CHAPTER I

Sources of Alkaloids and their Isolation

R. H. F. MANSKE

Dominion Rubber Research Laboratory,

Guelph, Canada

I. Natural Occurrence

The chemist who plans to work on the constitution of alkaloids is generally confronted with three main problems; first the location of a suitable plant source, second the isolation of the plant bases from this source, and third the resolution of this mixture of alkaloids into its pure components.

Alkaloid bearing plants have been found in virtually every habitat in which vascular plants grow, so no investigator need be short of plant material because of geographical limitations. There are, however, no taxonomic characteristics by which a plant may arbitrarily be assigned to a group suitable for alkaloid study. Well authenticated bases have been found to occur in some thirty-eight plant families and it may safely be said that the remaining families will provide only an occasional alkaloid bearing plant. As more plants are examined many more families will be included in the alkaloid bearing group for very recently two more genera (*Salsola* and *Anabasis*) in Chenopodiaceae have been found to yield alkaloids. This family along with others (Compositae, Boraginaceae, Convolvulaceae, etc.) is typical of those which only rarely yield such plants and it may safely be conjectured that the processes which gave rise to these genera or species

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involved mutations which are abnormal to the group as a whole.   An extreme is encountered in the Papaveraceae in which all species contain alkaloids and no mutations resulting in alkaloid free plants have yet occurred.   The majority of plants occupy an intermediate position in which most species within a genus or closely related genera do or do not contain alkaloids.   For example all *Aconitum* and *Delphinium* species elaborate them although most of the other genera (*Ranunculus, Trollius, Anemone,* etc.) in the same family (Ranunculaceae) do not.   It has been observed that the structure of the alkaloids elaborated in various genera exhibit a degree of similarity of an order commensurate with the relationship of the genera from which they are derived.   This is well exemplified by the occurrence of lycoctine in both *Delphinium* and *Aconitum.*   When the relationship of the two genera within a family is remote (*Aconitum* vs. *Hydrastis*) the contained alkaloids (aconitines vs. hydrastine and berberine) may differ markedly in their nuclear type.
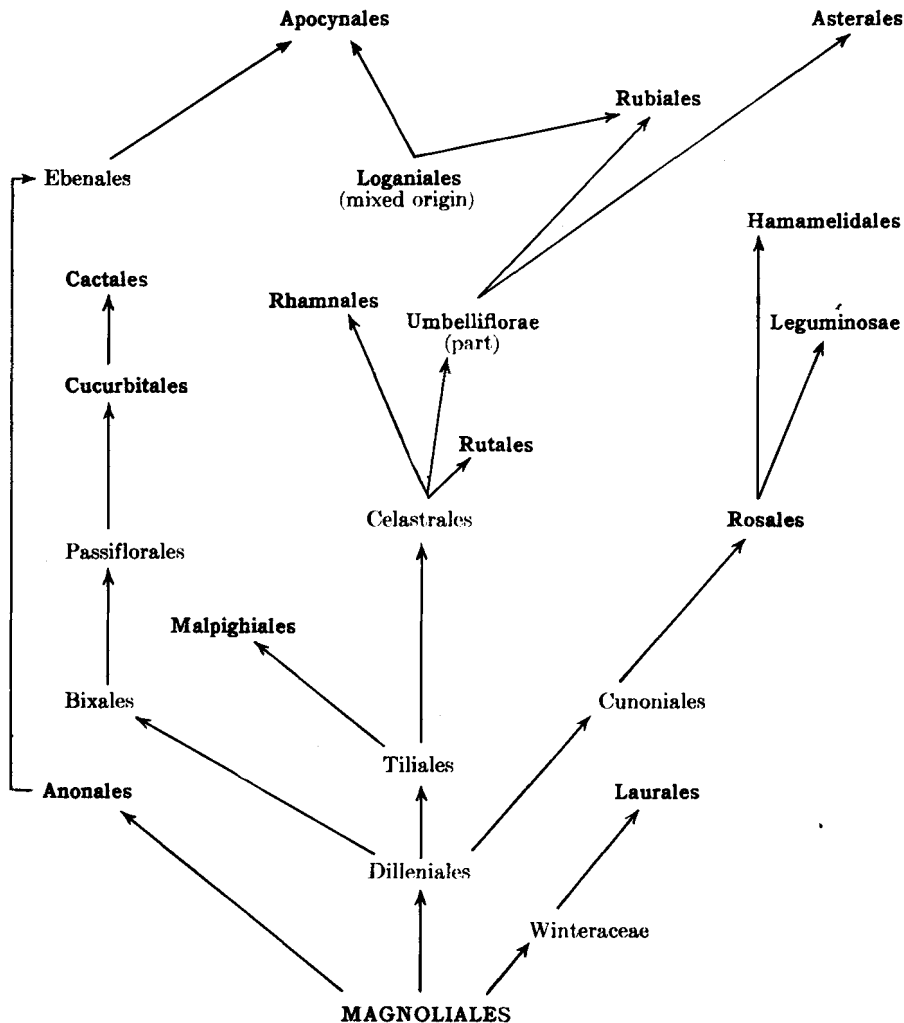
J. Hutchinson in his book, *The Families of Flowering Plants,* (MacMillan and Co. Ltd., London, 1926) has suggested a probable course of evolution of flowering plants based upon an original group of Archichlamydeae, which was assumed to evolve two basic orders, namely the Magnoliales, in which the arborescent habit is predominant, and the Ranales, in which the herbaceous habit is predominant.   Both orders contain alkaloid bearing families and indeed many of the early descendant orders (Anonales, Laurales, Berberidales, Rhoeadales) retain this power of synthesis.   On the other hand those orders which are near derivatives and do not yield alkaloids in the main give rise to orders which are equally impotent to elaborate them.   For example, the Dilleniales are considered to have given rise to some forty orders of which less than fifteen are known to be alkaloid bearing.   Charts I – III show Hutchinson's projected course of the evolution of the Gymnosperms.   Only those orders which yield alkaloid bearing plants or give rise to such orders are shown.   The orders and families are also given in Table 1.   The alkaloid chemist is not likely to dispute such a course of evolution for although many apparent chemical anomolies do exist, they have been adequately accounted for.   For example, the Anonales, the Laurales, and the Rhoeadales all elaborate aporphine alkaloids, yet the bases of the first two orders are secondary amines as opposed to the tertiary nature of members of the third order.   Furthermore, the aporphines in the Rhoeadales are always accompanied by other types of alkaloids not contained in the first two orders.

