PHYTOCHEMISTRY OF ALKALOIDS

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Introduction Two main source of medicine are, one is synthetic and another is naturally occurring. Synthetic drugs show rapid onset of action but having more side effects in comparison to naturally occurring drugs. The modern trend re back to choose natural medicine against synthetic medicine. Natural source of drugs are plants, animals, or minerals. About 8000 plants are listed in medicinal uses. In this 1800 in Ayurveda, 1100 in Shiddha, 750 in Unani, 300 in Tibetan and 4700 plants are used as traditionally ethno medicinally.

The world over the total trade of medicinal plants about 8800-lakh dollar, of which contribution of India in world trade less then 1%.
Out of this majority of plants are yet to be study photochemical, estimated of such pants to the extent of 5000 to 6000.

Phytochemistry is branch of Chemistry, which deals with the study of chemistry of plants. Where the term phytochemistry is comes out from phyta + chemistry (phyta⇒ plant).

Pharmaphytochemistry The word pharmaphytochemistry is derived from pharmakon⇒drugs, phyta⇒ plants i.e. chemistry of medicinal substance inside the plants.

Alkaloids Chemistry: Sertuerner in 1806 laid the foundation of Alkaloids Chemistry. It is the branch of Pharma Phyto Chemistry, which deals with the study of Alkaloids. He reported isolation of Morphine from opium.

What is Alkaloids: Alkaloids means Alkali likes. The Pharmacist **W.Meissner** proposed the term Alkaloids in 1819. Acc. to him "Alkaloids (alkali = base, oid=like sub) are basic nitrogenous compd. of plant origin which have complex molecular structure & many pharmacological activity."

Acc to Landenberg "*Alkaloids are defined as natural plant compounds that have a basic character and contain at least one nitrogen atom in a heterocyclic ring and having biological activities."*

Acc to characteristic features ***Alkaloids are basic nitrogenous plant origin, mostly optically active & possessing nitrogen hetero cycles as there structural units with physiological action.***

This definition not fully correct because not follow on all alkaloids for e.g.

Colchicine: Colchicine is regarded as an alkaloid although it is not Heterocyclic and is scarcely basic.

Thiamine: It is heterocyclic nitrogenous base but not as a alkaloid because it is universally distributed in living matter.

Nitrogen as side chain: Some compound is classed as in alkaloids but they do not contain nitrogen in heterocyclic ring, but contain nitrogen inside the chain e.g. ephedrine, hordenine, betanine, choline, muscarine, strychnine & tryptamine etc.

Naturally occurring open chain basic compound: These compounds have physiological activity but do not class in alkaloids e.g. Cholines, amino acid, phenylethylamines etc.

Piperine: It is neither basic character nor possessing any physiological activity but include in alkaloids.

Those compound, which fully satisfy the definitions, like physiological active, heterrocyclic basic nitrogenous ring but they do not classed in alkaloids e.g.- Thiamine, caffeine, purine, theobromine, and xanthenes.

Acc. To Pelletier 1983 "***an alkaloids is cyclic compounds containing nitrogen in negative of oxidation state. Which is of limited distribution in Living organisms"***.

"From above discussion, a conclusion can be drown quit safely that it is still difficult to define an Alkaloid"

Occurrence of Alkaloids

Alkaloids are chemically nitrogenous heterocyclic basic compound occur in nature, about15% of vascular plant & widely distributed in higher plant e.g.. - Apocynace, papaveraceae, papilanaceae, rananeulaceae, solenaceae.

They are present in the form of salts of organic acid, like acetic acid, oxalic acid, malic, lactic, tartaric, tannic, aconitic acid, few are with sugar e.g. Solanum, veratrum groups. Acc. to parts of plants:

Leaves: Nicotine

Bark: Cinchonine, Quinine.

Seeds: Strychnine, Nibidine.

Roots: Rawelfinine, Glycyrrhizin, Punarnavine I & II

NOMENCLATURE

There was no systematic nomenclature. But there are some methods for nomenclature are mention below.

1. According to their source: There are named according to the family in which they are found e.g. papavarine, punarnavin, ephedrin.

2. According to their Physiological response: There are named according to their physiological response e.g.. Morphine means God of dreams, emetine means to vomit.

