PHARMACOGNOSY

for 3rd year students 22 Public health 226 «Pharmacy, industrial parmacy», educational program «Pharmacy» Фм17(5,0д) англ 1, 2, 3, 4, 5, 6, 7, 8 groups

16.03 – 7, 8 groups

18.03 – 1, 2 group

19.03 – 3, 4 groups

20.03 – 5, 6 group

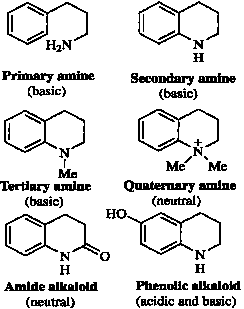
**LABORATORY CLASS. Topic:**  **«Chemical, morphological and anatomical analysis of MPM containing alkaloids – І.»**

**THE THEORETICAL PART.**

No other group of natural products has contributed more to medicines and pharmaceutical preparations than the alkaloids. Plants have been a rich source of alkaloids, but some are found in animals, fungi, and bacteria; practically all have been reproduced in the laboratory by chemical synthesis. As a group, they display an exceptionally wide array of biological activities and have an equally wide distribution, being present in plants, fungi, bacteria, amphibian, insects, marine animals and man. Plants and fungi rich in these natural product were used by early man to relieve pain, as recreational stimulants or, in religious ceremonies, to enter a psychological state to achieve ‘communication’ between his ancestors or God. The German pharmacist Karl Friedrich Wilhelm Meissner first coined the term ‘alkaloid’ in 1818, to describe substances that had alkaline (hence alkaloid) properties.

**An alkaloid** may be defined as a naturally occurring organic alkaline, containing one or more heterocyclic nitrogen atoms in its molecule. Alkaloids possess pharmacological activity and have economic importance as clinical agents.

The names of the alkaloids are obtained in various ways: from the generic name of the plant yielding them with the suffix ‘-***ine***’(*atropine)*; from the specific name of the plant yielding them, e.g. (*cocaine*); from the common name of the drug yielding them, e.g. *ergotamine*; from their physiologic activity, e.g. *emetine*; from the discoverer, e.g. *pelletierine*.

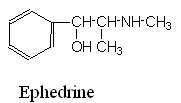
Many alkaloids are indeed alkaline in nature as they possess either a primary, secondary or tertiary amine functional group and the alkaline (basic) properties of these groups may be exploited to aid their extraction and purification. However, some alkaloids exist as quaternary amine salts in which a lone pair of electrons from the nitrogen atom is used to form a bond which another group (e.g. methyl) and therefore a positive charge resides on the nitrogen making this group essentially neutral (neither basic nor acidic). Care must therefore be taken with the alkali or base definition of alkaloids as some are neutral especially the amides, and some alkaloids possess phenolic groups which actually contribute to the acidity of the molecule. Biosynthetically, the alkaloids are produced from several different amino acids thereby giving rise to a diverse group of fundamental structure.

**Classification**

Alkaloids are usually classified according to the nature of their chemical structures. Generally, there are 3 broad divisions:

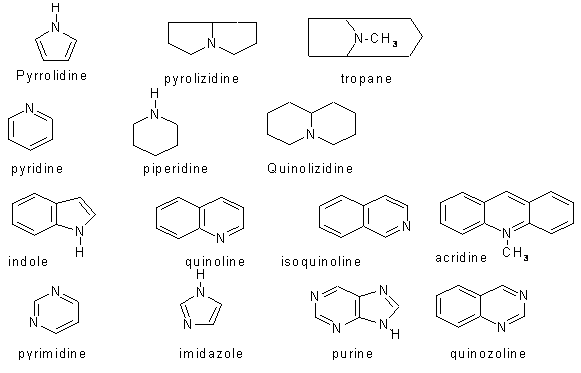
1. Protoalkaloids.
2. Typical alkaloids
3. Pseudoalkaloids

The name ***'protoalkaloid'*** or ‘biological amines' is sometimes applied to compounds such as capsaicin, ephedrine and colchicine which lack one or more of the properties of typical alkaloids.



The alkaloids in this group do not contain heterocyclic nitrogen atoms. Many are simple derivatives of phenylethylamine and as such, are derived from the common amino acids, phenylalanine or tyrosine.

***Typical alkaloids*** are derived from amino acid precursors, they are basic, they contain one or more nitrogen atoms (usually in a heterocyclic ring) and they usually have a marked physiological action on man or other animals. Typical alkaloids or heterocyclic, divided into **14 groups** according to their ring structure.



