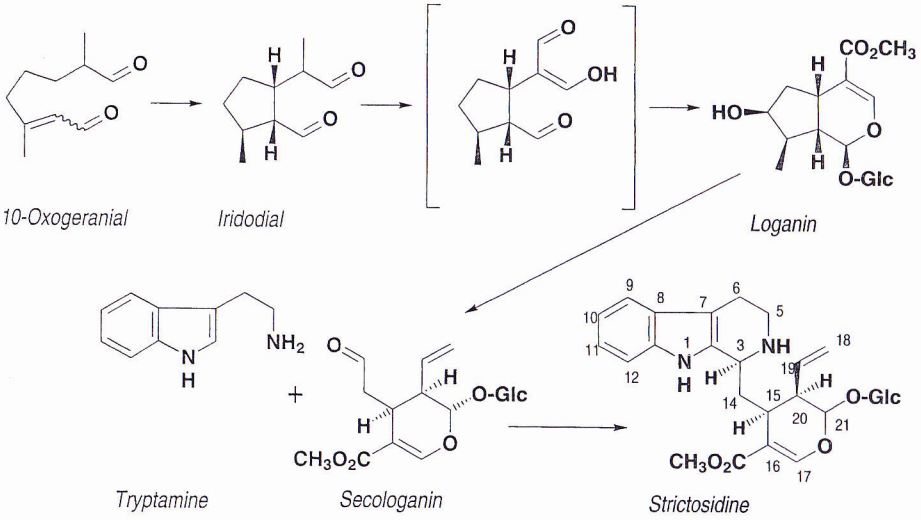
Monoterpenoid Indole Alkaloids

1. Introduction... 1000
2. Biosynthetic Origin. 1001
    A. From Tryptamine to Strictosidine, Origin of the Common Precursor 1001
    B. From Strictosidine to the Alkaloids: Principal Structural Types 1003
    C. Biosynthesis of Type I Indole Alkaloids 1005
    D. Biosynthesis of Type II and III Alkaloids. 1007
    E. Special Cases 1008
        1. Binary Alkaloids from *Catharanthus.* 1008
        2. Quinoline Alkaloids from Cinchonas.. 1009
3. Monoterpenoid Indole Alkaloid Distribution. 1010
4. Drugs Containing Indole Alkaloids.. 1013
    A. Loganiaceae: Nux Vomica (1013), Yellow Jessamine 1014
    B. Rubiaceae: Yohimbe. 1015
    C. Apocynaceae. 1016
        Madagascan Periwinkle 1016
        Semisynthetic Derivatives 1019
        Route to Binary Alkaloids: Biomimetic Synthesis.. 1021
        Common Periwinkle 1022
        Rauwolfia 1024
        Other Apocynaceae 1027
        Iboga (1027), *Voacanga* (1028), *Ochrosia* 1028
5. Drugs Containing Quinoline Alkaloids 1029
    Cinchonas 1029
    Camptotheca. 1037
6. Bibliography 1038

1. INTRODUCTION

As stated in the general introduction to alkaloids derived from tryptophan, the distribution of this very vast group of alkaloids is practically limited to three families of the order Gentianales: the Apocynaceae, Loganiaceae, and Rubiaceae, with the Apocynaceae containing the majority of the alkaloids that have been isolated or marketed, and have pharmacological applications or don't.

The most remarkable characteristic of the alkaloids in this group is probably their common biosynthetic origin: all of the known compounds arise from a unique precursor, namely strictosidine. This is a glycoside. It is formed by the condensation of one molecule of tryptamine with a monoterpenoid aldehyde, namely secologanoside, which, as described in the chapter on "iridoids", comes from mevalonic acid, *via* dimethylallyl and isopentenyl pyrophosphate, geraniol, and iridodial.

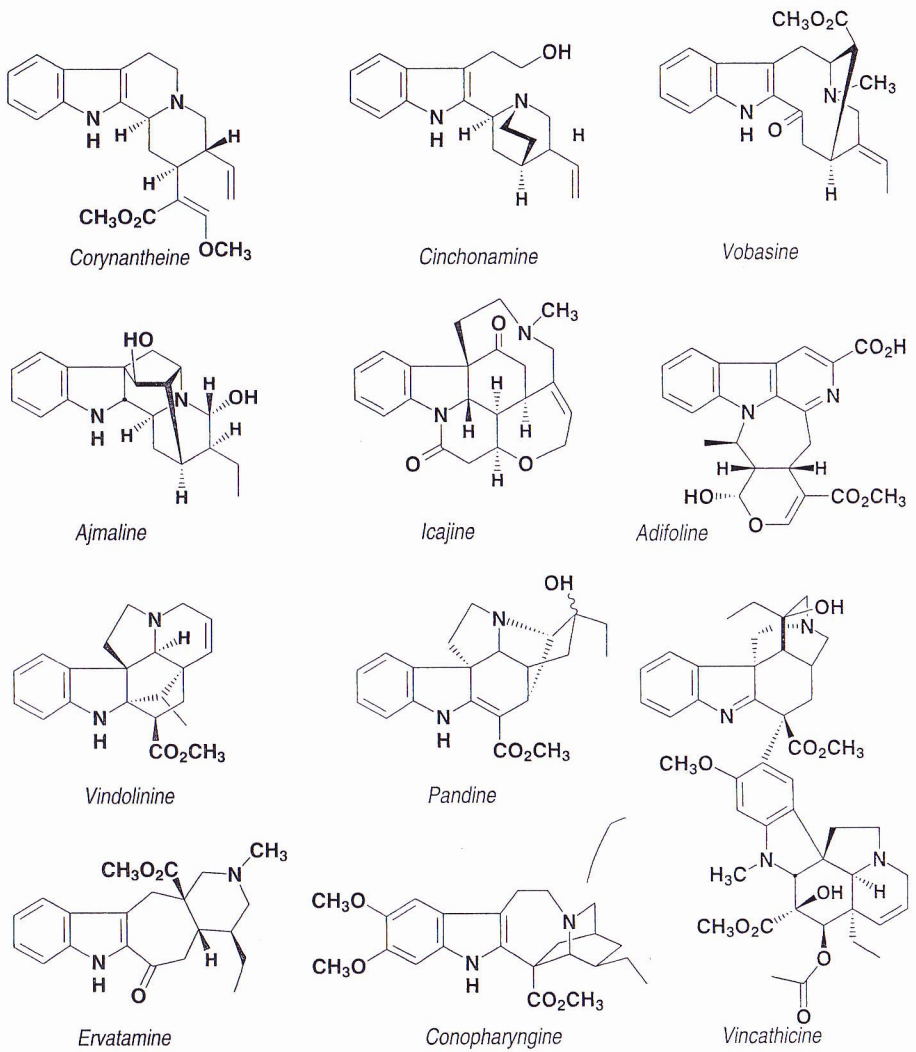


Origin of strictosidine

The structural diversity of this group, which probably comprises over 2,000 different compounds, far exceeds the scope of this book: we shall merely examine a few of the most representative examples.

The first source of structural variability is linked to the tryptamine moiety. For example, tryptophan, which in the majority of cases is incorporated as tryptamine, can fail to be decarboxylated, leading to the alkaloids of *Adina* and other Rubiaceae (see adifoline in the figure below). Another example is when the ethanamine chain of tryptamine loses a carbon atom, which is observed in the ellipticines or in uleine. In some rare cases, a rearrangement converts the initial indole to a quinoline (see cinchonas).

The other source of structural variability, which is in fact by far the most important one, is linked to the monoterpenoid moiety, which may undergo multiple rearrangements; the examples in the figure of p. 1004 illustrate some of the

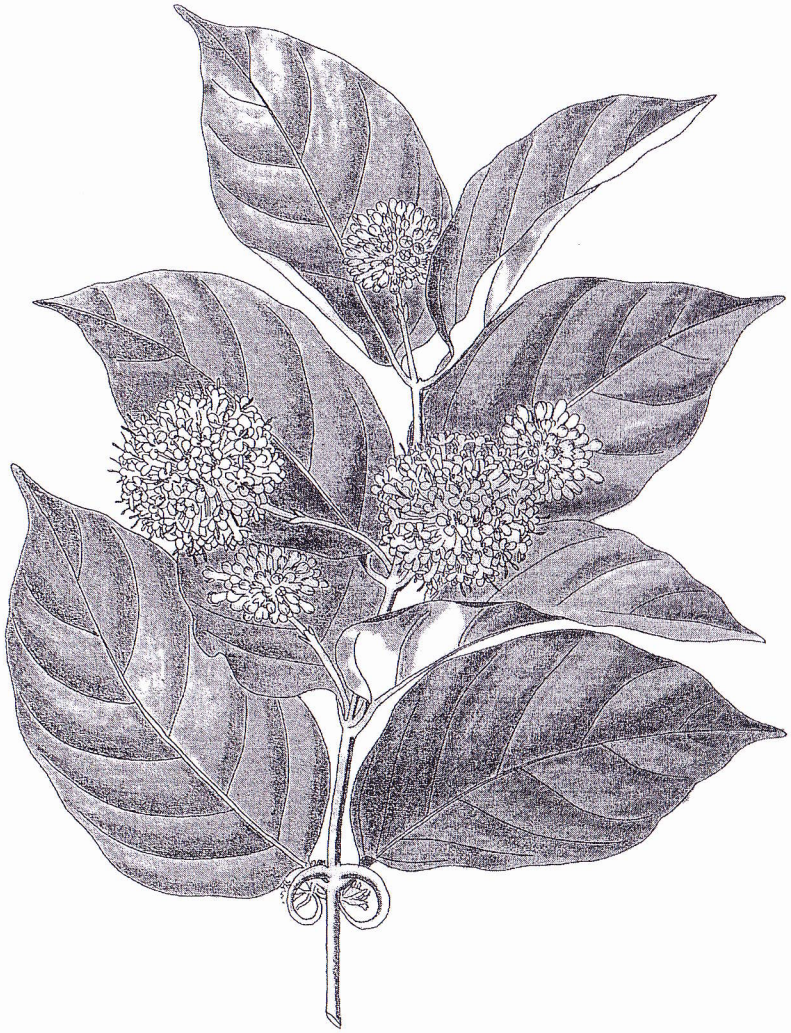


characteristic possibilities (in each structure, the monoterpenoid part arising from secologanin is drawn in boldface).

2. BIOSYNTHETIC ORIGIN

A. From Tryptamine to Strictosidine, Origin of the Common Precursor

The conversion of loganin to the alkaloids, i.e., its condensation with tryptamine, requires the cleavage of the cyclopentane ring, which results in the formation of secologanin. The mechanism of this cleavage remains obscure; the possibility of



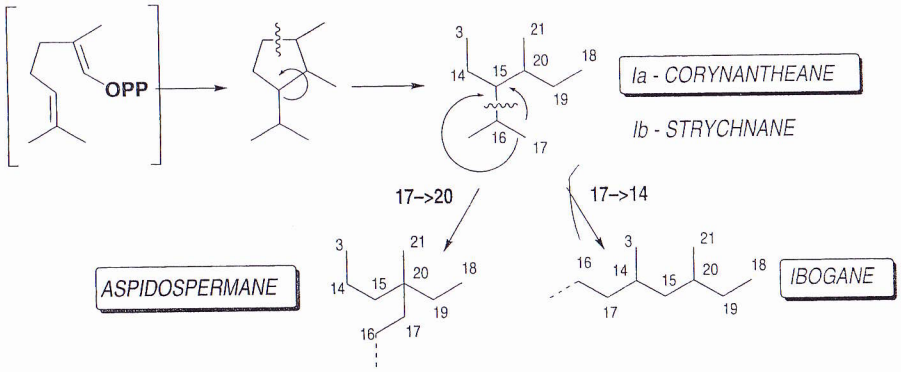
UNCARIA GAMBIR (Hunter) Roxb.

labelled precursors. The condensation of secologanin with tryptamine leads *in vitro* to two epimeric glycosides: vincoside (3β) and strictosidine (3α). In plants, the enzyme (strictosidine synthase) exclusively catalyzes the formation of strictosidine, the precursor of all of the monoterpenoid indole alkaloids.