The fact that berberine occurs in no less than six families has been the subject of frequent comment, and it is to be noted that three of these families (Berberidaceae, Ranunculaceae, and Papaveraceae) are either in the order Ranales or in orders derived therefrom and three (Rutaceae, Menisper-

maceae, and Anonaceae) are derived from Magnoliales. More remarkable still is the widespread distribution of nicotine. It is present not only in derivatives of the Ranales and Magnoliales but is to be found also in two

CHART I

ALKALOID BEARING DICOTYLEDONES DERIVED FROM MAGNOLIALES

Apocynales   Asterales

Rubiales

Ebenales   Loganiales
(mixed origin)

Hamamelidales

Cactales   Rhamnales

Leguminosae

Cucurbitales   Umbelliflorae
(part)

Rutales

Passiflorales   Celastrales   Rosales

Malpighiales

Bixales   Cunoniales

Tiliales   Laurales

Anonales   Dilleniales

Winteraceae

MAGNOLIALES

Cryptogamic orders, Lycopodiales and Equisetales. Since no reasonable taxonomic concessions could bring these orders into close relationship it must be assumed that parallel evolutions have occurred in plants that are

widely separated taxonomically. The occurrence of 3-methoxypyridine in *Equisetum* and in *Thermopsis*, and other closely related alkaloids occurring in widely separated families appear to give further support to the hypothesis of parallel mutation. In the main, the alkaloids of closely related

