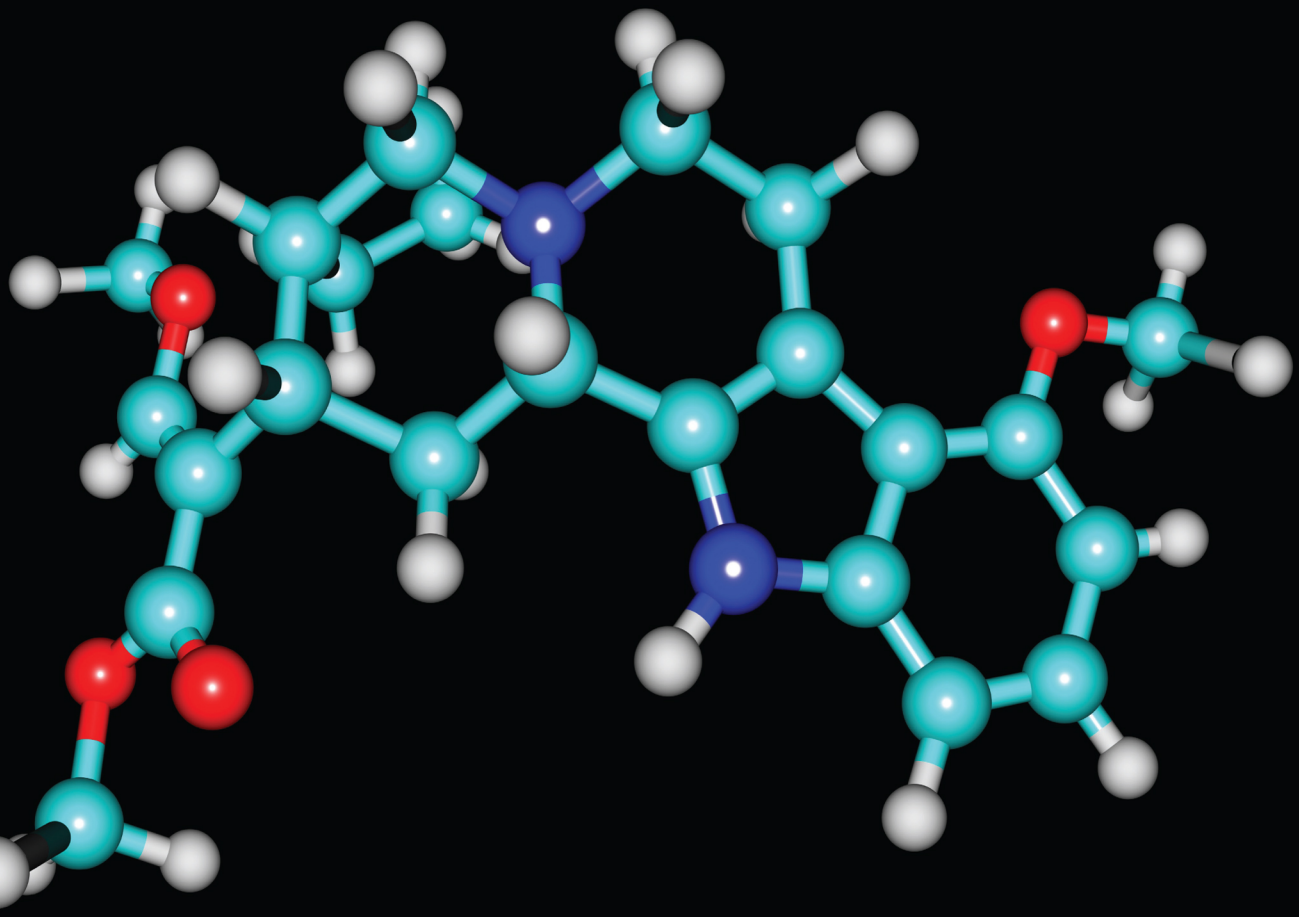

The Alkaloids

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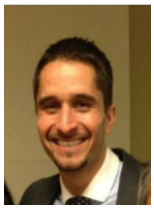

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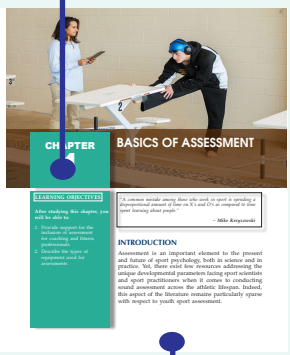
HOW TO USE THE BOOK

This book has been divided into many chapters. Chapter gives the motivation for this book and the use of templates. The text is presented in the simplest language. Each paragraph has been arranged under a suitable heading for easy retention of concept. Keywords are the words that academics use to reveal the internal structure of an author's reasoning. Review questions at the end of each chapter ask students to review or explain the concepts. References provides the reader an additional source through which he/she can obtain more information regarding the topic.

LEARNING OBJECTIVES

See what you are going to cover and what you should already know at the start of each chapter

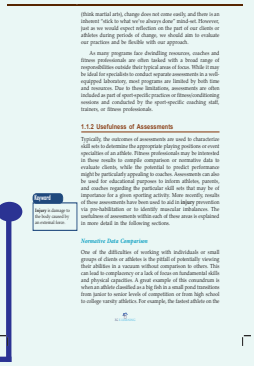


ABOUT THIS CHAPTER

An introduction is a beginning of section which states the purpose and goals of the topics which are discussed in the chapter. It also starts the topics in brief.

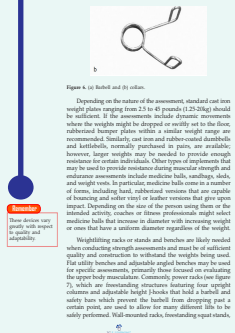
REMEMBER

This revitalizes a must read information of the topic.



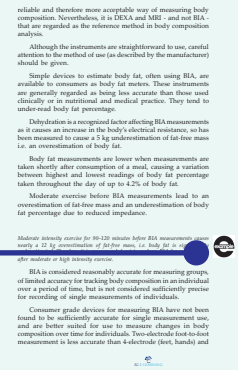
KEYWORDS

This section contains some important definitions that are discussed in the chapter. A keyword is an index entry that identifies a specific record or document. It also gives the extra information to the reader and an easy way to remember the word definition.



DID YOU KNOW?

This section equip readers the interesting facts and figures of the topic.



reliable and therefore more acceptable way of measuring body composition. Nevertheless, it is DEXA and MRI - and not BIA - that are regarded as the reference method in body composition analysis.

Although the instruments are straightforward to use, careful attention to the method of use (as described by the manufacturer) should be given.

Simple devices to estimate body fat, often using BIA, are available to consumers as body fat meters. These instruments are generally regarded as being less accurate than those used clinically or in nutritional and medical practice. They tend to under-read body fat percentage.

Dehydration is a recognized factor affecting BIA measurements as it causes an increase in the body's electrical resistance, so has been measured to cause a 5 kg underestimation of fat-free mass i.e. an overestimation of body fat.

Body fat measurements are lower when measurements are taken shortly after consumption of a meal, causing a variation between highest and lowest readings of body fat percentage taken throughout the day of up to 4.2% of body fat.

Moderate exercise before BIA measurements lead to an overestimation of fat-free mass and an underestimation of body fat percentage due to reduced impedance.

Moderate intensity exercise for 90-120 minutes before BIA measurements caused nearly a 12 kg overestimation of fat-free mass, i.e. body fat is reported as lower after moderate or high intensity exercise.

BIA is considered reasonably accurate for measuring groups, of limited accuracy for tracking body composition in an individual over a period of time, but is not considered sufficiently precise for recording of single measurements of individuals.

Consumer grade devices for measuring BIA have not been found to be sufficiently accurate for single measurement use, and are better suited for use to measure changes in body composition over time for individuals. Two-electrode foot-to-foot measurement is less accurate than 4-electrode (feet, hands) and

Did You Know?

According to Deming, during his lectures in Japan in the early 1950s, the Japanese participants shortened the steps to the now traditional plan, do, check, act.

a model stemming from the field of quality management, termed the Plan-Do-Check-Act (PDCA) cycle (see figure 1), may be used to illustrate the importance of assessments.

Figure 1. PDCA cycle.

The Plan, Do, and Act portions of this cycle represent the traditional qualitative strengths of coaches and fitness professionals. The Plan portion entails the initial strategic analysis and goal-setting procedure, Do is the execution of the plan, and Act is the summative response (i.e., evaluating or making sense of the available information) and adjustment to this implementation. The Check portion of the PDCA cycle represents formative feedback (i.e., bringing together or monitoring of the available information) from knowledge-based quantitative data collection via appropriate assessments that inform the decision-making process. This cyclical approach with integrated qualitative (via observation) and quantitative (via data) components allows for the management of both individual client or athlete needs as well as reflection on the strategic approach. For example, through use of the PDCA cycle, coaches and fitness professionals might determine if specific adjustments need to be made on an individual basis from a single cycle or, as a result of several cycles, if a change to the process employed by the training staff should be considered.

Assessments should allow for a properly informed decision-making process. The results of well-designed and appropriately selected assessments can be used by the coaching or training staff and other stakeholders to design and modify training

EXAMPLE

The book cabinets' examples to illustrate specific ideas in each chapter.

ROLE MODEL

A biography of someone who has/had acquired remarkable success in their respective field as Role Models are important because they give us the ability to imagine our future selves.

ROLE MODEL

EVGENIYA KANAEVA: RUSSIAN RHYTHMIC GYMNAST

Evgeniya Olegovna Kanaeva is a Russian retired individual rhythmic gymnast, known for her consistency, elegant routines and high level of technical difficulty. She is the only individual rhythmic gymnast in history to win two Olympic all-around gold medals, winning at the 2008 Summer Olympics, where she finished with 3.75 points ahead of silver medalist Inna Zhukova, and at the 2012 Summer Olympics, where she also became the oldest gymnast to win the Olympic gold. On 4 July 2013, Kanaeva received the International Fair Play Award for "Sport and Life".

Kanaeva holds the record for most World titles with seventeen and thirteen European titles. Kanaeva shares the record for most individual world all-around titles with Maria Petrova, Maria Gigova and fellow Russian gymnasts Yana Kudryavtseva and Dina Averina, and Kanaeva is the one of only three gymnasts to have won all three titles without being tied, impossible due to the tie breaking system even though she never was tied for a title.

At the 2009 World Championship in Mie, Japan, Kanaeva became the first rhythmic gymnast to win all six titles. She repeated the feat at the 2011 World Championship in Montpellier, France, equaling her own record.

Kanaeva is the only gymnast to receive a perfect score under the 30-point judging system, having done so twice: in the 2011 Grand Prix Final in Brno and in the 2012 Grand Prix in Vorarlberg.

In 2009, Kanaeva was awarded the title Merited Master of Sports in Russia. After the 2012 Summer Olympics, on 15 August at the Grand Kremlin Palace, Kanaeva, along with fellow Olympic gold medalists, was awarded the Merit for the Fatherland IV Degree. Russian President Vladimir Putin presented the honors.

CASE STUDY

This reveals what students need to create and provide an opportunity for the development of key skills such as communication, group working and problem solving.

CASE STUDY

EFFECT OF THERAGUN ON THE IMPROVEMENT OF BACK FLEXIBILITY

Muscle tightness may be connected to postural instability. Both can contribute to various musculoskeletal conditions. Reduced extensibility resultant from increased hamstring stiffness could be a probable causative factor to low back injuries. Considering that forward bending is one of the mainly common movements in daily activities, shortened hamstrings may increase the risk of injury to the spine from mechanical stresses. Flexibility dysfunction is a extensive problem faced by common as well as sportspersons, especially in case of hamstring group of muscle. Vibration therapy improves muscular strength, power improvement and kinesthetic awareness.

History

We describe a 25-year-old male patient. He is a dentist. His height was 162 centimeters, weight 65 kilograms and body mass index (BMI) was 24.8. The patient was seen by a female physiotherapist and enrolled for daily treatment. He complained of back pain that got aggravated with forwarding bending activity and prolonged sitting. He also complained of difficulty in horse riding. He belonged to a high socioeconomic class and fair family and social support. He had no history of trauma.

Physical Examination

His Back movements were restricted. There were a bilateral Hamstring tightness and reduced back flexibility.

Procedure

Ethical approval was granted from the Institutional Ethical Committee and the Patient gave informed written consent. His demographic data, physical examination and the intensity of pain was done with use of numeric Pain rating Scale score was noted. Flexibility measurement was done with the use of sit and reach test and hamstrings tightness measurement was done with the use of a 90-90 straight leg raising test. Activity difficulty was measure by the use of the patient -specific functional scale.

MULTIPLE CHOICE QUESTIONS

This is given to the students for progress check at the end of each chapter.

MULTIPLE CHOICE QUESTIONS

1. **A full domain name is a sequence of labels separated by _____.**
   a. semicolons
   b. dots
   c. colons
   d. none of the above
2. **A _____ server loads all information from the primary server.**
   a. primary
   b. secondary
   c. [illegible]
   d. none of the above
3. **The first level in the generic domains section allows _____ possible labels.**
   a. 10
   b. 12
   c. 16
   d. none of the above
4. **If a label is not terminated by a null string, it is called a _____.**
   a. FQDN
   b. FQDN
   c. SQDN
   d. none of the above
5. **What a server is responsible for or has authority over is called a _____.**
   a. domain
   b. label
   c. zone
   d. none of the above
6. **DNS can use the services of _____ using the well-known port 53.**
   a. UDP
   b. TCP
   c. either (a) or (b)
   d. none of the above

REVIEW QUESTIONS

This section is to analyze the knowledge and ability of the reader.

REVIEW QUESTIONS

1. What exactly is a domain name?
2. How to choose a domain name for business? Explain.
3. Discuss how to register a domain name? What will it cost?
4. What's the difference between my domain name and web hosting?
5. What is the best way to secure a domain name?
6. Which domain is best for business?

Answer to Multiple Choice Questions

1. (b) 2. (b) 3. (d) 4. (a) 5. (c)
6. (c) 7. (a) 8. (c) 9. (b) 10. (c)

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References refer those books which discuss the topics given in the chapters in almost same manner.

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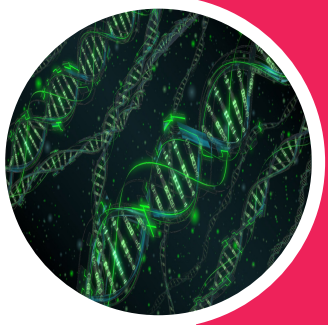

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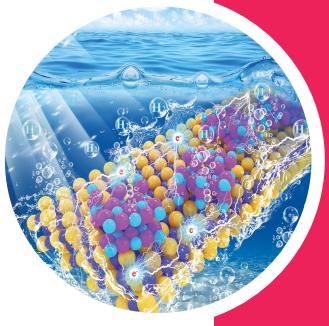

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PREFACE

Alkaloids are compounds needed for cell activity and gene code realization in the genotype. Alkaloids are a huge group of naturally occurring organic compounds which contain nitrogen atom or atoms in their structures. Alkaloids have been reported as one of the important groups of phytoconstituents obtained from natural sources. It plays an important role in the ecology of organisms which synthesize them. Alkaloids play an important role in the defense systems against pathogens and animals. These compounds play an important role in living organisms. Alkaloids occurred to be extremely important for human beings for ages, besides they are secondary metabolites, what could suggest that they are useless. Alkaloids showed strong biological effects on animal and human organisms in very small doses. Alkaloids are present not only in human daily life in food and drinks but also as stimulant drugs. Alkaloids are useful as diet ingredients, supplements, and pharmaceuticals, in medicine and in other applications in human life. Alkaloids are also important compounds in organic synthesis for searching new semisynthetic and synthetic compounds with possibly better biological activity than parent compounds.

Organization of the Book

This book is comprised of seven chapters. The book presents an overview of alkaloids. It also deals with the function, properties, classifications, extraction, and their importance in nature.

Chapter 1 presents the definition and occurrence of alkaloids. Alkaloids are a huge group of naturally occurring organic compounds which contain nitrogen atom or atoms in their structures. These nitrogen atoms cause alkalinity of these compounds. These nitrogen atoms are usually situated in some ring (cyclic) system.

Chapter 2 focuses on alkaloid chemistry. It determines alkaloids and their importance in nature and for human life.

Chapter 3 focuses on biology of alkaloids**.** It deals with bioactivity and biotoxicity of alkaloids.

Chapter 4 focuses on ecology of alkaloids. It deals with defense strategies in plants and describe the ecological roles of alkaloids. It also explain the animal sequestration of alkaloids.

Chapter 5 deals with alkaloids biosynthesis. You will understand the biosynthesis of tetrahydrobenzylisoquinoline alkaloids.

Chapter 6 aims to cover applied potential and current applications of alkaloids. It also focus on photochemistry and classification of alkaloids. The chapter also explains the alkaloids and their applications in pharmaceutical chemistry.

Chapter 7 focuses on alkaloids in arrow poisons. Arrow poisons are used to poison arrow heads or darts for the purposes of hunting and warfare. They have been used by indigenous peoples worldwide and are still in use in areas of South America, Africa and Asia. Notable examples are the poisons secreted from the skin of the poison dart frog, and curare, a general term for a range of plant-derived arrow poisons used by the indigenous peoples of South America.

CHAPTER 1

DEFINITION AND OCCURRENCE OF ALKALOIDS

LEARNING OBJECTIVES

After studying this chapter, you will be able to:

1. Definition of alkaloids
2. Discuss about alkaloids occurrence in nature

"Alkaloids are natural fungicides, insecticides, and pesticides. It has been estimated that, on average, each of us ingests about a gram and a half of natural pesticide every day, from the plants and plant products in our diet. The estimate for residues from synthetic pesticides is around 0.15 milligrams daily—about ten thousand times less than the natural dose!"

—Penny Le Couteur

INTRODUCTION

Alkaloids are basic nitrogenous plant products in which most of them are optically active. In most of their structure, nitrogen hetrocyclic unit is present and these compounds have pronounced physiological activity.

Alkaloids are a huge group of naturally occurring organic compounds which contain nitrogen atom or atoms (amino or amido in some cases) in their structures. These nitrogen atoms cause alkalinity of

these compounds. These nitrogen atoms are usually situated in some ring (cyclic) system. For example, indole alkaloids are those that contain nitrogen atom in indole ring system. Generally based on structures, alkaloids can be divided into classes like indoles, quinolines, isoquinolines, pyrrolidines, pyridines, pyrrolizidines, tropanes, and terpenoids and steroids. Other classification system is connected with a family of plant species that they occur. One of the examples is the opium alkaloids that occur in the opium poppy (*Papaver somniferum*). These two different classification systems cause confusion between their biological distribution and the chemical types of alkaloids, because there is not unmistakable correlation.

Alkaloids (whose name originally comes from "alkali-like") can react with acids and then form salts, just like inorganic alkalis. These nitrogen atoms can behave like a base in acid-base reactions. In general alkaloids, which are treated as amines, the same as amines in their names, have suffixine. Alkaloids in pure form are usually colorless, odorless crystalline solids, but sometimes they can be yellowish liquids. Quite often, they have bitter taste. Now more than 3000 of alkaloids are known in over different 4000 plant species.

These compounds are produced generally by many plant species, mainly by flowering plants and also by some animals. Plants produce and store many organic compounds like amino acids, proteins, carbohydrates, fats, and alkaloids, which are usually treated as secondary metabolites. They are stored in each part of the plant—leaves, stem, root, and fruits of plants—but in different amounts. It was suggested that they are plants' waste product, but now evidence suggests that they play some important biological function in plants.

Some groups of structurally related alkaloids are present in plants from few to even 30. These alkaloids belong to the same class but have some differences in their structure and one of them usually occurs in majority. Some plant families are very rich in alkaloids. For example, in plants like opium poppy (*Papaver somniferum*) and the ergot fungus (*Claviceps*), there are about 30 different alkaloid types. In plants, their function is still mostly unknown. Alkaloids because of their bitter taste are natural compound to deter herbivorous organisms. In some plants they are used as natural pesticides. It was suggested that alkaloids in plants have a function to protect them from destructive activity of some insect species. Alkaloids are also present in some animal species like frogs (poison dart frogs (*Phyllobates*)), New World beaver (*Castor canadensis*), and lizards, and they are produced by fungi species and ergot.

Besides having the same general name—alkaloids—they have an extreme variety of chemical structures. Some of these compounds seem to have people known for ages because of their wide range of activity on human organisms and also other animals. For thousand years, extracts from plants containing alkaloids had medicinal use as drugs, and they owe their powerful effects thanks to the presence of alkaloids. Morphine was the first alkaloid which was isolated about 1804 from opium poppy in

crystalline form. Alkaloids are an interesting group of compounds with a wide range of activities, undesirable and desirable, on animal and human organisms. Alkaloids have diverse physiological effects: antibacterial, antimitotic, anti-inflammatory, analgesic, local anesthetic, hypnotic, psychotropic, and antitumor activity and many others. Nowadays, alkaloids usually from plants rather than from animals are still of great interest to organic chemists, biologists, biochemists, pharmacologists, and pharmacists. Well-known alkaloids include morphine, strychnine, quinine, atropine, caffeine, ephedrine, and nicotine.

1.1 DEFINITION OF ALKALOIDS

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. This was in 1805, and proved a significant step forward in chemistry and pharmacology. 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. The term "alkaloid" was first mentioned in 1819 by W. Meißner, an apothecary from Halle. He observed that these compounds appeared "like alkali", and so named them alkaloids.

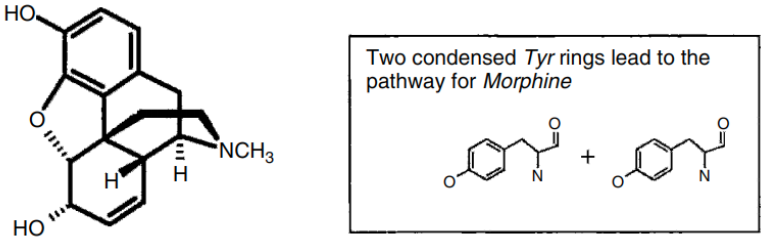


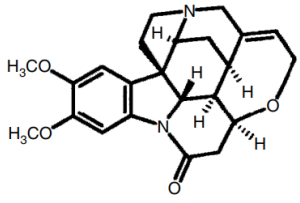
Figure 1: Contemporary scheme of morphine. Friedrich Sertürner, who first isolated this alkaloid in an impure form in 1805, did know that it was converted from the pathway of tyrosine, Tyr. The correct morphine structure was determined by Gulland and Robinson in 1923. Moreover, even 200 years after Sertürner's 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.

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

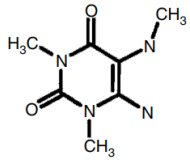
many cases, medicinal or ecological use. This definition, as a relatively wide one based on application, can be criticized as inexact. However, it presents a general picture of what kinds of compound are under consideration. The biological and chemical nature of this group of compounds leads to the conclusion 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. Sometimes, to avoid presenting this list of exceptions, the basic characteristics of alkaloids are given in the definition. Winterstein and Tier 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 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 simply stressed that alkaloids are a group of nitrogen-containing bases and that most of them are drugs.

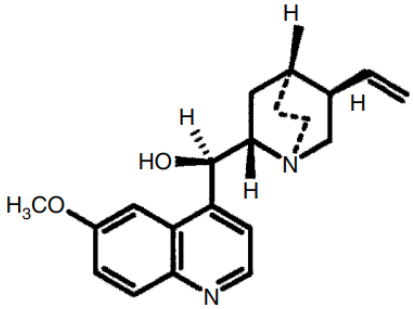
The most important points for the biologist are that alkaloids are a special group of chemicals that are active at different cellular levels of organisms, and that they take part in the biological processes of plants, animals and microorganisms.



Brucine



Caffeine



Quinine

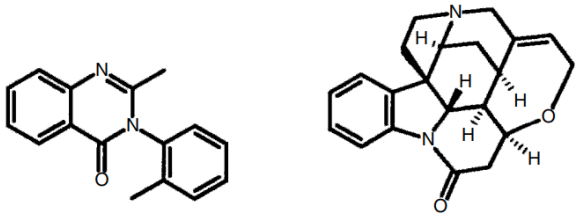


Figure 2: Some alkaloids isolated by pharmaceutists Pierre Joseph Pelletier and Joseph Beinamé Caventou during 1817–1821. They did not know the exact structures. Their compounds thus isolated are combinations of alkaloids rather than one pure alkaloid.

For the medical scientist, the term "alkaloids" means any group of nitrogenous substances of vegetable origin, often of complex structure and high molecular mass. 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. 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. 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. 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. In another chemical definition, it is stated only that alkaloids are nitrogen-containing compounds derived from plants and animals. 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.

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 antimalarian 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.

Keyword

Parkinson's disease is a progressive nervous system disorder that affects movement.

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 view of biological activity, 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).

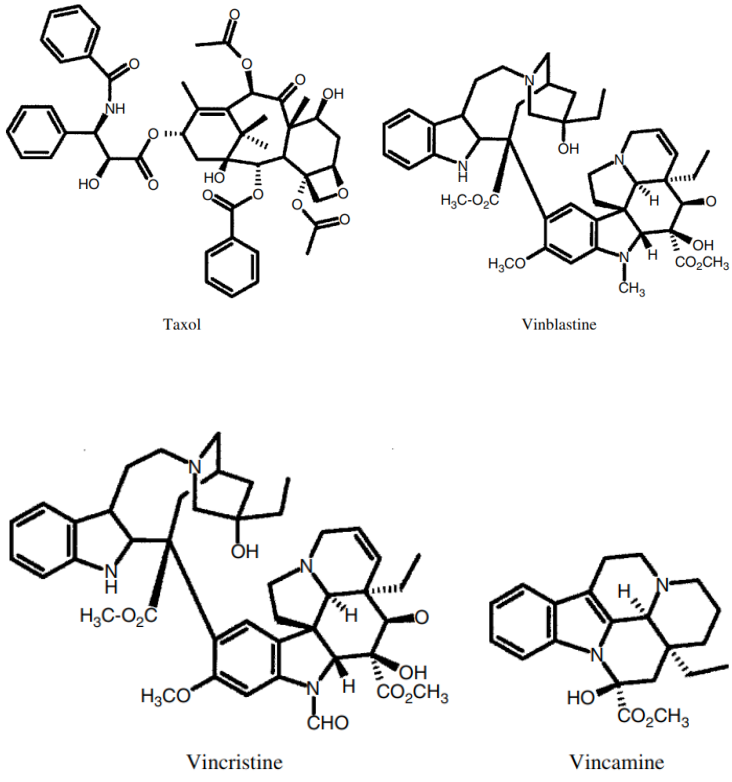


Figure 3: Schemes of taxol, vinblastine, vincristine and vincamine.

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).

1.1.1 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 nonnitrogenous 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. 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.

1.1.2 Protoalkaloids

Protoalkaloids are compounds, in which the N atom derived from an amino acid is not a part of the heterocyclic. Such kinds of alkaloid include compounds derived from L-tyrosine and L-tryptophan (see Table 1). Protoalkaloids are those with a closed ring, being perfect but structurally simple alkaloids.

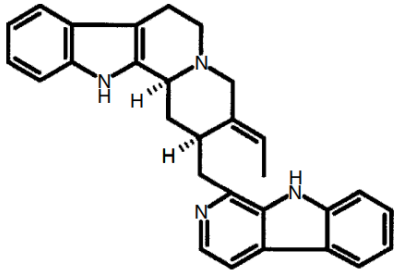
Table 1: Main types of alkaloids and their chemical groups

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
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

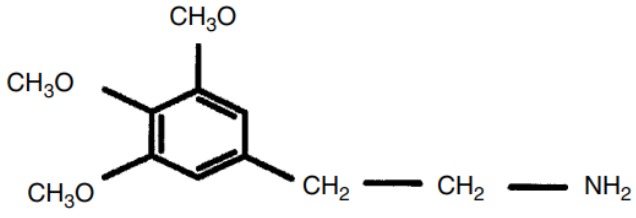
				Dihydrocoryn-antheine Corynanthine
		Ergot alkaloids		Ergobine Ergotamine Ergocryptine
	L-histidine	Imidazole alkaloids	Imidazole	Histamine Pilocarpine Pilosine
		Manzamine alkaloids	Xestomanz-amine	Xestomanz-amine A Xestomanz-amine B
	L-arginine	Marine alkaloids	β-carboline	Saxitoxin Tetrodotoxin
	Anthranilic acid	Quinazoline alkaloids	Quinazoline	Peganine
		Quinoline alkaloids	Quinoline	Acetylfolidine Acutine Bucharine Dictamnine Dubunidine γ-fagarine Flindersine Foliosidine Glycoperine 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

	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



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.

They form a minority of all alkaloids. Hordenine, mescaline (Figure 5) and yohimbine are good examples of these kinds of alkaloid. 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.

Keyword

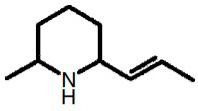
Capparidaceae is a family of dicotyledonous (Dicotyledoneae) plants most of which are shrubs and small trees.

1.1.3 Pseudoalkaloids

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



Pinidine

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

Did You Know?

The first complete synthesis of an alkaloid was achieved in 1886 by the German chemist Albert Ladenburg.

1.2 ALKALOIDS 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. 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 contemporaneously, only alkaloids are molecules of natural origin with highly important benefits and diagnostic uses.

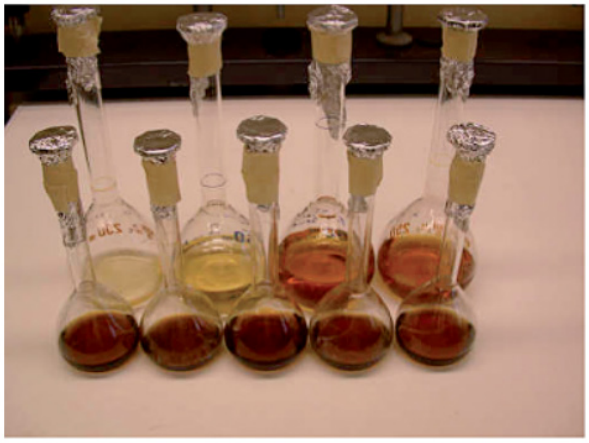


Figure 7: The raw extraction of quinolizidine alkaloids from different lupine species. Observe the different colours of the raw extracts, which signifies different concentrations of alkaloids in different species.

They can be characterized as the most useful and also the most dangerous products of nature. They can be extracted and purified (Figure 7).

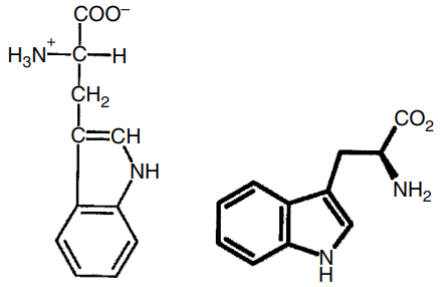
Alkaloids are most abundant in higher plants. At least 25% of higher plants contain these molecules. In effect this means that on average, at least one in fourth plants contains some alkaloids. In reality, it is not impossible that alkaloids occur more commonly. Using the latest equipment and technology, 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. 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.

1.2.1 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 genera: devil's-pepper (Rauvolfia L.), periwinkle (Catharanthus G. Don), milkwood (Tabernaemontana L.), strophanthus (Strophanthus DC.), voacanga (Voacanga U.) and alstonia (Alstonia R. Br.).

Table 2: General botanical characteristics of the Dogbane family

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.

The species belonging to these genera contain L-tryptophan-derived alkaloids (Figure 8). Indian snakeroot (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. 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 as a tranquilizer and also as a controller of other cardiac disorders. Deserpine is a compound with limited availability from natural sources.

Keyword

Reserpine is an adrenergic blocking agent used to treat mild to moderate hypertension via the disruption of norepinephrine vesicular storage.

Figure 9: The devil's-pepper genus contains L-tryptophan-derived alkaloids. Rauwolfia serpentina appears on flowers.

Reserpine usually occurs at about 0.10–0.16% of natural extracts and deserpine only at 0.04%. Furthermore, five new

indole alkaloids (Nb-methylajmaline, Nb-methylisoajmaline, 3-hydroxysarpagine, yohimbic acid and isorauhimbic acid) were isolated from the dried roots of Rauwolfia serpentina. 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, 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/Shydroxy-conodurine, tabernaelegantine A, B, C, and D. 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.

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. 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.

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.

From Aspidosperma megalocarpon Müll. Arg., growing in Colombia, three alkaloids were extracted – fendlerine, aspidoalbine and aspidolimidine. All display bioactivity and the potential for applications in medicine. Jokela and Lounasmaa 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:

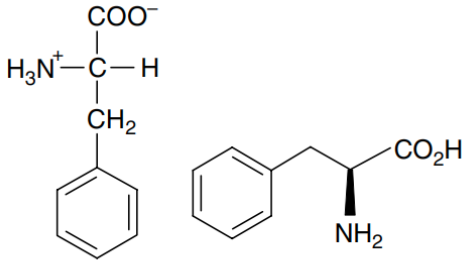
ajmaline, 17-O-acetylajmaline, isoajmaline, isosandwichine, rauflorine, vincamajine and vincamedine. Eleven indole alkaloids were isolated from the stem bark of Kopsia hainanensis Tsiang, which is one of for species of Kopsia, endemic in China. They are (−)-kopsinine, (−)-kopsinnic acid, (−)-kopsinoline, 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. Moreover, in the Dogbane plant family are also phenylalanine-derived alkaloids, such as -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.

1.2.2 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) and the genus Knapweed (Centaurea L.) in alkaloids derived from L-tryptophan, for example afzelin and apigenin.

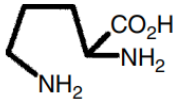


L-phenylalanine

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

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.

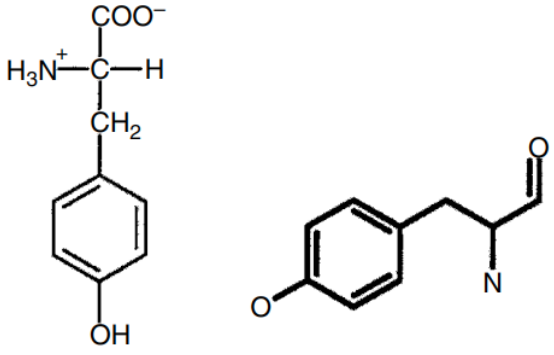
Alkaloid-containing species are distributed worldwide throughout the temperate areas. The Ragwort genus is endemic to Mediterranean and West Asian regions. From Senecio triangularis, other alkaloids were extracted. They are 9-O-acetyl-7-O-angelyl-retronecine, 7-O-angelyl-, 9-O-angelyl-, and 7-Oangelyl-9-O-sarracinylretronecine.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

Keyword

Usaramine is a pyrrolizidine alkaloid isolated from seeds of Crolatalaria pallida.

1.2.3 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. The Logan plant genus (Strychnos L.) is especially rich in many of alkaloids such as strychnine, brucine and curare.



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

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

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. 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. 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. Brucine and strychnine have been extracted from S. nux-vomica.

1.2.4 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

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. Phytochemical investigation of Glaucium leiocarpum Boiss. revealed 11 isolated alkaloids: (+)-glaucine, 6,6adehydronorglaucine, oxoglaucine, (+)--methylglaucine, (+)-lastourviline, (+)-predicentrine, (+)-dihydropontevedrine, secoglaucine, (−)-Nmethylcoclaurine, allocryptopine and protopine81. Glaucium paucilobum contains stylopine, protopine,)-allocryptopine, bulbocapnine, corydine, isocorydine, crabbine and arosine. Twenty-three isoquinoline alkaloids have been isolated from Corydalis bulleyana Diels. Hao and Qicheng 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, Corydalis 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. (±)-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, severzinine168, 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. These alkaloids are well known for their biological activity. For example, spallidamine has been found to display fungitoxic activity. Fumaria bracteosa Pomel is characterized by the presence of (+)-adlumidine, (+)-α-hydrastine, (+)-bicucullidine and protopine.

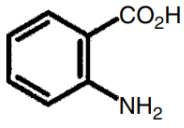
1.2.5 The Citrus Botanical Family (Rutaceae)

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 acid (Figure 13) and L-histidine (Figure 14) derived alkaloids.

Table 6: General botanical characteristics of the Citrus family

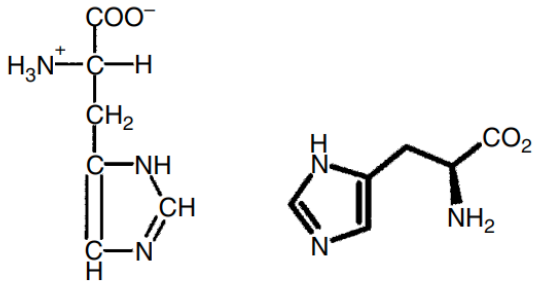
Botanical Forms and Parts	Characteristics
Botanical form	Shrubs Shrublets Trees Herbs
Some typical genera	*Agathosma* *Amyris* *Citrus* *Clausena* *Cneoridium* *Fagara* *Glycosmis* *Haplophyllum* *Helietta* *Poncirus* *Ptelea* *Pilocarpus* *Ruta* *Spathelia* *Zanthoxylum*
Special characteristics	Usually 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

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. These are acutine, acetylfolifidine, bucharidine, dubinidine, dubinine, glycoperine, evoxine, γ-fagarine, folifidine, foliosidine, haplophyline, haplopine, perfamine and skimmianine. 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.

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.

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. In earlier studies 1-hydroxyru taecarpine, rutaecarpine and 1-methoxyrutaecarpine have been reported from this plant. In Zanthoxylum hyemaline St. Hill two quinoline alkaloids (−)-R-geilbalansine and hyemaline were isolated. Bioassay-guided fractionation led to the isolation of three indolopyridoquinazoline alkaloids, 1-hydroxy rutaecarpine, rutaecarpine and 1-metoxyrutaecarpine, from the fruit of Z. integrifolium. Moreover, Melicope semecarpifolia produces

melicarpine and samecarpine. The genera Toddalia, Dictamus, Pelea and Stauranthus were also present in these furoguinoline alkaloids. Galipea officinalis Hancock, a shrub growing in tropical America and used in folk medicine as an antispasmodic, antipyreti, astringent and tonic, yields nine quinoline alkaloids, of which galipine, cusparine, cuspareine, demethoxycusparine and galipinine are the most important. 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(1H)-quinolone. In addition, the fruits of the similar species, Evodia rutaecarpa, contain four quinolone alkaloids: 1-methyl-2-tetradecyl-4(1H)-quinolone, evocarpine, 1-methyl-2-[(4Z,7Z)-4,7- decadienyl]-4(1H)-quinolone and 1-methyl-2-[(6Z,9Z)-6,9-pentadecadienyl]- 4(1H)-quinolone. 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, the new carbazole alkaloid 7-methoxy-glycomaurin, discovered in Glycosmis rupestris Ridely. 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. The isolation and identification of six 2-alkyl-4(1H)-quinolone alkaloids from the leaves of previously uninvestigated Spathelia excelsa (K. Krause). 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. Clausine structure was established by spectroscopic methods and its bioactivity was determined. According to this compound exhibits inhibitory activity against cyclin-dependent kinase 5 (CDK5) and shows protective effects on cerebellar granule neurons in vitro.

1.2.6 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. 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 mentioned in this context.

Table 7: General botanical characteristics of the Nightshade family

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.

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. Especially, steroid alkaloid solasodine may penetrate in animal body (experiments with rats), the placental and hematoencephalical barriers and impact the foetuses. According to 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.

1.2.7 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. Typical characteristics of this family are elliptic, light green leaves (4–7×3–4 cm), small, white flowers and small, reddish-orange drupes. 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. 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.

1.2.8 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. New alkaloids from 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 and Heliotrium esfandiarii europine N-oxide. 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. 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. 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.

Keyword

Erythroxylum is a genus of tropical flowering plants in the family Erythroxylaceae.

1.2.9 The Legume Botanical Family (Fabaceae)

Alkaloids derived from L-ornithine, L-lysine, and L-trypthophan occur in the Legume plant family (Fabaceae Juss.). 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. 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. 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:

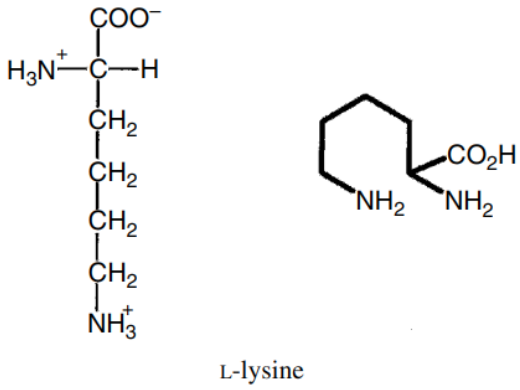
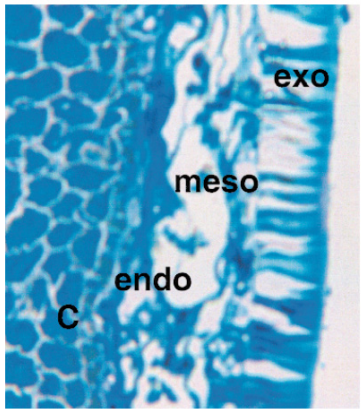


Figure 16: L-lysine is a precursor of piperidine, quinolizidine and indolizidine alkaloids.

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, 3-hydroxylupanine, multiflorine, 17-oxolupanine, 13α- hydroxylupanine, 3 13 α-dihydroxylupanine, 13α-angeloylolupanine, 13 α- tigloyloxylupanine and 4-tigloyloxylupanine. 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. Isolated two alkaloids, 2-methoxyl-3-(3-indolyl)-propionic acid and 2-hydroxyl-3-[3-(1-N-methyl)-indolyl] propionic acid, in peanut skins (Arachis hypogaea L.).



(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.

These alkaloids had not previously been found in natural sources. Moreover, 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, 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. Recently, from the flowers of broad beans (Vicia faba L.) N-[(3R, 7R)-(−)-jasmonoyl]-(S)-dopa and N- [(3R,7R)-(−)-

jasmonoyl]-dopamine were isolated. These alkaloids are tyrosine-derived compounds. All alkaloids occurring in Fabaceae have both biological and ecological significance.

1.2.10 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. The Monseed botanical family is large, containing about 70 genera and 450 species. 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. 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. 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. 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. Isolated tetrandrine from the root of a Chinese herb Stephania tetrandra S. Moore. This alkaloid showed to inhibit both cultureactivation and TGF-beta(1)-stimulated activation of quiescent rat hepatic stellate cells (HSCs) in vitro. From Stephania cepharantha Hayata, cepharathine, cepharanoline, isotetrandrine and berbamine have been isolated.

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) Troupin has also been reported. 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. 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.

1.2.11 The Berberry Botanical Family (Berberidaceae)

L-tyrosine-derived alkaloids occur also in the Berberry botanical family (Berberidaceae Torr., Gray, Juss.). 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. Moreover, it is also reported that, together with berberine, other alkaloids were detected, such as glaucine, hydroxyacanthin and berbamine. A natural alkaloid derived from Nandina domestica Thunberg, which was first isolated by Takase and Ohasi in 1926. Subsequently, extracts 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. This alkaloid shows actions of anti-arhythmia, anti-myocardial ischemia and antithrombosis.

1.2.12 The Buttercup Botanical Family (Ranunculaceae)

The Buttercup botanical family (Ranunculaceae Juss.) 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. 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. In Aconitum arcuatum (Maxim.), a new alkaloid, arcutin with antibacterial and medicinal impact, was located. In Aconitum coreanum (Levl.) Rapaics the tangutisine, acorone, acorridine, coryphine and coryphidine were found, all of which have powerful biological impact. Methyllycaconitine and barbine are typical in the genus Larkspur (Delphinium L.). Delphinium corymbosum contained delcorinine and delsonine, while Delphinium poltoratskii was found to hold a lot of alkaloids. These included methyllycaconitine, lycoctonine, anthranoyllycoctonine, ajacine, karacoline and delpoline.

1.2.13 The Lily Botanical Family (Liliaceae)

The Lily botanical family (Liliaceae Adans., Juss.) 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.). Four new steroid alkaloids (puqienine A, puqienine B, N-demethylpuqietinone, puqietinonoside) have been isolated from Fritillaria species. 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.

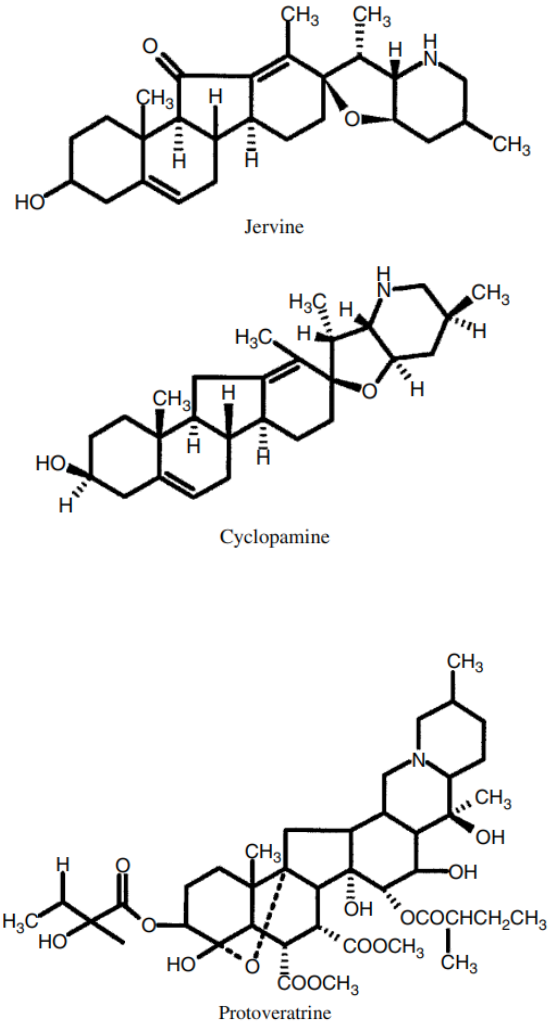


Figure 18: Jervine, cyclopamine and protoveratrine structures.

1.2.14 The Coffee Botanical Family (Rubiaceae)

The Coffee (syn. Madder) botanical family (Rubiaceae Juss.) 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- / guaninederived 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, 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. 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. This alkaloid has important biological potential.

Five alkaloids isolated from the species Corynanthe pachyceras K. Schum., a member of the Rubiaceae family. Corynantheidine, corynantheine, dihydrocorynantheine, L-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). 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. Moreover, mitragynine was also isolated from this plant 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. 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. These alkaloids showed a high toxicity on HTC.

Remember

The tropane alkaloids occur in several genera in the family Solanaceae (Atropa, Datura, Hyoscyamus, and Duboisia), while the ecgonine alkaloids are restricted to the genus Erythroxylum in the family Erythroxylaceae.

1.2.15 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 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. Isolating four alkaloids from Zephyranthes citrina (Baker) belonging to the Amaryllis plant family.

They were galanthine, haemanthamine, lycorine and lycorenine. More recently, 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 cells.

Haemanthidine also has a powerful bioimpact as a cytotoxic agent against various human tumoural cell lines, and galanthine has a high inhibitory capacity with ascorbic acid biosynthesis in the potato. Maritidine exhibits antineoplastic activity. 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. These alkaloids have antiviral, antitumoural, analgesic and insecticidal effects. Three alkaloids, lycorine, homolycorine and 2-O-acetyllycorine, were recently isolated from the bulbs of Leucojum vernum and two new alkaloids, leucovernine and acetylleucovernine, by Forgo and Hohmann. 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. Moreover, alkaloids from Crinum stuhlmannii Baker have also been reported. 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.

1.2.16 The Oleaster Botanical Family (Elaeagnaceae)

The Oleaster botanical family (Elaeagnaceae Lindl.) has 3 genera and 50 species. 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-tryptophanderived alkaloid elaeagine occurs in the Oleaster genus (Elaeagnus L.), and especially in the Russian olive (Elaeagnus angustifolia L.).

1.2.17 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). 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.). In Nitraria komarovii, komavine and acetylkomavine have been detected.

1.2.18 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. Psilocybe semilanceata and Phanaeolus subbalteatus proved to be the only psilocybincontaining fungi that can be gathered in middle and northern Europe in sufficient quantities to permit abuse.

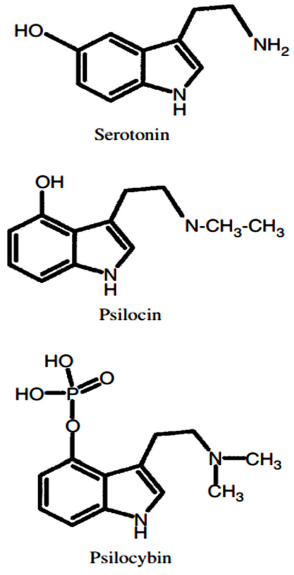


Figure 19: Basic alkaloids of mushrooms.

1.2.19 Moss

The moss alkaloids annotinine, lycopodine and cernuine occur in the genus Lycopodium 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). Moreover, 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.

1.2.20 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. 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. These compounds showed a low to moderate general toxicity.

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. Isolated perinadine A from the cultured broth of 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.

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

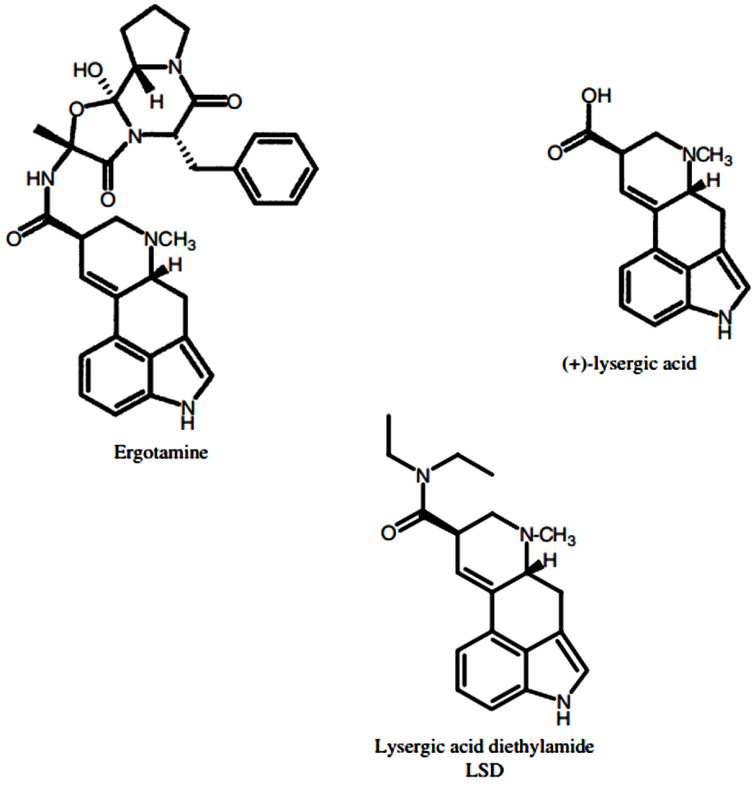


Figure 20: Ergotamine and LSD.

1.2.21 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. 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. Many of guanidine alkaloids display ichthyotoxicity, and antibacterial,

Remember

Ergonovine, an alkaloid from the fungus Claviceps purpurea, and the second alkaloid ephedrine isolated from Ephedra species both act as blood vessel constrictors. Also ephedrine is used in bronchial asthma and to relieve discomfort of hay fever, sinusitis, and common colds

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). 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. 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. 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. From Melanophryniscus montevidensis, alkaloids of the pumiliotoxin (PTX) group and indolizidines were isolated.

The lady bird (Coccinellidae) and other beetles also contain alkaloids. 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. Longitarsus lateripunctatus (Coleoptera, Chrysomelidae, Alticidae), a leaf beetle feeding on Pulmonaria abscura leaves, contained readily traceable quantities of pyrrolizidine alkaloids. 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. 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. Some pyrroloindole alkaloids, such as pseudophrynaminol, were found in the Australian frog (Pseudophryne coriacea). However, it is known that a diverse array of over 800 biologically active alkaloids have been discovered in amphibian skin. 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.

From North Sea Bryozoan (Flustra foliacea) several, brominated indole alkaloids have been isolated. 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). Erythrinan alkaloids (-erythroidine and dihydro--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. The spectrum of alkaloids in mammals ranges from isoquinoline derivatives, via -carbolines, through to thiazolidines, arising from vitamin B6, chloral and glyoxylic acid. For a long time, tetrahydroisoquinoline alkaloids were considered to be exclusively of plant origin. 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. 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. Although it is generally accepted or strongly suggested that alkaloids occur in animal species, even as a common matter, 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.

ROLE MODEL

ALBERT LADENBURG

Albert Ladenburg, a German chemist born on July 02, 1842 – died on August 15, 1911. Ladenburg along with Ghent another eminent chemist worked on structure of benzene.

He proposed a structure of benzene but in 1973, it was realised and confirmed as structure of prismane chemical compound.

Ladenburg received Hanbury medal for chemistry research work in the year of 1889, Davy medal for synthesis of natural alkaloids in the year of 1905.

Ladenburg discovered the chemical similarities between silicon and carbon. He also investigated pyridine bases, proved that the molecule of ozone consists of three atoms of oxygen, and also isolated hyoscine or scopolamine.

Early Life and Education

Ladenburg was a member of the well-known Jewish Ladenburg family [de] in Mannheim. He was educated at a Realgymnasium at Mannheim and then, after the age of 15, at the technical school of Karlsruhe, where he studied mathematics and modern languages. He then proceeded to the University of Heidelberg where he studied chemistry and physics with Robert Bunsen. He also studied physics in Berlin. He got his Ph.D. in Heidelberg.

Academic Career

In 1873, Ladenburg went to Kiel as professor of chemistry and director of the laboratory, remaining there until 1889 when he went to the University of Breslau in the same capacity. He was made an honorary member of the Pharmaceutical Society of Great Britain in 1886 and received the Hanbury Medal for original research in chemistry in 1889.

Ladenburg isolated hyoscine, also known as scopolamine for the first time in 1880. In 1900 Ladenburg founded

the *Chemische Gesellschaft Breslau*, which he managed until 1910. He was also awarded the prestigious Davy Medal in 1905 «for his researches in organic chemistry, especially in connection with the synthesis of natural alkaloids».

Ladenburg also addressed the relation of religion and science in a book he published in 1904, where he dealt with the topics of "Science and spiritual life" and Christianity.

Research

In Ghent, Ladenburg worked for 6 months with August Kekulé who introduced him to structural theory. They worked on the structure of Benzene. Ladenburg's theory that benzene was a prismatic molecule turned out to be wrong. His proposed structure was eventually realised in 1973 in the molecule prismane.

Ladenburg visited England, and then went on to work for 18 months in Paris with Charles-Adolphe Wurtz and Charles Friedel on organosilicon compounds and tin compounds. He then returned to Heidelberg to teach.

Family

His son, Rudolf (1882–1952), became an atomic physicist. Other Son Eric died in boating accident in the early 1900's.

SUMMARY

- Alkaloids are a huge group of naturally occurring organic compounds which contain nitrogen atom or atoms (amino or amido in some cases) in their structures.
- 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.
- Many alkaloids can influence an animal's nervous system, providing possible changes in the functionality of the organism.
- Alkaloids are generally classified by their common molecular precursors, based on the biological pathway used to construct the molecule.
- All true alkaloids have a bitter taste and appear as a white solid, with the exception of nicotine which has a brown liquid.
- Pseudoalkaloids are compounds, the basic carbon skeletons of which are not derived from amino acids.
- Alkaloids are substances very well known for their biological activity at the beginning of world civilization.

MULTIPLE CHOICE QUESTIONS

1. Which of the following is not an example of alkaloids?

a. Vinca
b. Benzoin
c. Rauwolfia
d. Belladonna

2. Alkaloids are__

a. Basic in nature
b. Contain heterocyclic nitrogen ring
c. Both A & C
d. None of the above

3. Which of the following Alkaloid give false positive result ?

a. True alkaloids
b. Proto alkaloids
c. False alkaloids
d. Pseudo alkaloids

4. Family of belladonna is__

a. Umbelliferae
b. Labitae
c. Leguminosae
d. Solanaceae

5. Family of opium is

a. Solanaceae
b. Papaveraceae
c. Umbelliferae
d. Labitae

REVIEW QUESTIONS

1. What is the definition of alkaloids?
2. Define the true alkaloids.
3. Explain about pseudoalkaloids.
4. What is the dogbane botanical family?
5. Describe the poppy botanical family (papaveraceae).

Answers to Multiple Choice Questions

1. (b) 2. (c) 3. (d) 4. (d) 5. (b)

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CHAPTER 2

ALKALOID CHEMISTRY

LEARNING OBJECTIVES

After studying this chapter, you will be able to:

1. Underline the alkaloid chemistry
2. Determine alkaloids and their importance in nature and for human life
3. Illustrate the chemical structures and biological properties of plant alkaloids

"A whole host of things that we now know are drugs turn out to be plant alkaloids."

—Gregory Petsko

INTRODUCTION

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. Alkaloid chemistry underlines the significance of the blocks, pathways and transamination reactions. Alkaloids are nitrogen containing chemical compounds. They are naturally accruing in a wide range of plants and other organisms (secondary metabolites) and they have often pharmacological effects.

Chemically, the alkaloids organic are chemical compounds that can be structured very differently and

exercise quite different functions in nature. A chemical classification is often on hand of the basic molecular structure, such as a particular ring system. Another classification is based on the occurrence in nature of (ergot alkaloids). Biochemically these natural substances are secondary metabolites.

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 behavior 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 behavior, 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.

2.1 UNDERLINE THE ALKALOID CHEMISTRY

Alkaloid may be classified according to the structural relationship between the nitrogen-containing structure such as pyrrolidine, piperidine, quinoline, isolquinoline and indole and the alkaloid skeleton. Generally, amino acids such as ornithine, lysine, phenylalanine, tyrosine, tryptophan and histidine are precursor for most of the alkaloids.

Alkaloids can be described as products of secondary plant metabolism like other complex natural compounds such as flavonoids, terpenoids, and steroids. The role of alkaloids in alkaloid-containing plants is still a matter of speculation. For a long time, it has generally been accepted that alkaloids are end products of metabolism and are waste products of the plant. However, more recent investigations have shown that alkaloids are involved in a dynamic process; for example, the amount of the alkaloid coniine in Conium maculatum varies during a single day as well as in the course of the development of the plant. There have been similar observations of the atropine alkaloids in A. belladonna and the opium alkaloids in P. somniferum. In isotopic labeling experiments it was demonstrated that the turnover rate (half-life) of alkaloids in plants is very fast. This clearly shows that plants do metabolize alkaloids, and therefore the theory that alkaloids are metabolic end products is no longer tenable.

However, whether alkaloids are of any use to the plant is still debatable since in only a few cases has it been shown that alkaloid-containing plants are protected against consumption by animals. On the other hand, both alkaloid-free and alkaloid-containing plants can be attacked by parasites, fungi, and bacteria.

In contrast to their role in plants, the function of alkaloids in insects is much more established; for example, coccinelline, the tricyclic alkaloid N-oxide, is produced by beetles of the Coccinellidae and is used as a defensive allomone against predators.

Decarboxylation of amino acid. Amino acids are the most common precursors in the biosynthesis of alkaloids. Decarboxylation of amino acids is first step towards alkaloid biosynthesis. It is performed by an enzyme often using the cofactor pyridoxal phosphate. The reaction is initiated with the nucleophilic attack of the amino function of amino acid to the aldehyde function of pyridoxal phosphate. The resulting imine intermediate undergoes decarboxylation giving a carbanion in which the negative charge can be delocalized.

Alkaloids derived from Ornithine. L-Ornithine is a natural amino acid not found in proteins. In animals, it is produced from arginine during urea cycle, a reaction catalyzed by the enzyme arginase. In plants, it is produced from L-glutamic acid. Ornithine contains two amino functions: α and δ-amino group, δ- amino group is a part of alkaloid structure along with the carbon chain excluding carboxyl group. Thus, pyrrolidine ring system (C4N) in alkaloid derived from ornithine. It is also present in tropane alkaloids

Biosynthesis of Polyamines. The simple polyamines such as putrescine, spermidine, and spermine play critical biological roles in eukaryotic cells and are synthesized from L-Ornithine and L-Methionine. Further, in animals, putrescine is synthesized from the ornithine through PLP dependent decarboxylation reaction. In plants and microorganisms, putrescine is also synthesized simultaneously from arginine. In this route, L-ornithine is converted to L-arginine which undergoes decarboxylated via PLP to form agmatine. The hydrolysis of imine function in guanidine in agmatine yields N-carbamoyl putrescine, which on further hydrolysis gives putrescine. At this point, alkylation of putrescine gives spermidine and spermine respectively. It is noted here that aminopropyl group comes from a decarboxylated SAM (dcSAM) to putrescine.

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, α-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. 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.

2.1.1 Alkaloids and their Biosynthesis

Since their first isolation in the beginning of the 19th century, alkaloids have stirred the imagination, the creativity, and the very souls of chemists. They remain passionately pursued, for their beautiful and seemingly endless structural variation, for the challenges their synthesis provides to even the most sophisticated and erudite organic chemists, for the diverse biological responses which they provide, and for the abundant novelty and acrobatics in the pathways of biosynthetic formation. No other group of natural products has provided such stimulation for chemists and biologists in the past 200 years.

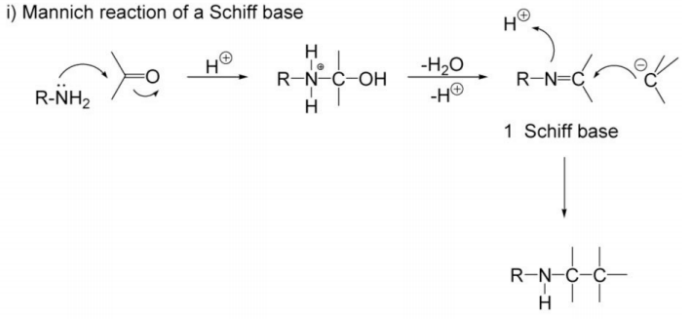
Probably the first, semi-purified alkaloid isolated was the "principium somniferum" from opium obtained by Serturner, and published in 1805 in the Journal der Pharmazie. Alkaloids really came of age though as a result of the efforts of French chemists Pelletier and Caventou. Between 1819 and 1821 they succeeded in isolating brucine, quinine, and strychnine, following their successful isolation of emetine in 1817. Other chemists took up the challenge of investigating the constituents of biologically significant plants, and piperine, atropine, caffeine, solanine, chelidonine, coniine, nicotine, aconitine, and colchicine were all isolated before 1833. By 1837, when the Swedish chemist Berzelius wrote his Lehrbuch der Chemie, he was able to list thirteen "Pflanzenbasen". Sparteine was isolated in 1851, and cocaine in 1860. Although Meissner first coined the term "alkaloid" in 1819, it was not until 1882 that it was brought into common usage by Jacobsen in a review. What an alkaloid is, in terms of a definition, however, has remained elusive, and no attempt will be made here to fill that perceived gap. As has been said…."You know one when you see one."

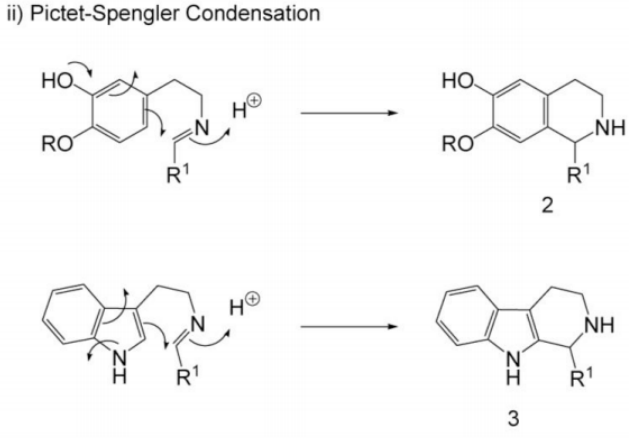
Initial alkaloid isolations were from higher plants, particularly those used as medicines or known to be highly toxic. As the natural world was investigated chemically in the late 20th century, "alkaloids" were isolated from many different terrestrial and marine sources, including amphibians, arthropods, mammals, insects, sponges, fishes, fungi and bacteria, and, of course, Homo sapiens. From higher plants alone there are now at least 22,000 alkaloids known, so at this point, the total from all sources is

probably in excess of 30,000. The early alkaloid isolations were achieved before there was a notion of the complexity of molecular structure, and before the concepts of stereochemistry and the three-dimensional nature of compounds were developed. One of the dominant challenges for the ensuing 160 years was to develop the techniques, first chemical and then spectral, for determining the detailed structures of these alkaloids.

The first alkaloid structure to be determined was that of xanthine in 1882, and in 1886 that the first synthesis of an alkaloid, that of (+)-coniine, was reported by Ladenburg. Structure determination frequently involved chemical degradation under harsh conditions. As a result, the core heterocyclic nucleus was sometimes all that survived, and this became the foundation of a new branch of organic chemistry involving heterocyclic nuclei; for example, the distillation of quinine with KOH led to the isolation of quinoline, and indigo afforded indole.

Prior to discussing a few of the amazing pathways to the diversity of alkaloids, it is pertinent to review three key reactions which form the cornerstone of the biosynthesis of alkaloids: i) the Mannich reaction of a Schiff base with a nucleophile, ii) the PictetSpengler condensation, and iii) the phenolic coupling reaction (fig. 1).





