Pharmacognosy III

ALKALOIDS

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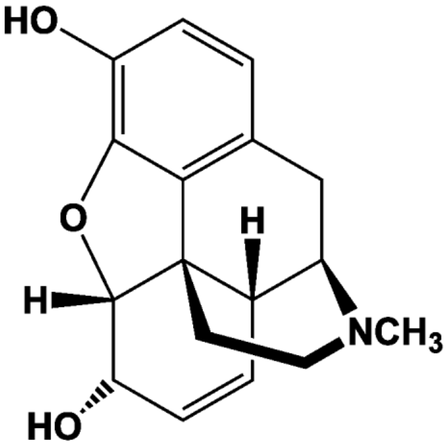
Generalities

The number of products have been described, their structural diversity, and the scope of their pharmacological activities make alkaloids one of the most important groups of naturally occuring substances of therapeutical interest, whose number is almost unfathomable.

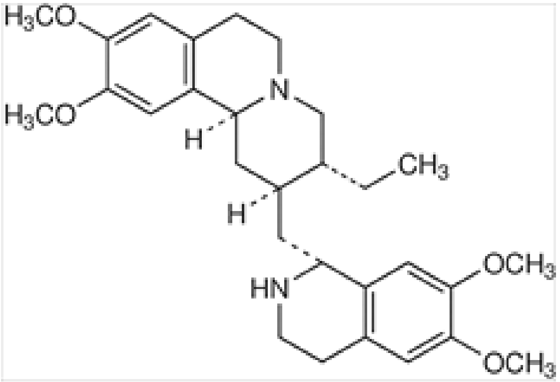
The term alkaloid was introduced by W. Meisner at the beginning of the nineteenth century to designate natural substances reacting like bases, in other words, like alkalis (from the Arabic *al kaly*, soda, and from the Greek *eidos*, appearance).

Initially defined as nitrogen-containing, basic substances, of natural origin and of limited distribution, alkaloids have a complex structure. Their nitrogen atom is part of a heterocyclic system and they possess a significant pharmacological activity. They are found as salts, and they are formed biosynthetically from an amino acid. These elements characterize what may be referred as **true alkaloids**, but many authors distinguish, in addition, protoalkaloids and pseudoalkaloids.

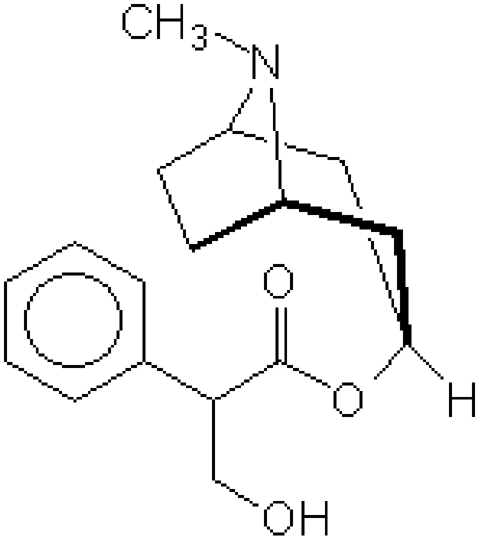
Some true alkaloids



Morphine



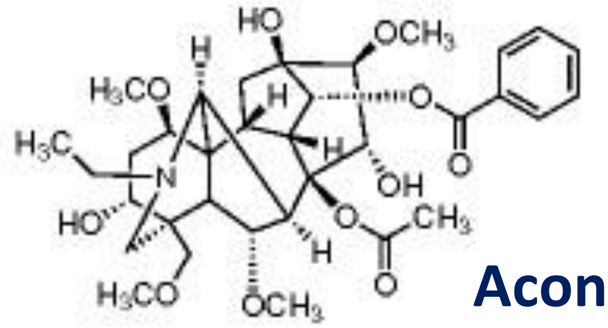
Emetine



Atropine

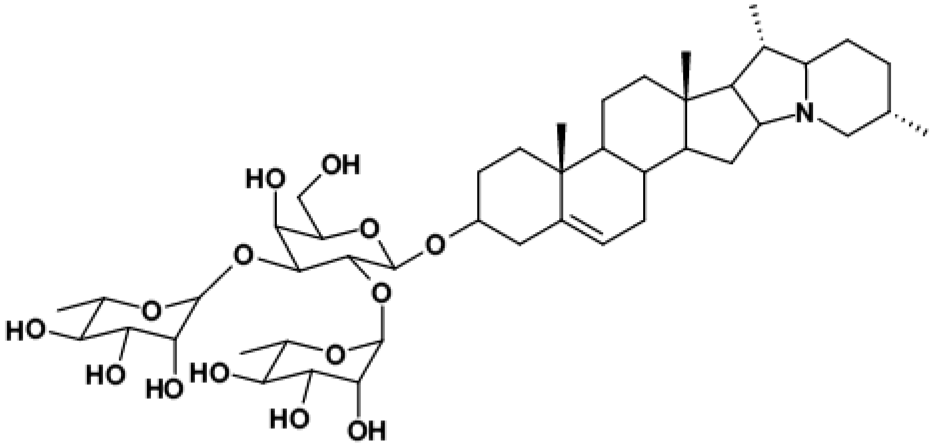
Pseudoalkaloids most often have all of the characteristics of the true alkaloids, but they are not derived from amino acids. Most of the known examples are isoprenoids and are referred to as terpenoid alkaloids, or steroidal alkaloids.

Diterpenoid alkaloid



Aconitine

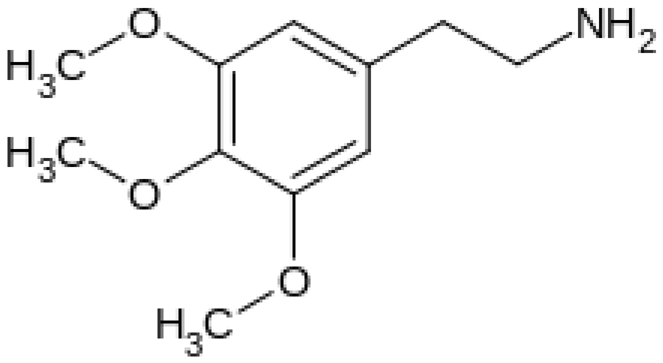
Steroidal alkaloid



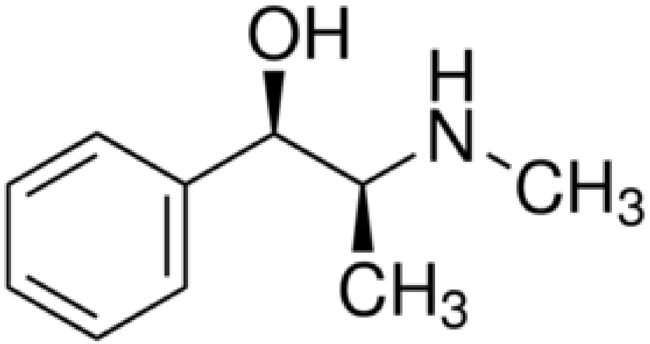
Solanine

Some pseudoalkaloids

Protoalkaloids are simple amines in which the nitrogen atom is not part of a heterocyclic ring; they are basic and are elaborated from amino acids.