The term *‘****pseudoalkaloid*’** has been introduced to cover alkaloids, which are formed from terpenes. They divide into 2 groups:

* 1. **Terpenoid** alkaloids. Included in the terpenoid alkaloids are monoterpenes, sesquiterpenes and diterpenes (e.g. the alkaloids of *Aconitum*, *Delphinium* and *Taxus spp.*) Aconite which has some medicinal interest contains aconitin. These alkaloids are: aconitine, lyctonine.

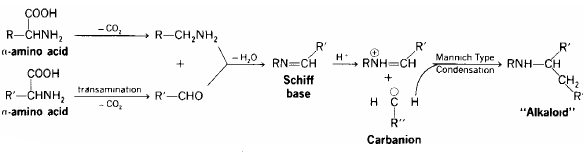


* 2. **Steroidal** alkaloids. The steroidal alkaloids are characterized by the cyclopentenoperhydrophenantherene nucleus. Solasodine, veratrum, alkamine esters and their glycosides are the main alkaloids of this group. They occur in genus: *Solanum*, *Veratrum*.



**Functions of alkaloids in plants and biosynthesis**

Some of the possibilities functions of alkaloids in plants include their functions as poisonous agents protecting the plant against insects and herbivores, end products of detoxification reactions representing a metabolic locking-up of compounds otherwise harmful to the plant, regulatory growth factors, or reserve substances capable of supplying nitrogen or other elements necessary to the plants economy.



**Figure 20.1. General reactions of alkaloidal biosynthesis**

The biosynthesis of many alkaloidal structures can be rationalized through simple chemical reactions that involve amino acids (Fig. 20.1). The amino acids that most often serve as alkaloidal precursors include phenylalanine, tyrosine, tryptophan, histidine, anthranilic acid, lysine and ornithine. Some of the general reactions that are of particular importance include the decarboxilation and transamination of the amino acids to yield a corresponding amine or aldehyde

**Occurrence and distribution**

The alkaloids are not equally distributed in plant kingdom. They are nearly absent in Algae and in the lower groups of plants with the exception of one or two families of the fungi. Fungal examples include ergot and various mushrooms. Streptomyces sp. produce the antibiotic alkaloids Chloromycetin (chloramphenicol) and erythromycin. Alkaloids are of common occurrence in the monocotyledons (Amaryllidaceae and Liliaceae). Among the angiosperms, Apocynaceae, Papaveraceae, Ranunculaceae, Rubiaceae, Solanaceae and Berberidaceae are outstanding for plants yielding alkaloids. The Lamiaceae and Rosaceae are almost free of them. It has been estimated that at least 15% of all vascular plants contain alkaloids.

The occurrence of alkaloids in plants does not appear to be confined to certain specific organs. Alkaloids are either found in all organs of the plant (Belladonna plant) or especially found in various different organs, e.g. in barks (Pomegranate, Cinchona), in roots (Aconite), in fruits (Conium, Black pepper), in seeds (Nux vomica, Areca), in leaves (Hyoscyamus), in rhizomes and roots (Ipecacuanha and Hydrastis).

It should be pointed out that in anyone particular species, usually only one or two organs and not all organs, possess the function of alkaloid formation. For instance, the alkaloids of the tobacco plant are formed in the root and are translocated to the leaves where the alkaloid accumulate. In the opium poppy the alkaloids occur in the latex of the fruit, while the seeds are devoid of alkaloids. In the autumn crocus (Colchicum autumnale), the alkaloids occur in the seed as well as in the corm.

In some cases, there is variety of alkaloid content in different organs of the plant during the growing season, or between day and night. In other cases, especially perennials, localization of the alkaloids in one or two particular organs, appears to become more marked with the increased age of the plant.

Accumulation of alkaloids depends on age and phase of development of plant and on factors of outside environment (light, soil, climate conditions, geography factor etc). In over-ground organs the largest content of alkaloids are accumulated in the phase of flowering: and in autumn their contents is decreased. The largest number of alkaloids are accumulated in southern regions, in northern ones their contents is decreased.