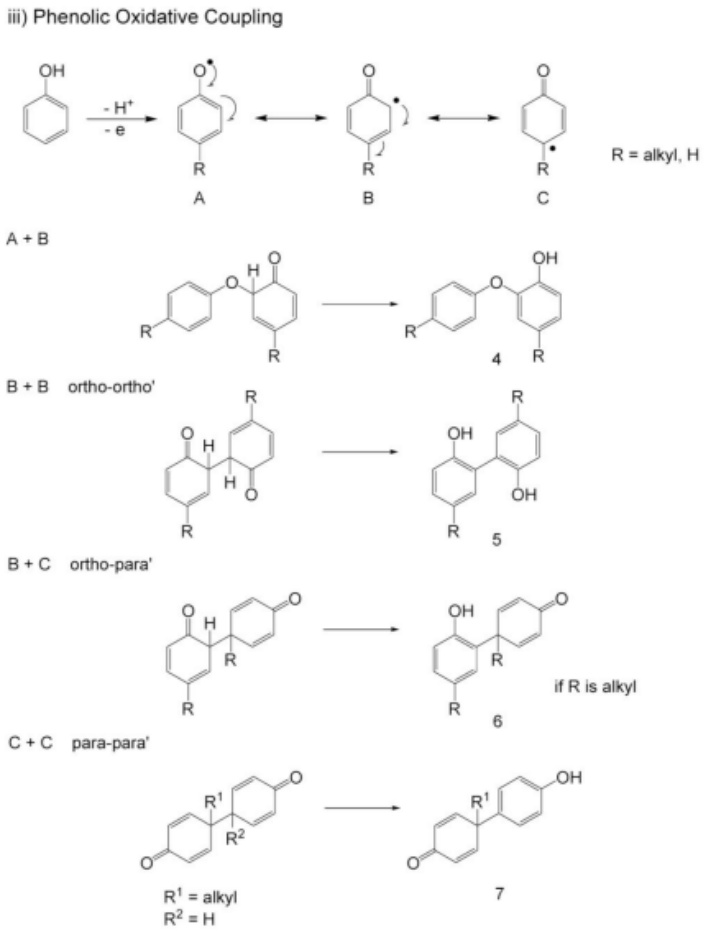


Figure 1. Important Reactions in Alkaloid Biosynthesis.

i) When an aldehyde or a ketone condenses with an amine and elimination of water occurs, the product is a Schiff base (e.g. 1). This species is a very powerful electrophile, and can attract a nucleophilic cation from any one of a number of sources. Frequently, the product represents a new, enhanced carbon skeleton with a heterocyclic nucleus.

ii) When the Schiff base is attacked intermolecularly by an aromatic nucleus, the product is often either a tetrahydroisoquinoline (e.g. 2) or a tetrahydro-beta-carboline (e.g. 3). This process is known as the Pictet-Spengler reaction.

iii) When a phenolic hydroxyl group is oxidized by the loss of a hydrogen radical, a highly reactive radical intermediate is produced which can be trapped either internally, or by another radical-containing unit, to form several different products, including those derived from carbon-oxygen bond formation (e.g. 4) and carbon-carbon bond formation at the positions ortho- (e.g. 5) or para- (e.g. 6 and 7) to the phenolic radical.

Metabolic engineering has become a major driving force for the reinvestigation of alkaloid biosynthesis. Through the overexpression of an enzyme that is rate-limiting in the pathway an important avenue to improve the availability of crucial intermediates or of pharmaceutically important products may be provided. Introducing new aspects to the pathway may produce metabolites that have not been observed previously. Or a "knock-out" strategy of deleting a key enzyme may allow a crucial intermediate in the pathway to accumulate. These techniques can evolve into significant approaches to increase the number of available metabolites which an organism can produce, and thus is of interest as an approach to increase the molecular diversity available for biological screening in pharmaceutical companies. Metabolic engineering can also be used to develop strains of plants which no longer produce undesired metabolites, such as caffeine in tea and coffee. Placing alkaloid-producing enzymes in a heterologous system, such as a microbial or insect system has been successful in enhancing the yield of the enzymic product more rapidly than in **plant tissue culture** systems, and in making the enzyme more available for crystallographic study. The future potential is very exciting as there is much to learn regarding the utilization of alkaloid biosynthetic pathways to produce medicinal agents more effectively for enhanced health care.

Keyword

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition

2.1.2 Alkaloids Derived from Ornithine

Ornithine (8) is a simple amino acid comprised of five carbons and two nitrogen atoms. In animals it is produced from arginine, through the action of the enzyme arginase; in plants it is derived from glutamic acid (9). In plants, not all of these atoms are incorporated into the secondary metabolic product. Typically, one of the nitrogen atoms, and often the carboxylic acid carbon, is lost, and the symmetrical intermediate putrescine (10) is often critical in the pathway. N-Methylation of putrescine with SAM is the first step in tropane alkaloid biosynthesis, and the cDNAs encoding for this transferase have been isolated from Atropa belladonna L. and Hyoscyamus niger L. Reaction of N-methyl putrescine (11) with diamine oxidase yields an equilibrium mixture of the amino-aldehyde 12 and the important Schiff base, the N-methyl-Δ 1 - pyrrolinium cation (13) (Fig. 2). A methylputrescine oxidase from Nicotiana tabacum has been

cloned and characterized. The N-methyl-Δ^1 -pyrrolinium cation (13) is readily attacked by nucleophilic centers and appears as a unit in alkaloids such as ficine (14), brevicolline (15), and cuscohygrine (16).

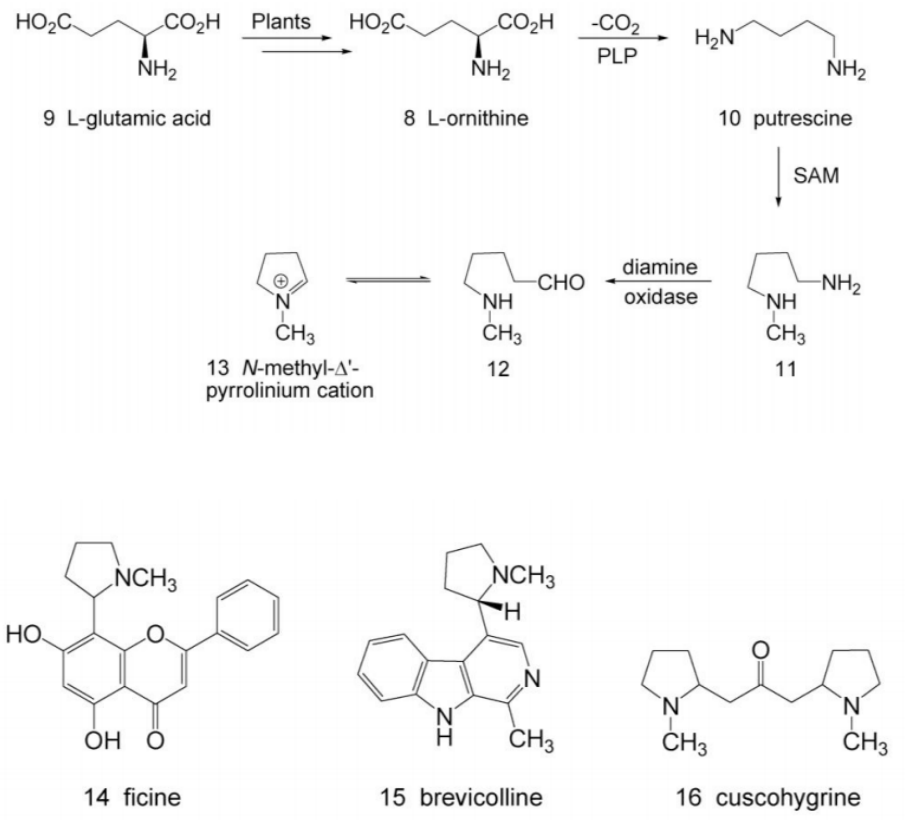


Figure 2. Formation of N-Methyl-Δ ′ -pyrrolinium Cation (13).

The tropane and ecgonine alkaloids are a biologically powerful group of alkaloids. They include (-)-hyoscyamine (17) (whose racemate is atropine) and scopolamine (18) in the tropane group, and cocaine (19) in the ecgonine group, each with a long history of traditional use over the millennia. Atropine (17) is used as a mydriatic agent and scopolamine (18) for motion sickness. Cocaine (19), besides its widespread illicit use, remains as a powerful anesthetic, and served as the model for several important synthetic anesthetic agents (benzocaine, lidocaine, etc.). The tropane alkaloids occur in several genera in the family Solanaceae (Atropa, Datura, Hyoscyamus, and Duboisia), while the ecgonine alkaloids are restricted to the genus Erythroxylum in the family Erythroxylaceae. They differ in structure by the presence in the ecgonine alkaloids of a carbomethoxy group at the C-2 position and an inversion of stereochemistry at C-3.

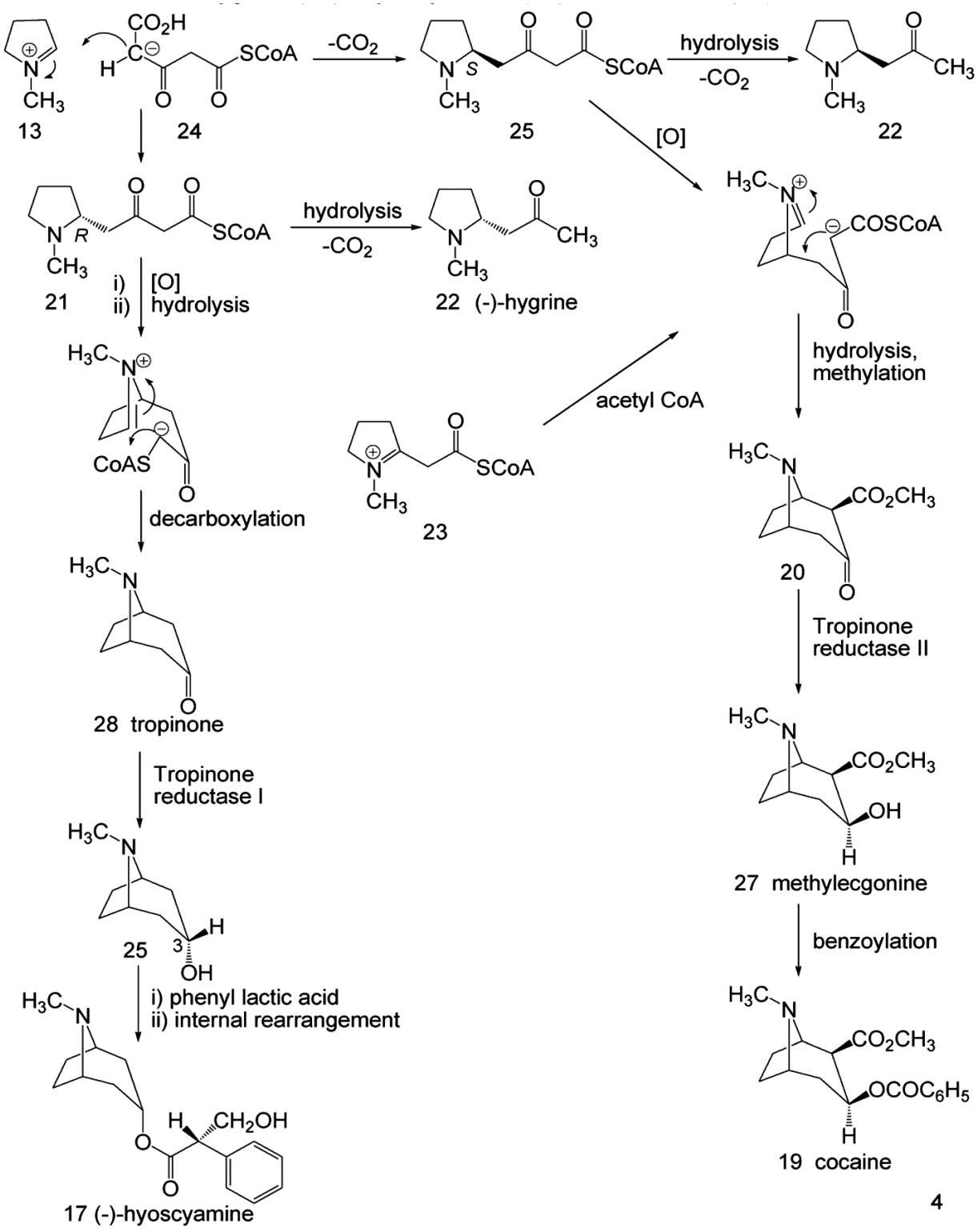


Figure 3. Formation of the Tropane Alkaloids Hygrine (22), Hyoscyamine (17) and Cocaine (19).

The pathway towards (-)-hyoscyamine (17) involves acetoacetate or the sequential addition of acetate to the N-methyl-Δ 1 -pyrrolinium species 13 to afford the ecgonine nucleus (20) (Figure 3). The acid 21 (as its CoA ester) is viewed as the next intermediate, after it was found that hygrine (22) and 2-(1-methylpyrrolidin-2-yl)acetate (23) were not precursors of hyoscyamine (17) in root cultures of D. stramonium. These results indicate that a four-carbon acetoacetate unit 24 is incorporated as a single unit.

Similar results were observed for scopolamine (18) thereby precluding the stepwise addition of acetate. However, studies in Erythroxylum coca Lam. on cocaine (19) biosynthesis did suggest a sequential build up of the four carbon chain from the ester 23, which as the labeled methyl ester was well incorporated into 19. Cuscohygrine (16) from Erythroxylum coca is derived from an acetoacetate 24 precursor and two Nmethyl-Δ 1 -pyrrolinium (13) species. The two series of tropane alkaloids differ in their stereochemistry at the C-3 position, an α-orientation in the formation of tropine (26) on the pathway to 17, and a β-orientation in the formation of methylecgonine (27) on the pathway to 19. The separate reductases for these processes, TRI and TRII, have been isolated and characterized. They selectively reduce the tropinone system of 28 or 20 to produce an α- or a β-hydroxy group, respectively for the tropane or ecgonine alkaloids. They co-occur in all Solanaceae plants producing tropane alkaloids. A very unusual intramolecular rearrangement occurs at the alkaloid level in the formation of (-)-hyoscyamine (17) from tropine (26) through the intermediate littorine (29) (Figure 4). The lactic acid portion of this alkaloid rearranges through a mutase reaction involving radical intermediates generated by P450 enzymes, followed by the action of a dehydrogenase to afford the tropic acid derivative 30, probably through a concerted carbonation process.

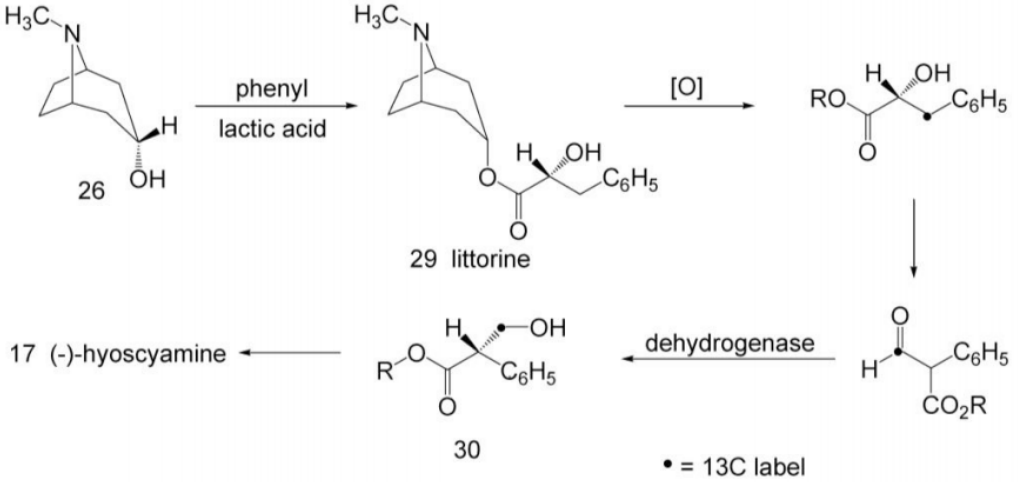


Figure 4. Formation of (-)- Hyoscyamine (17) from Littorine (29).

Other modifications to the tropane nucleus can involve hydroxylations at the 6β- and 7β- positions, followed by esterification, or oxidation to form a 6,7-oxido derivative, (-)-hyoscine (scopolamine) (18) (Figure 5). The gene for hyoscyamine 6β-hydroxylase has been expressed in Hyoscyamus niger and Atropa belladonna. Thus, 6- ^{18}O-6βhydroxyhyoscyamine (31) is incorporated intact into hyoscine (18) in Duboisia myoporoides R.Br. establishing that 6,7-dehydrohyoscyamine is not an intermediate; the dioxygenase for this process has been isolated. Evidence suggests that ornithine, as the N-methyl-Δ^1 -pyrrolinium cation (13), is incorporated in an unsymmetrical manner into hyoscyamine (17) in Datura, but through a symmetrical intermediate in Nicotiana, Hyoscyamus, and Erythroxylum. Detailed reviews of tropane alkaloid biosynthesis are available, including aspects of the architecture of the enzymes involved.

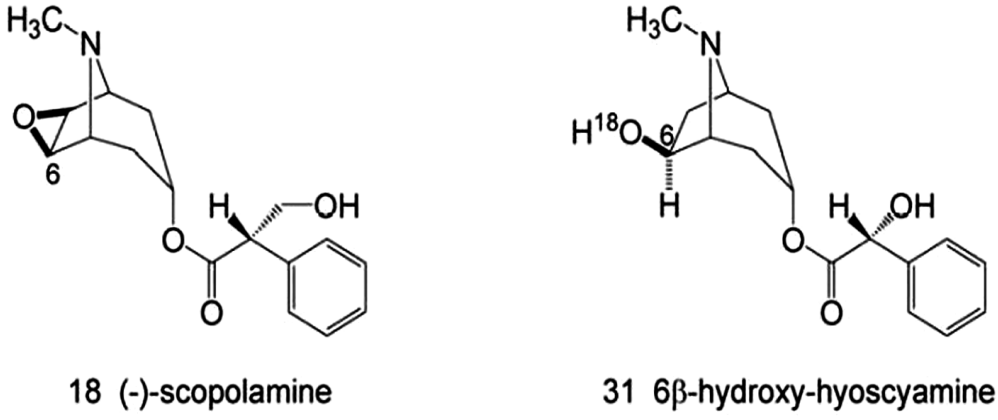


Figure 5. Scopolamine (18) and 6β-hydroxy-hyoscyamine (31).

2.1.3 Alkaloids Derived from Phenylalanine/Tyrosine

Phenylalanine (32) is a precursor of a very substantial number of alkaloids with a high level of structural diversity. 4′-Hydroxyphenylalanine (33), tyrosine, is produced from 32 through an oxidation reaction involving the NIH-shift of the 4′-proton. The alkaloids derived from 32 and 33 range in structure from simple derivatives, such as mescaline (34), to tetrahydroisoquinolines [pellotine (35)], the morphinan alkaloids [morphine (36)], and the complex bisbenzylisoquinoline alkaloids, such as tetrandrine (37). The aromatic nucleus can also be cleaved, yielding a system such as betanidin (38), or reduced and cyclized, as found in securinine (39) (Figure 6).

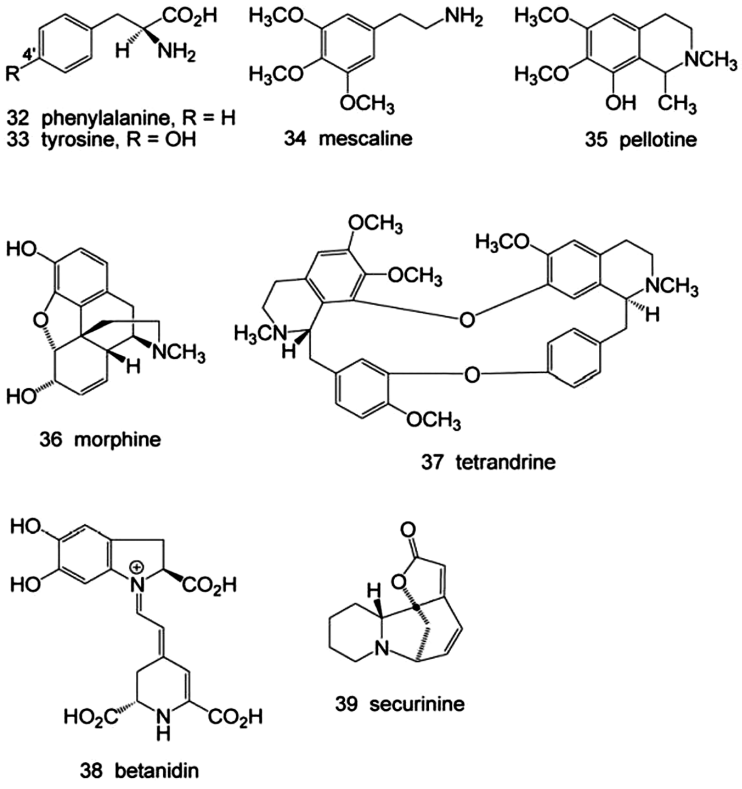


Figure 6. Representative Phenylalanine (32)/Tyrosine (33) Derivatives.

Keyword

Tyrosine is an amino acid. Amino acids are the building blocks of protein. The body makes tyrosine from another amino acid called phenylalanine.

There is a wide range of plant families associated with alkaloids derived from phenylalanine (32) and **tyrosine** (33), although some individual structural types do have a very limited distribution reflecting taxonomically-limited biosynthetic pathways.

2.2 ALKALOIDS - THEIR IMPORTANCE IN NATURE AND FOR HUMAN LIFE

In nature there are many natural compounds. From among many classes of naturally occurring organic compounds such as carbohydrates, lipids, proteins, amino acids, anthocyanins, flavonoids, and steroids, the one that seems to be quite special is alkaloids. What makes them special? They derived from amino acids and can be synthetized as secondary metabolites by plants and some animals. These compounds play an important role in living organisms. Alkaloids occurred to be extremely important for human beings for ages, besides they are secondary

metabolites, what could suggest that they are useless. Alkaloids showed strong biological effects on animal and human organisms in very small doses. Alkaloids are present not only in human daily life in food and drinks but also as stimulant drugs. They showed anti-inflammatory, anticancer, analgesics, local anesthetic and pain relief, neuropharmacologic, antimicrobial, antifungal, and many other activities. Alkaloids are useful as diet ingredients, supplements, and pharmaceuticals, in medicine and in other applications in human life. Alkaloids are also important compounds in organic synthesis for searching new semisynthetic and synthetic compounds with possibly better biological activity than parent compounds.

Alkaloids are a huge group of naturally occurring organic compounds which contain nitrogen atom or atoms (amino or amido in some cases) in their structures. These nitrogen atoms cause alkalinity of these compounds. These nitrogen atoms are usually situated in some ring (cyclic) system. For example, indole alkaloids are those that contain nitrogen atom in indole ring system. Generally based on structures, alkaloids can be divided into classes like indoles, quinolines, isoquinolines, pyrrolidines, pyridines, pyrrolizidines, tropanes, and terpenoids and steroids. Other classification system is connected with a family of plant species that they occur. One of the examples is the opium alkaloids that occur in the opium poppy (Papaver somniferum). These two different classification systems cause confusion between their biological distribution and the chemical types of alkaloids, because there is not unmistakable correlation.

Alkaloids (whose name originally comes from "alkali-like") can react with acids and then form salts, just like inorganic alkalis. These nitrogen atoms can behave like a base in acid-base reactions. In general alkaloids, which are treated as amines, the same as amines in their names, have suffix -ine. Alkaloids in pure form are usually colorless, odorless crystalline solids, but sometimes they can be yellowish liquids. Quite often, they have bitter taste. Now more than 3000 of alkaloids are known in over different 4000 plant species.

These compounds are produced generally by many plant species, mainly by flowering plants and also by some animals. Plants produce and store many organic compounds like amino acids, proteins, carbohydrates, fats, and alkaloids, which are usually treated as secondary metabolites. They are stored in each part of the plant—leaves, stem, root, and fruits of plants—but in different amounts. It was suggested that they are plants' waste product, but now evidence suggests that they play some important biological function in plants.

Some groups of structurally related alkaloids are present in plants from few to even 30. These alkaloids belong to the same class but have some differences in their structure and one of them usually occurs in majority. Some plant families are very rich in alkaloids. For example, in plants like opium poppy (Papaver somniferum) and the ergot fungus (Claviceps), there are about 30 different alkaloid types. In plants, their function is still mostly unknown. Alkaloids because of their bitter taste are natural

compound to deter herbivorous organisms. In some plants they are used as natural pesticides. It was suggested that alkaloids in plants have a function to protect them from destructive activity of some insect species. Alkaloids are also present in some animal species like frogs (poison dart frogs (Phyllobates)), New World beaver (Castor canadensis), and lizards, and they are produced by fungi species and ergot.

Besides having the same general name—alkaloids—they have an extreme variety of chemical structures. Some of these compounds seem to have people known for ages because of their wide range of activity on human organisms and also other animals. For thousand years, extracts from plants containing alkaloids had medicinal use as drugs, and they owe their powerful effects thanks to the presence of alkaloids. Morphine was the first alkaloid which was isolated about 1804 from opium poppy in crystalline form. Alkaloids are an interesting group of compounds with a wide range of activities, undesirable and desirable, on animal and human organisms.

Remember

Alkaloids have diverse physiological effects: antibacterial, antimitotic, anti-inflammatory, analgesic, local anesthetic, hypnotic, psychotropic, and antitumor activity and many others. Nowadays, alkaloids usually from plants rather than from animals are still of great interest to organic chemists, biologists, biochemists, pharmacologists, and pharmacists. Well-known alkaloids include morphine, strychnine, quinine, atropine, caffeine, ephedrine, and nicotine.

2.2.1 Classification of Alkaloids

An alkaloid has been defined by Pelletier as: "a cyclic organic compound containing nitrogen in a negative oxidation state which is of limited distribution among living organisms".

Most alkaloids have basic properties connected with a heterocyclic tertiary nitrogen. Notable exceptions are colchicine, caffeine, and paclitaxel. Most alkaloids are biosynthetically derived from amino acids such as phenylalanine, tyrosine, tryptophan, ornithine, and lysine. Alkaloids represent a wide variety of chemical structures. About 20000 alkaloids are known, most being isolated from plants. But alkaloids have also been found in microorganisms, marine organisms such as algae, dinoflagellates, and puffer fish, and terrestrial animals such as insects, salamanders, and toads.

Alkaloids are often classified according to their molecular skeleton, e.g., the two largest groups are the indole alkaloids and isoquinoline alkaloids (each more than 4000 compounds). Other important groups are tropane alkaloids (◎300 compounds), steroidal alkaloids (◎450 compounds), and pyridine and pyrrolizidine alkaloids (respectively, ◎250 and 570 compounds).

Classification based on botanical origin of the alkaloids are also used, e.g., Papaver (opium) alkaloids, Cinchona alkaloids, Rauvolfia alkaloids, Catharanthus alkaloids, Strychnos alkaloids, Ergot alkaloids, cactus alkaloids, and Solanum alkaloids. The structures of some alkaloids are shown in Figure 7.

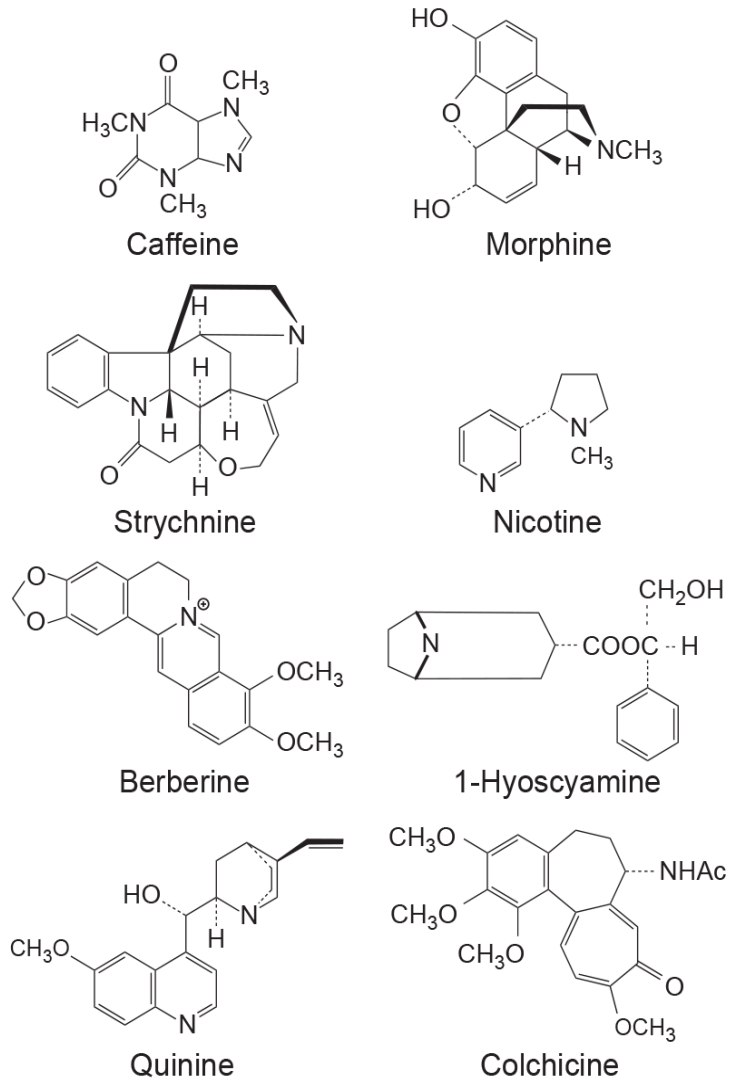


Figure 7. Structures of some different types of alkaloids.

Many alkaloids have strong biological activities in man. In part this can be explained by structural relationship with important signal compounds (neurotransmitters) such as dopamine, acetylcholine, noradrenaline, and serotonin. The fact that alkaloids are water soluble under acidic conditions and lipid soluble under neutral and basic

conditions give them unique properties for medicinal use, as they can be transported in the protonated form, and can pass membranes in the neutral form. In fact most synthetic medicines do contain one or more tertiary nitrogens.

A number of alkaloids have commercial interest as medicines of tools in pharmacological studies (see Table 1). Both pure compounds and plants (or extracts thereof) containing alkaloids are used. Furthermore some alkaloids are widely found as drugs of abuse (e.g., mescaline, cocaine, psilocybin, psilocin, morphine, and its semisynthetic derivative heroin), as doping compounds (e.g., strychnine, ephedrine, caffeine), and as poisons (e.g., strychnine, pyrrolizidine alkaloids, coniine, nicotine, tetrodotoxin). Consequently, methods for the determination of alkaloids can be found in quite different contexts, mainly dependent on the matrices in which the alkaloids are found.

Table 1. Some alkaloids of pharmaceutical interest

Ajmalicine	Physostigmine
Ajmaline	Pilocarpine
Quinine	Veratrine
Quinidine	Solasodine
Strychnine	Harringtonine
Reserpine	Ephedrine
Rescinnamine	Mescaline
Yohimbine	Aconitine
Vincamine	Nicotine
Vinblastine	Tetrodotoxin
Vincristine	Saxitoxin
9-Hydroxyellipticine	Sparteine
Camptothecine	Lobeline

Emetine	Muscarine
Atropine (1-hyoscyamine)	Serotonine
Scopolamine	Harmane
Cocaine	Psilocybine
Codeine	Caffeine
Morphine	Theophylline
Thebaine	Theobromine
Papaverine	Taxol
Narceine	Ergotamine
Narcotine	Ergonovine
Berberine	Ergosine
Sanguinarine	Ergocristine
Tubocurarine	Ergocryptine
Boldine	Ergocornine
Colchicine	Lysergic acid

2.2.2 Methods of Isolation

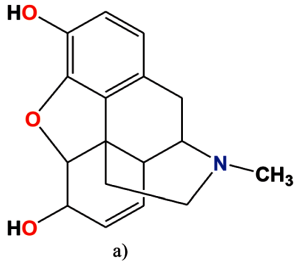
Extracts of plants containing alkaloids were known and used because of their diverse activity by people from ages. But ages ago people did not know direct methods to isolate pure compounds from specified plant species. Alkaloids in plants usually exist as aqueous solution in tissues. To isolate them the method called extraction is usually used. For commercially useful alkaloids, special extraction methods were developed. In general mixture containing alkaloid should be dissolved with some solvent with reagents. Extraction method allows recovery of alkaloids from solution. Then, each alkaloid can be separated from mixture and be obtained in pure form. To obtain crystalline form of alkaloids, certain solvents should be used. Another method is **chromatography**. It uses differences in degrees of adsorption of different alkaloids in some solvent system on solid materials such as silica or alumina.

2.2.3 Pharmaceutical and medicinal use of alkaloids

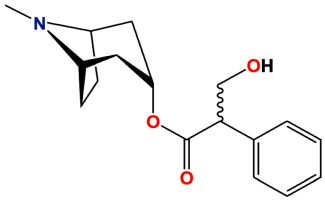
Keyword

Chromatography is a process for separating components of a mixture. To get the process started, the mixture is dissolved in a substance called the mobile phase, which carries it through a second substance called the stationary phase

Alkaloids showed quite diverse medicinal properties. Many of them possess local anesthetic properties, but their practical use is limited for clinical purpose. Morphine (Figure 8a) is one of the most known alkaloids which had been used and still is for medical purposes. This alkaloid is a powerful narcotic which is used for the relief of pain, but its usefulness is limited because of addictive properties.



a)



b)

Figure 8. Structures of alkaloids: (a) morphine and (b) atropine.

Methyl ether derivative of morphine—codeine—naturally occurring next to morphine in the opium poppy, possesses an excellent analgesic activity and is shown to be relatively nonaddictive. These alkaloids act as respiratory or cardiac stimulants. Next, the alkaloid which is used as medication in many clinical applications is atropine (Figure 8b). For example, injection with atropine is given to treat bradycardia (low heart rate).

Tubocurarine (Figure 9) is an alkaloid, is an ingredient of poison curare, and is used in surgery as muscle relaxant.

Alkaloids vincristine and vinblastine are used as chemotherapeutic agent in the treatment of many cancer types. Cocaine an alkaloid present in Erythroxylum cocais a potent local anesthetic. Ergonovine, an alkaloid from the fungus Claviceps purpurea, and the second alkaloid ephedrine isolated from Ephedraspecies both act as blood vessel constrictors. Also ephedrine is used in bronchial asthma and to relieve discomfort of hay fever, sinusitis, and common colds.

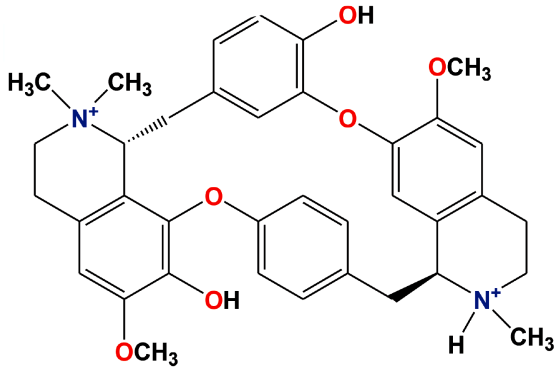


Figure 9. Structure of tubocurarine.

Quinine (Figure 10) is a powerful antimalarial agent and more often is replaced by synthetic drugs, which are more effective and less toxic. Another alkaloid from Cinchonaspecies is quinidine which has medical application as treatment of irregular rhythms of the heartbeat or arrhythmias.

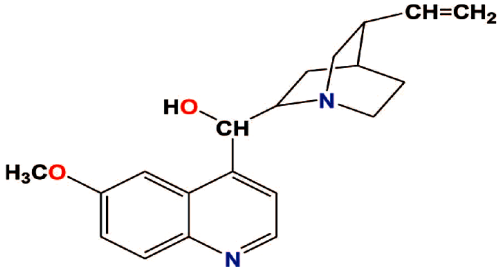


Figure 10. Structure of quinine fromCinchona species.

Colchicine (Figure 11) is another alkaloid, present in plants of Liliaceae family, known for ages to treat acute gout attacks. Another clinically used alkaloid is lobeline isolated from Lobelia inflata, which has multiple mechanisms of action.

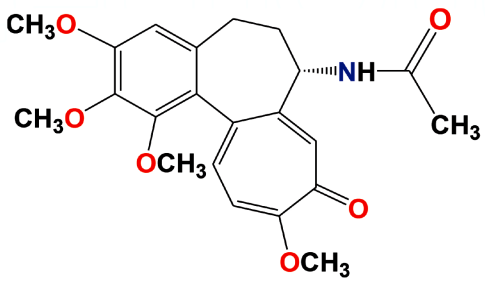


Figure 11. Structure of colchicine.

2.2.4 Alkaloids in Human Food and Drinks

Many alkaloids are elements of human diet, both in food and drinks. The plants in the human diet in which alkaloids are present are not only coffee seeds (caffeine, Figure 12), cacao seeds (theobromine and caffeine), and tea leaves (theophylline, caffeine) but also tomatoes (tomatine) and potatoes (solanine). The most common alkaloid is caffeine which has also application as an ingredient of soft drinks like Coca-Cola to improve their taste and in drinks for active people who do sport.

Figure 12. Plant source of caffeine and its structure and powdered caffeine (pure form) (author's own photos).

Other known alkaloid with bitter taste used as an ingredient of tonics is quinine (Figure 10) isolated from Cinchonaspecies.

2.2.5 Alkaloids as stimulants

Alkaloids stimulate human organisms, for example, central nervous system, or directly work on the human brain. Nicotine (Figure 13) is an alkaloid obtained from the tobacco

plant (Nicotiana tabacum) and is a potent stimulant and the main ingredient in tobacco smoked in pipes, cigars, and cigarettes. This alkaloid is highly addictive.

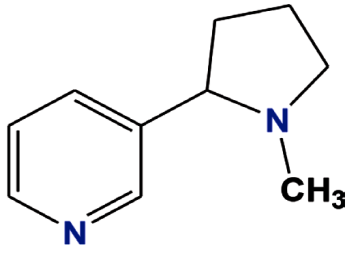


Figure 13. Structure of nicotine.

Cocaine is a narcotic drug, which activity is not suitable for medical purposes. This alkaloid has an opposite effect than morphine. This compound produces in the human body a euphoric hyperarousal state, but high doses of it may lead to fibrillation and death.

2.2.6 Dark Nature of Alkaloids

Some alkaloids are illicit drugs and poisons. Poisonous activities of some alkaloids are known for ages. One of these is strychnine (from Strychnosspecies, Figure 14). One of the well-known poison curare (obtained from Chondrodendron tomentosum) used in the South Africa as narrow poison contains alkaloid tubocurarine.

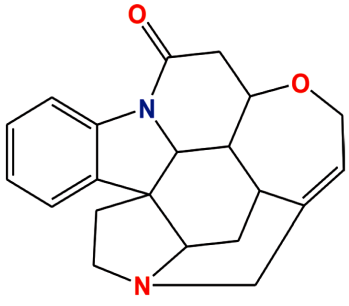


Figure 14. Structure of strychnine.

Coniine is an alkaloid isolated from Conium maculatum, which is an active ingredient of poison hemlock. Mescaline isolated from Anhaloniumspecies has hallucinogenic activity. Psilocybin is a naturally occurring drug isolated from fungi species Psilocybe mexicanaand possesses psychedelic activity. During the past decades, many semisynthetic derivatives of naturally occurring alkaloids with various activities have been synthesized.

Synthetic derivative of morphine is heroin, and, from lysergic acid naturally present in C. purpurea, LSD was produced.

2.3 CHEMICAL STRUCTURES AND BIOLOGICAL PROPERTIES OF PLANT ALKALOIDS

Plants possess a diverse array of metabolic products arising from both primary and secondary metabolisms. Primary metabolites exist in every living cell produced from vital metabolic reactions. Conversely, specialized metabolites are derived from the primary metabolism and are present only in prominent tissues required for specific functions. Alkaloids are naturally occurring specialized metabolites with nitrogen as a characteristic element present in their chemical structures. The treasure of the biological potency of alkaloids is attributed to the different arrangement of the atoms within their chemical structures.

Across the kingdoms, alkaloids occur with different chemical structures and attached functional entities, displaying wide-reaching biological properties. Organisms from the marine world such as shellfish and sponges contain alkaloids such as pinnatoxins, pinnamine, and halochlorine, which are found to be useful for treating cardiac and non-cardiac inflammatory ailments. Alkaloids act as defensive chemicals in many ladybird beetles, which secrete hemolymph-containing bitter alkaloids upon molestation. Few ant species have alkaloids like *cis-* and *trans*-2-methyl-6-alkylpiperidines along with proteinaceous substances in their poisonous venom, which are used for both defensive and offensive purposes.

Traditionally, plant extracts have been used as medicines in healthcare systems. Since the nineteenth century, the bioactivity of these compounds has been utilized for the production of therapeutic and psychoactive drugs. Recently, the compounds synthesized in different plant tissues have been extensively studied for their biosynthesis and biological activities. Pharmaceutical industries have utilized these naturally occurring compounds to develop formulations for better therapeutic potentials. These alkaloids show activities ranging from medicinal to acute toxicity, such as in the case of poppy alkaloids, depending upon the dosage of compounds. Higher plant species belonging to the Berberidaceae, Amaryllidaceae, Liliaceae, Leguminaceae, Papaveraceae, Ranunculaceae, and Solanaceae families are prominently rich in alkaloids, based on reports to date. Furthermore, different classes of alkaloids are found across different families, which in turn depends on the active biosynthetic pathway in a particular species. The detailed study of specialized metabolite **biosynthesis** through chemical and biotechnological approaches has created a comprehensive understanding of the diversity in alkaloids and their precursors.

Subsequently, five major and crucial chemical transformations to the backbone of alkaloids, such as glycosylation, acylation, reduction, oxidation, and methylation, have been discussed along with the diversified biological activities of alkaloids.

Keyword

Biosynthesis is a multi-step, enzyme-catalyzed process where substrates are converted into more complex products in living organisms.

2.3.1 Classification of Plant Alkaloids

The arrangement and combination of functional groups result in the production of a diverse range of alkaloids in both the plant and animal kingdoms. This broad class of specialized alkaloids has been further classified according to different aspects in plants, such as their biosynthesis pathways, chemical structures, and taxonomical groups (Table 2). Many alkaloids share a common skeleton within a particular genus of plants, however differ in their chemical and biological properties. The important groups of such alkaloids are depicted in Figure 15.

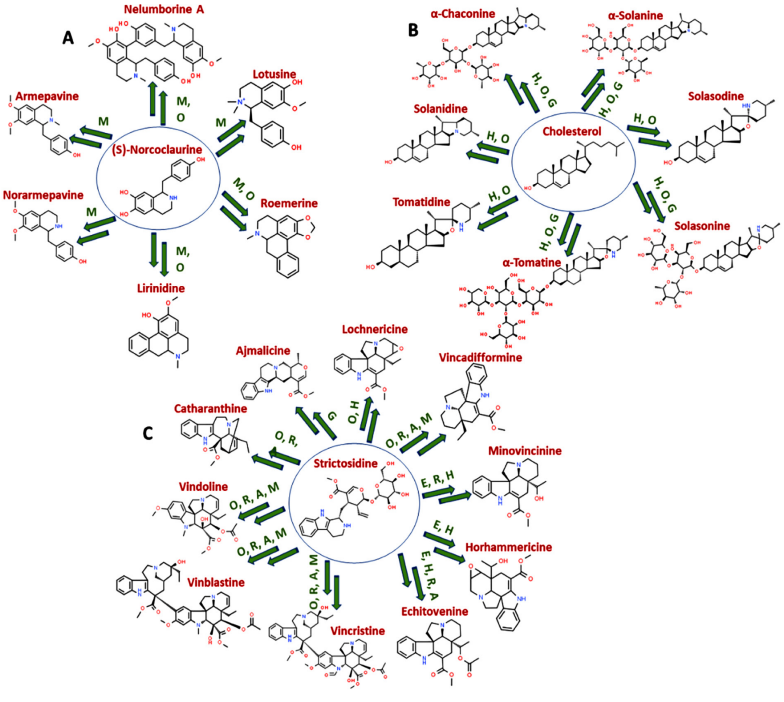


Figure 15. Examples of alkaloids biosynthesized from the common skeleton.

Table 2. Classification of alkaloids.

Group	Characteristic Feature	Representative Compounds	Plant Source
Tetrahydroisoquinoline alkaloids	Tyrosine acts as precursor	Berberine	*Argemone mexicana, Berberis aristata, B. aquifolium, B. heterophylla, B. beaniana, Coscinium fenestratum, C. chinensis, C. japonica, C. rhizome, Hydratis Canadensis, Chelidonium majus, Coptidis rhizome*
Indole alkaloids	Tryptophan acts as precursor	Ajmalicin	*Rauvolfia* spp., *Catharanthus roseus, Mitragyna speciosa*
		strychnine, brucine	*Strychnos nuxvomica*
Pyrrolizidine alkaloids	Ornithine acts as precursor	Senecionine	*Jacobaea vulgaris, Brachyglottis repanda, Emilia sp., Erechtites hieraciifolius, Petasites sp., Syneilesis sp., Crotalaria sp., Senecio sp., Cynoglossum sp., Symphytum sp., Heliotropium sp., Caltha leptosepala, Castilleja sp.*
Tropane alkaloids		Scopolamine	*Hyoscyamus niger, Datura* sp., *Brugmansia* sp., *Duboisia* sp.
Piperidine alkaloids	Lysine acts as precursor	Piperine	*Piper nigrum, P. longum*
Quinolizidine alkaloids		Lupinine	*Lupinus argenteus*
		Cytisine	*Laburnum anagyroides, L. alpinum, Cytisus canariensis*
Indolizidine alkaloids		Swainsonine	*Astragalus earlei, A. mollissimus, A. wootoni, A. pehuenches, Oxytropis lambertii, O. sericea, O. campestris, Swainsona luteola, S. greyana, S. galegifolia*
Pyridine alkaloids	L-Aspartate acts as precursor	Nicotine	*Nicotiana tabacum, Nicotiana rustica, Duboisia hopwoodii*
Pyridinone alkaloids		Cerpegin	*Ceropegia bulbosa, C. juncea,*
Quinoline alkaloids	Anthranillic acid acts as precursor	Skimmianine	*Skimmia japonica, Zanthoxylum nitidum Camptotheca acuminata*
		Camptothecin	
Quinazoline alkaloids		Vasicine	*Adhatoda vasica, Peganum harmala*
Xanthine alkaloid	Adenosine (SAM cycle) acts as precursor	Theobromine	*Theobroma cacao, Camellia sinensis, Cola acuminate, Paullinia cupana, Ilex guayusa*
		Caffeine	*Coffea arabica, C. canephora, C. liberica, C. racemose, Theobroma cacao, Camellia sinensis, Cola acuminate, Paullinia cupana, Ilex guayusa, I. vomitoria, I. paraguariensis*
Steroid alkaloids	Formed by the inclusion of one or two nitrogen atoms to a preformed steroid molecule	Veratridine, jervine and cyclopamine;	*Veratrum album, V. californicum, V. viride, Schoenocaulon officinale*
		Zygacine	*Toxicoscordion venenosum, Zigadenus glaberrimus*
Terpenoid alkaloids	Formed by the introduction of a nitrogen atom from methylamine, ethylamine, or β-aminoethanol to terpenoidal skeletons	Secodaphniphyllate	*Daphniphyllum macropodum, D. teijsmanni, D. humile*
		Aconitine	*Aconitum napellus, A. variegatum, A. noveboracense, A. vulparia, A. delphinifolium*

Chromone alkaloids	Formed by the linkage of a structure consisting of a nitrogen system to the "A" ring of chromone	Rohitukine	*Amoora rohituka, Dysoxylum binectariferum, Schumanniophyton magnificum, S. problematicum*
		Dysoline	*Dysoxylum binectariferum*
		Cassiadinine	*Senna siamea*
		Ficine and isoficine	*Ficus pantoniana*
Flavoalkaloids		capitavine	*Buchenavia capitata*
		Aquiledine, isoaquiledine	*Aquilegia ecalcarata*
Heterocyclic alkaloids (typical alkaloids)	Mononuclear	Hygrine	*Erythroxylum coca, Convolvulus hanadae*
		Boldine	*Peumus boldus, Lindera aggregata*
	Polynuclear	Atropine	*Atropa belladonna, Datura innoxia, D. metel, D. stramonium, Brugmansia* sp., *Hyoscyamus* sp.
		Reserpine	*Rauvolfia serpentine*
		Quinine	*Cinchona officinalis*
Non-heterocyclic alkaloids (atypical alkaloids)	Phenylethylamine skeleton	Ephedrine	*Ephedra sinica, E. viridis, E. fragilis, E. distachya, E. ciliate*
		Capsaicin	*Capsicum frutescens, C. annuum, C. chinense, C. baccatum*
	Tropolone skeleton	Colchicine	*Colchicum autumnale, Gloriosa superba*
	Modified diterpenes	Paclitaxel	*Taxus baccata, T. brevifolia, T. chinensis*
Opium alkaloids	Present in the Papaveraceae family	Morphine, codeine, papeverine, and thebaine	*Papaver somniferum*
Solanum alkaloids	Present in the Solanaceae family	Solanine, tomatidine, and solasodamine	*Solanum tuberosum, S. lycopersicum, S. melongena*
Daphniphyllum alkaloids	Present in the Daphniphyllaceae family	Daphniphylline, daphilactone-B	*Daphniphyllum macropodum*
Vinca alkaloids	Present in the Apocynaceae family	Catharanthine leurosine, vincristine, and vinblastine	*Catharanthus roseus*
Protoberberine alkaloids	Present in Annonaceae Ranunculaceae, Berberidaceae, Menispermaceae families; shares the same protoberberine skeleton	Berberine	*Berberis aristata, B. aquifolium, B. heterophylla*
		Jatrorrhizine	*Enantia chlorantha, Thalictrum lucidumm, Thalictrum revolutum*
		Palmatine	*Phellodendron amurense, Guatteria friesiana*
Ephedra alkaloids	Present in Ephedra genus of the Ephedraceae family	Ephedrine	*Ephedra sinica, E. viridis, E. fragilis, E. distachya, E. ciliate*

Biosynthetic Pathway

In this category, alkaloids are grouped on the criteria of being biosynthesized from the same/similar biochemical precursors, which after going through certain chemical

reactions gives rise to stable alkaloids (Table 2). Tyrosine, tryptophan, ornithine, and lysine are amino acid precursors, which undergo enzymatically catalyzed chemical reactions giving rise to tetrahydroisoquinoline, indole, pyrrolizidine, and piperidine alkaloids, respectively.

L-aspartate acts as a precursor for the biosynthesis of pyridine- and pyridinone-type alkaloids. Nicotinic alkaloids, such as nicotine and anabasine, are pyridine-type alkaloids containing nicotinic acid in their partial structure, whereas cerpegin is a pyridinone-type alkaloid, whose biosynthetic route is similar to the nicotine biosynthetic pathway.

Some non-amino acid compounds also act as precursors by supplying a nitrogen atom for alkaloid biosynthesis, such as anthranillic acid, which gives rise to quinoline and quinazoline alkaloids. Xanthine alkaloids include caffeine and theobromine, derived from a nucleoside, adenosine. Steroid and terpenoid alkaloids are pseudoalkaloids, whose carbon skeletons are derived from a mevalonic acid backbone and nitrogen sources are β-aminoethanol, ethylamine, and methylamine instead of amino acids. Terpenoid alkaloids include mono-, di-, and sesquiterpene alkaloids; amongst which diterpene alkaloids are the most important, as they are prominently used for pharmaceutical purposes. A less explored class of alkaloids includes chromone and flavoalkaloids, in which the nitrogen system is linked to "A" ring of chromone. In case of chromone alkaloids, chromone nucleus exists as noreugenin (5,7-dihydroxy-2-methylchromone), whereas flavoalkaloids bear an aryl substituent in the *C-2* position. Flavonoids including flavons, flavonols, flavanones, and flavan-3-ols are present in the structure of flavoalkaloids, making this an important class due to their distinctive amphoteric nature (basic and phenolic) as well as their biological properties.

Chemical Structure

In this category, alkaloids are grouped under heterocyclic and non-heterocyclic compounds based on the position of the nitrogen atom in their chemical structure. In the heterocyclic group, nitrogen is present in the main heterocyclic ring, as is the

case in alkaloids derived from L-tyrosine, L-phenylalanine, L-ornithine, L-tryptophan, L-lysine, and L-histidine, which are formed by the decarboxylation process of respective amino acid precursors. If a nitrogen atom occupies a position other than in the cyclic ring and is present in the aliphatic chain, non-heterocyclic alkaloids are formed. This non-heterocyclic group includes phenylethylamine- and tropolone-derived alkaloids, such as ephedrine, capsaicin, colchicine, and paclitaxel as main alkaloids (Table 2).

Taxonomy

Alkaloids produced by plant species of same genera are grouped under one category and this leads to broadened knowledge regarding the distribution of alkaloids in different plant species (Table 2). For instance, five main alkaloids including morphine, codeine, noscapine, thebaine, and papaverine produced in raw *Papaver somniferum* L. are grouped under opium alkaloids. Solanum alkaloids include steroidal alkaloids and their corresponding glycosides present in the *Solanum* plant species, including potato, tomato, eggplant, and various nightshades. Steroidal alkaloids and their glycosides include solanine, solasodine, solanidine, chaconine, tomatidine, tomatine, etc. Steroidal alkaloids are also found in the *Veratrum* genus, grouped as Veratrum alkaloids, including toxic veratridine, cyclopamine, and jervine.

Daphniphyllum alkaloids are structurally unique and diverse organic compounds produced in the plants of genus *Daphniphyllum*. These alkaloids are derived from six molecules of mevalonic acid through a squalene-like intermediate and are divided into six nitrogen heterocyclic skeleton types, namely daphniphylline, secodaphniphylline, daphnilactone-A, daphnilactone-B, yuzurinine, and daphnigracine. An important group of alkaloids include *Vinca* alkaloids derived from *Catharanthus roseus*, which include leurosine, vinblastine, and vincristine. Vinblastine and vincristine are chemotherapeutic agents for cancer treatment.

Their dimeric chemical structures are composed of two basic multi-ringed units; an indole nucleus (catharanthine) and a dihydroindole nucleus (vindoline) that are joined with other complex systems (Figure 15). Protoberberine alkaloids are another distinct class distributed among different genera of many plant families, including Annonaceae, Apocynaceae, Aristolochiaceae, Fabaceae, Lauracreae, Magnoliaceae, Menispermaceae, Ranuculaceae, Rutaceae, Berberidaceae, and Papaveraceae. These plant families comprise the highest number of plant species producing protoberberine alkaloids such as berberine, jatrorrhizine, and palmatine. These are tetracyclic alkaloids derived from benzylisoquinolines by the process of phenolic oxidation and coupling with the isoquinoline N-methyl group, resulting in the formation of "berberine bridge" carbon. Plant species from the genus *Ephedra* produce phenylalanine-derived alkaloids ephedrine, pseudoephedrine, phenylpropanolamine, and cathine. Of these ephedra alkaloids, ephedrine is the most potent thermogenic agent.

2.3.2 Chemical Reactions and Modification of Plant Alkaloids

Across the plant kingdom, modifying enzymes of different families act on many alkaloids to produce a diverse array of biologically important alkaloid derivatives having altered physical, chemical, and biological properties (Figure 16). Chemical modification reactions catalyzed by these enzymes, including mainly methylation, glycosylation, oxidation, reduction, hydroxylation, and acylation. Few examples of these reactions in alkaloid biosynthesis in plants are depicted in Figure 17.

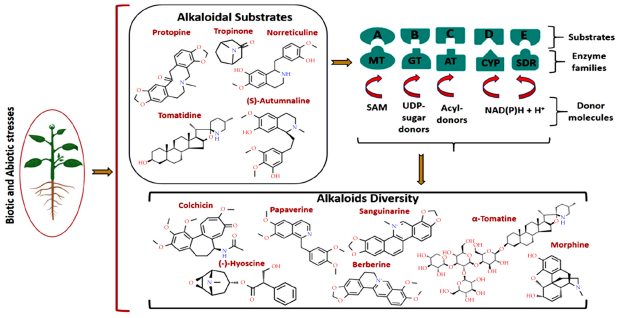
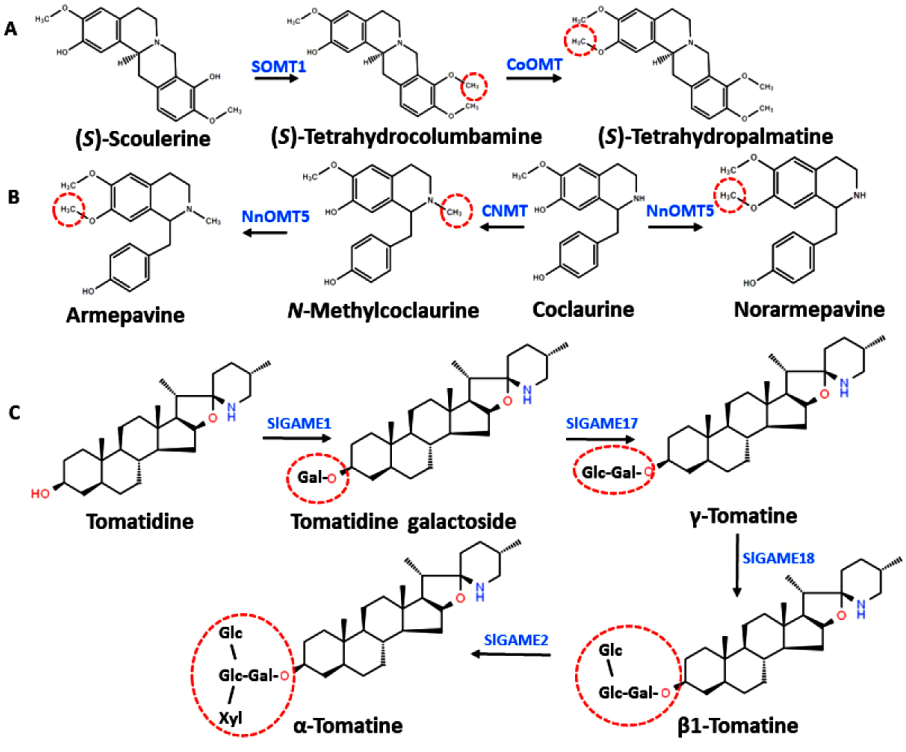


Figure 16. Alkaloid diversity in the plant kingdom.



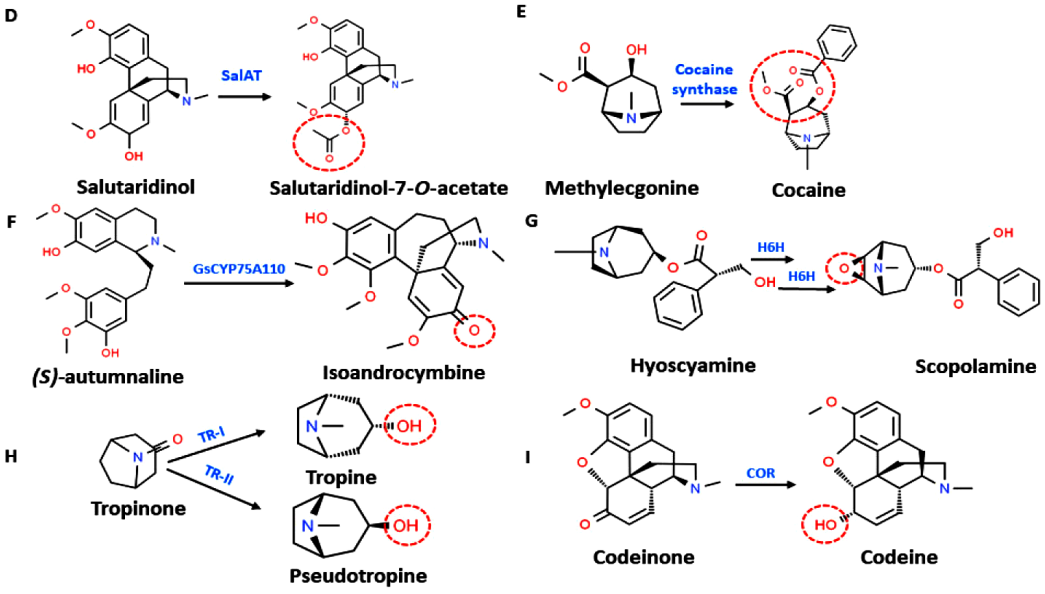


Figure 17. Examples of chemical reactions taking place in alkaloid biosynthesis in plants.

Methylation

Methylation plays a prominent role in developing the chemodiversity of alkaloids by functionalizing the parent compound with methyl groups. The methyl transferase (MT) gene family enzymes catalyze this type of reaction. *S*-adenosyl L-methionine acts as the classical donor of methyl group for over 95% of MTs; which after utilization undergo SN2 nucleophilic reaction and are converted into *S*-adenosyl L-homocystine (SAH) with the addition of a methyl group to the alkylated substrate. The C-terminal domain of the enzyme carries a Rossmann fold for substrate binding and catalysis, whereas its N-terminal region plays a prominent role in dimerization. In plants, *O*-methyltransferase (OMT) and *N*-methyltransferase (NMT) are well-known classes of MTs catalyzing the methylation of alkaloids. OMT represents the largest class of enzymes catalyzing the methyl transfer reaction at the hydroxyl position of the alkaloidal substrate. The differential selectivity of substrates with respect to stereochemistry is an important feature displayed by plant OMTs, especially those involved in benzylisoquinoline alkaloids (BIAs) metabolism (Figure 17A). For example, papaverine is an important BIA with antispasmodic activity. Several chemical modifications of intermediate alkaloids are reported during the biosynthesis of papaverine. Subsequent *O*-methylation steps of methylnorlaudanosoline by *4'OMT* and *N7OMT* produce *(S)*-norreticuline and *(S)*-norlaudanine, respectively. The methylation mediated by *3'-OMT* from the opium poppy (*Ps3'OMT*) has been implicated in the formation of two alkaloids, i.e., (*S*)-tetrahydropapaverine (a benzyltetrahydroisoquinoline alkaloid) and *(S)*-nororientaline (a tetrahydroisoquinoline alkaloid).

Remember

In Sacred lotus (Nelumbo nucifera Gaertn), methylation reactions generate a variety of alkaloids belonging to aporphin and bisbenzylisoquinoline structural categories. The methylation of norcoclaurine by NnOMT1 generates coclaurine, which is a nicotinic acetylcholine receptor antagonist having a tetrahydroi soquinoline structure similar to norcoclaurine.

Unlike OMT, NMT acts on nitrogen atom of substrate adding methyl group. The conversion of coclaurine to *N*-methylcoclaurine is catalyzed by coclaurine *N*-methyltransferase (NnCNMT1). NnOMT5 catalyzes 7-*O*-methylations of coclaurine and *N*-methylcoclaurine, generating norarmepavine and armepavine, respectively (Figure 17B). In *Laburnum anagyroides*, *S*-adenosyl L-methionine-dependent cytisine *N*-methyltransferase catalyzes the transfer of a methyl group from *S*-adenosyl L-methionine to cytisine, a quinolizidine alkaloid; the same enzyme further methylates cytisine to the less polar *N*-methylcytisine.

Glycosylation

Numerous glycosyl transferases catalyzing glycosylation reactions are reported in plants that transfer a glycosyl moiety from nucleotide sugar donors to different acceptor molecules such as hormones and specialized metabolites. These enzymes show less similarity in their primary sequences, although a 44-amino acid sequence, named as plant secondary product glycosyltransferase (PSPG)-box, is highly conserved. This box present in C-terminal region is believed to be involved in binding to the UDP moiety. These enzymes catalyze a SN2 nucleophilic displacement reaction between nucleotide sugar donors such as UDP-Glucose (UDP-Glc), UDP-rhamnose (UDP-Rha), UDP-xylose (UDP-Xyl), UDP-galactose (UDP-Gal), and UDP-glucuronic acid (UDP-GlcUA) and nucleophile acceptors. The resulting glycosylated compounds alter biological, physical, and chemical properties from the parent compounds along with distinct subcellular localization (Figure 17C). The glycosylation process has been proven to be helpful in improving the stability, solubility, and toxicity modulation of plant specialized metabolites.

In cultivated potato plants, solanidine undergoes glycosylation reactions at the 3-OH position by a set of glycosyltransferases, including solanidine galactosyltransferase (SGT1), solanidine glycosyltransferase (SGT2), and rhamnosyltransferase (SGT3), forming α-solanine and α-chaconine steroidal glycoalkaloids (SGAs). However, in wild potato plants, these SGAs are converted into leptines by a glykoalkaloid metabolism encoding gene—*GAME32*. The toxicity of the steroidal alkaloid tomatidine towards plant cells has been reported in tomato plants; therefore, to prevent self-toxicity, it is glycosylated with various sugar moieties

in the presence of uridine diphosphate-glycosyl transferases encoded by *SlGAME1, -2, -17,* and *-18* to generate α-tomatine (Figure 17C). It is the major SGA produced in the green leaves and unripe fruits of tomato plants and provide protection to plants against a plethora of microorganisms.

The in vitro enzymatic activity of three new GTs (UGT84A33, UGT71AE1, and UGT90A14) from *Carthamus tinctorius* has been tested for the synthesis of BIA glycosides from BIAs like berberines, berberrubine, jatrorrhizine, columbamine, palmaturbin, groenlandicine, protoberberines, etc. The glycosylating potential of these GTs can be helpful in generating novel alkaloids glycosides with improved bioactivity and reduced toxicity.

Acylation

The process of acylation in several classes of specialized metabolites contributes to the generation of chemodiversity in compounds. The acylation reaction involves the transfer of an acyl group from an activated donor to an acceptor molecule, catalyzed by acyltransferases (ATs). Two types of enzymes belonging to BAHD-acyltransferases (BAHD-ATs) and Serine CarboxyPeptidase-like (SCPL)-acyltransferases (SCPL-ATs) are known. These enzymes require "energy-rich" donor molecules; acyl-CoA thioesters serve as donors for BAHD-ATs, whereas SCPL-ATs utilize 1-*O*-β-glucose esters. The interplay between BAHD- and SCPL-ATs in different subcellular compartments is a necessary criteria for the secondary modification steps of different compounds. A cytosolic role has been reported for various members of BAHD-AT family; however, in plants, the acyl-CoA thioester is synthesized in vacuoles that are transported to cytosol to serve as donors for BAHD-ATs. On the other hand, SCPL-ATs carry out the downstream processing of metabolites in vacuoles, however, the formation of 1-*O*-β-glucose esters is catalyzed by UGTs in cytosol. This glycosylation step generates an active substrate for SCPL-ATs by adding a glucose tag to enable recognition by the transporters required for importation from cytosol to vacuole.

