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ALKALOIDAL DRUGS - A REVIEW

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ABSTRACT

An alkaloid is a type of plant-derived organic compound. Alkaloids are generally composed of oxygen, hydrogen, carbon, and nitrogen. Some alkaloids are considered toxic, but others are often used medicinally. Alkaloids one might encounter in everyday life include caffeine, the drugs atropine and quinine, and the deadly nightshade plant. Alkaloids are relatively rare in plants. They have what's considered to be a complex chemical structure, and they always contain a nitrogen molecule. An alkaloid may also contain molecules of oxygen, carbon, and hydrogen. Though their effects vary from medicinal to poisonous, all alkaloids cause a physiological effect on the human body. Some alkaloids, such as the ergot alkaloid, can be toxic and even deadly. Cocaine and caffeine are two plant-based alkaloids believed to be toxic in their effects. A class of alkaloids known as the tropane alkaloids is historically famous for their use as poisons. The alkaloid atropine belongs in this class. It is typically derived from the plant *atropa belladonna* or deadly nightshade. Many alkaloids can be used for medical purposes. Atropine, believed to have been used historically as a poison, is now sometimes used to stimulate the central nervous system and dilate the pupils of the eyes. Scopolamine, an alkaloid of the same class, is often used to treat motion sickness. Quinine, one of 31 alkaloid chemical compounds found in the plant *cinchona succirubra*, has antimalarial properties. Quinine is still used as a treatment of choice for malaria. Some alkaloids, such as the morphine alkaloids, can have narcotic effects. These alkaloids are typically derived from the opium poppy. Morphine alkaloids may be some of the oldest drugs in the world. Their use was first recorded in Sumeria circa 3500 B.C. Morphine alkaloids were often used to induce drowsiness in laudanum preparations from the 1830s onward, even though they were believed to be addictive. Today, morphine alkaloids are still sometimes used in medicine as an analgesic, or pain reliever. Vincristine, an alkaloid believed to have been first isolated from *catharanthus roseus* in the 1950s, is now used to treat non-Hodgkin's lymphoma and childhood leukemia. Vinblastine, also isolated from the same plant, is believed to be an effective treatment for Hodgkin's disease.

Key words: Antimalarial, physiological effect, Hodgkin's disease.

INTRODUCTION

Properties of Alkaloids

The properties of alkaloids may be grouped together in *two* categories, namely:

(*a*) Physical properties

(*b*) Chemical properties.

These *two* categories shall now be discussed individually in the sections that follows:

1.Physical Properties

A comprehensive knowledge of the solubility of complete range of alkaloids and their corresponding salts is of utmost pharmaceutical importance because of their extremely specific and potent pharmacological actions.[17,19]

It is pertinent to mention here that in general the solubilities of different alkaloids and their respective salts usually exhibit considerable variation, which may be attributed from their extremely complex and varied chemical structures. However, it has been observed that the free alkaloid bases as such are invariably found to be fairly soluble in organic solvents, such as: either, chloroform, relatively non-polar solvents (hexane, benzene, petroleum ether), immiscible solvent, lower alcohols (methanol, ethanol) but they are either practically insoluble or very sparingly soluble in water.

Interestingly, the alkaloidal salts are almost freely soluble in water, relatively less soluble in alcohol and mostly either insoluble or sparingly soluble in organic solvents:

Examples Atropine sulphate and morphine hydrochloride are much more soluble in water than their corresponding bases *i.e.*, atropine and morphine.

However, there are a few exceptions to the above stated

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generalizations, namely:

a. Certain alkaloid bases are water soluble, but these may be solely regarded as exceptions rather than any specific rule, such as: ephedrine, colchicine, pilocarpine ***(i)*** ;the quaternary alkaloid- ***(ii)***
base like berberine and tubocurarine; caffeine-base readily extracted from tea with water.

2. Narceine and pilocarpine are insoluble in organic solvents, whereas morphine is sparingly soluble in organic solvents viz., solubility in either 1:5000.

3. Certain alkaloidal salts, for instance: lobeline hydrochloride and apoatropine hydrochloride are found to be soluble in organic solvent like chloroform.

4. Some alkaloidal salts are sparingly soluble in water whereas others are extremely watersoluble, such as: Quinine sulphate-soluble in 1:1000 parts of water, Quinine hydrochloride soluble in 1:1 part of water. The physical characteristics of some potentalkaloids, such as: mp, optical rotation and solubility are enlisted below so as to have a glimps of the distinct variation in the observed parameters.

2. Chemical Properties

The chemical properties of the alkaloids are so broadly spread out, therefore, they shall be treated individually under the following heads, namely.

[A] N-in the Molecule Besides, the other normal elements *e.g.*, carbon, hydrogen, oxygen, the alkaloids must essentially contain at least one N-atom. The number of N-atoms vary from the bear minimum one in a molecule *e.g.*, cocaine, to even five in a molecule *e.g.*, ergotamine. It has been observed that these N-atoms are normally present as a part of the heterocyclic ring in the alkaloid molecule *e.g.*, quinine, reserpine, strychnine, vinblastine and yohimbine; whereas there are certain alkaloids that contain the N-atom in the aliphatic side chain *e.g.*, ephedrine, mescaline.Invariably, the alkaloids contain the N-atom in the tertiary-amine form (R_3N) *e.g.*,morphine, reserpine; lesser in the secondary-amine form (R_2NH) *e.g.*, ephedrine; and very rarely in the primary-amine form (RNH_2) *e.g.*, nor-pseudo-ephedrine. Furthermore, whenever N-atom occurs either in the *tertiary*- or *secondary*-form, it essentially constitutes as an integral part of the ringsystem, precisely the heterocyclic ring system.

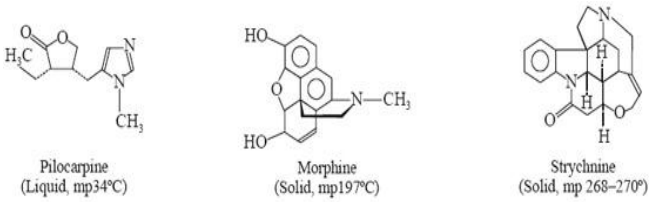
Noticeably, the tertiary N-atoms wherein only two of the bonds are involved in a ring, the methyl moiety is usually found as the third component, for instance: N-methyl group inmorphine, cocaine, colchicine, dextro methorphan, codeine, physostigmine, vinblastine, vindesine etc. Hence, methyl moiety seems to be the only alkyl group that has been found to be substituted on the N-atom.

However, in some very specific cases, the N-atom occurs in the quaternary ammonium form ($R_4N^+.X$) *e.g.*, tubocurarine chloride. Nevertheless, the quaternary ammonium compounds are logically and technically not regarded as alkaloids by virtue of the following *two* particular reasons, namely
N-atom does not possess a H-atom
Chemical properties are quite different.

As a matter of convenience, they are legitimately grouped along with the *alkaloids*.

