Surh, Y.-J. and Lee, S.S. (1996). Capsaicin in Hot Chili Pepper: Carcinogen, Co-carcinogen or Anticarcinogen? *Fd. Chem. Toxic.*, **34**, 313-316.

Annatto Tree

Scotter, M.J., Wilson, L.A., Appleton, G.P. and Castle, L. (1998). Analysis of Anatto (*Bixa orellana*) Food Coloring Formulations; 1. Determination of Coloring Components and Colored Thermal Degradation Products by High-performance Liquid Chromatography with Photodiode Array Detection, *J. Agric. Food Chem.*, **46**, 1031-1038.

Saffron

Ríos, J.L., Recio, M.C., Giner, R.M. and Máñez, S. (1996). An Update Review of Saffron and its Active Constituents, *Phytother. Res.*, **10**, 189-193.

Part 4
ALKALOIDS

Generalities

The number of products that have been described, their structural diversity, and the scope of their pharmacological activities make alkaloids one of the most important groups of naturally occurring substances of therapeutical interest, whose number "is almost unfathomable" (G.Richter*). Considering the structural and biosynthetic variety, this phrase is not as exaggerated as it sounds...

1. DEFINITION

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

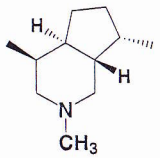
* Richter, G. (1993). *Stoffwechselphysiologie der Pflanzen*, 1988, Georg Thieme Verlag, Stuttgart.

(from the Arabic *al kaly*, soda, and from the Greek *eidos*, appearance). There is no simple and precise definition of alkaloids, and it is sometimes difficult to distinguish the thin line between alkaloids and other natural nitrogen-containing metabolites.

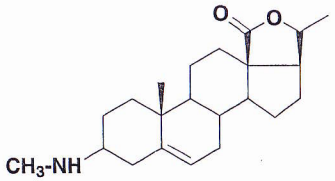
Initially defined as nitrogen-containing, basic substances of natural origin and of limited distribution, alkaloids have a complex structure. Their nitrogen atom is part of a heterocyclic system and they possess a significant pharmacological activity; according to some authors, they occur only in the vegetable kingdom. They are found as salts, and we may add that they are formed biosynthetically from an amino acid.

These elements characterize what may be referred to as ***true alkaloids***. Many authors distinguish, in addition, protoalkaloids and pseudoalkaloids.

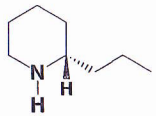
Pseudoalkaloids most often have all of the characteristics of the true alkaloids, but they are not derived from amino acids. Most of the known examples are isoprenoids and are referred to as terpenoid alkaloids: monoterpenoid alkaloids (e.g., β-skythantine), sesquiterpenoid alkaloids of the Nymphaeaceae, diterpenoid alkaloids, such as aconitine from the tubercle of the aconite, or steroidal alkaloids (e.g., paravallarine), to name only a few. Also known are heterocyclic nitrogen-containing substances arising from the metabolism of acetate, for example coniine, the toxic principle of hemlock.



β-*Skytanthine*



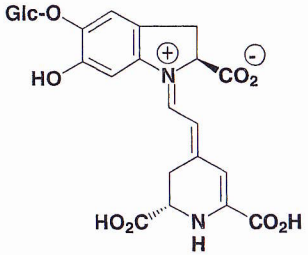
Paravallarine



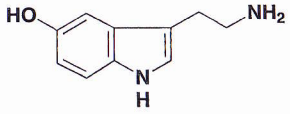
(+)-Coniine

Protoalkaloids are simple amines in which the nitrogen atom is not part of a heterocyclic ring; they are basic and are elaborated *in vivo* from amino acids. Various substances fulfill this definition: simple amines such as serotonin, mescaline from peyote, or cathinone from Abyssinian tea, but also betains (resulting from the quaternarization of the nitrogen atom of amino acids); some authors include betalains (sometimes referred to as chromoalkaloids) in this group (e.g., betanin).

Mescaline

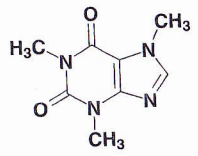


Betanin

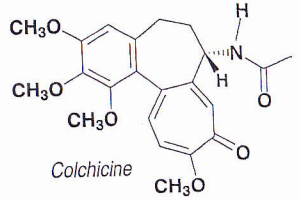


Serotonin

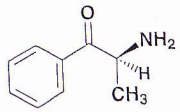
Although the distinction between true alkaloids, protoalkaloids, and pseudoalkaloids is intellectually appealing, it is not always easy to apply: where does colchicine belong, with its nitrogen atom within an amide function, but not within a heterocyclic ring? Where should caffeine and theophylline be classified? Are amine-containing glycosides such as the chaconines to be considered as no more than nitrogen-containing saponins?