CHART II

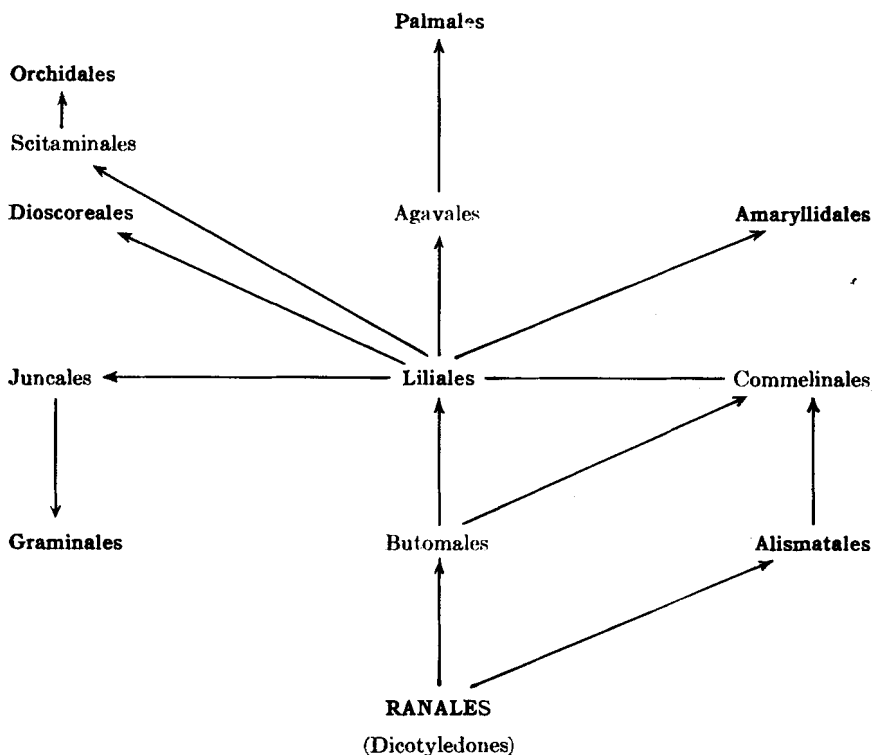ALKALOID BEARING DICOTYLEDONES DERIVED FROM RANALES
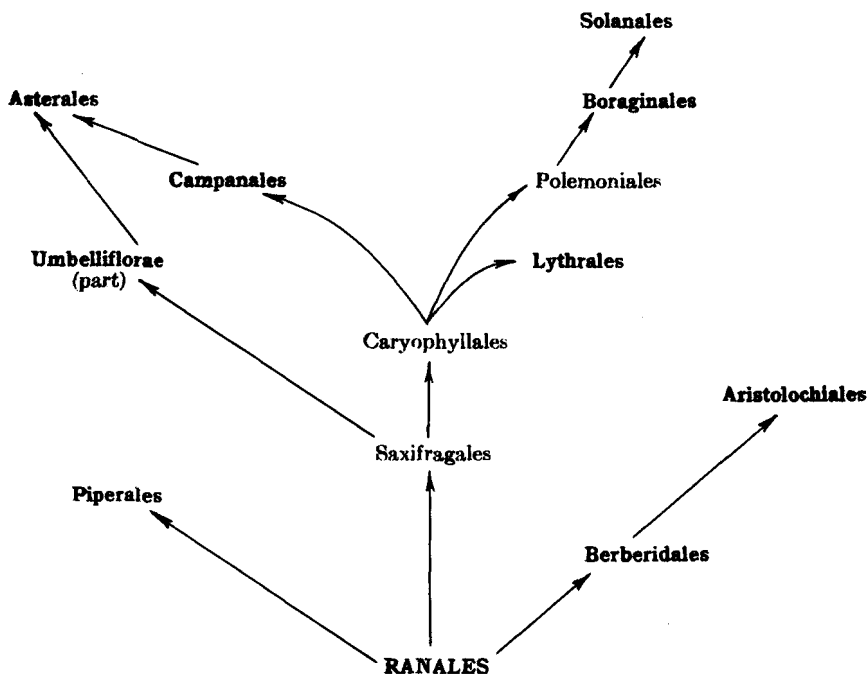
groups of plants show remarkably similar structural features. As this relationship becomes closer the contained alkaloids exhibit a greater similarity, but it is only in rare cases that two or more well defined species (*Stylophorum diphyllum* (Michx.) Nutt. and *Dicranostigma franchetianum* (Prain) Fedde) elaborate the same alkaloids. Elsewhere the author has pointed out that there is no justification chemically for establishing a new genus for the latter species since it was originally classified as *Stylophorum*. Within the divisions of a genus it is often possible by chemical methods to point to affinities and differences which taxonomic methods alone are unable to discern. The genus *Corydalis* has been subdivided into a number of sections by F. Fedde (Engler-Prantl, *Die Natürlichen Pflanzenfamilien*,