[B] O-in the Molecule In addition to the common elements C, H and N, a variety ofalkaloids normally contains O-atom. Invariably, these specific alkaloids are found in the solid state, with a few exceptions where the oxygenated alkaloids usually occur as non-volatile liquids, such as: pilocarpine.

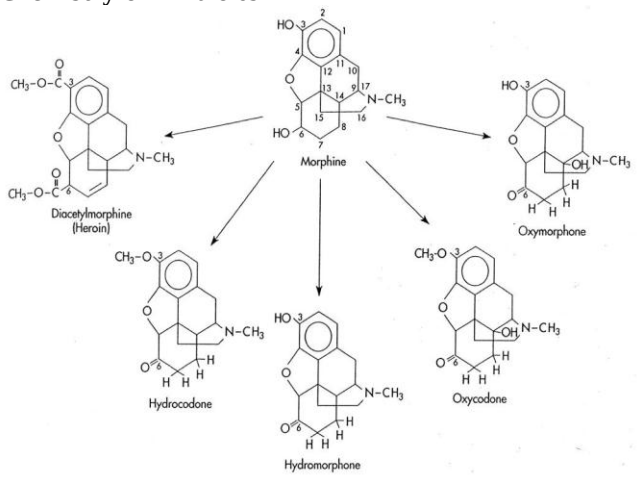
[C] Basicity (Alkalinity) In general, the alkaloids are basic (alkaline) in reaction, by virtue of the presence of N-atom present in the molecule. Hence, these are prone to the formation of their respective salts with various acids.



Degree of Basicity: The degree of basicity of the alkaloids mostly depends upon the prevailing influence caused due to the electrostatic status of the N-atom present in the alkaloid molecule, for instance, the number of N-atom present in the alkaloid, whether the N-atom is located in the ring or in the side-chain, the presence of alkyl group (*e.g.*, methyl) to the N-atom etc.

Another factor, which establishes the degree of basicity of an alkaloid, is the presence of *pri*-, *sec*-, *tert*-, or *quaternary* N-atom or atoms in it. In fact, such apparent differences in the degree of basicity arising from the various structural features, are eventually reflected by the different dissociation constant values (*i.e.*, pKa values) with regard to various alkaloids as stated below.

Chemistry of Alkaloids



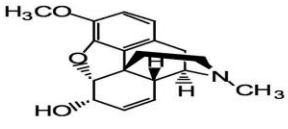
Salient Features

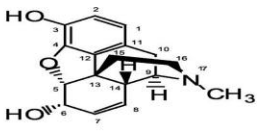
1. The weaker bases, *i.e.*, alkaloids having low pKa values, shall require a more acidic medium to form their respective salts with the corresponding acid.

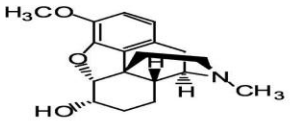
2. The strongly basic alkaloids *i.e.*, those possessing high pKa values, shall require comparatively low acidic medium to form their respective salts with the acid.

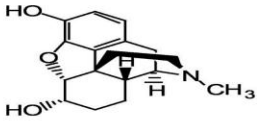
3. In a medium at a weakly acidic pH certain strongly basic alkaloids would be easily converted to their respective salt by interaction with the corresponding acid, whereas the alkaloids which are relatively weaker bases having lower pKa values shall still remain in their free-base form. Such a critical situation is skillfully exploited for the separation of a specific alkaloid or a group of alkaloids having closely identical pKa values, from other alkaloids that essentially possess either very low or very high pKa values.

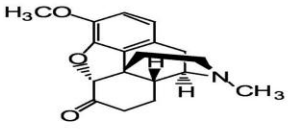
Parameters	Doses (*mg/kg* body weight)			
	Controls	250	500	1000
Number of implantation sites	10.26±0.22 [a]	6.78±0.43 [b]	6.21±0.61 [b]	6.28±0.95 [b]
Number of corpora lutea	10.66±0.14 [a]	7.62±0.69 [b]	7.14±0.83 [b]	7.22±1.03 [b]
Implantation index (%)	91.74	88.98	86.97	86.98
Pre- implantation losses (%)	3.75	11.02	13.02	13.01
Post- implantation loss (%)	0	100	100	100
Number of resorption sites	0.00±0.00 [a]	3.48±0.08 [b]	4.05±0.09 [c]	3.98±0.05 [b]
Resorption index (%)	0	51.33	65.22	63.38
*Weight of animal before pregnancy (*g*)	144.00±3.28 [a]	144.00±2.19 [a]	142.50±3.83 [a]	145.46±0.41 [a]
*Weight of animal after pregnancy (g)	185.50±3.83 [b]	166.00±3.89 [c]	162.00±2.69 [c]	167.33±1.03 [c]
Maternal weight gain (%)	28.82	15.28	14.08	15.04
Feed intake (*g/day* per rat)	20.32±0.75 [a]	14.50±0.87 [b]	15.33±0.47 [b]	14.89±0.70 [b]
Water intake (*ml/day* per rat)	19.83±1.45 [a]	16.63±0.75 [b]	14.57±1.89 [b]	11.99±1.83 [c]

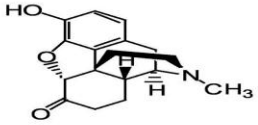

Codeine

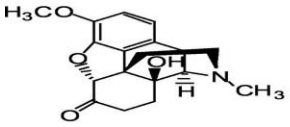

Morphine

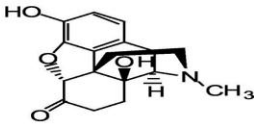

Dihydrocodeine (DHC)


Dihydromorphine (DHM)


Hydrocodone (HC)

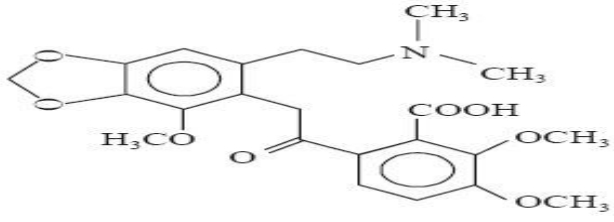

Hydromorphone (HM)


Oxycodone (OC)

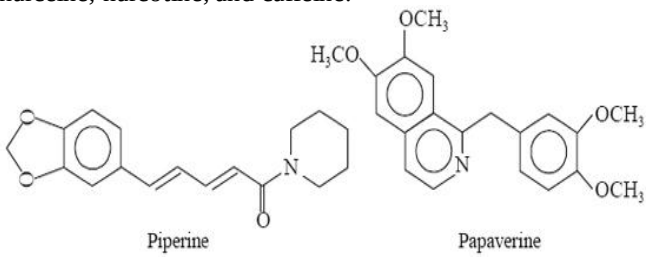

Oxymorphone (OM)

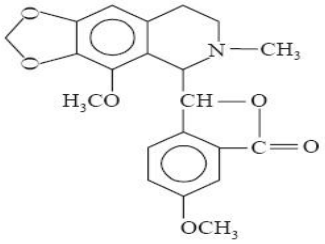
4. The alkaloids are usually neutrallized with acids to form salts that may be converted to the corresponding free-base by the cautious addition of selective weak bases, such as, ammonia, calcium hydroxide or sodium carbonate. The usage of either NaOH or KOH solutions must be avoided so as to prevent the decomposition or destruction of highly sensitive alkaloids.