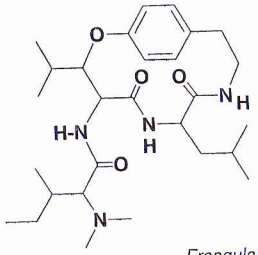
Caffeine



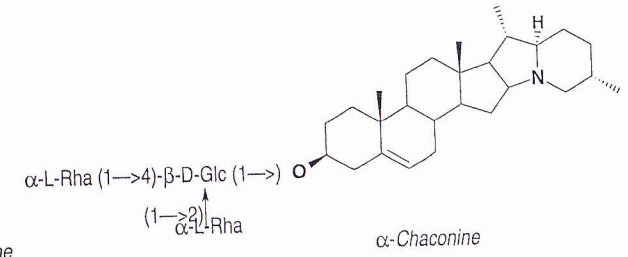
Colchicine



(–)-Cathinone



Frangulanine



α-Chaconine

In practice, it is widely accepted that the following are not alkaloids: simple amines, peptides *, amino sugars, porphyrins, alkylamines, and arylalkyl-amines, *at least those that are widely distributed* (in contrast, products with limited distribution such as ephedrine are most often recognized as alkaloids). All other compounds are commonly referred to as alkaloids: it would not occur to anyone to refer otherwise to colchicine, coniine, or aconitine!

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, at low doses, marked pharmacological properties. That this grouping has a sound basis is confirmed by the fact that these compounds have in common some reactions of precipitation with the "general reagents for alkaloids" (see below).

* Again this is often a thin line: there is no major difference between the cyclic polypeptides of amanitas (amatoxins, phallotoxins) and peptide alkaloids such as frangulanine: the latter have a macrocyclic ring attached onto a benzene ring in the 1,3- or 1,4 positions, whereas the former are generally classified as polypeptides in the strict sense of the term. In the same fashion, the macrocycles derived from spermidine or spermine may be considered alkaloids (their ring includes at least one non-peptidic bond).

To bring this paragraph to a close, note the interesting definition by Pelletier *: "An alkaloid is a cyclic organic compound containing nitrogen in a negative oxidation state which is of limited distribution among living organisms". From this point of view, amines and their oxides, amides, and quaternary ammonium salts are included, but nitrated derivatives (e.g., aristolochic acid) are excluded, and so are acyclic amides and polyamines. This definition allows, according to its author **, the inclusion of caffeine, colchicine, ephedrine, or ricinine in the alkaloids. Note that this definition does not include the activity criterion, and that the author comments that any compound administered at sufficient doses will eventually have an effect on a living organism (this tends to be forgotten, sometimes too easily).

2. HISTORY

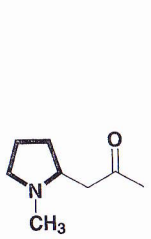
Although the concept of alkaloid is relatively recent, the knowledge of the toxicity and properties of the plants containing them dates back to ancient times: opium, coca, aconite, belladonna, colchicum, as well as cinchona, ipecac, and curare have been used for centuries, if not millennia.

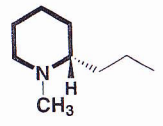
It was probably Derosne, who, while extracting a mixture of narcotine and morphine from opium in 1803, was the first to isolate a vegetable alkali. In 1806, Serturner recognized the alkaline nature of the somniferous principle of opium, which he named morphine about ten years later. Shortly afterwards, between 1817 and 1820, two French pharmacists, Pelletier and Caventou, "discovered" an impressive series of active compounds: caffeine, emetine from ipecac, strychnine from *nux vomica*, quinine and cinchonine from cinchona bark, followed a little later by coniine. Chemists attempted to elucidate the structure of these molecules very early on: in the most simple cases, they were successful (coniine, Schiff, 1870), but in other cases they had to wait until the second half of the twentieth century: the 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. The synthesis of these compounds also represented a challenge for chemists initially: from the synthesis of coniine at the end of the nineteenth century to that of morphine in 1952, this challenge played—and it continues to play—a key role in the development of organic chemistry.

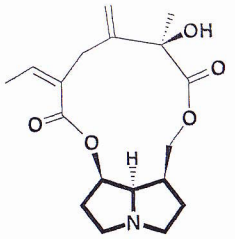
The isolation of reserpine at the beginning of the 1950s and its success in therapeutics inspired phytochemists to systematically explore the immense field of alkaloids: the number of structures described continues to increase, and structural, biosynthetic, synthetic, or pharmacological data have now reached considerable proportions. In a few cases, naturally-occurring compounds have been introduced in

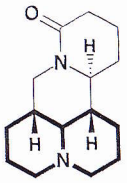
* Pelletier, S.W. (1983). The Nature and Definition of an Alkaloid, *in* "Alkaloids, Chemical and Biological Perspectives", *op. cit.*, **1**, p. 26 *sq.*