3. According to there discover: There are named according to there discover e.g.. pelletierine group has been named its discoverer, P.J. Pelletier.

4. Prefixes: There are named by some prefixes are fix in nomenclature of alkaloids, e.g. epi, iso, neo, pseudo, nor- CH_3 group not attach to Nitrogen.

CLASSIFICATION

Alkaloids are classified as:

Taxonomic based: According to their family e.g. solanaceous, papilionaceous without reference their chemical type of alkaloids present & another according to genus. e.g.. ephedra, cinchona etc.

Pharmacological based: Their pharmacological activity or response. For example:

1. Analgesic alkaloids
2. Cardio active alkaloids etc. Do not have chemical similarity in their group.

Bio Synthetic based: According to this alkaloids are classified on the basis of the type precursors or building block compounds used by plants to synthesise the complex structure. e.g.. Morphine, papaverine, narcotine, tubocurarine & calchicine in phenylalanine tyrosin derived base.

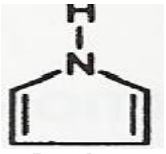
Chemical classification: This classification is universally adopted & depends on the fundamental ring structure. According to these two main groups.

1. **Non-heterocyclic Alkaloids:** In this group of alkaloid not has any one Heterocyclic ring in their structure. e.g.- Hordinine (*Hordeum vulgare*), Ephedrine(*Ephedra gerardiana)* Genateceae.

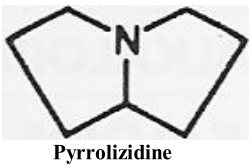
2. Heterocyclic Alkaloids: According to heterocyclic ring the alkaloids are sub divide in following: -

1) **Pyrrole-Pyrrolidine:** This type of alkaloids contains pyroll or pyrrolidine ring in their structure e.g.. Hygrines *Coca sp.*

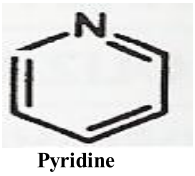
2) Pyrrolizidine: Alkaloids containing Pyrrolizidine Heterocyclic ring in their structure e.g.. - seneciphylline *Senecio sp.*



Pyrrole



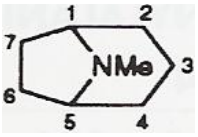
Pyrrolizidine



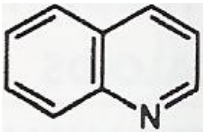
Pyridine

3) Pyridine & Piperidine: Alkaloids containing Pyridine Heterocyclic ring in their structure e.g. Nicotine, Lobaline, Piperidine, Ricinine.

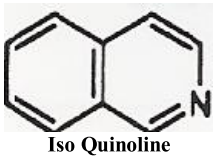
4) Piperidine (Tropane): Alkaloid containing tropone ring. e.g..- Hyoscyomine, Atropine Hyoscine- Solanceae Cocain- Coca sp.



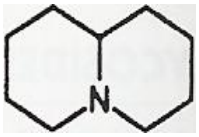
Tropane



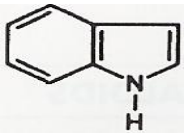
Quinoline



Iso Quinoline



Nor Lupinane



Iodole

5) Quinoline : Those Alkaloids containing quinolin ring in their structure e.g..- Quinine, Quinidine. (Cinchona bark) Cinchonine, Cinchonidine & cusparin -(cusparia bark

6) Iso Quinoline: Alkaloids containing iso quinoline ring in thier chemical structure e.g. Papavarine, Narceine Emetine & cephaline. (Cephalis sp Rubiaceae).

7) Reduced isoquinoline (Aporphine): The alkaloid contain reduced isoquinoline ring in their structure e.g. Baldine, (Peumus Baldus) (Manioniaceae)

8) Nor Lupinane: Alkaloids present in leguminoceae plants e.g. spartine, lupanine.

9) Indole Alkaloids: Alkaloids containing indole ring. e.g. Yohimbine, Aspidospermine (Apocynaceae) Vinblasine, vincristine (catheranthus roseus).