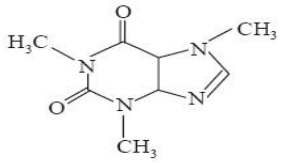
5. Amphoteric alkaloids: There are some alkaloids which are amphoteric in nature*i.e.*, they are neither acidic nor basic in character; this is due to the presence of phenolic (–OH) moiety in Morphine, or the presence of carboxylic (–COOH) function in Narceine,as shown below.


Narceine

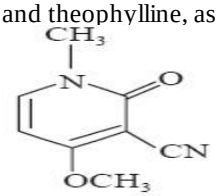
Unstable alkaloidal salts: There exists some specific alkaloids that inherently possess *weakbasic properties* and their salts are not so stable, for instance: piperine, papaverine, narceine, narcotine, and caffeine.

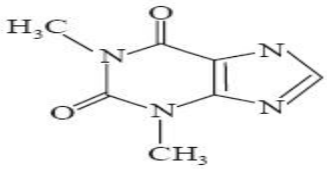

Piperine Papaverine


Narcotine


Caffeine

6. Neutral or slightly acidic alkaloids: There are a few typical naturally occurring alkaloids that almost behave as either neutral or slightly acidic character, namely: ricinine and theophylline, as depicted below:


Ricinine


Theopylline

[D] Precipitation by Specific Reagents A good number of alkaloids obtained from various plant sources invariably give a distinct precipitate with certain specific reagents to an extent as small as *one microgram*. Based on these observations, these alkaloid-precipitating reagents are sometimes employed for either detecting the presence or absence of alkaloids in:

(i) Crude extracts or plant materials

(ii) Ascertaining whether a specific extraction procedure has exhausted completely the alkaloidal contents or not.

However, a negative test *i.e.*, the absence of precipitation, may infer that the alkaloids are absent. It is pertinent to mention here that a positive test may not always indicate the presence of alkaloids, but may also be due to the presence of other plant constituents, such as: purines, proteins, betaines and ammonium salts etc. Therefore, it is always desired to rule out the possibility of a *false-test* by alkalifying the acidic solution with dilute ammonium hydroxide and subsequently extracting the liberated alkaloid with chloroform. The residue thus obtained, after the removal of the solvent (chloroform), is tested with the alkaloid-precipitating reagents. Now, if the test is positive, the presence of an alkaloid is almost confirmed.

Microcrystalline precipitates of alkaloids

Alkaloids, alike other amines, usually form *double salts* with salts of heavy metals, such as, gold (Au), mercury (Hg) and platinum (Pt). The resulting double salts are found to be possessing characteristic microcrystalline structures. It has been observed that under controlled and specific experimental parameters *viz*., profile of mixing and gradual evaporation, a drop of an alkaloidal solution reacting with a drop of an appropriate alkaloidalprecipitating reagent, such as: chloroplatinic acid (H_2PtCl_6) or chlorauric acid ($HAu . Cl_4$), on a microscopic-glass slide, gives rise to microcrystalline products having specific and characteristic shapes and structures solely based upon the manner of aggregation.* It may, however, be exploited skill fully as a convenient means of rapid-microscopical identification of an alkaloid.

Test for alkaloids:-

The various reagents that are invariably used either for the testing of alkaloids by precipitation or by the formation of microcrystalline complexes (salts) are as stated below along with their individual compositions, namely:

(*i*) Mayer's Reagent (Potassium-Mercuric Iodide Test Solution):

Mercuric chloride = 1.36 g
Potassium Iodide = 3.00 g
Distilled water to make = 100.00 ml [8]

(*ii*) Wagner's Reagent (Potassium Triiodide):

Iodine = 1.3 g
Potassium = 2.0 g
Distilled water to make = 100.00 ml

(*iii*) Kraut's Reagent (Modified Dragendorff's Reagent or Potassium Bismuth Iodide):
Bismuth Nitrate = 8.0 g
Nitric Acid = 20.0 ml
Potassium Iodide = 27.2 g
Distilled water to make = 100.00 ml

(*iv*) Marme's Reagent (Potassium-Cadmium Iodide Reagent):
Cadmium Iodide = 10.0 g
Potassium Iodide = 20.0 g
Distilled water to make = 100.00 ml

(*v*) Scheibler's Reagent (Phosphotungstic Acid Reagent):
Sodium Tungstate = 20.0 g
Disodium Phosphate = 70.0 g
Distilled water to make = 100.00 ml
Note: Acidify with nitric acid to litmus paper.

(*vi*) Hager's Reagent:
A saturated solution of *Picric Acid*.

(*vii*) Sonnenschein's Reagent (Phosphomolybdic Acid): A 1% (w/v) solution of phosphomolybdic acid in ethanol.
(*viii*) Bertrand's Reagent (Silicotungstic Acid):
A 1% (w/v) solution of silicotungstic acid in distilled water. [9]

(*ix*) Reineckate salt solution:
Ammonium Reineckate = 1.0 g
NH_4 [Cr. $(NH_3)_2$ $(SCN)_4$
Hydroxylamine HCl = 0.3 g
Ethanol = 100.00 ml
Note: Filter and store in a refrigerator.

[E] Colour Reactions with Specific Reagents Broadly speaking the colour reactions of the alkaloids are rather unspecific; however, they are certainly very sensitive so much so that even alkaloids present in microgram quantities invariably afford immediate and instant response. The ultimate development of a characteristic colour reaction is solely dependent upon either the dehydration or the oxidation of the alkaloid. Generally, a large number of these reagents essentially consist of concentrated sulphuric acid along with certain specific added compounds, such as, sulphomolybdic acid, formaldehyde, sulphovanadic acid, potassium arsenate, hydrogen peroxide, and selenious acid. A number of such specific reagents shall be described in the section that follows:

(*a*) Froehd's reagent: Dissolve 5 mg of molybdic acid or sodium molybdate in 5 ml of pure concentrated H_2SO_4.
Note: The reagent should be freshly prepared before use.
(*b*) Erdmann's reagent: A mixture of 10 drops of concentrates HNO_3, and 100 ml of water are added to 20 ml of pure concentrated H_2SO_4.
(*c*) Marqui's reagent: A mixture of 2-3 drops of formaldehyde solution (40%) with 3 ml of concentrated H_2SO_4.
(*d*) Mandalin's reagent: Dissolve 1 g of finely powdered ammonium vanadate in 200 g of pure conc. H_2SO_4.[27]
(*e*) Mecke's Reagent: Dissolve 1 g of selenious acid in 200 g of pure concentrated H_2SO_4.
(*f*) Modified Dragendroff's reagent: Dissolve 1.6 g of bismuth subnitrate in 60 ml of 20% glacial acetic acid, add to it 5 ml of 40% aqueous solution of KI, 5ml of glacial acetic acid and make up the volume to 100 ml of water.
(*g*) Rosenthaler's reagent: Dissolve 1 g of potassium arsenates in 100 g of pure concentrated H_2SO_4.
(*h*) Schaer's reagent: Mix carefully 1 volume of pure 30% H_2O_2 with 10 volumes of concentrated H_2SO_4.
Note: The reagent is always prepared afresh, before use.

