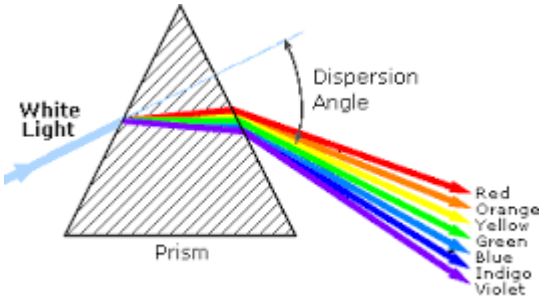
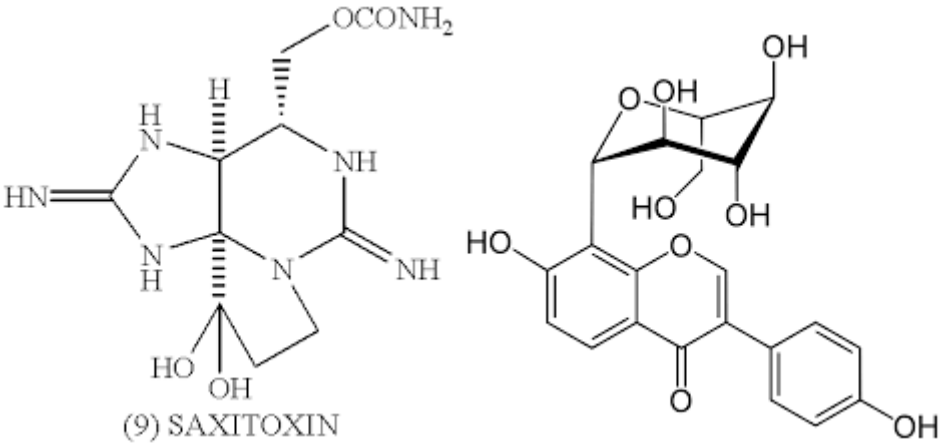
MScCH-08

Vardhman Mahaveer Open University, Kota





(9) SAXITOXIN

Natural Products, Heterocycles, Biogenesis and Spectroscopy

MScCH-08

Vardhman Mahaveer Open University, Kota

Natural Products, Heterocycles, Biogenesis and Spectroscopy

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Preface

The present book entitled “Natural Products, Heterocycles, Biogenesis andSpectroscopy ” has been designed so as to cover the unit-wise syllabus of MScCH-08 course for M.Sc. Chemistry (Final) students of Vardhman Mahaveer Open University, Kota. The basic principles and theory have been explained in simple, concise and lucid manner. Adequate examples, diagrammes , photographs and self-learning exercises have also been included to enable the students to grasp the subject easily. The unit writers have consulted various standard books and internet as their reference on the subject and they are thankful to the authors of these reference books.

Unit - 1

Alkaloids

Structure of unit

1.1 Objectives

The unit alkaloids is useful for the students to understand the importance of natural products, because alkaloids are obtained from natural sources (e.g. extracted from plants). This unit covers the following points.

- Definition
- Physiological effects of alkaloids
- Structure of important alkaloids
- Stereochemical aspects of alkaloids
- Use in daily life

1.2 Introduction

The word alkaloids refered as –

Alkal-alkali (basic), oid-like (same).

The alkaloids are isolated from plants and word alkaloid first time introduced by *W. meissner*. In early year no correct definition has been given for alkaloids, so in views of scientists many definition has suggested.

- According to Koings alkaloids are pyridine ring containing organic bases.
- According to Ladenburg alkaloids are organic bases having at least one heteroatom in ring.
- In keeping of above definition alkaloids may be defined as "alkaloids are optically active nitrogen containing heterocycles which possess effective physiological properties".

1.3 Nomenclature of alkaloids:

Alkaloids have complex molecular structure. So systematic nomenclature not refered for alkaloids. So following methods are proposed for nomenclature.

Source basis nomenclature : Name of alkaloids will be according to source (plant) from which they are obtained.

Source (Plant)	Name of alkaloid
Papaver somniferum	Papavarine
Berberis vulgaris L.	Berberine

Substituent basis nomenclature : during nomenclature of alkaloids such prefixes like Iso, Neo, Nor, Pseudo etc. are used for e.g. Nor prefix is used for the absence of methyl substituent.

1.4 Physiological action of alkaloids

Alkaloids are obtained from natural sources. Mostly alkaloids are isolated from plants. Alkaloids can perform one/more function in plant metabolism process which are described as follow :

a) Alkaloids may be act as food reservior for protein synthesis.
b) Many alkaloids have poisonous effect, so it is useful for plant protection from insects and herbivores.

c) Alkaloids can be used as nitrogen suppler.
d) Accumulation of alkaloids in plants show toxic effect and due to this plant can be damage.

Production of alkaloids in plant can affect plant metabolic process, plant growth and reproductive process.

1.5 Occurrence of alkaloids:

As we have already discussed that alkaloids are isolated from natural plant and found in bark, leaves, seeds, roots etc. Alkaloids are nitrogen containing heterogeneous substances which are found in families of dicotyledons e.g. Solanaceae, Rutaceae, Rubiaceae, Papilionaceae, Papaveraceae, Apocyanaceae etc.

Due to presence of nitrogen, alkaloids are basic in nature. So mostly alkaloids are found in their salt from as salt of succinic acid, oxalic acid, tannic acid, tartonic acid etc. Some alkaloids are found as their glycoside derivatives of sugar eg. piperine found as ester derivative atropine, cocaine etc.

1.6 Isolation of alkaloids:

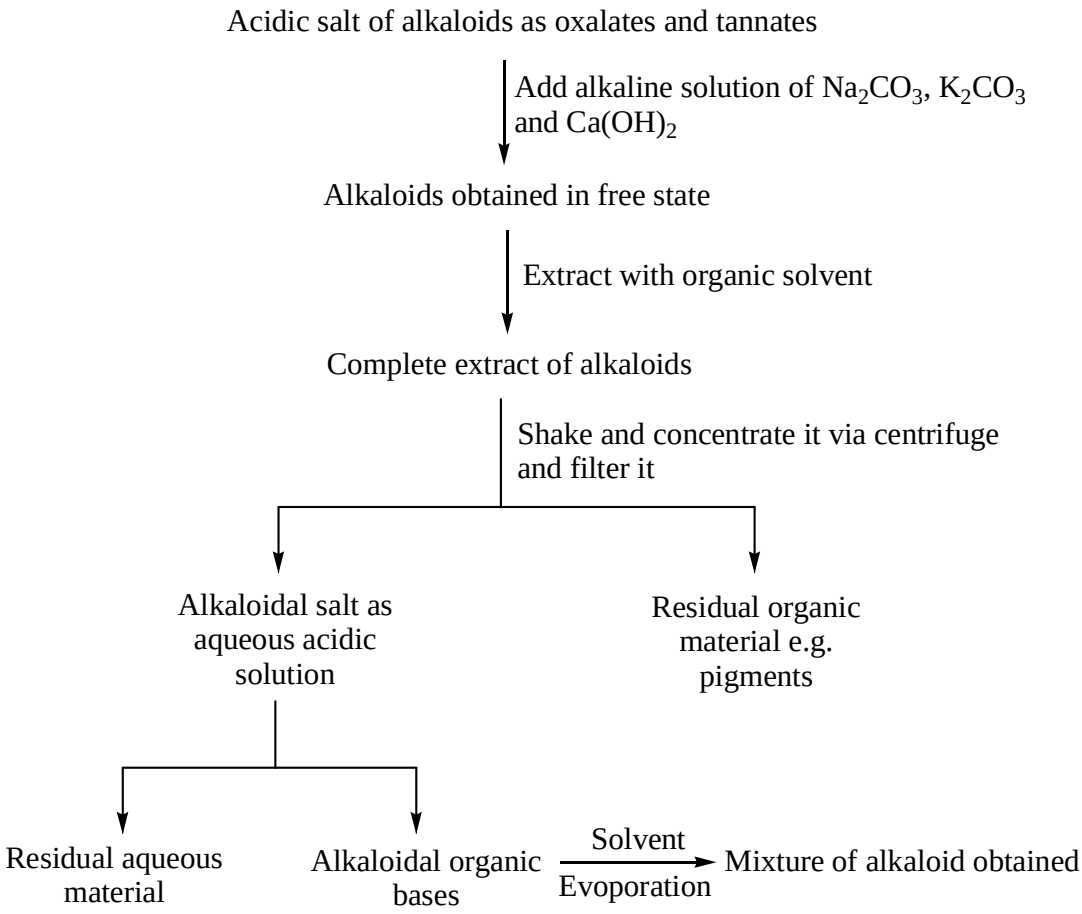
Isolation of alkaloids is a very complicated process because of a plant contains a mixture of alkaloids. So isolation of alkaloids have following procedure/methods.

Method-I:

(I) At first by using alkaloidal precipitating reagents e.g. Mayer's reagent (potassium mercuric iodide), Wegner's reagent (Iodine dissolve in potassium iodide) the presence of alkaloids is confirmed in plant. Precipitation of alkaloids can be done by using of colour reagents e.g. formaldehyde (Marquis reagent) and nitric acid (Erdman's reagent).

(II) When the presence of alkaloids in plant is confirmed then separation technique is applied as small percentage by dry weight basis from plant material e.g. Atropa belladonna contains 0.2% hyocyamine, Rauwolfia Serpentina root contains 0.1-0.2% reserpine, opium contains 10% morphine.

(III) Alkaloids are separated and purified from crude extract in the last step. The alkaloids have a heteroatom so these are soluble in acidic/alkaline solution but not in organic solvent. It is the basis of Stas-otto process. There are three basis of solvent dependent method involving Stas-otto process.



Method-II:

This method involve the extraction of powdered material with organic solvents like methanol, ethanol etc. and then repeat the steps of method-I.

Method (II) have some advantage over method (I).

(a) In this method alkali not used so, less number of extraction is applied then method (I).
(b) It is less hazardous for health.

Method III:

Water and alcohol are used for the extraction of alkaloids from plant. Other waste material is removed from extract. The alkaloids are precipitated by addition of alkali and separated by filteration of extract.

1.7 General methods of structure determination / elucidation:

(I) When a pure sample of alkaloid is obtained then applied qualitative analysis (presence of C, H, N, O) and followed by quantitative analysis for the determination of empirical formula. Then its molecular weight is calculated and followed by determination of molecular formula.

The mostly alkaloids are unsaturated so, no. of double bond equivalents (DBE) is calculated for alkaloids as follow:

$$= \frac{\text{no. of (H) atom in saturated alkane - No. of (H) atom in given compound}}{2}$$

e.g. Benzene (C_6H_6) Complete saturated alkane (C_6H_{14})

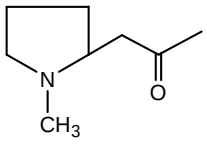
$$= \frac{14-6}{2} = \frac{8}{2} = 4$$

Note: DBE may be equivalent to double bond or ring.

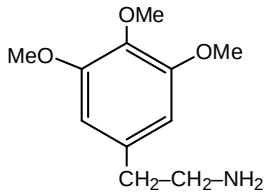
If the alkaloids contains other atoms like N, O then following formula is used for calculation of D.B.E.

$$a - \frac{1}{2}b + \frac{1}{2}c + 1$$

C H N O

Name of alkaloid	Molecular formula	Molecular structure	D.B.E.
1. Hygrine	$C_8H_{15}NO$		2 — 1 ring, 1-oxo group

2. Mescaline $C_{11}H_{17}NO_3$



4 → 3-double bond
4 → 1-ring

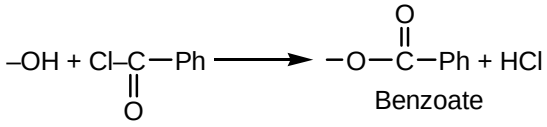
(II) When the alkaloids is optically active then its specific rotation is measured as.

(+) $\rightarrow$ dextrorotatory (d)

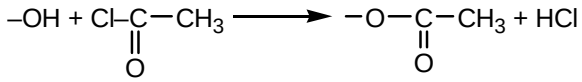
(–) $\rightarrow$ Leavorotatory (l)

(III) Mostly alkaloids have oxygen. So it can be present in following functional group. e.g. Hydroxyl group (alcoholic or phenolic), carboxylic acid, carbonyl and ether group. So presence of these functional group explained as –

(i) Oxygen as hydroxyl group: Presence of oxygen in hydroxyl group is confirmed by its derivative formation as ester e.g. benzoate, acetate etc.



Benzoate



Acetate

If the presence of hydroxyl group is confirmed then problem is that it is phenolic or alcoholic. If the hydroxyl group is soluble in NaOH and reprecipitated by carbondioxide, if colouration appear with $FeCl_3$ then hydroxyl group will be phenolic in nature. If the hydroxyl group does not behave as phenolic then it will be alcoholic in nature. The number of hydroxyl group is confirmed by acetylation and the estimation of active hydrogen is done by Zerewitinoff method by using of methyl magnesium bromide.

