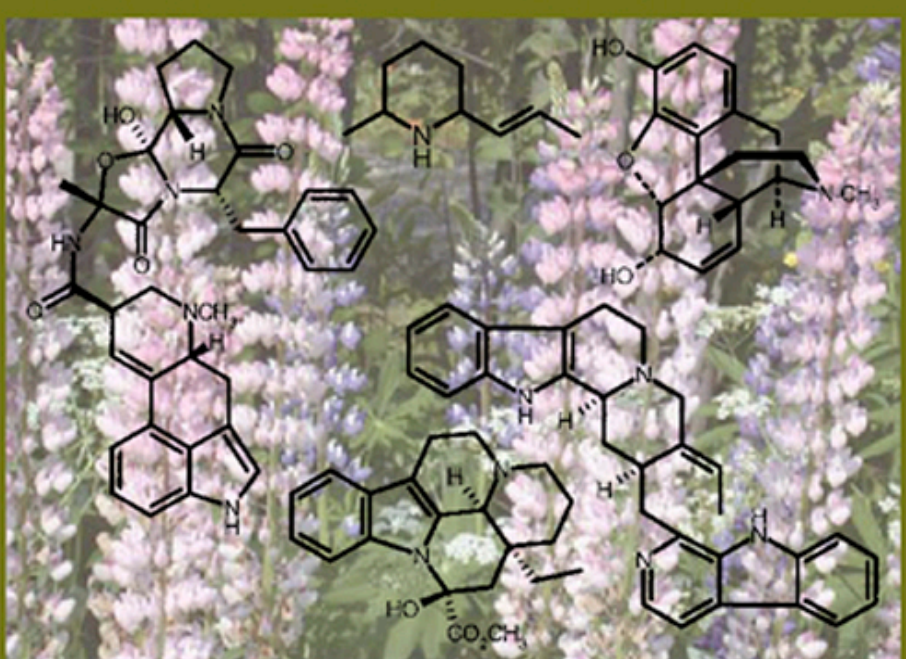
Alkaloids – Secrets of Life

Alkaloid Chemistry, Biological Significance, Applications and Ecological Role

Tadeusz Aniszewski



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ALKALOID CHEMISTRY, BIOLOGICAL SIGNIFICANCE, APPLICATIONS AND ECOLOGICAL ROLE

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Dedicated to my Family and Friends

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Preface

This book is intended to be a presentation of alkaloids from chemical, biological and ecological points of view. It is a text for chemists, biologists and ecologists alike. However, the intended audience of this work is not limited to scientists, teachers and other present and future specialists. In fact, I wrote this book because I felt the need for it as a university educator and as a scientific enthusiast on the subject. My purpose was to compose a beneficial text for an academic and professional audience that could also serve as a source of knowledge for anyone who is interested in the fascinating subject of alkaloids. As a subject, alkaloids represent a field of scientific investigation that attracts students and researchers from diverse academic disciplines and a large circle of professionals in clinical and university laboratories.

Alkaloids, the subject of this book, represent a group of very interesting and complex chemical compounds, produced by the secondary metabolism of living organisms in different biotopes. Alkaloids are relatively common chemicals in all kingdoms of living organisms in all environments. Two hundred years of scientific research has not yet fully explained the connections between alkaloids and life, nor has it explained why these diverse chemicals are produced and degraded by organisms, or why they have such a very large spectrum of biological activities. Alkaloids are the products of the life process, and their diversity is similar to the diversity of life on Earth. Therefore, they can be said to encapsulate the very secrets of life.

The literature on alkaloids is growing rapidly. Researchers are persistently attempting to decode the many secrets surrounding alkaloids. In June 2006, the Web of Science (WoS) database, produced by the Institute for Scientific Information (ISI), mentioned 11,066 research papers containing the keyword *alkaloid*. Each year hundreds of additional research papers are published on the subject. During a period of only 6 months (from January to June 2006), 302 papers were published in the scientific journals indexed by the ISI. Thus, the level of scientific research activity in connection to alkaloids is high internationally. Moreover, this activity is connected to the human aspiration and belief that drugs developed from alkaloids or by using natural models of these compounds could help in the search for future cures to serious diseases such as cancer or AIDS. Alkaloids also have the potential to improve human life and the economy through their applications in biotechnology, agriculture, food and research equipment

industries. The more that is known about alkaloids, the more possibilities are made available.

Alkaloids have been a direct or indirect subject of many books and academic works from various scientific fields. *Alkaloids – Secrets of Life* presents actual knowledge of alkaloids from an interdisciplinary point of view. Not only do I present the subject, but I also approach some unresearched areas and several questions that persist in this fascinating field of research. *Alkaloids – Secrets of Life* consists of five chapters, the first of which presents recent knowledge of alkaloid distribution among species and environments. The second chapter discusses alkaloid chemistry in biosynthesis, models and other methodological considerations and basic techniques used. Biological signification is presented in the light of recent research in Chapter 3, and concerning recent applications of alkaloids in Chapter 4. Finally, Chapter 5 outlines the ecological role of alkaloids through a case study. Each chapter features an abstract. The last portion of this book includes appendices, which include a listing of alkaloids, plants containing alkaloids and some basic protocols of alkaloid analysis.

I would like to thank Mervi Hannele Kupari, Aki Juhani Leinonen, Veli-Pekka Pennanen, Minna Marika Sinkkonen and Gaëlle Gabriel for their work in my laboratory. Pekka Piironen has participated actively in my research at the Botanical Garden of the University of Joensuu. Through their technical assistance, Kirsti Kyyrönen and Ilkka Konttinen aided me in the process of preparing several diagrams. My special thanks are also due to Kaisa Mustonen, who participated in the preparation of the chemical diagrams and indices featured in this book. Dr Peter Lawson, Adam Lerch, Kathryn Lessey and Dr Greg Watson have reviewed the language of the manuscript. While writing this book, I have drawn on research and study experiences from 30 years, covering many thousand hours in different laboratories and libraries. On 17 December 1993, it was my honour to participate in the ceremony of awarding the title of *Doctor Honoris Causa* to Professor Arnold Brossi, the eminent authority on the chemistry of alkaloids and the use of natural products in medicine and molecular biology. I would like to thank all the professors, teachers and scientists from whom I have had the pleasure to learn during these years. The International Summer School on Legumes, held in 1990s by the Department of Biology (presently the Faculty of Biosciences) of the University of Joensuu, was a forum that discussed alkaloids from many different points of view by experienced and young scientists from various countries and laboratories. I extend my sincere thanks to everyone for these fruitful years of study, cooperation and life.

Tadeusz Aniszewski
Midsummer white night
Juhannus Day, 24 June 2006

CHAPTER 1

Definition, Typology and Occurrence of Alkaloids

Docendo discimus.
Seneca

Abstract: Alkaloids are a group of molecules with a relatively large occurrence in nature around the Globe. They are very diverse chemicals and biomolecules, but they are all secondary compounds and they are derived from amino acids or from the transamination process. Alkaloids are classified according to the amino acids that provide their nitrogen atom and part of their skeleton. Similar alkaloids can have quite different biosynthetic pathways and different bioimpacts. Alkaloids are derived from L-lysine, L-ornithine, L-tyrosine, L-tryptophan, L-histidine, L-phenylalanine, nicotinic acid, anthranilic acid or acetate. The terpenoid, steroid and purine alkaloids are also important. Millions of people around the Globe use purine alkaloids every day whether starting the day with a cup of coffee or drinking a cup of tea in the afternoon. Alkaloids also occur in the animal kingdom. Differently from plants, the source of these molecules in an animal's body can be endogenous or exogenous. Alkaloids are molecules participating in both producer and consumer chains in nature. They are vital in feeding, and enjoy servations, agressivity and defence of the species. *Homo sapiens* is one of them.

Key words: alkaloid, alkaloid derivation, alkaloid occurrence, heterocycles, molecular precursors, protoalkaloids, pseudoalkaloids, true alkaloids

1. Definition

The definition of the term *alkaloid* is not a simple one, and is in many cases a source of academic controversy. Difficulties with the definition of such a group of secondary and natural molecules as alkaloids stem from similarities of alkaloids with other secondary compounds. Attempts to define the term "alkaloid" originated at the time of the discovery of these compounds. Friedrich Sertürner, an apothecary's assistant from Westphalia, first isolated morphine (Figure 1), one of the most important alkaloids in the applied sense[1]. This was in 1805, and proved a significant step forward in chemistry and pharmacology[2,3,4]. Using the method developed by Friedrich Sertürner, the pharmacists Pierre Joseph Pelletier and Joseph Benaimé Caventou isolated, from 1817 to 1821, a remarkable range of other alkaloids (Figure 2), such as brucine (a close relative of strychnine), febrifuge, quinine, caffeine and veratrine[1,5]. The term "alkaloid" was

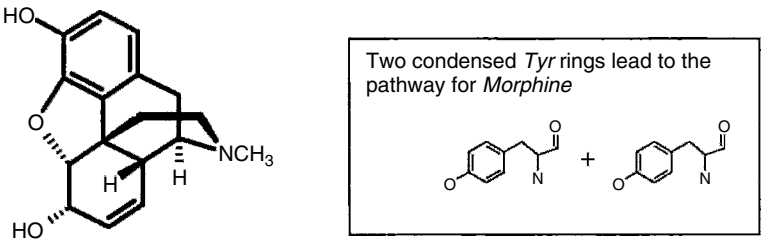


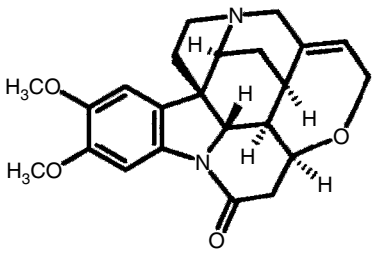
Figure 1. Contemporary scheme of morphine. Friedrich Sertürner, who first isolated this alkaloid in an impure form in 1805, did know that it was converted from the pathway of *tyrosine*, *Tyr*. The correct morphine structure was determined by Gulland and Robinson in 1923. Moreover, even 200 years after Sertürner's isolation, scientists are still discussing the synthesis of this alkaloid from a molecular point of view. This is a good example of the scientific evolution of knowledge of alkaloids.

first mentioned in 1819 by W. Meißner, an apothecary from Halle. He observed that these compounds appeared "like alkali", and so named them alkaloids[6].

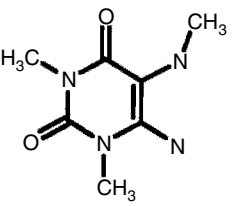
For the biologist, the alkaloid is a pure and perfect natural product. From the biological point of view, the alkaloid is any biologically active and heterocyclic chemical compound which contains nitrogen and may some pharmacological activity and, in many cases, medicinal or ecological use[7]. This definition, as a relatively wide one based on application, can be criticized as inexact. However, it presents a general picture of what kinds of compound are under consideration. The biological and chemical nature of this group of compounds leads to the conclusion that each definition of alkaloids is either too broad or too narrow. A short exact definition is not possible without a long list of exceptions[8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24]. Sometimes, to avoid presenting this list of exceptions, the basic characteristics of alkaloids are given in the definition. Winterstein and Tier[8] stressed that these compounds had such characteristics as (1) greater or lesser toxicity, which acts primarily on the central nervous system (CNS), (2) the basic character of a chemical construction, (3) heterocyclic nitrogen as an ingredient, (4) a synthesis from amino acids or their immediate derivatives and (5) a limited distribution in nature.

In another definition, Waller and Nowacki[16] mentioned many characteristics of alkaloids. They especially drew attention to the fact that alkaloids have nitrogen in the molecule and are connected to at least two carbon atoms. Moreover, this compound has at least one ring in the molecule, and its ring is not necessarily heterocyclic. The authors also stated that alkaloids could not be structural units of macromolecular cellular substances, vitamins or hormones. More recently, Sengbush[25] simply stressed that alkaloids are a group of nitrogen-containing bases and that most of them are drugs.

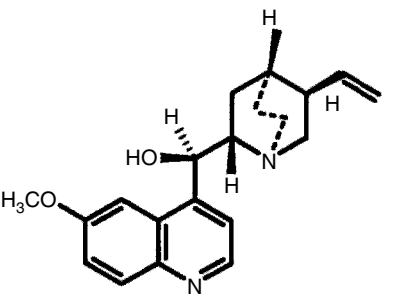
The most important points for the biologist are that alkaloids are a special group of chemicals that are active at different cellular levels of organisms, and



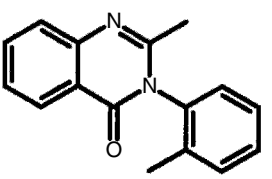
Brucine



Caffeine



Quinine



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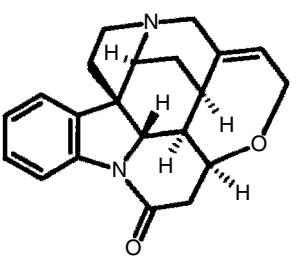


Strychnine

Figure 2. Some alkaloids isolated by pharmaceutists Pierre Joseph Pelletier and Joseph Beinamé Caventou during 1817–1821. They did not know the exact structures. Their compounds thus isolated are combinations of alkaloids rather than one pure alkaloid.

that they take part in the biological processes of plants, animals and microorganisms.

For the medical scientist, the term "alkaloids" means any group of nitrogenous substances of vegetable origin, often of complex structure and high molecular mass[26]. Moreover, it is important that alkaloids are often heterocycles, and may have primary, secondary or tertiary bases, or may contain quaternary ammonium groups. Certainly, the fact that alkaloids are only slightly soluble in water but soluble in ethanol, benzene, ether and chloroform is also extremely important, and highlighted in the medical definition. This long definition also notes that alkaloids exhibit some general characteristics which are revealed by the

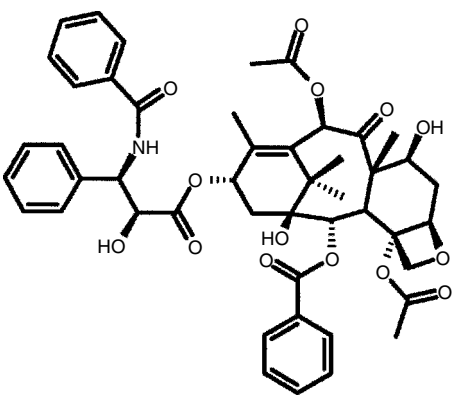
coloration or precipitation of alkaloid reagents. Finally, medicine draws attention to the fact that alkaloids create intense physiological action, and they are widely used in the medical fields as curative drugs. Some alkaloids can also be highly toxic, even in very small doses[26]. In the database of the National Library of Medicine it is possible to find the definition of alkaloids, according to which these compounds are nitrogenous bases and occur in animal and vegetable kingdoms, while some of them have been synthesized[27]. Another electronic database also provides a definition of alkaloids, stating that an alkaloid is a nitrogenous organic compound which has pharmacological effects on humans and other animals, and whose name is derived from the world alkaline[28]. As can be seen, the definition of alkaloids in the field of medicine also offers parameters of "may be", "often", "slightly" and "highly", which are not exact. This is typical of the scientific and practical fields, where alkaloids are well known and used in the bettering of human health, but where the term remains relatively difficult to define exactly and concisely.

Chemistry has provided a definition of alkaloids in purely chemical terms. Chemists stress that alkaloids are any group of complex heterocyclic nitrogen compounds, which have strong physiological activity, are often toxic, and retain their own basic chemical properties. It is also stated that there are a few exceptions to this definition[29]. In another chemical definition, it is stated only that alkaloids are nitrogen-containing compounds derived from plants and animals[30]. Later, chemists stressed that alkaloids were biogenic, nitrogen-containing and mostly *N*-heterocyclic compounds. In this definition it is also stated that amino acids, peptides, nucleosides, amino sugars and antibiotics are not considered as to be alkaloids[31].

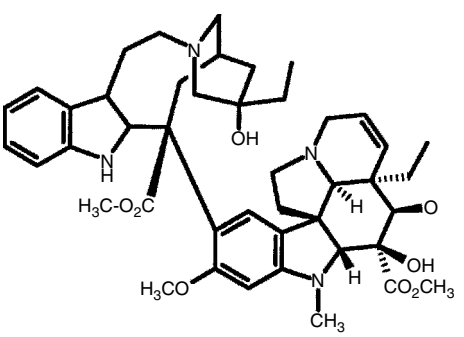
Inspite of differences between the research fields of biology, medicine and chemistry, and the fact that there remain some differences of accentuation in alkaloid definitions, such definitions are very similar, indeed almost identical. Scientists are recognizing the vital importance of these products for biology, medicine and chemistry. What has been learnt about alkaloids from the last 200 years of studies? It is fascinating that alkaloids are just a product of nature, and a very small unit of global nature both in the material sense and in processes as they occur. They are just a product of living cells, for other living cells. The alkaloid is a product of chemical molecules for the production of other molecules. It is synthesized, playing its own role in the metabolism after that. The alkaloid represents perfection in much the same way as perfection appears in life and nature. This is the reason why alkaloids were and are a fascinating subject of study. This is also the reason why definitions of these groups of molecules, provided by scientists of biology, medicine and chemistry, are acceptably imperfect. However, alkaloids are recognized as a large group of compounds with biological, pharmacological or physiological and chemical activity. Without alkaloids, stupendous achievements in the battle against malaria, leukaemia and cancer as well as Parkinson disease would be not possible. The pharmaceutical drug industry

has succeeded in the use of natural plant alkaloids for the development of anti-malarian agents (quinine and chloroquinine), anticancer agents (taxol, vinblastine and vincristine) and agents promoting blood circulation in the brain (vincamine) (Figure 3). Many alkaloids can influence an animal's nervous system, providing possible changes in the functionality of the organism. The activity of alkaloid molecules on a psychomental level (opium latex, papaverine, morphine, cocaine) is one of natural phenomena in the process of species self-protection, and the interactions between producers (plants) and consumers (herbivores). It is also a good example of natural selection mechanisms and results. Nowadays, there are more than 8000 natural compounds and their derivatives recognized as alkaloids. Each year, scientists around the Globe discover at least 100 new molecules. They frequently occur as acid salts, but some also occur in combination with sugars whereas, others appear as amides or esters. Alkaloids can also be quaternary salts or tertiary amine oxides[23].

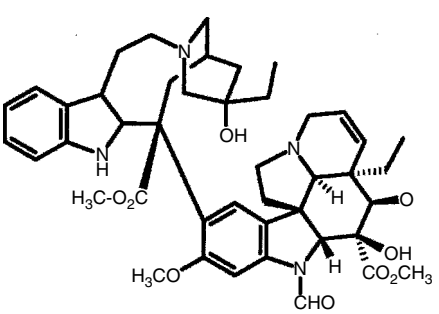
Alkaloids can be classified in the terms of their (1) biological and ecological activity; (2) chemical structures and (3) biosynthetic pathway. From the point of



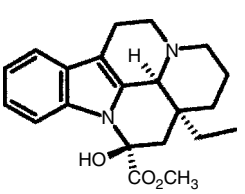
Taxol



Vinblastine



Vincristine



Vincamine

Figure 3. Schemes of taxol, vinblastine, vincristine and vincamine.

view of biological activity[23], it is possible to divide alkaloids into (1) neutral or weakly basic molecules (e.g., lactams such as ricinine, certain N-oxides such as indicine), (2) animal-derived alkaloids (e.g., anuran, mammalian and arthropod alkaloids), (3) marine alkaloids, (4) moss alkaloids, (5) fungal and bacterial alkaloids and (6) non-natural alkaloids (structurally modified or analogues).

Nowadays, the group of compounds mentioned as non-natural alkaloids is growing especially rapidly as a result of bio-organic and stereochemistry research. Pharmacological research and the drug industry rapidly advance and promote the most promising new molecules for possible production applications. This is necessary since the sources of infections (micro-organisms) are constantly changing their species and infection ability, becoming resistant to medicines and antibiotics.

Alkaloids are generally classified by their common molecular precursors, based on the biological pathway used to construct the molecule. From a structural point of view, alkaloids are divided according to their shapes and origins. There are three main types of alkaloids: (1) true alkaloids, (2) protoalkaloids and (3) pseudoalkaloids. True alkaloids and protoalkaloids are derived from amino acids, whereas pseudoalkaloids are not derived from these compounds (Table 1).

1.1. True alkaloids

True alkaloids derive from amino acid and they share a heterocyclic ring with nitrogen. These alkaloids are highly reactive substances with biological activity even in low doses. All true alkaloids have a bitter taste and appear as a white solid, with the exception of nicotine which has a brown liquid. True alkaloids form water-soluble salts. Moreover, most of them are well-defined crystalline substances which unite with acids to form salts. True alkaloids may occur in plants (1) in the free state, (2) as salts and (3) as N-oxides. These alkaloids occur in a limited number of species and families, and are those compounds in which decarboxylated amino acids are condensed with a non-nitrogenous structural moiety. The primary precursors of true alkaloids are such amino acids as L-ornithine, L-lysine, L-phenylalanine/L-tyrosine, L-tryptophan and L-histidine[23,32]. Examples of true alkaloids include such biologically active alkaloids as cocaine, quinine, dopamine, morphine and usambarensine (Figure 4). A fuller list of examples appears in Table 1.

1.2. Protoalkaloids

Protoalkaloids are compounds, in which the N atom derived from an amino acid is not a part of the heterocyclic[31]. Such kinds of alkaloid include compounds derived from L-tyrosine and L-tryptophan (see Table 1). Protoalkaloids are those

Table 1 Main types of alkaloids and their chemical groups

Alkaloid Type	Precursor Compound	Chemical Group of Alkaloids	Parent Compounds	Examples of Alkaloids
True alkaloids	L-ornithine	Pyrrolidine alkaloids	Pyrrolidine	Cuscohygrine Hygrine
		Tropane alkaloids	Tropane	Atropine Cocaine Hyoscyamine Scopolamine/ hyoscine
		Pyrrolizidine alkaloids	Pyrrolizidine	Acetyl- lycopsamine Acetyl-intermedine Europine Homospermidine Ilamine Indicine-*N*-oxide Meteloidine Retronecine
	L-lysine	Piperidine alkaloids	Piperidine	Anaferine Lobelanine Lobeline *N*-methyl pelletierine Pelletierine Piperidine Piperine Pseudopelletierine Sedamine
		Quinolizidine alkaloids	Quinolizidine	Cytisine Lupanine Sparteine
		Indolizidine alkaloids	Indolizidine	Castanospermine Swansonine
	L-tyrosine	Phenylethyl- aminoalkaloids	Phenylethyl amine	Adrenaline Anhalamine Dopamine Noradrealine Tyramine
		Simple tetrahydroiso- quinoline alkaloids	Benzyltetra- hydro-iso- quinoline	Codeine Morphine Norcoclaurine Papaverine Tetrandrine Thebaine Tubocurarine

(*continued*)

Table 1 (Continued)

Alkaloid Type	Precursor Compound	Chemical Group of Alkaloids	Parent Compounds	Examples of Alkaloids
	L-tyrosine or L-phenylanine	Phenethylisoquinoline alkaloids	Amaryllidaceae alkaloids	Autumnaline Crinine Floramultine Galanthamine Galanthine Haemanthamine Lycorine Lycorenine Maritidine Oxomaritidine Vittatine
	L-tryptophan	Indole alkaloids	Indole	
			Simple indole alkaloids	Arundacine Arundamine Psilocin Serotonin Tryptamine Zolmitriptan
			Simple β-carboline alkaloids	Elaeagnine Harmine
			Terpenoid indole alkaloids	Ajmalicine Catharanthine Secologanin Tabersonine
		Quinoline alkaloids	Quinoline	Chloroquinine Cinchonidine Quinine Quinidine
		Pyrroloindole alkaloids	Indole	A-yohimbine Chimonantheine Chimonantheine Corynantheine Corynantheidine Dihydrocoryn-antheine Corynanthine
		Ergot alkaloids		Ergobine Ergotamine Ergocryptine
	L-histidine	Imidazole alkaloids	Imidazole	Histamine Pilocarpine Pilosine

Table 1 (Continued)

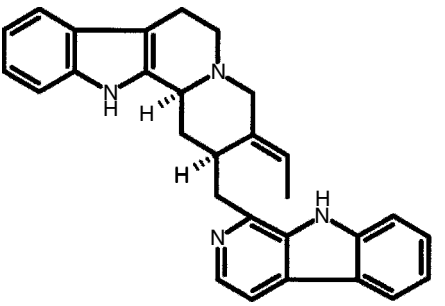
Alkaloid Type	Precursor Compound	Chemical Group of Alkaloids	Parent Compounds	Examples of Alkaloids
		Manzamine alkaloids	Xestomanz-amine	Xestomanz-amine A Xestomanz-amine B
	L-arginine	Marine alkaloids	β-carboline	Saxitoxin Tetrodotoxin
	Anthranilic acid	Quinazoline alkaloids	Quinazoline	Peganine
		Quinoline alkaloids	Quinoline	Acetylfolidine Acutine Bucharine Dictamnine Dubunidine γ-fagarine Flindersine Foliosidine Glycoperine Haplophyllidine Haplopine Helietidine Kokusaginine Maculosine Perfamine Perforine Polifidine Skimmianine
		Acridone alkaloids	Acridine	Acronycine Rutacridone
	Nicotinic acid	Pyridine alkaloids	Pyridine/ Pyrrolidine	Anabasine Cassinine Celapanin Evoline Evonoline Evorine Maymyrsine Nicotine Regelidine Wilforine
Protoalkaloids	L-tyrosine	Phenylethylamino-alkaloids	Phenylethyl-amine	Hordenine Mescaline
	L-tryptophan	Terpenoid indole alkaloids	Indole	Yohimbine

(*continued*)

Table 1 (Continued)

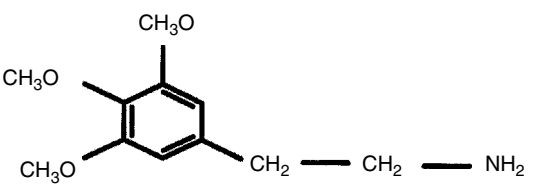
Alkaloid Type	Precursor Compound	Chemical Group of Alkaloids	Parent Compounds	Examples of Alkaloids
	L-ornithine	Pyrrolizidine alkaloids	Pyrrolizidine	4-hydroxy-stachydrine Stachydrine
Pseudoalkaloids	Acetate	Piperidine alkaloids	Piperidine	Coniine Coniceine Pinidine
		Sesquiterpene alkaloids	Sesquiterpene	Cassinine Celapanin Evonine Evonoline Evorine Maymyrsine Regelidine Wilforine
	Pyruvic acid	Ephedra alkaloids	Phenyl C	Cathine Cathinone Ephedrine Norephedrine
	Ferulic acid	Aromatic alkaloids	Phenyl	Capsaicin
	Geraniol	Terpenoid alkaloids	Terpenoid	Aconitine Actinidine Atisine Gentianine β-skytanthine
	Saponins	Steroid alkaloids		Cholestane Conessine Cyclopamine Jervine Pregnenolone Protoveratrine A Protoveratrine B Solanidine Solasodine Squalamine Tomatidine
	Adenine/ Guanine	Purine alkaloids	Purine	Caffeine Theobromine Theophylline

Sources: Refs [7, 23, 28, 31, 32, 33, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58].



Usambarensine

Figure 4. An example of a true alkaloid. L-tyrosine-derived alkaloid usambarensine has strong anti-malarial potential. Usambarensine was extracted from the root bark of African *Strychnos usambarensis*, a small tree in East and South Africa, and a small bush in West Africa.



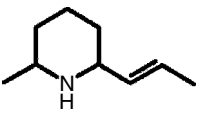
Mescaline

Figure 5. An example of protoalkaloids. Mescaline is the alkaloid derived from L-tyrosine and extracted from the Peyote cactus (*Lophophora williamsii*) belonging to the Cactus family (Cactaceae). Mescaline has strong psychoactive and hallucinogenic properties. Peyote cactus grows in the desert areas of northern Mexico and the southern parts of the USA. This plant was used in Pre-Columbian America in the shamanic practice of local tribes.

with a closed ring, being perfect but structurally simple alkaloids. They form a minority of all alkaloids. Hordenine, mescaline (Figure 5) and yohimbine are good examples of these kinds of alkaloid. Chini et al.[33] have found new alkaloids, stachydrine and 4-hydroxystachydrine, derived from *Boscia angustifolia*, a plant belonging to the Capparidacea family. These alkaloids have a pyrroline nucleus and are basic alkaloids in the genus *Boscia*. The species from this genus have been used in folk medicine in East and South Africa. *Boscia angustifolia* is used for the treatment of mental illness, and occasionally to combat pain and neuralgia.

1.3. Pseudoalkaloids

Pseudoalkaloids are compounds, the basic carbon skeletons of which are not derived from amino acids[31]. In reality, pseudoalkaloids are connected with amino



Pinidine

Figure 6. An example of a pseudoalkaloid. Acetate-derived alkaloid pinidine is extracted from the *Pinus* species, for example, from *Pinus penderosa*. (Photo: T. Aniszewski). Pinidine has antimicrobial activity.

acid pathways. They are derived from the precursors or postcursors (derivatives the indegradation process) of amino acids. They can also result from the amination and transamination reactions[32] of the different pathways connected with precursors or postcursors of amino acids.

These alkaloids can also be derived from non-aminoacid precursors. The N atom is inserted into the molecule at a relatively late stage, for example, in the case of steroidal or terpenoid skeletons. Certainly, the N atom can also be donated by an amino acid source across a transamination reaction, if there is a suitable aldehyde or ketone. Pseudoalkaloids can be acetate and phenylalanine-derived or terpenoid, as well as steroidal alkaloids. Examples of pseudoalkaloids include such compounds as coniine, capsaicin, ephedrine, solanidine, caffeine, theobromine and pinidine (Figure 6). More examples appear in Table 1.

2. Occurrence in nature

Alkaloids are substances very well known for their biological activity at the beginning of world civilization. They were used in shamanism, in traditional herbal medicine for the cure of diseases and in weapons as toxins during tribal wars and during hunting. They also had, and still have, socio-cultural and personal significance in ethnobotany[34]. Moreover, they have been and continue to be the object of human interest concerning new possibilities for their safe utilization and ensuing health benefits. Of all secondary compounds, historically and

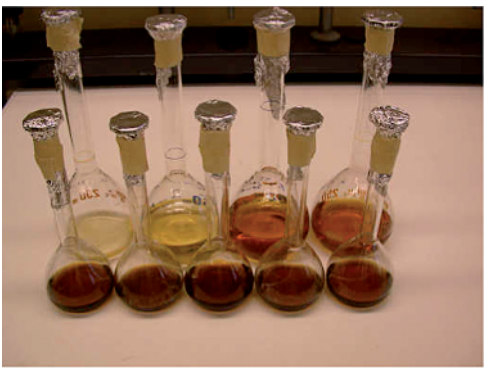


Figure 7. The raw extraction of quinolizidine alkaloids from different lupine species in the Research and Teaching Laboratory of Applied Botany of the University of Joensuu (Photo: T. Aniszewski). Observe the different colours of the raw extracts, which signifies different concentrations of alkaloids in different species.

contemporaneously, only alkaloids are molecules of natural origin with highly important benefits and diagnostic uses. They can be characterized as the most useful and also the most dangerous products of nature. They can be extracted and purified (Figure 7).

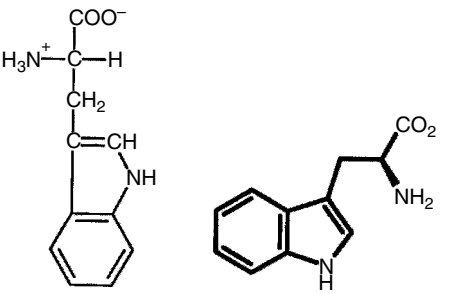
Alkaloids are most abundant in higher plants. At least 25% of higher plants contain these molecules. In effect this means that on average, at least one in fourth plants contains some alkaloids. In reality, it is not impossible that alkaloids occur more commonly. Using the latest equipment and technology, such slight traces of alkaloids may be detected (e.g., less than 10 gigagrams per kg of plant mass) that these have no real influence on biological receptors and activity. Generally these species are not considered as alkaloid species. Hegnauer[12,13] has defined alkaloid plants as those species which contain more than 0.01% of alkaloids. This is right from the point of view of the classification. From the genetic point of view, and the genetic mechanism of alkaloid synthesis, it is a real limitation. Paying attention to slight traces of alkaloids in plants, we see the members of the plant family which are relatives. They have a genetically determined alkaloid mechanism with a species expression. Moreover, this expression is also on the hybrid level[59].

2.1. The Dogbane botanical family (Apocynaceae)

Some plant families are especially rich in alkaloids. The Dogbane botanical family (Apocynaceae Lindl., Juss.) is a good example (Table 2). This family is distributed worldwide, especially in tropical and sub-tropical areas. The Dogbane family is a large botanical taxa containing at least 150 genera and 1700 species. Alkaloids are especially abundant in the following

Table 2 General botanical characteristics of the Dogbane family[312,313,315,316]

Botanical Forms and Parts	Characteristics
Botanical forms	Trees Shrubs Lianas Herbs Vines Sometimes succulents or cactus-like
Some typical genera	*Alstonia* *Amsonia* *Angadenia* *Apocynum* *Asclepias* *Catharanthus* *Ceropegia* *Cynanchum* *Echites* *Gonolobus* *Hoya* *Macrosiphonia* *Mandevilla* *Matelea* *Morrenia* *Pentalinon* *Rhabdadenia* *Rauvolfia* *Secamone* *Sarcostemma* *Skythantus* *Strophanthus* *Tabernaemontana* *Vallesia* *Voacanga*
Special characteristics	Milky juice or latex, hairs
Leaves	Opposite or verticillate with reduced stipules Pinnateveined
Flowers	Regular, radial Calyx with 5 sepals Tubular corolla Pollen grains usually tricolporate (dicolporate rarely) 2 carpels
Fruits	Ovary Follicles Sometimes berry-like or drupe-like
Seeds	Compressed with tufts of long hairs Albumen



L-tryptophan

Figure 8. L-tryptophan with its aromatic side chain is a precursor of indole, terpenoid indole, quinoline, pyrroloindole and ergot alkaloids.

genera: devil's-pepper (*Rauvolfia* L.), periwinkle (*Catharanthus* G. Don), milkwood (*Tabernaemontana* L.), strophanthus (*Strophanthus* DC.), voacanga (*Voacanga* U.) and alstonia (*Alstonia* R. Br.). The species belonging to these genera contain L-tryptophan-derived alkaloids (Figure 8). Indian snake-root *(Rauvolfia serpentina*) (Figure 9) contains reserpine and rescinnamine, the quinine tree (*Rauwolfia capra)* yields quinine, and iboga milkwood (*Tabernaemontana iboga)* produces iboganine. Deserpine has been isolated from the roots of *Rauwolfia canescens*[60]. This alkaloid differs from reserpine only by absence of a metoxy group but shows an interesting profile of biological activity. It has been employed in clinical practice for the treatment of hypertension and

Figure 9. The devil's-pepper genus contains L-tryptophan-derived alkaloids. *Rauwolfia serpentina* appears on flowers (Photo: T. Aniszewski).

as a tranquilizer and also as a controller of other cardiac disorders. Deserpine is a compound with limited availability from natural sources.

According to Varchi et al.[60] reserpine usually occurs at about 0.10–0.16% of natural extracts and deserpine only at 0.04%. Furthermore, five new indole alkaloids (N_b-methylajmaline, N_b-methylisoajmaline, 3-hydroxysarpagine, yohimbic acid and isorauhimbic acid) were isolated from the dried roots of *Rauwolfia serpentina*[61]. Srivastava et al.[62] reported on alkaloids isolated from heynana milkwood (*Tabernaemontana heyneana* Wall.). They discovered ervatine, tabersonine, coronaridine, heyneanine, voacristine, voacristine hydroxyindolenine, hydroxyibogamine and coronaridine hydroxyindolenine. These alkaloids show both bioimpact and uterotrophic activity. Moreover, Heijden et al.[63] have described the isolation of indole alkaloids from *Tabernaemontana elegans*, a species which occurs in southern part of Africa and is used in traditional medicine in Zimbabwe, Mozambique and Southern Africa. These alkaloids are apparicine, 16-*S*-hydroxy-16, 22-dihydroapparicine, tubotaiwine, vobasine, vobasinol, tabernaemontaninol, tabernaemontanine, isovoacangine, dregamine, dregaminol, dregaminol-methylether, 3-*R*/*S*-hydroxytabernaelegantine B, 3-methoxy-tabernaelegantine C, 3-*R*/*S*-hydroxy-conodurine, tabernaelegantine A, B, C, and D[63]. Alstonia plants produce menilamine, which is known as a new anti-malarial alkaloid isolated from alstonia trees growing in the Philippines, where this plant is common[64]. These plants are known as prospective medicinal plants and they are well distributed throughout tropical America, India and Malaysia as evergreen trees and shrubs. Many prospective liana plants from this family grow particularly in Amazonian America, tropical Africa and Madagascar. From *Alstonia macrophylla* Wall. Ex G. Don growing in Thailand, talcarpine, pleiocarpamine, alstoumerine, 20-Epiantirhine, alstonerine, alstophylline, macralstonine, villalstonine, alstomacroline and macrocarpamine were isolated[65]. All these alkaloids display strong bioactivity and are considered to be of potential use in medicine. Moreover, two other Thai *Alstonia* species, *Alstonia glaucescens* and *Alstonia scholaris* were also found to be indentical or similar to alkaloids such as *O*-methylmacralstonine[64,65]. It should be noted that more than 180 biologically active alkaloids have been isolated from the genus *Alstonia*. This makes this genus one of the most important in terms of potential alkaloid use. The *Alstonia*, Devil's pepper and Milkwood genera are endemic only in Asia and Australia, but they are distributed around the Globe in the tropics and subtropics. Ajmalicine, catharanthine, leurosine, vindoline, vindolinine, vinblastine, vincristine, vindesine and alioline are present in the periwinkle (e.g., *Catharanthus roseus and Vinca* spp.). From the leaves of *Vinca difformis* Pourr, vincamajine, vincamedine, vincadifformine, akuammidine, vellosimine, vincadiffine, difforlemenine, difforine and normacusine have been isolated[66]. From *Aspidosperma megalocarpon* Müll. Arg., growing in Colombia, three alkaloids were extracted – fendlerine, aspidoalbine and aspidolimidine[67]. All display bioactivity and the potential for applications

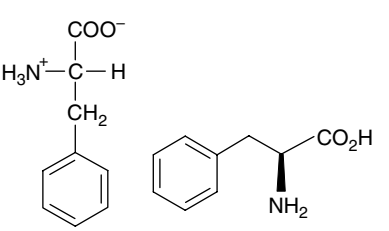
in medicine. Jokela and Lounasmaa[68] have presented ^{1}H and ^{13}C-NMR exact spectral data for seven types of ajmaline-type alkaloids from various species of the Dogbane family. These alkaloids are as follows: ajmaline, 17-*O*-acetyl-ajmaline, isoajmaline, isosandwichine, rauflorine, vincamajine and vincamedine. Eleven indole alkaloids were isolated from the stem bark of *Kopsia hainanensis* Tsiang, which is one of for species of *Kopsia*, endemic in China[69]. They are (−)-kopsinine, (−)-kopsinnic acid, (−)-kopsinoline, kopsinilam, kopsanome, (+)-5,22-dioxokopsane, eburnamenine, (+)-eburnamine, (−)-isoeburnamine, (+)-tubotaiwine and (+)-kopsoffine. *Kopsia officinalis* Tsiang seems to be very similar with respect to alkaloid content. In both species (−)-kopsinine is the principal alkaloids[69]. Moreover, in the Dogbane plant family are also phenylalanine-derived alkaloids, such as β-skytanthine in the *Skythantus* species (Figure 10, Table 2 and 10). All alkaloids from the Dogbane family have a strong biological and medicinal effect. Many of them are used in cancer chemotherapy.

2.2. The Aster botanical family (Asteraceae)

The Aster (syn. Daisy) botanical family (Asteraceae Dum.) is very large, containing over 900 genera and more than 20 000 species (Table 3).

Their distribution is worldwide, and species belonging to this family are found everywhere. The Aster plant family contains species yielded in similar ways to some natural alkaloids.

The genus Ragwort (*Senecio* L.) is especially rich in L-ornithine (Figure 11) derived alkaloids (senecionine, senecivernine, seneciphylline, spartioidine, intergerrimine, jacobine, jacozine, sekirkine, jacoline, dehydrosenkirkine, erucifoline, jaconine, adonifoline, neosenkirkine, dehydrojaconine, usaramine, otosenine, eruciflorine, acetylerucifoline, sennecicannabine, deacetyldoronine, florosenine, floridamine, doronine)[70] and the genus Knapweed (*Centaurea* L.) in alkaloids derived from L-tryptophan, for example afzelin and apigenin.

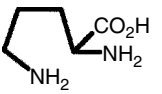


L-phenylalanine

Figure 10. L-phenylalanine is a precursor of alkaloids in *Skythantus* species belonging to the Dogbane plant family.

Table 3 General botanical characteristics of the Aster family[312,313,316,317,318]

Botanical Forms and Parts	Characteristics
Botanical form	Herbs Shrubs Trees (rarely)
Some typical genera	*Ambrosia* *Antennaria* *Artemisia* *Aster* *Baccharis* *Bidens* *Centaurea* *Chrysothamnus* *Cirsium* *Coreopsis* *Cousinia* *Elephanthopus* *Erigeron* *Eupatorium* *Gallardia* *Gamochaeta* *Gnaphalium* *Haplopappus* *Helianthus* *Helichrysum* *Hieracium* *Jurinea* *Liatris* *Mikania* *Rudbeckia* *Sussurea* *Senecio* *Solidago* *Verbensia* *Vernonia*
Special characteristics	Milky juice, hairs
Leaves	Alternate, opposite or whorled exstipulate
Flowers	Regular or irregular Bisexual or unisexual Sometimes sterile calyx reduced Corolla tubular or flattened
Fruits	Achene Pappus
Seeds	Exalbuminous



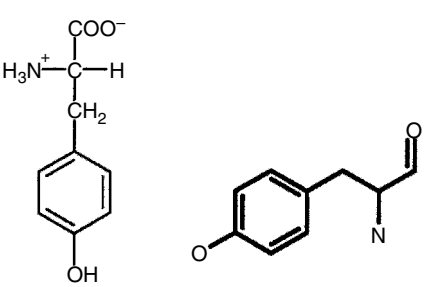
L-ornithine

Figure 11. L-ornithine is an important precursor of pyrrolidine, tropane and pyrrolizidine alkaloids.

Alkaloid-containing species are distributed worldwide throughout the temperate areas. The Ragwort genus is endemic to Mediterranean and West Asian regions. From *Senecio triangularis*, other alkaloids were extracted. They are 9-*O*-acetyl-7-*O*-angelyl-retronecine, 7-*O*-angelyl-, 9-*O*-angelyl-, and 7-*O*-angelyl-9-*O*-sarracinylretronecine. *Senecio pseudaureus* and *Senecio streptanthifolios* yield only retrorsine and senecionine[71]. However, a phytochemical investigation of *Senecio divarigata* L. (syn. *Gynura divaricata* DC.) has shown such alkaloids as intergerrimine and usaramine[72]. In Switzerland, the alkaloids of *Petasites hybridus*, found growing in many different places, have been studied[73]. Petasin, senecionine and intergerrimine were detected. Cheng and Röder[74] have been isolated two pyrrolizidine alkaloids (senkirkine and doronine) from *Emilia sonchifolia*.

2.3. The Logan botanical family (Loganiaceae)

The Logan plant family (Loganiaceae Lindl.) is abundant in species containing L-tyrosine (Figure 12) derived alkaloids (Table 4). Thirty genera and more than 500 species belong to this family although new systematic research has proposed that Loganiaceae should be divided into several families[319]. The Logan plant genus (*Strychnos* L.) is especially rich in many of alkaloids such as strychnine,



L-tyrosine

Figure 12. L-tyrosine, with its aromatic side chain, is a precursor of phenylethylamino- and isoquinoline alkaloids.

Table 4 General botanical characteristics of the Logan family[312,313,316,319]

Botanical Forms and Parts	Characteristics
Botanical forms	Herbs Shrubs Trees
Some typical genera	*Logania* *Mitreola* *Mitrasacme* *Strychnos* *Spigelia*
Leaves	Opposite Simple
Flowers	Regular in cymes or panicles Calyx Corolla 2 carpels
Fruits	Capsule Rarely a berry-like or a drupe
Seeds	Albuminous Sometimes winged

brucine and curare. From the genus *Strychnos* L., which contains 190 species, more than 300 different alkaloids have been isolated. This genus provides alkaloids which have important biological activities and strong medicinal impact. Species containing strychnine are as follows: *Strychnos nux-vomica* L., *Strychnos ignatii* P. Bergius and *Strychnos wallichiana* Steud ex DC. These are found throughout Asia, while *Strychnos lucida* R. Br. is located in Australia. *Strychnos icaja* Baillon and *Strychnos tienningsi* grow in Africa and *Strychnos panamensis* L. in South America. Curare alkaloid exists in *S. usambarensis*, the species distributed throughout tropical Africa, and *Strychnos guianensis*, the species found in the South American Amazonian region. Lansiaux et al.[75] report on sungucine and isosungucine, isolated from *S. icaja* Baillon, and their strong bioactivity. Sungucine and isosungucine interact with DNA, inhibit the synthesis of nucleic acids and induce apoptosis in HL-60 leukemia cells. Frédérich et al.[76] reported on the isolation and biological testing of isostrychnopentamine, an alkaloid in the leaves of *S. usambarensis* with strong antiplasmodial activity. Dolichantoside, strictoside and palicoside have been detected in the stem bark of *Strychnos mellodora*, a tree found growing in the mountainous rain forests of east Africa, particularly in Tanzania and Zimbabwe[77]. Brucine and strychnine have been extracted from *S. nux-vomica*[78].

2.4. The Poppy botanical family (Papaveraceae)

The Poppy botanical family (Papaveraceae) contains L-tyrosine (Figure 12) derived alkaloids such as morphine, codeine, thebanine, papaverine, narcotine, narceine, isoboldine and salsolinol. The Poppy family is relatively large, comprising 26 genera and about 250 species. The family is distributed in the sub-tropical and temperate regions of the northern hemisphere (Table 5). The opium poppy (*Papaver somniferum* L.) is a known source of opium from its latex. The Poppy family alkaloids have strong biological and medicinal impact. They are also strong narcotics.

Table 5 General botanical characteristics of the Poppy family[314,316,320]

Botanical Forms and Parts	Characteristics
Botanical forms	Herbs
Some typical genera	*Adlumia* *Arctomecon* *Argemone* *Canbya* *Chelidonium* *Corydalis* *Dendromecon* *Dicentra* *Eschscholzia* *Fumaria* *Hesperomecon* *Meconella* *Papaver* *Platystemon* *Romneya* *Sanguinaria* *Stylophorum*
Special characteristics	Milky juice Stem with vascular bundles
Leaves	Usually lobed or dissected
Flowers	Bisexual Regular Red Violet Yellow White 2 sepals
Fruits	Capsules
Seeds	Dark seed in the capsule

However, many new alkaloids have been reported on within this family. From greater celandine (*Chelidonium majus)*, widespread in Central Europe, such alkaloids as sanguinarine, cholidonine, hydrastine, berberine and chelerythine have been isolated[79,80]. Phytochemical investigation of *Glaucium leiocarpum* Boiss. revealed 11 isolated alkaloids: (+)-glaucine, 6,6a-dehydronorglaucine, oxoglaucine, (+)-*N*-methylglaucine, (+)-lastourviline, (+)-predicentrine, (+)-dihydropontevedrine, secoglaucine, (−)-*N*-methylcoclaurine, allocryptopine and protopine[81]. *Glaucium paucilobum* contains stylopine, protopine, α-allocryptopine, bulbocapnine, corydine, isocorydine, crabbine and arosine[82]. Twenty-three isoquinoline alkaloids have been isolated from *Corydalis bulleyana* Diels. Hao and Qicheng[83] have reported on such alkaloids as protopine, (+)-consperine, (+)-acetylcorynoline, dihydrosangunarine, (+)-acetylisocorynoline, (±)stylopine, (+)-corynoline, (+)-corynoloxine, (+)-isocorynoline, (−)-chelanthifoline, corycavanine, (+)-scoulerine, (+)-isoboldine, acetylcorydamine, allocryptopine, corydamine, bulleyamine, (+)-6-acetonylcorynoline, (+)-12-formyloxycorynoline, (+)-6-oxoacetylcorynoline, (+)-12-hydroxycorynoline, (+)-bulleyanaline and (+)-norjuziphine. *Corydalis bulleyana* Diels is used in traditional medicine as a febrifuge, antidote or analgesic. Moreover, other species of this genus such as *Corydalis amabilis* Migo, *Corydalis yanhusao* W. T. Wang, Cory*dalis ambigua* Cham and Schlecht, *Corydalis bungeana* Turcz. and *Corydalis incisa* Thunb. are also used in folk medicine in China. They contain identical or similar alkaloids as *C. bulleyana* Diels.[83]. Jain et al.[84] have reported on (±)-cheilanthifoline and hunnemanine from *Eschscholzia californica* Cham.

L-tyrosine (Figure 12) derived alkaloids such as bicuculline and metiodine occur in the genera Bleeding heart (*Corydalis* L.) and Dutchman's breeches (*Dicentra* L.). From the species *Corydalis flabellata* Edgew, many alkaloids have been isolated: sibiricine, severzinine[168], 6-(2-hydroxyethyl)-5-6-dihydrosanguinarine, 6-acetonyl-5,6-dihydrosanguinarine, 6-acetonyl-5,6-dihydrosanguinarine, *N*-methyl-2,3,7,8-tetramethoxy-6-oxo-5,6-dihydrobenzophenanthridine, oxosanguinarine, spallidamine, 6-acetonyl-5,6-dihydrochelerythrine, 6-oxochelerythrine and sanguidimerine[133]. These alkaloids are well known for their biological activity. For example, spallidamine has been found to display fungitoxic activity[169]. *Fumaria bracteosa* Pomel is characterized by the presence of (+)-adlumidine, (+)-α-hydrastine, (+)-bicucullidine and protopine[170].

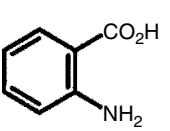
2.5. The Citrus botanical family (Rutaceae)

The Citrus (syn. Rue) botanical family (Rutaceae Juss.) contains more than 150 genera and over 900 species (Table 6). The distribution of these species is worldwide across tropical and sub-tropical areas. Many species contain both anthranilic

Table 6 General botanical characteristics of the Citrus family[312,313,316,318]

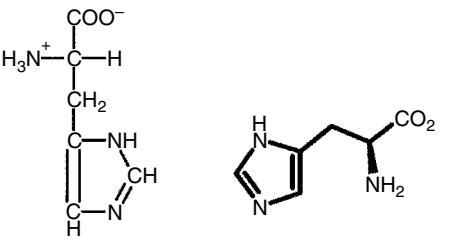
Botanical Forms and Parts	Characteristics
Botanical form	Shrubs Shrublets Trees Herbs
Some typical genera	*Agathosma* *Amyris* *Citrus* *Clausena* *Cneoridium* *Fagara* *Glycosmis* *Haplophyllum* *Helietta* *Poncirus* *Ptelea* *Pilocarpus* *Ruta* *Spathelia* *Zanthoxylum*
Special characteristics	Usually aromatic with resinous tissues
Leaves	Alternate Exstipulate Dotted with translucent in oil glands
Flowers	Bisexual or unisexual Small Regular Petals 3–5 Ovary superior, usually syncarpous
Fruits	Capsule Drupe Samara or berry

acid (Figure 13) and L-histidine (Figure 14) derived alkaloids. Anthranilic acid–derived alkaloids are dictamnine, skimmianine (in such species as *Dictamus albus* or *Skimmia japonica*), acronycine in *Acronychia baueri*, melicopicine in *Melicope fareana*, and rutacridone in *Ruta graveolens*. In the genus



L-anthranilic acid

Figure 13. L-anthranilic acid is a precursor of quinazoline, quinoline and acridine alkaloids.



L-histidine

Figure 14. L-histidine is a precursor of imidazole alkaloids.

Haplophyllum A. Juss., a lot of alkaloids with potential estrogenic activity were reported[54]. These are acutine, acetylfolifidine, bucharidine, dubinidine, dubinine, glycoperine, evoxine, γ-fagarine, folifidine, foliosidine, haplophyline, haplopine, perfamine and skimmianine. Moura et al.[56] reported on alkaloids from *Helietta longifoliata* Britt., a Rutaceae family plant, which grows in South America and is used in Brazilian folk medicine. Helietidine, γ-fagarine, flindrsine, kokusaginine and maculasine have been isolated and their antibacterial activity demonstrated. Alkaloids derived from L-histidine are, for example, pilocarpine and pilosine, in such species as *Pilocarpus microphyllus* and *Pilocarpus jaborandi*. Recent investigation has described fagaronine, the alkaloid extracted from *Fagara zanthoxyloides* Lam. There is evidence that this alkaloid induces erythroleukemic cell differentiation by gene activation[85].

From *Zanthoxylum integrifolium* Merr., an evergreen tree which grows in the northern Philippines and Taiwan, three new alkaloids have recently been isolated: 7,8-dehydro-1-methoxyrutaecarpine, isodecarpine and 8-*demethyloxychelerythine*[86]. In earlier studies 1-hydroxyrutaecarpine, rutaecarpine and 1-methoxyrutaecarpine have been reported from this plant[87]. In *Zanthoxylum hyemaline* St. Hill two quinoline alkaloids (−)-R-geilbalansine and hyemaline were isolated[56]. Bioassay-guided fractionation led to the isolation of three indolopyridoquinazoline alkaloids, 1-hydroxy rutaecarpine, rutaecarpine and 1-metoxyrutaecarpine, from the fruit of *Z. integrifolium*[87]. Moreover, *Melicope semecarpifolia* produces melicarpine and samecarpine[88]. The genera *Toddalia, Dictamus, Pelea* and *Stauranthus* were also present in these furoguinoline alkaloids[89,90,91,92,93,94,95]. *Galipea officinalis* Hancock, a shrub growing in tropical America and used in folk medicine as an antispasmodic, antipyreti, astringent and tonic[96,97,98], yields nine quinoline alkaloids, of which galipine, cusparine, cuspareine, demethoxycusparine and galipinine are the most important[99]. The fruits of *Evodia officinalis*, which has traditionally been used as a folk medicine in Korea for the treatment of gastrointestinal disorders, postpartum haemorrhage and amenorrhea, contain six quinoline alkaloids: (2-hydroxy-4-methoxy)-3-(3′-methyl-2′-butenyl)-quinoline, evocarpine, dihydroevocarpine, evodiamine, rutaecarpine, and

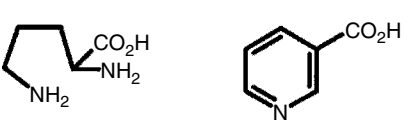
1-methyl-2-[(Z)-6-undecenyl]-4(1*H*)-quinolone[100]. In addition, the fruits of the similar species, *Evodia rutaecarpa*, contain four quinolone alkaloids: 1-methyl-2-tetradecyl-4(1*H*)-quinolone, evocarpine, 1-methyl-2-[(4Z,7Z)-4,7-decadienyl]-4(1*H*)-quinolone and 1-methyl-2-[(6Z,9Z)-6,9-pentadecadienyl]-4(1*H*)-quinolone[101]. Alkaloids occurring in *E. rutaecarpa* show various bioactivities, including angiotensin II antagonistic effects, an inhibitory effect on *Helicobacter pyroli* growth, and DGAT inhibition activity. Moreover, Rahmani et al.[102] reported on the new carbazole alkaloid 7-methoxy-glycomaurin, discovered in *Glycosmis rupestris* Ridely. Rahman and Gray[103] reported on carbazole alkaloids from *Murraya koenigii* (L.) Spreng., a small tree with dark grey bark, which grows in Asia. Mahanimbine has been reported to possess insecticidal and antimicrobial properties[103,104]. The isolation and identification of six 2-alkyl-4(1*H*)-quinolone alkaloids from the leaves of previously uninvestigated *Spathelia excelsa* (K. Krause) has been described by Lima et al.[105]. These data have chemosystematic significance in order to clarify the relationships of this species and *Rutaceae* plant family. Moreover, a new carbazole alkaloid, named clausine Z, has been isolated from stems and leaves of *Clausena excavata* Burm. by Potterat et al.[106]. Clausine structure was established by spectroscopic methods and its bioactivity was determined. According to Potterat et al.[106] this compound exhibits inhibitory activity against cyclin-dependent kinase 5 (CDK5) and shows protective effects on cerebellar granule neurons *in vitro*.

2.6. The Nightshade botanical family (Solanaceae)

The Nightshade plant family (Solanaceae Pers.), containing 90 genera and more than 2000 species distributed in all continents, particularly is abundant in alkaloids (Table 7). The plant species belonging to this family grow especially in the tropics and sub-tropics. However, the majority of the species occur in Central and South America. The L-ornithine (Figures 11 and 15) derived alkaloids occur in many species of this family. Hyoscyamine and hyoscine and cuscohygrine are in the genus Nightshade (*Atropa* L.). This genus is distributed in large areas from the Mediterranean to central Asia and the Himalayas. Deadly nightshade (*Atropa belladonna* L.) is a typical species containing tropan alkaloids[107]. Moreover, the genus Jimsweed (otherwise known as Thornapple) (*Datura* L.), from tropical and warm temperate regions, and the genus Pitura plants (*Deboisia* L.), native to Australia and New Caledonia, also contain these compounds. Further, rich in the above-mentioned L-ornithine-derived alkaloids are also the genus of Henbane plants (*Hyoscyamus* L.) occurring in Europe and North America, as well as the large area from northern Africa to central Asia. The black henbane (*Hyoscyamus niger* L.) is a good example of this alkaloid-containing genus, but there are many more genera with the ability to yield these alkaloids. The genera of Mandrake plants (*Mandragora* L.) and Scopolia plants (*Scopolia* L.) may be

Table 7 General botanical characteristics of the Nightshade family[312,313,316,318]

Botanical Forms and Parts	Characteristics
Botanical forms	Herbs Shrubs Small trees Vines
Some typical genera	*Atropa* *Capsicum* *Cestrum* *Datura* *Deboisia* *Hyoscyamus* *Lycianthes* *Lycium* *Mandragora* *Nicotiana* *Petunia* *Physalis* *Solanum*
Special characteristics	Sometimes climbing Hairs
Leaves	Alternate Exstipulate
Flowers	Regular or slightly irregular with tabular calyx Corola rotate Hermaphrodite Bisexual
Fruits	Berry or capsule Many seeded
Seeds	Albuminous Embryo straight or curved



L-ornithine L-nicotinic acid

Figure 15. L-ornithine and L-nicotinic acids are precursors of some alkaloids in the Nightshade family.

mentioned in this context. However, the Nightshade plant family (*Solanaceae*) also contains other alkaloids, such as the compounds derived from Nicotinic acid (Figure 15). The Tobacco plant genus (*Nicotiana* L.), with approximately 45 species native to the North and South Americas and 21 species native to Australia

and Polynesia, contains such alkaloids as nicotine and anabasine. Moreover, phenylalanine-derived alkaloids are also characteristic of the Nightshade plant family (Solaneceae). Capsaicin is a typical alkaloid of the paprika plant genus (*Capsicum* L.), which has approximately 50 species native to Central and South America. Steroidial alkaloids, such as solanidine, are very common in the potato plant genus (*Solanum* L.), with more than 1500 species distributed throughout the tropical, sub-tropical and temperate zones of the Globe. Certainly, the plant species belonging to the genus *Solanum* L. are endemic only in South America. *Solanum lycocarpum* St. Hill is an invasive and native shrub in Brazilian savanna. It is well known that this plant contains solamargine and solasodine, present in the unripe fruits[108]. Especially, steroid alkaloid solasodine may penetrate in animal body (experiments with rats), the placental and hematoencephalical barriers and impact the foetuses. According to Schwarz et al.[108] *S. lycocarpum* fruit may act as phytohormones, promoting perhaps some neural alterations that at adult age may impair the sexual behaviour of the experimental female without impairing the fertility and sexual hormone synthesis. Another steroid alkaloid is tomatine, characteristic of the Tomato plant genus (*Lycopersicon* L.), with 7 species, and native to the Pacific coast of South America.

2.7. The Coca botanical family (Erythroxylaceae)

Alkaloids also occur in many other plant families. It is relevant to mention the Coca plant family (Erythroxylaceae L.), distributed in the tropics and endemic to South America, especially in the regions of Peru and Bolivia, where the coca bush (*Erythroxylum coca*) has been known for at least 5000 years[109]. Typical characteristics of this family are elliptic, light green leaves ($4–7 \times 3–4$ cm), small, white flowers and small, reddish-orange drupes[318]. Nowadays, it is distributed in the Andean region, the African tropics and in Southern Asia. There are many L-ornithine-derived alkaloids in this plant family, from which three species, the aforementioned *E. coca* and also *Erythroxylum truxilense* and *Erythroxylum novagranatense*, contain cocaine, ecgonine, cinnamylcocaine, α-truxilline, truxilline, methylecgonine, tropine, hygrine, hygroline and cuscohygrine. These strong alkaloids are commonly used as drugs in mainstream medicine and are also, at times, the object of pathological or criminal activity – the source of many personal human tragedies. Zanolari et al.[110] reported on new alkaloids from *Erythroxylum vacciniifolium* Mart., a Brazilian endemic plant used in traditional medicine. From the bark of this plant, nine tropane alkaloids (catuabines H–I, three of their hydroxy derivatives and vaccinines A and B) have been isolated. These tropane alkaloids are interesting for their ester moieties. The genus *Erythroxylum* has some 250 species and apart from the cocaine-producing species has not been examined systematically by modern analytical methods.

2.8. The Borage botanical family (Boraginaceae)

The Borage plant (syn. Forget-me-not) family (Boraginaceae Lindl.) contains L-ornithine (Figure 11 and 15) derived alkaloids, such as indicine-N-oxide in the heliotrope (*Heliotropium indicum*) and southern hound's tongue (*Cynoglosum creticum*) species (Table 8). Farsam et al.[46] reported on new alkaloids from

Table 8 General botanical characteristics of the Borage family[312,313,316]

Botanical Forms and Parts	Characteristics
Botanical forms	Herbs Rarely shrubs or trees Lianas (rarely)
Some typical genera	*Amsinckia* *Anchusa* *Bourreria* *Cordia* *Cryptantha* *Cynoglosum* *Ehretia* *Hackelia* *Heliotropium* *Lappula* *Lithospermum* *Mertensia* *Myosotis* *Onosma* *Onosmodium* *Pulmonaria* *Tournefortia* *Plagiobothrys* *Symphytum*
Special characteristic	Stiff and bristly hairs
Leaves	Alternate Simple Usually rough-hairy Exstipulate
Flowers	Regular Calyx 5-parted Regular corola (5-lobed) Blue or white
Fruits	A drupe Rarely berry-like
Seeds	Straight or curved embryo Scant albumen

another heliotrope species, *Heliotropium crassifolium*: europine and ilamine and their N-oxides. These alkaloids have strong toxic effects.

Moreover, six pyrrolizidine alkaloids were detected in *Anchusa strigosa* Banks and Sol[111] and *Heliotrium esfandiarii* europine N-oxide[112]. Alkaloids of both species have bioimpact. *Anchusa strigosa* is a plant widely distributed in the Mediterranean region. It is used in local folk medicine as a diuretic, analgesic sedative, sudorific remedies and for treatment of stomach ulcers and externally for skin diseases[113,114]. Siciliano et al.[115] have analysed the qualitative and quantitative composition of alkaloids in flowers, leaves and roots of *A. strigosa*. This phytochemical study led to the isolation of nine pyrrolizidine alkaloids, from which three have been unidentified. Many pyrrolizidine alkaloids have been shown to be isolated from leaves, roots and rhizomes of the lungwort species (*Pulmonaria* spp.). In both *Pulmonaria officinalis* and *Pulmonaria obscura* such alkaloids as intermedine, lycopsamine and symphitine have been detected. This means that *P. officinalis* is not an exception among Boraginaceae in not having pyrrolizidine alkaloids, as had been previously claimed[116]. Haberer et al.[117]presented the evidence for this. Thus, they have advanced the theory of the botanical family base for alkaloid distribution. Acetyl-intermedine and acetyl-lycopsamine are alkaloids yielded in common comfrey (*Symphytum officinale* L.). Many species belonging to the Borage plant family are native to the Mediterranean area.

2.9. The Legume botanical family (Fabaceae)

Alkaloids derived from L-ornithine, L-lysine, and L-trypthophan occur in the Legume plant family (Fabaceae Juss.) (Table 9). This plant family is the third largest botanical family, with 650 genera and 18 000 species in the humid tropics, sub-tropics, temperate and sub-arctic zones around the Globe[118]. L-ornithine-derived alkaloids such as senecionine are present in the genus Crota (*Crotalaria* L.).

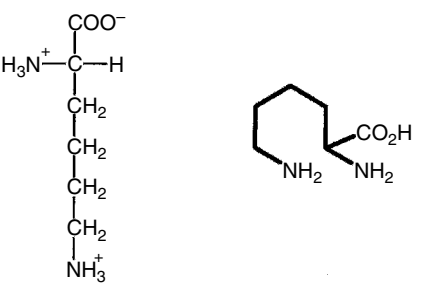
The most typical alkaloids for this botanical family are L-lysine (Figure 16) derived alkaloids, such as lupinine, sparteine, lupanine, angustifoline, epilupinine, anagyrine and so on. Lysine alkaloids occur in many species belonging to the legume family. They are quinolizdine alkaloids occurring in the large and very diverse genus Lupine (*Lupinus* L.) (Figure 17), and in the genus of Broom plants (*Cytisus* L.). Both the genus *Swainsona* (*Swainsona* L.) and the genus of Blackbean plants (*Castanospermum* L.) contain swainsonine and castanospermine. Przybylak et al.[119] have detected 46 compounds from 6 Mexican lupin species (*Lupinus rotundiflorus, Lupinus montanus, Lupinus mexicanus, Lupinus elegans, Lupinus madrensis, Lupinus exaltatus*). From among 46 detected compounds it was possible to identify unambiguously 24 of them. Most of the identified alkaloids are from lupanine group:

Table 9 General botanical characteristics of the Legume family[312,313,316]

Botanical Forms and Parts	Characteristics
Botanical forms	Herbs Shrubs Lianes Vines Trees
Some typical genera	*Acacia* *Adesmia* *Aeschynomene* *Albizia* *Arachis* *Astragalus* *Baptisia* *Bauhinia* *Caesalpinia* *Calliandra* *Cassia* *Cercis* *Chamaecrista* *Crotalaria* *Dalbergia* *Dalea* *Delonix* *Desmodium* *Erythrina* *Gleditsia* *Glycine* *Indigofera* *Inga* *Lathyrus* *Leucaena* *Lonchocarpus* *Lotus* *Lupinus* *Meliotus* *Milletia* *Mimosa* *Parkia* *Parkinsonia* *Phaseolus* *Pisum* *Pithecellobium* *Robinia* *Rhynchosia* *Senna* *Swartzia*

Table 9 (Continued)

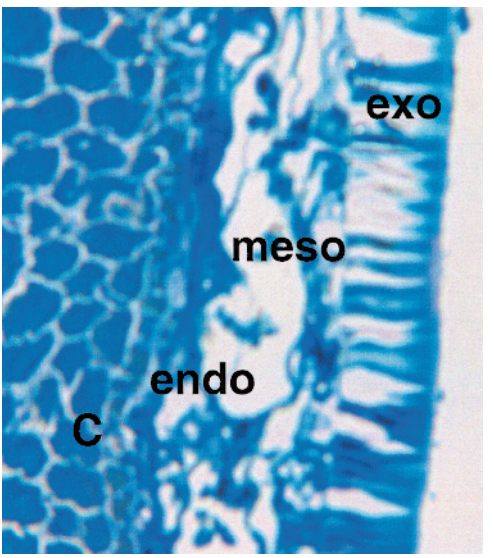
Botanical Forms and Parts	Characteristics
	Tamarindus *Tephrosia* *Trifolium* *Vicia* *Wisteria*
Special characteristic	Often twining or climbing Hairs
Leaves	Alternate Stipulate
Flowers	Regular or irregular Usually bisexual
Fruits	A legume
Seeds	Big or small seed



L-lysine

Figure 16. L-lysine is a precursor of piperidine, quinolizidine and indolizidine alkaloids.

sparteine, ammodendrine, epiaphyllidine, epiaphylline, tetrahydrorhombifoline, 17-oxosperteine, 5,6-dehydro-α-isolupanine, angustifoline, α-isolupanine, aphyllidine, 5,6-dehydrolupanine, lupanine, aphylline, 11,12-dehydrolupanine, dehydrooxosparteine, 3β-hydroxylupanine, multiflorine, 17-oxolupanine, 13α-hydroxylupanine, 3β, 13α-dihydroxylupanine, 13α-angeloylolupanine, 13α-tigloyloxylupanine and 4β-tigloyloxylupanine. Matrine has been isolated from *Sophora subprostata* Chun et T. Chen[149]. Moreover, in plants belonging to the Legume family (*Fabaceae* L.) alkaloids derived from L-tryptophan also occur. Eserine, eseramine, physovenine and geneserine are all examples of these kind of alkaloids, which occur, for example, in the Calabar bean (*Physostigma venenosum* L.). Erysovine and wrythraline are high toxic alkaloids in *Erythrina lysistemon*. Lou et al.[130] have isolated two alkaloids, 2-methoxyl-3-(3-indolyl)-propionic acid and 2-hydroxyl-3-[3-(1-*N*-methyl)-indolyl] propionic acid, in



(a)

(b)

Figure 17. (a) Structure of the seed testa of the Washington lupine (*Lupinus polyphyllus* Lindl.). Transmission electron microscope (TEM) research proved large structural diversity inside both the genus *Lupinus* L. and the species. The picture shows exotesta (exo), mesotesta (meso) and endotesta (endo), cotyledon (C), the parts which differ in the species and varieties *Lupinus* spp. In the testa and part of the storage cells, alkaloids are present; (b) Alkaloidal *Lupinus polyphyllus* Lindl. in flowering stage.

peanut skins (*Arachis hypogaea* L.). These alkaloids had not previously been found in natural sources[130]. Moreover, Wanjala et al.[138] have isolated and identified several new alkaloids in *Erythrina latissim*, widespread in Botswana, Zimbabwe and South Africa. One alkaloid named (+)-erysotrine shows bioimpact as an antimicrobial agent. Moreover, Tanaka et al.[150] have reported on a new alkaloid in *Erythrina poeppigiana*, a plant found in central and South America. This alkaloid, 8-oxo-α-erythroidine epoxine, is similar to other alkaloids previously found in this species, such as erysodine, erysovine, α-erythroidine, β-erythroidine and dihydro-β-erythroidine[151,152]. Recently, from the flowers of broad beans (*Vicia faba* L.) *N*-[(3R, 7R)-(−)-jasmonoyl]-(S)-dopa and *N*-[(3R,7R)-(−)-jasmonoyl]-dopamine were isolated by Kramell et al.[153]. These alkaloids are tyrosine-derived compounds. All alkaloids occurring in Fabaceae have both biological and ecological significance.

The occurrence of some important alkaloids in nature is shown in Table 10.