Specific alkaloids of complex structures are ordinarily confined to specific plant families (hyoscyamine in Solanaceae, colchicine in Liliaceae). A family may contain more than one structurally nonrelated alkaloids, e.g. Solanaceae contain nicotine in Tobacco, hyoscyamine in Hyoscyamtts, solanine in solanum and capsaicine in capsicum. Within a particular organ they may be concentrated in one tissue (e.g., Alropa leaf - epidermis, nerves). The contents of alkaloids in plants are small and ranges from thousandth shares of percent to some percents. For example, the bark of cinchona contains 15-20% of alkaloids. The content of alkaloids in medicinal plant raw material often ranges from 0,1 to 2%.

Alkaloids usually occur in plants, in the form of salts of acetic, malic, oxalic, tartaric, succinic, tannic or other plant acids. In some cases, the alkaloids occur in combination with special acids, e.g. aconitine occurs in combination with aconitic acid, the opium alkaloids with meconic acid, the cinchona alkaloids with quinic or cinchotannic acid, and the lobelia alkaloids with chelidonic acid. Salts with inorganic acids may be also present, e.g. morphine is partly present as sulfate in opium.

A few alkaloids occur in glycosidal combination with sugars, forming the gluco-alkaloids. The toxic solanine, found in sprouts of potato tubers, belongs to this group; it yields a mixture of sugars and the aglycone solanidine (an alkaloid) when hydrolysed.

**General physical and chemical properties**

Alkaloids are usually odorless, colorless crystalline, non-volatile, bitter solids. Only a few a colored, e.g. berberine and colchicine are yellow. A colorless alkaloid, however may have colored salts e.g. hydrastine salts, yellow and sanguinarine salts, red.

In addition to the elements, carbon, hydrogen and oxygen in most of the alkaloids, they must contain at least one nitrogen atom, although some like ergotamine may contain up to five. These nitrogen atom usually exist in a heterocyclic ring. But in a number of alkaloidal amines, (e.g. ephedrine, mescaline and colchicines) which are considered as alkaloids, the nitrogen in the molecule is not in the ring but in the side chain, i.e. of aliphatic character. The nitrogen usually occurs in the tertiary amine form (R3N), less in the secondary amine form (R2NH), e.g. ephedrine, rarely in the primary amine form (RNH2), e.g. norpseudo-ephedrine. When it occurs in the secondary or tertiary form, it usually constitutes a part of a ring system. In few cases, the nitrogen occurs in the quaternary ammonium form (R4N+(X)), e.g. tubocurarine chloride. Quaternary ammonium compounds are technically not alkaloids, since the nitrogen atom no longer carries a hydrogen atom and their chemical properties are quite different. However, as a matter of convenience, they are often grouped with alkaloids.

Many alkaloids contain one or more asymmetric carbon atoms in the molecule, and therefore show optical activity than the (+) – isomer (laevorotatory) has considerable greater pharmacological activity than the (+) isomer (dextrorotatory) of the some alkaloid.

The traditional designations l- and d- for the laevo- and dextro-rotatory isomers respectively are to be distinguished from the designations L- and D- which refer to the steric configuration in relation to a conventionally accepted reference compound.

For instance, D (-) ephedrine is about three and half times as active as D (+) isomer. Ergotamine (which is the (-) form) possess three to four times greater pharmacological action than the ergotaminine (which is the (+) isomer of ergotamine). Again there are exceptions: the medicinally useful d-tubocurarin is the (+) form.

In some alkaloids, both the (-) form and (+) form are medicinal useful. Quinine (the (-) form) and quinidine (the (+) form) are such examples. Quinine is primarily used as an antimalarial, and quinidine - for its action in restoring cardiac arrhythmia to normal rhythm.

Knowledge of the solubility of alkaloids and their salts is of considerably pharmaceutical importance. The difference in solubility between alkaloids and their salts, provide methods for their isolation from the plant and separation from the non-alkaloidal substances also present.

Whilst the solubility of different alkaloids and salts show considerable variation, the free alkaloid bases themselves, are usually fairly soluble in organic solvents, such as chloroform, ether or other relatively non-polar, solvents, but are practically insoluble in water.

On the other hand, the alkaloid salts are generally soluble in water, less in alcohol and mostly nearly insoluble or sparingly soluble in organic solvents.

There are many exceptions to the above generalization. Few alkaloidal bases are very sparingly soluble in organic solvents – as morphine in ether (1:5000); pilocarpine are insoluble. Few alkaloidal bases are soluble in water, but these are exceptions rather than a rule. Some examples of water soluble alkaloids are ephedrine, colchicines, pilocarpine and the quaternary alkaloid-bases like tubocurarine and berberine. Caffeine base is readily extracted from the tea with water.