W. Engelmann, Leipzig, 1936) and a number of species in the section "Eucorydalis Prantl" have been examined chemically. In general, it was found that the alkaloids of the several species were closely related or identical while plants of other sections yielded alkaloids of different nuclear

CHART III

ALKALOID BEARING MONOCOTYLEDONES DERIVED FROM RANALES

Palmales

Orchidales

Scitaminales

Dioscoreales   Agavales   Amaryllidales

Juncales ← Liliales → Commelinales

Graminales   Butomales   Alismatales

RANALES

(Dicotyledones)

structure. *Corydalis sempervirens* (L.) Pers., however, yielded alkaloids of a type foreign to those of other plants in the same section, e.g., *C. aurea* Willd., *C. caseana* A. Gray, *C. ophiocarpa* Hook. f. et Thoms., and others. The conclusion that *Corydalis sempervirens* (L.) Pers. is wrongly classified seems inescapable. Such inconsistencies of classification are slowly being recognized and it is highly probable that a reclassification of plants based upon their chemical constituents will be possible when their chemical assay becomes more all-inclusive. In this connection it should be pointed out that the chemical method of classification is not based on the choice of arbitrary characteristics without which taxonomic botany would not be systematic.

The orders which contain alkaloid bearing plants and the families in which these plants occur are listed in Table 1.   Charts I and II are adapted

TABLE 1

ALKALOID BEARING ORDERS AND FAMILIES

A. Dicotyledones

Orders	Families
Magnoliales	Magnoliaceae
Anonales	Anonaceae
Laurales	Lauraceae
	Monimiaceae
Ranales	Ranunculaceae
Berberidales	Berberidaceae
	Menispermaceae
Aristolochiales	Aristolochiaceae
Piperales	Piperaceae
Rhoeadales	Papaveraceae
Chenopodiales	Chenopodiaceae
Lythrales	Punicaceae
Cucurbitales	Caricaceae
Cactales	Cactaceae
Malpighiales	Erythroxylaceae
Rosales	Calycanthaceae
Leguminosae	Papilionaceae
Hamamelidales	Buxaceae
Rhamnales	Rhamnaceae
Rutales	Rutaceae
Umbelliflorae	Umbelliferae
Loganiales	Loganiaceae
Rubiales	Rubiaceae
Asterales	Compositae
Campanales	Lobeliaceae
Boraginales	Boraginaceae
Solanales	Solanaceae
	Convolvulaceae

B. Monocotyledones

Liliales	Liliaceae
Amaryllidales	Amaryllidaceae
Dioscoreales	Roxburghiaceae (Stemonaceae)
	Dioscoreaceae
Palmales	Palmaceae
Graminales	Graminaceae
Orchidales	Orchidaceae

C. Gymnospermae

Coniferae	Taxaceae
Gnetales	Gnetaceae

D. Pteridophytae

Equisetales	Equisetaceae
Lycopodiales	Lycopodiaceae

from Hutchinson's classification and illustrate a possible course of evolution of the Magnoliales and Ranales, respectively, and their derived orders from the Archichlamydeae.   Chart II schematically depicts a possible course

of evolution of the Monocotyledones from the Ranales. In these charts only those orders which contain alkaloid bearing plants (in bold-face type) or those orders (in light-face type) which are progenitors of orders containing such plants are shown.