B. From Strictosidine to the Alkaloids: Principal Structural Types

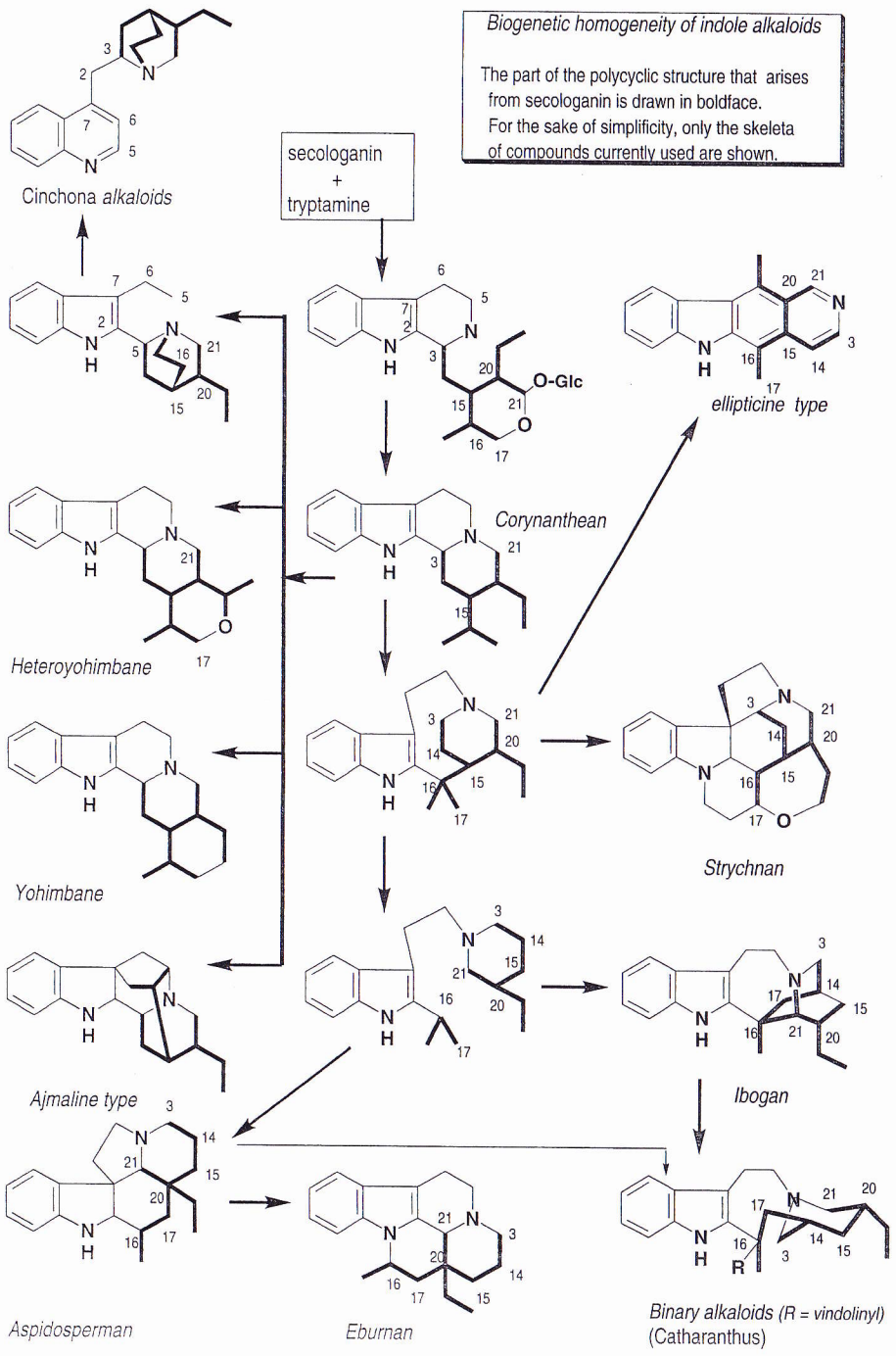
It is possible to classify indole alkaloids into different categories based on their biogenesis: type I alkaloids, including corynantheanes (Ia), strychnanes (Ib), and other skeletons in which the monoterpenoid unit has not been rearranged; type II alkaloids, including aspidospermanes and related skeletons, as well as type III alkaloids, including iboganes and related skeletons, in which a monoterpenoid unit has been rearranged. More complex (and more precise) classification systems have been proposed, and their detailed review belongs in specialized publications *. The two figures below ** summarize the fundamental characteristics of these groups and their chief skeletons:

• Group I-A: closure occurs between C-21 and Nb, and may be followed by a cyclization of the C-17-C-18-type (yohimbanes), or of the C-17-*O*-C-19-type (heteroyohimbanes), or of the C-16-C-7-type, among others.



* We have purposely opted for simplification and focussed on alkaloids of therapeutic interest. We recommend reading a publication which is not recent, but remains interesting even though many novel structures have been described since: Kisakürek, M.V., Leeuwenberg, A.J.M. and Hesse, M. (1983). A Chemotaxonomic Investigation of the Plant Families of Apocynaceae, Loganiaceae, and Rubiaceae by Their Indole Alkaloid Content, *in* "Alkaloids -Chemical and Biological Perspectives" (Pelletier, S.W., editor), vol. 1, pp. 211-376, John Wiley, New York. Also interesting are the references cited in the general introduction, particularly the excellent book by Geoffrey A. Cordell.

** The numbering shown is known as biogenetic, and was proposed in 1965 by Le Men and Taylor; it has the advantage of showing the remarkable structural homogeneity, which is not obvious because of the apparent diversity.



• Group **I**-B: the monoterpenoid unit remains intact; the C-2-C-3 bond is cleaved and replaced by two new bonds, namely C-2-C-16 and C-3-C-7 (strychnanes).

• Groups **II** and **III**: the C-2-C-3 bond and the C-15-C-16 bond of the monoterpenoid unit are cleaved. Reclosure may occur by formation of a C-17-C-20 bond (group II, aspidospermane, eburnanes) or of a C-17-C-14 bond (group III, iboganes).

In terms of chemotaxonomy *(p.1003), it is noteworthy that an increase in structural complexity leads to a higher specificity in the distribution. Thus, the alkaloids comprising a rearranged monoterpenoid unit are more evolved than those with an unrearranged unit: the former occur only in the Apocynaceae, whereas the Loganiaceae, considered the common ancestors of the Rubiaceae and Apocynaceae, only contain type I alkaloids (corynanthean, strychnan).

C. Biosynthesis of Type I Indole Alkaloids

Type Ia: corynantheans and related skeleta

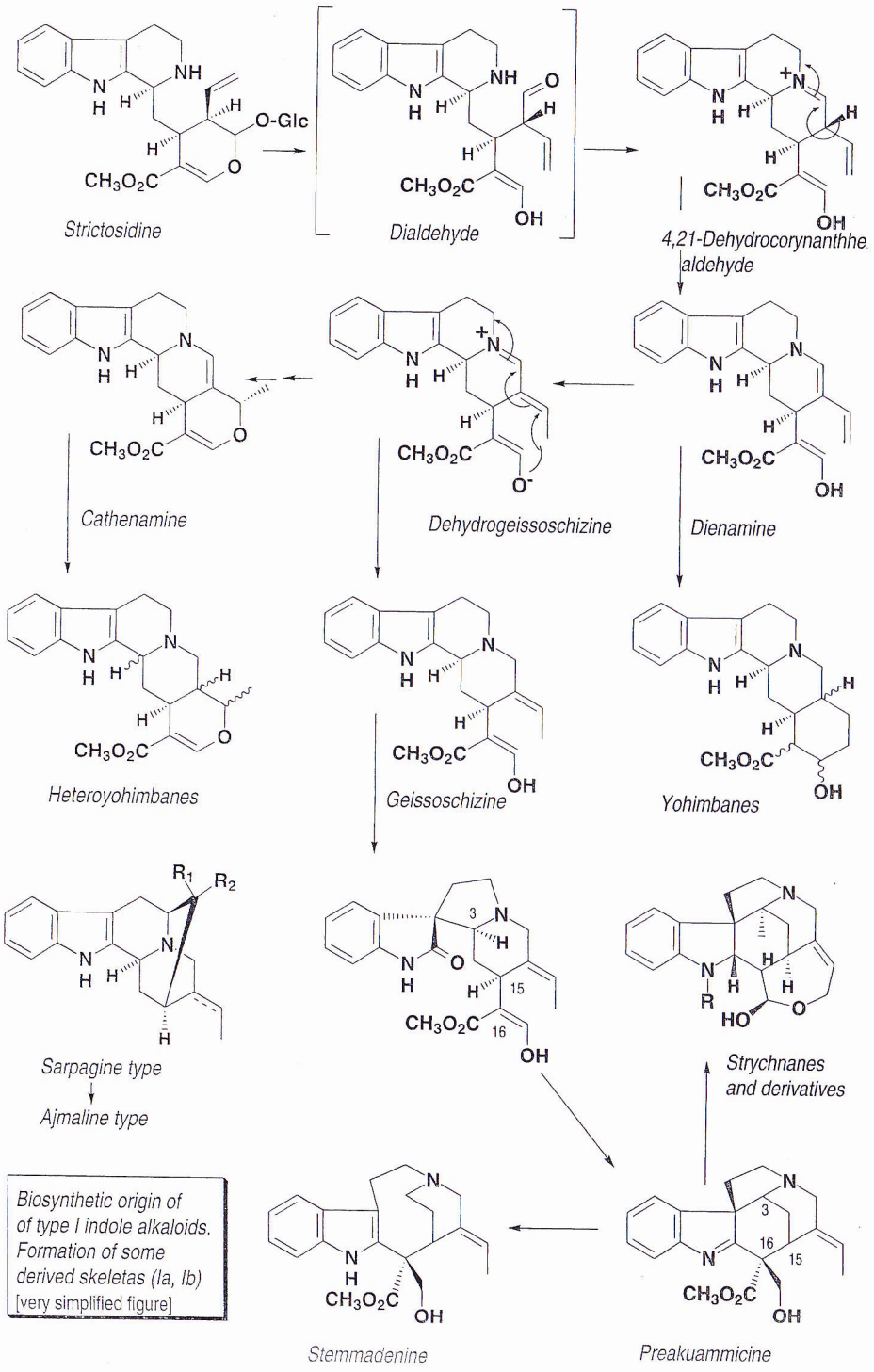
Formally, the formation of the corynanthean skeleton requires, as a first step, the enzymatic elimination of glucose. The free aglycone, a hemiketal, is in fact a highly reactive dialdehyde-containing intermediate: an intramolecular condensation leads to a carbinolamine, which is dehydrated to 4,21-dehydrocorynantheal.

Cathenamine, isolated from a Rubiaceae species, turns out to be an efficient precursor for ajmalicine: it is thought to be formed *via* a dienamine and 4,21-dehydro-geissochizine. The formation of C-19 and C-20 epimers is explained by the occurrence, for cathenamine, of an equilibrium between the enamine and the iminium ion.

The figure on the page 1006 summarizes the pathway which leads to the alkaloids related to yohimbane and heteroyohimbane. The high reactivity of the intermediates explains the occurrence of numerous skeletons derived from corynanthean (see vobasine, sarpagine, ajmaline, oxindoles, and also ervatamine and quinine) or of a more primitive stage (vincosan: adifoline). For example, the C-5-C-16 bond characteristic of vobasine and ajmaline requires an attack by C-16 (activated by the carbonyl functions) on an iminium ion: 4,5-dehydrogeissochizine.

Type Ia: strychnans

In these structures (e.g., strychnine), a C-3-C-7 bond replaces the C-3-C-2 bond: we can envision that a β-oxidation of the indole to a hydroxyindolenine leads to an oxindole, from which the formation of a C-2-C-16 bond becomes possible (formation of preakuammicine). The subsequent conversions are more obvious: loss of the carboxymethoxy fragment and formation of methylene indoline (nor-C-

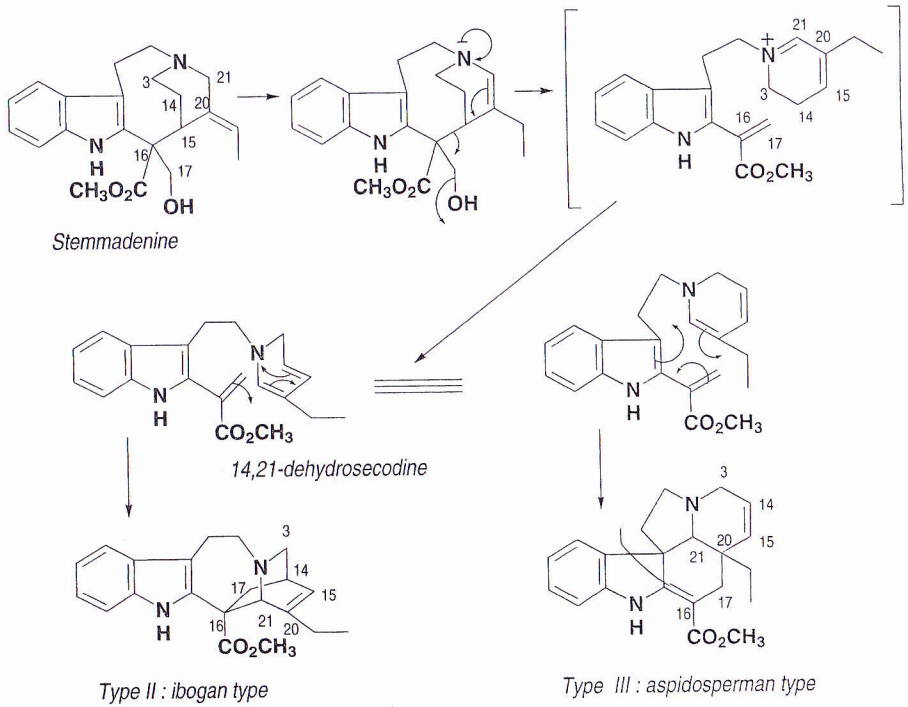


Biosynthetic origin of of type I indole alkaloids. Formation of some derived skeletas (Ia, Ib) [very simplified figure]

fluorocurarine). The latter leads to the Wieland-Gumlich aldehyde (= *N*a-desacetyl-diaboline, see nux vomica, p. 1013), as well as to its derivative dehydroxylated at C-18. In both cases, the 2,16-double bond is reduced. Both aldehydes may undergo isomerization: this reaction leads to the quaternary ammonium curares (see curares, p. 902). Strychnine itself arises from the alkylation by aceto-acetyl-CoA of the Wieland-Gumlich aldehyde as a hemiketal, followed by a cyclization involving the indoline nitrogen atom.