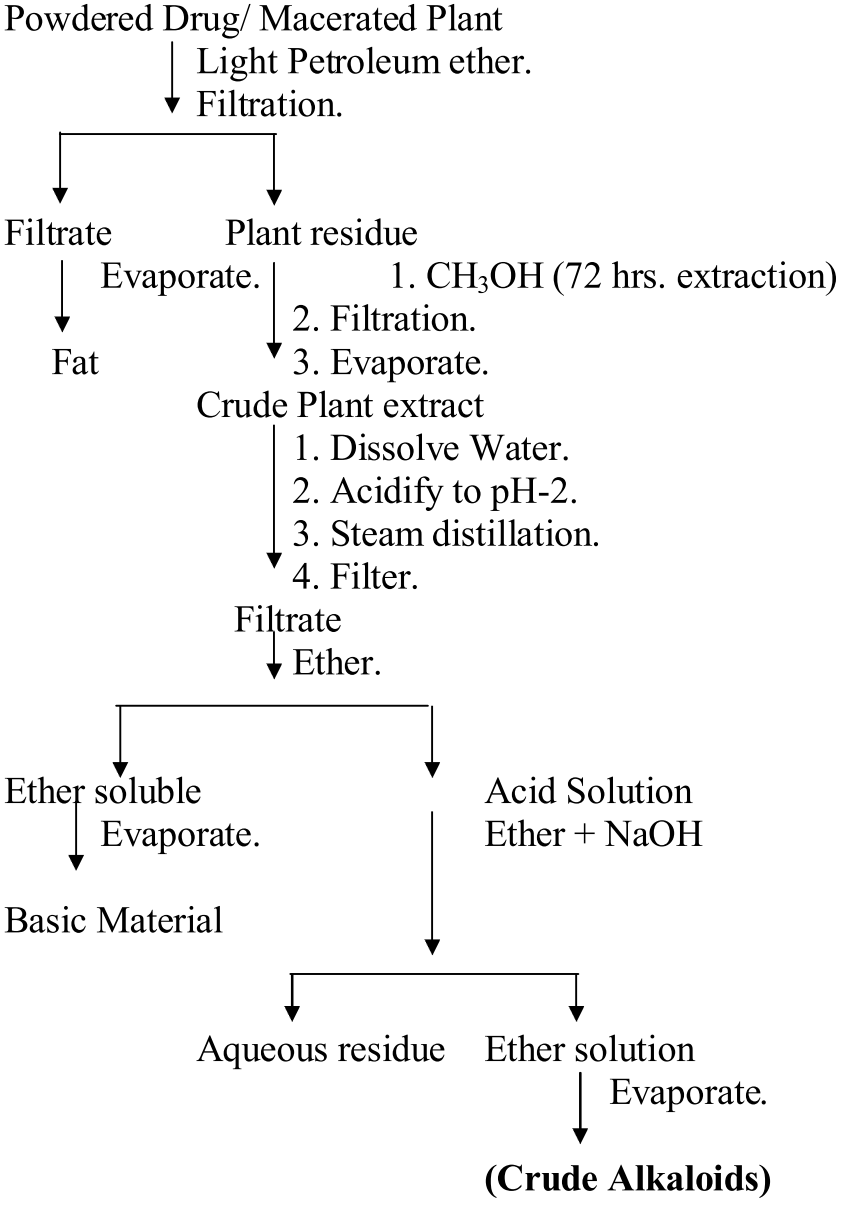
Extraction and Isolation

Purification or isolation of alkaloids from a plant is always difficult process because an alkaloids bearing plant generally contains a complex mixture of several alkaloids with glycoside organic acid also complicate the process. Following steps are involved in isolation process.

1. Detection of presence of Alkaloids: First of all confirm the presence of alkaloids in raw material or crud drugs by various reagents called Alkaloids reagents e.g.

I. Mayer (Cream Co lour) Test **II.** Marquis (Conc. HCHO) Test**. III**. Erdmann (Conc. HNO_3) Test **IV.** Hager's (Yellow Colour) Test **V.** Frohdes (Molybdic acid) Test

2. Extraction: - The plants is dried, then finally powdered and extracted with boiling methanol. The solvent is distilled off and the residue treated with inorganic acids, when the bases (alkaloids) are extracted as their soluble salts. The aqueous layer containing the salt of alkaloids and soluble plant impurities is made basic with NaOH. The insoluble alkaloids are set free precipitate out. The solid man (ppt.) so obtained is then extracted with ether when alkaloid pass into solution and impurity left behind. Flow Chart of extraction



3. **Separation of Alkaloids:** After detection of next step is separation of a relatively small percentage of alkaloids from large amount of crude drugs. E.g.- Opium contains 10% Morphine, Chincona contains 5-8 % Quinine, Belladona- 0.2% of Hyoscyamine.