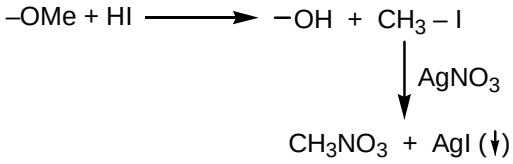
(ii) Oxygen as carbonyl group: Carbonyl group may be ascertained by its derivative formation e.g. oxime, semicarbazone and phenylhydrazone.

Carbonyl group may be aldehydic or ketonic, so it is confirmed by spectral technique e.g. UV, IR, NMR etc. and chemical process (reduction and oxidation).

(iii) Oxygen as carboxylic group:

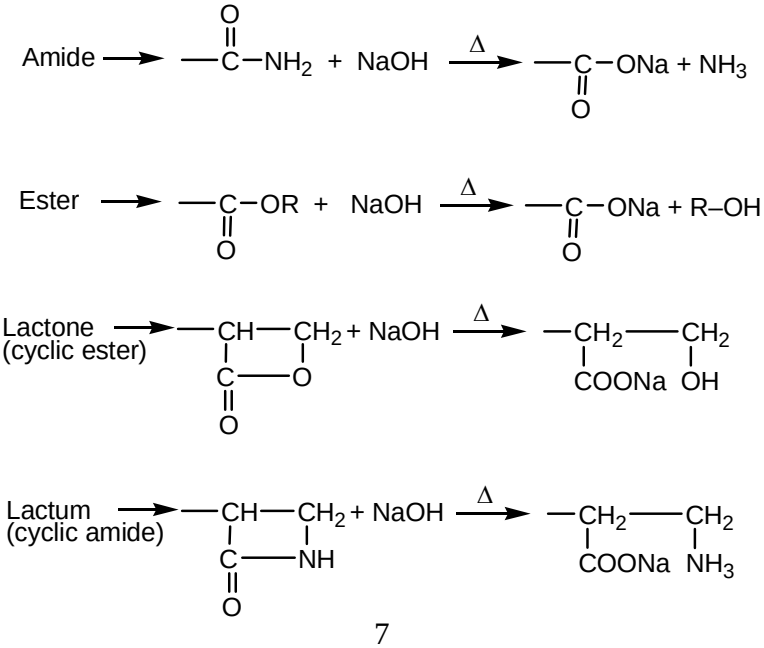
If the alkaloid is soluble in aqueous sodium carbonate or ammonia solution then it suggest the presence of carboxylic group. Presence of carboxylic group is also confirmed by its ester formation. When presence of carboxylic group is confirmed then its number is confirmed volumetrically by titration against barium hydroxide by using of phenolphthalein as an indicator.

(iv) Oxygen as methoxy group: Presence and number of methoxyl group is determined by Zeisel method which have following procedure. The known amount of alkaloid is heated with HI at its b.p. then methoxyl group converted in to methyl iodide which is absorbed by ethanolic solution of silver nitrate then precipitate of (AgI) setteled, filter, dried and weight it.



(v) Oxygen as methylenedioxyl group (–O–CH_2–O–): Its presence is confirmed by the formation of formaldehyde which is formed by the reaction of alkaloid with the hydrochloric acid or sulphuric acid.

(vi) The following functional groups are identified by their product when the alkaloid undergoes acidic or alkali hydrolysis.



Nature of functional nitrogen in alkaloids:

Nature of amine (primary, secondary and tertiary) can be confirmed by following points:

(a) Reaction with methyl iodide: Amine (primary, secondary and tertiary) react with excess amount of methyl iodide to form quartnary ammonium iodide salt. The primary amine react with three equivalent, secondary amine react with two equivalent and tertiary amine react with one equivalent of methyl iodide.

(b) Reaction with aqueous potassium hydroxide: It confirm the nature and number of alkyl groups which are attached with nitrogen atom. The product forms as methyl amine, dimethyl amine and trimethyl amine which confirm the nature of nitrogen.

(c) If the alkaloid have N-methyl group then it gives methyl amine on reaction with Sodalime.

(d) The hydrolysis of alkaloid having an amide, lactum is also useful in the determination of functional nature of nitrogen atom.

1.8 Methods of degradation of alkaloids:

Following methods are suggested for degradation of alkaloids.

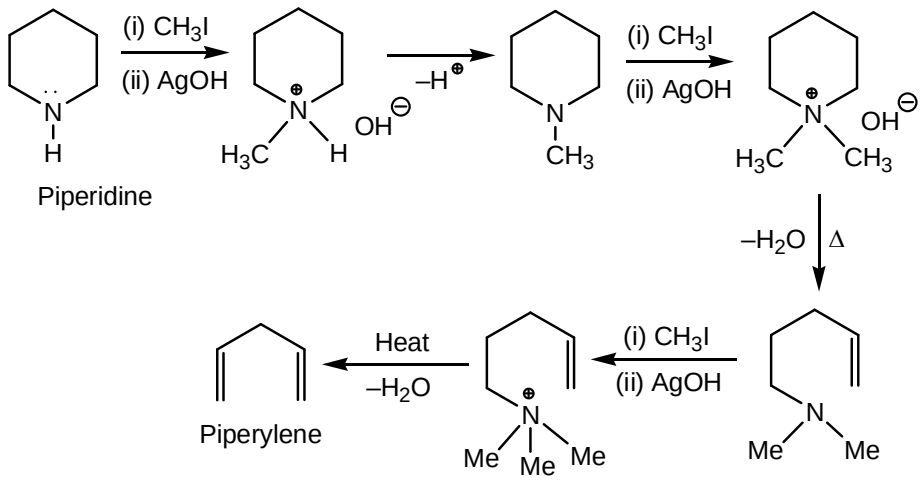
(I) Hoffmann exhaustive methylation method
(II) Emde's degradation
(III) Von Braun's degradation
(IV) Reduction degradation with zinc dust
(V) Oxidation

(I) Hoffmann exhaustive methylation method:

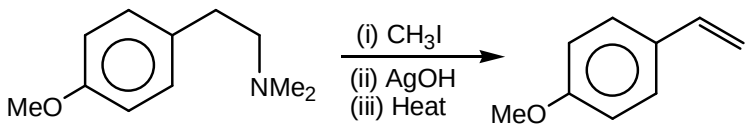
This method involve the reaction of alkaloid having nitrogen atom (amine with excess amount of methyl iodide to form quartnary ammonium iodide salt, which is undergoes pyrolysis at 200°C to give an olefin via β-elimination.

The above process will be continue until the nitrogen atom is eliminated from heterocyclic ring.

eg. (a)



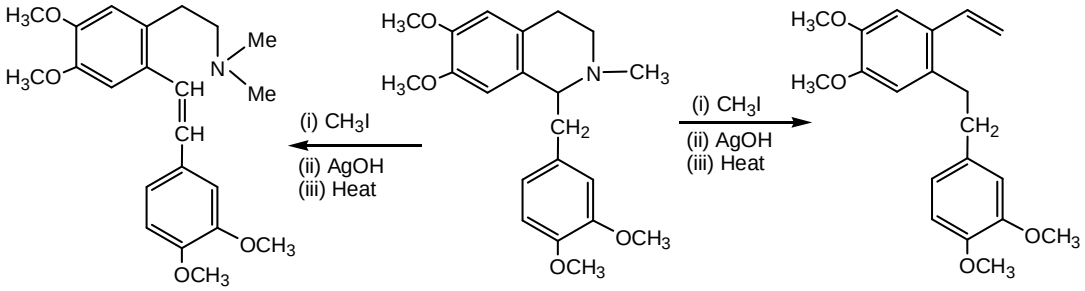
(b)



Hordenine methyl ether

p-methoxy styrene

(c) If the nitrogen atom is part of ring and having two β-hydrogen then the β-hydrogen will eliminate which gives more conjugated olefin. In given e.g. (A) is major product.

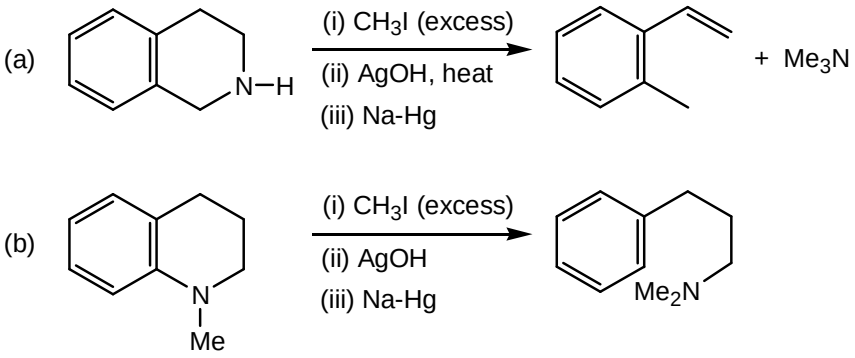


Note: If the nitrogen atom present in unsaturated ring or takes part in conjugation or β-hydrogen is absent then Hoffmann's exhaustive methylation fails. So, the Emde's degradation followed.

(II) Emde's degradation:

It involve the degradation of quartnary salt with sodium amalgam, sodium liq. NH_3 or by catalytic hydrogenation.

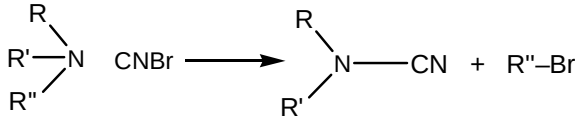
e.g.



(III) Von Braun's method:

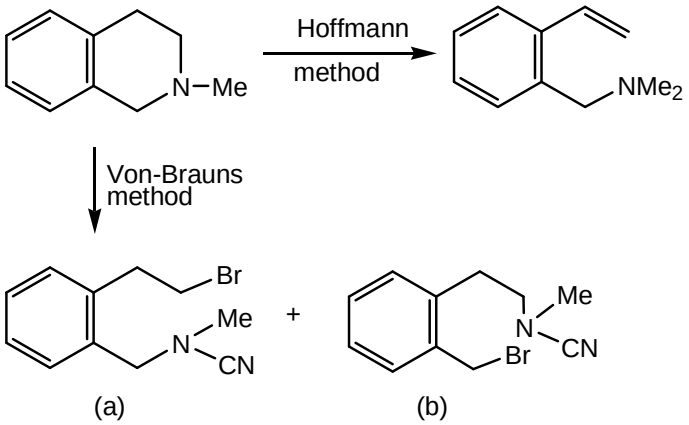
This method can be studied in two parts :

Part-A: It involve substitution reaction of tertiary amine (cyclic or acyclic) with cyanogen bromide to give alkyl bromide and cynamide.



Note: If alkyl groups on nitrogen atom are different then smaller group goes with halide.

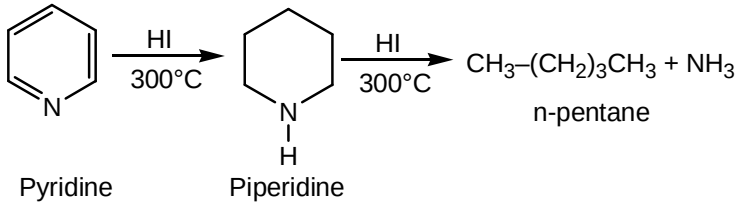
e.g.

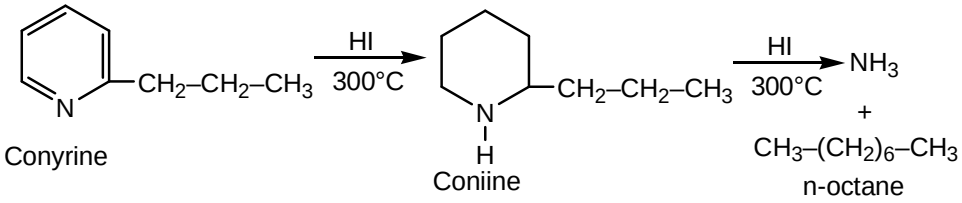


Product (b) formed as major product because it is formed by formation by benzyl carbocation.

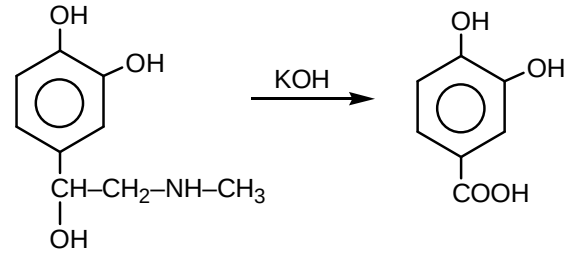
(IV) Reductive degradation with Zn-dust and HI:

(i) Reaction with HI: It involve reduction followed by ring opening.