Table 10 Occurrence of some important alkaloids in the nature

Precursor Compound of Alkaloid Derivation	Occurrence in Nature		
	Family	Species	Alkaloids
L-ornithine-derived alkaloids	Solanaceae	*Atropa belladonna*	(−)-hyoscyamine (−)-hyoscine Cuscohygrine
		Datura innoxia	As *Atropa*
		Datura stramonium	As *Atropa*
		Datura metel	As *Atropa*
		Datura sanguine	As *Atropa*
		Duboisia myoporoides	As *Atropa*
		Hyoscyamus niger	As *Atropa*
		Hyoscyamus muticus	As *Atropa*
		Mandragora officinarum	As *Atropa*
		Scopolia carniolica	As *Atropa*
		Withana somnifera	Withasomnine
	Erythroxylaceae	*Erythroxylum coca*	(−)-cocaine (−)-ecgonine
		Erythroxylum truxilense	Cinnamylcocaine α-truxilline Truxilline Methylecgonine Tropine Hygrine Hygroline Cuscohygrine

(*continued*)

Table 10 (Continued)

Precursor Compound of Alkaloid Derivation	Occurrence in Nature		
	Family	Species	Alkaloids
	Boraginaceae	*Heliotropium indicum*	Indicine-*N*-oxide
		Cynoglossum spp.	Indicine-*N*-oxide
		Symphytum officinale	Acetyl-intermedine Acetyl-lycopsamine
	Asteraceae	*Senecio vulgaris*	Senecionine
		Senecio jacobaea	Senecionine
	Fabaceae	*Crotalaria* spp.	Senecionine
	Capparidaceae	*Boscia angustifolia*	Stachydrine 4 -hydroxystachydrine
L-lysine derived alkaloids	Punicaceae	*Punica granatum*	Pelletierine Pseudopelletierine Methylpelletierine Anaferine
	Crassulaceae	*Lobelia inflata*	Obeline Lobelanine
		Sedum acre	Sedamine
	Piperaceae	*Piper nigrum*	Piperine Piperidine
	Fabaceae	*Baptisia alba*	Anagyrine Cytisine Sparteine Thermopsine
		Lupinus albus	Albine Angustifoline Lupanine Sparteine
		Lupinus luteus	Lupinine Sparteine
		Lupinus polyphyllus	Lupanine
		Lupinus angustifolius	Angustifoline
		Lupinus hispanicus	Lupinine Epilupinine
		Lupinus latifolius	Anagiryne
		Cytisus scoparius	Sparteine
		Swainsona canescens	Swainsonine
		Castanospermum australe	Castanospermine

Table 10 (Continued)

Precursor Compound of Alkaloid Derivation	Occurrence in Nature		
	Family	Species	Alkaloids
L-tyrosine derived alkaloids	Graminae	*Hordeum vulgare*	Hordenine Tyramine
	Cactaceae	*Lophophora williamsii*	Mescaline Anhalamine Anhalonine Anhalonidine
	Papaveraceae	*Corydalis* spp.	Salsolinol
	Menispermaceae	*Chondrodendron tomentosum*	Curare Tubocurarine
		Cissampelos pereira	Fangchinoline
		Cyclea barbata	Fangchinoline
		Cyclea peltata	Fangchinoline
		Stephania dinklagei	*N*-methyllirio dendronine 2-*O*-*N*-dimethyl liriodendronine Dicentrinone Corydine Aloe-emodin
		Stephania tetrandra	Tetrandrine Stephanine Fangchinoline
		Stephania harnandifolica	Fangchinoline
		Triclisia subcordata	Fangchinoline
	Loganiaceae	*Strychnos toxifera*	Tubocurarine
	Papaveraceae	*Papaver somniferum*	Morphine Codeine Thebaine Papaverine Narcotine Narceine Isoboldine
	Berberidaceae	*Berberis* spp.	Berberine Berbamine Hydroxyacanthin Glaucine
		Mahonia spp.	Berberine
		Nandina domestica	(+)-nantenine

(*continued*)

Table 10 (Continued)

Precursor Compound of Alkaloid Derivation	Occurrence in Nature		
	Family	Species	Alkaloids
	Ranunculaceae	*Hydrastis canadiensis*	Berberine Hydrastine
		Thalictrum orientale	Fangchinoline Fuzitine
	Fumariaceae	*Corydalis* spp.	Bicuculline Metiodine
		Corydalis flabellate	Spallidamine Sanguidimerine Oxosanguinarine
		Dicentra spp.	Bicuculline Metiodine
	Liliaceae	*Kreysigia multiflora*	Autumnaline Floramultine Kreysigine
		Colchicum autumnale	Colchicine
	Rubiaceae	*Cephaelis ipecacuanha*	Emetine Cephaeline Secologanin Ipecoside
		Mitragyna speciosa	Mitragynine
	Amaryllidaceae	*Leucojum vernum*	Lycorine Homolycorine 2-*O*-acetyllycorine Leucovernine Acetylleucoverine *N*-demethylgalanthamine
			Hippeasterine 9-*O*-demethylhomolycorine 5α-hydroxyhomolycorine 11-hydroxyvittatine
		Lycorus radiata	Lycorine
		Galanthus spp.	Galanthamine
		Galanthus plicatus ssp.	Galanthindole
		Pancratium sickenbergeri	Hippadine Trispheridine Pseudolycorine Haemanthidine Norgalanthamine Haemanthamine

Table 10 (Continued)

Precursor Compound of Alkaloid Derivation	Occurrence in Nature		
	Family	Species	Alkaloids
			Vittatine Pancracine Lycorine
		Zephyranthes citrina	Galanthine Haemanthamine Lycorine Lycorenine Oxomaritidine Martidine Vittatine
L-tryptophan-derived alkaloids	Agaricacea	*Psilocybe semilanceata*	Psilocin Psylocybin
		Cynocybe spp.	Psilocin Psilocybin
		Panaeolus spp.	Psilocin Psylocybin
		Stropharia spp.	Psilocin Psilocybin
	Graminae	*Hordeum vulgare*	Gramine
		Secale cereale (with *C. purpurea*)	Ergotamine
		Triticum aestivum (with *C. purpurea*)	Ergotamine
		Triticale (with *C. purpurea*)	Ergotamine
	Elaeagnaceae	*Elaeagnus angustifolia*	Elaeagnine
	Zygophyllaceae	*Peganum harmala*	Harman, harmine
	Rubiaceae	*Pausinystalia yohimbe* *Ophiorrhiza mungos*	Yohimbine Camptothecin
	Apocynaceae	*Alstonia macrophylla*	Talcarpine Pleiocarpamine Alstoumerine 2-*O*-epiantirhine Alstonerine Alstophyline Macralstonine Alstomacrophyline Villalstonine Alstomacroline Macrocarpamine

(*continued*)

Table 10 (Continued)

Precursor Compound of Alkaloid Derivation	Occurrence in Nature		
	Family	Species	Alkaloids
		Alstonia scholaris	Menilamine
		Aspidosperma megalocarpon	Fendlerine Aspidoalbine Aspidolimidin
		Rauvolia capra	Quinine
		Rauvolfia serpentina	Reserpine Rescinnamine Ajmalicine
		Rauvolfia canescens	Reserpine Rescinnamine Deserpine
		Rauvolfia vormitoria	Reserpine Rescinnamine
		Catharanthus roseus	Ajmalicine Catharanthine Vindoline Vinblastine Vincristine Vindesine Alioline
		Ochrosia elliptica	Ellipticine
		Ervatmia heyneana	Camptothecin
	Loganiaceae	*Strychnos icaja*	Sungucine Isosungucine
		Strychnos nux-vomica	Strychnine Brucine
		Strychnos toxifera	Curare C-toxiferine Alcuronium
		Strychnos usambarensis	Isostrychnopentamine
	Poaceae	*Arundo donax*	Arundamine Arundacine
	Rubiaceae	*Cinchona officinalis*	Quinine Quininidine Cinchonine Cinchonidine
		Cinchona succirubra	Quinine Cinchonidine Quinidine
		Cinchona calisaya	Quinine Cinchonidine Quinidine

Table 10 (Continued)

Precursor Compound of Alkaloid Derivation	Occurrence in Nature		
	Family	Species	Alkaloids
		Corynanthe pachyceras	Corynanthine α-yohimbine Dihydrocorynantheine Corynantheine Corynantheidin
		Ophiorrhiza mungos	Camptothecin
	Sterculiaceae	*Waltheria douradinha*	Waltherione-A
	Nyssaceae	*Camptotheca acuminata*	Camptothecin
	Icacinaceae	*Nothapodytes foetida*	Camptothecin
		Merilliodendron megacarpum	Camptothecin
		Pyrrenacantha klaineana	Camptothecin
	Fabaceae	*Arachis hypogaea*	2-methoxyl-3-(3-indolyl)-propionic acid 2-hydroxyl-3-[3-(1-N-methyl)-indolyl]propionic acid
		Physostigma venenosum	Eserine Eseramine Physovenine Geneserine
		Erythrina lysistemon	Erysovi ne Erythraline
		Erythrina latissima	(+)-erysotrine
		Erythrina poeppigiana	8-oxo-α-erythroidine-epoxine Erysovine α-erythroidine β-erythroidine Dihydro-β-erythroidine
	Convolvulaceae	*Ipomea violacea*	Ergoline Ergotamine Ergine
		Rivea corymbosa	Ergoline Ergotamine Ergine
	Asteraceae	*Centaurea schischkinii*	Afzelin Apigenin Arctigenin Astragalin Schischkinin

(*continued*)

Table 10 (Continued)

Precursor Compound of Alkaloid Derivation	Occurrence in Nature		
	Family	Species	Alkaloids
L-histidine-derived alkaloids	Cactaceae	*Dolichothele sphaerica*	Dolichotheline
	Rutaceae	*Pilocarpus microphyllus*	Pilocarpine Pilosine
		Pilocarpus jaborandi	Pilocarpine Pilosine
Anthranilic acid–derived alkaloids	Zygophyllaceae Acanthaceae Rutaceae	*Peganum harmala* *Justicia adhatoda* *Dictamus albus*	Harmin Vaccine Dictamnine Skimmianine
		Helietta longifoliata	Maculosine γ-fagarine Helietidine Flindersine Kokusaginine
		Skimmia japonica	Dictamnine Skimmianine
		Acronychia baueri	Acronycine
		Melicope fareana	Melicopicine
		Melicope semecarpifolia	Melicarpine
		Ruta graveolens	Semecarpine Rutacridone
Nicotinic acid–derived alkaloids	Solanaceae	*Nicotiana tabacum*	(−)-nicotine Anabasine
	Discoreaceae Euphorbiaceae Palmae	*Discorea dregeana* *Ricinus communis* *Areca catechu*	Discorine Ricinine Arecoline Pyridine
Acetate-derived alkaloids	Umbelliferae Pinaceae Zygophyllaceae	*Conium maculatum* *Pinus* spp. *Nitraria komarovii*	Coniine Pinidine Komavine Acetylkomavine
		Nitraria sibirica	Dihydroschoberine Nitrabirine *N*-oxide
Phenylalanine-derived alkaloids	Ephedraceae	*Ephedra intermedia*	Ephedrine Cathinone Cathine
		Ephedra geriardiana	Ephedrine Cathinone Cathine

Table 10 (Continued)

Precursor Compound of Alkaloid Derivation	Occurrence in Nature		
	Family	Species	Alkaloids
		Ephedra major	Ephedrine Cathinone Cathine
	Celastraceae	*Catha edulis*	Norpseudoephedrine
	Solanaceae	*Capsicum annuum*	Capsaicin
Terpenoid alkaloids	Apocynaceae	*Skytanthus acutus*	β-skytanthin
	Actinidiaceae	*Actinida polygama*	Actinidine
	Celastraceae	*Tripterygium wilfordii*	Dihydroagarofuran
	Gentianaceae	*Gentiana lutea*	Gentiopicroside
	Ranunculaceae	*Aconitum arcuatum*	Arcutin
		Aconitum coreanum	Acorone Accoridine Corypidine Coryphine Tangutisine
		Aconitum karacolicum	12-acetylnepelline Cammaconine Karacoline Karakanine Nepelline Songorine
		Aconitum napellus	Aconitine
		Aconitum sinomontanum	Sinomontanine
		Aconitum vulparia	Aconitine
		Delphinium berbeyi	Methyllycaconitine Barbine
		Delphinium corymbosum	Delcorine Delsonine
		Delphinium occidentale	Methyllycaconitine Barbine
		Delphinium glaucescens	Methyllycaconitine Barbine
		Delphinium poltoratskii	Ajacine Anthranoyllycoctonine Candelphine Delphyrine Delpoline Delsonine Karacoline Lycococtine

(*continued*)

Table 10 (Continued)

Precursor Compound of Alkaloid Derivation	Occurrence in Nature		
	Family	Species	Alkaloids
Steroid alkaloids	Solanaceae	*Solanum tuberosum*	Solanidine
		Lycopersicon esculentum	Tomatine
	Liliaceae	*Veratrum album*	Jervine Cyclopamine Cycloposine Protoveratrine A Protoveratrine B
		Veratrum lobelianum	*O*-acetylfervine
		Veratrum californicum	Jervine Cyclopamin Cycloposine Protoveratrine A Protoveratrine B
		Veratrum viride	Jervine Cyclopamin Cycloposine Protoveratrine A Protoveratrine B
	Apocynaceae	*Holarrhena floribunda*	Holaphyllamin
		Holarrhena antidysenterica	Conessine
Purine alkaloids	Rubiaceae	*Coffea arabica*	Caffeine Theophylline Theobromine
		Coffea canephora	Caffeine Theophylline Theobromine
		Coffea liberica	Caffeine Theophylline Theobromine
	Theaceae	*Camellia sinensis*	Caffeine Theophylline Theobromine
	Aquifoliaceae	*Ilex paraguensis*	Caffeine Theobromine
		Ilex cassine	Caffeine Theobromine Theophylline
		Ilex vormitoria	Caffeine Theobromine Theophylline

Table 10 (Continued)

Precursor Compound of Alkaloid Derivation	Occurrence in Nature		
	Family	Species	Alkaloids
	Sapinidaceae	*Paullinia cupana*	Guaranine Theophylline Theobromine
	Sterculiaceae	*Cola nitida*	Caffeine Theobromine
		Cola acuminata	Caffeine Theobromine
		Theobroma cacao	Theobromine Caffeine
Moss alkaloids	Lycopodiaceae	*Lycopodium annotinum*	Annotinine Lycodine
		Lycopodium complanatum	Lycopodine
		Lycopodium cernuum	Cernuine
		Huperzia serrata	Huperzine A Huperzine J Huperzine K Huperzine L Phlegmariurine
Fungus alkaloids		*Aspergillus terreus*	Asterrelenin Terretonin Territrem A Territrem B
		Rhizopus, Penicillium, Claviceps purpurea (with *Secale cereale*)	Chanoclavine Ergoline Ergotamine
Bacter alkaloids		*Pseudomonas tabaci* *Pseudomonas aeruginosa*	Tabtoxin Pyocyanine
Animal alkaloids	Bryozoa	*Flustra foliacea*	Deformylflustrabromin Lustrabromine Flustramine
	Saxidomus	*Saxidomus giganteus*	Saxitoxin Tetrodotoxin
	Salamandra	*Salamandra maculosa*	Samandarine Samandarone Samandaridine Cycloneosamandaridine Cycloneosamandione
		Salamandra samandarine	Samandenone

(*continued*)

Table 10 (Continued)

Precursor Compound of Alkaloid Derivation	Occurrence in Nature		
	Family	Species	Alkaloids
	Dendrobatidae	*Phyllobates aurotaenia*	Cardiotoxin Neurotoxin Batrachotoxin Homobatrachotoxin
		Dendrobates histrionicum	Histrionicotoxin Dihydroisohistrionicotoxin Gephyrotoxin
		Dendrobates pumilio	Pumiliotoxin Pumiolotoxin A Pumiliotoxin B
		Pseudophryne coriacea	Pseudophrynaminol
Mammals		*Castor fiberei* *Moschus moschiferus*	Castoramine Muscopyridine
Mymonoptera	Formicidae	*Solenopsis invicta* *Dontomachus hastatus* *Ontomachus brunneus*	Cassine Dialkylpyrazine Trialkylpyrazine
	Rhinotermitidae	*Reticuliterms flavipes* T*iculitermes virginica*	Norharman Norharmane
Diploda		*Glomeris marginata*	Glomerin Homoglomerin
		Polyzonium rosalbum	Polyzonimine
Coleoptera	Coccinellidae	*Adalia bipunctata*	Adaline
		Coccinella septempunctata	Coccinelline Podamine
		Pilachna varivestis	Epilachnene
		Podamia convergens	Convergine Podamine
		Myrrha octodecimguttata	Myrrhine Propeleine
		Propylea quatuordecimpunctata	Propyleine
		C*hilocorus cacti*	Stenusine
	Staphylinidae	*Paederus fuscipes*	(+)-pederin Pederone

Sources: Refs [7, 23, 28, 31, 32, 33, 35, 36, 37, 38, 39, 41, 42, 43, 44, 45, 47, 48, 49, 50, 51, 52, 53, 55, 56, 62, 64, 75, 85, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148].

2.10. The Monseed botanical family (Menispermaceae)

The Monseed plant family (Menispermaceae) contains L-tyrosine-derived alkaloids (Figure 12). Plant species belonging to this family are found throughout the tropics and, especially in, tropical lowland zones[154]. The Monseed botanical family is large, containing about 70 genera and 450 species (Table 11). The genus *Stephania* produces tetrandrine and stephanine, while the genus Curare (*Chondrodendron*) yields curare and tubocurarine. All are known to be medicinal alkaloids. More than 150 different alkaloids have been isolated from plants of *Stephania* genus. Camacho[126] reported on many of alkaloids found in *Stephania dinklagei*, a climbing shrub of the deciduous forest of Africa. They were methylliriodendronine, 2-*O*,*N*-dimetylliriodendronine, liriodenine, dicentronine, corydine and aloe-emodin. These alkaloids display strong biological impact with antiprotozal activity. A report by Gören et al.[155] noted that these plants also yielded liriodenine, corydine, isocorydine, atherospermidine, stephalagine and dehydrostephalagine. Liriodenine showed strong cytotoxic activity. Corydine and atherospermidine even revealed activity damaging to DNA. Zhang and Yue[156] reported on the isolation and structural elucidation of new alkaloids from *Stephania longa* Lour., a perennial herbaceous liana. They detected stephalonines A–I, norprostephabyssine, isoprostephabyssine, isolonganone and isostephaboline. Chen et al.[157] have isolated tetrandrine from the root of a Chinese herb *Stephania tetrandra* S. Moore. This alkaloid showed to inhibit both culture-activation and TGF-beta(1)-stimulated activation of quiescent rat hepatic stellate cells (HSCs) *in vitro*[157]. From *Stephania cepharantha* Hayata, cepharathine, cepharanoline, isotetrandrine and berbamine have been isolated[158].

Cepharanthine is a particularly active component of hair growth. Moreover, the isolation and characterization of alkaloids (cycleanine, cycleanine *N*-oxide, isochondodendrine, cocsoline and quinine) from *Epinetrum villosum* (Exell)

Table 11 General botanical characteristics of the Monseed family[312,313,316]

Botanical Forms and Parts	Characteristics
Botanical forms	Trees Lianas
Leaves	Alternate Usually palmately veined Often lobed
Flowers	Regular Small Unisexual
Fruits	Endocarp
Seeds	Curved embryo

Troupin has also been reported[159]. These alkaloids were found to exhibit antimicrobial and antiplasmodial activities. *Epinetrum villosum* is a twining liana, growing in secondary forests in the coastal areas in Congo and Angola and is used in traditional medical for the treatment of fever, malaria and dysentery[159,160]. The genus *Cissampelos* contains cissampareine, which has potential medicinal uses, but it is also psychoactive. It is a principal alkaloid of dawidjiewortel (*Cissampelos capensis*), which grows in South Africa.

2.11. The Berberry botanical family (Berberidaceae)

L-tyrosine-derived alkaloids occur also in the Berberry botanical family (Berberidaceae Torr., Gray, Juss.) (Table 12). Berberine is produced particularly by the Berberry genus (*Berberis* L.) and the Mahonia genus (*Mahonia* Nutt.). These alkaloids are found in such species as the common berberry (*Berberis vulgaris* L.), native to Euroasia; the Japanese berberry (*Berberis thunbergii* DC.), native to Asia and the Chita (*Berberis aristat* DC.) in the Himalayas. It is also present in Holly (*Mahonia aquifolium* Nutt.), native to Western America, and in the Creeping mahonia (*Mahonia repens* (Lindl.) G. Don., endemic to the North America. New research reports mention berberine, found in the oblonga berberry *(Berberis oblonga* Scheid), growing in Kazakhstan but native to Central Asia[161]. Moreover, it is also reported that, together with berberine, other alkaloids were detected, such as glaucine, hydroxyacanthin and berbamine. Orallo[162] reported on (+)-nantenine, a natural alkaloid derived from *Nandina domestica* Thunberg, which was first isolated by Takase and Ohasi in 1926. Subsequently, extracts

Table 12 General botanical characteristics of the Berberry family[312,316]

Botanical Forms and Parts	Characteristics
Botanical forms	Bushes Herbs Trees
Special characteristics	Small hairs Stems with vascular bundles
Leaves	Generally alternate Simple
Flowers	Regular Olitary or in cymes, racemes or panicles Bisexual
Fruits	A berry or pod
Seeds	Anatropous with endosperm Small embryo

containing this alkaloid were widely used in Japanese folk medicine for the treatment of whooping cough, asthma, pharynx tumours, uterine bleeding and diabetes. Berbamine was extracted from *Berberis poiretil* Echneid, a plant which grows in China[163]. This alkaloid shows actions of anti-arhythmia, anti-myocardial ischemia and antithrombosis.

2.12. The Buttercup botanical family (Ranunculaceae)

The Buttercup botanical family (Ranunculaceae Juss.) (Table 13) yields both L-tyrosine and terpenoid alkaloids. This plant family, which has 50 genera and nearly 2000 species, is situated around the Globe in the temperate zones. Tyrosine-derived alkaloids, such as berberine and hydrastine, occur in the Seal genus (*Hydrastis* L.). Fangcholine and fuzitine have been reported in the genus *Thalictrum* (*Thalictrum orientale*), growing in Turkey[127]. Terpenoid alkaloids, such as aconitine and sinomontanine, appear in the genus Hood (*Aconitum* L.). Many other alkaloids have been found in this genus. In *Aconitum karacolicum* (Rapaics) from Kyrgyzstan, karacoline, karakanine, songorine, nepelline, 12-acetylnepelline, cammaconine and secokaraconitine were detected[164]. In *Aconitum arcuatum (*Maxim.), a new alkaloid, arcutin with antibacterial and medicinal impact, was located[165]. In *Aconitum coreanum* (Levl.) Rapaics the tangutisine, acorone, acorridine, coryphine and coryphidine were found, all of which have powerful biological impact[166]. Methyllycaconitine and barbine are typical in the genus Larkspur (*Delphinium* L.). *Delphinium corymbosum* contained delcorinine and delsonine[167], while *Delphinium poltoratskii* was found to hold a lot of alkaloids[125]. These included methyllycaconitine, lycoctonine, anthranoyllycoctonine, ajacine, karacoline and delpoline.

Table 13 General botanical characteristics of the Buttercup family[312,313,316]

Botanical Forms and Parts	Characteristics
Botanical forms	Herbs Climbers (rarely) Trees (rarely) Vines (rarely)
Special characteristics	A medium-sized plants Stems with vascular bundles
Leaves	Alternate with sheathing bases or opposite
Flowers	Regular or irregular Usually bisexual
Fruits	Achene or follicle or berry-like (rarely)
Seeds	Seeds with endosperm A minute embryo

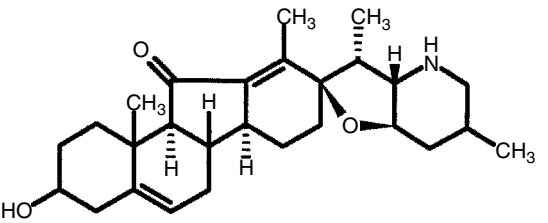
2.13. The Lily botanical family (Liliaceae)

The Lily botanical family (Liliaceae Adans., Juss.) (Table 14) is spread worldwide and contains more than 200 genera and around 3500 species. Some genera of this family produce L-tyrosine-derived alkaloids. The genus *Kreysigia* yields autumnaline, floramultine and kreysigine.

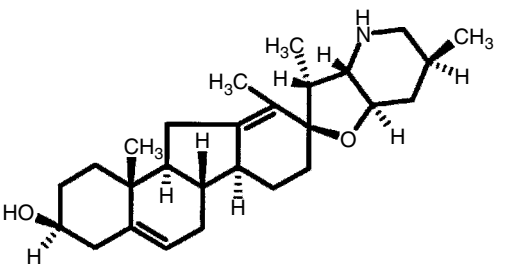
The genus *Colchicum* (*Colchicum* L.) produces colchicine. Stereoidal alkaloids in this family are found in the Hellebore genus (*Veratrum* Bernch.). Jervine, cyclopamine (Figure 18), cycloposine, protoveratrine A and protoveratrine B yield *Veratrum album*. O-acetyljervine has been reported in the false hellebore (*Veratrum lobelianum* Bernch.)[136]. Four new steroid alkaloids (puqienine A, puqienine B, *N*-demethylpuqietinone, puqietinonoside) have been isolated from *Fritillaria* species by Jiang et al.[171] The bulbs of these plants have been used as an antitussive and expectorant in folk Chinese medicine. All four new alkaloids have been reported to display the antitussive activity on mouse[171].

Table 14 General botanical characteristics of the Lily family[312,313,316]

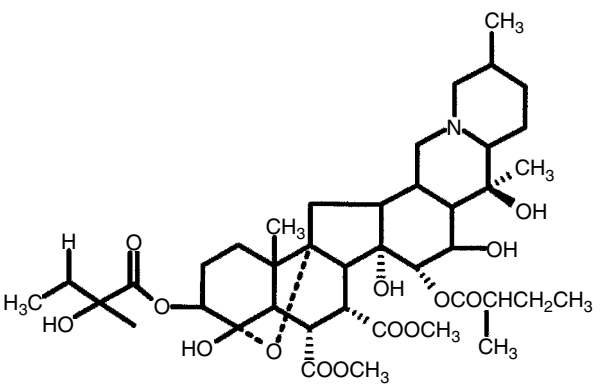
Botanical Forms and Parts	Characteristics
Botanical forms	Herbs Shrubs Trees Climbers (rarely)
Some typical genera	*Colchicum* *Erythronium* *Fritillaria* *Gagea* *Kreysigia* *Lilium* *Medeola* *Tulipa* *Veratrum*
Special characteristics	Cosmopolitan family Usually bulbs
Leaves	Alternate Parallel-weined
Flowers	Regular Solitary or in racemes, panicles or umbels
Fruits	Dehiscent capsule or a berry
Seeds	Small embryo Albumen



Jervine



Cyclopamine



Protoveratrine

Figure 18. Jervine, cyclopamine and protoveratrine structures.

2.14. The Coffee botanical family (Rubiaceae)

The Coffee (syn. Madder) botanical family (Rubiaceae Juss.) (Table 15) consists of more than 400 genera and over 6000 species. It grows in the tropics and the sub-tropics. Plants belonging to this family include trees, bushes and liane. The Coffee plant family contains two major purines of adenine- /guanine-derived alkaloids, the so-called "purine alkaloids". Purine is a nitrogenous base of nucleotide, which consists of just purine and pentose sugar (D-ribose or 2 deoxy-D-ribose). Typical purine alkaloids are caffeine, theophylline and theobromine. The same or similar purine alkaloids occur also in other plant families,

Table 15 General botanical characteristics of the Coffee family[312,313,316,318]

Botanical Forms and Parts	Characteristics
Botanical forms	Trees Shrubs Lianas Herbs Climbers
Some typical genera	*Borreria* *Casasia* *Catesbaea* *Cephalanthus* *Chiococca* *Coffea* *Dioidia* *Ernodia* *Erithalis* *Exostema* *Galium* *Gardenia* *Guettarda* *Hamelia* *Hedyotis* *Ixora* *Mitchella* *Morinda* *Mussaenda* *Pavetta* *Pentodon* *Pickneya* *Policourea* *Psychotria* *Randia* *Richardia* *Rondeletia* *Spermacoce* *Tarenna*
Special characteristics	Mainly tropical Some genera in temperate zone Lacking internal phloem Hairs
Leaves	Opposite or whorled Stipulate
Flowers	Regular Corolla with cylindric tube Usually bisexual
Fruits	A capsule or a berry or drupe or schizocarp
Seeds	Albumen Embryo straight to curved

such as the Tea plant family (Theaceae), the Guarana plant family (Sapinidaceae) and the Cola plant family (Sterculiaceae). The plants of the Guarana family have one additional alkaloid, guaranine (Table 10). Purine alkaloids have a biological and according to recent (still unpublished) clinical results, also a positive and prophylactic effect in decreasing the risk of Parkinson's disease, for example in the case of caffeine. From *Waltheria douradinha* St. Hill belonging to the Cola family, walterione A, a tryptophan-derived alkaloid, has been discovered[147]. This alkaloid has important biological potential. Staerk et al.[50] noted five alkaloids isolated from the species *Corynanthe pachyceras* K. Schum., a member of the *Rubiaceae* family. Corynantheidine, corynantheine, dihydrocorynantheine, α-yohimbine and corynanthine were isolated from the bark of this species and all these alkaloids demonstrate powerful bio and ecoimpacts (leishmanicidal, antiplasmodial and cytotoxic activity). Other L-tryptophan-derived alkaloids were found in the stem bark of *Cinchona officinalis*, belonging to same botanical family (*Rubiaceae*)[55]. These are quinine, quinidine, cinchonine and cinchonidine. Such alkaloids also show bioimpact. Moreover, the latest research focuses on mitragynine, an alkaloid in *Mitragyna speciosa* which grows in Thailand. This alkaloid has a powerful, opium-like effect[172]. Moreover, mitragynine was also isolated from this plant[173] and the effect of mitragynine on neurogenic contraction of smooth muscle was studied in guinea-pig vas deferens. The alkaloid inhibited the contraction of the vas deferens produced by electrical transmural stimulation. On the other hand, mitragynine failed to affect the responses to norepinephrine and ATP[173]. From the leaves of *Psychotria forsteriana* quadrigemine A, quadrigemine B, psychotridine and isopsychotridine C have been also isolated and their cytotoxic activity on cultured rat hepatoma cells (HTC line) have been reported[174]. These alkaloids showed a high toxicity on HTC.

2.15. The Amaryllis botanical family (Amaryllidaceae)

L-tyrosine-derived alkaloids are found in the Amaryllis (syn. Daffodil or Snowdrop) plant family (Amaryllidaceae Hill.), which is distributed throughout the world. This large botanical family (Table 16) comprises 50 genera and over 850 species. Lycorine has been detected in the Spider lily genus (*Lycorus* L.), and galanthamine in the Snowdrop genus (*Galanthus* L.). Galanthindole was isolated from *Galanthus plicatus* ssp. *byzantinus*[175,176]. Boit et al.[177] reported isolating four alkaloids from *Zephyranthes citrina* (Baker) belonging to the Amaryllis plant family. They were galanthine, haemanthamine, lycorine and lycorenine. More recently, Herrera et al.[51] also isolated oxomaritidine, maritidine and vittatine from this species. Oxomaritidine was reported for the first time by the authors. Alkaloids from *Z. citrina* (especially *haemanthamine*) have a clear bioimpact with inhibitory effects on the growth of HeLa cells and protein synthesis, as well as being a cytotoxic agent against MOLT 4 tumoural

Table 16 General botanical characteristics of the Amaryllis family[313,316]

Botanical Forms and Parts	Characteristics
Botanical forms	Herbs
Some typical genera	*Behria* *Crinum* *Cyrtanthus* *Haemanthus* *Hippeastrum* *Hymenocallis* *Leucojum* *Narcissus* *Zephyranthes*
Special characteristics	A medium-sized herbs Bulbs Reduced stems
Leaves	More or less linear from bulbs
Flowers	On a leafless stalk from the bulb or solitary flower (rarely) Corona Bisexual
Fruits	A capsule or a berry
Seeds	Small seeds Testa Embryo curved

cells[178,179]. Haemanthidine also has a powerful bioimpact as a cytotoxic agent against various human tumoural cell lines[180], and galanthine has a high inhibitory capacity with ascorbic acid biosynthesis in the potato[181]. Maritidine exhibits antineoplastic activity[182]. From *Pancratium sickenbergi*, hippadine, tris- pheridine, pseudolycorine, haemanthamine, norgalanthamine, haemanthidine, vittatine, 11-hydroxyvittatine, pancracine, lycorine, ent-6α-6β-hydroxybuphasine and (−)-8-*demethylmaritidine* have been isolated[183]. These alkaloids have antiviral, antitumoural, analgesic and insecticidal effects[183,184,185,186]. Three alkaloids, lycorine, homolycorine and 2-*O*-acetyllycorine, were recently isolated from the bulbs of *Leucojum vernum* by Szlávik et al.[187] and two new alkaloids, leucovernine and acetylleucovernine, by Forgo and Hohmann[141]. These alkaloids, similarly as many other new alkaloids from Amaryllidaceae, display antiviral activity. Shihunine and dihydroshihunine exist in *Behria tenuiflora* Greene. These alkaloids have been shown particularly to be inhibitors of Na^+/K^+ ATPase in the rat kidney[188]. Moreover, alkaloids from *Crinum stuhlmannii* Baker have also been reported. Machocho et al.[189] detected eight alkaloids (lycorine, kirkine, 9-*O*-demethylpluvine, ambelline, crinine, hamayne, crinamine and amabiline) in

this plant. Five alkaloids (lycorine, hamayne, vittatine, ismine and ungeremine) were isolated from *Hippeastrum solandriflorum* Herb[190].

2.16. The Oleaster botanical family (Elaeagnaceae)

The Oleaster botanical family (Elaeagnaceae Lindl.) has 3 genera and 50 species (Table 17). It is distributed around the world, mostly in the temperate climatic zone, and especially in the northern hemisphere. It also grows in eastern Australia, as well in some tropical and sub-tropical areas. The L-tryptophan-derived alkaloid elaeagine occurs in the Oleaster genus (*Elaeagnus* L.), and especially in the Russian olive (*Elaeagnus angustifolia* L.).

2.17. The Caltrop botanical family (Zygophyllaceae)

The L-tryptophan-derived alkaloid known as harman, and the Anthranilic acid–derived alkaloid known as harmine, occur in the Caltrop plant family (Zygophyllaceae R. Brown) (Table 18). It contains near 30 genera and more than 230 species, and grows worldwide, especially in the tropics, subtropics, warm temperate zones and dry areas. Harman and harmine occur in harmala pegan (*Peganum harmala* L.), the species belonging to the Pegan genus (*Peganum* L.). Alkaloids derived from acetate, dihydroschoberine and nitrabirine N-oxide have been found in the genus Nitraria (*Nitraria* Pall.) from the Siberian nitraria (*Nitraria sibirica* Pall.)[131]. In *Nitraria komarovii* (I. et. L.), komavine and acetylkomavine have been detected[132]

Table 17 General botanical characteristics of the Oleaster family[312]

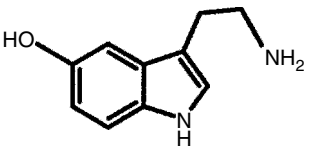
Botanical Forms and Parts	Characteristics
Botanical forms	Trees Shrubs
Leaves	Alternate or opposite Exstipule
Flowers	Regular Unisexual Calyx-tube
Fruits	Drupe-like
Seeds	With bony testa Ex-albuminous Straight embryo

Table 18 General botanical characteristics of the Caltrop family[312,313,316]

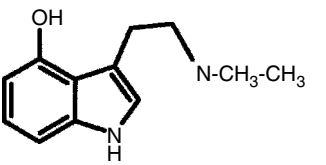
Botanical Forms and Parts	Characteristics
Botanical forms	Shrubs Some trees and sub-shrubs Annual herbs (rarely)
Some typical genera	*Balanites* *Guaiacum* *Fagonia* *Kallstroemia* *Larrea* *Porlieria* *Tribulus* *Zygophyllum*
Special characteristics	Mainly in warm and arid regions Stems often sympodial Stems joined at the nodes Xylem with vessels Tracheids and fibres Hairs
Leaves	Opposite 2-ranked Stipulate
Flowers	Regular with 4–5 free sepals and petals Bisexual
Fruits	Capsules Berries (rarely)
Seeds	One-seeded Endosperm present

2.18. Mushroom

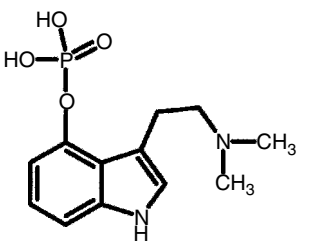
Alkaloids occur in many other botanical families. Moreover, there are alkaloids derived from L-tryptophan which occur in mushrooms genera: Psilocybe mushrooms (*Psilocybe*), Conocybe mushrooms (*Conocybe*), Haymaker's mushrooms (*Panaeolus*) and Stoparia mushrooms (*Stoparia*). Serotonin, psilocin and psilocybin are basic alkaloids derived from these mushrooms (Figure 19). They are powerful psychoactive and neurotransmitter compounds. Recreational use of hallucinogenic mushrooms has been reported in several European countries, including England, Norway, Finland, the Netherlands and Germany[191]. *Psilocybe semilanceata* and *Phanaeolus subbalteatus* proved to be the only psilocybin-containing fungi that can be gathered in middle and northern Europe in sufficient quantities to permit abuse[191].



Serotonin



Psilocin



Psilocybin

Figure 19. Basic alkaloids of mushrooms.

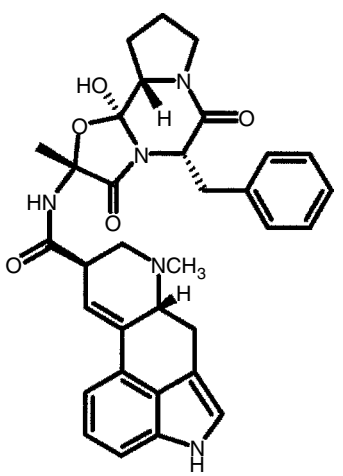
2.19. Moss

The moss alkaloids annotinine, lycopodine and cernuine occur in the genus *Lycopodium* L. Gao et al.[129] reported on the isolation of new alkaloids from the *Lycopodiaceae* family. Researchers working on *Huperzia serrata* (Thunb.), Trev. detected huperzine J, huperzine K and huperzine L. These alkaloids have potential effects on Alzheimer's disease. They occur not only in *H. serrata*, but also in other species belonging to the genus *Huperzia*. There are other similar alkaloids, such as huperzine A and its derivatives[124]). Moreover, Tan et al.[137] reported on several new alkaloids isolated from *H. serrata* (Thunb.). They are 11α-hydroxy-phlegmariurine B, 7α-hydroxyphlegmariurine B and 7α11α-dihydroxyphlegmariurine. Phlegmariurine was also reported in this species.

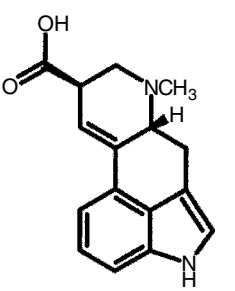
2.20. Fungus and bacter

The fungi *Aspergillus, Rhizopus, Penicillium* and *Claviceps* produce parasitic ergoline and ergotamine alkaloids. The ergot alkaloids derived from L-tryptophan in the fungus *Claviceps purpurea*, growing on grain in the ears of rye (*Secale*

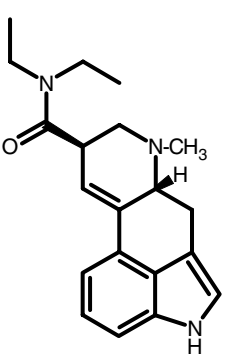
cereale), wheat *(Triticum aestivum) or* triticosecale (*Triticale*), are highly toxic (Figure 20). They have been used in the development of lysergic acid diethylamine, LSD, which is hallucinogenic and, in small doses, is used in the treatment of schizophrenia. Li et al.[192] reported isolating a new alkaloid, asterrelenin, from *Aspergillus terreus*. Moreover, from this fungi species, terretonin, territrem A and territrem B have been also isolated. Two new diastereomeric quinolinone alkaloids have recently been identified from fungus *Penicillium janczewskii* obtained from a marine sample[193]. These compounds showed a low to moderate general toxicity. Dalsgaard et al.[194] have recently reported of the isolation of communesins G and communesins H from the new species *Penicillium rivulum* Frisvad. The compounds were isolated by high-speed counter-current chromatography and preparative HPLC using UV-guided fractionation and subjected to antiviral, antimicrobial and anticancer activity tests. In contrast to all other known communesins, communesins G and H were found inactive in these activities studied[194]. Sasaki et al.[195] have isolated perinadine A from the cultured broth of



Ergotamine



(+)-lysergic acid



Lysergic acid diethylamide
LSD

Figure 20. Ergotamine and LSD.

the fungus *Penicillium citrinum* which was separated from the gastrointestine of a marine fish. Citrinadin A, a pentacyclic indolinole alkaloid, has been isolated from the cultured broth of this fungus, which was also separated from a marine red alga[196].

The bacteria *Pseudomonas* spp. produce tabtoxin and pyocyanine, alkaloids with a relatively powerful biological activity.

2.21. Animals

Alkaloids are also found in the animal kingdom, especially in millipedes, salamanders, toads, frogs, fish and mammals. They occur particularly in the genera *Saxidomus*, *Salamandra*, *Phyllobates*, *Dendrobates*, *Castor* and *Moschus*. Moreover, alkaloid molecules are found in such genera as *Solenopsis, Odontomachus, Glomeris* and *Polyzonium*. Many alkaloids have been recently isolated from marine environment, especially from the sponges[197]. The discovery of ptilomycalin A from the sponges *Ptilocaulis spiculifer* and *Hemimycale* spp. preceded the isolation of several analogues from other sponges such as *Crambe crambe, Monanchora arbuscula, Monanchora ungiculata* as well as from the some starfishes such as *Fromia monilis* and *Celerina heffernani*. From the Caribbean sponge *Monanchora unguifera* the guanidine alkaloids (batzelladine J, ptilomycalin A, ptilocaulin and isoptilocaulin) have been recently isolated[197]. Many of guanidine alkaloids display ichthyotoxicity, and antibacterial, antifungal and antiviral activity. Antiviral activity has been exhibited against Herpes Simplex virus (HSV-1) and also in inhibiting the HIV virus and cytotoxicity against murine leukemia cell lines (L1210) and human colon carcinoma cells (HCT-16). Segraves and Crews[198] reported on the isolation of six new brominated tryptophan derived alkaloids from two *Thorectidae* sponges: *Thorectandra* and *Smenospongia*. These alkaloids have also the wide ranging of biological activities and they are attractive compounds for potential applications.

Alkaloids occur in amphibians. These vertebrate animals are reliant on water for their reproduction. Some species live both in and out of water and others are exclusively aquatic species. There are three orders of amphibians: the Anura (syn. Salientia) with more than 4500 species of frogs and toads, the Urodela (syn. Caudata) with 450 species of newts and salamanders, and the Apoda (syn. Gymnophiona) with more than 160 species of worm-like organisms. The skin of amphibians contains alkaloids. Costa et al.[199] have reported on bufetenin from Anura species. This tryptamine alkaloid is widely spread as a component of chemical defence system in these species. Bufetenin acts as a potent hallucinogenic factor showing similar activity to LSD upon interaction with the 5HT2 human receptor[199]. This compound has been isolated from the skin of three arboreal amphibian species, *Osteocephalus taurinus, Osteocephalus oophagus* and *Osteocephalus langsdorfii*, from the Amazon and the Atlantic rain forests.

Moreover, it is known that toads belonging to the genus Melanophryniscus contain toxic alkaloids in their skin[200]. From *Melanophryniscus montevidensis*, alkaloids of the pumiliotoxin (PTX) group and indolizidines were isolated.

The lady bird (*Coccinellidae*) and other beetles also contain alkaloids. Examples are mentioned in Table 10. Conversely, some moths, such as the arctiid moth (*Utethesia ornatrix*), are dependent on alkaloids for defence. *Utethesia ornatix*, for example, sequesters pyrrolizidine alkaloids as a larva from the food plants of *Crotalaria*, belonging to the Fabaceae family[201]. *Longitarsus lateripunctatus* (Coleoptera, Chrysomelidae, Alticidae), a leaf beetle feeding on *Pulmonaria abscura* leaves, contained readily traceable quantities of pyrrolizidine alkaloids[117,202]. On the other hand, it is now known that some poisonous frogs (*Mantella*) digest alkaloids in their food. The ants *Anochetum grandidieri* and *Tetramorium electrum*, containing pyrrolizidine alkaloids, have been found in the stomachs of *Mantella* frogs[203]. The strawberry poison frog (*Dendrobates pumilio*) contains dendrobatid alkaloids that are considered to be sequestered through the consumption of alkaloid-containing anthropods distributed in the habitat[204]. Some pyrroloindole alkaloids, such as pseudophrynaminol, were found in the Australian frog (*Pseudophryne coriacea*)[205]. However, it is known that a diverse array of over 800 biologically active alkaloids have been discovered in amphibian skin[206]. With the exception of the samandarines and pseudophrynamines, all alkaloids appear to be derived from dietary sources. It has been discovered that the beetles are sources for batrachotoxins and coccinelline-like tricyclics and ants and mites for pumiliotoxins. Moreover, ants are sources for decahydroquinolines, izidines, pyrrolidines and piperidines. They are likely sources for histrionicotoxins, lehmizidines and tricyclic gephyrotoxins[206].

From North Sea Bryozoan (*Flustra foliacea*) several, brominated indole alkaloids have been isolated[207,208]. These include deformylflustramine and flustramine. Deformylfrustrabromine A and deformylfrustrabromine B have been shown to have affinities in the lower micromolar range with the neuronal nicotinic acetylcholine receptor (nAChR). As early as 1973, it was reported[151] that erythrinan alkaloids (β-erythroidine and dihydro-β-erythroidine) with neuromuscular transition blocking activity resembling the effects of curare had been found in the milk of goats (*Capra*) which grazed the leaves of *Erythrinia poeppigiana*[152]. The spectrum of alkaloids in mammals[209] ranges from isoquinoline derivatives, via β-carbolines, through to thiazolidines, arising from vitamin B_6, chloral and glyoxylic acid. For a long time, tetrahydroisoquinoline alkaloids were considered to be exclusively of plant origin. Bringmann et al.[209] suspected that the formation of such endogenous alkaloids occur naturally in man and mammals. The spontaneous formation of mammalian alkaloids, their further metabolic fate and their biological and medicinal roles are a key not only to a better understanding of metabolic diseases, but also to novel therapeutic concepts. In the case of animal species, it is necessary to check whether alkaloid molecules detected are endogenous or derived from exochemicals of

dietary origin. One example of this problem which could be mentioned occurs in the important alkaloid as morphine. The biosynthesis of this alkaloid by plants from the Poppy family (Papaveraceae) is practically resolved, and there are not many research problems. However, the opposite situation occurs in the case of animals. It was reported, and biochemical data was presented to prove, that this alkaloid can occur in animals and humans, in considerable quantities. The only question remaining concerns the origin of this alkaloid in the animal and human body: Is it endogenous? If yes, moreover, the evidence of existing enzymes needed for the biosynthesis of the alkaloid in animals should be presented and biosynthetical activity should be documented. Only after that can the occurrence of alkaloids in the animal species be accepted finally as an endogenous characteristic can without any conditions. On the other hand, there is evidence that animal and human bodies can produce endogenous alkaloids[210]. Mammalian alkaloids derive from L-tryptophan via biogenic amines such as dopamine, tryptamine and sorotonine. Small amounts of alkaloids are normal in mammals. When disease strikes, alkaloid levels rise steeply. The common mammalian alkaloids are harman, norharman, tetrahydroharman, harmalan, 6-metoxyharman, salsolinol, norlaudanosoline (THP), dideoxynorlaudanosoline 1-carboxylic acid and spinaceamines. Newly detected alkaloids are L-histamine derivatives[210,211]. Although it is generally accepted or strongly suggested that alkaloids occur in animal species, even as a common matter[209,210,211], the genetic origin of these compounds as purely animal is still under discussion. Many research groups are working on this problem. Certainly, alkaloid chemical and biological research is both very challenging and prospectively fascinating. Alkaloids in nature are a part of production and consumer (feeding) chains. Moreover, they contribute to species growth, pleasure and pathology. They are key to the processes of agressivity and defence by the species. Alkaloids are used in nature for many purposes, and by many species. *Homo sapiens* is just one of them.

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CHAPTER 2

Alkaloid Chemistry

Naturam mutare difficile
Seneca

Abstract: Alkaloid chemistry underlines the significance of the blocks, pathways and transamination reactions. The synthesis of the alkaloids is started from the acetate, shikimate, mevalonate and deoxyxylulose pathways. The main criterion for alkaloid precursor determination is the skeleton nucleus of the alkaloid. The following most important alkaloid nuclei exist: piperidine, indolizidine, quinolizidine, pyridon, pyrrolidine, imidazole, manzamine, quinazoline, quinoline, acridine, pyridine, sesquiterpene, phenyl, phenylpropyl, indole, β-carboline, pyrroloindole, iboga, corynanthe and aspidosperma. Their synthesis occur in different pathways, which consist of a series of reactions and compounds as well as enzymes. The sequence of all reactions leading to any alkaloid synthesis is divided into precursor, intermedia, obligatory intermedia, second obligatory intermedia, alkaloid and its postcursors. The structural development of piperidine, indolizidine, quinolizidine, pyrrolizidine, izidine, pyrrolidine, tropane, imidazole, quinazoline, acridone, pyridine, sesquiterpene pyridine, phenyl and phenylpropyl, indole and manzamine alkaloids is presented in this chapter. Moreover, chemistry, biochemistry and molecular biology models of alkaloid biogenesis in organisms is discussed and method of alkaloid analysis described. Alkaloids are natural products. They can be isolated, detected and modified. Modification of alkaloids by chemical and biological processes and bioengineering can produce new applications. Chemistry not only investigates alkaloids, their structures and activities, but also develops methods for their structural manipulation.

Key words: alkaloids, enzymes, genes, intermedia, metabolism, models, pathways, precursors, skeleton, synthesis

1. Alkaloids as secondary metabolism molecules

The precursors of true alkaloids and protoalkaloids are aminoacids (both their precursors and postcursors), while transamination reactions precede pseudoalkaloids (Tables 1 and 10). It is not difficult to see that from all aminoacids only a small part is known as alkaloid precursors (Table 19). Both true and proto alkaloids are synthesized mainly from the aromatic amino acids, phenylalanine, tyrosine (isoquinoline alkaloids) and tryptophan (indole alkaloids). Lysine is the

Table 19 Amino acids and their participation in alkaloid synthesis

Group of Amino Acids/Amino acids	Alkaloid Type	Participation in Alkaloid Synthesis
Protein amino acids		
L-alanine		
L-arginine	Arginine-derived alkaloids	True alkaloids
L-asparagine		Marine alkaloids
L-aspartic acid		
L-cysteine		
L-glutamine		
L-glutamic acid		
L-glycine		
L-histidine	Histidine-derived alkaloids	True alkaloids
		Imidazole alkaloids
		Manzamine alkaloids
L-isoleucine		
L-leucine		
L-lysine	Lysine-derived alkaloids	True alkaloids
		Piperidine alkaloids
		Quinolizidine alkaloids
		Indolizidine alkaloids
L-methionine		
L-phenylalanine	Phenylalanine-derived alkaloids	True alkaloids
		Phenylethylamino alkaloids
		Phenylisoquinoline alkaloids
		Amaryllidaceae alkaloids
L-proline		
L-serine		
L-threonine		
L-tryptophan	Tryptophan-derived alkaloids	True alkaloids
		Indole alkaloids
		Quinoline alkaloids
		β-carboline alkaloids
		Pyrroloindole alkaloids
		Ergot alkaloids
		Iboga alkaloids
		Corynanthe alkaloids
		Aspidosperma alkaloids
		Protoalkaloids
		Terpenoid indole alkaloids
L-tyrosine	Tyrosine-derived alkaloids	True alkaloids
		Phenylethylamino alkaloids
		Simple tetrahydroiso quinoline alkaloids

Table 19 (Continued)

Group of Amino Acids/Amino acids	Alkaloid Type	Participation in Alkaloid Synthesis
		Phenethylisoquinoline alkaloids Amaryllidaceae alkaloids
		Protoalkaloids Phenylethylamino alkaloids
L-valine		
Non-protein aminoacids		
L-ornithine	Ornithine-derived alkaloids	True alkaloids Pyrrolidine alkaloids Tropane alkaloids Pyrrolizidine alkaloids
Anthranilic acid	Anthranilic acid-derived alkaloids	True alkaloids Quinazoline alkaloids Quinoline alkaloids Acridine alkaloids
Nicotinic acid	Nicotinic acid–derived alkaloids	True alkaloids Pyridine alkaloids Sesquiterpene pyridine alkaloids

precursor for piperidine and quinolizidine alkaloid, and ornithine for pyrrolidine, pyrrolizidine and tropane alkaloids. Pseudoalkaloids are synthesized from other compounds, for example acetate in the case of piperidine alkaloids (coniine or pinidine). Alkaloids are derived from the amino acid in L-configuration (protein aminoacids) and from non-protein amino acids such as ornithine. However, it is important to note that alkaloids should be derived directly from the precursors of amino acids as, for example, in the case of anthranilic acid (the precursor of trypthophan from the shikimate pathway) or acetate (the precursor of lysine via α-ketoadipic acid and transamination in some algae and fungi). The precursors' substrata for alkaloids can also derive directly from the degradated parts of amino acids as, for example, in the case of nicotinic acid (Niacin or Vitamin B_3), which is the precursor and a key part of coenzymes NAD^+ and $NADP^+$ in the degradation process of tryptophan. Alkaloid chemistry is clearly directly connected with the protein aminoacids, their precursors or postcursors in different pathways. It is difficult to find the exception to this rule. Although ornithine is a non-protein amino acid, in reality its precursor is L-glutamate (in plants) and L-arginine (in animals). The importance of ornithine as the precursor of alkaloids is not that this amino acid is non-protein, but just that it is postcursor of the protein amino acid (L-glutamate). Although the pathways of alkaloids are at

present relatively well understood from the point of view of organic chemistry, there remain many questions relating to the biological nature of alkaloid synthesis. Mahler and Cordes[212] considered and discussed three general examples of the synthesis of alkaloids from amino acids: (1) synthesis of the pyrroline ring and derived alkaloids from ornithine; (2) synthesis of the piperidine ring and derived alkaloids from lysine and (3) synthesis of isoquinolizidine alkaloids from tyrosine.

Nowadays a lot of new data is available on chemical alkaloid research, but the above-mentioned three classic examples are still important in the understanding of alkaloid synthesis. Certainly, the present trend in alkaloid chemistry is to underline the significance of the blocks, pathways and transamination reactions in alkaloid synthesis[32]. However, a presentation of chemical pathways and the synthesis of true alkaloids, protoalkaloids, and pseudoalkaloids is in many cases impossible without the characterization of their precursors. As already stated, protein amino acids, with their precursors and postcursors in different pathways, with or without transamination reactions, are generally substrates for alkaloids. This concept is very important because it highlights the probable role of alkaloids in metabolisms and underlines the significance of protein amino acids, their synthesis and degradation. Alkaloids exist in some kind of balance between distribution and degradation within amino acid production in the organisms producing them. This claim may provoke some controversy, but the connection between the amino acid pathway and the alkaloid synthesis is so evident that it cannot be omitted.

The similarity of the alkaloid to each molecule from the secondary metabolism is a consequence of the derivation process in the constructed active block. There are only four basic active blocks for the secondary compounds. Acetyl coenzyme A (acetyl CoA) is used in the acetate pathway, and shikimic acid in the shikimate pathway. The third block, mevalonic acid, is active in the mevalonate pathway, and the last, 1-deoxyxylulose 5-phosphate, key to the deoxyxylulose phosphate pathway (Figures 21–22). The theory of secondary compound synthesizing blocks is one of the most important in chemistry, as well as being interesting from a biological perspective. The establishment of the block needs the energy, and the primary metabolism is the source of it. On the other hand, the building blocks for the secondary metabolism are strongly regulated and this regulation seems to be genetically determined. The building blocks link the primary and secondary metabolisms. Acetyl CoA is derived from pyruvic acid (the product of a glycolytic pathway) and used in the acetate pathway (Figure 21). Pyruvate is derived primarily from glucose 6-phosphate. Another source of this three-carbon α-keto acid are the conversion reactions of oxaloacetate, lactate and alanine (Figure 22). Acetyl CoA is synthesized by the oxidative decarboxylation of peruvate and the β-oxidation of fatty acids, as well as from ketogenic amino acids. A part of the acetyl CoA can be exported to the cytosol in the form of citrate, thus participating in the fatty acid synthesis.

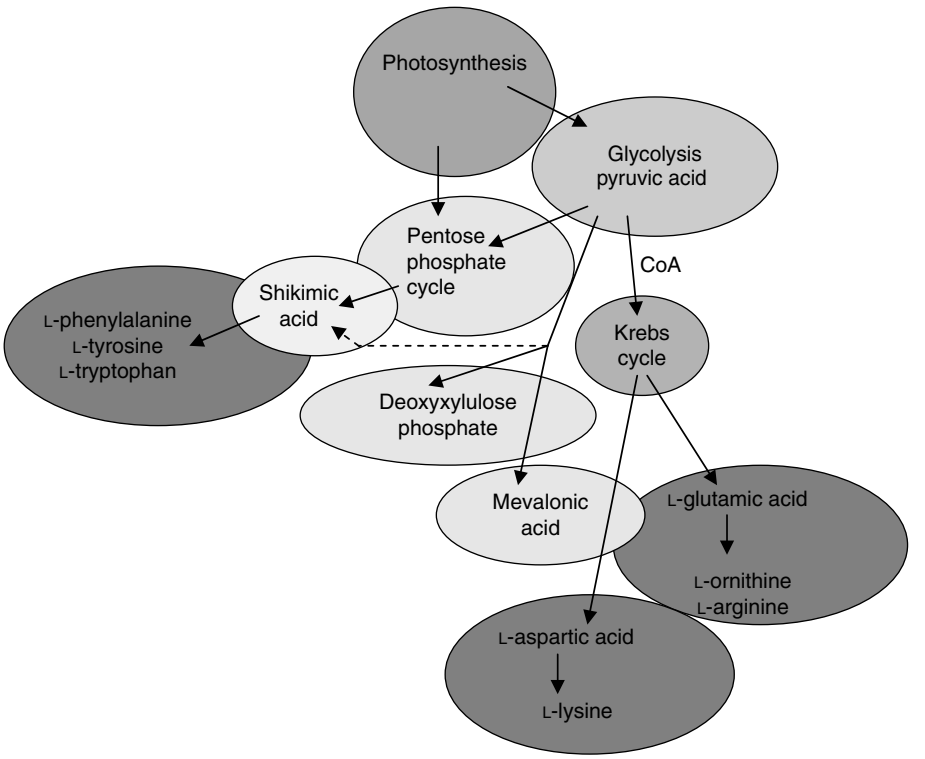


Figure 21. Secondary metabolism blocks and amino acid derivation. Note that shikimic acid can be derived directly from photosynthesis and glycolysis through the pentose phosphate cycle, or alternatively as a pyruvic acid postcursor.

However, in mammals acetyl CoA cannot be converted back into pyruvate. What is most important regarding alkaloid synthesis is that the pyruvate metabolism is a base for its alkaloid pathway precursors (Figure 22). As a group of specific molecules, part of the alkaloids is synthesized in the shikimic pathway. However, alkaloids are not the main product of this pathway, from which many phenols and lignans are also derived. Moreover, the shikimic pathway is only a source for aromatic amino acids such as phenylalanine, tyrosine and tryptophan. These amino acids are known to be the precursors of some alkaloids. Other amino acids are alkaloidal precursors from the different pathways. Ornithine is the postcursor of L-glutamic acid and L-lysine is postcursor of L-aspartic acid. Both glutamic and aspartic acids originate from the Krebs cycle. However, the shikimic and acetate pathways are very important as the original chain of alkaloids. Certainly, steroid alkaloids originate from the activity of mevalonate and deoxyxylulose phosphate pathways. This means that different alkaloids may derive from different secondary metabolism blocks and pathways. Alkaloid chemistry is, therefore, a part of the total secondary metabolism and has its roots

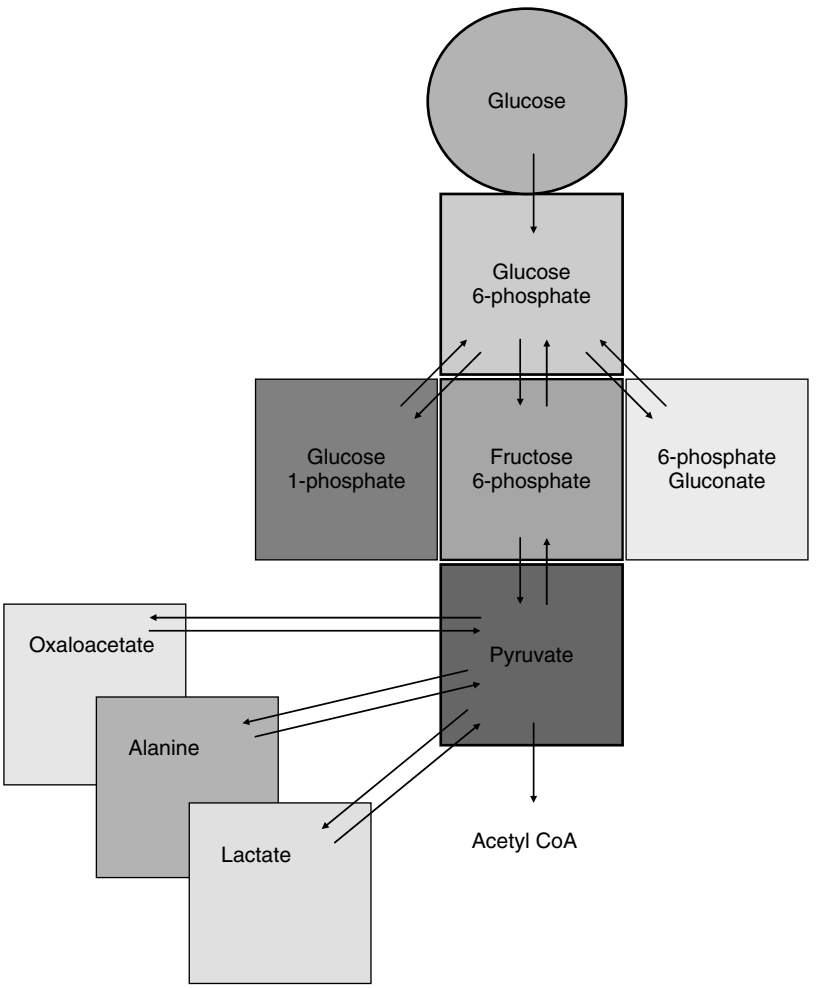


Figure 22. Pyruvate derivation and acetyl CoA synthesis. Observe that pyruvate, and subsequently the acetyl CoA pathway, has chain roots in the primary metabolism. Pyruvate can also be synthetized by conversion reactions. The secondary acetyl CoA is constructed as a building block on the pyruvate and glycolysis.

in the primary metabolism, photosynthesis and the Krebs cycle. The CoA and shikimic acid remain very important blocks for the alkaloids and their chemistry.

2. Synthesis and metabolism

Each biomolecule of a chemical nature in living organisms has its own synthesizing, transformational and interconverting processes. Therefore, the formation of the ring of the alkaloid molecule, and the flow of the nitrogen atom into this molecule, is the basic point for understanding alkaloid synthesis and its metabolism.

Alkaloid biosynthesis needs the substrate. Substrates are derivatives of the secondary metabolism building blocks: the acetyl coenzyme A (acetyl-CoA), shikimic acid, mevalonic acid and 1-deoxyxylulose 5-phosphate (Figure 21). The synthesis of alkaloids starts from the acetate, shikimate, mevalonate and deoxyxylulose pathways. The acetyl coenzyme A pathway (acetate pathway) is the source of some alkaloids and their precursors (e.g., piperidine alkaloids or anthranilic acid as aromatized CoA ester (antraniloyl-CoA)). Shikimic acid is a product of the glycolytic and pentose phosphate pathways, a construction facilitated by parts of phosphoenolpyruvate and erythrose 4-phosphate (Figure 21). The shikimic acid pathway is the source of such alkaloids as quinazoline, quinoline and acridine.

The mevalonate pathway is based on mevalonic acid (three molecules of acetyl-CoA) which is closely related to the acetate pathway, while the deoxyxylulose phosphate pathway is based on a combination of pyruvic acid and glyceraldehyde 3-phosphate (both from the glycolytic pathway). Together, mevalonate and deoxyxylulose phosphate pathways produce terpenoid and steroid compounds. However, it is important to note that the Krebs cycle pathway is also key to many precursors of alkaloids. Ornithine, a postcursor of L-arginine in animals and of L-glutamate in plants, and, for example, L-lysine, a principal protein amino acid, deriving from the Krebs cycle pathway compound, are useful examples of the role of the Krebs cycle for alkaloid precursors (Figure 21). Moreover, there are other sources of alkaloid substrates, particularly in purine alkaloids. Figure 23 represents the general scope of alkaloid synthesis in the metabolic system of organisms and their energy production. Enzymatic activity is very important in the primary metabolism of glycolysis and the Krebs cycle. Pyruvic acid and CoA are key compounds in the synthesis of alkaloid precursors. Moreover, these precursors (amino acids) can be derived from different points in the glycolysis and Krebs cycles. Consequently, the synthesis of alkaloids as a secondary metabolic activity is a very challenging research subject. Generally, it is recognized in the literature that alkaloid metabolism in animals, and especially in mammals, is closely related to that of plants[210]. However, some exceptions exist. Figure 23 shows two means of L-ornithine synthesis.

In plants, this non-protein amino acid is derived from L-glutamate and in animals from L-arginine. Moreover, Figure 23 demonstrates that synthesis of alkaloids is complicated by the ability of the same amino acid to synthesize many different alkaloids.

2.1. Skeleton diversity

The skeleton nucleus of the alkaloid is the main criterion for alkaloid precursor determination. Many skeletons are produced in the process of alkaloid synthesis. Figure 24 illustrates some nuclei and skeletons supplied in the synthesis. Alkaloid

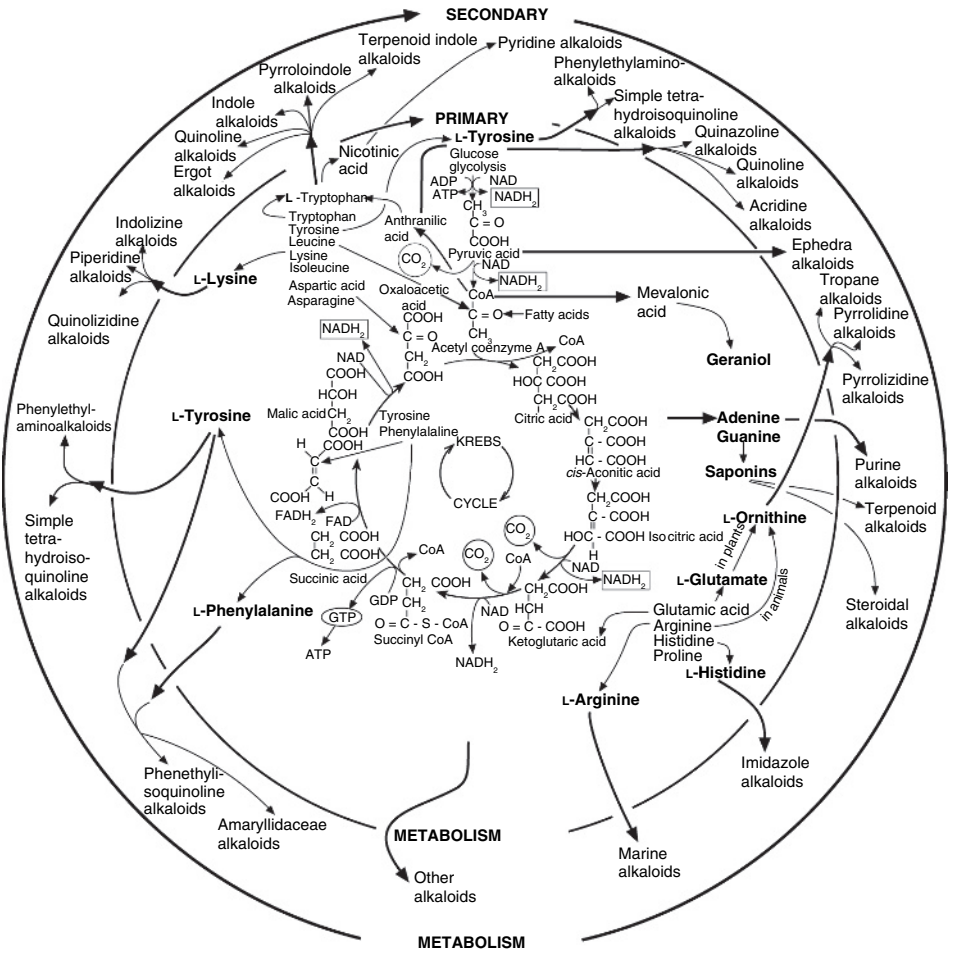


Figure 23. General scheme of alkaloid synthesis.

relates only to the reactions of this stage of the synthesis. Moreover, skeletons can change their form during the synthesis. A case in point is the synthesis of quinine, where the indole nucleus is reconstructed to form the quinoline nucleus.

During biosynthetic processes, L-lysine can produce at least 4 alkaloid skeletons with different alkaloid nuclei: piperidine nucleus (C_5N skeleton), indolizine nucleus (C_5NC_3 skeleton), quinolizidine nucleus (C_5NC_4 skeleton) and pyridon nucleus (with the variated quinolizidine nucleus and C_5NC_4 skeleton). The ability of L-lysine to provide different alkaloid nuclei is related to the role of this DNA amino acid in plant and animal organisms. In plants, this amino acid is an endogenous compound synthesis that is used in both primary and secondary metabolisms. In the animal kingdom, lysine is principally an exogenous amino acid, mainly of dietary origin. Consequently, L-lysine-derived

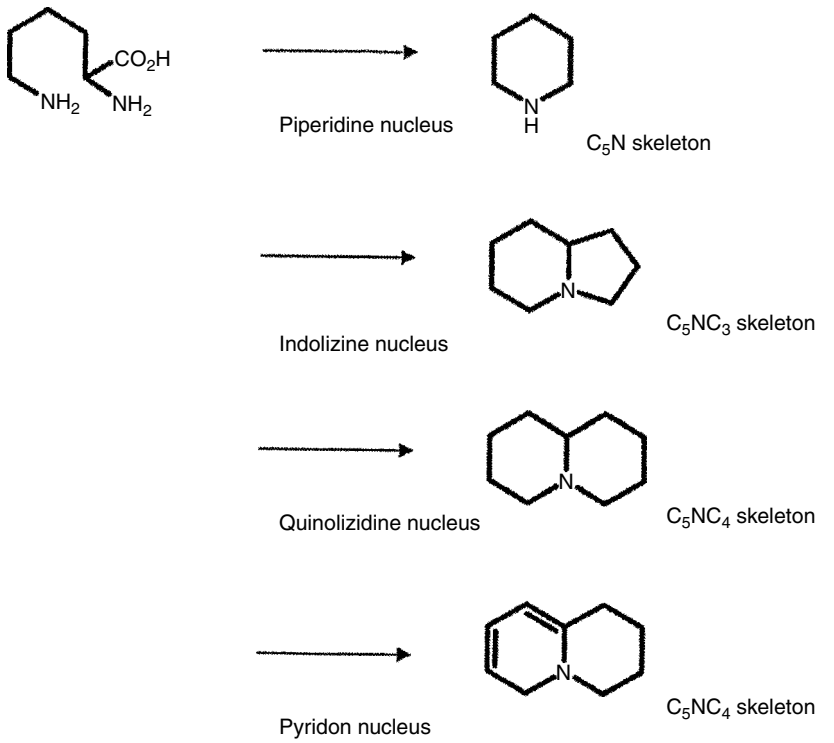


Figure 24. L-lysine-derived nuclei.

alkaloids with different type of skeletons have a very different biological impact on the organisms. Piperidine, indolizine, quinolizidine and pyridon alkaloids have different effects on the digestive and nervous systems of herbivores. Their acute toxicity and ability to temporarily or permanently change cell numbers or the functional metabolism differ markedly. Moreover, skeleton structure also influences taste. In this regard, the position of the N atom is important. In all the skeletons derived from L-lysine, the position of N is the same as in the substrate.

In the case of the izidine alkaloids (Figure 25) the position of the nitrogen atom is the same, but the number of C atoms should be different. The difference lies in the rings of these alkaloids. They represent different structural groups of alkaloids, although they have two rings and are two cyclic compounds. This structural point is key to their biological activity. Pyrrol-, indol- and quinolizidine rings display structural similarities but diversity in both their origin and, what is very important, bioimpact. Even small differences in nucleus can effect huge changes in the alkaloid activity[213].

L-ornithine (Figure 26) produces the pyrrolidine nucleus (C_4N skeleton). This nucleus is also constructed within tropane alkaloids (C_4N skeleton +) (Figure 26). Alkaloids which contain the pyrrolidine and tropane nuclei are

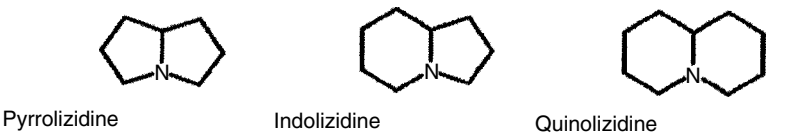


Figure 25. Nuclei and skeletons of izidine alkaloids.

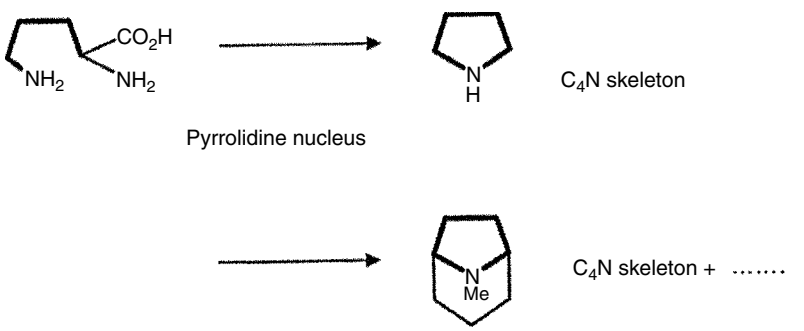


Figure 26. The source and forms of the pyrrolidine ring.

very vigorous in their biological activity. Common pyrrolidine nucleus alkaloids include hygrine, hyoscyamine, cocaine, cuscohygrine and so on. The best-known plants alkaloids with pyrrolidine nuclei are henbane (*Hyoscyamus niger*), deadly nightshade (*Atropa belladonna*) and Jamestown weed (*Datura stramonium*).