Mescaline



Ephedrine

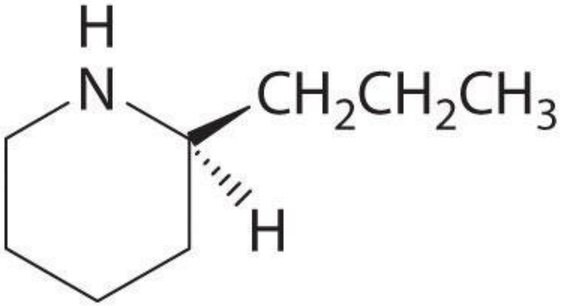
In practice, it is widely accepted that the following are not alkaloids : simple amines, peptides, amino sugars, porphyrins, alkylamines, and aryl-alkyl-amines. All other compounds are commonly referred to as alkaloids.

Thus we can state that an alkaloid is an organic compound of natural origin, which contains a **nitrogen** atom, is more or less basic, is of limited distribution, and has a low doses, marked pharmacological properties. That this grupping has a sound basis is confirmed by the fact that these compounds have in common some reactions with the “general reagents for alkaloids”.

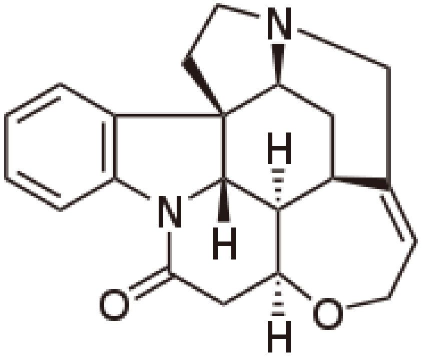
HISTORY

It was probably Derosne, who, while extracting a mixture of narcotine and morphine from opium in 1803, was the first to isolate a vegetable alkali. In 1806, Serturner recognized the alkali nature of the somniferous principle of opium, which he named morphine about ten years later. Shortly afterwards, between 1817 and 1820, two French pharmacists, Pelletier and Caventou discovered an impressive series of active compounds: caffeine, emetine, strychnine, quinine and cinchonine.

Chemists attempted to elucidate the structure of this molecules very early on : in the simple cases they were successful (coniine, Schiff, 1870), but polycyclic edifice of strychnine resisted the endeavors of chemical investigators for nearly 130 years. Today advanced NMR techniques and X-ray diffraction spectrometry allow the elucidation of the most complex structures.



Coniine



Styrichnine

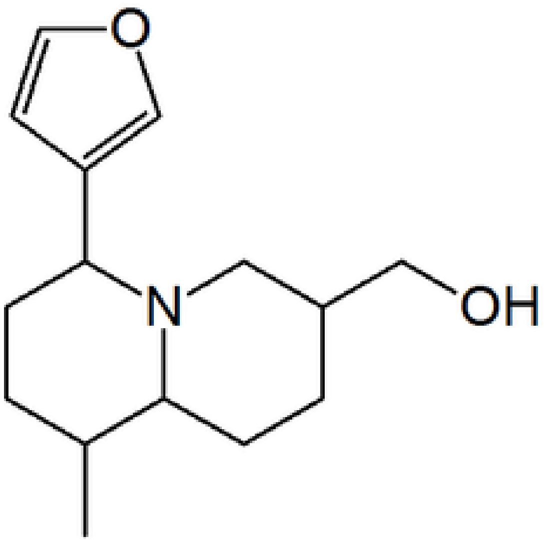
NATURAL OCCURENCE, DISTRIBUTION, AND LOCALIZATION

Alkaloids occur only exceptionally in bacteria (procyanine from *Pseudomonas aeruginosa*) and rather rarely in fungi (psilocin from the hallucinogenic mushrooms of Central Amerika, ergolines from *Claviceps purpurea*). The Pteridophytes rarely contain alkaloids and among them the Lycopodiaceae represent the main exception (alkaloids derived from lysine) ; the same comment applies to the Gymnosperms (alkaloids from *Cephalotaxus*).

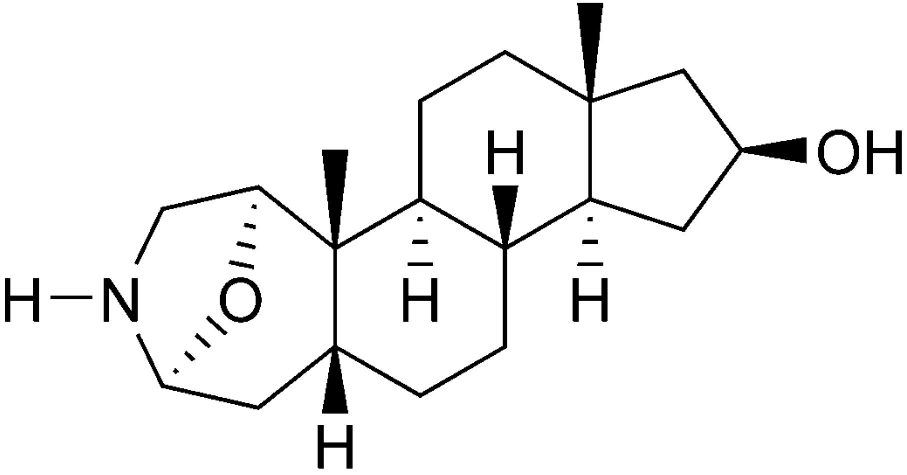
Thus, alkaloids are compounds , essentially found in the Angiosperms. Certain families have a marked tendency to elaborate alkaloids: this is true for the Monocotyledons (Amaryllidaceae, Liliaceae) as well as Dicotyledons (Annonaceae, Apocynaceae, Fumariaceae, Lauraceae, Loganiaceae, Magnoliaceae, Menispermaceae, Papaveraceae, Ranunculaceae, Rubiaceae, Rutaceae, Solanaceae, among others). Within these families, some genera produce alkaloids and others do not. Sometimes, they are found in all of the genera (Papaveraceae), although this far less common.