In the biosynthesis of morphinan alkaloids, salutaridinol 7-*O*-acetyltransferase plays a crucial role in the acylation of phenanthrene alkaloid salutaridinol, utilizing acetyl-CoA as the donor to form salutaridinol-7-*O*-acetate (Figure 17D), an immediate precursor for thebaine. In monoterpene indole alkaloids biosynthesis, the acetyl-CoA- or CoA-dependent reversible formation of vinorine (or 11-methoxy-vinorine) and 16-epi-vellosimine is catalyzed by vinorine synthase. These indole alkaloids act as a direct precursor for the ajmaline biosynthetic route. The tissue-specific expression patterns of BAHD-ATs have been reported, as in the case of the tabersonine derivative 19-*O*-acetyltransferase (TAT), which is able to acetylate minovincinine and horhammericine, the 19-hydroxytabersonine derivatives from roots. In coca plants, cocaine synthase is an important enzyme belonging to BAHD family that catalyzes the condensation of a pharmacologically inactive alkaloid, methylecgonine with benzoyl-

CoA derived from L-phenylalanine, to produce cocaine, which is a powerful stimulant (Figure 17E). Interestingly, littorine is a tropane alkaloid, which is structurally similar to cocaine, and both involve an **esterification** reaction in the last step of their respective biosynthesis pathways. However, this reaction is catalyzed by different enzymes in both cases, e.g., cocaine synthase catalyzes the formation of cocaine in coca plants, whereas littorine synthase belonging to SCPL family condenses phenyllactylglucose and tropine via esterification to form littorine in Solanaceous plants.

Keyword

Esterification is the process of combining an organic acid (RCOOH) with an alcohol (ROH) to form an ester (RCOOR) and water; or a chemical reaction resulting in the formation of at least one ester product.

Oxidation

Oxidation reactions taking place along the biosynthetic route of specialized metabolites occur in a stereo- and regio-specific manner. The formation of a diverse range of alkaloids and their parent compounds involves multiple oxidation reactions utilizing aromatic amino acid precursors. These reactions are catalyzed by cytochrome P-450 (CYP) enzymes, 2-oxoglutarate-dependent dioxygenases, and flavoproteins. CYPs containing haem as cofactor are a superfamily of enzymes exhibiting a broad diversity in their chemical structure and biological functions, occurring in different families and subfamilies across the plant kingdom. CYP450 monooxygenases perform hydroxylation reactions, which are the most common type of oxidation reactions in alkaloid formation. In Sacred lotus, two CYP450 monooxygenases belonging to CYP80G and CYP719A families are proposed to catalyze the conversion of *N*-methylcoclaurine to aporphins such as lirinidine and roemerine. The C–O coupling reaction required for the conversion of *N*-methylcoclaurine into Nelumboferine (a bisbenzylisoquinoline alkaloid) is possibly catalyzed by an enzyme encoded by the *CYP80A* family.

In SGA biosynthesis, in Solanaceous plants such as tomato and potato, hydroxylation and oxidation reactions are carried out by *GAME* genes that encode for CYP450 monooxygenases. These harmful SGAs are converted into non-toxic specialized metabolites by a series of chemical modifications involving hydroxylation, acetylation, and glycosylation. In tomato, the 2-oxoglutarate-dependent dioxygenase (2-ODD) enzyme encoded by *GAME31* catalyzes the hydroxylation of bitter α-tomatine to hydroxytomatine during ripening, which is an important step

towards the formation of non-bitter esculeosides. In potato, this enzyme is encoded by *GAME32*, which hydroxylates bitter SGAs to produce leptinines, which further produce leptines responsible for providing protection to plants against the Colorado potato beetle.

In the formation of colchicine in *Colchicum* and *Gloriosa* plants, *(S)*-autumnaline undergoes an oxidative para-para phenol coupling reaction catalyzed by GsCYP75A110 to form another isoquinoline alkaloid, namely isoandrocymbine (Figure 17F). The methylation of this compound by GsOMT4 yields *O*-methylandrocymbine. Furthermore, the expansion of the dienone ring of the previous compound and the formation of a tropolone ring takes place. These steps are catalyzed by GsCYP71FB1, which forms a tropolone-containing compound, N-formyldemecolcine.

For tropane alkaloids biosynthesis, the condensation of tropine with activated *(R)*-phenyllactate delivers the third ring intermediate to form littorine by littorine synthase. Further rearrangement by littorine mutase (a cytochrome P450) produces hyoscyamine aldehyde, which reduces to hyoscyamine. The enzyme hyoscyamine 6β-hydroxylase is a 2-oxoglutarate dependent dioxygenase showing bifunctional properties. Firstly, it carries out the hydroxylation of hyoscyamine to 6β-hydroxy hyoscyamine, and secondly, the epoxidation of 6β-hydroxy hyoscyamine to scopolamine (Figure 17G). These alkaloids possess differences in biological effects despite having a similar tropane ring structure.

The final step of papaverine biosynthesis involves the activity of *TPOX* (tetrahydropapaverine oxidase), which dehydrogenates the *O*-methylated and *N*-desmethyl alkaloid tetrahydropapaverine to yield papaverine. Expanding the papaverine biosynthetic pathway, a novel 2-oxoglutarate/Fe_2+-dependent dioxygenases (2ODD) catalyzing the efficient substrate- and regio-specific 7-*O*-demethylation of papaverine yielding pacodine (analogue of papaverine) has been reported.

Reduction

The cytochrome P450 reductase (CPR), short-chain dehydrogenase/reductase (SDR), and aldo-keto reductase (AKR) superfamilies are enzymes that carry out reduction reactions to several alkaloids. In the formation of tropane alkaloids in Solanaceae plants, the reduction of the keto group in the tropane ring is catalyzed by stereospecific tropinone reductases (TRs), which are NAD (P)(H)-dependent monomeric oxidoreductases belonging to the SDR enzyme family. Pathways to two distinct tropane alkaloid categories, scopolamine and calystegines, are decided upon by two reductases. Tropinone reductase I coverts tropinone to tropine (3α-tropanol), which is used to produce scopolamine. Tropinone reductase II reduces tropinone to pseudotropine (3β-tropanol) (Figure 17H), which further proceeds towards calystegine biosynthesis.

A short-chain alcohol dehydrogenase/reductase co-expressing with norbelladine 4'-*O*-methyltransferase from *Narcissus* and *Galanthus* spp. catalyzes a carbon-carbon

double bond reduction in noroxomaritidine to form oxomaritinamine, which is required for the biosynthesis of amaryllidaceae alkaloids. In several cases, CYP450 enzymes require the shared activity of CPR for two-electron transfer activity. It was studied in *C. roseus* that a class II CPR provides electrons for highly expressing P450s, which are involved in tissue-specific and induced specialized metabolism. The cloning and purification of first plant CPR was reported in *C. roseus*. Perakin reductase is the example of first aldo-keto reductase superfamily enzyme, isolated from *Rauvolfia* sp., and was found to be involved in monoterpene indole alkaloid biosynthesis.

2.3.3 Chemical Conversions in Plant Alkaloids

A series of biochemical modification reactions in plants bring with them new sequels of alkaloids with diverse arrays of chemical structures and biological activities. We have highlighted some plant alkaloids involving multiple chemical modifications that occur in the final steps of alkaloidal conversions.

Noscapine Alkaloids

The diverse class of the isoquinoline alkaloid contains noscapine as an important non-narcotic drug belonging to the phthalideisoquinoline subclass, which has been isolated from the Papaveraceae family plant species. Noscapine undergoes 3′-hydroxylation and a series of *O*- and *N*-methylations to form (*S*)-reticuline, which is one of the alkaloids found in the opium poppy. Methylene-bridge formation takes place in (*S*)-reticuline via the berberine bridge enzyme (BBE) to form the protoberberine alkaloid (*S*)-scoulerine, which undergoes 9-*O*-methylation catalyzed by an enzyme encoded by *S9OMT1* to form *(S)*-tetrahydrocolumbanine. *Canadine synthase* gene encoding CYP719A19 catalyzes the formation of the methylenedioxy bridge and generates *(S)*-canadine, which can block K (ATP) channels in dopamine neurons. Further, the oxidation of a cyclic narcotine hemiacetal (a benzylisoquinoline alkaloid and a cyclic acetal) to the lactone ring in noscapine is performed by noscapine synthase (NOS), also known as short-chain dehydrogenase/reductase (SDR1).

Morphinan Alkaloids

Another important class of BIAs in opium poppy includes morphinan alkaloids, which are strong narcotic analgesics. These comprise of natural opiates like morphine and codeine and their semisynthetic derivatives, such as dihydromorphine and hydromorphone. These are used for treating severe pain associated with cancer, rheumatism, and dental problems. The morphine pathway diverges from other BIA pathways as it utilizes *(R)*-reticuline instead of *(S)*-reticuline. The conversion of *(S)*-reticuline to *(R)*-reticuline is catalyzed by STORR ([*S*]-to [*R*]-reticuline), which is a P450 enzyme displaying separate domains for two enzymatic activities. In the first part of reaction, the cytochrome

P450 module of the enzyme converts *(S)*-reticuline to 1,2-dehydroreticuline, and in the latter part, oxidoreductase module converts 1,2-dehydroreticuline to *(R)*-reticuline. The salutaridine, which is the basic skeleton of opiates, is formed by the coupling reaction between the 20th position and 10th position of the carbon atoms of (R)-reticuline catalyzed by salutaridine synthase. Salutaridine reductase performs the reduction of salutaridine to produce thebaine, followed by acetylation with salutaridine 7-*O*-acetyltransferase and a spontaneously occurring deacetylation reaction. Thebaine undergoes demethylation by thebaine 6-*O*-demethylase and reduction by codeinone reductase to produce codeine (Figure 17I). Morphine is synthesized by the demethylation of codeine by codeine *O*-demethylase.

Sanguinarine, Protopines, and Berberine Type Alkaloids

Sanguinarine is a benzophenanthridine alkaloid, belonging to the BIA class of alkaloids extracted from many plant species, such as *Sanguinaria canadensis*, *Chelidonium majus*, and *Macleaya cordata*. The BIA alkaloid (*S*)-Scoulerine undergoes several modification reactions to generate a series of alkaloids possessing diverse biological properties. (*S*)-Scoulerine is synthesized from *(S)*-reticuline by the berberine bridge enzyme. Cheilanthifoline synthase, which is a member of the CYP719A subfamily, catalyzes the addition of a methylenedioxy bridge to form *(S)*-cheilanthifoline, which is used in Bhutanese traditional medicine for the treatment of fever. Further, the oxidation of *(S)*-cheilanthifoline is performed by another CYP719A enzyme, stylopine synthase, to form *(S)*-stylopine. Opium poppy TNMT catalyzes the *N*-methylation of *(S)*-stylopine to form *(S)-cis-N*-methylstylopine, which is further acted upon by *N*-methylstylopine 14-hydroxylase, a member of the CYP82 N subfamily, to produce protopine. Protopine acts as an analgesic and also inhibits histamine H1 receptors and platelet aggregation. Protopine undergoes 6-hydroxylation and gets converted into dihydrosanguinarine by *P6H* protopine 6-hydroxylase. *DBOX (*dihydrosanguinarine oxidase) and *SanR* (sanguinarine reductase) catalyze forward and backward reactions to produce sanguinarine, which is a toxic alkaloid. STOX, (*S*)-tetrahydroxy protoberberine oxidase, produces berberine, which is a protoberberine type of isoquinoline alkaloid. Berberine shows antimicrobial activity and antidiabetic effects in experimental animal and clinical diabetic patients.

Monoterpene Indole Alkaloids

The biological activities attributed to this group of alkaloids make them promising candidates for utilization in the pharmaceutical industry. Stemmadenine is an alkaloid which after second carbon-carbon cleavage forms dehydrosecodine (an acrylic ester). This intermediate possibly goes through a Diels Alder reaction towards an iboga-type alkaloid, catharanthine, and an aspidosperma-type alkaloid, tabersonine. Tabersonine is converted into vindoline by via seven-step pathway involving hydroxylation, *O*-methylation,

C-3-oxidation, C-2/C-3-reduction step, *N*-methylation, C-4-hydroxylation, and C-4-O-acetylation. Vindoline is the precursor for important the anticancerous metabolites, vincristine and vinblastine. The synthesis of these metabolites takes place in the leaf tissues of the plant.

In the roots of *C. roseus* and *Tabernaemontana divaricata*, lochnericine is a major MIA derived from the stereoselective C6, C7-epoxidation of tabersonine by tabersonine 6,7-epoxidase 1. In another biosynthetic route, *O*-acetylstemmadenine is acted upon by vincadiformine synthase 1 or 2 (VS1/2) to form vincadiformine, which undergoes hydroxylation to form minovincinine by vincadiformine 19-hydroxylase. This is followed by acetylation by minovincinine 19-hydroxy-*O*-acetyltransferase (MAT) to form echitovenine. In the contrasting biosynthetic route, the hydroxylated form of vincadiformine is methylated by 16-*O*-methyltransferase to form ervinceine. Vincadiformine and vincamine are reported at higher levels in the leaves of *Vinca minor*. Vincamine is synthesized in leaves of *V. minor* when 16-methoxy tabersonine undergoes epoxidation in the presence of tabersonine 3-oxidase (T3O), which after rearrangement forms an eburnamine-vincamine like skeleton (fundamental parent alkaloid) that further transforms into vincamine.

2.3.4 Role of Alkaloids in Plant Defense against Biotic and Abiotic Stresses

Alkaloids act as reservoirs for nitrogen storage and plants have evolved their metabolic diversity to cope with environmental stress conditions. For example, plants use specialized metabolites as an integral part of their defense system, including biotic and abiotic stress responses (Figure 18). Furthermore, these specialized metabolites are found in varying levels in different tissues of plants (e.g., leaf, stem, root, flower, seed, fruit, and storage organs), and offer protection against a diverse variety of pests, predators, and herbivores. Synthesized alkaloids are stored in specific cellular compartments and upon sensing different stress signals from environment they are released from the stored organelle/specific glands and exported to the target tissues.

Many alkaloids show potent antimicrobial activities against various pathogenic microorganisms. Specific alkaloids, such as α-tomatine from tomato, piperine from black pepper, and protoberberines and berberines from a wide range of plant species including *Berberis aristate*, exhibit both antimicrobial and antifungal properties. However, selective alkaloids possess only antibacterial activities, for example, squalamine acts against *Klebsiella pneumoniae*, lysergol acts against *Escherichia coli*, and tomatidine acts against *Staphylococcus aureus*, *Bacillus cereus*, *B. subtilis*, and *Listeria monocytogenes*. Some alkaloids specifically exhibit antifungal properties, such as the tomatidine glycoalkaloid from tomato acts which against yeast—*Saccharomyces cerevisiae*, quinoline from *Waltheria indica* L. which acts against *Candida albicans*, and Jatrorrhizine (a protoberberine alkaloid

from *Mahonia aquifolium*), β-carboline, and cocsoline which act against several fungal species. Other alkaloids (such as perivine, leurocristine, berberine, and acrimarine F) show antiviral activities against poliovirus, vaccinia, influenza, HIV, and Epstein-Barr virus (Figure 18).

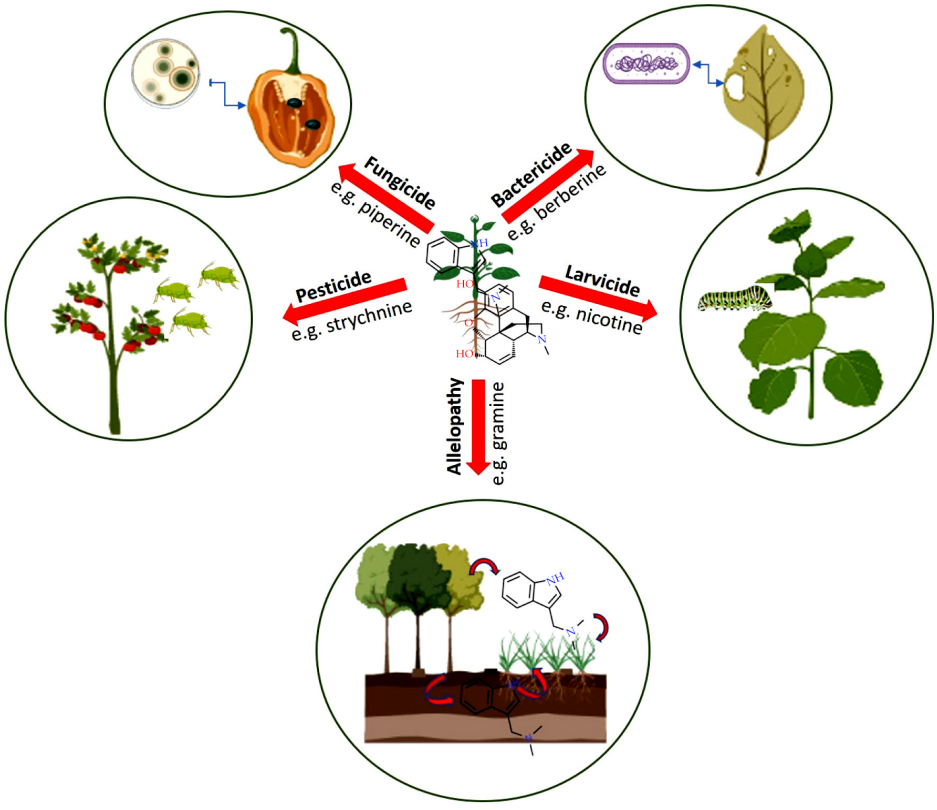


Figure 18. The role of alkaloids in plant defense.

Several alkaloids such as nicotine, α-chaconine, and α-solanine are known to possess toxicity against various chewing insects (e.g., *Spodoptera exigua, Manduca sexta*, and *Tecia solanivora*) as well as sucking insects such as whiteflies, aphids, and planthoppers (Figure 18). The aphicidal activity of alkaloids isolated from Amaryllidaceae plants has been reported against *Aphis citricola*. Colchicine produced by *Colchicum autumnale* inhibits the polymerization of tubulin and the depolymerization of microtubule during mitosis, and causes toxicity to predators such as the honeybee (*Apis mellifera*) and the honeycomb moth (*Galleria mellonella*) (Figure 19).

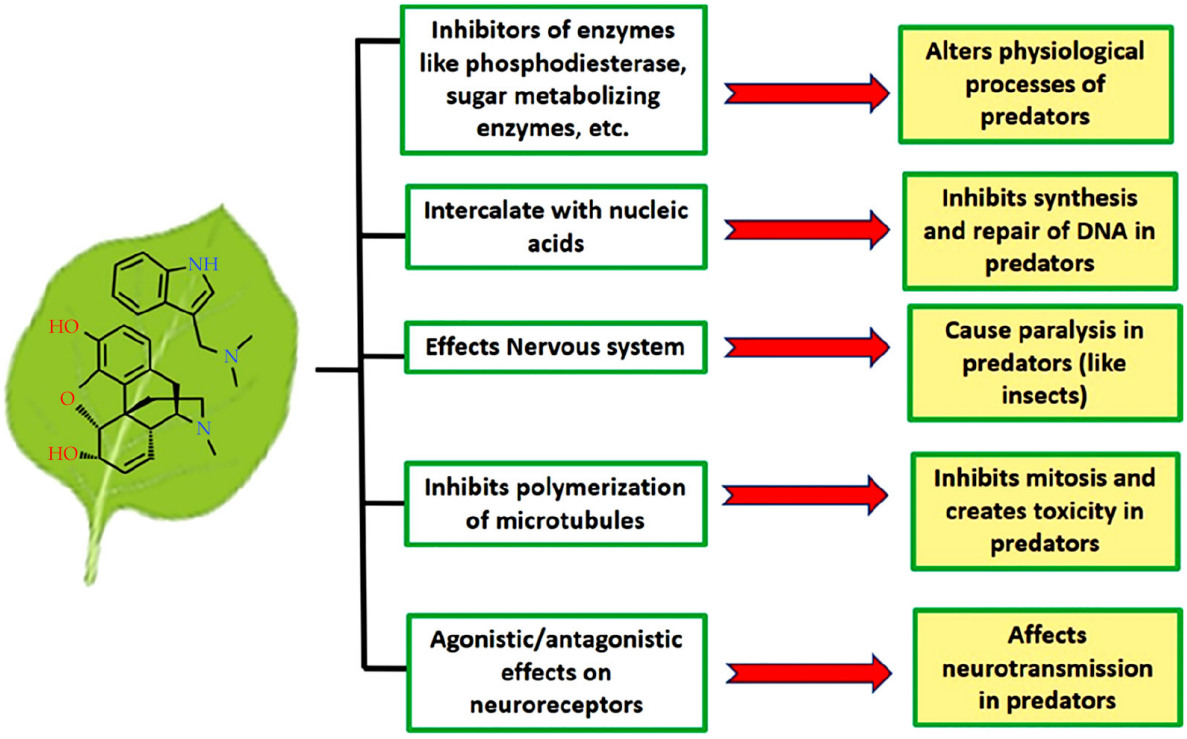


Figure 19. The mechanism of action of alkaloids.

Nitrogen-fixing plants, as well as plants grown on high-nitrogen contents, accumulate high levels of alkaloids in their leaves making htem more resistant to herbivory. Alkaloids that are derived from aromatic amino acids (e.g., isoquinoline, quinoline, and indole alkaloids) are known to exhibit antiherbivoral activities. Many alkaloids act on different enzymes of predators to disturb their physiological processes (Figure 19). Swainsonine from locoweed (*Astragalus* and *Oxytropis* species) is known to inhibit the activity of α-mannosidase, and thus, affects the synthesis of N-glycans in cellular membranes and the ingestion of respective plants causes intoxication amongst livestock. Alkaloids like morphine, codeine, and caffeine produce stimulatory effects on the central nervous system of predators, which can cause paralysis attack in predators (Figure 19). Some alkaloids are highly poisonous to mammals and other animals. For example, plants containing strychnine, brucine, and atropines upon ingestion by various predators produce serious effects on **neurotransmission** and the central nervous system, leading to the death of individuals (Figure 19). Specific plant alkaloids, such as quinine, emetine, β-carboline, furanocoumarin, and furanoquinoline, have nucleic acid intercalating properties, interfereing with DNA replication and repair mechanisms, which may lead to mutations and genotoxicity. Overall, alkaloids display distinct mechanisms to protect plants from predator attack.

Furthermore, certain alkaloids leached from leaves, roots, and other plant tissues exhibit allelopathic effects by affecting the growth potential of the roots and shoots of other competitor plants (Figure 18). For example, capsaicin, the pungent alkaloid produced mostly in the seeds of *Capsicum* species, has been shown to affect the germination, seedling growth, and chlorophyll accumulation in mung bean (*Vigna radiata*) plants. Several other alkaloids such as berberine, gramine, and sanguinarine exhibit allelopathic effects against *Lactuca sativa* and *Lepidium sativum* seedlings (Figure 18).

Keyword

Neurotransmission is the fundamental process that drives information transfer between neurons and their targets.

Various abiotic stress conditions such as drought, salinity, high temperatures, etc., are also known to influence the accumulation of alkaloids in many plant species. For example, drought stress alters the levels of chinolizidin alkaloids in *Lupinus angustifolius* and morphine alkaloids in *Papaver somniferum*. Moreover, emerging studies suggest that heat and drought conditions alone or in combination can alter the accumulation of alkaloids in plant species such as *Mentha piperita* and *C. roseus*. Tropane alkaloid levels in the young leaves of *Datura innoxia* could be induced by salt stress (153.8 moL/m^3 NaCl). Using a B5 suspension culture of *C. roseus*, it has been demonstrated that the total alkaloid yield could be enhanced during salinity stress (100 mM NaCl). An increased alkaloid content in *C. roseus* seedlings exposed to salt or salinity stress has also been reported previously. The effects of different nitrogen sources on the levels of indole alkaloid content in *C. roseus* seedlings has also been studied, wherein potassium nitrate (20 mM KNO_3) treatment led to an increased accumulation of alkaloid content in leaves compared to ammonium chloride (2 mM NH_4Cl). Moreover, it was earlier reported that UV-B significantly increased the content of terpenoid indole alkaloids (lochnericine and ajmalicine) in the hairy roots of *C. roseus*. Apart from abiotic factors, phytohormones are also known to influence the levels of alkaloids in plants.

2.3.5 Biological Activities of Alkaloids

Since ancient times, alkaloids have shown great effects on animal and human lives and are an inclusive part of the food and beverages consumed in daily life. Besides, these compounds are used in medicinal and stimulant drugs with core biological

activities (Table 3). Alkaloids with anticancerous activities, such as vincristine, vinblastine, and taxol, are being effectively used as chemotherapeutic drugs. Vincristine and vinblastine isolated from *Vinca rosea* act by binding to tubulin; however, they work on different tumor types. Vincristine is used to treat acute leukemia and other lymphomas, while vinblastine is mainly used for the treatment of Hodgkin lymphoma and advanced breast or testicular cancer. Other Vinca alkaloids, vinorellaine and vinflunine, are used to treat lung cancer and urothelial cancer, respectively. Berberine, an isoquinoline alkaloid, has been shown to possess anticancer potential through the inhibition cell proliferation by interacting with respective microRNAs and suppressing telomerase activity (Table 3). Evodiamine, the bioactive compound isolated from *Evodia rutaecarpa*, is a quinolone alkaloid which has been shown to exhibit anticancer activities both in vitro and in vivo by inhibiting angiogenesis, invasion, and metastasis in a variety of cancer cell lines. Piperine is an alkaloid from *Piper nigrum* and *P. longum* which is shown to exhibit chemopreventive effects. This dietary phytochemical can act against several kinds of carcinogen, such as 7,12-dimethyl benz(a)anthracene and benzo(a)pyrene. Colchicine extracted from Colchicum and Gloriosa plant species has been approved by the Food and Drug Administration (FDA, USA) for the treatment of acute cases of gout Mediterranean fever, pericarditis, and Behcet's disease. The bioactivity of colchicine is thought to result from its interaction with tubulin dimers and the subsequent inhibition of microtubule growth. However, colchicine alone or in combination with taxane and Vinca alkaloids is too potent to be used in chemotherapy (Table 3).

Table 3. The biological activities of alkaloids.

Alkaloid	Biological Activities	Mechanism of Action	IC50 Range
Paclitaxel	Antineoplastic and antimicrotubule	Suppresses microtubule dynamics by binding to β-tubulin subunits of microtubule and thereby inhibiting spindle function.	0.00126–12.3 μM
Vincristine	Antileukemic, antilymphoma, antineuroblastoma, and antisarcoma	Inhibits mitosis at the metaphase stage by interacting with tubulin; interferes with amino acids, cyclic AMP, glutathione metabolism, and calmodulin-dependent Ca_2^+-transport ATPase activity.	0.00126–1.01e + 3 μM
Camptothecin	Antitopoisomerase and anti-HIV	Causes DNA damage by binding to topoisomerase I and the DNA complex forming a ternary complex, stabilizing it, and preventing DNA re-ligation resulting in apoptosis.	0.00214–62.3 μM
Rohitukine	Anti-inflammatory, anti-fertility, anti-implantation, anti-cancer, and immuno-modulatory	Triggers apoptosis in lung cancer cells.	0.3–7.3 μM

Strychnine	Neurotoxic, pesticidal, and rodenticidal	Acts as an antagonist of glycine (an inhibitory neurotransmitter) and acetylcholine receptors, thereby preventing inhibitory signals and activating motor neurons in the spinal cord, resulting into spastic muscle contraction.	64–92 nM
Ephedrine	Promotes short-term weight loss, decreases motion sickness, possesses a cardiac stimulant, hyperglycaemic, hypertensive, bronchodilator	Indirectly stimulates the adrenergic receptor system by increasing the activity of norepinephrine at the postsynaptic α and β receptors. Acts as a CNS stimulant, due to its ability to cross the blood-brain barrier.	124 μM
Colchicine	Anti-gout, anti-inflammation, and treats familial Mediterranean fever	Inhibits mitosis by inhibiting microtubule polymerization; inhibits proinflammatory mechanisms and increases anti-inflammatory mediators; inhibits neutrophil motility and activity, interferes with superoxide formation, and thereby inhibits or prevents gout inflammation.	3–300 nM
Vasicine	Bronchodilator, mucolytic, antitussive, antibacterial, cytotoxic, abortifacient, and uterotonic	Acts as an acetylcholinesterase inhibitor and a butyrylcholinesterase inhibitor.	125 μM
Reserpine	Anti-hypertensive and anti-psychotic	Interferes with the sequestering of neurotransmitters into storage vesicles located in the presynaptic neuron by inhibiting their ATP/Mg^{2+} pump, causing a reduction in catecholamines, thereby causing antihypertensive effects.	1.7–2.8 μM
Ajmalicin	Anti-hypertensive	Acts as α1-adrenergic receptor antagonist and shows hypotensive effects.	3.5–5.44 μM
Tetrandrine	Anti-inflammatory, immunologic, anti-allergenic, and anti-tumour; used for treating Ebola virus infection in mice	Acts as a calcium-channel blocker, inhibits the degranulation of mast cells.	11.3 μM
Morphine	Analgesic and CNS stimulant	Acts as agonists for mu and kappa opioid receptors, on the ventral tegmental area of the brain; agonist of the delta-opioid receptor in the nucleus accumbens and activates the morphine reward pathway.	1–8.8 mM
Codeine	Analgesic, antidiarrheal, and antitussive	Acts as agonist for mu opioid receptors involved in the transmission of pain throughout the body and central nervous system.	60 μM

Papaverine	Vasodilatory and antispasmodic	Shows direct vasodilating action on cerebral blood vessels, increases cerebral blood flow and decreases cerebral vascular resistance.	2–37 μM
Berberine	Antimicrobial, antitumor, anti-hyperglycemic, antimalarial, and anti-inflammation; Alzheimer's disease treatment	Lowers cholesterol through LDL-receptor-mediated liver LDL cholesterol clearance, promotes LDL-receptor expression through the proprotein convertase subtilisin/kexin type 9 (PCSK9)-LDL-receptor pathway.	0.1–25 μM
Scopolamine	Depressant action on sympathetic nervous system; possesses mydriatic, spasmolytic, and local anesthetic effects; treats motion sickness, postoperative nausea, and vomiting	Acts as a non-selective competitive inhibitor of M1-M5 mAChRs (G-protein-coupled muscarinic acetylcholine receptors), shows anticholinergic effect, and alters signalling through CNS associated with vomiting.	928 μM
Piperine	Presents hemo-preventive, anti-carcinogenic, antioxidant, anti-inflammatory, anticarcinogenic, stimulatory, hepatoprotective, antihyperlipidemia, anti-asthmatic activities; gastro-intestinal stimulant, and appetite stimulant	Affects the plasma concentrations of P-glycoprotein in the (P-gp)-mediated transport of drugs and metabolizes enzyme CYP3A4 substrates in humans; lowers endogenous UDP-glucuronic acid contents and inhibits transferase activity, thereby modifying the rate of glucuronidation.	1–34 μM
Lupinine	Insecticidal	Reversible inhibitor of acetylcholinesterases; possesses a binding affinity for muscarinic and nicotinic acetylcholine receptors.	712 μM
Swainsonine	Chemotherapeutic	Acts as a golgi α-mannosidase II inhibitor	34 nM
Skimmianine	Analgesic, antispastic, sedative, and anti-inflammatory	Suppresses TNF-α and IL-6 gene transcription, inhibits the production of NO, prostaglandin E2, and superoxide anions.	8.6 μg/mL
Theobromine	Antitumor, bronchorelaxater, and antitussive	Acts as antagonist to adenosine-receptors within the plasma membrane of virtually every cell, which further promotes neurotransmitter release.	2500 μM
Caffeine	Autonomous nervous system stimulant, anti-inflammation; improves cognitive performance	Inhibits the activity of nucleotide phosphodiesterase enzymes, regulates calcium handling in cells, and participates in adenosine receptor antagonism, stimulating inotropic effects in the heart.	500–1000 μM
Nicotine	Antiherbivore, insecticide, teratogenic, addictive, stimulant, and anxiolytic effects; treatment of nicotine dependence	Acts as an agonist/antagonist of certain nicotinic acetylcholine receptors, binding with receptors leading to depolarization, activating voltage-gated calcium channels.	0.5–20 nM

Veratridine	Inhibitor of sodium channel inactivation and neurotoxic	Depolarizes cells by affecting sodium channels, can activate Nav 1.8 along with additional Nav channels; enhances protein tyrosine phosphorylation; can turn the membrane potential to a more positive one and can also modify the effect of progesterone on $(a_2+)i$ and sperm membrane potential.	27–84 μM
Aconitine	Analgesic, blood coagulant, anti-inflammatory, cardiotoxic, and neurotoxic	Interacts with voltage-dependent sodium-ion channels, binds to the channel at the neurotoxin binding site 2 on the α-subunit, suppressing the conformational change in the sodium-ion channel from an active state to an inactive state.	10–20 μM
Hygrine	Sedative, hypnotic laxative, and diuretics	Not known.	Not reported
Boldine	Antioxidant, antipyretic, anti-inflammation, hepatoprotectant, cytoprotectant, and neuroprotectant	Acts as an α-adrenergic antagonist in vascular tissues; it can cross the blood-brain barrier exhibiting neuroprotective effects.	8.5 μM
Atropine	Anticholinergic, antispasmodic, and antimuscarinic	Binds and inhibits muscarinic acetylcholine receptors, producing anticholinergic effects.	2–55 μM
Capsaicin	Anti-obesity, antifungal action, and chemical irritant; treating peripheral neuropathy, psoriasis, and non-allergic rhinitis	Induces a topical hypersensitivity reaction on the skin by carrying out the "defunctionalization" of nociceptor fibers. Pain mechanism is due to temporary loss of membrane potential, inability to transport neurotrophic factors, and the reversible retraction of epidermal and dermal nerve fiber terminals.	50 μM
α-Solanine	Antiallergic, anti-inflammation, antipyretic, and anti-carcinogen; treating gastrointestinal and neurological disorders	Inhibits cholinesterase activity, disrupts cell membranes; opens the potassium channels of the mitochondria increasing their membrane potential, followed by the transport of Ca_2+ from mitochondria into the cytoplasm leading to the an increased concentration of Ca_2+ in the cytoplasm triggering cell damage and apoptosis.	32.18 μM
α-Tomatine	Anti-leukemia, fungicide, antimicrobial, and insecticide	Causes the disruption of cellular membranes and the inhibition of acetylcholinesterase; stimulates the immune system by participation in a sequence of respiratory burst destroying bacteria.	7–10 μM
Jatrorrhizine	Antibacterial and antifungal	Blocks α-1 and α-2 adrenoreceptors and monoamine oxidase A and B.	4–62 μM

Palmatine	Antimicrobial, hypoglycemic, antiarrhythmic, and antioxidant	Intercalates with nucleic acids; induces apoptosis; inhibits proliferation.	0.07–22 μM
Quinine	Antimalaria, mild antipyretic, and analgesic	Interferes with a parasite's ability to break down and digest hemoglobin, leading to starvation in parasites.	13.4 μM
Cytisine	Teratogenic	Partial agonist of α4-β2 nicotinic acetylcholine receptors; causes a reduction in the effects of nicotine on dopamine release in the mesolimbic system when given alone, while simultaneously attenuates nicotine withdrawal symptoms accompanying cessation attempts.	27.3 nM

Did You Know?

The Term alkaloid was coined in 1819 by the German Scientist Carl F. W. Meissner and is derived from Arabic al qualja "means ashes of plants". Alkaloids are groups of natural occurring simple or complex, low molecular weight nitrogen containing compounds which are basic in nature.

The Vinca alkaloid category includes vincamine, which has vasodilatory activity and increases blood flow to the brain. It is sold in tablet form in Europe and has also been used as a nootropic supplement in diet to improve the brain function of healthy people. Some alkaloids show psychotropic effects by stimulating the central nervous system. These include cocaine, ephedrine, and strychnine, and their long-term use can cause addiction and serious adverse effects upon frequent consumption. Cocaine is frequently used as a recreational drug due to its stimulant property, causing mental effects of feelings of joy. Aside from harmful effects, a central nervous system stimulant such as ephedrine has a bronchial smooth muscle relaxant property, and therefore, it is used as a decongestant. Strychnine, a terpene indole alkaloid, is a highly toxic compound to humans as well as other vertebrate animals, such as rats and birds. It is mainly used as a rodenticide but varies in specificity and can kill other animals too. Quinazoline alkaloids such as vasicinone and vasicine show bronchodilatory activity and are respiratory stimulant used in various asthma medications.

Alkaloids also have the potential to reduce hypertension. Tetrandrine isolated from *Stephania tetrandra* has been reported to be useful for the treatment of hypertension in patients accompanied with poor sleep efficiency. Reserpine and Ajmalicine are recommended alternative drugs for treating hypertension. Alkaloids extracted from the opium poppy (*P. somniferum*) induce analgesic and narcotic effects by acting upon opioid receptors. The alkaloids from poppy include morphine, codeine, thebaine, noscapine, and their derivatives, which are used for treating moderate to severe pain. However, they may cause adverse side effects such as dizziness, sedation, nausea, vomiting, respiratory

depression, dependency, and tolerance. Noscapine has been used as safe cough suppressant and has evoked attention of pharmaceutical industries in cancer treatment (Table 3).

(S)-reticuline, the central pathway intermediate of most BIAs, is a potent central nervous system depressant and is also suggested to be responsible for atypical parkinsonism. Hyoscyamine extracted from *Datura stramonium* is an antagonist of muscarinic acetylcholine receptors and can control neuropathic pain; the efficacy of its analgesic effect is improved when used in combination with opioids. Scopolamine, also known as hyoscine, is actively produced in *Hyoscyamus niger* and has a strong hallucinogenic effect, making it a constituent of psychoactive drugs. Veratrum alkaloids are toxic compounds that can cause rapid heart failure by activating sodium ion channels. Although veratrum alkaloids are toxic, they have been used for the treatment of myasthenia gravis and hypotension (Table 3).

Another alkaloid in the protoberberine group is coptisine, which is used in Chinese herbal formulations. Coptisine exhibits a wide range of pharmacological properties such as antibacterial, hypoglycemic, anti-tumerogenic, and neuroprotectant effects. Palmitine is the major component of the alkaloidal extract of *Enantia chlorantha,* which has been studied for its use in the treatment of jaundice, hypertension, inflammation, dysentery, and liver-related diseases. Anti-inflammatory, antimicrobial, and antifungal activities have been reported for jatrorrhizine, which is also an important alkaloid in the above protoberberine-type alkaloidal group. Ephedrine and pseudoephedrine are used in decongestants and cold medicines. Ephedrine-related Chinese formulations are sold as dietary supplements for effective weight loss and to enhance athletic performance. Cytisine, a quinolizidine alkaloid from *Laburnum* and *Cytisus* plants in the Fabaceae family, acts as a partial agonist of nicotinic acetylcholine receptors (nAChRs) and has been used to help smoking cessation (Table 3).

Steroidal alkaloids and their glycosides present in Solanaceous plants pose various biological activities ranging from toxic to useful properties. α-tomatine produced in the green tissue of tomato plants is an antinutritional compound and its consumption in the range of 500 to 5000 mg/kg of dry weight in green tomato recipes shows toxicity in humans. On the other hand, tomatine can act as a powerful adjuvant, and is reported to elicit an antigen-specific cell-mediated immune response to a pre-erythrocytic stage malaria vaccine candidate antigen. α-chaconine present in green potatoes is a natural toxicant which causes harmful physiological effects in other organisms but provides protection to plants against fungi and insects.

Aporphin and bisbenzylisoquinoline alkaloids from Sacred lotus possess nutritional and medicinal values. Norcoclaurine, also known as higenamine, obtained from lotus seeds is reported to show notable pharmacological properties such as anti-inflammatory, anti-thrombotic, and β-adrenergic receptor agonist effects. Norarmepavine and armepavine have potential to be used in the cosmetic industry due to their melanogenesis inhibition activity. Due to the immunomodulatory effect of armepavine,

it has been used as potent herbal drug in the treatment of autoimmune disorders such as systemic lupus erythematosus and crescentic glomerulonephritis. Roemerine has been ascribed anti-fungal and anti-malarial properties. Nelumboferine, along with other bisbenzylisoquinoline alkaloids, such as neferine and liensinine, exhibited sedative effects in a mouse model (Table 3).

Hundreds of alkaloids have activities against bacteria (e.g., squalamine, lysergol, tomatidine, etc.); fungi (e.g., tomadine, quinoline, β-carboline, and cocsoline, etc.) and viruses (e.g., leurocristine, periformyline, perivine, and vincaleucoblastine). Thus, the antibiotic property of alkaloids has been efficiently utilized by human beings for pharmaceutical purposes. The commercialization of products containing alkaloids has created new opportunities in the market to treat various health ailments (Table 4). Many of them also act as stimulant drugs (e.g., nicotine, morphine, caffeine, codeine, etc.). The nutraceutical values of alkaloids have been employed for manufacturing dietary ingredients (e.g., caffeine, atropine, and cocaine), nutritional supplements in combination with other natural compounds (e.g., hyoscyamine, scopolamine, tigloiodine, and cocaine), as well as natural food preservatives (Table 4).

Table 4. Commercial applications of plant alkaloids

Application	Constituent Alkaloid	Formulation Names
Chemotherapy	Paclitaxel	Taxol®, Taxotere®;
	Vinorelbine prepared from vindoline and catharanthine	Navelbine®;
	Vinblastine	Velban®;
	Vincristine	Vincasar Pfs®, Oncovin®;
	Camptothecin	Camptosar®
Gout treatment	Colchicin	Colcrys, Mitigare, Gloperba
Respiratory ailments treatment	Vasicine	Ayusas Adulsa Cough syrup;
	Codeine	Ambenyl®, Calcidrine, Neo AC cough syrup;
	Capsaicin	Nasol Nasal spray™
Hypertension treatment	Reserpine	Diupres-250, Diupres-500, Regroton®, Demi-Regroton;
	Ajmalicine	Isosarpan, Iskedyl, Isquebral, Duxil, Duxor, Saltucin Co, Salvalion, Sarpan;

Anesthetic premedication, toxicity antidotes	Atropine	Atropen
Antimuscarinic agents	Atropine	Isopto Atropine, Vistatropine Eye Drops
Analgesic agents	Morphine	Kadian, Kadian ER, Morphabond, Oramorph SR,
		Roxanol;
	Codeine	Emperine 3;
	Capsaicin	Capsitop O Roll ON, Zostrix, Capzasin-HP, Axsain, Rid-A-Pain, Salonpas Hot, Medigrip Capsicum Plaster
Cardiac ailment treatment	Papaverin	Pavabid® (Marion), Papaver™
Malaria treatment	Quinine	Qualaquin,
Scalp repairment	Capsaicin	Thermascalp
Nutritional supplement	Ephedrine	ECA Stack;
	Berberine	Berberta, Myobery Tablet, Berberine Glucose Support, Berbitol Tablet;
	Piperine	Superb™ Qp, Rhodiola, Dezcumin
	Vincamine	Oxybral SR, Brain Ox, Vincabral SR
Smoking cessation	Cytosine	Tabex
	Nicotine	Nicotex Nicotine Gum,
		STOP-NIC Nicotine Gum, Nixit-Nicotine Gum
Pesticide	Strychnine	Boomer-Rid, Certox, Dog-button, Dolco mouse Ceral, Stricnina, Mole death, Mouse-nots, Strychnos

SUMMARY

- Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. Alkaloid chemistry underlines the significance of the blocks, pathways and transamination reactions. Alkaloids are nitrogen containing chemical compounds.
- 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 early alkaloid isolations were achieved before there was a notion of the complexity of molecular structure, and before the concepts of stereochemistry and the three-dimensional nature of compounds were developed.
- Alkaloids occurred to be extremely important for human beings for ages, besides they are secondary metabolites, what could suggest that they are useless. Alkaloids showed strong biological effects on animal and human organisms in very small doses. Alkaloids are present not only in human daily life in food and drinks but also as stimulant drugs.
- Alkaloids showed quite diverse medicinal properties. Many of them possess local anesthetic properties, but their practical use is limited for clinical purpose.
- Extracts of plants containing alkaloids were known and used because of their diverse activity by people from ages. But ages ago people did not know direct methods to isolate pure compounds from specified plant species.
- Methyl ether derivative of morphine—codeine—naturally occurring next to morphine in the opium poppy, possesses an excellent analgesic activity and is shown to be relatively nonaddictive.

MULTIPLE CHOICE QUESTIONS

1. Which of the following is not an example of alkaloids?

a. Vinca
b. Benzoin
c. Rauwolfia
d. Belladonna

2. Alkaloids are__

a. Basic in nature
b. Contain heterocyclic nitrogen ring
c. Both A & C
d. None of the above

3. Alkaloids does not respond _

a. Mayer's test
b. Wagner's test
c. Dragondroff test
d. Goldbeater's skin test

4. Main constitute of Dragondroff reagent is

a. Potassium Bismuth iodide solution
b. Potassium mercuric iodide solution
c. Aqueous iodine solution
d. Saturated picric acid solution

5. Hager's reagent gives__

a. White ppts
b. Yellow ppts
c. Green colour
d. Blue color

REVIEW QUESTIONS

1. Discuss on alkaloids and their biosynthesis.
2. How to alkaloids derived from ornithine?
3. Evaluate the alkaloids in human food and drinks.
4. Determine the chemical reactions and modification of plant alkaloids.
5. Examine the chemical conversions in plant alkaloids.

Answer to Multiple Choice Questions

1. (b) 2. (c) 3. (d) 4. (a) 5. (b)

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CHAPTER 3

BIOLOGY OF ALKALOIDS

LEARNING OBJECTIVES

After studying this chapter, you will be able to:

1. Discuss about alkaloids in biology
2. Focus on bioactivity
3. Explain the biotoxicity

"A whole host of things that we now know are drugs turn out to be plant alkaloids."

—Gregory Petsko

INTRODUCTION

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 and a part of an endogenous security and regulation mechanism. Some alkaloids have significance as hemoglobinizators of leukemia cells, and they can be biologically determined to be estrogenically active molecules. They display antimicrobial and antiparasitic properties. Biotoxicity is directed only toward 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 a lot of genes coding for the enzymes involved in alkaloid synthesis have been isolated. Alkaloid molecules are active agents in molecular evolutionary interactions.

3.1 ALKALOIDS IN BIOLOGY

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.

The biological functions of alkaloids within the plants are not clearly understood but it is clear that they are not produced in plants for a single function, but for many functions. The following functions have been observed in different plant species.

Alkaloids are considered as:

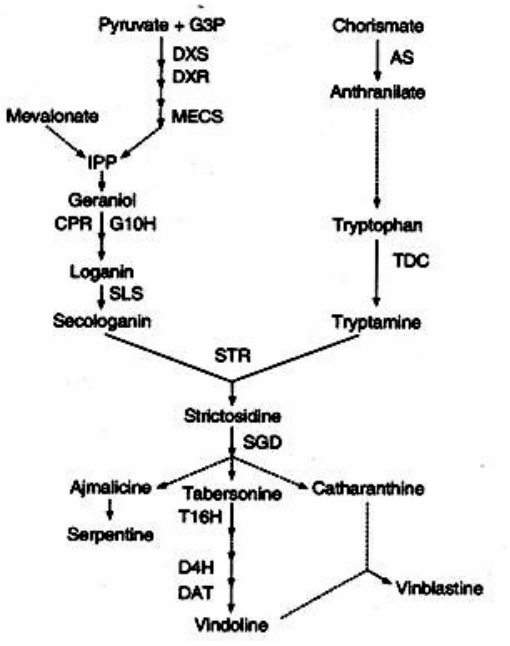


Figure 1. Biosynthetic pathway of indole alkaloid. broken lines represents several steps.

- Reserve substances to supply nitrogen, but very little evidence is available about this function.
- End products of the detoxification mechanism, otherwise their accumulation

in plants might cause damage to the plants, e.g., in tobacco, 10% of carbon metabolism is directed to synthesize nicotine biosynthesis. Thus, it is an energy expensive process.

- Poisonous substances to protect the plant itself from insects and animals. Nicotine has insecticidal properties. Sheep avoid grazing lupin plants with high alkaloid content. Some cacti repel fruit fly but Drosophila pachea is resistant and breeds on cactus.
- Plant stimulants or regulators, e.g., alkaloids inhibit rye and oat seedling growth. Colchicine inhibits cell division.
- Reservoirs for protein synthesis.
- Excretory products of plant.
- Inhibition of enzymatic activity by alkaloids is also known.

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 microorganisms 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. 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. 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.

Remember

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.

This suggests that alkaloids are compounds fundamental for cell activity and gene code realization in the genotype. 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.

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 conditions and different receptors. This process has to be genetically regulated (Figure 2). Future detailed studies will likely clear up this fascinating and complex matter concerning the biological nature of alkaloids.

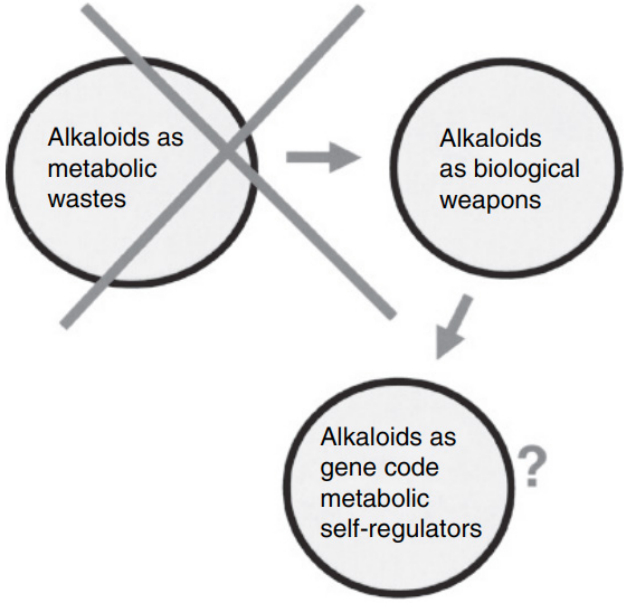


Figure 2. Three basic hypotheses on the biological nature of alkaloids.

3.1.1 From Stimulators to Inhibitors and Destroyers of Growth

Waller and Nowacki 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 signaling 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.

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, 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, 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.

There is evidence in literature that alkaloid biology is connected with regulation, stimulation and induction functions. The 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 3). It is known that caffeine has been found to enter the brain by both simple diffusion and saturable carrier-mediated transport. The hepatobiliary excretion of caffeine has also been reported in humans, rabbits and rats.

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

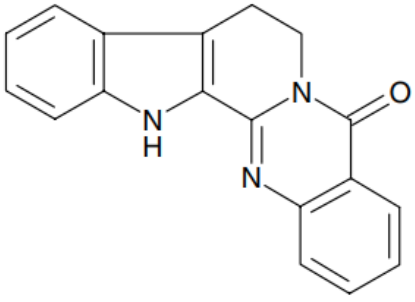


Figure 3. Rutaecarpine, an alkaloid from Evodia rutaecarpa.

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 4). Foliar application of lupine extract on several crops resulted in yield increases of 17–20% and 15–25%. 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%. 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. 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.

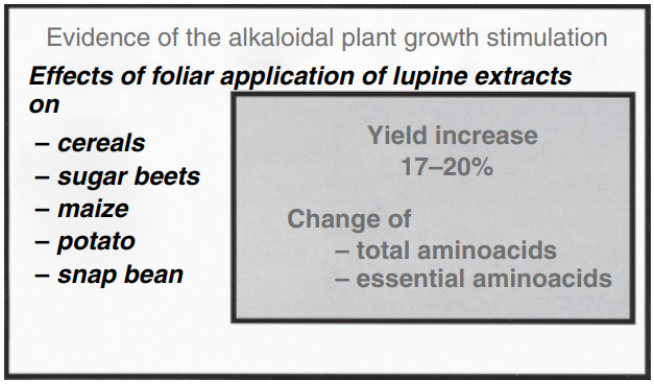


Figure 4. Effects of foliar application of lupine extracts.

3.1.2 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. The sources of alkaloid formation and changes in Tabernaemontana pachysiphon plants. 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. Clearly documented that higher leaf alkaloid content is thought to result from higher nitrogen and cation availability.

Keyword

Leaf is the principal lateral appendage of the vascular plant stem, usually borne above ground and specialized for photosynthesis.

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 5).

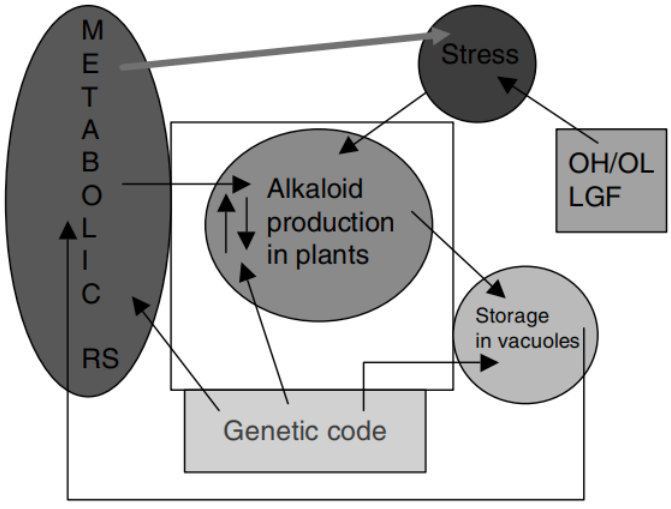


Figure 5. Mechanism of regulation of alkaloid content in plants. Abbreviations: RS – regulation system; OH – overhigh level; OL – overlow level: LGF – life growing factors. Observe that this regulation system is coded in genes. Life growing factors (light, water, CO_2, nutrients including nitrogen, temperature, etc.) influence on stress, which is also dependent on this system.

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 nitrification and may thus contribute to low leaf alkaloid accumulation. The above-mentioned experiments, 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.

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. The 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. Moreover, this also suggests that the basic biological function of alkaloids is endogenous. Many present research results suggest just this.

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 -carboline alkaloids in humans. These alkaloids occur in mammals. 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 0.5ng/g tissue. Norharman induces pro-conflict behavior in limbic-hypothalamic structures and alterations of motor activity. 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. 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.

3.2 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. Alkaloids are nitrogen-containing compounds that occur naturally not only in plants but also in microorganisms, marine organisms, and animals. Although it is not clear why alkaloids show significant biological activity, they are often useful as drugs or biological probes for physiological studies. As new and more complicated diseases are encountered worldwide, the importance of bioactive alkaloids has increased due to their potential application in chemotherapy. As the application of alkaloids has expanded, the definition of alkaloids has become less restricted.

Biological activity describes the beneficial or adverse effects of a drug on living matter. When a drug is a complex chemical mixture, this activity is exerted by the substance's active ingredient or pharmacophore but can be modified by the other constituents. Among the various properties of chemical compounds, pharmacological/biological activity plays a crucial role since it suggests uses of the compounds in the medical applications. However, chemical compounds may show some adverse and toxic effects which may prevent their use in medical practice.

Activity is generally dosage-dependent. Further, it is common to have effects ranging from beneficial to adverse for one substance when going from low to high doses. Activity depends critically on fulfillment of the ADME criteria.

Bioactivity is a key property that promotes Osseo integration for bonding and better stability of dental implants. Bioglass coatings represent high surface area and reactivity leading to an effective interaction of the coating material and surrounding bone tissues. In the biological environment, the formation of a layer of carbonated hydroxyapatite (CHA) initiates bonding to the bone tissues. The bioglass surface coating undergoes leaching/ exchange of ions, dissolution of glass, and formation of the HA layer that promotes cellular response of tissues. The high specific surface area of bioactive glasses is likely to induce quicker solubility of the material, availability of ions in the surrounding area, and enhanced protein adsorption ability. These factors altogether contribute toward the bioactivity of bioglass coatings. In addition, tissue mineralization (bone, teeth) is promoted while tissue forming cells are in direct contact with bioglass materials.

Remember

To be an effective drug, a compound not only must be active against a target, but also possess the appropriate ADME (Absorption, Distribution, Metabolism, and Excretion) properties necessary to make it suitable for use as a drug.

3.2.1 Secrets of life

Alkaloids are structurally very similar to plant growth hormones. Waller and Nowacki 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. 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. 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. 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, 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. 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. 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. In individual plants without such alkaloids, the biosynthetic pathway of the alkaloids with HTM/HLTase is blocked.

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 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 than alkaloid-poor plants? What empirical evidence exists that alkaloids also have the effect of growth regulators? Waller and Nowacki16 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. The alkaloid-rich plants grow at a higher rate and higher canopy than alkaloid poor plants. 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 favorable 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 CO2 dm^{-2} h^{-1}, and that of L. polyphyllus Lindl. (alkaloid-poor) was 10.04 mg CO2 dm^{-2} h^{-1}.

3.2.2 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. You 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-desmethyl8 β-ethoxypretazettine, N-desmethyl-8-ethoxypretazettine, lycorine, 1-Oacetyllycorine, 1,2-di-O-acetyllycorine and cherylline have been shown to inhibit AChE. Lycorine-type alkaloids are the most active against AChE. 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. Studies on steroid alkaloids such as saracocine, saracodine, saracorine and alkaloid-C isolated from Sarcococca saligna 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 and so on. These alkaloids can activate AChE or inhibit it by influence of enzyme AChE (Figure 6). 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. Galanthanine has analgesic, anticholinergic and anticholinesterase properties. The lycorine-type alkaloid (pratorinine) and the crininetype alkaloid (6α-hydroxybuphanisine) showed a moderate cytotoxic activity. Moreover, (−)-spectaline, a piperedine alkaloid isolated from the legume Cassia leptophylla Vog., has been proved in studies by Alexandre-Moreira et al. 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. This is a promising research direction when considering possible bioapplications of this alkaloid.

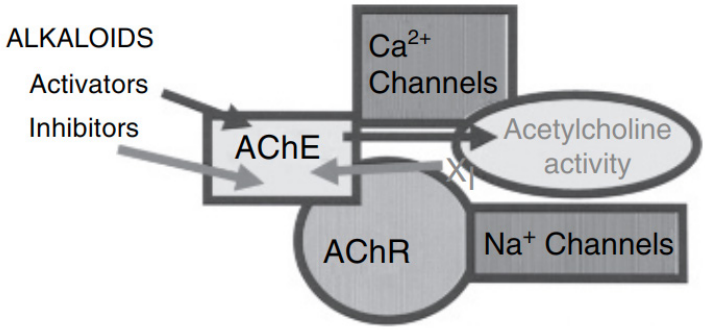


Figure 6. Alkaloids in the acetylcholine receptor. Abbreviations: AChE – acetylcholine enzyme; AChR – acetylcholine receptor; XI (red) – inactivity of AChE.

Neolitsine, dicentrine, cassythine and actinodaphine are aporphine alkaloids isolated from Cassytha filiformis. These alkaloids have been studied, 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. You 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. Very interesting results concerning the cytotoxicity of alkaloids isolated from the flowers of ornamental legume plant Senna spectabilis have been noted by Sriphong. N,O-diacetylcassine, 3(R)-benzoyloxy-2(R)-methyl (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. You 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 tested366. 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. Which is a monoterpene indole

alkaloid. This research investigated HeLA, HepG2, HL60, KB and MCF-7 cells in vitro and in mice. The anti-tumour properties of echitamine in vitro and in vivo. Moreover, Long and Li have noted the anti-tumourous characteristics of alkaloid extracted from **Oxytropis** ochrocephala, and concluded that the activity is dose dependent. This antitumour effect is associated with the expression of inhibition of proliferating cell nuclear antigen (PCNA) and mutant p53 protein.

Keyword

Oxytropis is a genus of plants in the legume family. It is one of three genera of plants known as locoweeds, and are notorious for being toxic to grazing animals.

The cytotoxic activity of phenanthroquinolizidine alkaloids has also been reported. 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, guattegaumerine exerts activity on B16 melanoma, which is a relatively resistant tumour. Cytotoxic activity of 8-OCinnamoylneoline, an alkaloid isolated from flower bud of Aconitum carmichaeli (Ranunculaceae). 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. The 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.

3.2.3 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. The receptor for estrogenic activity is located in the nucleus (Figure 7). 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. However, there were differences between the alkaloids studied concerning the intensity of estrogenic activity. The 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. Estrogenic activity depends on the heterocyclic skeleton, N and the nature of the substituent.

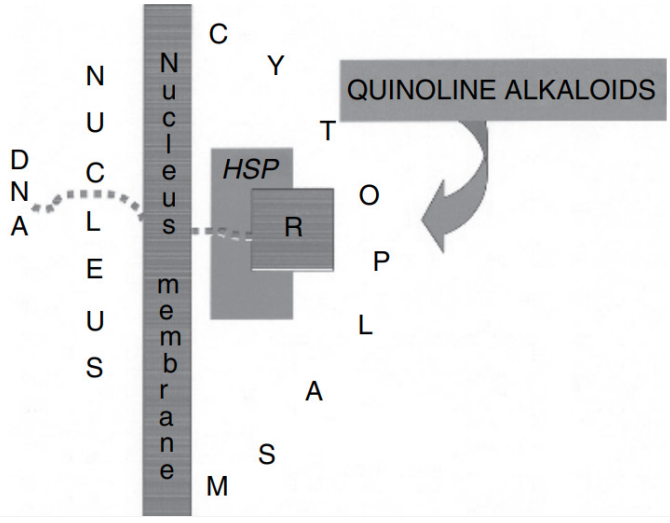


Figure 7. Diagram of estrogenic activity of the alkaloids. Abbreviations: R – receptor; HSP – heat shock proteins.

3.2.4 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 tage 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. You 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. These 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 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 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 aroused awareness of a problem. This has been studied in connection to indole alkaloids (Figure 8) from the Strychnos species (Loganiaceae) 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. You 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. Moreover, 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. 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.

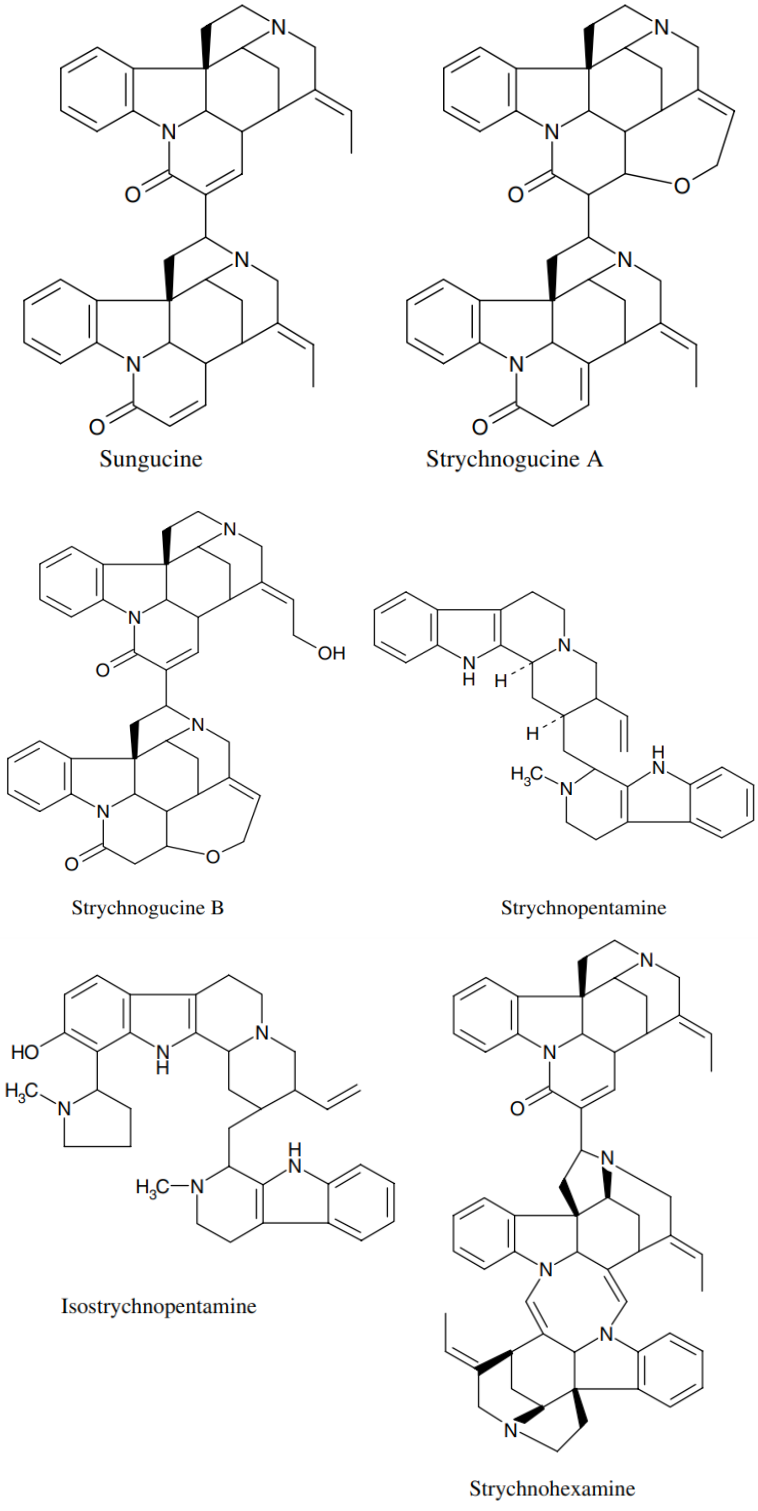


Figure 8. Some alkaloids from Strychnos species.

3.3 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. 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.

Keyword

An **organism** is any organic, living system that functions as an individual entity.

3.3.1 Research Evidence

Alkaloids are active bioagents in animal tissues. There is clear scientific evidence of this. Crawford and Kocan 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.

You 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.

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. Rabbits have reportedly experienced toxic effects. The classic toxic symptoms of coniine alkaloids range from paralysis, muscular tremors, muscle weakness and respiratory failure preceding death. It is not difficult to observe that the bioactivity of coniine alkaloids and especially their symptoms are similar to these of curare or of nicotine. You 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.

3.3.2 Influence on DNA

The phenethylisoquinoline alkaloids present in some members of the Lily family (Liliaceae) are known to be toxic. Wang and Wang 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. 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. Berberine was alone tested for possible genotoxicity, mutagenecity and recombinogenic activities in micro-organisms. 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. The berberine's cytotoxicity results from a mutational blockage in the DNA strandbreak repair pathway (rad52-1). The influence of this alkaloid on DNA is evident. You 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. You 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 9 presents the acute toxicities of berberine and the baine. 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.