The imidazole nucleus (Figure 27) is supplied during alkaloid biosynthesis by L-histamine. Typical alkaloids with the imidazole nucleus include histamine,

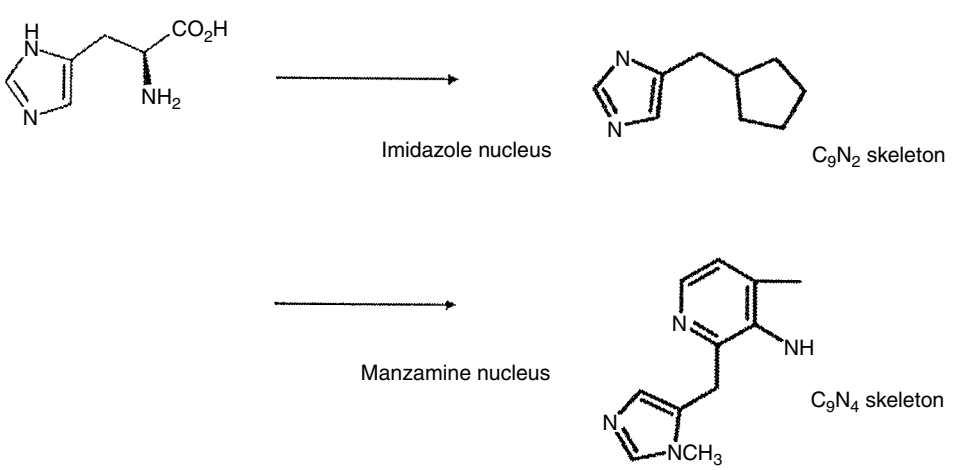


Figure 27. L-histidine and the nuclei of imidazole and manzamine alkaloids.

histidine, procarpine and pilosine. They are found as basic alkaloids in two principal families, Cactaceae and Rutaceae.

The basic alkaloid in *Pilocarpus jaborandi* (Rutaceae) is pilocarpine, a molecule of which contains an imidazole nucleus and is also used as a clinical drug. During alkaloid synthesis, L-histidine can produce the manzamine nucleus (Figure 27). These alkaloids are quite widespread, though they were first isolated in the late 1980s in marine sponges[57]. They have an unusual polycyclic system and a very broad range of bioactivities. Common alkaloids with this nucleus include manzamine A, manzamine B, manzamine X, manzamine Y, sextomanzamine A and so on.

In the case of C_6C_2N skeletons (Figure 28) converted from the antranilic acid into the alkaloids quinazoline, quinoline and acridine, nuclei are constructed inside the cyclic system. Only this part is derived from the precursor, while the rest of the ring system comes from other sources[32]. Alkaloids with the C_6C_2N skeleton occur in many species, such as *Peganum harmala, Dictamus albus, Skimmia japonica* and *Ruta graveolens*. The best known alkaloids containing these nuclei are peganine (vasicine), dictamine, skimmianine, melicopicine, acronycine and rutacridone.

All alkaloids with the C_6C_2N skeleton are bioactive; since they constitute a very large group of compounds, they display different properties. As already stated, anthranilic acid provides these alkaloids with a nucleus (Figure 28) but the rest of the skeleton comes from other donors. Simply this can have an influence on the characteristic activities of alkaloids.

Nicotinic acid (Figure 29) provides alkaloids with the pyridine nucleus in the synthesizing process. This nucleus appears in such alkaloids as anabasine, anatabine, nicotine, nornicotine, ricine and arecoline. Moreover, many alkaloids

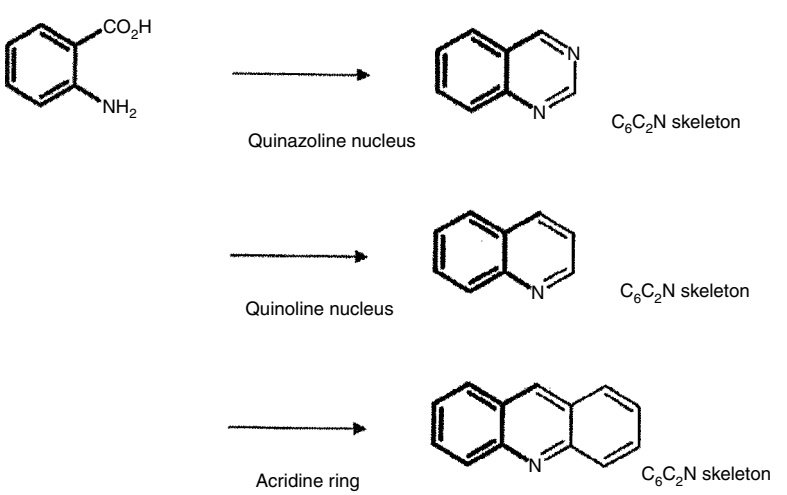


Figure 28. The nuclei produced by anthranilic acid in alkaloids.

Nicotinic acid = Niacin = Vitamin B_3

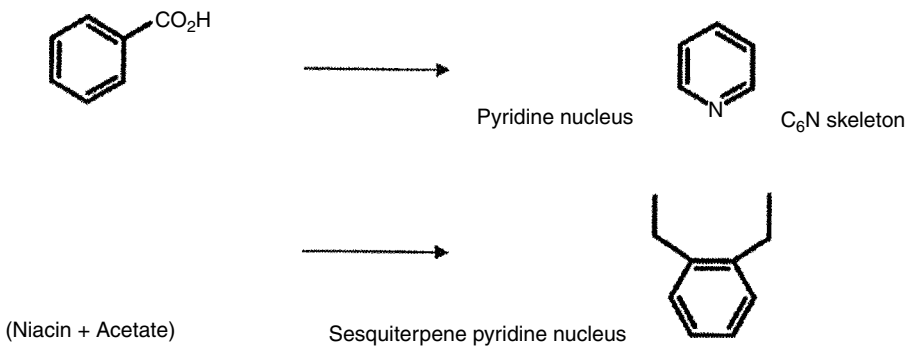


Figure 29. The nucleus of alkaloids derived from nicotinic acid.

contain the pyridine nucleus as part of their total skeleton. For example, anabasine is derived from nicotinic acid and lysine[18]. Alkaloids with the pyridine nucleus occur in such plants as tobacco (*Nicotiana tabacum*), castor (*Ricinus communis*) and betel nuts (*Areca catechu*). The sesquiterpene pyridine nucleus derives partly from nicotinic acid, and partly from the acetate pathway. There are more than 200 known alkaloids in this group[58].

In the alkaloid synthesis, L-phenylalanine (Figure 30) provides to alkaloid the phenyl or phenylpropyl nucleus. These kinds of nuclei occur in cathionine, cathine, ephedrine, pseudoephedrine and norpseudoephedrine. Such alkaloids are found especially in many species of *Ephedra*. Natural alkaloid molecules from these plants have similar properties to synthetic compounds used as narcotics (e.g., amphetamine).

L-tyrosine (Figure 31) is an aromatic amino acid (similar in compound from L-phenylalanine) which also provides phenyl (Figures 30–31) and phenylpropyl

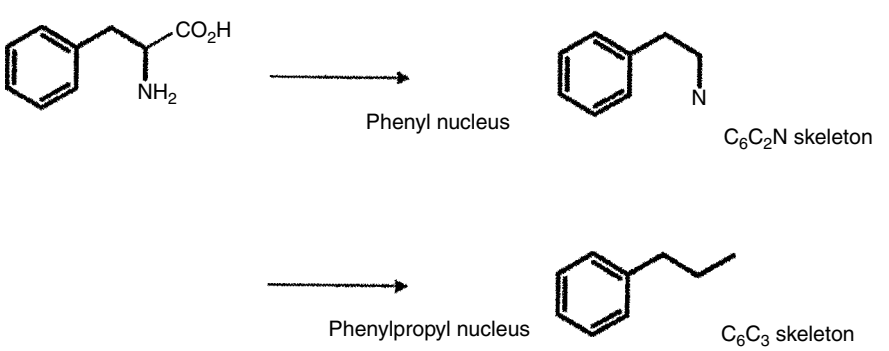


Figure 30. L-phenylanine-derived nuclei in alkaloid biosynthesis.

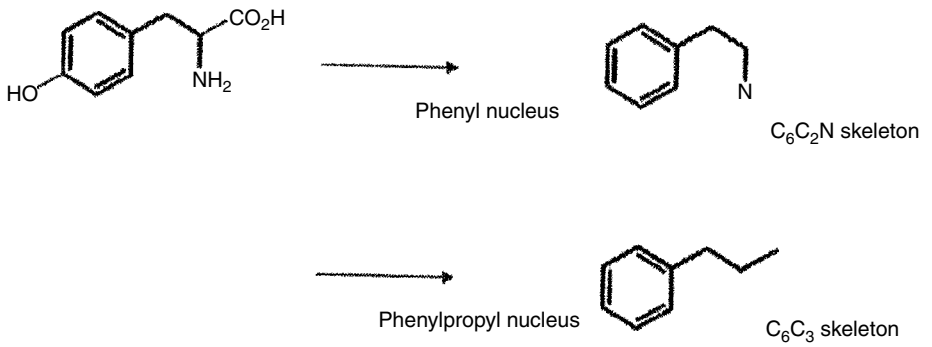


Figure 31. Nuclei supplied to alkaloids by L-tyrosine in the synthesizing process.

(Figures 30–31) nuclei for alkaloids. Molecules containing nuclei from L-tyrosine include, for example, mescaline, anhalamine, papaverine, curare and morphine. They are biologically very strong natural compounds and occur relatively widely in the plant kingdom (Table 10).

Another aromatic amino acid, L-tryptophan (Figure 32), contains the indole nucleus. This nucleus is synthesized in a large number of alkaloids, such as psilocin, psilocybin, harmine, catharanthine, reserpine, ajmalicine, vindoline, vincristine, strychnine, quinine, ergotamine and other ergot alkaloids. The alkaloid nucleus as a fragment of the precursor structure given to the new molecule during its synthesis is very interesting and relatively unknown. The evident original donor of each carbon in the alkaloid ring is still not exactly comprehended. However, along the alkaloid pathways from the secondary building blocks to the synthesis of alkaloids is a long chain of reactions. The nucleus translocation into the alkaloid molecule is the most important step in this alkaloid synthesis.

The indole nucleus can change during the synthesizing reaction into quinoline nucleus (Figure 32). Moreover L-tryptophan, the precursor, provides both β-carboline and pyrroloindole nuclei. Iboga, Corynanthe and Aspidosperma nuclei also originate from L-tryptophan (Figure 32). Alkaloids with nuclei derived from this amino acid tend to be very active compounds with a relatively widespread provenance in nature (Table 10).

2.2. Ornithine-derived alkaloids

Ornithine is a metabolically quite active amino acid, and the important precursor of pyrrolidine nucleus, which is found in pyrrolizidine alkaloids. Ornithine itself is a non-protein amino acid formed mainly from L-glumate in plants, and synthesized from the urea cycle in animals as a result of the reaction catalyzed by enzymes in arginine.

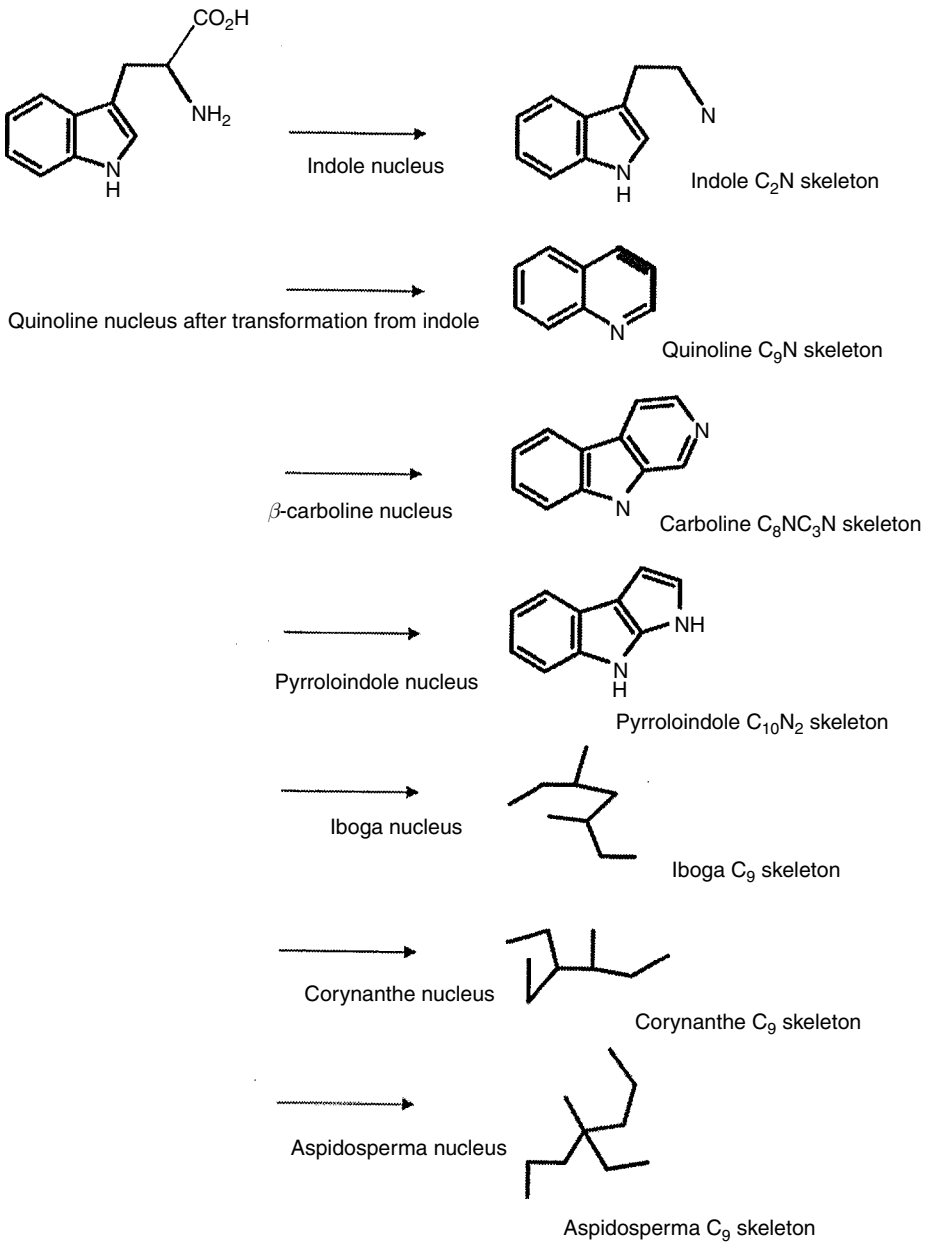


Figure 32. The L-tryptophan-supplied nucleus during synthesis.

The synthesis of alkaloids from L-ornithine starts with decarboxylation by the Pyridoxal Phosphate (PLP) to putrescine (Figure 33) and putrescine metylation by *S*-Adenosylmethionine (SAMe) to *N*-methylputrescine. The SAM is a naturally occurring reaction, when the departing groups convert

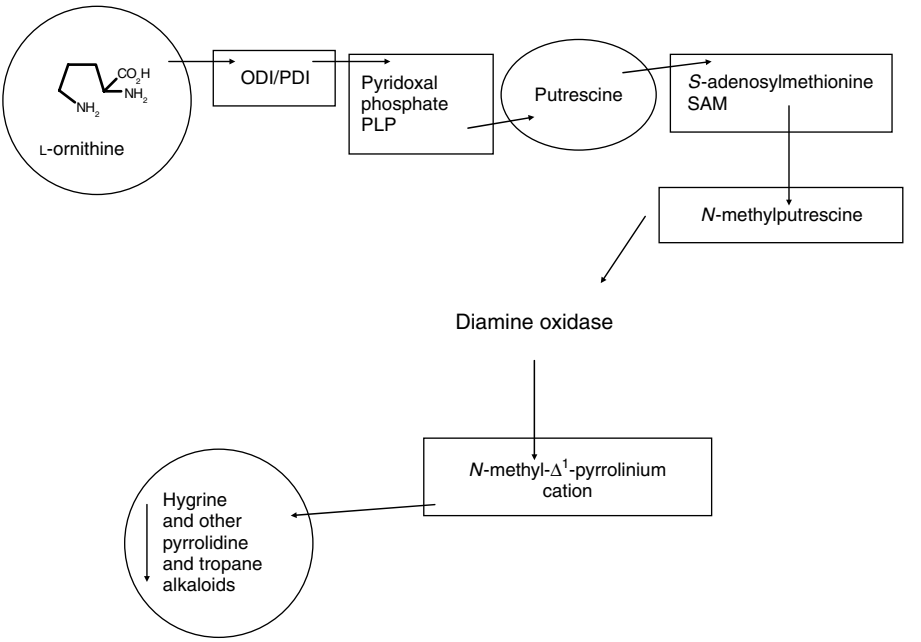
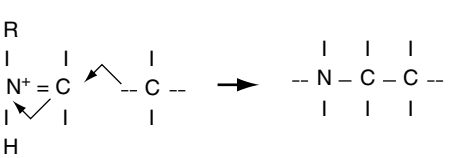


Figure 33. Synthesis of alkaloids from ornithine. Alkaloids are derived via putrescine or glutamic semialdehyde. At least two enzymes, ODL (Ornithine decarboxylase) or PDL (Pyrroline decarboxylase), are needed.

L-methionine to *S*-adenosylmethionine. In this process a positively charged sulphur is produced and facilitates the nucleophilic reaction. By the activity of diamine oxidase, the *N*-methyl-Δ^1-pyrrolinium cation is formed and after that the first alkaloid, hygrine. From hygrine, by way of acetyl CoA, hydrolysis and intramolecular Mannich reactions, other pyrrolidine and tropane alkaloids are synthesized: cuscohygrine, hyoscyamine or tropinone, tropine and cocaine. The Mannich reaction involves the combination of an amine, an aldehyde or a ketone with a nucleophilic carbon. This reaction is typical in alkaloid synthesis, and can be written as follows:



The synthesis of tropine from tropinione requires dehydrogenase $NADPH^+$. Similarly, the synthesis of cocaine requires the Mannich reaction, SAM and $NADPH^+$. Putrescine is a biogenic amine. Other biogenic amines also participate in alkaloid synthesis, for example cadaverine in the case of lysine alkaloids. Aniszewski et al.[214] drew attention to the fact that the various biogenic amines

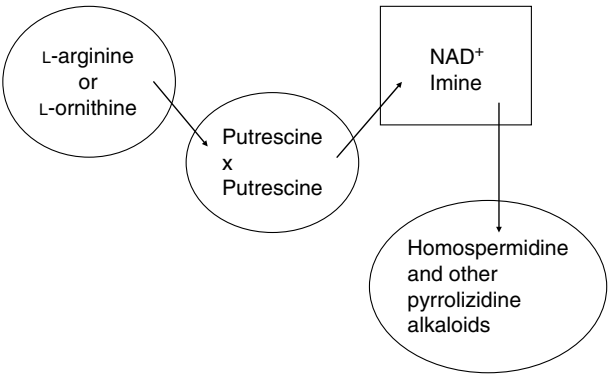


Figure 34. Synthesis pathway of the pyrrolizidine alkaloids from L-ornithine or L-arginine.

that actively participate in the biosynthetic process of alkaloids play a role in the equilibrium between basic nitrogen compounds. Moreover, enzyme participation in pyrrolidine and tropane alkaloid synthesis has also been noted. From the L-ornithine, and alternatively also from L-arginine pyrrolizidine alkaloids are synthesized (Figure 34). The L-arginine, alternative pyrrolizidine precursor is based on its ability to change into L-ornithine, and alternatively into putrescine, via coenzyme pyridoxal phosphate (PLP) and agmatine. In the synthetic pathway to homospermidine, which is the first pyrrolizidine alkaloid in this synthesis chain, two molecules of putrescine are condensed by the enzyme NAD^+ into imine before NADH converts it to homospermidine. From homospermidine, the synthesis chain continues across oxidative and base formation, and the Mannich reactions, to synthesize other alkaloids, such as retronecine and its diester senecionine. This synthesis pathway is also characteristic for heliotridine, laburine, lycopsamine and indicine-N-oxide. All these alkaloids contain the pyrrolidine nucleus, which is derived from ornithine or its precursors and postcursors.

2.3. Tyrosine-derived alkaloids

Tyrosine is an important precursor of alkaloids with the phenyl and phenylpropyl nuclei. There are four basic alkaloid pathways.

2.3.1. Mescaline pathway

This alkaloid pathway starts with PLP decarboxylation to tyramine, and subsequently via SAM dimethylation synthesizes hordeine (Figure 35).

The second synthesis pathway from L-tyrosine is to dopamine across hydroxylation patterns and PLP activity. Dopamine is a very important compound in the

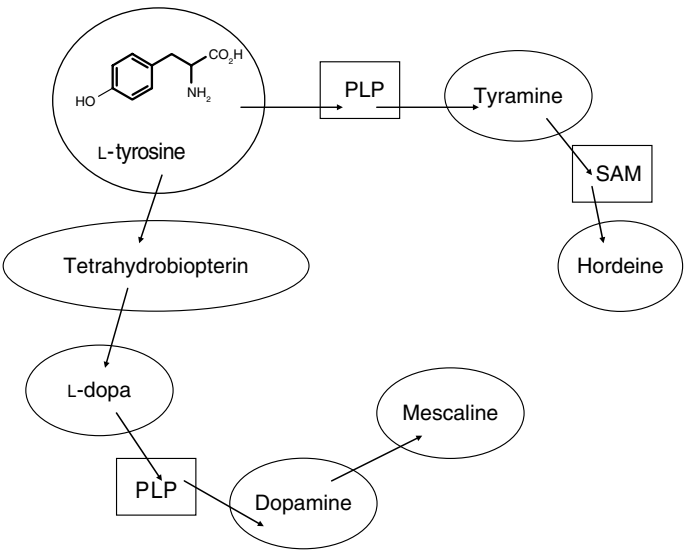


Figure 35. Synthesis of hordeine and mescaline.

synthesis of alkaloids, especially in animals. Only dopamine can be converted to an other alkaloid, for example mescaline. Anhalamine, anhalonine and anhalonidine can also be synthesized in this way. Like mescaline, they are typical of simple tetrahydroisoquinoline alkaloids.

2.3.2. Kreysigine and colchicine pathway

From L-tyrosine, and alternatively also from L-phenylalanine, kreysigine synthesis begins with dopamine (Figure 36). *S*-autumnaline is derived via a Mannich-like reaction. *S*-autumnaline is converted into floramultine by the oxidative coupling. Subsequently, the kreysigine is synthesized through the

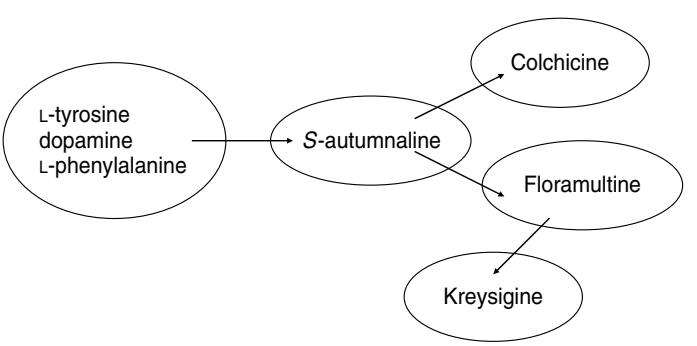


Figure 36. Synthesis pathway of kreysigine and colchicine.

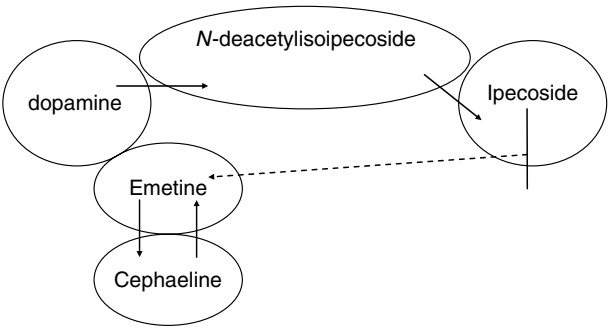


Figure 37. Emetine and cephaeline synthesis pathway.

activities of SAM. From *S*-autumnaline, other alkaloids can also be derived. The destination of this pathway is colchicine (Figure 36).

2.3.3. Dopamine – cephaeline pathway

From dopamine, the pathway of tetrahydroisoquinoline alkaloids, such as emetine and cephaeline (Figure 37), also begins. Dopamine and secologanin undergo a Mannich-like to produce *N*-deacetylisoipecoside and ipecoside, and after hydrolysis and transformation, this is converted to emetine and cephaeline.

2.3.4. Galanthamine pathway

From L-tyrosine, or alternatively from L-phenylalanine, there is one further alkaloid biosynthesis pathway. This is the galanthamine pathway (Figure 38). Galanthamine synthesizes with tyramine, norbelladine, lycorine, crinine, *N*-demethylnarwedine and *N*-demethylgalanthamine. Schiff base and reduction reaction, oxidative coupling and enzyme NADPH and SAM activity occur in this pathway. Schiff base is a reaction for the elimination of water in formation with the C−N bonds process.

From norbelladine, through the activity of the SAM, the 4′-0-methylnorbelladine synthesizes, and again is transformed to lycorine, crinine and, by oxidative coupling, to *N*-demethylarwedine, which is the object of enzyme NADPH activity. Galanthamine is synthesized by transformation trough the activity of the SAM from *N*-demethylgalanthamine.

2.4. Tryptophan-derived alkaloids

Alkaloids derived from L-tryptophan hold the indole nucleus in a ring system. The ring system originates in the shikimate secondary compounds building block and the anthranilic acid pathway. It is known that the shikimate block,

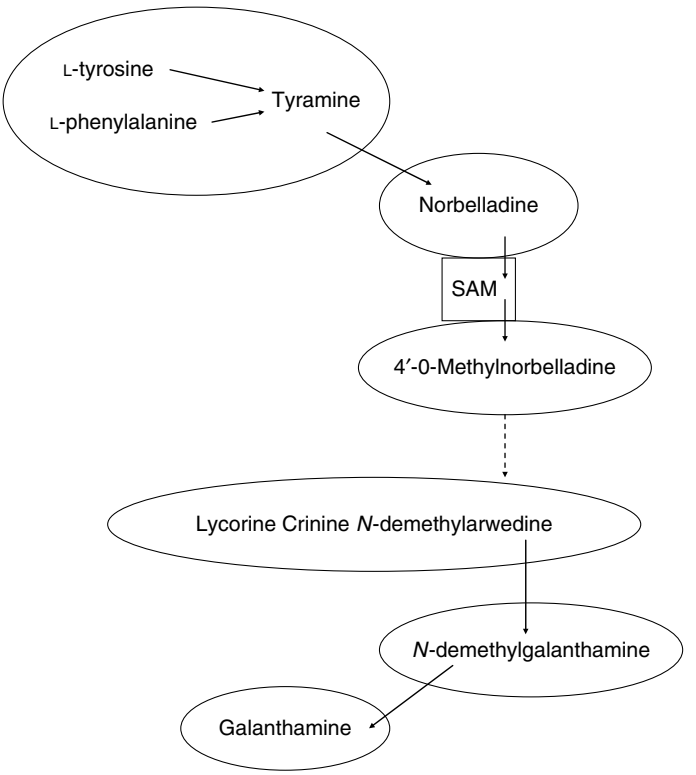


Figure 38. Galanthamine synthesis pathway.

in general, and anthranilic acid, in particular, are precursors to many indole alkaloids. However, there are many rearrangement reactions which can convert the indole ring system into a quinoline ring.

2.4.1. Psilocybin pathway

In this pathway, L-tryptophan is enzymatically transferred to tryptamine and subsequently, through the activity of SAM, to psilocin. By the reaction of phosphorylation, psilocin is converted into psilocybin (Figure 39).

Psilocybin and psilocin are psychoactive hallucinogenous alkaloids synthesized from the small mushroom genus *Psilocybe* spp. On average, the concentration of these alkaloids is $300\,g^{-3}$ in 100 g of mushroom mass. Structurally, these alkaloids are neurotransmitters 5-HT.

From L-tryptophan, the serotonin synthesis pathway also begins. Serotonin is 5-hydroxytryptamine. It is derived from L-tryptophan, which at first is simply hydroxylated to 5-hydroxy-L-tryptophan, and subsequently to the serotonin (Figure 39). Structurally, serotonin is also a 5-HT monoamine neurotransmitter.

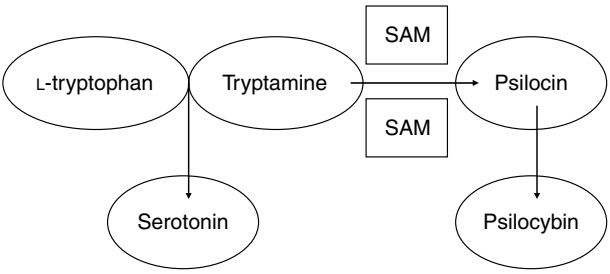


Figure 39. Psilocybin and serotonin synthesis pathway.

It is found in many cellular complexes, such as the CNS, the peripheral nervous system and the cardio vascular system, but it also appears in blood cells.

2.4.2. Elaeagnine, harman and harmine pathway

From tryptamine (derived from L-tryptophan, Figure 39), the synthesis pathway of harman and harmine, which are alkaloids based on a β-carboline ring, also starts. Using the Schiff base formation and Mannich-like reaction, the carboline ring is synthesized. Then, by a Mannich-like reaction using keto acid and oxidative decarboxylation, harmaline is synthesized. Harmaline is converted to harmine and tetrahydroharmine. Certainly, following the above-mentioned Mannich reaction and oxidative decarboxylation, a reduction reaction can ensue and this leads to the synthesizing of elaeagnine (Figure 40). Elaeagnine is synthesized in *Elaeagnus angustifolia* (Elaeagnaceae). Harmine and harman are alkaloids having fully aromatic β-carboline structures. They

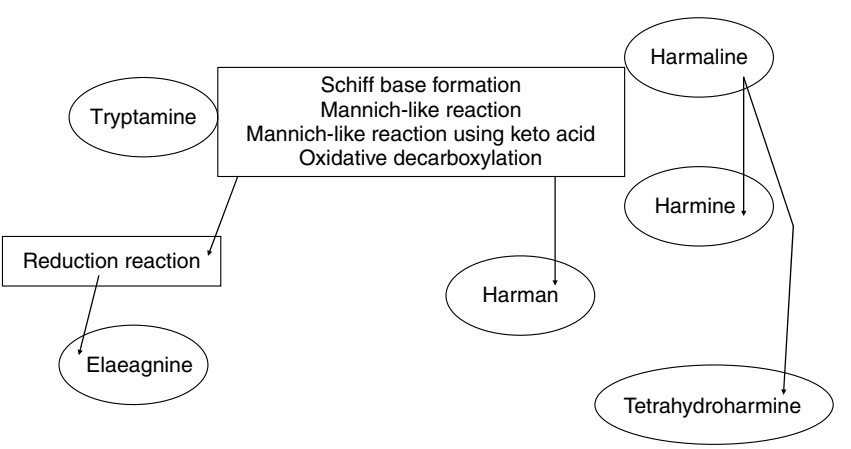


Figure 40. Scheme of elaeagnine, harman and harmine synthesis pathway.

have been detected in *Peganum harmala* (Zygophyllaceae). These alkaloids have psychoactive properties. Harman is a very important mammalian alkaloid[210].

2.4.3. Ajmalicine, tabersonine and catharanthine pathway

In this pathway, over 3000 alkaloids are synthesized. These are terpenoid indole alkaloids, one of the principal groups of alkaloids in the plant kingdom. Some of the most important alkaloids used widely in medicine belong to this group. As stated in Chapter 1, these alkaloids belong mainly to eight botanical families, of which Apocynaceae, Loganiaceae and Rubiaceae are the most important from the perspective of existing applications. Ajmalicine and akuammicine are typical alkaloids containing the Corynanthe nucleus, and tabersonine is typical for the Aspidosperma nucleus. The Iboga type of these alkaloids may be clearly seen in catharanthine and iboganine. All these alkaloids have C9 and C10 fragments in their structure, which derive from terpenoid. Molecules from these alkaloids originate partly from terpenoid, in combination with tryptamine. The synthetic pathway starts with geraniol and, via iridodial and iridotrial, is synthesized as loganin. Subsequently, through oxidation and formation of alkene and ring cleavage, loganin is converted to secologanin, which crosses tryptamine to form the Corynanthe-type nucleus. From this ring, akummicine or ajmalicine is synthesized in turn. Ajmalicine is derived from tryptamine (partly from geraniol) via secologanin, strictosidine and cathenamine. Reduction of cathenamine to ajmalicine is facilitated by enzyme NADPH activity. Again by transformation, the Aspidosperma-type tabersonine or Iboga-type catharanthine is synthesized (Figure 41). Yohimbine is a carbocyclic variant of ajmalicine. It has been found in species belonging to the Apocynaceae and Rubiaceae families. Yohimbine can be converted to reserpine and rescinnamine (trimethoxybenzoyl esters). Rescinnamine is a trimethoxycinnamoyl ester.

2.4.4. Vindoline, vinblastine and vincristine pathway

Vincamine, vinblastine and vincristine are very important clinic alkaloids. They are produced naturally by plants: vincamine by *Vinca minor*, and vinblascine and vincristine by Madagascar periwinkle (*Catharanthus roseus*). The vindoline synthesis pathway starts with strictosidine and, via dehydrogeissoschizine, preakuammicine, stemmadenine and tabersonine, is converted to vindoline and vincristine (Figure 42). Conversion from vindoline to vinblastine is based on the NADH enzyme activity. Vinblastine and vincristine are very similar alkaloids. The difference is that vincristine has CHO connected to N, whereas vinblastine in the same situation has only CO_3. This synthetic structural differences influence their activity. Vinblastine is used to treat Hodgkin's disease (a form of lymphoid cancer), while vincristine is used clinically in the treatment of children's leukaemia. Vincristine is more neurotoxic than vinblastine.

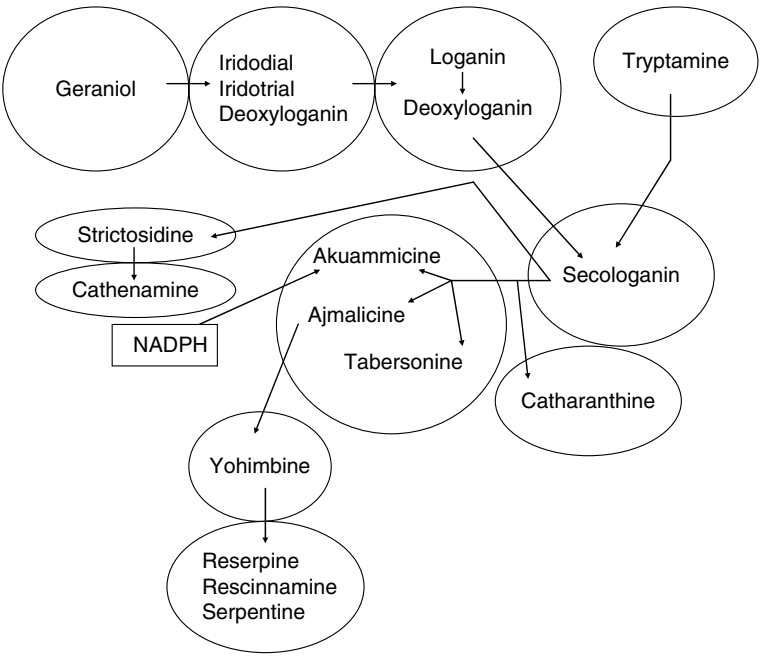


Figure 41. Pattern of the ajmalicine, tabersonine and catharanthine pathway.

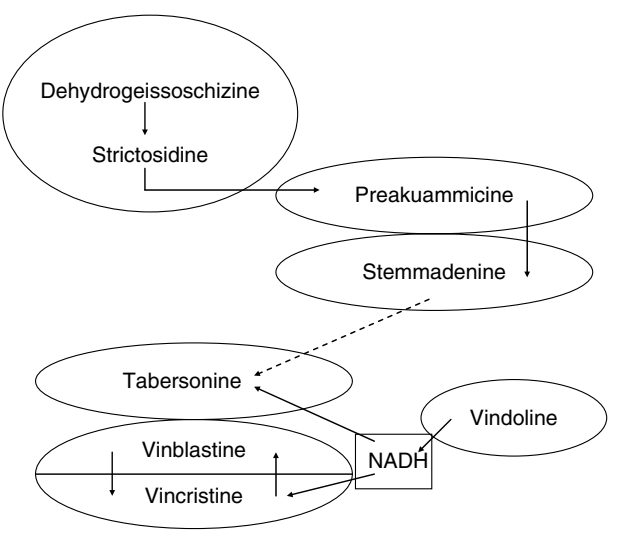


Figure 42. Diagram of the vindoline, vinblastine and vincristine pathway.

2.4.5. Strychnine and brucine pathway

The synthetic pathway starts with the preakuammicine structure (Figure 42) by hydrolysis, decarboxylation and condensation reactions to aldehyde (Wieland-Gumlich), and subsequently reacts with acetyl-CoA to make a hemiacetal form of aldehyde (Wieland-Gumlich) and strychnine (Figure 43).

Strychnine and brucine are extremely toxic alkaloids. Strychnine binds itself to receptor sites in the spinal cord and accommodates glycine. Brucine is a dimethoxy form of strychnine, and is less toxic.

2.4.6. Quinine, quinidine and cinchonine synthesis pathway

As noted in Chapter 1, quinine, quinidine, cinchonidine and cinchonine alkaloids are found particularly in the genus *Cinchona* from the botanical family Rubiaceae. They have a powerful bioimpact and are important anti-malarial drugs. Quinidine is also used to treat cardiac arrhythmias because it inhibits fibrillation, where there is no coordinated contraction of muscle fibres in the heart. During the synthesis of these alkaloids, a change occurs in the nucleus. The indole nucleus is transformed into the quinoline nucleus. This is one reason why these compounds are known as quinoline alkaloids. The synthesis pathway starts with strictosidine, which is transformed by hydrolysis and the decarboxylation reaction into corynantheal (Figure 44). At this point, the indole nucleus and cinchoamine are synthesized. Cinchonamine is an indole derivative and an intermediate compound in the quinine pathway. At the next stage of the synthesis, the transformation of the nucleus occurs and the resultant intermediate cinchoninone no longer contains the indole nucleus. By the enzymatic reaction of NADPH, cinchonidine is synthesized from cinchoninone and subsequently changes to quinine. Cinchonidine and quinine are similar alkaloids. The difference is only that cinchonidine has H while quinine has OCH_3 in the same position. By epimerization and NADPH activity, cinchonine or quinine are synthesized from cinchoninone. The difference between cinchonine and quinidine is very similar to that between cinchonidine and quinine.

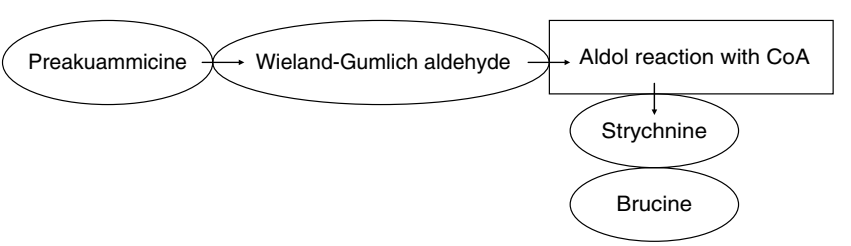


Figure 43. Diagram of the strychnine and brucine pathway.

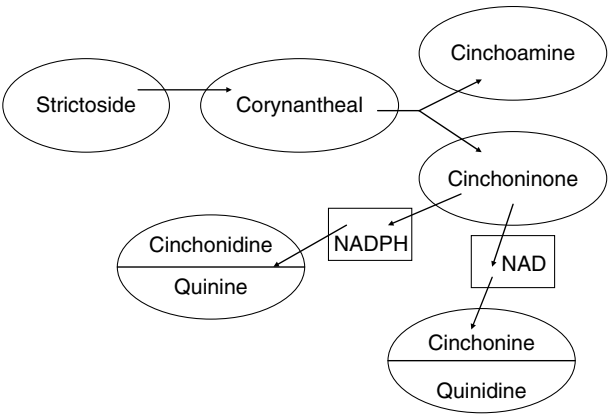


Figure 44. Diagram of the quinine, quinidine and cinchonine synthesis pathway.

2.4.7. Eserine synthesis pathway

Eserine (physostigmine) has a pyrroloindole skeleton. This alkaloid is used as an anticholinesterase drug, which is fairly important in the treatment of Alzheimer's disease. Eserine is synthesized in *Physostigma venenosum* and stored in the seeds of this leguminous plant. The synthesis pathway starts with tryptamine, which is transformed into eserine (Figure 45).

2.4.8. Ergotamine synthesis pathway

Ergotamine is one of the ergot alkaloids produced by the fungus genus *Claviceps*, which lives on cereal kernels and grass seeds. Toxicity of ergot kernels and grass seeds is extreme. The ergotamine synthesis pathway starts from L-tryptophan and, continuing via chanoclavine-I and chanoclavine-2, then agroclavine, elymoclavine and pispalic acid, is converted into D-(+)-lysergic acid. This compound is very important in ergotamine synthesis. By powerful enzymatic activity (ATP and SH) and hydroxylation, ergotamine is synthesized (Figure 46). Ergotamine also contains a peptide fragment in its structure.

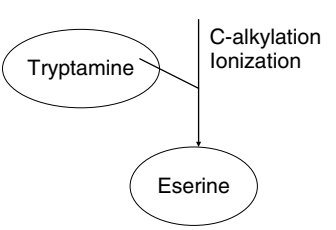


Figure 45. Diagram of the eserine synthesis pathway.

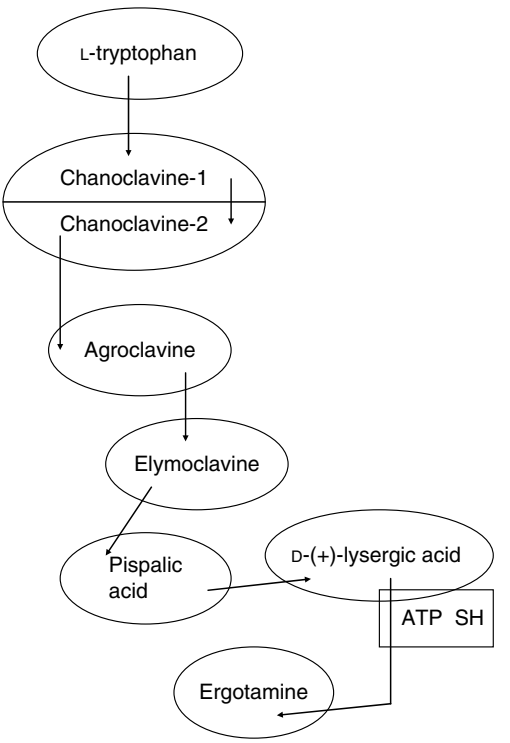


Figure 46. Diagram of the ergotamine synthesis pathway.

2.5. Nicotinic acid–derived alkaloids

Alkaloids derived from nicotinic acid contain a pyridine nucleus. Nicotinic acid itself is synthesized from L-tryptophan via *N*-formylkynurenine, L-kynurenine, 3-hydroxykynurenine, 3-hydroxyanthranilic acid and quinolinic acid.

The pyridine nucleus is passed to alkaloids via dihydronicotinic acid, moving from dihydropyridine to nicotine and nornicotine (Figure 47). Dihydronicotinic acid is synthesized by the enzymatic activity of NADP and subsequently becomes 1,2-dihydropyridine by a reduction reaction. At the next stage, nicotine is synthesized by the reactions of ionization and enzyme $NADP^+$. Nornicotine synthesis is achieved by hydroxylation, NADPH activity and, in its final stage, by non-enzymatic decomposition. Interestingly, during the ionization reaction the residue cation from putrescine (a derivate of L-ornithine) appears. The synthesis of other alkaloids derived from nicotinic acid is illustrated in Figure 48. Anabasine is synthesized from nicotinic acid using the Δ^1-piperidinium cation (from L-lysine), and anatabine from nicotinic acid (as with nicotine) via dihydronicotinic acid and 1,2–dihydropyridine. However, the synthesis of ricine from nicotinic acid is accomplished through nicotinamide.

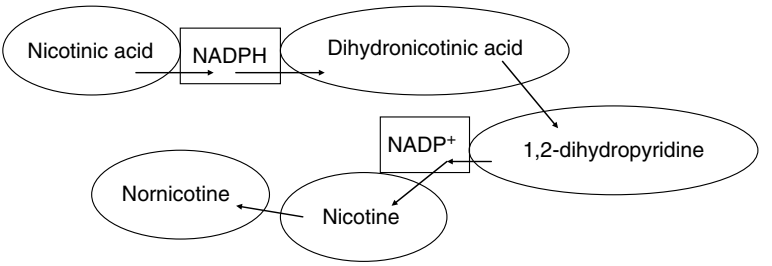


Figure 47. Scheme of nicotine and nornicotine synthesis pathway.

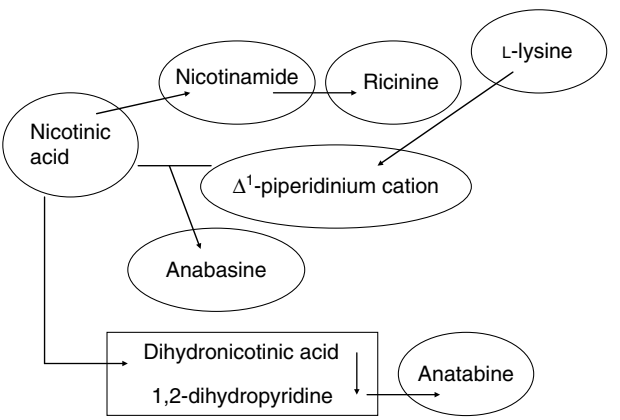


Figure 48. Diagram of anatabine, anabasine and ricinine synthesis pathway.

The other alkaloids derived from nicotinic acid, with pyridine nucleus such as arecoline, arecaidine and guvacoline, are tetrahydronicotinic acid (guvacine) derivatives.

Nicotinic acid also forms part of the sesquiterpene pyridine nucleus. As is well known, aliphatic and aromatic acids esterify hydroxyl groups of fundamental sesquiterpene. Some sesquiterpene forms bridges with pyridine rings of nicotine acid derivatives, and then the sesquiterpene pyridine nucleus appears. More than 220 sesquiterpene pyridine nucleus alkaloids have been documented[58]. Cathedulin alkaloids, isoevoniate, hydroxyisoevoniate, epimeric, norevoniate, wilfordate, hydroxywilfordate, isowilfordate, benzoyloxy- and furanoyloxywolfordate, edulinate and cassinate constitute the groups of these reported alkaloids. In nature, sesqiterpene pyridine alkaloid formation involves a mixed biosynthetic route. The sesquiterpene moiety originates from the acetate metabolism via the mevalonic acid pathway. Sesquiterpene pyridine alkaloids are synthesized when nicotinic acid and (3*S*)-isoleucine form evoninic acid, which is configured with the sesquiterpene moiety.

2.6. Lysine-derived alkaloids

L-lysine furnishes alkaloids with at least four different nuclei. It is a protein amino acid, one of the most important alkaloid precursors. L-lysine-derived alkaloids have a basic skeleton with C_5N (the piperidine nucleus) and $C_5N+\ldots$. (indolizidine, quinolizidine and pyridon nuclei).

2.6.1. Pelletierine, lobelanine and piperine synthesis pathway

Alkaloids with the piperidine nucleus, such as pelletierine (*Punica granatum*), lobelanine (*Lobelia inflata*) and piperine (*Piper nigrum*), have a typical biosynthesis pathway. It starts with L-lysine and continues via cadaverine (biogenic amine), Δ^1-piperideine and Δ^1-piperidinium cations and lobelanine, to be synthesized as lobeline. Piperine is synthesized from Δ^1-piperideine via piperidine (Figure 49). For the transformation from Δ^1-piperideine to Δ^1-piperideine cation, the residue from acetyl-CoA is needed, together with SAM activity in the transformation to lobelanine. Piperine is synthesized from piperidine through the formation of amide.

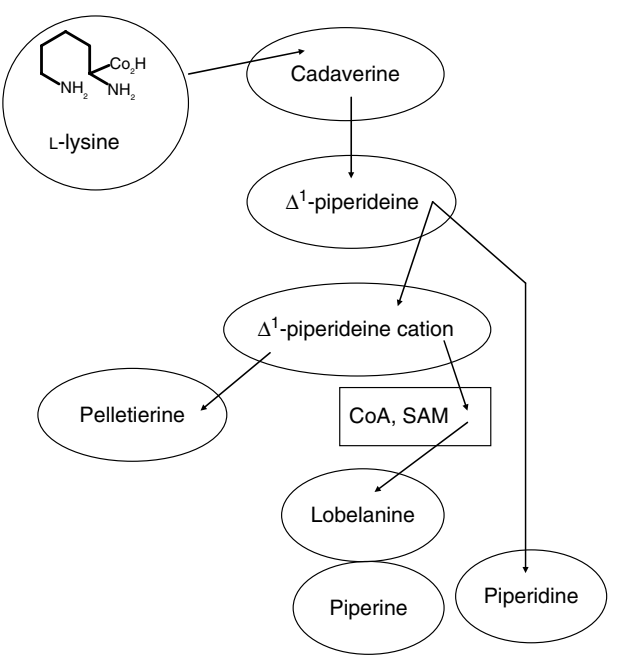


Figure 49. Diagram of the pelletierine, lobelanine and piperine synthesis pathway.

2.6.2. The swansonine and castanospermine synthesis pathway

Swansonine and castanospermine synthesis starts with the α-aminoacid, γ-semialadehyde and, via piperidine-6-carboxylic acid synthetases, L-pipecolic acid. This compound is a substrate to HSCoA and acetyl-CoA. As a result of this activity, the second ring is established. Subsequently, it changes to 1-indolizidinone and, by oxidation reaction, produces castanospermine or swansonine (Figure 50).

Both castanospermine and swansonine occur in some legume plants, such as *Castanospermum australe* and *Swainsona canescens* respectively. They are hybrid molecules compounded of pyrrolizidine and quinolizidine alkaloids, and have shown some resistance to the AIDS virus. Certainly, the above-mentioned alkaloids are also toxic for animals.

2.6.3. The lupinine, lupanine, sparteine and cytisine synthesis pathway

L-lysine is a very important precursor for alkaloids with the quinolizidine nucleus. This group of alkaloids can be divided according to their construction into three sub-groups as follows: bicyclic alkaloids (1st sub-group), tricyclic (2nd sub-group) and tetracyclic alkaloids (3rd sub-group)[7]. In the older studies, a division of quinolizidine alkaloids was established according to alkaloid type. Kinghorn and Balandrin[215] divided these alkaloids into (1) quinolizidines with simple substituents, (2) the leontidine-type, (3) the sparteine/lupanine-type, (4) the esters of sparteine/lupanine-type, (5) the tricyclic degradation products of sparteine/lupanine-type, (6) the pyridine bases-type, (7) the matrine-type, (8) the Ormosia-type and (9) quinolizidine alkaloids having miscellaneous structures.

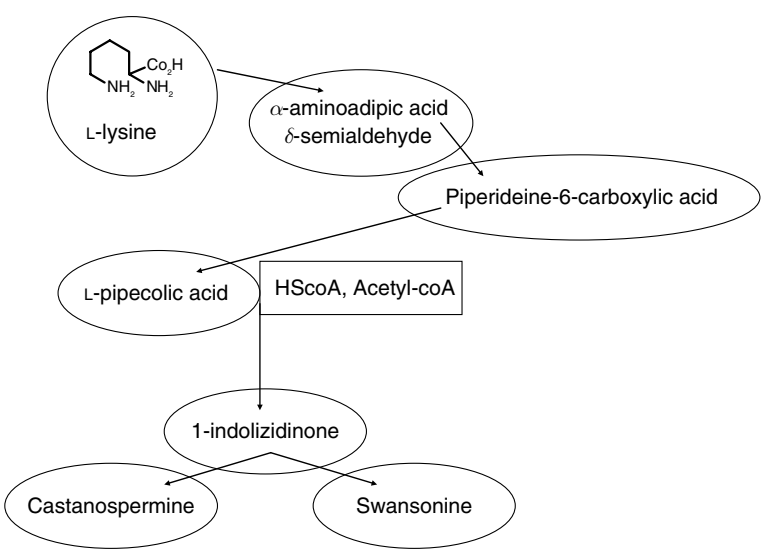


Figure 50. Diagram of the swansonine and castanospermine synthesis pathway.

The synthesis pathway of quinolizidine alkaloids is based on lysine conversion by enzymatic activity to cadaverine in exactly the same way as in the case of piperidine alkaloids. Certainly, in the relatively rich literature which attempts to explain quinolizidine alkaloid synthesis[32,216,217,218,219,220], there are different experimental variants of this conversion. According to new experimental data[32], the conversion is achieved by coenzyme PLP (pyridoxal phosphate) activity, when the lysine is CO_2 reduced. From cadeverine, via the activity of the diamine oxidase, Schiff base formation and four minor reactions (Aldol-type reaction, hydrolysis of imine to aldehyde/amine, oxidative reaction and again Schiff base formation), the pathway is divided into two directions. The subway synthesizes (−)-lupinine by two reductive steps, and the main synthesis stream goes via the Schiff base formation and coupling to the compound substrate, from which again the synthetic pathway divides to form (+)-lupanine synthesis and (−)-sparteine synthesis. From (−)-sparteine, the route by conversion to (+)-cytisine synthesis is open (Figure 51). Cytisine is an alkaloid with the pyridone nucleus.

This pathway clearly proves that the first quinolizidine alkaloid to be synthesized is (−) lupinine (two cycling alkaloids) and subsequently both (+)-lupanine and (−)-sparteine. This is a new approach to the synthesis of this type of alkaloids because in the older literature just four cycling alkaloids (lupanine and sparteine) were mentioned as the first synthesized molecules[216,217,219,220]. In the cadaverine conversion, the participation of diamine oxidase is more reliable than the oxosparteine synthase mentioned by some older studies[219,220].

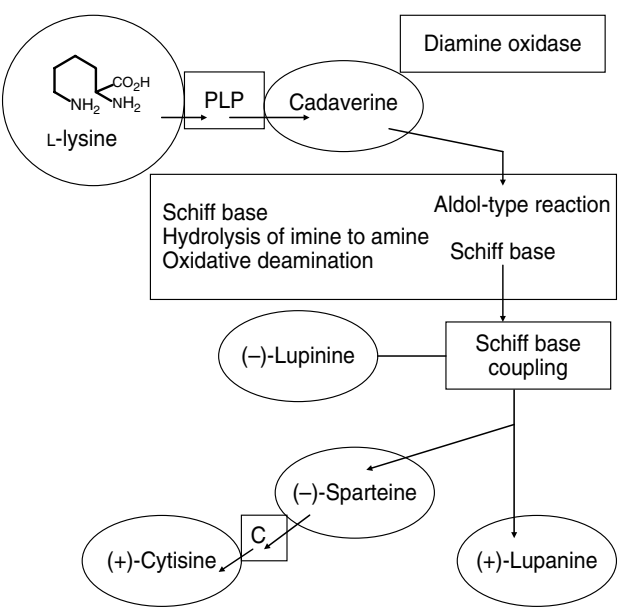


Figure 51. Diagram of the lupinine, sparteine, lupanine and cytisine synthesis pathway. Abbreviations: PLP = coenzyme pyridoxal phosphate; C = cleavage of C_4 unit.

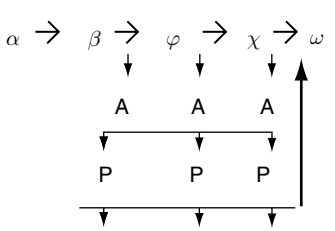
2.7. Methods of analysis

This presentation of basic alkaloid synthesis pathways clearly reveals the diversity and complicity of this process in nature. Moreover, the large number of different pathways and synthesis routes proves the status of alkaloids as a phenomenon of the metabolic activity of organisms. Here, we have seen only the basic pathways and routes. In reality, each alkaloid has its own synthesis route. It is possible to find or to place it on one of the basic pathways. Certainly, experimental data is required for this, the obtaining of which necessitates deep research into molecular structure. Although the technical level of research in the leading laboratories is very high, deep structural and synthesis research is not easy. It is a very expensive and complex job. Pure chemical structure analysis does not suffice today to explain the nature of alkaloid behavioural synthesis in depth. Reactions require a lot of the energy derived from the Krebs cycle and, generally, enzymatic activity. Alkaloid studies use chemical methods of analysis to clarify the constructional and taxonomical nature of these compounds, together with biological and semi-biological methods to describe the role and behaviour of these molecules in life processes. It is well known that natural product molecules are biosynthesized by a chain of reactions which, with very few exceptions, are catalysed by enzymes[32,221]. This is especially important in the case of alkaloids – biologically very active secondary compounds, which have genetic background and environmental oscillators[7]. A discussion on the synthesis of alkaloids derived from different substracts in the metabolic system of a living organism should cover the form and construction blocks of substrate and the changes occurring in the synthesis. The most important step is to provide the answer to the question of origin and the link of the different compounds in the synthesis reactions. An alkaloid metabolism is concerned with the formation of new molecular substances or with the degradation of synthesized molecules. This metabolism is in reality connected with the active transport of metabolites, inorganic ions and organic atoms. Moreover, the converting of energy in biosynthetic and degradative processes is also a base for the reaction chains. The metabolic system of alkaloid pathways is clearly regulated. Preiss and Kosuge[222] have emphasized that enzyme synthesis or degradation, and enzyme activity, are integrated to produce a more efficient modulation. Simpkins[223] notes that there must be a positive correlation between the rate of overall physiological processes and the kinetic and regulatory properties of key enzymes. Moreover, he contends that the specific activity of key enzymes involved in a metabolic pathway would be expected to be high. Therefore, alkaloid synthesis is regulated by a mechanism linked to enzymatic strategy. This strategy seems to be one of the fundamental blocks in alkaloid analysis. It is generally known that enzymes are proteins with catalytic activity in the life system and metabolic processes. They were discovered by Sumner, who first isolated the enzyme urase in crystalline form from jack bean meal in 1926. This historic event was very important for

both chemistry and biology. However, from today's perspective it is difficult to understand why the first isolation and crystallization of enzymes met with such strong criticism, and even derision, among large groups of leading scientists. Only the methodological development and subsequent works with crystallization, and the identification of many new enzymes, published more than 20 years later by Northrup et al.[224], confirmed Sumner's discovery and finally established that the enzyme was a protein. The enzyme as a catalytic unit has a direct relation to alkaloid synthesis research. Even nowadays, not all metabolic enzymes have been isolated and named in alkaloid synthesis pathways. The development of enzymatic methods for the detection of the steps of the synthesis and degradation of alkaloid molecules seems to be very challenging for contemporary research. Mahler and Cordes[212] have shown that enzymes catalyse reactions in four directions: (1) increasing reaction efficiency and speed; (2) increasing of utilization of substrate; (3) broadening of the spectrum of reactions; and (4) controlling cellular metabolism. All these catalyzing purposes are applicable to alkaloid synthesis and degradation reactions. Methods of alkaloid analysis are therefore focused on the molecular-level biology inside and outside the organism. On the other hand, any analysis of alkaloids is impossible without pure classical chemistry and bioorganic chemistry methods.

2.7.1. Methodological considerations

Alkaloids generally, and especially investigations into them throughout history, retain some kind of mystery. This is connected with their strong biological activity, which was observed and used even in ancient times, but without any explanation. Even in the middle of the 20th century, the scientific explanation of alkaloids was based on the opinion that these compounds were the products of waste organic metabolic processes. Only in 1960s and 1970s did alkaloids begin to be seen as evolutionary and biogenetic markers of living organisms[225]. Inspite of the fact that alkaloids are very different compounds, they can be classified and harmonized according to morphology and metabolism. As stated earlier, it is evident that an alkaloid pathway consists of a series of reactions and compounds as well as enzymes[4,7,221]. The sequence of all reactions leading to any alkaloid synthesis can be presented as follows:



α = precursor
β = intermedia
φ = obligatory intermedia
χ = second obligatory intermedia
ω = final product of metabolism
A = alkaloid synthesis
P = postcursor of alkaloid.

The alkaloid is not the final product of a secondary metabolism. This new conception explains why alkaloids were traditionally considered to be unnecessary and undesirable compounds in organisms. Although the "waste" theory is no longer seriously entertained, there are many questions which remain unanswered. It is not quite clear why plants, animals and particularly micro-organisms produce alkaloids. Certainly, there are many hypotheses and theories regarding this problem, with compelling arguments but also with points open to criticism. A similar question is also related to biosynthesis: why does an alkaloid need its intermediate molecule in the synthesis process and why is it not derived directly from the precursor? In natural processes, there is a tendency to cut corners in developing links and pathways. However, in the alkaloid synthesis pathway just the opposite occurs, although this is also a process occurring in nature. Take, for example, the synthesis of quinolizidine alkaloids. From a theoretical point of view, the structural transformation of L-lysine into (−)-lupinine should be simpler; but according to empirical studies into ring and carbon spectra, a direct reaction does not exist[32]. This transformation occurs only by intermedia (cadaverine) reactions.

2.7.1.1. The precursor

The alkaloid precursor is a substrate for the alkaloid molecule, as well as being the source of the alkaloid's nitrogen and skeleton. Precursors of alkaloids are very diverse in their structure, type and metabolic function. Generally, alkaloid precursors may be non-protein aminoacids (e.g., ornithine, nicotinic acid, anthranilic acid), protein aminoacids (e.g., lysine, tyrosine, tryptophan, histidine, phenylalanine) and various compounds (e.g., acetate and malonate), L-phenylalanine in the case of providing nitrogen from amination (geraniol, cholesterol, adenine, guanine in transamination reactions). In some cases there may be more that one precursor, when alkaloids can be synthesized via alternative pathways. This is typical, for example, of L-tyrosine and L-phenylalanine. Both are protein amino acids with aromatic side chains, but L-phenylalanine is not so frequently used in alkaloid synthesis. Moreover, L-phenylalanine generally contributes only carbon atom units (e.g., C_6C_3, C_6C_2, C_6C_1) to the alkaloid skeletons, without providing a nitrogen atom. Phenethylisoquinoline alkaloids, for example autumnaline, floramultine, kreysigine and colchicine, are derived from both these amino acids.

Although L-phenylalanine is a protein amino acid, and is known as a protein acid type of alkaloid precursor, its real role in biosynthesis (providing C and N atoms) only relates to carbon atoms. L-phenylalanine is a part of "magic 20" (a term deployed by Crick in his discussion of the genetic code) and just for this reason should also be listed as a protein amino acid type of alkaloid precursor, although its duty in alkaloid synthesis is not the same as other protein amino acids. However, in relation to "magic 20" it is necessary to observe that only part of these amino acids are well-known alkaloid precursors. They are formed from only two amino acid families: Histidine and Aromatic[226,227] and the Aspartate family[226].

2.7.1.2. The intermedia

The intermedia is a compound formed from the precursor in each alkaloid synthesis pathway. In the case of non-protein aminoacids as precursors of alkaloids, the intermedia is generally a biogenic amine (e.g., putrescine in hygrine and other pyrrolidine and tropane alkaloid pathway (Figure 33), dihydronicotinic acid in the nicotine pathway (Figure 47) or nicotinamide in the ricinine pathway (Figure 48) and the amide formation compound in peganine or the dictamnine pathway). Generally speaking, the transformation of a precursor to an intermedia is done by an enzyme (e.g., PLP in the hygrine pathway, and NADPH in the nicotine pathway) or by CoA with part of another substrate (e.g., antranilloyl-CoA with part of L-ornithine in the peganine pathway). If differences in the formation of intermedia are sought, the intermedia derived from anthranilic acid should be found in this group of precursors. As stated above, for intermedia formation the CoA and part of the other precursor is needed. In many other cases, the metabolism of anthranilic acid as an alkaloid precursor is slightly different from standards of non-protein amino acids. It is related to the origin of this precursor. Anthranilic acid can be an intermediate compound (precursor) in tryptophan biosynthesis and also the postcursor of the tryptophan during the degradation process.

In the case of protein amino acids as precursors of alkaloids, the intermedia is biogenic amine (e.g., L-dopa in the mescaline pathway or tyramine in the hordeine pathway (Figure 35), putrescine in the homospermidine pathway (Figure 34), dopamine in the kreysigine and colchicine pathways (Figure 36), tyramine in the galanthamine pathway (Figure 38) and tryptamine in the psilocybin pathway (Figure 39), etc. Each DNA amino acid as a precursor of alkaloids has a clearly determined intermedia. The transformation of a precursor to an intermedia is achieved by PLP or DC enzymes.

Other precursors of alkaloids form intermedia as acids (e.g., capric acid in the coniine pathway, 26-hydroxycholesterol in the solasodine pathway and piperidine in the jervine pathway). Moreover, in the case of purine as an alkaloid precursor, the intermedia is inosine monophosphate (IMP).

One of the characteristics of intermedia is that in many cases it is not a stable compound (e.g., cadaverine). Intermedia is a compound which can be the final product of any pathway. However, an alkaloid can convert from an intermedia (e.g., norbelladine from tyramine in the galanthamine pathway), though this process is restricted. Generally, the synthesis pathway continues to establish the next compound, the obligatory intermedia.

2.7.1.3. Obligatory intermedia

An obligatory intermedia is a compound which follows the intermedia in the synthesis process of the alkaloid and metabolism pathway. The synthesis of this, generally not stable compound, is obligatory or alternative for alkaloid formation. In the case of non-protein and protein amino acids as precursors of alkaloids, the obligatory intermedia is derived, in most instances, from biogenic amine (intermedia) by SAM-dependent *N*-methylation (e.g., the conversion from putrescine to *N*-methylputrescine in the hygrine pathway), enzyme NAD^+ in the conversion of putrescine to imine in the homospermidine pathway or enzyme DAO in the conversion of cadaverine to Δ^1-piperideine in the quinolizidine alkaloids pathway. In the case of non-amino acid precursors, the conversion from intermedia to obligatory intermedia occurs by a coupling reaction, for example from piperidine to protoverine in the jervine pathway, or from IMP to XMP in purine alkaloids.

The basic characteristic of obligatory intermedia synthesis is that there cannot be an alkaloid between intermedia and obligatory intermedia, but in some cases the obligatory intermedia can be obligatory alkaloid needed for synthesis of other alkaloids, for example protoverine in the jervine pathway. In these cases, the obligatory intermedia has biological activity as, for example, with protoverine. After the obligatory intermedia, the alkaloid can be synthesized or the obligatory intermedia converts to the second obligatory intermedia.

2.7.1.4. Second obligatory intermedia

In the case of protein amino acid–derived alkaloids, the second obligatory intermedia is synthesized from the obligatory intermedia by chemical reactions. In the pelletierine synthesis pathway started with L-lysine, the second obligatory intermedia is Δ^1-piperidinium cation. It is formed by a Mannich reaction from Δ^1-piperidine (obligatory intermedia) and COSCoA. The second obligatory intermedia, by hydrolysis decarboxylation, produces pelletierine.

In the case of non-protein amino acid–derived alkaloids, the second obligatory intermedia is derived from the obligatory intermedia enzymatically and by the Schiff base formation as, for example, in the hygrine pathway. The second obligatory intermedia is, in this case, the *N*-methyl-Δ^1-pyrrolinium cation.

The general characteristic of the second obligatory intermedia is that this compound is not stable. It is poison and biologically active. Subsequently, the second intermedia alkaloid is synthesized.

2.7.1.5. Final product

In many cases, alkaloid synthesis is not the end of the metabolic pathway as part of the secondary metabolism block. An alkaloid is generally the sub-product of this metabolism, and in many cases can be used by living cells in neuro-physiological activity. Part of the alkaloid substrate can again be metabolized to the alkaloid postcursors in the metabolic sub-pathway in the form of synthesis or degradation. In the case of a primary metabolism it is relatively simple to show the final product. In the case of a secondary metabolism, it is not possible in every case to predict the final product, because there are many possibilities of stopping or prolonging the reaction chain according to physiological needs and signallings. Although alkaloid pathways exist, they are parts of the more general secondary metabolism. Alkaloids are not the final products of this metabolism, and their nature is to be a part of the metabolic chains.

2.7.2. Structural approach

Alkaloids as non-final products of the secondary metabolism are very different in their structure and life functions in organisms. Many different groups of alkaloids are known. As mentioned, they have different precursors and rings. They also have different sub-pathways and intermedia.

2.7.2.1. Piperidine alkaloids

Piperidine alkaloids contain the piperidine nucleus. The structural development of this group of alkaloids in synthesis is presented in Figure 52. Here α is L-lysine and β is cadaverine. The basic ring of β is the same as in α, although the activity of PLP reduces carbon dioxide. The β is biogenic amine, neither a stable nor a poisonous compound

By oxidative deamination, in which diamine oxidase (DAO) is active together with Schiff base formation, the β converts into φ. The φ is Δ^1-piperideine, which can not be substituted by other compounds, although several hypothetical obligatory intermedia such as glutardialdehyde and 5-aminopentanal have been proposed[225]. In the case of piperidine alkaloids, these hypothetical substitutes of φ are ruled out. φ has slowly changed the basic ring, but it is no longer piperidine. The deeper change in the ring occurs in the next reaction, the Mannich Reaction (MR), the result of which is χ. For this purpose, the nucleophilic acetoacetyl-CoA with $(-)$ is used for $(+)$ closing the ring. In this case, the intermolecular Mannich reaction is under question. Subsequently, χ, which is Δ^1-piperidinium cation, has a ring similar to piperidine, though not identical. Only after hydrolysis and decarboxylation will the compound be A (pelletierine) with the piperidine nucleus. By the activity of SAM, A is converted to P, which is *N*-methylpelletierine. P can be converted by the intramolecular Mannich reaction to the next postcursor, P_1, which is pseudopelletierine. Other postcursors of pelletierine are possible,

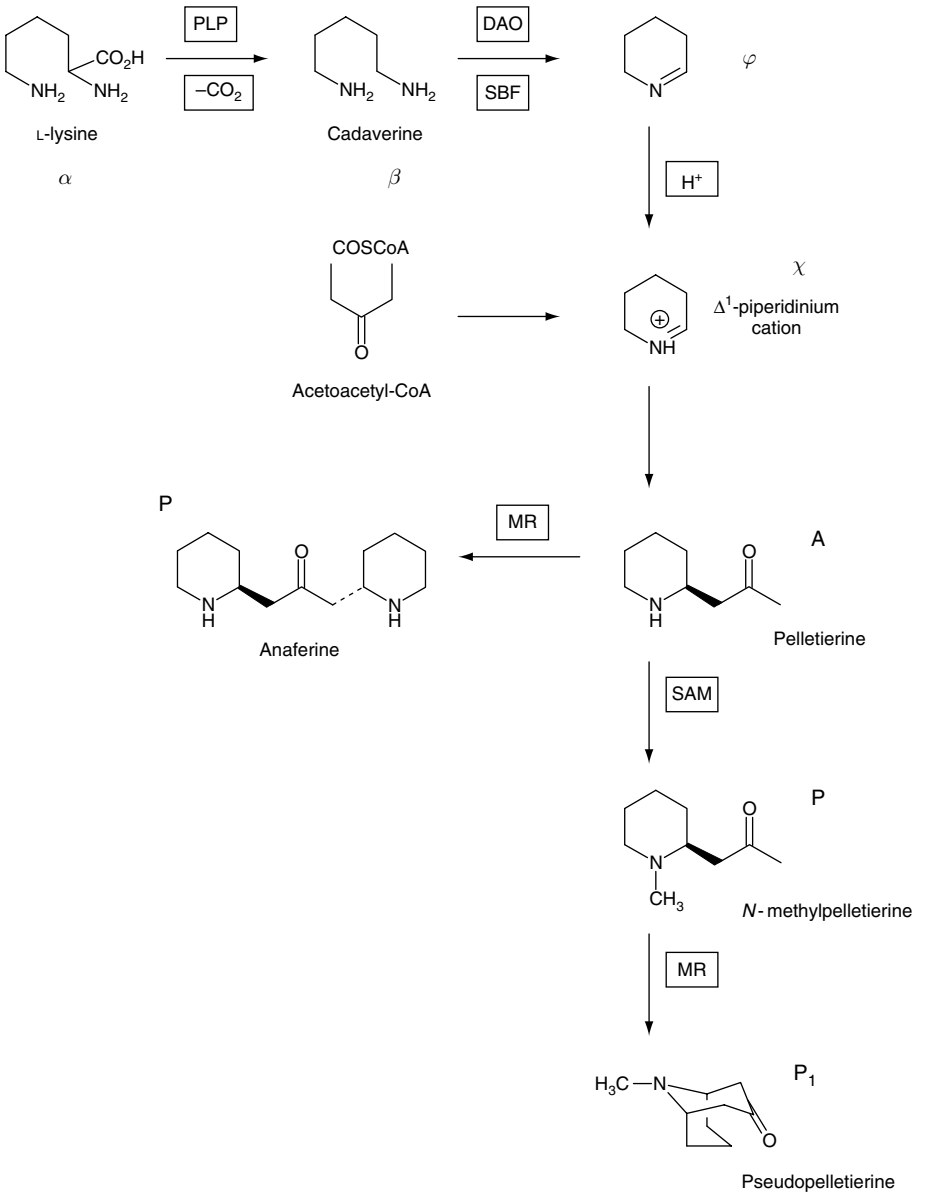


Figure 52. Structural development of piperidine alkaloids.

for example anaferine, in the intermolecular Mannich reaction. True piperidine alkaloids have one-cycle compounds with the C_5N nucleus.

2.7.2.2. Indolizidine alkaloids

Indolizidine alkaloids contain the indolizidine nucleus with two different cycles. The structural development of this kind of alkaloid is presented in Figure 53.

