine, tyrosine, and tryptophan, may have UV-absorbing properties, besides antiherbivoral and antimicrobial activities.

Only the defensive properties of alkaloids will be discussed in more detail in this chapter.

1.3 Modes of Action

In order to deter, repel, or inhibit the diverse set of potential enemies, ranging from arthropods and vertebrates to bacteria, fungi, viruses, and competing plants, alkaloids must be able to interfere with important cellular and molecular targets in these organisms. A short overview of these potential targets is given in Figure 1.4a and b. The modulation of a molecular target will negatively influence its communication with other components of the cellular network, especially proteins (cross-talk of proteins) or elements of signal transduction. As a consequence, the metabolism and function of cells, tissues, organs, and eventually the whole organism will be affected and an overall physiological or toxic effect achieved. Although we know the structures of many secondary metabolites, our knowledge of their molecular modes of action is largely fragmentary and incomplete. Such knowledge is, however, important for an understanding of the functions of secondary metabolites in the producing organism, and for the rational utilization of secondary metabolites in medicine or plant protection [10,25].

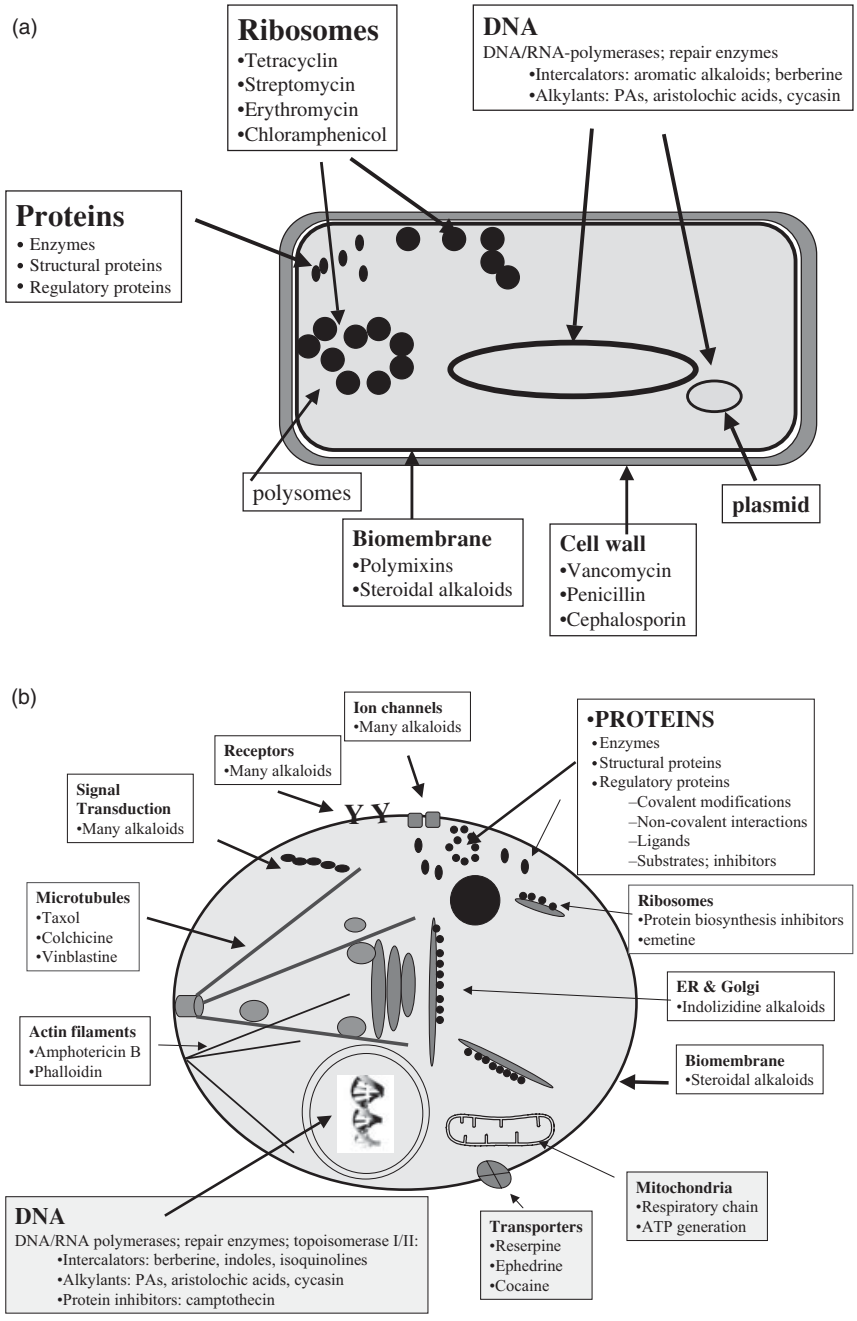


Fig. 1.4 Molecular targets for secondary metabolites, especially alkaloids. (a) Targets in bacterial cells, (b) targets in animal cells.

Whereas many secondary metabolites interact with multiple targets, and thus have unspecific broad (pleiotropic) activities, others, especially alkaloids, are more specific and interact exclusively with a single particular target. Secondary metabolites with broad and nonspecific activities interact mainly with proteins, biomembranes, and DNA/RNA which are present in all organisms.