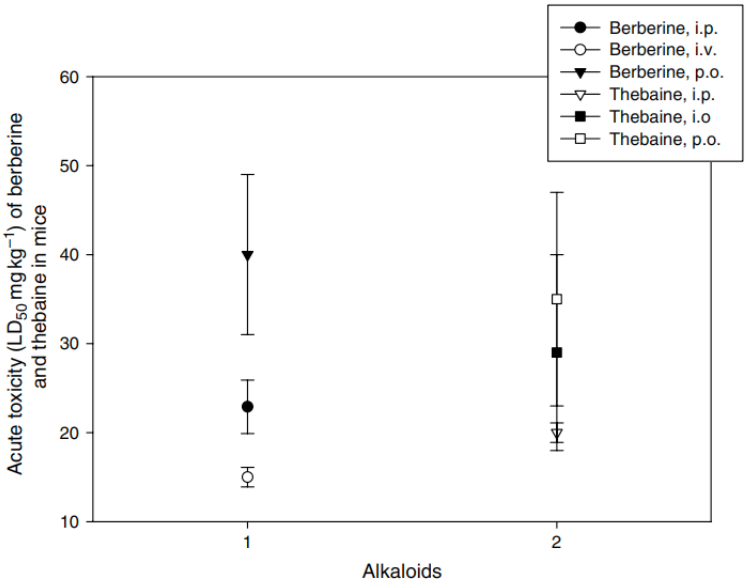


Figure 9. Acute toxicity of berberine and thebaine on mice in relation to form administration. Abbreviations: 1 – berberine; 2 – thebaine.; i.p. – intraperitoneal; i.v. – intravenous; p.o. – oral.

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. 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, 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. These suggest that the lack of higher DNA damage in smokers compared to non-smokers is connected only with nicotine dose.

3.3.3 Selective Effectors of Death

One of the most known toxic alkaloid is strychnine. You are examples of those who have studied its toxicity, although it is practically rather evident. This alkaloid has been used as a strong rodenticide. 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. This is interesting in the sense that there is clear evidence of the selectivity of strychnine sub-chronic dietary toxicity being species dependent. 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. These 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 colored legends of the use of alkaloids for acute effects of death (murders, executions, weapons etc.). 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.

Keyword

Strychnine is a highly toxic, colorless, bitter, crystalline alkaloid used as a pesticide, particularly for killing small vertebrates such as birds and rodents.

3.3.4 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. The biotoxicity of alkaloids has for some time been considered to be connected with their bitter taste. 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. 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. 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. 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 10), 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}. 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, labored breathing, trembling, convulsions and respiratory paralysis in sheep.

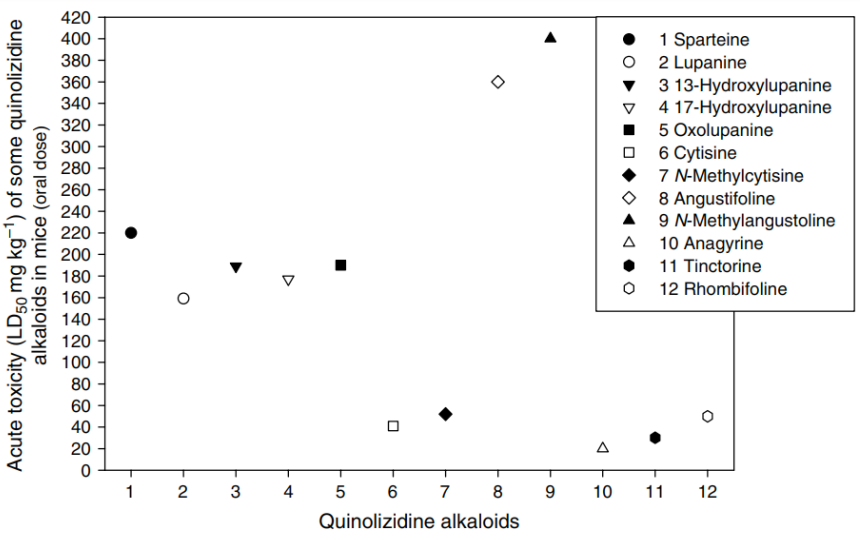


Figure 10. Acute toxicity (LD_{50}) of some quinolizidine alkaloids in mice

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. On the other hand, some quinolizidine alkaloids are used as a drug in folk medicine. They probably have chronic toxicity. 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.

3.3.5 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. You 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 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, 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, 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. 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. None of these studies question the symptoms of acute and chronic toxicity of nicotine. Moreover, 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. Nicotine toxicity is also considered a health risk for agricultural workers on tobacco plantations in India. Nicotine toxicity is also connected to pica disease. Many symptoms of nicotine toxicity were observed in smokers in numerous studies. 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. The combination of a nicotine patch and tobacco smoking induced an overdose of nicotine in this case. Some studies also claim that a chronic administration of high doses of nicotine results in axonal degeneration in the central core. 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. 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.

3.3.6 Aberrations in cells

Pyrrolizidine alkaloids are toxic to foreign organisms (Figure 11). This problem was largely studied in the 1960s–1980s. 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. The toxicity of pyrrolizidine alkaloids to livestock was considered coincidental. You 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). You have reported cases of calves being poisoned, 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}. Lasiocarpine doses equivalent to 0.2 mg kg^{-1} body weight per day lead to the development of tumors 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.

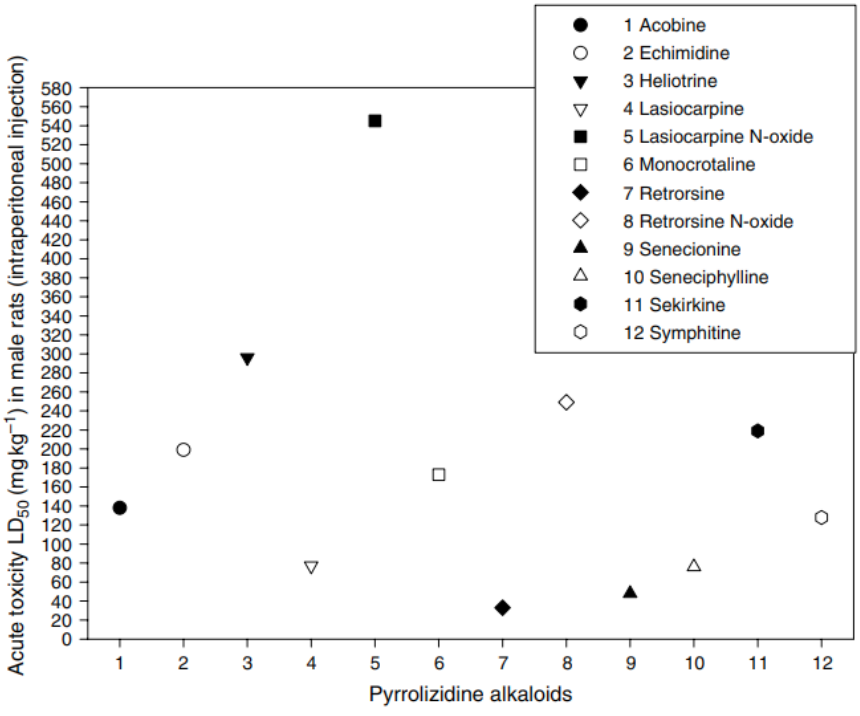


Figure 11. Acute toxicity (LD_{50}) of some pyrrolizidine alkaloids in male rats.

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. According to Elbein and Molyneux 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. It is known as a good inhibitor of several α-glucosidases. This is due to the suppression of digestive enzymes.

3.3.7 Narcotics

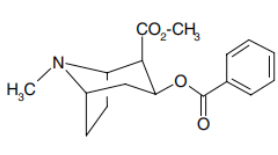
All alkaloids are neurotransmitters and active agents in the nervous system. Many alkaloids from natural plants and also modified alkaloids can impress euphoric, psychomimetic and hallucinogenic properties on humans. Some of them can influence narcosis, states of stupor, unconsciousness, or arrested 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 12) are stimulants which are active on the central nervous system causing disorders and some temporary or permanent changes in this system and behavior. Serious negative consequences of narcotics include dependence, a chronic disorder.

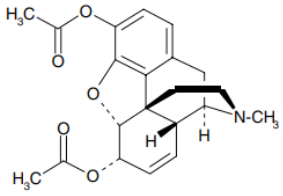
The most known narcotics are the opium alkaloids such as morphine, codeine, the baine, papaverine, noscapine and their derivatives and modified compounds such as nalmorphine, apomorphine, apomopholcodine, dihydrocodeine, hydromorphone and heroine, also known as diamorphine. Synthetic narcotics share 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 the baine model. The compounds serve various purposes in clinical practice.

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

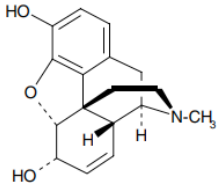
ripening capsule of papaver when it is in the process of changing color from blue-green to yellow. When the tubs are cut, it is possible to procure the milk. During coagulation, the milk's color changes to brown. Fresh opium is soft but it hardens during storage. Crude opium has been used in the past 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.



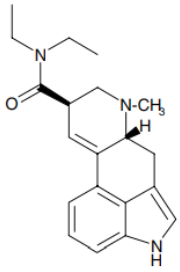
Cocaine



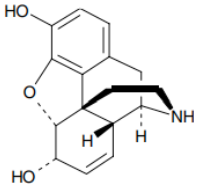
Heroine (diamorphine)



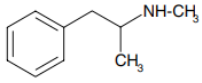
Morphine



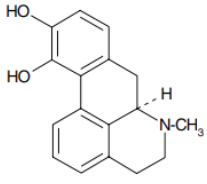
LSD



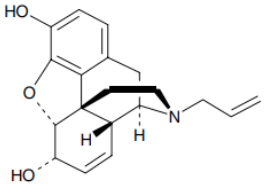
Normorphine



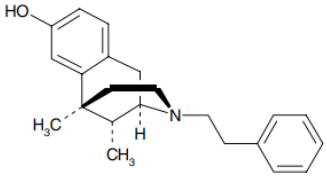
Metamphetamine



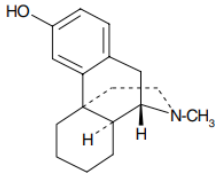
Apomorphine



Nalmorphine



Phenazocine



Dextromethorphan

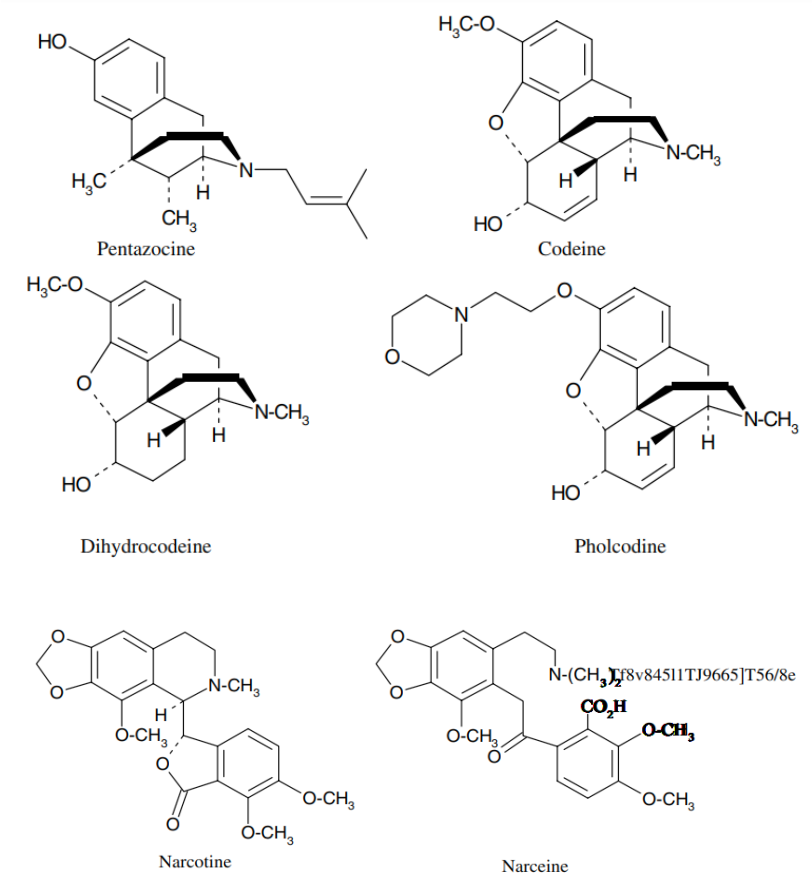


Figure 12. Some narcotics and their derivatives.

3.3.8 Genetic Approach to Alkaloids

Alkaloid biogenesis in an organism is determined genetically. This means that many specific genes participate in alkaloid metabolism, and gene participation in metabolism is a very important basis for understanding the alkaloids.

The Genetic Approach to Learning and Memory

The genetic approach to understand the molecular processes mediating learning and memory was founded upon the principles of genetics and molecular biology that were discovered in the first half of the twentieth century. Studies of mutant organisms with physical defects, along with knowledge of the structure of DNA, the hereditary material, led to the realization that a mutation in a single nucleotide of DNA offered the ultimate way of performing a biological dissection. In other words, a mutation that inactivates a single gene of an animal offers the biologist a way of studying the biological consequences of removing but a single

building block from the animal. This genetic approach has been used to study many different questions in biology.

This approach for dissecting behavior is reductionist in the sense that it reduces the problem to the molecular level. It begins with the idea that a mutation in an animal that produces a learning or memory deficiency identifies a gene and its protein product required for normal learning in nonmutant animals. With the identification of a sufficient number of genes and proteins that are involved, some mechanistic understanding of the molecular dynamics underlying the process can be gained. While this is true, it is now accepted that a genetic connection between gene and behavior is insufficient to gain the necessary depth of understanding. This is because the behavior of an animal emerges from complex interactions at levels other than the molecular. Molecules mediate learning through their biological functions and interactions but this occurs within the context of certain neurons. These neurons, in turn, can be part of complex neural networks that convey information or are involved in behavioral output. Thus, research in the late twentieth century revealed that a multilevel analysis of learning and memory is essential for a complete understanding of the process. In other words, it is necessary to understand learning and memory at the genetic level, the cellular level, the neuroanatomical level, and the behavioral level, since behavior emerges from biological processes at all of these functional levels.

3.3.9 Evolutionary Influences on Alkaloid Biology

Biological evolution is the change in inherited traits over successive generations in populations of organisms. Adaptation is a key evolutionary process in which variation in the fitness of traits and species are adjusted by natural selection to become better suited for survival in specific ecological habitats. The environment acts to promote evolution through changes in development. Therefore, determining how developmental changes are mediated is critical for understanding the mechanisms of evolution.

Biological processes are often studied in model organisms. A model organism is a species that is studied extensively in the laboratory with anticipation that the results can be applied to biological phenomena in general. Cave animals can serve as excellent models to study the relationships between the environment, evolution, adaptation, and development. Troglomorphic (cave-related) traits, including elongated appendages, lowered metabolism, specialized sensory systems, and loss of eyes and pigmentation have evolved as a response to the effects of perpetual darkness. The characid fish *Astyanax mexicanus*, as a vertebrate model system for studying the developmental basis of evolution and adaptation to the cave environment.

SUMMARY

- Some alkaloids have significance as hemoglobinizators of leukemia cells, and they can be biologically determined to be estrogenically active molecules.
- Biotoxicity is directed only toward foreign organisms or cells, and it is selective. Alkaloids can alter DNA, selectively deform cells, and cause locoism.
- Alkaloids play a very important role in organism metabolism and functional activity. They are metabolic products in plants, animals and micro-organisms.
- The biological functions of alkaloids within the plants are not clearly understood but it is clear that they are not produced in plants for a single function, but for many functions.
- 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 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.
- 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.
- 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.
- Biological activity, although typical for alkaloids, can be very different and dependent on the chemical structure of alkaloid molecules.
- 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.
- This alkaloid has been used as a strong rodenticide. It is also known for being dangerous to humans. One general characteristic of strychnine is its chemical stability.
- 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.

- These alkaloids act via inhibition of ganglionic impulse transmissions of the sympathetic nervous system. It is evident that each alkaloid has its own effect.
- The most known narcotics are the opium alkaloids such as morphine, codeine, the baine, papaverine, noscapine and their derivatives and modified compounds such as nalmorphine, apomorphine, apomopholcodine, dihydrocodeine, hydromorphone and heroine, also known as diamorphine.
- Alkaloid biogenesis in an organism is determined genetically. This means that many specific genes participate in alkaloid metabolism, and gene participation in metabolism is a very important basis for understanding the alkaloids.

MULTIPLE CHOICE QUESTIONS

1. Which is not the biological source of Cinchona?

a. Cinchona calisaya
b. cinchona officinalis
c. Cinchona succirubra
d. Cinchona indica

2. Which ergot alkaloid is water-soluble?

a. Ergotamine
b. Ergosine
c. Ergocristine
d. Ergometrine

3. Ergometrine alkaloid shows:

a. Laevo
b. Dextro
c. Racemic mixture
d. Trans

4. Alkaloids are compounds needed for cell activity and gene code realization in the genotype.

a. True
b. False

5. Alkaloids play a very important role in organism metabolism and functional activity.

a. True
b. False

REVIEW QUESTIONS

1. Explain the stimulators to inhibitors and destroyers of growth.
2. Discuss the effects of stress and endogenous security mechanisms.
3. Focus on life regulation through the high and low cytotoxicity.
4. Explain the anti-parasitic activity.
5. Discuss about genetic approach to alkaloids.

Answer to Multiple Choice Questions

1. (d) 2. (d) 3. (a) 4. (a) 5. (a)

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CHAPTER 4

ECOLOGY OF ALKALOIDS

LEARNING OBJECTIVES

After studying this chapter, you will be able to:

1. Define defense strategies in plants
2. Describe the ecological roles of alkaloids
3. Identify the modes of action
4. Explain the animal sequestration of alkaloids

"Ecology is not somebody's work; it's everybody's work."

—Sadhguru Jaggi Vasudev

INTRODUCTION

Plant secondary metabolites are well recognized to provide protection against harmful organisms. Alkaloids are one of the most diverse groups of secondary metabolites. They are produced by a large variety of organisms, including bacteria, fungi, plants, and animals. Positive or negative effects of alkaloids can be seen among different herbivores and insects. Alkaloids being toxic in nature help the plants, herbivores, and insects to ward off their enemies or competitors and facilitate their own survival in the ecosystem. Some herbivores

and insects sequester them to defend against their own enemy illustrating the flow of alkaloids through different trophic levels. Among plants, alkaloids work as allelopathic compounds, and this property makes some of the plants a potential natural weedicide. This chapter highlights the ecological roles of alkaloids and their possible role in chemical management of pests.

4.1 DEFENSE STRATEGIES IN PLANTS

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

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

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

Keyword

Trichomes are fine outgrowths or appendages on plants, algae, lichens, and certain protists.

Secondly, plants are masters of chemical defense, with a fascinating ability to produce a high diversity of chemical defense compounds, also known as secondary metabolites or allelochemicals. Chemical defense involves macromolecular

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

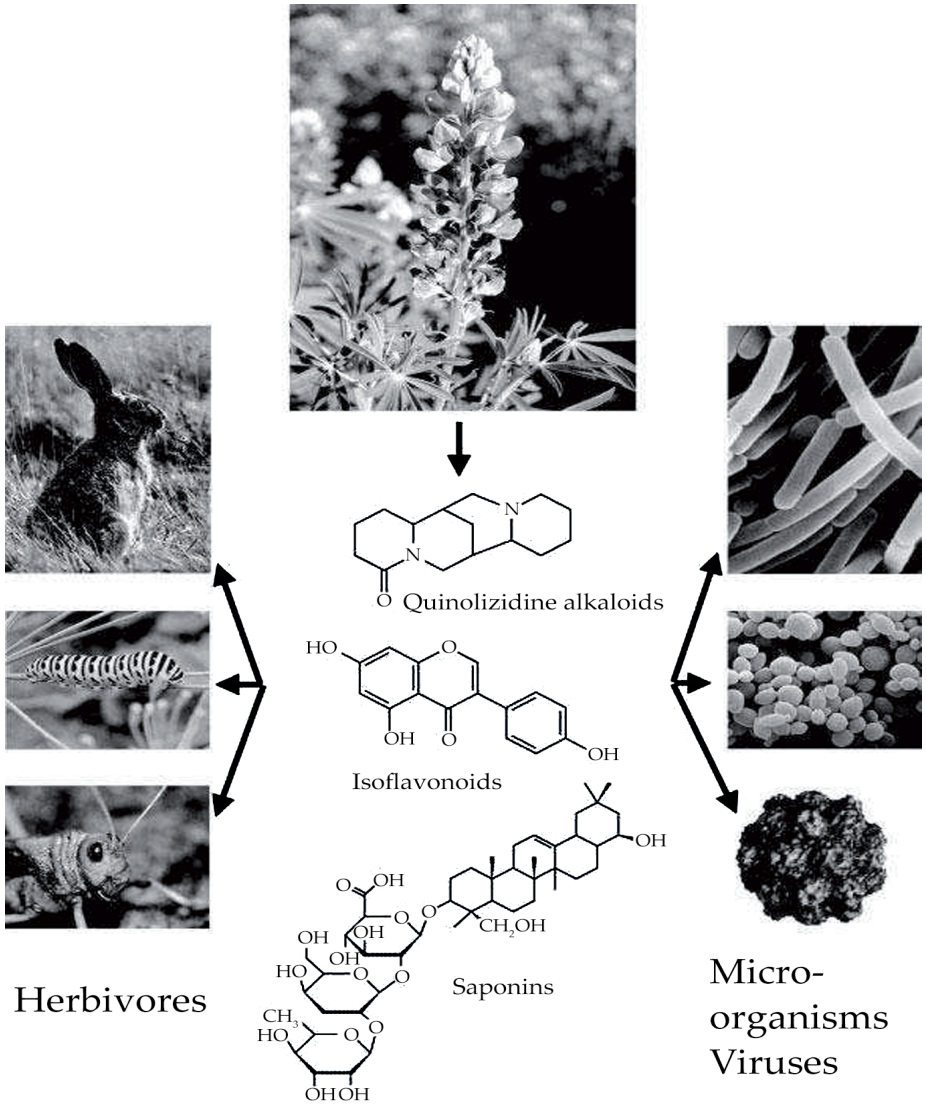


Figure 1. Relationships between plants, their secondary metabolites, and potential enemies (herbivores, microorganisms, and viruses). Example: Lupins produce quinolizidine alkaloids, isoflavonoids, and saponins as main defense compounds.

Among the secondary metabolites that are produced by plants, alkaloids figure as a very prominent class of defense compounds. Over 21 000 alkaloids have been

identified, which thus constitute the largest group among the nitrogen-containing secondary metabolites (besides 700 nonprotein amino acids, 100 amines, 60 cyanogenic glycosides, 100 glucosinolates, and 150 alkylamides). However, the class of secondary metabolites without nitrogen is even larger, with more than 25 000 terpenoids, 7000 phenolics and polyphenols, 1500 polyacetylenes, fatty acids, waxes, and 200 carbohydrates.

4.1.1 Plant-Herbivore Interactions

Invertebrates

Insects are extremely successful from the evolutionary point of view and represent the largest class of organisms; they comprise at least 1 million and perhaps as many as 20- 30 million species.

Most insects are herbivores and their adaptation to host plants and their chemistry is often very close and complex. As many plants need insects for pollination and seed dispersal, but try to avoid insect herbivory, the interplay between attraction and deterrence can be very complicated. In the latter context it can be observed that plants attract insects by chemical means (colors, fragrances, sugar, amino acids) and at the same time other secondary metabolites are employed to prevent herbivory on flowers and seeds. The close association between plants, especially the Angiospermae, and insects evolved during the last 200 million years. Some scientists have called this phenomenon a "coevolutionary" process, but it has to be recalled that the associations seen today are not necessarily those in which the chemical interactions originally evolved, i.e., the current associations may be quite recent. Insect herbivores can be divided in two large groups, whose strategies with respect to the plant's defense chemistry differ substantially: The polyphagous species exploit a wide range of host plants, whereas the mono-/oligophagous insects often specialize on one or a small number of host plants which are often systematically related and accumulate the same class of secondary compounds. For these "specialists" the originally noxious defense compounds are no longer toxic but often attractive feeding and oviposition stimulants.

Did You Know?

The earliest animal fossils appear to be those of invertebrates. 665-million-year-old fossils in the Trezona Formation at Trezona Bore, West Central Flinders, South Australia have been interpreted as being early sponges.[

Insects cope with dietary allelochemicals using one of several strategies:

1. Insects are commonly endowed with fantastic and powerful olfactory receptors and can select between plants with high or low amounts of "toxins" and also can ascertain the food quality present, such as lipid, protein, or carbohydrate contents. The polyphagous "generalists" are usually deterred from feeding on plants sequestering high amounts of toxic allelochemicals and either select those with less active ones or change host plants rapidly, consuming small portions of a particular poison at a given time and thus avoid severe intoxication.
2. A species "learns"; or more accurately, during evolution variants have been favored by natural selection which can tolerate a noxious defense compound: (a) by developing a mechanism to avoid toxin resorption in the gut. (b) Ifresorption cannot be prevented, to eliminate the toxin quickly via the Malpighian tubules or degrade it by detoxifying microsomal and other enzymes. Most polyphagous species have evolved active detoxification mechanisms, such as microsomal oxidases, glutathione transferase, and peroxidase, which promote rapid detoxification and elimination of dietary secondary products. (c) By developing a target site that is resistant to the toxin, i.e., a receptor that does not bind the exogenous ligand any longer. For example, in the monarch butterfly (Dana us plexippus) which stores dietary cardiac glycosides, the ouabain binding site of Na^+ /K^+ -ATPase has been made ouabain insensitive by a single point mutation.
3. Alternatively, a species not only tolerates a plant's defense compound, but it also exploits it for its own chemical protection or for other purposes, such as pheromones.

Insect Feeding Deterrence and Alkaloid Toxicity

In general, we would expect that alkaloids are active feeding deterrents against most insects. Given the choice, polyphagous insects tend to select a diet with no or only a small dose of alkaloids. Also the specialists avoid most "toxins" except those of their host plants. These findings indicate that under natural conditions, plants with a high load of alkaloids should be safe from most herbivorous insects (which is indeed the case) with the exception of particular monophagous species or a few very resistant polyphagous ones.

If animals have no choice or if they are very hungry, the deterrency threshold value is much reduced and they often feed on a diet containing alkaloids that they would normally avoid. In this case the toxicity of an ingested alkaloid can be assessed. Alternatively, alkaloid toxicity can be determined to some degree by topical application, although such data are of limited ecological relevance. A substantial number of alkaloids display significant insect toxicity: examples include nicotine, piperine, lupin alkaloids, caffeine, and rayanodine. The toxic effects of alkaloids on insects can be caused by their interference with a diversity of cellular and intracellular targets.

Sequestration of Alkaloids by Insects

Plants that defend themselves effectively constitute an ecological niche, almost devoid of herbivores and pathogens. It is not surprising that during evolution a number of organisms were selected that have specialized on a particular host plant species and found ways to tolerate or even to exploit the defense chemistry of their hosts. As compared to the huge number of potential enemies, the number of adapted specialists is usually small and in general a "status quo" or equilibrium can be observed between specialists (or parasites) and their hosts. A specialist is well advised not to kill its host, for to do so would destroy its own resources; a mutualism is more productive.

Superficially, these observations seem to contradict the working hypothesis, that secondary metabolite s are primarily defense compounds. But these specialists are only the exceptions to the general rule. In this context we should recall that our immune system is fantastic in warding off bacteria, fungi, viruses, and parasites. We usually take notice of its existence only when it fails, i.e., when a specialized pathogen has found a way to undergo the immune response. Nobody would call the immune system ineffective because of this! Considering the specialized herbivores that have overcome the chemical defense barrier of plants a similar logic applies.

On a basic evolutionary level we find insects that can tolerate the defense chemistry of their host plants. One such example is Manduca sexta, whose larvae live on Nicotiana and other solanaceous plants. The tobacco hom worm can even grow on a diet with more than 1% nicotine without any adverse effects. The alkaloids present, such as nicotine or hyoscyamine, are not stored by the insects but degraded or directly eliminated with the feces. In order to avoid toxicity it has been postulated either that nicotine may not diffuse into nerve cells or that the ACH receptor no longer binds nicotine, as in "normal" animals. Recent experiments from my laboratory have shown that Manduca has ACh receptors that can bind nicotine. Furthermore, we have sequenced the alpha subunit of the receptor which does not show a substantial target site modification as compared to other moths. The potato beetle (Leptinotarsa decemlineata) lives on Solanum species containing steroidal alkaloids, which are tolerated but not stored by this species. The bruchid beetle,

Keyword

Quinolizidine alkaloids are natural products that have a quinolizidine structure; this includes the lupine alkaloids.

Bruchidius villosus, predates seeds of **quinolizidine alkaloid (QA)**-rich plants, such as Laburnum anagyroides. This beetle eliminates most of the dietary cytisine with the feces.

In a number of plants alkaloids are translocated via the phloem. If aphids live on these plants, they come in direct contact with the alkaloids present. A few adapted aphids can store the dietary alkaloids. Examples are QA in Aphis cytisorum, A. genistae, and Macrosiphum albifrons and pyrrolizidine alkaloids (PA) in Aphis jacobaeae and A. cacaliaster. For alkaloid-storing M. albifrons it was shown experimentally that the QA stored provide protection against carnivorous beetles, such as Carabus problematicus (Fig. 2), or Coccinella septempunctata or syrphids (Episyrphus balteatus). Acyrthosiphon spartii prefers sparteine-rich Cytisus scoparius plants; it is likely that this species also stores QA.

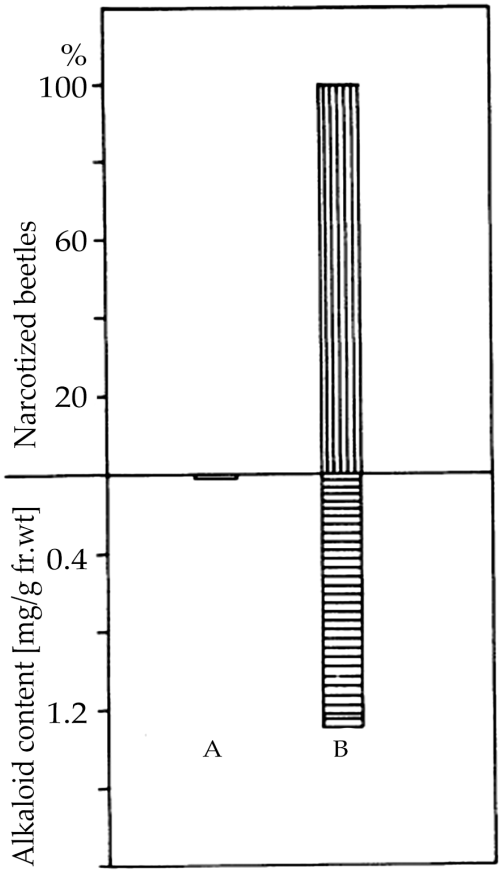
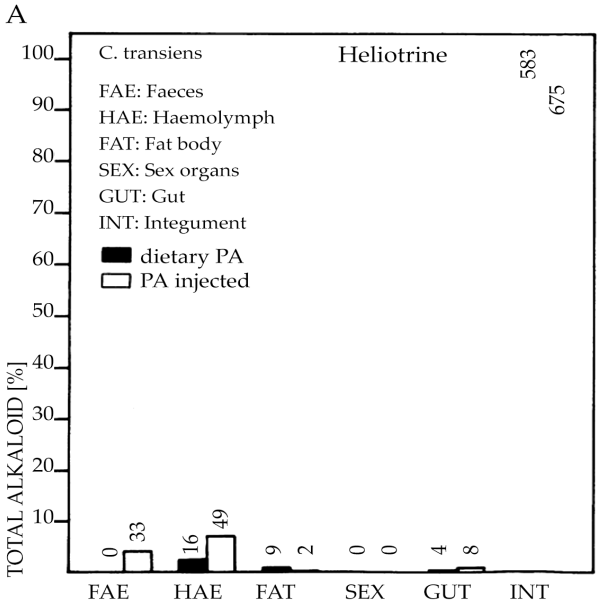


Figure 2. Effects of quinolizidine alkaloids stored by the lupin aphid, Macrosiphum albifrons , on a carnivorous beetle (Carabus problemataicusi. About 12 individually kept beetles were given aphids without QA (control: A) or aphids with a QA content of 1.3 mg g^{-1} fresh weight (B). Experiments were evaluated after 16 hr; in B all beetles lay on their backs and remained narcotized for more than 48 hr, whereas the control beetles showed no symptoms.

A further (tritrophic) interaction has also become evident: Aphis cytisorum and A. genistae colonies are regularly visited by ants, which collect honey dew. Regarding Lasius niger collected from an A. cytisorum colony, the ants contained about 45 μg cytisine g-l fresh weight. As cytisine is a very toxic alkaloid, it would be interesting to find out whether the ants gain protection from the alkaloids obtained from aphids. Regarding PA a tritrophic interaction has also been reported: Ladybirds (Coccinella) sequestered PA from Aphis jacobaeae feeding on PA-rich Senecio jacobaea. It is likely that, in ladybirds, besides the endogenously produced coccinellines the PA also serve as chemical protectants Larvae of the pyralid moth, Uresiphita reversalis, live on QA-producing plants, such as Teline monspessulana. The larvae store some of the dietary alkaloids, especially in the integument and silk glands. The uptake is both specific and selective and achieved by a carrier mechanism: Whereas alkaloids of the 10-oxosparteine type dominate in the plant, it is the more toxic cytisine that is accumulated by the larvae and the 10- oxosparteines are eliminated with the feces. These larvae gain some protection from storing QA as was shown in experiments with predatory ants (lridomyrmex humilis) and the paper wasp (Mischocyttarus flavitarsus). When the larvae pupate, most of their stored alkaloids are used to impregnate the silk of the cocoon, thus providing defense for this critical developmental stage. The emerging moth lives cryptically and has no aposematic coloring and does not contain alkaloids. In contrast, the alkaloid-rich larvae were aposematically colored and live openly on the plants.



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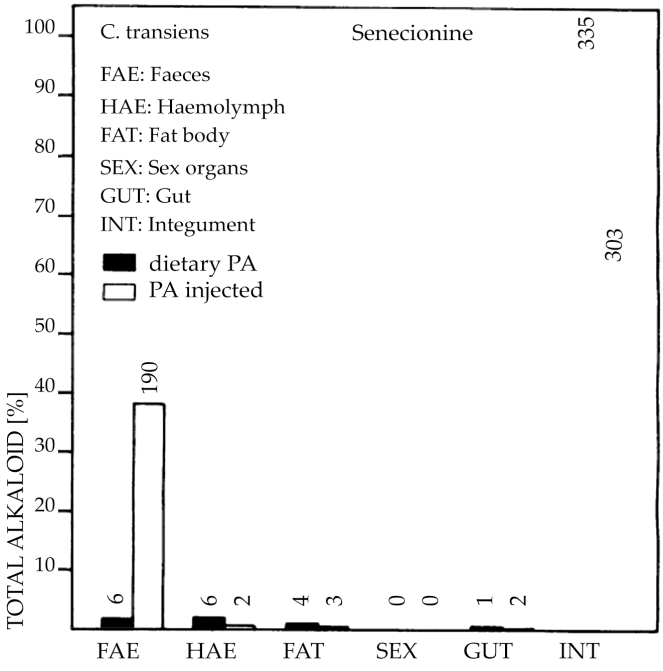

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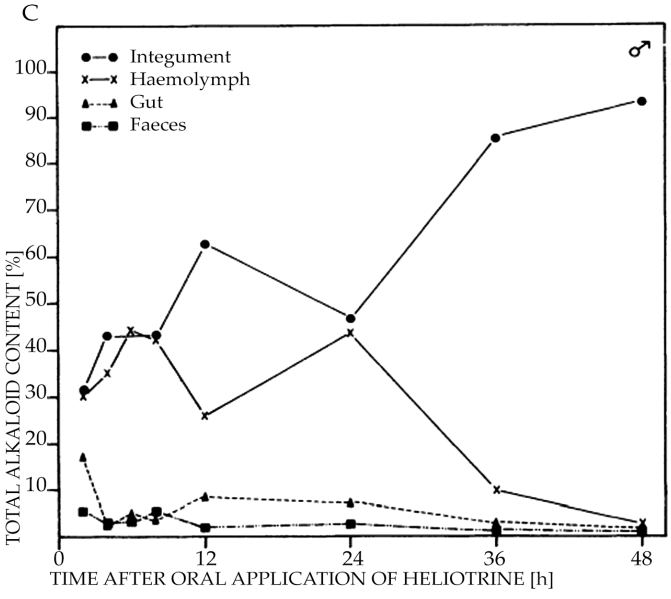


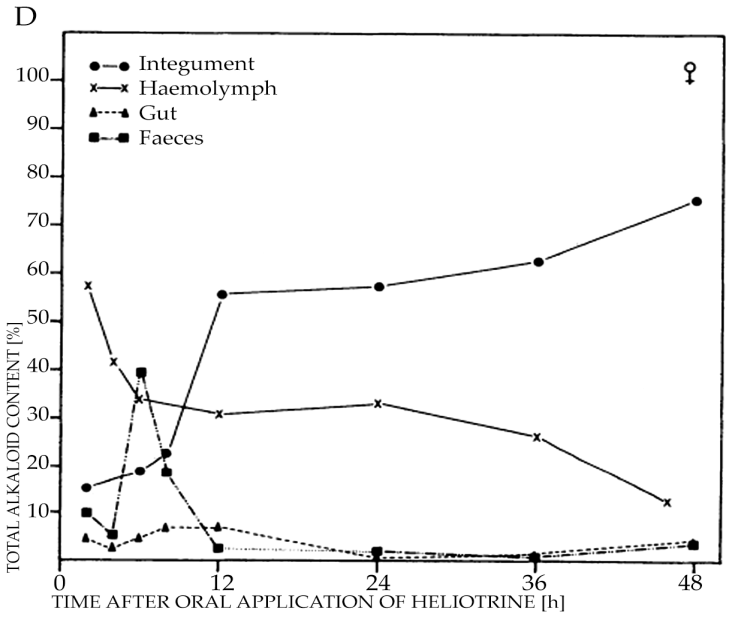


Figure 3. Distribution of pyrrolizidine alkaloid in larvae of the arctiid moth, ereatonotos transiens. (A, B) PAs [heliotrine (A) or senecionine (B)] were administered orally or via injection into the hemolymph . After 48 hr PA distributions in the different parts of the insects were determined. (C, D) Kinetics of heliotrine within the first 48 hr after feeding, differentiated for males (C) and females (D).

Especially among lepidopteran larvae, many examples have been reported of insects sequestering PA of their host plants and probably making use of them for their own defense. PA are almost always stored as their N-oxides and not as a free base. Because PA N-oxides cannot diffuse freely across biomembranes, they can be easily stored and retained in specific organs or tissues, in general, the integuments. In addition, because they also affect muscarinic ACh and serotonergic receptors, the N-oxides probably contribute to the deterrence and toxicity of PA. Following resorption from the midgut, PA remain transiently in the hemolymph before they are transferred to the integuments (Fig. 3). In larvae of the arctiid moth, Creatonotos transiens, we could show that during metamorphosis the PA are partly transferred to the ovary in females and to the spermatophore in males. During copulation, females obtain the PA-rich spermatophore (as a "nuptial gift") and transfer the alkaloids to the eggs. Thus, both sexes contribute their PA to the clutch which may benefit from the PA as a chemical protectant. A similar phenomenon has been reported for Utetheisa ornatrix.

Besides their use as chemical defense compounds, some insects exploit PA as precursors for pheromones (Fig. 5). In C. transiens PA are converted into hydroxydanaidal (the hydroxyl group at C-7 is R-configured), which is dissipated via the inflatable scent organs of the male (Fig. 4). Because the pheromone content depends on the

storage of dietary PA during the larval stages, PA-rich males should be especially attractive to females. If males with a high content of hydroxydanaidal are selected, this behavior would ensure that females can obtain a PA-rich nuptial gift during copulation. Normally, PA are 7R-configured in nature. In the event that PA are present in the 7S configuration, larvae of C. transiens and a few other species can invert the configuration to the correct 7R form. In androconial organs of Danainae and Ithomiinae, PA-derived male courtship pheromones have been found, such as hydroxydanaidal, danaidal, danaidone, and ithominae lactone. In these butterflies PA are often acquired as adults while feeding on nectar or wilting PA-containing plants.

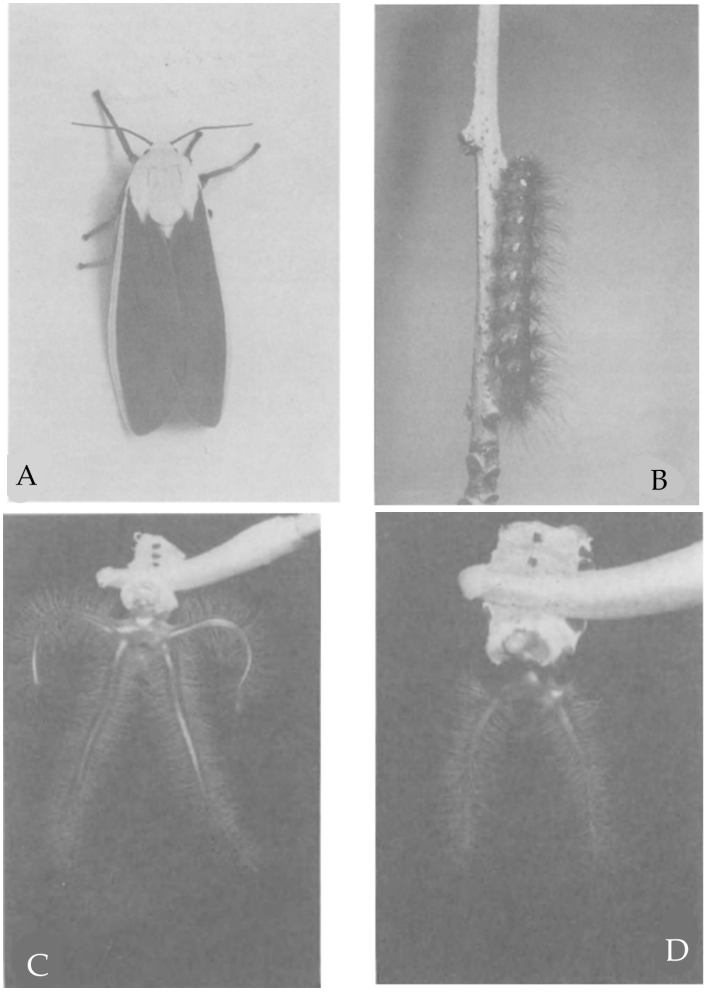


Figure 4. Corema development in Creatonotos transiens and alkaloid feeding. (A) Adult moth; (B) larva; (C) male corema (larva had PA as a diet); (D) corema of an insect raised without PA.

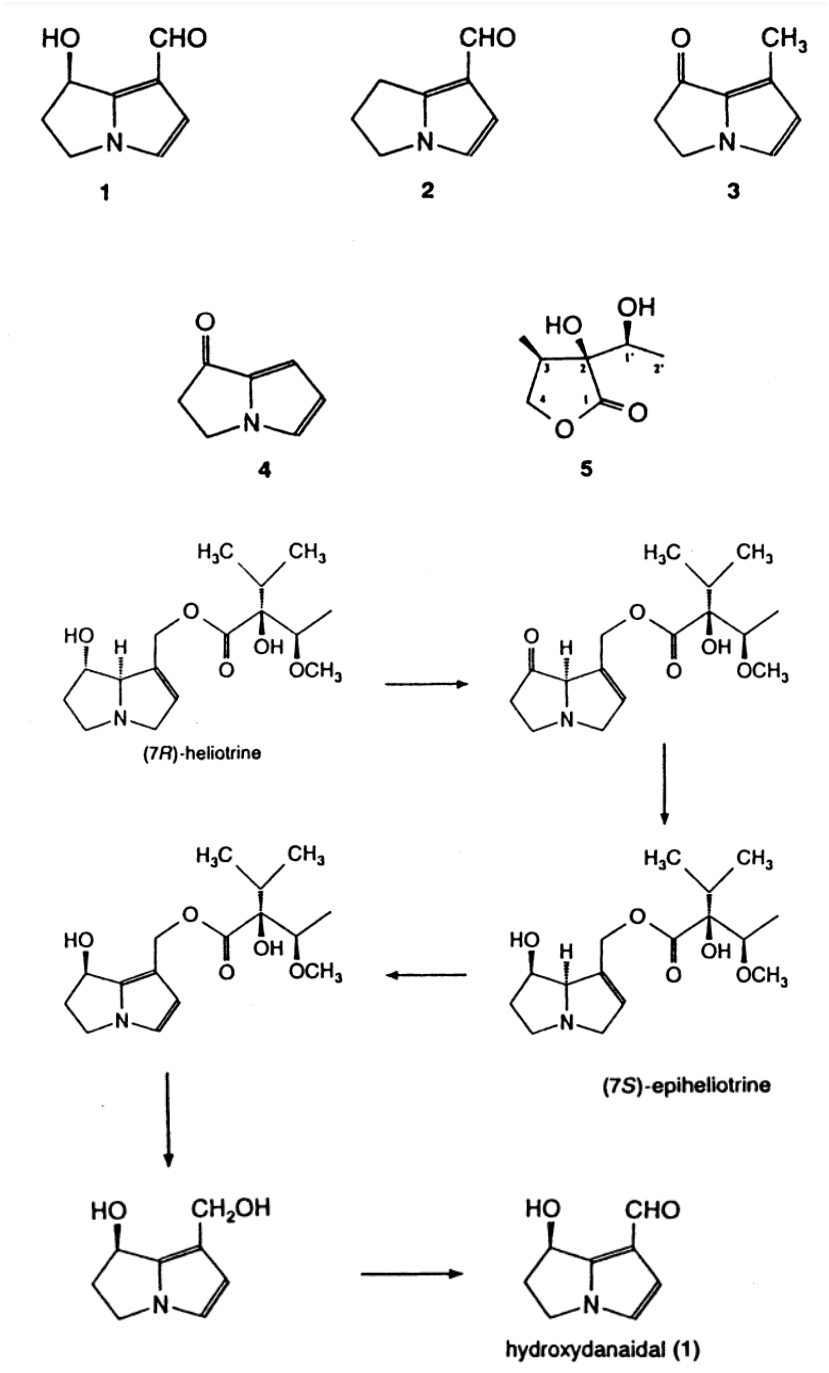


Figure 5. Structu res of PA-derived phero mones [1 = (7R) hydroxydanaidal, 2 = danaidal, 3 = danaidone, 4 = nordanaidone, 5 = ithominae lactone] and pathway of heliotrine transformation in Creatonotos transiens and other arctiid moths.

In C. transiens the PA adaptation even went one step further: Only if the larvae feed on a PA-rich diet are the males able to develop their big inflatable scent organs (corema) (Fig. 4). PA serve as a morphogen which triggers the morphological development of the coremata.

A number of other alkaloids (e.g., aconitine, cinchonine, aristolochic acid, cocaine, polyhydroxy alkaloids, l3-carbolines, cycasin) have also been reported to be sequestered by insects.

It is worth recalling that a number of animals (sponges, nudibranchs, worms, insects, toads, frogs, and salamanders) are able to synthesize their own defense compounds, among which are several alkaloids. The endogenously produced and the acquired alkaloids appear to serve as chemical defense compounds, in analogy to the situation found in plants. In animals we observe the trend that sessile species, such as bryozoa, or slow moving ones without armor, such as worms, nudibranchs, frogs, toads, and salamanders, produce active allelochemicals but not so animals with weapons, armor, or the possibility of escape by flight. Plants just share their strategy with sessile animal species. In this context it seems amazing that hardly anyone has doubted the defense role of animal alkaloids, whereas people did and still do doubt the defense role regarding alkaloids in plants.

Vertebrate Herbivores

As man and his livestock are herbivores rather than carnivores, a large body of information on adverse effects of alkaloids and other dietary secondary metabolites has been accumulated over the centuries.

Many alkaloids exhibit a bitter or pungent taste for vertebrates and a bitter or pungent diet is normally instinctively avoided. Examples of bitter-tasting alkaloids (at least in man) are quinine, strychnine, brucine, emetine, and sparteine and for pungent alkaloids, capsaicine and piperine. It should be recalled that these taste properties are not identical for all animals. For example, geese, which are obligate herbivores, hardly avoid food with alkaloids or smelly compounds (amines, mercaptoethanol) which are strong repellents for humans. On the other hand, fragrances that are attractive to us, are highly repellent to geese. Even within a population taste can differ significantly: a substantial proportion of humans cannot detect the smell of HCN, whereas others are highly sensitive. Furthermore, olfactory sensitivity can differ with age, sex, and hormonal cycles.

The bitterness varies with the chemical structure: in the case of QA the following scale was determined for man: Mean detection levels are 0.00085% for sparteine, 0.0021% for lupanine, and 0.017% for hydroxylupanine. Whereas we know most parameters of olfactory qualities in Homo sapiens, much less or hardly anything is known for most other vertebrates.

Alkaloids are infamous for their toxic properties in vertebrates and plants that produce alkaloids are often classified as poisonous or toxic. For a number of alkaloids the respective LDso values have been determined with laboratory animals, especially mice, but also with rats, guinea pigs, cats, rabbits, dogs, or pigeons. As rodents are herbivores (and thus adapted to **allelochemicals**), they are not especially sensitive to alkaloids as toxins and some of the data may be misleading. The toxic effects observed with complete animals have their counterpart in the cytotoxic effect determined for some alkaloids. Most of these data have been obtained by screening many natural products for anticancer activity. But an alkaloid that can kill a cancer cell is usually also toxic for "normal" cells and the complete animal. Therefore, the data shown in Table V are another indication of the general toxicity of alkaloids toward animals. The mechanisms underlying the toxic effects have been elucidated in some detail. Often molecular targets and processes are involved that are important for all cells, such as DNA, RNA, proteins, replication, transcription, protein biosynthesis, membrane assembly and stability, electron chains, or metabolically important enzymes or proteins, such as receptors, hormones, signal compounds.

Keyword

Allelochemicals are a subset of secondary metabolites, which are not required for metabolism (i.e. growth, development and reproduction) of the allelopathic organism.

Whereas many insect herbivores are "specialists," vertebrate herbivores are rather polyphagous, although some specialization may occur. For example, grouse lagopus) or capercaillies (Tetrao urogallus) prefer plants of the Ericaceae or Coniferae; crossbills, the seeds of Picea and Abies which are rich in terpenes. The Australian koala is oligophagous and consumes certain terpene-rich species of the genus Eucalyptus. While a single plant can be a host for hundreds of insect larvae, hundreds of plants comprise a daily menu for a larger grazing mammal. Vertebrates share a few strategies with insects in coping with allelochernicals. But a sequestration and storage of dietary alkaloids has hardly been reported; the storage of quinolizidine-type alkaloids in castoreum (derived from food plants) is an exception rather than a rule. Strategies of vertebrates include:

- Avoidance of alkaloid-rich plants (usually labeled toxic or poisonous by man) which is facilitated by the bitter or pungent taste of most alkaloids.
- Sampling of food from a wide variety of sources and thus minimizing the ingestion of high amounts of a single toxin.

- Detoxification of dietary alkaloids, which can be achieved by symbiotic bacteria or protozoa, living in the rumen or intestines, or by liver enzymes, which are specialized for the chemical modification of xenobiotics. Carnivorous animals, such as cats, are known to be much more sensitive than herbivores toward plant poisons. It has been suggested that animals that do not face the problem of toxic food are not adapted to the handling of allelochemicals.
- Some animals such as ungulates, monkeys, parrots, or geese ingest soil (so-called "geophagy"). For geese and chimpanzees it was shown that the ingested soil binds dietary allelochemicals, especially alkaloids.
- Animals are intelligent organisms, able to learn. The role of learning in food and toxin avoidance is rather important but is not understood in most species.

4.2 ECOLOGICAL ROLES OF ALKALOIDS

Alkaloids are widely distributed in the plant kingdom, especially among angiosperms (more than 20 % of all species produce alkaloids). Alkaloids are less common but present in gymnosperms, club mosses (Lycopodium), horsetails (Equisetum), mosses, and algae.

Alkaloids also occur in bacteria (often termed antibiotics), fungi, many marine animals (sponges, slugs, worms, bryozoa), arthropods, amphibians (toads, frogs, salamanders), and also in a few birds, and mammals.

Alkaloids are apparently important for the well-being of the organism that produces those (Figures 6). One of the main functions is that of chemical defense against herbivores or predators. Some alkaloids are antibacterial, antifungal, and antiviral; and these properties may extend to toxicity towards animals.

Alkaloids can also be used by plants as herbicides against competing plants. The importance of alkaloids can be demonstrated in lupins which – as wild plants – produce quinolizidine alkaloids (''bitter lupins''), that are strong neurotoxins (Table 1). Since lupin seeds are rich in protein, farmers were interested in using the seeds for animal nutrition.

This was only possible after the alkaloids (seed content 2–6 %) had been eliminated. Plant breeders created so-called sweet lupins with alkaloid levels below 0.02 %. If bitter and sweet lupins are grown together in the field it is possible to study the importance of alkaloids for defense. For example, Figure 7 shows that rabbits strongly discriminate between sweet and bitter lupins and prefer the former. This is also true for insects, as aphids and mining flies always favor sweet lupins. In the wild, sweet lupins would not survive because of the lack of an appropriate chemical defense.

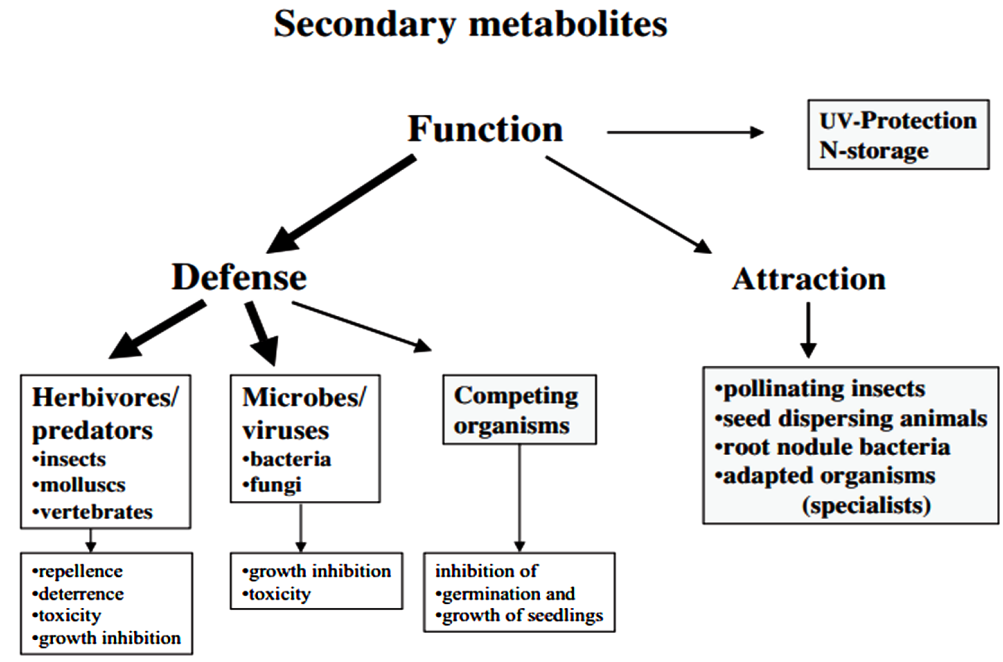


Figure 6. Overview of the ecological functions of secondary metabolites.

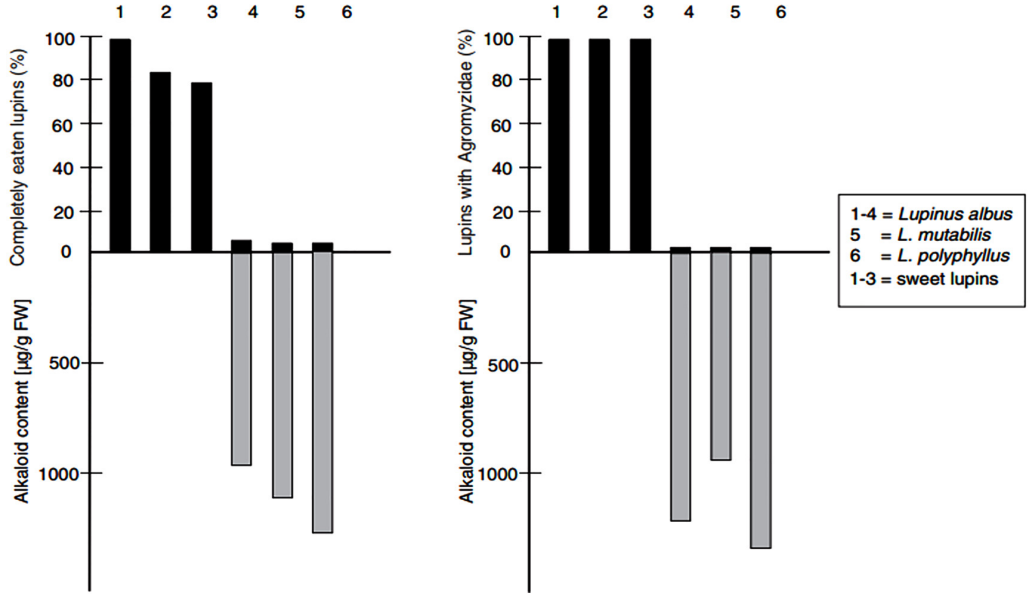


Figure 7. Importance of quinolizidine alkaloids for lupins against herbivores. lupins with or without alkaloids were grown in the field. When rabbits got into the field, they preferentially consumed the sweet, alkaloid-free lupins. Also larvae of mining flies preferred sweet lupins.

Table 1. Molecular targets of alkaloids in neuronal signal transduction

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

Serotonine receptor	Akuaminine and related indole alkaloids (A); annonaine, boldine, liriodenine and related aporphine alkaloids (AA); berberine and related protoberberine alkaloids; ergotamine, ergometrine, and related ergot alkaloids (AA); psilocin, psilocybine (A); bufotenine, N,N-dimethyltryptamine, and related indoles (A); harmaline and related b-carboline alkaloids (A); kokusagine and related furoquinoline alkaloids (AA); mescaline (A); ibogaine and other monoterpene indole alkaloids (A); gramine; N,N-dimethyltryptamine and derivates (AA)
Adenosine receptor	Caffeine, theobromine, and other purine alkaloids (AA
Opiate receptor	Morphine and related morphinan alkaloids (A); akuammine, mitragynine (A), ibogaine and related indole alkaloids
Acetylcholine esterase	Galanthamine (AA); physostigmine and related indole alkaloids (AA); berberine and related protoberberine alkaloids (AA); vasicinol and related quinazolines (AA); huperzine (AA); harmaline and related β-carboline alkaloids (AA); demissine and related steroidal alkaloids (AA)
Monoamine oxidase	Harmaline and related b-carbolines (AA); carnegine, salsolidine, O-methylcorypalline, and related isoquinolines (AA); N,N-dimethyltryptamine and related indoles (AA);
Neurotransmitter uptake (transporter)	Ephedrine and related phenylalkyl amines (AA); reserpine, ibogaine, and related indole alkaloids (AA); cocaine (AA); annonaine and related aporphine alkaloids (AA); arecaidine (AA); norharman and related β-carboline alkaloids (AA); salsolinol and related isoquinolines (AA)
Na^+, K^+ channels	Aconitine and related diterpene alkaloids (A); veratridine, zygadenine, and related steroidal alkaloids (A); ajmaline, vincamine, ervatamine, and other indole alkaloids (AA); dicentrine and other aporphine alkaloids (AA); gonyautoxin (AA); paspalitrem and related indoles (AA); phalloidin (AA); quinidine and related quinoline alkaloids (AA); sparteine and related quinolizidine alkaloids (AA); saxitoxin (AA); strychnine (AA); tetrodotoxin (AA)
Ca^{2+} channels	Ryanodine (A); tetrandrine, berbamine, antioquine, and related bis-isoquinoline alkaloids (AA); boldine, glaucine, liriodenine, and other aporphine alkaloids (AA); caffeine and related purine alkaloids (A/AA); cocaine (AA); corlumidine, mitragynine, and other indole alkaloids (A/AA); bisnordehydrotoxiferine (AA)
Adenylate cyclase	Ergometrine and related ergot alkaloids (AA); nuciferine and related aporphine alkaloids (AA)

cAMP phosphodiesterase	Caffeine and related purine alkaloids (AA); papaverine (AA); chelerythrine, sanguinarine, and related benzophenanthridine alkaloids (AA); colchicines (AA); infractine and related indole alkaloids (AA)
Protein kinase A (PKA)	Ellipticine and related indole alkaloids (AA)
Protein kinase C (PKC)	Cepheranthine and related bis-isoquinoline alkaloids (AA); michellamine B and related isoquinoline alkaloids (AA); chelerythrine and related benzophenanthridine alkaloids (AA); ellipticine and related indole alkaloids (AA)
Phospholipase (PLA_2)	Aristolochic acid and related aporphine alkaloids (AA); berbamine and related bis-isoquinoline alkaloids (AA)

Secondary metabolites are not only mono- but usually multifunctional. In many cases, even a single alkaloid can exhibit more than one biological function. During evolution, the constitution of alkaloids (that are costly to produce) has been modulated so that they usually contain more than one active functional group, allowing them to interact with several molecular targets and usually more than one group of enemies. Many plants employ secondary metabolites (rarely alkaloids, mostly colored phenolics and fragrant terpenoids) to attract pollinating and seed-dispersing animals; the compounds involved are usually both attractant and feeding deterrents. Attracted animals are rewarded by nectar or fleshy fruit tissues but should leave seeds or flowers undamaged. Hence, a multifunctional or pleiotropic effect is a common theme in alkaloids and other secondary **metabolites**.

Keyword

Metabolites are the intermediate products of metabolic reactions catalyzed by various enzymes that naturally occur within cells.

An alkaloid never occurs alone; alkaloids are usually present as a mixture of a few major and several minor alkaloids of a particular biosynthetic unit, which differ in functional groups. Furthermore, an alkaloid-producing plant often concomitantly accumulates mixtures of other secondary metabolites, mostly those without nitrogen, such as terpenoids and polyphenols, allowing them to interfere with even more targets in animals or microorganisms.

The multiple functions that alkaloids can exhibit concomitantly include a few physiological tasks: sometimes, alkaloids also serve as toxic nitrogen storage and nitrogen transport molecules. Plants that produce few and large seeds, nearly always invest

Remember

When considering the total benefits to a plant from secondary metabolites or the pharmacological activities of a drug, the potential additive or even synergistic effect of the different groups of secondary metabolites should be taken into account.

in toxic defense compounds (often alkaloids) that are stored together with proteins, carbohydrates, or lipids. Since nitrogen is a limiting factor for plant growth, nitrogen apparently is a valuable asset for plants.

In many species that store nitrogen in proteins and/or secondary metabolites in seeds or tubers, a remobilization has been observed after germination or regrowth in spring. In plants that shed their leaves, alkaloids are usually exported to storage organs prior to leaf fall.

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

4.3 MODES OF ACTION

In order to deter, repel, or inhibit the diverse set of potential enemies, ranging from arthropods and vertebrates to bacteria, fungi, viruses, and competing plants, alkaloids must be able to interfere with important cellular and molecular targets in these organisms. A short overview of these potential targets is given in Figure 8a and b.

The modulation of a molecular target will negatively influence its communication with other components of the cellular network, especially proteins (cross-talk of proteins) or elements of signal transduction. As a consequence, the metabolism and function of cells, tissues, organs, and eventually the whole organism will be affected and an overall physiological or toxic effect achieved.

Although we know the structures of many secondary metabolites, our knowledge of their molecular modes of action is largely fragmentary and incomplete. Such knowledge is, however, important for an understanding of the functions of secondary metabolites in the producing organism, and for the rational utilization of secondary metabolites in medicine or plant protection.

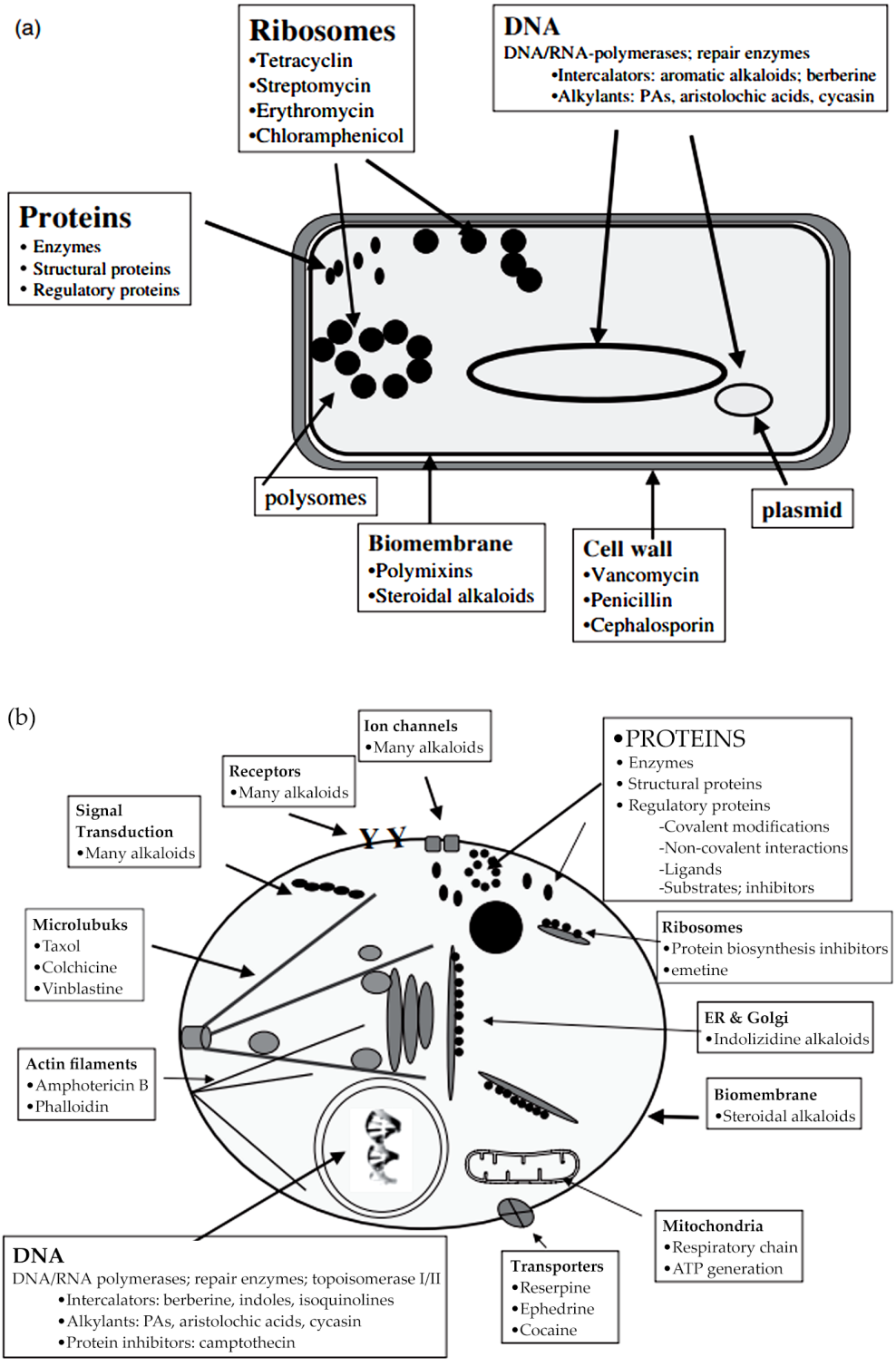


Figure 8. Molecular targets for secondary metabolites, especially alkaloids. (a) Targets in bacterial cells, (b) targets in animal cells.