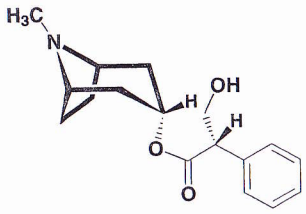
** Pelletier is careful to specify, about the term "cyclic": "a cyclic structure in some part of the molecule", which broadens the definition substantially

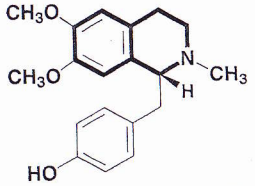

Hygrine **A**

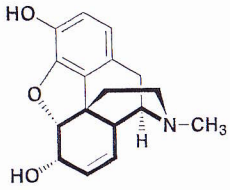

N-Methyl coniine **B**

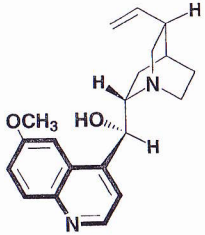

Seneciphylline **C**

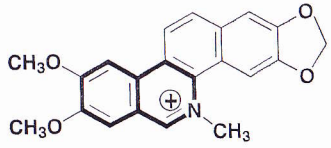

Matrine **D**

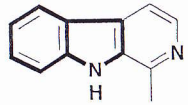

Hyoscyamine **E**

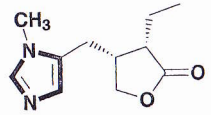

(-)-Armepavine **F**

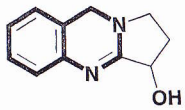

Morphine **F**

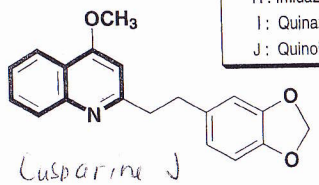

Quinine **J**


Nitidine **F**


Harmane **G**


Pilocarpine **H**

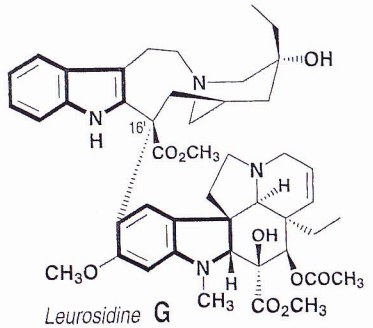

Vasicine **I**

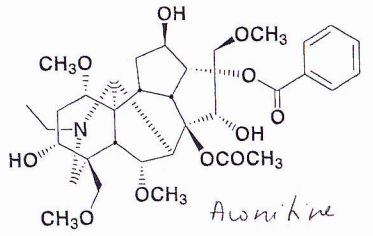

Cuspareine J

From the simplest...
... to the most complex:

Examples of alkaloid structures illustrating the chief heterocyclic system encountered. The basic heterocyclic system is in boldface

A : Pyrrolidine,
B :Piperidine,
C : Pyrrolizidine,
D : Quinolizidine,
E : Tropane,
F : Isoquinoline,
G : Indole
H : Imidazole,
I : Quinazoline,
J : Quinoline.


Leurosidine **G**


Aconitine

clinical trials—for example the binary alkaloids of *Catharanthus*—and in many other cases, structural analogs have been synthesized and marketed (derivatives of ergot alkaloids); interesting potential pharmacological activities have been uncovered and have inspired developments in a variety of directions (synthesis, structure-activity relationships, receptor experiments, and more).

3. NATURAL OCCURRENCE, DISTRIBUTION, AND LOCALIZATION

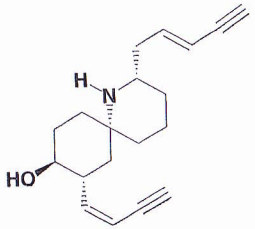
Alkaloids occur only exceptionally in bacteria (pyocyanine from *Pseudomonas aeruginosa*) and rather rarely in fungi (psilocin from the hallucinogenic mushrooms of Central America, ergolines from *Claviceps* and other actinomycetes, sporidesmins, roquefortine, and others). The Pteridophytes rarely contain 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, and some authors estimate that 10 to 15% of these synthesize those products. Certain families have a marked tendency to elaborate alkaloids: this is true for the Monocotyledons (Amaryllidaceae, Liliaceae) as well as the 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 is far less common.

Certain alkaloids occur in several genera that belong to different families; sometimes these are quite distant taxonomically (caffeine), but most often they are fairly close (reticuline, yohimbine). Other alkaloids are characteristic of a limited number of genera within one family (hyoscyamine), or of a group of species within one genus (thebaine); some are highly specific (morphine).

