The Alkaloids

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THE ALKALOIDS
Chemistry and Biology

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PREFACE

Once again, this volume of *"The Alkaloids: Chemistry and Biology"* is comprised of four quite different chapters, from three different continents, on mechanisms of cytotoxic action, the calystegines, strychnine synthesis, and substituted quinoline alkaloids. This diversity reflects the need to see alkaloids as a class of natural product having tremendous biological potential and of continued broad scientific interest.

Numerous alkaloids have over the past 35 years shown cytotoxic activity. For the first time, Wink, in Chapter 1, discusses the diverse mechanisms through which various alkaloid classes, and individual compounds within those classes, effect their activity. It is also made apparent that alkaloids offer opportunities to overcome drug resistance, which is the nemesis of many therapeutic regimens and requires more detailed studies.

While the tropane alkaloids are best known for pharmaceutical agents such as cocaine and atropine, the development of the polyhydroxy tropane alkaloids, the calystegines, has brought new challenges. In Chapter 2, the advances in the isolation, structure determination, synthesis, biosynthetic pathways, and biology of this relatively new group of alkaloids are discussed by Biastoff and Dräger.

Small molecules with a high number of stereocenters offer challenges to synthetic organic chemists which are almost irresistable, and thus become model systems for the evolution of new methodologies. In Chapter 3, one of these archetypical alkaloids, strychnine, is reviewed solely from the perspective of recent synthetic efforts by Shibasaki and Ohshima.

Finally, in Chapter 4, a new group of alkaloids, those with a quinoline nucleus and various alkyl, aryl, and alkylaryl side chains, are discussed by the Brazilian group of da Silva, Soares, Fernandes, and Vieria from the perspectives of biosynthesis, biogenesis, and distribution in the plant, marine, and fungal environments.

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CHAPTER 1

Molecular Modes of Action of Cytotoxic Alkaloids: From DNA Intercalation, Spindle Poisoning, Topoisomerase Inhibition to Apoptosis and Multiple Drug Resistance

Michael Wink*

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I. INTRODUCTION

Plants produce a high diversity of secondary metabolites (SM), and among them, alkaloids are a most prominent class. Over 21,000 alkaloids have been identified,

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which constitute the largest group among the nitrogen-containing SM (including 700 non-protein amino acids, 100 amines, 60 cyanogenic glycosides, 100 glucosinolates, and 150 alkylamides). However, the class of SM without nitrogen is even larger (more than 25,000 terpenoids, 7000 phenolics and polyphenols, 1000 polyacetylenes, fatty acids, waxes, and 200 carbohydrates) (1–4). Alkaloids are widely distributed in the plant kingdom, especially among angiosperms (more than 20% of all species produce alkaloids), and are less common in gymnosperms, lycopods, horsetails, mosses, and algae (3,5,6). Alkaloids also occur in bacteria, fungi, many marine animals (sponges, slugs), arthropods, amphibians, birds, and mammals (3,7–9).

Alkaloids are apparently important for the fitness of the organism that produces them. One of the main functions is that of chemical defence against herbivores or predators. Some alkaloids have antibacterial, antifungal, and antiviral activities in addition. In many cases, a single alkaloid can exhibit more than one biological function. During evolution, the constitution of alkaloids has been modulated so that they usually contain more than one active functional group allowing them to interact with several molecular targets. Therefore, a pleiotropic effect is a common theme in alkaloids and other SM (2,3,7,8,10–13). The multiple functions that alkaloids can exhibit include a few physiological tasks: sometimes, toxic alkaloids also concomitantly serve as nitrogen-storage and nitrogen-transport molecules. The ecological functions will not be reviewed in this chapter as they were discussed in previous reviews (2,7,8,10,11).

II. MOLECULAR TARGETS OF SECONDARY METABOLITES

In order to deter, repel, or inhibit the diverse range of potential enemies, ranging from arthropods, and vertebrates to bacteria, fungi, and viruses, alkaloids must be able to interfere with important cellular and molecular targets. A short overview of these potential targets is given in Figures 1 and 2. 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, or signal transducers. As a consequence, the metabolism and function of cells, tissues, organs, and eventually the whole organism will be affected. Although we know the structures of many SM, our knowledge concerning their molecular mode(s) of action is largely fragmentary and incomplete. Such knowledge is, however, important in order to understand the functions of SM for the producing organism, and for the rational utilization of SM in medicine or plant protection. Whereas many SM interact with multiple targets and thus have unspecific broad activities, others are highly specific, and interact exclusively with a particular target. SM with broad activities interact mainly with proteins, biomembranes, and DNA/RNA (Table I).