The required alkaloid is separated from the mixture from fractional, crystallization, chromatography and ion exchange method.

Physical-Property

I) They are colorless, crystalline solid. Exception - Berberin (Yellow), Nicotine Coniine (liquid).

II) They are insoluble in water (exception liquid alkaloids soluble in water), soluble in organic solvent ($CHCl_3$, Ethyl alcohol ether)

III) Taste: They are bitter in taste.

IV) Optically active, Most of levo ratatory but few are -Dextro rotatory e.g. Coniine, some inactive- e.g.- papaverine.

General Methods for Structure Determination of Alkaloids

Molecular formula of majority of Alkaloids is complex so very little achievement in their elucidation of structure. During 19th Century. Now general procedures for elucidation of structure of alkaloids are adopted.

1. Molecular formula molecular weight: A pure specimen of alkaloids its empirical formula and molecular weight by elemental or combustion analysis. No. Of double bond is determined by double bond equivalent method.

2) Number of Double bond: - Number of Rings present in an alkaloids can be determine by following formula- $C_a H_b N_c O_d$

Then number of double bond present in Ring= a-b/2 + C/2 + 1

3) Functional group Analysis:

a) Functional Nature of Oxygen: - Oxygen presents in alkaloids as: - OH (Phenolic/ Alcoholic), - OCH_3 Methoxy, - $OCOCH_3$ (Acetoxy), - $OCOC_6H_5$ (Benzoxyl), -COOH (Carboxylic),- COOK (carboxylate),>C=O (Carbonyl),= C-O-O (Lactones Ring).It can be determined by infra red or organic analysis method.

(1) Hydroxyl group: - Formation of Acetate on treatment with Acetic anhydride /Acetyl chloride or benzoate on treatment with Benzyl chloride.

$R\text{-}OH + (CH_3CO)_2O \longrightarrow ROOCCH_3 + CH_3COOH$
$R\text{-}OH + CH_3COCl \longrightarrow ROOCCH_3 + HCl$
$R\text{-}OH + C_6H_5COCl \longrightarrow ROOCC_6H_5 + HCl$

If Primary amines are present in an alkaloids also give this test. Then Hydroxyl group is can be determined by zerewitinoff method.

$$R\text{-}OH + CH_3COCl \longrightarrow R\text{-}OCOCH_3 \xrightarrow{NaOH} R\text{-}OH + CH_3COONa$$

Excess of Alkali is estimated by titration with standard HCl. Number of -OH group can be calculated from the volume of Alkali used for Hydrolysis. Grignard-method.

$-OH + MeMgI \longrightarrow -OMgI + CH_4$
$-NH + MeMgI \longrightarrow -NMgI + CH_4$

1- OH= 1>NH group= 22.4 liter of N_2 STP

1^0, 2^0-OH, 3^0 - OH group. By oxidation or dehydration to unsaturated compound. If OH group is phenolic, and then Alkaloid is -

- Soluble in NaOH
- Re precipitated by CO_2
- Giving coloration with $FeCl_3$

(2) Carboxylic group: - soluble in aqueous solution sodium carbonate or ammonia on treat with alcohol form ester.

Number of -COOH group can be determined by volumetrically by titration against a standard. $Ba(OH)_2$ solution by using phenolphthalein as an indicator.

(3) Oxo-group: - On treatment with Hydroxylamine. Semicanbezide, phenylhydrazide ,oxime ,semicarbazone phenyl Hydrazine

$>C=O + H_2NOH \longrightarrow >C=N\text{-}OH$

$>C=O + H_2NNHCONH_2 \longrightarrow >C=NNHCONH_2$

The distinction between aldehyde and ketone is done by oxidation or reduction, also by NMR, IR, and UV techniques.