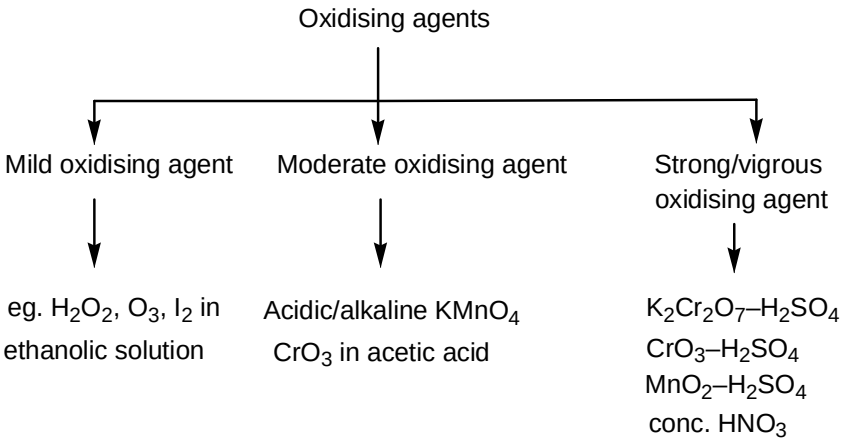




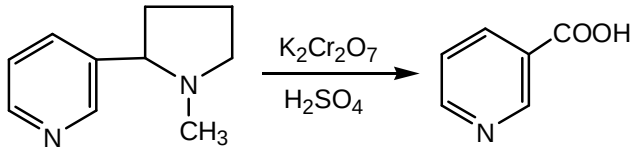
(ii) Zinc-duct distillation: It causes dehydrogenation and deoxygenation to generate unsaturation.



(V) Oxidation: It is the useful method for determination of alkaloid structure. Various products are formed which are depend on the nature of oxidizing agents.



e.g. Nicotine undergoes oxidation with $K_2Cr_2O_7$-H_2SO_4 then it gives nicotinic acid, it confirm that nicotine have pyridine ring.



Nicotine Nicotinic acid

Now a days some spectral techniques also useful in the determination of alkaloid structure which are listed below:

(a) UV-spectroscopy
(b) IR-spectroscopy
(c) NMR-spectroscopy
(d) Mass-spectrometry
(e) Optical rotatory dispersion (ORD) and circular dichroism (CD)

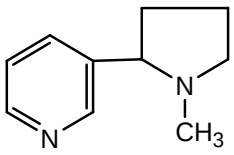
1.9 Detailed study of some alkaloids:

1.9.1 Nicotine:

It is a pyridine-pyrrolidine alkaloid. The name nicotine given in the honour of J. Nicot. Nicotine occurs in tobacco plant (*Nicotiana tobacum*) and other Nicotiana species in the form of salt of acid. Tobacco is the commercial source of nicotine and mainly found in the leaves of plant.

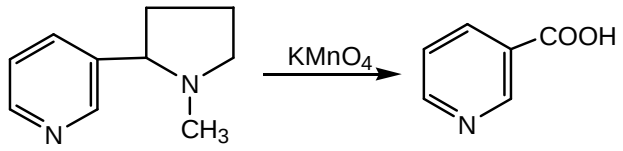
(I) Molecular formula: $C_{10}H_{14}N_2$

(II) Molecular structure:



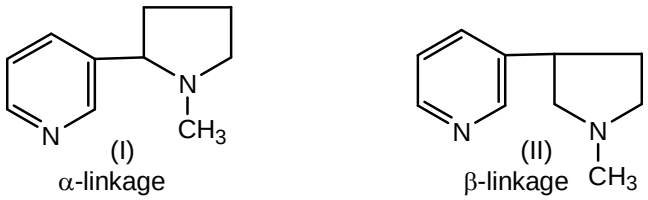
(III) Presence of two tertiary N-atom: It is confirmed by the reaction of Nicotine with methyl iodine, that it will react with two equivalent of methyl iodide.

(IV) If the oxidation of nicotine is done by $KMnO_4$ or HNO_3 then it yields nicotinic acid (pyridine-3-carboxylic acid).



Nicotine Nicotinic acid

(V) Attachment of pyrrolidine ring with pyridine nucleus: Pyrrolidine ring in nicotine which is attached with pyridine ring can be attached in two ways as follows:

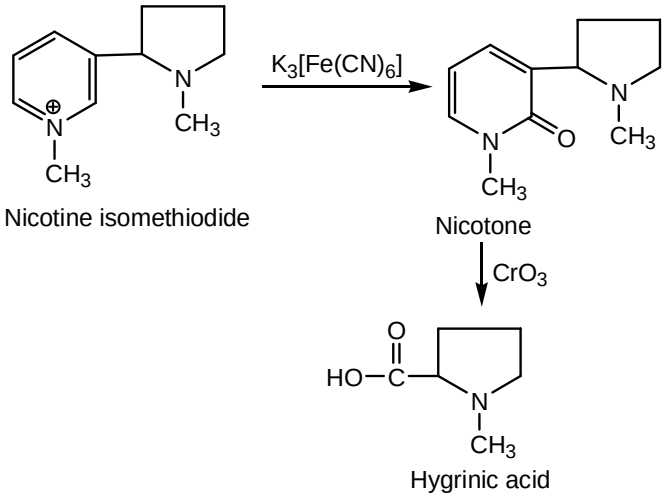


There are such chemical reaction which are support and attachement of pyrrolidine ring.

(a) When the nicotine zinc chloride is distilled then yields pyridine, pyrrole and methylamine. This reaction confirm that side chain is pyrrole ring derivative.

(b) Secondary if the nicotine is heated with HI at 150°C then methyl iodide is obtained. This confirm that nicotine side chain possess N-methyl group.

(c) When the nicotine isomethiodide is undergoes reaction with CrO_3 or $K_3[Fe(CN)_6]$ then it gives hygrinic acid.



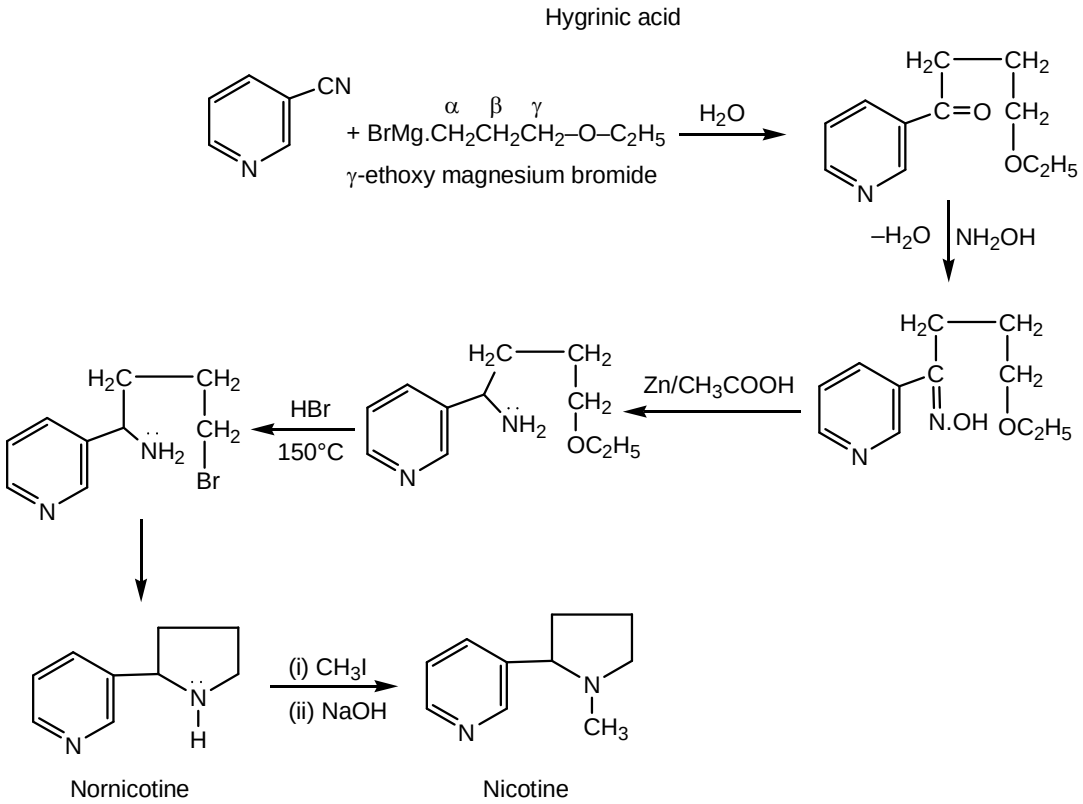
Nicotine isomethiodide

Nicotone

Hygrinic acid

Further the correct structure of nicotine is supported by the synthesis.

(I) Craig's synthesis:

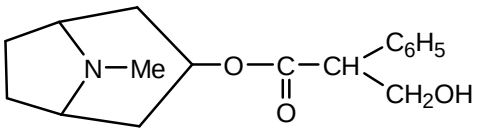


1.9.2 Atropine:

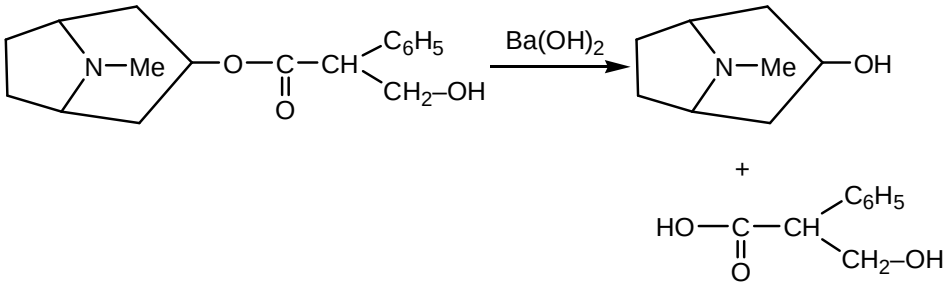
Atropine is known as Solanaceous alkaloid. Atropine is mostly found in *Atropa belladonna, Datura stramonium,* and other plants of Solanaceae family with the hyocyamine. Atropine is optically active that is exist in form of (±) – hyocyamine.

(I) Molecular formula: $C_{17}H_{23}NO_3$

(II) Molecular structure:



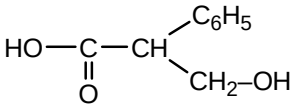
When the atropine is treated with barium hydroxide solution then it yields racemic (±) tropic acid and optically inactive tropine alcohol (tropanol). So, we can say that atropine is a tropane ester of tropic acid.



(±) - Tropic acid

For the structure elucidation of atropine we have to study separately tropic acid and tropine.

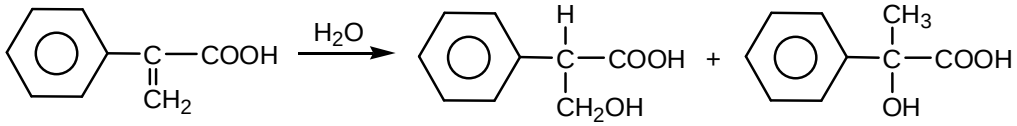
Tropic acid: Molecular formula: $C_9H_{10}O_3$

Molecular structure: 

Following points should be studied for the explanation of structure of tropic acid:

(a) **It has monocarboxylic acid:** Tropic acid react with one equivalent of alkali and forms monoester with alcohol.

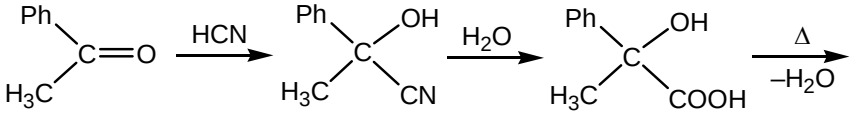
(b) **It has one primary hydroxyl group:** Tropic acid react with one equivalent of acetic acid to form monoacetate and with one equivalent of benzoic acid to give monobenzoate. When the tropc acid is heated then removal of water molecule and yields an unsaturated carboxylic acid (atropic acid). When the atropic acid react with H_2O then it gives two products.

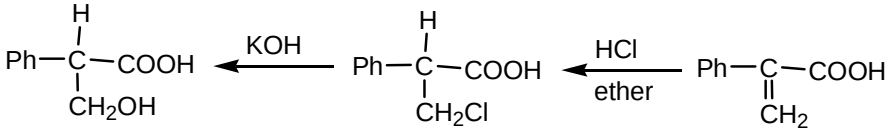


Atropic acid (a) (b)

So, the correct structure of atropic acid is confirmed by its synthesis.