1.3.1 Unspecific Interactions

Among broadly active alkaloids, a distinction can be made between those that are able to form covalent bonds with proteins and nucleic acids, and those that modulate the conformation of proteins and nucleic acids by noncovalent bonding.

Covalent modifications are the result when the following functional groups interact with proteins [18,25]:

- reaction of aldehyde groups with amino and sulfhydryl groups;
- reaction of exocyclic methylene groups with SH groups;
- reaction of epoxides with proteins and DNA (epoxides can be generated in the liver as a detoxification reaction);
- reaction of quinone structures with metal ions (Fe^{2+}/Fe^{3+}).

Noncovalent bonds are generated when the following groups interact with proteins [18,25]:

- ionic bonds (alkaloids with phenolic hydroxyl groups, that can dissociate as phenolate ions; alkaloid bases that are present as protonated compounds under physiological conditions);
- hydrogen bonds (alkaloids with hydroxyl groups, carbonyl, or keto groups);
- van der Waals and hydrophobic interactions (lipophilic compounds).

Noncovalent bonds, especially hydrogen bonds, ionic bonds, hydrophobic interactions, and van der Waals forces are weak individually, but can be powerful if they work cooperatively. For example, alkaloids with phenolic properties (found in several isoquinoline and indole alkaloids) usually have two or more phenolic hydroxyl groups that can form hydrogen bonds with proteins and nucleic acids. Furthermore, these OH groups may dissociate under physiological conditions to form phenate ions that can form ionic bonds with positively charged amino acid residues, such as those from lysine, arginine, and histidine. These OH groups are crucial for the biological activity of phenolics [18,25].

Molecules of nitrogen-containing compounds, such as alkaloids, amines, and peptides, usually contain (under physiological conditions) positively charged N-atoms that can form ionic bonds with negatively charged amino acid residues of glutamic and aspartic acid in proteins. Both the covalent and the noncovalent interactions will modulate the three-dimensional protein structure, that is, the conformation that is so important for the bioactivities of proteins (enzymes,

receptors, transcription factors, transporters, ion channels, hormones, cytoskeleton). A conformational change is usually associated with a loss or reduction in the activity of a protein, leading to inhibition of enzyme or receptor activity or interference with the very important protein–protein interactions [17,18,25].

Lipophilic compounds, such as the various terpenoids, tend to associate with other hydrophobic molecules in a cell; these can be biomembranes or the hydrophobic core of many proteins and of the DNA double helix [10,18,24,25]. In proteins, such hydrophobic and van der Waals interactions can also lead to conformational changes, and thus protein inactivation. A major target for terpenoids, especially saponins, is the biomembrane. Saponins (and, among them, the steroid alkaloids) can change the fluidity of biomembranes, thus reducing their function as a permeation barrier. Saponins can even make cells leaky, and this immediately leads to cell death. This can easily be seen in erythrocytes; when they are attacked by saponins these cells burst and release hemoglobin (hemolysis) [1,6,17]. Among alkaloids, steroidal alkaloids (from Solanaceae) and other terpenoids have these properties.

These pleiotropic multitarget bioactivities are not specific, but are nevertheless effective, and this is critical in an ecological context. Compounds with pleiotropic properties have the advantage that they can attack any enemy that is encountered by a plant, be it a herbivore or a bacterium, fungus, or virus. These classes of compounds are seldom unique constituents; quite often plants produce a mixture of secondary metabolites, often both phenolics and terpenoids, and thus exhibit both covalent and noncovalent interactions. These activities are probably not only additive but synergistic [10,25].

1.3.2 Specific Interactions

Plants not only evolved allelochemicals with broad activities (see Section 1.3.1) but also some that can interfere with a particular target [3,6,17–19,25]. Targets that are present in animals but not in plants are nerve cells, neuronal signal transduction, and the endocrinal hormone system. Compounds that interfere with these targets are usually not toxic for the plants producing them. Plants have had to develop special precautions (compartmentation: resin ducts, trichomes, laticifers) in order to store the allelochemicals with broad activities that could also harm the producer.

Many alkaloids fall into the class of specific modulators and have been modified during evolution in such a way that they mimic endogenous ligands, hormones, or substrates [1,3,18,19]. We have termed this selection process "evolutionary molecular modeling" [12,13,19,23]. Many alkaloids are strong neurotoxins that were selected for defense against animals [2,3,19]. Table 1.1 summarizes the potential neuronal targets that can be affected by alkaloids. Extensive reviews on this topic have been published [2,3,19].

Neurotransmitters derive from amino acids; most of them are amines that become protonated under physiological conditions. Since alkaloids also derive from amino acids (often the same ones as neurotransmitters) it is no surprise that several alkaloids have structural similarities to neurotransmitters. They can be considered as neurotransmitter analogs (Figure 1.5a–c).