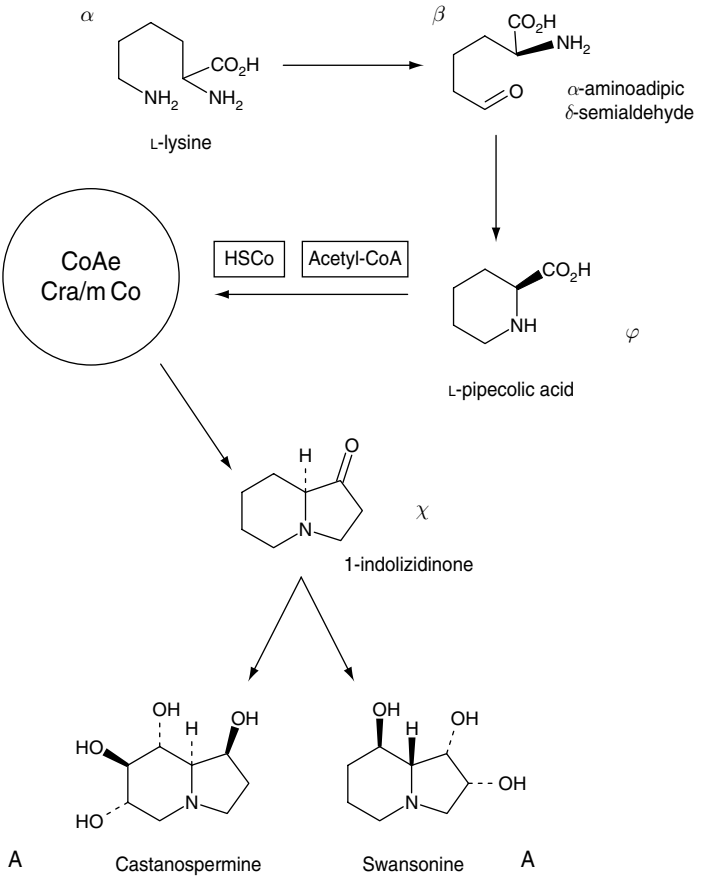


Figure 53. Structural development of indolizidine alkaloids.

The α is L-lysine, as in the case of piperidine, but the β is different. The β is α-aminoadipic acid δ-semialdehyde. The φ is L-pipecolic acid, which is synthesized in plants from piperideine-6-carboxylic acid. In the case of many other organisms, the obligatory intermedia (φ) is derived from the β. The φ retains one ring structure. The indolizidine nucleus will be formed only in the synthesis of the χ. The deep structural change occurs when φ is transformed by a chain of reactions: the formation of CoA ester (CoAe), the Claisen reaction with acetyl or malonyl CoA (Cra/mCoA) and the ring closure process (by amide or imine) to 1-indolizidinone, which is the χ. The second obligatory intermedia (χ) only has the indolizidine nucleus.

The χ is transformed by hydroxylation to A, which is castanospermine. The A is a typical sub-way product. The main pathway is transformed to the χ by hydroxylation and the ring fusion to another A, which is swansonine.

Both alkaloids (castanospermine and swansonine) have the ability to inhibit glycosidase enzymes (GE), the activity of which is necessary in glycoprotein biosynthesis.

2.7.2.3. Quinolizidine alkaloids

The third structural group of alkaloids, from the same α, are quinolizidine alkaloids (QAs). It is a large group of compounds with very different abilities[7,16,40,119,213,215,218,219,220,228,229,230,231,232,233,234,235,236,237,238]. The structural development of quinolizidine alkaloids is presented in Figure 54. The α (L-lysine) provides the basic components of the quinolizidine nucleus and skeleton. The β is cadaverine and is synthesized in the same way as piperidine alkaloids (by the activity of PLP). The transformation from β to φ also occurs through the activity of diamine oxidase (DO). The φ is Δ^1-piperideine, which develops by the Schiff base formation, the aldol-type reaction between enamine and iminium, the hydrolysis of imine to aldehyde, oxidative deamination and, again, the Schiff base formation. During this stage, the quinolizidine nucleus is formed. From φ as a subway product the (−)-lupinine is synthesized, which is a two-cycle quinolizidine alkaloid. The first A is therefore a two-cycle quinolizidine alkaloid[32], although in previous studies four-ring quinolizidine alkaloids have been said to form first[216,217,218,220]. The main way that the product will be formed is by the step reaction of the Schiff base formation, for which the molecule of the β or the φ is again needed. The four-cycle quinolizidine skeleton is formed in this stage by the molecule coupling with cationed nitrogen in the χ (second obligatory intermedia), which is simply two transformed molecules of the φ with cationed nitrogen. In reality, this χ is two molecules of (−)-lupinine connected together in an opposite molecule order with H atom reduction. This strongly suggests that there is also an alternative way for four-cycle quinolizidine alkaloids synthesis from (−)-lupinine.

The χ is transformed in two directions: (−)-sparteine and (+)-lupanine, the two basic quinolizidine alkaloids which occur in nature and which have an important role in the ecosystem. The (−)-sparteine is transformed by the cleavage of the C_4 unit to (+)-cytisine, a three-cycle quinolizidine alkaloid with a pyridon nucleus, and from this step to the other pyridon quinolizidine alkaloids (P, P_1). The (+)-lupanine converts to the lupanine derivatives, angustifoline, α-isolupanine and 13α-hydroxylupanine (P) (Figure 54).

Bicyclic quinolizidine alkaloids

Bicyclic quinolizidine alkaloids have the simplest chemical structure, based only on the quinolizidine nucleus[7]. Typical representatives of this type of alkaloids are lupinine, epilupinine, and lusitanine[7,239,240]. Lusitanine is an alkaloid derived from *Genista lusitanica* L.[241], *Lupinus excubitus* and *Lupinus holosericeus*[242]. In their absolute configurations, the melting-point of Lusitanine is 184–186 °C, of dihydro 96–100 °C and of epidyhydrolusitanine 140–144 °C[241]. Lupinine and epilupinine are typical bicyclic quinolizidine alkaloids in lupines, especially in

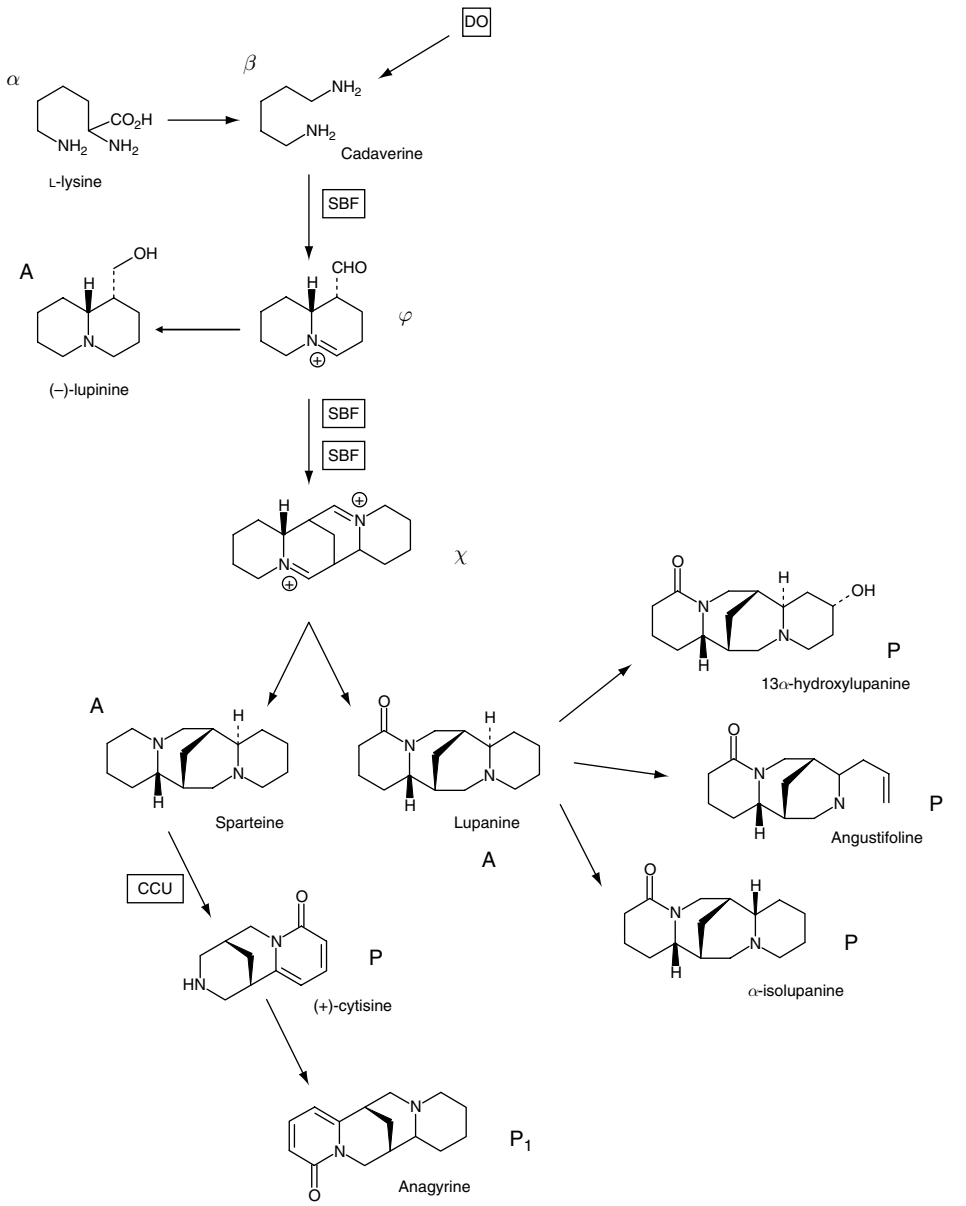


Figure 54. Structural development of quinolizidine alkaloids.

Lupinus luteus L., *Lupinus hispanicus* L., *Lupinus hirsutus* L. They have also been found in *Anabasis aphyla*. In absolute configuration, lupinine is in its (−)-form, which is non-stable thermally, and is easily epimerized to epilupinine, which is a stable (+)- form of lupinine[240,241]. The melting point of (−)-lupinine is 70–71 °C, of mixed (+ and −)-lupinine 63–64 °C, and of (+)-lupinine (synthetic)

167–168 °C. Lupinine and epilupinine contain esters, which have been found in *L.luteus* seedlings[7,240,241].

Tricyclic quinolizidine alkaloids

Tricyclic quinolizidine alkaloids occur in lupines. Angustifoline, with its derivatives and albine, are examples of this structural group of alkaloids. Angustifoline is identical with jamaicensine, an alkaloid isolated from *Ormosia jamaicensis*. Angustifoline is a compound which has been found in *L. angustifolius* L., *L. polyphyllus* Lindl. and *Lupinus albus* L.[243]. Angustifoline is in the (−)-form in absolute configuration, with a melting point of 79–80 °C[241]. From *L. albus* L. and from viable seeds of *Lupinus termis* L. (+)-angustifoline as a diasrereoisomer of (−)-angustifoline has been isolated by Wysocka and Przybył[244,245] in the Alkaloid Chemistry Laboratory in Poland. Other derivatives of angustifoline are dihydroangustifoline, with a melting point of 82–83 °C[241], and isoangustifoline, with a melting point of 96–97 °C[120,234]. Albine has been found in *L. albus*[246] and structurally reinvestigated by Wysocka and Brukwicki[247,248], Wysocka et al.[249,250], Wysocka and Przybył[245].

Tetracyclic quinolizidine alkaloids

Tetracyclic quinolizidine alkaloids can be divided into two types, both according to chemical structure and, especially, biological activity. These are tetracyclic alkaloids, which contain a quinolizidine nucleus, and others with a pyridone nucleus. Here, the first type of alkaloids (with a quinolizidine nucleus) will be discussed. The second type will be considered in the next sub-chapter as pyridone alkaloids.

Sparteine is one of the basic, and probably most important, tetracyclic alkaloids with a quinolizidine nucleus. In absolute configuration, sparteine occurs as (−)-sparteine, which is lupinidine. Lupinidine, with a melting point of 181 °C, occurs in all lupine species, although in different concentrations. In *L. luteus* sparteine (lupinidine) is a major alkaloid, and consequently this yellow lupine has been described as a "typical" sparteine species. However, sparteine is also found in *Lupinus mutabilis*[251], *L. polyphyllus*[120,121,234] and *Genista tinctoria*[252]. (+)-Sparteine has been detected in *Lupinus pussilus*, *Cytisus caucasicus* and many other plants[253,254]. Most recently this alkaloid was discoverd in *Hovea linearis*[255], *Maackia amurensis*[256], *Termopsis mongolica*[257], *Lygos raetam*[258] and in *L. albus*[259]. The melting point of (+)-sparteine is 173–174 °C, and it is also known as pachycarpine[241]. Sparteine is also familiar in the form of (±)-sparteine. Its melting point is 231 °C. According to the literature, this alkaloid form does not occur in the lupin species[241].

Lupinus sericeus contains (−)-7-hydroxy-β-isosparteine and 10, 17-dioxo-β-isosparteine, which are also sparteines: tetracyclic quinolizidine alkaloids with a quinolizidine nucleus. Moreover, other alkaloids from this group include epiaphylline and aphylline, alkaloids from *L. latifolius*, and (−)-lindenianine, an alkaloid from *Lupinus lindenianus* and *Lupinus verbasciformis*[240,260]. Nuttalline

(4β-hydroxy-2-oxosparteine) is a tetracyclic quinolizidine alkaloid from *Lupinus nuttalli*[240]. An alkaloid, sparteine can be converted to α-isosparteine or β-isosparteine, which occurs particulary in *Cytisophyllum sessilifolium*[243,252,261]. In contrast to aphylline, 17-oxosparteine is known to be synthesized only under energetic conditions[243].

One of the most important tetracyclic quinolizidine alkaloids with a quinolizidine nucleus is lupanine, which is in fact 2-oxo-11α-sparteine. In absolute configuration, lupanine is (+)-lupanine with a molecular weight of 248 and melting point of 127 °C[7,241]. Lupanine occurs in *L. polyphyllus, L. albus* and *L. angustifolius* and is, like sparteine, probably found in all lupine species in different concentrations, from main compounds to mere traces. Lupanine is the main alkaloid in the seeds of *Lupinus rotundiflorus*, *Lupinus exaltatus* and *Lupinus mexicanus*. It has been discovered in considerable amounts in *Lupinus montanus* and *Lupinus madrensis*, but only traces were noted in *Lupinus elegans*[119]. Moreover, lupanine occurs in *Cytisus scoparius* and *Leontice eversmannii*[241].

The (−)-lupanine is hydrorhombinine and has been isolated from *L. pussilus* and *Lupinus macouni* as well as other species, such as *Baptisia versicolor* and *Podalyria calyptrata*. The melting point of (−)-lupanine is 190 °C[241]. In such species as *L. albus* and *L. termis* lupanine occurs as (±)-lupanine with melting points of 127–128 °C and 250–252 °C[241]. In *L. polyphyllus* Lindl., *L. angustifolius* L. and *L. albus* L., 17-oxolupanine has also been detected[243,245,252]. Hydroxylupanine and their esters occur in *Lupinus bicolor, Lupinus densiflorus, L. latifolius, Lupinus polycarpus, Lupinus ruber, Lupinus burkei*[251], *L. rotundiflorus, L. montanus, L. exaltatus, L. mexicanus, L. madrensis*[119] and *L. polyphyllus*[120,121,234].

Pyridone alkaloids

This group of alkaloids has a pyridone nucleus and generally takes the tetracyclic or tricyclic form. The α for pyridone alkaloids is L-lysine, while the β, φ and χ are the same as for other quinolizidine alkaloids. Quinolizidine alkaloids containing the pyridone nucleus are the P from the (−)-sparteine by cleavage of the C_4 unit[32]. The first quinolizidine alkaloid with the pyridone nucleus is tricyclic cytisine, which converts to four cyclic alkaloids. In this synthesis the anagyrine, the most poisonous quinolizidine alkaloid with a pyridone nucleus, has its own synthesis pathway.

Anagyrine has a molecular weight of 244 and a melting point of 264 °C. It only takes a (±)-form[241]. This alkaloid occurs in *L. latifolius*[260], *Lupinus arboreus, Lupinus caudatus, L. densiflorus, L. sericeus, Lupinus argenteus, Lupinus leucophyllus*[242]. Anagyrine was found in neither bitter nor sweet *L. polyphyllus* Lindl., which grows in Finland[120,121,234].

2.7.2.4. Pyrrolizidine alkaloids

The pyrrolizidine nucleus is characteristic of this group of alkaloids. The α is either L-ornithine or L-arginine, and the β is a biogenic amine, the putrescine. Oxidative deamination by enzyme NAD^+ converts two molecules of putrescine

into the imine (φ). By the activity of NADH, imine is reduced to homospermidine (χ). Then, the pyrrolizidine nucleus is formed via a chain of reactions such as oxidative deamination, Schift base formation, oxidative reaction again and the intramolecular Mannich reaction. Retronecine (A), which is simple pyrrolizidine alkaloid, commonly occurs in nature. The formation of the pyrrolizidine structure is presented in Figure 55. Retronecine and its P, the senecionine, necine, heliotrine, indicine-*N*-oxide, malaxine, monocrotaline and absulin, are typical representatives of this group of alkaloids[32,40,262]. Pyrrolizidine alkaloids are widely dispersed throughout the natural world. According to Robins[262] these

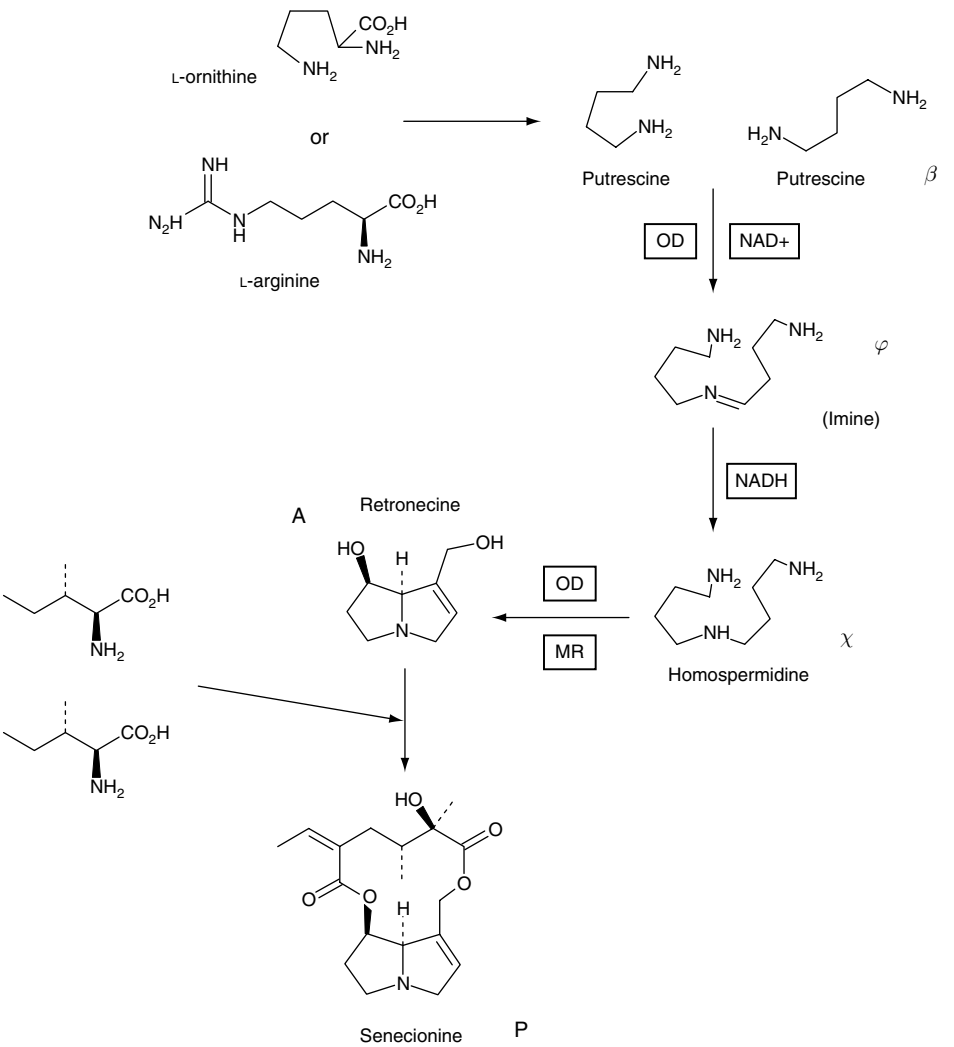


Figure 55. Structural development of pyrrolizidine alkaloids.

alkaloids have been found in 15 different families, although three plant families (Boraginaceae, Asteraceae and Fabaceae) are the most important sources of these compounds[32]. In Fabaceae, the genus *Crotalaria* is particularly representative of pyrrolizidine alkaloids, and in Asteraceae, the genus *Senecio*[40]. The characteristics of these alkaloids are as follows: (1) they are accumulated in plants as *N*-oxides; (2) they are poisons; (3) some of them have a bioimpact (e.g., indicine-*N*-oxide).

2.7.2.5. Izidine alkaloids

Izidine alkaloids cover compounds, which contain one of the izidine skeletons. There are three different skeletons in this group: pyrrolizidine, indolizidine and quinolizidine. Izidine alkaloids are, therefore, compounds with a bicyclic nucleus, which have different α, β, φ and χ. Izidines present structural similarities, but organic and functional differences. These alkaloids include more than 800 compounds. Several of them show interesting physiological and pharmacological behaviour. Izidine alkaloids with the pyrrolizidine nucleus (e.g., hellosupine, senecionine, retronecine, acetyl-intermedine, acetyl-lycopsamine and indicine-*N*-oxide) are toxic and known to affect the liver[24]. Izidine alkaloids containing the indolizidine nucleus (e.g., slaframine, elaeocanine, securitinine, tylophorine, swansonine and castanospermine) play an important role as actual and potential drugs in the fight against viruses, including AIDS. Some of these compounds, for example, pumiliotoxin B, have an ecological function in nature. Izidine alkaloids with the quinolizidine nucleus (e.g., lupinine, lusitanine, lamprolobine, angustifoline, lindenianine, sparteine, lupanine, nuttalline, aphylline, sophoridine, isomatrine, albertidine, aloperine and nitraramine) contain toxic compounds with strong selective ecological impact[7].

2.7.2.6. Pyrrolidine alkaloids

Pyrrolidine alkaloids have a pyrrolidine (C_4N skeleton) nucleus. The structural α of these alkaloids is L-ornithine (in plants) and L-arginine (in animals). The pyrroline skeleton is synthesized after β (putrescine) and φ (*N*-methylputrescine), when DO activity and Schiff base reaction forms χ, which is *N*-methyl-Δ^1-pyrrolinium cation. Subsequently, A (hygrine) is formed. Typical pyrroline alkaloids are (−)- and (+)- hygrines (Figure 56).

2.7.2.7. Tropane alkaloids

Tropane alkaloids have a tropane (C_4N skeleton +) nucleus. Structurally, these alkaloids synthesize as postcursors of pyrrolines (Figure 57). α, β, φ and χ in the tropane pathway are the same as in pyrrolines. Typical tropane alkaloids (e.g., atropine, hyoscyamine, cocaine, tropinone, tropine, littorine and cuscohygrine) have a strong biological activity, especially as neurotransmitters.

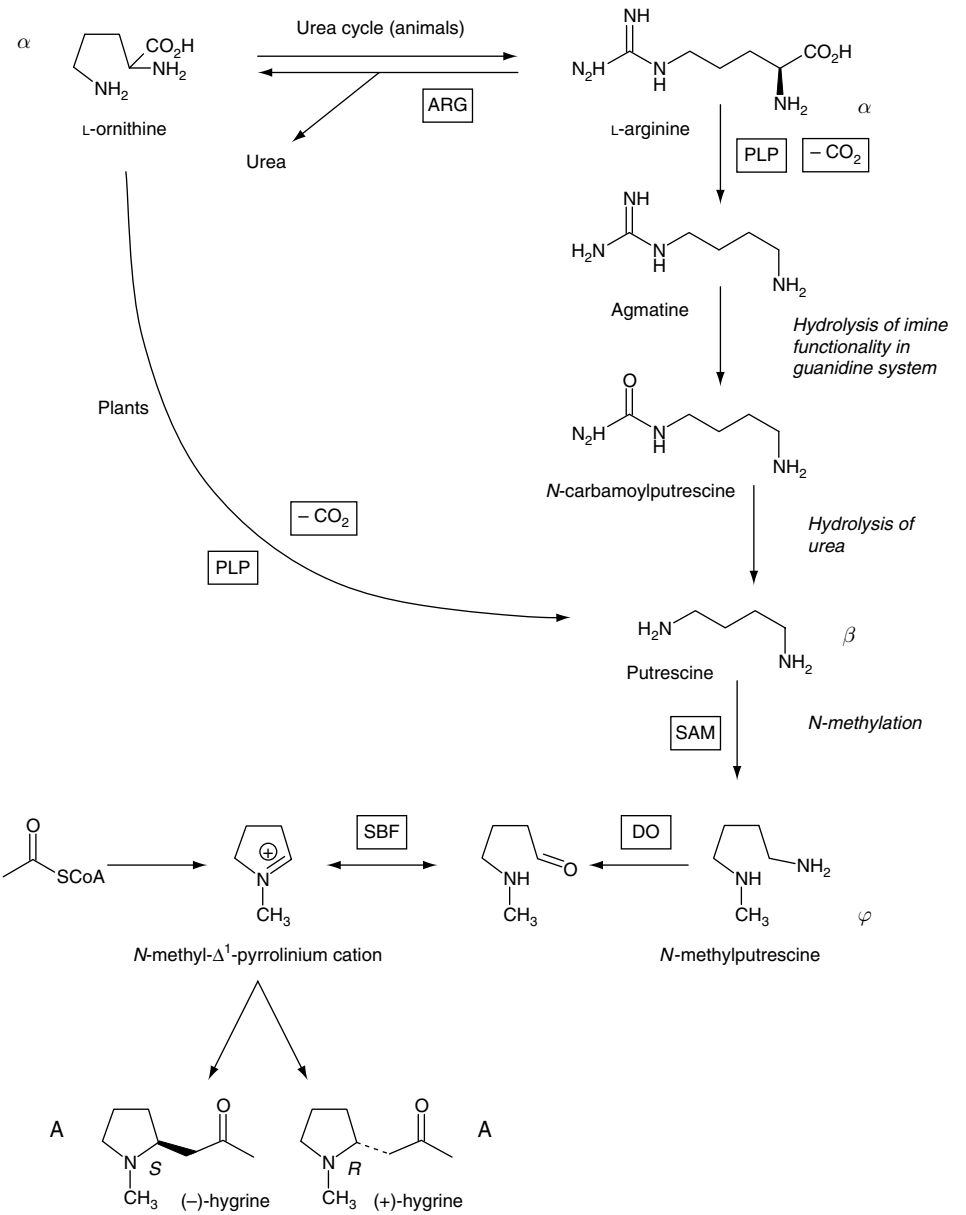


Figure 56. Structural development of pyrrolidine alkaloids.

2.7.2.8. Imidazole alkaloids

This group of alkaloids is an exception in the transformation process of structures, because the imidazole nucleus is already made at the stage of the precursor. The α of these alkaloids is L-histidine, and the first A is developed in a decarboxylation process by histidine decarboxylase (HDC). The histamine is a product of

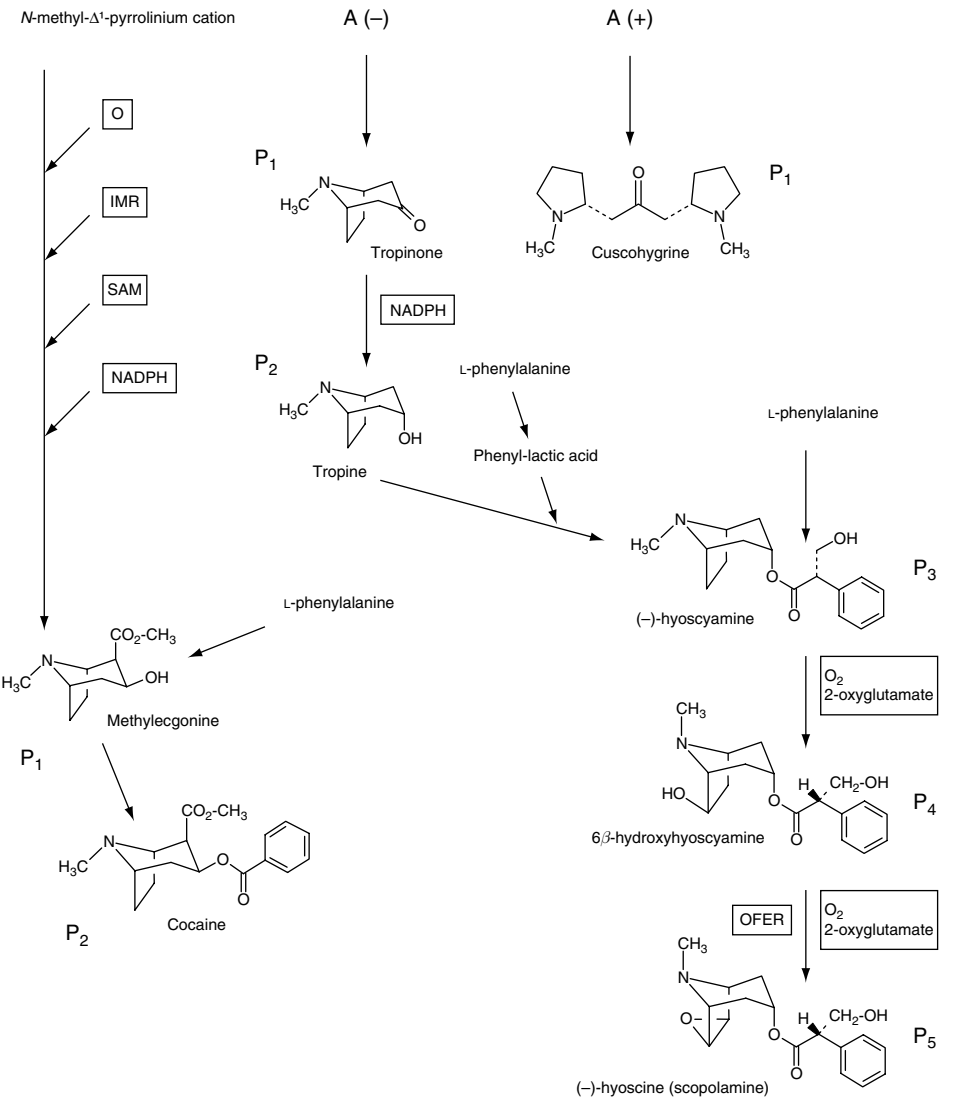


Figure 57. Structural development of tropane alkaloids.

this reaction (Figure 58). Other alkaloids from this group include, for example, dolichotheline, pilocarpine and pilosine.

2.7.2.9. Quinazoline alkaloids

Quinazoline alkaloids contain more than 100 compounds. They have been isolated from animal and plant sources. The plant family Rutaceae is especially rich in these alkaloids. Typical quinazoline alkaloids include, for example, arborine, glomerin, homoglomerin, glycorine, glycosminine, febrifugine and

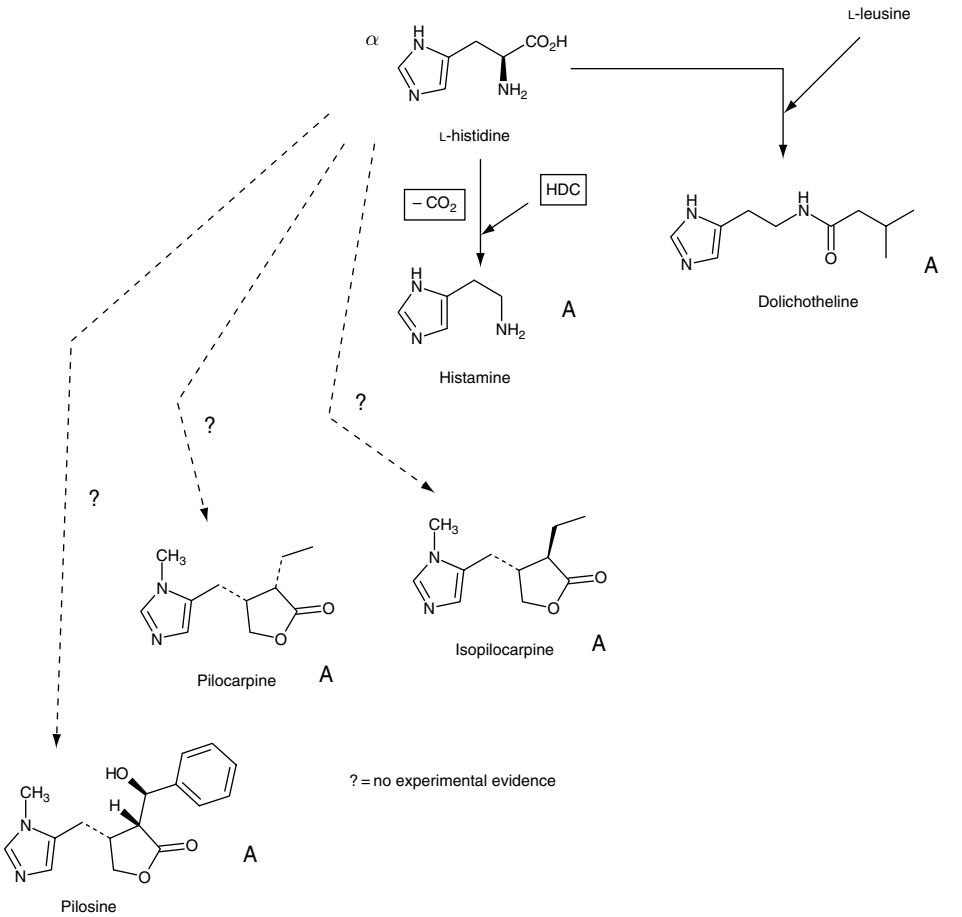


Figure 58. Structural development of imidazole alkaloids.

vasicine (Figure 59). The α of quinazoline alkaloids is anthranilic acid, though there are also in some cases alternative α, such as phenylalanine in the case of arborine or ornithine. The β is anthranoylphenylalanine.

Quinazoline alkaloids are known as biologically active compounds. Arborine inhibits the peripheral action of acetylcholine and induces a fall in blood pressure. Febrifugine is an anti-malarial agent and vasicine acts as a uterine stimulant. Glomerin and homoglomerin are alkaloids of the defensive system in some organisms (e.g., in the glomerid millipede).

2.7.2.10. Acridone alkaloids

Fewer than 100 acridone alkaloids are known. Typical compounds include, for example, atalaphylline, acronycine and preacronycine. Acridone alkaloids occur in plants and animals. They are especially characteristic of the Rutaceae plant

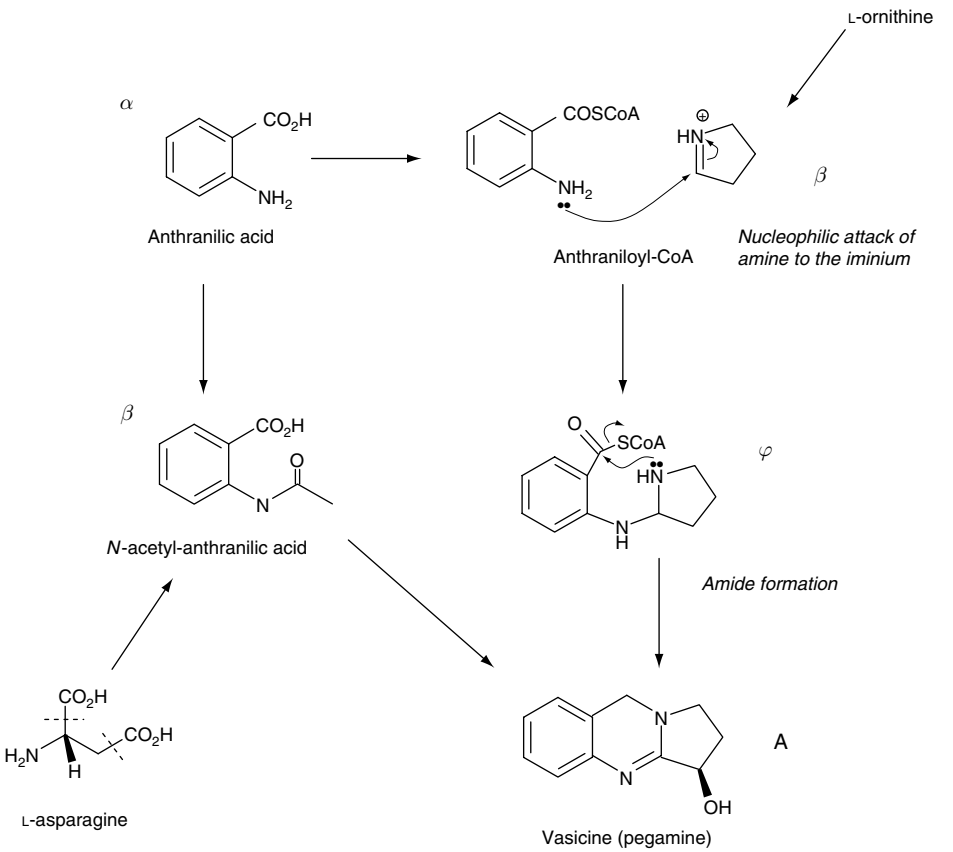


Figure 59. Structural development of quinazoline alkaloid vasicine.

family. The α is anthranilic acid, the β is *N*-methyl anthraniloyl-CoA, and the φ is 1,3-dihydroxy-*N*-methylacridone (Figure 60). Acridone alkaloids are biologically active. Acronycine is also known for its antitumour activity.

2.7.2.11. Pyridine alkaloids

Pyridine alkaloids are compounds with a pyridine nucleus and a pyrrolidine or piperidine unit. The pyrrolidine ring appears in nicotine and the piperidine ring in anabasine. Typical alkaloids from this group are nornicotine and anatabine. The α of pyridine alkaloids is nicotinic acid, the β is dihydronicotinic acid, the φ is 1,2-dihydropyridine (Figure 61). The A is nicotine and its P is nornicotine[32].

2.7.2.12. Sesquiterpene pyridine alkaloids

Compounds belonging to this group of alkaloids are sourced from the Celastraceae and Hippocrateaceae families and contain the sesquiterpene nucleus. More than 220 alkaloids are known in this group[58]. Sesquiterpene pyridine

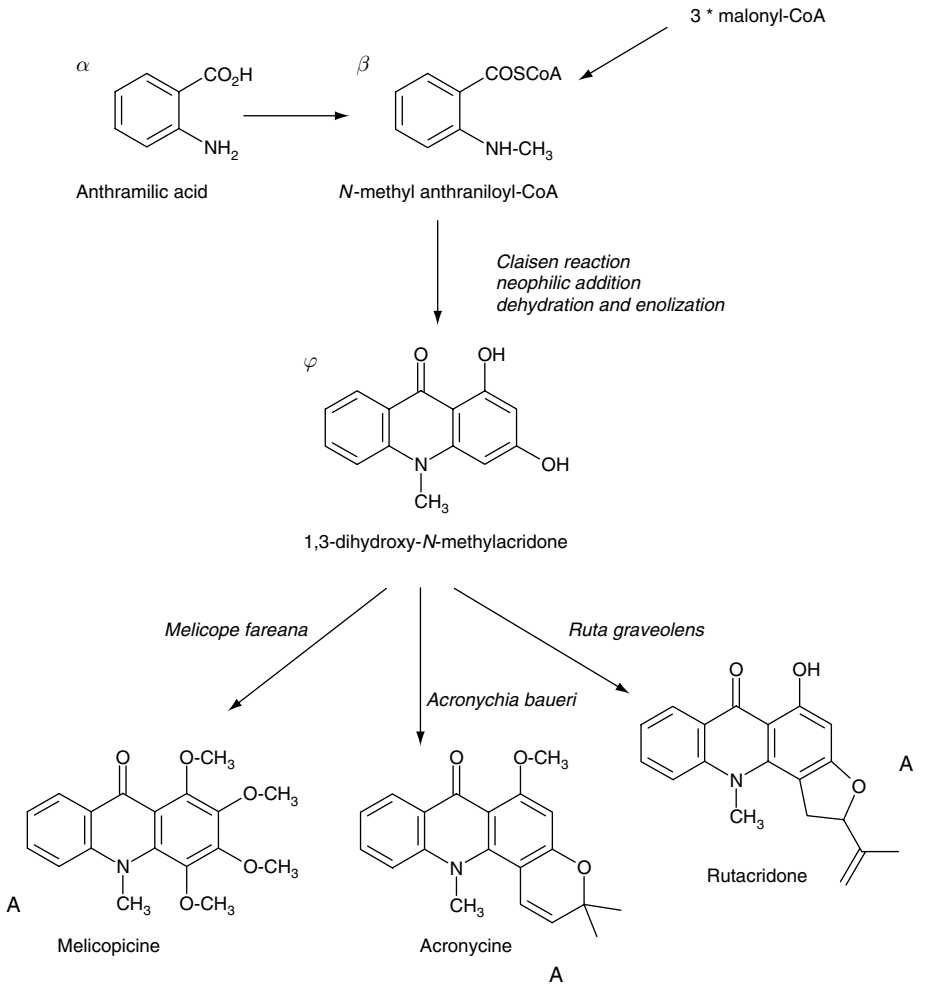


Figure 60. Structural development of acridone alkaloids.

alkaloids can be divided into several types, such as evoninate, wilfordate, edulinate, cassinate, lower molecular weight sesquiterpene pyridine and non celastraceous sesquiterpene pyridine alkaloids[58]. Evoninate sesquiterpene alkaloids can again be divided into evoninate, isoevoninate, hydroxyisoevoninate, epimeric evoninate and norevoninate compounds. Evoninate sesquiterpene alkaloids are compounds in which evonic acid esterifies the sesquiterpene nucleus. Typical alkaloids of this sub-group are evonimol derivatives (e.g., evonine), euonyminol derivatives (e.g., euonymine and hippocrateine), isoeuonyminol derivatives (e.g., emarginatine), 4-Deoxysesquiterpene cores (e.g., chuchuhuanine), cathedulin alkaloids (e.g., cathedulin), dimacrocyclic sesquiterpene pyridine alkaloids (e.g., triptonine). A typical isoevoninate sesquiterpene alkaloid

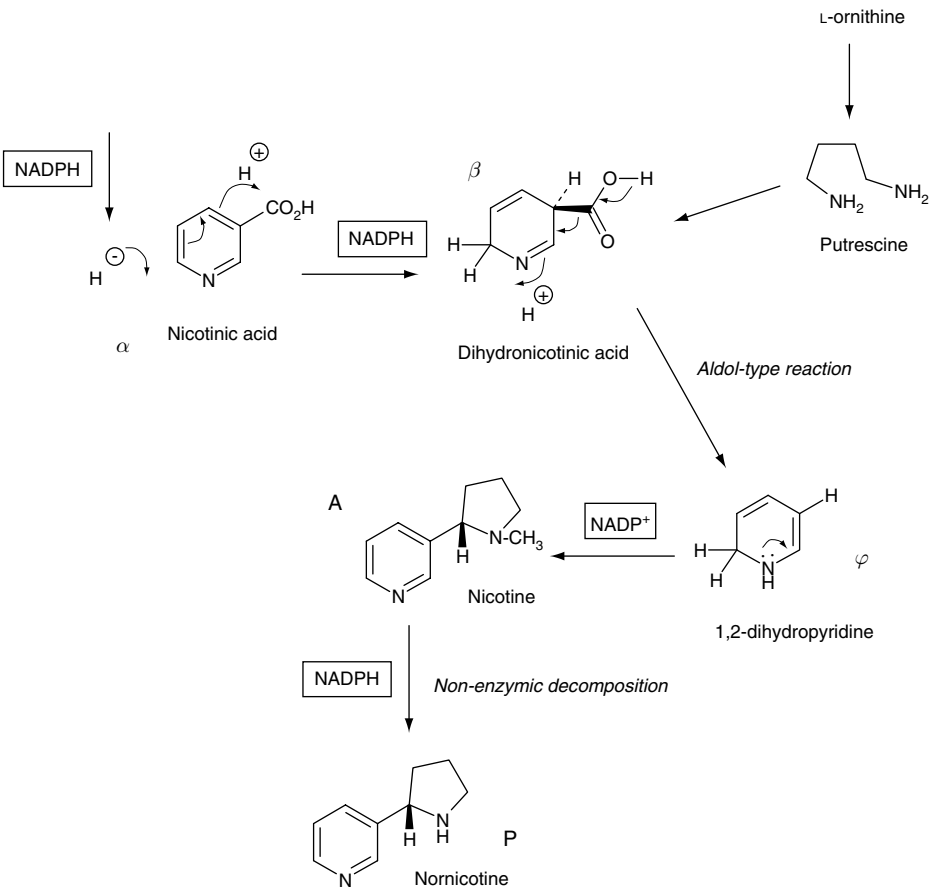


Figure 61. Structural development of pyridine alkaloids.

is hippocrateine, and a typical hydroxyisoevoninate one is hypoglaunine. Acanthothamine is a representative compound of epimeric evoninate alkaloid. Norevoninate alkaloid has only been isolated from *Hippocratea excelsa*[58].

Wilfordate alkaloids are another type of sesquiterpene pyridine compound. They are sesquiterpenes, macrocyclic compounds esterified by wildorfic acid. Wilforine is an example of this kind of alkaloid. Edulinate sesquiterpene pyridine alkaloids are sesquiterpene compounds esterified by edulinic acid. Cathedulin is one example. The cassinate group of sesquiterpene alkaloids contains orthosphenine and cassinine. These alkaloids have dihydroagarofuran sesquiterpenes esterified by cassinic acid. Lower-molecular-weight sesquiterpene pyridine alkaloids have only been isolated from plants of the Celastraceae family, and are characterized by the absence of a macrocyclic ring. They have a sesquiterpene core and a nitrogenous base through esterification. Lower-molecular-weight sesquiterpene pyridine alkaloids are also known as nocotinoyl sesquiterpene alkaloids[58].

Non-celastraceous sesquiterpene pyridine alkaloids are those compounds which have been isolated from other plants not belonging to the Celestraceae family. Rotundine, for example, has been isolated from *Cyperus rotundus* (Cypraceae). This is a structurally interesting alkaloid because it has a sesquiterpene skeleton containing a cyclopentane ring attached to the pyridine ring[58].

Natural sesquiterpene pyridine alkaloid formation needs two precursors, one for the pyridinium moiety and another for the sesquiterpene moiety. The α for formation of the pyridinium moiety is nicotinic acid, which reacts with isoleucine and, by oxidative reaction, produces evoninic acid, wilfordic acid or edulinic acids. α for the sesquiterpene moiety is still open to question, but E, E-famesyl cation has been suggested as one possibility and hedycarylol as a second. This moiety is dihydroagarofuran. Therefore, α for the sesquiterpene pyridine alkaloids is nicotinic acid and E, E-famesyl cation and, controversially, hedycaryol. The β is amacrocycling ring formation substance (two moieties), from which the alkaloid forms (Figure 62).

The sesquiterpene pyridine alkaloids have antifeedant and insecticidal activities. Some alkaloids, such as triptonine B, hypoglaunine B, hyponine B and wilfortrine, have antivirus activity potential. Others, such as emarginatines A–B, E–G and emarginatine, have cytotoxic activity. Ebenifoline and cangorinine have immunosuppressive activity.

2.7.2.13. Phenyl and phenylpropyl alkaloids

These alkaloids have a phenyl or phenylpropyl nucleus. The group includes simple phenyl amine (tyramine, hordenine), catecholamine (dopamine, noradrenaline, adrenaline), simple tetrahydroisoquinoline (mescaline, anhalamine, anhalonine, anhalonidine), benzylisoquinoline (e.g., papaverine), phthalideisoquinoline (e.g., noscapine), phenethylisoquinoline (autumnaline, floramultine and kreysigine), tetrahydroisoquinoline (emetine and cephaeline) and terpenoid tetrahydroisoquinoline (secologanin and ipecoside) alkaloids.

The α for this group of alkaloids is L-tyrosine (in some cases, also phenylalanine) and the β is L-dihydroxyphenylalanine (L-DOPA). Simple phenylamines, such as tyramine and hordenine, are derived from α by PLP (Figure 63). The A is tyramine and by activity of the SAM its P is hordenine. Phenyl and phenylpropyl alkaloids form a very large and diverse group. Many of the compounds belonging to them have very important commercial implications and are used in pharmacology and medicine. The alkaloids from Amaryllidaceae (e.g., norbelladine, lycorine, crinine and galanthamine) are also considered to be a part of this large group, although they are structurally very different. However, the pattern of biosynthesis, especially for α, is the same as for other alkaloids in this large group[32]. The term "isoquinoline alkaloids" was previously used for the group of phenyl and phenylpropyl alkaloids[18].

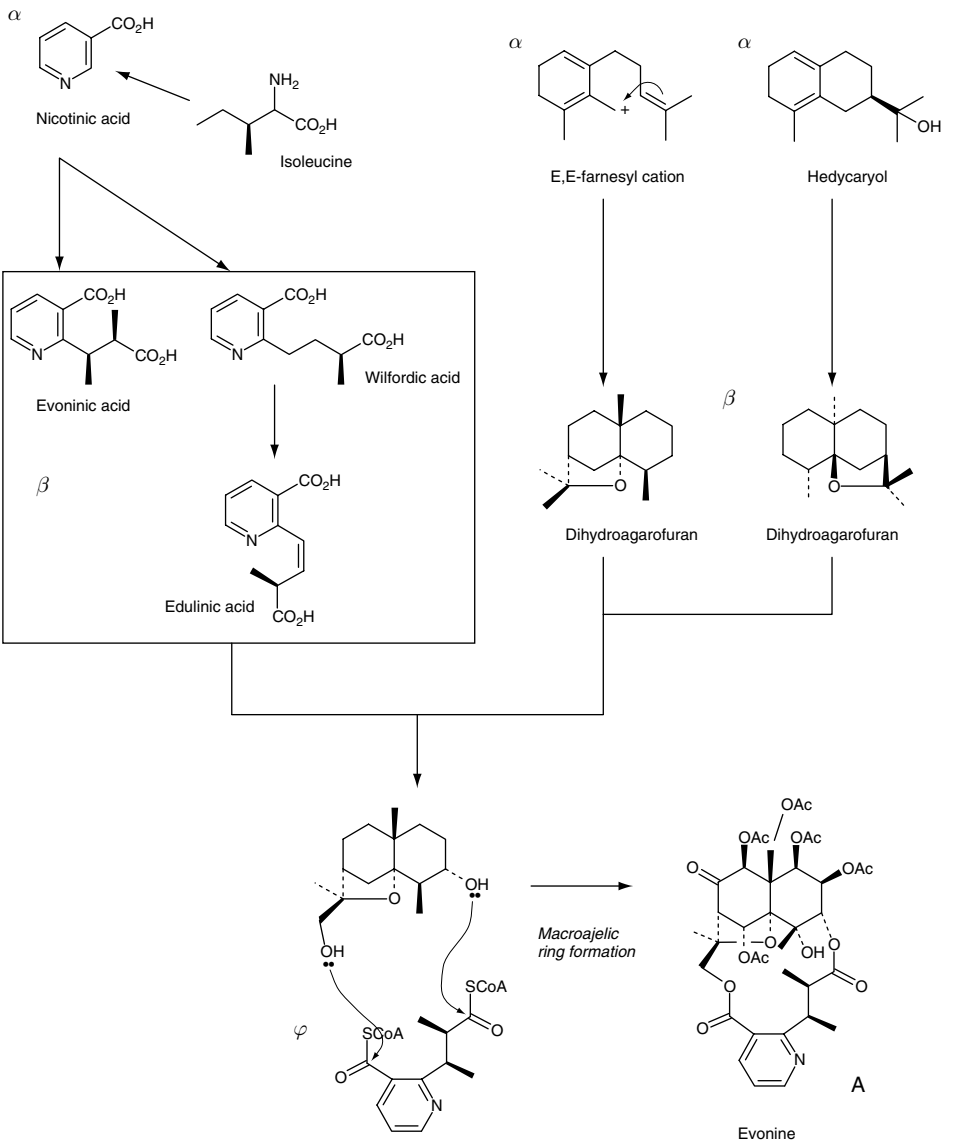


Figure 62. Structural development of sesquiterpene pyridine alkaloids.

2.7.2.14. Indole alkaloids

This structural group of indole alkaloids covers simple indole alkaloids (e.g., tryptamine, serotonin, psilocin and psilocybin), β-carboline alkaloids (e.g., harmine), terpenoid indole (e.g., ajmalicine, catharanthine and tabersonine), quinoline alkaloids (e.g., quinine, quinidine and cinchonidine), pyrroloindole

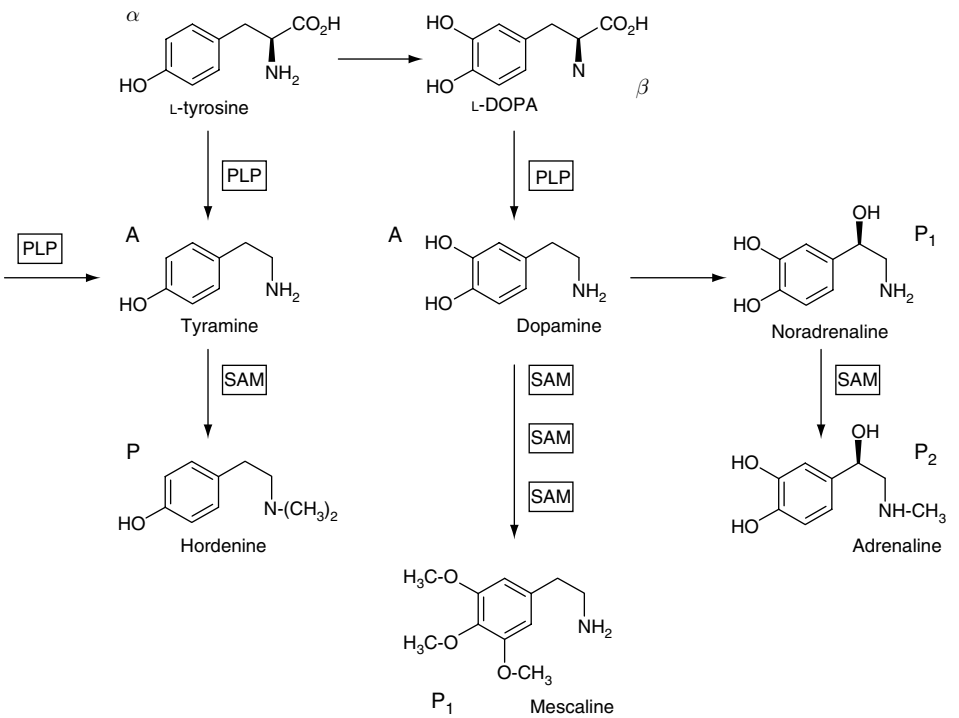


Figure 63. Structural development of phenyl and phenylpropyl alkaloids.

alkaloids (e.g., eserine) and ergot alkaloids (e.g., ergotamine). Indole alkaloids form a very important group from the perspective of their application.

Simple indole alkaloids

The α for structural development of serotonin (simple indole alkaloid) is L-tryptophan, and the β is 5-hydroxy-L-tryptophan (Figure 64). Serotonin is a monoamine. It is a bioactive alkaloid known as a neurotransmitter. It has been found in the cardiovascular system, in blood cells and the peripheral and CNS.

The α for the structural development of psilocin and psilocybin is L-tryptophan and the β is tryptamine. Psilocin is A and psilocybin is P. A and P are the main alkaloids in hallucinogenic mushrooms belonging to the genus *Psilocybe*.

Carboline alkaloids

The α of carboline alkaloids is L -tryptophan and the β is tryptamine, while the φ is dihydro-β-carboline. The carboline nucleus is formed at the stage of the φ. The A is elaeagnine, harman and harmaline, and the P is tetrahydroharmine or harmine (Figure 65).

Carboline alkaloids, and especially β-carbolines, are common in mammals[211].

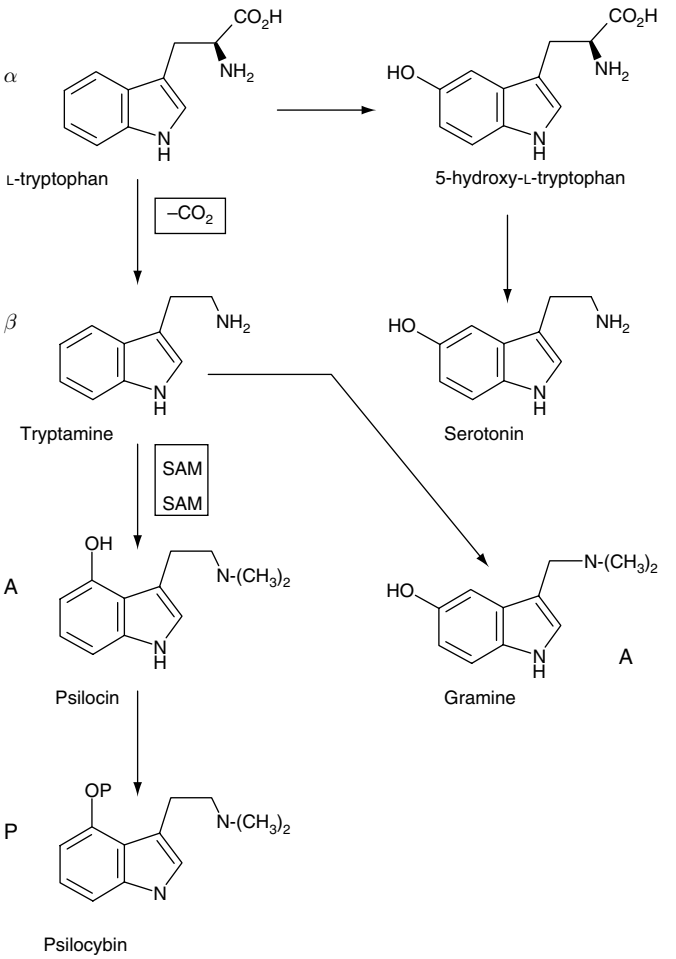


Figure 64. Structural development of simple indole alkaloids.

Terpenoid indole alkaloids

This group of alkaloids is very large and contains more than 3000 compounds. Three types of nucleus occur here: the corynanthe, iboga and aspidosperma.

1. *Corynanthe alkaloids*: The α of structural development is L-tryptophan and the β is tryptamine. The strictosidine is the φ. Moreover, the χ is dehydrogeissoschizine and the A is cathenamine. Ajmalicine is the P of cathenamine (Figure 66).
2. *Iboga alkaloids*: The common monomeric iboga alkaloids are ibogamine, ibogaine, coronaridine, voacangine and catharanthine. Ibogamine and catharanthine are prototypical structures. The α for cathenamine is L-tryptophan and the β is strictosidine, as in the case of *Corynanthe* alkaloids. Ibogaine is

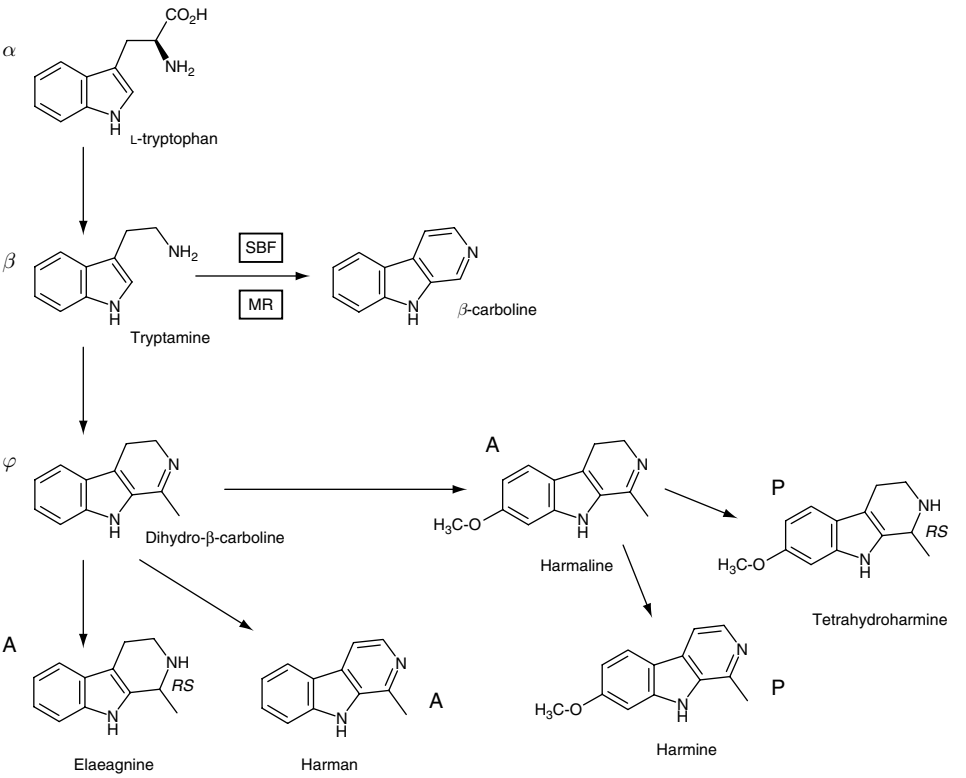


Figure 65. Structural development of carboline alkaloids.

the P of cathenamine (Figure 67). The iboga-type nucleus is derived from the Corynanthe type[32].

3. *Aspidosperma alkaloids*: These alkaloids have an aspidosperma-type nucleus. The α and β are the same as in corynanthe type. Catharantine is the P_4 from cathenamine (Figure 68).

Quinoline alkaloids

This group of alkaloids has two structurally different α. The α of alkaloids found in the genus *Cinchona* (Rubiaceae), such as quinine, quinidine, cinchonidine and cinchonine, is L-tryptophan. The β is tryptamine and the φ is strictosidine. The corynantheal is the χ. A is cinchonamine and cinchoninone. Cinchonine, quinidine, quinine and cinchonidine are the Ps of cinchoninone (Figure 69).

The second type of quinoline alkaloid, found especially found in the Rutaceae family, has α as anthranilic acid. Typical compounds from this group are edulitine, halfordamine, folifidine, folinine, casimiroin, foliosidine and swietenidine. The β is 3-carboxyquinoline and the A is graveoline in the case of those alkaloids from *Ruta angustifolia*.

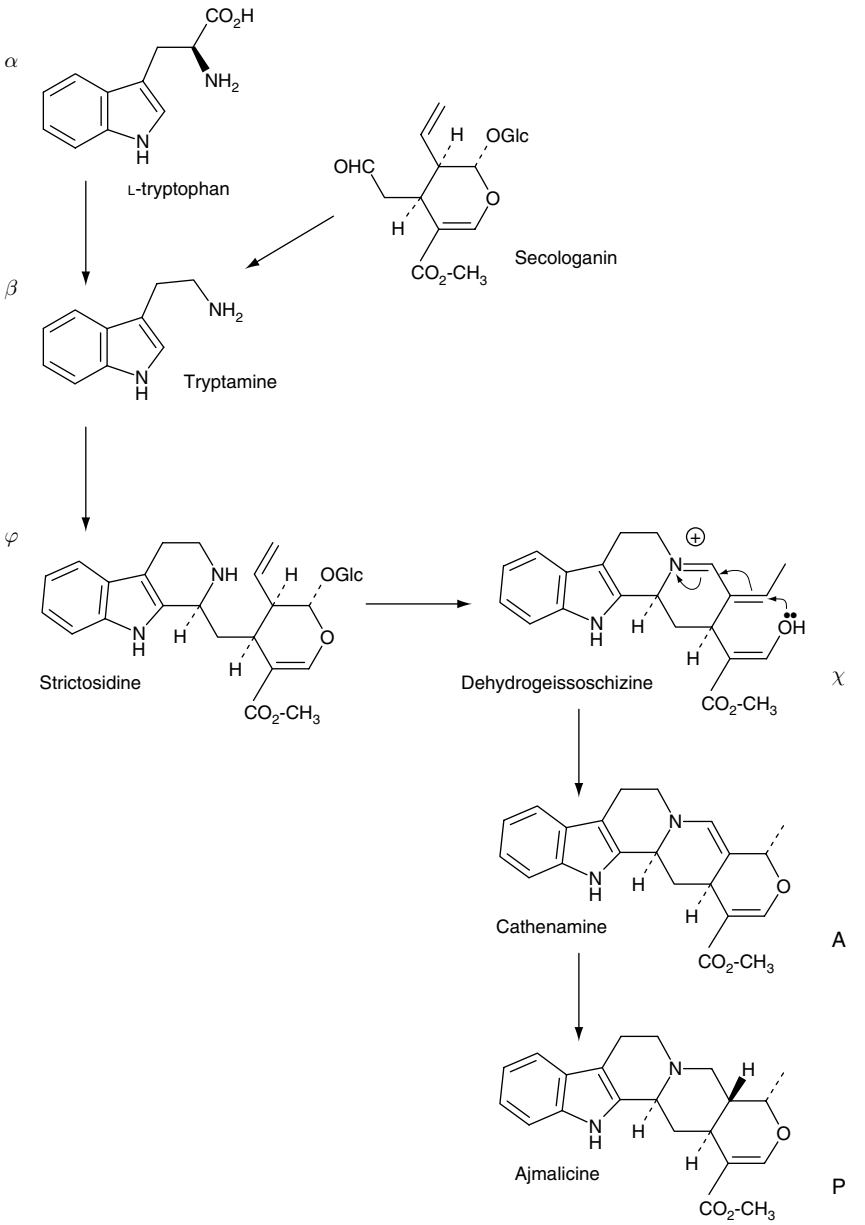


Figure 66. Structural development of corynanthe alkaloids.

In the case of *S. japonica*, the A is eduline and is formed directly from α in the decarboxylation process. In the case of micro-organisms, for example *Pseudomonas aeruginosa*, the α is incorporated directly to the A, which is 2-heptyl-4-hydroxyquinoline.

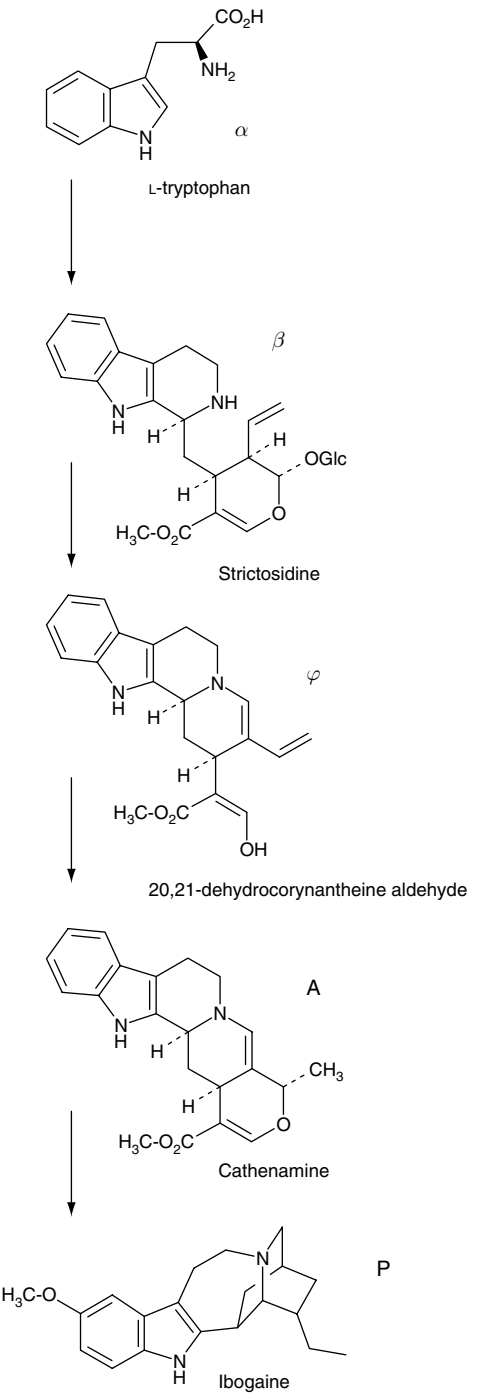


Figure 67. Structural development of iboga alkaloids.

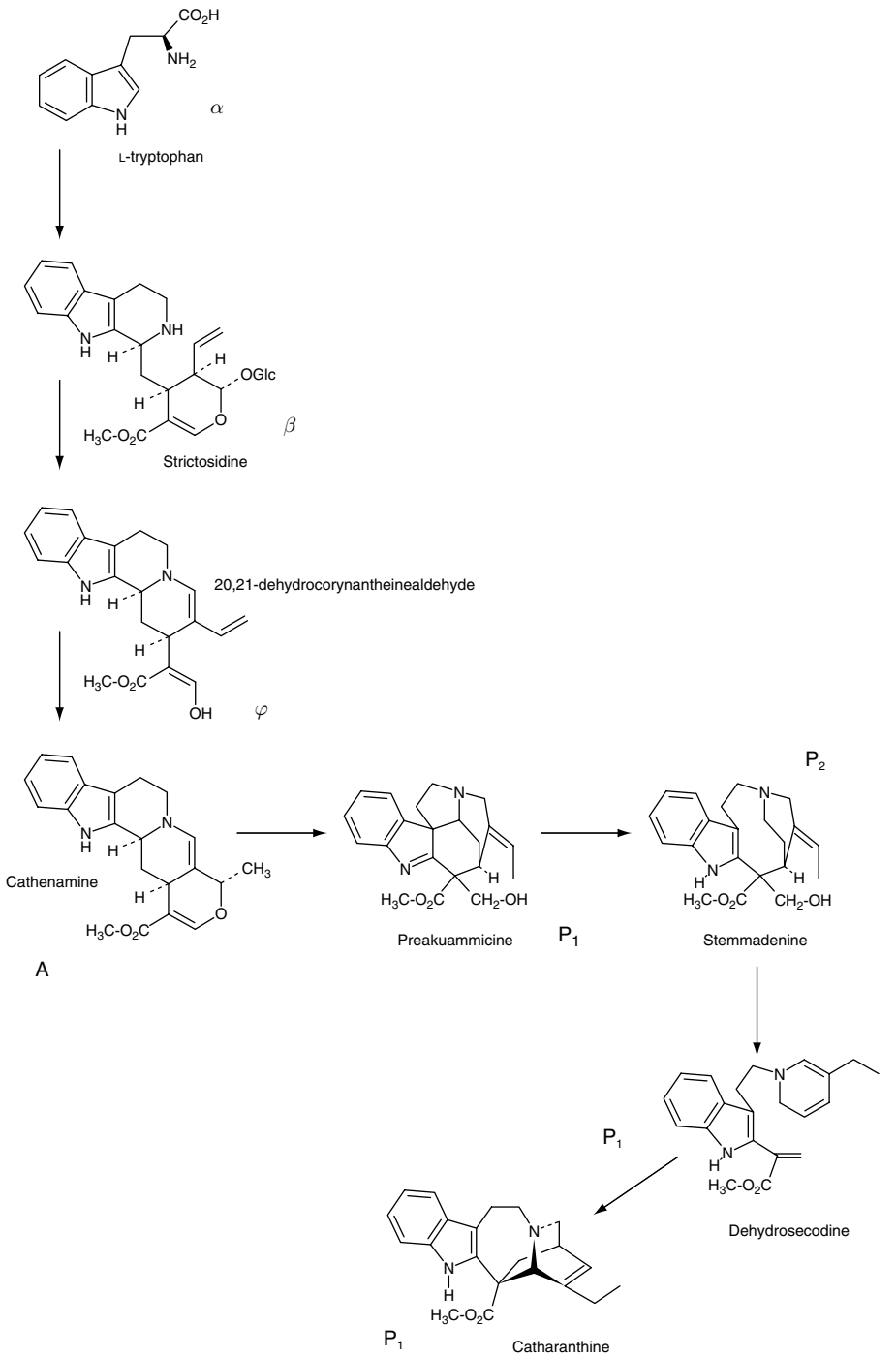


Figure 68. Structural development of aspidosperma alkaloids.