II. Distribution in the Plant

The elaboration of alkaloids is not localized in certain specific organs but appears to be a characteristic of all organs (including the seeds) although it must be emphasized that not all organs of any one species possess such function. Noteworthy amongst organs which are devoid of alkaloids are the seeds of the tobacco plant and of the opium poppy. Although the seeds of these plants do not store detectable quantities of alkaloids yet on germination alkaloids are to be found in the very young seedling. On the other hand the author failed to detect alkaloids in the leaves and bark of *Calycanthus floridus L.* while the seeds contained 1% of basic products.

In the first year of growth alkaloids seem to be quite evenly distributed amongst the various organs, but with increased age (perennials) there appears to be a localization of these bases in a few organs. The bark of arborescent plants is generally richer in alkaloids than are the leaves or shoots (*Cinchona*) and this may be attributed to their accumulation in the bark year after year. The bark of old barberry roots may contain as much as 10% of berberine whereas that of very young plants contains little more than is found in the leaves. As in all generalizations there are exceptions to the statement that in biennials and perennials there is a preponderance of these nitrogenous products in the root. Notable amongst these exceptions are the aerial portions of *Dicentra, Aconitum,* and *Delphinium,* which in some instances prove to be a rich source of alkaloids. However, the difficulties attendant upon their extraction (due to fats and other extractives in the leaves) often offset the advantage of the abundance of these bases in the aerial portions.

While localization of the alkaloids in various organs does not appear to occur in the annuals yet there is a marked fluctuation of alkaloid content in all the organs throughout the growing season. The period of maximum output of these bases in *Papaver, Senecio, Corydalis,* etc. appears to be coincident with the early flowering stage. It is obvious then that the alkaloids are intermediates in plant metabolism though it is not possible to state the fate of the nitrogen. When radioactive nitrogen of sufficiently long life becomes available the problem may then be resolved by tracer techniques.

When plants elaborate more than one alkaloid their ratio in the plant need not necessarily be the same at all stages of growth. Isothebaine, for

example, is virtually absent in the young oriental poppy (*Papaver orientale L.*) but increases to isolable amounts as the plant approaches maturity. This observation may explain why different investigators report the isolation of different alkaloids from the same species. It is to be noted, however, that the cultivated oriental poppy may be in part a hybrid with *Papaver bracteatum Lindl.* In any case it is markedly heterozygous and discrepancies in alkaloid content may be attributable to variations in the source plants. In order that alkaloids with a transient existence in the plant (e.g., isothebaine) be not overlooked it is recommended that plant material at all stages of growth be examined. If this is not possible then the record of the investigation should include a statement of the stage of the growth of the plants examined.

Cultural and climatic conditions have only a moderate effect on the alkaloid content of plants. It is known that the amount of alkaloids in opium varies with the source, but some of this variation is undoubtedly due to varietal differences in the poppies in question. A lone example, frequently cited to illustrate the catastrophic effect of soil or climatic conditions upon the type of alkaloid elaborated, is the California poppy (*Eschscholtzia californica Cham.*). Records show that when this plant was grown in Brittany it yielded but a single base, ionidine, which, if correctly characterized, differed from those bases characteristic of the plant when it is grown in other habitats. In a systematic study of the true species and of cultivated varieties grown both in very rich and very poor soils, the author (unpublished results) failed to detect the presence of ionidine although several new alkaloids were found.

There are ample a priori reasons to suspect that different plants of a single species may elaborate different amounts of alkaloids. It is well known that strains of tobacco and of lupines can be selected to yield greater or lesser amounts of alkaloids. That mutants occur is also highly probable. In this connection it is not unreasonable to suspect that the opium poppy may in fact be a mutant which was selected and cultivated because of its unique alkaloids. Neither morphine nor codeine have been found in any other species of *Papaver* and the basic ring system in these alkaloids has been found in part only in alkaloids derived from plants of a family (Menispermaceae) whose affinities with Papaveraceae can only be remote.

III. Isolation

Work on the constitution of alkaloids is often prefaced by the problem of their isolation from plant material or from residues after commercially important constituents have been removed. While the isolation of each alkaloid ultimately proves to be an individual problem there are never-

theless a variety of procedures which may be entitled to generic rank.