Alkaloid concentrations have a wide range of variation: from a few ppm as in the case of the anticancer alkaloids of the Madagascan periwinkle (*Catharanthus roseus*: the level barely reaches 3 g of vinblastine for one metric ton of leaves) to more than 15% in the bark of the trunk of *Cinchona ledgeriana*. Only rarely do alkaloid-containing plants contain only one alkaloid: sometimes they do contain virtually only one constituent (e.g., hyoscyamine from the leaves of belladonna), but, most often, they yield a complex mixture, which may be dominated by one major constituent. It is not uncommon to find several dozen alkaloids in one drug (nearly one hundred in the case of the Madagascan periwinkle). As a general rule, all of the alkaloids of a given plant have a common biogenetic origin, even if their structures may at first seem quite different. In a given plant, the concentration of alkaloids can vary widely from part to part, and some parts may contain none. Qualitative variations are also frequent: it is not uncommon for different parts of one plant to contain dissimilar alkaloids. One example is quinine, which accumulates in the trunk bark of *Cinchona*, but is completely absent from the leaves;

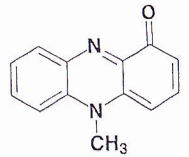
similarly, conessine accumulates in the seeds and the bark of kurchi (*Holarrhena pubescens*), but is absent from the leaves. The occurrence of chemotypes is well known (*Rauwolfia, Duboisia*).

For a long time, alkaloids used to be considered products of the metabolism 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 urodele (salamanders) or anurous Amphibia (*Bufo, Phyllobates, Dendrobates*, and other toads). Alkaloids occur frequently in the Arthropods, who secrete them in very small quantities in their exocrine glands. They are well known in Hymenoptera (e.g., solenopsine of Myrmicides), but they are also elaborated and used by Coleoptera, Neuroptera, and some of the Myriapoda. They

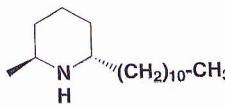


Histrionicotoxine
(Dendrobates histrionicus)

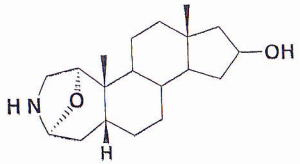
Glomerine
(Glomeris marginata, *Myriapodes*)



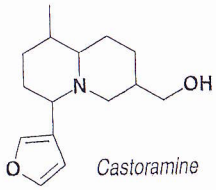
Pyocyanine



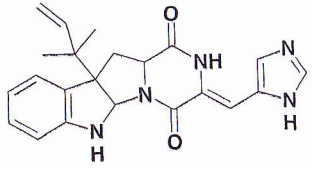
Solenopsine A
(ants)



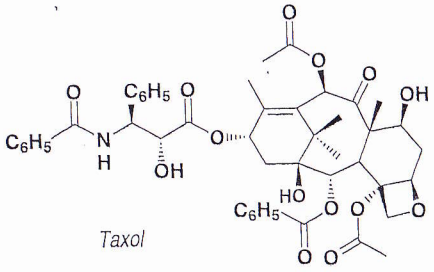
Samaderine
(salamander)



Castoramine



Roquefortine



Taxol

have low molecular weights (pyrrolidines, piperidines, pyrroles, indolizidines, pyrazines), and they are volatile enough to act as chemical signals, defense compounds (allomones), or communication compounds (pheromones). In recent years, progress in marine biology has led to the isolation of many nitrogen-containing heterocyclic structures, particularly in the sponges.

Localization

In the plant, alkaloids occur as soluble salts (citrates, malates, tartrates, meconates, isobutyrates, benzoates), or in combination with tannins. Microchemistry shows that alkaloids are most often localized in the peripheral tissues: external layers of the bark of the stems and roots, or seed tegument. Most of these compounds have a basic character and an antimetabolite activity which make their compartmentalization necessary: they are normally stored in the cell vacuoles, which may be specialized (laticiferous) or not. Most often, alkaloid synthesis takes place at specific sites (growing root, laticiferous cells, chloroplasts); the compounds are subsequently transported to the storage site.

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: if we accept the notion that structural diversity reflects constant adaptation, it reinforces this hypothesis. Although some authors believe that alkaloids are terminal metabolites, in other words unusable waste, this is highly unlikely: in several cases, they have been shown to act as intermediate metabolites. Storage substances? Growth regulators? The question remains unanswered.