Interestingly, there are some instances where in the intensity of the colour so produced is in *linear proportion* under standardized experimental parameters. Therefore, such specific colour reactions may be used exclusively for the quantitative determination of certain groups of alkaloids, such as:

***(i)* For Ergot Alkaloids:**
The blue colour produced by the ergot alkaloids with the Van Urk Reagent (or Ehrlich Reagent) *i.e., para*-dimethylaminobenzaldehyde in 65% H_2SO_4, is employed for the quantitative estimation of ergot alkaloids.

***(ii)* For Belladona Alkaloids:**
The violet colour caused by the belladona alkaloids with fuming HNO_3 and alcoholic KOH solution is employed for their assay.

[F] Stability of Alkaloids Alkaloids,

In general, are not very stable. They normally undergo degradation or decomposition on being exposed to air, light, moisture and heat, besides chemical reagents. A few typical examples of alkaloids *vis-a-vis* their stability are stated below, namely:

(i) Ergotamine gets destroyed by prolonged treatment with alkali, whereas strychnine can stand such vigorous action.

(ii) An aqueous solution of alkaloids undergo rapid decomposition or degradation as compared to their solid forms.

(iii) Storage of alkaloids in pure form or their dry extracts is usually done in a vacuum desiccator over a dehydrating agent *e.g.*, phosphorous pentoxide (P_2O_5) or calcium chloride ($CaCl_2$) anhydrous for their better stability.

(iv) During the course of extraction of alkaloids followed by isolation, the solvent is preferably removed effectively by distillation under vacuum** (or reduced atmospheric pressure) or by subjecting it to evaporation in a Rotary Thin-Film Evaporator under vacuum so that the desired product is not exposed to excessive heat, thus avoiding decomposition.

Alkaloids, are stored in amber-coloured glass bottles preferably in a vacuum desiccator.

[G] Acid salts of Alkaloids A plethora of alkaloids are strongly alkaline in nature and most of them form well-defined salts. However, in certain instances the basicity of analkaloid is quite weak and feeble, and hence the formation of the corresponding salts with either acetic or other weak acids is practically insignificant and rare. The salts formed with stronger acids *e.g.*, HCl, H_2SO_4 etc., get decomposed in the presence of water to liberate the free base and the acid. It has been observed that only a few of the alkaloids form carbonates, and consequently either the alkali carbonates or the alkali hydrogen carbonates are invariably used to liberate them from the aqueous solutions of their corresponding salts.

Alkaloids, in general, containing either one or more than one N-atom usually behave asmonoacidic bases; and, therefore, form only one series of salts with acids as designated by '*BA*' (where: B = base; and A = acid). It is pertinent to mention here that quinine in particular and the cinchona alkaloids in general are an exception to the earlier concept and found to behave as *diacidic bases*. Besides, a number of alkaloids to behave as monoacidic bases, even though they contain two N-atoms in their molecule. It is worthwhile to mention here that the basicities of the alkaloids is of utmost importance with regard to their quantitative volumetric estimation.

In common practice the salts of alkaloids are prepared by using cold and dilute solutions of the mineral acid specifically, *e.g.*, morphine hydrochloride, atropine sulphate, quinine sulphate, ephedrine hydrochloride etc. It may be pointed out that the use of concentrated mineral acids, or heating an alkaloid even with a dilute acid under

pressure may ultimately lead to profound changes in them. Noticeably, the concentrated mineral acids invariably give rise to characteristic colour changes, that are usually used as a means of identification and characterization of the alkaloids. In addition to the complete decomposition of alkaloids by strong acids to result the various colour changes, the chemical changes caused by the mineral acids on them may be categorized into *three*different types, namely:

(*a*) Dehydration: Dehydration of alkaloids give rise to either *anhydro-* or *apo*-alkaloids, such as:

Apomorphine obtained from Morphine

Apoatropine obtained from Atropine

(*b*) Demethoxylation: The removal or elimination of the methoxyl groups from the alkaloids by treatment with either concentrated HCl or HI to produce methyl chloride (CH3Cl) or methyl iodide (CH_3I) while giving rise to the corresponding *hydroxy base*. The methoxyl group (s) are present in a variety of alkaloids, for instance: codeine, quinine, narcotine andpapaverine.

Example:

NARCOTINE + 3HI -> NORNARCOTINE + 3CH3I

(*c*) Hydrolysis: A good number of naturally occurring alkaloids are obtained as*esters*. They easily undergo hydrolysis on being heated with either alkalies or mineral acids thereby resulting into the formation of the corresponding acids along with respective alcohols or phenols of the alkaloids.

A few typical examples are as give below:

(*i*) ATROPINE + H2O -> TROPINE + TROPIC ACID

(*ii*) COCAINE + 2H2O -> ECGONINE + BENZOIC ACID + METHANOL

[H] Action of Alkalies *e.g.*, NaOH and KOH on the alkaloids are found to be varying in nature as enumerated below:

(*a*) Dilute alkaline solutions of KOH or NaOH normally decompose most alkaloidal salts and finally liberate the free alkaloids.

(*b*) Certain alkaloids containing phenolic hydroxyl groups *e.g.*, morphine, on being treated with alkaline solutions yield, their corresponding soluble sodium or potassium salts.

(*c*) The ester alkaloids usually undergo hydrolysis on being treated with dilute alkalies, such as: atropine, cocaine.

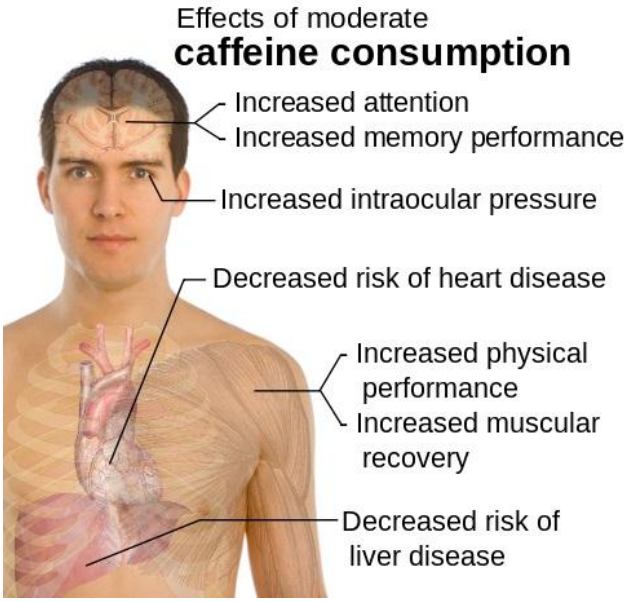
(*d*) Racemic Isomeride: The action of alkali hydroxides on hyoscyamine in alcohol gives rise to the racemic isomeride atropine.