There are very few plants which elaborate but a single alkaloid so that the main problem is the separation of mixtures. Fortunately, the isolation of the total alkaloids reasonably free from inorganic or other organic matter may be reduced to a simple procedure. The fact that most alkaloids are basic (rutaecarpine is a notable exception) and therefore soluble in aqueous acids (colchicine is non-basic but is soluble in water) suffices to separate them from the host of water insoluble products found in all plants. Although a number of procedures for preparing such solutions are available the simplest expedient is to extract the plant material with acidified water. Such extracts, however, are usually contaminated with organic and inorganic materials which render the solutions difficult to handle. They tend to foam, are often difficult to filter, and with immiscible liquids they generally form stubborn emulsions. These inconveniences, however, may often be circumvented if the alkaloids (sparteine and nicotine) are volatile in steam.

If an organic solvent is selected purification of the solvent before use is a prime prerequisite, for many such impurities as acetone in ethanol form condensation products which, in many instances, prove difficult to remove. When possible low boiling solvents (methanol) should be selected. Alcohol has many advantages over water as an extraction solvent but when the plant material (seeds) is very rich in fats, removal of these glycerides by a preliminary extraction with a petroleum solvent is often advantageous. In either case the alcoholic extract is concentrated to a small volume (reduced pressure is preferable if frothing can be avoided). The extract, largely free from solvent, may then be acidified to a pH of 2 or less and steam distilled to remove the adhering solvent. The aqueous solution remaining in the steam distillation flask is usually quite dark and contains suspended organic matter. Although it is desirable to keep the volume of this aqueous solution to a minimum yet it should be sufficiently dilute so that the further addition of water does not produce an appreciable turbidity. In general it is possible to get satisfactory solutions when 1 liter of water is used for every 1 – 3 kg. of plant material. Removal of the suspended matter from this aqueous solution in many instances offers serious difficulties which filter aids help little to overcome. In the writer's experience there is only one infallible procedure which has, as well, the added advantage of simplicity. If the boiling aqueous extract is set aside without being disturbed for 24 hours, and then is placed in a refrigerator for two to three days, the now clear aqueous solution can often be decanted from the separated resins and fats, and can be entirely freed from insoluble matter by filtering through a layer of charcoal or other material. Should this simple procedure fail, then in those cases where the foreign material

does not melt at 100°, paraffin may be added to entrap the suspended matter and the procedure followed as outlined above.

Removal of suspended matter is usually followed by extraction with an immiscible solvent to remove water soluble organic material which may interfere in subsequent operations. Addition of an organic solvent to this clear aqueous solution usually discharges a resin which collects at the interface of the two liquids (similar resins generally separate when these clear aqueous solutions stand for some time). The choice of organic solvent for such extractions is limited in practice almost entirely to ether or chloroform. The latter solvent offers the advantage that the extract can be drawn from the bottom of the separatory funnel, thus minimizing the manipulation of large volumes. Caution must be exercised since many alkaloid hydrochlorides as such are extracted from their aqueous solutions by chloroform but not by ether.

These aqueous solutions, when rendered alkaline with ammonia or sodium carbonate (sodium hydroxide when phenolic alkaloids are known to be absent), may yield the basic constituents in filterable form. However, experience has shown that it is more convenient to recover these by extraction with ether or chloroform. Chloroform is to be preferred for it extracts all alkaloids except the quaternary bases (curine), which in any case must be recovered in the form of an insoluble complex salt. This procedure fails when the alkaloids are unstable under the conditions of the experiment, and is conspicuously unsuccessful with the ergot bases. It is also probable that some alkamine esters suffer change under the conditions imposed.

A somewhat milder technique, and one occasionally preferred, is to extract the plant material with a water immiscible solvent (chloroform, ethylene dichloride, benzene, etc.) after it has been basified with ammonia or aqueous sodium carbonate solution. In this case too, prior defatting (petroleum solvent) is often advantageous. The alkaloids are extracted from the organic layer by the use of dilute solutions of acids.

At this stage a cursory test for the presence of alkaloids in the extract is in order. Mayer's reagent (potassium mercuric iodide solution) has proved very satisfactory, for it yields a precipitate with virtually all alkaloids. Unfortunately, the formation of precipitates by this reagent is not diagnostic for this group of natural products alone since plant extracts which are entirely free of alkaloids often give precipitates. Hence a positive test with this reagent must be interpreted with caution.