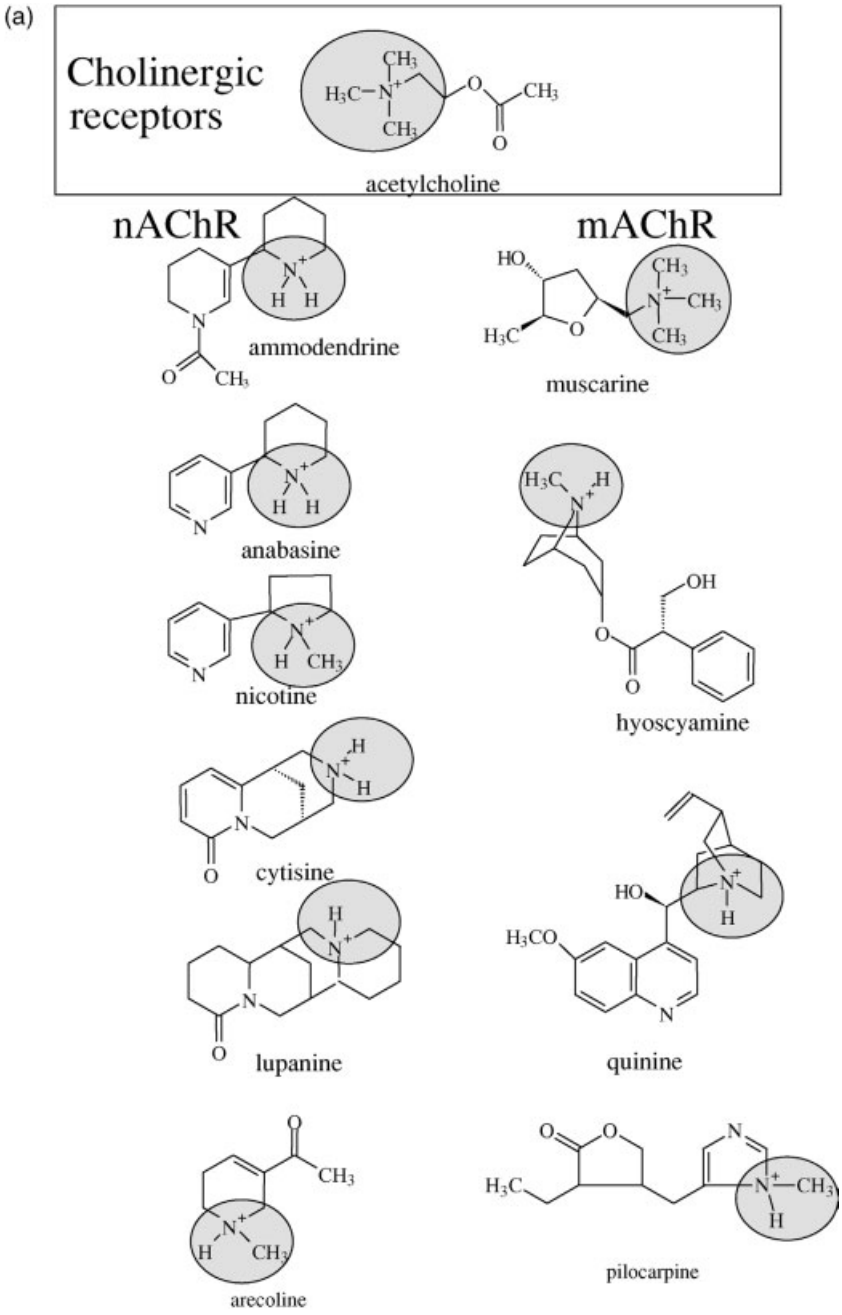
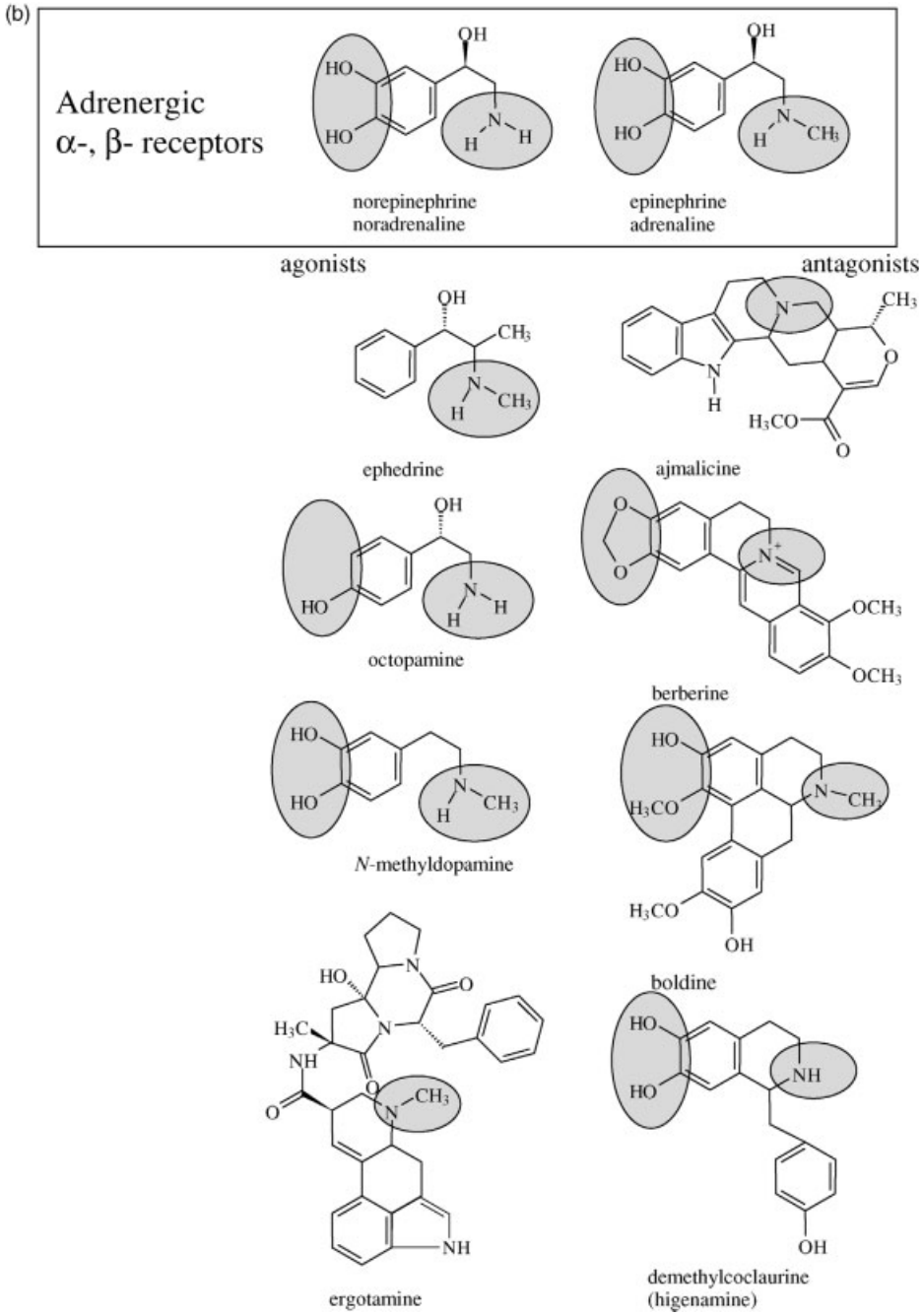