(I) **Mackenzie and Wood synthesis:**

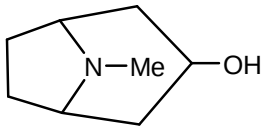




Tropic acid

Tropine (Tropanol): Molecular formula: $C_8H_{15}NO$

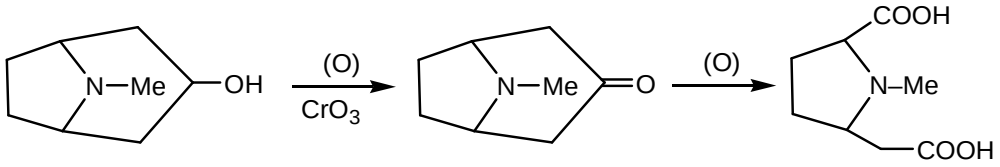
Molecular structure:



Following points should be studied for structure determination of tropine.

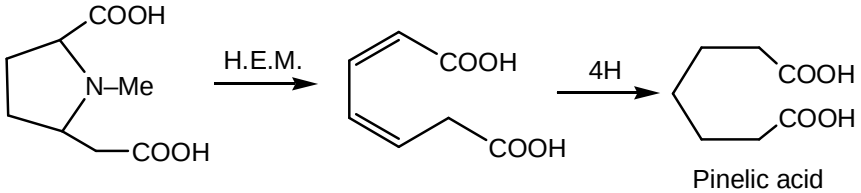
(a) **Presence of tertiary N-atom:** Tropine react with one equyivalent of methyl iodide to give quartnary ammonium Iodide salt.

(b) **Presence of alcoholic group:** Tropine react with one equivalent of acetic acid to give monoacetate derivative. The secondary nature of (OH) group is confirmed by that tropine yields tropinone on oxidation which further undergoes oxidation to give tropinic acid (i.e. dicarboxylic acid).



Tropanol Tropinone Tropinic acid

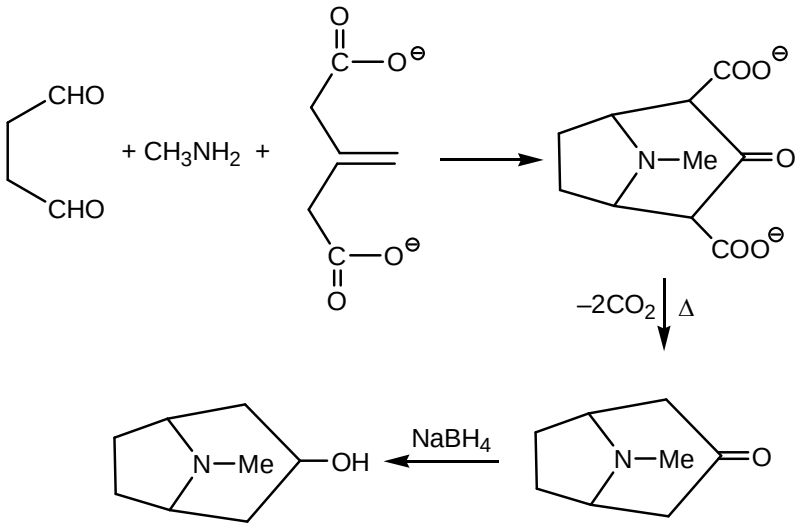
When the tropinic acid undergoes Hoffmann exhaustive methylation then it yields pimelic acid.



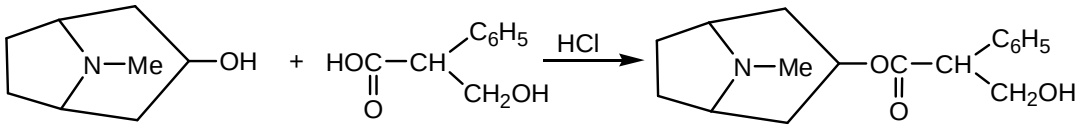
Pinelic acid

The original structure of tropine is confirmed by its synthesis.

Robinson synthesis:

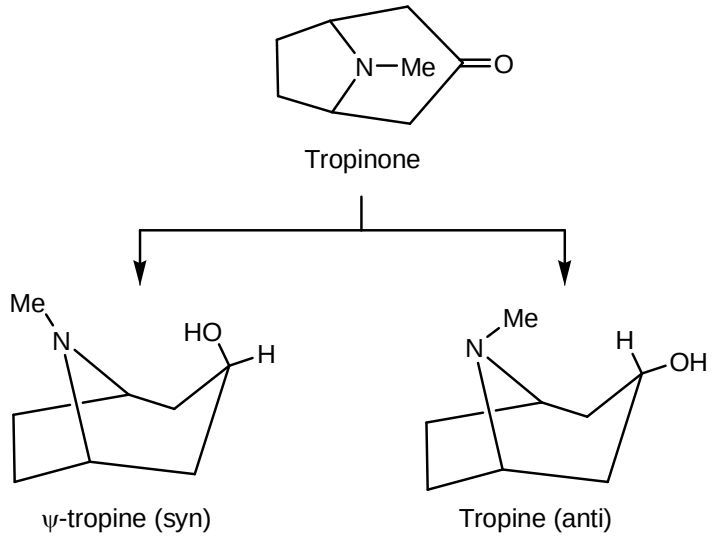


Finally tropic acid and tropanol are heated in presence of HCl to give atropine.



Tropanol Tropic acid Atropine

Streochemistry of tropine: When the tropinone undergoes reduction then it yields mixture of two alcohols (a) Tropine (b) ψ-tropine (Pseudotropine). The formation of product depend on the nature of reducing agent which are explain as follow:



Reducing agents

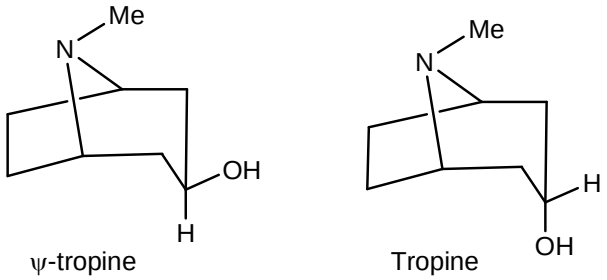
(a) Catalytic hydrogenation (Pt, H_2)
(b) Electrolytic reduction
(c) Zinc dust / HI
(d) $LiAlH_4/NaBH_4$

Reducing agents

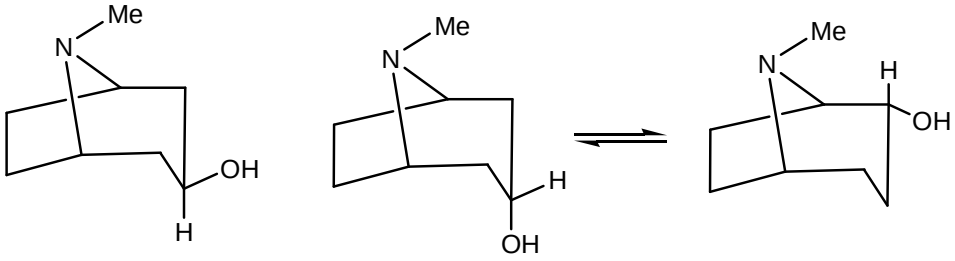
(a) Na-Hg
(b) Na/C_2H_5OH
(c) $LiAlH_4/NaBH_4$

The tropine and ψ-tropine both are epimer of each other in which one have N-methyl and (OH) insame side and another have in opposite side. The both forms possess plane of symmetry, so both are optically inactive (due to internal compensation).

Bose *et al.* suggested the chair conformation of tropine and ψ-tropine in which (OH) group is equatorial in ψ-tropine and at axial in tropine.



For tropine the predominant conformation is chair form but it also exist in minor amount in boat form.



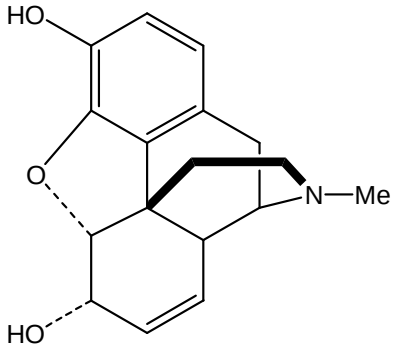
ψ-tropine Tropine chair form Boat form

1.9.3 Morphine:

Morphine is an opium alkaloid which contain phenanthrene nucleus. So morphine also called phenanthrene alkaloid.

(I) Molecular formula: $C_{17}H_{19}NO_3$

(II) Molecular structure:



(III) Nature of N-atom: Morphine react with one equivalent of methyl iodide, so it is confirmed that nature of N-atom is tertiarly. Morphine also undergoes Hoffmann exhaustive methylation.

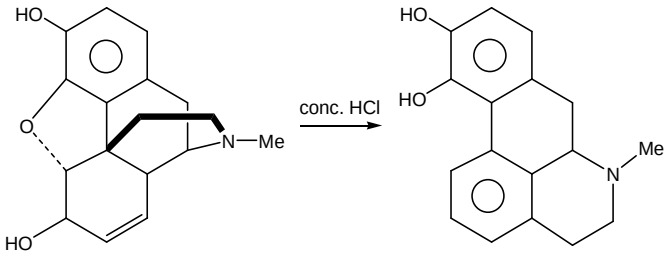
(IV) Nature of oxygen atom: When Morphine react with acetyl chloride and benzoyl chloride then it yields diacetyl (heroin) and dibenzoyl derivative. It confirm the presence of two hydroxyl group. Now it is not clear that (OH) group is phenolic or alcoholic. Morphine gives monosodium salt and also produce colour with $FeCl_3$, the both reaction reveals that one of the (OH) group is phenolic in nature. When the another (OH) group is treated with halo acid then yields halogen derivative and also react with methyl iodide to form methoxyl derivative (codeine). The methoxyl derivative undergoes oxidation to give a ketone, means it is secondary in nature. The third oxygen in morphine is unreactive so, it is present as ether linkage.

(V) Presence of benzene ring: When morphine undergoes bromination then it gives monobrominated product not addition product.

(VI) Presence of phenanthrene nucleus: When the morphine distilled with zinc dust then yield phenanthrene. It can be confirmed on the basis of following facts:

When codeine methiodide is boiled with NaOH then α-methyl morphinethine is obtained which further heated with acetic anhydride to give methylmorphol and ethanol dimethyl amine.

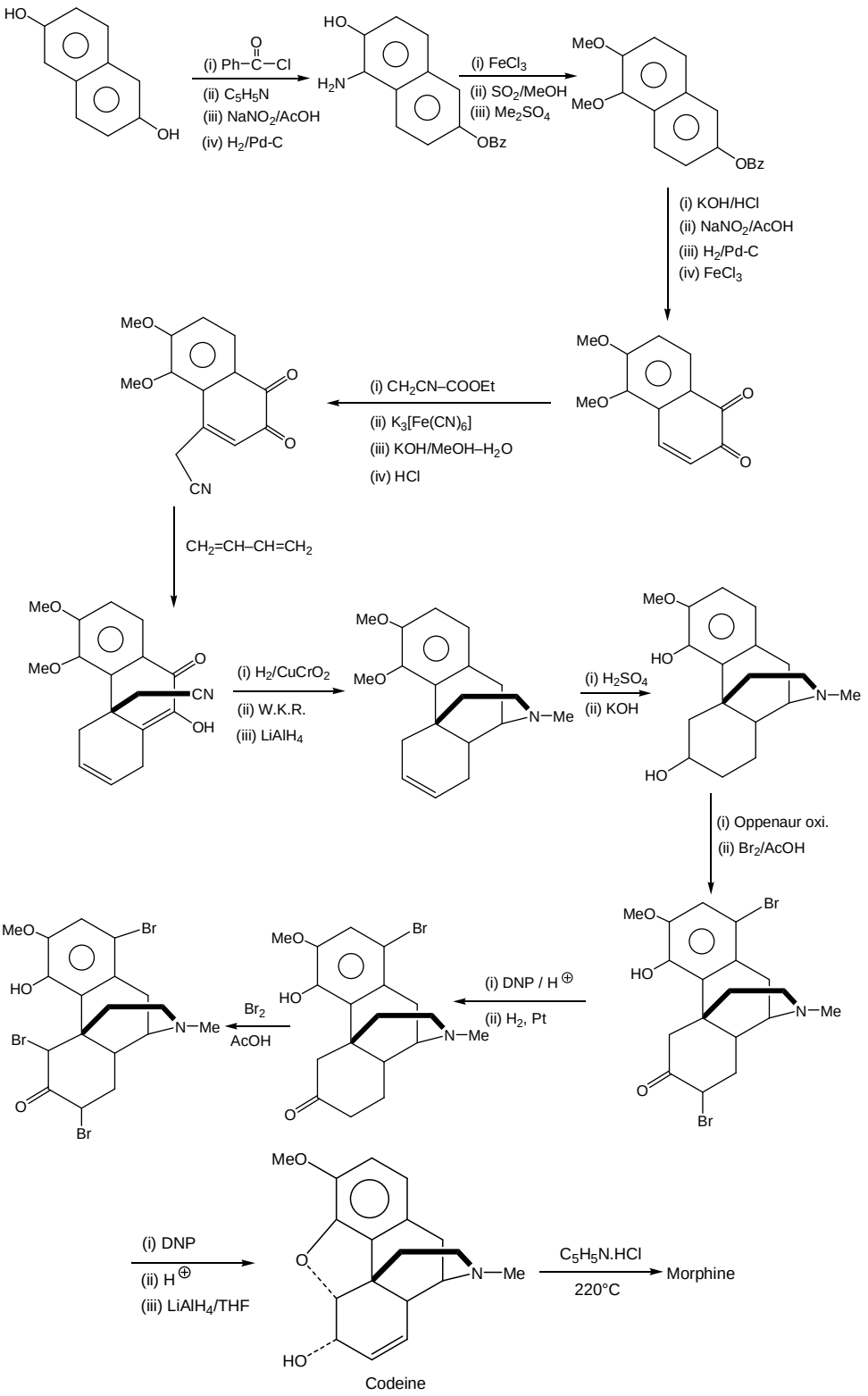
Morphine undergoes a chemical change with concentrated HCl to produce apomorphine via rearrangement which takes place through dehydration.