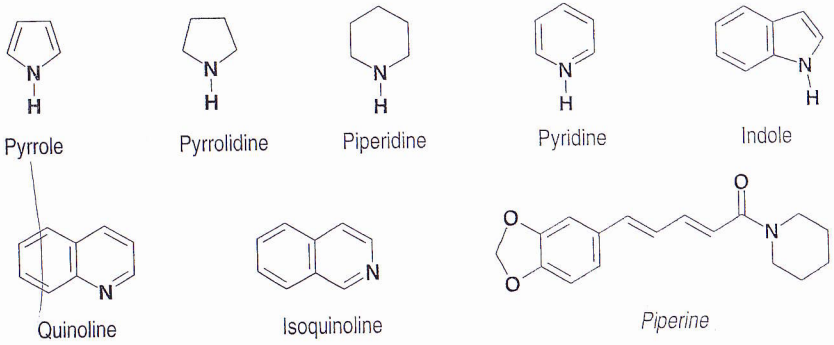
4. PHYSICO-CHEMICAL PROPERTIES

Alkaloids have molecular weights ranging from 100 to 900. Although most of the bases that do not contain oxygen atoms are liquid at ordinary temperatures (nicotine, sparteine, coniine), those that do contain oxygen atoms—virtually all of the known structures—are normally crystallizable solids, and in rare cases they are colored (berberine). Almost all of the crystallized bases rotate the plane of polarized light, and have sharp melting points, without decomposition, especially below 200°C. As a general rule, alkaloids as bases are not soluble or are sparingly soluble in water, soluble in apolar or only slightly polar organic solvents, and are soluble in concentrated hydroalcoholic solutions.

The basicity of alkaloids varies greatly, since this property depends entirely on the availability of the lone pair of electrons on the nitrogen atom. Electron-withdrawing groups in close proximity to the nitrogen atom decrease the basicity, whereas

presence of the carbonyl group on the amide, practically neutral. The basic character of the heterocyclic ring itself varies: in the molecule of pyridine, with 6 π electrons, and in the case of quinoline and isoquinoline, the lone pair of electrons on the nitrogen atom is available and the basicity is clear. In the case of pyrrole or indole, the lone pair of electrons on the nitrogen atom plays a role in the aromatic character, and the compounds are not basic (they are acidic). Another example is pyrrolidine, which is saturated, and is a strong base. The basicity is also influenced by steric constraints (at least in the complex polycyclic molecules). Finally, let us emphasize that their basic character renders these compounds unstable, so that as bases in solution they are sensitive to heat, light, and oxygen.

The basic character of alkaloids allows the formation of salts with mineral acids (hydrochlorides, sulfates, nitrates) or organic acids (tartrates, sulfamates, maleates). Alkaloid salts are generally soluble in water and in dilute alcohols, and they are, except in rare cases, not soluble in organic solvents. The crystallized salts can be conserved fairly well and are the common commercial form of these compounds.



5. DETECTION AND CHARACTERIZATION

A detection technique ought to be, to the extent possible, rapid, simple, reproducible, and sensitive; it must be applicable to a small sample. The detection methods currently in use are preceded by an extraction and consist, most generally, in precipitating the alkaloids by using fairly specific reagents: the "general reagents for alkaloids". The preliminary extraction can be a "classic" alkaloid extraction (see below) or an alcoholic maceration, which takes less time: the alcoholic solution is evaporated and the residue redissolved in acidic water; after filtering, the alkaloids are characterized in the filtrate.

The general reactions of precipitation are based on the fact that alkaloids form combinations with metals and metalloids: bismuth, mercury, tungsten, and iodine. In practice, what is used is a solution containing iodine and iodide, or a solution containing potassium iodide and mercuric chloride—known as Mayer's reagent—or a reagent containing bismuth nitrate and potassium iodide, better known as Dragendorff's reagent. It is also possible to use silicotungstic acid (a mixture of

CONIUM MACULATUM L.

of these reagents is not absolute: proteins, α-pyrones, some coumarins, hydroxyflavones, lignans, and other compounds can give false positive reactions with Dragendorff's reagent.

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

- *p*-dimethylaminobenzaldehyde for the ergot alkaloids and pyrrolizidine alkaloids;
- cerium and ammonium sulfate, which differentiate indoles (yellow), dihydroindoles (red), β-anilinoacrylates (blue), oxindoles;
- ninhydrin for arylalkylamines;
- 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*).

The reactions listed above show the presence of alkaloids, but are not sufficient to verify the identity of a drug; they also do not provide information on the composition of mixtures. To this end, and as in the case of many other secondary metabolites from plants, the methods currently used are TLC and HPLC, on normal or reverse phase (with solvents of the water-methanol or water-acetonitrile type). Dragendorff's reagent, the iodine-iodide solution (or iodine vapors), potassium iodoplatinate, or cerium and ammonium sulfate are commonly used to visualize TLC plates. Due to the lack of volatility of virtually all of the alkaloids, GC is reserved for a few special cases. For more examples, see the specialized literature.

6. EXTRACTION OF ALKALOIDS

The extraction of alkaloids is based, as a 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 (this is particularly true for 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. These technical problems can be more or less completely avoided by a 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.

A. Extraction *per se*

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.