The residue of mixed alkaloids, after removal of the solvent, usually takes the form of a resinous mass and further purification is necessary before crystalline material is obtained. Towards this end the crude alkaloids may be dissolved in dilute acids, filtered from insoluble material, and

reprecipitated. Extraction of the aqueous acid solution with ether often proves advantageous, especially when the bases are dissolved in oxalic acid. Sparingly soluble salts often separate from the aqueous phase in a relatively pure and crystalline form during the ether extraction.

Many alkaloids may be only sparingly soluble in ether but, with the exception of pentane and hexane, their purification is more readily effected from this solvent than from any other. It is possible to prepare ether solutions of these bases of a concentration far in excess of their equilibrium value which then often yield a crystalline solid in reattaining a state of equilibrium. A supersaturated ethereal solution of these bases is readily prepared as follows: The relatively dilute aqueous acid solution of the alkaloids, in a separatory funnel, is covered with a layer of ether, *and* a layer of water interposed between the two layers by the cautious addition of water down the side of the funnel. Aqueous ammonia is run in, in a like manner, the stopper inserted in the funnel, and the mixture shaken very vigorously. Often the more insoluble alkaloids will crystallize from the ether layer before it can be separated. In any case the wet turbid ethereal solution can be brought to brilliant clarity by treatment with charcoal. Removal of the ether leaves a residue which is now amenable to fractional crystallization from other solvents, to fractional distillation, or to salt formation.

IV. Separation and Purification

There is no known procedure by which all mixtures of alkaloids can be separated into their components. Nevertheless a number of helpful methods of attack may be outlined.

Fractional crystallization should be the first mode of attack on the separation of these mixtures, but in the event that this method fails it may be necessary to resort to other methods. Virtually all solvents have proved useful. For most alkaloids the order of increasing solubility is the following: hexane, benzene, ether, ethyl acetate, methanol, acetone, and chloroform. An alkaloid that is too sparingly soluble in chloroform will generally dissolve quite easily in hot dioxane. When an alkaloid has solubilities in various solvents different from the order given its separation is relatively simple, as will be seen from the following example. Protopine and chelidonine are both readily soluble in chloroform and sparingly so in methanol, while their hydrochlorides are sparingly soluble in water. A naturally occurring mixture of these two bases is easily separated by extracting the mixture of bases with cold ether in which chelidonine is readily soluble and in which protopine is less soluble than in methanol. When the ether solution is evaporated to a thin sirup and treated with methanol pure chelidonine crystallizes. If the residual protopine is dis-

solved in chloroform and this solution is evaporated to a thin sirup, the
addition of methanol yields a solution from which protopine crystallizes
with great facility.   If no single solvent should prove suitable for the
separation, resort must then be made to a mixture of miscible solvents.
The most satisfactory results are obtained by this method when the mixture
of alkaloids is dissolved in the solvent in which it is more soluble, concen-
trated to a convenient consistency, and the second solvent (also hot) then
added portionwise.   For reasons not entirely obvious there are certain
combinations of solvents that, in the writer's experience, are much more
satisfactory than others in this field.   Methanol or ethanol are the only sol-
vents which work well in combination with chloroform. Acetone-methanol,
ethyl acetate-methanol, dry ether containing only a few per cent of methanol,
and hexane-dry ether are other useful combinations.   Benzene, on the
other hand, either alone or in combination with other solvents, rarely
proves satisfactory.   The use of methanol-water and acetone-water mix-
tures is limited to cases where large crystals of an already pure alkaloid
are desired.   It cannot be too strongly emphasized that only adequately
purified and freshly distilled solvents should be used.   Ether *must* be free
from peroxides and alcohol free from acetone.

    In the event that separation by fractional crystallization fails, and
that one or more of the components of the mixture are volatile, then
fractional distillation is recommended.   In view of the relatively small
losses in this method recent micro techniques may be applied conveniently
to as little as 1 mg. of alkaloid.   This method of separation has been
applied with success to the *Lupine* and *Lycopodium* bases which crystal-
lize only with difficulty.