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 which modulate the conformation of proteins and nucleic acids by non-covalent bonding. Covalent bonds can be formed with reactive functional groups of SM, such as

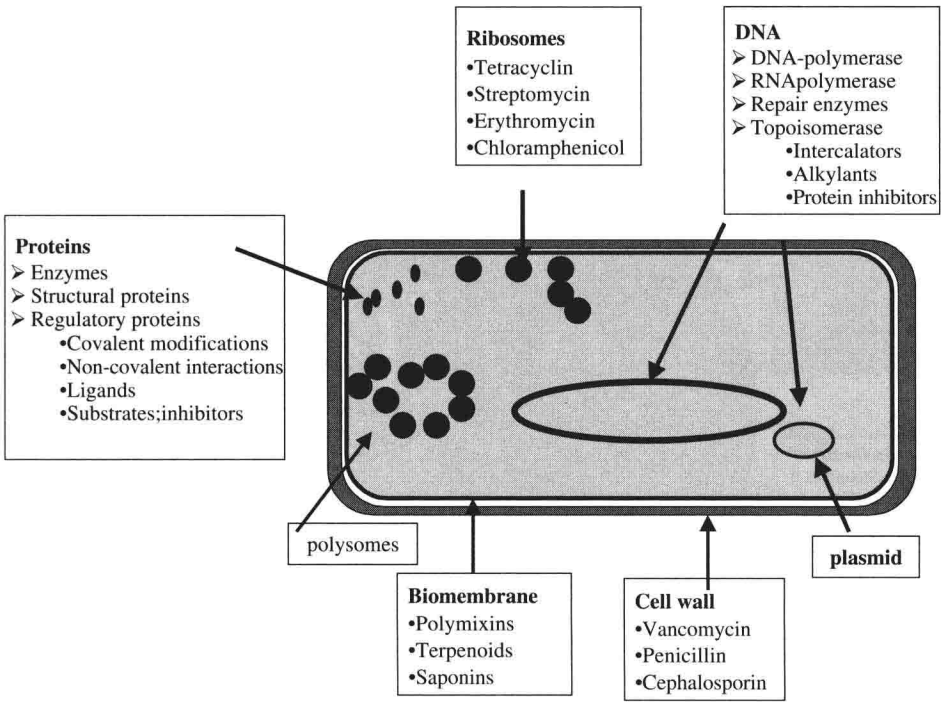

Figure 1 Molecular targets in bacteria that can be affected by natural products.

aldehydes, epoxides, SH groups, phenolic radicals, activated double bonds, and exocyclic or terminal methylene groups (Table I) (4,13,14).

Non-covalent bonds, especially hydrogen bonds, ionic bonds, hydrophobic interactions, and van der Waals forces are weak individually, but can be powerful if they work co-operatively together. For example, a tannin typically has several (more than 10) phenolic hydroxyl groups that can form hydrogen bonds with proteins and nucleic acids. Furthermore, these OH groups may dissociate under physiological conditions to form phenolate ions that can form ionic bonds with positively charged amino acid residues, such as those from lysine, arginine, and histidine. Phenolic hydroxyl groups are a common theme in many SM, mostly in phenylpropanoids, flavonoids, tannins, and polyketides, and they also occur in some alkaloids (especially in the isoquinoline and quinoline alkaloids). These OH groups are crucial for the biological activity of phenolics.