Morphine Apomorphine

Synthesis of morphine:

Gates *et al.* synthesis:


HO
OH
(i) Ph–C(=O)–Cl
(ii) C_5H_5N
(iii) $NaNO_2$/AcOH
(iv) H_2/Pd-C
H_2N
(i) $FeCl_3$
(ii) SO_2/MeOH
(iii) Me_2SO_4
MeO
OBz
(i) KOH/HCl
(ii) $NaNO_2$/AcOH
(iii) H_2/Pd-C
(iv) $FeCl_3$
O
(i) $CH_2CN–COOEt$
(ii) $K_3[Fe(CN)_6]$
(iii) KOH/MeOH–H_2O
(iv) HCl
CN
$CH_2=CH–CH=CH_2$
(i) $H_2/CuCrO_2$
(ii) W.K.R.
(iii) $LiAlH_4$
OH
N–Me
(i) H_2SO_4
(ii) KOH
HO
(i) Oppenaur oxi.
(ii) Br_2/AcOH
Br
(i) DNP / $H^{\oplus}$
(ii) H_2, Pt
Br_2
AcOH
(i) DNP
(ii) $H^{\oplus}$
(iii) $LiAlH_4$/THF
C_5H_5N.HCl
220°C
Morphine
Codeine

1.10 Summary

Alkaloids are obtained from natural sources mostly from plants. The separation of alkaloid from plant involve many chemical process, so it is a time consuming process. When the alkaloidal extract is obtained then it will be purified by many extraction process. The alkaloids may affect many plant activities like growth, metabolism and reproductive process. This chapter covers the study of alkaloids e.g. nicotine, atropine and morphine under molecular structure, separation, chemical reaction, stereochemistry and synthesis.

1.11 Glossary

- Alkaloids are natural plant sources which are found in leaves, bark, seed and roots.
- Alkaloids are optically active compounds (heterocycles) which posses effective physiological properties.
- Nomenclature of alkaloids can be done on the basis of their source, discoverer, physiological action and substituents.
- Many physiological action are carried out in human body and in plant by alkaloids.
- Alkaloids in plant are obtained in a mixture of alkaloid which are further purified by many chemical and physical process.
- In alkaloids (N) and (O) both heteroatoms are found in many functional groups.
- Spectroscopic techniques are also useful in the structure determination of alkaloids.
- This chapter covers whole study of nicotine, atropine and morphine.

1.12 Review questions / comprehensive questions

1. What are alkaloids ? Give the details of nomenclature of alkaloids
2. Discuss the separation techniques of alkaloids
3. Write short note on following points
   (i) Hoffman exhaustive methylation method
   (ii) Emde's degradation

(iii) Von-Braun's method

4. Give the details of general methods of structure elucidation of alkaloids.
5. Explain the following in nicotine
   (a) Presence and nature of nitrogen atom
   (b) Presence of pyridine-pyrrolidine nucleus
   (c) Presence of benzene ring
   (d) Synthesis of nicotine
6. Explain the occurrence, general structure and stereochemistry of atropine
7. Discuss the presence of following in morphine –
   (a) Presence of hydroxyl group
   (b) Presence of ethylenic bond
   (c) Presence and nature of N-atom
   (d) Presence of phenanthrene nucleus

1.13 References and suggested readings

- Organic chemistry, Volume 2 : Stereochemistry and the chemistry of natural products, fifth edition – I.L. Finar.
- Organic chemistry – J. Calyden, Greeve, S. Warren and Others (Oxford University Press) 2001.
- Chemistry of the alkaloids, Pelletier ed., Van Nostrand Reinhold Co. (1970).
- The plant alkaloids, Churchill (1949, 4th ed.).

Unit- 2

Steroids and Hormones

Structure of Unit

2.1 Objectives

This unit introduces the students with steroids and sex hormones. The steroids (cholesterol) and sex hormones (testosterone, aldosterone, progestrone and oestrone) are obtained from human beings and animals. But these are obtained in very minute amount. This unit explain the following points.

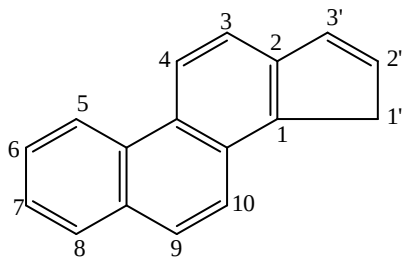
- Definition (steroids and sex hormones)

- Physiological effects / importance
- Structure of cholesterol
- Stereochemical information
- Importance of steroids

2.2 Introduction

Steroid are any class of natural or synthetic organic compounds which comprise a group of cyclical organic compounds whose basis is a characteristic arrangement of seventeen carbon atoms in a four-ring structure linked together from three 6-carbon rings followed by a 5-carbon ring and an eight-carbon side chain on carbon 17. Steroids are important in biology, chemistry, and medicine. Among the synthetic steroids of therapeutic value are considered as large number of anti-inflammatory agents and anabolic (growth-stimulating) agents.

The steroids basically introduces the sterols, bile acid, vitamin D and sex hormones (androsterone, testosterone, progestrone, estrogen and estrone). All the steroids have basic skeleton i.e., 1,2-cyclopentophenanthrene nucleus which is also basic skeleton of Diel's hydrocarbon.



Molecular formula ($C_{18}H_{16}$)

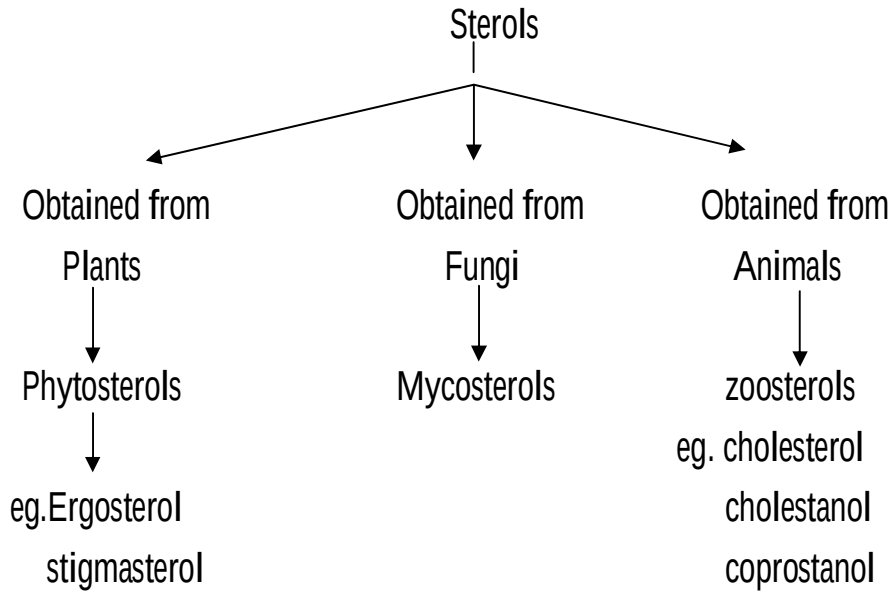
So, we can say that any compound which produce Diel's hydrocarbon on distillation with selenium consider as steroids. If the distillation carried out at 420°C, then chrysene and picene both are obtained.

$$\text{Chrysene + Picene} \xleftarrow[420°C]{\text{Se}} \text{All steroids} \xrightarrow[360°C]{\text{Se}} \text{Diel's hydrocarbon}$$

2.3 Occurrence of steroids

All the steroids possess sterol. Sterol is mainly found in plants and animals. The sterols are crystalline organic compound which possess an alcoholic group. Due to

presence of alcoholic group sterols are obtained in esterified state with fatty acids.



Some important sterols and their sources are listed below:

common name	**systematic name**	**Occurrence**
Cholesterol	5-cholesten-3β-ol	principal sterol of most animals and all vertebrate tissues
Coprostanol	5β -cholestan-3β -ol	feces of vertebrates
Cholestanol	5α -cholestan-3β-ol	minor vertebrate sterol: guinea pig and rabbit adrenal
dehydrocholesterol	5,7-cholestadien-3β-ol	mammalian skin, intestine
zymosterol	5α-cholesta-8,24-dien-3β-ol	minor sterol of yeasts
ergosterol	5,7,22-ergostatrien-3β-ol	principal sterol of yeasts, ergot (*Claviceps purpurea*), and other fungi
stigmasterol	5,22-stigmastadien-3β-ol	most green plants, soybeans
sitosterol	5-stigmasten-3β-ol	most green plants, wheat germ

fucosterol	5,24(28)-stigmastadien-3β-ol	principal sterol of marine brown algae (*Fucus* species)
lanosterol	8,24-lanostadien-3β-ol	skin, sheep wool, fat, yeasts

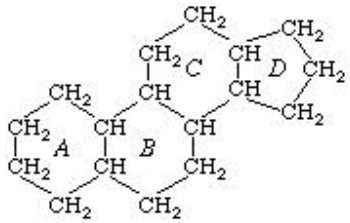
2.4 Steroid nomenclature and numbering system

All steroids are related to a characteristic molecular structure composed of 17 carbon atoms which are arranged in four rings conventionally denoted by the letters *A*, *B*, *C*, and *D* having 28 hydrogen atoms in four rings.

Names of fundamental structures most often used in steroid nomenclature

	carbon atoms present (as numbered in structure 6)	**naturally occurring general classes**	**examples shown in text**
gonane	1–17	None	Gonane
androstane	1–19	androgens	Androstane, testosterone androstanedione
pregnane	1–21	gestogens and adrenal steroids	Progesterone, cortisol aldosterone
cholane	1–24	bile acids	cholic acid, sodium sodium glycocholate
cholestane	1–27	Sterols	Cholesterol, scymnol
ergostane	1–28	Sterols	Ergosterol, cyasterone
stigmastane	1–29	Sterols	Stigmasterol, antheridiol

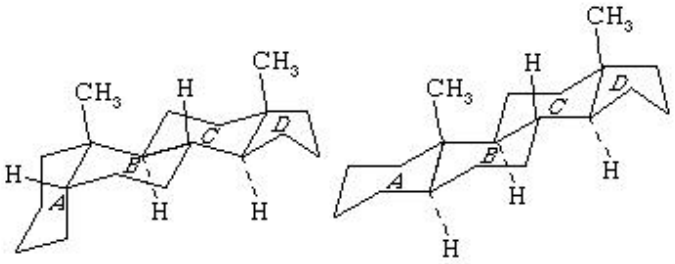
*Gonane and androstane themselves do not occur in nature.



gonane (1)

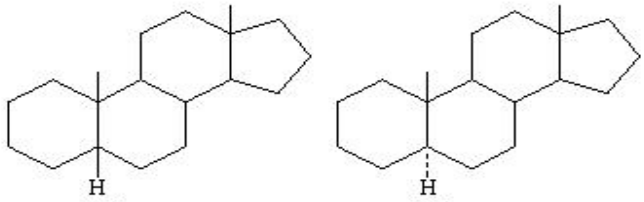
This parent structure (1), named gonane (also known as the steroid nucleus), may be modified in a practically unlimited number of ways by removal, replacement, or addition of a few atoms at a time; hundreds of steroids have been isolated from plants and animals.

The steroid nucleus is a three-dimensional structure, and atoms or groups are attached to it by spatially directed bonds. Although many stereoisomers of this nucleus are possible (and may be synthesized), the saturated nuclear structures of most classes of natural steroids are alike, except at the junction of rings *A* and *B*. Simplified three-dimensional diagrams may be used to illustrate stereochemical details. For example, androstane, common to a number of natural and synthetic steroids, exists in two forms (2 and 3), in which the *A*/*B* ring fusions are called *cis* and *trans,* respectively.