Alkalinization is very often achieved with aqueous ammonia. If the structure of the alkaloids to be extracted contains a fragile element, for example, an ester or lactone function, aqueous ammonia must be replaced by an alkaline carbonate solution. In some special cases, a mixture of calcium hydroxide and sodium hydroxide is used, for example for *Cinchona* bark, in which the alkaloids are combined with tannins. When using sodium hydroxide, remember that it will turn phenolic alkaloids into phenolates, which will remain in solution in water: if necessary, this can be used to fractionate the total alkaloids.

The organic solvent can be a chlorinated solvent (dichloromethane, chloroform), ethyl acetate, or diethyl ether. In the extraction industry, the selection criteria for determining the solvent generally include toxicity, safety, cost, ease of recovery and recycling (a mixture of Diesel oil and kerosene may well be an efficient solvent).

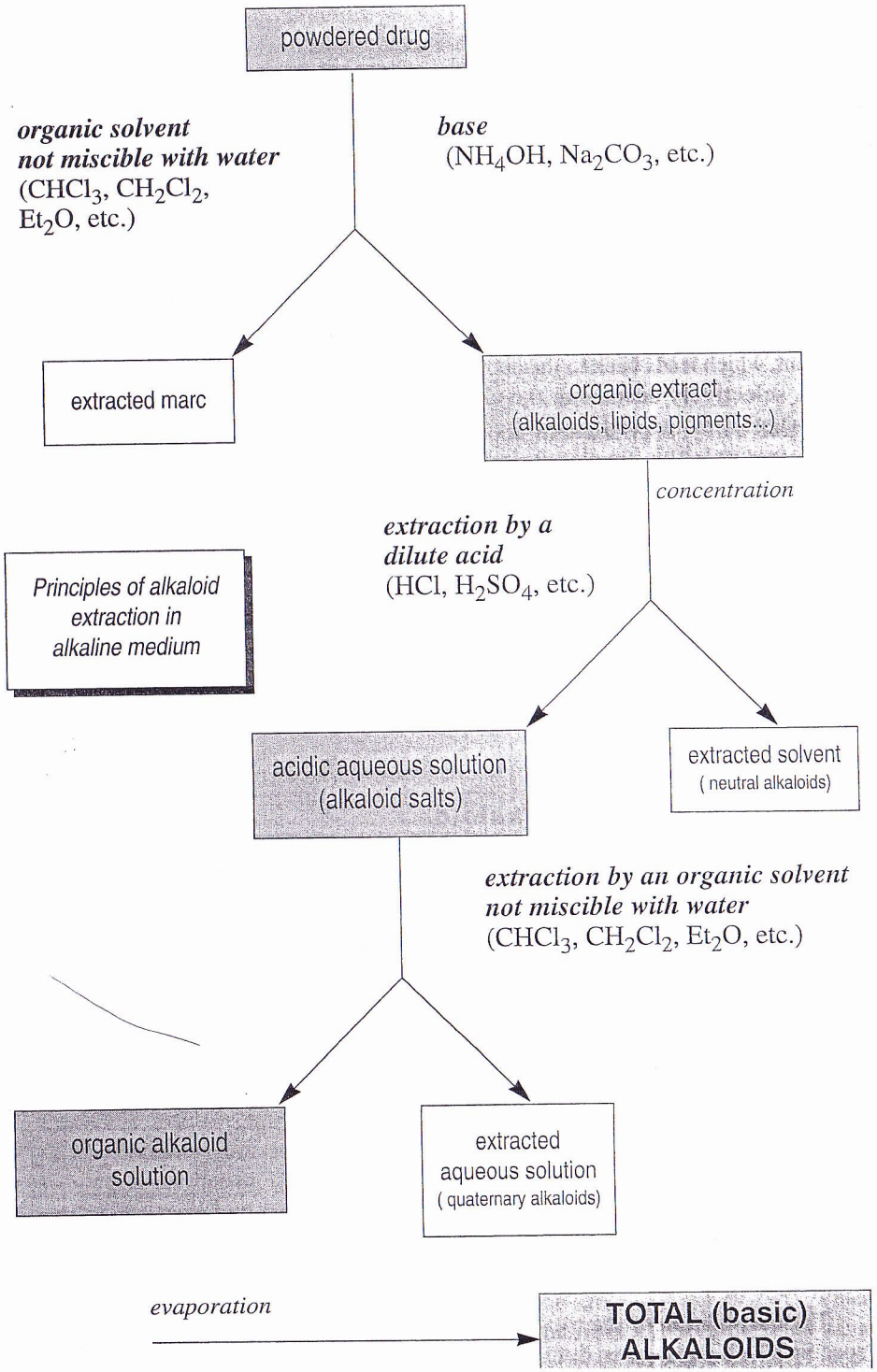
The extraction may be achieved by mere contact, or, much better, by multiple contacts within set-ups operating on the principle of the Soxhlet apparatus. The industry uses such, or even more efficient solid-liquid extractors based on the principle of counter-current extraction.