    Should both fractional crystallization and distillation fail in the
resolution of these mixtures then they may be converted into any one
of a number of salts in the hope that one of the component salts may be
insoluble.   There are a number of cases where certain special salts crystal-
lize remarkably well but preliminary trials should be limited largely to
the use of such acids as hydrochloric, hydrobromic, perchloric, picric, and
oxalic, although sulfuric acid frequently affords acid or neutral sulfates
that are sparingly soluble in alcohol or water.   Instead of aqueous hydro-
chloric or hydrobromic acid absolute methanolic solutions of the reagents
are recommended, since methanol is a good solvent for many bases.   The
methanolic solutions offer the added advantage that the excess hydrogen
halide is readily removed by precipitating the salt with an excess of dry
ether.   Hydrochlorides, thus prepared, often crystallize readily from boil-
ing acetone, or acetone containing just enough methanol to effect solution.
Excesses of perchloric, picric, and oxalic acid may also be removed by
precipitating the salts with dry ether and repeatedly triturating the gener-

ally pasty precipitate with fresh portions of the same precipitant. These salts are often appreciably soluble in acetone, from which they may be crystallized by the judicious addition of ethyl acetate (but not ether, benzene, or chloroform). In many instances perchlorates and oxalates crystallize satisfactorily from water but picrates, on the other hand, are generally too insoluble. Sparingly soluble nitrates (or iodides) are best prepared by dissolving the base in dilute acetic acid followed by the gradual addition of potassium nitrate (or potassium iodide) to the solution. The remarkable solubility of some alkaloid hydrochlorides in chloroform has formed the basis for an almost quantitative method for separating the Papaveraceous alkaloids into two groups. Some of these hydrochlorides (glaucine, bicuculline as well as some *Lobelia* alkaloids, and others) are so readily soluble in chloroform that this solvent readily extracts them, *as such*, even from dilute aqueous solutions. Methylene dichloride is almost equally good for this purpose, but no case has been reported of the solution of one of these hydrochlorides in carbon tetrachloride. Some alkaloid hydrochlorides (dicentrine and isocorydine), although they are readily soluble in chloroform saturated with water, are only sparingly soluble in the anhydrous solvent.

That phenolic alkaloids are soluble in aqueous solutions of fixed alkalies need not be stressed but it is important to note that some free bases (notably corydine and ochotensine) are extracted from such solutions by ether while others (bulbocapnine and hunnemanine) are not. A convenient method for recovery of these phenolic bases is to saturate their aqueous alkaline solutions with carbon dioxide or ammonium chloride. The latter reagent, however, is not invariably satisfactory (e.g., hunnemanine).

Separation of a mixture of secondary and tertiary amines is best accomplished by acetylation followed by trituration with dilute mineral acids. The acid soluble fraction contains the tertiary amine. The secondary amine may be regenerated from its insoluble acetyl derivative by mild acid hydrolysis. Benzoylation, however, cannot be applied with equal impunity for under the conditions for this reaction certain isoquinoline alkaloids (aporphines) suffer ring fission and benzoylation at the nitrogen atom.

The criteria for purity of alkaloids, as for other organic compounds, are constancy of melting point and of optical rotation. If, as is the case with many complex salts, melting is preceded by decomposition then a sharp melting point may often be obtained by the use of melting point tubes evacuated to oil pump vacua. The optical activity of an alkaloid may vary somewhat, depending upon solvent and concentration. Furthermore, many alkaloids, though otherwise pure, occur as partial racemates or may be partly or completely racemized during isolation (peganine).

The characterization of alkaloids has been limited, in most instances,

to the preparation of methiodides and a number of salts. Phenolic bases are best characterized as their methyl ethers (diazomethane), and secondary amines by their benzoyl derivatives (calycanthine is a notable exception).

Color and precipitation reactions may be quite helpful in forensic chemistry but as a means of identifying alkaloids they suffer from lack of specificity. Although the absence of certain colors with specific reagents shows the absence of a certain alkaloid, yet when the anticipated color is obtained confirmatory evidence for the alkaloid's presence is necessary.