Fig. 1.5 Agonistic or antagonistic modulation of neuroreceptors by alkaloids that mimic neurotransmitters. (a) Interaction at cholinergic neurotransmitters that bind acetylcholine: nicotinic acetylcholine receptor (nAChR) and muscarinic acetylcholine receptors (mAChR), (b) interaction at adrenergic receptors that bind noradrenaline and adrenaline, (c) interaction at serotonergic receptors that bind serotonin.



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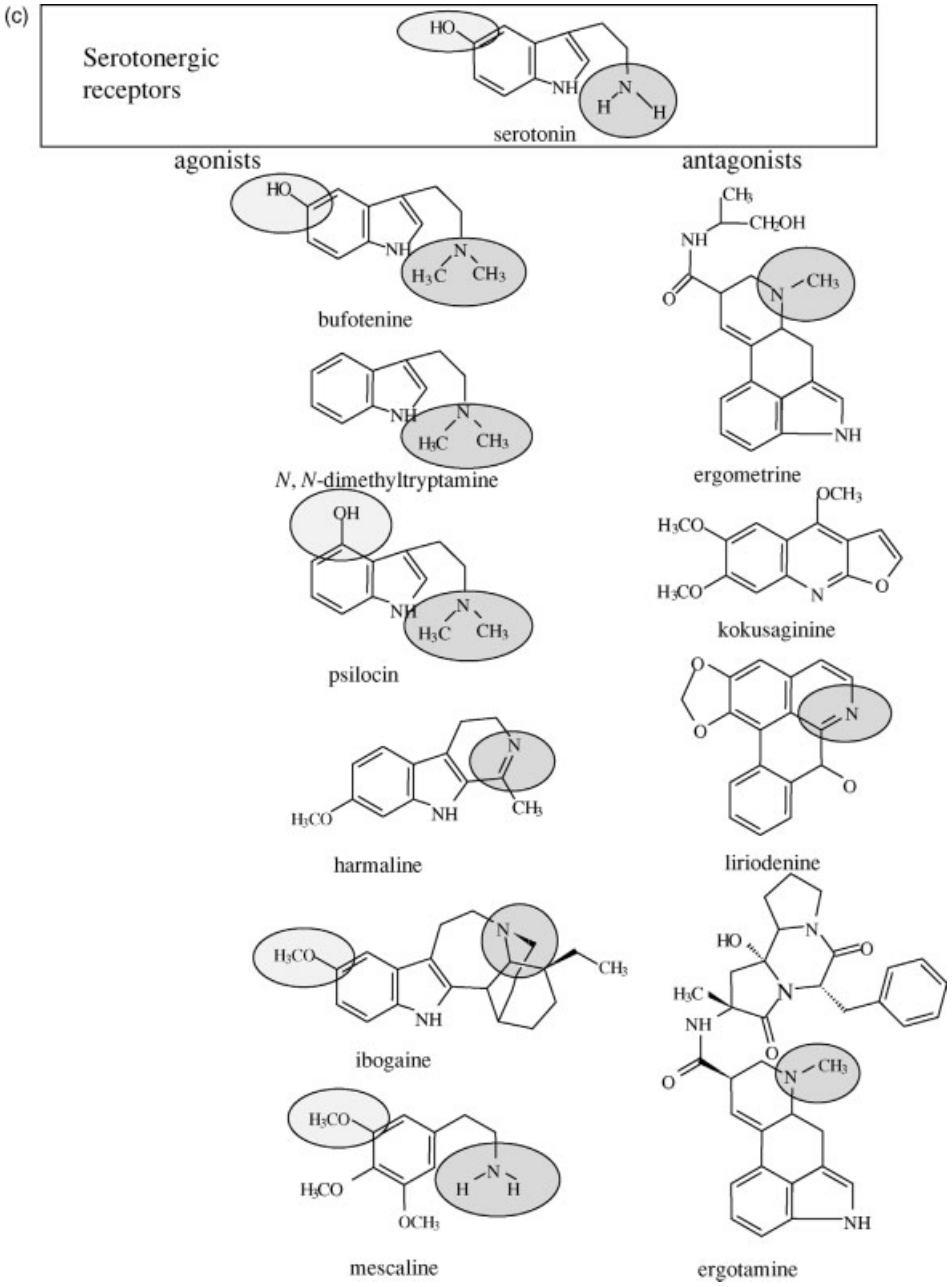