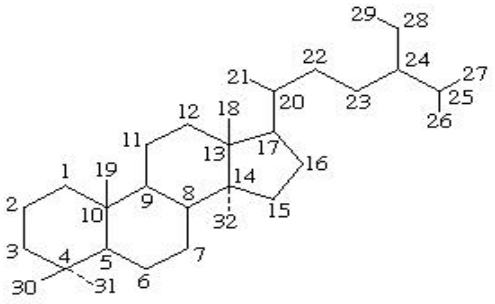
cis-androstane (2) *trans*-androstane (3)

In the *cis* isomer, bonds to the methyl group (CH_3) and to the hydrogen atom (H) both project upward from the general plane defined by the rest of the molecule, whereas in the *trans* isomer, the methyl group projects up and the hydrogen projects down. Usually, however, steroid structures are represented as plane projection diagrams such as 4 and 5, which correspond to 2 and 3, respectively.



The stereochemistry of rings *A* and *B* must be specified by showing the orientation of the hydrogen atom attached at C5 (that is, carbon atom number 5; steroid numbering is explained below) as either above the plane of the diagram (designated β) or below it (α). The α-, β- symbolism is used in a similar manner to indicate the orientation of any substituent group that is attached to a saturated (fully substituted) carbon within the steroid ring system. Bonding of β-attached substituents is by a full line, while of α-substituents by a broken line and if not known then shown by a wavy line.

Each carbon atom of a steroid molecule is numbered, and the number is reserved to a particular position in the hypothetical parent skeletal structure (6) whether this position is occupied by a carbon atom or not.

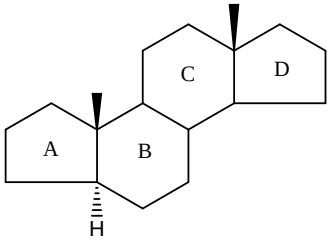


(6)

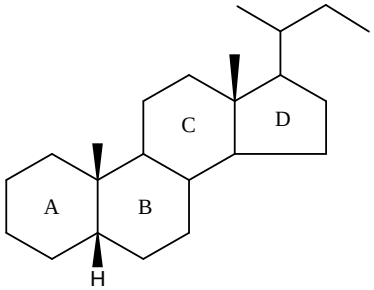
Systematic rules agreed by the International Union of Pure and Applied Chemistry for the nomenclature. By attaching prefixes and suffixes to the name of the appropriate root structure, the character of substituent groups or other structural modification is indicated. The prefixes and suffixes include numbers, called locants, indicative of the position in the carbon skeleton at which the modification occurs, and, where necessary, the orientation of a substituent is shown as α or β. The carbon atom at position 3, for example, is referred to as C3; a hydroxyl group attached to C3 is referred to as a 3-OH group or, more specifically, as a 3α-OH or

3β-OH group. In addition to differences in details of the steroid nucleus, the various classes of steroids are distinguished by variations in the size and structure of an atomic group (the side chain) attached at position 17.

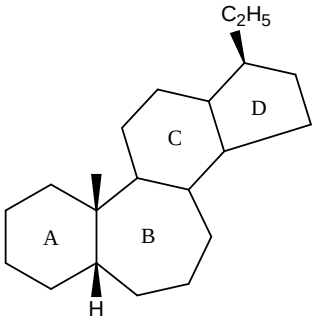
In addition to the usual chemical notations for substituent groups replacing hydrogen atoms (e.g., methyl-, chloro-, hydroxy-, oxo-), the following prefixes are commonly used: dehydro- (lacking two hydrogen atoms from adjacent positions); dihydro- (possessing two additional hydrogen atoms in adjacent positions); deoxy- (hydroxyl group replaced by a hydrogen atom); epi- (differing in configuration of a carbon atom bonded to two other carbon atoms); iso- (differing in configuration of a carbon atom bonded to three other carbon atoms); nor- (lacking one carbon atom); homo- (possessing one additional carbon atom); cyclo- (with a bond between two carbons that are normally not united); and seco- (with a carbon-carbon bond of the nucleus broken).



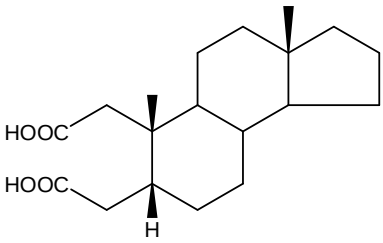
A-nor-5α-androstane



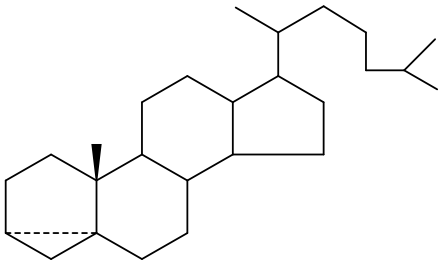
23-nor-5B-cholane



B-Homo-5β-pregnane



2,3-Seco-5β-androstane-2,3-dioic acid

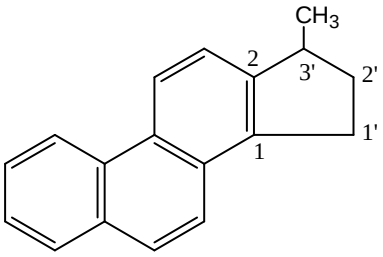


3α, 5α-Cyclocholestane

2.5 Basic skeleton of steroids

The basic skeleton of all steroids posses 1,2-cyclopentophenanthrene nucleus i.e. known as Diel's hydrocarbon.

Structure of Diel's hydrocarbon:



3'-Methyl-1,2-cyclopento phenanthrene

Molecular Formula: $C_{18}H_{16}$

2.6 Isolation of steroids

The isolation of the considerable amount of pure steroids required for structure elucidation,degradation, biological testing, and other research needs (generally

milligrams to grams). The methods of isolation generally involve extraction, precipitation, adsorptions, chromatography, and sometimes crystallizations. The isolated matter is purified to chemical homogeneity. The structure determination methods that are applied to determine the chemical structure of an isolated steroid, a process that involves an array of chemical and physical methods that included NMR and small molecule crystallography. Analytical methods involve in determining of structure if a steroid is present in an analytical mixture.

Procedures for isolation of steroids differ according to the chemical nature of the steroids and the scale and purpose of the isolation. Steroids are isolated from natural sources by extraction with organic solvents, in which they usually dissolve more readily than in the aqueous fluids of tissues. The source material often is treated initially with an alcoholic solvent, which dehydrates it, denatures , proteins associated with the steroids, and dissolves many steroids. Saponification either of whole tissues or of substances extracted from them by alcohol splits the molecules of sterol esters, triglycerides, and other fatty esters and permits the extraction of the sterols by means of water-immiscible solvents, such as hexane or ether, with considerable purification. Intact sterol esters or hormonal steroids and their metabolites (compounds produced by biological transformation) that are sensitive to strong acids or alkalies, however, require essentially neutral conditions for isolation, although some procedures for analysis of urinary steroids employ acid treatment, milder hydrolysis, as by enzymes, is preferred. The acidity of some steroids allows them to be held in alkaline solution, while nonacidic impurities are extracted with organic solvents.

Commercially, abundant steroids usually are purified by repeated crystallization from solvents. Small-scale laboratory isolations for investigative or assay purposes usually exploit differing polarities of the steroid and of its impurities, which may be separated by partitioning between solvents differing in polarity or by chromatography. Occasionally, special reagents may selectively precipitate or otherwise sequester the desired steroid. A classical example is the precipitation of 3β-hydroxy sterols such as cholesterol by the natural steroid derivative digitonin. New steroids of great physiological interest often are isolated from tissue only with extreme difficulty, because they are usually trace constituents.

The percentage recovery of known steroid hormones during their assay in small biological samples usually is assessed by adding a trace of the same steroid in

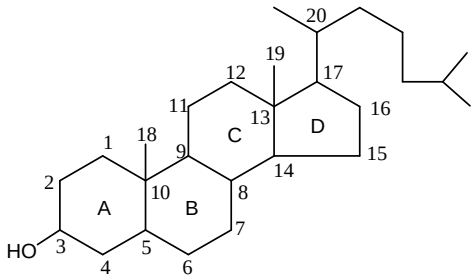
radioactive form to the initial sample, followed by radioassay (analysis based on radioactivity) after purification is complete.

2.7 Stereochemistry of steroids

For the study of steroids stereochemistry we will take the e.g. of sterol. Sterol has 8 disimilar chiral centre at carbon, C-3,5,8,9,10,13,14 and 17. So the total number of optical isomer can be calculated as follow:

Formula = 2^n

Where n = number of chiral centre



If we consider the chiral centre of main nucleus then number of optical isomer will be

$$n = 8, \text{ So } 2^8 = 256$$

If we consider the chiral centre that is outside of nucleus (C-20) then the number of optical isomer will be –

$$N = 9, \text{ So, } 2^9 = 512$$

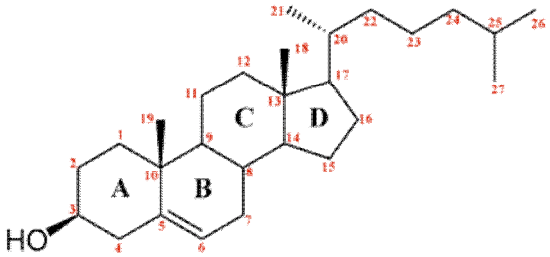
In sterol such chiral centre which are produced due to ring junction. If we count these chiral centre (5,8,9,10,13 and 14) then number of optical isomer are $2^6 = 64$, however many of these are not possible because of some steric hindrance.

2.8 Structure determination and synthesis of some steroid (cholesterol) and sex hormones (testosterone and oestrone)

2.8.1 Cholesterol:

Molecular formula: $C_{27}H_{46}O$

Molecular Structure:



The three cyclohexane rings are designated as rings A, B and C in the figure to the right and the one cyclopentane ring as ring D. Individual steroids vary, first and primarily, by the oxidation state of the carbon atoms in the rings and by the chains and functional groups attached to this four-ring system; second, steroids can vary more markedly via changes to the ring structure. Sterols are a particularly important form of steroids, with sterols having a cholestane-derived framework and an hydroxyl group at the C-3 ring position.

Study of cholesterol is done under following points:

(I) Structure of nucleus (pressence and size of four ring)

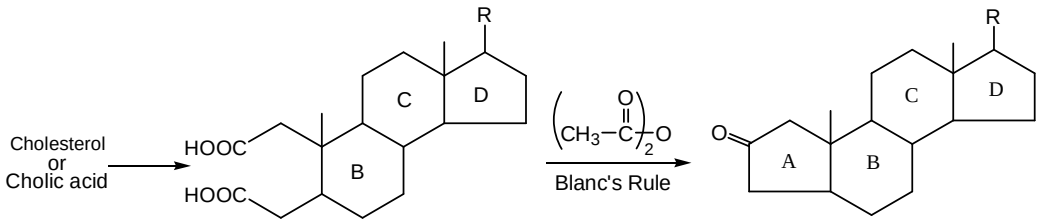
(II) Presence and position of hydroxyl group and double bond

(III) Nature and position of side chain

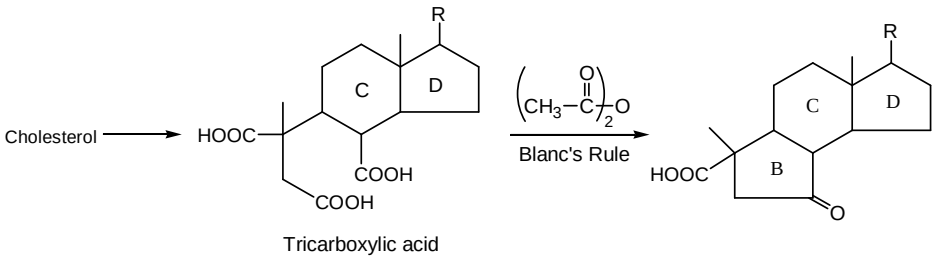
(IV) Position of two angular methyl group

(I) Nature of nucleus (presence of four rings A,B,C,D):

(a) Size of ring A: When the cholesterol and cholic acid decomposed into dicarboxylic acid, then it converted into cyclopentanone via ring contraction. This suggest that ring (A) is six membered.