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

4.3.1 Unspecific Interactions

Among broadly active alkaloids, a distinction can be made between those that are able to form covalent bonds with proteins and nucleic acids, and those that modulate the conformation of proteins and nucleic acids by noncovalent bonding. Covalent modifications are the result when the following functional groups interact with proteins:

reaction of aldehyde groups with amino and sulfhydryl groups;

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

Noncovalent bonds are generated when the following groups interact with proteins:

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

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

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

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

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

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

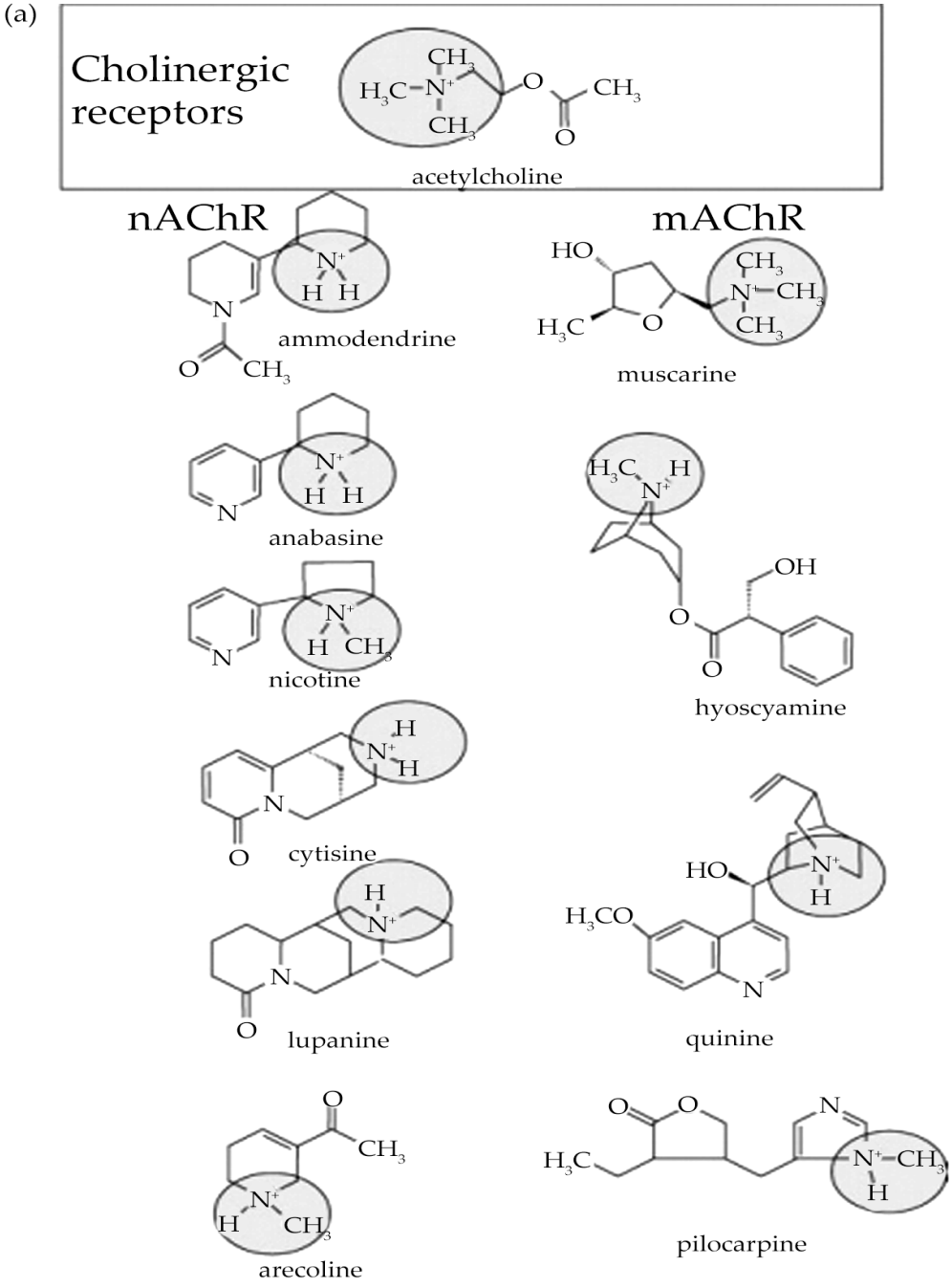
4.3.2 Specific Interactions

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

Many alkaloids fall into the class of specific modulators and have been modified during evolution in such a way that they mimic endogenous ligands, hormones, or substrates. We have termed this selection process ''evolutionary molecular modeling''. Many alkaloids are strong neurotoxins that were selected for defense against animals. Table 1 summarizes the potential neuronal targets that can be affected by alkaloids.

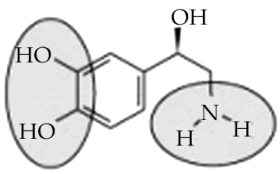
Neurotransmitters derive from amino acids; most of them are amines that become protonated under physiological conditions. Since alkaloids also derive from amino acids

(often the same ones as neurotransmitters) it is no surprise that several alkaloids have structural similarities to neurotransmitters. They can be considered as neurotransmitter analogs (Figure 9a–c).

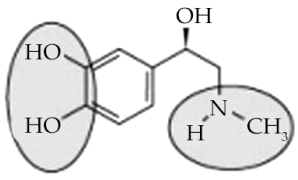


(b)

Adrenergic
α-, β- receptors



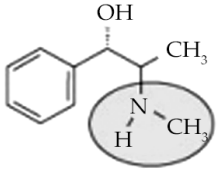
norepinephrine
noradrenaline



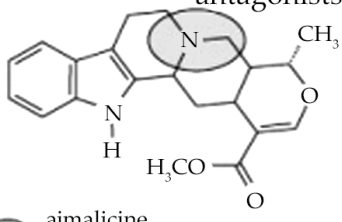
epinephrine
adrenaline

agonists

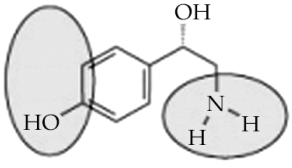
antagonists



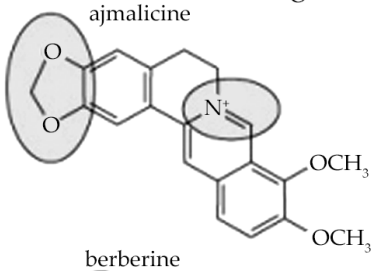
ephedrine



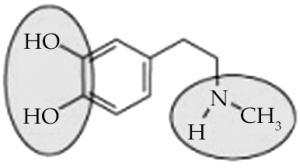
ajmalicine



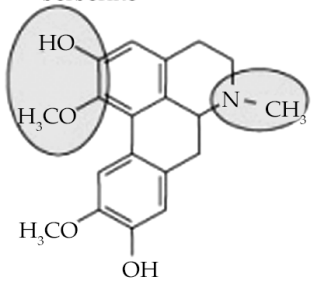
octopamine

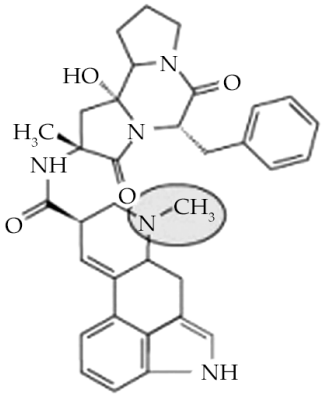


berberine



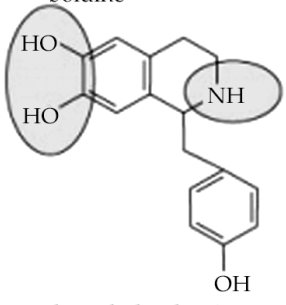
N-methyldopamine





ergotamine

boldine



demethylcoclaurine
(higenamine)

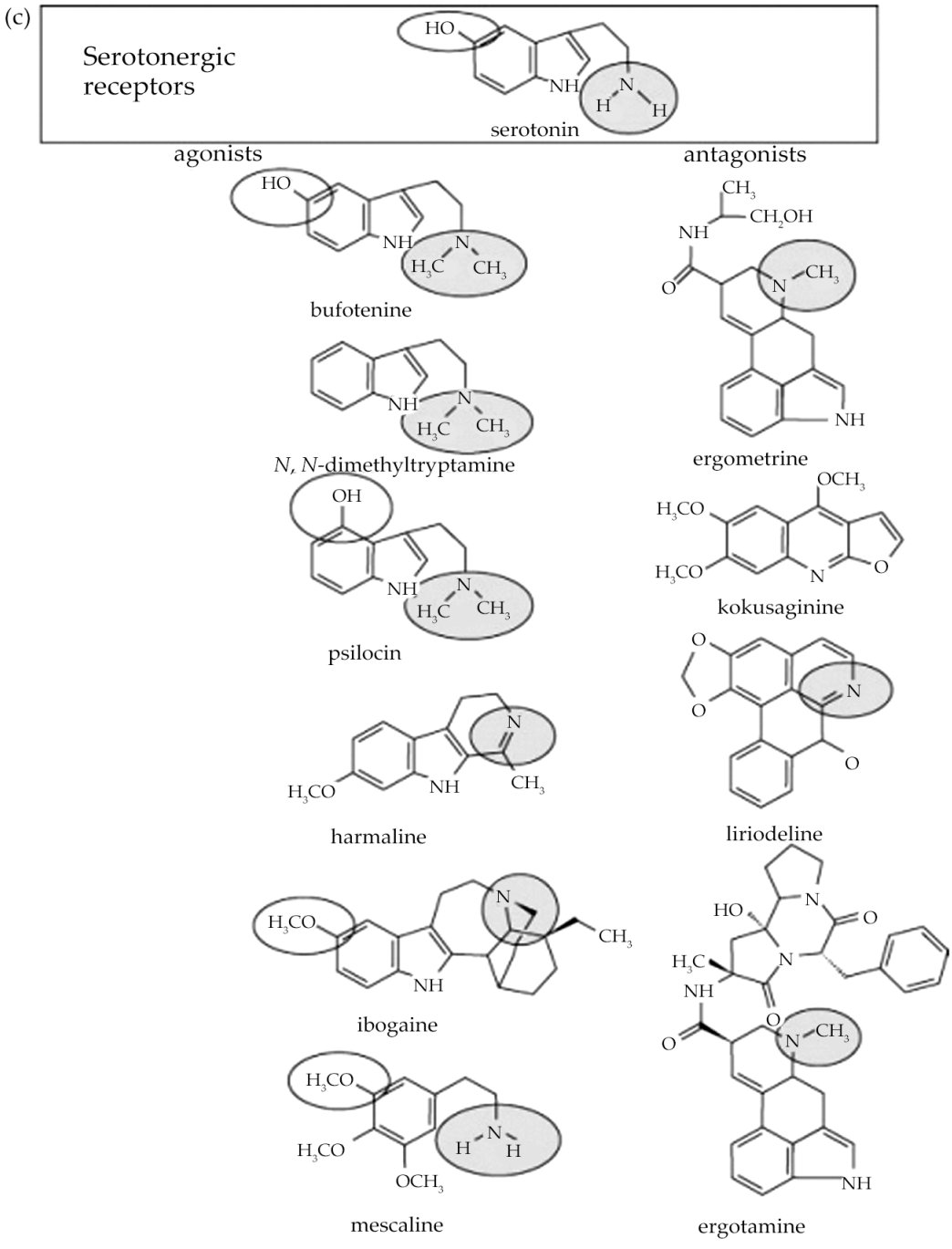


Figure 9. Agonistic or antagonistic modulation of neuroreceptors by alkaloids that mimic neurotransmitters. (a) Interaction at cholinergic neurotransmitters that bind acetylcholine: nicotinic acetylcholine receptor (nAChR) and muscarinic acetylcholine receptors (mAChR), (b) interaction at adrenergic receptors that bind noradrenaline and adrenaline, (c) interaction at serotonergic receptors that bind serotonin.

Alkaloids that structurally mimic neurotransmitters can bind to neuroreceptors and either activate (agonists) or inactivate (antagonists) them (Table 1). Additional important targets are ion channels, such as the Na^+, K^+, and Ca^{2+} channels; several alkaloids are known that inhibit or activate these ion channels (Table 1).

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

4.3.3 Cytotoxicity of Alkaloids

Many alkaloids are infamous for their strong toxicity towards animals and humans. Most of the deadly alkaloids fall into the class of neurotoxins. The others have cytotoxic properties (Table 2). A cytotoxic effect can be generated when cell membranes are made leaky (as by saponins or steroidal alkaloids), or when elements of the cytoskeleton are inhibited. The spindle poisons vinblastine, vincristine, colchicine, and taxol are particularly famous. Actin filament formation is blocked by fungal poisons such as phalloidin from **Amanita phalloides**.

DNA can also be a target for alkaloids: planar and lipophilic alkaloids, such as berberine and sanguinarine (Figure 10) are intercalating compounds that assemble between the stacks of paired nucleotides in the DNA double helix. DNA intercalation can disturb replication, DNA repair, and DNA topoisomerases. Frameshift mutations are one of the adverse consequences of intercalating compounds.

Some alkaloids, such as pyrrolizidine alkaloids, aristolochic acids, cycasin, and furoquinoline alkaloids, are known to form covalent adducts with DNA bases. Mutations and tumor formation can be the result of such interactions. DNA alkylation occurs in some alkaloids only after activation by liver enzymes, such as cytochrome p450 oxidases (pyrrolizidine alkaloids, aristolochic acids).

Keyword

Amanita phalloides, commonly known as the death cap or the death cap amanita, is a deadly poisonous basidiomycete fungus, one of many in the genus Amanita.

Table 2. Molecular modes of action of selected cytotoxic alkaloids.

Alkaloid	Toxicity (animals)	Cyto-toxicity	Apoptosis	Micro-tubules	DNA topoiso-merase	Telo-merase	Membrane lysis	DNA inter-calation	DNA alkylation	Mutagenic	Inhibition of protein biosynthesis
Alkaloids derived from tryptophan											
Camptothecin	X	X	X		X			X			
Cinchonine, cinchonidine	X	X						X			
Cryptolepine	X	X	X		X	X		X			
Dictamnine	X	X						X	X	X	
Ellipticine	X				X			X		X	
Ergotamine	X		X					X			
Evodiamine	X	X		X							
Fagarine	X	X						X		X	
Harmine	X	X	X		X					X	
Quinine	X	X	X					X			
Vincristine	X	X	X	X				X			
Alkaloids derived from phenylalanine, tyrosine											
Aristolochic acids	X	X								X	X
Berbamine	X								X		
Berberine	X	X	X		X		X		X		
Chelerythrine	X	X	x						X	X	
Chelidonine	X	X	X	X					X		
Colchicine	X	X	X	X							
Coralyne	X	X			X				X		
Dicentrine	X	X			X						X
Emetine	X	X	X					X			X
Fagaronine	X	X			X			X			
Liriodenine	X	X	X		X					X	
Lycorine	X	X	X								
Noscapine	X	X	X	X							
Piperine		X	X								
Salsolinol	X	X									
Sanguinarine	X	X	X					X	X	x	
Tetrandrine	X	X	X				X				
Alkaloids derived from ornithine, arginine											
Pyrrolizidine alkaloids	X	X	X						X	X	
Miscellaneous alkaloids											
Acronycine	X	X	X					X		X	
Cycasin	X								X	X	
Cyclopamine	X	X	X							X	
Lobeline	X							X			
Maytansine	X	X		X							
Paclitaxel	X	X	X	X							
Solarmargine	X	X	X				X				

Ribosomal protein biosynthesis is often inhibited by alkaloids that interact with nucleic acids. There are also more specific inhibitors, such as emetine. Disturbances of the cytoskeleton, DNA replication, and DNA topoisomerase, or DNA alkylation and

intercalation usually lead to cell death by apoptosis (Table 2). The cytotoxic properties are usually not specific for animals but also affect bacteria, fungi, other plants, and even viruses. Alkaloids thus defend plants against a wide diversity of enemies. They have the disadvantage that a producing plant could theoretically kill itself by its own poison. Compartmentation, target-site insensitivity, and other mechanisms (which are largely unknown) must have evolved to overcome such problems.

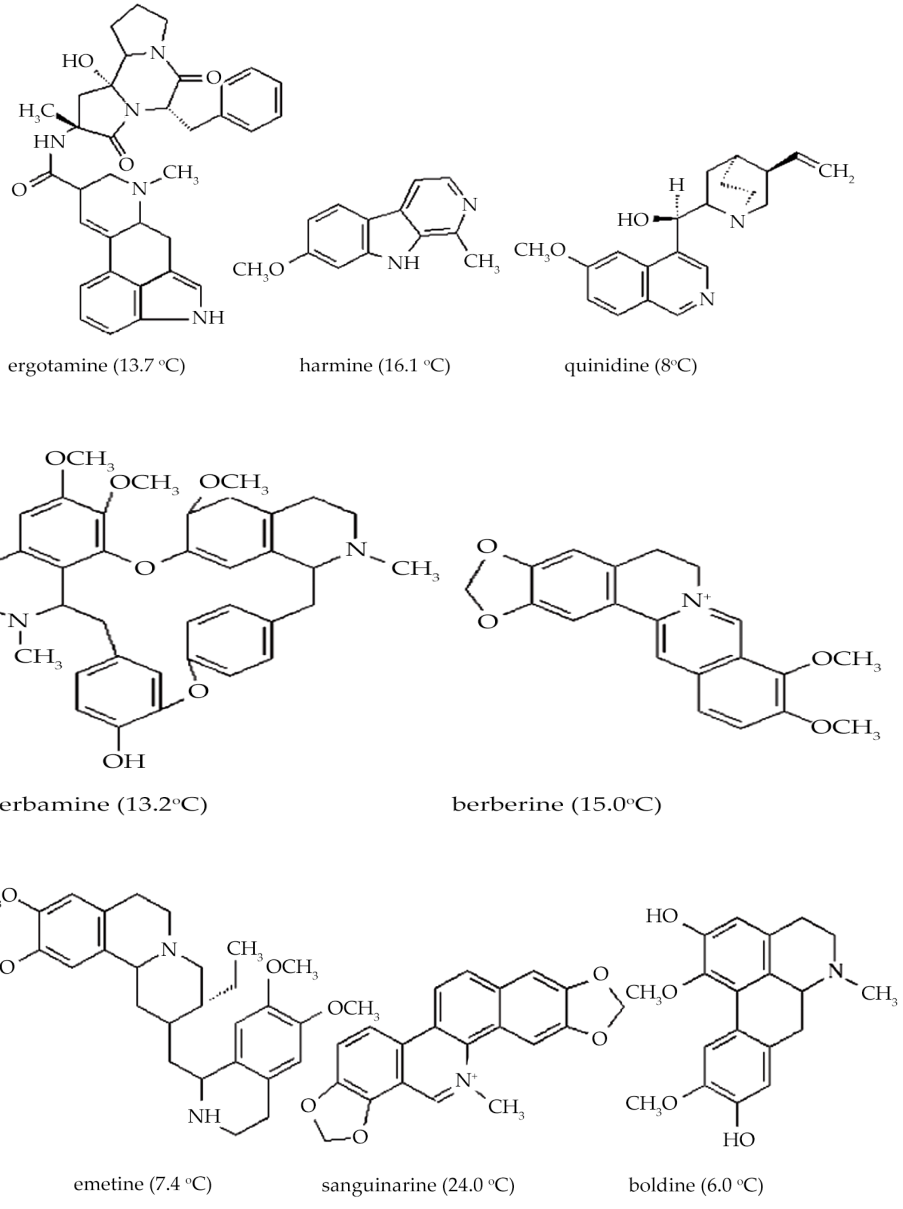


Figure 10. Examples of alkaloids that intercalate DNA. Intercalation increases the melting temperature of DNA; relevant Tm values are shown in parentheses.

4.4 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. 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. Some animals, including dairy cows, have been shown to selectively feed on only alkaloid-poor green plants394. 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 behavioral responses were very important for researchers. The chemical analysis of the green matter clearly confirmed field observations and the animals' consumption behavior. 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 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 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. The coefficients of the selective toxicity show that alkaloids are very dominantly selective toxins to vertebrates (Table 3). Vertebrate very strong selectivity (<0.01) is observed in such alkaloids as ajmalicine, brucine, ephedrine, ergometrine, harmaline, lupanine, lupinine, scopolamine and strychnine (Table 3). 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 3). 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 3).

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. Some examples of this kind of insect behaviour have been seen in Aphis cytisorum, Aphis genistae and Macrosiphum albifrons. Wink has mentioned that M. albifrons stores alkaloids in order to provide protection against carnivorous beetles, such as Carabus problematicus or Coc-cinella septempunctata. However, the protection provided by the sequestration of alkaloids seems to be still more conjecture than scientifically proven. Stermitz 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, Wackers underlined that insect behavior 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 3. 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. 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.

Table 3. Selective Toxicity Coefficients (STC) of some alkaloids and selective toxicity in the ecosystem

Alkaloid	STC	Selective Toxicity in Ecosystem
Ajmalicine	0.0015[MS]	vvss
Ajmaline	0.014[MS]	vss
Anabasine	0.013[MS]	vss
Arecoline	0.014[MS]	vss
Atropine	0.75[RB]	msv
Berberine	1.0[MBE]	ns
Boldine	0.12[MS]	vss
Brucine	0.005[RBE]	vvss
Caffeine	0.7[MBE]	msv
Castanospernine	0.1[RA]	msv
Chaconine	0.15[RA]	msv
Chelidonine	0.45[RS]	msv
Cinchonidine	3.6[RBE]	msiv
Cinchonine	3.1[MBE]	msiv
Codeine	5.0[MF]	msiv
Colchicine	0.003[MABE]	vvss
Coniine	0.112[AGP]	msv
Cytisine	0.03[MF]	vss
Emetine	0.044[MS]	vss
Ephedrine	0.001[MBE]	vvss
Ergometrine	0.0003[MS]	vvss
Ergotamine	0.11[MS]	msv
Gramine	0.1[MS]	msv
Harmaline	0.006[MS]	vvss
Harmine	0.7[MBE]	msv

Heliotrine	0.45[RBE]	msv
Hyoscyamine	0.3[RBE]	msv
Jacobine	15.0[RL]	msiv
Lobeline	2.5[RBE]	msiv
Lupanine	0.008[MA**]	vss
Lupinine	0.009[MA]	vvss
Nicotine	0.08[MBE***]	vss
Papaverine	3.0[MF]	msiv
Pilocarpine	0.9[MF]	vsss
Quinine	0.01[ABE]	vss
Reserpine	0.1[AB]	msv
Sanguinarine	0.9[MS]	vsss
Scopolamine	0.003[MBE]	vvss
Senecionine	0.63[MBE]	msv
Solanidine	0.09[MC]	vss
Solanine	0.06[MC]	vss
Sparteine	0.01[MBE]**	vss
Strychnine	0.005[MBE]	vvss
Tomatidine	0.08[MC]	vss
Vinblastine	0.8[MBE]	msv
Vincamine	1.0[MBE]	ns
Vincristine	0.9[MBE]	vsms
Yohimbine	0.45[MBE]	msv

Alkaloids are a part of plant-based sustenance for herbivores, omnivores and according to the latest research, also for carnivores. The literature mentions that alkaloids and other secondary compounds also occur in floral nectar, pollen, honeydew, leaves, stems and roots of plants. 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

Keyword

Lobeline is a pyridine alkaloid found in a variety of plants, particularly those in the genus Lobelia, including Indian tobacco, Devil's tobacco, great lobelia, Lobelia chinensis, and Hippobroma longiflora.

and behavior. The sequestration and accumulation of these compounds in the liver strengthen this hypothesis. The possible protective role of alkaloids seems to be secondary. 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.

There exists evidence that some insects store dietary alkaloids derived from natural sources. Figure 11 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.

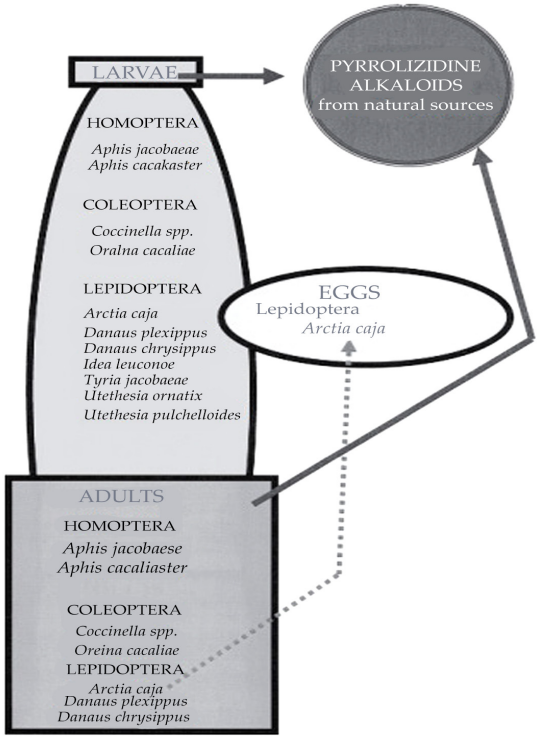


Figure 11. A diagram of the accumulation of pyrrolizidine alkaloids in some insect species during various developmental stages. It should be noted that it is not often that these alkaloids are present in

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).

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 behavior. 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. 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.

There are two sources of alkaloids in animals. 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 behavior 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. 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.

ROLE MODEL

EUGENE ODUM

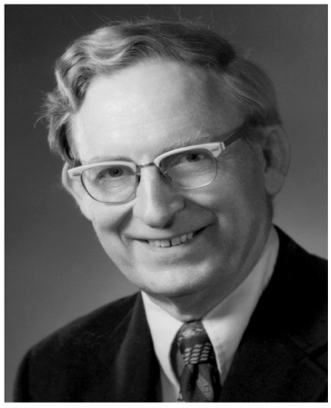


Photo by Gittings

Eugene Pleasants Odum, called "the father of modern ecology," brought the word *ecosystem* into common parlance by making it the organizing concept in his 1953 *Fundamentals of Ecology*. Through that textbook, which was translated into twelve languages, and through his many other books and articles, he led the way toward the study of nature in terms of ecosystems, and with his brother, the ecologist Howard Thomas Odum, powerfully influenced the development of ecosystem ecology.

Life

Odum was born in Newport, New Hampshire, on September 17, 1913. His parents, Anna Louise Kranz and Howard Washington Odum, were vacationing there to escape the summer heat of Athens, where the senior Odum served on the faculty of the University of Georgia. Howard W. Odum later gained national prominence as a sociologist at the University of North Carolina at Chapel Hill, as founder of the journal *Social Forces*, and as one of the founders of the Southern Regional Council.

Eugene Odum grew up with his younger siblings, Mary Frances and Howard Thomas, in Chapel Hill, where he developed his lifelong interest in ornithology. He obtained his A.B. and A.M. in zoology from the University of North Carolina in 1934 and 1936, respectively, and his Ph.D. in zoology, with a major in ecology, from the University of Illinois in 1939. He married Martha Ann Huff in 1939, served the 1939-40 academic year as resident naturalist at the Edmund Niles Huyck Preserve in Rensselaerville, New York, and joined the Department of Zoology at the University of Georgia in the fall of 1940. He and Martha had two sons, William Eugene and Daniel Thomas. Martha, who had graduated from the University of Illinois with a degree in design, became a leader in the Athens art community, and William followed his father and his uncle into ecology, ending his career at the University of Virginia in 1991 when he died of liver cancer. Martha died of cancer in 1995. Odum retired from the University of Georgia in 1984, leaving

his position as director of the Institute of Ecology, Alumni Foundation Distinguished Professor of Zoology, and Callaway Professor of Ecology. He had been instrumental in the founding of the university's Savannah River Ecology Laboratory, University of Georgia Marine Institute on Sapelo Island, and the Institute of Ecology, which in 2007 was renamed the Eugene P. Odum School of Ecology in his honor.

Odum died of natural causes after tending his garden at his Athens home on August 10, 2002. "He was the best-known ecologist in the world, there's no question about that," said a colleague, University of Georgia ecology professor Whit Gibbons.

Contributions to Ecosystem Ecology

During the 1950s Odum took advantage of the U.S. government's interest in building atomic weapons facilities and in commissioning preinstallation environmental inventories of their sites to engage University of Georgia graduate students and faculty in ecological field studies. With a grant in 1951 from the U.S. Atomic Energy Commission (AEC), he initiated at the Savannah River nuclear plant a program of long-term ecological research that would eventually become the university's Savannah River Ecology Laboratory. A decade later, encouraged by the growing national interest in radiation ecology, or radioecology, Odum and his colleagues began planning an on-campus Institute of Radiation Ecology, which would become the University of Georgia Institute of Ecology.

In 1954 Eugene Odum and his brother acquired another grant from the AEC to study the effects of nuclear fallout in the Eniwetok Atoll of the South Pacific, where the U.S. government had been testing atomic weapons. In a paper that won the 1956 Mercer Award from the Ecological Society of America, they proved that the coral reef was maintaining itself in equilibrium because of the symbiotic relationship of the coral and the algae. Odum was to use that discovery again and again in his argument that symbiosis promotes stability.

In 1964, as president of the Ecological Society of America, Odum announced in the journal *BioScience* the establishment of a "new ecology," a "systems ecology" that dealt with the world as a whole, bringing all of the ecosystem sciences together. In the article he laid out the discipline's fundamental assumptions: that the ecosystem is the basic unit of nature; that biological diversity increases ecosystem stability; that "homeostasis" is important at all levels of the biological spectrum; that "the whole is greater than the sum of its parts"; and that therefore reductionist scientific methods cannot adequately explain living systems.

These were the premises on which Odum based his science and with which he would thereafter be identified. The statement "the ecosystem is greater than the sum of its parts" is inscribed on the bust of him that adorns the entrance to the Ecology Building at the University of Georgia.

SUMMARY

- Plants are autotrophic organisms and serve as both a major and the ultimate source of food for animals and microorganisms. Plants cannot run away or fight back when attacked by an herbivore, nor do they have an immune system to protect them against pathogenic bacteria, fungi, viruses, or parasites.
- The polyphagous species exploit a wide range of host plants, whereas the mono-/oligophagous insects often specialize on one or a small number of host plants which are often systematically related and accumulate the same class of secondary compounds.
- Many alkaloids exhibit a bitter or pungent taste for vertebrates and a bitter or pungent diet is normally instinctively avoided.
- Alkaloids are infamous for their toxic properties in vertebrates and plants that produce alkaloids are often classified as poisonous or toxic.
- Vertebrates share a few strategies with insects in coping with allelochernicals. But a sequestration and storage of dietary alkaloids has hardly been reported; the storage of quinolizidine-type alkaloids in castoreum (derived from food plants) is an exception rather than a rule.
- Alkaloids are widely distributed in the plant kingdom, especially among angiosperms (more than 20 % of all species produce alkaloids). Alkaloids are less common but present in gymnosperms, club mosses (Lycopodium), horsetails (Equisetum), mosses, and algae.
- An alkaloid never occurs alone; alkaloids are usually present as a mixture of a few major and several minor alkaloids of a particular biosynthetic unit, which differ in functional groups.
- The modulation of a molecular target will negatively influence its communication with other components of the cellular network, especially proteins (cross-talk of proteins) or elements of signal transduction.

MULTIPLE CHOICE QUESTIONS

1. **Animal adopt a similar state like sleep to reduce their metabolic rate, it is called:**
   a. Migration
   b. Transpiration
   c. Hibernating
   d. None of these
2. **Which is not a feature of heliophyte among the following?**
   a. Stem with long internodes
   b. Numerous rootlets
   c. Long lateral roots
   d. Vigorous fruiting and flowering
3. **Plants are**
   a. producers.
   b. consumers.
   c. herbivores.
   d. omnivores.
4. **Plants that defend themselves effectively constitute an ecological niche, almost devoid of herbivores and pathogens.**
   a. True
   b. False
5. **The endogenously produced and the acquired alkaloids appear to serve as chemical defense compounds, in analogy to the situation found in plants**
   a. True
   b. False

REVIEW QUESTIONS

1. Define the insect feeding deterrence and alkaloid toxicity.
2. What is vertebrate herbivores?
3. What are ecological roles of alkaloids?
4. Define the cytotoxicity of alkaloids.
5. Explain the animal sequestration of alkaloids.

Answer to Multiple Choice Questions

1. (c) 2. (a) 3. (a) 4. (a) 5. (a)

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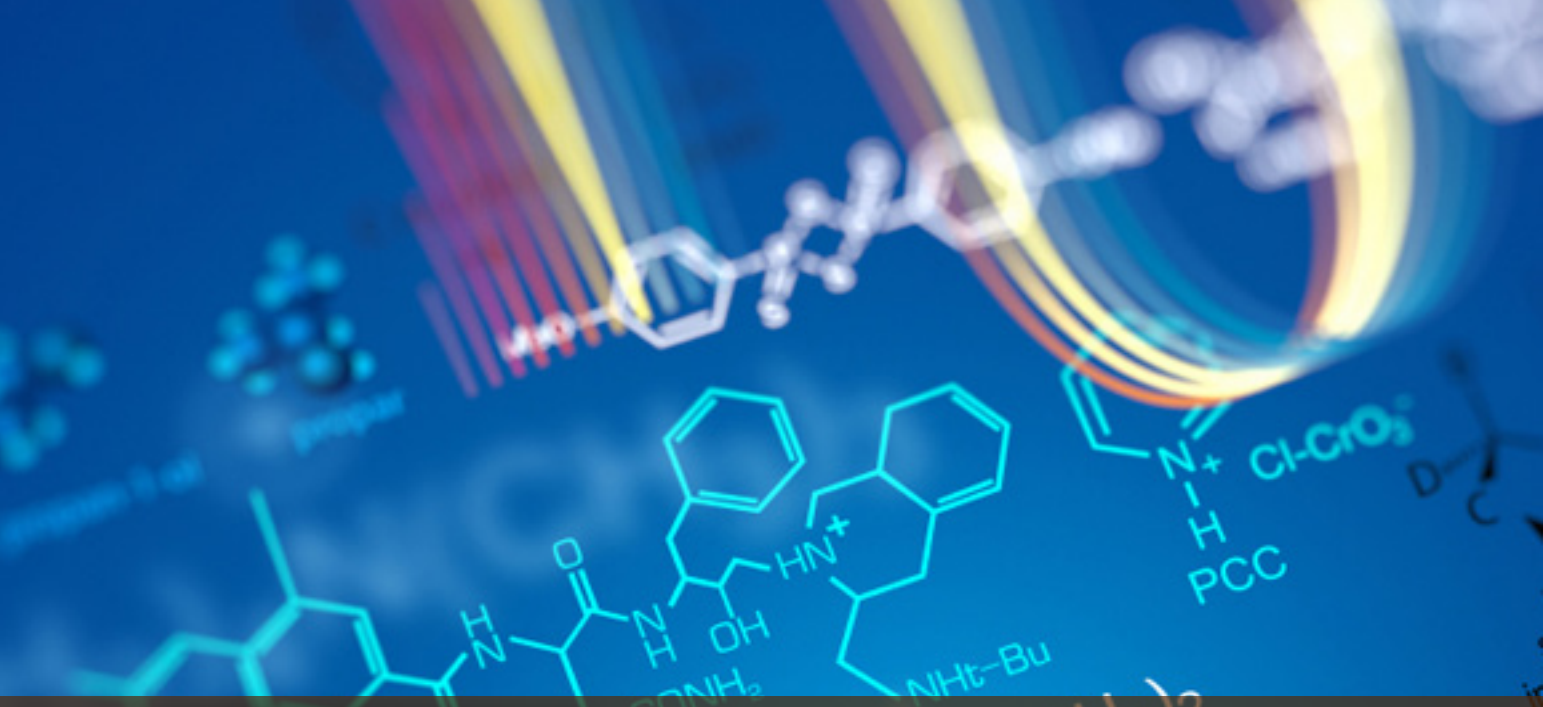


CHAPTER 5

ALKALOIDS BIOSYNTHESIS

LEARNING OBJECTIVES

After studying this chapter, you will be able to:

1. Understand biosynthesis of tetrahydrobenzylisoquinoline alkaloids
2. Explain biosynthesis of terpene indole alkaloids
3. Describe biosynthesis of tropane alkaloids
4. Focus on purine alkaloids biosynthesis

"The poison in the arrow that had struck her was, in chemical structure, like curare; it paralyzed first, killed second. It is not a merciful death: one dies fully conscious and aware of one's surroundings."

—Douglas Preston

INTRODUCTION

Alkaloids are a highly diverse group of natural products related only by the presence of a basic nitrogen atom located at some position in the molecule. Even among biosynthetically related classes of alkaloids, the chemical structures are often highly divergent. Although some classes of natural products have a recognizable biochemical paradigm that is centrally applied throughout the pathway – for example, the "assembly line" logic of polyketide biosynthesis – the biosynthetic pathways of alkaloids are as diverse as the

structures. It is difficult to predict the biochemistry of a given alkaloid based solely on precedent, making alkaloid biosynthesis a challenging, but rewarding, area of study.

Hundreds of alkaloid biosynthetic pathways have been studied by chemical strategies, such as isotopic labeling experiments. However, modern molecular biology and genetic methodologies have facilitated the identification of alkaloid biosynthetic enzymes. This review focuses on pathways for which a significant amount of genetic and enzymatic information has been obtained. Although alkaloid natural products are produced by insects, plants, fungi and bacteria, this review focuses on four major classes of plant alkaloids: the tetrahydrobenzylisoquinoline alkaloids, the terpene indole alkaloids, the tropane alkaloids and the purine alkaloids.

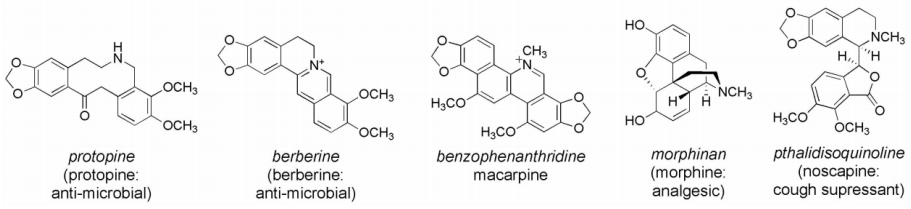
In general, plant biosynthetic pathways are poorly understood when compared to prokaryotic and fungal metabolic pathways. A major reason for this is that genes expressing complete plant pathways are not typically clustered together on the genome. Therefore, each plant enzyme is often individually isolated and cloned independently. However, a number of enzymes involved in plant alkaloid biosynthesis have been successfully cloned, and many more enzymes have been purified from alkaloid producing plants or cell lines. Identification and study of the biosynthetic enzymes has a significant impact on understanding of the biochemistry of the pathway. Furthermore, genetic information can also be used to understand the complicated localization patterns and regulation of these plant pathways.

5.1 BIOSYNTHESIS OF TETRAHYDROBENZYLISOQUINOLINE ALKALOIDS

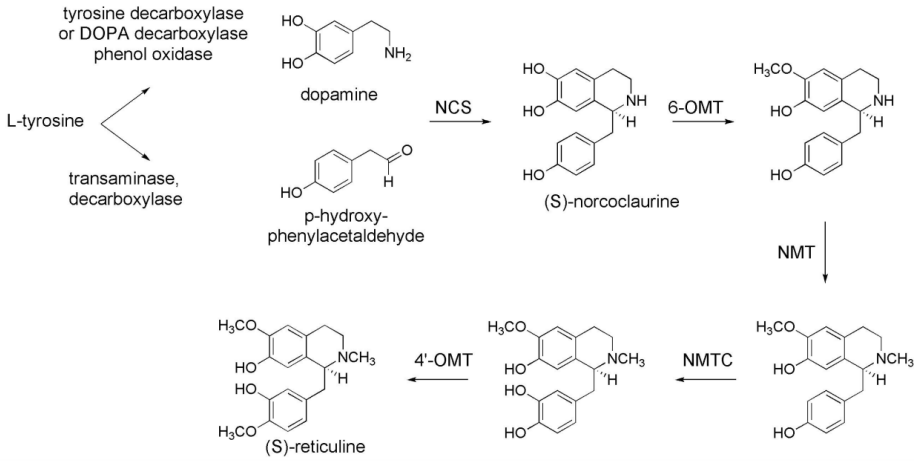
The tetrahydrobenzylisoquinoline alkaloids include the analgesics morphine and codeine, and the antibiotic berberine (Figure 1A). Morphine and codeine are two of the most important analgesics used in medicine, and plants remain the main commercial source of the alkaloids. Development of plant cell cultures of *Eschscholzia californica*, *Papaver somniferum* and *Coptis japonica* has aided in the isolation and cloning of many of the enzymes involved in the biosynthesis of tetrahydrobenzylisoquinoline alkaloids.

5.1.1 Early steps of tetrahydroisoquinoline biosynthesis

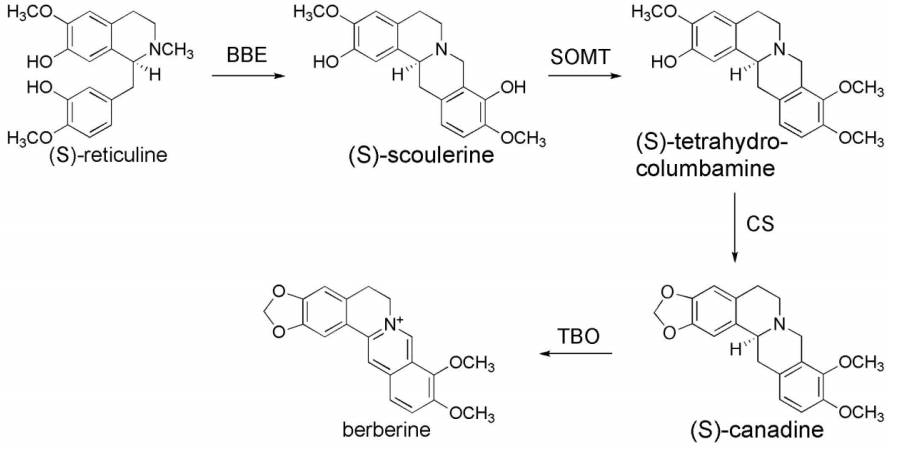
Tetrahydrobenzylisoquinoline biosynthesis begins with the substrates dopamine and p-hydroxyphenylacetaldehyde (Figure 1B). **Dopamine** is made from tyrosine by hydroxylation and decarboxylation. Enzymes that catalyze the hydroxylation and decarboxylation steps in either order are present in the plant, and the predominant pathway for formation for dopamine from tyrosine is not clear. The second substrate, p-hydroxyphenylacetaldehyde, is generated by transamination and decarboxylation of tyrosine.



(A)



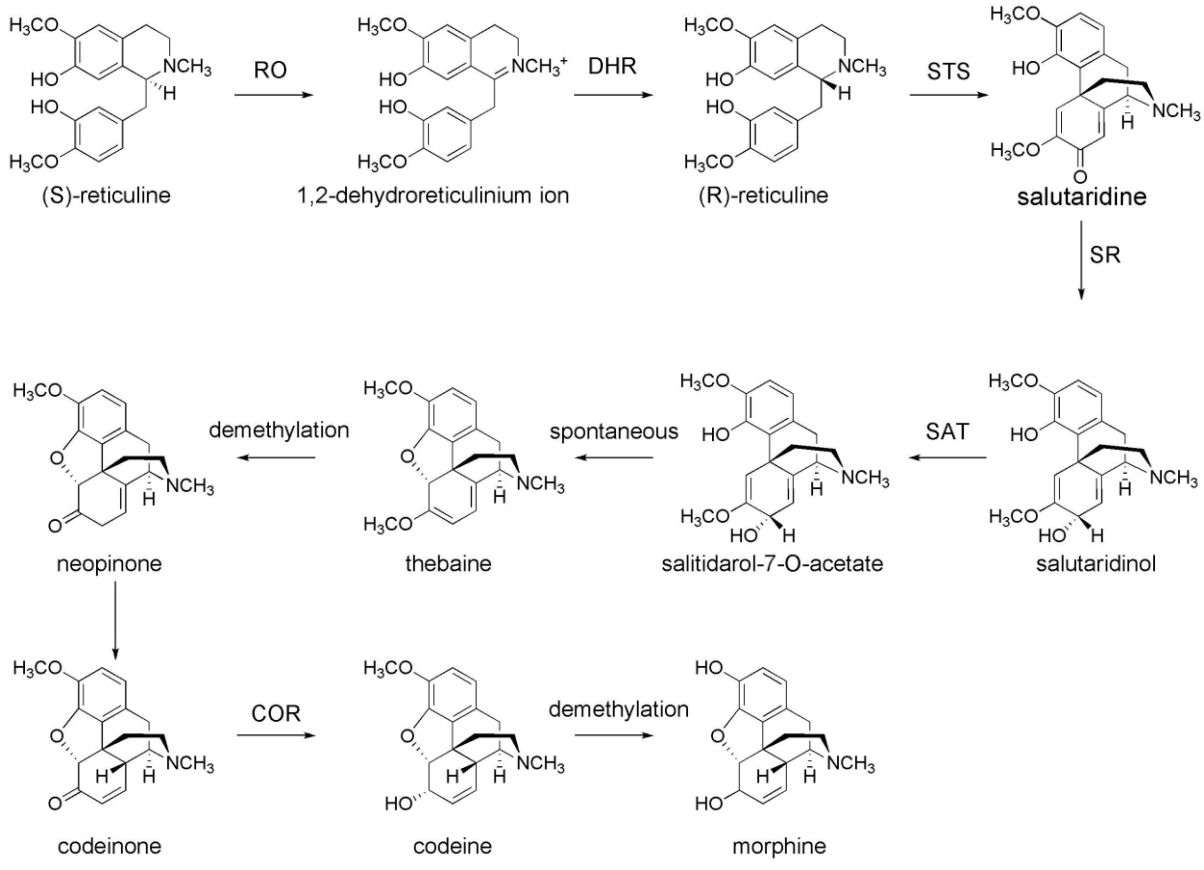
(B)



(C)

Keyword

Dopamine is a neurotransmitter that plays several important roles in the brain and body. It is an organic chemical of the catecholamine and phenethylamine families. Dopamine constitutes about 80% of the catecholamine content in the brain.



(D)

Figure 1: A. Representative tetrahydrobenzylisoquinoline alkaloids. B. Early biosynthetic steps of the tetrahydrobenzynlisoquinoline pathway yield the biosynthetic intermediate (S)-reticuline, the central biosynthetic intermediate for all tetrahydroisoquinoline alkaloids.C. Berberine biosynthesis pathway. D. Morphine biosynthesis. NCS, norcoclaurine synthase; 6-OMT, norcoclaurine 6-Omethyltransferase; NMT, coclaurine N-methyltransferase; NMTC, Nmethylcoclaurine 3′-hydroxylase; 4′-OMT, 3′-hydroxy-N-methylcoclaurine 4′-OmethyltransferaseBBE, berberine bridge enzyme; SOMT, scoulerine 9-Omethyltransferase; CS, canadine synthase; TBO, tetrahydroprotoberberine oxidase; RO, reticuline oxidase; DHR, dihydroreticulinium ion reductase; STS, salutaridine synthase; SR, salutaridine reductase; SAT, salutaridinol acetyltransferase; COR, codeinone reductase.

Condensation of dopamine and p-hydroxyphenylacetaldehyde is catalyzed by norcoclaurine synthase to form (S)-norcoclaurine (Figure 1B). Two norcoclaurine synthases with completely unrelated sequences were cloned (Thalictrum flavum and Coptis japonica) and heterologously expressed in E. coli. One is homologous to iron dependent diooxygenases, while the other is homologous to a pathogenesis related

protein. Undoubtedly, future experiments will shed light on the mechanism of these proteins and how two such widely divergent sequences can catalyze the same reaction.

One of the hydroxyl groups of (S)-norcoclaurine is methylated by a SAM (S-adenosyl methionine) dependent O-methyl transferase to yield (S)-coclaurine. This enzyme has been cloned and heterologously expressed enzyme exhibited the expected activity. The resulting intermediate is then N-methylated to yield N-methylcoclaurine, an enzyme that has been recently cloned. Nmethylcoclaurine is in turn hydroxylated by a P450 dependent enzyme (CYP80B), N-methylcoclaurine 3'-hydroxylase that has been cloned. The 4' hydroxyl group is then methylated to yield (S)-reticuline, the common biosynthetic intermediate for the berberine, benzophenanthridine, and morphinan alkaloids (Figure 1B). This methyl transferase has also been cloned. These **gene sequences** were also used to identify the corresponding T. flavum genes that encode the biosynthetic enzymes for reticuline from a cDNA library.

Keyword

Gene sequences specify the initial amino acid sequences of proteins as expressed by the ribosomes.

5.1.2 Berberine Biosynthesis

(S)-reticuline is converted to (S)-scoulerine by the action of a wellcharacterized flavin dependent enzyme, berberine bridge enzyme (Figure 1C). This enzyme has been cloned from several plant species and the mechanism of this enzyme has been extensively studied. (S)-scolerine is then Omethylated to yield (S)-tetrahydrocolumbamine. Heterologous expression of a clone in E. coli revealed an enzyme having the expected substrate specificity. A variety of O-methyl transferases have also been cloned from P. somniferum cultures. A cytochrome P450 oxidase then generates the methylene dioxy bridge of (S)-canadine. The final step of berberine biosynthesis is catalyzed by an oxidase that has not been cloned.

Overproduction of berberine in C. japonica cell suspension cultures was achieved by selection of a high producing cell line, with reported productivity of berberine reaching 7 g/L. This was one of the first demonstrations of production of a benzylisoquinoline alkaloid in cell culture at levels necessary for economic production. This cell line greatly facilitated the identification of the biosynthetic enzymes.

5.1.3 Morphine biosynthesis

The later steps of morphine biosynthesis has been investigated exclusively in P. somniferum cells and tissue. Notably, in morphine biosynthesis, (S)-reticuline is converted to (R)-reticuline, thereby epimerizing the stereocenter generated by norcoclaurine synthase at the start of the pathway (Figure 1D). (S)- reticuline is converted to (R)-reticuline through a 1, 2-dehydroreticuline intermediate. Dehydroreticuline synthase catalyzes the oxidation of (S)-reticuline to 1,2-dehydroreticulinium ion. This enzyme has not been cloned, but has been partially purified and shown to be membrane associated. This intermediate is then reduced by dehydroreticuline reductase, an NADPH dependent enzyme that stereoselectively transfers a hydride to dehydroreticulinium ion to yield (R)- reticuline. This enzyme has not yet been cloned, but has also been purified to homogeneity.

Next, the key carbon-carbon bond of the morphanan alkaloids is formed by the cytochrome P450 enzyme salutaridine synthase. Activity for this enzyme has been detected in microsomal preparations, but has not been cloned. The keto moiety of the resulting product, salutaridine, is then stereoselectively reduced by the NADPH dependent salutaridine reductase to form salutardinol.

The enzyme has been purified, and a recent transcript analysis profile of P. sominiferum has resulted in the identification of the clone. Salutaridinol acetyltransferase, also cloned, then transfers an acyl group from acetyl-CoA to the newly formed hydroxyl group, resulting in the formation of salutaridinol-7-Oacetate.

This modification sets up the molecule to undergo a spontaneous reaction in which the acetate can act as a leaving group. The resulting product, thebaine, is then demethylated by an as yet uncharacterized enzyme to yield neopinione, which exists in equilibrium with its tautomer codeinone. The NADPH dependent codeinone reductase catalyzes the reduction of codeinone to codeine and has been cloned. Finally, codeine is demethylated by an uncharacterized enzyme to yield morphine.

The localization of morphine biosynthesis has been investigated at the cellular level in intact poppy plants using immunoflouresence microscopy. The localization of 4’-OMT (reticuline biosynthesis), berberine bridge enzyme (berberine), salutaridinol acetyltransferase (morphine biosynthesis) and codinone reductase (morphine biosynthesis) were probed by immunolocalization. 4’-OMT and salutaridinol acetyltransferase were localized to parenchyma cells, while codeinone reductase is localized to laticifer cells in sections of capsule (fruit) from poppy plants. Berberine bridge enzyme was found in parenchyma cells in roots. Therefore, there are two cell types involved in tetrahydrobenzylisoquinoline biosynthesis in poppy. Another study implicates a third cell type (sieve elements) in morphine biosynthesis, though this is somewhat controversial.

5.1.4 Metabolic Engineering of Morphine Biosynthesis

One strategy to increase production of the morphinan alkaloids in poppy plants is to prevent (S)-reticuline from being converted to other alkaloids. Production of berberine bridge enzyme (Figure 1C) was suppressed in opium poppy plant and changes in the alkaloid profile resulted in the latex, though not the roots.

In attempts to accumulate thebaine, and decrease production of morphine (a precursor to the recreational drug heroine), codeinone reductase in opium poppy plant was down regulated using RNAi. Silencing of codeinone reductase resulted in the accumulation of (S)-reticuline, but not the substrate codeinone or other compounds on the pathway from (S)-reticuline to codeine. However, the over expression of codeinone reductase in opium poppy plants did in fact result in an increase in morphine and other morphinan alkaloids, such as morphine, codeine, and thebaine compared to control plants. Natural variants of low morphine producing poppy plants have also been analyzed.

The cytochrome P450 responsible for the oxidation of (S)-Nmethylcoclaurine to (S)-3′-hydroxy-N-methylcocluarine was over expressed in opium poppy plants, and morphinan alkaloid production in the latex was increased to 4.5 times the level in wild type plants. Additionally, suppression of CYP80B3 resulted in a decrease in morphinan alkaloids to 16% of the wild type level. Notably, analysis of a variety of biosynthetic gene transcript levels in these experiments support the hypothesis that this P450 enzyme plays a regulatory role in the biosynthesis of benzylisoquinoline alkaloids.

Remember

Collectively, these studies highlight that the complex metabolic networks found in plants are not always easily or predictably redirected.

5.2 BIOSYNTHESIS OF TERPENE INDOLE ALKALOIDS

The terpene indole alkaloids, produced primarily by the Apocynaceae and Rubiaceae, effectively illustrate the complexity of alkaloid biosynthesis. The terpene indole alkaloids are a particularly diverse class of natural products, comprising approximately 3000 members possessing a range of chemical structures and a wealth of biological activities (Fig. 2). A number of terpene indole alkaloids are used as anticancer, anti-malarial

and anti-arrhythmic agents (Fig. 2). In the US, vinblastine (Velban) and vincristine (Oncovin) are used clinically to treat cancers including Hodgkin's disease, non-Hodgkin's lymphoma 14 and Kaposi's sarcoma. Notably, 500 kilograms of the plant Catharanthus roseus are required to produce 1 gram of vincristine (a yield of 0.0002%), and total synthesis of this compound is not practical on an industrial scale.

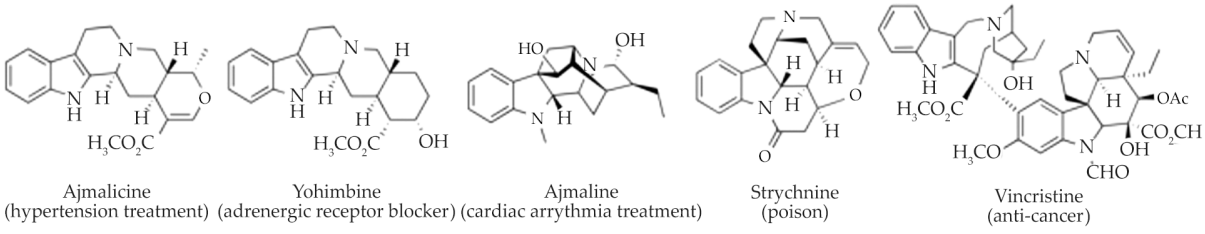


Figure 2. Representative terpene indole alkaloids.

5.2.1 Early Enzymes of Terpene Indole Alkaloid Biosynthesis

The first few steps of TIA biosynthesis are well known and are outlined in Fig. 3. Terpene indole alkaloids are derived from tryptophan, which is decarboxylated to yield tryptamine. The involvement of the monoterpene iridoid secologanin has been established. Strictosidine (S stereochemistry at C5) is a common intermediate for all terpene indole alkaloids.

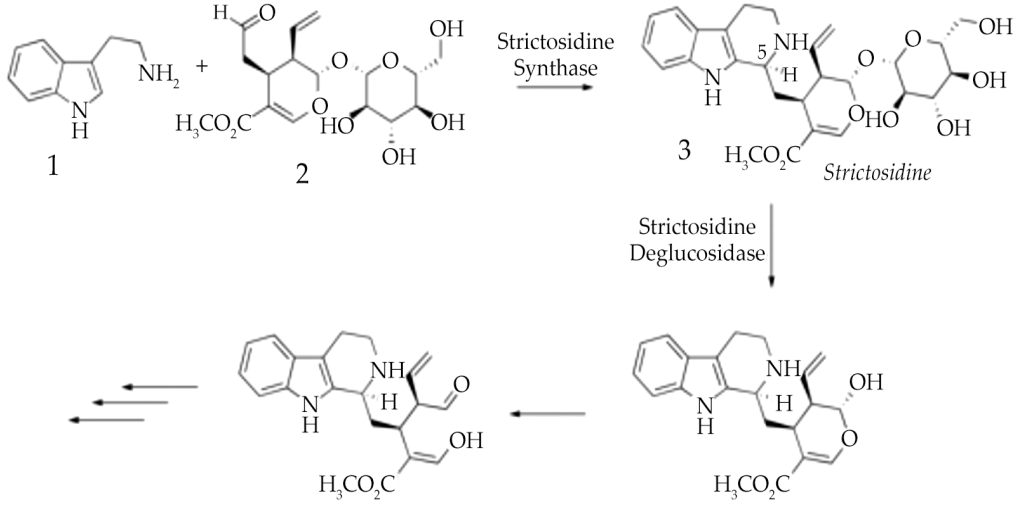


Figure 3. The early steps of terpene indole alkaloid biosynthesis. Strictosidine synthase sets the stereochemistry at C5.

The enzymes that catalyze these first steps of terpene indole alkaloid biosynthesis are known and have been cloned. Tryptophan decarboxylase, a pyridoxal dependent enzyme, converts tryptophan to tryptamine. Strictosidine synthase catalyzes the stereoselective Pictet-Spengler condensation of tryptamine (1, Fig. 3) and the aldehyde secologanin (2) to yield strictosidine (3). Secologanin is a natural product in its own right, and a few of the enzymes responsible for secologanin biosynthesis have also been isolated. Previous studies with strictosidine synthase from the plants Catharanthus roseus and Rauwolfia serpentina have reported Km values of 20-200 μM for tryptamine (no reported Km for secologanin) and a range of Vmax values. A limited number of alternate substrates have been tested with strictosidine synthase, including Nsubstituted tryptamine, **tryptophan**, phenylethylamine, tyramine, and a variety of iridoid aldehydes.

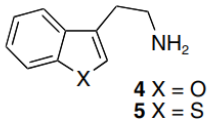
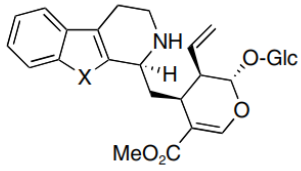
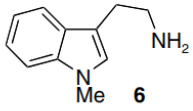
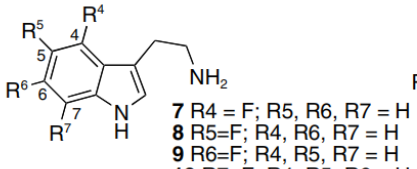
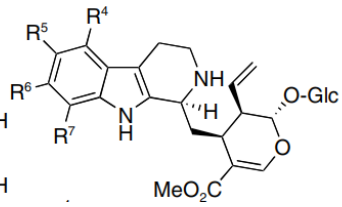
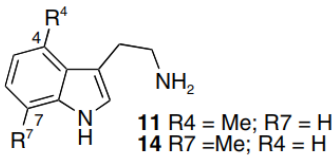
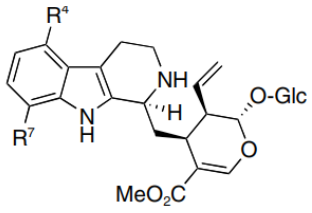
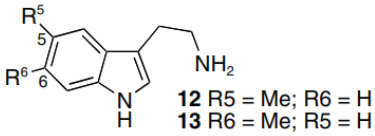
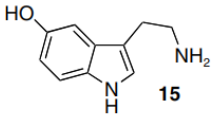
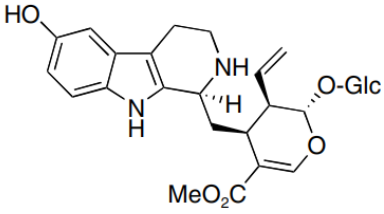
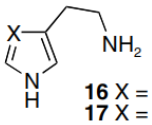
Keyword

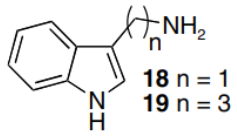
Tryptophan is an amino acid needed for normal growth in infants and for the production and maintenance of the body's proteins, muscles, enzymes, and neurotransmitters. It is an essential amino acid. This means your body cannot produce it, so you must get it from your diet.

Strictosidine Synthase Tryptamine Substrate Specificity

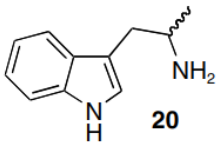
The Pictet-Spengler cyclization is critical for the biosynthesis of thousands of alkaloids. We sought to expand our understanding of strictosidine synthase by systematically probing the electronic and steric requirements of the indole substrate and quantifying the steady state kinetics for each of these substrates. Both the 3-(2- aminoethyl)-benzofuran (4) and benzothiophene (5) analogs have been assayed. Precedence exists for benzofuran and benzothiophene heterocycles with interesting biological properties. Both compounds are turned over by strictosidine synthase in the presence of the aldehyde substrate secologanin 2, and a single diastereomer is observed, indicating that enantioselective enzymatic catalysis is not compromised (Table 1). The alternate heterocycles are turned over by strictosidine synthase at a diminished rate relative to the tryptamine 1 substrate. Although the low activity of the thiophene substrate precluded a quantitative comparison of 4 and 5, the rate of reaction of benzothiophene 5 is significantly slower than benzofuran 4. Notably, no chemical reaction of 3-(2-aminoethyl)-benzofuran (4) and 3-(2-aminoethyl)-benzothiophene (5) occurred at 40 mM concentration under mild acidic conditions, demonstrating that the enzyme can catalyze product formation with relatively chemically inactive substrates with complete enantioselective control.

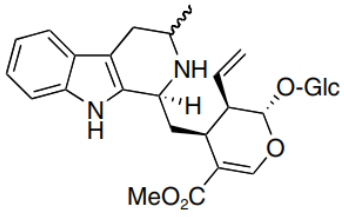
Table 1. Substrates tested with strictosidine synthase and the resulting products.

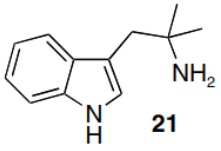
Unnatural substrate	Strictosidine analog
 **4** X = O **5** X = S	
 **6**	No reaction
 **7** R4 = F; R5, R6, R7 = H **8** R5=F; R4, R6, R7 = H **9** R6=F; R4, R5, R7 = H **10** R7=F; R4, R5, R6 = H	
 **11** R4 = Me; R7 = H **14** R7 =Me; R4 = H	
 **12** R5 = Me; R6 = H **13** R6 = Me; R5 = H	No reaction
 **15**	
 **16** X = CH **17** X = N	No reaction



No reaction







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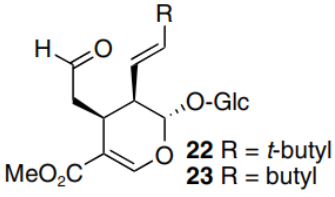

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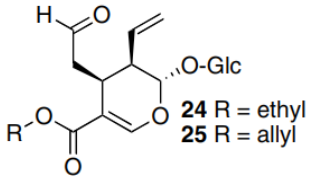
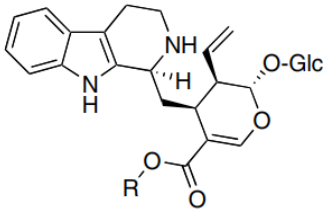




Since the Pictet-Spengler cyclization is inherently dependent on an electron rich aminoethylarene substrate, the decreased electron density of the benzofuran and benzothiophene rings may cause the slower rate compared to indole. Alternatively, strictosidine synthase may utilize a specific hydrogen bonding interaction to the indole nitrogen. The benzofuran 4 exhibits a K_m value close to that of tryptamine, but displays a significantly reduced k_{cat}, suggesting that the electron deficient nature of the heterocylic ring is slowing catalysis (Table 2). N-methyl tryptamine 6 (Table 1) was not a competent substrate, suggesting that the enzyme tolerates only small steric perturbations at the indole nitrogen. To further explore the effect of electron density on catalysis, the indole ring was substituted with electron-withdrawing substituents (fluoro=F) at each of the indole ring positions (Table 1).