Fig. 1.5 (*Continued*)

Alkaloids that structurally mimic neurotransmitters can bind to neuroreceptors and either activate (agonists) or inactivate (antagonists) them (Table 1.1). Additional important targets are ion channels, such as the Na^+, K^+, and Ca^{2+} channels; several alkaloids are known that inhibit or activate these ion channels (Table 1.1).

Neuronal signal transduction is a very critical target in animals, since all organs are controlled by either the parasympathetic or the sympathetic nervous system. Its disturbance stops organ function (heart and circulation, respiration), mobility, orientation, and ability for flight in most animals. Many alkaloids are indeed strong (even deadly) neurotoxins or have mind-altering and hallucinogenic properties [1–3,17,19].

1.3.3 Cytotoxicity of Alkaloids

Many alkaloids are infamous for their strong toxicity towards animals and humans. Most of the deadly alkaloids fall into the class of neurotoxins (see above). The others have cytotoxic properties (Table 1.2). A cytotoxic effect can be generated when cell membranes are made leaky (as by saponins or steroidal alkaloids), or when elements of the cytoskeleton are inhibited. The spindle poisons vinblastine, vincristine, colchicine, and taxol are particularly famous. Actin filament formation is blocked by fungal poisons such as phalloidin from *Amanita phalloides*.

DNA can also be a target for alkaloids: planar and lipophilic alkaloids, such as berberine and sanguinarine (Figure 1.6) are intercalating compounds that assemble between the stacks of paired nucleotides in the DNA double helix [2,3,18,23]. DNA intercalation can disturb replication, DNA repair, and DNA topoisomerases. Frameshift mutations are one of the adverse consequences of intercalating compounds. Some alkaloids, such as pyrrolizidine alkaloids, aristolochic acids, cycasin, and furoquinoline alkaloids, are known to form covalent adducts with DNA bases. Mutations and tumor formation can be the result of such interactions. DNA alkylation occurs in some alkaloids only after activation by liver enzymes, such as cytochrome p450 oxidases (pyrrolizidine alkaloids, aristolochic acids) [17,18,24].

Ribosomal protein biosynthesis is often inhibited by alkaloids that interact with nucleic acids [23]. There are also more specific inhibitors, such as emetine.

Disturbances of the cytoskeleton, DNA replication, and DNA topoisomerase, or DNA alkylation and intercalation usually lead to cell death by apoptosis [18] (Table 1.2). The cytotoxic properties are usually not specific for animals but also affect bacteria, fungi, other plants, and even viruses. Alkaloids thus defend plants against a wide diversity of enemies. They have the disadvantage that a producing plant could theoretically kill itself by its own poison. Compartmentation, target-site insensitivity, and other mechanisms (which are largely unknown) must have evolved to overcome such problems.

Tab. 1.2 Molecular modes of action of selected cytotoxic alkaloids [3,18].

Alkaloid	Toxicity (animals)	Cyto-toxicity	Apoptosis	Micro-tubules	DNA topoiso-merase	Telo-merase	Membrane lysis	DNA inter-calation	DNA alkylation	Mutagenic	Inhibition of protein biosynthesis
Alkaloids derived from tryptophan											
Camptothecin	X	X	X		X			X			
Cinchonine, cinchonidine	X	X						X			
Cryptolepine	X	X	X		X	X		X			
Dictamnine	X	X						X	X	X	
Ellipticine	X				X			X		X	
Ergotamine	X		X					X			
Evodiamine	X	X		X							
Fagarine	X	X						X		X	
Harmine	X	X	X		X					X	
Quinine	X	X	X					X			
Vincristine	X	X	X	X				X			
Alkaloids derived from phenylalanine, tyrosine											
Aristolochic acids	X	X							X	X	
Berbamine	X							X			
Berberine	X	X	X		X	X		X			
Chelerythrine	X	X	x					X	X		
Chelidonine	X	X	X	X				X			
Colchicine	X	X	X	X							
Coralyne	X	X			X			X			
Dicentrine	X	X			X					X	

(*continued*)