(4) Methoxyle group: - BY Zeisel determination method. When methoxy group present in a alkaloids treated with HI at 126^0C perform methyl iodide which can treated further with silver nitrites to perform silver iodide precipitate. Which estimated gravimetrically e.g.. Papavarine.

$$C_{20}H_{40}O_4N \xrightarrow{HI} 4MeI \xrightarrow{AgNO_3} 4AgI \quad \text{(estimated gravimetrically)}$$

(5) Methylenedioxy group: - On heated with concentrated with HCL or H2SO4 to form formaldehyde and further formation of dime done derivative, which estimated gravimetrically.

$$\text{-}OCH_2O\text{-} \xrightarrow{\text{heat/HCl/H2SO4}} \textbf{HCHO} \longrightarrow \textbf{dime done derivative}$$

(Estimated gravimetrically)

b) Ester Amide lacton & Lactum group: These groups are identified by the estimation of product.

$>CONH_2 + NaOH \xrightarrow{Heat} \text{-}COONa + NH_3$

$>COOR + NaOH \xrightarrow{Heat} \text{-}COONa + ROH$

c) Nature of Nitrogen

Majority of nitrogen presence in alkaloids are secondary and tertiary: If tertiary when treated with H_2O_2 (50%) form.

$\bar{=}N + H_2O_2 \longrightarrow \equiv N\rightarrow O + H_2O$

If alkaloids react with one molecule of methyl-iodide to form N-methyl derivative, it means secondary e.g.

$(C_8H_{16}O_4)\ NH + CH_3I \longrightarrow (C_8H_{16}O_4)\ NCH_3 + HI$

General reaction of alkaloids with acetic-anhydride, methyl-iodide, and nitrous acid are often showing the nature of nitrogen

If react with one molecule of methyl-iodide to form crystalline quaternary salt this indicates that nitrogen is tertiary e.g.

$N{=}(C_{10}H_{24}){=}N + 2CH_3I \longrightarrow IH_3C^{+}N{=}(C_{10}H_{24}){=}N^{+}CH_3I^{-}$

N-methyl group:

On distillation with soda lime if methylamine is produce show the presence of N-methyl group for e.g.

$$(C_{10}H_{14}N){=}N\text{-}CH_3 \xrightarrow{\text{Soda-lime}} CH_3NH_2$$

Nicotine CaO

Determination of Number of CH_3-groups Attached to N-atom: By Herzig-Mayer's method when a sample is treated with hydrogen iodide at 150^0-300^0C and than treated silver nitrate, which form silver iodide, which estimated gravimetrically.

$$\text{N- methylamine} + HI \xrightarrow{150^0\text{-}300^0} >NH + MeI \xrightarrow{AgNO3} AgI$$

Estimated gravimetrically

d) Estimation of C-Me Group by Kuhn –Roth Oxidation: When treated with $K_2Cr_2O_7$ or H_2SO_4 an acid is produce, which estimated gravimetrically.

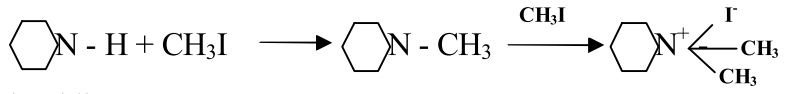
$$\text{-}\overset{|}{\underset{|}{C}}\text{-Me} \xrightarrow{K_2Cr_2O_7 \text{ or } H_2SO_4} MeCOOH$$

Estimated gravimetrically

e) Degradation of Alkaloids: For discovering the structural system which incorporate these substituents groups & is tackled by degradation of the molecules by following methods:

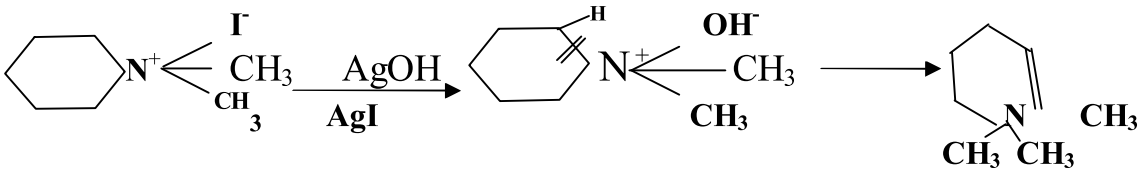
1) Hoffmann's exhaustive methylation: - This is a composite reaction of alkaloid (Heterocyclic amines). This involves following steps:

a) The alkaloid is treated with excess of CH_3I to form quartertionarey - ammoniumiodide.