- **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 (e.g., hydrochloric, sulfuric, sulfamic, citric, tartaric), but always in very dilute solutions (1-5%).

- **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). This purification step may be carried out, like the previous one and depending on the quantity, in an separation funnel, or in more or less complex apparatuses: centrifugal extractors and other types of on-line set-ups.

- 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.



Extraction in Acidic Medium

Two approaches are possible: in the first one, the pulverized drug is extracted directly with acidified water; in the second case, it is extracted with an acidified alcoholic or hydroalcoholic solution. In the latter case, the extraction is followed by a distillation under vacuum which eliminates the alcohol and leaves behind an acidic aqueous solution of the alkaloid salts.

In both cases, the result is an aqueous solution of alkaloid salts requiring purification. This can be accomplished by:

1. alkalinizing the solution and extracting the bases with an immiscible organic solvent, which leads back to the above step;
2. selectively adsorbing the alkaloids contained in the solution on an ion-exchange resin, then eluting them with a strong acid;
3. precipitating the alkaloids as iodomercurates. The resulting complex is recovered by filtration, dissolved in a mixture of water, alcohol, and acetone, and decomposed by passing it through an ion-exchange resin. This technique can be used to extract quaternary ammonium salts.

We shall not describe here the special techniques optimized for one particular type of alkaloid (steam distillation of sparteine, extraction of the very weak bases of *Rauwolfia*, decaffeinating coffee), for difficult cases (adsorption on a styrene-divinylbenzene resin, on diatomaceous earths), or for extraction from biological matrices (as ions pairs).

B. Isolation of the 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 bases that must be separated. In the best conditions, one of the alkaloids is the major constituent and can be obtained by direct crystallization: one example is quinine which is crystallized as a basic sulfate by simply neutralizing the acidic extraction medium with sodium carbonate to pH 6. In other cases, the various alkaloids in the mixture have different basicities and this allows the design of back-extractions by non miscible solvents at various pHs. 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, ion-exchange resins, and so forth). In research laboratories these techniques, as well as HPLC and preparative TLC, are most often used.

7. QUANTITATION

The quantitation of the total alkaloids must be distinguished from that of one specific alkaloid in a given drug.

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

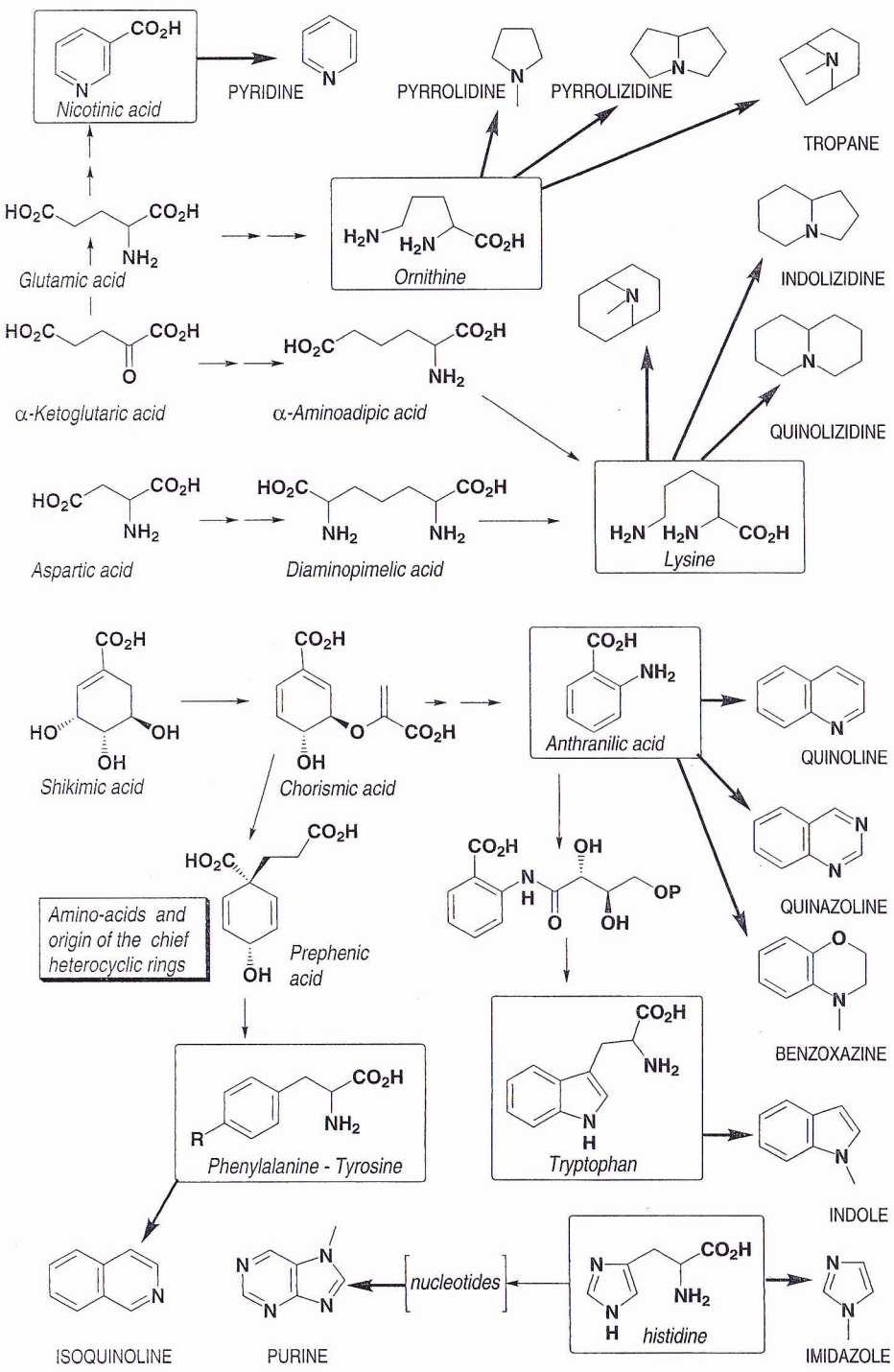
Next, the total alkaloid residue is estimated by a gravimetric method or a volumetric quantitation. The gravimetric methods are easy to implement, but the simple weighing of the total alkaloid residue lacks precision: it leads to non-trivial overestimates and should be replaced by other procedures. Volumetric methods are based either on direct acidimetry, or, most often, on back titration of the acid: after dissolving the residue in an excess of titrated acid, the acid in excess is quantitated by a base of known concentration in the presence of an indicator (mydriatic Solanaceae, Boldo). If needed, acid titration in non-aqueous medium can be used (weak bases).