Tab. 1.2 (*Continued*)

Alkaloid	Toxicity (animals)	Cyto-toxicity	Apoptosis	Micro-tubules	DNA topoiso-merase	Telo-merase	Membrane lysis	DNA inter-calation	DNA alkylation	Mutagenic	Inhibition of protein biosynthesis
Emetine	X	X	X					X			X
Fagaronine	X	X			X			X			
Liriodenine	X	X	X		X					X	
Lycorine	X	X	X								
Noscapine	X	X	X	X							
Piperine		X	X								
Salsolinol	X	X									
Sanguinarine	X	X	X					X	X	x	
Tetrandrine	X	X	X				X				
Alkaloids derived from ornithine, arginine											
Pyrrolizidine alkaloids	X	X	X						X	X	
Miscellaneous alkaloids											
Acronycine	X	X	X					X		X	
Cycasin	X								X	X	
Cyclopamine	X	X	X							X	
Lobeline	X							X			
Maytansine	X	X		X							
Paclitaxel	X	X	X	X							
Solarmargine	X	X	X				X				

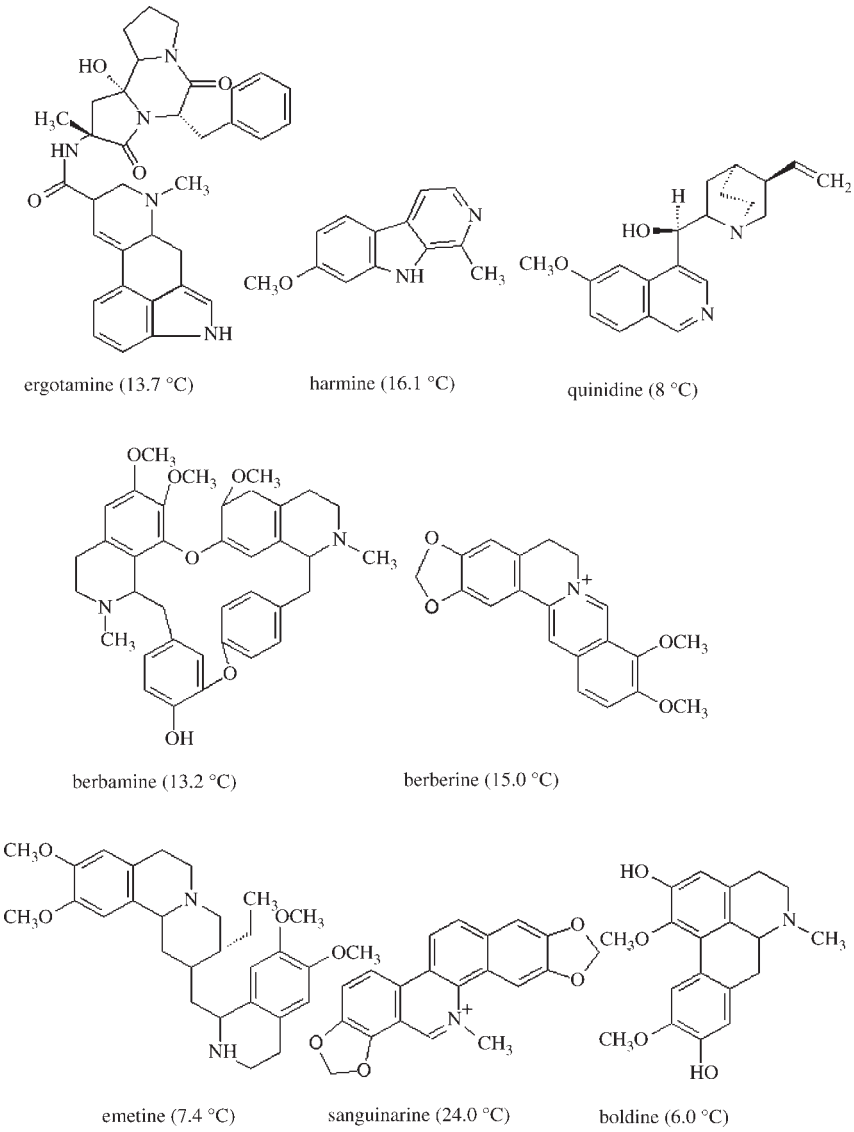

Fig. 1.6 Examples of alkaloids that intercalate DNA. Intercalation increases the melting temperature of DNA; relevant T_m values are shown in parentheses.

1.4 Evolution of Alkaloidal Defense Systems

Alkaloids are apparently well-adapted molecules that can serve plants as potent defense chemicals which are used on their own or together with other mostly