Remember

Substitution with a fluoro moiety results in a decrease in kcat in each case, suggesting that the enzymatic reaction is inherently dependent on the electron density of the substrate.

Table 2. Kinetic parameters for the most highly active amine strictosidine synthase substrates. k_{cat} and K_m were measured using a purified E. coli preparation of strictosidine synthase.

Substrate	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m (M^{-1}/s^{-1})
1	7.4	0.9	2,030
4	7.7	0.023	50
7	42	0.35	139
8	7.1	0.043	101
9	8.9	0.056	105
10	13	0.11	141
11	80	0.19	40
14	198	0.29	24
15	1,200	0.096	1.3

Early qualitative studies, performed after strictosidine synthase was first isolated, indicated that some substitution on the indole ring was tolerated. To rigorously quantify the effect of indole ring substitution on catalysis, each position of the indole ring was systematically substituted with a methyl group (compounds 11-14, Table 1), and the kinetic parameters of active substrates were measured. In general, reactivity of substrates with methyl substitutions in the 4 (compound 11) and 7 (compound 14) indole positions was significantly higher than substrates with substitutions at the 5 (compound 12) and 6 (compound 13) positions (Tables 1 and 2). The K_m for the 4-substituted tryptamine analog 11 was approximately 2-fold lower than the K_m for 7-substituted compound 14, while substrates with methyl moieties in the 5 and 6 positions– compounds 12 and 13– were completely inactive. Substitution with a hydroxyl group in the 5 position (compound 15, Table 1 and Table 2) did yield an active substrate, although the Km was the highest measured in this series– a 60-fold increase compared to the native substrate tryptamine. Therefore, the binding pocket of the enzyme can better tolerate a hydrophilic hydroxyl substituent than a hydrophobic methyl group at the 5 position.

The 2-pyrrole-3-ethylamine analog (19) has been shown to undergo a nonenzymatically catalyzed Pictet-Spengler reaction. Surprisingly, this smaller substrate along with the isosteric histamine (20) were not turned over by strictosidine synthase, indicating that the benzyl moiety is absolutely required for recognition by the enzyme (Table 1). It was previously established that tryptophan, phenylethylamine and tyramine are not accepted by strictosidine synthase, and since pyrrole substrates are also not tolerated, we conclude that the basic indole framework is required for recognition by this enzyme. Interestingly, the only other sequenced "Pictet-Spenglerase" (norcoclaurine synthase), which utilizes tyrosine derived amine and aldehyde substrates, exhibits no sequence homology to strictosidine synthase.

Strictosidine Synthase Secologanin Substrate Specificity

Several naturally occurring iridoid terpenes had been previously shown to fail to serve as competent aldehyde substrates in place of secologanin. Therefore, we modified two of the key functional groups of the secologanin substrate to assess the aldehyde substrate requirements. A streamlined gram-scale isolation protocol of secologanin from a local source of Lonicera tatarica enabled a semisynthetic approach to yield secologanin derivatives. Olefin cross metathesis was used to introduce a variety of alkyl groups at the vinyl position of secologanin (i.e. compounds 21 and 22, Table 1). Since a reduced version of secologanin, in which the vinyl group is hydrogenated to yield a saturated single C-C bond, had been previously shown to be a competent substrate, we were optimistic that this position could be derivatized. However, our assays indicated that bulkier groups at the vinyl position completely prevented turnover (Table 1). In contrast, trans-esterification at the methyl ester with larger alkyl groups gave substrates 23 and 24 that were turned over by the enzyme to yield the corresponding strictosidine analogs, suggesting that this is a more promising position for **derivatization** (Table 1).

Keyword

Derivatization is the process by which a compound is chemically changed, producing a new compound that has properties more. amenable to a particular analytical method. Some samples analyzed by GC require derivatization in order to make them suitable for.

5.2.2 Enzymes after Strictosidine Synthase

In the first enzymatic step after strictosidine formation, the glucose of strictosidine is enzymatically hydrolyzed to reveal a reactive hemi-acetal (Fig. 3). In essence, the glucose moiety is serving as a protecting group to mask a reactive species, a strategy that is utilized in other plant biosynthetic pathways such as the cyanogenic glucosides and the glucosinolates. The dedicated glycosidase, strictosidine-β-glucosidase has been isolated and cloned from Catharanthus roseus and Rauwolfia serpentina. Based on its amino acid sequence, strictosidine glucosidase is predicted to be a type 1 beta glycosyl hydrolase with a retaining mechanism. Some biochemistry of the glucosidase from the plants C. roseus and R. serpentina has been investigated. K_m values of ~100-200 μM for strictosidine, a pH optimum of 5-8.5, and a range of V_{max} values have been reported for this enzyme.

Fig. 4 summarizes much of what is known about the enzymes of alkaloid biosynthesis that act after strictosidine

deglycosylation. In ajmaline biosynthesis, at least eight enzymes are predicted to catalyze the subsequent steps after strictosidine deglycosylation. Two of these enzymes have been cloned, and the remainder have been either purified or detected in crude cell extracts. The pathway for ajmaline biosynthesis is arguably the best-characterized terpene indole alkaloid pathway. Five enzymes are predicted to catalyze the transformations leading from tabersonine (23) to vindoline (24) . Three of these enzymes have been clonedand the remainder have been partially purified.

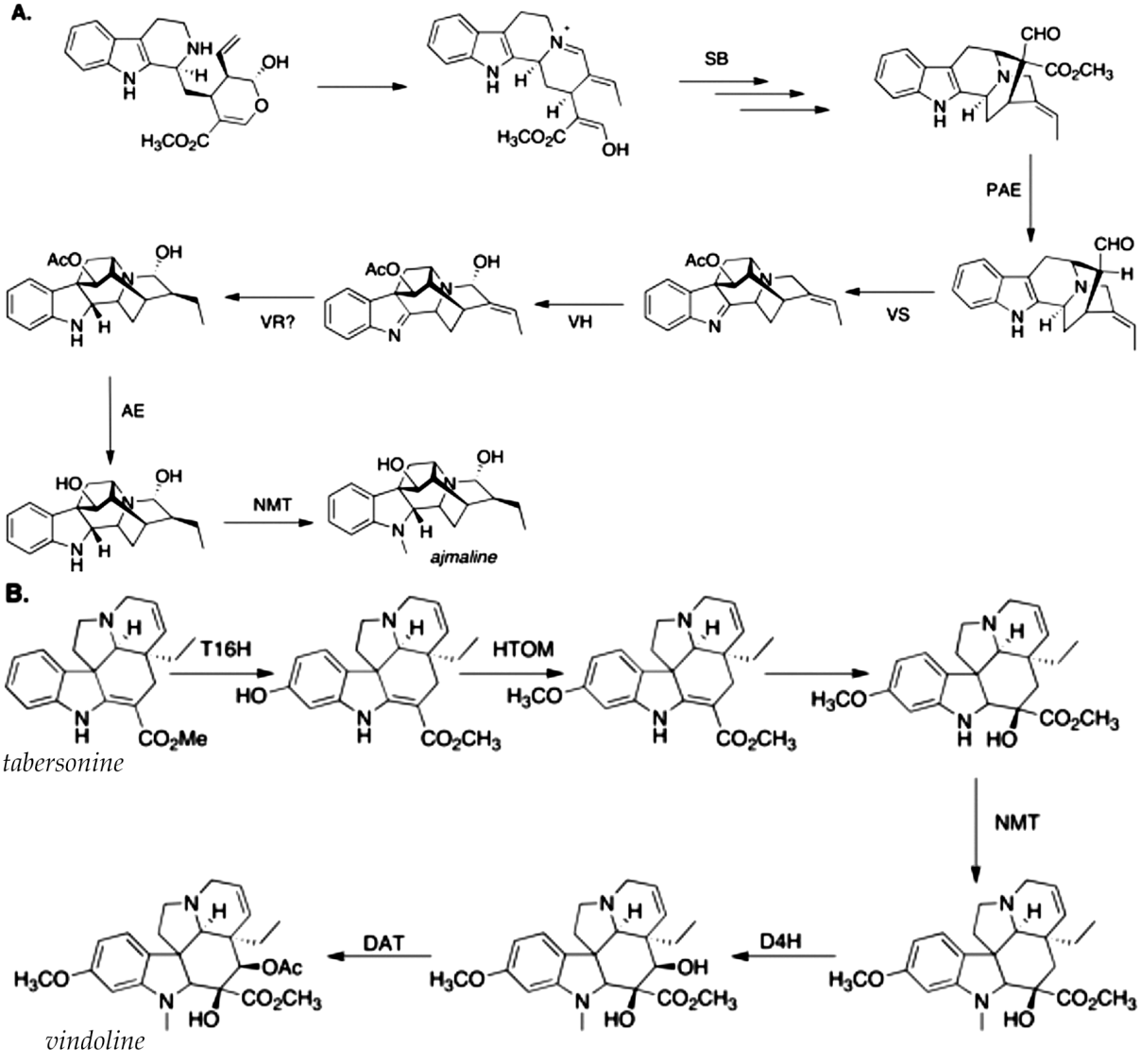


Figure 4. This scheme summarizes much of the current knowledge of TIA enzymes that act after the deglycosylation of strictosidine. A. Ajmaline biosynthesis. SB, sarpagan bridge enzyme; PAE, polyneuridine aldehyde reductase; VS, vinorine synthase; VH, vinorine hydroxylase; VR, vomilenine reductase(s); AE, 17-O-acetyl-ajmalan acetylesterase; NMT, norajmaline-N-methyltransferase. Only

polyneuridine aldehyde reductase and vinorine synthase have been cloned. B. Vindoline biosynthesis from tabersonine. T16H, tabersonine-16-hydroxylase; HTOM, 16-hydroxytabersonine-16-Omethyltransferase; NMT, N-methyltransferase; D4H, desacetoxyvindoline-4- hydroxylase; DAT, desacetylvindoline O-acetyltransferase. Tabersonine-16-hydroxylase, desacetoxyvindoline-4-hydroxylase and desacetylvindoline O-acetyltransferase have been cloned.

5.2.3 Structural Diversity of Terpene Indole Alkaloids

A number of examples of the terpene indole alkaloid classes, each arising from rearrangement of strictosidine, are shown in Fig. 5. How the wealth of TIA structures each derive from the deglycosylated strictosidine intermediate remains one of the most fascinating problems in secondary metabolism. Extensive feeding studies and biomimetic syntheses executed in the 1960's and 1970's yielded chemical information about how this branching process might occur. After deglycosylation of strictosidine (3) the resulting aglycone (25) opens to form an intermediate often referred to as a dialdehyde (26). The resulting aldehyde then reacts with the secondary amine to form a six membered ring to yield dihydrocorynanthe aldehyde (27). Dihydrocorynanthe aldehyde can undergo allylic isomerization and enolization to produce either the enol (28) or keto (29) forms of dehydrogeissoschizine. Dehydrogeissoschizine is believed to be a central intermediate in TIA biosynthesis. Dehydrogeissoschizine can be reduced by a dehydrogenase enzyme to yield geissoschizine (30), an intermediate that may also play a role in TIA biosynthesis.

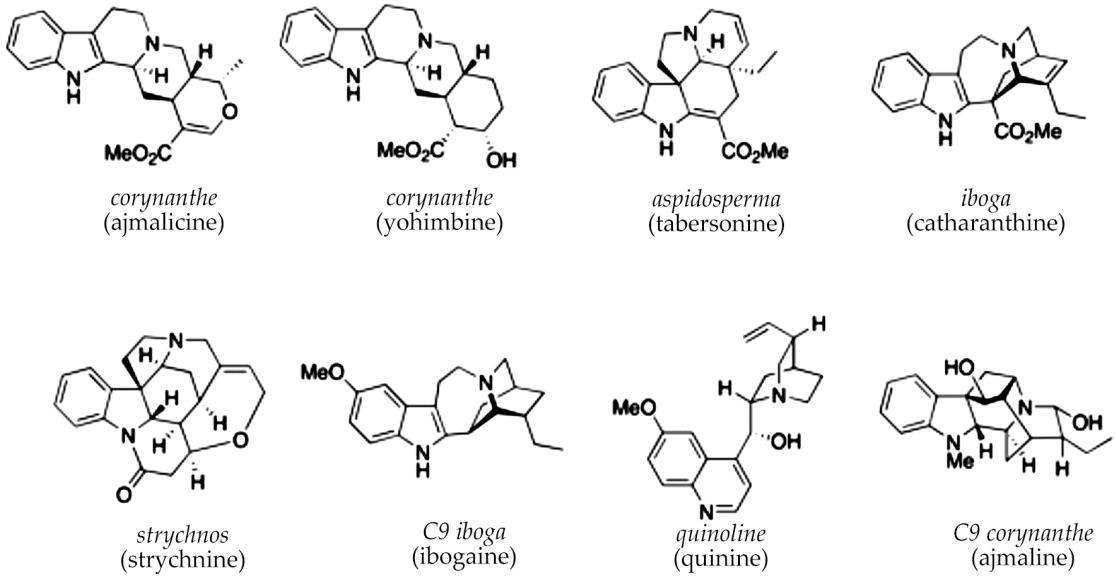


Figure 5. Representative classes of the terpene indole alkaloids. The name of the alkaloid class is given in parentheses below the name of the molecule. Each of these alkaloids is derived from the common intermediate strictosidine.

It is likely that the enol form of dehydrogeissoschizine 28 will undergo 1,4 conjugate addition to produce the heteroyohimbine cathenamine (33). Early biomimetic syntheses support the hypothesis that cathenamine can be produced from dehydrogeissoschizine. An equilibrium between cathenamine and dehydrogeissoschizine has also been observed. Stereoselective reduction of cathenamine will yield ajmalicine 34, and further oxidation will yield serpentine 35. Cathenamine 9 is the major product isolated after incubation of strictosidine with strictosidine-β-glucosidase in vitro. Therefore, an enzymatic pathway to the corynanthe skeleton from strictosidine appears to be relatively straightforward.

The enzymatic conversion of deglycosylated strictosidine to the other classes of alkaloids remains much less clear. For yohimbine (38) formation, a direct biosynthetic route could involve homoallylic isomerization of the keto dehydrogeissoschizine 29 followed by 1,4 conjugate addition. The structurally more complex aspidosperma, strychnos and iboga alkaloids may each be derived from the corynanthe alkaloids. This hypothesis is indirectly supported by observation that the corynanthe alkaloids are produced early in the lifetime of the Catharanthus roseus plant, with the aspidosperma and iboga alkaloids appearing after the plant ages. Deglycosylated strictosidine can rearrange to form the strychnos, aspidosperma and iboga alkaloids. Although the details of the pathway are not absolutely certain, it is generally agreed that dehydrogeissoschizine (28) can rearrange to form a strychnos-like intermediate termed preakkumacine (39). Stemmadenine (40) could then in turn rearrange to form an acrylic ester (41) that could serve as a common intermediate for the aspidosperma (i.e. tabersonine 43) and the iboga skeletons (i.e. catharanthine 42).

The branching among these enzymes is in part controlled by the species of plant. While the corynanthe, iboga, and aspidosperma alkaloids are observed in Catharanthus roseus plants, strychnine (Strychnos nux vomica) and ajmaline (Rauwolfia) are not. Moreover, the aspidosperma and iboga alkaloids appear to be concentrated in the ariel portions of C. roseus, while the corynanthe appear primarily in the roots. The coexistence of multiple pathways–the corynanthe, aspidosperma and iboga– makes Catharanthus an intriguing system to monitor alkaloid biosynthesis.

5.2.4 Turnover of Strictosidine Analogs by Strictosidine Glucosidase

Chemical synthesis of complex natural products is often impractical on a commercial scale, and isolation of these compounds from the environment can also be an expensive and low yielding process. Furthermore, isolation procedures provide limited opportunities to modify the chemical and biological properties of the natural product. Understanding the enzymes that catalyze natural product synthesis may enable production in more tractable host organisms and may also facilitate reprogramming of biosynthetic pathways to produce “unnatural” natural products with potentially improved pharmacological

activities. Natural products from **polyketide**, non-ribosomal peptide, terpene, and saccharide biosynthetic pathways have been heterologously expressed in organisms that are faster growing or easier to culture. Although metabolic engineering has proven remarkably successful in polyketide biosynthetic and nonribosomal peptide pathways, in terpene indole alkaloid biosynthesis, the backbone of strictosidine is significantly rearranged over the course of several steps, whereas polyketides and nonribosomal peptides are synthesized by an iterative, "assembly-line" process, in which a linear chain is successively elongated. Can a "nonmodular" pathway process unnatural substrates to yield novel alkaloids?

While initial results suggest that strictosidine synthase can produce a range of strictosidine analogs, it remains to be established whether these alternate intermediates can be processed by the downstream terpene indole alkaloid machinery to produce novel, biologically active alkaloids. In the next step of the pathway, a dedicated glucosidase hydrolyzes the glycosidic linkage of strictosidine to yield cathenamine 33.

To evaluate whether cathenamine derivatives could be enzymatically produced from the corresponding strictosidine analogs, we incubated all enzymatically generated strictosidine derivatives with the second enzyme of the pathway, strictosidine-β-glucosidase. All strictosidine derivatives were processed by strictosidine glucosidase as monitored by the disappearance of the strictosidine derivative peak by HPLC. These results suggest that the substrate specificities of strictosidine synthase and glucosidase are sufficiently complementary to produce a variety of terpene indole alkaloid intermediate analogs.

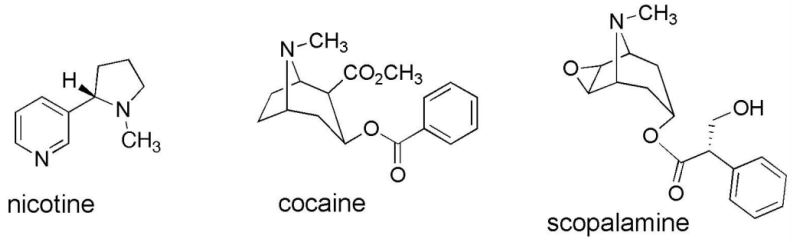
Keyword

Polyketides are a large group of secondary metabolites which either contain alternating carbonyl groups and methylene groups (-CO-CH2-), or are derived from precursors which contain such alternating groups.

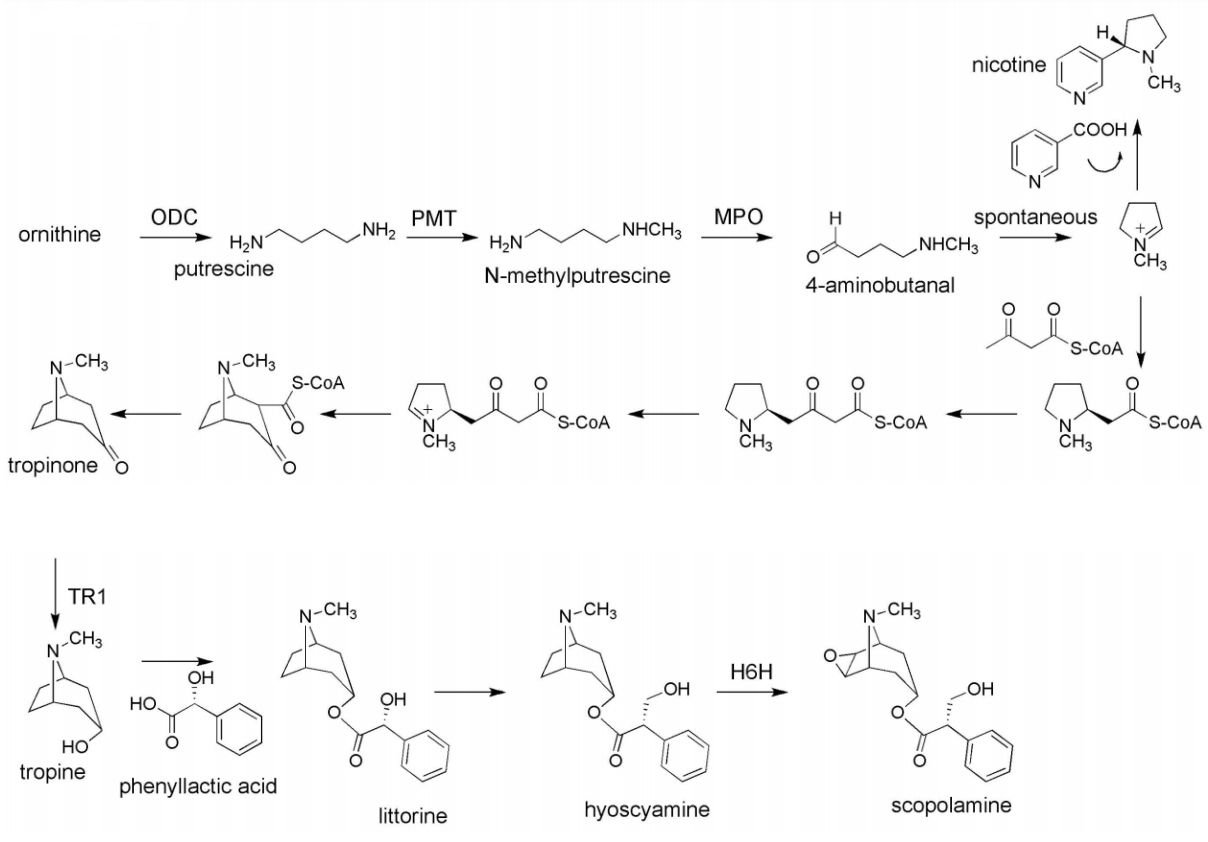
5.3 BIOSYNTHESIS OF TROPANE ALKALOIDS

The tropane alkaloids hyoscyamine and scopolamine (Figure 6A) function as acetylcholine receptor antagonists and are clinically used as parasympatholytics. The illegal drug cocaine is also a tropane alkaloid. The tropane alkaloids are biosynthesized primarily in plants of the family Solonaceae, which includes Hyoscyamus, Duboisia, Atropa, and Scopolia; each of these species are capable of biosynthesis of both nicotine and tropane alkaloids. Nicotine, although perhaps not immediately apparent

from its structure, is biosynthetically related to the tropane alkaloids and is also found in Solonaceae plants (Figure 6B).



(A)



(B)

Figure 6. A. Representative tropane and nicotine alkaloids. B. Tropane biosynthesis. ODC, ornithine decarboxylase; PMT, putrescine Nmethyltransferase; MPO, diamine oxidase; TR1, tropinone reductase 1; H6H, hyocyamine 6b-hydroxylase.

Tropane alkaloid biosynthesis has been studied at the biochemical level and several enzymes from the biosynthetic pathway have been isolated and cloned, although the pathway has not been completely elucidated at the genetic level (Figure 6B). L-arginine is converted to the non-proteogenic amino acid L-ornithine by the urease enzyme arginase. Ornithine decarboxylase then decarboxylates ornithine to yield the diamine putrescine. In Hyoscyamus, Duboisia, and Atropa puterscine serves as the common precursor for the tropane alkaloids.

Putrescine is N-methylated by a SAM dependent methyl transferase that has been cloned to yield N-methylputrescine. N-methylputrescine is then oxidized by diamine oxidase to form 4-methylaminobutanal, which then spontaneously cyclizes to form the N-methyl-D-pyrrolinium ion. This enzyme, which has recently been cloned, appears to be a copper dependent amine oxidase. Immunoprecipitation experiments suggest that this enzyme associates with S-adenosylhomocysteine hydrolase. The pyrrolinium ion is then converted to the tropanone skeleton by as yet uncharacterized enzymes (Figure 6B). Although no enzymatic information is available, chemical labelling studies have indicated that an acetate derived moiety condenses with the pyridollium ion; one possible mechanism is shown in Figure 6B.

Tropanone is then reduced via an NADPH dependent reductase to tropine that has been cloned from Hyoscyamus niger. All tropane producing plants appear to contain two tropinone reductases, a branch point. Tropinone reductase I yields the tropane skeleton (Figure 6B) while tropinone reductase II yields the opposite stereocenter, pseudotropine. These two tropinone reductases were crystallized, and site directed mutagenesis studies showed that the stereoselectivity of the enzymes could be reversed.

The biosynthesis of scopolamine is the best characterized of the tropane alkaloids. After action by tropinone reductase I, tropine is condensed with phenyllactate through the action of an uncharacterized enzyme to form littorine. The phenyllactate moiety is believed to arise from an intermediate involved in phenylalanine metabolism. Littorine then undergoes rearrangement to form hyosamine. It is believed that the enzyme that catalyzes this rearrangement, which has been partially purified, works via a radical mechanism using Sadenysylmethione

Did You Know?

Since the structure of the tropane ring system was first elucidated in 1901, organic chemists and biochemists have been interested in how these mysterious tropane alkaloids are assembled in vitro and in vivo.

as the source of an adenosyl radical. Labeling studies have been used to study the mechanism of rearrangement. Hyoscyamine 6B-hydroxylase (H6H) catalyzes the hydroxylation of hyoscyamine to 6B-hydroxyhyoscyamine, as well as the epoxidation to scopolamine (Figure 6B). H6H, which has been cloned and heterologously expressed, is a non-heme iron dependent oxoglutarate dependent protein. It appears that the epoxidation reaction occurs much more slowly than the hydroxylation reaction.

Early studies revealed that tropane alkaloids are formed in the roots and then transported to the aerial parts of the plant. Protein blot analysis indicated that H6H is found only in the roots. The levels of tropane alkaloid production in a variety of hairy root cultures were altered by overexpression of methyltransferase putrescine-N-methyltransferase (PMT) and hyoscyamine 6β-hydroxylase (H6H). Overexpression of both of these enzymes in a hairy root cell culture resulted in significant increases in scopolamine production. Fluorinated phenyllactic acid substrates could be incorporated into the pathway, and several substrates derived from putrescine analogues were turned over by the enzymes of several Solonaceae species.

5.4 PURINE ALKALOIDS BIOSYNTHESIS

Accumulation of purine alkaloids occurs in several plant species used for beverages and foods.

5.4.1 Caffeine biosynthesis

Caffeine, a purine alkaloid, is one of the most widely known natural products. Caffeine is ingested as a natural component of coffee, tea and cocoa, and the impact of caffeine on human health has been extensively studied. The biosynthetic pathway of caffeine has been recently elucidated on the genetic level. Caffeine biosynthesis has been most widely studied in the plant species Coffea (coffee) and Camellia (tea).

Xanthosine, which is derived from purine metabolites, is the first committed intermediate in caffeine biosynthesis (Figure 7). Xanthosine can be formed from de novo purine biosynthesis, S-adenosylmethione (SAM) cofactor, the adenylate pool and the guanylate pool. It is believed that de novo purine biosynthesis and the adensosine from SAM are the most important sources of xanthosine.

The biosynthesis of caffeine begins with the methylation of xanthosine to yield N-methylxanthosine by the enzyme 7-methylxanthosine synthase (MXS). N-methylxanthosine is converted to N-methylxanthine by methylxanthine nucleosidase, an enzyme that has not yet been cloned. Nmethylxanthine is converted to theobromine by theobromine synthase, a second N-methyltransferase. Theobromine is converted to

caffeine by a final N-methyltransferase, caffeine synthase. Coffee and tea plants appear to contain a variety of N-methyltransferase enzymes having varying substrate specificity. For example, a caffeine synthase enzyme isolated from tea leaves catalyzes both the N-methylation of Nmethylxanthine and theobromine. The substrate specificity of the methyltransferases can be changed by site directed **mutagenesis**.

Mutagenesis is the process by which an organism's deoxyribonucleic acids (DNA) change, resulting in a gene mutation. A mutation is a permanent and heritable change in genetic material, which can result in altered protein function and phenotypic changes.

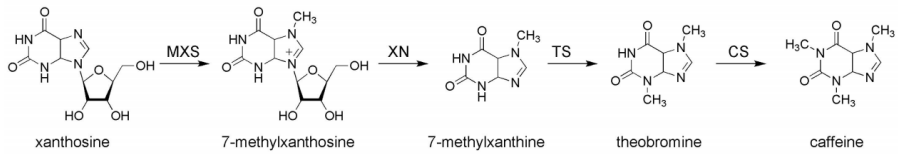


Figure 7. Caffeine biosynthesis. MXS, 7-methyl xanthosine synthase; XN; methylxanthosine nucleotidase; TS, theobromine synthase; CS, caffeine synthase.

Keyword

Mutagenesis is the process by which an organism's deoxyribonucleic acids (DNA) change, resulting in a gene mutation. A mutation is a permanent and heritable change in genetic material, which can result in altered protein function and phenotypic changes.

5.4.2 Metabolic engineering of caffeine biosynthesis

Caffeine may act as a natural insecticide in plants. When these three Nmethyltransferase genes were overexpressed in tobacco, the resulting increase in caffeine production improved the tolerance of the plants to certain pests. Conversely, coffee beans with low caffeine levels would be commercially valuable, given the demand for decaffeinated coffee. A natural variant of coffee deficient in caffeine production has been discovered. Moreover, since the caffeine pathway has been cloned, genetically engineered coffee plants with reduced caffeine content can now be constructed. For example, a 70% reduction in caffeine content in Coffea was obtained by downregulating theobromine synthase using RNAi. Additionally, the promoter of one of the N-methyltransferases has been recently discovered, which may allow transcriptional gene silencing.

ROLE MODEL

PIERRE JEAN ROBIQUET : FRENCH CHEMIST

The French chemist and pharmacist Pierre-Jean Robiquet (1780–1840) made major strides in analytical chemistry in the early 19th century. In addition to recognizing the connection between protein and amino acids, he identified the chemical components of everyday substances ranging from caffeine to mustard seed to strychnine.

Pierre-Jean Robiquet was the first to isolate an amino acid, thereby discovering one of the fundamental building blocks of proteins, and he determined the chemical composition of numerous substances in common use today. Through his investigations, he isolated the painkiller codeine and the stimulant caffeine, working independently of colleagues who were pursuing these studies concurrently. Beyond these firsts, Robiquet was notable for the range and imagination of his scientific activities. His inquiring mind compelled him to the laboratory, where he investigated the properties of dyes and what made them work; studied the chemical basis of aromas; worked to isolate chemical compounds in bitter almonds, mustard seeds, and other foods; and investigated the properties of useful poisons such as strychnine and cantharides. Robiquet's work was important not only in the realm of pure chemistry but in the development of France's chemical and pharmaceutical industries.

Family Disrupted by Revolutionary Activities

Robiquet was born in Rennes, France, on January 13, 1780; coming of age during the violent epoch defined by the French Revolution was to shape his life significantly. His father, Jean-François Robiquet, was a printer who, as a social conservative (Girondin), became ensnared in the Jacobin-Girondin controversy of 1793, was condemned by Jacobin activists, and arrested. Robiquet attended a free, church-affiliated school whose staff of clergy likewise ran afoul of the Jacobin proto-communist revolutionaries, with the result that the school was closed down. After studying architecture briefly, he was taken in by family friends and apprenticed to a carpenter. Another relative

managed to find him an apprenticeship in the more lucrative trade of pharmacy, and for a year he worked in a pharmacy owned by a man named Clary. In 1795, Robiquet advanced in his new career when he was employed by the pharmacy of the French Navy, which had been crippled by the revolution's penchant for beheading aristocratic officers.

Robiquet rejoined his family in Rennes after his father's release from prison, working his way through a classical education. He supported himself by performing pharmacist duties for the French Army of the West, a force then engaged in pacifying the civil war in western France. In 1796, when the revolutionary force was reconfigured by the First French Republic, the 16-year-old returned to Paris for another set of pharmacy courses, at one point studying under eminent chemist Antoine François, a colleague of Antoine Lavoisier (1743–1794). This course so impressed Robiquet that he decided to pursue a career as a chemist. With his father's help, he got a job in a chemical lab owned by Fourcroy and another prominent chemist, Louis Nicolas Vauquelin. Still in his teens, he now participated in cuttingedge research on the composition of urinary tract deposits. A registered pharmacist at Paris's École Polytechnique after 1808, Robiquet became a lecturer in chemistry in 1811. That same year, by decree of Emperor Napoleon Bonaparte, he was appointed assistant professor of pharmaceuticals at the city's Faculté de Pharmacie, and he became full professor in 1814. At that point, he was given an assistant, Pierre-Joseph Pelletier (1788–1842), who would draw on Robiquet's work in future collaborations with other chemists at the Faculté de Pharmacie.

Investigated Spanish Fly

Between 1805 and 1835, Robiquet published more than 60 scientific papers on an impressive variety of subjects. Some of his early research, on vegetable dyes and mineral pigments, had practical results—his discoveries improved manufacturing processes in the French dye industry—but it also oriented him toward pure analytical chemistry as he tried to isolate the components that gave plants the ability to impart a color that could affix itself to cloth. Some of his research focused on the madder root (*rubia tinctorum*), which only one other scientist had tried to break down to its constituent parts. Robiquet succeeded in isolating the coloring agent, which he called alizarin.

Robiquet also investigated the pharmaceutical properties of cantharides, an extract from a beetle that was known as Spanish fly. While cantharides was famed for its aphrodisiac qualities, Robiquet was primarily interested in its capacity for irritating the skin and producing blisters. Another area of his interest was the investigation of aromas—the specific substances that gave an orange flower a particular scent when water distilled from that flower had no scent. Robiquet discovered that, for many plants, such odor-causing agents developed only through combination with other chemical substances.

In other experiments, Robiquet analyzed foods such as bitter almonds, mustard seeds, black and white pepper, and asparagus. Breaking them down and processing them by boiling, evaporation, and separation of the resulting liquids and solids, he extracted their essences—the substances that gave them their essential flavors—and studied how these essences interacted with water when a food was cooked. His first important paper, published in 1806, was based on his work with Vauquelin and dealt with their discovery of the amino acid asparagine.

In experiments performed in 1822 and 1831, together with the chemist Antoine Boutron-Charlard, Robiquet processed bitter almond extracts with various substances, including ammonia, and closely observed the results. The seeds of *Prunus dulcis* var. *amara* contain a recessive gene that produces a bitter-tasting seed. Isolating the essential oil of these bitter nuts, Robiquet and BoutronCharlard isolated an enzyme they named amygdalin. The basis of laetrile, which was promoted as a cancer treatment beginning in the early 1950s, amygdalin contains a component that can be released as the toxic substance cyanide.

Robiquet's analyses of discrete foodstuffs generated detailed and specific results that surpassed those achieved by earlier chemists, and his work alerted scientists to the existence of amino acids, fundamental building blocks of organic matter. Asparagine was the first amino acid ever identified; amygdalin is a derivative of another amino acid, phenylalanine. German scientists Friedrich Wöhler (1800–1882) and Justus von Liebig (1803–1883) would build on Robiquet's research and their discoveries of the compounds benzamide and benzoin would be important stepping stones in the development of modern organic chemistry.

Independently Discovered Caffeine

Using similar methods to those he developed during his research on foods, Robiquet isolated caffeine, the stimulant component of coffee, in an experiment he described to the Paris Society of Pharmacy in 1821. Three other scientists, Friedlieb Ferdinand Runge (1795–1867), and the team of Pelletier and Joseph Bienaimé Caventou (1795–1877), independently discovered caffeine at almost the same time.

In 1817 Robiquet followed a line of study begun by Jean-François Derosne (1774-1855) and Friedrich Wilhelm Sertürner (1782–1841) in isolating the active ingredient of the opium poppy, *Papaver somniferum*. Derosne had produced a white salt from raw opium that he named narceine, and Sertürner provided a fresh interpretation, deducing that this crystalline distillate, which he called morphia, could only be effective in combination with meconic acid. Robiquet substantiated Derosne's work and built on it, doing his own experiments on narcotine (another opium distillate) and morphia (morphine) while overseeing the work of other scientists under the direction of the Société de Pharmacie.

In 1830, together with an assistant, J.B. Berthemont, Robiquet was validating the ongoing work involving opium when he recognized the uniqueness of a byproduct of morphine extraction. He called this substance codeine, which also had psychotropic effects. Discussing this new opiate, a rival to morphine, Robiquet wrote in a medical journal (as quoted by Jaime Wisniak in *Educación Quím*ica) that, "Dr. Kunckel, to whom I have provided with a small sample, is convinced that (codeine) has a very strong action on the spinal chord and that does not paralyse the back parts (as morphine does). It approximates very much the action that opium has on animal economy." Codeine remains a central component of modern pain management.

SUMMARY

- Alkaloids are a highly diverse group of natural products related only by the presence of a basic nitrogen atom located at some position in the molecule.
- Morphine and codeine are two of the most important analgesics used in medicine, and plants remain the main commercial source of the alkaloids.
- Tetrahydrobenzylisoquinoline biosynthesis begins with the substrates dopamine and p-hydroxyphenylacetaldehyde.
- Overproduction of berberine in C. japonica cell suspension cultures was achieved by selection of a high producing cell line, with reported productivity of berberine reaching 7 g/L. This was one of the first demonstrations of production of a benzylisoquinoline alkaloid in cell culture at levels necessary for economic production. This cell line greatly facilitated the identification of the biosynthetic enzymes.
- The localization of morphine biosynthesis has been investigated at the cellular level in intact poppy plants using immunoflouresence microscopy.
- One strategy to increase production of the morphinan alkaloids in poppy plants is to prevent (S)-reticuline from being converted to other alkaloids.
- The terpene indole alkaloids, produced primarily by the Apocynaceae and Rubiaceae, effectively illustrate the complexity of alkaloid biosynthesis. The terpene indole alkaloids are a particularly diverse class of natural products, comprising approximately 3000 members possessing a range of chemical structures and a wealth of biological activities.
- Several naturally occurring iridoid terpenes had been previously shown to fail to serve as competent aldehyde substrates in place of secologanin. Therefore, we modified two of the key functional groups of the secologanin substrate to assess the aldehyde substrate requirements.
- Chemical synthesis of complex natural products is often impractical on a commercial scale, and isolation of these compounds from the environment can also be an expensive and low yielding process.
- The tropane alkaloids hyoscyamine and scopolamine function as acetylcholine receptor antagonists and are clinically used as parasympatholytics.
- Accumulation of purine alkaloids occurs in several plant species used for beverages and foods.
- Caffeine, a purine alkaloid, is one of the most widely known natural products. Caffeine is ingested as a natural component of coffee, tea and cocoa, and the impact of caffeine on human health has been extensively studied.

MULTIPLE CHOICE QUESTIONS

1. **The example of source of gum containing a glucomannans**
   a. tragacanth
   b. guar
   c. acacia
   d. xanthan
2. **The gum which also contains oxidase enzyme**
   a. tragacanth
   b. sterculia
   c. acacia
   d. guar
3. **The example of plant as source of mucilage**
   a. linseed
   b. tragacanth
   c. inulin
   d. acacia
4. **The method of extraction wherein the plant material is kept in contact with whole menstrum in closed vessel and allowed to stand for 7 days shaking it occasionally.**
   a. Percolation
   b. Maceration
   c. Decoction
   d. Soxhlet
5. **The following is the example of proteolytic enzyme obtained from microbial origin**
   a. Papain
   b. Bromelain
   c. Seratiopeptidase
   d. Pepsin
6. **Flax is an example of fibre belonging to the class**
   a. carbohydrate fibre
   b. protein fibre
   c. regenerated fibre
   d. mineral fibre

7. Keratin is the chemical constituent present in the fibre of natural origin

a. asbestos
b. silk
c. wool
d. hemp

8. The chemical constituents which are present in sodium alginate

a. D-mannuronic acid and L-glucoronic acids
b. amylose and amylopectin
c. pentosan and aldobionic acid
d. D-mannose and D-galactose

9. Out of the following which plant contains maximum percentage of pectin

a. Lemon
b. Orange
c. Beets
d. Papaya

10. The following is the example of cellulose ether

a. Cellulose acetate propionate
b. Cellulose acetate
c. Cellulose acetate phthalate
d. Hydroxypropyl methyl cellulose

REVIEW QUESTIONS

1. How alkaloids are biosynthesis in plants?
2. How are alkaloids metabolized?
3. What is the process of biosynthesis?
4. Which was the first alkaloid synthesized?
5. What is mean of biosynthesis class for alkaloids?
6. Which precursor is used for biosynthesis of tropane alkaloids?
7. How will you identify tropane alkaloids?

Answer to Multiple Choice Questions

1. (d)	2. (c)	3. (a)	4. (b)	5. (c)
6. (a)	7. (c)	8. (a)	9. (c)	10. (d)

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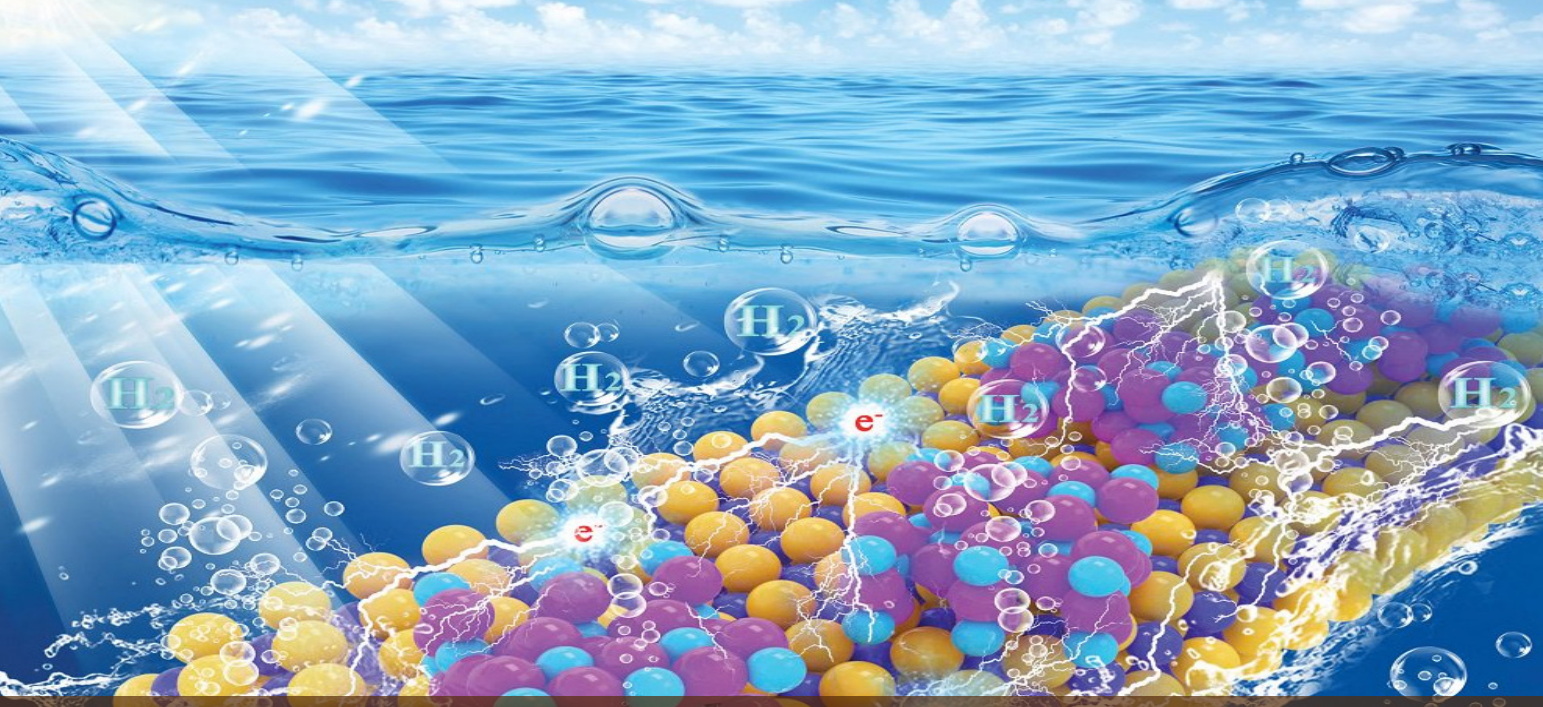

CHAPTER 6

APPLIED POTENTIAL AND CURRENT APPLICATIONS OF ALKALOIDS

LEARNING OBJECTIVES

After studying this chapter, you will be able to:

1. Explain the method of alkaloids
2. Describe the analysis of alkaloids (indole alkaloids, isoquinoline alkaloids, tropane alkaloids)
3. Focus on phytochemistry and classification of alkaloids
4. Explain the alkaloids and their applications in pharmaceutical chemistry
5. Brief the alkaloids isolated from natural herbs as the anticancer agents

"The alkaloids are bitter-tasting, natural nitrogen-containing compounds found particularly in plants. The name is derived from their characteristic basic properties (alkali-like), which are induced by the lone electron pair of nitrogen. As with acyclic amines, the (Lewis) basic nature of the alkaloids, in con junction with their particular three-dimensional architecture, gives rise to often potent phys iological activity. We have already noted some examples of this behavior in the narcotics morphine and heroin, the psychoactive lysergic acid and LSD, and the antibiotic penicillins."

—K. Peter C. Vollhardt

INTRODUCTION

In nature there are many natural compounds. From among many classes of naturally occurring organic compounds such as carbohydrates, lipids, proteins, amino acids, anthocyanins, flavonoids, and steroids, the

one that seems to be quite special is alkaloids. What makes them special? They derived from amino acids and can be synthetized as secondary metabolites by plants and some animals. These compounds play an important role in living organisms. Alkaloids occurred to be extremely important for human beings for ages, besides they are secondary metabolites, what could suggest that they are useless. Alkaloids showed strong biological effects on animal and human organisms in very small doses. Alkaloids are present not only in human daily life in food and drinks but also as stimulant drugs. They showed anti-inflammatory, anticancer, analgesics, local anesthetic and pain relief, neuropharmacology, antimicrobial, antifungal, and many other activities. Alkaloids are useful as diet ingredients, supplements, and pharmaceuticals, in medicine and in other applications in human life. Alkaloids are also important compounds in organic synthesis for searching new semisynthetic and synthetic compounds with possibly better biological activity than parent compounds.

6.1 METHOD OF ALKALOIDS

Alkaloids are a huge group of naturally occurring organic compounds which contain nitrogen atom or atoms (amino or amido in some cases) in their structures. These nitrogen atoms cause alkalinity of these compounds. These nitrogen atoms are usually situated in some ring (cyclic) system. For example, indole alkaloids are those that contain nitrogen atom in indole ring system. Generally based on structures, alkaloids can be divided into classes like indoles, quinolines, isoquinolines, pyrrolidines, pyridines, pyrrolizidines, tropanes, and terpenoids and steroids. Other classification system is connected with a family of plant species that they occur. One of the examples is the opium alkaloids that occur in the opium poppy (*Papaver somniferum*). These two different classification systems cause confusion between their biological distribution and the chemical types of alkaloids, because there is not unmistakable correlation.

Keyword

Alkaloids are a class of basic, naturally occurring organic compounds that contain at least one nitrogen atom

Alkaloids (whose name originally comes from “alkali-like”) can react with acids and then form salts, just like inorganic alkalis. These nitrogen atoms can behave like a base in acid-base reactions. In general alkaloids, which are treated as amines, the same as amines in their names, have suffix ine. Alkaloids in pure form are usually colorless, odorless crystalline solids,

but sometimes they can be yellowish liquids. Quite often, they have bitter taste. Now more than 3000 of **alkaloids** are known in over different 4000 plant species.

These compounds are produced generally by many plant species, mainly by flowering plants and also by some animals. Plants produce and store many organic compounds like amino acids, proteins, carbohydrates, fats, and alkaloids, which are usually treated as secondary metabolites. They are stored in each part of the plant—leaves, stem, root, and fruits of plants—but in different amounts. It was suggested that they are plants' waste product, but now evidence suggests that they play some important biological function in plants.

Some groups of structurally related alkaloids are present in plants from few to even 30. These alkaloids belong to the same class but have some differences in their structure and one of them usually occurs in majority. Some plant families are very rich in alkaloids. For example, in plants like opium poppy (*Papaver somniferum*) and the ergot fungus (*Claviceps*), there are about 30 different alkaloid types. In plants, their function is still mostly unknown. Alkaloids because of their bitter taste are natural compound to deter herbivorous organisms. In some plants they are used as natural pesticides. It was suggested that alkaloids in plants have a function to protect them from destructive activity of some insect species. Alkaloids are also present in some animal species like frogs (poison dart frogs (*Phyllobates*)), New World beaver (*Castor canadensis*), and lizards, and they are produced by fungi species and ergot.

Besides having the same general name—alkaloids—they have an extreme variety of chemical structures. Some of these compounds seem to have people known for ages because of their wide range of activity on human organisms and also other animals. For thousand years, extracts from plants containing alkaloids had medicinal use as drugs, and they owe their powerful effects thanks to the presence of alkaloids. Morphine was the first alkaloid which was isolated about 1804 from opium poppy in crystalline form. Alkaloids are an interesting group of compounds with a wide range of activities, undesirable and desirable, on animal and human organisms. Alkaloids have diverse physiological effects: antibacterial, antimitotic, anti-inflammatory, analgesic, local anesthetic, hypnotic, psychotropic, and antitumor activity and many others. Nowadays, alkaloids usually from plants rather than from animals are still of great interest to organic chemists, biologists, biochemists, pharmacologists, and pharmacists. Well-known alkaloids include morphine, strychnine, quinine, atropine, caffeine, ephedrine, and nicotine.

6.1.1 Methods of Isolation

Extracts of plants containing alkaloids were known and used because of their diverse activity by people from ages. But ages ago people did not know direct methods to isolate pure compounds from specified plant species. Alkaloids in plants usually exist

as aqueous solution in tissues. To isolate them the method called extraction is usually used.

Another method is chromatography. It uses differences in degrees of adsorption of different alkaloids in some **solvent system** on solid materials such as silica or alumina.

Remember

For commercially useful alkaloids, special extraction methods were developed. In general mixture containing alkaloid should be dissolved with some solvent with reagents. Extraction method allows recovery of alkaloids from solution. Then, each alkaloid can be separated from mixture and be obtained in pure form. To obtain crystalline form of alkaloids, certain solvents should be used.

6.1.2 Pharmaceutical and Medicinal Use of Alkaloids

Alkaloids showed quite diverse medicinal properties. Many of them possess local anesthetic properties, but their practical use is limited for clinical purpose. Morphine (Figure 1a) is one of the most known alkaloids which had been used and still is for medical purposes. This alkaloid is a powerful narcotic which is used for the relief of pain, but its usefulness is limited because of addictive properties.

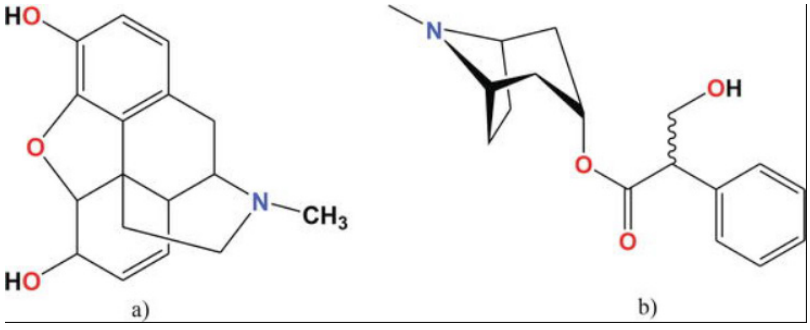

Figure 1: Structures of alkaloids: (a) morphine and (b) atropine.

Keyword

Solvent captions when dissolved in that solvent are called acids while the substances which give solvent anions when dissolved in that solvent are called bases.

Methyl ether derivative of morphine—codeine—naturally occurring next to morphine in the opium poppy, possesses an excellent analgesic activity and is shown to be relatively nonaddictive. These alkaloids act as respiratory or cardiac stimulants. Next, the alkaloid which is used as medication in many clinical applications is atropine (Figure 1b). For example, injection with atropine is given to treat bradycardia (low heart rate).

Tubocurarine (Figure 2) is an alkaloid, is an ingredient of poison curare, and is used in surgery as muscle relaxant. Alkaloids vincristine and vinblastine are used as chemotherapeutic agent in the treatment of many cancer types. Cocaine an alkaloid

present in *Erythroxylum coca* is a potent local anesthetic. Ergonovine, an alkaloid from the fungus *Claviceps purpurea*, and the second alkaloid ephedrine isolated from *Ephedra* species both act as blood vessel constrictors. Also ephedrine is used in bronchial asthma and to relieve discomfort of hay fever, sinusitis, and common colds.

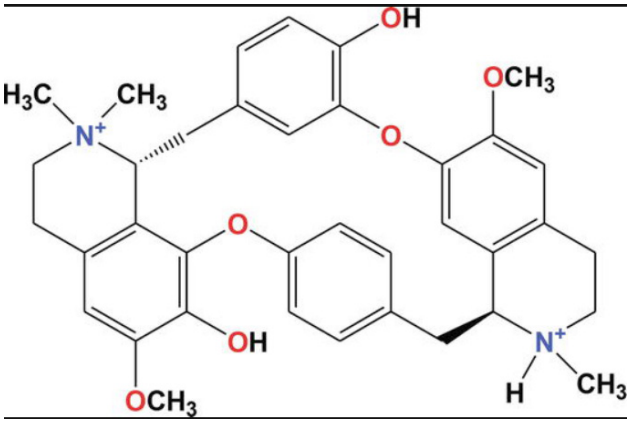

Figure 2: Structure of tubocurarine.

Quinine (Figure 3) is a powerful antimalarial agent and more often is replaced by synthetic drugs, which are more effective and less toxic. Another alkaloid from *Cinchona* species is quinidine which has medical application as treatment of irregular rhythms of the heartbeat or arrhythmias.

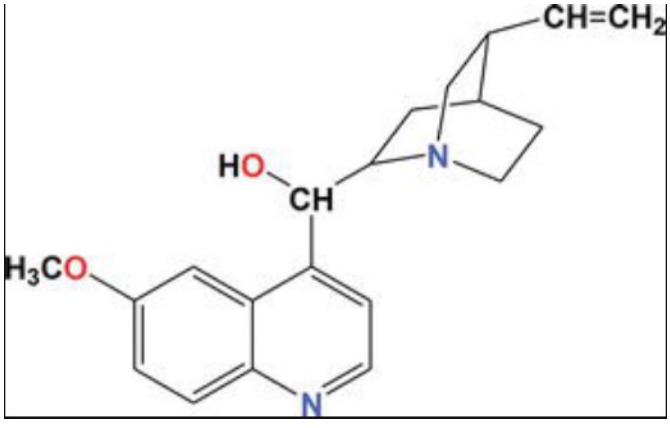

Figure 3: Structure of quinine from Cinchona species.

Colchicine (Figure 4) is another alkaloid, present in plants of Liliaceae family, known for ages to treat acute gout attacks. Another clinically used alkaloid is lobeline isolated from *Lobelia inflata*, which has multiple mechanisms of action.

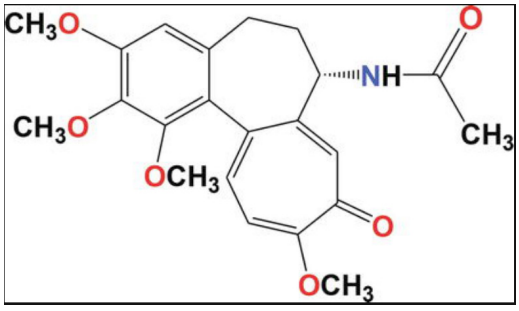

Figure 4: Structure of colchicine.

6.1.3 Alkaloids in Human Food and Drinks

Many **alkaloids** are elements of human diet, both in food and drinks. The plants in the human diet in which alkaloids are present are not only coffee seeds (caffeine, Figure 5), cacao seeds (theobromine and caffeine), and tea leaves (theophylline, caffeine) but also tomatoes (tomatine) and potatoes (solanine). The most common alkaloid is caffeine which has also application as an ingredient of soft drinks like Coca-Cola to improve their taste and in drinks for active people who do sport.

Keyword

Alkaloids are a class of basic, naturally occurring organic compounds that contain at least one nitrogen atom.

Figure 5: Plant source of caffeine and its structure and powdered caffeine (pure form) (author's own photos).

Other known alkaloid with bitter taste used as an ingredient of tonics is quinine (Figure 3) isolated from *Cinchona* species.

6.1.4 Alkaloids as Stimulants

Alkaloids stimulate human organisms, for example, central nervous system, or directly work on the human brain. Nicotine

(Figure 6) is an alkaloid obtained from the tobacco plant (*Nicotiana tabacum*) and is a potent stimulant and the main ingredient in tobacco smoked in pipes, cigars, and cigarettes. This alkaloid is highly addictive.

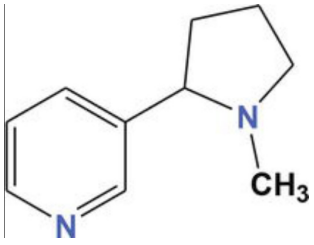

Figure 6: Structure of nicotine.

Cocaine is a narcotic drug, which activity is not suitable for medical purposes. This alkaloid has an opposite effect than morphine. This compound produces in the human body a euphoric hyper arousal state, but high doses of it may lead to **fibrillation** and death.

6.1.5 Dark Nature of Alkaloids

Some alkaloids are illicit drugs and poisons. Poisonous activities of some alkaloids are known for ages. One of these is strychnine (from *Strychnos* species, Figure 7). One of the well-known poison curare (obtained from *Chondrodendron tomentosum*) used in the South Africa as narrow poison contains alkaloid tubocurarine.

Keyword

Fibrillation is the rapid, irregular, and unsynchronized contraction of muscle fibers. An important occurrence is with regard to the heart.

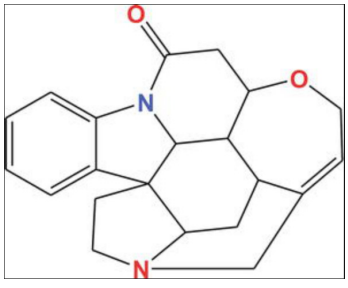

Figure 7: Structure of strychnine.

Coniine is an alkaloid isolated from *Conium maculatum*, which is an active ingredient of poison hemlock. Mescaline isolated from *Anhalonium* species has hallucinogenic activity.

Psilocybin is a naturally occurring drug isolated from fungi species *Psilocybe mexicana* and possesses psychedelic activity. During the past decades, many semisynthetic derivatives of naturally occurring alkaloids with various activities have been synthesized. Synthetic derivative of **morphine** is heroin, and, from lysergic acid naturally present in *C. purpurea*, LSD was produced.

Keyword

Morphine is a pain medication of the opiate family that is found naturally in a dark brown, resinous form, from the poppy plant

6.1.6 Other Practical Use of Alkaloids

Besides activities mentioned above, alkaloids from many different plant species have many other useful applications such as antiparasitic, antiplasmodial, anticorrosive, antioxidative, antibacterial, anti-HIV, and insecticidal activities.

6.2 ANALYSIS OF ALKALOIDS (INDOLE ALKALOIDS, ISOQUINOLINE ALKALOIDS, TROPANE ALKALOIDS)

Remember

Alkaloids have antiproliferative, antibacterial, antioxidant potential, which can be used for the development of drugs. This therapeutic potential of alkaloids grows up their industrial application. Numerous research have been carried out in pharmaceutical properties of different alkaloids extracted from plants.

Plants are the renowned cradle of traditional medicine system that assuages human diseases and promotes health for thousands of years. Plants are a rich reservoir of a vast array of active constituents that have significant therapeutic applications like antiviral, anticancer, analgesic, antitubercular. Among them, alkaloids are the important secondary metabolites that were initially discovered and used as early as 4000 years ago and are well recognized for their rich therapeutic potential. Based on their heterocyclic ring system and biosynthetic precursor, alkaloids are classified into diverse categories, viz. indole, purine, quinoline, isoquinoline, tropane, imidazole, among others.

Alkaloids are an assembly of naturally occurring chemical composites, which typically comprise basic nitrogen atoms. They may also contain some neutral or weakly acidic compounds. Few synthetic compounds are also considered as alkaloids too. Apart from carbon, nitrogen, or hydrogen, alkaloids may comprise sulfur and rarely bromine, phosphorus, or chlorine.

These secondary metabolites are formed by a large variety of entities, including plants, animals, fungi, and bacteria. Because of their vast array of pharmacological actions (anticancer,

antimalarial, anesthetic, stimulant), they are purified from the crude extract by **acid-base extraction**.

Alkaloids are mostly solids and are known to occur in higher plants. They are prevalent in the plants belonging to the following botanical families: Apocynaceae, Annonaceae, Amaryllidaceae, Berberidaceae, Boraginaceae, Gnetaceae, Liliaceae, Leguminoceae, Lauraceae, Loganiaceae, Magnoliaceae, Menispermaceae, Papaveraceae, Piperaceae, Rutaceae, Rubiaceae, Ranunculaceae, Solanaceae, etc.

Did You Know?

The word "alkaloid" was first coined by the German chemist Carl F. W. Meissner in 1819, derived from the Arabic name al-qali, which is associated to the plant from which soda was first sequestered. Alkaloids are low-molecular-weight structures and form approximately 20% of plant-based secondary metabolites. So far, approximately 12,000 alkaloids are isolated from various genera of the plant kingdom.

6.3 PHYTOCHEMISTRY AND CLASSIFICATION OF ALKALOIDS

Alkaloids illustrate large diversity not only in their botanical and biochemical origin but also in structure and pharmacological action. In this connection, various systems of classification are possible. From a structural perception, alkaloids can be classified, based on their molecular precursor, structures, and origins or on the biological pathways used to obtain the molecule.

There are three central types of alkaloids: (1) true alkaloids, (2) protoalkaloids, and (3) pseudoalkaloids. True alkaloids and protoalkaloids are produced from amino acids, whereas pseudoalkaloids are not derived from these compounds.

True alkaloids

This type of alkaloids are obtained from amino acids and they share a nitrogen-containing heterocyclic ring. They are highly reactive in nature and have potent biological activity. They form water-soluble salts, and many of them are crystalline in nature, which conjugates with acid and forms a salt. Almost all true alkaloids are bitter in taste and solid, except nicotine, which is a brown liquid.

Their occurrence in plants occurs in three forms: (a) in Free-state, (b) as N-oxide, or (c) as salts. Various amino acids like l-phenylalanine/l-tyrosine, l-ornithine, l-histidine, l-lysine are the main sources of true alkaloids (Table 1) Cocaine, morphine, quinine are the common true alkaloids found in nature.

Keyword

Acid–base extraction is a procedure using sequential liquid–liquid extractions to purify acids and bases from mixtures based on their chemical properties.

Table 1: Amino acid and their involvement in alkaloid synthesis

Alkaloid type	Major group of alkaloid	Chemical group of alkaloid	Amino acid precursor
Tryptophan-derived alkaloids	True alkaloid	Ergot alkaloids Pyrroloindole alkaloids Indole alkaloids Aspidosperma alkaloids Quinoline alkaloids	l-Threonine l-Proline l-Tryptophan l-Serine
	Protoalkaloids	Terpenoid indole alkaloids	
Arginine-derived alkaloids	True alkaloid	Marine alkaloids	l-Asparagine l-Alanine l-Aspartic acid l-arginine
Ornithine-derived alkaloids	True alkaloid	Pyrrolizidine alkaloids Tropane alkaloids Pyrrolidine alkaloids	l-Ornithine
Histidine-derived alkaloids	True alkaloid	Manzamine alkaloids Imidazole alkaloids	l-Histidine
Nicotinic acid-derived alkaloids	True alkaloid	Sesquiterpene pyridine alkaloids Pyridine alkaloids	Nicotinic acid
Lysine-derived alkaloids	True alkaloid	Indolizidine alkaloids Quinolizidine alkaloids Piperidine alkaloids	l-Lysine l-Leucine l-Isoleucine
Anthranilic acid-derived alkaloids	True alkaloid	Acridine alkaloids Quinoline alkaloids Quinazoline alkaloids	Anthranilic acid
Tryptophan-derived alkaloids	Protoalkaloids	Terpenoid indole alkaloids	l-Threonine l-Proline l-Tryptophan l-Serine
Tyrosine-derived alkaloids	Protoalkaloids	Phenylethylamine alkaloids	l-Tyrosine

Protoalkaloids

This type of alkaloids contains a nitrogen atom, which is derived from an amino acid but is not part of the heterocyclic ring system. l-Tryptophan and l-tyrosine are the main precursors of this type of alkaloids. This minor group is structurally composed of simple alkaloids. Yohimbine, mescaline, and hordenine are the main alkaloids of

this type. They are used in various health disorders, including mental illness, pain, and neuralgia.

Pseudoalkaloids

The basic carbon skeleton of pseudoalkaloids is not directly derived from amino acids; instead, they are connected with amino acid pathways where they are derived from by amination or transamination reaction from forerunners or postcursors of amino acid. Nonamino-acid precursors can also produce pseudoalkaloids. They can be phenylalanine or acetate derived. Capsaicin, caffeine, ephedrine are very common examples of pseudoalkaloids (Table 2).

Table 2: Involvement of parent compound in pseudoalkaloids synthesis

Parent compounds	Precursor compound	Chemical group of alkaloids	Examples
Terpenoid	Geraniol	Terpenoid Alkaloids	Gentianine Aconitine β-Skytanthine Actinidine
Sesquiterpene	Acetate	Sesquiterpene Alkaloids	Evonoline Cassinine Evorine Celapanin
Phenyl	Ferulic acid	Aromatic Alkaloids	Capsaicin
Piperidine	Acetate	Piperidine Alkaloids	Pinidine Coniceine Coniine
Purine	Adenine/Guanine	Purine alkaloids	Theophylline Theobromine Caffeine

6.3.1 Classification Established Upon the Ring Structure

This is the most comprehensively established classification, based on the presence of a basic heterocyclic nucleus in their structure.

Tropane alkaloid

This category of alkaloids has tropane (C4N skeleton) nucleus. They are abundantly found in the Solanaceae family. They are derived from ornithine and acetoacetate. Structurally, pyrrolines are the precursor of these type of alkaloids. Maximum of them are esters of mono, di, trihydroxytropane, having a wide range of hydroxylation

arrangements. Cocaine, atropine, scopolamine, and their derivatives are widely studied since the 19th century because of their enormous pharmacological actions (Fig 8).