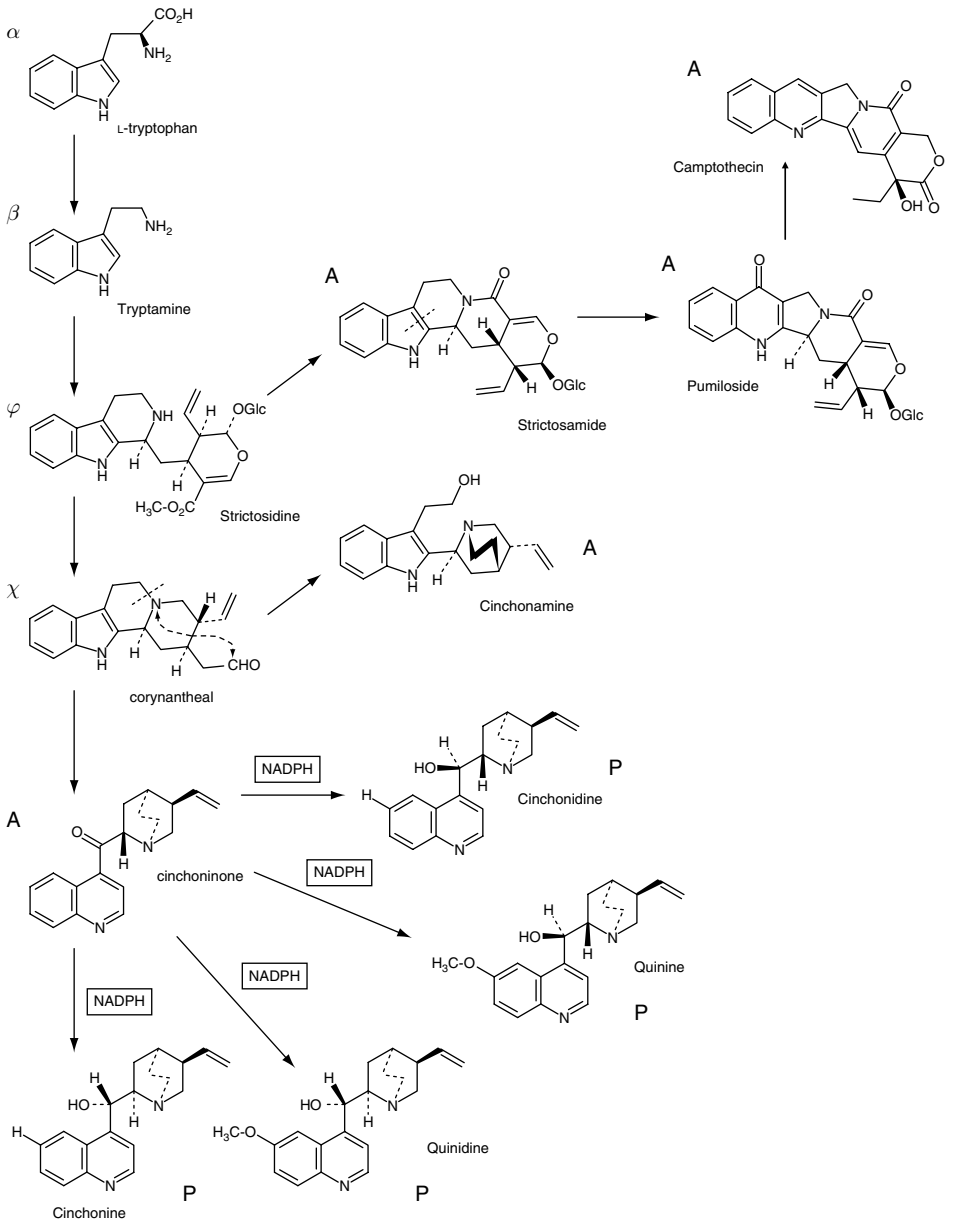


Figure 69. Structural development of quinoline alkaloids.

Quinoline alkaloids from the Rutaceae family (α = L-anthranilic acid) show antimicrobial activity. Moreover, quinoline alkaloids of the *Haplophyllum* species are known for their powerful biological properties. For example, skimmianine has sedative, hypothermic and antidiuretic uses. Haplophyllidine is a strong depressant of the CNS.

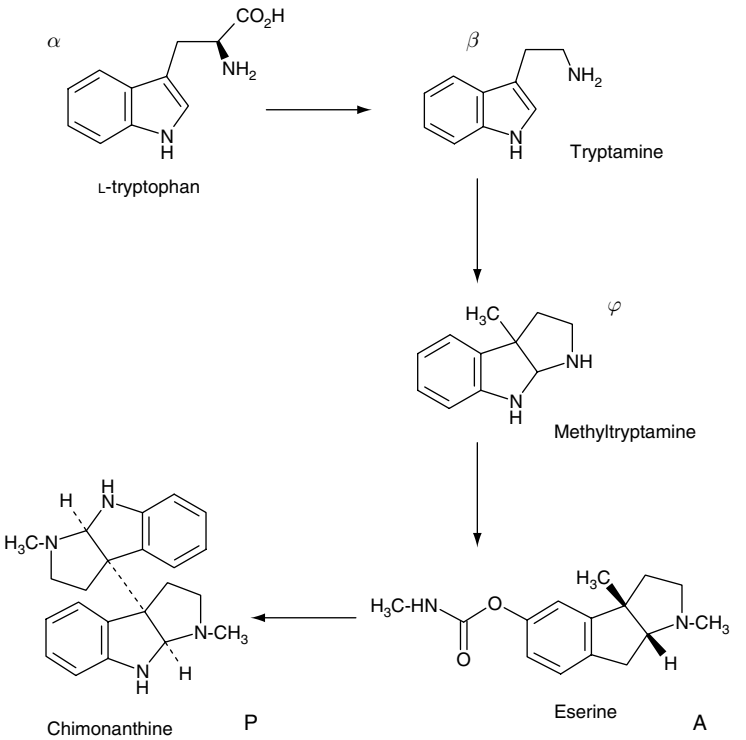


Figure 70. Structural development of pyrroloindole alkaloids.

Pyrroloindole alkaloids

The α of this group of alkaloids is L-tryptophan and the β is tryptamine, and the φ is methyltryptamine (Figure 70). The best-known alkaloids belonging to this group are eserine (A), chimonanthine (P), eseramine, physovenine, rivastigmine, eptastigmine, neostigmine, pyridostigmine and distigmine.

Ergot alkaloids

The α for ergot alkaloids is L-tryptophan and the β is four-steps alkaloid reaction chain. The φ is paspalic acid which converts to the D-(+)-lysergic acid (Figure 71). Ergotamine and ergometrine are the best-known members of this group.

2.7.2.15. Manzamine alkaloids

Manzamine alkaloids can be isolated from marine sponges. They often contain β-carboline. This group has a diverse range of bioactivities. It also has its own way of establishing its structures. An intramolecular Diels-Adler reaction for manzamines has been proposed. The α is bisdihydropyridine (derived probably from amonia), and the β is intramolecular cycloaddition in a pentacyclic

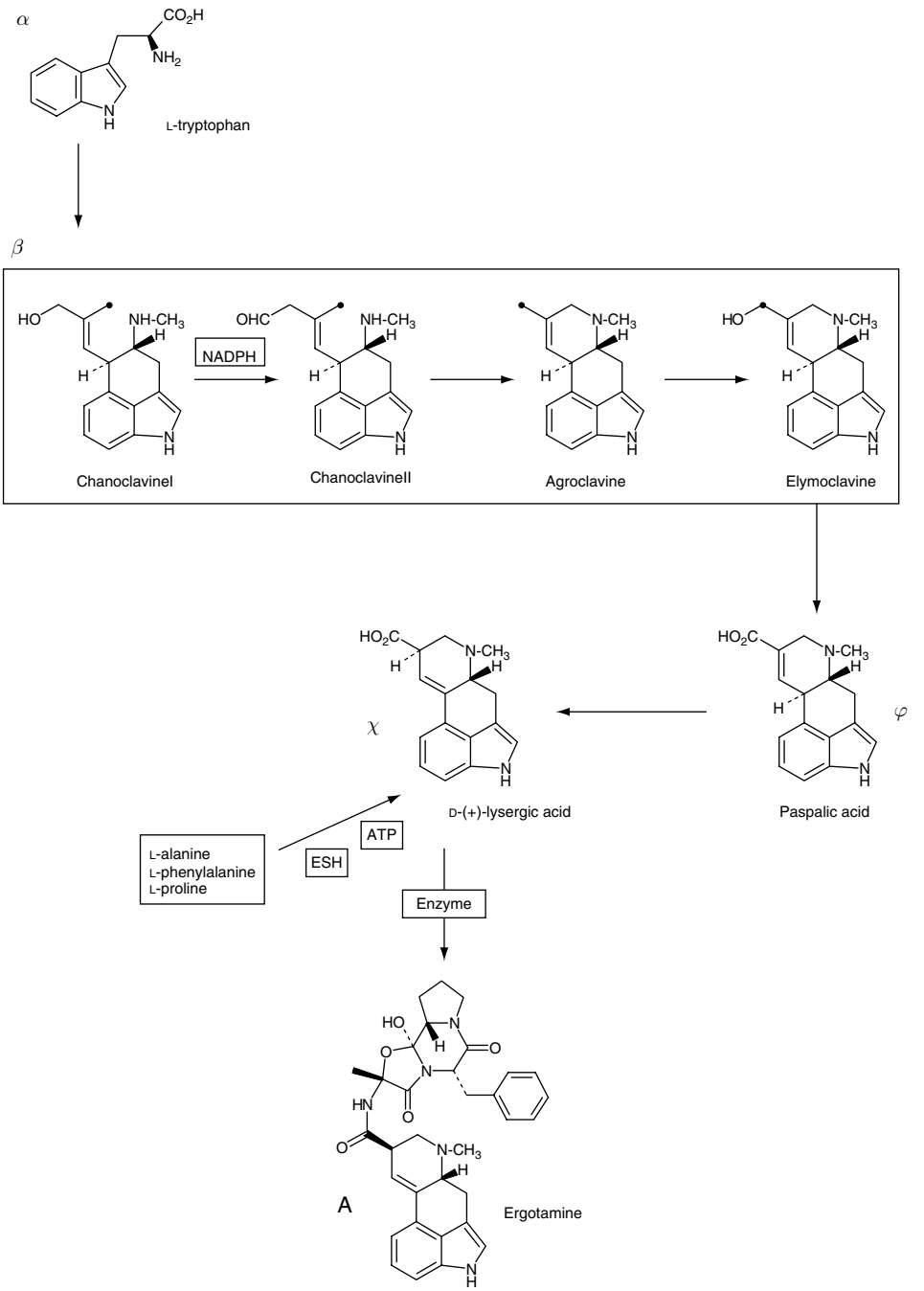


Figure 71. Structural development of ergot alkaloids.

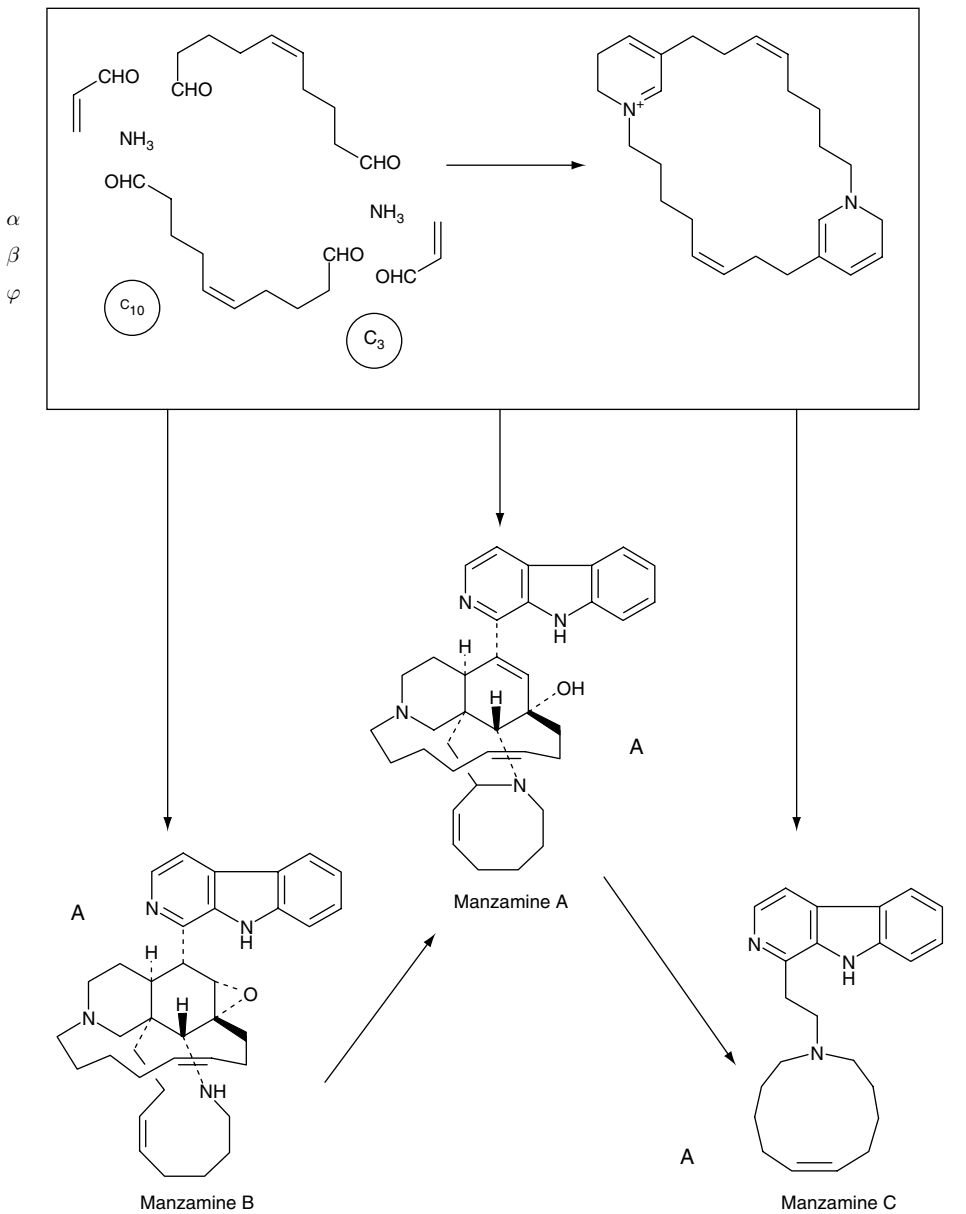


Figure 72. Structural development of manzamine alkaloids.

structure[57]. The φ is a tetracyclic intermedia, and the A is manzamine A and manzamine B and manzamine C (Figure 72).

The best-known manzamines are ent-8-hydroxymanzamine A, manzamines B–M and X, keramamine, kauluamine and xestomanzamines A and B.

2.8. Biogenesis of alkaloids

The synthesis and structural analysis of alkaloids leads to the following basic questions: why are alkaloids synthesized in an organism and on which mechanism is alkaloid formation and degradation dependent in the life cycle? It is known that alkaloids have a genetic nature[59] and that alkaloid content is diverse inside and between the species[16]. In nature the same species of plants may have both high and low alkaloid content[120,121]. Natural hybridization has been successfully used in plant breeding for the development of the so-called "sweet cultivars" in crop production. "Sweet cultivars", however, are not without alkaloids. The total removal of alkaloids is impossible. "Sweet cultivars" are therefore plants, in useful organs of which alkaloids are present at a very low level, the bioactivity of which is not of any significant or observable level. However, alkaloid decrease by hybridization is an undirect but strong argument for the case that alkaloids have an heredity nature and that their presence in plants is of an evolutional character. This is fundamental in answering the first question connected with the biogenesis of alkaloids. Alkaloids have a strong genetic–physiological function and background in the organisms which produce them. The biogenesis of alkaloids is therefore a part of the total genetic-functional strategy of such metabolisms.

2.8.1. Chemistry models

From the year 1805, when alkaloid chemical research started, the problem of the biogenesis of alkaloids proved central for chemists. The background to this problem was the fact that chemical compounds are synthesized by plants, used by plants and degradaded by plants. In the case of alkaloids, it was still difficult in the middle of the 20th century to truly ascertain the purpose of alkaloids in plants. Certainly, the use of these compounds in many applications outside of the organisms producing them was well recognized. Their role within the plants, especially in the metabolism, was not known. The general consensus was that alkaloids were "the waste" product of metabolisms and had no active role to play[16]. Therefore, chemical chains of alkaloid production were explained as chemical reactions, the "technical" process of life. Later, especially from the late 70s of the 20th century, the theory of "wastes" was debated and corrected[16]. However, chemical research has now extensively proved the existence of new alkaloids, the pathways of their biosynthesis and structural modification. Three directions in this research have been followed, one purely chemical, the second, biochemical, and the third purely biomolecular, or the molbiological direction.

The chemical explanation of alkaloid biogenesis is based on the consideration that all reactions are of a chemical nature and that the energy needed for life is produced by chemical reactions. Figure 73 shows a diagram of the chemical explanations for alkaloid biogenesis. From this diagram, it is clear that alkaloid

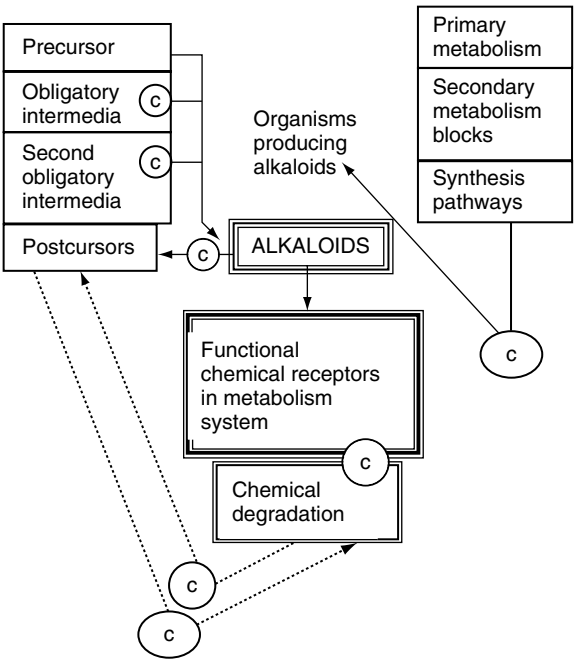


Figure 73. Chemical explanation for alkaloid biogenesis in organisms (c = catalysers).

is one of the metabolic objects in the system. It has a long chemical chain, which includes chemical synthesis before and chemical degradation after its functional activity in the metabolism. Biogenesis is, therefore, considered by chemistry to be the chain of the reactions between chemical molecules and by chemical means, in which reactions, conditions and catalysers are of special importance. Chemistry and organic chemistry consider alkaloid biogenesis to be the transformation of organic material with reaction catalysers. Different alkaloids have their own biogenesis and they are used, separately or together, with biochemical models in developing the methods for synthetic reactions and the modification of structures. Moreover, these models are also used in biotechnology[263,264]. Figure 74 presents the chemical model for the synthesis of *Catharanthus* alkaloids. It shows the primary metabolism as the background for alkaloid formation, although the *Catharanthus* alkaloids are the yields of a secondary metabolism. The connection between primary and secondary metabolisms is an important area for future studies in chemistry. From the model presented, it is clear that *Catharanthus* alkaloids are postcursors from three basic compounds: acetate, glucose and tryptophan. In the *Catharanthus* alkaloids, three types of ring nucleus are presented. The chemical model describes biogenesis from the point of view of the formation nucleus and skeleton of alkaloids, together with connected chemical molecule reactions in their structural and dynamic changes. Torssell[225] has used the term "mechanistic approach" for the secondary metabolism to describe the chemical approach

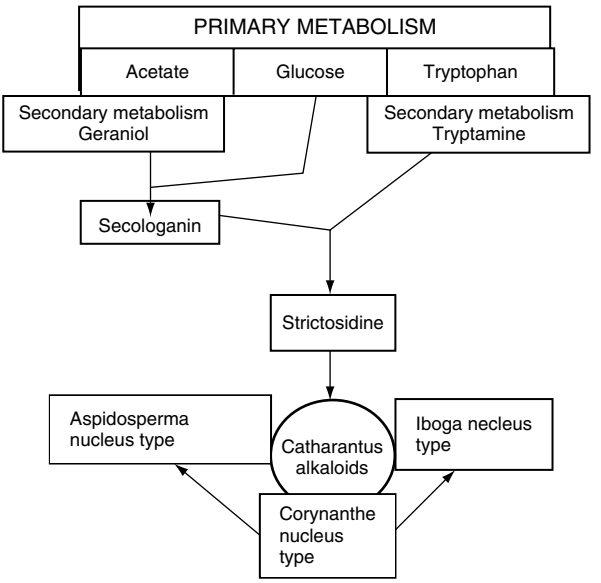


Figure 74. Chemical model of indole alkaloid formation in *Catharanthus roseus*. The arrows represent the direction of formation, the flux of compounds skeleton construction.

to this metabolism. Chemical routes, and alternative routes and their options, are very important for chemical models. In particular, the tendency of natural processes and reactions to shorten synthesis pathways is significant. Nowadays, the principles of alkaloid biochemistry and their biosynthetic means are widely recognized by specialists, and chemical, or mechanistic approaches to synthesis and biosynthesis, from a basic part of research. Without carbons, nucleus, skeleton, ring and moiote, the alkaloid will not exist. To research these structural components of alkaloids, chemical models of approach are the most effective.

2.8.2. Biochemistry models

The description of single enzyme activity in chemical reactions, together with the activity of other biomolecules, is typical for biochemical models of alkaloid biogenesis. There is no contradiction between chemical and biochemical, which serve to enrich one another. In many cases, typical chemical and biochemical models are unified in papers today[263,264].

Biochemical reactions are basically the same as other chemical organic reactions with their thermodynamic and mechanistic characteristics, but they have the enzyme stage. Laws of thermodynamics, standard energy status and standard free energy change, reduction–oxidation (redox) and electrochemical potential equations are applicable to these reactions. Enzymes catalyse reactions and induce them to be much faster[225,265]. Enzymes are classified by international

convention into six classes on the basis of the chemical reaction which they catalyse. According to Enzyme Commission (EC) rules, the enzyme classes are (1) oxidoreductases (transfer of hydrogen or oxygen atoms and electron forms), (2) transferases (transfer of chemical groups), (3) hydrolases (catalysing of hydrolytic reactions), (4) lyases (cleaving substrates by other reactions than hydrolysis, (5) isomerases (intramolecular rearragements), (6) synthases (catalyse covalent bond formation). The best-known enzymes and coenzymes active in alkaloid biogenesis are presented in Table 20.

The biochemical model contains the pathways of the enzymatic reactions in the synthetic routes. Model can be constructed for each alkaloid. Figure 75 presents biochemistry model of *Catharantus* alkaloids. The most important enzymes on this model are TDC (tryptophan decarbocylase), G10H (geraniol 10-hydroxylase) and SS (strictoside synthase). $NADPH^+$, PO (Peroxidase), O (oxidase) and $NADH^+$ are all active in different *Catharantus* alkaloid formations. The biochemical models are subject to both qualitative and quantitative alkaloid

Table 20 Some well-known enzymes and coenzymes active in alkaloid biogenesis

Enzyme Type	Reactions
Decarboxylases (DC) Tryptophan decarboxylase (TDC) Phenylalanine decarboxylase (PDC)	Decarboxylation
Dimerases (DM)	Dimerisation
Hydroxylases (H)	Hydroxylation
Methylases (MT)	$+CH_3$
Synthases	Synthesis
Oxidases (O)	Removing hydrogen from a substrate
Peroxidases (PO)	Using hydrogen peroxide
N-methyltransferase (MT)	Transfer of methyl group
Amine oxidases (AO)	Oxidizing reactions
Monoamine oxidase (MO)	Dehydrogenation to an imine
Diamine oxidase (DO)	Oxidizing to aldehyde
Dehydrogenases (DHG)	Removes two hydrogen atoms from the substrate
NAD^+(nicotinamide adenine dinucleotide)	Tends to be utilized as hydrogen acceptor
$NADP^+$(nicotinamide adenine dinucleotide phosphate)	Tends to be utilized as hydrogen acceptor
Pyridoxal phosphate (PLP)	Coenzyme in transamination and decarboxylation
CoA	Involves biological reactions
S-adenosylmetionine (SAM)	Provides positively charged sulphur and facilitates nucleophilic substitution
Dimethylallyl diphosphate (DMAPP)	Nucleophilic substitution
Transaminases (TA)	Transamination
Reductases (RD)	Reduction

Sources: Refs [32, 308, 529, 553, 639, 677, 769, 770, 771, 772, 773, 774, 775].

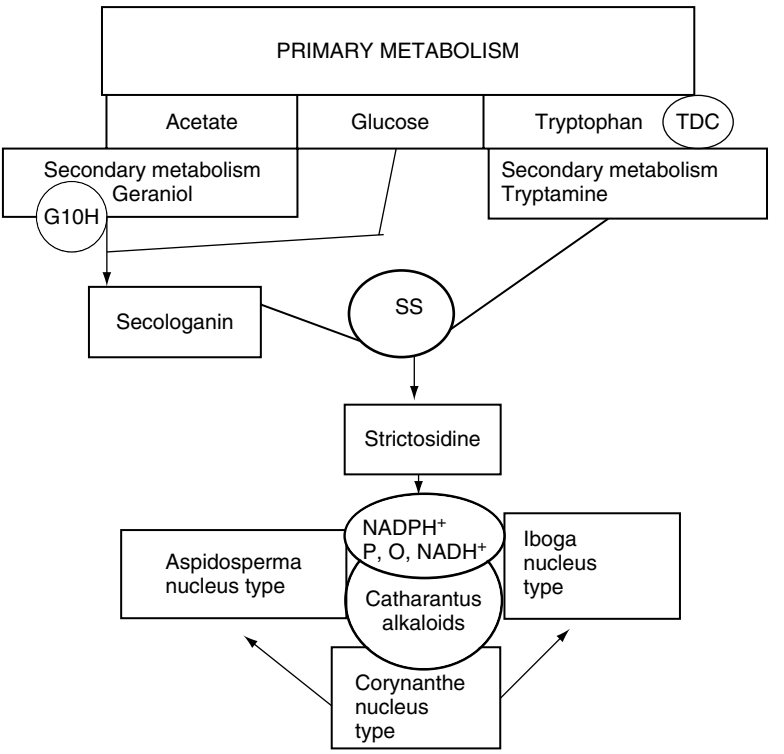


Figure 75. The biochemical model for indole alkaloid formation in *Catharanthus roseus*. The arrows represent the direction of the formation and the flux of compounds in skeleton construction. On the diagram, enzymes are shown by a circle.

analysis. Not all enzymes participating in alkaloid synthesis and degradation are yet known. Alkaloid enzymatology is, therefore, a growing research area.

2.8.3. Molecular biology models

Alkaloid research and bioanalysis of central-processing molecules (DNA and RNA) led to the important concept of the heredity nature of alkaloid metabolisms. Recent investigations have proved empirically that alkaloids have a genetic background and that all their biogenesis is genetically determined[266,267,268,269,311]. According to Tudzynski et al.[266], cpd1 gene coding for dimethylallyltryptophan syntase (DMATS) catalyses the first step in the biosynthesis of ergot alkaloids from *Claviceps purpurea*. The second gene for ergot alkaloid biosynthesis is cpps1, which encodes for a 356-kDa polypeptide showing significant similarity to fungal modular peptide synthetases. According to Tudzynski and his research group[266], this protein contains three amino acid–activating modules, and in the second module a sequence is found which matches that of an internal peptide

(17 amino acids in length) obtained from a tryptic digest of lysergyl peptide synthase 1 (LPS1) of *C. purpurea*. The authors proved that cpps1 encodes LPS1. Cpd 1 is also involved in ergot alkaloid biogenesis. Cpox 1 probably encodes for an FAD-dependent oxidoreductase (which could represent the chanoclavine cyclase), while the second putative oxidoreductase gene, cpox2, is closely linked to it in inverse orientation[266].

At least some genes of ergot alkaloid biogenesis in *C. purpurea* were found to be clustered. This means that detailed molecular genetic analysis of the alkaloid pathway is possible[266]. These results were confirmed by the research of Haarmann et al.[269]. Moreover, Huang and Kutchan[267] found three genes (cyp80b1, bbe1 and cor1) which encode the enzymes needed for sanguinarine synthesis. Molecular biology models may be constructed for each alkaloid biogenesis. An example of this kind of model is presented in Figure 76.

Molecular biology research on alkaloids is very revealing. Its results can be used in the construction of alkaloid biogenetic models. At present, only a few alkaloid metabolism genes are known.

2.8.4. Analytical dilemmas

Chemical, biochemical and biological models of alkaloid biogenesis can only be constructed according to scientific research on the small chains of the synthesis of each alkaloid, and enzyme and gene involved in these chains. Models are constructed from the experimental data on synthesis and degradation of alkaloids.

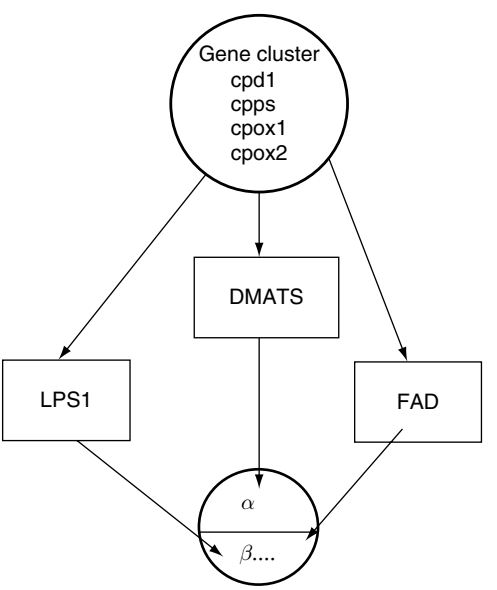


Figure 76. Molecular biology model of *Claviceps purpurea* alkaloids.

Despite the fact that a high technical level of analytical equipment exists and research is exact, the results do not give a direct answer to some questions of biogenesis. For structural (chemical), enzymatic (biochemical) and genetic (molbiology) research, different techniques are deployed. They work in different conditions and the results received are from these different conditions, although from the same places in the pathway. In model construction, a researcher should unify these results for a logical chain. In many cases, there are theoretical or hypothetical conclusions. Certainly, the common analysis in the same trial of the structural, biochemical and biological aspects of alkaloids will be the best method. Such kinds of supertechnique are still not developed. Therefore, the primary analytical dilemma concerns the question of separate or common analysis[4]. On the one hand, this question covers the chemical, biochemical and molbiology aspects and, on the other, the isolation of alkaloids. The dominant way is separate analysis for separate alkaloids. Separate alkaloid analysis gives exact results in microscale, microplace and microimportance. In many cases, such results would be compromised in macroscale analysis (the biogenesis model). However, in the extraction of some group of alkaloids, the same method (common in this sense) is accepted in many papers. One example is quinolizidine alkaloids. Although quinolizidine alkaloids are very similar, they also have differences in optimal dissolving (temperature and pH-value), purity, stability and (+)- and (−)-forms. The same method of extraction for all alkaloids is a great compromise and causes compromised results, which have been regarded as sufficient.

Another analytical dilemma is the problem of *in vitro* and *in vivo* conditions. Alkaloids should be studied in their physiological conditions in organisms. This is not possible in many cases. *In vitro* experiments give compromised data. Theoretical conclusions and hypotheses in analysis, although they are in many cases indicators of a new breakthrough, also have some problems and some risks.

Analytical dilemmas are one reason why continuous novelization of structural, biochemical and molbiological results is necessary. These dilemmas merit attention nowadays, more than 200 years after the first alkaloid was isolated.

2.9. Methods of alkaloid analysis

These analytical dilemmas interfere with the methods of alkaloid analysis. Each group of alkaloids has its own methods of extraction, isolation and crystallization, as well as detection in structure, molecule and dynamicity. Not all these stages are still possible in the majority of alkaloids. In recent years, many techniques have been used in alkaloid detection. There are atomic and molecular electronic spectroscopy, vibration spectroscopy and electron and nuclear spin orientation in magnetic fields, mass spectroscopy, chromatography, radioisotope and electrochemical techniques. Although important developments in methodology and

methods of alkaloid analysis have occurred over the last 200 years, the most efficient methods are still awaited. The oldest parts of these methods seen to be the extraction and isolation stages.

2.9.1. Methods in history

The first method of alkaloid analysis was developed in 1805, in the case of morphine. This method of isolation, with minor and major variations, is still used today. By this method, the first quinolizidine alkaloids were also extracted: sparteine in 1851, lupinine in 1865 and lupanine 2 years later. At the beginning of the 20th century, the extraction and determination of total quinolizidine alkaloids in the same analysis (common) was carried out by Jurkowski[270], Nowotnówna[271], Trier[272], Ivanov[273,280], Sengbusch[274,275,276,277], Łukaszewicz[278], Wuttke[279]. Reifer and Niziołek[281] and Wiewiórowski and Skolik[282] initiated research in which the sum of the contents of the different and separate alkaloids is the total alkaloid content. The method of isolation of quinolizidine alkaloids was developed next by Wysocka et al.[249,250] and Wysocka and Przybył[244,245].

2.9.2. Basic methods and instruments

The first step in the development of methods was the evidence that molecules synthesized and degradated, and that intermediate compounds existed. Initial methods have provided molecule isolation, and subsequently the place in the metabolic chains. The basic methods of alkaloid determination developed historically as follows: iodine, taster, seed colour, Dragendorf reagent, fluorescence, calorimetry, photometry, electrophotometry, spectrometry, paper chromatography, thin layer chromatography, high-performance liquid chromatography, gas chromatography, gas liquid chromatography-mass spectrometry, nuclear magnetic resonance, X-ray, enzyme-like immunosorbent assay, radio immuno assay and scintillation proximity assay methods[4]. The most effective method for establishing a metabolic pathway is the use of isotopes in radiotracing and mass spectrometry methods. The basic instruments that have been developed are photometers, calorimeters, analyzers, spectrometers, chromatographs and different mass spectrometers. These instruments have subsequently been improved to be more exact, and have been through many generations in their development by many different producers.

2.9.3. From iodine to enzyme

Alkaloid analytical methods were developed by applications based on different hypotheses, from the simple to the very complicated. Subsequently, corresponding instruments were developed. This development can be seen by considering the example of quinolizidine alkaloids.

2.9.3.1. Iodine

Iodine was discovered by Barnard Courtois in 1811 in France. It has been successfully researched and used in biochemistry and clinical research since 1825. In analytical work, however, it is necessary to draw attention to the fact that iodine can occur in biological material as free iodine, or in some other forms such as iodoaminoacids and iodoproteins. Three chemical methods for the quick determination of quinolizidine alkaloids were developed for use in practical breeding in 1927[277]. All of these methods were based on the use of iodine. In the first of Sengbusch's methods, the alkaloids were extracted from whole seeds or leaves by means of hot water, and precipitated with iodine-quicksilverpotash of iodine. By this method of alkaloid analysis, the first "sweet" plants (without indication of alkaloid content) of *L. luteus* were found[277]. With material of low alkaloid content, the second method could be evolved in which the alkaloids were extracted with hydrochloric acid instead of hot water. According to Sengbusch[277], this method is mainly suitable for the investigation of leaf material, whereas in the testing of seeds, precipitation of non-alkaloid substances apparently also occurs. The hydrochloric acid method with cold water extraction was then developed. In this method, the alkaloids are extracted from the seed with cold water and are then precipitated with iodine-potash. According to Sengbusch[277], this method permits the testing of seed-material without damage to germination so that the tested seeds can be sown. This is very important, for example, in plant breeding work.

The use of iodine in determining total alkaloid content has been developed by plant breeders in field conditions up to the present. The basic iodine solution contains 100 g J and 140 g KJ as well as 1000 ml water. Before application, the basic iodine solution is diluted by 1:3 or 1:5. The colour of leaves without alkaloids is not changed after application of the iodine solution. Following this method, the leaf colour changes to red-brown[277,279].

Very similar to the method of Sengbusch was that developed by Schwarze, during which the juice of leaves was transferred onto blotting paper. After that, the blotting paper was put into the iodine–potash–iodine solution. The brown colour which appeared on the blotting paper was caused by alkaloids, and the colour of the blotting paper in which alkaloids were absent was slightly yellow or green[4]. A similar solution is also known as the old Dragendorff reagent, and as the KI/I_2 test. The use of KI/I_2 needs 2–3 hours for sample determination.

2.9.3.2. Tester method

This is a non-chemical, and probably the first biological, method of determining the presence of alkaloids. It was first used particularly with quinolizidine alkaloids in lupine plants. The tasters were men or animals, even in ancient times. It is based on the fact that quinolizidine alkaloid has a bitter taste. This method is qualitative. Taste is a subjective and individual category, especially in the

case of animals. It is known generally that hares and sheep are more tolerant of quinolizidine alkaloids than other animals. This method can be described as a simple attempt to determine bitterness (alkaloid) in lupine plants.

2.9.3.3. Seed colour method

The simple observation that white seeds are sweeter that black seeds was used in the construction of a practical method of judging lupine seeds qualitatively. This method cannot be used with confidence, because, especially in white lupine, even very white seeds can have a high alkaloid content. On the other hand, plants from the same species are "sweet". In some species, for example in the case of L. *angustifolius* or L. *luteus*, the tendency of white seeds to be "sweet" is more likely but not absolutely certain.

2.9.3.4. Dragendorff reagent

Dragendorff reagent (DRG) was developed as a reagent for detecting alkaloids, heterocyclic nitrogen compounds and quartenary amines. It is used particularly in plant drug analysis[283]. Alt least six different Dragendorff reagents are known. Each one also contains potassium iodine.

2.9.3.5. Fluorescence method

This method is based on the fluorescence characteristics of lupanine and its derivatives in L. *albus*. They have a fluorescence capacity to light of 366 nm[284,285,286]. Fluorescence is an emission of light from a molecule which is returning to its normal ground state from the lowest vibrational level of an excited singlet state light[285]. Fluorescence is closely related to absorption, because absorption must precede fluorescence emission. For this method, a UV lamp with light of 366 nm is necessary. Bitter seeds are fluorescent and sweet seeds are not. Generally, the fluorescence method of lupine seed analysis is considered to be qualitative only[286]. In reality, this method can, after development, also be quantitative. For this purpose, it is necessary to ensure (1) that the intensity of fluorescence is directly proportional to the molecular absorptivity, (2) that the intensity of fluorescence is directly proportional to the concentration of the fluorescent species and (3) that the intensity of fluorescence is directly proportional to the intensity of the incident light[285]. This method, with some innovations, is also used currently in the detection of other alkaloids, especially for detection of a 9-acridone moiety in UV of 401, 352, 323, 285, 275 and 269 nm and a xanthone skeleton. The fluorescence method is used in the process of lupine seed qualification. This method is relatively easy. The possible risk of the destruction of seeds does not exist. However, this method is not perfect. The humidity of seeds is a very important factor in their fluorescence. The best results have been obtained with 90–92% dryness of seeds.

2.9.3.6. Calorimetry method

The idea of calorimetry is based on the chemical reaction characteristic of molecules. The calorimetry method does not allow absolute measurements, as is the case, for example, with volumetric methods. The results given by unknown compounds must be compared with the calibration curve prepared from known amounts of pure standard compounds under the same conditions[285]. In practical laboratory work there are very different applications of this method, because there is no general rule for reporting results of calorimetric determinations. A conventional spectrophotometry is used with a calorimeter[285]. The limitations of many calometric procedures lie in the chemical reactions upon which these procedures are based rather than upon the instruments available[287]. This method was first adapted for quinolizidine alkaloid analysis in 1940 by Prudhomme, and subsequently used and developed by many authors. In particular, a calorimetric microdetermination of lupine and sparteine was developed in 1957[281]. The micromethod depends upon the reaction between the alkaloid bases and methyl range in chloroform.

2.9.3.7. Photometry method

The basis of the photometry method is a comparison of the extent of the absorption of radiant energy at a particular wavelength in a solution of the test material with that in series of standard solutions. Filter photometers are suitable for routine methods that do not involve complex spectra[287]. In practical laboratory work the photometric micromethod was developed for determination of sparteine, lupanine, lupinine, hydroxylupanine and angustifoline[282]. This method, tested on model solutions, is suitable for the determination of alkaloids in vegetal material of very low alkaloid content.

2.9.3.8. Electrophotometry method

The use of electrophotometry requires a sample preparation with a coloured solution. Together with an electrophotometer for alkaloid analysis, a constant light intensity and filter, as well as an electronic installation for measurement, must be used. The electrophotometry method is an application of both calorimetry and photometry in the same analysis.

2.9.3.9. Paper chromatography

Paper chromatography as a method of alkaloid analysis has a long history. It was first proposed in Russia by M.S. Tswett in 1903 after the successful separation of a mixture of plant pigments[288,289]. The solution containing the alkaloid is transferred onto tissue-paper. The colour of the tissue-paper is very important and can be compared against a standard. This is a qualitative and also a quantitative method of analysis if the standard is scaled. The paper used is very similar to that for thin layer chromatography, but without the need of special coatings[290]. Modern chromatographic methods are based on the principles of this

first method, in which the different distribution and behaviour of compounds or their parts in the stationary and mobile phases of the solid are fundamental considerations.

2.9.3.10. Thin layer (planar) chromatography

Thin layer chromatography (TLC) is widely adopted for the analysis of alkaloids. The basic characteristics of thin layer chromatography as a method are as follows: qualitative and also semi-quantitative analysis, speed of analysis and a chromatographic fingerprint (R_f values and colours, colour photography, densitometry or fluorometry of the chromatogram at certain wavelengths and a photographic alkaloid atlas). At the mobile phase, the mixture moves across the layer from one side to the opposite. This movement of the solid transfers analyte placed on the layer at the rate determined by its distribution coefficient (K) between the stationary and mobile phases[289]. The movement of the analyte, therefore, can be expressed by factor R_f, which is the relation between the distance moved by the analyte from its origin to the distance moved by the solvent from its origin ($R_f = K_a/K_s$). The sample applied to the TLC should contain at least 50–100 μg of alkaloids. This method was used recently for alkaloid metabolite extraction, analysis and purification[308].

2.9.3.11. High performance liquid chromatography

High performance liquid chromatography (HPLC) is a modern application of liquid chromatography. High performance liquid chromatography guarantees a high sensitivity and, at the same time, this technique has its gas analogue. The principle of HPLC is the same as that of liquid chromatography (LC), liquid–solid chromatography (LSC) and liquid–liquid chromatography (LLC). High performance liquid chromatography is the most recent technique.

The stationary phase may be a solid or liquid on a solid support. The mechanisms responsible for distribution between phases include surface absorption, ion exchange, relative solubilities and steric affects[289,290,291,292]. High performance liquid chromatography is a useful method for quinolizidine alkaloid analysis, especially when pure standards are available[4]. This method was recently used for alkaloid metabolite extraction and analysis[308,309]. A simple reversed-phase liquid chromatographic method has been developed for the simultaneous quantitation of four anticancerous alkaloids vincristine, vinblastine, and their precursors catharanthine and vindoline using a specific HPLC column[310].

2.9.3.12. Gas chromatography

Gas chromatography is a similar method to that of HPLC. It provides a quick and easy way of determining alkaloids in a mixture. The only requirement is some degree of stability at the temperature necessary to maintain the substance in the gas state[290].

Gas chromatography is divided into two subclasses, according to the nature of the stationary phase. One of this in GSC (gas–solid chromatography). The fixed phase consists of a solid material such as granual silica, alumina or carbon. Gas–solid chromatography is an important method in the separation of permanent gases and low-boiling hydrocarbons[290]. The second subclass, more important for lupine alkaloid analysis, is gas–liquid chromatography (GLC). A gas chromatograph is needed for the analysis. Basically, a gas chromatograph consists of six parts as follows: (1) a supply of carrier gas in a high-pressure cylinder with attendant pressure regulators and flow meters, (2) a similar injection system, (3) the separation column, (4) detectors, (5) an electrometer and strip-chart recorder (integrator), and (6) separate thermostated compartments for housing the columns and the detector so as to regulate their temperature. Helium is the preferred carrier gas[287]. For alkaloid analysis, a nitrogen detector is needed.

Gas–liquid chromatography is a qualitative, but also quantitative, method of alkaloid analysis. It is very sensitive. The only problem concerns the distribution of the alkaloid mixture in the chromatographic process and the identification of alkaloids, which must be achieved by a different technique[120,121]. A very positive characteristic is the possibility of totally computerizing this method of alkaloid detection.

2.9.3.13. Gas–liquid chromatography–mass spectrometry

Capillary gas–liquid chromatography (GLC) combined with mass spectrometry (MS) has been successfully used for the separation of complex mixtures of alkaloids. The aim of Gas–liquid chromatography–mass spectrometry (GLC/MS) is to operate both a gas chromatograph and a mass spectrometer. Gas chromatography is an ideal separator, whereas the mass spectrometer is an identificator[287,289,290,291,293,294,295,296]. The technique of mass spectrometry was discovered in 1912, and developed to become one of the most effective methods for biomolecular research. The mass spectrometer or mass spectrograph, as it is also called, generally consists of four units: (1) an inlet system, (2) an ion source, (3) an electrostatic accelerating system, and (4) a detector and readout system. Different mass spectrometers exist. The mass spectrometer determines the mass spectrum from the alkaloid analysis. The mass spectrum of a compound contains the masses of the ion fragments and the relative abundance of these ions plus, often, the parent ion. Mass spectrometer can be used for electron impact (EI^+) or for EI^+ and chemical ionisation (CI) of compounds. This molecular fragmentation is the basis for alkaloid identification. The basis of mass spectrometry analysis is that in the same conditions the molecular fragments of the alkaloid mass must be identical.

2.9.3.14. Nuclear magnetic resonance

The nuclear magnetic resonance (NMR) method is based on the interaction between matter and electromagnetic forces, and can be observed by subjecting

a sample simultaneously to two magnetic fields: one stationary and the other varying at a certain radio frequency. At particular combinations of fields, energy is absorbed by the sample, and this absorption can be observed as a change in the signal developed by a radio frequency detector and amplifier[290]. Nuclear magnetic resonance spectrometry was discovered in 1946, and became one of the basic methods in organic chemistry. In quinolizidine alkaloids, two techniques of NMR are currently used: ^{1}H-NMR and ^{13}C-NMR. In the case of ^{1}H-NMR analysis, the basis is that energy absorption can be related to the magnetic dipolar nature of spinning nuclei. Quantum theory is used in this case. In ^{1}H-NMR analysis, the H-nuclei from the alkaloid molecule is very important. In the case of ^{13}C-NMR, the sensitivity of the ^{13}C isotope is used. ^{13}C-spectra show chemical shifts that are more sensitive to details of structure than proton shifts. ^{13}C–^{1}H spin–spin interaction is capable of being tested. For example, non-protonated ^{13}C gives a singlet, ^{13}CH a doublet, $^{13}CH_2$ a triplet and so on. Nuclear magnetic resonance spectroscopy is today a basic method in the structural studies of alkaloids.

2.9.3.15. X-ray method

As structure and function are intimely related, X-ray crystallography is the most comprehensive technique, which elucides the three-dimensional structure of the molecule. X-ray crystallographic study provides an accurate and complete chemical characterization of the compound. This method has successfully been used for the analysis of such opioid alkaloids as morphine and has evaluated as very precise and even suitable for the research of novelizations of compounds. The use of this method can also help the estimation of the receptor, because compound structure is important in binding to the receptor.

Quinolizidine alkaloid analysis also utilizes the X-ray method, which is based on the absorption of X-rays, diffraction of X-rays, wavelength, and radiant power measurements of X-rays. When an atom is excited by the removal of an electron from an inner shell, it usually returns to its normal state by transferring an electron from some outer shell to the inner with the consequent emission of energy as an X-ray. The X-ray method is applied to quinolizidine alkaloids which have a crystalline form. In this sense it is the same as the RTG methods, which can be applied only to crystalline materials. X-rays can be absorbed by material and this gives rise to X-ray absorption spectra[296]. The spectrum provides material for the identification of compounds.

2.9.3.16. Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) is a new method in alkaloid studies. The application of ELISA to alkaloid study is based on antibody incubations. It differs from classical precipitation-based methods in that specific antigen–antibody interactions are recognized by assaying an enzyme label conjugated to one reactant, usually an antibody. Because of the sensitivity with which enzyme

markers can be detected by their reaction with an appropriate substrate, ELISA offers several possibilities of greater sensitivity. In this technique, an enzyme is labelled with an alkaloid molecule. The labelled enzyme is then bound by an antialkaloid antibody. In the complex, the enzyme is rendered inactive. When a free alkaloid (e.g., hydroxylupanine) is present, it competes with the enzyme alkaloid for antibody-binding sites again and reacts with the bacterial substrate present in the tube. The enzyme activity is directly related to the concentration of free alkaloid in the sample[297].

Enzyme-linked immunosorbent assay is a heterogenous immunoassay. Reactions involve a solid phase to which components are sequentially presented and successively bound. This method is very effective in the determination of the total alkaloid content. The positive characteristics of this method are the use of non-toxic reagents and basic equipment with low costs, a small sample volume and the ability to measure alkaloids in crude sample extracts. According to the literature, compared with results obtained from GLC, the precision of ELISA for quinolizidine alkaloids is not as high as that of the gas chromatography procedure, but is adequate for plant breeding purposes. The use of enzymes in developing the methods of quinolizidine alkaloids analysis looks likely to increase in the future.

2.9.3.17. Radioimmunoassay

Radioimmunoassay (RIA), like ELISA, is based on the radioactive labelling of the antibody molecules. The labelled antibody reacts with the antigen present in the tube; the amount of radioactivity present in the bound complex is directly proportional to the amount of antigen added to the tube[297].

2.9.3.18. Scintillation proximity assay

Scintillation proximity assay (SPA) is a variety, or part, of RIA. This method is based on the measurement of the scintillation of radioactive molecules. On the basis of the power of the scintillation, it is possible to determine the amount of radioactivity. Then, it is possible to count the alkaloid content. This method was used recently in the biosynthesis of communesin alkaloids[308].

2.9.3.19. Capillary zone electrophoresis

Capillary electrophoresis is suitable for use to separate a wide spectrum of both large and small biological molecules. This method was used for analysis of opium alkaloids such as thebanine, codeine, morphine, papaverine and narcotine.

2.9.4. Choice of method and confidence

There exists a long list of different methods of quinolizidine alkaloid analysis (Table 21). These methods are of a chemical and biological nature. The development of methods of alkaloid analysis has been a long and difficult process.

Table 21 General characteristics of the methods and techniques of quinolizidine alkaloid analysis

Method of Technique	Nature of Method	Kind of Measurements	Sensitivity
Iodine	ch	qual	y/n, c
Taster	bio	qual	y/n, nc
Seed colour	bio	qual	y/n,nc
DRG	ch	qual	y/n, nc
Fluorescence	ph, ch	qual, quant	y/n, nc
Calorimetry	ph, ch	qual, quant	1 μg, c
Photometry	ph, ch	qual, quant	1–50 μg
Electrophotometry	ph, ch	qual, quant	1–50 μg
Spectrophotometry	ph, ch	qual, quant	1–50 μg
PC	ph, ch	qual, quant	y/n, ±1 mg
GLC	ch	qual, quant	1 μg, c
GLC/MS	ch	qual, quant	1 μg, c
HPLC	ch	qual, quant	1 μg, c
NMR	ph, ch	qual, quant	±0.017%
X-Ray, RTG	ph, ch	qual, quant	±0.04%
ELISA	bio	qual, quant	y/n, 0.001%
RIA	bio, ph	qual, quant	y/n, 0.001%
SPA	bio, ph	qual, quant	y/n, 0.001%

Abbreviations: ch – chemical; bio – biological; ph – physical; qual – qualitative; quant – quantitative; y – yes alkaloid exists; n – no alkaloid absent; c – confident; nc – non-confident.

Each method has its drawbacks. The various methods for the determination of alkaloids are very diverse, and the results of measurements are not comparable. This is a particular problem when plant material is being compared.

A problem of great importance is the isolation of alkaloids. Traditionally, very strong solvents have been used. This presents some difficulties connected with the confidence with which the results can be treated. The isolation of all alkaloids from the sample, and the purity of this isolation, is also a significant problem.

A further problem which must be resolved is the resistance of quinolizidine alkaloids to other amines and nitrogen compounds during the analysis. The general conclusion is that a perfect method of alkaloid analysis does not exist. Therefore, the explanation of results in the light of the above-mentioned factors is vital in each case.

The history of the use and development of methods of analysing quinolizidine alkaloids shows a move away from the deployment of iodine towards the use of complicated biological processes, such as antialkaloid antibody and enzymatic processes. It seems to be necessary to incorporate biological methods of alkaloid analysis into the system of analytic-chemical monitoring used in modern laboratories.

2.9.5. Chemical modification of alkaloids

Chemical modification is a process in the change of the structure, skeleton, configuration, moiety groups, biosynthetic ability or form of a compound. Modification is connected with structural changes, including the changes in bioactivity of alkaloids[214]. By chemical modification, many medicines may be developed for the pharmacological market.

Modification of alkaloids can be considered in three aspects: chemical, biochemical and molbiological. Mechanical changes (chemical) cover structural alterations in all possible parts of compounds. Biochemical changes are connected with the modifications of enzyme activity, while molbiological changes cover biofactor manipulation inside the alkaloid. The latter is a new approach to the modification of alkaloids. The modification of an enzyme and its transfer to an alkaloid molecule is currently a growing research area. Such a modification can be achieved by changes in alkaloids directly, or by changes in their precursors, postcursors or connected proteins. These changes remain possible, but very challenging in alkaloid research. He et al.[298] and Teng et al.[299,300] have reported on chemical modification of tryptophan enzymes, which also have potential significance for alkaloid research and modifications. Similar studies have also been carried out by Masuda et al.[301] in Japan and by Januszewski et al.[302] in the USA. Phenylalanine enzymes have been modified as well [303]. Moreover, some solvent-stabilized Pt (2.3–2.8 nm) and Pd (83.7–3.8 nm) nanoparticles can accelerate and modify alkaloids, as in the case of *Cinchona* alkaloids[304]. Krasnov et al.[305] have reported on chemical modification of plant alkaloids, and especially the reaction of cotarnine with bifunctional NH- and CH-acids. In this research, substituted 1,2,3,4-tetrahydroisoquinoline systems were prepared by the reaction of cotarnine with the NH- and CH-acids methyl- and acyl derivatives of pyrazole and 1,3-dicarbonyl reagents. According to Krasnov et al.[305], bifunctional pyrazole nucleophiles can deliver substitution products in the N atom, methyl or acyl group, depending on the structure and reaction conditions.

2.9.5.1. Basic techniques

Basic techniques of alkaloid modification are grounded on the following reactions: (1) the Schiff formation and Mannich reaction, (2) Aldol and Claisen Reactions, (3) Wagner–Meerwein Rearragements, (4) Nucleophilic substitution, (5) Electrophilic addition, (6) Decarboxylation, (7) the Transamination reaction, (8) Enzymatic reactions (oxidation, reduction and dehydrogenation), (9) Elimination reactions, (10) Coupling reactions, (11) Reactions with reagents. The Schiff formation is a reaction in the formation of C−N bonds, with a nucleophilic addition followed by the elimination of water and the given imine (Schiff base). The Mannich reaction is also connected with C−N bonds formation. Meanwhile, the protonated form of imine reacts with the nucleophilic addition.

The Aldol and Claisen reactions are connected with C−C bond formation. Wagner–Meerwein rearrangements are related to the generation of more stable

carbocations. Nucleophilic substitution is connected with SAM, which produces positively charged sulphur and promotes nucleophilic substitution (S_N2). Electrophilic addition is connected with the C_5 isoprene unit in the form of DMAPP, which can ionize to generate a resonance-stabilized allylic carbocation and can then react with IPP. Decarboxylation is the reduction of carbon, while transamination is the exchange within the amino group of an amino acid to a keto acid (the introduction or removal of nitrogen). Enzymatic reactions change the oxidation and hydroxylation state of the molecule through the activity of enzymes. Elimination reactions are connected to exchange within hydroxyl, amino or mercapto groups. Coupling reactions are connected with the unification of two or more phenolic systems in a process readily rationalized by means of free radical reactions. The solvent reaction of NH- and CH-acids with alkaloid can produce modification. Some other reagents and some solvent-stabilised Pt and Pd nanoparticles can accelerate and modify alkaloids, as for example in the case of *Cinchona* alkaloids[304].

The above mentioned reactions are widely used in alkaloid modification. A good example of alkaloid modifications for clinical curation purposes are opioides. Morphine and codeine are natural products of *Papaver somniferum*. However, the codeine is naturally produced in small amounts. This is one reason why it is produced synthetically from morphine by modification. As codeine is the 3-O-methyl ether of morphine, the mono-O-methylation occurs in the acidic phenolic hydroxyl. Pholcodine is obtained by modification of morphine through alkylation with *N*-(chloroethyl)morpholine. Moreover, dihydrocodeine, hydromorphone and heroine are also obtained from morphine through modifications.

Modification of alkaloids is very important for their use in medicine[306,307,397]. In particular, modification through biological processes and bioengineering may lead to a new generation of compounds for medical applications.

2.9.5.2. Chemical achievements

Alkaloid chemistry is a small part of chemistry, whose history began in 1805, when the first alkaloid was isolated. Since this time, there have been many famous achievements in research and product development. A host of excellent scientists have been working successfully in this field. Alkaloid chemistry has saved many millions of lives by producing the knowledge, on the bases of which alkaloid-based medicines have been developed against malaria and other diseases. Chemistry has not only investigated alkaloids, their structures and activity, but also developed methods for their modifications and structural manipulation. These methods are successfully used in both the pharmaceutical industry and biotechnology.

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CHAPTER 3

Biological Significance of Alkaloids

Natura non facit saltus.
Carl von Linné

Abstract: Alkaloids are compounds needed for cell activity and gene code realization in the genotype. They are biologically significant as active stimulators, inhibitors and terminators of growth, a part of an endogenous security and regulation mechanism. Some alkaloids have significance as haemoglobinizators of leukaemia cells and they can be biologically determined to be estrogenically active molecules. They display antimicrobial and anti-parasitic properties. Recent research has proved that they are not toxic to the organisms that produce them. Biotoxicity is directed only towards foreign organisms or cells and it is selective. Alkaloids can alter DNA, selectively deform cells and cause locoism. Some alkaloid molecules, both natural and synthetic, can act as narcotics. Moreover, they play a very important role in the immune systems of animals and plants. Alkaloid metabolism is genetically coded, and to date more than 30 genes coding for the enzymes involved in alkaloid synthesis have been isolated. Alkaloid molecules are active agents in evolutionary interactions.

Key words: alkaloids, antimicrobial activity, anti-parasitic activity, biology, cytotoxicity, DNA, endogenous security mechanism, estrogenic effect, evolution, genes, haemoglobinization, immune system, inhibitor, locoism, narcotics, regulation, stimulator

Alkaloids play a very important role in organism metabolism and functional activity. They are metabolic products in plants, animals and micro-organisms. They occur in both vertebrates and invertebrates as endogenous and exogenous compounds. Many of them have a distributing effect on the nervous systems of animals. Alkaloids are the oldest successfully used drugs throughout the historical treatment of many diseases[321].

1. Alkaloids in biology

For many years, the nature of alkaloids in biology was a mystery. It has been difficult to understand the function of these compounds in plant metabolism. There are many explanations for why plants, animals and micro-organisms produce alkaloids.

Nowadays, when genomes, DNA and genes serve as the basis for biological explanations, this issue is of great importance and still open for discussion and for deep scientific analysis. Despite the advanced research in the field, a final comprehensive biological explanation of the nature of alkaloids is still on the way. In this sense the alkaloid mystery continues to exist. New compounds are being discovered all the time; however, their biological significance remains unexplained. The slow pace of science and scientific research requires a lot of time to arrive at such explanations. Moreover, the structures of many of these compounds are unexpected and their bioactivity is surprising. The molecular mechanisms and metabolic roles of newly discovered compounds have remained unclear. Alkaloids from marine environments and those produced by micro-organisms and animal skin are, in particular, objects of current chemical and biological research. Moreover, the nature and role of alkaloids has been based on a throng of theoretical hypotheses compiled during the last 200 years. It has even been hypothesized that alkaloids are plant wastes and an end product of metabolism[322]. Today, the role of alkaloids can be explained by two factors: the functions of these compounds inside and outside the organism producing them. The external function of alkaloids is presently a particularly strong and growing research area[323,324,325,326]. This trend in alkaloid research is based on the hypothesis that alkaloids are compounds that solely play a protective role in interaction with other organisms (as some kind of organic bio-weapons). This seems to be a rather limited oversimplification of the issue. Although there is strong evidence of this kind of activity, it is not entirely clear if it is a basic function of these compounds in the organisms producing them. The idea that this ecologically important role may only be a secondary function and that alkaloids primarily function in connection with the regulation of metabolism as the result of gene expression should not be dismissed. It is known that in the case of quinolizidine alkaloids the total removal of these compounds by genetic means leads to the death of the lupine plant[327]. This suggests that alkaloids are compounds fundamental for cell activity and gene code realization in the genotype[328]. This also means that alkaloids basically function in connection with genes, enzymes and proteins inside the organism. Moreover, it is also known that quinolizidine alkaloids are able to change their structural chemical configurations under changing cellular pH conditions. This observation and experimentally measured effect first noted in the 1990s has unfortunately been given little literary attention by other scientists. Although this self-regulation process is still not understood in detail, there are many recent studies which prove that chemical structural changes influence large changes in the biological activity of chemical compounds[54,213,329,330].

Alkaloids are non-toxic in vacuoles where they are stored but toxic when they escape from the vacuoles. They have to change their chemical configurations and biological activity in different cells and tissues according to pH changes. This means that some alkaloids can have different biological activity in different cell

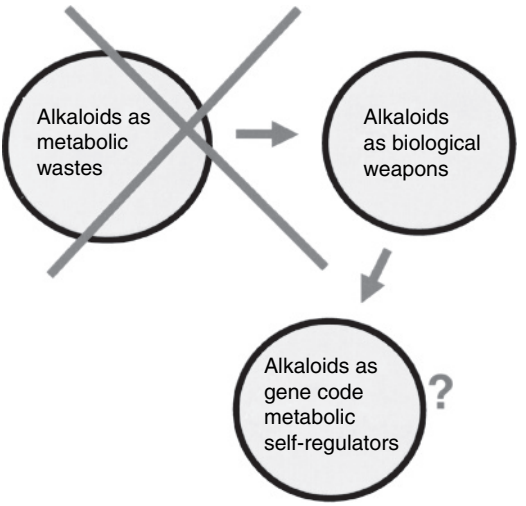


Figure 77. Three basic hypotheses on the biological nature of alkaloids.

conditions and different receptors. This process has to be genetically regulated (Figure 77). Future detailed studies will likely clear up this fascinating and complex matter concerning the biological nature of alkaloids.

1.1. From stimulators to inhibitors and destroyers of growth

Waller and Nowacki[16] distinguished the role of alkaloids in plants as growth stimulators and inhibitors and also as protective agents and reservoirs of nitrogen. Some alkaloids are known neurotransmitters in animals and can also be considered part of the signalling system. This system is constructed as a part of cell and metabolic operations controlled by functional mechanisms of biological membranes, channels, receptors and enzymes. It is known that some alkaloids, for example purine and steroidal alkaloids, can bind to some compounds presented on cell membranes. As a result of this interactive process, the moiety segment of alkaloids can be changed by addition to different parts (e.g. lipophilic, hydrophilic etc.) of the molecule, which assists in binding to the receptor. There are different receptors for different compounds transported in the organism. Alkaloids can promote receptor activity or inhibit it. This is also in many cases connected with alkaloid moiety. The steroidal alkaloid gagamine, which has been isolated from the roots of *Cynanchum wilfordi* Hamsley (Asclepiadaceae), can be mentioned as an example. This alkaloid is known to have an inhibitory effect on the activity of aldehyde oxidase, which metabolizes heterocyclic rings[336].

Alkaloids have their own signalling system. Receptors and membranes play an active role in this system. The role of biological membranes in alkaloid signalling is also connected with the action of the specific ion channels of

Ca^{2+}, Na^{+} and K^{+} and their active pumps (e.g. Ca^{2+}-ATPase). Only alkaloids can promote or inhibit activity of ion channels and their active pumps. Therefore, these channels are important in an alkaloid signalling system. This mechanism is connected directly or indirectly to receptor proteins. Alkaloids such as dopamine, histamine or serotonin are well-known neurotransmitters with their own receptors. The stimulation of a neurotransmitter system (especially ion channels) is caused by an influx of Na^{+}-ions. This large-scale and rapid influx activates a so-called voltage gate of Na^{+} and K^{+}-channels, which is essential for alkaloids. Neurotransmission is one of the most important biological characteristics of alkaloids. However, the latest research data published by Pineda et al.[337] presents information about the effects of the crude extracts of lupine quinolizidine alkaloids, which were intracerebroventricularly administrated in adult rat brain tissue. These extracts were administrated to the right lateral ventricle of adult rats through a stainless steel cannula for five consecutive days. The researchers stated in their report that immediately after the administration of quinolizidine alkaloid from *Lupinus exaltatus* and *Lupinus montanus* seeds, the rats began grooming and suffered from tachycardia, tachypnea, piloerection, tail erection, muscular contractions, loss of equilibrium, excitation and an unsteady gait. Moreover, Pineda et al.[337] reported that the rats treated with alkaloids had damaged neurons. Although there was no statistical significance, damages were observed and may suggest a histo-pathological influence on neurons. The most frequent abnormalities observed in this brain tissue were the "red neurons" with a shrunken eosinophilic cytoplasm, strongly stained pyknotic nuclei, neuronal swelling, spongiform neuropil, "ghost cells" (hypochromasia) and abundant neuronophagic figures in numerous brain areas. If these results will be proved in the future by no direct administration of alkaloids to the brain, they will serve as evidence of the destructive role of alkaloids in the animal body. Although the research of Pineda et al.[337] is interesting in many aspects, the results cannot be considered as evidence of such destruction caused by alkaloids. It is evidently known that crude extracts cannot be physiologically transported to the animal brain. The direct administration of crude extracts to the brain tissues from outside the liver affects the influence of all components of these extracts, from which alkaloids are only one part.

There is evidence in literature that alkaloid biology is connected with regulation, stimulation and induction functions. Tsai et al.[338] proved that caffeine levels in the blood, brain and bile of rats decreased when given a treatment of rutaecarpine, an alkaloid from *Evodia rutaecarpa* (Figure 78). It is known that caffeine has been found to enter the brain by both simple diffusion and saturable carrier-mediated transport[339]. The hepatobiliary excretion of caffeine has also been reported in humans[341], rabbits[340] and rats[338].

A treatment of rutaecarpine causes an increase in renal microsomal enzymes related to CYP1A and enhances the activity and protein levels of CYP1A. It is known that caffeine is a mild stimulant. It is metabolized in the liver by

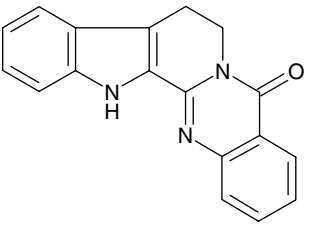


Figure 78. Rutaecarpine, an alkaloid from *Evodia rutaecarpa*.

CYP1A2, and it also has been shown to be an inducer of CYP1A2 in rodents on account of the increase in hepatic microsomal CYP1A2[342]. Rutaecarpine is an inducer of cytochrome P450(CYP)1A in mouse liver and kidney[343].

There is evidence that alkaloids influence plant growth, as both stimulators and regulators. A large series of applied studies in Germany and in Poland started in the 1980s proved that quinolizidine alkaloids in crude lupine extracts had effects on both yield amount and quality (Figure 79). Foliar application of lupine extract on several crops resulted in yield increases of 17–20%[331,332] and 15–25%[235,333]. Moreover, these results proved that crude lupine extract with quinolizidine alkaloids influenced the balance of nitrogen compounds in plants. Increases in protein concentration and changes in amino acid contents have been observed. Snap bean (*Phaseolus vulgaris* L.) seed yield after foliar application of the extract increased by 16.4% and the biological value of protein measured with essential amino acid coefficients increased by 2.87%[333]. The stimulation role of alkaloids can be explained by more intense nitrogen metabolism after application. In the 1950s a case of applying pure lupanine solution to the leaves of alkaloid-poor *Lupinus albus* L. was shown to have a growth-stimulating effect[16]. However, there are also old findings indicating some plants exhibited no effects at all when treated. In some cases they exhibited growth inhibition or the effects of poisoning.

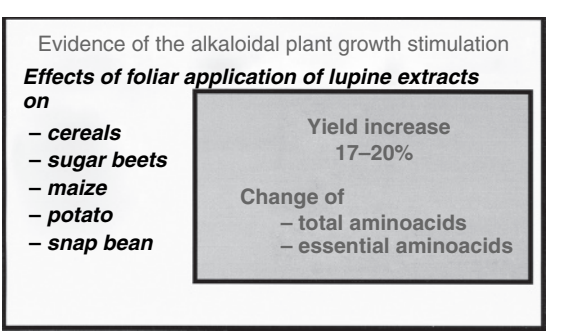


Figure 79. Effects of foliar application of lupine extracts.
Sources: Refs [331, 332, 333, 334, 335].

1.2. The effects of stress and endogenous security mechanisms

In biology today, the basic question concerning alkaloids is connected with the relation between their internal and external roles. It appears that the external role is only secondary, and the endogenous use of alkaloids as genetically coded is the primary function. Höft et al.[344] have studied the sources of alkaloid formation and changes in *Tabernaemontana pachysiphon* plants. In this research the endogenous factors were leaf age, plant age, leaf position in the crown and teratological leaf dwarf growth on leaf alkaloid contents. Environmental factors were soil and other climatic factors controlled in a greenhouse in the case of young plants. In the case of the old trees, environmental factors were measured in natural habitat. Höft et al.[344] clearly documented that higher leaf alkaloid content is thought to result from higher nitrogen and cation availability.

The relationship between nutrients in the soil and changes in alkaloid amount occurring in plants is one of the most important topics in alkaloid biology and furthermore in plant physiology and biochemistry. Alkaloid content in plants, for example in tobacco (*Nicotiana*) or lupine (*Lupinus*), may increase with treatments high in nitrogen. There are, however, many exceptions to this. Amounts of indole, purine and steroid alkaloids in plants do not change rapidly in response to such treatments. It does seem that alkaloid content is generally related to nitrogen levels available to plants. Two basic factors seem to influence this relation: (1) the biosynthetic nature of alkaloids themselves, and (2) the balance of nitrogen and other nutrients in the soil. The alkaloid biosynthetic pathway is important in this sense that during synthesis the nitrogen existing in the precursor can be liberated, or additional nitrogen may bind. Some precursors are richer in nitrogen than alkaloids, for example in the case of morphine, nicotine, hyoscyamine and so on. In the case of gramine or caffeine the amount of nitrogen is the same as in their precursors. In alkaloids such as tomatidine or coniine the amount of nitrogen is higher than in their precursors. This is the reason why some alkaloids are more sensitive to nitrogen availability than others. Moreover, the balance of nitrogen in the soil seems to be very important. High or low concentrations of nitrogen in soil seem to influence alkaloid content in the plant despite the biosynthetic nature of alkaloids (Figure 80). In both mentioned cases, the plant suffers from nutritional stress and the production of alkaloids seems to increase. Nutritional stress seems to be the reason for this. It is affected by absences and a high demand for nitrogen during metabolism. Plant stress in this sense can be determined as a force which strengthens alkaloid production for both continuing storage in vacuoles and for continuing their departure from vacuoles for the metabolic regulation of stress. It is necessary to mention that this topic has not been yet the object of larger specialized laboratory studies. Therefore, this explanation remains a strong hypothesis to be investigated in future studies. It is, however, known that nitrate uptake promotes alkaloid accumulation and is preferred over ammonium uptake. Soil acidity and temporary drought stress are also known to block

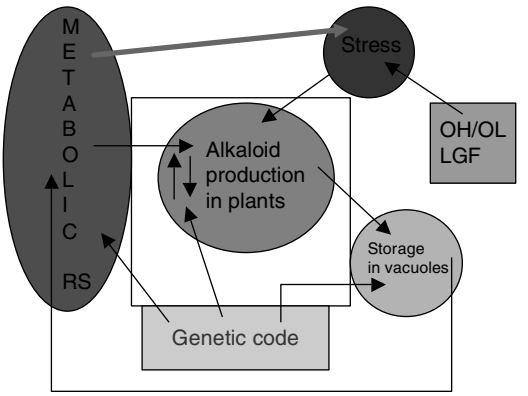


Figure 80. Mechanism of regulation of alkaloid content in plants. Abbreviations: RS – regulation system; OH – overhigh level; OL – overlow level: LGF – life growing factors. Observe that this regulation system is coded in genes. Life growing factors (light, water, CO_2, nutrients including nitrogen, temperature, etc.) influence on stress, which is also dependent on this system.

nitrification and may thus contribute to low leaf alkaloid accumulation[345]. The above-mentioned experiments by Höft et al.[344] proved that in *Tabernaemontana pachysiphon*, the differences in alkaloid levels due to endogenous factors such as leaf age or dwarf growth were much more pronounced than any other difference caused by environmental factors. The influence of age or tetratological leaf growth differed depending on the alkaloid. Apparicine content was enhanced in very young leaves and equally high contents in dwarf-leaves of old trees[344]. The different positions of leaves may have been caused either by small differences in leaf age or by a plant's internal nutrient and water fluxes. According to Höft et al.[344] differences in alkaloid levels according to tree age were rather marginal. Although their research does not directly answer the question of the internal and external roles of alkaloids, it does show the factors influencing alkaloid production. These factors also indirectly mean that alkaloids become more needed in a plant when the factors influencing their production are present. When summarizing and generalizing empirical results, it can be stated that stress and stressful situations in plants induce alkaloid production and their needs in regulatory processes of metabolism. The high content of alkaloids in old leaves suggests a metabolism regulation function similar to growth hormones, although it is also known that plant hormones such as cytokinins were found to stimulate the alkaloid synthesis[346]. Moreover, this also suggests that the basic biological function of alkaloids is endogenous. Many present research results suggest just this. For example, research by Henriques et al.[347] has shown that alkaloid production and accumulation in plants of *Psychotria leiocarpa* (Rubiaceae) increased with plant age and light exposure. The alkaloids in this case are needed for physiological and metabolic regulation by a plant. Another good example of

alkaloid production and accumulation and its function can be observed in the case of β-carboline alkaloids in humans. These alkaloids occur in mammals[211]. As neurotransmitters they play a regulative role in various metabolic processes. The natural concentration of harman, an endogenous inhibitor of monoamine oxidase sub-type A with a high affinity in brain and peripheral organs, in rat brain is reported to be less than 0.5 ng/g tissue. Norharman induces pro-conflict behaviour in limbic-hypothalamic structures and alterations of motor activity[211]. This also proved that endogenous activity seems to be a basic function of some alkaloids. Alkaloids are therefore some kind of natural endogenous medicines needed for ordering metabolic processes by inhibiting or accelerating other active molecules. In this sense the external role, especially in growing environments and species interaction, seems to be secondary.