- To quantitate one constituent, or one group of constituents, in a drug, the available techniques include spectrophotometry, colorimetry, fluorimetry, and densitometry. The spectrophotometric methods are very sensitive and fairly often recommended, for example to quantitate quinine- and cinchonine-type alkaloids in *Cinchona* bark by measuring the absorbance at two different wavelengths, or to quantitate caffeine in tea leaves. 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 (e.g., quantitation of ajmalicine in *Catharanthus* roots). The colorimetric methods can also be applied to the quantitation of one alkaloid (or of a group of alkaloids), for example the weak bases of *Rauwolfia*.

Of course, HPLC tends to advantageously replace the "classic" methods. This is true in the daily work in specialized laboratories and in the industry, but also in the latest edition of the French Pharmacopoeia where the (complex) gravimetric quantitation of morphine in opium has been replaced with an HPLC method.

8. BIOSYNTHETIC ORIGIN

The biosynthetic origin cannot be discussed in general terms: it will be covered for each major group of alkaloids, in the corresponding chapter. Here, we shall merely note that the precursor is, for true alkaloids, an amino acid: ornithine, lysine, phenylalanine, tyrosine, tryptophan, histidine, or anthranilic acid. The figure on page 798 shows the chief basic heterocyclic skeleta and links them to their precursors. The formation of the heterocyclic system generally proceeds through a simple inter- or intramolecular mechanism: formation of a Schiff base, or, in many cases, Mannich reaction. The formation of the alkaloid may require the involvement of only one molecule of amino acid (hygrine, cathine), or two molecules of the same amino acid (quinolizidines, benzylisoquinolines), or, less commonly, of two different amino acids (tubulosine), or else of several molecules of the same acid (sparteine). When the alkaloid has additional carbon atoms, these come from intermediates that have major roles in other metabolic pathways: acetate (tropane), dimethylallylpyro-



phosphate (ergolines, furoquinolines), or intermediates more specific to a particular group of plants, like secologanin (monoterpenoid indole alkaloids). Allylic oxidations, oxidative couplings, oxidation of the aromatic rings, esterifications, and etherifications explain the occurrence of many structural variations. In the particular case of the terpenoid alkaloids, the precursors are strictly of terpenoid origin and the formation of an amine function occurs late in the pathway.

9. 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 (quinine), antibacterials (berberine), and amebicides (emetine).

These various activities (among others) lead to the 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 *Catharanthus* roots, vincamine 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, serpentine to ajmalicine, and tabersonine to vincamine; tropane alkaloids are quaternized. 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 (see especially the derivatives of ergot alkaloids and those of the binary alkaloids of *Catharanthus*).

10. BIBLIOGRAPHY

General references

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* Minute quantities are still used to prepare galenicals.