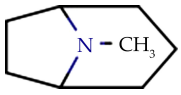

Figure 8: Basic structure of the tropane nucleus.

Pyrrolizidine alkaloids

The pyrrolizidine nucleus is distinctive of this group of alkaloids. They occur in the plants from Asteraceae and Fabaceae family. Majority of pyrrolizidine alkaloids occur in the plants as N-oxides, whose role being lost during the isolation process. They have extensively reviewed alkaloids because of their toxic effects, especially liver damage. These alkaloids enter into the food chain and become antifeedants for the animals who eat them. Senecionine is the popular alkaloid of this type (Fig 9).

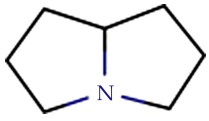

Figure 9: Basic structure of the Pyrrolizidine nucleus.

Piperidine alkaloids

Piperidine nucleus is the basic ring system of this group of alkaloids. Monocycle compounds with the C5N nucleus is the important feature of true piperidine alkaloids. Presence of odor is the common feature of piperidine alkaloids. They exert chronic neurotoxicity. Many of them are originated from plants. Although piperidine itself is a lysine-derived alkaloid, some of the piperidine alkaloids also derived from acetate, acetoacetate, in an analogous fashion to the simple pyrrolidine alkaloids. Lobeline is one of the important alkaloids in this group (Fig 10).

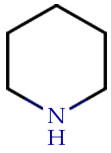

Figure 10: Basic structure of the piperidine nucleus.

Quinolines alkaloid

This type of quinolone-nucleus-containing alkaloid is achieved exclusively from the bark of the Cinchona plant. But a variety of simple heteroaromatic quinolines are also isolated from various marine sources (4, 8-quinolinediol from cephalopod ink and 2-heptyl-4-hydroxyquinoline from a marine pseudomonad). The major alkaloid of this specific group is cinchonine, cinchonidine, quinine, and quinidine (Fig. 11).

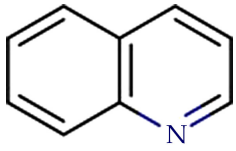

Figure 11: Basic structure of the Quinoline nucleus.

Isoquinoline alkaloids

Isoquinoline alkaloids are an extremely large group of alkaloids mostly occurring in higher plants, but few groups are also isoquinolinoid marine alkaloids. Isoquinoline nucleus is the basic structural feature. These groups of alkaloids have huge types of medicinal properties like antiviral, antifungal, anticancer, antioxidant, antispasmodic, and an enzyme inhibitor. Morphine and codeine are the major and widely studied isoquinoline alkaloids. They are derived from tyrosine or phenylalanine. They are made from a predecessor of dopamine (3, 4-dihydroxytryptamine) associated with a ketone or aldehyde. This group of alkaloids is further classified as follows: Simple isoquinoline alkaloids (e.g., salsoline, mimosamycin), benzylisoquinoline alkaloids (e.g., reticuline, imbricatine), bisbenzylisoquinoline alkaloids (e.g., fumaricine), manzamine alkaloids (e.g., manzamine a), pseudobenzylisoquinoline alkaloids (e.g., polycarpine, ledecorine), secobisbenzylisoquinoline alkaloids (e.g., baluchistanamine), bisbenzylisoquinoline alkaloids containing one ether link (e.g., dauricine), bisbenzylisoquinoline alkaloids containing two ether links (e.g., berbamine), bisbenzylisoquinoline alkaloids containing aryl links only (e.g., pisopowetine), bisbenzylisoquinoline alkaloids containing one aromatic link and one or two ether links (e.g., rodiasine) (Fig. 12).

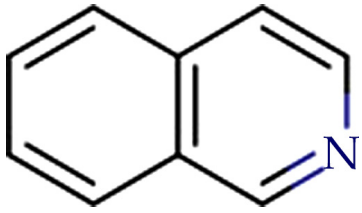

Figure 12: Basic structure of the isoquinoline nucleus.

Indole alkaloids

This is the largest and most interesting alkaloid group derived from tryptophan. The important alkaloids from this group include simple tryptamine derivatives, carbazoles (where the ethanamine chain has been lost), a diversity of alkaloids where one or more prenyl residues are combined with tryptamine, and others where integration of regular monoterpenoid or diterpenoid units occurred. Although structural diversity varies according to the terrestrial and marine source, classical research studies have been carried out on alkaloids from both origins and the fungal source. Polyhalogenation is a common feature of these alkaloids. They are further classified as follows: simple indole alkaloids (e.g., Aplysinopsin, Gramine), bisindoles (e.g., Indirubin, 6, 6′-Dibromoindigotin), simple tryptamine alkaloids (e.g., Tryptamine), cyclotryptamine alkaloids (e.g., Physostigmine), quinazolinocarbazole alkaloids (e.g., Rutaecarpine), β-carboline alkaloids (e.g., Harman), carbazole alkaloids (e.g., Ekeberginine), indolonaphthyridine alkaloids (e.g., Canthin-6-one), ergot alkaloids (e.g., ergotamine) (Fig. 13).

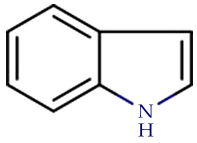

Figure 13: Basic structure of the indole nucleus.

Steroidal alkaloids

1, 2-Cyclopentane phenanthrene ring system is the characteristic of this type of alkaloids. They are typically originated from higher plants, which belong to Liliaceae, Solanaceae, Apocynaceae, Buxaceae families, but some are also isolated from amphibians too. These alkaloids are divided into various other subtypes, among them various types of aminopregnanes are the simplest type. The others types of steroidal alkaloids are Salamandra type (e.g., cycloneosamandione), jerveratrum type (e.g., jervine), spirosolane type (e.g., soladulcidine), solanidine type (e.g., rubijervine), cerveratrum type (e.g., 3,6-cevanediol), conanine type (e.g., didymeline), Buxus type (e.g., cyclobuxine), pregnane type (e.g., 20α-dimethylamino-3β-senecioylamino-16β-hydroxy-pregn-5-ene), cephalostatins/ritterazines (e.g., ritterazines a), miscellaneous steroidal alkaloids (e.g., bufotoxin) (Fig. 14).

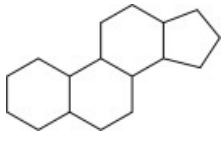

Figure 14: Basic structure of the steroidal alkaloid nucleus.

Imidazole alkaloid

The imidazole ring structure is the characteristic of this type of alkaloid. The imidazole ring of these alkaloids is made at the stage of the precursor, so they are an exemption in the transformation procedure of structures. This type of alkaloids contains numerous structurally different examples, particularly among marine and microbial alkaloids. They display a wide array of biological activities and significant pharmaceutical potential. Pilocarpine is the most pharmaceutically significant imidazole alkaloid (Fig. 15).

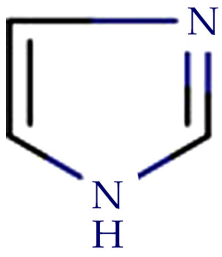

Figure 15: Basic structure of the imidazole nucleus.

Purine alkaloids

Purine is the nitrogenous base of nucleotide (building block of DNA and RNA), which consist of purine ring and pentose sugar along with another base pyrimidine. Caffeine, Theophylline and Theobromine are typical examples of purine alkaloids. They are popular as plant alkaloids, but they can be also originated in marine organisms with substituted purines (e.g., Phidolopin) and a variety of terpenoid-purine alkaloids, such as the age lines and others (Fig. 16).

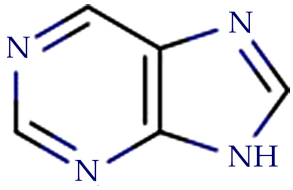

Figure 16: Basic structure of the purine nucleus.

Pyrrolidine alkaloids

Pyrrolidine (C4N skeleton) nucleus constitutes the basic nucleus of pyrrolidine alkaloids. Many pyrrolidine alkaloids are known from plants. Hygrine (biosynthesized from ornithine), ficine (where the pyrrolidine ring is involved to a flavone nucleus), and

brevicolline (wherein it is attached to a β-carboline unit) are some examples of this type of alkaloids.

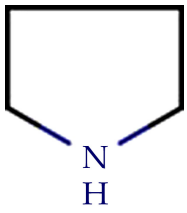

Figure 17: Basic structure of the pyrrolidine nucleus.

6.4 ALKALOIDS AND THEIR APPLICATIONS IN PHARMACEUTICAL CHEMISTRY

Most alkaloids contain oxygen in their molecular structure; those compounds are usually colorless crystals at ambient conditions. Oxygen-free alkaloids, such as, nicotine or coniine, are typically volatile, colorless, oily liquid some alkaloids are colored, like berberine (yellow) and sanguinarine (orange) Most alkaloids are weak bases, but some, such as the bromine and theophylline, are amphoteric. Many alkaloids dissolve poorly in water but readily dissolve in organic solvents, such as diethyl ether, chloroform or 1, 2- dichloroethane. Caffeine, cocaine, codeine and nicotine are water-soluble (with a solubility of ≥1g/L), whereas others, including morphine are highly water-soluble (0.1–1 g/L). Alkaloids and acids form salts of various strengths. These salts are usually soluble in water and ethanol and poorly soluble in most organic soluble.

6.4.1 Biological Role

The role of alkaloids for living organisms that produce them is still unclear. It was initially assumed that the alkaloids are the final products of nitrogen metabolism in plants, as urea in mammals. It was later shown that alkaloid concentrations varies over time, and this hypothesis was refuted. Most of the known functions of alkaloids are related to protection. However, some animals are adapted to alkaloids and even use them in their own metabolism. Such alkaloid-related substances as serotonin, dopamine and histamine are important neurotransmitters in animals. Alkaloids are also known to regulate plant growth. Another example of an organism that uses alkaloids for protection is the Utetheisa ornatrix, more commonly known as the ornate moth. Pyrrolizidine alkaloids render these larvae and adult moths unpalatable to many of their natural enemies like coccinelid beetles, green lacewings, insectivorous hemiptera and insectivorous bats.

6.4.2 Applications in Medicine

Medical use of alkaloid-containing plants has a long history, and, thus, when the first alkaloids were isolated in the 19th century, they immediately found application in clinical practice. Many alkaloids are still used in medicine, usually in the form of salts, including the following:

Alkaloid	Action
Ajmaline	antiarrhythmic
Atropine, scopolamine, hyoscyamine	anticholinergic
Caffeine	stimulant, adenosine receptor antagonist
Codeine	cough medicine, analgesic
Colchicine	remedy for gout
Emetine	antiprotozoal agent
Ergot alkaloids	sympathomimetic, vasodilator, antihypertensive
Morphine	analgesic
Nicotine	stimulant, nicotinic acetylcholine receptor agonist
Physostigmine	inhibitor of acetylcholinesterase
Quinidine	antiarrhythmic
Quinine	antipyretics, antimalarial
Reserpine	antihypertensive
Tubocurarine	muscle relaxant
Vinblastine, vincristine	antitumor
Vincamine	vasodilating, antihypertensive
Yohimbine	stimulant, aphrodisiac

Many synthetic and semisynthetic drugs are structural modifications of the alkaloids, which were designed to enhance or change the primary effect of the drug and reduce unwanted side-effects. For example, naloxone, an opioid receptor antagonist, is a derivative of the baine that is present in opium.

6.5 ALKALOIDS ISOLATED FROM NATURAL HERBS AS THE ANTICANCER AGENTS

Alkaloids are a highly diverse group of compounds that contain a ring structure and a nitrogen atom. In most cases, the nitrogen atom is located inside the heterocyclic ring structure. A classification based on biosynthetic pathways is mostly used to categorize different alkaloid. Alkaloids have a wide distribution in the plant kingdom and mainly exist in higher plants, such as those belonging to Ranunculaceae, Leguminosae, Papaveraceae, Menispermaceae, and Loganiaceae. Moreover, several alkaloids exhibit significant biological activities, such as the relieving action of ephedrine for asthma, the analgesic action of morphine, and the anticancer effects of vinblastine. In fact, alkaloids are among the most important active components in natural herbs, and some of these compounds have already been successfully developed into chemotherapeutic

drugs, such as camptothecin (CPT), a famous topoisomerase I (TopI) inhibitor, and vinblastine, which interacts with tubulin.

Herein, we searched the PubMed database and the naturally derived alkaloids, such as berberine, evodiamine, matrine, piperine, sanguinarine, and tetrandrine (Figure 18), which have relatively more anticancer studies, have been selected for reviewing. Other alkaloids (such as chelerythrine, chelidonine, fagaronine, lycorine, nitidine chloride, and solanine) lacking systematic anticancer investigations have also been mentioned. The aim of this paper is to summarize and investigate the mechanisms of action of these compounds to accelerate the discovery of anticancer drugs derived from alkaloids. We propose that the development of alkaloids into new anticancer agents has a bright future despite some difficulties.

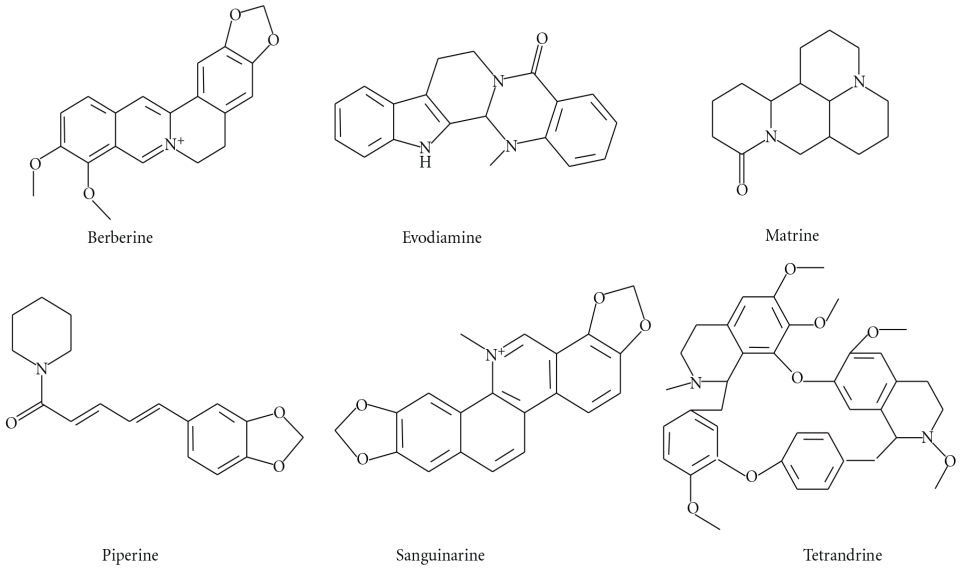

Figure 18: The chemical structures of berberine, evodiamine, matrine, piperine, sanguinarine, and tetrandrine.

6.5.1 Alkaloids with Anticancer Effects and the Related Mechanisms

Berberine (Figure 18) is an isoquinoline alkaloid widely distributed in natural herbs, including *Rhizoma Coptidis*, a widely prescribed Chinese herb. It has a broad range of bioactivities, such as antiinflammatory, antibacterial, antidiabetes, antiulcer, sedation, protection of myocardial ischemia-reperfusion injury, expansion of blood vessels, and inhibition of platelet aggregation, hepatoprotective, and neuroprotective effects. Berberine has been used in the treatment of diarrhea, neurasthenia, arrhythmia, diabetes, and so forth.

Several studies have shown that berberine has anticancer potentials by interfering with the multiple aspects of tumorigenesis and tumor progression in both *in vitro* and *in vivo* experiments. These observations have been well summarized in the recent reports. Berberine inhibits the proliferation of multiple cancer cell lines by inducing cell cycle arrest at the G_1 or G2/ M phases and by apoptosis. In addition, berberine induces endoplasmic reticulum stress and autophagy in cancer cells. However, compared with clinically prescribed anticancer drugs, the cytotoxic potency of berberine is much lower, with an IC_{50} generally at 10 μM to 100 μM depending on the cell type and treatment duration *in vitro*. Besides, berberine also induces morphologic differentiation in human teratocarcinoma cells. Inhibition of tumor invasion and metastasis is an important aspect of berberine's anticancer activities. A few studies have reported berberine's inhibition of tumor angiogenesis. In addition, its combination with chemotherapeutic drugs or irradiation could enhance the therapeutic effects. Recently, a study reported that berberine also showed promising chemopreventive efficacy in hamster buccal pouch carcinogenesis.

The potential molecular targets and mechanisms of berberine are rather complicated. Berberine interacts with DNA or RNA to form a berberine-DNA or a berberine-RNA complex, respectively. Berberine is also identified as an inhibitor of several enzymes, such as N-acetyltransferase (NAT), cyclooxygenase-2 (COX-2), and telomerase. Other mechanisms of berberine are mainly related to its effect on cell cycle arrest and apoptosis, including regulation of cyclin-dependent kinase (CDK) family of proteins and expression regulation of B-cell lymphoma 2 (Bcl-2) family of proteins (such as Bax, Bcl-2, and Bcl-xL), and caspases. Furthermore, berberine inhibits the activation of the nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) and induces the formation of intracellular reactive oxygen species (ROS) in cancer cells. Interestingly, these effects might be specific for cancer cells. The effect of berberine on invasion, migration, metastasis, and angiogenesis is mediated through the inhibition of focal adhesion kinase (FAK), NF-κB, urokinase-type plasminogen-activator (u-PA), matrix metalloproteinase 2 (MMP-2), and matrix metalloproteinase 9 (MMP-9); reduction of Rho kinase-mediated Ezrin phosphorylation; reduction of the expression of COX-2, prostaglandin E, and prostaglandin E receptors; downregulation of hypoxia-inducible factor 1 (HIF-1), vascular endothelial growth factor (VEGF), proinflammatory mediators, and so forth.

6.5.2 Evodiamine

Evodiamine (Figure 18), a quinolone alkaloid, is one of the major bioactive compounds isolated from the Chinese herb *Evodia rutaecarpa*. It possesses antianxiety, antiobese, antinociceptive, antiinflammatory, antiallergic, and anticancer effects. Besides, it has thermoregulation, protection of myocardial ischemia-reperfusion injury and vessel-relaxing activities. Evodiamine exhibits anticancer activities both *in vitro* and *in vivo* by

inducing the cell cycle arrest or apoptosis, inhibiting the angiogenesis, invasion, and metastasis in a variety of cancer cell lines. It presents anticancer potentials at micromolar concentrations and even at the nanomolar level in some cell lines *in vitro*. Evodiamine also stimulates autophagy, which serves as a survival function. Compared with other compounds, evodiamine is less toxic to normal human cells, such as human peripheral blood mononuclear cells. It also inhibits the proliferation of adriamycin-resistant human breast cancer NCI/ADR-RES cells both *in vitro* and in Balb-c/nude mice. Evodiamine (10 mg/kg) administrated orally twice daily significantly inhibits the tumor growth. Moreover, treatment with 10 mg/kg evodiamine from the 6th day after tumor inoculation into mice reduces lung metastasis and does not affect the body weight of mice during the experimental period

Evodiamine inhibits TopI enzyme, forms the DNA covalent complex with a similar concentration to that of CPT, and induces DNA damage. However, TopI may not be the main target of this compound. Cancer cells treated with evodiamine exhibit G2/ M phase arrest rather than S phase arrest, which is not consistent with the mechanism of classic TopI inhibitors, such as CPT. Therefore, other targets aside from TopI may also be important for realizing the anticancer potentials of evodiamine. This statement is supported by the fact that evodiamine has effect on tubulin polymerization. Exposure to evodiamine rapidly increases intracellular ROS followed by an onset of mitochondrial depolarization. The generation of ROS and nitric oxide acts in synergy and triggers mitochondria-dependent apoptosis. Evodiamine also induces caspase-dependent and caspase-independent apoptosis, downregulates Bcl-2 expression, and upregulates Bax expression in some cancer cells. The phosphatidylinositol 3-kinase/Akt/caspase and Fas ligand (Fas-L)/NF-κB signaling pathways might account for evodiamine-induced cell death. Moreover, these signals could be increased by the ubiquitin-proteasome pathway.

6.5.3 Matrine

Matrine (Figure 18) is a major alkaloid found in many *Sophora* plants, including *Sophora flavescens* Ait. It exhibits a wide range of pharmacological properties such as antibacterial, antiviral, antiinflammatory, antiasthmatic, antiarrhythmic, antiobesity, anticancer, diuretic, choleretic, hepatoprotective, nephroprotective, and cardioprotective effects. It has been used for treatment of bacillary dysentery, enteritis, malignant pleural effusion, and so forth in China, and the anticancer effects have also been widely studied. Although the needed concentration of matrine to inhibit cancer cell proliferation is relatively high (i.e., at millimolar level), it has no significant effects on the viability of normal cells. Matrine inhibits the proliferation of various types of cancer cells mainly through mediation of G_1 cell cycle arrest or apoptosis. Apoptosis and autophagy could be both induced by matrine in human cancer cells, such as hepatoma G2 cells and SGC-7901 cells. Matrine also induces the differentiation of K562 cells and presents antiangiogenesis activities. The *in vivo* anticancer efficacy of matrine has already been

evaluated in H22 cells, MNNG/HOS cells, 4T1 cells and BxPC-3 cells in BALB/c mice, among others. For example, matrine at 50 mg/kg or 100 mg/kg inhibits MNNG/HOS xenograft growth, and it reduces the pancreatic tumor volumes compared to those of control at the similar doses.

6.5.4 Piperine

Piperine (Figure 18), a piperidine alkaloid isolated from Piper nigrum and Piper longum, is a compound found in famous spices that have been used for centuries. It exhibits antioxidant, antiinflammatory, antidiarrheal, anticonvulsant, antimutagenic, hypolipidemic, promoting bile secretion, and tumor inhibitory activities. It is also a known antidepressant of the central nervous system. The chemopreventive effects of piperine against several kinds of carcinogen, such as benzo(a)pyrene, and 7,12-dimethyl benz(a)anthracene, show its potential as a cancer preventive agent

6.5.5 Sanguinarine

Sanguinarine (Figure 18) is a benzophenanthridine alkaloid isolated from the Papaveracea family, which includes *Sanguinaria canadensis* L. and *Chelidonium majus* L. It has antibacterial, antifungal, antischistosomal, antiplatelet, and antiinflammatory properties, and is used for schistosomiasis control. Sanguinarine also exhibits anticancer potentials and is currently receiving attention from researchers. Data from *in vitro* studies indicates that this alkaloid presents anticancer effects at concentrations less than ten micromoles in most cases. Sanguinarine induces cell cycle arrest at different phases or apoptosis in a variety of cancer cells. It remarkably sensitizes breast cancer cells to tumor necrosis factor (TNF)-related apoptosis-inducing ligand-mediated apoptosis. Sanguinarine also shows antiangiogenic effects in mice (5 mg/kg), presents anti-invasive effects, and overcomes P-gp-mediated MDR phenotype. A strategy involving the coadministration of COX-2 inhibitors and sanguinarine has been recommended for the management of prostate cancer. It has also been suggested that sanguinarine may be developed as an agent for the management of conditions elicited by ultraviolet exposure such as skin cancer

The most possible mechanism responsible for the anticancer effects of this compound is its ability to directly interact with glutathione (GSH). This interaction severely depletes cellular GSH and induces ROS generation.

6.5.6 Tetrandrine

Tetrandrine (Figure 18), a bisbenzylisoquinoline alkaloid from the root of *Stephania tetrandra*, exhibits a broad range of pharmacological activities, including immunomodulating,

antihepatofibrogenetic, antiinflammatory, antiarrhythmic, antiportal hypertension, anticancer and neuroprotective activities. It generally presents its anticancer effects in the micromolar concentrations. Tetrandrine induces different phases of cell cycle arrest, depends on cancer cell types, and also induces apoptosis in many human cancer cells, including leukemia, bladder, colon, hepatoma, and lung. *In vivo* experiments have also demonstrated the potential value of tetrandrine against cancer activity.

The survival of mice subcutaneously inoculated with CT-26 cells is extended after daily oral gavage of 50 mg/kg or 150 mg/kg of tetrandrine. Tetrandrine also inhibits the expression of VEGF in glioma cells, has cytotoxic effect on ECV304 human umbilical vein endothelial cells, and suppresses in vivo angiogenesis.

Tetrandrine-treated mice (10 mg/kg/day) have fewer metastases than vehicle-treated mice, and no acute toxicity or obvious changes can be observed in the body weight of both groups

Coadministration of tetrandrine restores the sensitivity of MDR cancer cells to doxorubicin, paclitaxel, docetaxel, and vincristine [133–135] through the inhibition of P-gp. In mice with MDR MCF-7/adr or KBv200 cell xenografts, co-administration of tetrandrine increases the anticancer activity of doxorubicin and vincristine without a significant increase in toxicity [133, 135]. Hence, tetrandrine holds a great promise as a MDR modulator for the treatment of P-gp-mediated MDR cancers. Tetrandrine appears to be a promising candidate for combining with several chemotherapeutic agents, such as 5-fluorouracil and cisplatin, *in vitro* or *in vivo*. It enhances tamoxifen-induced antiproliferation by inhibiting phosphoinositide-dependent kinase 1. Tetrandrine also enhances the radio sensitivity of various cancer cells mainly by affecting the radiation-induced cell cycle arrest and redistributing the cell cycle. All these observations are rational evidence supporting the application of tetrandrine as an adjunct for cancer chemotherapy or radiotherapy.

6.5.7 Other Alkaloids with Anticancer Effects

Aside from the aforementioned alkaloids, other alkaloids such as chelerythrine isolated from *Toddalia asiatica* (L.) Lam, chelidonine isolated from *Chelidonium majus* L., fagaronine isolated from *Fagara zanthoxyloides* Lam., lycorine isolated from *Lycoris*, nitidine chloride isolated from *Zanthoxylum nitidum* (Roxb.) DC., solanine isolated from *Solanum tuberosum*, sophocarpine isolated from *Sophora alopecuroides* L., trigonelline isolated from *trigonella foenum-graecum* also present anticancer potentials with diversiform mechanisms. However, reports on the anticancer activities and underlying mechanism of actions of these compounds are limited.

SUMMARY

- In nature there are many natural compounds. From among many classes of naturally occurring organic compounds such as carbohydrates, lipids, proteins, amino acids, anthocyanins, flavonoids, and steroids, the one that seems to be quite special is alkaloids.
- Alkaloids are a huge group of naturally occurring organic compounds which contain nitrogen atom or atoms (amino or amido in some cases) in their structures. These nitrogen atoms cause alkalinity of these compounds.
- Plants produce and store many organic compounds like amino acids, proteins, carbohydrates, fats, and alkaloids, which are usually treated as secondary metabolites. They are stored in each part of the plant—leaves, stem, root, and fruits of plants—but in different amounts. It was suggested that they are plants' waste product, but now evidence suggests that they play some important biological function in plants.
- Extracts of plants containing alkaloids were known and used because of their diverse activity by people from ages. But ages ago people did not know direct methods to isolate pure compounds from specified plant species. Alkaloids in plants usually exist as aqueous solution in tissues. To isolate them the method called extraction is usually used.
- Cocaine is a narcotic drug, which activity is not suitable for medical purposes. This alkaloid has an opposite effect than morphine. This compound produces in the human body a euphoric hyper arousal state, but high doses of it may lead to fibrillation and death.
- Alkaloids are an assembly of naturally occurring chemical composites, which typically comprise basic nitrogen atoms. They may also contain some neutral or weakly acidic compounds. Few synthetic compounds are also considered as alkaloids too. Apart from carbon, nitrogen, or hydrogen, alkaloids may comprise sulfur and rarely bromine, phosphorus, or chlorine.
- Alkaloids are mostly solids and are known to occur in higher plants. They are prevalent in the plants belonging to the following botanical families: Apocynaceae, Annonaceae, Amaryllidaceae, Berberidaceae, Boraginaceae, Gnetaceae, Liliaceae, Leguminoceae, Lauraceae, Loganiaceae, Magnoliaceae, Menispermaceae, Papaveraceae, Piperaceae, Rutaceae, Rubiaceae, Ranunculaceae, Solanaceae, etc.

MULTIPLE CHOICE QUESTIONS

1. Which forms of the ergot alkaloids are especially important?

a. Laevo
b. Dextro
c. cis
d. trans

2. How many pairs of alkaloid are present Ergot?

a. Five
b. Four
c. Three
d. Six

3. Ergot powder gives blue color with:

a. P-dimethylaminobenzaldehyde
b. Benzaldehyde
c. Cinnamaldehyde
d. Formaldehyde

4. Van-Urk's reagent chemically is:

a. P-dimethylaminobenzaldehyde
b. Benzoic acid+Cinnamic acid
c. P-dimethylbenzoic acid
d. Cinnamaldehyde

5. Ergometrine gives fluorescence in water:

a. Yellow
b. Green
c. Red
d. Blue

REVIEW QUESTIONS

1. Describe the classification of alkaloids.
2 .Explain the method of alkaloids.
3. Explain the alkaloids in human food and drinks.
4. Brief about alkaloids and their applications in pharmaceutical chemistry
5. Discuss about evodiamine.

Answer to Multiple Choice Questions

1. (a) 2. (d) 3. (a) 4. (a) 5. (d)

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CHAPTER 7

ALKALOIDS IN ARROW POISONS

LEARNING OBJECTIVES

After studying this chapter, you will be able to:

1. Describe the African arrow poisons
2. Know the south American arrow poisons
3. Learn about Asian arrow poisons

"Opiates are compounds derived from opium and include morphine, codeine, and a variety of related alkaloids. The term opioid is broader and includes all compounds (alkaloids or peptides) that have affinity for opioid receptors."

— Author: Alex S. Evers

INTRODUCTION

Arrow poisons are used to poison arrow heads or darts for the purposes of hunting and warfare. They have been used by indigenous peoples worldwide and are still in use in areas of South America, Africa and Asia. Notable examples are the poisons secreted from the skin of the poison dart frog, and curare (or 'ampi'), a general term for a range of plant-derived arrow poisons used by the indigenous peoples of South America. Poisoned arrows have featured in mythology, notably the Greek story of Heracles slaying the centaur Nessus using arrows

poisoned with the blood of the Lernaean Hydra. The Greek hero Odysseus poisons his arrows with hellebore in Homer's *Odyssey*. Poisoned arrows also figure in Homer's epic about the Trojan War, the *Iliad*, in which both Achaeans and Trojans used toxic arrows and spears. Poisoned arrows are referred to in the Book of Job in the Bible, descriptive of the sufferings experienced by the just man, Job.

The modern terms "toxic" and "toxin" derive from the ancient Greek word for "bow", *toxon*, from Old Persian **taxa-*, "an arrow".

A prime example of applied alkaloids-doubtless before medicinal use-are poisons for weapons, especially arrow poisons. The use of poisoned weapons is a fascinating aspect of man's attempts to gain mastery over a hostile environment, to provide food, and to protect himself from his animal and human enemies. Not least important, the biologically active plants yielded drugs and remedies for his diseases. A great deal of anecdotal information has already been lost, but, in spite of their often-prognosticated disappearance, plants containing toxic principles are by no means a thing of the past. Poisons derived from plants continue to be utilized not only in hunting food and against the depredations of wild animals, but also in tribal warfare, especially in Africa. Associated with this, at present, is the extensive use of constituents of the plants providing the hunting poisons as sources of medicines; in Africa these are among the most renowned plants of traditional medicine. The dreaded arrow poisons have provided medicine for effective therapy, or have been applied as tools in research. The best-known examples are ouabain and k-strophanthin for acute cardiac insufficiency, physostigmine for treatment of glaucoma and myasthenia gravis, d-tubocurarine as a muscle relaxant in anesthesia, reserpine as an antihypertensive and psychotropic drug, and ajmaline for cases of cardiac rhythm disturbances. Often, however, the therapeutic effects of the plant extract with its complex of compounds may be more beneficial than the effects of an individual compound. Certainly, the highly biologically active arrow poisons are many times more likely to possess therapeutically valuable compounds than are extracts from higher plants selected at random.

Sooner or later, arrow poisons will disappear, and therefore there is a real need to research these poisons and to evaluate their potential medicinal use before they disappear completely.

Two wider reviews exist that deal with arrow poisons, but because of the lack of detailed facts, both are more like general discussions: Perrot and Vogt's book (1913) in France and that of the toxicologist Lewin (1923) in Germany. Both are out-of-date, but they are of historical value in showing the extent to which such poisons have been used. Lewin's work is the more concrete and useful because he investigated the poison activity of many poisoned arrows from Africa, Asia, and South America (Leipzig, Museum of Ethnology). Finally, the literature is vast, very scattered, often incorrect, and of doubtful scientific value. A new, comprehensive review of African arrow poisons is given by Neuwinger (1996).

Peoples in all parts of the world, with the possible exception of Australia and New Zealand, have used poisoned weapons. Early evidence of that use comes from Egypt. In a tomb from the First Intermediate Period (about 2181-2050 B.C.), an arrow was found with largely water-soluble poison, the aqueous extract of which was cardioactive in mice like the cardenolide strophanthin and muscle-relaxant like the alkaloids in curare.

Arrow poisons can be roughly classified as follows:

- Africa, with a clear predomination of cardiac poisons (cardenolides)
- South America, with almost exclusively muscle-paralyzing (curarizing) poisons (alkaloids)
- Asia, mainly with cardiac poisons followed by tetanizing poisons (cardenolides, alkaloids)

With a few exceptions, African and most Asian arrow poisons as cardiac poisons are absolutely deadly; there is no antidote, in contrast to the South American curare poisons, which normally can be survived with the use of antidotes or artificial respiration.

7.1 AFRICAN ARROW POISONS

The first European killed by an African poisoned arrow was probably Nuno Tristan in 1447, at the mouth of the Gambia River in West Africa. The next centuries showed that poisoned arrows were nowhere else as common or used to such an extent as in Africa.

There are poisons containing essentially one plant extract, but more often the poisons consist of a mixture of plant materials with up to a dozen ingredients. Strongholds of such complex poisons are the forest zones. Generally, one or two of the components are the primary source of activity (base poison) and others are added for a variety of reasons: to increase the toxicity and effectiveness of the poison, e.g., by promoting its absorption from the wound; to enable it to adhere better to the arrowhead, but also for magical purposes and perhaps also to mask the real composition of the **poison** and to divert attention from the real toxic components. Today, most poisons consist of two to four components.

Keyword

Poisons are substances that can cause death, injury or harm to organs, tissues, cells, and DNA usually by chemical reactions or other activity on the molecular scales, when an organism is exposed to a sufficient quantity.

Depending on ethnic origin, there are a great variety of poisons, often with complicated compositions. The unique variety and complexity of African hunting poisons, together with a secrecy that appeared totally insuperable, has made their identification and investigation difficult. Emergence of scientifically useful results started only after the Second World War. In certain areas, even today, the composition and preparation of arrow poison is a closely guarded secret of certain individuals.

Lewin (1923) made a toxicological investigation, using laboratory animals, of many of the poisoned arrows in the Leipzig Museum. He showed for the first time the wide range of cardiac poisons among the African arrow poisons. Little was known, however, about the botanical base and almost nothing about the chemistry of the poisons and their active principles. Since the end of the Second World War, a few detailed and scientifically useful findings on the arrow poisons from certain areas have been published.

In 25 years of research (1968-1993), it has been possible to demonstrate the use of 265 different plants in the preparation of arrow poisons from all over the African continent. This comprehensive treatment of the botany, chemistry, pharmacology, and toxicology of the plants also discusses their use in traditional medicine.

7.1.1 Poisoned Weapons

The bow and arrow is a common weapon. The arrows consist either of hardwood, with the tip hardened in a fire (forest type) (Fig. 1), or they have an iron tip and plant material is wrapped around behind it for better adhesion of the poison (savannah type) (Fig. 2).

Experienced hunters never smear the poison on the iron tip but always behind it, so that fresh poison cannot be stripped off when the arrow penetrates the skin. Often the shaft behind the head is notched; when the wounded animal is running through the bush, the shaft easily breaks off and the poisoned tip stays lodged in the wound.

The same effect can be achieved by subdividing the arrow; a thin shaft as the holder of the poison is inserted into a hollow in the main shaft (Fig. 3 and 4). When bolting, the animal brushes off the main shaft which juts out a long way and the thin shaft with the poison stays in the animal and takes effect. In central Africa (southeast Cameroon, southwest Central African Republic, north Congo, north Gabon, and Equatorial Guinea), some tribes, mainly the pygmies, use the crossbow, a very efficient weapon for poisoned arrows (Fig. 5). It was also found among tribes in Southeast Asia in former times.

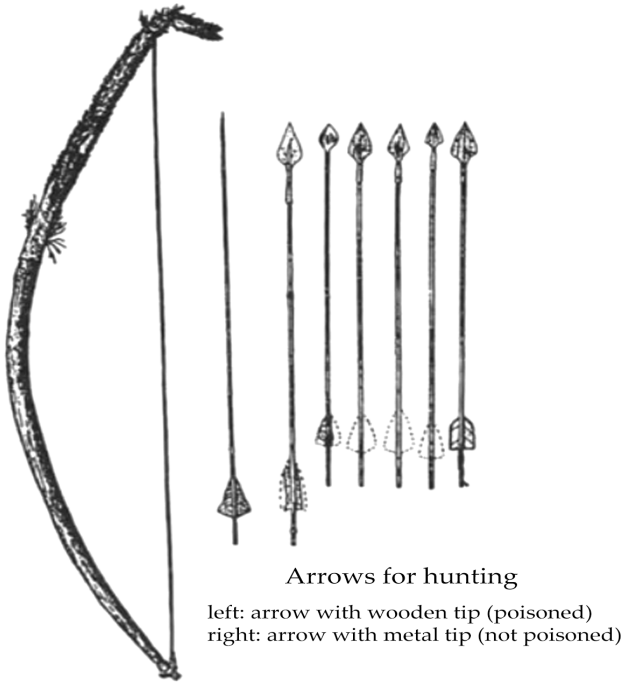


Figure 1. Bow and arrows for hunting of the Mbuti Pygmies in northeast Zaire.

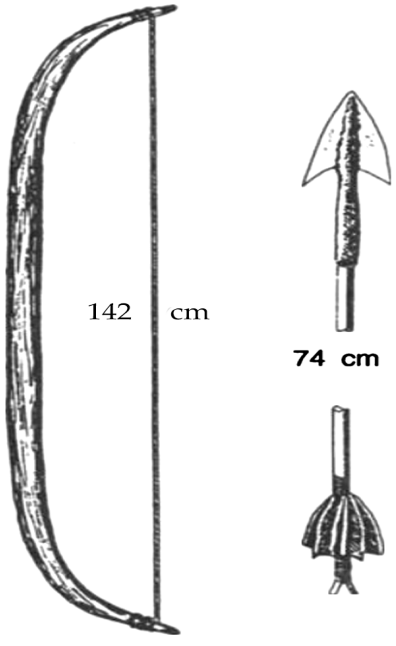


Figure 2. Bow and poisoned arrow of the Tjimba (Namibia/Angola).

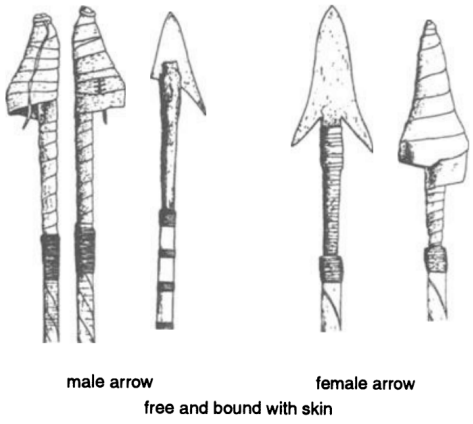


Figure 3. Poisoned arrows of the Hadza (Tanzania. Lake Eyasi).

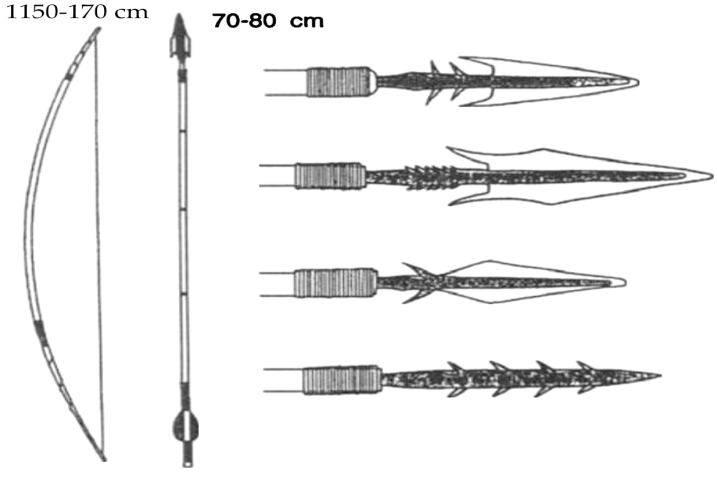
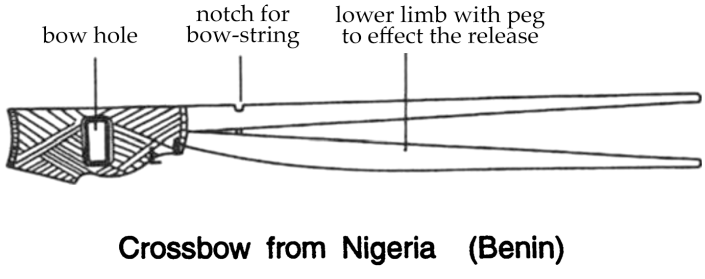
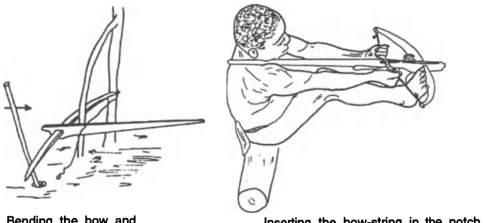


Figure 4. Bow and poisoned arrows of the Bambote (Zaire. Lake Tanganyika).



Crossbow from Cameroon, Gabon, CAR, Equatorial Guinea



African crossbow

Figure 5. The stock is split laterally throughout the greater part of its length. By bringing the upper and lower limbs together using a squeezing action, the square-sectioned peg fixed to the lower limb forces out the bowstring as it rises in the notch.

7.1.2 Poison Preparation

The secret composition and preparation of poisons is known by the medicine man, magician, herbalist, a certain individual or family and often today by the hunter himself. Poison preparation is considered to be a medicinal-magical art and even today poison making is sometimes still accompanied by mysterious rituals and taboos.

A poison-maker would never prepare poison while ill; in this condition his weakness could be transmitted to the poison and make it weak and ineffective.

example

Three principal methods of poison preparation are usual:

1. Boiling in water (aqueous decoction). The most common method of poison preparation is boiling the ground plant material in water. Often the boiling time is long, from some hours to a few days, with continuous addition of water. After concentration, a tarry mass remains; it is smeared in a thick layer on (wooden tip) or behind

(iron tip) the arrowhead. The technique of preparation ranges from primitive boiling in an open pot to a complicated poison extraction using specially built equipment.

2. Pounding of fresh ingredients and addition of glutinous sap. This method of poison extraction is mostly restricted to oil-rich plant parts, e.g., seeds and roots. The addition of glutinous sap helps to hold the poisonous mass together and to adhere it to the arrowhead. The adhesive power of the latex of cactiform Euphorbia, an almost constant component of arrow poisons, is especially strong. Euphorbia species are highly toxic on their own account, and are found almost everywhere.
3. Squeezing out fresh plant material. This method for wooden-tipped arrows is typical of the forest tribes, especially the pygmies. There is a large stationary press and a small mobile press for poison-making during the hunt. The plant material must be fresh and rich in sap: thin twigs, stem bark, and roots of young bushes and trees, Hanas, and herbaceous plants.

7.1.3 Plant Sources and Their Active Principles

Almost all of the primary active ingredients come from plants, only a few from animal sources (toads, snakes). Only the Bushmen of the central Kalahari Desert use exclusively an animal poison. It is prepared from the highly toxic larvae of several leafeating beetles (Diamphidia, Polyclada) and the larvae of their flesh-eating parasites (Lebistina), found deep in the ground beneath certain host bushes and trees.

Figure 6. Poison packed in a cigar-shaped parcel (12 x 2 cm) for sale (Kenya).

Bushmen from other areas mix the larvae with plant extracts. In African arrow poisons, cardiac poisons clearly dominate. At least 80% of the poisons are based on cardioactive components, mostly cardiac glycosides. The main plant sources are Acokanthera, Parquetina, Strophanthus (APS poisons, Fig. 7), and, from time to time or in special cases, Adenium, Mansonia, Calotropis, Pergularia, **Corchorus**, and Erythrophleum, the last an exception in that it contains cardiac alkaloids.

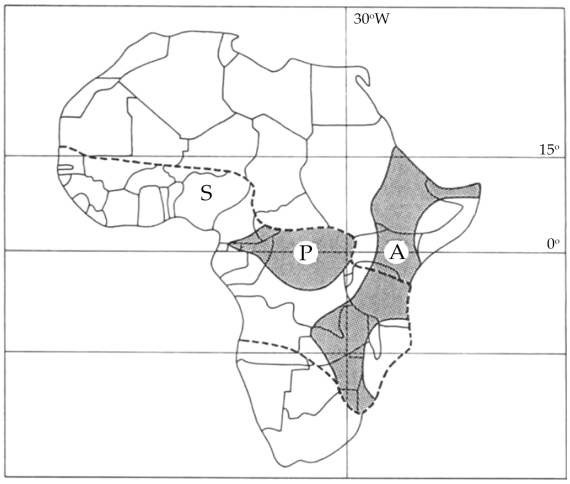


Figure 7. Distribution area of the APS (Acokanthera-Parquetina-Strophanthus) hunting poisons in Africa.

Keyword

Corchorus is a genus of about 40–100 species of flowering plants in the family Malvaceae, native to tropical and subtropical regions throughout the world.

Two other large groups of poisonous principles of African hunting poisons are triterpenoids, especially saponins (many plant genera), and diterpenoids, e.g., the highly irritant esters of diterpenoid alcohols (Euphorbia. Gnidia, Jatropha). Most saponins are able to cause hemolysis of erythrocytes using only a few micrograms per milliliter. They also have other toxic properties that do not always run parallel to their hemolytic properties. Saponin-bearing plants have been extensively employed as fishing poisons, e.g., plants with diterpenoid esters (Euphorbia species). Euphorbia latex is highly esteemed for its glutinous properties. Beyond that, the extremely irritant diterpene esters present may promote the absorption of the toxic principle through stimulation of peripheral blood circulation, in addition to being highly toxic in their own right. Capsicum frutescens has similar properties; its fruit or seeds are found in many arrow poisons throughout Africa. Its pungent capsaicinoid complex (related vanillylamides of carboxyl acids with capsaicin as the major compound) not only causes strong irritation externally and inflammation around the arrow wound but is also toxic, parenterally administered (LD_{50} = 1.1 mg kg^{-1} guinea pig i.p.).

Plants containing cyanogens often are used in the preparation of arrow poison; they release volatile hydrogen cyanide when the plant cell is damaged, but it does not survive the poison-making process. The same thing occurs with most animal venom, e.g., snakes, scorpions, or insects; they contribute nothing to

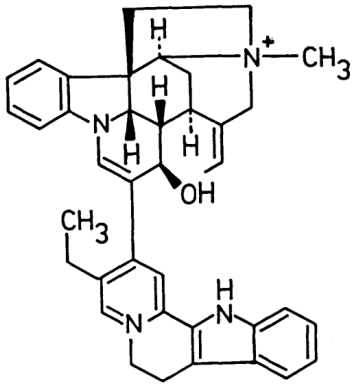
the toxic properties of the final product; they may be highly toxic in laboratory tests but they do not survive the usual long-lasting boiling process. Finally, such venoms are few in number. Only toad toxins (bufadienolides, similar to the cardenolides) do survive the boiling process and are active in the finished poison. Many other plant toxins used have a variety of activities, but their toxic effects are long term rather than acute, e.g., sesquiterpene lactones, iridoids, pyrrolizidine alkaloids, tannins.

It is unclear, and difficult to evaluate, to what extent the adjuvant plants contribute to the activity of the poison. It is known that the finished poison is often essentially more active than the concentrated extracts of the corresponding plants; this may be related to possible interactions between the various compounds during the elaboration process of the poison. The restriction of this review to alkaloid-bearing plant poisons thus gives a fairly one-sided and distorted picture of African arrow poisons. With a few exceptions, alkaloid bearing plants play a dominant role as adjuvants; several could well be-and some, indeed, are-base plants of a poison.

Strychnos species are a major group of arrow poison adjuvants, their roots being used especially in the equatorial forest regions of central Africa.

Keyword

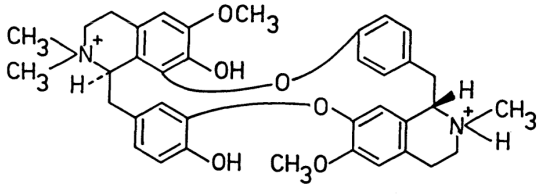
Strychnos is a genus of flowering plants, belonging to the family Loganiaceae (sometimes Strychnaceae). The genus includes about 100 accepted species of trees and lianas, and more than 200 that are as yet unresolved.



1

The Nyambo, a small tribe of hunters in Rwanda living along the Akagera River which forms the border with Tanzania, use one of the most surprising plants in preparing arrow poison. Their poison is famous because of its powerful action. Base plants are the cardenolide-bearing Acokanthera schimperi and the alkaloid-

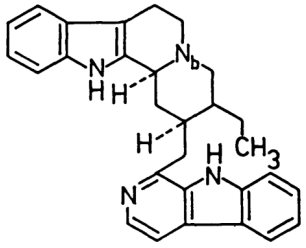
bearing Strychnos usambarensis. The root bark of the latter plant, widespread but until that time little noticed, provided a chemical and toxicological surprise: it was found to act like a high-quality curare poison; indeed, alkaloids previously only known in South American calabash curare and Strychnos species were obtained from this African Strychnos and the poison derived from its roots: C-dihydrotoxiferine, C-curarine, C-calebassine (see structures 50, 53, and 56, respectively), and a new one, which was named afrocurarine (1) because of its occurrence only in "African curare." The strongly curarizing alkaloids are bis-quaternary dimeric perfectly symmetrical indole bases. In contrast, afrocurarine is the first example of a bis-quaternary dimeric asymmetrical indole base; it is composed of a strychnine part (type dihydrofluorocurarine) and a corynane part joined via an enamine bridge. The four bases are potent neuromuscular blocking agents with the final result being complete paralysis of the skeletal or striated muscle apparatus. Afrocurarine was found to be less potent than the other three, probably because of its plane, asymmetrical structure and its stereochemistry. For pharmacology and mechanism of action of these compounds. C-curarine is the most active alkaloid, followed by C-dihydrotoxiferine, C-calebassine, and afrocurarine; the last has only 5% of the activity of (+)- tubocurarine (2). In addition to the high toxicity of the four alkaloids as quaternary bases, they are easily water soluble and therefore the plant is equally suited to the poison-makers as a plant with easily water-extractable cardenolide-glycosides.



2

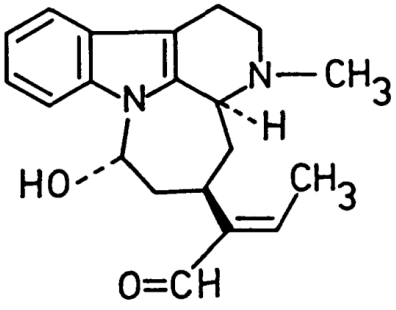
Currently, S. usambarensis is chemically the best-known Strychnos species; more than 60 alkaloids are indicated and 37 characterized. Beyond the 4 curare alkaloids, the roots of the plant contain many other tertiary and quaternary bases with multiple activities. The main representative of the tertiary root alkaloids is usambarensine (3) with an atropine-like activity. The other plant parts used in the Nyambo poison, especially the leaves, contain other alkaloids which, with a single exception, are tertiary dimeric asymmetrical bisindole alkaloids of the corynanthe type (usambarine and usambarensine derivatives). These alkaloids do not differ markedly in their structures, and have different biological activities than do the root alkaloids. The exception is akagerine (4), which belongs to a new type of alkaloid. Only two quaternary bisindoles have been found in the leaves to date; they correspond to the Nb-methyl derivatives of 10- and l l-hydroxyusambarine. In pharmacological studies akagerine was reported to be a

convulsant agent, but was 100 times less potent than strychnine (5). A number of the leaf alkaloids possess high in vitro activity against pathogenic protozoa (amoebiasis, malaria) and can exert a strong cytotoxicity on cancer and noncancer cell lines.



3

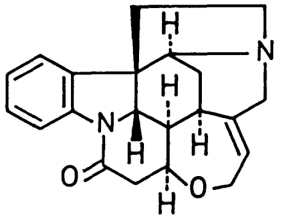
Another Strychnos species, S. camptoneura, whose root bark we have found as an arrow poison in the southwest of the Central African Republic, has toxicological analogy with S. usambarensis roots. It is not clear which of the ten compounds isolated to date is responsible for the strong muscle-paralyzing activity, but camptoneurine (6) may contribute to the effect.



4

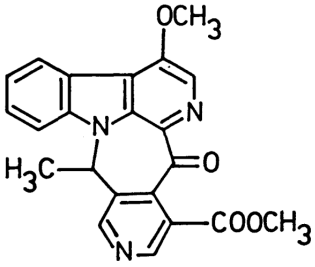
Triclisia dictyophylla (Menispermaceae), an arrow poison plant from central Africa, has curarizing activity similar to that of the Strychnos species; 25 mg kg^{-1} of raw quaternary alkaloid mixture of the stem bark caused death in rats after 3 min. Triclisia species have been found to be excellent sources of bisbenzylisoquinoline alkaloids with the quaternary bisbenzylisoquinoline N,N'-dimethylphaeanthine (7), the main compound of this type in T. dictyophylla, responsible for the curarizing effects (LD_{50} = 2.63 mg kg^{-1} mouse i.v.). It has about 9% of the activity of d-tubocurarine (2) (LD_{50} = 0.14 mg kg^{-1} mouse i.v.); 25 mg kg^{-1} i.p. kills rats in 1 min by asphyxia. Another **Menispermaceae**, Cissampelos mucronata, a thin twiner or trailer, which

enters into the preparation of Strophanthusbased arrow poison in northern Nigeria, also produces (root extract) curariform paralysis in animal muscle preparations. The root contains a number of tertiary and quaternary bisbenzylisoquinoline and protoberberine alkaloids. dl-Hayatine (8), dl-hayatinine (9), d-isochondodendrine, and I-bebeerine (I-curine), the major representatives, have a tubocurarine skeleton. Some quaternary alkaloids are responsible for the curarizing action.



5

In India, the quaternary N,N′ -dimethylhayatine (hayatine methiodide) was tested clinically in 100 cases as a substitute for d-tubocurarine. At the same dosage the duration of effect is about the same while the strength of effect is about half that of d-tubocurarine.

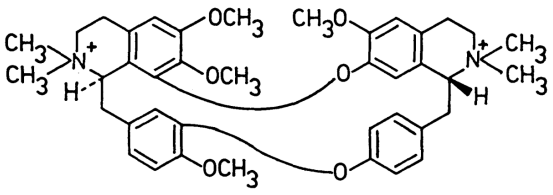


6

The aporphine bulbocapnine (9), isolated from stem bark and leaves, acts peculiarly on the CNS. It induces catalepsy, the so-called "bulbocapnine rigidity" movement disorder, while maintaining the muscle tonus and the startle reflexes. It also acts hypnotically. Death results from paralysis of the muscles of respiration. It is used therapeutically in the treatment of tremor and of Meniere's disease.

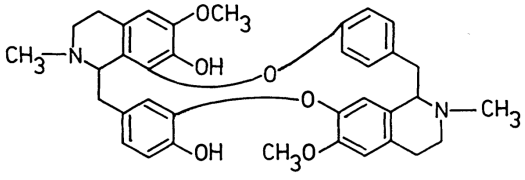
Keyword

Menispermaceae is a family of flowering plants. The alkaloid tubocurarine, a neuromuscular blocker and the active ingredient in the 'tube curare' form of the dart poison curare, is derived from the South American liana Chondrodendron tomentosum.



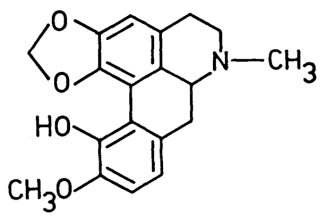
7

The active principles from S. icaja, a Strychnos species, are quite different from those of S. usambarensis. The red root of this large liana forms an important ingredient of arrow poisons in the Central African Republic and Zaire. S. icaja is, however, primarily an ordeal poison and at present is still used in the Central African Republic, Gabon, Congo, and Zaire. Strychnine (5) (LD_{50}. = 0.47 mg kg^{-1} mouse s.c.) and 12-hydroxystrychnine (LD_{50} = 0.56 mg kg^{-1} mouse s.c.) are responsible for the high toxicity of the roots. Strychnine is always accompanied by minor alkaloids, but with only a weak tetanizing activity. e.g., brucine and u- and l3-colubrine.



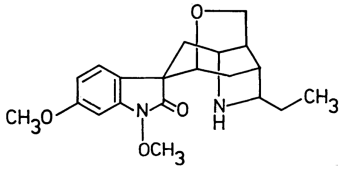
8

Strychnine belongs to the analeptics. It acts selectively as a competitive agonist and blocks postsynaptic receptors of the inhibitory transmitter glycine in the spinal cord and motoneurons. It may also have a potent presynaptic action preventing release of the inhibitory transmitter, thus, strychnine (like tetanus toxin) acts by inhibition of inhibitory actions: the excitatory synapses can then exert their unrestrained action, which results in hyperexcitability and tonic convulsions and finally death through respiratory or spinal paralysis. With intravenous injection, the onset of convulsions in the case of brucine is almost instantaneous and death occurs in tonic seizures, but its toxicity is distinctly lower (LD_{50} = 12 mg kg^{-1} mouse i.v.). Recent investigation clearly showed that the aqueous extract of the root bark has, besides the predominantly convulsive effect, a conflicting effect as well, which paralyzes the neuromuscular junction and the cardiac muscle. Indeed, a muscle-relaxing compound has been isolated from the roots: bisnordihydrotoxiferine. This compound is also a sedative, having a marked depressant effect on the CNS. The aqueous quaternary alkaloidal extract of S. icaja root bark exhibited a high cardiotoxicity. The leaves of S. icaja contain quite different alkaloids with lesser activity (derivatives of icajine and vomitine). Only pseudostrychnine (3-hydroxystrychnine) acts in a similar but weaker way to strychnine.



9

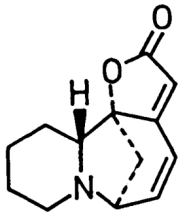
Another Loganiaceae used as an arrow poison plant in central Africa is Mostuea (brunonis and hirsuta). M. brunonis is reported to contain the highly toxic oxindole alkaloid gelsemicine (10) (LD_{50} = 0.05 mg kg^{-1} rabbit i.v.) as a major compound of the leaves and twigs. It affects the respiratory center alone, has no effect on the vagus, but depresses the motoneurons of the brain and spinal cord, resulting in a generalized muscle weakness. Respiratory arrest after fatal doses is not caused by paralysis of the center but is attributable to paralysis of the spinal motoneurons innervating the respiratory muscles. The potent cholinesterase blocker sempervirine, an anhydronium base with the yohimbine nucleus, has been isolated from the roots.



10

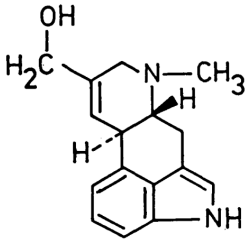
A very interesting ingredient of hunting poisons (Nigeria, Zambia) is Securidaca longepedunculata. The root juice, however, is more commonly used as an ordeal poison, especially in the Central African Republic and southeast Zaire. This Polygalaceae is a common suicide poison and probably the best-known African abortifacient. The reputation of its roots as a snakebite antidote is legendary. In 1980, this activity was scientifically confirmed and the active principle was identified as a protein similar to that found in the venom of the spitting cobra (Naja nigricollis). The strong-smelling roots contain methylsalicylate and toxic triterpenoid saponins and the stem bark contains alkaloids. The major alkaloid isolated from the bark is the tricyclic securinine (11r) with an α, β-unsaturated butenolide ring as in cardenolides. Securinine is highly toxic (LD_{50} = 6.23 mg kg^{-1} t mouse i.v.); it has two conspicuous characteristics: an extremely rapid initiation of action both orally and by injection and an unusually rapid inactivation in the organism. In mice and rats killed immediately after parenteral administration, 5-30 min after an i.p. injection, no securinine could be detected. It acts primarily on the spinal cord, causing an enhancement of reflex activity and increase in muscular tone in animals; it also influences the functions of the autonomic nervous system. In toxic

doses, securinine induces powerful tonic convulsions involving all skeletal muscles, similar to strychnine convulsions. The dosage causing convulsions is very close to that causing death (respiratory arrest). In rats, i.v., it acts 25 times more strongly than strychnine as a CNS stimulant. Surprisingly, ergot alkaloids were found in the roots in 1992, among them elymoclavine (12). Such alkaloids are very rare in higher plants. Elymoclavine belongs to the "excitor" group of ergot alkaloids which cause central stimulatory syndromes consisting of sympathetic excitation like those of LSD; it is the most potent stimulant in this group. Dihydrogenation, however, converts excitation to depression: dihydroelymoclavine belongs to the "inhibitor" group and causes mainly central depression.



11

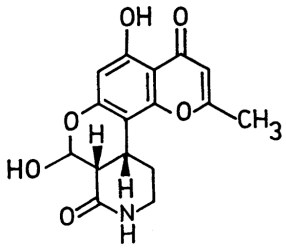
Derivatives of securinine, e.g., virosecurinine, norsecurinine, allosecurinine, are to be found in a plant of quite another family, the Euphorbiaceae Flueggea virosa, an ingredient of a Strophanthus-based arrow poison in Nigeria and Benin. The toxicity of allosecurinine, at least, is lower than that of securinine. Some of these derivatives demonstrated significant cytotoxicity in tumor cell lines.



12

Another commonly used arrow poison plant, which has a good reputation in Nigeria for the treatment of snakebite, is Schumann iophython magnificum (Rubiaceae). For this purpose the stem bark juice is applied to a small incision made at the site of the snakebite. Recent studies have demonstrated that polar extracts of the stem bark show activity against cobra cardiotoxin, both in vivo and in vitro, but not against a

curaremimetic neurotoxin component. Crude extracts caused contracture of muscle accompanied by inhibition of the cardiotoxin effect. Ten related chromone alkaloids based on the chromone noreugenin were isolated from root and stem bark. The direct contracture was related to the alkaloid N-methyl-schumannificine (13, N-Ch_3) , while the anticardiotoxin effect was caused by a peptide that is similar in amino acid composition to the cardiotoxins present in snake venom. Another alkaloid, schumannifoside, was also reported to reduce the lethal effect of black cobra venom through a chemical inactivation reaction.

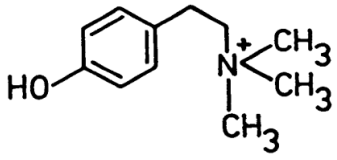


13

InTanzania, west of Kilimanjaro, Masai-Ndorobo hunters prepare an arrow poison from the herbaceous Compositae Vernonia hildebrandtii and the Rutaceae Zanthoxylum chalybeum. The former is of special interest because it contains an alkaloid of stillunknown structure which in laboratory animals, especially rabbits s.c., caused a continuous decrease in cardiac contraction, toxic vagal stimulation, profound movement disorders as far as paralysis similar to that of a curarized animal, and death. A narcotic stage lasting several hours was also observed. Zanthoxylum (Fagara) species are often found as ingredients of arrow poisons, e.g., Z. gilletii in the Ivory Coast and Cameroon, Z. chalybeum in Rwanda and Tanzania. Zanthoxylum species proved to be rich sources of tertiary and quaternary alkaloids, primarily isoquinolines of various structure types (derivatives of l-benzyltetrahydroisoquinoline, benzophenanthridines, aporphines, and, to a lesser extent, quinolines). They exhibit a wide range of biological activity and some of them are without doubt highly toxic; the quaternary alkaloids, in particular, are responsible for the toxicity. The simple phenylalkylamine candicine (14), perhaps the most toxic Zanthoxylum alka loid, causes a curarelike effect in dogs and frogs i.v, at higher doses (6 mg kg^{-1} dog i.v.), with death by asphyxia. The aporphines magnoflorine (15), N-methylisocorydine (menisperine) (16), and N-methylcorydine (17) also act as neuromuscular blocking agents antagonized by cholinesterase inhibitors such as physostigmine. The stem bark alkaloid berberine also antagonizes the **curare** effect and shows many pharmacological activities. The quinoline alkaloid quinidine exerts exclusively negative actions on the heart.

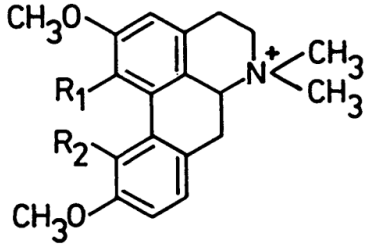
Keyword

Curare is a common name for various plant extract alkaloid arrow poisons originating from indigenous peoples in Central and South America.



14

Many of these tertiary and quaternary isoquinoline and quinoline alkaloids are also found in the Annonaceae Annickia polycarpa (Enantia polycarpa), an adjuvant to Mansonia altissima-based hunting poisons of the Guere tribes in the southeast Ivory Coast.



Three other Annonaceae also make a significant contribution to African arrow poisons: Annona senegalensis in Nigeria (base: Strophanthus hispidus), Pachypodanthium staudtii in the Ivory Coast (base: Mansonia altissima), and Cleistopholis glauca in Zaire (base: Parquetina nigrescens).

Almost all of the alkaloids contained in them possess an isoquinoline-derived structure. In Cleistopholis species especially , I-bisbenzylisoquinolines have been found.

A 95% alcoholic extract of the stem bark of Annona senegalensis was fatal to rats with an MLD of 100 mg kg^{-1} i.p., death occurring in 3 hr. The stem bark of Pachypodanthium staudtii showed significant toxicity in rats: 500 mg kg^{-1} of crude bark i.p. killed rats within 30 min. Most of its alkaloids are toxicologically unknown.