Certain alkaloids occur in several genera that belong to different families; sometimes these are quite distant taxonomically (caffeine Coffeae semen-Rubiaceae, Theae folium, Theaceae). Other alkaloids are characteristic of a limited number of genera within one family (hyoscyamine-Solanaceae), or of a group of species within one genus (thebaine-*Papaver*); some are highly specific (morphine-*Papaver somniferum).*

For a long time alkaloids used to be considered products of methabolism of plants only. In fact, alkaloids also occur in animals. In some cases , they are products formed from the alkaloids contained in the plants on which the animals feed : examples are castoramine, which arises from the metabolism of the alkaloids of the water-lilies consumed by beavers, and the pyrrolizidine alkaloids found in some butterflies. In other cases, the alkaloids appear to be the products of the metabolism of the animal : this is true for the urodole (salamander) or anurous Ambfibia (*Bufo, Phyllobates, Dendrobates,* and other toads).



Castoramine



Samandarine

Some animal-alkaloids

Localization : In the plant, alkaloids occur as soluble salts (citrates, maleates, tartrates, maconates, benzoates), or in combination with tannins. Microchemistry shows that alkaloids are most often localized in the peripheral tissues : external layers of the bark and stems and roots, or seed tegument. They are normally stored in the cell vacuoles, which may be specialized (laticiferous) or not.

Function : As in the case of many other secondary metabolites, almost nothing is known of the role of alkaloids in plants. Some may be involved in plant-predator relationships, by protecting the former against the latter. Are the alkaloids storage substances?, growth regulators? The question remains unanswered.

PHYSICO-CHEMICAL PROPERTIES

Alkaloids have molecular weights ranging from 100 to 900. Although most of the bases that do not contain oxygen atoms are liquid at ordinary temparatures (nicotine, sparteine, coniine) (volatile alkaloids), those that do contain oxygen atoms, are normally crystallizable solids, and in rare cases they are colored (berberine yellow, sanguinarine red). Almost all of the crystallized bases rotate the plane of the polarized light, and have sharp melting points, without decomposition, especially below 200°.

As a general rule, alkaloids as bases are not soluble in water, soluble in apolar or only slightly polar organic solvents, and are soluble in concentrated hydroalcoholic solutions.

The basic character of alkaloids allows the formation of salts with mineral acids (hydrochlorides, sulfates, nitrates) or organic acids (tartrates, maleates). Alkaloid salts are generally soluble in water and in dilue alcohols, and they are, except in rare cases, not soluble in organic solvents

DETECTION AND CHARACTERIZATION

The detection methods currently in use are preceted by an extraction and consist, mostly generally, in precipitating the alkaloids by using firy specific reagents «the general reagents»

DRAGENDORFF'S REAGENT– orange precipitation

(Bismuth nitrate, (KI) potassium iodide, water)

MAYER'S REAGENT– yellowish-white precipitation
(mercuric chloride, KI, water)

BOUCHARDAT'S REAGENT– brownish-red precipitation

((I) iodine, KI, water)

Other reagents are avelaible to characterize alkaloids, particularly those that give color reactions characteristic of subgroups of alkaloids

-*p*-dimethylaminobenzaldehyde for the ergot alkaloids and pyrrolizidine alkaloids

- the Vitali-Morin reaction for the esters of tropic acid
- reagents containing ferric chloride in the presence of hydrochloric acid (tropolones) or perchloric acid (*Rauwolfia*)
- cerium and ammonium sulfate, which differentiate indoles (yellow), dihydroindoles (red), ß-anilinoacrylate (blue), oxindoles
- ninhydrin for alkylamines

EXTRACTION

The extraction of alkaloids is based, as general rule, on the fact that they normally occur in the plant as salts and on their basicity, in other words on the differential solubility of the bases and salts in water and organic solvents.

The plant material often contains substantial quantities of fats (in the seeds), and also waxes, terpenes, pigments, and other lipophilic substances, which may interfere with the extraction procedure, for example by causing the formation of emulsions. This technical problems can be more or less completely avoided by preliminary defatting of the crushed drug. Petroleum ether and hexane are well suited for this step : alkaloids are soluble in these solvents only in exceptional cases, when the medium is neutral.

Extraction Methods

Solvent extraction in alkaline medium

- First step : The powdered defatted drug is mixed with an alkaline aqueous solution which displaces the alkaloids from their combinations as salts; the free bases are then extracted with an organic solvent (dichloromethane,chloroform, ethyl acetate or diethyl ether).