(*e*) Fusion of alkaloids with dry KOH or NaOH by the application of heat ultimately leads to drastic decomposition of the former thereby yielding ultimately the simple heterocyclic bases, for instance: pyridine, quinoline, pyrrolidine etc.

(*f*) Simple fusion of alkaloids with alkali hydroxides may give rise to distinct and visible colour changes.

[I] Pharmacological Activity:

The alkaloids exhibit a wide-spectrum and complete diversity of complex structures which ultimately is responsible for their extra ordinary broad-range of pharmacological activities covering both the cardio-vascular and central nervous system. It has been observed beyond any reasonable doubt that most alkaloids usually exert certain specific and definite pharmacological action. Moreover, a small quantity of an alkaloid (0.1–1.0 mg) may bring about a marked and pronounced pharmacological action on various organs and tissues both of animal and human origin. However, the potency of an individual alkaloid varies from one another widely and profusely.A few typical pharmacological actions of some alkaloids are stated below showing their broadspectrum of activities, namely:



CLASSIFICATION OF ALKALOIDS

The alkaloids, as an important and enormously large conglomerate of naturally occurring nitrogencontaining plant substances having very specific as well as most diversified pharmacological properties may be classified in a number of modes and means. Hegnauer* (1963) conveniently classified alkaloids into *six* important groups, corresponding to the six amino-acids legitimately considered as the starting points for their biosynthesis, such as: anthranilic acid, histidine, lysine, ornithine phenylalanine and tryptophan. Price* (1963) further took a leading clue from the earlier observation and considered in details the alkaloids present in one of the families, (*Rutaceae*) and logically placed them in the following *nine* chemical-structural categories, namely: acridines, amides, amines, benzyl isoquinolines, canthinones, imidazoles, indolquinazolines, furoquinolines, and quinazolines.

Another school of thought classifies alkaloids in the following *four* heads, namely:

(*a*) Biosynthetic Classification In this particular instance the significance solely lies to the precursor from which the alkaloids in question are produced in the plant biosynthetically. Therefore, it is quite convenient and also logical to group together all alkaloids having been derived from the same precursor but possessing different taxonomic distribution and pharmacological activities.

Examples:-

(i) Indole alkaloids derived from *tryptophan*.
(ii) Piperidine alkaloids derived from *lysine*.
(iii) Pyrrolidine alkaloids derived from *ornithine*.
(iv) Phenylethylamine alkaloids derived from *tyrosine*.
(v) Imidazole alkaloids derived from *histidine*.

(*b*) Chemical Classification:- It is probably the most widely accepted and common mode of classification of alkaloids for which the main criterion is the presence of the basic heterocyclic nucleus (*i.e.*, the chemical entity).

Examples

1. Pyrrolidine alkaloids *e.g.*, Hygrine
2. Piperidine alkaloids *e.g.*, Lobeline
3. Pyrrolizidine alkaloids *e.g.*, Senecionine
4. Tropane alkaloids *e.g.*, Atropine
5. Quinoline alkaloids *e.g.*, Quinine
6. Isoquinoline alkaloids *e.g.*, Morphine
7. Aporphine alkaloids *e.g.*, Boldine
8. Indole alkaloids *e.g.*, Ergometrine
9. Imidazole alkaloids *e.g.*, Pilocarpine
10. Diazocin alkaloids *e.g.*, Lupanine
11. Purine alkaloids *e.g.*, Caffeine
12. Steroidal alkaloids *e.g.*, Solanidine
13. Amino alkaloids *e.g.*, Ephedrine
14. Diterpene alkaloids *e.g.*, Aconitine.

(*c*) Pharmacological Classification:-The alkaloids exhibit a broad range of very specific pharmacological characteristics. Perhaps this might also be used as a strong basis for the general classification of the wide-spectrum of alkaloids derived from the plant kingdom, such as: analgesics, cardio-vascular drugs, CNS-stimulants and depressants, dilation of pupil of eye, mydriatics, anticholinergics, sympathomimetics, antimalarials, purgatives, and the like. However, such a classification is not quite common and broadly known.

Examples:-

1. Morphine as Narcotic analgesic
2. Quinine as Antimalarial
3. Strychnine as Reflex excitability
4. Lobeline as Respiratory stimulant
5. Boldine as Choleretics and laxatives
6. Aconitine as Neuralgia
7. Pilocarpine as Antiglaucoma agent and miotic
8. Ergonovine as Oxytocic
9. Ephedrine as Bronchodilator

(i) Narceine as Analgesic (narcotic) and antitussive.

(*d*) Taxonomic Classification:- This particular classification essentially deals with the 'Taxon' *i.e.*, the taxonomic category. The most common *taxa* are the genus, subgenus, species, subspecies, and variety. Therefore, the taxonomic classification encompasses the plethora of alkaloids exclusively based on their respective distribution in a variety of Plant Families, sometimes also referred to as the 'Natural order'. A few typical examples of plant families and the various species associated with them are stated below, namely:

(*i*) Cannabinaceous Alkaloids:

e.g., *Cannabis sativa* Linn., (Hemp, Marijuana).

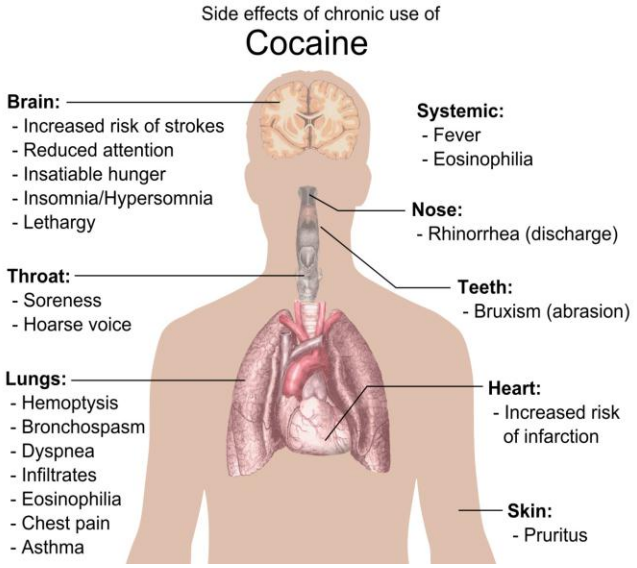
(*ii*) Rubiaceous Alkaloids:

e.g., *Cinchona Sp.* (Quinine)
Mitragyna speciosa Korth (Katum, Kratum, Kutum)
Pausinystalia johimbe (K. Schum) (Yohimbe).