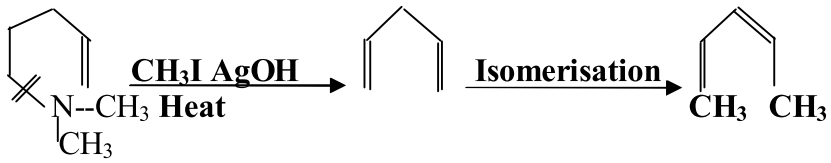


Piperidine

b) 4^0-ammonium iodide is converted to the hydroxide and heated. The -OH of hydroxide extracts hydrogen atom from beta position and eliminate a water molecule and also the ring is cleaved at the N-atom to give an open chain 3^0-amine.

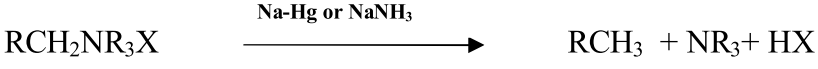


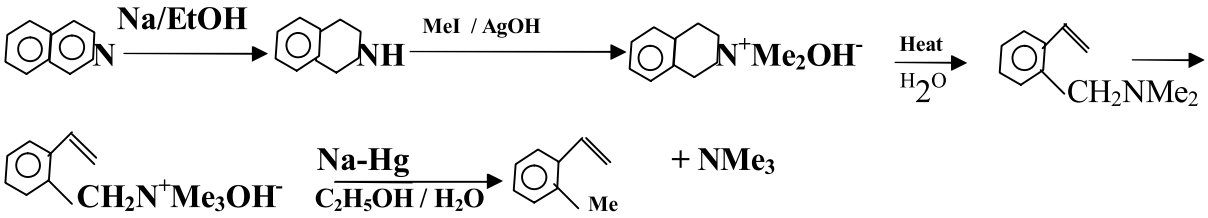
c) The step I^{st} and II^{nd} are repeated when a second cleavage at the N-atom given an unsaturated hydrocarbon which isomerase's to conjugated derivative.



The exhaustive methylation of an alkaloid is an important method for the investigation of the nature of the C-skeleton in the heterocyclic system.

2) Emde Degradation Method: If alkaloids do not contain beta Hydrogen atom then Hoffmann exhaustive degradation method is failed. In such cases Emde degradation are applied, in this final step involve the reductive cleavage of quartertionarey ammonium salt either by Na-Hg or $NaNH_3$ or by catalytic hydrogenation e.g.





2) Von Braun's Method: - This method is of two types:

A. Tertiary amine, which contains at least one-alkyl substituents, is treated with cyanogen bromide. The results in cleavage of an alkyls nitrogen bond to give an alkyl halide and a substituted Cyanamid.

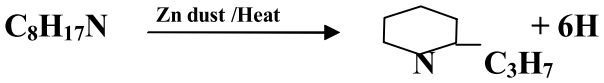
$R_3N + CN - Br \longrightarrow R - Br + R_2N - CN$

This method is often applicable to such compounds that do not respond to Hofmann's method e.g. – Cocaine

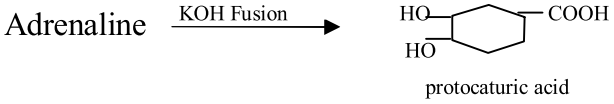
B. Secondary cyclic Amine is treated with Benzoyle chloride in presence of NaOH to yield the Benzoyle derivative which on treatment with phosphorus followed by distillation under reduced pressure yield di Halo compund.

$$R_2NH + C_6H_5COCl \xrightarrow{NaOH} R_2N\text{-}COC_6H_5 \xrightarrow{Br_2} R_2NCBr_2C_6H_5 \xrightarrow{Distillation} Br\text{-}RRBr + C_6H_5CN$$

4) Reductive degradation & Zinc dust distillation: In some case ring may open by Heating up to 300^0C with Hydroiogic acid e.g. Coniine



5) Alkali fusion: This is very drastic method, which is often employed to break down the complex of complex alkaloids molecule to simpler fragment. The nature of fragment, which will give information type of nucleus present in alkaloid molecule e.g. Adrenaline, Papaverine, Cinchonine



6) Oxidation: - This method gives quite information about the structure of alkaloids by varying the strength of the oxidizing agents it is possible to obtain a variety of product e.g.