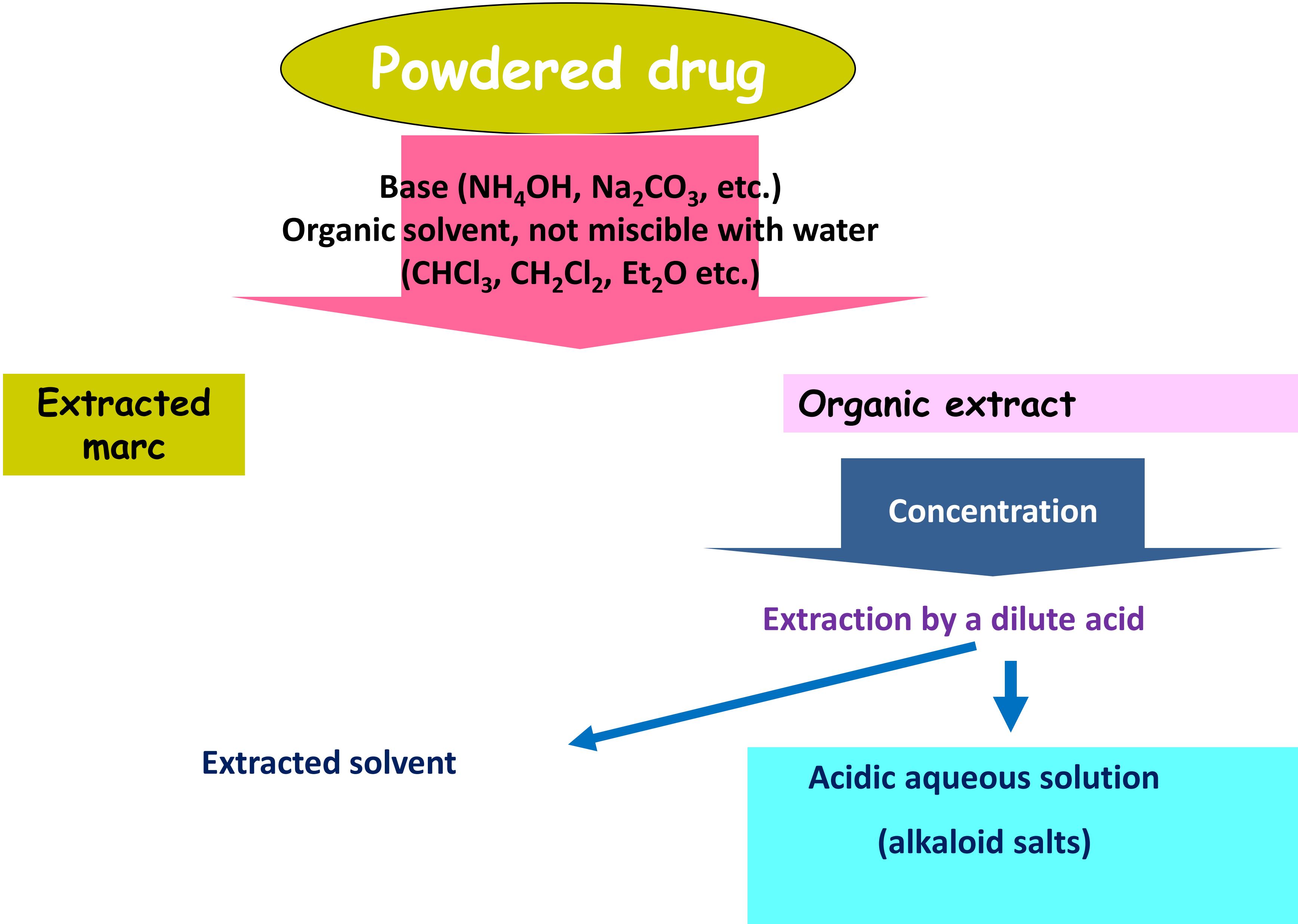
- Second step : The organic solvent containing the alkaloids as bases is separated from the residue and if necessary, partially concentrated by distillation under reduced pressure. The solvent is then stirred with an acidic aqueous solution: the alkaloids go into solution in the aqueous phase as salts, whereas the neutral impurities remain in the organic phase. The operation is repeated as many times as necessary until the organic phase no longer contains any alkaloids.

Many acids are used (hydrochloric, sulfuric, citric, tartaric), but always in very dilute solutions.

- Third step : The aqueous solutions of the alkaloid salts, combined, and if necessary, “washed” with an apolar solvent (hexane, diethyl ether) are alkalinized with a base in the presence of an organic solvent not miscible with water. The alkaloids as bases precipitate and dissolve in the organic phase. The extraction of the aqueous phase continues until the totality of the alkaloids has gone into the organic phase (which is easy to verify as Mayer’s reaction on the aqueous phase becomes negative).

- Finally, the organic solvent containing the alkaloids as bases is decanted, freed from possible traces of water by drying over an anhydrous salt (for example sodium sulfate), and evaporated under reduced pressure. A dry residue is left : **the total basic alkaloids**

1- Solvent Extraction in Alkaline Medium



Powdered drug

Base (NH_4OH, Na_2CO_3, etc.)
Organic solvent, not miscible with water
($CHCl_3$, CH_2Cl_2, Et_2O etc.)