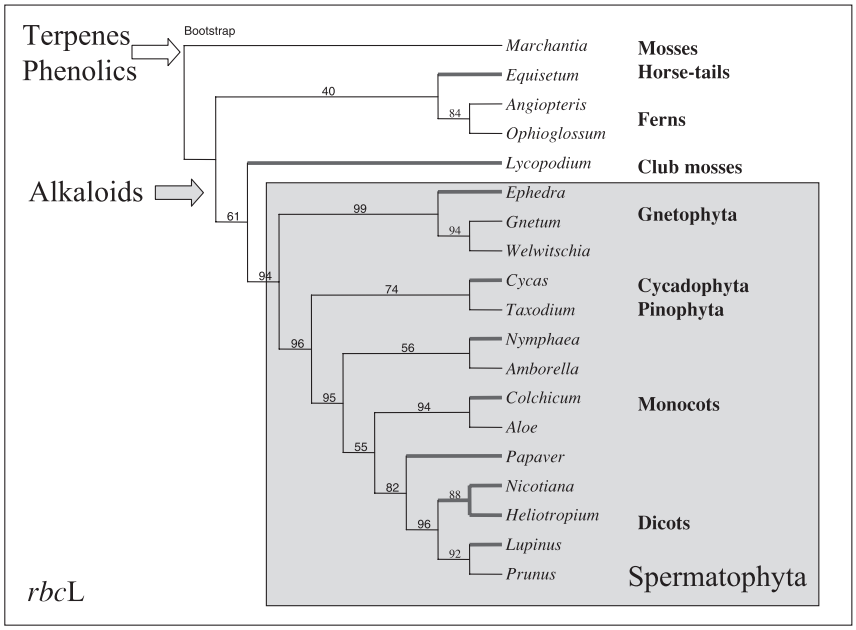

Fig. 1.7 Evolution of alkaloids in the phylogeny of plants. Using nucleotide sequences of the chloroplast gene *rbc*L a phylogenetic tree was computed with Maximum Parsimony. A bootstrap cladogram is shown with bootstrap values shown at the nodes. Branches leading to taxa that accumulate alkaloids are shown in bold.

co-occurring secondary metabolites, such as phenolics or terpenoids. We can speculate on the question of when alkaloid defense systems first arose during the evolution of plants. Figure 1.7 maps the character ''alkaloid defense'' on a phylogenetic tree that covers the whole plant kingdom. Alkaloids are present already in early branches of land plants, such as lycopods and horse-tails. They are also present in some members of the gymnosperms, especially in the Gnetales (i.e., *Ephedra*) and Cycadales (i.e., families Cycadaceae, Zamiaceae). Only a few conifers produce alkaloids (e.g., *Taxus*, *Harringtonia*). Within the angiosperms, however, alkaloid formation is a widely distributed trait and especially abundant in the families Solanaceae, Convolvulaceae, Fabaceae, Strychnaceae, Loganiaceae, Apocynaceae, Asclepiadaceae, Ranunculaceae, Papaveraceae, Berberidaceae, Fumariaceae, Buxaceae, Punicaceae, Celastraceae, Erythroxylacae, Zygophyllaceae, Rutaceae, Gelsemiaceae, Colchicaceae, Iridaceae, Boraginaceae, and Asteraceae. It can be speculated that the ancestors of present day plants developed alkaloids as defense chemicals early on because they had to face the attack of herbivorous animals (which were present already in the Cambrian) [2,12,26]. This would mean that genes for the biosynthesis of alkaloids are not only present in plants that actually produce the particular alkaloid but that they may be much more widely distributed among the plant kingdom [2,12,26].

Secondary metabolites with similar structural types and pharmacophoric groups can be seen in several bacteria (where they are often termed antibiotics if they have antimicrobial or cytotoxic properties). Since eukaryotic cells had taken up α-proteobacteria (which became mitochondria) and cyanobacteria (which became chloroplasts), they also inherited a number of genes that encode enzymes for pathways leading to secondary metabolites. Therefore, we may speculate that early plants already had the capacity of building defense compounds and that alkaloids were among the first. Since the numbers and types of herbivores and other enemies have increased within the last 100 million years, angiosperms have had to face more enemies and as a consequence have developed a more complex pattern of defense and signal compounds.

Alkaloids can assume their defense role if they are present at the right place, concentration, and time. Since alkaloids are costly for the plants to produce [2,12], usually only the most important plant tissues and organs (such as young leaves, flowers, seeds, and storage organs, such as roots and tubers) are heavily defended by them. For the same reason, alkaloids are not discarded with falling leaves or senescing tissues but remobilized and stored in seeds, roots, or tubers. Many secondary metabolites accumulate in special cells and tissues.

Several plants produce milk juice sequestered in laticifers; in several plant genera alkaloids are mainly stored in latex vesicles, such as isoquinoline alkaloids in *Papaver* and *Chelidonium*, or piperidine alkaloids in *Lobelia*. If herbivores wound such a plant, the latex will spill out and the herbivore will immediately be confronted with alkaloids. Since most of them are strong poisons, a deterrent effect is usually achieved. Another strategic way to store alkaloids is their sequestration in epidermal vacuoles or in trichomes. These tissues have to ward off not only herbivores (especially small ones) but also microorganisms in the first place. Several classes of alkaloids have been found in epidermal tissues, such as quinolizidine and tropane alkaloids [2,3].

Most plants produce an alkaloid in one organ and transport the alkaloids after synthesis, via either xylem or phloem (Table 1.3), to other plant tissues in which the alkaloids are stored for defense or signaling [2,11,21].

- Xylem transport has been reported for tropane alkaloids and nicotine, which are synthesized in roots but accumulate in aerial parts.

Tab. 1.3 Transport of alkaloids in plants [2].