Nowadays, knowledge of alkaloid biological function is based on empirical results. The most important biological function in plants involves the chemical and biological protection of cells. They protect plant bodies from physical stresses like ultraviolet light and heat[580]. Other biological functions are protection against pathogens and herbivores, protection of generative reproduction, an acute source of nitrogen, nitrogen storage and the stimulation of growth and adaptation to the local environment.

2. Bioactivity

The general characteristics of alkaloids are their chemical flexibility in regards to structure, and as a consequence of this, the biological activity. Individual alkaloids do not play only one role. The same alkaloid in different cell conditions is able to change its structure and thereby its biological activity. This ability makes the alkaloids a special group of secondary compounds.

2.1. Secrets of life

Alkaloids are structurally very similar to plant growth hormones. Waller and Nowacki[16] have critically considered the possibility that alkaloids have a hormonal influence on plant growth. This old hypothesis is still open for discussion; examples in literature attempt to both prove and disprove it. The contradictory results derive from the diversity of alkaloids, not to mention plant diversity and that of other organisms producing alkaloids. There are alkaloid-rich and alkaloid-poor plants from the same species. One such plant is Washington lupine (*Lupinus polyphyllus* Lindl.), which is capable of growing under various climatic conditions in both the Northern and the Southern Hemispheres[327,348]. The freely growing genotypes of this plant contained 1.74–3.15 mg of alkaloids in 100 mg of seeds, whereas one hybrid contained only 0.0004 mg. The alkaloid

content in leaves was about 1.6 mg in natural genotypes and 0.05 mg in hybrids. The content in shoots was 1.7 and 0.1 mg, respectively[348]. The Washington lupine is known also by many other common names such as *Blomsterlupin* (in Swedish), *Dauerlupine* (in German), *De belle lupine* (in French), *Komea lupiini* (in Finnish), *Łubin wieloletni* (in Polish) and *Mnogoletnii liupin* (in Russian). As a wild plant it is originally from North America, where its distribution extends from California to Alaska. This plant was brought to Europe in the 19th century and it distributed rapidly in numerous countries as a decoration and animal fodder in pastures and game animal farming[327,348]. As a perennial and cross-pollinated species, it has many different geno- and ecotypes with different alkaloid levels. It has been hypothesized that the role of alkaloids in alkaloid-rich and alkaloid-poor geno- and ecotypes differs because their amounts and structure vary. Diversity of alkaloid content in the same species and hybrids is one of the most interesting secrets of life. Chemical diversity generally constitutes an intrinsic property of biosynthesis, which is an inherent property. This diversity-oriented strategy is widespread in biosynthesis. Schwab[349], when discussing the diversity of secondary compounds, concludes that the number of metabolites in one species often exceeds the number of genes involved in their biosynthesis, and that increasing compound diversity does not correlate with increasing gene number. It has also been suggested that multifunctional enzymes are ubiquitous in the plant kingdom[349]. In the case of alkaloids, the diversity in content among plants is connected to the genetic code. The proof of this is evident in hybridization, where it is possible to noticeably decrease the alkaloid level of the Washington lupine. This has been done over the period 1982–1990 in Finland[348]. The mechanism of determining the alkaloid-rich and alkaloid-poor plants is connected with enzymatic activity and production of alkaloid precursor. In the case of quinolizidine alkaloids, an alkaloid is plant specific and their occurrence in individual plants is connected to the metabolism of lysine. In expanded vegetation, there is a surplus of lysine which leads to the production of quinolizidine alkaloids through the activity of HMT/HLTase and ECTase[350]. In individual plants without such alkaloids, the biosynthetic pathway of the alkaloids with HTM/HLTase is blocked[351].

The difficulty of studying the effect of alkaloids as growth regulators is similar to the problem of alkaloid content variation in plants. Waller and Nowacki[16] clearly took up this issue for methodological discussion. Level of alkaloid richness will affect further addition of alkaloids to a plant. However, environmental growth factors such as light, moisture, temperature, nutrition and the genetic factors such as genotype and photosynthesis capacity of a species influence alkaloid precursors and their derivation to alkaloids. The concentrations of these compounds in plants influence their activity as growth regulators. However, many questions arise in the light of this. Do alkaloid-rich plants grow better and faster then alkaloid-poor plants? What empirical evidence exists that alkaloids also have the effect of growth regulators? Waller and Nowacki[16] mentioned that

alkaloids are growth regulators. They mentioned differences in regulator activity and also pointed out exceptions. The answer to the first question nearly 30 years later is certainly not exactly the same. Research has advanced during this time as the development of techniques and equipment illustrates. According to my studies and observations carried out in experiments in Finland, the answer is just opposite to the one given by Waller and Nowacki[16]. The alkaloid-rich plants grow at a higher rate and higher canopy than alkaloid poor plants[327,352]. However, when ripening period is compared, alkaloid-rich plants ripen more slowly than alkaloid-poor plants. The growing conditions in the Boreal zone of Finland are generally very favourable for perennial lupines and especially for the Washington lupine. The populations of this species have been large and this species has had no factors reducing populations (e.g. herbivory or disease). Rapid growth and higher growth rate per day can be considered a result of regulator activity. Empirical studies support this. *Lupinus angustifolius* cult. Mirela (alkaloid-rich plant) grows more rapidly than alkaloid-poor species. In chamber experiments the mean photosynthetic uptake of *L. angustifolius* cult. Mirela (alkaloid-rich) was $12.71\,mg\,CO_2\,dm^{-2}\,h^{-1}$, and that of *L. polyphyllus* Lindl. (alkaloid-poor) was $10.04\,mg\,CO_2\,dm^{-2}\,h^{-1}$ [353].

2.2. Life regulation through the high and low cytotoxicity

Alkaloids from the plant family *Amaryllidaceae* are known to have a wide range of biological activities. They have analgesic, antiviral, anti-malarial, antineoplastic properties and display effects on the CNS. Elgorashi et al.[354] have studied 25 *Amaryllidaceae* alkaloids for possible inhibitory activity of their acetylcholinesterase enzyme (AChE). This enzyme is biologically very important. According to the cholinergic hypothesis Alzheimer's disease symptoms result from AChE activity, which reduces brain acetylcholine activity. Crinine, crinamidine, epivittatine, 6-hydroxycrinamine, *N*-desmethyl-8α-ethoxypretazettine, *N*-desmethyl-8β-ethoxypretazettine, lycorine, 1-*O*-acetyllycorine, 1,2-di-*O*-acetyllycorine and cherylline have been shown to inhibit AChE[354]. Lycorine-type alkaloids are the most active against AChE[354,355]. The action mechanism of these alkaloids on AChE inhibition is still not exactly known, although it has been reported that the crystal structures of the acetylcholinesterase inhibitors such as galanthamine, huperzine A, tacrine and edrophonium demonstrated binding to the active site gorge of AChE[354,356]. Studies on steroid alkaloids such as saracocine, saracodine, saracorine and alkaloid-C isolated from *Sarcococca saligna*[357] suggest that these alkaloids are also calcium antagonists and AChE inhibitors. The AChE is known to be located on the acetylcholine receptor (AChR), which is also bound by such alkaloids as anabasine, arecoline, coniine, C-toxiferine, cytisine, hyoscyamine, lobeline, lupanine, muscarine, nicotine, pilocarpine, tubocurarine, scopolamine, sparteine

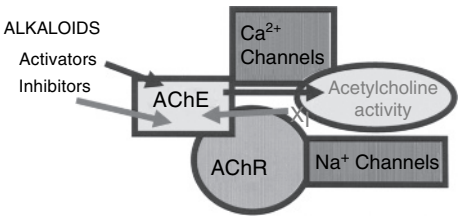


Figure 81. Alkaloids in the acetylcholine receptor. Abbreviations: AChE – acetylcholine enzyme; AChR – acetylcholine receptor; X_I (red) – inactivity of AChE.

and so on. These alkaloids can activate AChE or inhibit it by influence of enzyme AChE (Figure 81). As in cases of the Amaryllidaceae alkaloids, AChE can be inhibited. As a result of this, acetylcholine activity increases. Acetylcholine activity is needed for human brain function. It seems that Amaryllidaceae alkaloids have a wide biological regulatory ability. It is known that lycorine, one of the most important Amaryllidaceae alkaloids, is actively antiviral. Pseudolycorine and pretazettine are active against several types of leukaemia by the inhibition of protein synthesis and prevention of peptide-bond formation[358]. Galanthanine has analgesic, anticholinergic and anticholinesterase properties. The minor Amaryllidaceae alkaloids studied by Abd El Hafiz et al[358] are also biologically active. The lycorine-type alkaloid (pratorinine) and the crinine-type alkaloid (6α-hydroxybuphanisine) showed a moderate cytotoxic activity. Moreover, (−)-spectaline, a piperedine alkaloid isolated from the legume *Cassia leptophylla* Vog.[359], has been proved in studies by Alexandre-Moreira et al.[360] to have no significant toxicity effects but rather antinociceptive traits. In these experiments conducted on mice, (−)-spectaline was able to significantly inhibit abdominal writhing in the mice in comparison to the control animals. It was suggested that this bioactivity of (−)-spectaline was connected to a direct interaction of the binding of the vanilloid system or excitatory amino acid on its receptors[360]. This is a promising research direction when considering possible bioapplications of this alkaloid.

Neolitsine, dicentrine, cassythine and actinodaphine are aporphine alkaloids isolated from *Cassytha filiformis*. These alkaloids have been studied by Stévigny et al.[361] for their cytotoxic activities on cancerous and non-cancerous cell lines *in vitro*. Neolitsine was very active against HeLa and 3T3 cells and cassythine and actinodaphnine showed activity against Mel-5 and HL-60 cells. Dicentrine was previously reported to be cytotoxic against several tumour cells and has been shown to inhibit DNA and RNA biosynthesis[775]. Dicentrine also acts as a topoisomerase II inhibitor[362]. Chen et al.[363] have researched aporphine alkaloids isolated from the trunk bark of *Hernandia nymphaeifolia*. These alkaloids also showed potent cytotoxities against P-388, KB16, A549 and HT-29 cell lines. Similar results have been previously noted in the case of dimeric aporphine alkaloids isolated from the same species[364].

Very interesting results concerning the cytotoxicity of alkaloids isolated from the flowers of ornamental legume plant *Senna spectabilis* have been noted by Sriphong et al.[365]. N,*O*-diacetylcassine, 3(*R*)-benzoyloxy-2(*R*)-methyl-6(*R*)-(11′-oxododecyl)-piperidine and 5-hydroxy-2-methyl-6-(11′-oxododecyl)-pyridine *N*-oxide exhibited cytotoxicity against KB cell lines.

One of the most common biological properties of alkaloids is their cytotoxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines. Wu et al.[366] have studied the cytotoxicity of 53 isoquinoline alkaloids and their *N*-oxides against A-549, HCT-8, KB, P-388 and L-1210 cells. The isoquinoline alkaloids represent a different structural type of alkaloids. Among all structural types investigated (tetrahydroprotoberbirines, protoberberines, aporphines, morphinadienone, oxoaporphines, phenanthrenes and their *N*-oxides), the most active were some of the oxoaporphines. Liriodenine especially showed potent and wide spectrum activity against all the cell lines tested[366]. Moreover, it has been evident in this research that human KB cells appear to be the most sensitive in detecting active compounds of different alkaloids. The same result has been noted by Jagetia et al.[367] in the case of echitamine, which is a monoterpene indole alkaloid. This research investigated HeLA, HepG2, HL60, KB and MCF-7 cells *in vitro* and in mice. Jegetia et al.[367] reported anti-tumour properties of echitamine *in vitro* and *in vivo*. Moreover, Long and Li[368] have noted the anti-tumourous characteristics of alkaloid extracted from *Oxytropis ochrocephala*, and concluded that the activity is dose dependent. This anti-tumour effect is associated with the expression of inhibition of proliferating cell nuclear antigen (PCNA) and mutant p53 protein[368].

The cytotoxic activity of phenanthroquinolizidine alkaloids has also been reported[369]. Of two studied alkaloids (boehmeriasin A and boehmeriasin B) isolated from *Boehmeria siamensis* Craib (Utricaceae), only boehmeriasin A possessed cytotoxicity against 12 cell lines from 6 types of cancer, including lung, colon, breast, prostate, kidney cancer and leukaemia. The anti-mitotic and cytotoxic activities of guattegaumerine, a bisbenzylisoquinoline alkaloid isolated from the bark of *Guatteria gaumeri*, have been studied by Leclerq et al.[370] According to the results, guattegaumerine exerts activity on B16 melanoma, which is a relatively resistant tumour. Cytotoxic activity of 8-*O*-Cinnamoylneoline, an alkaloid isolated from flower bud of *Aconitum carmichaeli* (Ranunculaceae), was studied by Taki et al.[371] This alkaloid was detected only in flower buds. These acute toxicity and analgesic activities are connected with the presence of C-8 substituent in its ring. The tubers of the *Aconitum* species have been known to be biologically very active. In China and Japan these species are known as herbs with strong bioactive potential. They contain masconitine, hypaconitine and aconitine that are extremely toxic[371]. Taki et al.[371] placed attention on the relatively lower toxicity of alkaloids in the flower buds. The research also suggests that alkaloids are in other above ground

parts of this plant such as flowers, stems and leaves. Alkaloids from other plant parts may have lower acute toxicities compared to the tubers. Biologically active alkaloids are regulators not only of endogenous life processes in the organisms that produce them, but also in the organism to which has consumed them.

2.3. Haemoglobinization of leukaemia cells

The biological activity of alkaloids can be demonstrated by fagaronine (Figure 82), an alkaloid isolated from *Fagara zanthoxyloides* Lam. (Rutaceae). This alkaloid alone has been tested by many research groups as biological agent of the haemoglobinization of human leukaemic cells[372,373,374,375,376]. One of the characteristics of leukaemic cells is escaping the normal regulatory pathway controlling cell proliferation and differentiation. As early as in 1972, Messmer et al.[372] reported on fagaronine anti-leukaemic activity against murine leukaemia P388. Four years later, Sethi[373] published a study with evidence indicating that fagaronine inhibits DNA polymerase activity in murine embryos. It was also found that fagaronine inhibits human DNA ligase I[377] and reverse transcriptases from RNA viruses[378]. This last finding was also confirmed by Tan et al.[379] in the case of human HIV-1 reverse trascriptase *in vitro*. In the year 1983, Pezzuto et al.[374] reported that fagaronine inhibits nucleic acid and protein synthesis in KB cells. Fagaronine was also reported to induce the haemoglobinization of leukaemic K562 cells[375]. Later studies pointed to the ability of this alkaloid to intercalate DNA, to interact with the ribosomal system[380] and to inhibit the activities of the DNA topoisomerase I and II[381,382]. The research group of Dupont in France has examined the effect of fagaronine on erythroid differentiation and growth of leukaemic K562 cells[376].

The results of this deep research can be considered promising in the field and they are also in agreement with results obtained in previous studies by Comoë et al.[375]. Dupont et al.[376] observed that fagaronine induces the homoglobinization of K562 cells and inhibits leukaemic cell growth in 80% of cells. Moreover, fagaronine has no acutely toxic effects on the K562 cell line[376]. The mechanism of this biological influence was explained by Dupont et al.[376] as resulting from

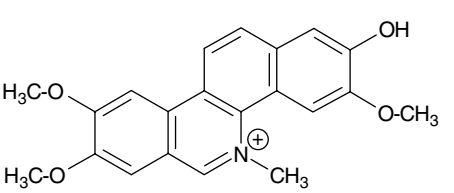


Figure 82. Fagaronine, an alkaloid from *Fagara zanthoxyloides* Lam.

the action of several genes. Haemoglobin synthesis mediated by fagaronine treatment was preceded by the increased transcription of several genes known as the markers of erythroid differentiation. They are α- and β-globins, PBGD and EPO-R. Moreover, haemoglobin synthesis in leukaemic cells was preceded also by an over-expression of GATA-1 and NF-E2 mRNAs as well as by GATA-1 protein accumulation[376]. Haemoglobin is a known tetramer of protein sub-units with two α and two β sub-units, myoglobin and two glutamic acid residues in β sub-units. A haeme is an iron-containing porphyrin acting as a prostethic (Figure 83). Moreover, fagaronine has caused increased transcriptional activity of the luciferase gene downstream of the α-globin, EPO-R and GATA-1 promoters. This study is very interesting and proved that alkaloids have strong biological activities in foreign organisms. In this respect, fagaronine has probably more activity than in *F. zanthoxyloides*, a plant producing it, although this is only a hypothesis. The biological activity of fagaronine in plant cells is under-studied in comparison to that in human cells. Its activity in plant cells should not be dismissed, for it may provide a better understanding of fagaronine activity in human cells.

There are presently many other studies on alkaloid bioactivity in human leukaemia cells and the role of alkaloids as competitive antagonists of cytotoxic agents[383,384]. Sampangine, for example, is an alkaloid extracted from the stem bark of *Cananga odorata*. Kluza et al.[383] show that the treatment of human HL-60 leukaemia cells for 48 hours with sampangine induced oxidative processes and proved that this alkaloid has anticancer properties. Another alkaloid, voacamine, extracted from *Peschiera fuchsiaefolia* has been evidenced as an inhibitor to P-glycoprotein action and a competitive antagonist of cytotoxic agents[384].

Pitzalis et al.[385] have studied the influence and molecular alternation of retrorsine on rat hepatic cell cycle. Retrorsine has been found to block proliferation of resident cells. Cyclin D1 mRNA and protein levels were found to be elevated in rats treated with this alkaloid. The PCNA was also elevated[385].

Figure 83. Model of haemoglobin. Explanation: H – heme. Peptides are part of myoglobin.

The conclusion from this study is that such a persistent block outside the resisting phase may contribute to selective replacement of resident cells during liver repopulation.

The haemoglobinization of human leukaemic cell lines by alkaloids demonstrated through *in vitro* means that these compounds are biologically very active. Alkaloids are therefore a promising botanical to be used in future applications.

2.4. Estrogenic effects

Biological activity, although typical for alkaloids, can be very different and dependent on the chemical structure of alkaloid molecules. Quinoline alkaloids extracted from the plant belonging to the genus *Haplophyllum* A. Juss. (family Rutaceae) have strong biological activity with an estrogenic effect[54]. The receptor for estrogenic activity is located in the nucleus (Figure 84). Therefore, this activity can be considered initiated with these receptors.

Empirical results with 15 quinoline alkaloids have been received in the study with mature intact rats. It was found that all the alkaloids studied (γ-fagarine, haplopine, skimmianine, glycoperine, evoxine, dubinidine, dubinine, perforine, haplophyllidine, perfamine, bucharidine, folifidine, acetylfolifidine, foliosidine and acutine) cause the uterus to hydrate. Some alkaloids changed the menstrual cycle of mature intact rats by lengthening the oestrus phase. If the average duration of a single menstruation was 1 day, the alkaloids extended it to 1.4 days[54]. However, there were differences between the alkaloids studied concerning the intensity of estrogenic activity. According to the results,

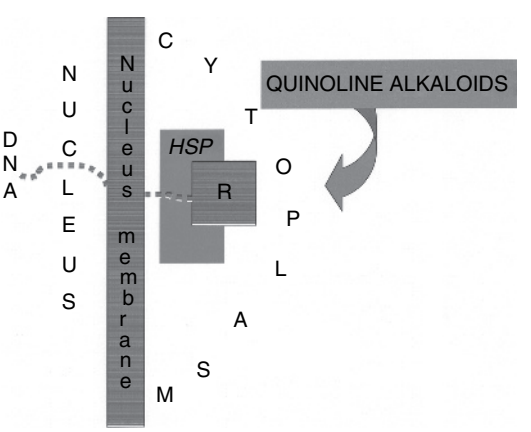


Figure 84. Diagram of estrogenic activity of the alkaloids. Abbreviations: R – receptor; HSP – heat shock proteins.

quinoline alkaloids have the highest estrogenic activity at doses from 50 to 100 mg kg^{-1}. This interesting study also observed that the estrogenic activity of perforine was many times greater than that of haplophyllidine. According to Nazrullaev et al.[54], estrogenic activity depends on the heterocyclic skeleton, N and the nature of the substituent.

2.5. Antimicrobial properties

It is generally recognized that alkaloids have strong antimicrobial, antibacterial and antifungal biological properties[329,383,386,387,388,389,390,391]. Moreover, some studies have evidenced anti-parasitic activity in this group of compounds[392,393]. Caron et al.[329] have investigated 34 quasi-dimeric indole alkaloids for antimicrobial activity using 8 different test micro-organisms. It was found that all of the studied alkaloids showed activity against *Staphylococcus aureus* and *Bacillus subtilis*, which are Gram-positive bacteria. Caron et al.[329] found that 31 alkaloids showed biological activity against micro-organisms. The micro-organisms tested by Caron et al.[329] were *B. subtilis, S. aureus, Mycobacterium smegmatits, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Aspergilus niger*. This study concluded that antimicrobial activity of alkaloids is connected with the stereochemistry of the carbon ring, its aromatic substitution and oxidation[329].

The antimicrobial activity of pendulamine A, pendulamine B and penduline isolated from the root extract of *Polyalthia longifolia* var. *pendula* was studied by Faizi et al.[388]. All three alkaloids showed bioactivity, though the most active was found to be pendulamine A. In this study, the following Gram-positive organisms were used: *B. subtilis, Corynebacterium hoffmanii, S. aureus, Streptococcus faecalis, Streptococcus pyogenes, Streptococcus viridans* and *Micrococcus lysodicklycus*. The Gram-negative micro-organisms were *E. coli, K. pneumoniae, P. aeruginosa, Proteus mirabilis, Salmonella paratyphi* A., *S. paratyphi* B. and *Salmonella typhi*[388]. Antibacterial properties of the alkaloids isolated from *Zanthoxylum rhifolium* have also been noted in the study of Gonzaga et al.[390]. Significant bioactivity was displayed by 6-acetonyldihydronitidine, 6-acetonyldihydroavicine and zanthoxyline. Gonzaga et al.[390] used the following Gram-positive bacteria in their research: *S. aureus, Staphylococcus epidermilis, Micrococcus luteus*. The Gram-negative bacteria were *K. pneumoniae, Salmonella setubal* and *E. coli*. Antifungal, antibacterial and anti-malarial properties are mentioned in the case of sampangine as a result of *in vitro* studies[383]. This alkaloid has also shown other bioactivities, especially a novel opportunity to be used as anticancer agent.

Figure 85 presents antibacterial activity by some alkaloids. There are clearly different minimum inhibitory amount of alkaloids for this activity. It is also clear that alkaloid antibacterial activity is selective.

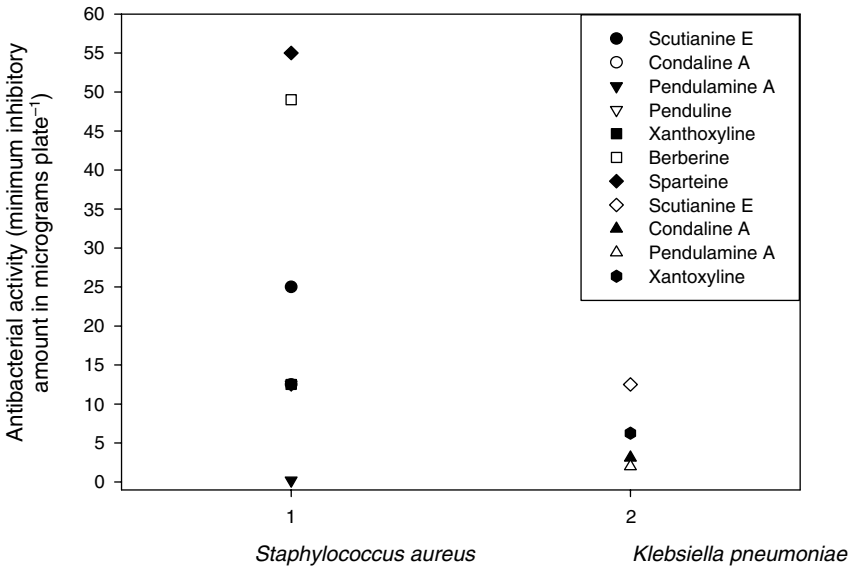


Figure 85. Activity of some alkaloids on Gram-positive (*Staphylococcus aureus*, 1) and Gram-negative (*Klebsiella pneumoniae*, 2) bacteria. Explanations: Observe that the value for Scutianine E and Xanthoxyline is the same (12.5 μg). Alkaloids indicated in 1 but not in 2 have values of more than 100 μg. In the case of Penduline there is no data available (Refs [388, 389, 390, 394]).

Morel et al.[389] reported on the antimicrobial activity of cyclopeptide alkaloids isolated from *Scutia buxifolia* Reiss (Rhamnaceae). The organisms used in this research were identical to those used in the study of Gonzaga et al.[390]. Condaline and scutianine have shown the widest range of bioactivity. It is necessary to mention that the antimicrobial activity of alkaloids is one of the many possible biological activities of these molecules. Recent studies provide vast amounts of new information about the status of marine environments as large reservoirs for biologically active alkaloids. The indole alkaloids from marine environments are a promising and active group of molecules. Their biological activity covers cytotoxic, antiviral, anti-parasitic, anti-inflammatory, serotonin and antagonistic realms[395]. Another alkaloid group from marine environments consists of the pyridoacridone alkaloids. They have been reported as having a wide range of biological properties[386,387,391,393]. These alkaloids have been known as having anti-tumourous and antifungal ability. Moreover, Sas-Piotrowska et al.[231] addressed the fungistatic effects of the quinolizidine alkaloid fractions from extract of *Lupinus* spp. on potato pathogens. The alkaloid fractions had the strongest effect on the colonization of certain potato leaf fungi, such as *Altenaria solani, Cladosporium herbarum, Colletotrichum coccodes* and

Vertricillum albo-atrum. Moreover, this study has evidenced that alkaloids from *Lupinus luteus* L. strongly inhibited the growth of potato tuber fungi such as *Rhizoctonia solani* and *Phoma exigua*. This study also suggested that alkaloid influence is stronger in the case of facultative fungi than in the case of specialized fungi. However, the clearly different sensitivities of various potato pathogen species observed in the experiments carried out by Sas-Piotrowska et al.[231] to alkaloid preparations have been explained by pathogenic cell structures and by the chemical structure of the alkaloids. On the other hand, it was also possible that the fungistatic action of the preparation was based on the influence of the different synergetic levels of various compounds found in fungi species.

Bringmann et al.[396] have researched naphthylisoquinoline alkaloids isolated from the tropical lianas *Ancistrocladus abbreviatus* and *Triphyophyllum peltatum*. The most prominent naphthylisoquinoline alkaloid is dioncophylline A, which has a variety of bioactivities. It was evidenced that this alkaloid possesses high fungicidal and insecticidal properties. It has proven very active in limiting the fungus *Botrytis cinerea* and inhibiting the growth of the insect *Spodoptera littoralis*. Moreover, this alkaloid has anti-parasitic bioactivity against *Plasmodium falciparum* and larivicidal in the case of *Anopheles stephensi*. Bringmann et al.[396] have studied naphthylisoquinoline alkaloids for their molluscicidal activity against the tropical snail *Biomphalaria glabrata*. This study indicated a strong molluscicidal activity by the alkaloid.

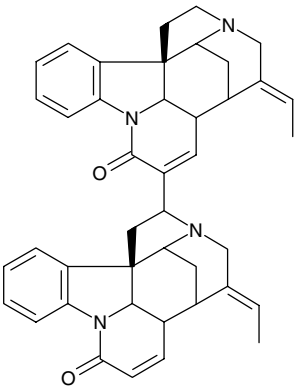
It is necessary to mention that the antimicrobial activity of alkaloids has been studied relatively extensively even during the 1940s–1980s. These studies have reported nearly 50 different steroid[397,398,399,400,401,402,403,404,405,406,407,408,409] and over 100 different isoquinolizidine[402,404,407,410,411,412,413,414,415,416,417,418,419,420,421,422,423,424,425,426], and at least 90 different terpenoid indole alkaloids to have antimicrobial activity[329,427,428,429,430,431,432,433,434,435,436,437,438,439,440,441,442]. Research has reputed large diversity in antimicrobial activity against bacteria, yeasts and fungi. In the 1990s, the most known and widely used alkaloids were berberine and sanguinarine, due to their antimicrobial activity. Berberine has anti-diarrhoetic and sanguinarine anti-carries properties.

2.6. Anti-parasitic activity

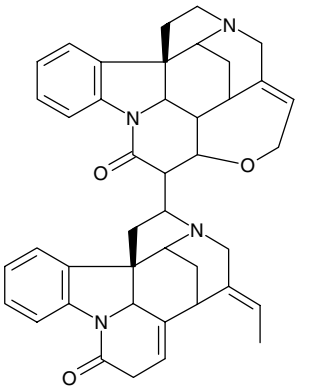
A parasite is an organism living in or on, and metabolically depending on, another organism. Endoparasites live inside an organism, and ectoparasites live on the surface of the host. Parasites can be carnivorous if living with animals or herbivorous if living with plants. Analyses of parasite/host suggest strong evidence of anti-carnivorous anti-herbivorous action of alkaloids. A good example is with protozoan parasites (*Plasmodium* spp.) injected into humans by mosquitoes of the genus *Anopheles*. The life cycle of this parasite includes a sexual reproductive

stage with multiplication (sporogony) occurring in the mosquito gut lumen and an asexual reproductive phase with multiplication (schizogony) occurring in the human host. Symptoms of this protozoan injection to humans and resulting symptoms of its asexual multiplication are known as malaria. Copp et al.[393] have investigated the anti-parasitic potential of alkaloids against this kind of organism. In *in vitro* studies of anti-parasitic activity on *Plasmodium falcicarum, Leishmania donovani, Trypanosoma cruzi* and *Trypanosoma brucei rhodesiense*, evidence arose that connected pyridoacridone alkaloids with anti-parasitism. However, these alkaloids also exhibit high cytotoxic activity, which can limit the use of their bioactivity in possible anti-malarial product development. Kapil[443] has studied the bioactivity of piperine on *L. donovani* promastigotes *in vitro* and received very promising results. According to this study, piperine exhibited a concentration-dependent inhibition of *L. donovani* promastigotes.

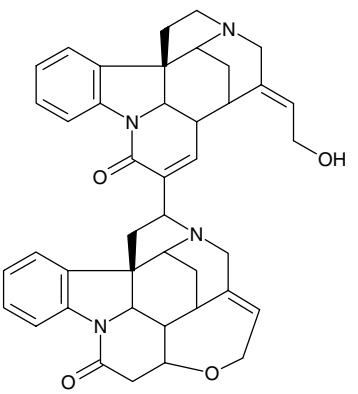
More recently Wright[444] has analysed bioactive possibilities of cryptoleptine, the main alkaloid from *Cryptolepis sanguinolenta*, as an anti-malarial agent. The bioactivity of alkaloids against parasites is becoming increasingly important because some parasites (e.g. *P. falciparum*) are presently resistant to traditional malarial medication. Cryptoleptine was considered by Wright[444] as an alkaloid having possibility to be an anti-malarial bioagent. However, more research in this direction is very important. As is known, the first alkaloid to be used against malaria was quinine obtained from the bark of *Cinchona*. Treatment was later commonly focused on quinoline-based drugs such as chloroquine, quinine, mefloquine, primaquine and fansidar. Observations that *P. falciparum* became resistant to chloroquine, mefloquine and halofartine[445,446] aroused awareness of a problem. This has been studied in connection to indole alkaloids (Figure 86) from the *Strychnos* species (Loganiaceae) by Frederich et al.[447] Sungucine presented very little activity, but some compounds (strychnogucine B and 18-hydroxyisosungucine) displayed more active qualities against quinine- and chloroquinine-resistant strains of *P. falciparum*. Anti-parasitic alkaloid activity against *Leishmania* spp. has also been reported in other studies[448,449,450,451]. Montenegro et al.[451] have studied alkaloids (xylopine, nornanteine, cryptodorine, nornuciferine, lysicamine and laudanosine) from *Guatteria amplifolia* Triana and Planch (Annonaceae). Their results provide evidence that xylopine, cryptodorine, nornanteine and nornuciferine have significant bioactive properties against *Leishmania mexicana* and *Leishmania panamensis*. Xylopine was the most active compound[451]. Moreover, Sari et al.[452] have studied the bioactivity of alkaloids from *Papaver lateritium* Koch, a plant endemic to Turkey. The quaternary alkaloid fraction with (−)-mecambridine showed the highest lethality to brine shrimp larvae. Moreover, a study of the bioactivity of *Stemona* alkaloids provides evidence that these alkaloids have anti-tussive activity[330]. This study also demonstrates a clear structure–bioactivity relationship in such alkaloids. Through substitution of a constituent of the alkaloid ring structure, it is possible to change bioactivity.



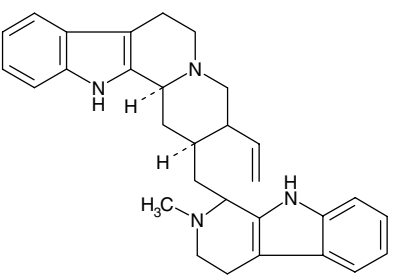
Sungucine



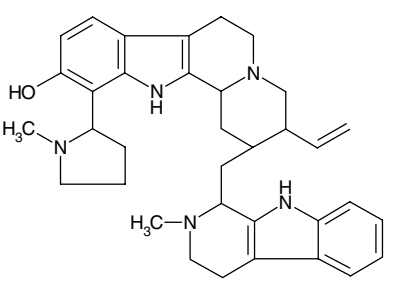
Strychnogucine A



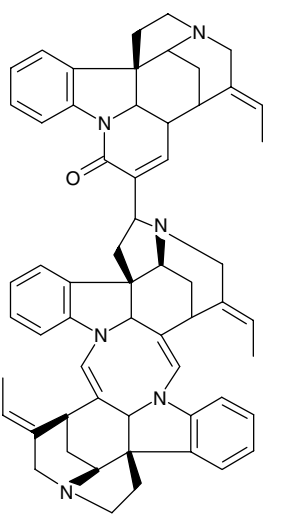
Strychnogucine B



Strychnopentamine



Isostrychnopentamine



Strychnohexamine

Figure 86. Some alkaloids from *Strychnos* species.

3. Biotoxicity

Many alkaloids are toxic to foreign organisms. Toxicity is a secondary function of the alkaloids, because they are generally non-toxic to the organisms producing them. This is very important for understanding alkaloid nature. There are many studies on alkaloid toxins published in recent years[453,454,455,456,457,458,459,460]. The biotoxicity of alkaloids is selective and dependent on different organisms and the chemical structure of alkaloids themselves. Multiple bonds and different bond groups and sub-groups especially directly or indirectly influence toxicity mechanisms.

3.1. Research evidence

Alkaloids are active bioagents in animal tissues. There is clear scientific evidence of this. Crawford and Kocan[454] have tested the toxicity of steroidal alkaloids from the potato (*Solanum tuberosum*), such as α-chaconine, α-solanine, solanidine and solasodine, and *Veratrum* alkaloid, jervine on fish. The results of Crawford and Kocan's research proved that rainbow trout exhibited a toxic response to chaconine, solasidine and solanine, while medaka only did so to chaconine and solanine. Embryo mortality was observed as an effect of toxicity in both species. Many other alkaloids are known to disturb or cause disorder in animal reproductive systems. For example, gossypol from cotton-seed oil is known as a clear reducer of spermatogenesis and premature abortion of the embryo.

Schneider et al.[455] have studied ergot alkaloid toxicity in cattle. The observed symptoms of the toxicity were hyperthermia, loss in milk production, loss of body mass and reduced fertility. The toxicity symptoms were affected by ergotamine, ergosine, ergocornine and ergocryptine. These ergot alkaloids caused gangrenous necrosis of extremities in young cattle. Their impact on livestock production is realized in significant financial losses each year[460].

Piperidine alkaloids such as coniine and (−)-coniceine are very poisonous. They occur in hemlock (*Conium maculatum* L.), known as a very toxic plant. One of the characteristics of these piperidine alkaloids is smell. Moreover, they are neurotoxins which have acute effects such as chronic toxicity.

There are known cases of death by respiratory failure resulting from coniine alkaloids. Pregnant cattle habitually ingesting amounts of plants with these alkaloids, for example from hay, gave birth to deformed offspring[461]. Rabbits have reportedly experienced toxic effects[462]. The classic toxic symptoms of coniine alkaloids range from paralysis, muscular tremors, muscle weakness and respiratory failure preceding death[461]. It is not difficult to observe that the bioactivity of coniine alkaloids and especially their symptoms are similar to these of curare[461] or of nicotine[463]. Piva et al.[456] have studied the toxicities of pyrrolidine and tropane alkaloids. In this research a synthetic scopolamine and hyoscyamine mixture in different concentrations was used on test pigs. Toxicity

was observed in the gastrointestinal tracts, where the mucous membrane showed lymphocytic infiltration and a loss of epithelium. The villi were necrotic. It was also observed that the high levels of alkaloids increased the blood concentration of total lipids, cholesterol and increased concentrations of urea and uric acid in the blood. Moreover, some alkaloids can inhibit digestive enzymes. Such kinds of alkaloids are, for example, swansonine or castanospermine.

3.2. Influence on DNA

The phenethylisoquinoline alkaloids present in some members of the Lily family (Liliaceae) are known to be toxic. Wang and Wang[457] have researched the activity of veratridine on rats. This alkaloid causes persistent opening of the voltage-gate Na^+ channel and reduces its single-channel conductance by 75%. However, its toxicity is concentration dependent. The toxicity of isoquinoline alkaloid berberine is low in concentrations 0.05% for living plant cells[464]. In these concentrations berberine did not kill onion, corn or broad bean cells, although it did reduce the growth rates of corn and bean. Moreover, in these concentrations berberine is used as a mobile apoplastic tracer. Sequential application of berberine hemisulphate and potassium thiocyanate to plant tissue affects crystal formation in unmodified walls and in the lumina of dead cells. However, berberine does not affect crystals in lignified and suberized cell walls[464]. Berberine was alone tested for possible genotoxicity, mutagenecity and recombinogenic activities in micro-organisms[465]. An SOS-chromotest with this alkaloid shows that there is no genotoxic activity nor significant cytotoxic, mutagenic or recombinogenic effects in *in vitro* (non-growing) conditions. However, Pasqual et al.[465] have observed the metabolic activity of this alkaloid in dividing cells. It has induced important cytotoxic and cytostatic effects in proficient and repair-deficient *Saccharomyces cerevisiae* strains. According to Pasqual et al.[465], berberine's cytotoxicity results from a mutational blockage in the DNA strand-break repair pathway (rad52-1). The influence of this alkaloid on DNA is evident. Pasqual et al.[465] have observed the same cytotoxicity in a triple mutant blocked in the excision (rad2-6), in the mutagenic (rad6-1) and in the recombinogenic (rad52-1) repair pathways. Although this toxicity has been observed in dividing cells, Pasqual et al.[465] concluded in their discussion of results that berberine is not a potent mutagenic agent although one cannot rule out possible implications of DNA topoisomerases in berberine toxicity mechanisms. The results presented by Pasqual et al.[465] have sufficiently characterized the nature of alkaloid activity and its potential toxicity. These characteristics are also typical for other alkaloids in general, although there may be some exceptions and reservations. Figure 87 presents the acute toxicities of berberine and thebaine. These alkaloids are very selective in their toxicity. There are also strong differences in acute toxicities according to the form in which these alkaloids were administrated to mice.

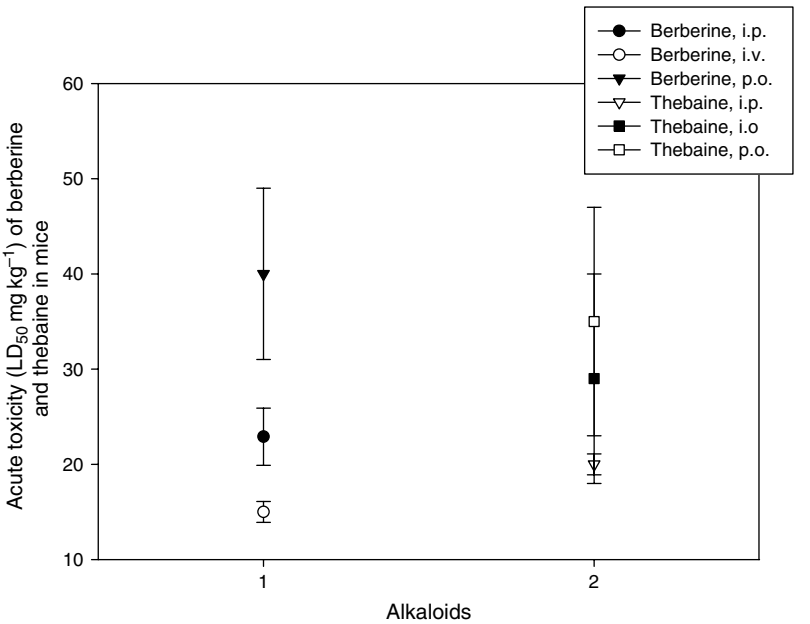


Figure 87. Acute toxicity of berberine and thebaine on mice in relation to form administration. Abbreviations: 1 – berberine; 2 – thebaine.; i.p. – intraperitoneal; i.v. – intravenous; p.o. – oral.

There are studies suggesting that nicotine influences human carcinogenesis. One such study was carried out by the Kleinsasser research group from the University of Regensburg in Germany[466]. To assess the genotoxicity of this alkaloid, researchers tested the DNA-damaging effect on human lymphocytes and target cells from lymphatic tissue. The experimental data by Kleinsasser et al.[466] evidently indicated that nicotine significantly and directly causes genotoxic effects in human target cells *in vitro*. However, there were no differences in DNA damage observed in cells from smokers and non-smokers incubated without nicotine. Kleinsasser et al.[466] suggest that the lack of higher DNA damage in smokers compared to non-smokers is connected only with nicotine dose.

3.3. Selective effectors of death

One of the most known toxic alkaloid is strychnine. Vanderkop[467] and Sterner et al.[468] are examples of those who have studied its toxicity, although it is practically rather evident. This alkaloid has been used as a strong rodenticide[225]. It is also known for being dangerous to humans. One general characteristic of strychnine is its chemical stability. This is some kind of exception in the alkaloids, which are generally flexible heterogeneous compounds. In cases of

poisoning this alkaloid can be detected in exhumed bodies even many years after death. However, in the case of strychnine some selectivity has been observed. The study of Sterner et al.[468] is interesting in the sense that there is clear evidence of the selectivity of strychnine sub-chronic dietary toxicity being species dependent. Sterner et al.[468] studied the sub-chronic toxicity of strychnine on the northern bobwhite quail (*Collinus virginianus*) and the mallard duck (*Anas platyrhynchos*). The authors evidenced that strychnine toxicity was much lower in *C. virginianus* than in *A. platyrhynchos*. Others have also investigated strychnine. The research of Altememi et al.[469] deserves mentioning when considering the species selectivity of strychnine. This study has evidenced that the addition of two acetylenic triazole derivatives has increased the potentiation of strychnine toxicity and lethality in mice. Strychnine may also cause convulsions and disorders of the CNS. This is a result of the strychnine activity mechanism. It is known that the strychnine binds to a receptor site in the spinal cord that normally binds with glycine. Some selectivity of strychnine to different species can be considered as a new point of view to the consideration of alkaloid toxicity in general. Some of the selectivity is also possibly present in other very toxic alkaloids. This means that the poisonous nature of alkaloids as immediate death effectors may not hold true for all species and individuals. Therefore, the exotic and coloured legends of the use of alkaloids for acute effects of death (murders, executions, weapons etc.) presented by some scientific books need to be critically checked in the light of present research data. The lethal dose (LD_{50}) should be explained very carefully and critically. Alkaloid toxicity is not absolute; it is dependent on species, individuals, presence of other chemicals and on its own concentration.

3.4. Non-toxic to self but deformer for others

Quinolizidine alkaloids are non-toxic to the legumes which produce them. On the other hand, the quinolizidine alkaloids can be toxic and in some cases very toxic to other organisms[7]. The biotoxicity of alkaloids has for some time been considered to be connected with their bitter taste[470,471]. The quinolizidine alkaloids are certainly bitter in taste to humans. However, not all alkaloids are. Literature states that some pyrrolizidine and indolizidine alkaloids are not bitter in their pure forms[471]. Furthermore, there are many non-alkaloid compounds, such as flavonoids, that are bitter in taste but non-toxic. Therefore, although quinolizidine alkaloids are bitter, the connection between biotoxicity and bitter taste is not absolute.

The most toxic quinolizidine alkaloids are tetracyclic with a pyridone nucleus. One of these is anagyrine. One case mentions in anagyrine being passed to the human body via milk from goats foraging on *Lupinus latifolius*[260]. The anagyrine caused severe bilateral deformities of the distal thoracic limbs in a baby boy.

The literature presents terrible cases of the poisoning of humans, adults and children by lupine alkaloids[476]. According to results, the acute toxicity of a mixture of quinolizidine alkaloids varies. The lethal dose (LD_{50}) for the extract of *L. angustifolius* L. is 2279 mg kg^{-1}, and for extract with lupanine 1464 mg kg^{-1}. In other studies the oral LD_{50}-value of sparteine was 220 mg kg^{-1} and of lupanine 410 mg kg^{-1}. According to the newest results (Figure 88), the LD_{50}-value for sparteine is 60 mg kg^{-1}, lupanine 159 mg kg^{-1}, 13-hydroxylupanine 189 mg kg^{-1}, 17-hydroxylupanine 177 mg kg^{-1} and oxolupanine 190 mg kg^{-1} [476]. The biological effect of the quinolizidine alkaloids is on the nervous system. Tremors, convulsions and pulmonary arrest have been noted in laboratory animals. Quinolizidine alkaloids cause depression, laboured breathing, trembling, convulsions and respiratory paralysis in sheep[236].

Yovo et al.[238] stated that these alkaloids act via inhibition of ganglionic impulse transmissions of the sympathetic nervous system. It is evident that each alkaloid has its own effect. Anagyrine caused skeletal deformity in foetuses when pregnant cows consumed toxic lupines[236]. On the other hand, some quinolizidine alkaloids are used as a drug in folk medicine[477]. They probably have chronic toxicity[7]. However, adequate knowledge about the chronic toxicity of these alkaloids and especially of chronic toxication across generations is not available. The premise that quinolizidine alkaloids have not produced hereditary symptoms has not been checked with total reliability.

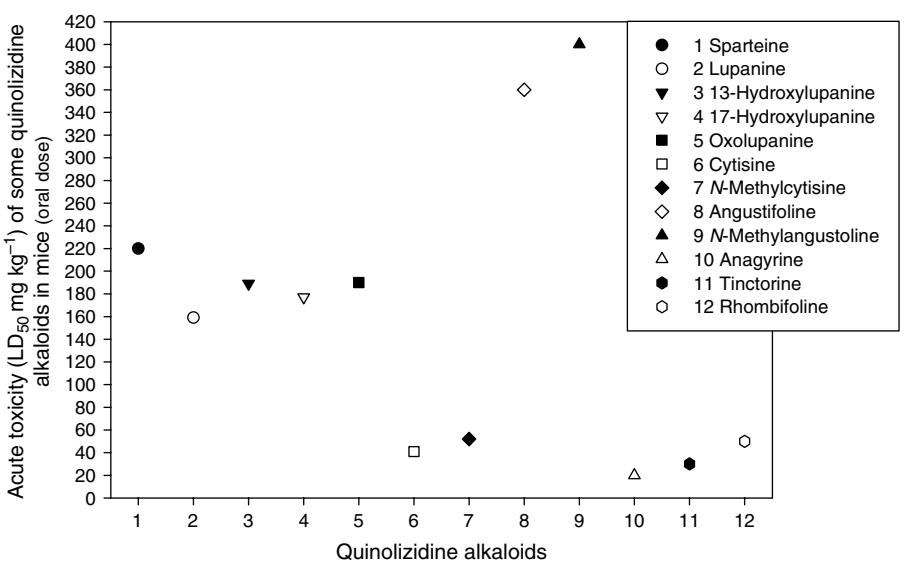


Figure 88. Acute toxicity (LD_{50}) of some quinolizidine alkaloids in mice (Refs [394, 472, 473, 474, 475]).

3.5. Degenerators of cells

The biotoxicity of pyridine alkaloids is well studied and the toxicity of nicotine is one of the best examples of the very active alkaloids study area. Aydos et al.[478] have studied 20 rats injected daily with nicotine at doses 0.4 mg 100 g^{-1} of body weight during 3 months and made comparisons to a control group of 20 rats. The researchers concluded that ultra-structural alternations in rats exposed to nicotine occurred.

Aydos et al.[478] underlined the particularly detrimental effects of nicotine on germ cells, peritubular structures and Sertoli cells. The germ cells were degenerated, and spermatids retained excess cytoplasm and accumulated electron-dense lipid droplets in the cytoplasm. Moreover, the results of Aydos et al.[478] proved that the acrosomes in rats exposed to nicotine were irregular and abnormally configured. It is not difficult to interpret these results as evidence of active nicotine toxicity. Moreover, this chronic toxicity is reported also by Sener et al.[479], who have studied aqueous garlic extract as an antioxidant. In this research, male Wistar albino rats were injected with nicotine, which led to increased collagen contents in tissues. Although Sener et al.[479] reported the aqueous garlic extract was a protector of rat tissues, there is evidence of nicotine-induced oxidative damage. Nicotine toxicity has been studies also on humans[480,481,482,483,484,485,486,487,488,489,490,491,492]. None of these studies question the symptoms of acute and chronic toxicity of nicotine. Moreover, the study by D'Alessandro et al.[483] points to evidence of the risk of nicotine toxicity for tobacco harvesters. They absorbed approximately 0.8 mg of nicotine daily. Harvesters had higher levels of nicotine in their blood and urine, and urine nicotine levels were also elevated[483]. Nicotine toxicity is also considered a health risk for agricultural workers on tobacco plantations in India[489]. Nicotine toxicity is also connected to pica disease[484]. Many symptoms of nicotine toxicity were observed in smokers in numerous studies[480,482,485,492]. A dramatic case of nicotine toxicity is presented by Rogers et al.[486], who mention a case of acute ingestion of this alkaloid by a child. Hypoxia and irreversible encephalopathy ensued in this rare and tragic emergency case. Berthier et al.[491] reported on a case of a 30-year-old woman with symptoms of acute edematous pancreatitis. The combination of a nicotine patch and tobacco smoking induced an overdose of nicotine in this case[491]. Some studies also claim that a chronic administration of high doses of nicotine results in axonal degeneration in the central core[490]. Studies of the efficacy of nicotine replacement therapy have produced mixed findings. Moreover, nicotine toxicity is also a topic of the latest clinical and theoretical studies[487,488]. The mechanism of this toxicity is still not completely known in details, but the research in the field is advanced and promising. On the other hand, it is also a difficult research area because of the large industry and large amount of trade involved with tobacco plants as a part of commercial products.

3.6. Aberrations in cells

Pyrrolizidine alkaloids are toxic to foreign organisms (Figure 89). This problem was largely studied in the 1960s–1980s[493,494,495,496,497,498,499,500]. Serious livestock poisoning episodes are mentioned in literature from the effects of the pyrrolizidine alkaloid of the *Senecio* genus especially *Senecio riddellii, Senecio douglasii* and *Senecio jacobaea*[471]. The toxicity of pyrrolizidine alkaloids to livestock was considered coincidental. Johnson and Molyneux[501] and Johnson et al.[502] have stated that experimental feedings of pyrrolizidine alkaloids to cattle empirically proved that the threshold level of ingesting alkaloids must be excessive for toxicity to occur. On the other hand, there are also known cases of animal poisoning from pyrrolizidine alkaloids found in *Cynoglossum officinale* (Boraginaceae). Baker et al.[503] have reported cases of calves being poisoned, and Knight et al.[504] connected the deaths of two horses to poisoning by pyrrolizidine alkaloids. The acute toxicity of these alkaloids varies widely; it is recognized by the International Programme on Chemical Safety (IPCS) that for rats the LD_{50} of most alkaloids is 34–300 $mg\,kg^{-1}$ [475]. Lasiocarpine doses equivalent to 0.2 $mg\,kg^{-1}$ body weight per day lead to the development

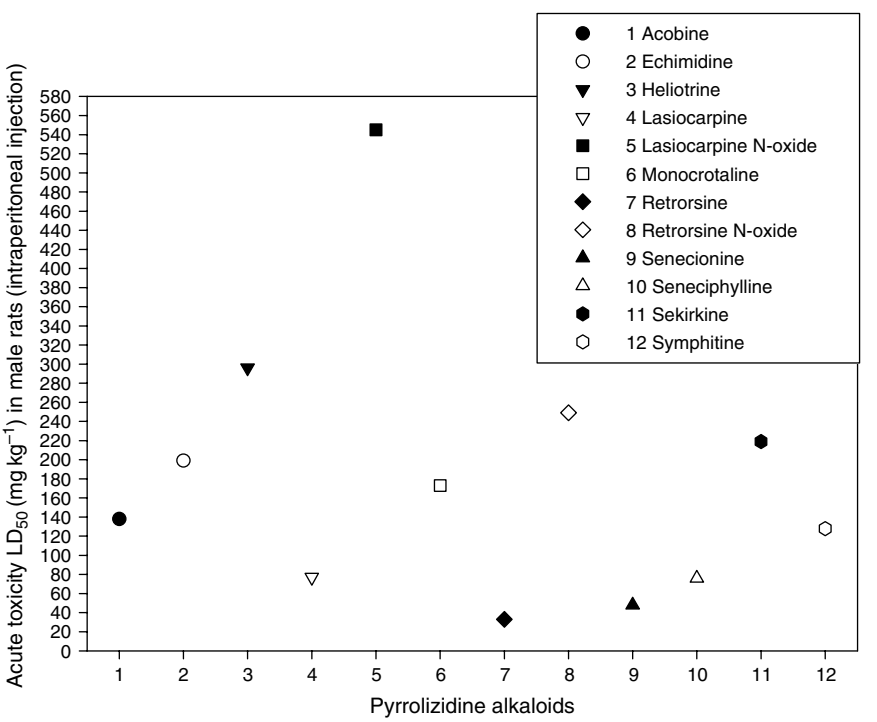


Figure 89. Acute toxicity (LD_{50}) of some pyrrolizidine alkaloids in male rats. (Sources: Refs [472, 473, 474, 475]).

of tumours in rats. For pigs, $1.8\,mg\,kg^{-1}$ doses cause chronic liver damage. For humans, the lowest reported intake level causing veno-occlusive disease (VOD) is estimated to be $0.015\,mg\,kg^{-1}$ body weight per day[475]. Some pyrrolizidine alkaloids are thought to cause lung damage, affect blood pressure and lead to secondary effects of the functioning of the right side of the heart. Moreover, according to the WHO data, pyrrolizidine alkaloids produce chromosomal aberrations in mammalian cells. Some pyrrolizidine alkaloids and their *N*-oxides are active as tumour inhibitors[500]. They also can induce genetic changes and produce cancer in the livers of rats. The toxic effects of these alkaloids can be acute or chronic. Toxicity laboratory trials with retrorsine reported by White et al.[505] resulted in centrilobular necrosis in rats, mice and guinea pigs, periportal necrosis in hamsters and focal necrosis in fowl and in monkeys. Even in the 1940s, Wakim et al.[506] reported that senecionine produces necrosis in the periportal and midzonal areas of liver lobules. Later, Dueker et al.[453] studied monocrotaline metabolism using rat and guinea pig hepatic microsomes. These results suggest that guinea pigs are resistant to pyrrolizidine alkaloid toxicity. Esterase hydrolysis was observed in the metabolism of the guinea pig, and in the case of rats, there was no esterase activity. This explains the guinea pig's resistance to pyrrolizidine alkaloid toxicity. Monocrotaline was also researched by Vaszar et al.[459] Their results showed statistically significant increases in proteases in rats as a result of the activity of this alkaloid toxin. Moreover, Smith et al.[458] have researched pyrrolizidine alkaloid toxicity in horses. They concluded that these alkaloids led to chronic active hepatitis and furthermore chronic heart damage, including right ventricular hypertropy as a consequence of pyrrolizidine lung damage. However, it is important to note that pyrrolizidine alkaloid toxicity depends on alkaloid structure and its possible reduction. The mechanism of toxic activity of these alkaloids is connected to metabolism in the parenchymal cells, where pyrrolizidine alkaloids change to pyrroles acting on hepatocytes and blood vessels in the liver or lungs. McLean[507] reported that as a consequence of this, disaggregation of polyribosomes, absence of pyruvate oxidation and lysosomal activity and necrosis occur. It is important to note that pyrrolizidine alkaloids are inactive as a cell poison by themselves.

3.7. Causers of locoism

Indolizidine alkaloids are also known as active biotoxins. Swansonine is especially cited in literature as a cause of locoism. This is a neurological lesion, especially in horses, cattle and sheep[508]. According to Elbein and Molyneux[509] swansonine is toxic due to the imbibition of α-mannosidase, an enzyme needed for proper functioning of mammalian cells. It is also known that swansonine inhibits several hydrolases. In addition, *Astragalus lentiginous* produces lentiginosine, which is an alkaloid related to swansonine[510]. It is known as a good inhibitor of several α-glucosidases. This is due to the suppression of digestive enzymes[471].

4. Narcotics

All alkaloids are neurotransmitters and active agents in the nervous system. Many alkaloids from natural plants and also modified alkaloids can impress euphoric, psychomimetic and hallucinogenic properties on humans. Some of them can influence narcosis, states of stupor, unconsciousness, or arrested activity. Some of them in moderate doses dull the senses, relieve pain and induce sleep. In excessive doses they can cause stupor, coma or convulsions. They are known as "narcotics", a term derived from the *narcoticus* in Latin, *narkotics* in Greek and *narcotique* in French. In the 1920s, lysergic acid diethylamide (LSD) was developed on the structural basis of ergotamine, the alkaloid produced by the fungus *Claviceps purpurea* living with rye (*Secale cereale* L.). Lysergic acid diethylamide has been developed and used primarily for treatment of schizophrenia. This compound is hallucinogenic. In the small doses it causes psychedelic effects. It is for this reason LSD has been and is used as a narcotic.

Narcotics (Figure 90) are stimulants which are active on the central nervous system causing disorders and some temporary or permanent changes in this system and behaviour. Serious negative consequences of narcotics include dependence, a chronic disorder.

The most known narcotics are the opium alkaloids such as morphine, codeine, thebaine, papaverine, noscapine and their derivatives and modified compounds such as nalmorphine, apomorphine, apomopholcodine, dihydrocodeine, hydromorphone and heroine, also known as diamorphine. Synthetic narcotics share the structural skeleton of morphine and include dextromethorphan, pentazocine, phenazocine meperidine (pethidine), phentanyl, anfentanil, remifentalin, methadone, dextropropoxyphene, levoproxyphene, dipipanone, dextromoramide, meptazinol and tramadol. Thebaine derivatives are also modified narcotics and include oxycodone, oxymorphone, etorphine, buprenorphine, nalbuphine, naloxone or naltrexone. Narcotics can be semi-synthesized or totally synthesized from the morphine and thebaine model. The compounds serve various purposes in clinical practise.

The natural source of these narcotics is *Papaver somniferum* L. and papaveretum, a mixture of purified opium alkaloids. Papaveretum is approximately 85.5% morphine, 8% codeine and 6.5% papaverine. Only purified alkaloids are considered here, as the total alkaloid content of ripe poppy capsules is only 0.5%. It is recovered from the ripening capsule of papaver when it is in the process of changing colour from blue-green to yellow. When the tubs are cut, it is possible to procure the milk. During coagulation, the milk's colour changes to brown. Fresh opium is soft but it hardens during storage.

Crude opium has been used in the past as a sleep-inducer and in folk medicine for many purposes and smoked for the feeling of pleasure. The last use has lead to drug dependence and unpleasant withdrawal symptoms.

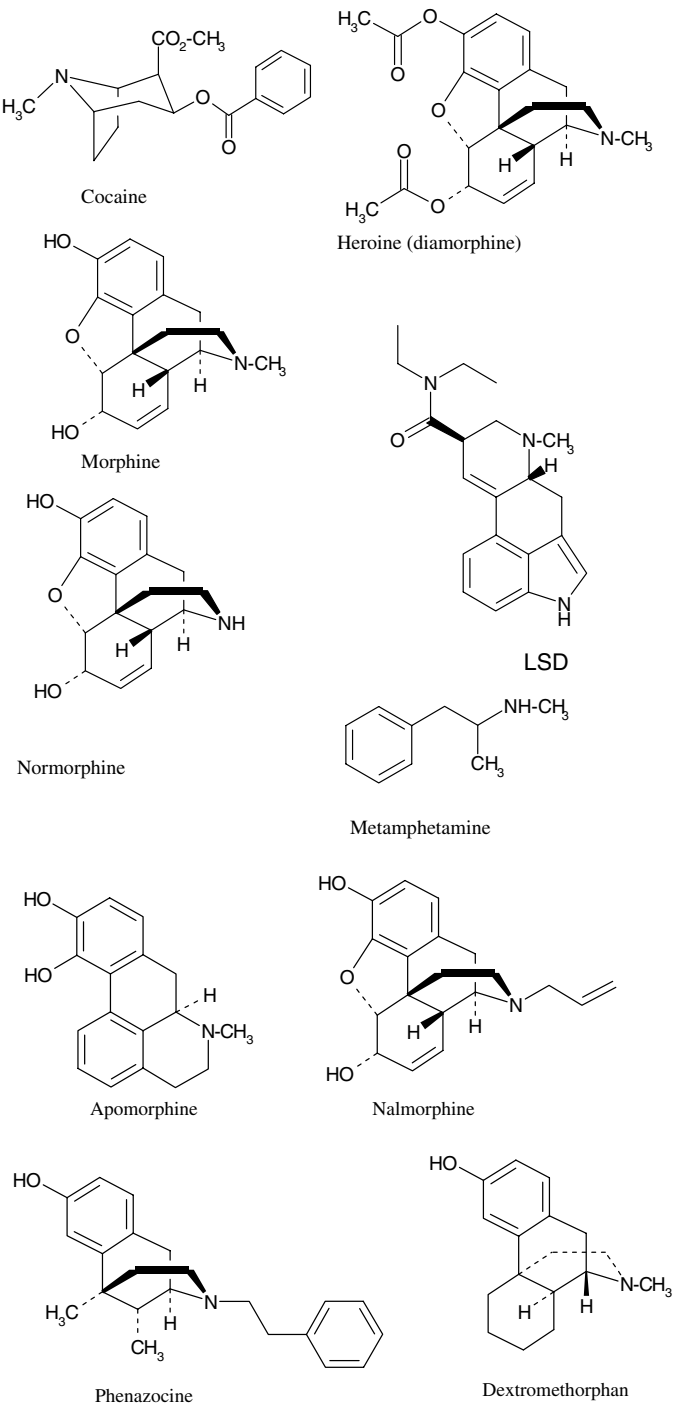


Figure 90. Some narcotics and their derivatives.

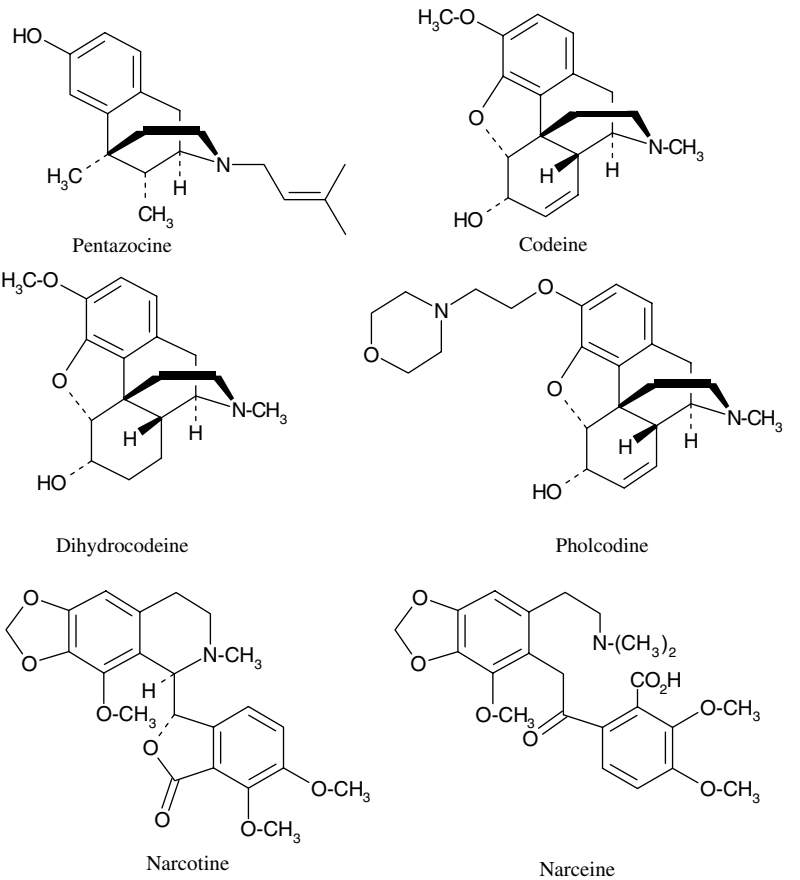


Figure 90. (Continued)

Narcotics are reportedly among the most widely abused substances in the world, particularly the CNS stimulants cocaine and methamphetamine[511]. These narcotics are a very serious problem because they may lead to strong drug dependence. Possible treatments for this dependence are relatively difficult. Common ones are based on the so-called "dopamine hypothesis", according to which stimulants have the ability to increase extracellular dopamine, which has an additional narcotic effect[511,512,513,514]. Cocaine has similar affinity for the dopamine transporter (DAT), norepinephrine transporter (NET) and serotonin transporter (SERT)[515]. Narcotic dependence can be treated with the use of the synthetic compounds, chemically similar to narcotic. Dependence, therefore, is a very serious problem. Although there are new findings that indicate κ opioid receptors may be involved in the modulation of some abuse-related effects and dopamine levels, problem-free therapy exists only in an optimistic future. It is known that the administration of cocaine upregulates κ opioid

receptors[516,517,518,519]. However, κ opioid receptor agonists offer an indirect possibility to modulate some of the abuse-related effects of narcotics. Presently, more research is needed on this subject.

Humans have known of and used opium for nearly 5000 years, mainly for medical purposes. Its abuse has been around as long. Governmental and international laws and licences regulate the production of *P. somniferum*, and only the clinical use of narcotics is legal and reasonable.

5. Alkaloids in the immune system

As has been stated in this book, alkaloids are special secondary compounds. This is due to a general metabolic dependence on the genetic code and their expression through both the genetic code and metabolic scale mediated by growing factors. It is known that alkaloids can interact with DNA or DNA-processing enzymes and can inhibit protein synthesis, as has been mentioned in this book when referring to bioactivity. Moreover, alkaloids can influence electron chains in metabolism and can modulate enzyme activity. As has been presented, these compounds are biologically very active. Another important role alkaloids play is in the immune systems of living organisms. The amount of empirical research on this role is scant.

Immunity is here defined as the ability to resist infections. Infections caused by micro-organisms can be avoided in many ways. There are external and internal barriers to possible infections. The external barriers are skin (impermeable to many infectious agents), cuticles, skin secretions and pH-value and washing. The internal barrier to infection is namely phagocytosis, a process of killing the infectious micro-organisms by special cells. This ability, first discovered by Metchnikov in Russia in the 1880s, is the basis for immune systems in living organisms.

Immune systems in animals and plants are quite different. There are two types of immune systems in animals: (1) innate, so-called “non-specific” or passive immunity; (2) adaptive, so-called “specifically acquired”, active, or cell-mediated immunity. Innate immunity is based on barriers to infectious agents and adaptive immunity is based on multiplicative and specific antibody release after contact with an antigen (infectious agent). The so-called “memory cells” in animals respond to secondary contact with an antigen.

Immune systems in plants are based on passive, structural immunity, such as a waxy surface or cuticle, and active immunity exists in the expression of some chemicals. The mechanism of this system is to prevent infectious agents from gaining access to plant cells. Plant immunity may also be protoplasmic. This means that the protoplast in cells is an unfavourable environment for pathogenic development. Plants do not, however, produce antibodies like animals do. The protoplasmic immunity is arranged generally by phytoalexins, non-specific

compounds whose concentrations increase in response to infections. Some alkaloids may act in a similar way to phytoalexins or in the direct chemical prevention of the infectious agent its growth[520].

Although there are many differences between immunity systems in plants and animals, there are similarities. Both systems have two kinds of immunity: passive and active. Alkaloids may take part in both systems.

Many recent studies have proved that many alkaloids have antiviral properties. This is directly connected with the immune systems of organisms. It is known that the surfaces of viruses contain haemagglutinin, which helps adhering to cells prior to the infection[520]. It is also known that viruses continually change the structure of their surface antigens through processes of antigenic drift and antigenic shift. Drift is a mutation in the viral genome and the shift process is the change of a virus in the host. These processes lead to alterations in haemagglutinin. Infection can occur only when alterations in haemagglutinin are sufficient to render previous immunity ineffective. The potential role of alkaloids becomes apparent in this stage. These compounds break the alterations in the haemagglutinin. Moreover, alkaloids strengthen antiviral cytotoxic T-cells. Viruses normally work to inhibit these cells by haemagglutinin. Alkaloids seem to benefit the immune system when they decrease haemagglutinin's ability to alter, and furthermore when they strengthen and protect T-cells. $CD8^+$T cells have an especially crucial role in an organism's pathogenic resistance[521]. These cells can kill malignant cells. Moreover, it is stated that some critical functions of these $CD8^+$T cells depend on helper activity provided by $CD4^+$T cells. The cooperation of these immunity cells subsets involves recognition of antigens[521]. Some alkaloids may weaken antigens and malignant cells. It is also known that NK cells are cytotoxic to cells infected with viruses[520]. In the immune system, the interaction between protective cells and chemicals is constant.

T-cells also have a very important role in antifungal activity. Fungal infections are very serious problems for many organisms. Fungi try to go across passive immune systems, in which some alkaloids are also important. Many alkaloids have fungistatic properties. In the defence process, the T cells and the NK cells are very important, because they are cytotoxic to fungi. The influence of alkaloids on fungi is evident in the reduction of their growth and in the advancement of T and NK cells.

Bacterial infections are problematic for many organisms, both animals and plants, because bacteria physiologically try to avoid phagocytosis by surrounding themselves with capsules. Capsule bacteria excrete exotoxins meant to kill phagocytes and destroy the immune system. Antibody defence neutralizes the toxins. Bacteria growing in intracellular spaces are killed by cell-mediated immunity (CMI) through specific synthesis of T cell helpers, which powerfully activate the formation of nitritic oxide (NO^-), reactive oxygen intermolecules (ROI) and other microbicidal mechanisms. The role of the alkaloids is in the prevention of bacterial growth and replication. Therefore, alkaloids help immune system activity.

The biological activity of alkaloids against parasites has been mentioned. Many authors have reported on this kind of activity. There are a lot of parasites that cause infections and resulting diseases. One group of these parasites is protozoa. Malaria, as previously mentioned, is caused by the protozoan *Plasmodium* spp. (*Plasmodium vivax, P. falciparum, Plasmodium ovale, Plasmodium malariae*). Leishmaniasis (Tropical sare, Kala azar, Espundia) is caused by *Leishmania* spp. (*Leishmania tropica, L. donovani, Leishmania brazilensis*). Chagas's disease results from infection by *Trypanosama cruzi* and sleeping sickness by *Trypanosama rhodesiense* and *Trypanosama gambiense*. Helminths and trematodes (flukes) make up another group of protozoa and cause the schistosomiasis. This disease results from infection by *Schistosoma mansoni, Schistosoma haematobium* and *Schistosoma japonicum*. Nematodes (roundworms) make up a third group and cause the diseases trichinosis (*Trichinella spiralis*), hookworm (*Straongyloides duodenale, Necator americanus*) and filariasis (*Wuchereria bancrofti, Onchocerca volvulus*). These three groups of parasites are especially connected to infections in humans and animals. In the case of plants there are many parasites in the form of micro-organisms and nematodes. There is large diversity among these parasites. In the steppe grasslands in Eastern Austria, 58 nematode genera were found, including the dominating species *Acrobeloides, Anaplectus, Heterocephalobus, Prismatolaimus, Aphelenchoides, Aphelenchus, Tylenchus* and *Pratylenchus*[522]. Moreover, an average of two to six individuals lived in one gram of soil. Although Zolda's[522] study found the plant-feeding nematodes to be third in numeral comparison (after the bacterial- and fungal-feeding), this group of nematodes is known to stress plants. Moreover, recent studies demonstrate that important linkages exist between dwarf mistletoe infection, host plant vigour and the ectomycorhizal colonisation and fungal community composition of pinyon pine (*Pinus edulis*)[523]. This study demonstrated that high levels of dwarf mistletoe infection were not associated with an increased mortality of infected trees. The infected trees only showed lower shoot growth. This is a good example of both plant adaptation to parasites and also indirectly of the immune system of the trees. Plant immunity seems to be imperfect, because parasites did establish infections in cells. The reduced growth of shoots was a means of preventing cell death. However, it is necessary to mention the numerous amounts of species of micro-organisms that have intimate, beneficial and sometimes essential relationships. Only a small fraction of these organisms are harmful. Therefore, the action of alkaloids as possible part of plant immunity is connected with strong selectivity and specifics. Although parasites live in the host organism and generally impart no benefits to the host, they have little or no harmful effects on the host in some cases, and their presence may be unapparent. However, micro-organisms that do damage host organism are pathogens. Pathogens and pests should be foremost considered when addressing the potential influence of alkaloids on the immune systems of plants. However, it is known that *Cuscuta reflexa* and *Cuscuta platyloba*

parasitize *Berberis vulgaris* but not *Mahonia aquifolium*. Moreover, *Cascuta reflexa* parasitized *Datura arborea* but not *Datura stramonium*[524]. The alkaloid patterns in *Berberis* and *Mahonia* are similar but not identical. The same can be stated in the case of *D. arborea* and *D. stramonium*. Quinolizidine, pyrrolizidine and indole alkaloids are known to have toxic, repellent, deterrent, neutral and stimulating activities depending on the specific aphid–plant relations[525]. The alkaloid gramine taken from *Hordeum* is recognized as a chemical that disturbs the feeding activities of cereal aphids.