Nitrogen-containing compounds, such as alkaloids, amines, and peptides usually have positively charged *N*-atoms (under physiological conditions) in their molecules that can form ionic bonds with negatively charged amino acid residues of glutamic and aspartic acid in proteins. Both the covalent and non-covalent interactions will modulate the three-dimensional protein structure, i.e., the conformation that is so important for their bioactivities. A conformational change is usually associated with a loss or reduction in the activity of a protein,

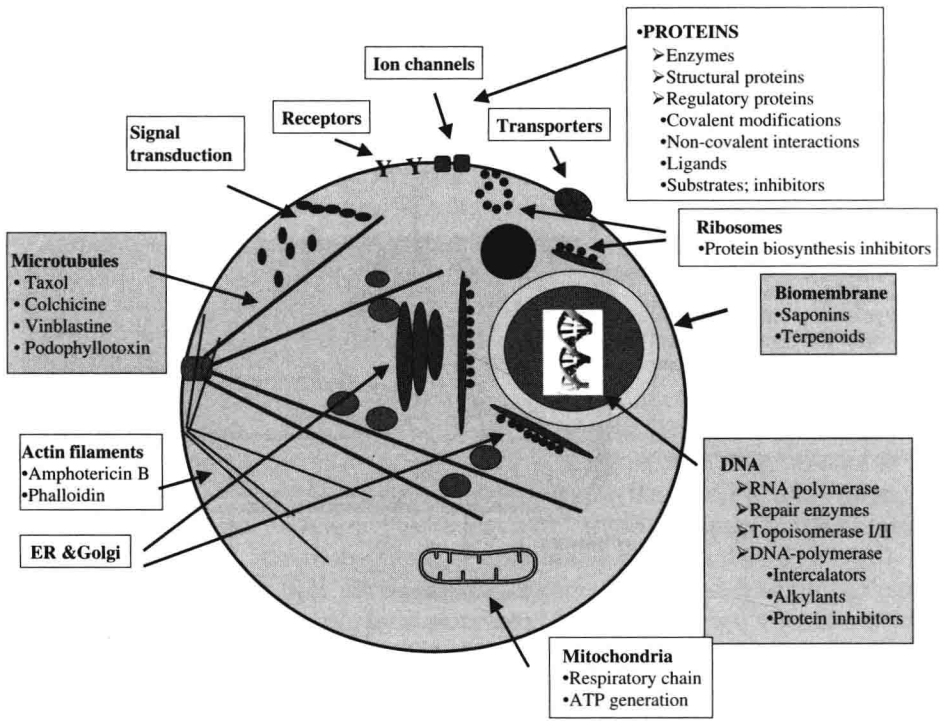

Figure 2 Molecular targets in animal and human cells that can be affected by natural products.

inhibiting enzyme, or receptor activity, or interference with the very important protein–protein interactions (4,13,14).

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 (4,13,14). 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 can also change the fluidity of biomembranes, thus reducing their function as a permeation barrier. Saponins can even make cells prone to leak, which 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) (4,13,14).

These pleiotropic, multitarget bioactivities are not specific, but are nevertheless effective, and this is what 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, a bacterium, fungus, or virus. These classes of compounds are seldom unique constituents; quite often plants produce a mixture of SM, often both phenolics and terpenoids, and thus exhibit both covalent and non-covalent interactions. These activities are probably both additive, and synergistic (13,14).

Table I Interactions of secondary metabolites with proteins and biomembranes

Mode of action	Class of secondary metabolite
Covalent modifications	
Reaction of aldehyde groups with amino groups	Iridoids, terpenoids with aldehyde groups
Reaction of isothiocyanate groups with amino and SH groups	Mustard oils, (isothiocyanates)
Reaction of α,β-unsaturated carbonyl groups with SH groups	Sesquiterpene lactones, phenylpropanoids, monoterpenes
Reaction of allylsulfides with SH groups	Allicin
Reaction of epoxides with proteins and DNA	Valepotriates, metabolically activated metabolites
Reaction with metal ions	Quinones, naphthoquinones
Non-covalent bonds	
Ionic bonds	Phenolics, tannins, bases, acids
Hydrogen bonds	Phenolics, tannins, anthraquinones
Van der Waals and hydrophobic interactions	Lipophilic compounds, such as terpenoids
Disturbance of membrane fluidity	
Hydrophobic/amphiphilic interactions	Saponins and other terpenoids

Pharmacologists clearly prefer SM that interact with a single target in a specific way (monotarget substances) because dose response and structure activity relationships can be much more easily determined than for non-specific, multitarget compounds. Many alkaloids fall in the class of specific modulators, and have been modified during evolution in such a way that they mimic endogenous ligands, hormones, or substrates. We have termed this selection process "evolutionary molecular modeling" (7,8,11,12). Many alkaloids are neurotoxins that were selected for defence against animals. These compounds have the advantage for the plants producing them that they are usually not toxic for the producing organism (as plants have no nerves). On the other hand, plants need special capacities to produce and store the non-specific multitarget SM (7). Table II lists the potential neuronal targets that can be affected by alkaloids. Extensive reviews on this topic have been published (8,10,11). Since neuronal signal transduction is a very critical target in animals, and its modulation usually leads to toxic effects, many alkaloids are indeed strong (even deadly) poisons, or have mind-altering and hallucinogenic properties.