D. Biosynthesis of Type II and III Alkaloids

The steps and mechanisms that lead from a corynanthean type intermediate to the aspidosperman and ibogan skeleta are only partially known. Note, however, that the pathway which led to preakuammicine and stemmadenine can go further: after migration of the 19,20-double bond to the 20,21-position, the cleavage of the C-15-C-16 bond affords an acrylic ester, namely dehydrosecodine. The involvement of an intermediate such as 14,21-dehydrosecodine in the formation of aspidosperman and ibogan type alkaloid is only a hypothesis: we must emphasize that conversions of this type have been achieved *in vitro* and tend to validate this theory.



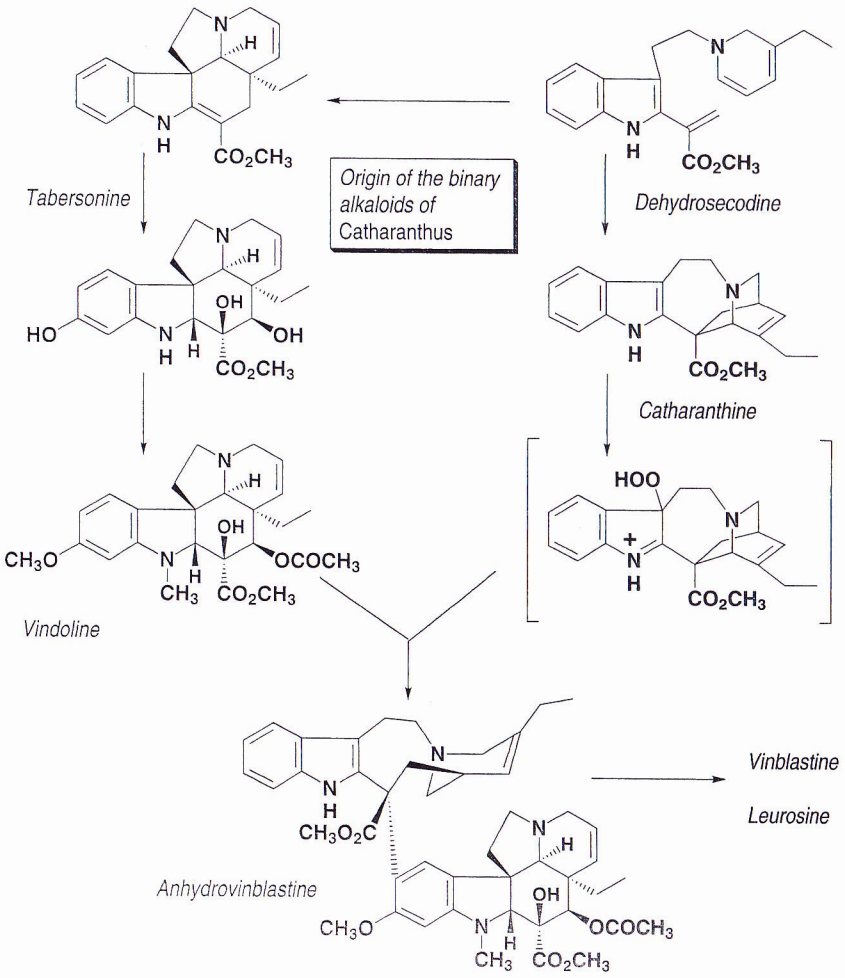
Pathway to types II and III: cyclisation of 14,21-dehydrosecodine

E. SPECIAL CASES

1. Binary Alkaloids from *Catharanthus*

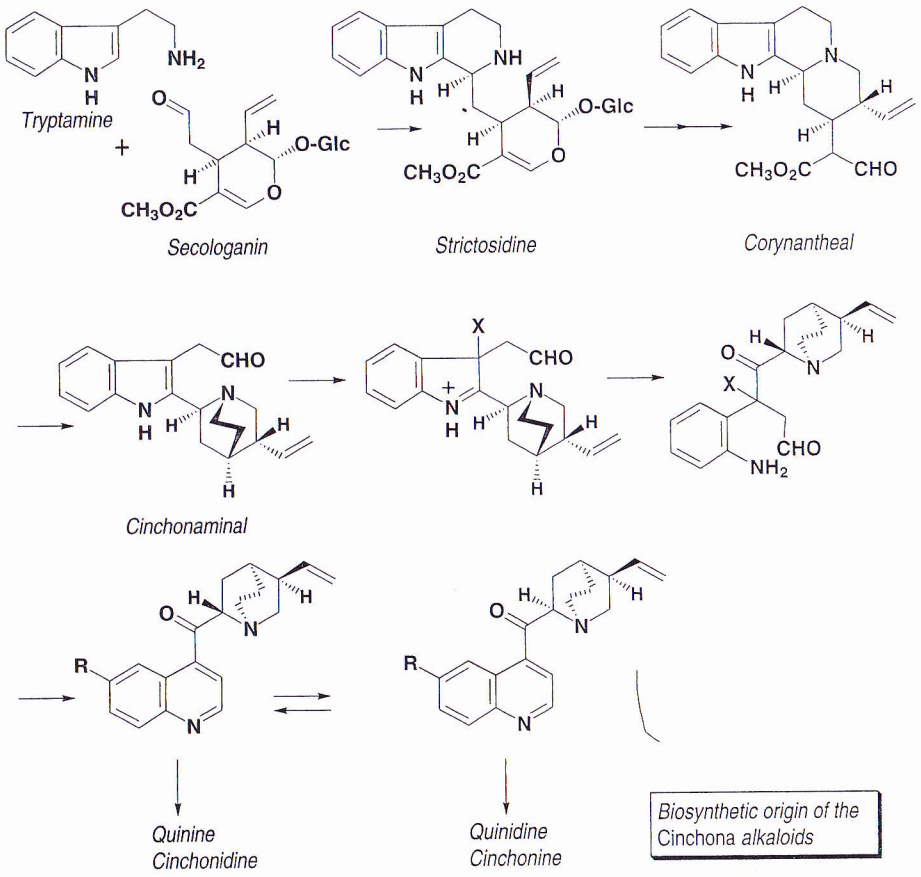
It is accepted that the first aspidosperman alkaloid to be formed, tabersonine, is the precursor of vindoline in the following sequence: hydroxylation and *O*-methylation of the aromatic ring, *N*a-methylation, 16,17-dihydroxylation, and *O*-acetylation.

It has also been shown that the two halves of the molecule are incorporated intact: labeled vindoline and catharanthine lead to the formation of labeled anhydrovinblastine and leurosine, and the intermediate could be a 7-peroxyindolenine, corresponding to catharanthine. Several mechanisms can be envisioned to explain the formation of vinblastine from its anhydro homolog: direct hydration of the double bond, or reduction and hydroxylation with or without inversion of configuration.



2. Quinoline Alkaloids from Cinchonas

The occurrence of indole alkaloids in cinchona leaves suggested a biosynthesis from tryptophan: labeling experiments have shown that this amino acid, as well as geraniol, loganin, or strictosidine, are indeed incorporated. The incorporation of strictosidine and the retention of the proton at C-3 prove that the rearrangement occurs late; using ^{15}N indicates that the nitrogen atom of the quinoline moiety is the indole *N*a of tryptophan, and using ^{14}C shows that the carbon joining the quinoline nucleus to the quinuclidine nucleus is the C-2 of the indole moiety. These elements, among others, have allowed the proposal of the pathway shown below, with the expansion of the cycle probably going through an indolenine intermediate.



3. MONOTERPENOID INDOLE ALKALOID DISTRIBUTION

A. Loganiaceae

Some *Gardneria* and *Mostuea* species contain alkaloids, but only a small number of species in the genera *Gelsemium* and *Strychnos* present a (low) pharmaceutical interest.

Over 200 alkaloids have been isolated from various species of *Strychnos*, a genus chiefly represented in the tropical areas of Africa and South America. Some of these alkaloids have an interesting pharmacological potential, others have been the starting points for semisynthetic derivatives used in anesthesiology, for example the quaternary bisindole bisammonium salts of the alcuronium type (see curares, p. 905). Among the species which contain strychnine, which grow wild mostly in southeast Asia, only one, nux vomica, provides a drug which is the subject of a monograph in the latest edition of the French Pharmacopoeia. Others, not currently in use, were formerly arrow poisons (India and Malaysia) or ordeal poisons (Africa).

B. Rubiaceae

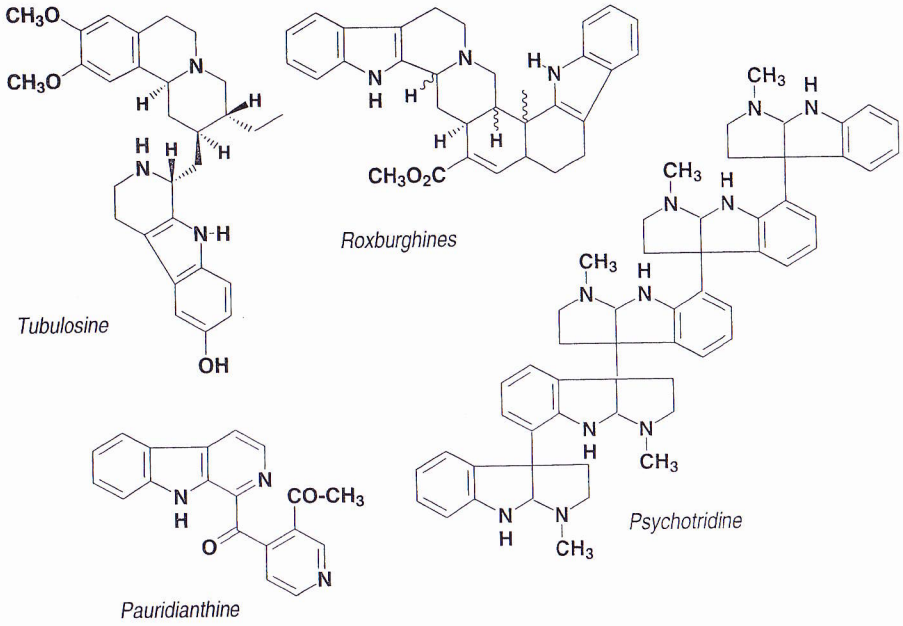
In the current state of knowledge, less than 10% of the genera in this family are known to elaborate alkaloids from a monoterpenoid unit. These are mainly genera in the most primitive tribes of the subfamilies Rubioideae (Psychotrieae: *Cephaelis*) and Cinchonoideae (Naucleae: *Adina, Nauclea*; Cinchoneae: *Cinchona, Corynanthe, Pausinystalia, Remijia, Mitragyna, Uncaria*).

In this family, secologanin can become combined with:

- two molecules of dopamine (formation of emetine—an isoquinoline alkaloid—in *Cephaelis* (*Psychotria*): see the previous chapter);
- one molecule of dopamine and one molecule of tyrosine (e.g., tubulosine of *Pogonopus* spp.);
- one molecule of tryptophan (e.g., alkaloids of *Adina rubescens* Hemsl.);
- two molecules of tryptamine (e.g., roxburghines of *Uncaria* spp.);
- only one molecule of tryptamine: this is most often the case. Type Ia alkaloids are then formed (yohimbanes, heteroyohimbanes), as in the Loganiaceae and Apocynaceae.

In some species, strictosidine can also react by incorporating a nitrogen atom, and lead to pyridine indole quinolizidinones and pyridine-type derivatives of harman (*Pauridiantha*). One particular case is that of the *Cinchona* and *Remijia* in which the indole moiety is converted to a quinoline (see below).

The Rubiaceae can also* elaborate indole alkaloid structures whose biosynthesis does not involve secologanin, for example, the polyindolenines of the Psychotrieae (*Palicourea, Psychotria*), the harmans (*Pavetta, Ophiorrhiza*), or the isopentenyl-tryptamines of the *Borreria*.



C. Apocynaceae

In this family, the genera that contain indole alkaloids are in the different tribes which all belong to the subfamily Plumerioideae: Carisseae (*Hunteria, Melodinus, Picralima*), Plumerieae (*Alstonia, Aspidosperma, Catharanthus, Vinca*), Tabernaemontaneae (*Crioceras, Tabernaemontana, Tabernanthe, Voacanga*), Rauwolfieae (*Kopsia, Ochrosia, Rauwolfia, Vallesia*). Although all of the genera elaborate type I alkaloids, they are not all capable of achieving the rearrangement of the non-tryptamine moiety to yield type II (aspidospermane) or III (ibogane). For example, with a few exceptions (*Catharanthus* among others), the only species that elaborate the ibogan skeleton belong to the tribe Tabernaemontaneae; the Rauwolfieae chiefly elaborate corynantheans and their derivatives; aspidospermans are common in most of the Plumerieae (rare in the *Alstonia*), and often found in the

*As the starting point of a literature review, we recommend: Hemingway, S.R. and Phillipson, J.D. (1980). Alkaloids of the Rubiaceae, *in* "Indole and Biogenetically Related Alkaloids" (Phillipson, J.D. and Zenk, M.H., Eds.), pp. 63-90, Academic Press, London, as well as chapter 3 in the same book (Alkaloids of the Loganiaceae, by Bisset, N.G., pp. 27-61).

STRYCHNOS NUX-VOMICA L.