A. Mild oxidation- H_2O_2, HI in ethanolic solution & alkaline potassium ferric-cyanide.

B. Moderate oxidation: $KMnO_4$ (Alkali) $Cr2O_3$ (Acetic Acid)

C. Vigorous oxidation: $K_2Cr_2O_7$, (H_2SO_4), $Cr2O_3$ (H_2SO_4), conc. HNO_3, MnO_2 (H_2SO_4)

$$C_{10}H_{14}N_2 \xrightarrow{\mathbf{K_2Cr_2O_7\ (H_2SO_4)}} C_6H_5COOH$$

7) Dehydrogenation: - When Alkaloid is distilled with catalyst such as sulphur, selenium or palladium dehydrogenation takes place to form relatively simple & easily recognizable product which provide the clue to gross skeleton.

4) Synthesis: - The structure of the alkaloids arrived at by the exclusive analytical evidence based on going method is only tentative. The final conformation of the structure must be done by the unambiguous synthesis.

Physical method

These following physical methods are applied to elucidate the structure of alkaloids:

- U.V. Spectroscopy
- IR Spectroscopy
- Nuclear Magnetic resonance spectroscopy
- Mass spectroscopy
- Optical rotatory dispersion & circular dichroism.
- Conformational Analysis
- X-Ray diffraction

Biosynthesis of Alkaloids

As more and more structure of alkaloids were elucidated, it become increasingly probable that the precursor in the biosynthesis of many alkaloids were amino acids and amino-aldehyde or amines derived from them, **Woodward** 1948 proposed a biosynthesis of strychnine.

Because the great diversity of structure of alkaloids, it not possible to develop only one hypothesis of biosynthesis of alkaloids. Thus many pathways have been proposed, each one accounting for the biosynthesis of a number of alkaloids of related structure.

The most common aminoacids that act as precursor in biosynthesis of alkaloids are:

i. Ornithine **$H_2N\ (CH_2)\ CHNH_2COOH$**

ii Lysine **$H_2N\ (CH_2)_3\ CHNH_2COOH$**

iii. Phenylalanine (R=H)

$RC_6H_5CH_2CH(NH_2)COOH$

iv. Tyrosine (R=OH)

vi. Methionine **$MeSCH_2CH_2CH(NH_2)COOH$**

vii Trytophen

Some common reactions are:

Decarboxylation : formation of amine

$RCH\ (NH_2)\ COOH \longrightarrow RCH_2NH_2 + CO_2$

Oxidation: formation of aldehyde

$RCH\ (NH_2)\ COOH \longrightarrow RCOCOOH \longrightarrow RCHO$

Shiff base formation:

$R_1CHO + R_2NH_2 \longrightarrow R_1CH{=}NR_2$

Chemical test of alkaloids

1. Mayer's Test: Specimen with Mayer's reagent give Cream or pale yellow ppt.

2. Dragendroff Reagent Test: Specimen with Dragendroff Reagent give orange ppt.

3. Wagners Test: Specimen with Wagner's Reagent give brown or reddish brown ppt.

4. Hager's Test: Specimen with Hager's reagent give yellow ppt. (Special Type)

5. Amonium Rinker Test: Specimen with Ammonium Rinket solutions with HCL give flocculent pink ppt.

Functions

Alkaloids functions -

- As reservoir of **nitrogen**
- As reservoir for **protein synthesis**
- As **detoxicating** agents
- As **toxicating** agents
- As **harm one** for many activity of plant.

Alkaloids have many **physiologically biological** and **pharmacological** properties

Conclusion

It can be concluded that:

1. Alkaloids are **naturally occurring heterocyclic complex** compounds.
2. Alkaloids have **indefinite** definition.
3. Alkaloids have mainly **nitrogen in heteroatom**.
4. Alkaloids have **complex molecular structure**.
5. Alkaloids are **bio & physiologically** and **pharmacologically** active.

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