III. CYTOTOXICITY OF ALKALOIDS

In this review, the emphasis is placed on the cytotoxic properties of alkaloids and their underlying modes of action. For other properties of alkaloids, see the

Table II Molecular targets of alkaloids in neuronal signal transduction (10–12,16)

Target	Selected alkaloids
Neuroreceptor	
Muscarinic acetylcholine receptor	Hyoscyamine and other tropane alkaloids; arecoline; berbamine and other isoquinoline alkaloids; sparteine and other quinolizidine alkaloids; cryptolepine; pilocarpine
Nicotinic acetylcholine receptors	Nicotine; boldine and other aporphine alkaloids; C-toxiferine; coniine and other piperidine alkaloids; cytisine and other quinolizidine alkaloids; epibatidine; tubocurarine
Adrenergic receptors	Berbamine, berberine, and other isoquinoline alkaloids; cinchonidine and other quinoline alkaloids; corynanthine, yohimbine, and other indole alkaloids; emetine; ephedrine; ergometrine and related ergot alkaloids
Dopamine receptor	Ergocornine and related ergot alkaloids; bulbocapnine and related aporphine alkaloids; anisocycline, stylopine, and related protoberberine alkaloids; salsolinol and related isoquinolines
GABA receptor	Bicuculline, cryptopine, hydrastine, and related isoquinoline alkaloids; securinine; harmaline and related beta-carboline alkaloids
Glycine receptor	Corymine, strychnine, and related indole alkaloids
Glutamate receptor	Histrionicotoxin and related piperidines; ibogaine and related indole alkaloids; nuciferine and related aporphine alkaloids
Serotonin receptor	Akuammine and related indole alkaloids; annonaine, boldine, and related aporphine alkaloids; berberine and related protoberberine alkalodis; ergotamine, LSD, and related ergot alkaloids; psilocybine, bufotenine, *N,N*-dimethyltryptamine, and related indoles; harmaline and related beta-carboline alkaloids; kokusagine and related furoquinoline alkaloids; mescaline; ibogaine and other monoterpene indole alkaloids
Adenosine receptor	Caffeine, theobromine, and other purine alkaloids
Opiate receptor	Morphine and related morphinan alkaloids; akuammine, mitragynine, ibogaine, and related indole alkaloids
Acetylcholine esterase	Galanthamine; physostigmine and related indole alkaloids; berberine and related protoberberine alkaloids; vasicinol and related quinazolines;

Table II. (*Continued*)

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

previous reviews (3,7,8,10,11). The body of information regarding the cytotoxic activities of SM and alkaloids is fast-growing since many compounds have been, and are presently, being screened in many academic and industrial laboratories for potential anticancer and antiviral activities. Data published during the last 12 years (i.e., after the 1993 review; 10) are so numerous (more than 1,000 entries in data bases) that a tabulation would be beyond the scope of this review. Rather, this review will highlight the molecular modes of action that underlie the cytotoxicity of alkaloids. Cytotoxic compounds are potentially interesting directly on their own or as lead compounds for the development of new anticancer drugs, for drugs against parasites, such as trypanosomes and *Plasmodium* sp. (12,15), and against viral infections.

IV. MOLECULAR MODES OF ACTION OF CYTOTOXIC ALKALOIDS

Cytotoxicity occurs as a result of the molecular interactions of an alkaloid with one or several important targets present in a cell (Figures 1 and 2). The main targets include DNA, RNA, and the associated enzymes and processes (i.e., replication, repair, transcription, DNA polymerase, RNA polymerase, reverse transcriptase, repair enzymes, topoisomerase, telomerase), protein biosynthesis, protein conformation, biomembranes, and membrane proteins (for reviews, see (10,11,16)).