Carisseae and the Tabernaemontaneae (cf. Kisakürek, M.V., Leeuwenberg, A.J.M. *et al.* (1983). See footnote *, p. 1003).

4. DRUGS CONTAINING INDOLE ALKALOIDS

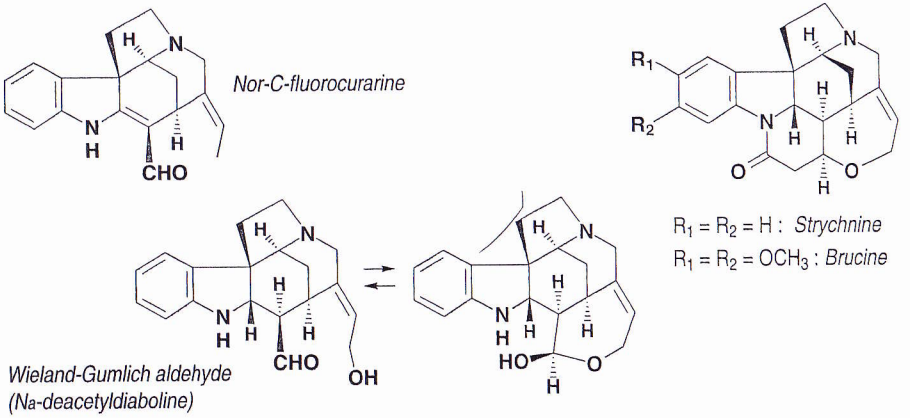
A. Loganiaceae

- **NUX VOMICA,**
  Strychnos nux-vomica L.

According to the 10th edition of the French Pharmacopoeia, "the part of nux vomica * that is used consists of the dried seed of *S. nux vomica* L.". This is a source of strychnine, a particularly toxic alkaloid (lethal dose in adults: 0.2 mg/kg) formerly used as a stimulant, and still used to destroy pests.

The nux vomica tree is a species from the south of Asia, with indeciduous leaves, whose fruit, a corticose berry with an orangy epicarp, contains two to five seeds swimming in a white pulp. The seed, known as nux vomica, is disc-shaped, and has a rounded edge, somewhat like a button. With a diameter of 20-25 mm and an average thickness of 5 mm, it is generally light gray and has a silky aspect due to a downy cover of tightly pressed, fine hairs radiating from a central point on each side of the seed. One side is marked by a radial ridge: the raphe.

The drug contains from 1 to 3% total alkaloids chiefly represented by strychnine and its dimethoxylated derivative, brucine. The minor alkaloids have a similar structure: colubrines, icajine, vomicine, novacine, pseudo- and isostrychnine.



* The wording is traditional, but surprising: to be perfectly rigorous one should refer to the seeds of the tree (how could the seed be a part of ...the seed!).

The assay includes the characterization of brucine and strychnine by TLC of a macerate in 70% ethanol. Quantitation is achieved by spectrophotometry of an alkaloid extract: it takes into account the difference between the absorbances measured at 258 and 300 nm. The strychnine concentration of the dried drug must be not less than 1.2%.

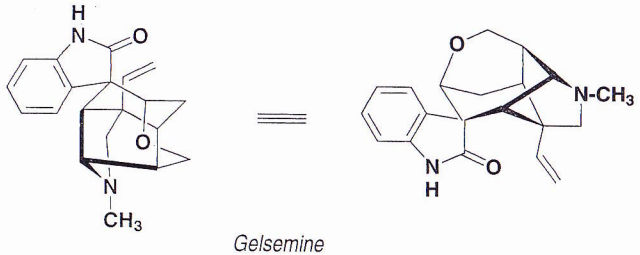
Introduced in Europe during the sixteenth century to eliminate pests, strychnine produces excitation of all portions of the CNS. Strychnine intoxication is reminiscent of tetanus; symptoms include anxiety, increased sensitivity to noise and light, and periodic convulsive attacks, triggered by some noise or light contact. Death occurs by asphyxia following the contraction of the diaphragm.

Strychnine was formerly used mainly to poison rodents, and the galenicals obtained from the drug were ingredients of replenishing and invigorating "tonic" preparations. It is a barbiturate antagonist which is no longer used in therapy. Only a few rare proprietary products based on nux vomica tincture are still available. In the past, a closely related species, the St.-Ignatius poison nut, *S. ignatii* Berg., was also used, and it is still used in homeopathy.

- **YELLOW JESSAMINE,**
  Gelsemium sempervirens (L.) Ait. *f.*

This species is a shrub with indeciduous leaves and yellow flowers, which grows wild in the damp woods of the south eastern United States. The drug is actually seldom used, and consists of the subterranean parts, including rhizome and roots. The alkaloids (0.5%) have a complex, oxindole structure: gelsemine, gelsemicine, gelsedine, and their hydroxylated derivatives.

Gelsemine and the preparations based on *Gelsemium* were formerly used to treat neuralgia, pain, and spasms (facial and dental neuralgia). As an antispasmodic, the tincture and extract of *Gelsemium* are still used as ingredients of some cough syrups.



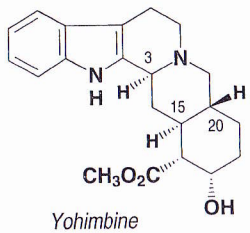
Gelsemine

B. Rubiaceae

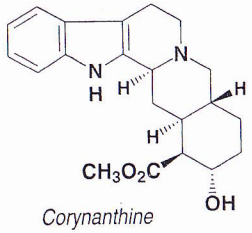
- **YOHIMBE,**
  Pausinystalia yohimbe (K. Schum.) Pierre

Yohimbe is a tall tree widespread in the forests of Cameroon, Gabon, and Congo. The part that is used is the bark of the trunk; stripped in long strips, it is cut and dried in the sun. The drug consists of quills with a reddish-brown external surface covered with grayish lichen patches, and with a satin-like inner surface, finely striated, and golden brown.

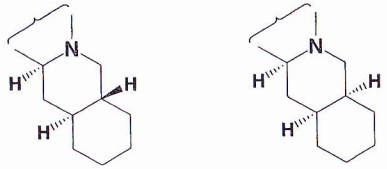
The majority of the 1 to 6% indole alkaloids contained in the trunk bark is of the yohimbane type. Alongside yohimbine, which is the chief constituent (*normal* series H-3α, H-15α, H-20β), note the presence of several of its isomers, in the same series (corynanthine, the 16-epimer) or in other series: *pseudo*-yohimbine (the 3-epimer), *allo*-yohimbine (the 20-epimer), and more. The drug also contains heteroyohimbanes such as ajmalicine and tetracyclic derivatives (corynantheine and related structures).



Yohimbine

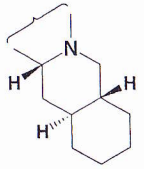


Corynanthine

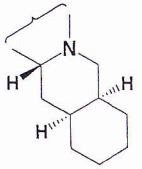


normale

allo



pseudo



epiallo

Yohimbine is a selective inhibitor of the presynaptic α-2-adrenergic receptors and is a sympatholytic. At low doses, it is hypertensive, and at higher doses, it is hypotensive, and it is a peripheral vasodilator: it is the vasodilation of the corpus cavernosum which is behind the reputation of yohimbe as an aphrodisiac. Its activity on smooth muscle results in an increase in intestinal tone and motility. Yohimbine is also active on the α-2-adrenergic receptors of adipocytes: their blockade is thought to result in an increase in lipolysis.

The clinical trials designed to define the usefulness of yohimbine in the treatment of impotence are not all conclusive and the methodology of some of them has been criticized. Some attempts at using yohimbine to treat obesity have led to contradictory results, most often negative.

Yohimbine hydrochloride has been on the market since the beginning of the twentieth century. Used for some time for the treatment of chronic constipation, this compound currently claims the following indications: impotence, especially in diabetic patients. It is also proposed for orthostatic hypotension, especially when it is induced by tricyclic antidepressants. It is contraindicated in case of severe hepatic and renal insufficiency, and it can cause, especially at the higher doses, a drop in blood pressure, lasting priapism, CNS symptoms (irritability, insomnia, tremors, dizziness, migraine), and digestive symptoms (nausea, vomiting, diarrhea).

C. Apocynaceae

• Madagascan Periwinkle, *Catharanthus roseus* G. Don.

The **aerial parts** of this pantropical species have been used for about thirty-five years for alkaloid extraction: they contain alkaloids that are prescribed in anticancer chemotherapy, most often as part of combination chemotherapy protocols. Also used are semisynthetic derivatives of structure closely related to that of the naturally occurring alkaloids.

The dried **root** is listed in the 10th edition of the French Pharmacopoeia, and is an industrial source of ajmalicine (like the roots of other species of the genus *Catharanthus*).

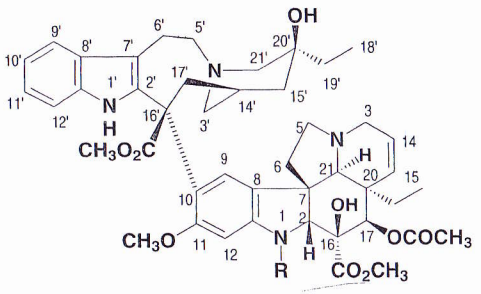
The Plant. *C. roseus* (= *Vinca rosea* L.) is a perennial subshrub with stems that are lignified at the base, and with opposite leaves with an oval and entire blade generally rounded at the apex. The flowers are showy and reminiscent of those of the periwinkle: constructed as a type 5, they are pink, purple, white, sometimes ocellate (literally, *Catharanthus* is the pure [*katharos*] flower [*-anthos*]). *C. roseus*, which is probably indigenous to Madagascar, is widespread in all of the tropical regions of the globe; it is planted in western Europe, and the U.S.A., as an ornamental species, but the more rigorous climate requires its cultivation as an annual plant. Many countries cultivate it to supply the extraction industry.

Chemical Composition. The aerial parts contain from 0.2 to 1% alkaloids. These form a very complex mixture in which about 95 constituents have been identified. All of them have an indole or dihydroindole structure (vindoline [the principal constituent], catharanthine, ajmalicine, akuammine, lochnerine, tetrahydroalstonine). The substances of pharmacological interest are the alkaloids formed by the coupling of two "monomeric" alkaloids, an indole and a dihydroindole. This particular structure has led to referring to them as "dimer" alkaloids or else "bisindole" alkaloids. Chemically, they are not dimers, therefore it is better to speak of "binary" alkaloids.

About twenty binary alkaloids have been isolated from the various *Catharanthus* (*C. roseus*, but also *C. lanceus* [Boj.] ex DC, *C. ovalis* Mgf., *C. longifolus* Pichon, and others). Several have cytostatic properties, especially:

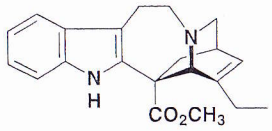
- vincristine (INN) (= leurocristine); its level does not exceed 0.0003% (i.e., 3 g per t of dried drug);
- vinblastine (INN) (= vincaleucoblastine), which is a little more abundant.

These two alkaloids formally comprise a dihydroindole moiety of the "aspidospermane" type (vindoline) and an indole moiety, velbanamine *. They differ by the nature of the substituent on the N_a of the dihydroindole moiety, which is either a formyl group (vincristine) or a methyl group (vinblastine).

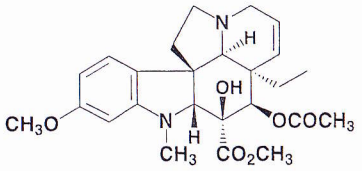


R = CH3 : Vinblastine
R = CHO : Vincristine

* biogenetic numbering



Catharanthine



Vindoline

Other binary alkaloids are active (e.g., leurosidine [= 20'-epivinblastine], leurosine [= 15',20'-epoxyvinblastine]); yet others can be extracted and converted, which improves the yields (e.g., formylation of desmethylvinblastine to vincristine).

Tests (for the official drug). The roots are identified by their macro- and microscopic characteristics, and by characterizing the indole alkaloids (with dimethyl-aminobenzaldehyde) after extraction ($CHCl_3$) under alkaline conditions (NH_4OH).

The assay per se consists of characterizing serpentine and ajmalicine (= raubasine) by two TLC analyses: the first one is applied to a 60% alcoholic extract (for serpentine), and the second one to a dichloromethane extract in alkaline conditions (for ajmalicine). The purpose of the quantitation is to estimate the concentration of total ajmalicine, in other words that which preexists, as well as that which can be derived from serpentine (the quaternary base corresponding to ajmalicine). Essentially, it includes a methanol extraction followed by a reduction of serpentine by sodium borohydride. After dilution (H_2O) and chloroform extraction, ajmalicine is quantitated by TLC: a known volume of the above chloroform solution

* Arising in reality from the fragmentation of a true "ibogane", namely catharanthine (see the paragraph on semisynthesis).

is applied to the TLC plate, and after development, the spot is scraped off the TLC plate, eluted with methanol, and the absorbance measured at 282 nm. This value is interpreted against a standard curve based on a blank and reference standards of known and increasing concentrations, subjected to the same conditions. The official root must contain not less than 0.4% serpentine and ajmalicine.