All alkaloids don’t have the same degree of alkalinity which depends on the influence exerted on the electronic disposition of the nitrogen in the alkaloid molecule by side chains and various substitutions, structure of the molecule and the presence and location of other functional groups. One important factor contributing to the different degrees of alkalinity among different alkaloids, is whether a given alkaloid contains primarily, secondary, tertiary or quaternary nitrogen atom or atoms. Such difference in the degree of alkalinity arising from the various structural characters, are reflected in the different dissotiation constant exponents (pKa values) for the different alkaloids. The weaker basis (low pKa values) would require a more acidic medium to form salts with the acid when would the strongly basic alkaloids (high pKa values). Therefore, at a weakly acidic pH, some strongly basic alkaloids may be converted to their salt form by reacting with the acidic present, while the alkaloids which are weaker base may still be in their free base form. Such a situation is sometimes used in the separation of particular alkaloids with closely similar pKa values, from other alkaloids which have very much higher or very much lower pKa values.

**Extraction and isolation of alkaloids**

There are several methods that can be used for the extraction of the alkaloids from plant materials. However, the common procedures are largely based on: the basic nature of most alkaloids; the subsequent ability to form salts with acids; the ease by which the free bases can be liberated from their salts by alkalinization and finally the relative solubility of the alkaloids and their salts in water and various organic solvents.

Before any extraction is begun, the plant material needs to be reduced to a coarse powder by suitable means, to facilitate effective contact of solvent with alkaloid containing tissues and cells. The alkaloids occur in the plant mostly as salts of acids. When the plant material is placed in the alkaline medium, the alkaloid salts are converted to the correspondent alkaloid bases; as usually a suitable mineral alkali solution is used for this purpose. Ammonia hydroxide solution is the most widely used alkali. It is sufficiently basic to liberate most of the common alkaloids with a minimum of difficulty. It also has advantage of being volatile and easily removed on evaporation of the solvent. Because of its considerable tendency to be extracted from aqueous solution by ether; it is important that ammonia be thoroughly removed by washing and evaporation. Other alkalies that have been used include sodium carbonate and bicarbonate, calcium hydroxide and magnesium oxide. Extraction of the alkaloid base can be achieved by organic solvents like chloroform, ethyl ether and isopropyl ether.

The conventional process involved in the alkaloids separation and isolation may be divided as follows:

**Method 1**

1. Preparation of the sample.
2. Liberation of the free alkaloidal base, by treating the dried material with suitable alkali.
3. Extraction of the **alkaloidal base** with an organic solvent.
4. Purification of the alkaloidal extract.

The drug could be extracted with acidified water, which is then made alkaline and the alkaloids are removed with organic solvent.

**Method 2**

1. Preparation of the sample.
2. Converting all alkaloids in salts, by treating the dried material with suitable acid.
3. Extraction of the **alkaloidal salts** with water.
4. Purification of the alkaloidal extract.

Extraction methods vary with the scale and purpose of the operation as well as with raw material. For many research purposes, chromatography gives both speedy and accurate results. Ion exchange resins, have also been used to strip plant materials of their alakloids. In industry aqueous extracts are pumped through columns of cationic resins, which pick up all bases as cationes. The alkaloids can then be washed off by use of a strong acid and the same column can be used over again.

Liquid volatile alkaloids can be obtained by steam distillation after adding water and excess of potassium hydroxide, to the powdered drug or to the residue of crude alkaloids.

Practically, each of the alkaloid bearing plants contains a mixture of closely related alkaloids. The separation of the required individual alkaloids becomes necessary. The process of separation is often difficult and tedious. Until recent times, the method of separation relied on were fractional crystallization, preparation of derivatives with low solubility in certain solvents and, in very few cases, fractional distillation (for volatile alkaloids). Among the modern method used are countercurrent distribution and chromatographic methods.

To identify purified alkaloid obtained the following steps are usually undertaken: determination of the melting point of the free alkaloid and eutectic melting point with other substances; determination of its solubility in different solvents; crystallographic characters of alkaloid salts and complexes with different precipitations, color reactions with special reagents, ultra-violet and infra red absorption spectra; specific rotation; determination of molecular formula; chromatographic constants using different techniques – paper, thin-layer, gas chromatography, ets.