Cell biologists distinguish between necrotic and apoptotic cell death. If a cell is lysed by saponins or other detergents, or when it is mechanically wounded or exposed to physical stress (heat, freezing, hypoxia) then it dies quickly by necrosis. Although macrophages have to rush in to clear away the debris, often an inflammation results. Apoptosis, or programmed cell death, was discovered about 35 years ago in 1972 by Kerr, Wyllie, and Currie (17), and is a central mechanism in the development of most organisms. The pathway functions naturally to generate shapes and to control the number of cells in various tissues.

Apoptosis is characterized by nuclear chromatin condensation, cytoplasmic shrinking, a dilated endoplasmic reticulum, membrane blebbing, and the formation of apoptotic bodies. Programmed cell death is clean, quick, and involves a predictable sequence of structural changes that cause a cell to shrink and to be rapidly digested by macrophages or neighboring cells.

Apoptosis can be induced by many substances, among them several natural products, such as alkaloids, that primarily interact with an important molecular target such as DNA or microtubules. Most of the anticancer drugs presently used in cancer therapy lead to apoptosis. The molecular targets associated with cell death and cytotoxicity are discussed in the next section.

A. Interactions with DNA, RNA, and Associated Enzymes

1 DNA Alkylation and Intercalation

DNA is a central target of all organisms, which can be affected by certain molecules (Figures 1 and 2). Some molecules can form covalent bonds with DNA bases, the so-called alkylating agents. Alkylating compounds often attach to the

*N*6 of guanine. Some anticancer chemotherapeutic agents act as alkylating agents, including cisplatin, nitrosourea derivatives, and nitrogen mustards. These compounds lead to cytotoxicity by alkylating guanine units in their *N*6-position, which eventually leads to strand breaks. Such modifications are removed by the repair enzyme, alkylguanine–DNA alkyl transferase (AGT). In this process, the alkyl residue is transferred to a cysteine in the active site of the enzyme (18). If these modifications are not repaired by the abundant and active repair enzymes of a cell, mutations arise. These mutations include nucleotide exchanges (transitions, transversions) and deletions. Deletions are especially harmful, as they result in frameshift mutations. The corresponding proteins (in coding genes) are then translated in the wrong frame and result in nonsense proteins. Therefore, frameshift mutations mostly lead to a loss of function. Several alkaloids with alkylating properties have been described, among them pyrrolizidine alkaloids, aristolochic acids, and cycasin are the most prominent cases (16) (Table III). These alkaloids are known to be mutagenic and teratogenic, and may induce cancer (10,11,16).

Intercalating compounds, that are planar and lipophilic, can insert between base pairs and thereby stabilize the double helix in a way that the replication and transcription process is disturbed. Intercalation usually leads to frameshift mutations. If these mutations occur in the heterochromatin, which does not code for genes, they are usually without much consequence. In protein coding genes, a nucleotide exchange in the third codon position (a so-called silent mutation) is also without much consequence because of the degenerated genetic code. However, a mutation which changes the amino acid sequence of a protein or which influences promoters and other regulatory sequences can have detrimental effects. These negative effects can include disregulation of metabolism, tumor growth, or even cell death.

Intercalating compounds also directly inhibit DNA replication and transcription. RNA is basically single stranded, but most RNA molecules have double-stranded stem structures because of complementary base pairing. These double-stranded regions can also be intercalated. Therefore, many intercalating SM are also inhibitors of DNA and RNA polymerases, of reverse transcriptase, and even of DNA topoisomerases and possibly telomerase.

Table III provides an overview of alkaloids that interfere with DNA, RNA, and associated proteins. Alkaloids with a planar and polycyclic structure are good candidates for DNA intercalation. Protonable ring nitrogens can stabilize the alkaloid–DNA complex by binding to the negatively charged DNA surface (19,20). Such properties are abundant in isoquinoline, quinoline, and indole alkaloids that are synthesized from the aromatic amino acids phenylalanine, tyrosine, and tryptophan (11,12,16).

2 DNA Topoisomerase I and II

DNA topoisomerase I and II play important roles in DNA replication and transcription. Their inhibition usually leads to cell-cycle arrest and cell death by apoptosis. During replication, DNA needs to be uncoiled. In order to avoid torsions and rotations, DNA is cleaved by DNA topoisomerase I, which forms