Pharmacological Activity. Vinblastine and vincristine are antimitotics. They bind to tubulin and prevent the formation of the microtubules whose role is well known in the formation of the mitotic spindle. Thus these compounds block mitosis and cause an accumulation of cells in the metaphase. The microtubule assembly also plays a role at other levels, particularly in neurotransmission (axon microtubules), hence the neurotoxicity of these alkaloids. They are generally *in vitro* inhibitors of the biosynthesis of proteins and nucleic acids. The treatment of cell populations with vincristine or vinblastine leads to an accumulation of cells in the M and G_2 phase, and the effect is lethal in the S phase.

Toxicity. Like most compounds with antitumor activity, the binary alkaloids of *Catharanthus* have a high toxicity.

• Vinblastine is highly leucopenic, and this limits the posology. In addition, it induces gastrointestinal distress (nausea, vomiting, constipation with apparent occlusion). Neurological symptoms can also be observed (headaches, neuritis, loss of the tendon reflexes, depression), as well as respiratory difficulties and alopecia.

• Vincristine mainly has central neurotoxic effects (possible convulsive episodes), peripheral neurotoxic effects (paresthesia, neuralgia, myalgia), and digestive effects (constipation up to paralytic ileus, which is rare). There are multiple side effects: alopecia (frequent) and less frequently, dyspnea, bronchospasm, headaches, transient blindness, buccal ulcerations, amenorrhea, and azoospermia, among others.

Pregnancy and breast feeding are contraindications for both alkaloids. Both are very irritating: accidental extravasation leads to cellulite, phlebitis, and eventually necrosis; contact with the eye can cause substantial irritation.

Uses. The aerial parts of the Madagascan periwinkle are only used to extract alkaloids. The binary alkaloids, which are marketed as a lyophilisate or a solution of a salt designed for the sole intravenous route (direct IV or through the infusion tubing; risk of tissue necrosis in case of extravasation).

• Vincristine sulfate (a controlled substance on French *liste I*, i.e., a prescription drug which may not be renewed; a prescription drug in the United States) is indicated in single-drug therapy for acute leukemia. In combination chemotherapy, it is indicated for the treatment of Hodgkin's disease, non-Hodgkin's lymphoma, breast cancer, uterine and cervical cancer, small cell bronchial cancer, rhabdomyosarcoma, and various sarcomas (normal dose: 1.4 mg/sq m of body surface area per injection

[adult]; in combination chemotherapy the frequency of the injections is approximately once a month).

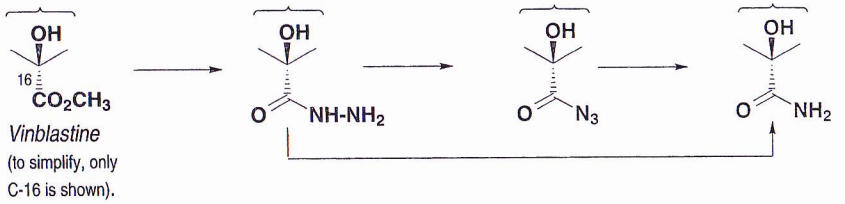
• Vinblastine sulfate (a controlled substance on French *liste I*, i.e., a prescription drug which may not be renewed; a prescription drug in the United States) is indicated in the treatment of Hodgkin's disease, of non-Hodgkin's lymphoma, of advanced testicular cancer, of breast and ovary epithelioma, of Kaposi's sarcoma, of choriocarcinomas, and in some cases of histiocytosis (normal dose: 5-7 mg/sq m of body surface area/week in adults).

Establishing the posology, administering the alkaloids, conducting and monitoring the treatment pharmaceutical as well as preventing side effects, is the responsibility of competent and specialized services. In most cases, vincristine and vinblastine are included in complex combination chemotherapy protocols (e.g., vinblastine, adriamycin, bleomycin, dacarbazine; vinblastine, cis-platinum, bleomycin).

Semisynthetic Derivatives of the Binary Alkaloids

1. Vindesine (INN)

Of the numerous structural analogs studied in the last thirty years, only one, vindesine, has been marketed. This alkaloid can be prepared from vinblastine (formation of the hydrazide of 16-desacetylvinblastine [with hydrazine] and reduction of the acylhydrazide [by Raney nickel in methanol]; a variation of this procedure consists of forming the acylazide [by reaction with nitrous acid] then the amide [by treatment with anhydrous NH_3]).



Vinblastine
(to simplify, only C-16 is shown).

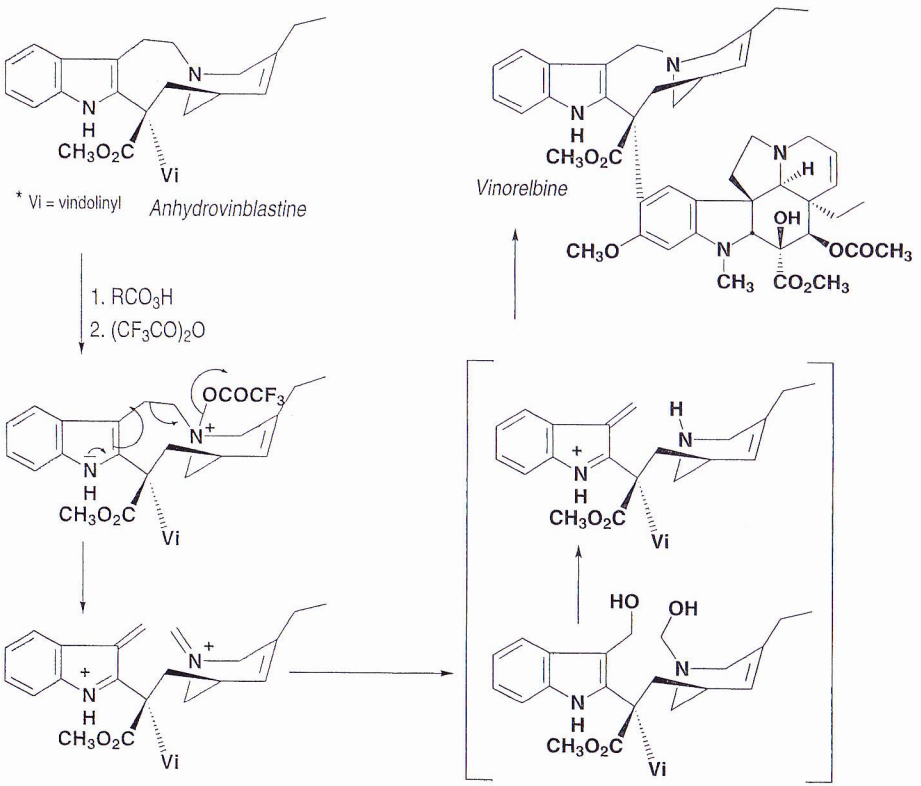
Vindesine is a potent antimitotic. Marketed as a sulfate (a controlled substance on French *liste I*, i.e., a prescription drug which may not be renewed), it is indicated in the treatment of acute lymphoblastic leukemia and refractory lymphomas. Certain solid tumors are also indications: breast, esophagus, upper respiratory and digestive tract, bronchopulmonary cancer (normal adult dose: 3 mg/sq m of body surface area every 7-10 days for one month, then every 15 days, when used alone in chemotherapy; can be used at lower doses in combination chemotherapy protocols; exclusively for IV injection).

Like vinblastine and vincristine, this derivative is toxic, and its side effects include a transient granulopenia (which limits the doses), gastrointestinal effects identical to those caused by the naturally occurring alkaloids (constipation

vomiting), neurological symptoms less marked than those induced by vincristine, reversible alopecia, weight loss, and muscular aches.

2. VINORELBINE (INN) = noranhydrovinblastine

This is a semisynthetic derivative characterized by the replacement of the tryptamine moiety of the "upper half" (indole-CH_2-CH_2-N-) with a "gramine" type moiety (indole-CH_2-N-), in other words by the elimination of one carbon atom. This derivative is obtained, *via* a bisiminium ion, by the Polonovski reaction on anhydrovinblastine (see figure) or by going through the bromoindolenine of anhydrovinblastine.



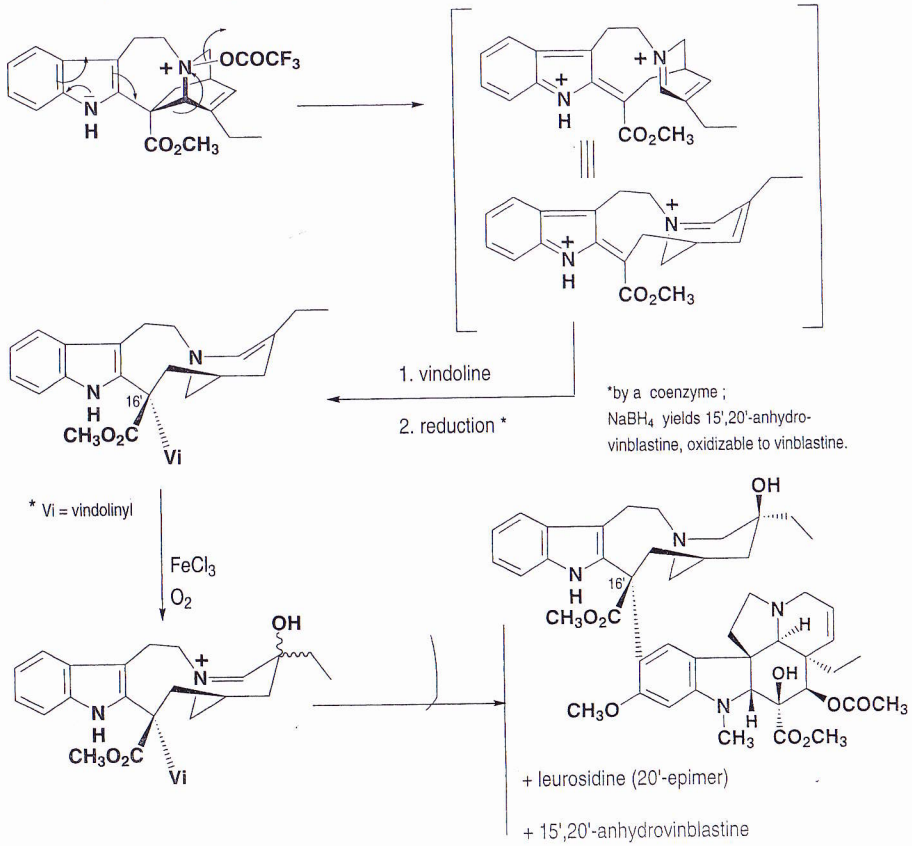
Vinorelbine, marketed as a bitartrate for injectable solutions (a controlled substance on French *liste I*, i.e., a prescription drug which may not be renewed) acts preferentially on mitotic microtubules and not so much on neuronal microtubules. Its current indications are metastatic breast cancer and bronchial cancer (not with small cells) (normal dose: 25-30 mg/sq m, adult, monotherapy; can be used in combination chemotherapy protocols [particularly with cis-platinum]). Although the neurological toxicity is limited (constipation by intestinal paresis, loss of tendon reflexes), and the frequency of severe adverse effects lower than with compounds such as vindesine, the hematological toxicity (granulopenia) is substantial and limits the posology

Vinorelbine is more efficacious than vindesine for the treatment of inoperable non small cell lung cancer, and on average it improves survival significantly, especially in combination with cis-platinum (average: 40 weeks; average for other chemotherapy: 28 weeks; average in case of symptomatic treatment: 16 weeks). For precautions and other side effects, see vincristine and vinblastine.

Semisynthetic routes to binary alkaloids

In addition to the possibility (currently exploited) of transforming vinblastine into vincristine by oxidation of the *N*a-methyl to *N*a-formyl (by chromic oxidation at very low temperature), the possibility of accessing binary derivatives by a biomimetic synthesis has been the object of many endeavors: it is now conceivable to obtain vinblastine from starting materials that are not rare and not too expensive, such as catharanthine and vindoline.

Theoretically one can hope to obtain binary alkaloids by activating the 16 position of an adequate tetracyclic iboga-type alkaloid to allow nucleophilic attack of carbon C-10 of vindoline. This method does not yield the desired isomer: only the 16'(*S*) isomer is pharmacologically active.



As seen above, the binary alkaloids arise biosynthetically from the coupling of an aspidosperma-type alkaloid (vindoline) with an iboga-type alkaloid (catharanthine): the reaction includes the cleavage of the C-16-C-21 bond of the ibogane moiety.

It is on the basis of this observation that the most efficient route was developed: a modified * Polonovski reaction applied to the *O*-trifluoroacetate of the *N*b-oxide of catharanthine leads to the cleavage of the C-16-C-21 bond; vindoline, if present, is attacked at C-10, and if the conditions are well selected (low temperature, anhydrous conditions), the reaction yields the desired 16'-configuration. After regioselective reduction of the intermediate dihydropyridinium at very low temperature, the resulting enamine may be directly oxidized at the desired position by mere aeration in the presence of ferric chloride in a dilute medium. The reduction of the mixture of immonium ions leads to a mixture of vinblastine (major product), leurosidine (20' epimer), and a small amount of anhydrovinblastine. What distinguishes this process from the syntheses previously published is the absence of toxic reagents for the anhydrovinblastine oxidation (osmium or thallium derivatives).

One of the advantages of this type of reaction is that it opens a route to various binary structures that may be pharmacologically interesting. This is a research avenue, and with the knowledge brought by the ongoing studies on analogs of these alkaloids (such as 20'-epi-20'-deoxy-vinblastine [vinepidine] and vinzolidine) and with the efforts expanded to attach these binary alkaloids to monoclonal antibodies, it allows hope for new advances on the theme of *Catharanthus* alkaloids.