**Qualitative identification**

**Precipitation by certain reagent.** There are several general reagents, which may be used to test the presence of alkaloids or to help their identification. This includes the alkaloidal precipitating reagents and the alkaloidal coloring reagents. In addition, there are some special reagent that can be used for recognizing and confirming the identity of each alkaloid.

Many alkaloids in small quantities in solution may form precipitates or turbidity with certain reagents. Most alkaloids are also precipitated by tannins. These alkaloid precipitating reagent are sometimes used in testing the presence or absence of alkaloids in crude extracts of plant materials, and in testing whether a particular step in an extraction procedure has exhausted the alkaloidal contents. A negative response (no precipitate or turbidity) can be taken to mean absence of alkaloids, but a positive test may or may not be due to the presence of alkaloids, e.g. proteins, purines, ammonium salts, betaines may also give such a positive response to these reagents.

Among the reagents most commonly used for the testing alkaloids by precipitation or micro-crystal formation are:

1. Mayer's reagent (Potassium-mercuric chloride iodide test solution)
2. Wagner's and Bushard’s reagent (Potassium triiodide)
3. Dragendorff’s reagent (Potassium Bismuth iodide)
4. Marme’reagent (Potassium cadmium iodide test solution)
5. Scheibler’s reagent (Phosphotungestic acid)
6. Hager’s reagent (Saturated solution of picric acid)
7. Bertrand’s reagent (Silicotungestic acid)
8. Tannins solution

**Color reaction with** **certain reagent.** These color reactions are rather unspecific, but they are often very sensitive. These color test usually depend on dehydration or oxydationof the alkaloid with a resultant characteristic color. Most of these reagent consist of concentrated sulfuric acid, to which has been added compounds such as selenic acid, ammonium vanadate, formaldehyde, dimethylaminobenzaldehyde and others.

Alkaloidal coloring reagents are:

1. Froed’s reagent (Sulphomolybdic acid)
2. Marqui’s reagent (Formaldehyde-sulfuric acid)
3. Mandalin’s reagent (Sulphovanadic acid)
4. Erdmann’s (Nitric acid-sulfuric acid)
5. Mecke’s reagent (selenious acid-sulfuric acid)

**Specific reaction.** Certain group of alkaloids give more or less characteristic colorscertain specific reagents. In some cases , under standardized conditions, the intensity of the color so formed is in linear proportion and may be used in quantitative determination of those groups of alkaloids. The blue color formed by the ergot alkaloids with the Van Urk reagent (Erlich reagent, p- dimethylaminobenzaldehyde in 65% sulfuric acid); Vitali Morin color reaction of the belladonna alkaloids(with fuming nitric acid and alcoholic potassium hydroxide solution) are specific reaction.

**Quantitative determination**

For quantitative determination of alkaloids volumetric, gravimetric and physico-chemical methods of analysis are used.

All method of quantitative test has 3 steps:

1. Extraction of alkaloids
2. Purification of alkaloids
3. Test of alkaloids

The dried powdered material is completely extracted in quantitative manner by one of the methods described under extraction and isolation of alkaloids. The alkaloidal residues obtained after purification process is either titrated directly or usually a measured volume of standard acid is added and the excess back-titrated with standard alkali.

Sometimes, non-aqueous titration method is also adopted, completing the titration in non-aqueous solvent as glacial acetic, chloroform or other solvents.

In the gravimetric methods, the dried residue or the dried complex compound formed with a suitable alkaloidal precipitant is weighed.

Volumetric determination is generally preferred to gravimetric determination because it is more rapid.

Colorimetric and spectrophotometric methods are used when a color is developed by a special reagent, and is measured in a suitable colorimeter or spectrophotometer at a certain wave length. For example, in case of solanaceous alkaloids, the color is developed by Vitali Morin reaction; for ergot alkaloids - the p- dimethylaminobenzaldehyde reagent; for morphine - the nitrous acid and for aliphatic amines – diazotization and coupling.

**Pharmacological activity**

The pharmacologic action of alkaloids varies widely: some (morphine, codeine) are analgesics and narcotics whereas others (strychnine, brucine) are central stimulants. Some (atropine) are mydriatics, whereas others (physostigmine, pilocarpine) are mytotics. Some (ephedrine) will cause a rise in blood pressure, but others (reserpine) produce a fall in excessive hypertension. The alkaloids are capable of extensive physiologic activity. Potency varies among different alkaloids.