Extracted marc

Organic extract

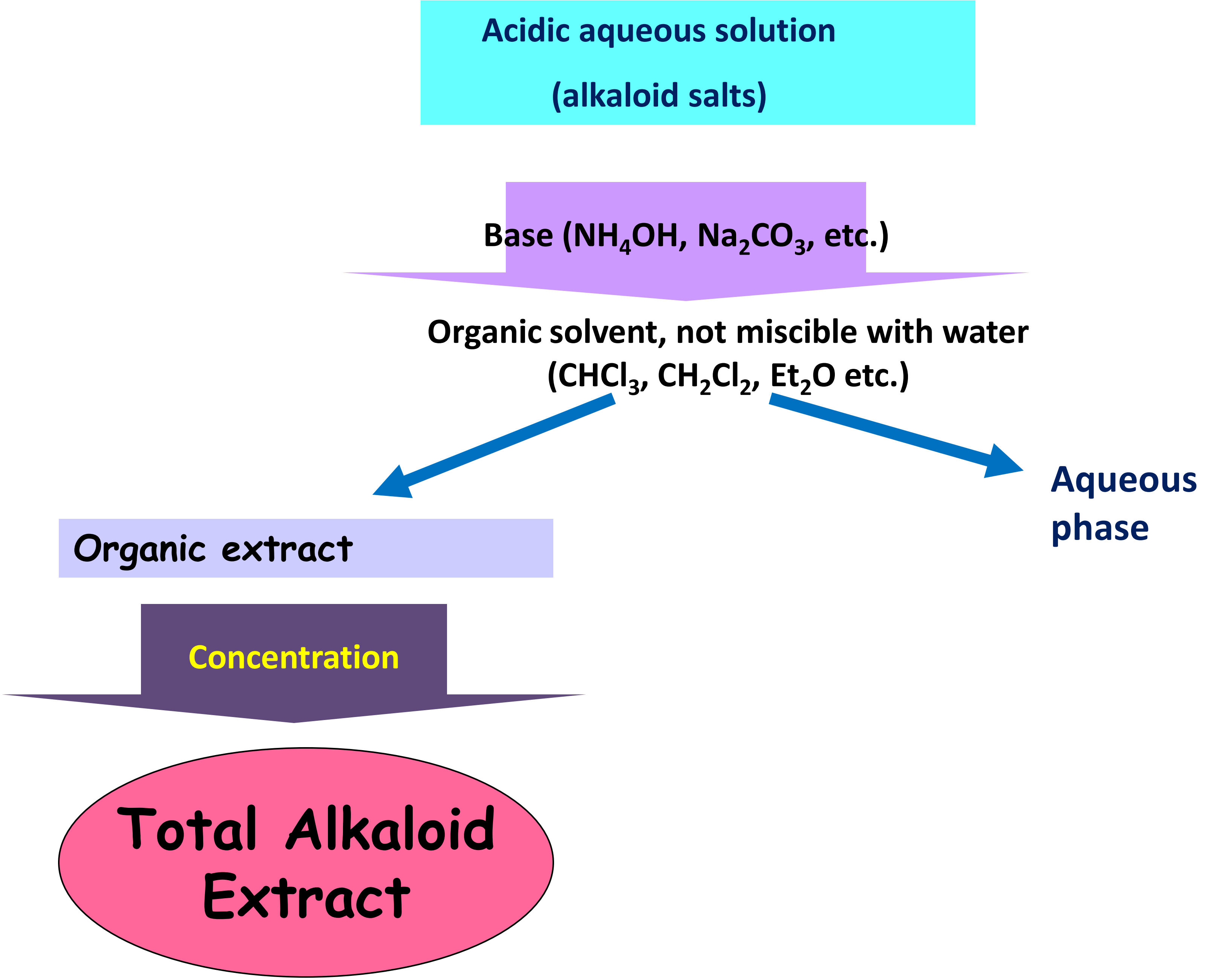
Concentration

Extraction by a dilute acid

Extracted solvent

Acidic aqueous solution

(alkaloid salts)



Acidic aqueous solution

(alkaloid salts)

Base (NH_4OH, Na_2CO_3, etc.)

Organic solvent, not miscible with water

($CHCl_3$, CH_2Cl_2, Et_2O etc.)

Organic extract

Aqueous phase

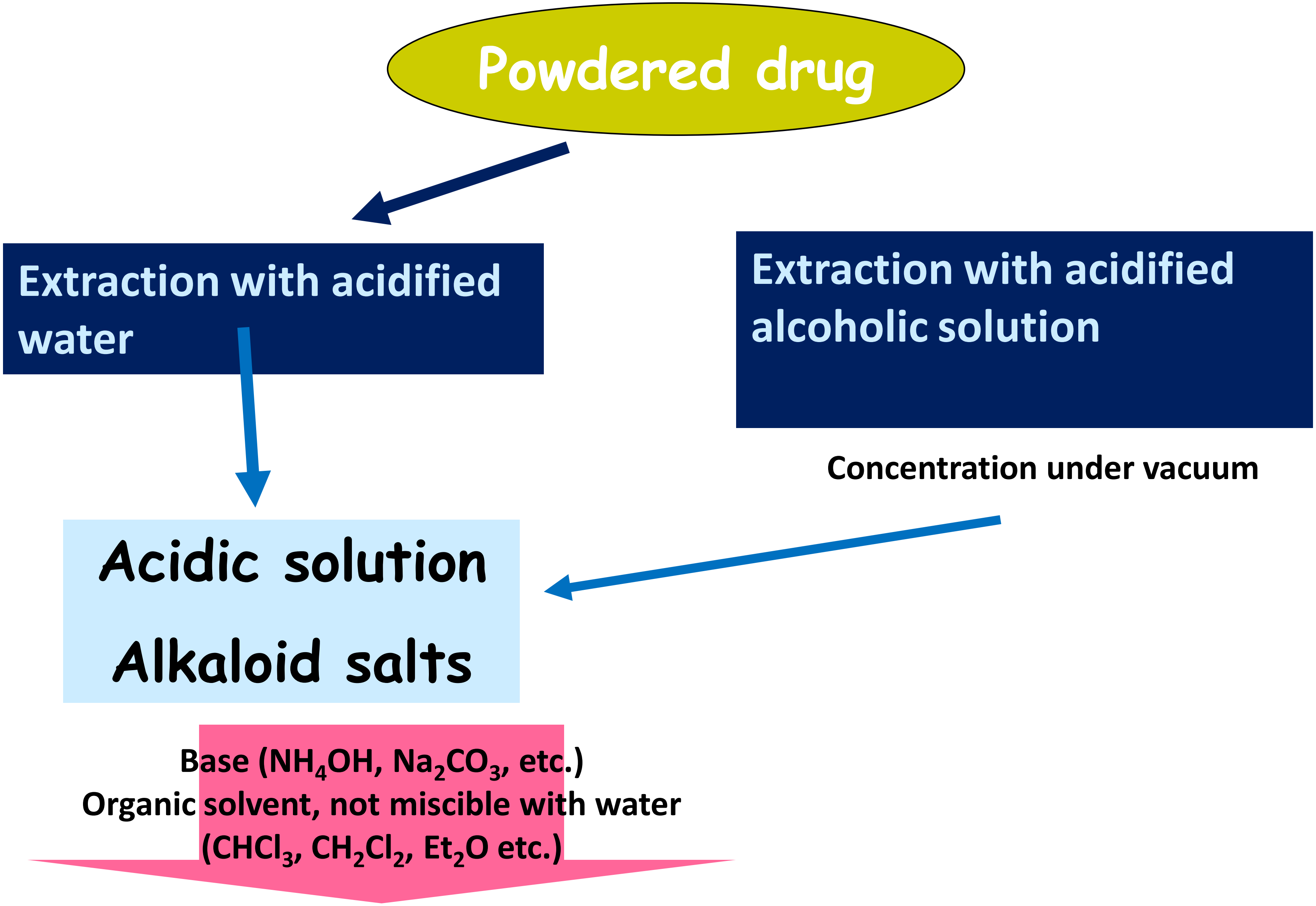
Concentration

Total Alkaloid Extract

Extraction in Acidic Medium

The pulverized drug is extracted with a-acidified water b-an acidified alcoholic solution. In the latter case, the extraction is followed by a distillation under vacuum. In both cases the result is an aqueous solution of alkaloid salts requiring purification. This can be accomplished by alkalinizing the solution and extracting the bases with immiscible organic solvent.

2 – Extraction in Acidic Medium



Powdered drug

Extraction with acidified water

Extraction with acidified alcoholic solution

Concentration under vacuum

Acidic solution

Alkaloid salts

Base (NH_4OH, Na_2CO_3, etc.)
Organic solvent, not miscible with water
($CHCl_3$, CH_2Cl_2, Et_2O etc.)

Organic solvent phase

Concentration

Total Alkaloid Extract

Isolation of Alkaloids

No matter what method is chosen to extract the alkaloids, it does not yield pure compounds, but total alkaloids, which are complex mixtures of base that must be separated. In many cases, it is necessary to resort to the classic methods of resolution of complex mixtures, particularly to chromatographic techniques (on silica gel, alumina, sometimes ion-exchange resins, and so forth). In research laboratories these techniques, as well as HPLC and preparative TLC, are most often used.

Quantitation

The quantitation of the total alkaloids requires beginning their extraction using a general method: generaly the alkaline medium approach is preferred, and at each step the completeness of the extraction must be verified.