• Common Periwinkle, *Vinca minor* L.

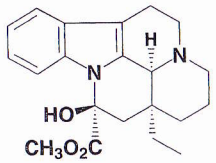
Known since ancient times, this plant, sometimes referred to in French as "the violet of witches", has been heralded for properties as numerous as they are unproven: astringent, wound-healing, antidermatosic, antigalactic... Today the leaf of this species (listed in the 10th edition of the French Pharmacopoeia) is one of the industrial sources of vincamine, an alkaloid still used in geriatrics.

The Plant, the Drug. The common periwinkle is a herbaceous plant with trailing stems that occasionally root into the ground, bearing persistent, opposite, and tough leaves with shiny blades. The flowers have a corolla that is tubular at the base, with five deep blue, spread out, truncated lobes. The fruit consists of two follicles. Common in all of Europe, the common periwinkle grows preferentially in cool woods and on shady rocks. It is cultivated. The drug is identified by its macro-

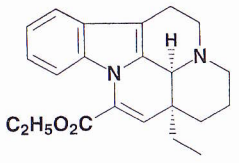
* The Polonovski reaction is a reaction between an acid anhydride and an *N*-oxide: it induces either the cleavage of a C-H bond, or that of a C-C bond; it is governed by steric and electronic factors. In the case of the *N*b-oxide of catharanthine the C-16-C-21 and C-5-C-6 bonds are antiparallel to the N—>O bond and therefore can be cleaved. Since the immonium ion formed during the reaction is conjugated in the case of a C-16-C-21 cleavage, that is the one that is favored.

and microscopic characteristics and by the presence of alkaloids. The French Pharmacopoeia does not require quantitation, but does require a TLC analysis of the total alkaloids (internal standard method, visualization by ceric ammonium sulfate).

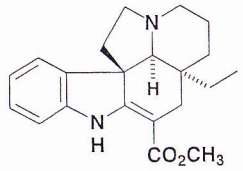
Chemical Composition. The drug contains 0.3 to 1% total alkaloids. Vincamine, which represents about 10% of the total alkaloids, occurs alongside approximately thirty other indole alkaloids of the eburnan type (vincine, epivincamine, eburnamonine), of the aspidospermane type and related compounds (vincadifformine, minovincine, quebrachamine, vincadine), or else of the corynanthean type.



Vincamine



Vinpocetine



Vincadifformine

Pharmacological Activity. Pharmacology experiments in animals show that vincamine increases cerebral blood flow. This circulatory activity could be the result of a metabolic activity: increase in oxygen and glucose consumption. This increase in glycolysis, by causing an increase in pCO_2, is thought to be the origin of the vasodilation. The decrease of the lactate/pyruvate ratio reflects the increase in aerobic glucose metabolism. Several studies in humans tend to confirm the activity on cerebral blood flow. Note in addition that this alkaloid can induce ventricular arrhythmia (especially when administered parenterally), and note the absence of a long term hypotensive action.

Uses. The drug is only used to extract vincamine. The psychological and behavioral problems of cerebral senility (attention deficit, memory loss, dizziness) constitute the chief indication of this compound (40-60 mg/day, *per os*, a controlled substance on French *liste II*, i.e., a prescription drug). This alkaloid is also proposed, alone or in combination (e.g., with rutin), to improve certain symptoms of senility (e.g., attention and memory problems, dizziness, tinnitus), to treat the sequelae of cerebrovascular accidents, the sequelae of recent cranial trauma, for disorders of vascular origin in ophthalmology and otorhinolaryngology (cochlear and vestibular problems). Vincamine is contraindicated in case of cerebral tumor with intracranial hypertension. It must not be taken concomitantly with medicines (antiarrhythmic or not) that might cause wave-burst arrhythmia (e.g., quinidine-type drugs, amiodarone) and the simultaneous use of potassium-wasting drugs or stimulant laxatives is discouraged. The posology must be adapted gradually in case of abnormalities of the cardiac rhythm and infarction sequelae; hypokalemia needs to be corrected prior to the start of the treatment. The low bioavailability of this

compound has led to the design and the marketing of continuous-release forms which permit regular absorption in the digestive tract.

Comments. For all chronic central and sensory manifestations thought to be of ischemic origin or else ill-defined, the French national agency ANDEM recently emphasized the following about "vasodilators and anti-ischemics": 1. that their efficacy in preventing cerebrovascular accidents has not been proved for the treatment of either the acute phase or the recovery from sequelae; 2. that there is a need to define the chronic alterations of cognitive function and behavior that are to be considered pathological, and the treatments that can be used for this indication; 3. that for the chronic alterations of cognitive function and behavior, these compounds should be used only on a case-by-case basis, only if there is true hope of clinical benefit for the patient, and with regular assessments of whether or not to continue such treatment.

Other Alkaloids Derived from Eburnane

Vinburnine. This alkaloid (a controlled substance on French *liste II*, i.e., a prescription drug) is used orally (60 mg/day) or by IM injection (15-30 mg/day). It is used for the same indications as vincamine; the contraindications are identical.

Vinpocetine (carboxyethyl derivative of eburnamenine). This is a product of comparable activity, which is not marketed in France.

Sources of vincamine

Besides the extraction from the leaves of cultivated periwinkles, it is possible to synthesize this alkaloid (there are several patented methods), or to semisynthesize it from tabersonine, an alkaloid with an aspidosperman skeleton found in various Apocynaceae, particularly in the seeds of *Voacanga* (see p. 1028).

Rauwolfia

The rauwolfia are lignified plants of variable size which grow wild in all of the tropical regions of the globe. The root of a genus of uncertain scope, namely *R. serpentina*, was the subject of a monograph in the 9th edition of the French Pharmacopoeia. Other species can be used for the extraction of the alkaloids.

The Indian plant known as *sarpagandha* in Sanskrit is a drug used since ancient times in Ayurvedic medicine to treat snake bites, and also mental disease and epilepsy. Although the demonstration of the antihypertensive and tranquilizing properties of reserpine largely contributed to the renewal of the interest in natural products in the 1950s, the appearance of more manageable compounds has led to a large decrease in its impact in therapeutics.

• RAUWOLFIA = SNAKEWOOD, *Rauwolfia serpentina* (L.) Benth. ex Kurz

The Plant. Rauwolfia is an evergreen shrub with a big root system and slender stems (0.5-1 m). The leaves are verticillate in groups of three to five and have a membranous blade. The flowers are small, white or pinkish, pentamerous, and grouped into cymes. The fruit is a black drupe normally 1-seeded.

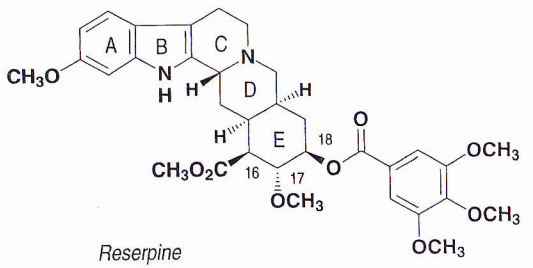
Growing wild in India, Pakistan, Myanmar, Thailand, Malaysia, and all the way to Indonesia, *R. serpentina* is a forest species which can be cultivated. Since the natural populations have been overexploited, restrictions have been imposed on the harvest of the roots to protect the survival of those populations.

The Drug. The commercial samples of the drug measure up to 15 cm in length for a diameter lower than 2 cm. The roots are tortuous and of low density. They often have an exfoliated suber, a yellowish color, a cross-section showing a substantial proportion of finely radiated wood, and a thin cortex. Under the microscope, the cut shows, among other features, wood containing numerous medullary rays and low caliber vessels.

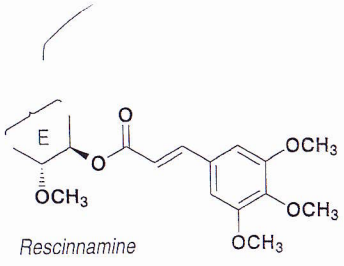
Chemical Composition. The total alkaloids (0.5-2.5%) constitute a complex mixture of nearly thirty different compounds, mostly indoles.

Of note is the occurrence of three main groups of alkaloids.

1. Yohimbane-type Derivatives. The most interesting ones have six asymmetrical centers (they are substituted at C-16, C-17, and C-18): reserpine (trimethoxybenzoate of methyl reserpate) and rescinnamine (trimethoxycinnamate of methyl reserpate) occur alongside related derivatives (e.g., deserpidine). Note also the presence of yohimbine, corynantheine, and some of their isomers. Reserpine and rescinnamine are weak bases: salts such as reserpine sulfate are soluble in chloroform. On treatment with sodium nitrite in the presence of sulfuric acid, these alkaloids are oxidized to the corresponding 3,4-dehydro derivatives: these are colored and can be used for quantitation.



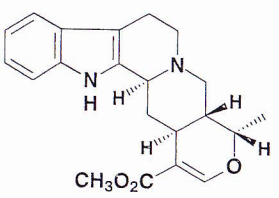
Reserpine



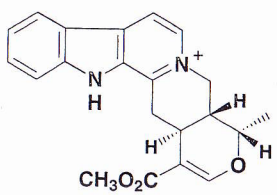
Rescinnamine

2. Heteroyohimbane Derivatives. These alkaloids, very closely related to the previous ones, have a heterocyclic E ring: Ajmalicine (also known as raubasine)

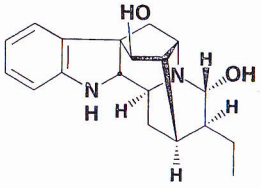
occurs alongside its methoxylated derivatives (reserpiline, 10,11-dimethoxy-ajmalicine), some of their isomers (reserpiline, isoreserpiline), and the corresponding quaternary bases, namely serpentine and alstonine.



Ajmalicine (raubasine)



Serpentine



Ajmaline

3. Dihydroindole derivatives are chiefly represented by ajmaline, a polycyclic indoline alkaloid.

Tests. Scrutinizing the anatomical characteristics allows the elimination of possible adulterations by other species in the genus: absence of sclerified cells in the cortical parenchyma and the phloem fibers (they are always found in the species of the group *tetraphylla* and in *R. vomitoria*); homogeneous wood with rare vessels of small caliber (in the species of the group *tetraphylla* and in *R. vomitoria* vessels are numerous and the wood is heterogeneous). As far as quantitation is concerned, the monograph of the 9th edition of the French Pharmacopoeia required one, by a classic approach, of the total alkaloids (gravimetry after extraction by the general procedure: >1%) and a specific quantitation of the "weakly basic" alkaloids, in other words of reserpine and related compounds (>0.1%). This determination was colorimetric ($NaNO_2/H_2SO_4$), and was carried out after a selective extraction ($CHCl_3$) of the weak bases present in a aqueous sulfuric acid solution of the sulfates of the total alkaloids.

Pharmacological Activity

Reserpine. Reserpine is currently of secondary interest, although it was formerly widely used, beginning in the 1960s, for its neuroleptic properties and mostly for its antihypertensive activity. By causing a peripheral catecholamine depletion, this alkaloid induces a lasting drop in blood pressure and heart rate. The central neurotransmitter depletion would explain its sedative and neuroleptic activity. Rescinnamine and deserpidine have the same activities.

Ajmalicine (note that the references that list proprietary products tend to use the denomination raubasine). Ajmalicine is an α-blocking spasmolytic, which at high doses inverts the effects of adrenaline, ajmalicine (= raubasine), and moderates the activity of the vasomotor centers, especially in the brain stem. It causes a transient increase of the blood flow to the brain and is slightly anxiolytic.

Ajmaline. Ajmaline is toxic and no longer marketed in France. Pharmacologically, it is an antiarrhythmic which decreases the rate of depolarization of atrial and ventricular cells substantially (decrease in excitability, decrease in conduction velocity, increase in refractory period).

Uses. Rauwolfia, or to be more accurate the rauwolfias, are used for the extraction of alkaloids. Although these species are indeed a source of ajmalicine, we must emphasize that ajmalicine is mostly obtained from the subterranean parts of various *Catharanthus*, particularly *C. roseus* (the official drug must contain not less than 0.4% ajmalicine).

Reserpine is still marketed in France, in a combination with a thiazide diuretic (bendroflumethiazide, INN); it is prescribed (0.1-0.3 mg/day, *per os*) in the treatment of arterial hypertension. The presence of reserpine in a medication of this type leads to the following contraindications: depression (especially in case of suicidal tendencies), combination with MAO inhibitors or levodopa, peptic ulcer, and hypersensitivity to the rauwolfia alkaloids. Reserpine contributes to the side effects of the medication, including drowsiness, nasal congestion, salivary and gastric hypersecretion, paradoxical anxiety and depression. In the United States, reserpine is marketed in combinations with different diuretics (chlorothiazide, chlorthalidone, hydroflumethiazide, methyclothiazide).

Ajmalicine is not used by itself. It is currently an ingredient of proprietary products used to treat the sequelae of cerebrovascular accidents and proposed to treat the symptoms of senility. It is combined with dihydroergocristine or almitrine (see comments, p. 995).