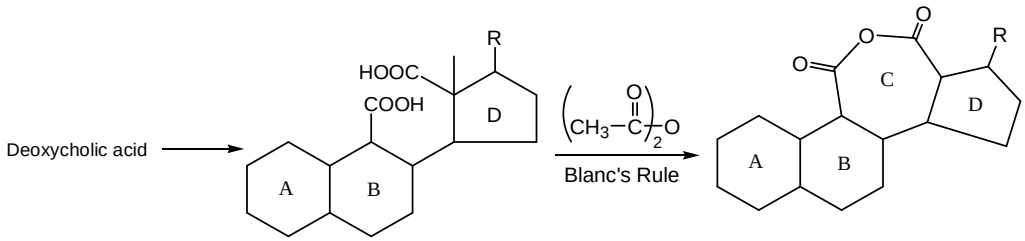


(b) Size of ring B: When cholesterol undergoes decomposition then ring (B) is decomposed to give tricarboxylic acid which further undergoes cyclisation to give cyclopentanone. This reaction suggested that ring (B) is six membered.

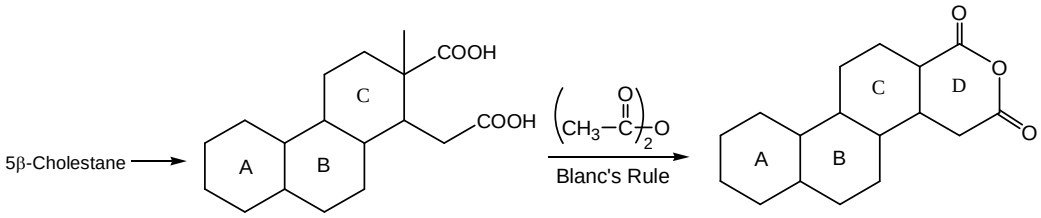


Tricarboxylic acid

(c) Size of ring C: When the deoxycholic acid is converted into dicarboxylic acid, which yields seven membered cyclic anhydride. This reaction also suggested that ring (C) is also six membered.



(d) Size of ring D: When the 5β-cholestane is converted into dicarboxylic acid i.e. etiobilianic acid then it yields six membered cyclic anhydride. This reaction suggest that ring (D) is five membered.

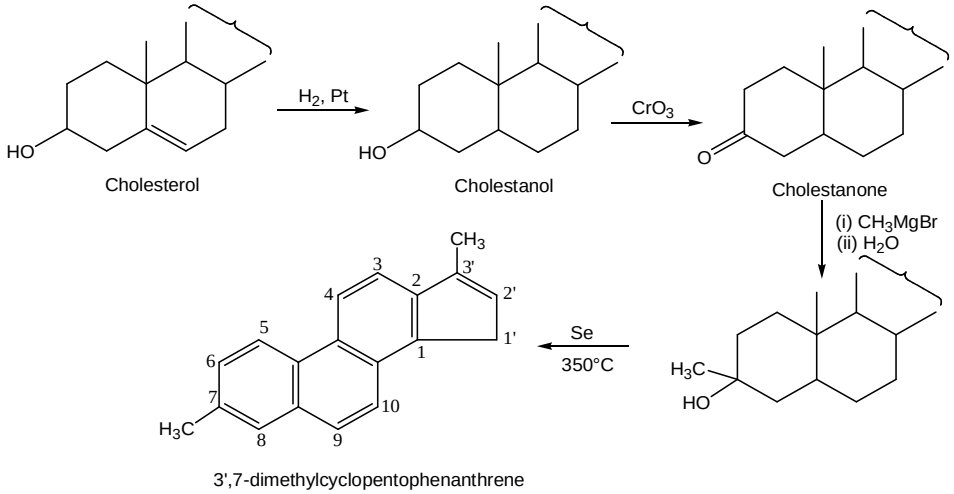


Etiobilianic acid

(II) Presence and position of hydroxyl group and double bond:

(a) Presence of hydroxyl group: When the cholesterol undergoes esterification with acetic acid and benzoic acid then it yields monoacetate and monobenzoate. Presence of hydroxyl can also be confirmed that cholesterol gives cholestanone on oxidation.

(b) Position of hydroxyl group: When the cholesterol undergoes reduction then it yields dihydroderivative (i.e. cholestanol). The later compound react with CrO_3 to give a saturated ketone (i.e. cholestenone). The cholestanone (a ketone) react with methyl magnesium bromide followed by hydrolysis and aromatization(dehydrogenation)to give 3',7-dimethylcyclopentophenanthrene. The above reaction confirm that hydroxyl group present in ring (A) at C-3 position.



Cholesterol Cholestanol Cholestanone

3',7-dimethylcyclopentophenanthrene

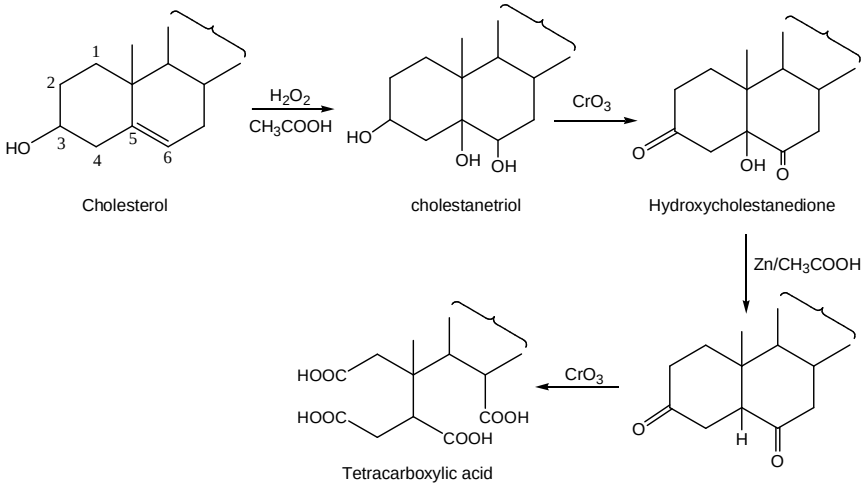
(c) Presence of double bond: Cholesterol react with one equivalent of hydrogen (H_2) to give its dihydroderivative, also react with (Br_2) to give dibromoderivative.

$$C_{27}H_{46}Br_2O \xleftarrow{Br_2} C_{27}H_{46}O \xrightarrow{H_2,\ Pt} C_{27}H_{48}O$$

Cholesterol Dihydrocholesterol

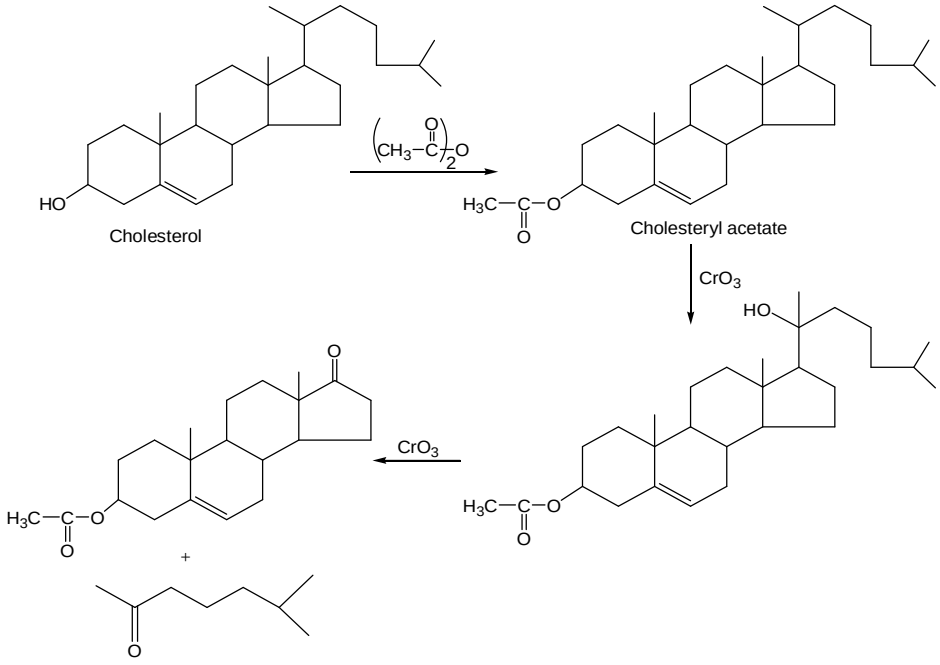
The above reaction suggested that cholesterol contains one double bond.

(d) Position of double bond: Position of double bond in cholesterol can be confirmed by these given set of reactions. When the cholesterol undergoes reaction with H_2O_2 / CH_3COOH then it undergoes dihydroxylation to give cholestane triol. Which further react with CrO_3 to give hydroxyl cholestane dione (diketone). The later compound undergoes reaction with Zn/CH_3COOH to produce cholestanedione which further undergoes oxidation to produce a tetracarboxylic acid. The above whole set of reaction suggested that position of double bond in cholesterol is in between (C_5-C_6).



(III) Nature and position of side chain:

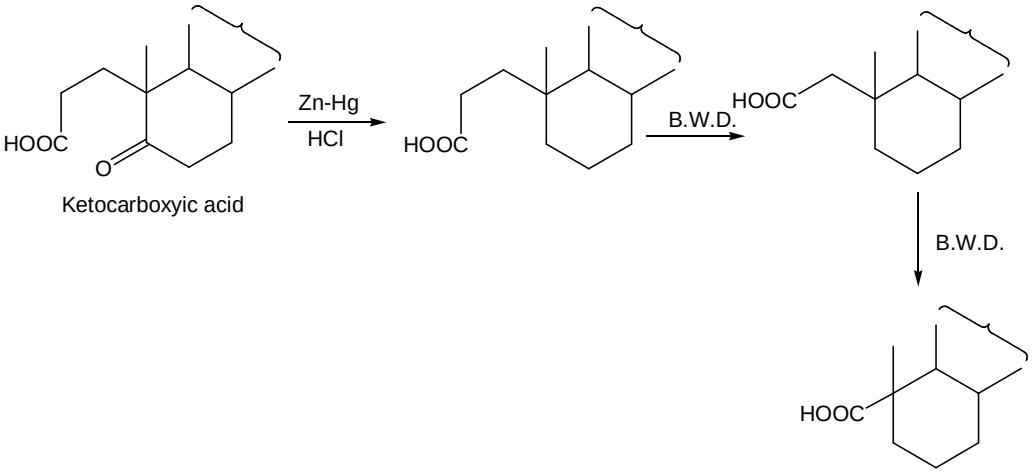
The nature and position of side chain in cholesterol can be explained by these set of chemical reactions. The cholesterol has a hydroxyl group when it undergoes acetylation then cholesteryl acetate will be formed.The later compound is undergoes the oxidation at the side chain to give a ketone of cholesteryl acetate and isohexyl methyl ketone. The formation of isohexyl methyl ketone reveals that the point of attachment of side chain will be at ring (D) with 3' carbon.



(IV) Position of two angular methyl group:

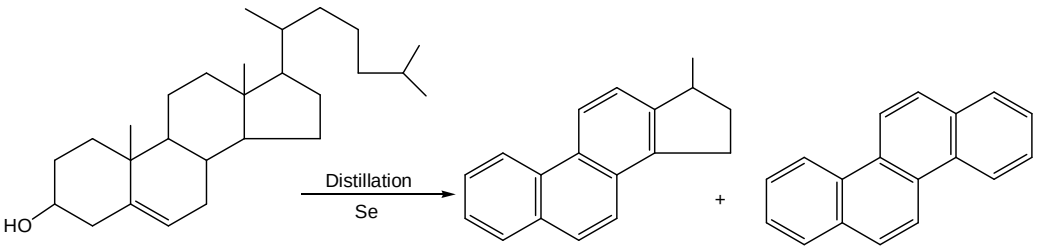
The molecular structure and molecular formula of cholesterol reveals that it contains 27 carbon atoms. The main nucleus (1,2-cyclopentophenanthrene) contains only 17 carbon atoms, the side at ring (D) contains 8 carbon atoms, so these are (17+8 = 25) 25 carbon atoms. So the remaining two carbon atoms are present as angular methyl group. These can be explained as follow:

(a) When the keto-carboxylic acid is undergoes Clemenson reduction which further subjected to the successive Barbier-Wieland degradation (Involve loss of one C-atom). So the keto-carboxylic acid undergoes only two times Barbier-Wieland degradation (B.W.D.). In the second last structure the carboxylic acid present at tertiary carbon atom, so it does not undergoes B.W.D. It confirm that one angular methyl group is present at that carbon.