(*iii*) Solanaceous Alkaloids:

e.g., *Atropa belladona* L., (Deadly Nightshade, Belladona)
Brunfelsia uniflorus (Pohl) D. Don (Manaca, Manacan)
*Capsicum annuum*L., (Sweet Peppers, Paprika)
Datura candida (Pers.) Saff. (Borrachero, Floripondio)
Duboisia myoporoides R. Br. (Corkwood Tree, Pituri)
Hyoscyamus niger L. (Henbane, Henblain, Jusquaime)
Mandragora officinarum L. (Mandrake, Loveapple)
Nicotiana glauca R. Grah. (Tree Tobacco)
Seopolia carniolica Jacq. (Scopolia)
Solanum dulcamara L.(Bittersweet, Bitter Nightshade, Felonwood)
Withania somniferum (L.) Dunal (Ashwagandha), etc.

Invariably, they are grouped together according to the name of the *genus* wherein they belong to, such as: coca, cinchona, ephedra.Some 'phytochemists' have even gone a step further and classified the alkaloidsbased on their chemotaxonomic classification.In the recent past, the alkaloids have been divided into *two* major categories based on the analogy that one containing a *non-heterocyclic nucleus*, while the other having the*heterocyclic nucleus*.



It is, however, pertinent to mention at this juncture that the enormous volume of authentic information accumulated so far with regard to the isolation of alkaloids from a variety of plant species and their subsequent characterization by the help of latest analytical techniques they may be classified as follows:

A. Alkaloids derived from Amination Reactions

1. Acetate-derived Alkaloids
2. Phenylalanine-derived Alkaloids
3. Terpenoid Alkaloids
4. Steroidal Alkaloids

B. Alkaloids derived from Anthranilic Acid

1. Quinazoline Alkaloids
2. Quinoline Alkaloids
3. Acridine Alkaloids

C. Alkaloids derived from Histidine

(i) Imidazole Alkaloids

D. Alkaloids derived from Lysine

1. Piperidine Alkaloids
2. Quinolizidine Alkaloids
3. Indolizidine Alkaloids

E. Alkaloids derived from Nicotinic Acid

(i)Pyridine Alkaloids

F. Alkaloids derived from Ornithine

1. Pyrrolidine Alkaloids
2. Tropane Alkaloids
3. Pyrrolizidine Alkaloids

G. Alkaloids derived from Tyrosine

1. Phenylethylamine Alkaloids
2. Simple Tetrahydro iso-quinoline Alkaloids
3. Modified Benzyl Tetrahydro iso-quinoline Alkaloids

H. Alkaloids derived from Tryptophan

1. Simple Indole Alkaloids
2. Simple b-Carboline Alkaloids
3. Terpenoid Indole Alkaloids
4. Quinoline Alkaloids
5. Pyrroloindole Alkaloids
6. Ergot Alkaloids

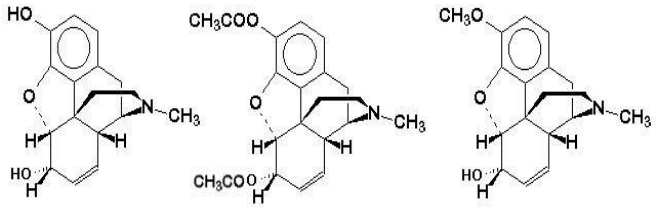
General Methods of Extraction and Isolation of Alkaloids

The general methods of extraction and isolation of the alkaloids from the plant sources one has to take into consideration the following steps in a sequential manner, namely:

(i) Separation of the alkaloid(s) from the main bulk of the non-alkaloidal substances,

(ii) Most of the alkaloid-containing plants, several alkaloids having closely related chemical structures are normally present, such as: the cinchona alkaloids consist of more than twentyfive alkaloids. There is hardly any known plant source that contains only one alkaloid exclusively,

(*iii*) Separation of each individual alkaloid from the mixture of alkaloids obtained from a particular plant source (*e.g*., cinchona bark) using latest separation techniques, for instance, preparative high-performances liquid chromatography, (HPLC) column chromatography, by the help of chromatotron, and high-performance thin-layer chromatography (HPTLC).Nevertheless, the general methods of isolation of alkaloids largely depend upon several vital factors, for instance:



Morphine Heroin Codeine

1. The alkaline nature of most alkaloids
2. The ability and ease of formation of alkaloidal salts with acids
3. Relative solubilities of the resulting alkaloidal salts either in polar organic solvents

e.g., ethanol, chloroform, isopropanol etc., or in aqueous medium.

The general methods of extraction of alkaloids from the plant sources solely depend upon the purpose and scale of the operation (*e.g*., pilot scale or commercial scale). It is also based on the quantum and bulk of the raw material to be employed in the operation. Of course, for research purposes column chromatography using ion-exchange resins have been used successfully and effectively to strip the plant materials of their alkaloidal contents. However, in the commercial scale large volumes of aqueous extracts of plant materials are normally pumped through huge metallic columns packed with cationic resins, which in turn pick up all basic components (cations). Subsequently, the alkaloids (*i.e*., the basic components are conveniently washed off by flushing the column with a moderately strong acid. The column having the cationic resins can be reused once again for the next drug substances.[7,9,16,33,23,34]. By the advent of the latest separation techniques and the copious volume of informations accumulated through the intensive and extensive research carried out with regard to the conventional processes essentially associated with the separation as well as isolation of the hundreds of alkaloids from the natural plant sources, the following *five*steps are most important and vital, namely:

1. **Sample preparation**
2. **Liberation of free alkaloidal base**
3. **Extraction of alkaloidal base with organic solvent**
4. **Purification of crude alkaloidal extract**
5. **Fractionation of crude alkaloids**

All these *five* steps shall be discussed individually as under:

Sample Preparation

The first and foremost step is the sample preparation. The plant material is reduced to a moderately coarse powder by appropriate means using grinders and sieves, to facilitate maximum effective contact of the solvent with the ruptured alkaloid bearing tissues and cells. In the case of plant substances that are rich in oils and fats, such as: seeds, kernels, these non-alkaloidal chemical components need to be eliminated completely by extraction with a suitable non-polar solvent like nhexane, light petroleum ether, in a soxhlet apparatus, which would not extract the alkaloids in question.

However, it is always advisable to shake the light-petroleum ether or n-hexane fraction with a dilute mineral acid and subsequently test the acidic solution for the presence of alkaloids.

Liberation of Free Alkaloid

It has been observed that the alkaloids invariably occur in the plant sources as the salt of acids, such as: oxalates, tannates etc. Therefore, when the plant substance is exposed to an alkaline medium, the alkaloidal salts are readily converted to the corresponding alkaloid bases.[5,9,11] Choice of Alkali Indeed, the choice of a suitable *mineral base* (alkali) for the ease of liberation of the alkaloid from the salts is not only very vital but also equally significant and largely depend on the following factors, namely:

(*a*) Natural state of the alkaloids: It has been observed that the salt of a *strongly basic alkaloid* with a mineral acid usually tends to undergo cleavage under the influences of a stronger base. Likewise, the corresponding salt of a *weakly basic alkaloid* and a relatively weak organic acid shall require a rather weaker base for its cleavage.