(15) $R_1 = R_2 = OH$
(16) $R_1 = OCH_3$, $R_2 = OH$
(17) $R_1 = OH$, $R_2 = OCH_3$

In the Central African Republic we found a poison hitherto only known as a poison in native judicial procedure: Physostigma venenosum. The bean-shaped seeds, known as "Calabar beans" or esere (both the ordeal poison and the seeds are called esere in Calabar) is one of the most notorious trial by ordeal poisons in West Africa, although the area of use is relatively small. It is mainly found in southeast Nigeria and neighboring Cameroon. Although strictly forbidden, the trials were still being held occasionally in the 1970s. Presently, the poison is mostly given to an animal as a representative of the accused.

The principle in this type of ordeal was as follows: The poison was given to a suspect to drink or eat. He was assumed to be innocent if he could reject, i.e., vomit, the poison. If the poison was retained, the person was considered to be guilty and was allowed to die from the effects of the poison or was disposed of in some other way. On the other hand, the fetishists who conducted the trials were by no means scrupulous; they had quite a number of "variables" to hand and could control the strength of the poison and therefore could direct the results of "ordeals" to a great extent.

In 1952-1953, the anthropologist Simmons (1956) researched in Calabar and produced the following more recent description of the esere trials:

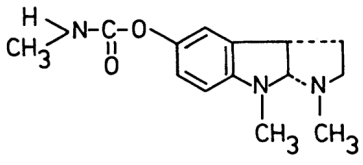
The Efik believe that the esere or Calabar bean possesses the power to uncover witchcraft and to destroy it. A suspect is given 8 ground beans in water to drink. If he is guilty, his mouth trembles and foam flows out of his nose. He is innocent if he raises his right hand and vomits everything up. If the poison still has an effect in spite of his innocence, an antidote is given in the form of a decoction of excrement mixed with water which has been used to wash female genitalia. If someone dies from the ordeal, the eyes are removed, a perforated clay pot is inverted on the head and the corpse is normally thrown into the forest, or it is buried head-down or burned and the ashes buried. By these methods it is hoped to stop the spirit of the witch returning. If the trouble continues in a village, however, suspicion falls on a recently-dead relative as an undiscovered witch. The family exhumes the corpse, in order to determine the extent of the putrefaction. If the corpse appears fresh with very little decay, then the dead person is seen as a witch. The corpse must then be burned.

P. venenosum is a further African poison plant that achieved medicinal importance and is a prime example of the transformation of an extensively used deadly poison to a

highly beneficial medicine and an important tool in experimental pharmacology. Up to now, seven indole alkaloids have been reported as occurring in the seeds. They are understood as tricyclic methylcarbaminates.

Did You Know?

The main alkaloid present in the beans was first isolated in a pure form in 1864 in Germany and called physostigmine and I year later in France as eserine.



Physostigmine

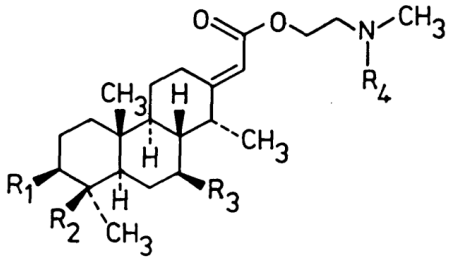
18

Physostigmine (18), or more correctly (-)-physostigmine, is an indirect-acting para sympathomimetic, a reversible cholinesterase inhibiting agent. It is the strongest cholinesterase blocker known. Alkaloids with such an activity are very rare among plants. As a tertiary amine it can penetrate the blood-brain barrier and thus produce central effects. Death occurs from respiratory arrest, in high doses from heart failure (LD_{50} = 0.45 mg kg^{-1} mouse i.v.).

Physostigmine was the first alkaloid proven to act through inhibition of an enzyme. It has contributed most to our understanding of neurohumoral chemical transmission; it played an important role in understanding the kinetics of the enzyme, cholinesterase, and has contributed to the elucidation of the configuration of the active center of this enzyme. Although replaced by synthetic compounds in many cases, physostigmine has had several uses in clinical medicine: in ophthalmology, as a miotic and in glaucoma; in myasthenia gravis; in testicular paralysis; as an antidote to central poisoning with atropine and other parasympatholytics. Alone or together with atropine, it is protective against organophosphate poisoning. Lastly, it is considered as the prototype of the carbamate insecticides, but is itself a poor insecticide. Because of its high toxicity and its very small therapeutic index, LD_{50}/ED_{50}, physostigmine is a dangerous substance. Norphysostigmine and physovenine are similarly toxic.

A plant of still more importance than Physostigma venenosum as an ordeal poison for determining innocence or guilt is the Caesalpiniaceae Erythrophleum, especially the species E.

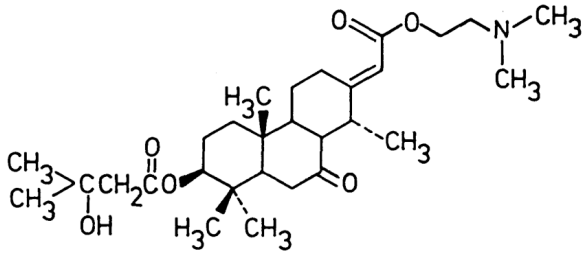
suaveolens, but also E. ivorense and E. africanum . If macerated or boiled, the red inner stem bark provides a blood-red extract which gives the tree its common name of "red water tree." This legendary extract is the most common and most notorious ordeal poison in Africa. The stem bark or the seeds are used as an arrow poison or poison ingredient mainly by the Mbuti Pygmies in northeast Zaire but also by other tribes in other African countries.



Erythrophleum-Alkaloids

19

Chemically the Erythrophleum alkaloids are tricyclic compounds with a semicyclic, unsaturated side chain as the carrier of the nitrogen, built up from a diterpenic acid which is mostly esterified, more rarely an amide bonded to a secondary or tertiary aminoethanole (19). Both series of ester types, N-methylaminoethyl esters and N,N-dimethylaminoethyl esters, are divided into groups according to different substitutions at C-3, C-4 and C-7 of the tricyclic nucleus.

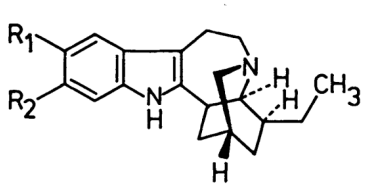


Coumingine

20

The Erythrophleum alkaloids are quite similar to Strophanthus cardenolides in their action as cardiac stimulants (positively inotropic and negatively chronotropic) and in their high degree of toxicity. Some of these alkaloids, especially coumidine and coumingine (20), have almost the same potency as ouabain. The therapeutic index of

Erythrophleum alkaloids is higher and the enteral absorption better than that of the cardenolides, but their effect is very brief. Change of the ester to an amide causes an opposite effect: these noralkaloids have a negative inotropic action on the heart. Some of the alkaloids are very potent local anesthetics, e.g., cassaine and cassaidine. It is remarkable that the watersoluble extract of the stem bark has been shown to have strong spasmogenic effects on smooth muscle, potentiated by physostigmine and blocked by atropine; in very high doses it causes contracture of skeletal muscle.

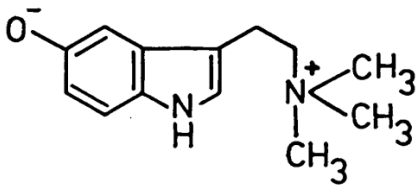


One of the most spectacular plants used in central African arrow poison is Tabernanthe iboga. The iboga plant is the most important African hallucinogen and up to now the only Apocynaceae to be utilized as such. This psychoactive drug is of great social importance, especially in south Cameroon, Gabon, eastern Equaitorial Guinea, the Congo, and western Zaire. T. iboga has the reputation of being a truly divine plant. It is employed by secret societies in initiation rites and messianic-prophetic associations for magicalreligious purposes. The most famous of those are the Bwiti cult of the Fang and the Ombudi initiation association of the Mitsogo in Gabon. Sorcerers take the drug before communicating with the spirit world or seeking advice from ancestors. The drug liberates from anxieties and conveys messages from ancestors and spirits to the iboga-eaters. Recently, cannibalism has been found in connection with the iboga cult in Gabon.

(21) $R_1 = H, R_2 = OCH_3$

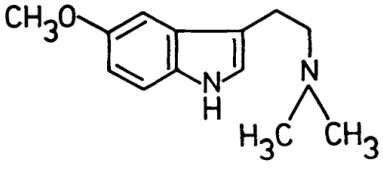
(22) $R_1 = OCH_3, R_2 = H$

The roots contain indole alkaloids, the so-called iboga alkaloids; they comprise up to 6% of the dried root bark. The major representatives are the alkaloids that mostly occur together-ibogaine, ibogamine, and tabernanthine. The exhaustively studied ibogaine (21) mainly exhibits CNS and cardiovascular activities. It acts as a cholinesterase inhibitor, a strong central stimulant, and a hallucinogen; its hallucinogenic effect is similar to that of LSD but weaker. The LD_{50} of tabernanthine (22) and ibogaine is 0.38 and 0.42 mg kg^{-1} mouse respectively.



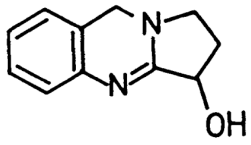
23

A well-investigated arrow poison adjuvant in Nigeria and Cameroon is Mucuna pruriens. The plant is characterized by indole alkaloids and indole-3-alkylarnines: N,Ndimethyltryptamine and derivatives, bufotenidine (23), 6-methoxyharmane, nicotine, and choline . Mucuna seeds are the richest source of t-dopa known. It is very interesting that Indians on the upper Orinoco river utilize a dart poison with the sole main ingredient of 5-methoxy-N,N-dimethyltryptamine (24) (up to 8%), as it occurs in M. pruriens. The tOXICIty LD_{50} of the most toxic representatives, bufotenidine and 5-hydroxy-N,N-dimethyltryptamine, is 1.3 mg kg^{-1} mouse i.v, N,N-dimethylated tryptamine derivatives are very potent psychornimetic drugs.



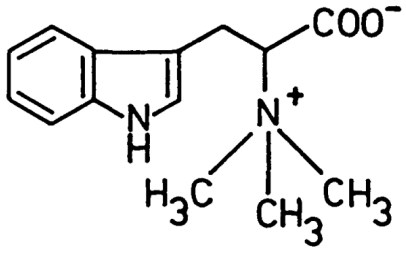
24

Three types of alkaloidal constituents-β-phenylalkylarnines (e.g., ephedrine, β-phenethylamine), quinazoline s (e.g., vasicine, vasicinone), and carboxylated tryptarnines (e.g., hypaphorine)-in addition to betaine and choline have been isolated from Sida cordifolia, a plant that is an ingredient of arrow poisons in northern Nigeria. Their toxicity is moderate. In the last few years, vasicine (25) has achieved importance as a powerful uterine stimulant, like oxytocin in activity. It is very selective in its action and accumulation in the uterus. The methylester iodide of hypaphorine (26) has curarizing properties.



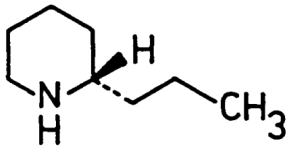
25

Phenylalkylamine alkaloids, as well as piperidine alkaloids, contained in the Aloe species are often observed as arrow poison adjuvants. In some of them the simple piperidine alkaloids (+-)-(S)-coniine (27) and -γ-coniceine (28), both highly toxic, were detected. In 399 B.C., Socrates died from poisoning by coniine, which has nicotinic as well as curarising activities leading to a painful death: vomiting followed by paralysis of the striated musculature and nerve centers, beginning at the legs and ascending until finally death occurs from central respiratory arrest while fully conscious. γ-Coniceine is still more powerful than coniine with mainly nicotinic actions and with an oral LD_{50} for mice reported as 12 mg kg^{-1}.



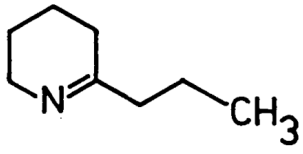
26

A very toxic plant that enters into the preparation of arrow poisons in the southwest Ivory Coast is the alkaloid-bearing Apocynaceae Tabernaemontana crassa. A crude ethanol extract of the stem bark at a dosage of 250 mg kg^{-1} i.p. killed rats in 30 min; similar results were found with a root extract at a dosage of 175 mg kg^{-1}. The stem extract causes reduction of motor activity and muscle paralysis; this is the reason for its use in traditional medicine to calm fits and insanity.



27

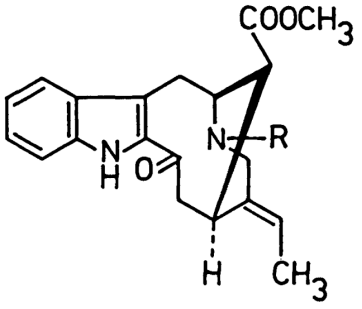
Some of the 34 indole alkaloids from the root bark, stem bark, and seeds have been investigated pharmacologically, but their physiological effects do not match the activity of the general extract. The majority of the bases belong to the iboga type and within that to the coronaridine series. One of the main compounds is ibogaine. Most of the alkaloids tested (ibogaine, ibogamine, coronaridine, voacangine, isovoacangine, voacristine, and vobasine) are central stimulants; four of the seven compounds caused catalepsy. All of the bases tested showed a characteristic two-stage hypotensive effect in rats. Perivine, coronaridine, and vobasine caused muscle-relaxant activity in rat muscle preparations. The effect is not nondepolarizing like curare, but like that shown by succinylcholine, caused by a depolarizing neuromuscular blocking mechanism. The three alkaloids have only a moderate grade of toxicity. The leaf decoction is in popular use as a local anesthetic during painful traditional orthopedic procedures, especially bone resetting. Recently the effect was scientifically confirmed. The alkaloids vobasine (29) and perivine (30) may be responsible; the anesthetic activity of the latter is twice that of cocaine.



28

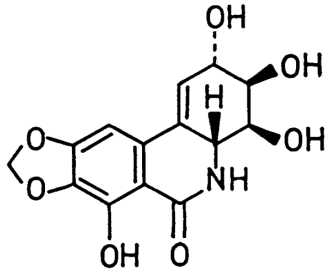
Scadoxus, Crinum, and Boiiphone are arrow poison plants that bear in their tubers the so-called "Amaryllidaceae alkaloids," a unique large group of bases that have so far been found only in that family. A review is given by Martin (1987). The numerous alkaloids with a great variety of structure types are mostly derived from a 5,10β-ethanophenanthridine nucleus. The most common representative is lycorine. They are mostly neurotoxins similar to the tropane alkaloids with high to moderate toxicity. The toxicity of the most toxic alkaloid narciclasine (31) (pyrollo[de]phenanthridine type) from Scadoxus multijlorus is 5 mg kg^{-1} mouse s.c. Galanthamine (32) (dibenzofuran

type, LD_{50} = 11.1 mg kg^{-1} mouse s.c.) acts as an antagonist to curare arrow poison and exhibits analgesic activity in mice comparable to that of morphine. S. multijlorus and S. cinnabarinus as well as Crinum zeylanicum are arrow poison ingredients in west and central Africa. Boiiphone disticha, formerly widely used in southern Africa, is now used only in southern Zaire for this purpose. Boophone poisoning resembles acute drunkenness with impaired vision and hallucinations related to poisoning with tropane alkaloids of Datura. The old Boer name "malgif" refers to this mad and confused behavior of animals poisoned with the Boophone bulb.



(29) R = H
(30) R = CH_3

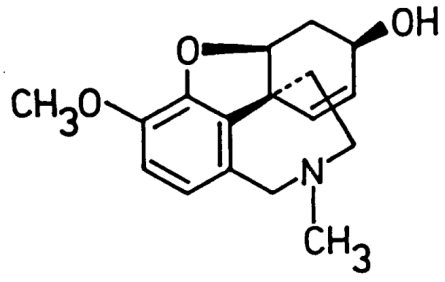
The tropane alkaloids, another type present in arrow poisons and also in ordeal poisons, come from Datura species. Main representatives are (-)-hyoscyamine and (-)-scopolamine (hyoscine) (33). They differ in the β-oxido group at position C-6, C-7.



31

Besides these, phenylalkylamines and indole-alkylamines, e.g., noradrenaline, 5-hydroxytryptamine (serotonin), 5-hydroxyindoleacetic acid, were found in different plant parts. Climatic variations have a very considerable influence on the alkaloid content. Arrow poisons based on Strophanthus hispidus or Acokanthera schimperi with fruit of D. stramonium or the very similar D. metel have been found in Kenya and Burkina Faso. Tropane alkaloids are widely distributed in the Solanaceae family. Both compounds are isomerized to the racemic forms. Thus, the well-known commercial atropine is racemic dlhyoscyamine. Hyoscyamine and scopolamine are competitive

antagonists of the actions of acetylcholine and other muscarine agonists, both have similar peripheral, parasympatholytic properties, but they differ quantitatively in antimuscarinic actions and in action on the CNS. Hyoscyamine and atropine have CNS-stimulating but also -depressing activities, with toxic doses resulting in irritability, disorientation, hallucinations, and delirium; with still larger doses, stimulation is followed by depression, coma, and medullary paralysis. Scopolamine tends to be more CNS-depressant. In both cases, death occurs from respiratory paralysis. Only a moderate grade of toxicity, however, was found for the two alkaloids: LD_{50} = 95 mg kg^{-1} for (-)-hyoscyamine and 163 mg kg^{-1} for (-)-scopolamine in mice i.v. On the other hand, the maximum tolerated dose of the alcoholic extract of D. metel (whole plant) for mice i.p. is 100 mg kg^{-1}. Its use as an ordeal poison is remarkable in Uganda (Banda tribe) and Tanzania (Pare and Chagga), Senegal, and Botswana. In Uganda, the accused is supposed to divulge his crimes under the influence of the central, psychoactive effects of Datura alkaloids (truth or chatter drug). In the various plant parts, both alkaloids are to be found in varying proportions. There is also a direct relationship between plant growth and alkaloid content; in young plant parts, scopolamine predominates, in mature parts hyoscyamine. In flowers and seeds scopolamine is usually the major constituent. When scopolamine predominates, the accused will go into a state of being half-awake and in a trance; he will answer all of the sorcerer's questions about things that he would rather keep secret. This psychic state is especially aspired to by the sorcerers. In Botswana, losing consciousness is a sign of guilt. In Senegal, the leaves are pounded in water and the extract drunk. There have been cases of such poisoning s treated in the Dakar neurological clinic in recent times.



32

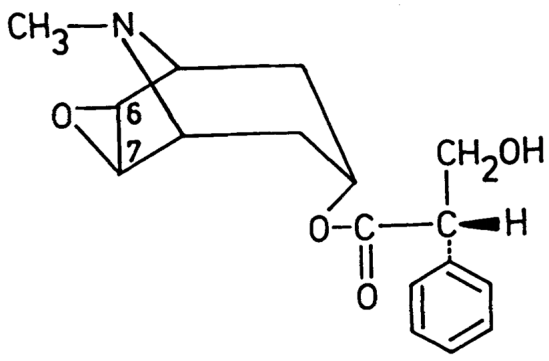
Most African wild species of Dioscorea (yams) are known to be highly toxic.

Five species have been positively proved as ingredients of arrow poisons: D. dumetorum, D. bulbifera, D. quartiniana, D. sansibarensis, and D. smilacifolia. The Dioscorea is best known for the steroid glycosides its species contain. Many of them, however, also contain large amounts of alkaloids based on an isoquinuclidine ring system, such as dioscorine and dihydrodioscorine, the major poisonous principles of such species of

Remember

The tubers must be carefully detoxified by soaking for a couple of days in running or salt water or boiling overnight to degrade the toxic alkaloids, unstable in heat.

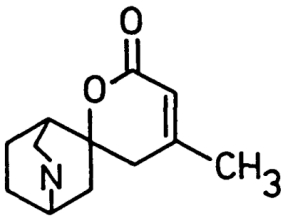
Dioscorea. Dioscorine (34) contains an N-methyl group and a lactone ring at C-5. D. dumetorum bears dume torine as a further alkaloid, an α,β-unsaturated γ-lactone joined to a piperidine ring via a CH2 bridge. Dioscorine has a picrotoxin-like convulsant effect and induces a general paralysis of the CNS. The ethanol extract of the tuber exhibited in mice generalized convulsions, followed by tonic-clonic convulsions, and, in lethal doses, death by extensor spasm within 10 min. The alkaloid-containing fraction is hyperglycemic; the crude hydroalcoholic extract, especially the glycosidic fraction, is contrastingly hypoglycemic. In Nigeria, a decoction of the tuber, prepared by steeping the peeled tuber in native gin for 3 days and boiling until the color changes from yellow to brown, is successfully used in the treatment of clinical diabetes mellitus.



33

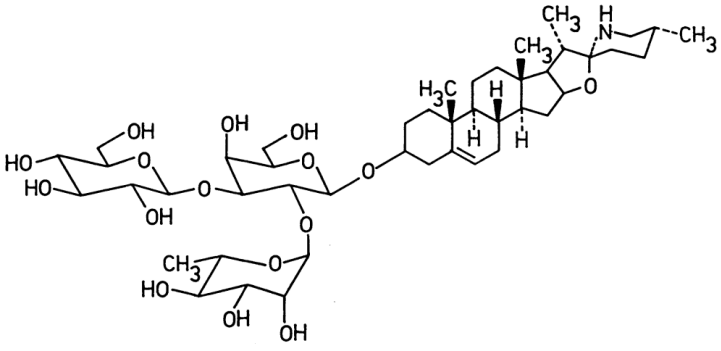
Solanum species have a very close relationship with man; many are important food plants. Several wild-growing Solanum species are used in preparing arrow poisons, especially the unripe fruit or roots of S. incanum (Nigeria, Niger, Rwanda, Kenya), S. torvum (Cameroon) and S. kwebense (Namibia). Many, if not all, of the Solanum species produce mainly poisonous steroidal alkaloids of saponin character ("basic saponins"). Predominant are the glycoalkaloids solasonine (35) and solamargine; both are based on the genine solasodine, which is the nitrogen analogue of the sapogenine diosgenine. Both compounds can easily be converted to 16-dehydropregnenolone, a key intermediate in the synthesis of steroid drugs. Besides the steroidal alkaloids,

the corresponding steroidal saponins are almost always found in Solanum. The fruit contain the highly toxic N-dimethylnitrosamine. In basic actions, the Solanum steroidal alkaloids are apparently very similar. A number of glycoalkaloids have been shown to produce cardiotonic effects similar to those of cardiac glycosides (cardenolides). Severe hemorrhagic gastroenteritis is generally seen. Death is caused by cardiac or respiratory arrest.



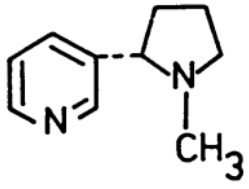
34

One of the best-known Solanaceae, the tobacco plant Nicotiana tabacum and also N. rustica, are ingredients of Strophanthus-based arrow poisons in Cameroon and Nigeria. The leaves are mostly used. In southeast Cameroon some poison-makers mix pounded Strophanthus seeds with juice of the tobacco plant, sometimes hunters smear the thickened juice alone on the arrowhead. N. tabacum contains a great number of simple alkaloids, nicotinoids, and others. The most abundant compound by far of N. tabacum and N. rustica is the pyridine-N-methylpyrrollidine nicotine (36), one of the few naturally liquid alka loids and an extremely strong poison (LD_{50} = 0.5 mg kg^{-1} mouse i.v.). It is very rapidly absorbed and in fatal doses death ensues within a few minutes. It has great variety and complexity of action. The symptoms of acute nicotine poisoning involve most bodily functions, by actions both at the periphery and on the CNS. It has both stimulant and depressant phases of action. Fatal doses lead to convulsions and respiratory paralysis. Its major mechanism of pharmacological action is an interaction with cholinoceptive receptors at many sites in the body. On the other hand, nicotine is quickly detoxified (by secretion in urine, bile, and feces or by metabolism in the liver). Recently it was recognized that the central actions of nicotine have a considerable therapeutic potential, e.g., in the treatment of Alzheimer's disease and as an anxiolytic. Anabasine (37), the major alkaloid in some species (e.g., N. glauca), is as toxic as nicotine.



35

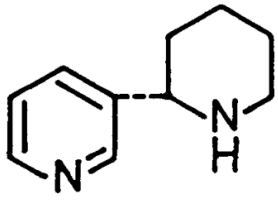
One of the plants that is a common ingredient in traditional medicine and also of therapeutic value for modem medicine is Rauwolfia vomitoria (Apocynacceae). Its roots often appear in the preparation of arrow poisons in Zaire and the Central African Republic.



36

R. vomitoria became an important article of commerce as an alternative to the Indian species R. serpentina as a source of the indole alkaloid reserpine, therapeutically widely used. It is chemically and pharmacologically one of the most thoroughly investigated African plants. Eighty-six indole alkaloids classified into 20 structure types have been isolated from the roots, stem bark, leaves, and unripe fruit. In 1982, the occurrence of 43 indole alkaloids was indicated in a single batch of stem bark and 39 of them identified. The stem bark and roots mainly yield heteroyohimbine- and yohimbine-type alkaloids (especially reserpiline, reserpine, rescinarnine) as well as dihydroindoles (especially ajmaline). The major alkaloid of the leaves is caranaupine, an oxindole. Reserpine (38) is a widely used drug for treatment of mild and moderately severe high blood pressure-it has a long-lasting effect-and in psychiatric disorders (neuroleptic). Because of its unpleasant side effects, it is mostly used nowadays in low dosage in combination with other drugs or replaced by drugs with lesser side effects. Decrease of blood pressure occurs as a consequence of decreased sympathetic tone because of a long-lasting depletion of peripheral catecholamine stores. The sedative or tranquillizing

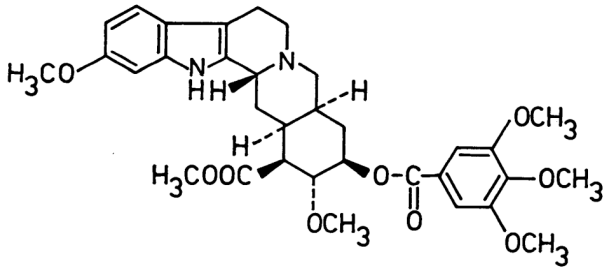
activity is in connection with the depletion of serotonin in the stores of brain tissues. Death ultimately results from central respiratory depression (LD_{50} = 0.5 mg kg^{-1} dog i.v., 10 mg kg^{-1} mouse i.v.), Ajmaline (39) (LD_{50} = 21 mg kg^{-1} mouse i.v.) exerts no CNS action, it acts like quinidine and is widely in use for cardiac disturbances, especially arrhythmia. The major alkaloids are poorly water soluble. The water-soluble root bark extract, as used as an arrow poison, caused severe bradypnea in dogs in high doses, followed by death through respiratory and cardiac arrest. The findings on the interaction between cardiac glycosides and reserpine in the heart and CNS are very remarkable. Ouabain and other cardiac glycosides produce a marked potentiation of the central depressant effects of reserpine: reserpine given simultaneously with ouabain potentiates the toxicity of ouabain and increases mortality from 5 out of 10 dogs (ouabain alone) to 9 out of 10. These synergistic toxic effects and the potentiation of toxicity may be of great interest with regard to arrow poisons: Rauwolfia is always used in combination with the cardenolide-bearing Parquetina nigrescens or Strophanthus species.



37

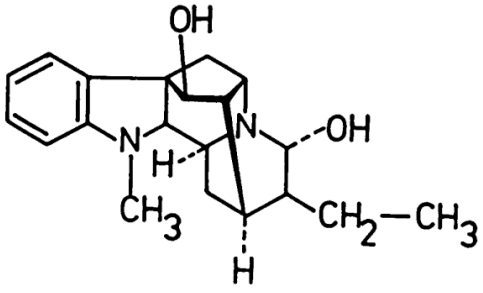
Two arrow poison plants that are chemically closely related to Rauwolfia vomitoria are Corynanthe pachyceras and Picralima nitida. The former is an ingredient of Mansonia altissima-based arrow poisons in the Ivory Coast; fruit and roots of the latter are often found as adjuvants to arrow poisons in Zaire and the Central African Republic, based on Parquetina nigrescens or Strophanthus. Corynanthe pachyceras bears yohimbines and oxindoles. The lethal dose of the most poisonous compound, corynanthe ine (40), is 1.4 mg kg^{-1} mouse i.v.; it exerts excitatory effects, severe convulsions, dyspnea, and death by respiratory arrest. Most representatives are sympatholytics. The 15 characterized bases of Picralima nitida, mostly tertiary, belong to various structure types of the indole alkaloids. In a wide range the principal alkaloid is akuammine (41); it is a strong sympathomimetic with effects similar to those of cocaine. Akuammidine, on the contrary, is a sympatholytic; its local anesthetic action was found to be three times that of cocaine hydrochloride. The only Picralima alkaloid of the strychnine type is akuammicine, very closely related to nor-C-fluorocurarine, which exhibits a strychnine-like activity. Pericine and apparicine (42) (pericalline) are CNS-active and cause tonic-clonic convulsions. The leaves also contain quaternary alkaloids of still

unknown structure, melinonine A excepted. P. nitida is considered to have potential in drug-resistant malaria chemotherapy; the antimalarial activity at least partly resides in akuammine. The decoction of the stem bark is trypanocidal, similar to diminazine aceturate.



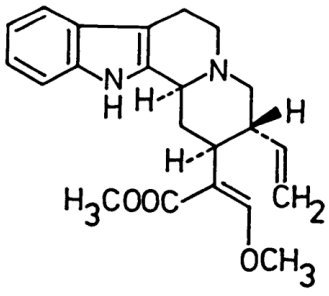
38

Sarcocephalus esculentus was formerly used as the sole constituent of arrow poisons in the Rio Nunez area of West African Guinea. Nowadays, the very bitter stem bark or the roots are ingredients of Strophanthus hispidus-based arrow poisons in northern Nigeria and in the northern part of the Ivory Coast (Senufo tribes).



39

From the root bark, several indoloquinolizidine (indolopyridine) alkaloids have been isolated: angustine (43) and angustoline, known from various Strychnos spp., and the six new bases nauclefine, naucletine (44), naufoline, descarbomethoxynauclechine, naucleidinale, and epi-19-naucleidinale. Root bark, stem bark, and hardwood contain, moreover, large amounts of the structurally-related alkaloid glycoside strictosamide (45) (stric tosidine lactarn, isovincoside lactam). The aqueous-alcoholic extract of 1 g of stem bark kills a lOO-g guinea pig in 15 min. Death occurs from respiratory arrest. In rats, significant central effects, decreased motor activity, and paralysis have been observed.



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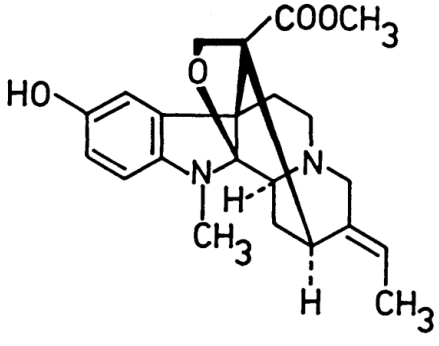


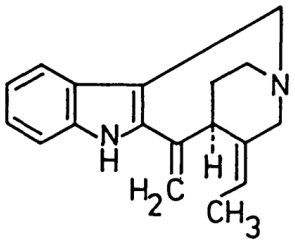
41

The tuber of an ornamental known worldwide, the Superb lily, Gloriosa superba, is an ingredient of Strophanthus-based arrow poisons in northern Nigeria. The individual parts of this plant are highly toxic. From the tuber and seeds, a great number of neutralphenolic tropolone and some basic nontropolone alkaloids were isolated. The principal alkaloid is mostly colchicine, rarely N-formyl-N-desacetylcolchicine. Colchicine (46), one of the most fascinating natural compounds, shows a great number of pharmacological activities.

It is highly toxic with a small therapeutic index. It is a unique anti-inflammatory agent selective against gouty arthritis, in acute attacks and as a prophylactic. It is also an antimitotic agent and has been widely employed as an experimental tool in the study of cell division and cell function; it can arrest plant and animal cell division. The cytostatic colchicine is probably the oldest cancer chemotherapeutic, but too toxic for the treatment of tumors.

Colchicine exhibits a variety of other pharmacological effects: it is one of the strongest poisons (LD_{50} = 0.25 mg kg^{-1} cat i.v., 4.1 mg kg^{-1} mouse i.v.), slow but longlasting in action. Gastrointestinal disorders, nausea, vomiting, diarrhea, and abdominal pain are

the most common effects of colchicine poisoning. In fatal doses, muscular depression is pronounced, an ascending paralysis of the CNS develops, and death results from respiratory arrest.



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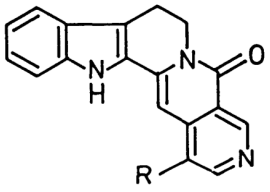
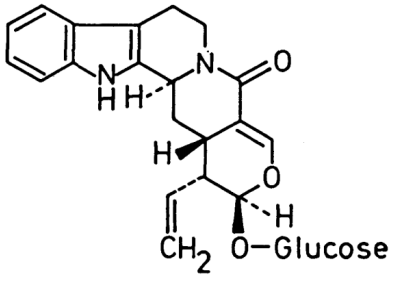


(**43**) R = HC = CH_2
(**44**) R = $COCH_3$

Another alkaloid that is fundamentally an antimitotic agent is maytansine (47), isolated from many Maytenus species, among them M. senegalensis. The stem bark of the twigs of this plant have repeatedly been found as an ingredient of arrow poisons in Burkina Faso, on the base of Strophanthus hispidus. It is not clear which compounds are responsible for its toxicity. Wilforine, an alkaloid with a complicated structure, has been isolated, too, The ansa macrolide maytansine (47) has been extensively studied; it was found to be an exceptionally interesting antitumor agent, which gave rise to a hopeful anticipation, as it is active at the level of micrograms per kilogram in various tumor test systems. Maytansine binds to tubulin and prevents formation of the microtubules necessary for mitotic spindle formation. This leads to arrest of cell division in metaphase. Phase I and phase II clinical trials, however, finally led to the conclusion that maytansine shows too little activity and is too toxic. The most common toxicities seen were gastrointestinal, nausea, vomiting, and diarrhea; furthermore, neurological toxicities include numbness, jaw and muscle pain, weakness, lethargy, and general malaise. For male mice the toxicity with a single dose is LD_{50} = 1.53 mg kg^{-1} i.v. Maytansine has, however, a cumulative effect: given for 5 days the LD_{50} is approximately 0.30 mg $kg^{-1.}$



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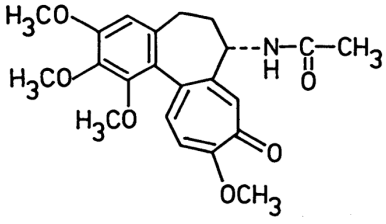
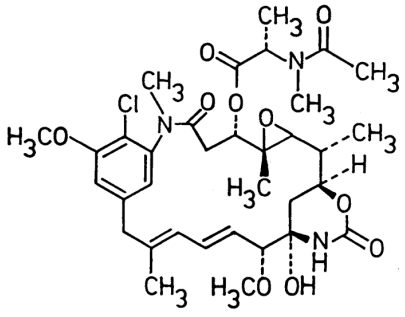


46

Alkaloids of unusual structure have been found in the genus Ziziphus. The roots or stem bark of Z. mauritiana enter into the preparation of Strophanthus-based arrow poisons in Niger and Burkina Faso. The fruit are used as a fishing poison in Ethiopia. The stem bark yields a number of cyclopeptide alkaloids. They contain a l4-membered ring system and are based on two structural types: (1) frangulanine type, a ring system formed by the amino acids p-hydroxystyrylamine, β-hydroxyleucine and an α -amino acid; the main representative is frangufoline (48); (2) Amphibine B system, a ring system formed by p-hydroxystyrylamine, trans-3-hydroxypyroline and an α-amino acid; main representatives are amphibine B, D, E, F and mauritine A-F, H. Nothing is known about the pharmacology and toxicology of these compounds.



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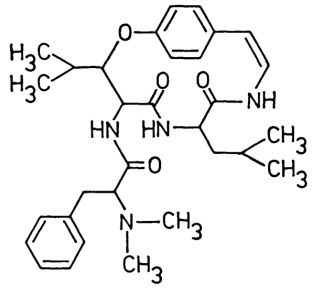

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7.2 SOUTH AMERICAN ARROW POISONS

The northern half of South America has always been a center of many plant derived hunting poisons. By far the most famous and most common of the South American arrow and dart poisons is curare, a name attributed to the large group of poisons with muscle paralysis of a special mechanism . "Curare" and "curarizing" are phenomenological terms describing neuromuscular block of impulse transmission of the motor end plates as a result of inhibition of acetylcholine with the consequence of complete paralysis of skeletal musculature. Curare is essentially a blowpipe dart poison of the forest tribes living in a roughly semicircular area (about 5000 × 4000 km) stretching from French Guyana to the Mato Grosso and including the northern and western parts of the Amazon basin, the middle and upper parts of the Orinoco basin, the Montana of Ecuador and Peru and northeast Bolivia and adjacent parts of the Brazilian plateau. It has also been prepared for arrows by tribes in the savannah of the Mato Grosso plateau. Curare poisons are still a necessary tool for the survival of a number of Indian tribes in the rain forest of South America.

7.2.1 Classification and Composition

In 1895, Rudolf Boehm in Germany classified curare poisons, more ethnographically than chemically, into three categories according to the relationship between incineration residues and poison content and the container: tubo-curare (in bamboo tubes), calabashcurare (in gourds), and pot-curare (in earthenware pots) (Fig. 8).

- Calabash curares are usually obtained from Loganiaceae (Strychnos species) and come principally from the region between the Orinoco in the north and the Negro and lower reaches of the Amazon in the south. A minor type of curare found in use by the Ticuna Indians in Brazil, the so-called bag curare, has similar properties to calabash curare.

Figure 8. Types of curare poison classified according to their containers (left to right: tubo-curare, pot-curare, calabash-curare, and bag-curare).

- Tuba curares are derived from Menispermaceae (chiefly Chondodendron and Curarea species) and predominate in Peru at the foot of the Andes in the eastern Lowlands ("La Montana"), the region bounded by the rivers Napo, Maranon, and Ucayali.
- Pot curares are mixed Loganiaceae/Menispermaceae products and are found mainly in the area covered by the middle reaches of the Amazon and to a lesser extent also in Guyana.

For many decades, all research on curare was based on Boehm's classification. In the course of time, it was found that this classification runs more or less parallel to that based on botanical sources and geographic areas and the term tubo, pot, or calabash curare often does not depend on its content. Many ethnographic observations did not take into consideration the origin of a curare: the poison is merchandise that is transported and marketed by Indian tribes throughout very large areas and it is often sought by tribes 1000 miles away, who send messengers with gifts to obtain a particularly active curare. The curare markets in Venezuela were well known. Curiously enough, in the 1920s, some of the Ecuadorean Indians smeared their arrow tips with curare imported from the German market, like the curare from the American market. Nowadays, the poison may simply be kept in the nearest handy container (e.g., bottle, tin can) or is smeared directly on the arrow tips and not stored.

Most curares comprise several components, which may be added for various reasons and which differ widely in accordance with local flora and tribal superstitions. The poisons are mostly obtained by squeezing the pounded plant parts, more rarely by long maceration or boiling and concentration. The following procedure is a common one: drip water through a leaf funnel that is filled with the pulverized plant material and concentrate the extract by low heat over the fire.

Among the plants mentioned as adjuvants are Dioscorea, Nicotiana, Capsicum, Tabernaemontana, Hura, Ficus. Picrasma, Picrolemma. Guadua. Euphorbia. Cocculus, Guatteria, Spigeila, and Abuta. According to R. E. Schultes, the Kofan Indians of

Colombia/ Ecuador make use of more than 75 different plant species in preparing their various poisons; one-third of them are known to contain highly active compounds, e.g., cardenolides.

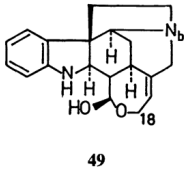
7.2.2 Chemistry

The scientific investigation of the complex curare produced about 70 alkaloids. A great part of them could be separated and characterized, but some of them have only a letter of the alphabet. Curare poisons differ very much in the composition of the alkaloids, but all curares always have in common the presence of dimeric quaternary alkaloids, although of different structures, as the main curarizing principles. A good curare contains up to 12% quaternary alkaloids. The distance between the two protonated N for maximum curare activity is about 0.85-0.9 nm.

In 1970, the structure of (+)-tubocurarine (2), the major alkaloid of the tubo or Chondodendron curare, was revised to be monoquaternary and not diquaternary as hitherto accepted: it possesses both a tertiary and a quaternary (one ammonium group) basic function, but the second tertiary nitrogen is easily protonated. The main bases of calabash or Strychnos curare are easily water-soluble bis-quaternary dimeric indole alkaloids formed by the union of two equal or different monomeric molecules of strychnine type and containing two quaternary ammonium groups.

The bases can be divided into three alkaloid "families" (the prefix C refers to isolation from calabash material):

- The C-toxiferine family (2 × N_b-metho-Wieland-Gumlich aldehyde) (49, N_b - CH_3); C-toxiferine (52), C-alkaloids A (58) and E (55) as main compounds.
- The C-dihydrotoxiferine family (2 × N_b-metho-18-deoxy-Wieland-Gumlich aldehyde); C-curarine (53), C-alkaloid D, C-calebassine (56) as main compounds.
- The C-alkaloid-H family (l × N_b-metho-Wieland-Gurnlich aldehyde + 1 × N_b-metho-18-deoxy-Wieland-Gurnlich aldehyde); C-alkaloids H (51), G (54), and F (57) as main compounds.



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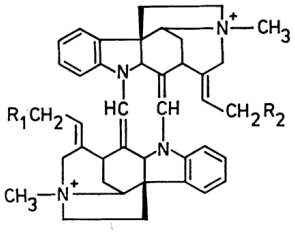
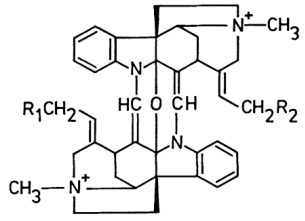
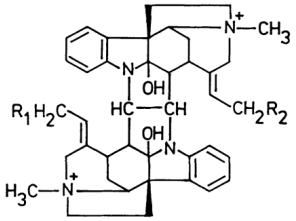


(**50**) C-Dihydrotoxiferine $R_1 = H$ $R_2 = H$
(**51**) C-Alkaloid H $R_1 = OH$ $R_2 = H$
(**52**) C-Toxiferine $R_1 = OH$ $R_2 = H$



(53) C-Curarine $R_1 = H$ $R_2 = H$
(54) C-Alkaloid G $R_1 = OH$ $R_2 = H$
(55) C-Alkaloid E $R_1 = OH$ $R_2 = H$



(56) C-Calebassine $R_1 = H$ $R_2 = H$
(57) C-Alkaloid F $R_1 = OH$ $R_2 = H$
(58) C-Alkaloid A $R_1 = OH$ $R_2 = H$

7.2.3 Pharmacology, Toxicology

When introduced into the bloodstream, all of the dimeric quaternary curare alkaloids are highly active muscle relaxants, acting by a nondepolarizing and competitive mecha- nism at the neuromuscular junction, competing with ACh for the active surface of the receptor and thus blocking the nerve-muscular transmission. Blockade of the postsynaptic nicotinic acetylcholine receptors causes progressive paralysis of voluntary movement and, as a final result, complete paralysis of the skeletal or striated muscle apparatus. The muscle involvement follows a definite order: eye and ear muscles are the first to be affected, then neck, limb, trunk, intercostal muscles, and diaphragm; death results from anoxia caused by respiratory failure . Artificial respiration prevents death. Physostigmine from the African arrow poison plant Physostigma venenosum, immediately given, antagonized all paralytic symptoms.

The toxicities (LD_{50}) of the three curare types for mice i.p, are reported to be 0.8-25 mg kg^{-1} for pot curares, 2-15 mg kg^{-1} for calabash curares, and 5-10 mg kg^{-1} for tubo curares. Calabash or Strychnos alkaloids have the greater curarizing potency and moreover, the greater the polarity, the greater the curarizing activity. The most active alkaloids belong to the group with an ether oxygen in the central eight-membered ring: alkaloid E (55) (LD_{50} = 0.007-0.012 mg kg^{-1} mice i.v.), alkaloid G (54) (LD_{50} = 0.001-0.008 mg kg^{-1} mice i.v.), and C-toxiferine (52) (LD_{50} = 0.023 mg kg^{-1} mice i.v.). Some are shortacting, some extremely long-acting. The duration of action of C-toxiferine is outstanding: it takes nearly 2 hr for the muscle to recover after a slow, progressive onset of paralysis; with a 20-fold paralytic dose total paralysis lasts 8 hr. Alkaloid E shows similar properties. The action of C-curarine (53) (LD_{50} = 0.050 mg kg^{-1} mouse i.v.) is comparable to that of (+)-tubocurarine (2) (LD_{50} = 0.14 mg kg^{-1} mouse i.v.), C-Calebassine (56) belongs to the weaker compounds with an LD_{50} = 0.32 mg kg^{-1} mouse i.v,

Curare poisons are very stable, once prepared. Although more than 140 years old, curare samples present in various European museums were shown to have remained very effective. The lethal doses for mice i.p. of such arrow poisons lie between 0.8 and 20 mg kg^{-1}, mostly 2-5 mg kg^{-1}. Rather curiously, the oldest arrow poisons (130-

140 years old) were the most active. Thus a pot curare of the Mayoruna in Brazil, 130 years old, had a lethal dose of 0.8 mg kg^{-1} mouse i.p. and mainly contained the bases C-alkaloids G (54) and A (58) and C-toxiferin (52). Moisture did not decrease the activity of curare. One of the most important unanswered questions concerning all arrow poisons is: especially, what kind of changes or modifications occur in the structure of alkaloids during the elaboration of curare? In the case of Macu curare elaborated with Chondodendron, Marini-Bettolo and his "group of Rome" demonstrated that the curarizing activity of the alkaloids obtained from tubo curare are tenfold greater than those extracted from the plant. Experiments indicated that, in the case of the bisbenzylisoquinoline alkaloids, some methylation reactions may occur with the more toxic, curarising quaternary alkaloids during the elaboration of the tubo curare.

7.2.4 Medicinal Use

The transformation of curare from deadly poison to a valuable medicinal agent is one of the best examples of the refinement of a crude natural product to an indispensable aid in modern medicine. In 1935, King announced the isolation of a pure crystalline alkaloid, (+)-tubocurarine chloride (2), from a museum sample of tubo curare of unknown botanical derivation. Seven years later, the same compound was found in a crude curare known to have been prepared from the single plant Chondodendron tomentosum. In 1939, curare was introduced in clinical medicine, first as a preventive of traumatic complications in metrazol convulsive shock therapy and electric shock therapy. Since 1942 the pure alkaloid (+)-tubocurarine has become a useful neuromuscular relaxant in all forms of anesthesia (Bennett, 1968). It continues to be used today. The structure of (+)-tubocurarine was taken as a model for elaborating synthetic curare and semisynthetic curare-like products with fewer side effects; it produced the molecular features necessary for muscle-relaxant activity and more exact knowledge of distribution, metabolism, and mechanism of action on the end plates and the transmission of stimulus to the muscles.

Keyword

Tubocurarine is a toxic alkaloid historically known for its use as an arrow poison.

Two other curare alkaloids, C-calebassine (56) and C-toxiferine (52) from calabash curare, are also used in clinical medicine, e.g., in Switzerland, especially for tetanic convulsions; their duration

of effect is longer-lasting than that of (+)-**tubocurarine** with fewer effects on respiration. Most alkaloids of the curare complex lower blood pressure in varying degrees.

It is of interest that the Auca Indians of eastern Ecuador apply their curare (made from Curarea tecunarum) directly to bacterial or fungal infections of the skin with good results reported. An extract of the same plant is used by the Deni of the Rio Cunhua in Brazil as a reputedly effective male and female contraceptive.

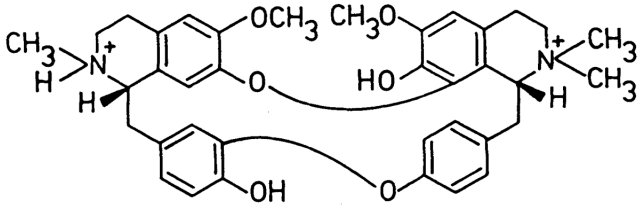
7.2.5 Other Plant Arrow Poisons

There have also been reports about "curares" based on plant sources other than Strychnos and Chondodendron, especially Virola (Myristicaceae) and Abuta (Menispermaceae) species. The use of both species as arrow poisons is ethnologically and pharmacologically remarkable. In 1931 and 1960, the use of Virola theiodora and to a lesser extent V. elongata (considered by some botanists to be synonymous) as a hallucinogenic snuff by the Yanoama (Waika) of southern Venezuela and northern Brazil was reported, with the remarks that the same plants were applied alternatively as arrow poisons. In 1977, Schultes and Holmstedt established the use of Virola theiodora resin as the sole ingredient of the poison made by the Yanoama (Waika) of the Rio Tototobi: the resin of the heated stem bark is applied repeatedly to bamboo arrow tips. An almost identical procedure was observed among the Sanama tribe living more than 300 krn north of the Rio Tototobi; both plants were used in the preparation of snuff and arrow poison. The Indians know that these are slow-acting arrow poisons and that the wounded animal must be followed for some time.

Although there was a considerable variation between samples, the indole alkaloids N-methyltryptamine, N,N-dimethyltryptamine, and 5-methoxy-N,N-dimethyltryptarnine were the main compounds. It is very interesting that the same components were found as the biologically active constituents of the African arrow poison plant Mucuna pruriens. Galeffi et al. (1983) confirmed the findings: the poison on Yanoama dart tips in Roraima territory, Brazil, did not contain any curarizing alkaloid, but a single substance in high concentration (8% by weight; 12 mg $dart^{-1}$), which was identified as 5-methoxy-N,N-dimethyltryptamine (= O-methylbufotenine)(24).

These and similar indole alkaloids have a number of peripheral and central activities, foremost their psychomimetic activity, especially their ability to elicit hallucinations. virola elongata bark, at least, does not contain highly toxic constituents which might explain its use as an arrow poison. Its use for hunting purposes can be explained by the incapacitation of the wounded animal; the predominating 5-methoxy-N.N-dimethyltryptamine (24), especially, causes much higher behavioral disturbances than other indolalkylamines, in addition to being considerably toxic (LD_{50} = 1.5-2.0 mg kg^{-1} sheep i.v.), It is the most active hallucinogen after LSD. In sheep, guinea pigs, rats, and mice, parenterally injected, it caused death in a short time from cardiac failure.

In 1965, Biocca et al. (1965) described a dart-tip curare, which is not stored in particular containers, being used directly for the preparation of dart points. In this curare, two types of active ingredients have been identified: bisindole alkaloids (Yanoama tribes of the Upper Orinoco) and bisbenzylisoquinoline alkaloids (area of Maria de los Guaicas). The same bisbenzylisoquinoline alkaloids, whose sole monoquaternary component is the muscle-relaxant macoline (59), have been isolated from Abuta grisebachii (Menisper maceae), which is the single ingredient of the "macoli" curare of the Sanama Indians.



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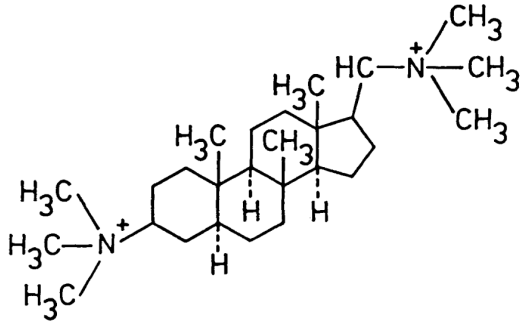
In Venezuela, arrow poisons derived from Malouetia species (M. nitida, M. schomburghiii) exert a paralyzing action similar to curare. A related plant from Africa, M. bequaertiana, affords the alkaloid malouetine (60), an unusual pregnane alkaloid with two quaternary ammonium groups. In frogs, it produces a curarizing effect different from other natural curarizing compounds.



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7.3 ASIAN ARROW POISONS

Poisoned weapons have been extensively utilized in Southeast Asia. This is the region in which the blowpipe and dart is the principal hunting weapon (Fig. 9). There is a lot of literature and information on the hunting poisons of this area but, as in Africa, there are two main difficulties regarding information gathered:

1. It is not always clear from the accounts which ethnic group was visited; moreover, changes in ethnological classification and designation make it difficult to relate the findings described in the earlier accounts to those acquired more recently.
2. Almost all of the botanical data and identifications, particularly of Strychnos, are incorrect, because in former times the plants collected were generally of poor quality, incomplete, and not well known, but some data have been amended in the course of revisions.

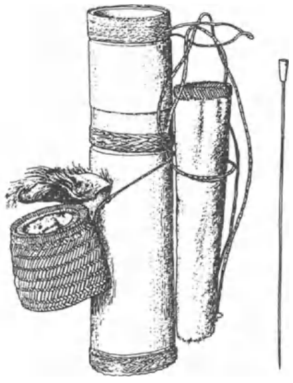


Figure 9. Dart for blowpipe and quiver from Sulawesi (Indonesia).

From Burma to China and Indonesia (as far as Timor), the major source is the latex of the highly toxic cardenolide-bearing Moraceae Antiaris toxicaria, the "upas" or "ipoh" tree of the Malays and Indonesians. Another important source is the root and stem bark of various species of alkaloid-bearing Strychnos, but they have a more restricted use. Their use is known with certainty only in Java, Borneo, Malaya, and Indochina. Arrow and dart poisons based on Lophopetalum (Celastraceae), Strophanthus (Apocynaceae), Beaumontia (Apocynaceae), and Sapium (Euphorbiaceae) species are also known in Southeast Asia.

7.3.1 Indonesia, Borneo, Philippines, Hainan, Vietnam, Cambodia

Hunting poisons are still being made, particularly in Borneo and no doubt in other places as well, but generally there is little recent information available. Strychnos species (S. ignatii and S. nux-vomica) have been revealed as important components of the common Antiaris toxicaria poisons. Other adjuvants are Derris. Dioscorea, Sapium, and Nicotiana. In Cambodia, an arrow poison is prepared from the bark of Sindora cochinchinensis, the sap of a Xylia sp., Capsicumfrutescens, and snake poison; it is still in use.

In the Philippines, a slow-acting arrow poison, formerly also used in India, is made from the seeds of the Papilionaceae Abrus precatorius:the seeds are powdered and formed into a paste with which the darts or arrows are smeared. Wounds caused by arrows poisoned with this paste are generally fatal within 24 hr. Boiling the seeds renders the poison ineffective. Local tribes, however, seldom avail themselves of this poison, probably because of its slow effect. The seeds contain abrine, a highly toxic glycoprotein, and 0.6-0.9% indole alkaloids, among them N-methyltryptamine, N-methyltryptophan, and hypaphorine (26) (the betaine of tryptophan) as well as saponins. The methylester iodide of hypaphorine has curarizing properties; by itself, it is less toxic for warm-blooded animals. In Zimbabwe (Africa), the plant is one of the most commonly used plants against haematuria caused by Schistosoma haematobium infections. A single dose of the powdered seeds is a long-lasting contraceptive, the effect lasting up to 13 menstrual cycles. The seed extracts also have activity against skin cancer. Complexes of human tumor cells with the lectins from A. precatorius have been prepared as antitumor vaccines. Injection of abrine-a into mice bearing Meth-A tumors inhibited the tumor growth by 90%.

Besides Antiaris toxicaria, there are very scant data available on arrow poisons or adjuvants in the Philippines. Some other plants that are used in the preparation of hunting poisons are Diospyros multiflora, Temstroemia sp., Dioscorea hispida, Lophopetalum javanicum, Mallotus floribundus, and Strophanthus sp.

7.3.2 Burma. Thailand, Malaysia

Malaysia was, and still is, the center of poisoned arrows and darts in southeast Asia. The Strychnos species used, solely or as adjuvants, are S. ignatii, S. nux-vomica, S. vanprukii (S. quadrangularis), S. rufa, and S. axillaris . Less is known about the alkaloids of the latter three species. In S. axillaris, the strychnine-type alkaloids spermostrychnine, strychnospermine, and its deacetyl derivative were detected; they are less toxic and cause clonic (but not tonic) convulsions only in high doses. S. vanprukii, in contrast to most other Asian Strychnos species, has a paralyzing rather than a tetanizing activity. Up to now, only the three common, weakly toxic, pentacyclic indole bases angustine (43), angustidine, and angustoline of the vallesiachotaman type have been found.

The alkaloid composition of S. ignatii is very variable. The root bark was shown to contain tertiary and also quaternary alkaloids. Both S. ignatii and S. nux-vomica contain the strong convulsant, strychnine (5); S. nux-vomica contains the major tertiary base, 12- hydroxystrychnine, as well.

The seco-alkaloid diaboline, from S. ignatii, is not as “diabolical” as its name suggests; the opening (seco) of the amide ring of the basic strychnine skeleton and the introduction of a hydroxyl group at the 17-position produced a marked decrease in toxicity: high doses have a weak curare effect. One of the identified quaternary bases

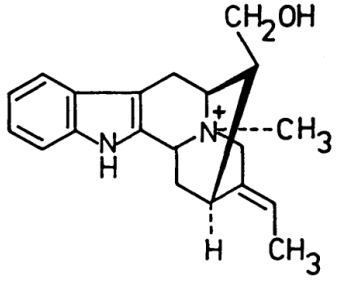
is the monoquaternary C-mavacurine (S. nux-vomica), which has a very low curare activity.

Macusine B (61) (S. ignatii) was found to act on both adrenergic and tryptamine receptors; it also caused clonic convulsions in vivo. The occurrence of N-cyano-sec-pseudo strychnine and -brucine in S. ignatii is remarkable.

Remember

One of these mixed poisons from western Malaysia has been shown to act as a weak curare.

The poison was most certainly derived from S. ignatii. Recent examination of nine Malayan dart poisons (1883,1924) from the Museum of Ethnology in Vienna has confirmed that their main active compounds are cardenolides from Antiaris toxicaria and Strychnos alkaloids, probably from S. ignatii, the most common Strychnos in such poisons. Only two poisons contained diaboline as the alkaloidal component.



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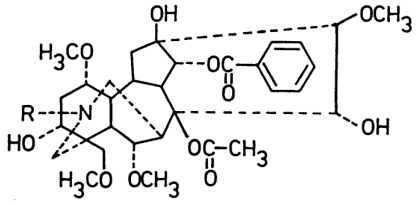
For the other minor alkaloids, see Aimi et al. (1989), for the chemistry and pharmacology/toxicology of strychnine and derivatives, see Strychnos icaja, above. Alkaloidbearing plants in Malayan dart poisons are: Atalantia kwangtungensis, Calamus javensis, Coscinium sp., Ervatamia sp., Gnetum gnemon , Gomphandra sp., Micromelum pubescens, Popowia tomentosa , Uvaria sp., Glycosmis sp., Dioscorea triphylla.

The bark and roots of the Apocynaceae Tabernaemontana corymbosa have been reportedly used to make arrow poison in Thailand; it also enters into the composition of the dart poison made by the Mantra (Temuan or Belanda) in Malaysia. The plant causes strong alkaloid reactions, but nothing is known about the compounds. Aconitum is a plant genus that has been widely used for arrow poisons in Asia (Burma, China, Himalaya, Kamchatka, Kuril islands, Sakhalin, Hokkaido, far eastern Siberia) as well as

in the United States (Alaska, Aleutian islands) and Europe. Among the species so used have been the notorious A. ferox, A. spicatum, and A. laciniatum . Aconitum species have been employed as arrow-poison plants throughout a large part of China, especially in the mountainous southwestern part of the country. The toxicity of these plants has also long been recognized by the Han Chinese. The Ainu of Sakhalin, Hokkaido, and the Kuril islands used predominately aconitum-based poison.

Aconitine (62) and mesaconitine (63) (both with an LD_{50} of about 0.12 mg kg^{-1} mouse i.v.) are the main toxic constituents of the tubers of the Aconitum species used in arrow poisons. Both are oxygenized C_{19}-diterpenoidester alkaloids. The ester functions are important for full toxicity. Given orally, 300-400 g of the fresh roots, is lethal to a horse.



(62) R = CH_2–CH_3
(63) R = CH_3

Both alkaloids cause prolonged nerve depolarization by binding to the nerve-cell membrane and, through allosteric interaction, preventing normal closure of the Nar channels. They have almost the same affinity for the Na^+ channels of brain synaptosomes, heart and skeletal muscle cells. They paralyze the skeletal muscles "curare style," but in contrast to curare and other neuromuscular blocking drugs, they act presynaptically by decreasing the ACh release. The effect is not antagonized by physostigmine.

The principal effect of the two alkaloids is on the cardiovascular and respiratory systems. They act cardiotoxically and neurotoxically, both peripherally and centrally, decreasing blood pressure and body temperature, causing cardiac arrhythmia, giddiness and weakness related to loss of muscle power. Death is from cardiac and respiratory failure.

SUMMARY

- Arrow poisons are used to poison arrow heads or darts for the purposes of hunting and warfare. They have been used by indigenous peoples worldwide and are still in use in areas of South America, Africa and Asia.
- The first European killed by an African poisoned arrow was probably Nuno Tristan in 1447, at the mouth of the Gambia River in West Africa. The next centuries showed that poisoned arrows were nowhere else as common or used to such an extent as in Africa. There are poisons containing essentially one plant extract, but more often the poisons consist of a mixture of plant materials with up to a dozen ingredients.
- The bow and arrow is a common weapon. The arrows consist either of hardwood, with the tip hardened in a fire (forest type), or they have an iron tip and plant material is wrapped around behind it for better adhesion of the poison (savannah type).
- The secret composition and preparation of poisons is known by the medicine man, magician, herbalist, a certain individual or family and often today by the hunter himself. Poison preparation is considered to be a medicinal-magical art and even today poison making is sometimes still accompanied by mysterious rituals and taboos.
- Plants containing cyanogens often are used in the preparation of arrow poison; they release volatile hydrogen cyanide when the plant cell is damaged, but it does not survive the poison-making process.
- A very interesting ingredient of hunting poisons (Nigeria, Zambia) is Securidaca longepedunculata. The root juice, however, is more commonly used as an ordeal poison, especially in the Central African Republic and southeast Zaire. This Polygalaceae is a common suicide poison and probably the best-known African abortifacient.
- P. venenosum is a further African poison plant that achieved medicinal importance and is a prime example of the transformation of an extensively used deadly poison to a highly beneficial medicine and an important tool in experimental pharmacology.
- The northern half of South America has always been a center of many plant derived hunting poisons. By far the most famous and most common of the South American arrow and dart poisons is curare, a name attributed to the large group of poisons with muscle paralysis of a special mechanism .
- “Curare” and “curarizing” are phenomenological terms describing neuromuscular block of impulse transmission of the motor end plates as a result of inhibition of acetylcholine with the consequence of complete paralysis of skeletal musculature.
- Poisoned weapons have been extensively utilized in Southeast Asia. This is the region in which the blowpipe and dart is the principal hunting weapon.

MULTIPLE CHOICE QUESTIONS

1. Which of the following is a clinical use for a muscarinic agonist?

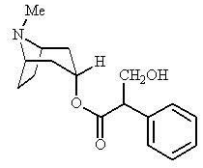
a. Treatment of myasthenia gravis

b. 'Switching off' the gastrointestinal tract prior to surgery

c. 'Switching on' the urinary tract after surgery

d. Increasing heart muscle activity in certain heart defects

2. Atropine has been used to decrease gastrointestinal motility and to counteract anticholinesterase poisoning.



What function does this molecule have?

a. Muscarinic agonist

b. Muscarinic antagonist

c. Nicotinic agonist

d. Nicotinic antagonist

3. What sort of receptor is the nicotinic receptor?

a. A G-protein coupled receptor

b. A kinase linked receptor

c. An intracellular receptor

d. An ion channel

4. What sort of receptor is the muscarinic receptor?

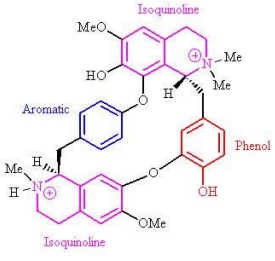
a. A G-protein coupled receptor

b. A kinase linked receptor

c. An intracellular receptor

d. An ion channel

5. Tubocurarine is a lead compound for a class of medicinally useful compounds.



What feature of the above structure is crucial to its activity?

a. The phenol ring
b. The isoquinoline rings
c. The aromatic ring
d. The two positively charged nitrogen atoms

REVIEW QUESTIONS

1. Why is arrow poison used?
2. Write the name of poisonous weapon.
3. Why are plants containing cyanogen used?
4. What was the first alkaloid proven to act through inhibition of an enzyme?
5. When did Rudolf Boehm in Germany classify the poisons of Kurare as chemically more ethnographic?

Answer to Multiple Choice Questions

1. (c) 2. (b) 3. (d) 4. (a) 5. (d)

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Level: Beginner to Advanced
Subject: Chemistry

The Alkaloids

Alkaloids are compounds needed for cell activity and gene code realization in the genotype. Alkaloids are a huge group of naturally occurring organic compounds which contain nitrogen atom or atoms in their structures. Alkaloids have been reported as one of the important groups of phytoconstituents obtained from natural sources. It plays an important role in the ecology of organisms which synthesize them. Alkaloids play an important role in the defense systems against pathogens and animals. These compounds play an important role in living organisms. Alkaloids occurred to be extremely important for human beings for ages, besides they are secondary metabolites, what could suggest that they are useless. Alkaloids showed strong biological effects on animal and human organisms in very small doses. Alkaloids are present not only in human daily life in food and drinks but also as stimulant drugs. Alkaloids are useful as diet ingredients, supplements, and pharmaceuticals, in medicine and in other applications in human life. Alkaloids are also important compounds in organic synthesis for searching new semisynthetic and synthetic compounds with possibly better biological activity than parent compounds.

This book is comprised of seven chapters. The book presents an overview of alkaloids. It also deals with the function, properties, classifications, extraction, and their importance in nature.

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