Next, the total alkaloid residue is estimated by a gravimetric method or a volumetric quantitation.

The gravimetric methods are easy to implement, but the simple weighing of the total alkaloid residue lacks precision. Volumetric methods are based either on direct acidimetry, or, most often, on back titration of the acid.

To quantitate one constituent, or one group of constituents, in a drug, the available techniques include spectrophotometry, colorimetry, fluorimetry, and densitometry.

If the quantitation cannot be carried out directly, it is possible to isolate the compound to be measured by TLC and to measure the absorbance after eluting the spots. Of course HPLC tends to advantageously replace the “classic” methods.

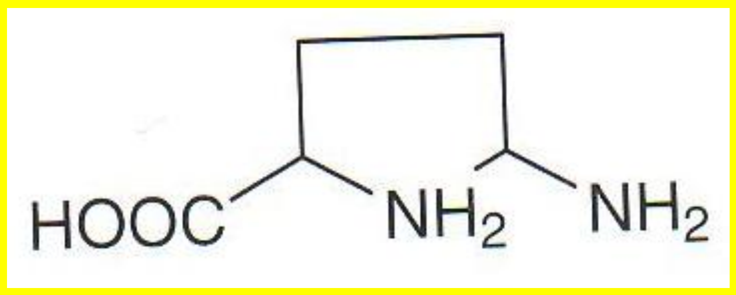
BIOSYNTHETIC ORIGIN

True- and protoalkaloids are formed biosynthetically from an amino acid (ornithine, lysine, phenylalanine, tyrosine, tryptophan, histidine, or anthranilic acid). Pseudoalkaloids are formed biosynthetically like terpenoids (isopentyl pyrophosphate + dimethylallylpyrophosphate).

BIOSYNTHESIS OF ALKALOIDS

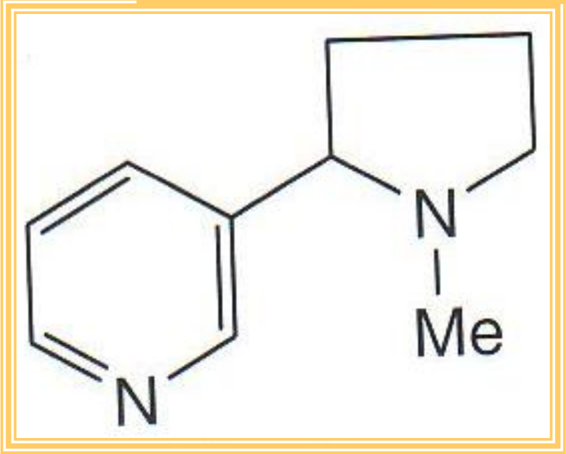
True alkaloids are formed from amino acids