6. Genetic approach to alkaloids

Alkaloid biogenesis in an organism is determined genetically[16,526,527,528,529,530,531,532,533,534,535]. This means that many specific genes participate in alkaloid metabolism, and gene participation in metabolism is a very important basis for understanding the alkaloids. As is widely recognized, the gene is a unit of hereditary information encoded in a discrete segment of a DNA molecule, which carries an enormous amount of genetic information. It has been generally estimated that human cells contain from 50 000 to 100 000 genes on 23 chromosomes. The initial results of the Human Genome Project have been published beginning in June 2000 and finally in 2003. As one result of the project, it became clear that the human genome has only 30 000–40 000 genes, which was less that expected in previous estimations[536,537]. The mouse (*Mus musculus*) has about 25 000 genes, the nematode (*Caenorhabditis elegans*) 19 000 genes, the fruit fly (*Drosophila melanogaster*) about 13 700 genes and the common wall cress plant (*Arabidopsis thaliana*) has 25 500 genes[538]. Genetic information connecting to the metabolism of alkaloids signifies that these secondary compounds are more important for the life cycle of organisms as they are not coded in the genome. Lal and Sharma[539] have studied alkaloid genetics in *P. somniferum*. The alkaloids of this plant are determined by dominant and recessive genes. The inheritance of morphine, codeine and thebaine content from parent plants to the next generation is 21–36%, and that of narcotine only 10.5–14.5%[539]. The authors[539] have also cited previous work of Briza, according to which the heritability of morphine content in *P. somniferum* ranged from 43% to 68%. When considering narcotine content, some degree of epistasis is reflected[539]. The dominant and recessive gene determination of alkaloid content makes alkaloid genetics a very difficult research topic. However, to date more than 30 genes coding for enzymes involved in alkaloid biosynthesis pathways have been isolated and cloned (Table 22). Recently, acetylajmalan esterase (AAE) was isolated and purified together with a full-length AAE cDNA clone from *Rauvolfia* cells[534]. This enzyme plays an essential role in the late stages of ajmaline biosynthesis. This was the eighth functional alkaloid gene extracted from this plant. This was also the sixth identified

Table 22 Enzymes specifically involved in alkaloid biosynthesis

Alkaloids of Plant Species	Enzymes	Coded by DNA
Purine alkaloids	Caffeine synthase	*Camellia sinensis, Coffea arabica*
	Xanthosine 7-*N*-methyltransferase	*Coffea arabica*
	7-Methylxanthine 3-*N*-methyltransferase	*Coffea arabica*
	Caffeine xanthinemethyltransferase 1 (CaXMT1)	*Coffea arabica*
	Caffeine methylxanthinemethyltransferase 2 (CaMXMT2)	*Coffea arabica*
	Caffeine Dimethylxanthinemethyltransferase (CaDXMT1)	*Coffea arabica*
	Theobromine 1-*N*-methyltransferase	*Coffea arabica*
Pyrrolizidine alkaloids	Homospermidine synthase	*Senecio vernalis, Senecio vulgaris*
Indole alkaloids	Tryptophane decarboxylase	*Catharanthus roseus*
		Camptotheca acuminata
	Secologanin synthase	*Catharanthus roseus*
	Strictosidine synthase	*Catharanthus roseus*
		Rauvolfia serpentina
	Polyneuridine aldehyde esterase	*Rauvolfia serpentina*
	Taberosine 16-hydrolase	*Catharanthus roseus*
	Desacetoxyivindoline acetyltransferase	*Catharanthus roseus*
	Geraniol/nerol 10-hydroxylase	*Catharanthus roseus*
Isoquinoline alkaloids	Tyrosine/DOPA decarboxylase	*Papaver somniferum*
		Arabidopsis thaliana
	Berberine bridge enzyme	*Eschscholtzia californica*
	Norcoclaurine 6-*O*-methyltransferase	*Coptis japonica*
	Coclaurine *N*-methyltransferase	*Coptis japonica*
	3′-Hydroxy-*N*-methylcoclaurine 4-*O*-methyltransferase	*Coptis japonica*
	Scoulerine 9-*O*-methyltransferase	*Coptis japonica*
	Columbamine *O*-methyltransferase	*Coptis japonica*
	O-methyltransferases	*Thalictrum tuberosum*
	N-methylcoclaurine 3′-hydroxylase	*Eschscholzia californica*
	Berbamunine synthase	*Berberis stolonifera*
	Codeinone reductase	*Papaver somniferum*
	Salutaridinol 7-*O*-acetyltransferase	*Papaver somniferum*
Tropane alkaloids	Hyoscyamine 6β-hydroxylase	*Hyoscyamus niger*
		Atropa belladonna
	Tropinone reductase-I	*Hyoscyamius niger*
		Datura stramonium
	Tropinone reductase-II	*Hyoscyamius niger*
		Datura stramonium
		Solanum tuberosum
Acridone alkaloids	Acridone synthase	*Ruta graveolens*

Sources: Refs [527, 532, 541, 542, 543, 544, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563].

ajmaline biosynthetic pathway-specific gene. Strictosidine synthase with cDNA and Genomic DNA (str1) has been isolated from *Rauwolfia serpentina* and *Rauwolfia mannii*[527,540,541]. This enzyme coded in cDNA has been isolated also from *Catharanthus reseus*[542,543]. Tryptophan decarbocylase endoded by cDNA has also been isolated from this plant and then described[544]. Moreover, Cane et al.[533] recently discovered that the synthesis of the nicotine in the roots of *Nicotiana tabacum* is strongly influenced by the presence of two non-allelic genes, A and B.

Hibi et al.[545] have reported on putrescine *N*-methyltransferase isolated from the nicotine biosynthetic pathway coded by cDNA. Recent advances in cell and molecular biology of alkaloid biosynthesis have heightened awareness of the genetic importance.

Biosynthetic genes involved in the formation of tropane, benzylisoquinoline and terepenoid indole alkaloids have been isolated[561]. Hyoscyamine 6-β-hydrolase[563] and tropinione reductases[548] encoded in cDNA and involved with tropane alkaloids have been isolated as well. Moreover, some genes involved in the metabolism of isoquinoline alkaloids and encoded in cDNA are also known. The berberine bridge enzyme from *Eschscholtzia californica*[547] and berbamurine synthase from *Berberis stolonifera*[551] belonging to this group of enzymes are encoded in cDNA. Coclaurine *N*-methyltransferase and columamine *O*-methyltransferase involved in the biosynthetic pathways of the isoquinoline alkaloids, berberine and palmatine respectively have been found and cloned in *Coptis japonica*[553,554]. Salutaridinol 7-*O*-acetyltransferase forms an immediate precursor of thebaine along the morphine biosynthetic pathway, and its cDNA was obtained from a cell suspension culture of the opium poppy (*P. somniferum*)[553,554]. Caffeine is formed from xanthosine through three successive transfers of methyl groups and a single robose removal in coffee plants. The methylation is catalysed by three *N*-methyltransferases: xanthosine methyltranserase (XMT), 7-methylxanthine methyltransferase (MXMT) and 3,7-dimethyltransferase (DXMT), which participates in the caffeine synthetic pathway[552].

There is the evidence that genes involved in alkaloid metabolism can be isolated and engineered to new plants. The biotechnological potential is apparent especially in cytochrome P450 genes isolated from *Catharanthus roseus*. P450 genes involve in the 16-hydroxylation of tabersonin in this plant and establish recombinant system CY71D12 as a tabersonine 16-hydroxylase. In *Lonicera japonica* P450 genes involve in the conversion of loganin into secologanin systems as CYP72A1 as a secologanin synthase and CYP76B6 as geraniol/nerol 10-hydroxylase. CYP72A1 from higher plants catalyse ring-opening reactions. CYP76B6 and CYP71D12 catalyse alkaloid moiety. In the indole alkaloid biogenesis P450 genes catalyse a large of number of reactions, for example P450 genes are important in the formation of parent ring systems of alkaloids. Engineered plant defence and herbicide tolerance is developed by transferring

of some 450 genes. Animal enzymes encoded by P450 indicate potential use in plant defence system after their translocation by biotechnological engineering[557]. Knowledge of these key genes can be used to enhance alkaloid production in the cell cultures[557,562]. The biological importance of alkaloids is connected with the structural, metabolic, functional and evolutionary role of these compounds in living organisms. The present research on the genes involved in the biosynthesis of alkaloids is advanced and many enzymes have been isolated and cloned. However, the major challenge in the near future is to isolate new genes and new enzymes. Research needs to uncover more information about the regulation of metabolism at different levels, such as genes, enzymes, alkaloid production and accumulation. A challenge in this research area will be to provide more data on genetic information as a means of mapping metabolic networks between these levels. This could help develop better models for alkaloid biosynthesis and production, which will support the metabolic engineering of alkaloids in the future.

7. Alkaloids in the evolution of organisms

Alkaloids hold many secrets of life. They are toxic and many of them can be used as narcotics. As important secondary compounds, they categorically determine much about life. They also play a large role in evolution due to their characteristics.

Charles Darwin's theory of evolution revolutionized biology and has motivated biologists to make empirical studies of evolutionary phenomena in nature and in the laboratory. As a result of this, a fundamental science presently exists based on this theory. A serious biologist must pay heed to Darwin's statements along with later neo-Darwinistic developments and Mendelism when searching for a deeper understanding of life. Molecular biology, which is presently powering all the biological sciences, is strengthened by Darwin's and Mendel's theories and has completely supported them.

There are many recent studies that consider evolution and co-evolutionary interactions between plants and insects[564,565,566,567,568,569]. Many of these proved that there is interdependence between plant chemistry and the animals which feed on these plants, especially with insects[570,571,572,573,574,575,576,577,578]. Literature is accordant in pointing out the importance of plant and animal chemistry in both evolutionary and co-evolutionary processes. Alkaloids are good examples of this chemical role. The classical example is the potato beetle *Leptinotarsa decemlineata* living on the *S. tuberosum* and other *Solanum* species. These species contain solanine, solanidine and other minor steroid alkaloids. Solanine and solanidine are toxic. However, *L. decemlineata* tolerates these alkaloids when feeding (on the green mass of potato). Moreover, *L. decemlineata* does not store these alkaloids in its body and they are eliminated during

metabolism. The study concerning the effects of quinolizidine alkaloids on the potato beetle (*Leptinotarsa decemlieata*) proved that these alkaloids reduce populations of *Leptinotarsa* and the development of their larvae[232]. Elsewhere, in a case concerning steroid alkaloids (solanine, solanidine), the Colorado beetle has not adapted to the alkaloid lupin. Moreover, in co-evolutionary development some aphids not only feed on alkaloid plants, but also sequester the alkaloids and keep them in the own body. Examples of this are the case of *Macrosiphum albifrons* with quinolizidine alkaloids or the case of *Aphis jacobaeae* or ladybirds (*Coccinella*) with pyrrolizidine alkaloids. On the other hand, it is necessary to pay attention to the fact that aphids, lady birds and other insects are feeding on the alkaloid poor, or alkaloid-free forms of the same species. This can be explained as some example of co-evolutionary development. Alkaloids are molecules developed in co-evolutionary processes with environment. The evolution of the ability to use some alkaloids by some insects is a consequence of this (Figure 91). When food sources change, an organism needs to adapt to the new conditions. This is a basic matter of evolution. Moreover, cytochrome c, one of the basic enzymes, exists largely in plants, animals, fungi and some bacteria as, for example, *Rhodospirillum rubrum*. Nearly 60% of amino acids of cytochrome c in the homogenic position are identical in wheat and human and even 30% are identical in *R. rubrum* and human. This is only one piece of evidence that genes for cytochrome c have evolved from the first gene of

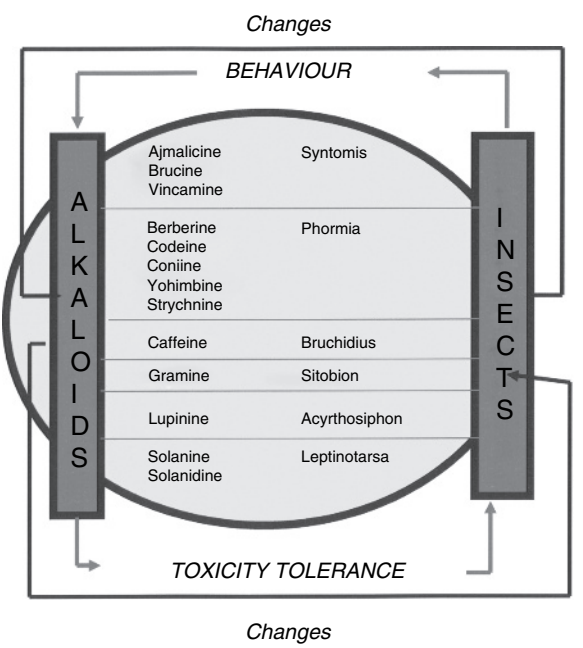


Figure 91. Model of evolutionary interaction between alkaloids and insects.

pre-bacteria in the history of life on the Globe. Genes for alkaloids should be also evolved in a similar way.

New evidence of evolution is presently available. Evolution of viruses as complex genomes[564] and the development of nucleoprotein into RNA and after that to DNA are two hypotheses considered very important for understanding the mechanisms of life[565]. It has been stated that alkaloids can influence DNA and RNA as well as protein synthesis in general because their metabolism is encoded genetically. Even the smallest changes in the gene code influence this mechanism. The starting point for all changes is the cell.

The chemical behaviour of one organism is affected that in another. Jackson et al.[566] have described the evolution of anti-predator traits in response to a strategy by predators, and Lion et al.[579] addressed the evolution of parasite manipulation of host dispersal behaviour. This study reveals that parasites can manipulate their host's dispersal. The evolution of herbivore-host plant specialization requires low levels of gene flow between populations. Leonardo and Mondor[567] showed that the facultative bacterial symbiont *Candidatus regiella insecticola* alters both dispersion and mating in the pea aphid *Acyrthosiphon pisum*. Changes in dispersal and mating associated with symbionts are likely to play a key role in the initiation of genetic differentiation and in the evolution of pea aphid-host plant specialization.

The evidence of the participation of alkaloids in the evolution of organisms is observed in interactions with numerous micro-organisms. As it has been stated, many alkaloids have antimicrobial activity. However, there are several alkaloids without this characteristic. Some micro-organisms as symbionts of *Bradyrhizobium* spp. can live in both alkaloid-rich and alkaloid-poor plants with the some level of activity. The same can be stated in connection to some fungi, for example mycorhiza. Adaptation processes in nature lead to permanent evolution and co-evolution between alkaloids as a part of biochemistry and organisms (as a part of environment). The evolution and ongoing co-evolution of alkaloids and organisms is an example that alkaloidal defence of a plant is only a secondary function of these molecules that also changes in the evolutionary process.

CHAPTER 4

Applications

Dictum sapienti sat est.
Plautus

Abstract: Alkaloid applications can be found in different areas. Some alkaloids are still used in modern medicine today as natural or modified compounds. Their use is connected to the regulation of Na^+ ions and channels, mescaric, cholinergic receptor, acetylcholine esterase, opioid and opiate receptors, glycine and other receptors, as well as the regulation of micro-tubules of the spindle apparatus. Moreover, alkaloids are used in the regulation of microbial and schizonticide activity and as pharmaceuticals. Alkaloids are also generally a problem in food; there are some applications in agriculture, especially in plant breeding (alkaloid-rich and alkaloid-poor cultivars). Genetically modified organisms (GMOs) can be considered to hold possibilities for vaccine development, especially in plants. Some alkaloids are used in food receptors as additional components or are consumed as a part of the final product (caffeine, theophylline, piperine, capsaicin). The use of alkaloids as a supplement in some products on the market is presently a matter of discussion, as they are considered a health risk (the case of ephedrine). Alkaloids can be used as biological fertilizers and as control agents in plant protection. Biotechnology opens new possibilities of alkaloid applications. The productional achievements are found in cell and organ cultures. The most well-known applications in root cultures are with anabasine, nicotine, harmine, harmaline, hyoscyamine, calystegine, scopolamine and senecionine. Alkaloidal enzymes can be purified from these cultures.

Key words: agriculture, alkaloids, biofertilizers, biotechnology, channels, drugs, food, ions, medicine, plant protection, production, receptors, root cultures

Alkaloidal applications can be found in different areas of the economy, industry, trade and services. The applicable characteristics of alkaloids are both chemical ones and the ability to be isolated as pure molecules or to be modified. The specific activity and utilization is a basis for the applications. Alkaloids have been used throughout history in folk medicine in different regions around the world. They have been a constituent part of plants used in phytotherapy. Generally speaking, many of the plants that contain alkaloids are just medicinal plants and have been used as herbs. From the times of Hippocrates (460–377 BCE),

herbs were known in Europe as a very important way of improving health. In ancient China, herbs were known and used even since 770 BCE, and in Mesopotamia approximately since 2000 BCE. In Mesopotamia alone, plants such as *Papaver somniferum* and *Atropa belladonna* have served a purpose, and the use of *Datura metel, Cannabis sativa* and the mushroom *Amanita muscaria* can be traced to ancient India. Moreover, plants containing alkaloids have been historically used for other purposes. Hunters, priests, medicine men, witches and magicians have all been known to use alkaloidal plants. Humans have used alkaloids as poisons in weapons[581]. The most poisonous alkaloids such as aconitine and tubocarine were used in ancient time as poisons for arrows. Especially in Africa, these weapons have been used in tribal warfare, where the poisons (alkaloids) were generally prepared from plants but also from animal sources as toads, snakes and frogs[582,583]. Poisoned arrows have also been used in Asia, especially in the large region including Indonesia, Burma, Thailand and Cambodia. Three methods were used in preparing poisons[583,584]. The first involved boiling arrows in water with a ground up plant. The second method used pounded fresh ingredients with glutinous sap added (especially in the case of oil-rich plants). The third method involved applying freshly squeezed plant material onto wooden-tipped arrows. Literature also refers to the fact that different alkaloid groups have been used as arrow poisons in different parts of the world. People in Africa and Asia predominantly used cardiac poisons, while South Americans almost exclusively preferred muscle-paralyzing (curarizing) poisons.[584]

Alkaloids and especially plants containing alkaloids were also used in the Middle Ages as a basic and practical human and animal cure for various ailments. Some cases of using alkaloids in executions are also known[321,581,582,585,586]. Some alkaloids that have played an important role in this sense include aconitine, atropine, colchicine, coniine, ephedrine, ergotamine, mescaline, morphine, strychnine, psilocin and psilocybin. Although alkaloids have been used throughout history, their isolation from plants as relatively pure compounds occurred only in the beginning of the 1800s, and their exact molecule structures were not determined until the 1900s.

1. Medicinal applications

Some alkaloids (Table 23) are still used in medicine today[586,587,588,589,590]. They are used as natural or modified compounds. They can also be totally synthesized based on the model of the natural molecule. Alkaloidal application in clinical practice is connected with biological activity in human and animal bodies (Figure 92).

Table 23 The most important alkaloids used in modern medicine

Alkaloid Name	Example of Natural Source
Aconitine	*Aconitum napellus*
Ajmaline	*Catharanthus roseus*
Atropine	*Atropa belladonna*
Berberine	*Berberis vulgaris*
Boldine	*Peumus boldo*
Caffeine	*Coffea* spp., *Cola* spp.
Cathine	*Catha edulis*
Cocaine	*Erythroxylon coca*
Codeine	*Papaver somniferum*
Colchicine	*Colchicum autumnale*
Emetine	*Cephaelis acuminate*
Ephedrine	*Ephedra sinica*
Ergotamine	*Claviceps purpurea*
Eserine[a]	*Physostigma venenosum*
Galanthamine	*Galanthus nivalis*
Hydrastine	*Hydrastis canadiensis*
Hyoscine	*Duboisia*, *Datura*, *Hyoscyamus* spp.
Hyoscyamine[b]	*Atropa belladonna*
Lobeline	*Lobelia inflata*
Morphine	*Papaver somniferum*
Narceine	*Papaver somniferum*
Nicotine	*Nicotiana* spp.
Noscapine[c]	*Papaver somniferum*
Papaverine	*Papaver somniferum*
Pilocarpine	*Pilocarpus* spp.
Quinidine	*Cinchona* spp.
Quinine	*Cinchona* spp.
Rescinnamine	*Rauvolfia* spp.
Reserpine	*Rauvolfia serpentina*
Sanguinarine	*Sanguinaria canadiensis*
Sparteine	*Cytisus scoparius**
Strychnine	*Strychnos nux-vomica*
Taxol	*Taxus brevifolia*
Theobromine	*Theobroma cacao*
Theophylline	**
Tubocurarine	*Chondodendron tomentosum*
Vinblastine	*Catharanthus roseus*
Vincamine	*Vinca minor*
Vincristine	*Catharanthus roseus*
Yohimbine	*Rauvolfia* spp.

Notes: [a] Physostigmine as synonymous name; [b] Scopolamine as synonymous name; [c] Narcotine as synonymous name; * In small concentrations in many species especially in *Lupinus* spp. The main alkaloid in *Cytisus scoparius*; ** Low concentrations in natural sources. Synthetical theophylline is used.

Sources: Refs [587, 588, 589, 590].

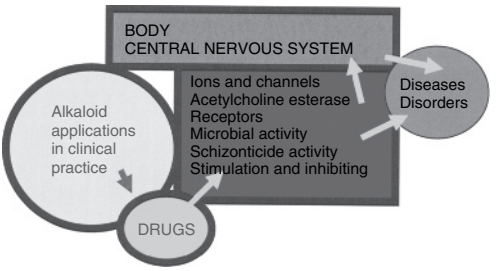


Figure 92. General diagram of alkaloidal applications in clinical practice.

1.1. Regulation of Na^+ ions and channels

As previously mentioned in this book, alkaloid biological activity is rather diverse, and alkaloids, therefore, have biological significance as histological regulators of metabolism. This significance is pertinent to the development of the applications. One such important role of some alkaloids is their influence on Na^+ channels and interaction with receptors. Aconitine, ajmaline, sanguinarine (Figure 93) and sparteine have clinical uses in this respect. Aconitine causes an influx of Na^+ ions across membranes. Therefore, aconitine can first activate and in later stages also block the nerve of the receptor. This alkaloid regulates the activity of Na^+ channels and consequently the receptor activity and enzymatic activity regulated by the receptor. Applications of this alkaloid are connected with the regulation of defects in neuro- and local disturbances in signalling and receptor nerve activity.

Although ajmaline also influences Na^+ channels, its activity is to block these channels. This influences the refraction phase of the heart beat and also decreases heart rate. Ajmaline may be used to correct arrhythmic defects. Sanguinarine also influences Na^+ ions and particularly inhibits esterase activity. This alkaloid has several other activities in the body and specifically in tissues. Possible applications are linked to the promotion discharge of mucus from the respiratory tract.

Sparteine is an alkaloid that inhibits N^+ channels and Na^+ ion flux and activating a muscarinergic acetylcholine receptor. In small doses this alkaloid acts as

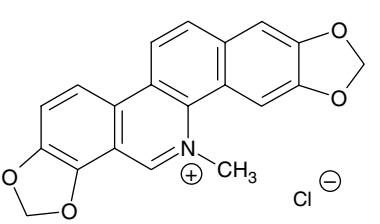


Figure 93. Sanguinarine, an alkaloid from *Sanguinaria canadiensis*.

a stimulant, while in large doses it has a paralyzing effect on autonomic ganglia. Medical applications may include possible correction of cardiac arrhythmia.

The regulation of Na^+ ions and channels are the means for possible correction of abnormalities in tissue functioning and ganglic integrations. However, it is necessary to pay attention to the possible unwanted side effects. Alkaloids are complex agents. Smaller doses are safer when considering cell toxicity or other possible metabolic effects. Alkaloids and alkaloidal substances should be given serious consideration and precautions before use.

1.2. Regulation of mescarinic cholinergic receptor

The clinical application of alkaloids involves them as agents to regulate mescaric cholinergic receptor activity and repairing possible disturbances or to correct possible defects. Atropine, hyoscine and hyoscyamine may all have these kinds of applications.

Atropine blocks muscaric cholinergic receptor competitively and has a large spectrum of clinic applications. Atropine acts as a parasympatholytic on parasymphathetically innervated organs. Therefore, the possible applications of atropine are as a general anaesthetic and include its use in pure form or as a component.

The muscarinic cholinergic receptor is competitively blocked by hyoscine and hyoscyamine. Both of these alkaloids are more active than atropine. The applications of these alkaloids for clinical purposes are connected with the induction of general anaesthesia. Clinical consideration should be paid to the fact that these alkaloids also affect the brain and thereby the central nervous system. Atropine crosses the blood–brain barrier. Hyoscine and hyoscyamine depress the motor areas of the cerebral cortex.

1.3. Regulation of acetylcholine esterase

The alkaloids which influence acetylcholine esterase activity are eserine, galanthamine, nicotine, lobeline and tubocurarine. All of these alkaloids are very active.

Eserine blocks acetylcholine esterase. This alkaloid may be used to decrease possible negative side effects connected to the use of other drugs, for example that of atropine.

Galanthamine also blocks acetylcholine esterase activity. One of the new applications of galanthamine is in a treatment of Alzheimer's disease. Moreover, this alkaloid can be used in decreasing negative side effects caused by applications of non-depolarizing alkaloids, for example those of tubocurarine.

Nicotine is an alkaloid that can be applied in clinical practice as a factor which agonistically activates nicotinic acetylcholine receptors. This means that

in very low doses it is a stimulant and in high doses it is a depressant. The use of this alkaloid is prevalent in treating smoking dependence. Lobeline has both similar activity and application potential as nicotine. It can also be used in treating vascular disorders.

Tubocurarine acts as a competitive inhibitor in the nicotinic acetylcholine receptor, meaning that the nerve impulse is blocked by this alkaloid. Tubocurarine is used in surgical practice as a muscle relaxant. These alkaloids have an observably large spectrum of activity and possible applications. Their utilization in the development of new applications is therefore relatively active in modern medicine.

1.4. Regulation of opioid and opiate receptors

Alkaloids such as boldine, codeine, narceine and morphine are active factors in their receptors. Boldine has morphine-like properties and is active on opioid receptors. It may be used to treat stomach disorders and as metabolic stimulant. As it is similar to morphine, boldine can also be considered in the possible development of treatments for narcotic dependence. Codeine also binds to opiate receptors, and specifically functions to reduce bronchial secretions. Codeine can also be used as a cough suppressant when acting on the centre of the medulla oblongata and as a sedative agent.

Morphine and narceine are active on μ-opiate and κ-receptors. They are also known as analgesic agents. These alkaloids may be used as pain relievers. Narceine is also known to be used in the treatment of a cough.

1.5. Regulation of glycine receptors

Strychnine, a very poisonous alkaloid to animals, binds to glycine receptors. Applications of strychnine can be considered only in clinical doses. Their purpose is to activate neurotransmitters in the spinal cord, which is generally suppressed by glycine. Strychnine competes only with glycine in the receptor. This alkaloid may be used to stimulate respiration and circulation in cases of physical weakness. Moreover, strychnine products are used in the treatment of eye and optic nerve disorders. Larger doses are lethal.

1.6. Regulation of other receptors

Some alkaloids are active in α and β-adrenergic receptors (e.g. ephedrine), α receptors (e.g. ergotamine), uterine α_2 receptors (e.g. ergometrine) or presynaptic α_2 adrenoreceptors (e.g. yohimbine). Ephedrine increases blood pressure

by elevating cardiac output. It is also known to have some stimulant activity on the respiratory centre. Ephedrine may be applied in the treatment of edema in insulin-dependent diabetics.

Ergotamine blocks α receptors. It has an inhibitory effect on the cardiovascular system. Ergometrine also has a high affinity to uterine α_2 receptors, and it therefore influences uterine muscle activity. Ergometrine is used in the treatment of postpartum or postbortal haemorrhaging. Yohimbine blocks presynaptic α_2 adrenoreceptors and increases the release of noradrenaline at sympathetic nerve endings. It may be used to increase heart rate and blood pressure and even in the treatment of severe cases of male impotency.

1.7. Regulation of microtubules of the spindle apparatus

Alkaloids such as vinblastine and vincristine are known to bind to the microtubules of the spindle apparatus. They are active agents that influence DNA synthesis and amino acid metabolism. They are also known to reduce mitosis at metaphase. Vinblastine and vincristine also have some immunosuppressive activity. There are many applications of these alkaloids. They have been used in the treatment of Hodgkin's disease, cancers and blood disorders. Vincristine is a basis for the development of clinic agents used to treat cerebral and pulmonary disorders. Vinblastine and vincristine are well-known anticancer agents.

1.8. Regulation of microbial activity

Alkaloids such as berberine are known to be anti-microbial. They inhibit estarases as well as DNA and RNA polymerases. Moreover, berberine inhibits cellular respiration and acts in DNA intercalation. As a strong anti-microbial agent, berberine may be used in the treatment of AIDS, as it inhibits HIV-1 reverse transcriptase. Berberine also has uses in the treatment of infections, specifically eye infections and hepatitis.

1.9. Regulation of stimulation

Many alkaloids are generally known to have stimulating properties, such as caffeine, cathine, theobromine and theophylline. These alkaloids are considered in many different medical applications.

Caffeine is a known inhibitor of phosphodiesterase. Caffeine has an effect on calcium-mediated signalling; namely it causes an increase of cAMP activity. Caffeine also has a competitive effect on the central adenosine receptor and is thought to increase analgesic activity. It is also known to be somewhat effective

in treating some forms of dermatitis as well as headaches. Headache relief is connected to caffeine's diuretic and vasodilatory properties, specifically on renal and brain blood vessels. Caffeine is therefore considered in many other applications.

Cathine is a known inhibitor of monoamine oxidase and a central stimulant as an indirect sympathomimetic. It is found in anorectic products.

Theobromine is an alkaloid used in medicine much like caffeine, due to the similar properties of these two alkaloids. Theobromine is not as potent as caffeine. Theobromine stimulates the CNS and increases blood flow.

Theophylline also has similar possible clinical applications. This alkaloid has a high level of purines, and because of this it can act as a relaxant. It has also been used in the prevention of bronchospasms.

1.10. Regulation of schizonticide activity

Quinidine and quinine have schizonticide activity due to the inhibition of nucleic acid synthesis through DNA intercalation. This activity is also based on carbohydrate metabolism. The action of both of these alkaloids is a result of their binding to sarcoplasmic reticular vesicles and the resulting reduced uptake of Ca^{2+}. These alkaloids are also active on Na^{+} and K^{+}-ATPase, both in an encouraging or inhibiting sense. Both alkaloids therefore have been developed as strong anti-malarial products. The basic product used has been quinine, and quinidine has been an alternative. These applications are currently decreasing in popularity because some malaria-causing agents (e.g. *Plasmodium* spp.) exhibit resistance. Modifications of these alkaloids or the finding of new molecules with anti-malarial properties is necessary for the process to develop. These alkaloids may have other applications as well. Quinidine is an antiarrhythmic agent, and quinine is used in the treatment of myotonic disorders.

2. Alkaloids as drugs

Medical applications of alkaloids have led to the production of drugs and drug components. They can be based on pure natural alkaloids, as in the case of extracts. Purified alkaloids, partially and even totally synthesized compounds based on the natural alkaloid structure, are also used. Chemically modified alkaloids are yet another example. Chemically modifying the structure affects biological activity. The general trend in modern medicine is to develop compounds that are biologically more active than those found in nature. This is achieved in many cases by alkaloid modifications and synthesis. However, natural compounds themselves are very important because they are the basis for artificial drugs. Moreover, alkaloids used as natural products are important

in phytomedicine, alternative medicine and homeopathy. Still today, folk- and ethnomedicine rely heavily on their use.

Aconitine-, ajmaline- and sanguinarine-based drugs have medicinal importance and are dosed clinically. The drugs are medical products developed by the pharmaceutical industry. Physicians have the right to determine the prescription, that is the use of these products and determination of dosage. Pharmecuticals based on these alkaloids are relatively strong. Several researched and patented drugs such as Aconitysat (aconitine) and Rauwopur (ajmaline) can be found on the pharmaceutical market today. Sanguinarine is generally used in toothpastes. Toothpaste generally has no side effects along with its anti-cavity properties.

Atropine-, hyoscine- and hyoscyamine-based drugs are developed on a large scale and they also have a variety of clinical purposes. Atropinol, for example, is based on atropine. This drug contains atropine sulphate. Another example is Buscopan, based on hyoscine. Hyoscyamine is used in transdermal plasters. Bella sanol also contains hyoscyamine. The therapeutic use is similar to that of atropine. At least 50 different products from these alkaloids have been developed and introduced on the pharmaceutical market.

Drugs based on eserine, galanthamine, nicotine, lobeline and tubocurarine are also prominent. Two examples of drugs containing eserine are Anticholium and Pilo-Eserin. There are at least 20 different products based on this alkaloid.

Nivalina is one drug that contains galanthamine. There are others as well, but less so than with the eserine drugs. Galanthamine-containing medicines have potential uses in the treatment of Alzheimer's disease. Because of this, a greater number of products containing galanthamine is expected to reach the market.

Nicotine is used in many products on the pharmaceutical market, for example Nicorette or Nicoderm. At least 20 different products are known to contain nicotine. These drugs are delivered in different forms. One of these is a transdermal plaster. Nicotine chewing gum and tablets are also available. These drugs are used especially to reduce nicotine cravings.

The drugs that contain lobeline are, for example, Stopsmoke or Lobatox. These products are used for similar purposes as drugs that contain nicotine.

Tubocurarine-containing drugs such as Tubarine or Jexin are used in surgical procedures as muscle relaxants.

Alkaloids such as boldine, codeine, narceine and morphine are also important in clinical practice. Boldosal and Oxyboldine are good examples of boldine-based drugs with morphine-like properties.

Codeine is a component of at least 250 pharmeaceutical products on the market. Codicaps or Codipront can be mentioned as examples. All of these products are opium derivatives.

Narceine-containing drugs are similar to those of codeine. They are used to treat coughs. Paneraj is an example of a typical trademark.

There is a long list of morphine-containing drugs. These drugs are used in serious instances, for example in cases of surgical operations and post-operation treatments. Morphalgin and Spasmofen are examples.

The alkaloids ephedrine, ergotamine, ergometrine and yohimbine are used in many forms. Products like Dorex or Endrine contain ephedrine as their major component. They are used for many purposes, including treating nasal cold symptoms or in bronchial asthma. More than 25 different drugs containing ephedrine have been developed today.

Ergotamine is an active alkaloid produced by fungi with many applications on the market. Pharmauceticals such as Ergostat or Migral are typical examples of ergotamine-based products. Drugs containing this alkaloid are used widely in the treatment of migraines.

Ergometrine is also an ergot alkaloid. It can be found in several products on the market, including Ergometron and Syntometrine.

Yohimbine can be found in drugs such as Aphrodyne or Yohimex. About 20 different products containing this alkaloid have been developed. These drugs are aphrodisiacs used to treat impotency and impotency-related problems in men.

Many other alkaloids are used with various applications. One of them is strychnine, an alkaloid known to be strongly toxic to animals. Drugs such as Dysurgal or Pasuma contain strychnine in clinical doses. Strychnine-containing drugs are used in many disorders, including those of the eye.

Vinblastine and vincristine are two further examples of important drug constituents. Vinblastine is found in such drugs as Periblastine or Velban, and vincristine in such drugs as Norcristine or Pericristine. These drugs are used in oncology as cancer treatments. Drugs with vinblastine are also used in the treatment of Hodgkin's disease, and those containing vincrastine in the treatment of Burkitt's disease as well as brain and lung tumours.

Many other alkaloid-based medicines exist. Drugs based on berberine are used in the treatment of infections as well as in the treatment of AIDS.

Stimulant drugs are often based on caffeine, cathine, theobromine and theophylline. Caffeine is a component of more than 300 different drugs. It is also a minor component in many other pharmaceuticals. Analgen or Panax are examples of caffeine-based drugs. Cathine is found in drugs such as Amorphan or Recatol. They have an anorectic influence of the liver. More than 25 different drugs have been developed from theobromine, for example Atrofed and Seominal. These drugs serve many clinical purposes including treating asthma and *Angina pectoris*. Moreover, 200 different drugs have been developed from theophylline. Theochron and Euphyllin are examples. Theophylline-containing drugs are used to treat bronchitis and asthma.

The applications containing quinidine and quinine are very well known and relatively old. Drugs such as Quinidex or Quinalan are good examples of quinidine applications. Quinine can be found in drugs such as Adaquin or Biquinate. These drugs are important in the prevention and treatment of malaria.

3. Agricultural applications

The alkaloids are known in food and agricultural production. Many crops contain these compounds. In food crops these molecules are a problem and topic of discussion due to possible health hazards and the fact that they have to be removed from plants by breeding and especially hybridization. Alkaloid-rich (bitter) and alkaloid-poor (sweet) cultivars are developed as a result. Both kinds of cultivars are needed for different purposes (Table 24). The total removal of alkaloids through breeding is not currently possible. However, it is possible to reduce alkaloid content to such levels that plant material is suitable for use in foraging and feed. The other way of the removing of alkaloids from plant material is via industrial processing. It is possible to completely remove alkaloids from raw plant material via technological means. Such kinds of industrial processing can be found in the food industry. Alkaloids are no longer problematic in the case of processed feed or fodder. However, problems still exist when fresh or unprocessed raw material is used.

The possibility of developing physiologically functional foods using botanicals is presently a major discussion point in many countries. Alkaloids as such botanicals are difficult compounds solely for the reason of their strong bioactivity. On the other hand, this bioactivity can be used in the development of feed and food. However, physiologically functional foods should not be too chemically strong, and doses have to be much more slight that clinic doses. Moreover, one of the possibilities for the potential use of the alkaloids in the food is developing of the so-called "natural, plant-based vaccines". The development of these kinds of applications is notable. Genetically modified plants

Table 24 Potential usage of alkaloid-rich and alkaloid-poor Washington lupine (Lupinus polyphyllus Lindl.) in agriculture

Potential Product	Alkaloid-Rich Genotype	Alkaloid-Poor Genotype
Biological pesticides	Lupanine as major alkaloid	
Biological fertilizers	Alkaloid extract	Organic matter
Biogas heat composts	Biomass with alkaloids	Biomass
Plant management	Seeds, saplings, vaccines of *Bradyrhizobium*	Seeds, saplings, vaccines of *Bradyrizobium*
Plant industry	Seeds, sapling	Seeds, saplings
Amino acids	Protein tablets[a]	Protein tablets[a]
Fibre	Fibre diet tablets[a]	Fibre diet tablets[a]
Aroma substances	Flavourings[a]	Flavourings[a]
Feed components	High-content protein feed[a]	High-content protein feed

Note: [a] Using technological processing. More details in the source (Ref. [348]).

(GMP) can contain additional genes and produce new proteins[594]. Alkaloids as botanicals can be therefore produced in the plants in which they usually do not exist. In the treatment of diseases such as cancer or AIDS, plant (food) vaccines are reasonable to develop.

3.1. Alkaloids in food

Alkaloidal plants used as food are small in number. The reason for this is the bioactivity and traditional use of alkaloids in medicines and drugs. Moreover, food is checked and controlled with the purposes of keeping alkaloid-contaminated food off the market. However, cases where pyrrolizidine alkaloids were found in the honey produced by bees that had foraged on the flowers of *Echium* and *Senecio* species are documented[475,495,591]. In these relatively old studies, bees had been feeding only on the pollen of one species, which is not typical of bee behaviour or natural honey production. More recent studies have reported that many alkaloids have been detected in the pollen of many species[592,593]. However, it is known that nectar and pollen contain considerably lower levels of alkaloids in comparison to other plant parts[592]. Therefore, the acute toxicity to the bees and the accumulation of alkaloids in honey should be not high, if not miniscule in natural environmental conditions. Moreover, a recent study by Wäckers[595] criticizes studies on unsuitable nectars for insects that are based only on laboratory results, as they do not necessarily translate to ecosystems and natural environments. According to literature, alkaloids have also been detected in other animals and animal products. Pyrrolizidine alkaloids were found in the livers and kidneys of domesticated animals, as well as in milk and eggs[493,596,597,598]. Food contaminated by alkaloids is generally considered to be a health risk[599].

However, some alkaloids are used as additional components of food. The most well known is the use of the quinine as a bitter in tonic water according to an established procedure. Theophylline is an important component of black tea. Caffeine is a well-known component of coffee. Theobromine is found in cacao plants but not in the final products based on cacao seeds, such as cacao drinks or chocolate. Theobromine is removed during fermentation and processing. Processing is very important for the production of the final alkaloid product consumed. In the case of coffee, a high-quality product is possible only from the ripened berries[600,601]. There are two methods of processing coffee. The first one is the so-called “dry method”, used especially in Brazil and in tropical Africa. It is based on the simple drying of berries in the sun. In humid areas this method cannot be used, as sun-drying can prove difficult. In these places, the so-called “wet process” is preferred. Berries are first crushed to a pulp in this method. Seed flesh and skin are then separated. At this stage, a mucilaginous remnant from the fruit flesh is stuck to the skin. It can be removed via fermentation or by

treatment with pectinase, an alkali, or mechanically. The coffee is washed, dried, hulled and polished. Next, the coffee is sorted according to size and colour. The green coffee can be stored for a long time. It contains caffeine as the main alkaloid. Raw coffee has no aroma. It is developed only during the roasting process. Roasting is done just before the coffee is ready for the market. Roasting occurs at temperatures around 200 °–250 °C. Instant coffee is produced from roasted coffee. Roasted coffee is grounded and extracted with water. After that it is powdered and dried. The drying process is crucial when considering the quality of instant coffee. Lower quality is achieved in the spray-drying procedure, and freeze drying produces the highest quality[600,601,602,603,604]. Coffee quality depends on caffeine percentage, aroma and taste. Decaffeinated coffee and coffee surrogates are available on the market. One of these surrogates is roasted lupin beans containing nearly 200 micrograms of quinolizidine alkaloids per gram[605].

Taste and caffeine content together with the catechins are also important quality parameters for tea. Moreover, a tea's quality also depends on the chosen processing method. One such method is based on fresh leaves (water content of 75–80% and drying up to 58–64%). After rolling in heavy machines, the cell structure is destroyed. Polyphenoloxidases, enzymes from the plant, come into contact with catechins. Next, fermentation takes place in temperatures kept below 25 °C. Here, the leaves change to a copper colour, which means that the catechins transform to theaflavin and thearubigin. These compounds are in the complex with caffeine and protein[601,606,607]. In the case of the so-called "green tea" produced in Asia, the phenoloxidases in the fresh leaves are first inactivated by steaming or in heating pans. This means that composition of the catechins has not changed. The rolling that takes place after inactivation is done as in the case of normal black tea. The colour of the tea is first olive green, but subtly turns to a golden colour. Green tea is more refreshing because there is more free caffeine, and is considered to be healthier because of the effect of catechins. There are also other different teas such as red or yellow teas from China. Different colours are indicative of when fermentation ended. In earlier times, pure caffeine was extracted from coffee and tea. Caffeine was a product that could be used as a food additive. Nowadays, both caffeine and theophylline are chemically synthesized[608]. They are used as additives to a large list of different products. Moreover, according to recent research data, consumption of green tea and coffee was linked to a decreased risk of type 2 diabetes in Japanese adults[609].

Theobromine is an alkaloid found in the raw material of the cacao. The processing of this product involves many steps beginning with the cutting open of harvested cacao fruits. The beans are then fermented in boxes together with the white, mucilaginous pulp. The rapid development of yeasts, acetic acid and lactic acid bacteria ensues. The temperature in this stage may reach 45 °C. During fermentation the alcohol received is oxidized to acetic acid, which kills the embryos of the seeds. Phenoloxidases oxidizes catechins and other phenolic compounds. As a result of this, the brown chocolate colour appears. During

the drying, the aroma and the bitter taste of cacao develop. Theobromine is extracted and cacao is then edible. In the case of chocolate production, cacao seeds are roasted at temperatures of 90 °–140 °C for 10–45 minutes. Cacao butter is extracted and used directly in chocolate production. Theobromine is also extracted in this stage.

Alkaloids are well known in food spices and herbs. The black, white, green (*Piper nigrum* L.) and long pepper (*Piper longum* L.) containing piperine are widely used in food. Other alkaloid plants used in food are Capsicum peppers such as chilli or red pepper (*Capsicum annuum* L.), Peruvian pepper (*Capsicum baccatum* L.), ají pepper (*Capsicum chinese* Jacq.), bird pepper or tabasco (*Capsicum frutescens* L) and rocoto pepper (*Capsicum pubescens* Ruiz et Pav.). According to recent studies, piperine is non-toxic and has a great deal of physiological activity. It has been recently documented that piperine interacts with a mammalian protein. This alkaloid is efficiently taken by calyx of bovine beta-lactoglobulin, which is the major whey protein in milk. Moreover, the piperine molecule can be also detected in the beta-barrel of human tear lipocali, human serum retinol–binding protein and human neutrophil gelatinase–associated lipocalin[610]. Capsaicin, from *Capsicum* ssp., has similar effects. Some products containing ephedrine alkaloids are well-known dietary supplements. These alkaloids, namely epinephrine and norepinephrine, naturally occur in low concentrations in the human body. They have sympathominetic effects and cause weight loss and enhanced athletic performance. The products are generally botanicals but ephedrine alkaloids may also be synthesized. There are risks connected with the use of these as supplements. Determining a risk-free dosage is currently a topic of discussion[611]. Dietary supplements containing ephedrine alkaloids are on the market[612,613].

3.2. Alkaloids as biological fertilizers

Alkaloids from many plants are considered to be used as biological fertilizers in ecological cultivation. This is very important especially in cases when more attention is given to these plants, which play not only a role in production but also in the cyclical maintenance of a field, garden or forest ecosystems[614,615,616]. Plants containing alkaloids, for example lupines, have the ability to establish complexes with the soil and with the rhizosphere. The excretion of many chemicals from roots to soil occurs in this complex. Plant mediation with the soil environment is the result. The alkaloids play a major role in this plant–soil interaction system.

Alkaloids are used as fertilizers for some crops. Mittex AG in Germany has developed a natural product, Lupinex, which contains quinolizidine alkaloids, minerals and carbohydrates[333]. Lupinex has more than 9% N, 1% P and 2% K. The raw material for this natural product is a waste received from the lupin alkaloid removing process, when the edible and non-edible components of food

are separated. According to the research data obtained at the University of Hohenheim (Germany), the use of Lupinex increased yields of cereals, legumes, oil plants, tubers and vegetable crops[331]. Moreover, later research confirmed that the increase in yield have also been observed in sunflowers, soybeans and Chinese cabbage[332]. Alkaloid extracts of lupin as promoters of yields have also been noted by other authors[235,333].

Lupin plants with quinolizidine alkaloids are good examples of alkaloids used in agriculture. As ecology friendly plants, lupines can be used for production of food after processing (especially proteins and oligosaccharides), and by-products containing alkaloids can be used as fertilizers. Moreover, in organic cultivation, alkaloid-rich plant material can be composted and alkaloids in this case serve as nitrogen and carbon sources. In ecological soil management, the maintenance of relations between macro and micronutrients is of great importance, foremost the relation between carbon and nitrogen. According to the field test carried out at the Botanical Gardens (62° 36′ N and 29° 43′E) of the University of Joensuu during 1988–1993 on fine, sandy silt soil (21.5% silt, 39.5% fine sand, 10.1% sand, 5.9% clay and 23.0% organic matter) with four different soil preparations, the carbon to nitrogen soil ratio (C:N) was highest when fertilized by alkaloids. Soil 2 was fertilized by a canopy of alkaloid-rich *Lupinus mutabilis*; the total C:N of the treated alkaloid-rich soils was the highest (5.3) and double in comparison with the control soils (2.4, 2.9)[615,616]. Moreover, the nitrogen content of soil with alkaloid-rich canopy addition was at the level of $30.6 \pm 12.3\,g\,L^{-1}$ after 1 year, the highest figure in comparison with other soils (Figure 94). The nitrogen content increase and larger changes of SD in Soil 2 were connected with the high alkaloidal content and composting process of the lupin in the soil. Nitrogen content in this soil treated with alkaloid-rich lupin was 20–44% more than in the standard Soils 1 and 4 (without manure) and 5% more than Soil 3, which was treated with grasses growing in the same place with lupine[615]. The total quinolizidine alkaloid content of the *L. mutabilis* canopy used in the experiments was $2.1 \pm 0.3\,g\,100\,g^{-1}$. Besides nitrogen, carbon and microbial carbon content were also relatively high in the soil fertilized by alkaloid-rich lupine[615,616].

3.3. Alkaloids in plant protection

Many investigations have been carried out with the purpose of investigating the possibility of using alkaloids in plant protection[617,618,619,620,621]. Organic farming requires new possibilities to protect plants without strong synthetic pesticides. Natural botanicals and natural compounds extracted from plants are considered as possibilities. Alkaloids are considered useful for this purpose.

Quinolizidine alkaloids, pure or in mixtures of plant extract, can be used to protect plants against noxious insects. Scientific data has shown that quinolizidine alkaloids play a role in the resistance of some lupine varieties to the pea

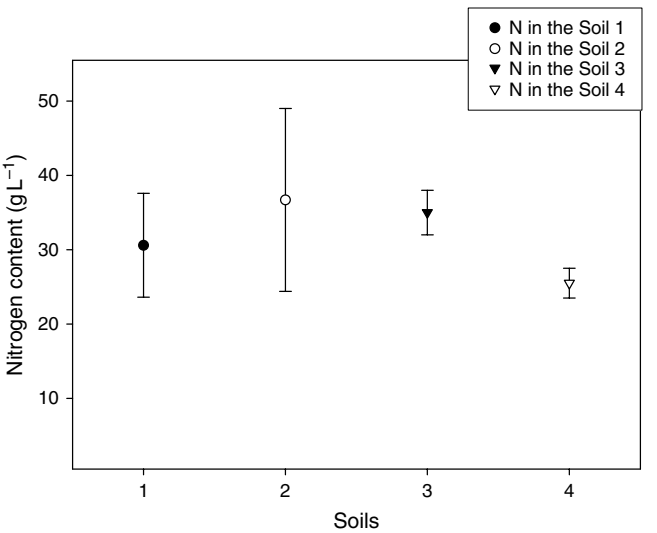


Figure 94. Nitrogen content in different soils after 1 year from sample preparations. (Notes: Soil 1 = control soil after lupine cropping, no canopy addition, stored as a large sample in a laboratory with a constant temperature of −15 °C; Soil 2 = soil identical to control with the lupine canopy and water addition (5 kg canopy, 13.5 kg standard soil, 20 l water), stored as a large sample in the experimental area; Soil 3 = soil identical to control 1 with lawn plant canopy (dominant species *Poa*, *Festuca*, *Lolium*) addition (5 kg canopy, 13.5 kg control soil and 20 l of water), stored as a large sample in the experimental area; Soil 4 = control soil after lupine cropping stored in natural conditions in the experimental area, stored as a part of natural soil; Bars indicate standard deviations (SD)).
(Sources: Refs [615, 616])

aphid (*Acyrthosiphon pisum* Harris) due to their ability to inhibit the development of this pest population[617]. Evidence also points out that alkaloidal extracts have an important influence on the feeding and development of larvae and on potato beetle mortality. Researchers concluded that the toxicity and restricted larval development was correlated with alkaloid content[618]. In this research, the strongest action of the extract contained 1.6–3.3% alkaloids. Spraying potatoes with lupine extract seems to be effective and hence a very promising possible application for alkaloids as natural plant-protecting agents. Other alkaloids toxic to insects can also be tested and used for this purpose. Kahnt and Hijazi[332] have suggested that Lupinex, a natural product which contains 5% quinolizidine alkaloids, is not only a fertilizer but also a protector against insects.

An extract taken from the composted straw of the alkaloid-rich lupin plants has produced very promising results in the development of biological control agents[622]. The fungistatic activity of straw compost extracts increased markedly when the lupin straw used for composting was enriched with alkaloid extract.

Alkaloids are botanicals. Their use in plant control is a very promising technological challenge in today's world, wrought with ecological problems connected to production and farming. The alkaloids are one group of compounds that seem

to play a role in the natural resistance mechanism of some plant species. The possibilities of using natural resistance in plant protection and in the development of resistance cultivars are both considered relatively large in number[623]. This is of importance when considering molecular biology possibilities in plant breeding. It is theoretically feasible that in the future alkaloid-immune and alkaloid-resistance models will be mimicked in new cultivars without decreasing the quality of food and feed. Toxic compounds will be replaced by non-toxic ones, which will have selective toxicity only to the noxious plant insect species.

4. Biotechnology

Alkaloids used as strongly bioactive molecules are vital in biotechnology for the development of new production methods. It is possible to produce more effective alkaloids via biotechnology, and also possible to produce them on a very large scale.

Biotechnology can be defined generally as the application of organisms, biomaterials and systems or processes in manufacturing and production. In the case of alkaloids, biotechnology is a process of effective production of alkaloids *in vitro* and *in vivo* (Figure 95). Biotechnology can be divided into chemical and industrial biotechnology depending on the method used. Furthermore, it can be thought of in a biomedical and pharmaceutical sense when medical objectives are concerned. Lastly, genome and proteomic biotechnology uses genetic and protein engineering. Biotechnology attempts to produce cells and molecules of animals, plants and micro-organisms. Alkaloids are one group of molecules which can be produced via biotechnological means. Plants are the basis for this

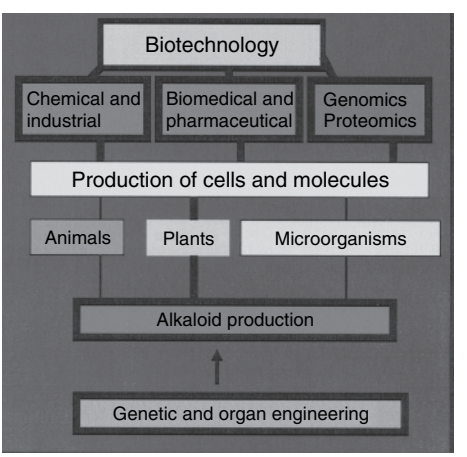


Figure 95. Diagram of the links between areas connected to alkaloidal applications produced by biotechnology.

type of production; however, alkaloids can also be produced in animal cells or in those of micro-organisms. Genetic or organ manipulation is needed in this production. Generally speaking, the best results in alkaloid production by biological methods are presently achieved using plant organs. However, it is also possible from micro-organisms as well as animal cells and tissues (Figure 95).

4.1. Cell cultures

Alkaloids can be produced by using cell culture techniques in the same or a similar way as with other molecules[624]. Alkaloids such as ajmalicine, serpentine (*C. roseus*) and berberine (*Coptis japonica*) can produce remarkable quantities of alkaloids in *in vitro* cultures[625,626]. Generally, cell cultures produce species-specific alkaloids. In some cases cell cultures can produce more alkaloids than the whole plant, in relation to dry weight. This occurs, for example, in the cases of nicotine, ajmalicine and berberine. According to estimations, cell cultures of *Nicotiana tabacum* can produce up to 3.40% alkaloids of cell dry matter when the plant of this species can produce only 2.5% of its dry matter. In a similar manner, ajmalicine in *C. roseus* can be produced as 0.26% of whole plant dry matter, but as 1.3% of the dry matter of cell cultures. This is possible by the addition of enzymes to the cultures. These enzymes catalyse the biosynthesis of alkaloids by cells. Furthermore, cell suspension cultures offer the possibility to produce alkaloids in a truly biological method quite rapidly, on a large scale in an artificial environment. The research of cell physiology, molecular biology, gene engineering, genetics and other branches of biology often depends on cell cultures. They have also an important role in plant breeding and vegetative reproduction of crops and decorative plants. The term "cell cultures" is very similar with the previously widely used term "tissue cultures." Cell culture production is a routine laboratory technique from the 1950s, when it was common in animal cell and later in plant cell research. The idea of the possibility of maintaining and growing living cells was established in 1885 by Wilhelm Roux, who had experimented with parts of an embryonic chicken. However, the first mass-produced products using cell-culture techniques were polio vaccines in the 1950s. The cell-culture technique is based on the maintenance of cultures with nutrients and growth hormones in the medium (Figure 96). Sterility is a basic requirement in the cell culture. Presently, cell cultures can be stored *in vitro* for a long time using cryopreservation techniques[627].

Cell-culture technique use in alkaloid production contains the following stages: (1) solid medium preparation and sterilization of the container for growing, (2) explantation of cells or tissues from plant and the start of growth, (3) primary callus formation, (4) isolation of growing callus and (5) initiating organogenesis of callus directly or using a protoplast cultivation technique by way of hybrid to initiate organogenesis (Figure 97).

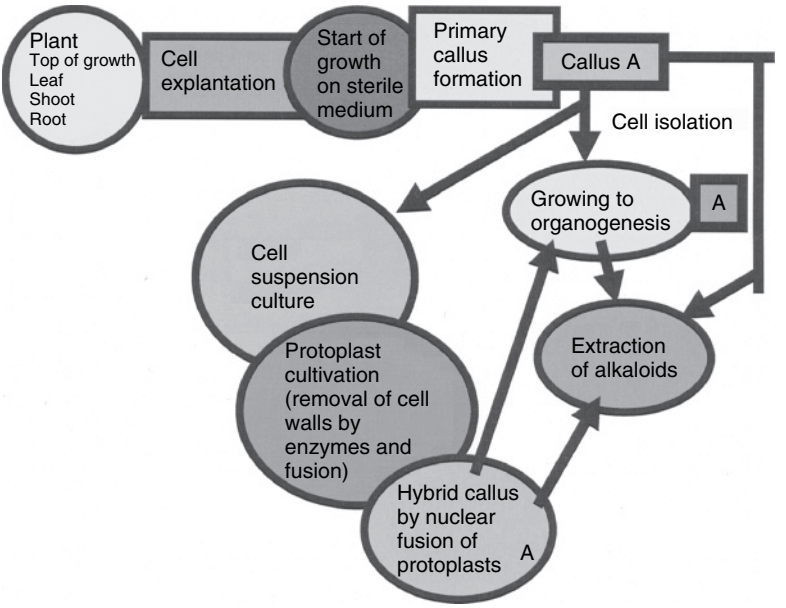


Figure 96. Diagram of alkaloid production by cell culture. Abbreviations: A – alkaloid synthesis.

Figure 97. Cell-culture techniques in the organogenesis stage. Arrows indicate medium. (Photo: T. Aniszewski)

The hybrid is able to produce more alkaloids than the basic callus, which is an undifferentiated mass of cells. Alkaloid production in cell cultures can be more successful with the immobilization of plant cells and enzymes and by using bioreactor systems[628,629,630]. Alkaloid produced in cell cultures can be isolated directly from this culture or from young plants grown from this culture. More than 250 alkaloids are reported to be produced by cell-culture techniques. Only a limited number of species have been researched in this respect. The species studied are known to produce alkaloids with special use in applications. The most researched alkaloids produced by cell cultures are mentioned in Table 25.

Alkaloids can be produced in batch suspension cultures, semi-continuous and continuous cultures. Batch suspension cultures multiply the cells in a liquid medium by using a closed system in which only gases are changed. Generally,

Table 25 Some alkaloids produced by cell cultures

Alkaloid Name	Cell Origin
Ajmalicine	*Rauvolfia serpentina* *Catharanthus roseus*
Akuammicine	*Catharanthus roseus* *Catharanthus ovalis* *Rhazya stricta*
Anabasine	*Duboisia hopwoodii* *Duboisia hopwoodii* *Nicotiana tabacum* *Nicotiana rustica*
11-Allylcytisine	*Cytisus canariensis*
Berbamine	*Stephania cepharantha*
Berberine	*Berberis wilsoniae* *Coptis japonica* *Thalictrum minus*
Biscoclaurine	*Stephania cepharantha*
Caffeine	*Coffea arabica*
Camptothecin	*Camptotheca acuminata*
Canthin-6-ones	*Ailanthus altissima* *Brucea javanica*
Catharanthine	*Catharanthus roseus*
Cephalotaxine	*Cephalotaxus harringtonia*
Cepharanone	*Stephania cepharantha*
Cinchonidine	*Cinchona ledgeriana*
Cryptopine	*Papaver somniferum*
(+)-Eburnamine	*Tabernaemontana divaricata* *Rhazya stricta*
Ellipticine	*Ochrosia elliptica*
Emetine	*Cephaelis ipecacuanha*
Harringtonine	*Cephalotaxus harringtonia*
Quinine	*Cinchona ledgeriana* *Cinchona pubescens*
Harman	*Peganum harmala*
(−)-Lupanine	*Genista pilosa* *Cytisus scoparius*
Magnoflorine	*Coptis japonica* *Corydalis incisa* *Eschscholzia californica* *Fumaria capreolata* *Papaver bracteatum* *Papaver somniferum* *Thalictrum minus*

Table 25 (Continued)

Alkaloid Name	Cell Origin
N-Methylcoclaurine	*Fumaria capreolata*
Morphine	*Papaver somniferum* *Papaver setigerum*
Nicotine	*Nicotiana tabacum* *Nicotiana rustica* *Duboisia hopwoodii* *Duboisia myoporoides*
Noscapine	*Papaver rhoas* *Papaver somniferum*
Rutacridone	*Ruta graveolens*
Sanguinarine	*Chelidonium majus* *Corydalis ophiocarpa* *Eschscholzia californica* *Fumaria cordata* *Macleaya cordata*
Skimmianine	*Choisya ternata*
Solasodine	*Solanum dulcamara* *Solanum nigrum* *Solanum laciniatum*
Stephanine	*Tinospora caffea* *Tinospora cordifolia*
Strictosidine	*Rauvolfia serpentina* *Catharanthus roseus*
(−)-Tabersonine	*Catharanthus ovalis* *Stemmademia tomentosum* *Voacanga africana*
Theobromine	*Theobroma cacao*
(−)-Tinctorine	*Cytisus canariensis*
Tomatidine	*Lycopersicon esculentum*
Vinblastine	*Catharanthus roseus*
Vincristine	*Catharanthus roseus*
Vindoline	*Catharanthus roseus*
Voafrine A	*Voacanga africana*
Voafrine B	*Voacanga africana*

this method contains the following phases: (1) inoculation of cells into the medium, (2) a lag phase in which cells slowly grow, (3) a rapid division phase and (4) slower division. The duration of Phase 2 is from 0 to 9 days and of Phase 3 is 9 days. In semi-continuous cultures, the medium is removed after Phase 3 and replaced by a new medium. In this way cell growth is continuous,

although there are cells from different mediums. In continuous cultures, a fresh medium is continuously added. There are two open systems in the arrangement of continuously growing cultures: chemostat and turbidostat. In the chemostat arrangement a steady state is achieved, controlled and monitored. The turbidostat arrangement is based on the maintenance of a constant cell concentration. Plant cells in cultures typically form cell aggregates. These aggregates can form small groups of cells.

In cell suspension cultures producing alkaloids, it is possible to choose and grow high-yielding cell lines. Cells and their metabolic capacity may differ as a result of genetic variability, the explant determination and cell culture acting adaptation[631,632,633,634,635]. Therefore clones and hybrids are generally used in cell cultures because of their higher capacity to produce alkaloids. They also have more stable and similar cells. Such clones and hybrids have been developed with nicotine (*N. tabacum*) and in hyoscyamine production (*Duboisia leichhardtii*)[636]. Moreover, there is evidence that the conversion of nicotine to nornicotine occurs in tobacco cell cultures[637].

One of the problems of alkaloid production in cell cultures is not only the different capacities of diverse cells resulting from somaclonal and cell-to-cell variation or the necessity to use clones and hybrids for this purpose, but also low alkaloid yield. This problem is necessary in avoiding enzyme addition and the establishment of specific cells. In the case of quinolizidine alkaloids, especially in lupins, there are also some problems with cell cultures establishing and growing beyond the callus. According to recent observations and measurements it is difficult to obtain quinolizidine alkaloids from *L. polyphyllus* cell cultures without special treatment. However, in the cell cultures of *L. polyphyllus* Lindl., the production level of lupanine is only 0.5% of the level found in the growing plant[638,639]. Hybrids and enzymatic treatments of cell cultures increase this rate if the cells explanted for inoculation in cultures do not come from alkaloid-poor plants, which genetically block the production of lysine, a precursor of the quinolizidine alkaloids[7,348,640,641]. Morphine production in *P. somniferum* cell cultures without special treatment is known to be low. However, there is also data which shows that the yield of morphine from *P. somniferum* suspension cultures was $2.5\,mg\,g^{-1}$ dw and that of codeine was $3\,mg\,g^{-1}$ dw[642]. According to this research, the removal of hormones increases the alkaloid production rate. However, it is necessary to mention that despite some problems in alkaloid production in cell cultures, there is evidence that some alkaloids (e.g. serpentine, nicotine, canthin-6-one etc.) can be produced in cell cultures at higher levels than in whole plants.

The special treatment of cell cultures for increasing the yield of alkaloids can be linked to improvements in medium composition, the addition of enzymes and alkaloid precursors, the addition of the so-called “elicitors” and changes in environmental factors. The medium can be improved by changes in the relations between its components: sucrose and carbon components, nitrogen, phosphorus

and hormones[639,643,644,645]. Higher alkaloid yields have been achieved by using this method[642]. The addition of enzymes and alkaloid precursors improves alkaloid yields[263,264,646]. The addition of tryptophan to *Cinchona ledgeriana* cell cultures increased the production of quinine and quinidine by up to 25%. Similarly, the addition of tryptophan to *C. roseus* cell cultures increases ajmalicine and serpentine production by more than 20%. Moreover, by immobilizing cells and enzymes it is possible to increase alkaloid yield in cultures and improve the culture system activity. Matrices such as agarose, alginate or polyacrylamide are used in immobilization techniques. It is known that sanguinarine, chelerythrine and macarpine production in the cells of *Eschscholtzia californica* was enhanced by sodium alginate and by entrapment in Ca^{2+}-alginate[647]. Tyrosine decarboxylase, which is a key enzyme for alkaloid biosynthesis in this species, has especially been induced by both sodium and calcium alginate. Moreover, it is also known that the immobilized cells of *C. roseus* produced ajmalicine and serpentine over 4 months[629,648,649]. Immobilized cells can also influence alkaloid conversion reactions such as the transformation of (−)-codeinone to (−)-codeine (*P. somniferum*). Alginate-entrapped cells can enhance alkaloid biosynthesis in cell cultures even up to 800-fold[647].

Better yields of alkaloids in cell cultures have been obtained by the addition of elicitors, that is micro-organisms. This had been reported already in the 1980s and 1990s[650,651,652,653,654]. The detailed mechanism of an elicitor's influence on alkaloid synthesis is a challenge for future studies. However, in cell cultures producing alkaloids, environmental factors are of great importance. For example, dissolved oxygen concentrations up to 50% in mediums increased berberine production in cultures of *Thalictrum minus*[655]. The influence of light on alkaloid production in cell cultures of *C. roseus* is also noted[656], though its influence is considered to be more cross the temperature and energy connected with the light. There is evidence that alkaloid production occurs in the dark in quinolizidine alkaloids, although older literature notes that their formation is associated with chloroplasts[638]. If all other alkaloids are produced in the dark, quinolizdine alkaloids should be also produced in such environments. No reason why quinolizidine alkaloids should be an exception exists. There is evidence that the transfer of cell cultures from dark to light environments causes a decrease in alkaloid production, and the movement of cell cultures from light to dark environments influences the increase of alkaloid production[657].

4.2. Root cultures

In vitro production of alkaloids is possible also with the use of another *in vitro* technique: organ culture. Root cultures are the most common concerning alkaloids, as this part of plants is of great importance for alkaloid synthesis. Root cultures that produce alkaloids have been studied as far back

as the 1950s[658], but this subject did not come under wide scrutiny until the 1970s–1990s[659,660,661,662,663,664,665,666,667,668,669,670]. Root cultures with the ability to produce alkaloids have importance nowadays in biotechnology and genetic engineering[671,672,673,674,675]. Modifications of roots by hybridization, gene transfer and the transformation of root cultures led to establishing organs with high alkaloid production potential. This research area is growing all the time. Richter et al.[673] reported an increase of total tropane alkaloids in the transformed root cultures of *A. belladonna*. Moreover, the transformation with cDNA of tropinone reductases successfully altered the ratio of tropine-derived alkaloids versus pseudotropine alkaloids. Similar results have also been reported by Dechaux and Boitel-Conti[671]. The optimization of the medium led to an increase of scopolamine production in hairy root cultures of *Datura innoxia* by increasing the produced biomass. Genetic engineering is the best way to increase the accumulation of scopolamine. Hong et al.[674] have studied *Catharantus roseus* hairy root cultures transgenic for the rol ABC genes from T-L-DNA of the agropine-type *Agrobacterium rhizogenes* strain A4. They concluded that the three genes rol ABC are sufficient to induce high-quality hairy roots in *Catharantus roseus*. A recent study by Hu and Du[675] proved that hairy root cultures have proved to be an efficient means of producing secondary metabolites that are normally biosynthesized in the roots of differentiated plants. Moreover, a transgenic root system offers tremendous potential for introducing additional genes along with the Ri plasmid, especially with the modified genes. Root cultures can be used to elucidate the intermediates and key enzymes involved in the biosynthesis of secondary metabolites. A prototype basket-bubble bioreactor has been designed and built to upgrade the scale of *Genista tinctoria* hairy root cultures[672]. The industrial mass scale production of alkaloids from bioreactors with transformed hairy root cultures is possible.

Many alkaloids can be produced by root cultures. The most known applications are with anabasine, nicotine, harmine, harmaline, hyoscyamine, calystegine, scopolamine, senecionine[263,670,673,676,677,678,679,680,681,682,683,684]. Enzymes such as putrescine *N*-methyltransferase[545], *N*-methylputrescine oxidase[685], tropinione reductase I and II[548,683], pseudotropine acyltransferase, hyoscyamine 6β-hydroxylase[553], homospermidine synthase[686] and tropine reductase[673] can be purified from root cultures producing alkaloids. The root cultures are useable in the production of enzymes active in alkaloid biosynthesis pathways.

CHAPTER 5

The Ecological Role of Alkaloids

Naturam mutare difficile est.
Seneca

Abstract: Alkaloids are a special group of secondary compounds and are part of an organism's adaptation mechanism to its living environment. They are not toxic when stored, but become toxic as a result of cell pH change. The defensive function of alkaloids is only secondary, and connected to internal immune and regulation processes. Animal responses to alkaloids are very diverse. Some animals can tolerate alkaloids relatively well, while others are harmed or even poisoned by them. Animal behaviour in relation to alkaloids depends on evolutionary and co-evolutionary factors. Sequestration of alkaloids is connected with these processes. Alkaloids are a part of plant-derived nutrition. A selective toxicity of these compounds in vertebrates is clearly observed. Vertebrates have the capacity to recognize alkaloids.

Alkaloids take part in the life processes of some invertebrates as pheromones, inducers of sexual behaviour, and in reproduction. A case study of quinolizidine alkaloids and population changes proved that these alkaloids occur in all legume species studied but not, however, in all individuals. The distribution and frequency changes of alkaloidal and non-alkaloidal plants in populations is a direct expression of natural selection; natural hybridization and micro-evolution can be considered as an evidence of current evolutionary responses by ecological and genetic systems.

Key words: alkaloids, attraction, case study, deterrence, ecology, food, MEC, micro-evolution, pheromones, quinolizidine alkaloids, selective toxicity, sexual life

Alkaloids have long represented a research subject in organic chemistry and pharmacology. The main object of research has been to recognize the profound chemical structure and the physical characteristics of these compounds. Advances in alkaloid chemistry also served to advance biological studies of alkaloids, since the chemical structure of these compounds defines their biological activity. As outlined in previous chapters, some areas remain unclear and under-researched; however, the knowledge of alkaloids is relatively large and very detailed in many areas. Moreover, this knowledge has been utilized in the development of many contemporary applications important to human life and society.

From the beginnings of alkaloid research (from the discovering of morphine) to today, one of the most interesting questions has been and remains the function

of alkaloids. In particular, the external function of alkaloids has been a popular area of study from both ecological and evolutional points of view. The basis for ecological studies of alkaloids is adaptation theory, according to which all living organisms adapt to environmental change[588,687,688,689]. Adaptation is the ability of an organism to use newfound conditions to increase its own chances of survival and reproduction. Adaptation has genetic and chemical levels. The short-term (during the life of one generation or of one population) adaptation can be described as acclimatization and in longer timeframes as evolution. During the acclimatization processes, physiological and biochemical adaptations occur[588,689,690,691,692]. The mechanism of adaptation influences both the primary metabolism (enzymes and proteins) and the secondary metabolism[588]. Alkaloids, as a special, genetically depended group of secondary compounds, function as a part of this mechanism. Moreover, plants and insects radiate and speciate in association with one another in a process referred to as co-evolution[577,693,694,695]. The basis for this co-evolution is the realization of the nutritional necessity of insects feeding on related species of plants[696]. This necessity leads to the co-evolution of interacting organisms: plants and insects. However, this co-evolution has also a reciprocal character. It is a consequence of the chemical interaction[575,577,697,698,699]. On the basis of these theories, secondary compounds have been recognized as plants' defence agents against herbivores. This also has an influence on studies on alkaloids. Many studies have mentioned the main role of alkaloids as defenders of organisms and mediators with the proximate environment[323,325,588,700,701,702,703,704,705,706,707,708]. However, it is still debatable whether this is the primary function of alkaloids in organisms. As mentioned above, alkaloids are not toxic when stored. They only become toxic as a result of plant cell pH change. This means that alkaloids have a primary role of non-toxic compounds in plants. The defence function is only secondary and related to internal immune and regulation processes. Although this topic needs more empirical research in cell biology, the connection between the physiological, inside-organism function of alkaloids and the outside, ecological function is evident. Alkaloids are produced by the organism primarily for its internal metabolism and activity purposes. Moreover, alkaloids represent only one group of the compounds needed in the defence process. Other compounds, such as non-alkaloid compounds, and phenolics[709,710,711,712] specifically, also seem to be important in this function.