(*b*) Chemical characteristics of the alkaloidal base: The usage of strong alkali *e.g.*, NaOH or KOH should be avoided as far as possible by virtue of the fact that certain alkaloids undergo hydrolysis on prolonged contact with a strong base.

Example

(*i*) Hydrolysis of ester-alkaloids,

e.g., cocaine, hyoscyamine;

(*ii*) Phenolic alkaloids

e.g., cephaeline, morphine. These alkaloids normally get solubilized while in contact with a strong alkali and, therefore milder alkaline reagents *e.g.*, dilute ammonia solution are necessary for their liberation.

(*c*) Presence of fatty substances: The usage of strong alkali is strictly prohibited in the case of fat containing plant materials because of the formation of saponified products causing troublesome emulsions. In such cases, it is always preferred to defat the plant substance before proceeding for the liberation of free alkaloids.

Ammonium Hydroxide Solution Dilute aqueous ammonium hydroxide solution is one of the choicest alkali most frequently used for the liberation of alkaloids from the plant sources. It enjoys a two-fold advantage. First, being its adequate alkalinity to liberate most of the common alkaloids, and second by, its volatile nature so that it may be removed by evaporation of the solvent. As it has a tendency to be extracted by solvent ether from the aqueous solution, therefore, it is almost necessary to get rid of it by evaporation and subsequent washing repeatedly. In normal practice, usually even the last traces of ammonia are removed when the combined ethereal extract is reduced to half of its original volume under vacuum.

NaOH or KOH Solution The alkaloids that occur naturally as their tannate salts specially require either NaOH or KOH solution for their subsequent liberation. In certain typical instance even the use of KOH or NaOH fails to cleave the tannate salts because of their intimately strong bondage with the alkaloid and extremely insoluble nature.

Example

(*i*) Cinchona Bark: It has got to be treated first by heating with dilute HCl so as to decompose the salts and liberate the alkaloids in the form of water soluble hydrochlorides, and

(*ii*) Pomegranate Bark: It does not have the tannin so tenaciously bound to the alkaloids as in the case of cinchona bark. Hence, NaOH solution is strong enough to cause on effective split of the alkaloidal salts. It also acts to control the solubility of the water-soluble pomegranate alkaloids by preventing their dissociation.

Extraction of Alkaloid

The extraction of alkaloidal base may be accomplished by *three* different types of solvents that are discussed below, namely:

[A] Extraction with Water-Miscible Solvents A plethora of alkaloids and their respective salts are soluble in alcohols, such as: methanol, ethanol, isopropanol; therefore, these very solvents may also be employed for the extraction of the plant substances. The usual pretreatment of the crude drug with alkali may be avoided completely, because alcohol appears to affect dissolution of not only the *alkaloidal salts*but also the *free bases* found in the plant substances. It is, however, believed that alcohol predominantly exerts a *hydrolyzing effect* upon the alkaloidal tannates and other salts. In actual practice, neither pretreatment of the crude drug with an alkali nor acidification of the alcohol with a small amount of a mineral acid or an organic acid is required.

1. The penetration and hence the subsequent extraction of the crude drug is almost complete with the help of four successive extractions with an alcohol. Further, the loss of

solvent is comparatively less than the chlorinated solvents e.g., chloroform.

2. The extraction of total alkaloids with alcohol is highly recommended because of its maximum efficiency and economical viability.

[B] Extraction with Water-Immiscible Solvents In reality, the most widely used water-immiscible solvents for the extraction of alkaloids are: chloroform, diethyl ether (solvent ether) and isopropyl ether. However, a few other specific organic solvents, namely: ethylene chloride, carbon tetrachloride and benzene* may be employed with an evident advantage for certain specific alkaloids. Interestingly, *chloroform* is regarded as the choicest water-immiscible solvent for a broad-spectrum of alkaloids present in the plant kingdom and extracts them with varying degrees of ease.

[C] Extraction with Water The crude drug is subjected to extraction with water previously acidified with dilute solution of HCl, H_2SO_4 or CH_3COOH, which is subsequently rendered alkaline, preferably with dilute NH_4OH solution and finally extracted with a water-immiscible solvent as stated in [B] above.

Undoubtedly, water being an excellent and absolutely inexpensive polar solvent for the extraction of alkaloids, but if offers an enormous volume of disadvantages because it carries along with it a large number of other plant components, for instance: sugar, pigments (*e.g.*, chlorophylls), starches, tannins, proteins etc., which ultimately puts across a collosal waste of time, energy and chemicals. Hence, its usage has been resulting to a bear minimum level.

In general, the alkaloids may be extracted by any of the following *three* well-defined and widely accepted processes, namely:

(*a*) Soxhlet Extraction Process
(*b*) Stas-Otto Process
(*c*) Kippenberger's Process.

All these three processes shall now be discussed briefly in the sections that follows:

***(a)* Soxhlet Extraction Process:-** The soxhlet assembly is a continuous extractor which is generally suitable for the extraction of alkaloids from powdered plant materials with the help of organic solvents. In this instance, the powdered drug is usually moistened with dilute ammonia solution and then packed loosely in the thimble of the Soxhlet apparatus; and the organic solvent affords a deep penetration of the moist drug thereby allowing the greatest possible extraction of the alkaloids from the exposed surfaces of the cells and tissues of the crude drug. Once, the extraction is ascertained to have completed, the solvent is filtered and evaporated in a Rotary Thin-Film Evaporator and the residue is treated further for the isolation of individual alkaloids.

(*b*) Stas-Otto Process:- The Stas-Otto process essentially consists of treating the powdered and sieved drug substance with 90–95% (v/v) ethanol, previously acidified with tartaric acid. The proportion of crude drug to solvent should be maintained as 1 Kg to 1 L. The alcohol is distilled off under vacuum and the resulting aqueous residue is treated with petroleum-ether (60-80°C) to remove the fatty components completely. If any alkaloid is removed by the petroleum ether, it must be recovered by treating it with dilute mineral acid. Thus, the resulting aqueous extract is mixed with the main bulk of aqueous extract. The combined aqueous extract is filtered and evaporated to dryness preferably in a Rotary *Thin*-Film Evaporator under vacuum. The residue is extracted with absolute ethanol thereby dissolving the total alkaloids.

(*c*) Kippenberger's Process:- In Kippenberger's process the powdered and sieved plant substance is first and foremost digested with solution of tannin (100 g) in glycerol (500 g) at a constant temperature of 40°C for a duration of 48 hours. The resulting mixture is further heated to 50°C so as to help in the complete coagnlation of proteinous substances, cooled to ambient temperature and finally filtered. The resulting filtrate is thoroughly shaken with petroleum ether to get rid of faulty materials (oils, fats and waxes), and the last traces of petroleum ether is removed from the extract by heating either on a water-bath (electric) or exposure to Infra-Red Lamp. The fat-free crude plant extract is subsequently acidified and shaken with chloroform, successively to remove the bulk of the alkaloids, namely, atropine, codeine, colchicine, narcotine, nicotine, papaverine, spartenine and thebaine.