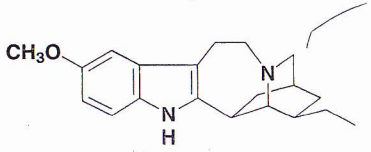
Other Rauwolfias

The extraction industry mostly uses various species in the genus, particularly *R. vomitoria* Afz., an African species with bulky roots and high alkaloid concentrations (7-10% of about forty different alkaloids), and *R. tetraphylla*, a collective species of northern South America and Central America, to name only two.

Other Apocynaceae

- **IBOGA,**
  Tabernanthe iboga H. Bn.

Iboga is a shrub indigenous to equatorial Africa, particularly the clearings of the rain forest of Congo and Gabon (delta and banks of the Ogooué river). It is prized for its big roots whose bark contains 5 to 6% indole alkaloids: ibogaine (principal constituent), tabernanthine, ibogaline, and ibogamine. In Gabon, the root is used for its antisoporific properties and its ability to increase resistance to fatigue. With a

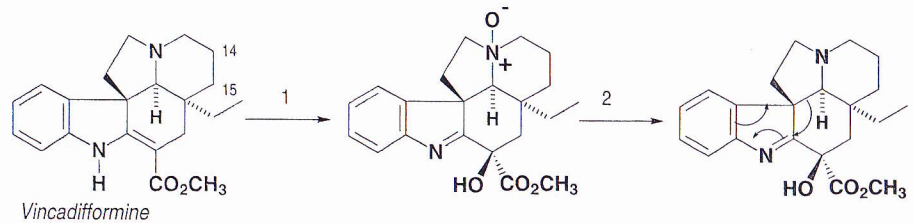


reputation as an aphrodisiac, it has been used during initiation ceremonies. Ibogaine is a CNS stimulant, either amphetamine-like or hallucinogenic depending on the dose; it is presented by some as useable in the treatment of opiate and cocaine dependence. High doses in humans can cause paralysis and even respiratory arrest. Its neurotoxicity is also well known (high doses induce Purkinje cell degeneracy). Ibogaine is prohibited in several countries.

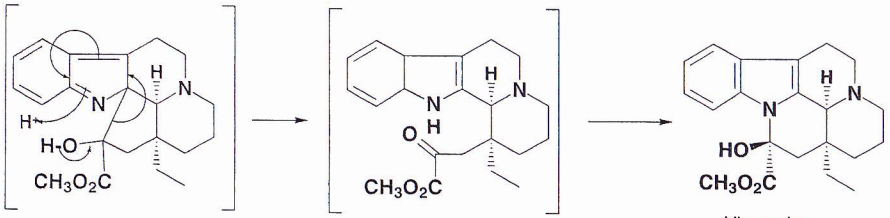
- *Voacanga* spp.

The main interest of these African plants (*V. africana* Stapf, *V. thouarsii* Roem. and Schuldt.) is that they produce seeds rich in tabersonine.

Tabersonine is an aspidosperman-type alkaloid: we can see (in the figure below) that a 90° rotation of the non-tryptamine carbon atoms leads, in theory, to an analog of vincamine. This conversion can be achieved *in vitro*. Since the tabersonine molecule has no 16-hydroxyl group and has a 14,15 double bond, the required first step is to hydrogenate the double bond. The resulting product, vincadifformine, is treated with 4-nitroperbenzoic acid, and the resulting *N*-oxide subsequently undergoes a rearrangement in the presence of triphenylphosphine and acetic acid. This produces vincamine in a yield of 66%; 16-epivincamine is formed simultaneously (yield = 21%). Other procedures include ozonization. These procedures are said to be "biomimetic" and are used to produce vincamine. This type of application shows, if needed, the point of studying biosynthetic mechanisms.



Vincadifformine
Dehydro-14(15) : tabersonine *1 : 4-nitroperbenzoic acid ; 2 : $(Ar)_3$-P, AcOH*



Vincamine

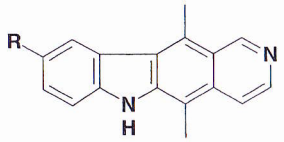
Semisynthesis of vincamine

- *Ochrosia* spp.

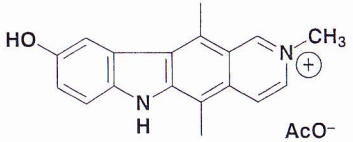
All of the species in the genus *Ochrosia* (*O. elliptica* Labill., *O. borbonica* Gmelin, *O. balansae* Baill.) are shrubs or trees of the islands of the Indian Ocean

and Pacific Ocean, and of the Australian continent. The trunk bark contains specific alkaloids: ellipticine, 10-methoxyellipticine, and heteroyohimbane-type alkaloids (reserpiline, isoreserpiline). Ellipticine and 10-methoxyellipticine have an antitumor activity, but also possess side effects that preclude their use in therapy. Research on their metabolism has led to the synthesis of more active and less toxic derivatives. A derivative that is structurally close, olivacine (initially isolated from an Aspidosperma), has similar properties.

***N*-Methyl-10-hydroxyellipticinium acetate**. This compound (elliptinium acetate, INN, a controlled substance on French *liste I*, i.e., a prescription drug which may not be renewed) is an anticancer agent which acts by DNA intercalation and resulting stabilization of the complexes formed by topoisomerase II. Note also that the oxidation of the phenol function at C-10 leads to an electrophilic quinone-imine able to form covalent bonds with biological macromolecules with nucleophilic sites. The compound has been used to treat metastatic breast cancer. Its use soon became limited because of its serious side effects (risk of hemolysis) and modest activity. Other synthetic derivatives (quaternary ammonium salts and olivacine derivatives) have undergone clinical trials or preclinical development.



R = H : Ellipticine
R = OCH_3 : Methoxyellipticine



N-methyl 10-hydroxy-ellipticinium acetate

The therapeutic indication for this compound is metastasized breast cancer. It is contraindicated in case of preexisting renal disease, as well as during pregnancy and breast-feeding. The risk of hemolysis leads to a strict requirement for close medical supervision (slow infusion, 80 mg/sq m of body surface area per day x 3 days, in repeated treatments). The most frequent side effects are digestive, neurovascular, and local venous reaction problems.

5. DRUGS CONTAINING QUINOLINE ALKALOIDS

- **CINCHONA**, *Cinchona* spp., Rubiaceae

"Cinchona consists of the dried bark of *C. pubescens* Vahl. (= *C. succirubra* Pavon) or of its varieties, or of its hybrids" (Eur. Ph., 3rd Ed.). Although only one species is official, others, such as *C. ledgeriana*, are used for the extraction of quinine and quinidine. The former is an antimalarial, and remains the treatment of choice for pernicious malaria. For now, the resistance of the parasite to this alkaloid

CINCHONA PUBESCENS Vahl.

According to research published in the late 1970s *, it was while observing Indian miners shivering after exposure to cold and damp conditions, and consuming bark powder macerated in water that the Jesuit missionaries had the idea, at the beginning of the seventeenth century, of using this powder to treat fevers.

Several years later, the "Countess's powder **" arrived in Spain where the virtues of these barks were rapidly recognized, from this "tree of the fever of the region of Loxa". Rapidly, and due to the influence of the members of the Company of Jesus, the "jesuits' powder" became known all over Europe. Because physicians gave credit to the drug, and because of the specificity of its action on malaria, it was recognized officially even while the identity of the producing species remained unknown: the genus *Cinchona* was going to be created by C. Linné in 1742 upon examination of the samples brought back from Peru by C.M. de la Condamine a few years earlier. In 1820, Pelletier and Caventou isolated quinine, thus opening the way for the isolation of other alkaloids: nearly thirty have been described in the various cinchonas. Quinine was synthesized in 1944; since then, other synthesis, of academic interest, have succeeded, and a biogenetic scheme has been in large part proposed.

The Plants. The genus *Cinchona* comprises about forty species, which are trees reaching, in their natural habitat, 15 to 20 meters in height. The leaves, opposite and decussate, have pinnate veins, often reddish, and a petiole, also reddish. The flowers are regular, white or pinkish, pentamerous, and have a corolla with lobes covered with white hairs; they are grouped in racemes of apical cymes.

All cinchonas are indigenous to the eastern slopes of the Amazonian area of the Andes *** where they grow between 1,500 and 3,000 m (about 5,000 ft and 10,000 ft) on either side of the equator (from Colombia to Bolivia, from latitudes 10 degrees north to 20 degrees south) in areas with substantial rainfall and humidity, and constant temperature.

Several species **** can be exploited:

- *C. pubescens* Vahl. (= *C. succirubra* Pavon) is the official species, indigenous to Ecuador, and particularly hardy;
- *C. officinalis* L. grows wild from Colombia to Peru, and is an aromatic species that can be used in the liquor industry;

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* Guerra, F. (1977), cited by Gramiccia, G. (1987). Notes on the Early History of *Cinchona* Plantations, *Acta Leidensia*, **55**, 5-13.

** The bark is said to have cured the Countess of Chinchon, wife of the Viceroy of Peru. Even if this story is only a legend, the Countess has reached posterity: Linné dedicated to her the genus *Cinchona*, unfortunately dropping an *h* in the process.

*** Cinchonas are also indigenous to the northern part of the Andes, on the eastern slopes of the central and western ranges.

**** As noted by Verpoorte *et al.* (1988), the fact that cultivation dates back to ancient times

- *C. calisaya* Weddell grows wild in Peru and Bolivia, and is fairly rich in alkaloids;
- *C. ledgeriana* Moens, considered by some as a variety of *C. calisaya* and by others as a *C. calisaya* (*C. calisaya* Wedd. = *C. calisaya* var. *ledgeriana* Howard = *C. ledgeriana* [Howard] Bern. Moens ex Trimen = *C. officinalis* auct. mult.), it is the cultivar exploited to produce quinine;
- Various hybrids can be cultivated.

The Drug.

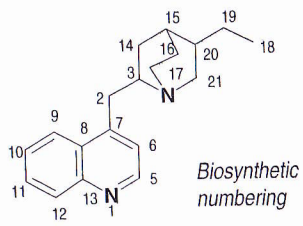
Sources of cinchona. The drug is derived exclusively from cultivated cinchonas. It was during the second half of the nineteenth century that cultivation was set up in India and Java. In India, *C. succirubra* and *C. officinalis* were grown, whereas in Java it was *C. ledgeriana*: the high concentrations of quinine of this species indigenous to the north of Bolivia ensured the rapid development of the plantations, which were all Dutch. In 1918, the production of quinine was under the total control of the "kina bureau" in Amsterdam. The geographical and political upheavals due to the second world war led to profound changes in the market: Indonesia still cultivates cinchonas, but Zaire has become the top supplier of a world market also supplied by other African countries (Burundi, Cameroon, Kenya) and various South American countries (Peru, Bolivia, Ecuador). The barks are obtained by beating and peeling the trees; the bark is partially regenerated and after a few years and several cycles of removing the bark and letting it grow back, the trees are uprooted.

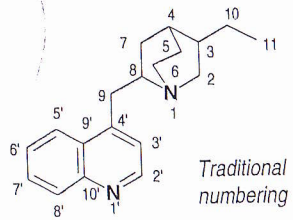
Characteristics. The trunk and stem bark of *C. succirubra* consists of small quills (stems) or large quills (trunk) with an external surface which is brownish-gray, rough, finely cracked in the transverse direction, has longitudinal grooves, and is frequently covered with lichen; the internal surface is dark reddish-brown. The external and internal surfaces of the root bark are the same color as the internal surface of the stem bark. The root bark breaks with a fibrous fracture whereas the stem bark breaks with a clean, short fracture in the external layers, and a fibrous fracture in the internal layers.

The microscopic analysis reveals the presence, in the phloem parenchyma, of fibers whose walls, thick, are highly striated and show infundibuliform pits. In the powder, these phloem fibers appear spindle-shaped (600-700 x 40-70 µm), yellow, striated, and pitted. Large secretory cells (100-350 µm) are disseminated in the internal part of the cortical parenchyma of the trunk and stem barks; they do not occur in the root bark.

Chemical Composition. Cinchona barks are often rich in phenolics. Thus, those of *C. succirubra* contain cinchonaines Ia-d (flavan-3-ols substituted by a caffeic acid), cinchonaines IIa and IIb (cinchonaines Ia [or Ib]-(4—>8)epicatechin) and proanthocyanidin dimers (B-2, B-5, A-2) and trimers (C-1). They also contain organic acids (quinic acid), dicarboxylic triterpenoid saponins, and essential oil.

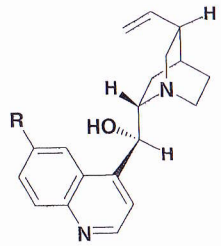
α-terpineol, linalool, limonene, and other terpenes, it also contains 11% of 2-hexyl-3-maleic anhydride.



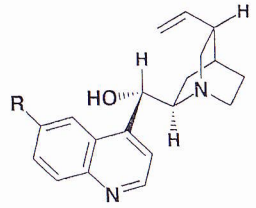


The concentration of total alkaloids and of quinine varies depending on the species:

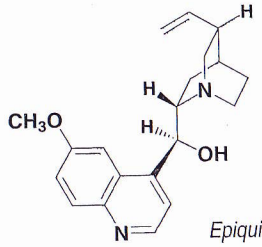
Species	Total alkaloids * (%)	Quinine * (%)
C. calisaya	3-7	0-4
C. pubescens	4.5-8.5	1-3
C. officinalis	5-8	2-7.5
C. ledgeriana	5-14	3-13



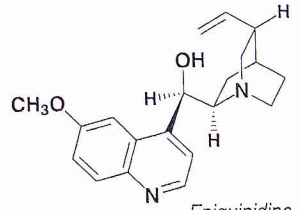
R = OCH_3 : *(–)-Quinine* (8*S*, 9*R*)
R = H : *(–)-Cinchonidine* (8*S*, 9*R*)



R = OCH_3 : *(+)-Quinidine* (8*R*, 9*S*)
R = H : *(+)-Cinchonine* (8*R*, 9*S*)



Epiquinine



Epiquinidine

* Figures from Paris, R.-R and Moyse, H. (1971). Précis de matière médicale, vol. **3**, p. 341, Masson, Paris.