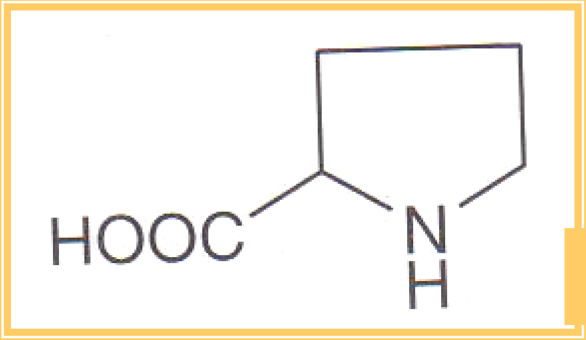
ornithine



Nicotinic acid + Me

nicotine

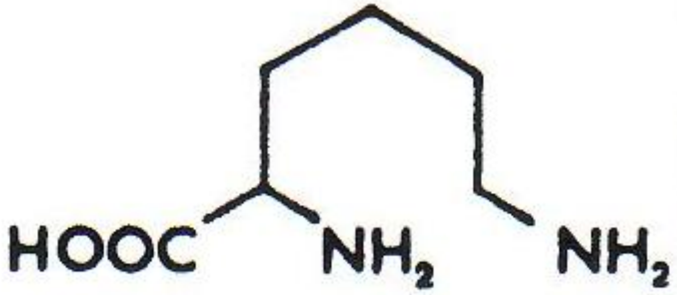




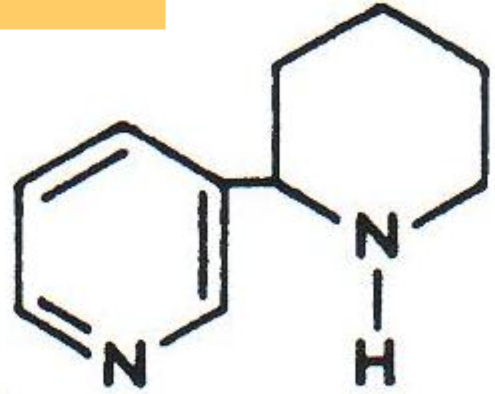
pyroline

True alkaloids are formed from amino acids

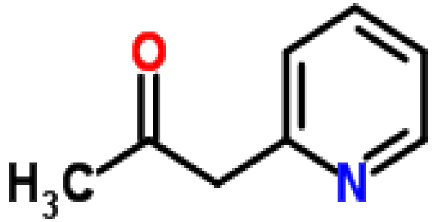
lysine



anabasine

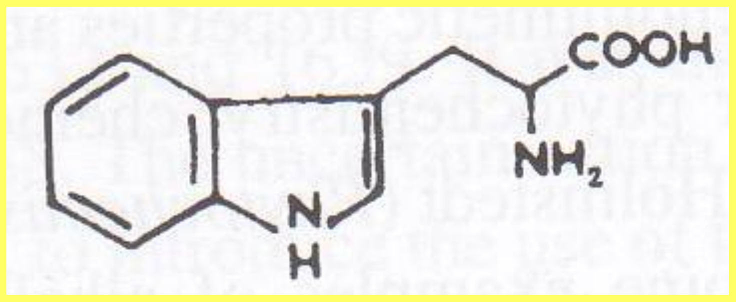


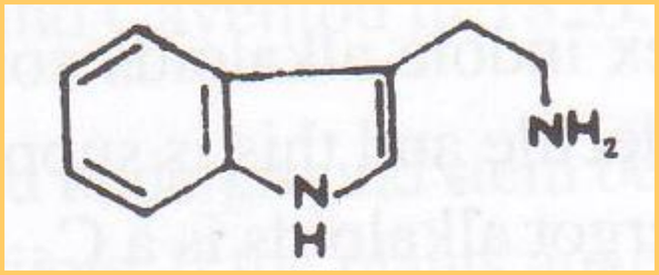
isopelletierine



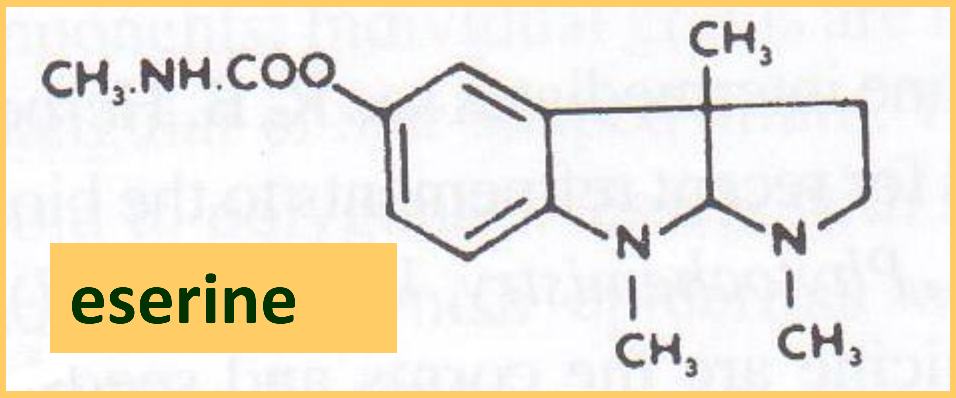
True alkaloids are formed from amino acids