The resulting residual extract may still contain narceine, curarine and morphine. However, narceine and morphine may be isolated by passing freshly generated CO2 directly into extract so as to convert the alkali hydroxide into their corresponding carbonate, which is then ultimately subjected to solvent extraction using a mixture of alcohol and chloroform. Finally, the third alkaloid, curarine, may be extracted by agitation with a mixture of equal volumes of ether and chloroform.

However, a combination of Kippenberger's process and Stas-Otto process by its application to the final alcoholic extract obtained by the latter process is found to give better separation of alkaloids.

Purification of Alkaloid

The main bulk of the crude alkaloidal extract is invariably subjected to further purification by means of either anyone or combination of the following methods:

(*a*) Extraction with Acid Solution The extraction of the alkaloid from the bulk of the crude alkaloid solution in immiscible organic solvent is invariably carried out by shaking with an acid solution. In usual practice, the use of HCl is restricted when chloroform remains as the solvent because of the fact that quite a few alkaloidal hydrochlorides are distinctly soluble in the latter. However,

dilute H_2SO_4 is always preferred over HCl for general use in the extraction of alkaloids. Subsequently, the acid solution is rendered alkaline with dilute NH4OH solution to liberate the alkaloids which is then extracted with an organic solvent. The solvent is removed under reduced pressure and the traces of moisture is removed with anhydrous sodium sulphate.

(*i*) To avoid the formation of stubborn and troublesome emulsions a solution of gumtragacanth is often added to the aqueous-phase. In case, it still persists the two phases may be got separated by centrifugation, and

(*ii*) To discard the presence of foreign interfering extractive components present in plant substances, such as: pigments, resins, waxes, oils and fats, the use of a 2.5-5% (w/v) solution of lead acetate is made to the alkaloidal extract which precipitates them effectively. The excess of lead present in the filtrate is removed by either passing H_2S gas through the Kipp's Apparatus or by adding sodium phosphate.

(*b*) Precipitation of Alkaloid with Precipitating Reagent The usual precipitation of the alkaloid as a complex compound is accomplished by the addition of a suitable precipitating reagent. The resulting alkaloidal complex is further purified by filtration, recrystallization and ultimately decomposed to obtain the desired free alkaloid(s). *(i)*

(ii)

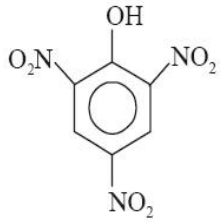
Example

(***i*) Tannic-acid Complex:** It is normally decomposed by treatment with freshly prepared $Pb(OH)_2$ or $Pb(CO_3)_2$.

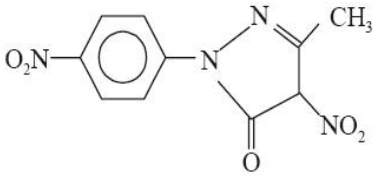
(*ii*) Precipitates obtained with HgC_{l2}, $AuCl_3$, $PtCl_4$, Mayer's Reagent: These precipitates are decomposed by passing a stream of H_2S gas through its suspension.

(*iii*) Precipitates with Double Salts: The double salt obtained with Dragendorff's Regent is quickly boiled with 5% (w/v) $BaCO_3$ solution.

(*iv*) Precipitates with Nitrogenous Acids: The precipitates obtained with nitrogenous acids like picric acid and picrolonic acid are normally decomposed by treatment with either NH_4OH or NaOH.



Picric acid



Picrolonic acid

(*v*) Reineckate Complex: The complex obtained from alkaloid with *Reinecke Salt*, $NH_4[Cr(NH3)_2 (SCN)_4]$, is normally decomposed by treating its solution in a mixture of acetone and water (1:1) with a silver sulphate solution. It is pertinent to mention here that thefree liberated alkaloid from the complexes stated above, (*i*) through (*v*), may be further extracted for its final recovery with an appropriate organic solvent, such as: chloroform.

(*c*) The purification of alkaloids may also be accomplished by the formation of its crystallised alkaloidal salt by the addition of an appropriate mineral or organic acid, such as: hydrochloric, hydrobromic, perchloric, sulphuric, oxalic and tartaric acids.

(*d*) Various known separation techniques, namely: partition, ion-exchange andcolumn chromatography are invariably used for the purification of a host of alkaloids.

Besides, various physical parameters like: specific rotation, melting point, solubility are frequently used as a definite criteria of ascertaining the purity of alkaloids.

Fractionation of Crude Alkaloids

It has been observed largely that most of the alkaloid-bearing plant materials usually contain a mixture of closely-related alkaloids. Therefore, it has become almost necessary to carry out an effective fractionation of crude alkaloids from the extract or solution of total crude alkaloids.

However, the traditional and orthodox methods of separation are not only difficult but also tedious and cumbersome. The commonly employed techniques of separation that were found to the reliable and dependable may be short-listed as follows:

Fractional crystallization Fractional distillation

Derivatization with low solubility products.

The latest methods employed for the separation of alkaloids are the preparative high performance liquid chromatography *(HPLC)*, high performance thin-layer chromatography *(HPTLC)*, chromatotron, counter-current distribution and other chromatographic techniques including column chromatography, ion-exchange chromatography.

Following are some of the typical situations whereby the mixture of alkaloids may be separated effectively, such as:

(*a*) A larger section of the alkaloids are easily soluble in chloroform and relatively less soluble in other organic solvents. In general, the order of solubility is as stated below *chloroform > acetone > ethanol > methanol > ethyl acetate > ether > n-hexane*. Keeping in view the above solubility profile of alkaloids in organic solvents, if one of the alkaloids is much less soluble in ethanol than chloroform, the fractional crystallization of this alkaloid is possible. In this particular instance the chloroform-fraction is concentrated to an appropriate level, and hot ethanol added in small proportions at intervals. Thus, upon cooling the alkaloid, which is less soluble in ethanol, separates out conveniently.

(*b*) In case, the fractional crystallization of the mixture of closely related alkaloids become tedious and ineffective, one may try to form their respective salts, and then carry out the separation indicated above.

(c) The various acids, namely: HCl, HBr, HI, $HClO_4$, HNO_3, $C_2H_2O_4$, and $C_6H_3N_3O_7$, may either be employed in aqueous or methanolic solution. Thus, from the resulting

methanolic solution, the salts of the respective alkaloids may be precipitated by the addition of ether. The precipitated crude alkaloidal salts may be further recrystallized from hot acetone containing a small proportion of methanol.

(*d*) In certain other specific instances, the salts of the respective oxalates, picrates and perchlorates may be precipitated from their solutions in acetone, by the addition of ethyl acetate.

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