** Since the majority of publications about cinchona use this numbering, so shall we; phytochemists also use the biogenetic numbering (see the previous chapter): it presents one [illegible] the biosynthetic (indole) origin of these alkaloids.

The principal alkaloids of the bark have a quinoline structure in which a quinoline nucleus, sometimes substituted at C-6' **(p. 1033), is linked by a carbon atom bearing a secondary hydroxyl group to a bicyclic, quinuclidine moiety. The principal alkaloids are stereo-isomers, quinine and quinidine, and their 6'-demethoxylated homologs: (–)-quinine and (–)-cinchonidine (8*S*,9*R*), (+)-quinidine and (+)-cinchonine (8*R*,9*S*). (See formulae p. 1033). Epi-bases (9-epimers) are also found, as well as hydrobases (reduction of the 10,11 double bond), and epi-hydrobases. The asymmetric centers C-3 and C-4 always have the same configuration (3*R*,4*S*). Alongside these quinoline alkaloids, minor alkaloids with an indole structure (e.g., cinchonamine) are also present; these indole derivatives are by far the principal alkaloids of cinchona leaves.

Physico-chemical Properties of the Alkaloids. Quinine gives two series of salts, the "basic" and the "neutral" salts. The "basic" salts in solution actually have a neutral reaction: they correspond to the formula Q^{2+},X^{-} (monovalent acid) or $(Q^{2+})_2,X^{2-}$(divalent acid) and are sparingly soluble in water. On the other hand, the "neutral" salts in solution have an acidic reaction: they correspond to the formula $Q^{2+},2X^{-}$ (monovalent acid) or Q^{2+},X^{2-}(divalent acid) and are far more soluble in water than the "basic" salts. This difference in solubility between the two series of salts can be the basis for the extraction and purification of quinine.

In acidic solutions made with oxygen-containing acids, quinine displays intense blue fluorescence under UV light, which disappears on addition of hydrochloric acid.

Color reactions have long been known and are still useful. Quinine and quinidine in solution in dilute sulfuric acid can be treated by bromine until the fluorescence disappears. The addition of aqueous ammonia causes the development of an emerald green color, which can be extracted with chloroform (thalleioquin test); the addition of potassium ferrocyanide in alkaline medium leads to a purplish-red color that can also be extracted with chloroform.

Tests. Cinchona is identified by its morphological characteristics and by the microscopic examination of the bark powder. The TLC analysis of a chloroform extract in the presence of aqueous ammonia shows the principal alkaloids: the TLC plate is sprayed with formic acid, examined under UV light, and the spots visualized with iodoplatinate.

The assay *per se* includes verifying the absence of foreign elements, total ash (<6%), ash insoluble in hydrochloric acid (<1%). The quantitation of quinine-type and cinchonine-type alkaloids is achieved by measuring the absorbance at two different wavelengths (316 and 348 nm) of a solution of the hydrochlorides of the total alkaloids. This solution is obtained by a conventional procedure: extraction of the drug powder with hot dilute HCl, alkalinization (NaOH), and alkaloid free base extraction by $CHCl_3$; after solvent evaporation, the residue is redissolved in ethanol, then in 0.1N HCl. Official cinchona must contain not less than 6.5% total alkaloids, of which not less than 30% and not more than 60% must consist of quinine-type alkaloids.

Pharmacological Activity.

Quinine. This alkaloid is most of all an antimalarial. It is active on the erythrocytic forms, up to the young trophozoite stage (*in vitro*, older trophozoites and schizonts are resistant). It is active on *Plasmodium vivax, falciparum, malariae,* and *ovale,* inactive on the sporozoites and the tissue stages, and practically inactive on the gametocytes.

The action of quinine on the myocardium, as well as of quinidine, but to a lesser extent, is a decrease in excitability, conductibility, and contractility. Quinine is only modestly antipyretic and analgesic; it has a weak curare-type activity on the motor end-plate.

Quinidine. This compound, essentially produced by semisynthesis from quinine, is an antiarrhythmic described by American pharmacologists as class I (sodium channel blockade), subclass A (moderate phase-0 depression and slow conduction; prolongs repolarization). By interfering directly with the electro-physiological properties of the cardiac cells, it inhibits the rapid sodium influx, decreases cell excitability, decreases the rate of depolarization, and increases the refractory periods; it decreases cardiac automaticity, contractility, and decreases the atrial and intraventricular conduction velocity.

Uses. The scope of the cinchona and quinine market is difficult to grasp: 300 to 500 t of quinine are thought to be extracted annually from 5,000 to 10,000 t of bark. Nearly half of the cinchona harvest is directed to the food technology industry*, and 30 to 50% of quinine is converted to quinidine. In 1989, France imported nearly 1,100 t of cinchona bark, to which we must add the importation of processed products (quinine and salts).

Cinchona galenicals are only used sporadically and their use in homeopathy (China) only accounts for a small amount of bark: quinine and quinidine constitute the major part of the cinchona products currently used in pharmacy. Note, however, that the 1998 French Explanatory Note allows the manufacture of phyto-pharmaceuticals from cinchona bark. In the context of the abridged application dossier for a French government marketing authorization or *dossier abrégé d'AMM,* these may claim several "traditional" indications: to stimulate the appetite, to facilitate weight gain, and externally, to relieve scalp itching and dandruff; they may also be used to treat fevers and flu symptoms.

Quinine. The current indications of quinine include the following.

1. The treatment of malaria attacks (particularly in case of resistance to other antimalarials) is the current indication of quinine. It is also proposed, although this

* The bitterness of tonic waters is due to quinine or to the cinchona extracts which are often among their ingredients (maximum concentration: 70 mg/L).

is debated, for prophylactic treatment in case of resistance to other antimalarials. The normal dose for a curative treatment in adults is, *per os*, 25 mg/kg, in other words 1.5 to 2 g/day in 3 doses for at least 3 days (5-6 days on average). Normal prophylactic doses rarely exceed 0.5 g/day. For a pernicious malaria attack, a quinine salt * can be administered by slow infusion.

Quinine is contraindicated in case of A-V conduction abnormalities, and can cause, at high doses, nervous and sensory side effects (partial loss of hearing, ringing in the ears, visual problems), abnormalities of intraventricular and A-V conduction, and gastrointestinal distress. Only a very small amount of quinine is excreted in breast milk, so it can be administered to nursing mothers, but in pregnant women, it is reserved for cases resistant to chloroquine. It must not be combined with astemizole and it is advisable to monitor glycemia during the treatment (risk of hypoglycemia during IV administration).

2. The symptomatic treatment of fevers and aches as well as flu-like states. In this case, the proprietary products that are available are always combinations with one or several active compounds: ascorbic acid, caffeine, camphor, codeine, eucalyptol, paracetamol, phenacetin, or pholcodine, among others.

3. Other pharmaceutical uses include older medications with miscellaneous indications: cardiac rhythm abnormalities, palpitations, anguish, precordial pain; quinine is generally combined with papaverine, phenobarbital, or hawthorn extract. Also marketed are quinine and urea hydrochloride (for injection sclerotherapy of bleeding hemorrhoids, treatment of anal fissures by the local submucosal route) and quinine ascorbate, combined with vitamins, used in programs to quit smoking (60-80 mg/day in four doses).

Note also that quinine is used, in combination with thiamine, to relieve muscle cramps (orally and rectally), which is not without risks (induction of thrombopenia).

Quinidine. The different quinidine salts (sulfate and long-acting derivatives such as polygalacturonate and arabogalactane-sulfate, controlled substances on French *liste I*, i.e., prescription drugs which may not be renewed; gluconate, polygalacturonate, sulfate, prescription drugs in the United States) are prescribed to treat cardiac arrhythmias, to maintain the sinus rhythm after normalization of atrial fibrillation, flutter, or tachycardia; they are also indicated in the case of atrial and ventricular extrasystoles, as well as in the preventive treatment of paroxysmal supraventricular tachycardia. The physician must take into account the contraindications (wave burst arrhythmia, A-V blocks, uncompensated cardiac insufficiency, hypersensitivity to quinidine) and the drug interactions which constitute further contraindications (ritonavir, antiarrhythmic or other drugs that cause wave burst arrhythmia: bepridil, amiodarone, sotalol, bretylium, disopyramide, vincamine, sultopride), or which are subject to precautions (urinary alkalinizing

* It is also possible, for this indication, to use a mixture of the principal cinchona alkaloids.

agents, digoxin, beta-blockers, drugs that lower the blood potassium level, enzyme inducers). The posology must be individually adjusted (0.7-1 g/day) following the administration of a test dose (watching for hypersensitivity) and taking into consideration the individual data on the patient (cardiac, renal, or hepatic insufficiency). The principal side effects are minor gastrointestinal symptoms, abnormalities in cardiac rhythm or conduction (extrasystoles, wave burst arrhythmia), and hematologic effects. Massive overdose is marked by dizziness, sensory problems (photophobia), respiratory difficulties (apnea), as well as serious abnormalities of the ECG, and requires hospitalization.

Semisynthetic Derivatives:

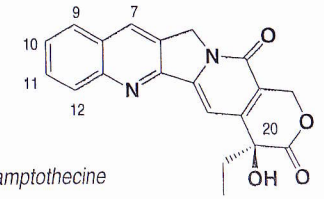
Quinicine. Quinicine is an alkaloid found in small quantities in cinchona barks, and is an oxidation product formed by cleavage of the quinuclidine ring C-8-*N*-1 bond and oxidation of the 9-hydroxyl group to a ketone. It has been proposed to treat cerebrovascular accidents (viquidil, INN).

Hydroquinidine. Hydroquinidine has the same properties as quinidine; it is an anticholinergic and an arterial vasodilator. Used for the same indications, it has the same contraindications and precautions; its side effects are identical.

• *CAMPTOTHECA ACUMINATA* Decsne., Nyssaceae

The Plant, Composition. The genus *Camptotheca* is a monotypic genus of the family Nyssaceae, a small family of the order Cornales (Rosidae). The trunk bark, root bark, and the fruits of this large tree indigenous to southeastern China contain 0.01, 0.02, and 0.03% camptothecine, respectively; this is a compound that has been found in an Icacinaceae (*Nothapodytes foetida* [Wight] Sleumer, roots, 0.1%) and in a Rubiaceae (*Ophiorrhiza mungos* L.).

The neutral lactam (it does not react with the general reagents for alkaloids and does not form stable salts) is particularly insoluble in conventional solvents. Camptothecine is characterized by a pyrrole[3,4*b*]quinoline sequence. Although this is not immediately apparent, it is biogenetically related to the indole group: strictosidine and strictosamide (the corresponding lactam) are its precursors; the passage from indole to quinoline is thought to involve a 10-membered macrocyclic ketolactam.



Camptothecine

Pharmacological Activity. The recognized cytostatic and antitumor activity of camptothecine led, in the 1970s, to preliminary clinical trials which were abruptly interrupted because of the substantial toxicity that was observed. Subsequently, there was evidence that this compound had some activity on topoisomerase I (an enzyme involved in the uncoiling of DNA, a prerequisite for replication and transcription), and in view of this, research resumed, especially to obtain synthetic analogs with reduced toxicity.

Several products are now the focus of attention and have in fact undergone preclinical or clinical investigations, or both (two of them were marketed recently):

- 9-amino-20*S*-camptothecine;
- 9-dimethylaminomethyl-10-hydroxy-20(*S*)-camptothecine (topotecan);
- 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecine (irinotecan);
- 9-nitro-20(*S*)-camptothecine;
- derivatives substituted at C-7 by a polar group, such as GL147211, and more.

Uses. Irinotecan was marketed in France in 1996 with the following indication: second-line treatment of metastatic colorectal cancer after failure of a previous valid treatment including 5-fluorouracil.

Irinotecan is metabolized to an active metabolite. During human trials, it had a response rate of nearly 25% with a mean survival time of 12 months for metastatic colon cancer refractory to 5-fluorouracil. Irinotecan is particularly toxic: neutropenia (in three out of four patients) and in over 80% of patients, acute cholinergic syndrome, nausea, vomiting, and delayed diarrhea (in 87% of patients). In half the cases, this diarrhea was severe and had to be treated (loperamide); it can make hospitalization in an intensive care unit necessary. The delayed diarrhea and neutropenia can be life-threatening.

Topotecan was approved by the FDA in May 1996 and recently marketed in France. It is indicated for metastatic ovarian cancer after failure of one or more lines of chemotherapy. Becase of its toxicity (severe neutropenia and thrombopenia), it is necessary to monitor hematological parameters regularly.

The clinical trials have shown the best response rates for small cell bronchial cancer (39% for first-line treatment); response rates for other types of tumors are more modest (e.g., 14% for ovarian cancer).

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