tryptophan

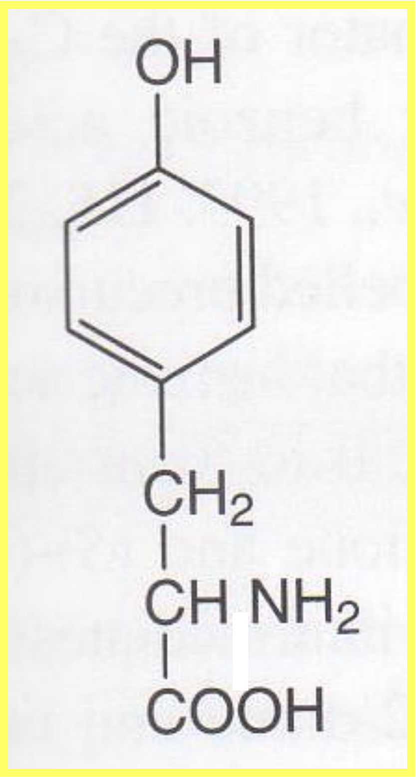




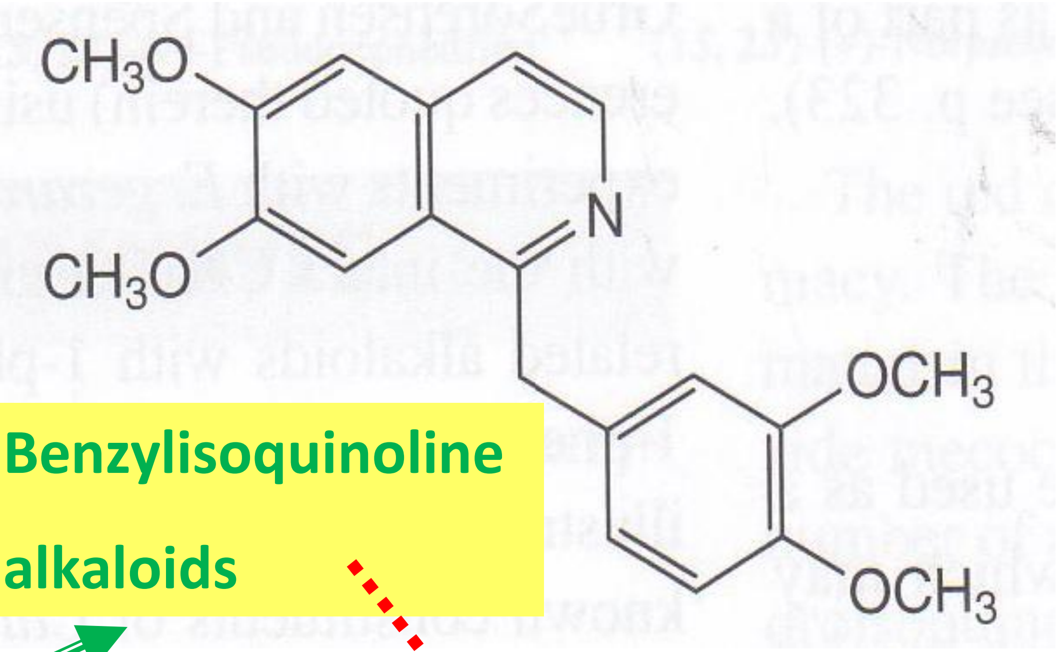
tryptamine



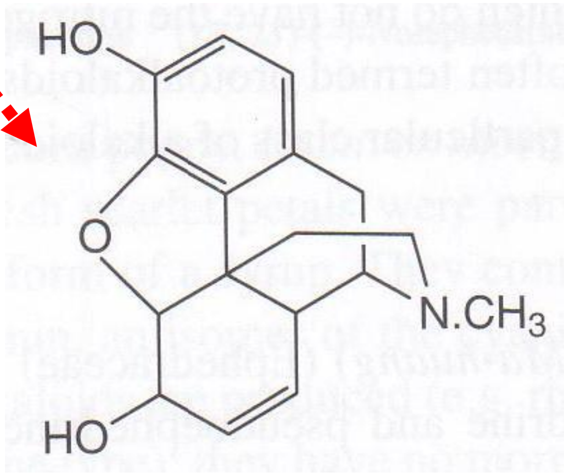
eserine



tyrosine

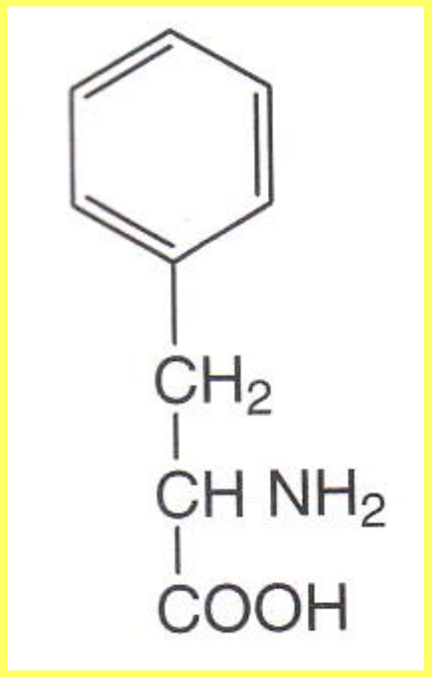


Benzylisoquinoline alkaloids

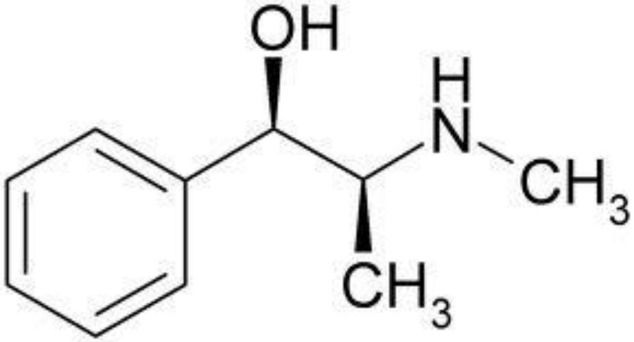


Opium alkaloids

Proto alkaloids are also formed from amino acids



phenylalanine



ephedrine

Ephedra major

PHARMACOLOGICAL ACTIVITY AND USES

Alkaloids are particularly interesting substances because of their multiple pharmacological activities :

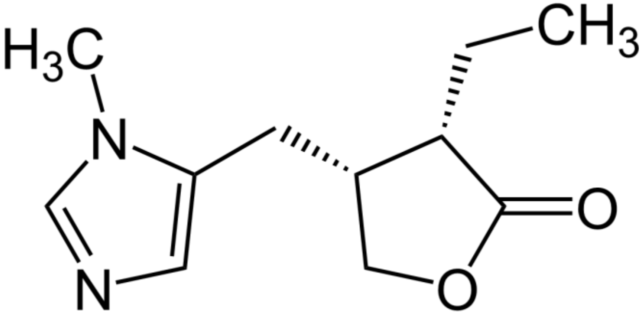
-on the CNS, whether they are depressants (morphine, scopolamine) or stimulants (strychnine, caffeine)

-on the autonomic nervous system : sympathomimetics (ephedrine) or sympatholytics (yohimbine, certain ergot alkaloids), parasympathomimetic (eserine, pilocarpine), anticholinergics (atropine, hyoscyamine), or ganglioplegics (sparteine, nicotine).

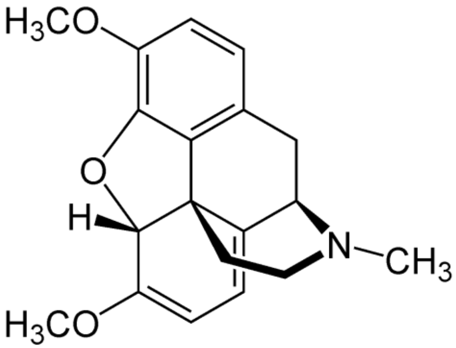
In addition, alkaloids include curare, local anesthetics (cocaine), agents to treat fibrillation (quinidine, antitumor agents (vinblastine, ellipticine), antimalarials (quinie), antibacterials (berberine), and amebicides (emetine).

These various activities (among others) lead to extensive use of alkaloid-containing drugs. Although some are only used as galenicals (belladonna, datura, henbane), many others are only used as starting materials for industrial extraction, for example, morphine from poppy straw or opium, scopolamine from *Duboisia*, ajmalicine from *Rauwolfia* roots, vincamine from from periwinkle leaves, and quinine from *Cinchona* bark.

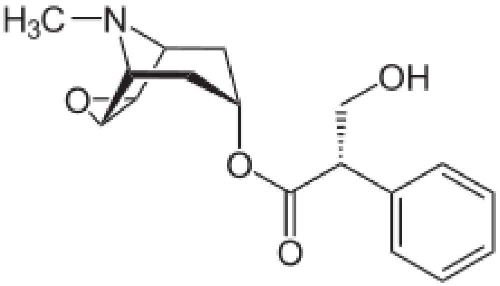
Some of the extracted alkaloids may undergo transformations : codeine is produced mostly by methylating morphine, quinine is converted to quinidine, sparteine to ajmalicine. In a few rare cases, the industry prefers direct synthesis : theophylline and papaverine are easily obtained that way. The drive to optimize therapeutic efficacy has sometimes resulted in achieving deeper transformations, or even total syntheses of analogous molecules, making use or not of starting materials of natural, plant, or fermentation origin (especially the derivatives of ergot alkaloids and those of the binary alkaloids of *Catharanthus*.)



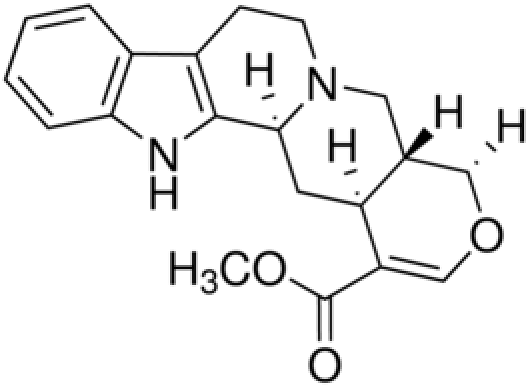
Pilocarpine



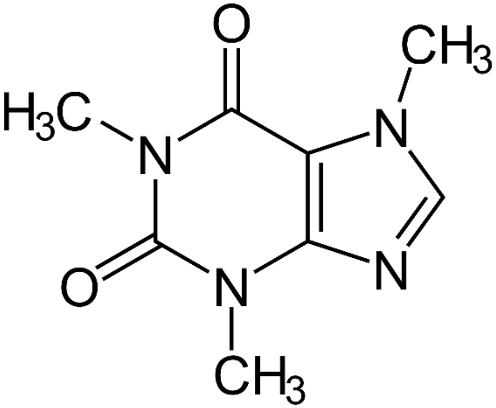
Thebaine



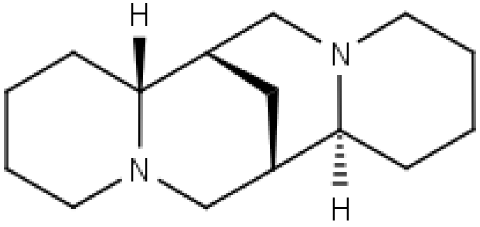
Scopolamine



Ajmalicine



Caffeine



Sparteine

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