1. Animal sequestration of alkaloids

Animal responses to secondary compounds, including alkaloids, are as diverse as natural chemicals. In the case of alkaloids produced by plants, animal responses depend on evolutionary and co-evolutionary factors. Some animals tolerate alkaloids relatively well, while others have well-developed detoxification systems.

Some animals, especially mammals, can be harmed or even poisoned by these compounds. There are many known cases of symptoms of poisoning in cattle by pyrrolizidine alkaloids (senecionine) from the *Senecio* species[475,493,588,713]. Anagyrine, from the quinolizidine alkaloid group with pyridone nucleus, has been known to cause skeletal deformities in the foetuses of pregnant cows consuming toxic lupines[7,236]. Some animals, including dairy cows, have been shown to selectively feed on only alkaloid-poor green plants[394]. Similar results were observed in a field test trial in 1983 at the Central Finland Research Station in Torikka (Laukaa), where lupin green mass of three cultivars, two bitter and one sweet, were offered to outdoor-grazing dairy cows. One cow approached the sweet green mass of one cultivar, tasted it and continued to consume it for approximately 20 minutes. Afterwards, it grazed on grass. Two other cows tried the bitter mass, tasted it and in both cases spait it out. They were restless during the spitting and did not consume any more lupin mass. These behavioural responses were very important for researchers. The chemical analysis of the green matter clearly confirmed field observations and the animals' consumption behaviour. Therefore, this is also a proof that a tester method of alkaloid analysis can be useful in some cases, especially when it is necessary to do so quickly, as in the decision on green mass quality as fodder. This simple test has also provided interesting for discussion. One might ask what was the cow's mechanism for recognizing the alkaloids in the green matter. Although there do not exist deep investigations into this question, the mechanism is most likely based on the recognition of bitterness by the animal's taste receptors. Moreover, the configuration of alkaloid skeletons might not be an adequate fit for the configuration possibilities of taste receptors, since the lupin juice from green matter started the bitter taste reaction mechanism of a cow.

Animal sequestration of alkaloids is connected not only with taste but also with the toxicity of these compounds. It has been stated that the toxicity of alkaloids is very selective. Aniszewski[328] has published data with some LD_{50} coefficients for some alkaloids and some pesticides and compared their toxicity from a selectivity point of view. There was clear evidence that alkaloids (sparteine and lupanine) are much more toxic for vertebrates than are some pesticides (e.g. malatione, phenitrothione, etc.). For invertebrates, pesticides were clearly more toxic than alkaloids. Selective toxicity coefficients (STC) were counted by dividing the LD_{50} for vertebrates by the LD_{50} for invertebrates. When the STC is 1.0 there is no selectivity; when STC is >1 there is invertebrate selectivity; and when <1 there is vertebrate selectivity. Selectivity simply means there exists more ability to toxify the organism.

Generally speaking, alkaloids are more toxic for vertebrates than for invertebrates[328]. The coefficients of the selective toxicity show that alkaloids are very dominantly selective toxins to vertebrates (Table 26). Vertebrate very strong selectivity (<0.01) is observed in such alkaloids as ajmalicine, brucine, ephedrine, ergometrine, harmaline, lupanine, lupinine, scopolamine and

Table 26 Selective Toxicity Coefficients (STC) of some alkaloids and selective toxicity in the ecosystem

Alkaloid	STC	Selective Toxicity in Ecosystem
Ajmalicine	$0.0015^{M,S}$	vvss
Ajmaline	$0.014^{M,S}$	vss
Anabasine	$0.013^{M,S}$	vss
Arecoline	$0.014^{M,S}$	vss
Atropine	$0.75^{R,B}$	msv
Berberine	$1.0^{M,BE}$	ns
Boldine	$0.12^{M,S}$	vss
Brucine	$0.005^{R,BE}$	vvss
Caffeine	$0.7^{M,BE}$	msv
Castanospernine	$0.1^{R,A}$	msv
Chaconine	$0.15^{R,A}$	msv
Chelidonine	$0.45^{R,S}$	msv
Cinchonidine	$3.6^{R,BE}$	msiv
Cinchonine	$3.1^{M,BE}$	msiv
Codeine	$5.0^{M,F}$	msiv
Colchicine	$0.003^{MA,BE}$	vvss
Coniine	$0.112^{AG,P}$	msv
Cytisine	$0.03^{M,F}$	vss
Emetine	$0.044^{M,S}$	vss
Ephedrine	$0.001^{M,BE}$	vvss
Ergometrine	$0.0003^{M,S}$	vvss
Ergotamine	$0.11^{M,S}$	msv
Gramine	$0.1^{M,S}$	msv
Harmaline	$0.006^{M,S}$	vvss
Harmine	$0.7^{M,BE}$	msv
Heliotrine	$0.45^{R,BE}$	msv
Hyoscyamine	$0.3^{R,BE}$	msv
Jacobine	$15.0^{R,L}$	msiv
Lobeline	$2.5^{R,BE}$	msiv
Lupanine	$0.008^{M,A**}$	vvss
Lupinine	$0.009^{M,A}$	vvss
Nicotine	$0.08^{M,BE***}$	vss
Papaverine	$3.0^{M,F}$	msiv
Pilocarpine	$0.9^{M,F}$	vsss
Quinine	$0.01^{A,BE}$	vss
Reserpine	$0.1^{A,B}$	msv
Sanguinarine	$0.9^{M,S}$	vsss
Scopolamine	$0.003^{M,BE}$	vvss
Senecionine	$0.63^{M,BE}$	msv
Solanidine	$0.09^{M,C}$	vss
Solanine	$0.06^{M,C}$	vss
Sparteine	$0.01^{M,BE**}$	vss
Strychnine	$0.005^{M,BE}$	vvss

Table 26 (Continued)

Alkaloid	STC	Selective Toxicity in Ecosystem
Tomatidine	$0.08^{M,C}$	vss
Vinblastine	$0.8^{M,BE}$	msv
Vincamine	$1.0^{M,BE}$	ns
Vincristine	$0.9^{M,BE}$	vsms
Yohimbine	$0.45^{M,BE}$	msv

Abbreviations: ^{R} = rat; ^{M} = mouse; ^{B} = *Bruchidius*; ^{BE} = bee; ^{MA} = man; ^{S} = *Syntomis*; ^{A} = Aphids; ^{F} = Formia; ^{AG} = *Agelaius*; ^{L} = *Locusta*; ^{C} = *Choristoneura*; * = 0.02–0.007 for other animals [328]; ** = 0.004–0.01 for other animals [328]; *** = 0.04 for other animals [328]; vvss = vertebrate very strong selectivity; vss = vertebrate strong selectivity; msv = more selectivity for vertebrate; ns = no selectivity; msiv = more selectivity for invertebrate; vsss = vertebrate slight selectivity.

strychnine (Table 26). Vertebrate strong selectivity (<0.1) exists in the case of ajmaline, anabasine, arecoline, boldine, cytisine, emetine, nicotine, quinine, solanidine, solanine, sparteine and tomatidine. More selectivity to vertebrates (<0.9) is observed in alkaloids such as atropine, caffeine, castanospermine, chaconine, chelidonine, ergotamine, gramine, harmine, heliotrine, hyoscyamine, reserpine, senecionine and vinblastine. Vertebrate slight selectivity (0.9–0.95) has been observed in pilocarpine and sanguinarine. No selectivity (1.0) was observed in the case of berberine and vincamine (Table 26). There are only a small number of alkaloids which have more selectivity to invertebrates (>1.0). These are alkaloids such as cinchonidine, cinchonine, codeine, jacobine, lobeline and papaverine (Table 26).

The sequestration of alkaloids by insects is considered to be a form of defence. Insects sequester alkaloids and accumulate them for protection against their enemies[701,702,703,704,714,715,727]. Some examples of this kind of insect behaviour have been seen in *Aphis cytisorum, Aphis genistae* and *Macrosiphum albifrons*. Wink[703] has mentioned that *M. albifrons* stores alkaloids in order to provide protection against carnivorous beetles, such as *Carabus problematicus* or *Coccinella septempunctata*. However, the protection provided by the sequestration of alkaloids seems to be still more conjecture than scientifically proven. Stermitz[524] has stated that there exists no proof in field conditions that the sequestration of alkaloids by certain insect species provides them with any defensive purpose. More recently, Wäckers[595] underlined that insect behaviour and the function of secondary compounds should be proven only under field conditions. This is very important when analysing the STC data presented in Table 26. The alkaloids have, in general, no special selective toxicity to insects. Only a very small number of these compounds with reduced distribution in plants are more toxic for invertebrates than vertebrates. Therefore, alkaloids are not strong selective toxins against insects, and their defence ability after sequestration seems problematic. Moreover, the lack of toxin selectivity to insects suggests that between

alkaloid-containing plants and insects a type of mutualism exists rather than an antagonist relationship[595]. By sequestering alkaloids through food, insects fulfil their physiological needs. Therefore, insects use alkaloids in their metabolism and life cycle more than in their direct defence. There exists no evidence in nature that insects sequester cinchonidine, cinchonine, codeine, jacobine, lobeline or papaverine, alkaloids that do have selective toxicity for insect enemies. Field studies on this topic are indispensable.

Alkaloids are a part of plant-based sustenance for herbivores, omnivores and according to the latest research, also for carnivores[595,716,717,718,719,720,721,722,723,724,725]. The literature mentions that alkaloids and other secondary compounds also occur in floral nectar, pollen, honeydew, leaves, stems and roots of plants[592,593,595,703,707,726,728]. Herbivores, omnivores and carnivores benefit from their interactions with plants, and alkaloids are a part of this benefit, having a valuable role in animal metabolism and behaviour. The sequestration and accumulation of these compounds in the liver strengthen this hypothesis. The possible protective role of alkaloids seems to be secondary, as previously stated. It is possible to see the interaction between plant and herbivore not only from the point of view of antagonistic theory (plant defends itself and the herbivore consumes it), but also from the point of view of a mutually beneficial relationship between plant and herbivore. In the case of insects, this relationship is more evident than an adversarial relationship. Alkaloids have an important role in this mutual relationship. As seen in the insect liver, alkaloids can be metabolized or accumulated (stored). Both can have the effect of mutualism between insects and plants. Although, more empirical studies are needed within this field, it can also be said that the present direction of ecological thinking is oriented more towards mutualism than towards older antagonistic approaches[595].

There exists evidence that some insects store dietary alkaloids derived from natural sources. Figure 98 presents insect species that are known to accumulate pyrrolizidine alkaloids during different developmental stages. The larvae and adults of these insects can metabolize pyrrolizidine alkaloids in current physiological activities. These alkaloids are not toxic for these organisms. Moreover, there is observed trace accumulation of a portion of these compounds in the liver. There is no definitive purpose for these traces. Generally, the opinion presented in 1888 by Stahl in Germany that the accumulation of alkaloids is for defensive purposes[703,731] has been most often cited in the research literature.

However, in the case of dietary alkaloids, it would seem that more than only traces of alkaloids, which do not exhibit selective toxicity to antagonist organisms, would be needed for defensive purposes. These trace alkaloids probably have a role in the organism's metabolism, development and behaviour. The traces of alkaloids in the eggs of *Arctia caja* also suggest a potential participation of these compounds in reproduction. Moreover, attention should be given to the fact that alkaloids are dietary sequestrations acquired from feeding on plants.

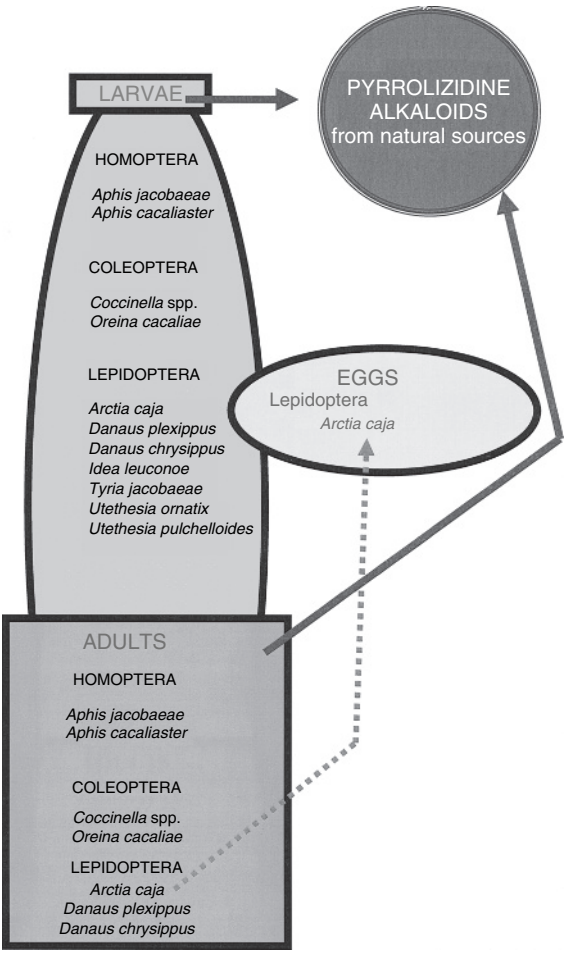


Figure 98. A diagram of the accumulation of pyrrolizidine alkaloids in some insect species during various developmental stages. It should be noted that it is not often that these alkaloids are present in the eggs, as in the case of the *Arctia caja*. Natural sources are pyrrolizidine alkaloid-rich plant species (*Senecio* spp., Homoptera; *Senecio* and *Adenostyles* spp., Coleoptera; and *Senecio, Adenostyles, Petasites, Crotalaria* and *Heliotropium* spp., Lepidoptera).
(Sources: Refs [588, 703, 704, 729, 730])

They are not, therefore, endogenous chemicals produced by the activity of the organism's genes or regulated by its metabolism. It could be then concluded that the pyrrolizidine alkaloids sequestered and accumulated in these cases are needed along with other nutrients, and therefore they function as a source of vitality and metabolic activity for these organisms. Future studies should strive to further elucidate this enduring secret of alkaloids.

There are two sources of alkaloids in animals, as already mentioned in the text. The first is the ability to synthesize them, and the second is through dietary

intake. Food and the food chain is the most important factor in the development, growth and dynamics of populations in the ecosystem. Alkaloids are for some species very desirable, while for others they go unwanted. Therefore, there exist different means of animal behaviour in relation to the intake, metabolizing and accumulation of these compounds. Alkaloids, like other secondary compounds, can be avoided when they are undesirable to certain animals. As stated earlier in this chapter, cows are able to avoid ingesting these compounds by virtue of taste. Other animals also avoid alkaloid-rich plants by taste, by olfactory recognition or by the first effects of neurotransmission activity of these compounds. Vertebrates have the ability to recognize alkaloids and the compounds that have selective toxicity to these organisms. Ecological interaction between vertebrates and plants is fundamentally based on this ability.

2. Sexual behaviour

Some insects ingest plant matter containing alkaloids and use alkaloids in its own metabolism. For example, *Ithomiine* butterflies utilize pyrrolizidine alkaloids. Moreover, it was reported even in 1970s that some butterflies (*Danaus* spp.) use pyrrolizidine alkaloids from *Senecio* spp. directly in their sexual life[729]. They sequester and accumulate alkaloids during their entire adulthood and use them as pheromones. The males of the species intake alkaloids and use them for this purpose. It is known that the females are attracted to the males by strong pheromones: danaidal, hydroxydanaidal and danaidon. These pheromones are postcursors of pyrrolizidine alkaloids. In practice, the male of *Danaus plexippus* and *Danaus chrysippus* invites the female to enter into sexual contact by flying near her body and spraying danaidal, hydroxydanaidal and danaidon. The stronger the pheromones are, the better the male's possibility of copulation. The pheromones prepare the female to accept the male's proposition. As a result, the female is in the best possible position for sexual contact and for the opening of the copulation canal. Without pyrrolizidine alkaloids, the sexual life of these butterflies and their eventual reproduction would be limited. There are at least 40 different known insect species that possess the ability to collect and accumulate plant toxins including alkaloids[700,729] (Figure 99).

Moreover, it is generally known that in some species of insects the location of eggs by females on plants is bio-chemically determined by plant–insect interaction. Some alkaloids play a very important role in the interaction and reproduction of insects. A good example is the egg-laying behaviour of *Leptinotarsa decemlineata* on potato plants. Potato alkaloids influence the female *Leptinotarsa decemlineta* choice of egg placement on the plant. This behaviour is determined by the chemical signals emitted by the female's taste and olfactory sense receptors during feeding or coming into contact with the parts of the plant[623]. The sexual and reproductive behaviour of some insects is connected

Figure 99. The copulation of butterflies. (Photo: T. Aniszewski)

with the acceptance of the plant as a beneficial environment for life. Alkaloids represent one group of compounds that are used as indicators of this acceptance.

3. Feeding attraction and deterrence

It has been mentioned earlier in this chapter that alkaloids have no selective toxicity to invertebrates. Therefore, for many insects, these compounds are more attractive than toxins (Figure 100). The cases of butterfly or beetle behaviours mentioned above are also a very evident example of the importance of plants for invertebrates. In both cases, alkaloids as secondary compounds take part in the

Figure 100. The butterfly's interaction with alkaloidal plants. (Photo: T. Aniszewski)

attraction for feeding. Moreover, it is also known that *Manduca sexta* flourish on alkaloid-rich *Nicotiana* plants and bruchid beetle (*Bruchidius villosus*) on quinolizidine alkaloid-rich plants[703,732]. Such examples are a consequence of the herbivore model for choosing a specific plant diet. This model is based on the plant–herbivore interaction. The plant can either attract or deter the insect or its larvae. Alkaloids represent only one group of the secondary compounds through which plants interact with insects and other animals.

The feeding attraction and deterrence by alkaloids are determined by the historical processes of the cell and cellular mutations, which lead to the evolution of mutualisms in ecosystems. Plant-provided food is an important base for the mutual benefit of the species[595]. Insects are not only consumers of alkaloids, but they are also vehicles for the transmission of alkaloids. Aphids ingest nutrients through plant juice, taking alkaloids from one plant and distributing them to the environment at large. Therefore, aphids function as alkaloid vectors. Through mediating alkaloid distribution from plants to the wider environment, aphis and other such organisms contribute to the evolution of life and the proliferation of alkaloidal plants. This puts increased pressure on the populations of animals that prefer non-alkaloidal plants. Evolution, in this sense, has not only a historical lens but also a future-oriented perspective. Evolution and micro-evolution perpetually occur in biocoenosis. Alkaloids are one group of chemicals that have central importance for and in the evolution of life.

4. A case study: Alkaloids and population changes

4.1. Introduction

There is no question that plants are very important for animals and humans with respect to photosynthesis[733]. This importance also applies to the Legume plant family. It is the third largest family of flowering plants, containing three sub-families, more than 650 genera with 18 000 species, including trees, shrubs, herbs, climbers and crops[734,735]. The legume species are well recognized for their good adaptation systems and their growth in various climates and environments around the world, ranging from the humid tropics to sub-arctic zones[736,737,738]. This means that legume species have a large degree of plasticity and ecological breadth, due to the fact that the ecological distribution of these species is influenced by individual patterns of response to environments for traits that contribute to fitness[739]. Moreover, legumes also have the genetic ability to produce secondary compounds[7,741]. This genetic trait carries an ecological significance[740]. Many types of secondary compounds in this large family have been found and described[741]. Quinolizidine alkaloids (QAs) are one group of secondary compounds that can be found in legume plants[7,742,743,744,745]. The QAs are secondary metabolites synthesized from lysine and contain one or several nitrogen atoms as

constituents of heterocycles[749]. Previous studies on QAs have shown by means of genetic experimentation (breeding by pollen crossing) that QAs have a genetic character. By means of hybridization, it is possible to noticeably decrease the alkaloid level[746,747]. It has also been shown that alkaloid-poor plants do not, over time, become bitter[748]. Moreover, QAs are biologically active compounds and are known as anti-nutritive factors for animals, but on the other hand, as protective factors for plants and their metabolisms[736]. As QAs have a hereditary nature controlled by genes expressed through HMT/HLTase and ECTase, they are evolutionary traits of plants and species.

Alkaloid distribution in plants has to interact with the plants' habitats and herbivores. This is a factor for both population and ecosystem stability, and the adaptation of plants to their habitats[736,748]. In Nordic ecosystems, the adaptation of plants to the cold and to short growing seasons is characteristic for legumes. In sub-arctic and arctic ecosystems, the adaptation of plants to the cold is a typical survival process. Therefore, the adaptation to very stressed and altered life factors in legume habitats is considered to be a micro-evolutionary process in the long-term evolution of plants. Many studies have described plant adaptation to the environment[689,739,750,751,752,753,754]. However, little is known about the variations in genetic traits by plants in wild or semi-wild populations to produce alkaloids, especially in legumes. Our knowledge in this field is important because these plants enrich the diversity of natural ecosystems and agro-ecosystems around the world, including sub-arctic ecosystems. They also represent an important protein source in the wild and for human and animal nutrition. In this case study, the frequencies of QAs and their distribution in wild and semi-wild Fabaceae populations for 5-years were tabulated during a 5-year period, using valid techniques. A total of 4000 legume plants growing wild in Finland were analysed and found to exhibit certain trends. As there has been no previous study in this exciting subject, therefore, my analysis gives special attention to establishing a formula for possible micro-evolution coefficients for the Fabaceae species. The interpretation of these coefficients is an important part of this work. The micro-evolution is a trend of process in the self-regulation of plant populations. This part of this book documents how alkaloid frequencies differ among wild-growing, Nordic legumes and how QAs are distributed in relation to evolutionary trends in these legume populations.

4.2. Material and methods

This case study was carried out in the Research and Teaching Laboratory of Applied Botany under the auspices of the Department of Biology of the University of Joensuu, Finland (N 62° 36′ E 29° 40′) from 1999 to 2003 as a part of the larger project, "Quinolizidine alkaloids in arctic and sub-arctic flora". This large project explores the broader problem of QAs occurring in

plants growing in northern ecosystems. This case study presents and discusses the research results from the first part of this project.

It has been qualitatively checked wild (X) and semi-wild (B) plants and some crops (?) of 40 legume species growing wild in Finland for alkaloids. The species studied were botanically determined with the use of a stereo light microscope at the beginning of the project, and for the experimental trials, the naturally growing vegetation places were pre-marked. In this way, the established trial area was 1 m^2 for each species in each habitat. In the case of a genus with a high and broad habit of growth, an additional trial area was provided (Table 27). For each year, there were at least 20 plants within the pre-marked areas.

For each year, the botanical purity and identity of the studied species were monitored using a stereo microscope. During these microscope investigations, no significant overwintering or disease damage were observed. The populations studied had wild or semi-wild distributions. They were growing under wild conditions in naturally competitive habitats. These habitats were (a) roadside, (b) forest border stand, (c) former field and (d) in a garden area. The legume populations in the trial areas were observed and tested for QAs each year for 5 years. Leaves of plants were taken from a total of 4000 legume individuals, belonging to 13 different legume genera, of which 40 species and sub-species were studied (Table 27). The natural distribution of legume species in northern conditions served as the basis for the size of the genus studied. Some species studied are rare and others are very common in Finland (Table 27).

Table 27 Systematic division and habitat characteristics of the studied legume species

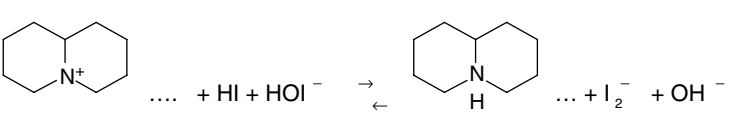
Genus	Number of Species	Number of Individuals	Habitat characteristics
Astragalus	500	5	(1) *d***B**$\vee \alpha$ r^+
Coronilla	100	1	(1) *a***X**$\vee$ r
Cytisus	100	1	(2) *b***XB**$\vee$ r
Lathyrus	600	6	(1), 3*bc***XB**$\vee$ r^+
Lotus	100	1	(1) *a***X**$\vee^+$
Lupinus	100	1	(1) *a***B**$\vee \vee \alpha^+$
Medicago	300	3	(1), 2*ac***B**?$\vee$ r^+
Meliotus	100	1	(1) *ac***XB**$\vee$ r
Ononis	100	1	(2) *c***X**$\vee$ r
Ornithopus	100	1	(1) *d*?$\vee$ r
Oxytropis	100	1	(1) *b***X**$\vee$ r
Trifolium	900	9	(1),(2) *ac*?**BX**$\vee\vee$ r^+
Vicia	900	9	(1),(2) *abcd***BX**?$\vee\vee$ r^+
Total 13	4000	40	

Notes: (1) = eastern Finland, (2) = southern Finland, (3) = western Finland; a = roadside, b = forest border stand, c = former field, d=garden area; **X** = wild plant, **B** = semi-wild plant, ? = crop; $\vee$ = vegetative growth advanced, $\vee\vee$ = vegetative growth advanced, the first phase of flowering; α = additional trial area; r = rare species; r^+ = both rare and common species; $^+$ = common species.

The number of species studied was the highest for the genera *Trifolium* and *Vicia, Lathyrus, Astragalus* and *Medicago*. Eight of the studied genera were represented by only one species (Table 27). The analysed samples were taken from plants in the stages of expanded vegetation, pre-flowering or at the beginning of flowering. The time of sampling fell between 20 June and 30 June of each year, which is known in sub-arctic flora ecosystems as a period of very active photosynthesis during the extended daylight conditions of the "white nights". During this time of year, light and temperature conditions were generally very similar at each experimental site. Each year, leaf samples were randomly selected from the same habitat and the same populations.

4.2.1. Method of QAs$^{(+)}$ indications

A DRG indicator has been used in the test for QAs in all the species studied. This quick and accurate qualitative method was developed and used to detect alkaloids and heterocyclic nitrogen compounds, especially in plant drug analysis[755]. A DRG indicator has been successfully used to obtaining qualitative indications of total QAs in previous studies[7,740,746,748,756]. This method was re-evaluated before being implemented in this study. Pure alkaloids and alkaloid-rich extracts were placed separately on TLC plates and, together with a TLC plate without an alkaloid (the control TLC), they were precipitated by the DRG. On all of the TLC plates, with the exception of the control TLC, a colour change occurred. This establishes that the DRG method functions well. In the sample-testing process, indications were obtained from the juice of the leaves on the TLC plates precipitated by the DRG. A colour change from yellow to orange signalled a positive indication (+), whereas no colour change served as a negative indication (−). At this juncture, some critical remarks on this method should be mentioned. The DRG represents a means of indicating the total amount of QAs as a specific class of alkaloids as chemical compounds but not in terms of specific alkaloids. The DRG indication reflects only the presence or absence of this group of compounds. In reality, there can be different alkaloids or their derivatives in different plant species. A DRG positive indication was studied and qualified this in comparison with GC and GC MS^{+} methods. The (+) indication mechanism is based on a reaction between N^{+} constructed in the QA ring as a result of a DRG final reaction with H^{+}



The orange colour change on the TLC plates was caused by I_2^{-} after a reaction with N^{+} in the alkaloid ring with H^{+} from HI. All QAs and their derivatives have

a heterocyclic structure and N^+ in the ring. The intra-molecular hydrogen bond N−H has been discussed in the literature[757], and the alkaloid indicator method used in this research worked very well. Although differences in colour change intensity were observed, this did not lead to any methodological problems. The concentration of QA skeletons in the plants and species varied. Only indications of the presence or absence of total compounds has been studied in this work. All test indications were clearly readable for colour change or absence of change. One hundred tests per species were conducted during the research period (i.e. 20 individual plants from each species per year).

4.2.2. Mutational trajectories

Populations follow mutational trajectories that move them upwards within the genotypic fitness landscape[758,759]. Changes in plants first result in short-term evolutionary (micro-evolutionary) dynamics and subsequently, in a much more extended time-scale, the mutagenesis process. A succession of novel, beneficial mutations may appear and become fixed during the later macro-evolutionary process. Many authors consider both processes important in understanding natural selection[758,759,760,761]. For these categories changes in both individual alkaloid$^{(+)}$ and individual alkaloid$^{(-)}$ plants within the populations were found. This question has not been previously studied. On the basis of changes and trends in the species' populations, the micro-evolution coefficient is expressed according to following established formula:

$$\mathrm{MEC} = (1 + \Sigma_{Fy}/Y_n) - 1$$

where MEC = micro-evolution coefficient; Σ = sum; F_y = average trait frequency of a plant with indication per year; Y_n = number of years; 1 = constant hypothetical coefficient, which means that the trait of an individual plant is the same from year to year (i.e. no trends, no mutations, no trajectories).

The MECs for a species are coefficients for real trends of plant traits during the period studied. Numerically, it can have a value from 0.00 to 1.00 and can also be interpreted, if needed, as a percentage. The lower the MEC, the weaker the rate of micro-evolution for the period of time studied. A maximum value (1.00) for the MEC signifies that a micro-evolution (trait change) has occurred in all the plants studied and, therefore, macro-evolution has occurred in the population. In this case, the new hypothetical constant coefficient is valid (no trends, no mutations, no trajectories). The MECs can be positive or negative. Positive MECs show that the traits are developing in a micro-evolutionary process, and negative MECs indicate the reverse. The MECs represent very useful parameters for measurement of micro-evolution in plant species.

All statistics and graphs were generated using SPSS and SigmaPlot 2002 for Windows Version 8.02 (SPSS Inc.). ANOVA was used to analyze the experimental data on alkaloid$^{(+)}$ frequencies. The average frequency and standard

deviation for each species were calculated, and five groups of species with different frequencies were established. The relative frequencies for each species with annual standard deviations for the populations of the species were found. The statistical significance of the variations in relative frequencies and the MECs for each species were calculated. Moreover, using ANOVA, the statistical significance of these coefficients and correlations with r between species were checked. To determine whether the MECs are similar or different, the BSR with R^2 as the best criterion, and the significance of possible different variables with the model were checked. Using the best criterion, it was possible to study the position of different MECs in relation to MECs for other species and evaluate their significance.

4.3. Results

4.3.1. QAs$^{(+)}$ occurrence and frequency

The QAs occurred in all the legume genera, species and sub-species studied. There were both QAs$^{(+)}$ and QAs$^{(-)}$ plants (Table 28). The frequency of QA occurrence mostly varied (0.1–0.8) between species ($P < 0.001$). The smallest frequency was found in only two species, *Trifolium arvense* and *Trifolium pratense*, in which positive test results were not found more frequently than every tenth individual (Table 28).

Conversely, the highest frequency [F(A)] was found in 14 species, in which at least every second individual tested positive for QAs (Table 29). Seven species exhibited a frequency range of 31–50% [F(B)] and 12 species and sub-species 21–30% [F(C)]. Moreover, 5 species had a group frequency F(D) range of 11–20% (Table 29). The largest relative frequency (RF) changes occurred in *Vicia* spp. and *Ononis repens*, and the smallest changes occurred in *L. polyphyllus* and *L. corniculatus* (Figures 101–106). Statistically significant frequency fluctuation (RF) was also observed during different years and in different species. The number of positive and negative individuals fluctuated according to species ($P < 0.001$). The average number of frequency fluctuation (RF) was also observed during different years and in different species. The number of positive and negative individuals fluctuated according to species ($P < 0.001$).

The average number of positive individuals clearly doubled for *Vicia hirsuta* during 1999–2001, after which the increase became smaller (Figure 106).

4.3.2. Tendencies of QAs$^{(+)}$ plants to evolve and their MECs

Thirty-three species had a tendency to decrease the number of individuals with QAs$^{(+)}$ ($P < 0.001$) but in the case of four species there was a tendency to increase the number of individuals with QAs$^{(+)}$ ($P < 0.001$). Three species

Table 28 Quinolizidine alkaloid frequencies in plant populations of Fabaceae in the boreal zone during 1999–2003

Species	Average Frequency in Populations $QAs^{(+)}$ Mean ($X_1/X_{n=100}$)
Astragalus alpinus	0.8 (0.03)
Astragalus arenarius	0.9 (0.04)
Astragalus cicer	0.9 (0.05)
Astragalus frigidus	0.9 (0.06)
Astragalus glycyphyllos	0.8 (0.04)
Coronilla varia	0.4 (0.03)
Cytisus scoparius	0.4 (0.02)
Lathyrus japonicus	0.7 (0.05)
Lathyrus linifolius	0.7 (0.04)
Lathyrus palustris	0.7 (0.04)
Lathyrus pratensis	0.7 (0.04)
Lathyrus sylvestris	0.8 (0.06)
Lathyrus vernus	0.7 (0.04)
Lotus corniculatus	0.6 (0.04)
Lupinus polyphyllus	0.9 (0.07)
Medicago sativa ssp. sativa	0.2 (0.02)
Medicago sativa ssp. falcate	0.3 (0.02)
Medicago lupulina	0.3 (0.03)
Meliotus officinalis	0.3 (0.03)
Ononis repens	0.3 (0.04)
Ornithopus perpusillus	0.4 (0.01)
Oxytropis campestris	0.6 (0.05)
Trifolium arvense	0.1 (0.02)
Trifolium aureum	0.3 (0.04)
Trifolium hybridum	0.3 (0.02)
Trifolium fragiferum	0.3 (0.03)
Trifolium medium	0.4 (0.05)
Trifolium montanum	0.4 (0.04)
Trifolium pratense	0.1 (0.01)
Trifolium spadiceum	0.4 (0.03)
Trifolium repens	0.4 (0.04)
Vicia cassubica	0.3 (0.03)
Vicia cracca	0.2 (0.03)
Vicia hirsuta	0.2 (0.03)
Vicia lathyroides	0.3 (0.03)
Vicia sativa	0.2 (0.03)
Vicia sepium	0.2 (0.02)
Vicia villosa	0.3 (0.03)
Vicia sylvatica	0.3 (0.04)
Vicia tetrasperma	0.3 (0.05)

Abbreviations: X_1 – number of individuals with $QAs^{(+)}$; X_n – total number of individuals; In parenthesis standard deviation of mean frequency; $P < 0.001^{***}$.

Table 29 Frequencies of QAs$^{(+)}$ in the legume species studied

Species with F(A)	Species with F(B)	Species with F(C)	Species with F(D)	Species with F(E)
Astragalus alpinus	*Coronilla varia*	*Medicago sativa* spp *falcata*	*Medicago sativa* ssp *sativa*	*Trifolium arvense*
Astragalus arenarius	*Cytisus scoparius*	*Medicago lupulina*	*Vicia cracca*	*Trifolium pratense*
Astragalus cicer	*Ornithopus perpusillis*	*Meliotus officinalis*	*Vicia hirsuta*	
Astragalus frigidus	*Trifolium medium*	*Ononis repens*	*Vicia sativa*	
Astragalus glycyphyllos	*Trifolium montanum*	*Trifolium aureum*	*Vicia sepium*	
Lathyrus japonicus	*Trifolium spadiceum*	*Trifolium hybridum*		
Lathyrus linifolius	*Trifolium repens*	*Trifolium fragiferum*		
Lathyrus palustris		*Vicia cassubica*		
Lathyrus pratensis		*Vicia lathyroides*		
Lathyrus sylvestris		*Vicia villosa*		
Lathyrus vernus		*Vicia sylvatica*		
Lotus corniculatus		*Vicia tetrasperma*		
Lupinus polyphyllus				
Oxytropis campestris				

Abbreviations: F(A) = frequency >50%; F(B) = frequency 31–50%; F(C) = frequency 21–30%; F(D) = frequency 11–20%; F(E) = frequency 1–10%.

had no tendency to change ($P < 0.001$) the number of individuals with QAs$^{(+)}$ (Figures 107–112). Only four species (*Astragalus arenarius* [$R^2 = 0.65$], *Astragalus cicer* [$R^2 = 0.552$] *Astragalus frigidus* [$R^2 = 0.64$] and *L. polyphyllus* [$R^2 = 0.706$]) had positive MECs ($P < 0.001$), which indicates that these species, more clearly than other species, are increasing and will consequently increase the number of individuals with QAs$^{(+)}$ (Figures 107–108). *Astragalus alpinus* ($R^2 = 0.551$), *Astragalus glycyphyllos* ($R^2 = 0.065$) and *Lathyrus sylvestris* ($R^2 = 0.91$) (Figures 107 and 109) had the MEC value of 0.0, indicating that these species have no tendency towards micro-evolution in the case of QAs$^{(+)}$ and QAs$^{(-)}$. All other species studied have a micro-evolutionary tendency to reduce their QAs$^{(+)}$ frequencies (Figures 107 and 112). This trend was highest in *T. arvense* ($R^2 = 0.87$) and *T. pratense* ($R^2 = 0.94$) (in both cases 0.7) and *Vicia cracca, V. hirsuta, Vicia sativa* and *Vicia sepium* ($R^2 = 0.079$) (−0.6 in each case) (Figures 111 and 112). Statistical analysis strongly confirmed the validity of these results.

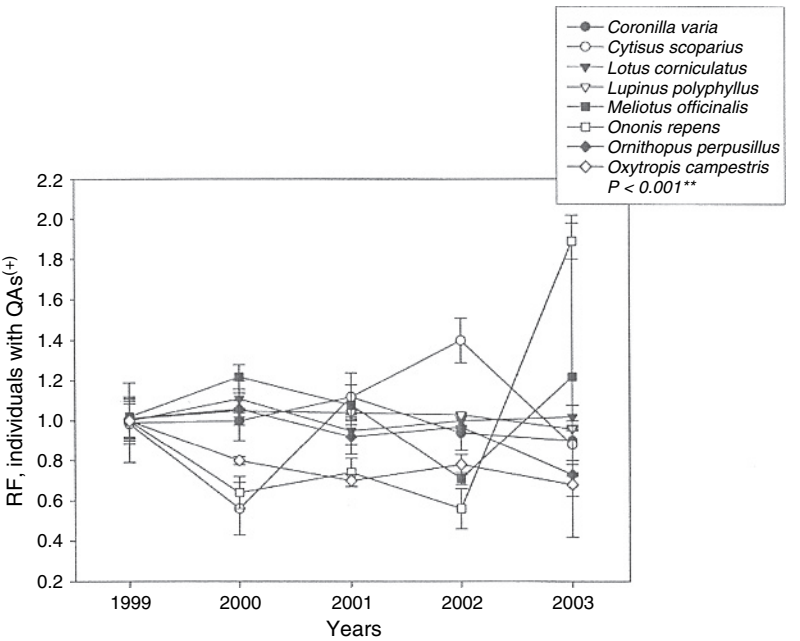


Figure 101. RF of QAs(+) individuals in *Coronilla varia, Cytisus scoparius, Lotus corniculatus, Lupinus polyphyllus, Meliotus officinalis, Ononis repens, Ornithopus perpusillus, Oxytropis campestris* during 1999–2003.

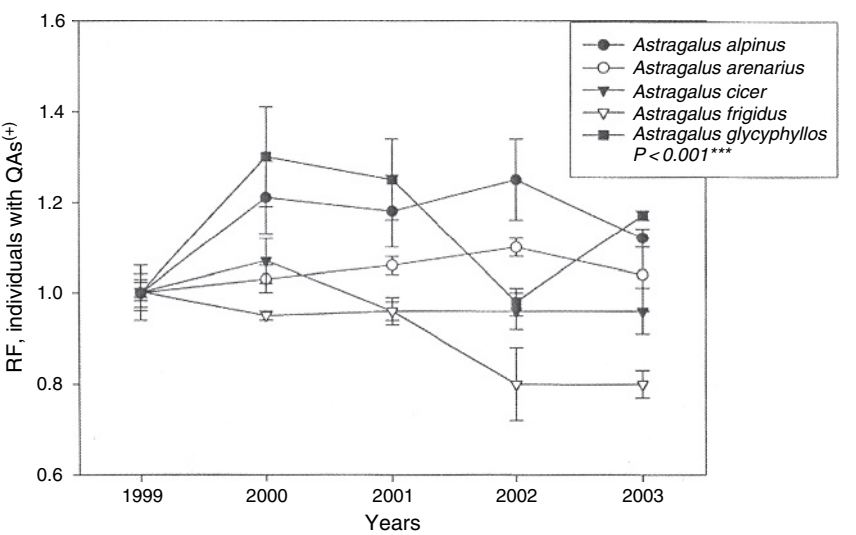


Figure 102. RF of QAs(+) individuals in *Astragalus* spp. during 1999–2003.

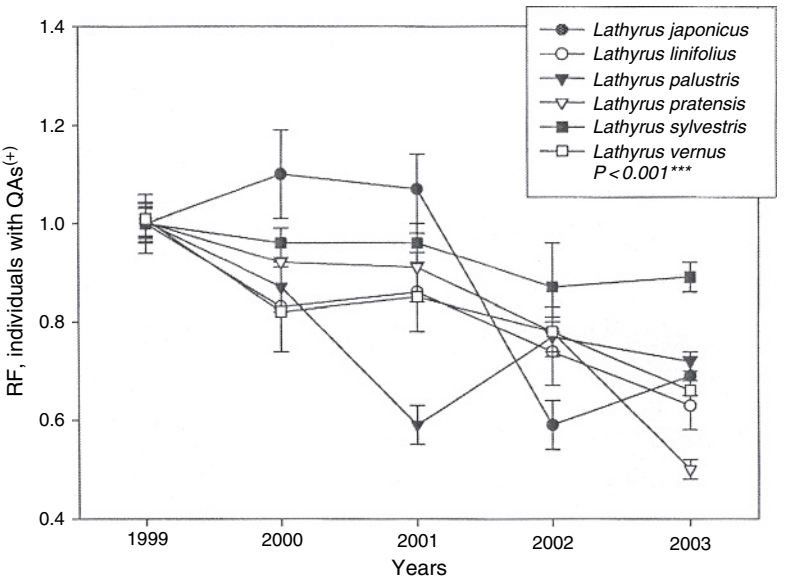


Figure 103. RF of QAs$^{(+)}$ individuals in *Lathyrus* spp. during 1999–2003.

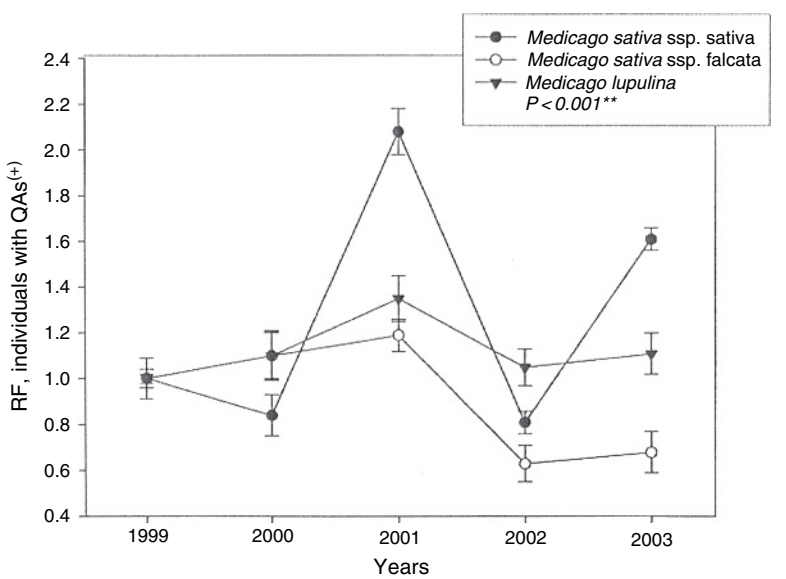


Figure 104. RF of QAs$^{(+)}$ individuals in *Medicago* spp. during 1999–2003.

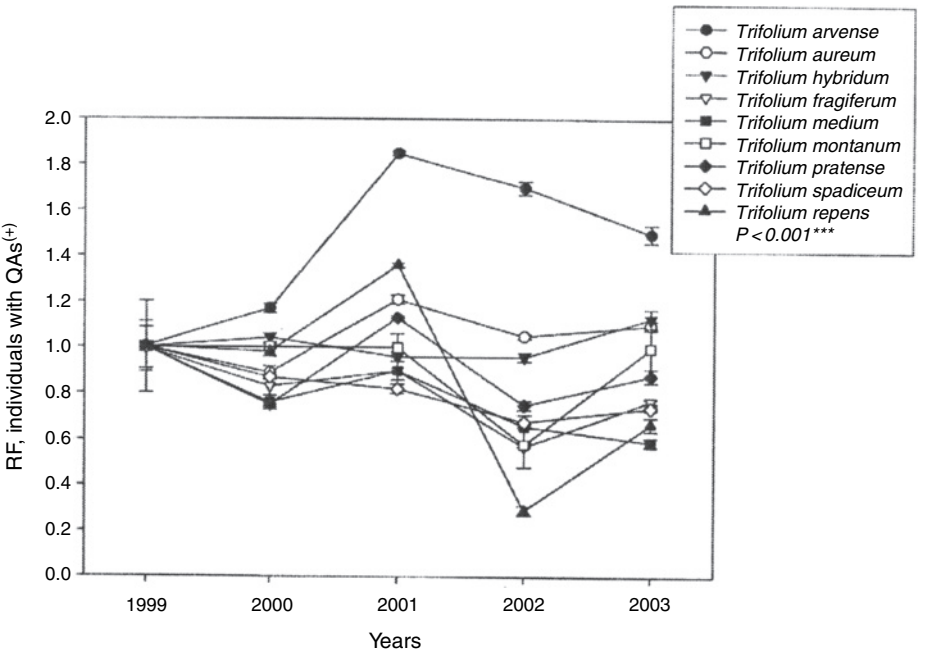


Figure 105. RF of QAs$^{(+)}$ individuals in *Trifolium* spp. during 1999–2003.

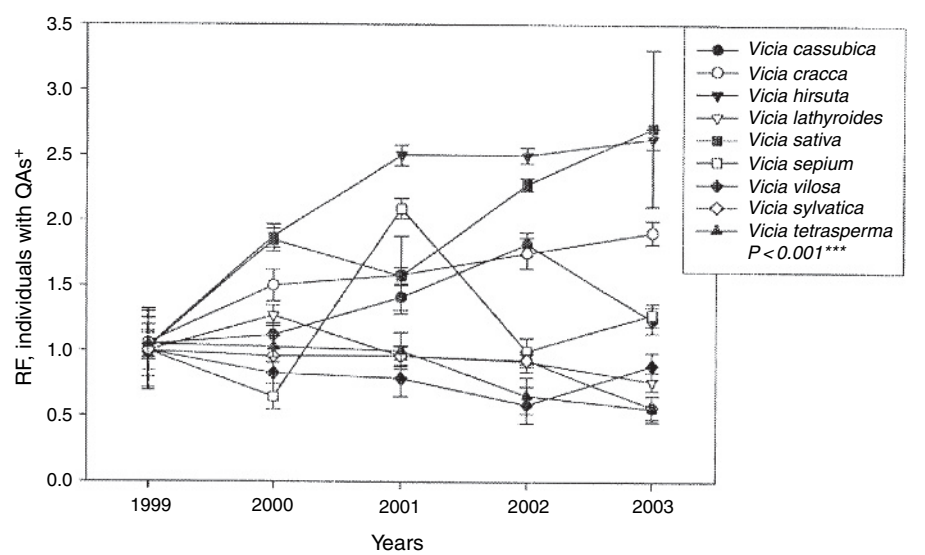
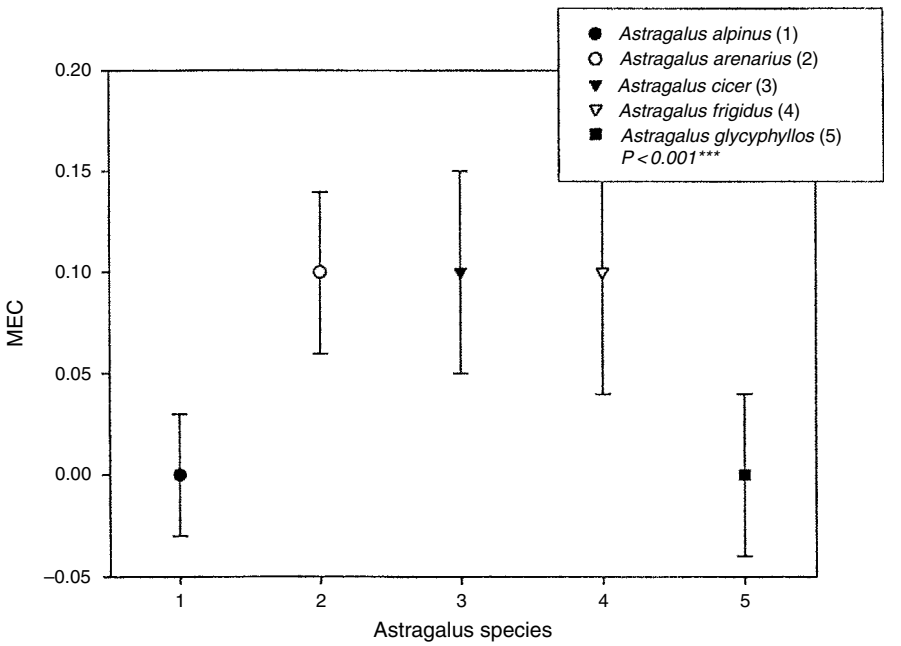


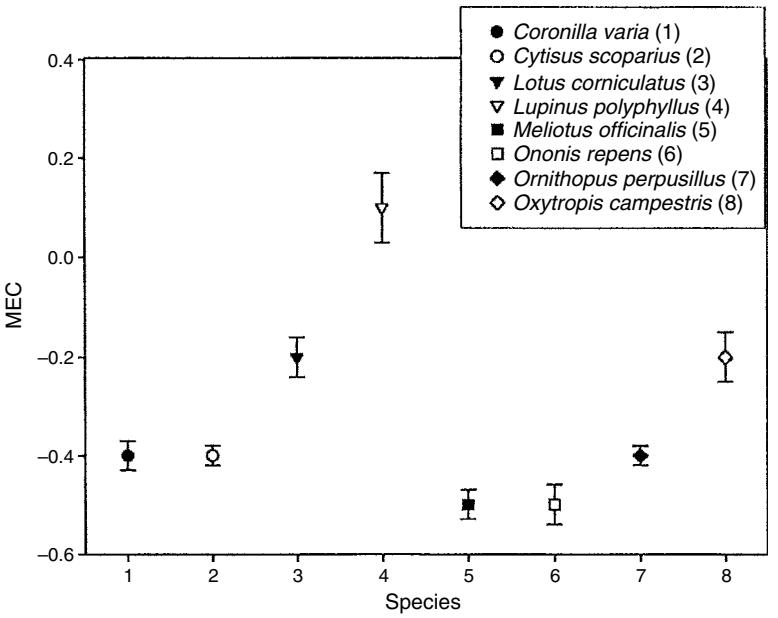
Figure 106. RF of QAs$^{(+)}$ individuals in *Vicia* spp. during 1999–2003.



Species *r*	2	3	4	5
1	–0.094	–0.037	0.33***	–0.11
2		0.495***	0.42***	–0.28**
3			0.49***	–0.32**
4				–0.29**

Species model ABCDEF	R^2
A. *cicer**** A	0.24***
A. *cicer*** – A. *glycyphyllos*** B	0.46***
A. *alpinus*** – A. *cicer*** – A. *glycyphyllos*** C	0.551***
A. *alpinus*** – A. *arenarius* – A. *cicer*** – A. *glycyphyllos*** D	0.552***
A. *arenarius*** – A. *frigidus*** – A. *glycyphyllos*** E	0.64***
A. *alpinus*^{ns} – A. *arenarius*** – A. *frigidus***– A. *glycyphyllos*** F	0.65***

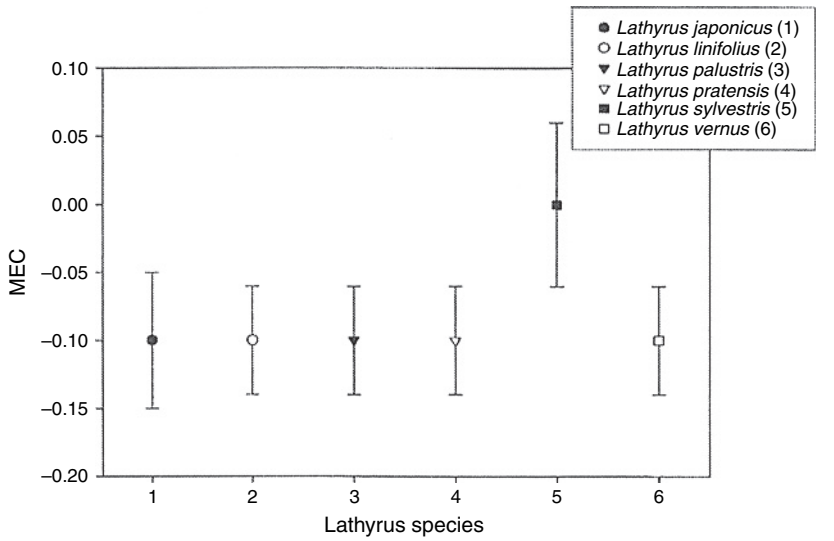
Figure 107. MEC of *Astragalus* species.



Species *r*	2	3	4	5	6	7	8
1	0.104	–0.109	0.164	0.065	0.495***	0.066	0.207
2		0.326***	–0.167	–0.304**	0.411***	0.319***	0.395***
3			–0.099	–0.088	–0.312**	–0.009	–0.086
4				–0.045	0.03	–0.032	–0.031
5					–0.512***	0.101	0.396***
6						0.286**	0.389***
7							0.030

Species model ABCDEFG	R^2
*Oxytropis campestris**** A	0.555***
*Oxytropis campestris**** – *Coronilla varia* *** B	0.605***
*Oxytropis campestris**** – *Coronilla varia* *** – *Lotus corniculatus** C	0.623***
Ononis repens – *Oxytropis campestris* *** – *Coronilla varia**** – *Lotus corniculatus*** D	0.637***
*Ononis repens*** – *Ornithopus perpusillus**** – *Oxytropis campestris**** – *Coronilla varia** ** – *Lotus corniculatus**** E	0.672***
*Ononis repens**** – *Ornithopus perpusillus**** – *Oxytropis campestris**** – *Coronilla varia** ** – *Cytisus scoparius*** – *Lotus corniculatus**** F	0.696***
*Ononis repens**** – *Ornitbhopus perpusillus**** – *Oxytropis campestris**** – *Coronilla varia** **– *Cytisus scoparius** – *Lotus corniculatus**** – *Lupinus polyphyllus* G	0.706***

Figure 108. MEC of *Coronilla, Cytisus, Lotus, Lupinus, Meliotus, Ononis, Ornithopus, Oxytropis* spp.



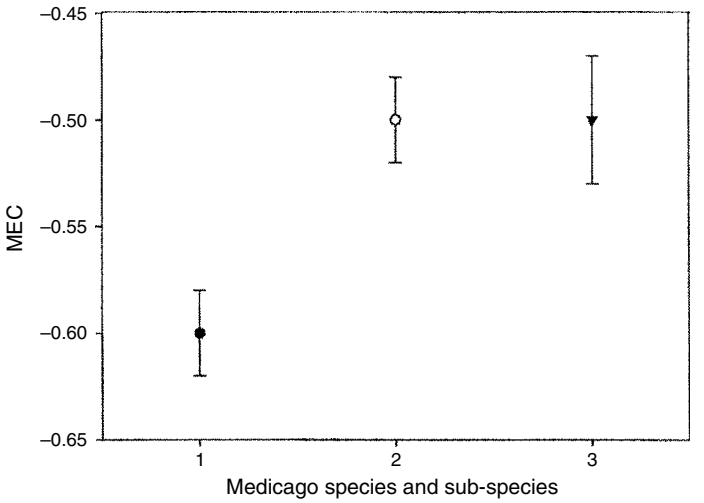
Species *r*	2	3	4	5	6
1	0.06	0.061	0.015	–0.06	–0.06
2		0.053	0.047	0.07	0.052
3			0.58***	0.163	–0.47
4				–0.36***	0.08
5					0.18

Model ABCDE	R^2
*Lathyrus japonicus – Lathyrus palustris**** A	0.95***
*Lathyrus japonicus – Lathyrus palustris**** *– Lathyrus sylvestris**** B	0.97***
*Lathyrus japonicus – Lathyrus palustris**** *– Lathyrus pratensis – Lathyrus sylvestris**** C	0.97***
*Lathyrus japonicus – Lathyrus palustris**** *– Lathyrus pratensis – Lathyrus sylvestris**** *– Lathyrus vernus* D	0.97***
*Lathyrus japonicus – Lathyrus linifolius – Lathyrus palustris**** *– Lathyrus pratensis – Lathyrus sylvestris**** *– Lathyrus vernus* E	0.91***

Figure 109. MEC of *Lathyrus* species.

4.3.3. Tendencies and dependencies of species

The genus *Astragalus* (Figure 107) clearly had different tendencies in evolutionary trait variations ($P < 0.001$). *Astragalus arenarius, A. cicer* and *A. frigidus* had similar evolutionary tendencies (MEC = 0.10) but their SDs differed, the



Medicago sativa ssp. sativa (1)
Medicago sativa ssp. falcata (2)
Medicago lupulina (3)

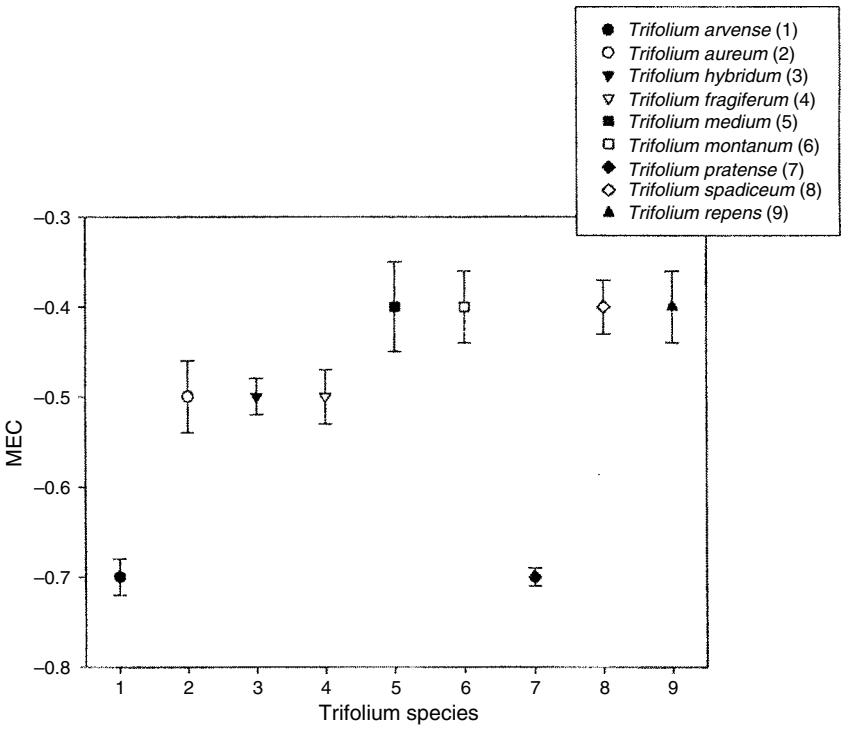
Species and sub-species *r*	2	3
1	−0.15	−0.05
2		0.49 ***

Model AB	R^2
Medicago sativa ssp. *sativa* *** – *Medicago sativa* ssp. *falcata* A	0.022
Medicago sativa ssp. *sativa* *** – *Medicago sativa* ssp. *falcata* – *Medicago lupulina* B	0.023

Figure 110. MEC of *Medicago sativa* and *Medicago lupulina*.

most stable being the case of *A. arenarius*. Changes in the MECs of this species correlated significantly with the MECs of *A. cicer* ($r = 0.495$) and *A. frigidus* ($r = 0.42$). However, *A. glycyphyllos* displayed a negative correlation ($r = -0.28$). Changes in *A. cicer* correlated positively with *A. frigidus* ($r = 0.49$) and negatively with *A. glycyphyllos* ($r = -0.32$). Moreover, *A. alpinus* also had a negative correlation with *A. arenarius* ($r = -0.094$) and *A. cicer* ($r = -0.037$).

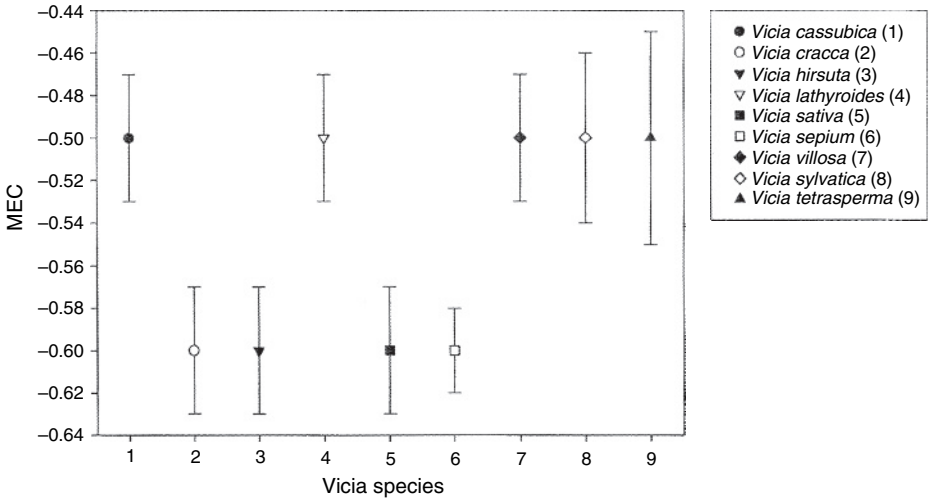
Lupinus polyphyllus had negative correlations with other species (Figure 108). Within genus *Lathyrus*, there were similar tendencies with the exception of *L. sylvestris* (Figure 109). *Medicago sativa* ssp. *sativa* and *Medicago sativa* ssp. *falcata* had a slight negative correlation, and they both had negative MECs.



species *r*	2	3	4	5	6	7	8	9
1	−0.22	−0.139	−0.43***	−0.88	−0.05	−0.06	0.08	−0.004
2		0.17	−0.06	−0.07	−0.07	0.19	−0.17	0.06
3			−0.057	0.141	−0.005	−0.111	−0.15	−0.07
4				−0.49***	−0.48***	0.22	0.48***	0.05
5					0.07	0.15	−0.37***	0.008
6						0.01	−0.31***	−0.05
7							0.27***	0.18
8								0.13

Model ABCDEFGH	R^2
*T. arvense**** – *T. pratense**** A	0.42***
*T. arvense**** – *T. aureum**** – *T. pratense**** B	0.54***
*T. arvense**** – *T. aureum* – *T. montanum**** – *T. pratense**** C	0.61***
*T. arvense**** – *T. aureum**** – *T. medium**** – *T. montanum**** – *T. pratense**** D	0.65***
*T. arvense**** – *T. aureum**** – *T. medium**** – *T. montanum**** – *T. pratense**** – *T. spadiceum**** E	0.68***
*T. arvense**** – *T. aureum**** – *T. hybridum* – *T. fragiferum**** – *T. montanum**** – *T. pratense**** – *T. spadiceum**** F	0.87***
*T. arvense**** – *T. hybridum**** – *T. fragiferum**** – *T. medium**** – *T. montanum**** – *T. pratense**** – *T. spadiceum**** – *T. repens* G	0.94***

Figure 111. MEC of *Trifolium* species.



Species *r*	2	3	4	5	6	7	8	9
1	–0.01	0.05	–0.06	0.09	–0.09	0.07	0.14	0.01
2		0.29**	0.09	0.50***	0.08	0.04	0.24*	0.45***
3			0.23*	0.40***	–0.39***	0.01	0.09	0.33
4				0.78	–0.27**	0.21*	0.18	–0.08
5					0.07	–0.26**	0.50***	0.26**
6						0.22*	–0.08	–0.05
7							–0.07	–0.05
8								–0.14

Model ABCDEFGH	R^2
*V. cassubica*** – *V. sylvatica* A	0.02
*V. cassubica*** – *V. villosa* – *V. sylvatica* B	0.03
*V. cassubica*** – *V. lathyroides* – *V. villosa* – *V. sylvatica** C	0.05
*V. cassubica*** – *V. cracca* – *V. lathyroides* – *V. sepium** – *V. villosa* D	0.055
*V. cassubica** – *V. cracca* – *V. lathyroides* – *V. sepium* – *V.villosa* – *V.sylvatica* E	0.06
*V. cassubica*** – *V. cracca* – *V. lathyroides* – *V. sepium* – *V.villosa* – *V.sylvatica* – *V. tetrasepium* F	0.076
*V. cassubica*** – *V.cracca* – *V. birsuta* – *V. lathyroides* – *V. sepium** – *V.villosa** – *V .sylvatica* – *V. tetrasepium* G	0.078
*V. cassubica** – *V. cracca* – *V. birsuta* – *V. lathyroides* – *V. sativa* – *V. sepium** – *V. villosa* – *V. sylvatica* – *V. tetrasepium* H	0.079

Figure 112. MEC of *Vicia* species.

The latter-mentioned *Medicago* sub-species exhibited a positive correlation ($r = 0.49$) with *M. lupulina* (Figure 110). Differences were also found within the genus *Trifolium* and the genus *Vicia*, and the evolutionary tendencies of both species were shown to be similar (Figures 111–112). The analyses of the correlations and the best R^2 strongly confirmed the utilization of MECs as a method of evolutionary analysis.

4.4. Discussion of case study results

The QAs occur in all the legume species included in this study but not in all individuals. This suggests additional evidence of the evolutionary role of secondary compounds, which are important for plant survival and reproductive fitness[7,325]. Thus, the mechanism for production of QAs has hereditary characteristics[740,746,747]. In some respects, the occurrence of alkaloids in legumes was disputed by previous literature. However, there was no dispute over whether QAs occur in Fabaceae. Bohlmann and Schumann[742] observed that occurrences of QAs are restricted to the Sophorae, Podalyrieae and Genistae tribes. However, Aslanov et al.[744] stated that a quinolizidine structure is found occasionally in molecules of complex alkaloids belonging to the indole, isoquilinone or other families of alkaloids not belonging to the quinolizidine series. Subsequently, Wink and Mohamed[762] stated that the distribution of QAs is restricted to the genistoids, whereas many of the other legumes produce non-protein amino acids. This analysis of the QAs based on a N^+-ring clearly demonstrates that plant individuals with $QAs^{(+)}$ were found in all the legume species studied. The frequencies of positive and negative alkaloid individuals certainly fluctuated according to plant species, confirming the validity of the study hypothesis. Therefore, the basic finding of this research is that QAs occur during a period of vegetation expansion and that their selectivity occurs not at the species level but rather according to individual, genetically regulated metabolisms. This is a logical explanation in the light of the fact that there are well-known alkaloid-rich and alkaloid-poor species of plants that have been developed by genetic selection (artificial evolution)[740,746,747,748]. There exists evidence that an alkaloid is plant specific and that the occurrence of QAs in plant individuals is connected with the metabolism of lysine. There exists a surplus of lysine in expanded vegetation, which leads to the production of QAs through the activity of HMT/HLTase and ECTase[350]. In individual plants without QAs, the biosynthetic pathway of the alkaloids with HTM/HLTase and ECTase is blocked[351]. Therefore, QAs and probably all other secondary compounds can be subjected to natural selection. These results clearly show that alkaloid distribution and frequencies among legume species and individuals indicate natural selection. Five groups of different frequencies [F(A), F(B), F(C), F(D), F(E)] have been

found, and inside these groups different trends are apparent. This also supports the basic hypothesis of this study.

The distribution and frequency changes of alkaloids is a direct expression of natural selection. It is widely recognized that an understanding of biochemical diversity will facilitate the interpretation of the evolution of plants[763,776]. Moreover, evolutionary changes can occur rapidly and may be an important means by which species could escape extinction in the face of global change[764]. The fluctuation of $QAs^{(+)}$ and $QAs^{(-)}$ plants in legume populations is also relevant in this respect as QAs are known to be metabolism-protective secondary compounds[7,736,765]. The $QAs^{(+)}$ and $QAs^{(-)}$ plants are products of natural hybridization, which is based on ecological distribution and evolutionary diversification. Specifically, the occurrence of mutations as the result of the discrete changes in the mutant is evidence of diversification in short-term evolutionary dynamics. Although most of the species studied have only discrete MECs, there are clearly observed trends that show an increase in the number of $QAs^{(+)}$ plants in the case of 4 species and a decrease of this parameter in the case of 33 species. Three species had no tendency in this direction. As QAs are plant-protective compounds and also important for herbivores, a micro-evolution towards a decrease in the number of $QAs^{(+)}$ plants in a species can weaken the protective ability of the species and curtail the overall population size. In turn, this will certainly place pressure on herbivores and their populations. Moreover, some alkaloid-containing plant genera are often readily ingested by livestock[767]. In addition, plant toxins from some legumes have applications in human bio-medicine[766]. The micro-evolution of alkaloid distribution in legume plants is therefore a crucial topic.

Four species have positive MECs: *A. arenarius, A. cicer, A. frigidus* and *L. polyphyllus*. One might ask why these species differ in this respect to other legumes. Future studies will hopefully provide an exact answer to this question. Presently, it can be mentioned that these species do share some characteristics. For example, all of these species are perennials with very rapid growth-rates in Nordic ecosystems. They also have a very competitive capacity to proliferate in the areas where they grow. These plants also have a strong pollination-based mating system, being high rate outcrossers. On the other hand, the species with negative MECs (e.g. *T. arvense* and *T. pratense*) are common plants in Nordic ecosystems. They also have a pollination-based mating system and a high rate of outcrossing. Natural selection is probably in both cases connected with heterozygosity and other life characteristics of the species and with their modification reasoning. Darwin already recognized a difference between the types of modifications due to diversification[768]. Evolutionary trait variations in alkaloid distribution among wild and semi-wild Fabaceae species can also be discussed from this point of view. Of the 40 legume species and sub-species studied here, only 3 species did not have a tendency to change (micro-evolutionary coefficients of zero). According to Darwin's theory, if a species diversifies in order

to occupy more places in the polity of nature, the change is depicted by relative distance along the horizontal dimension between different species. Alternatively, if a species is modified in order to become better adapted to its habitat within the polity of nature, there is no movement along the horizontal dimension[768]. In this sense, the species not perfectly adapted to their habitats and growing conditions are more disposed towards modifications and micro-evolutionary changes.

The variations in the distribution of the alkaloid ring in legume individuals is an evolutionary trait, which can be measured by an MEC. Prior to its development for the purpose of this study, this assertion has not been previously mentioned in the research literature. The question which follows is what do MECs, in fact, measure? They directly measure trends in trait change, and they indirectly measure the presence or absence of a mutational trajectory in the population of species. Therefore, MECs are useful coefficients for determining trends in the genetic oscillation of plants during a relatively short evolutionary period. Moreover, MECs also clearly manifest a tendency towards polymorphism in the biosynthesis of $QAs^{(+)}$, which has evolutionary implications. The genes that encode enzymes for the ring-closure step are repressed in alkaloid-negative plants and expressed in alkaloid-positive plants[351]. Polymorphism can create some difficulties in systematic plant diagnostics[753]. In addition, the current responses of ecological systems to climate may also represent an evolutionary role. It is possible that $QAs^{(+)}$ trends in individual plants can be regarded as a result of this kind of adaptive response in plant populations. Either way, $QAs^{(+)}$ and $QAs^{(-)}$ individuals in plant populations can be considered as evidence of current evolutionary responses by ecological and genetic systems.

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APPENDIX

Alkaloid Extraction Protocol

One can find a number of methods of quinolizidine alkaloid extraction within existing literature. The most frequently applied methods are extraction by alcohols (50% methanol or 96% ethanol, or these alkaloids with a 1% addition of glacial acetic acid).

Wysocka and Przybył presented an efficient extraction method of quinolizidine alkaloids[245]. The authors themselves developed and investigated this method, in which includes the following steps of extraction:

- maceration of the ground lupin seeds with 25% aq. KOH for about 36 hours in order to destroy the tissues and release the alkaloids from their salts;
- excess water present in the alkaline pulp is absorbed by diatomaceous earth;
- mass prepared in this way is poured into a linen sack and then placed in an extractor followed by elution with ethyl ether and then with methylene chloride;
- extracts are condensed to a volume of about 100 cm^3 and the alkaloids are eluted with 2 N hydrochloric acid;
- in order to degrease, the acidic alkaloid solution is extracted with petroleum ether (bp 40–60 °C) until the fats have been removed from the organic layers;
- after degreasing, the aqueous solution is made alkaline with 50% aq. KOH and then eluted with ethyl ether and methylene chloride.

This method has since been modified for efficiency, resulting in the following steps:

1. Grinding of seeds
2. Degreasing of the ground seeds with petroleum ether
3. Drying of the seeds in the air
4. Maceration of the meal with 25% aq. KOH
5. Mixing of the macerated meal with diatomaceous earth
6. Elution of alkaloids with methylene chloride
7. Purification of the extract by filtration through a column with aluminium oxide (activity grade II)
8. Evaporation of the extract to a constant weight
9. Yellow solidifying oil.

This method is more sensitive than other established methods. The amount of alkaloids obtained through this method is considerably higher than that through alcohol extraction.

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