Alkaloid	Type	Occurrence	Phloem	Xylem
Lupanine	Quinolizidine	*Lupinus, Genista, Cytisus Laburnum, Spartium*	Yes	No
Senecionine	Pyrrolizidine	*Senecio, Petasites, Adenostyles*	Yes	No
Aconitine	Diterpene	*Aconitum*	Yes	?
Swainsonine	Indolizidine	*Astragalus*	Yes	No
Nicotine	Pyrrolidine	*Nicotiana*	No	Yes
Hyoscyamine	Tropane	*Atropa, Datura, Hyoscyamus*	No	Yes
Rutacridone	Quinoline	*Ruta*	No	Yes

- Phloem transport is known for quinolizidine, pyrrolizidine alkaloids, and aconitine.

The transport of toxic alkaloids in the phloem can be an advantage for plants against phloem-feeding insects, such as aphids [8]. For example: alkaloid-rich lupins are avoided by aphids, whereas sweet lupins with very low alkaloid contents are preferred by polyphagous aphids [8,22].

No defense system is 100 % safe. This is also true for alkaloidal defense against herbivores. A few herbivores, mostly insects, have overcome the chemical defense system of their host plants by adapting to it [1,20,27]. A common theme in mono- and oligophagous insects is their ability not only to tolerate alkaloids but also to store them in their body (often the integuments). Alkaloids sequestered by insects include pyrrolizidine, quinolizidine alkaloids, and aconitine [1,3–5,20,27–29]. These specialized insects, which are often aposematically colored, employ the acquired alkaloids for their own defense against predators. The mechanisms by which these specialized insects overcome alkaloid toxicity remain open questions. It could be a target-site modification as observed for cardiac glycosides at the Na^+/K^+-ATPase in Monarch butterflies [30] or simply sequestration in tissues or cells without corresponding targets.

A comparable situation to insect specialists can be found in parasitic and hemiparasitic plants, another example of multitrophic interactions. In several instances it can be shown that the parasites can tap the xylem or phloem of their host plants and sequester the host alkaloids into their own system [2,31]. The parasites would gain chemical defense against herbivores by such a process (Table 1.4). In *Osyris alba* it can be shown that plants exist that can sequester the alkaloids of more than one host plant: that is, pyrrolizidine and quinolizidine alkaloids [32]. The situation of *Lolium* is even more complex [33]. If the grass *Lolium temulentum* is infected by an endophytic

Tab. 1.4 Transfer of alkaloids from host plants to parasitic and hemiparasitic plants [30–32].

Alkaloid	Type	Host plant	Hemiparasite/parasite
Sparteine	Quinolizidine	*Cytisus scoparius*	*Orobanche rapum-genistae*
Retamine	Quinolizidine	*Retama shaerocarpa*	*Viscum cruciatum*
Cytisine	Quinolizidine	*Genista acanthoclada*	*Cuscuta palaestina*
Lupanine	Quinolizidine	*Lupinus* spp.	*Cuscuta reflexa*; *Castilleja integra*
		Lupinus texensis	*Castilleja indivisa*
Thermopsine	Quinolizidine	*Lupinus argenteus*	*Castilleja miniata*
N-Methylcytisine	Quinolizidine	*Spartium junceum*	*Osyris alba*
Isolupanine	Quinolizidine	*Lupinus falcata*	*Pedicularis semibarbata*
Anagyrine	Quinolizidine	*Lupinus* spp.	*Pedicularis semibarbata*
Senecionine	Pyrrolizidine	*Senecio triangularis*	*Pedicularis semibarbata*
Senecionine	Pyrrolizidine	*Senecio* spp.; *Liatris punctata*	*Castilleja integra*
cis-Pinnidol	Piperidine	*Picea engelmannii*	*Arceutholobium microcarpum*
Norditerpene		*Delphinium occidentale*	*Castilleja sulphurea*
Loline	Pyrrolizidine	*Lolium temulentum*	*Rhinanthus minor*

fungus, it acquires the fungal toxins (the pyrrolizidine alkaloid loline). If *Lolium* was parasitized by *Rhinanthus minor*, a second transfer was observed into the hemiparasite, which could increase its fitness through this sequestration [33].

1.5 Conclusions

Alkaloids are not waste products but have evolved mainly as defense compounds against herbivores, but also against microbes, competing other plants, and even viruses. Although the production and storage of alkaloids is costly for plants, they are apparently a good investment against enemies. Alkaloid structures have been shaped during evolution so that they can interfere with critical targets in potential enemies. A disturbance of DNA/RNA and related enzymes, of the cytoskeleton, of ribosomal protein biosynthesis, and of membrane permeability by several alkaloids can be interpreted as a defense against all types of organism, ranging from bacteria to animals. The interference of alkaloids with neuroreceptors, ion channels, and other elements of the neuronal signal transduction chain is more specific and certainly a measure against animal herbivores.

Alkaloids do not only serve as poisons against herbivores and microorganisms; they can also be interesting and important in medicine as pharmaceutical agents. Given at a lower dose (than the plants use for defense) these alkaloids no longer work as poisons but can mediate useful pharmacological activities, such as reducing blood pressure, relieving pain and spasms, stimulating circulation and respiration, or killing tumor cells [10,14,25].

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