Ketocarboxyic acid

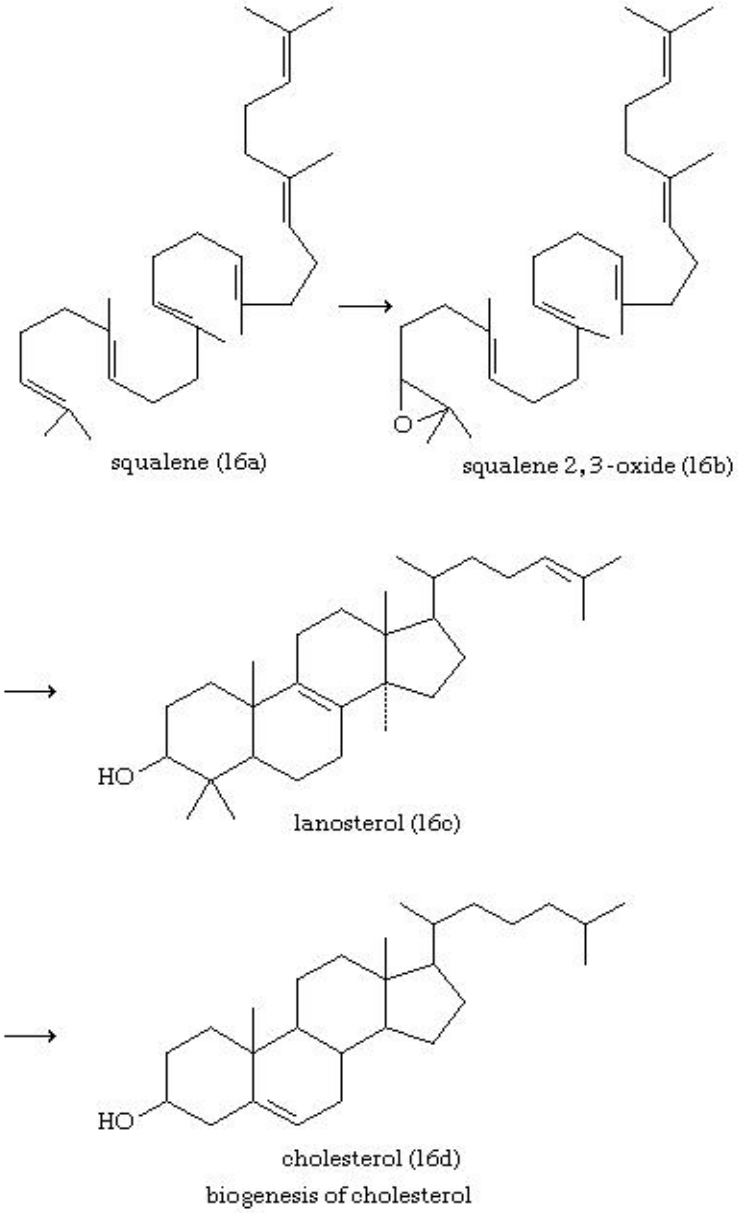
(b) The position of another angular methyl group can be confirm as: When the cholesterol skeleton is undergoes distillation with (Se) then it yields, Diel's hydrocarbon and chrysene. The chrysene have four six membered ring. The conversion of ring (D) from five membered to six membered suggested that the angular methyl group involve in cyclization.



Diel's hydrocarbon Chrysene

Synthesis of cholesterol:

In plants and animals, steroids is biosynthesized by similar reactions, beginning with acetic acid, assisted by a type of enzyme. The isoprenoid hydrocarbon called squalene, which occurs widely in nature, is thought to be the starting material from which all steroids are made. Enzymatic transformation of squalene produces lanosterol in animals and cycloartenol in plants, which yield cholesterol in both animals and plants. Cholesterol is then converted to bile acids and steroid hormones in animals and to steroids such as alkaloids in plants. Cholesterol and other steroids are biosynthesized by extension of the enzyme pathway by which terpenoids are synthesized. Acetate fragments derived from common nutrient materials are converted into mevalonic acid, from which the terpenoid hydrocarbon squalene is formed. One end of the squalene molecule is then oxidized, giving squalene 2,3-oxide, which, by an intramolecular cyclization reaction and structural rearrangement, yields the steroid lanosterol. This enzyme-controlled reaction may be initiated by introduction of a positive charge into the oxide ring, because it is remarkably similar to the nonenzymic, acid-catalyzed cyclizations of certain unsaturated hydrocarbons similar in structure to squalene. Cholesterol is formed from lanosterol by further structural changes.



biogenesis of cholesterol

Sex hormones: Sex hormones can be classified in two categories

Male sex hormones (Androgens): Testosterone and androstenedione are the major testicular androgens. Several other less-active androgens occur naturally. Major metabolites of testosterone are androsterone and etiocholanolone.

2.8.2 Testosterone:

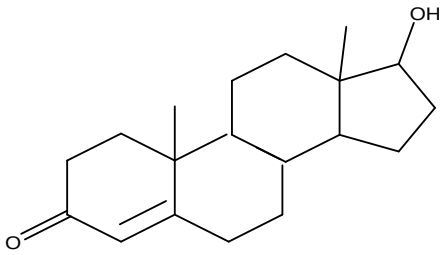
Testosterone is the principal male sex hormone of androgen group and found in mammals, reptiles, birds, and other vertebrates. In mammals, testosterone is secreted by the testicles of males and the ovaries of females, although small

amounts are also secreted by the adrenal glands. Androgens promote male sexual behaviour and aggressiveness, muscular development, and, in humans, the growth of facial and body hair and deepening of the voice. In men, testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate as well as promoting secondary sexual characteristics such as increased muscle, bone mass, and the growth of body hair. In addition, testosterone is essential for health and well-being as well as the prevention of osteoporosis. Testosterone has several major actions. It provides negative feedback inhibition on the secretion of gonadotropin-releasing hormone from the hypothalamus and the secretion of luteinizing hormone from the pituitary gland. It also directs the development of the embryonic Wolffian ducts into the vas deferens (ductus deferens) and seminal vesicles and stimulates the formation of muscle and bone. Dihydrotestosterone is responsible for sperm maturation during spermatogenesis, for the formation of the prostate gland and external genitalia, and for sexual maturation at puberty.

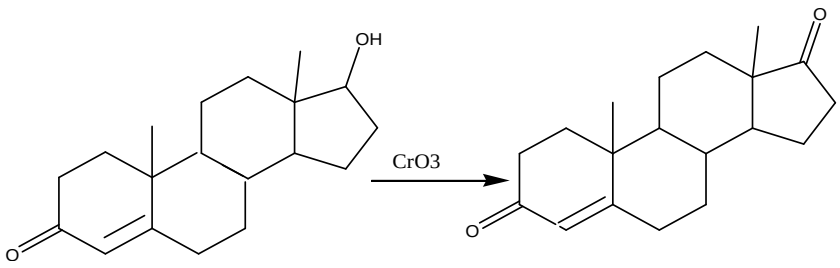
Testosterone can be manufactured by chemical and microbiological modification of inexpensive steroids, such as diosgenin. It is used clinically to treat testicular insufficiency, to suppress lactation (milk production), and to treat certain types of breast cancer.

(I) Molecular Formula: $C_{19}H_{28}O_2$

(II) Molecular Structure:



(III) Presence of secondary alcoholic group: When testosterone react with the carboxylic acid then forms monoester (monoacetate and monobenzoate). Testosterone also undergoes to oxidation with CrO_3 to give androst-4-ene-3,17-dione (i.e. diketone).

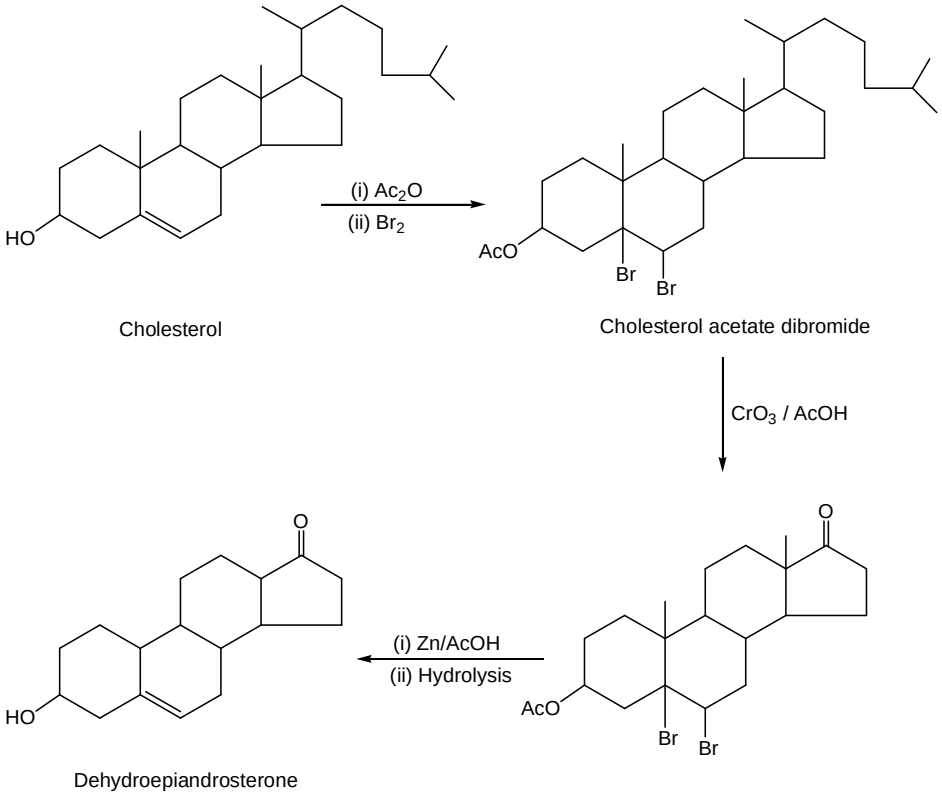


Testosterone Androst-4-ene-3,17-dione

(IV) Presence of α,β-unsaturated ketonic group: Testosterone undergoes to conjugate addition (Michael addition), and it is sensitive to alkali. Presence of the α,β-unsaturated ketonic group is also confirmed by the UV spectroscopy that it absorb at the 240 nm.

(V) Presence of tetracyclic ring system: Testosterone possess the tetracyclic ring system as we have already discussed in the case of the cholesterol (i.e. 1,2-cyclopentophenanthrene nucleus).

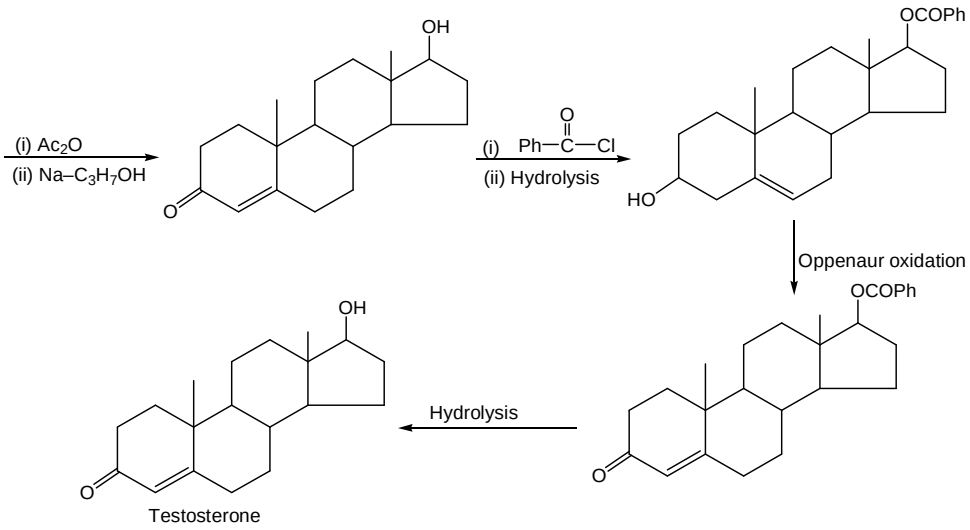
(VI) **Synthesis of testosterone:** From cholesterol.



Cholesterol

Cholesterol acetate dibromide

Dehydroepiandrosterone



Testosterone

Female sex hormones:

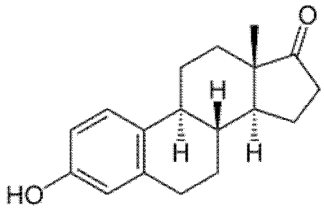
2.8.3 Oestrone (E1 or estrone):

Estrone is an estrogenic hormone secreted by the ovaries as well as adipose tissue. Estrone is an odorless, solid crystalline powder, white in color with a melting point of 254.5 °C. Estrone is one of several natural estrogens, which also include estriol and estradiol. Estrone is the least abundant of the three hormones. Estradiol is present almost always in the reproductive female body, and estriol is abundant primarily during pregnancy.

Estrone is known to be a carcinogen for human females as well as cause breast tenderness or pain, nausea, headache, hypertension, and leg cramps. In men, estrone has been known to cause anorexia, nausea, vomiting, and erectile dysfunction. Estrone is relevant to health and disease states because of its conversion to estrone sulfate, a long-lived derivative. Estrone sulfate acts as a reservoir that can be converted as needed to the more active estradiol. It is the predominant estrogen in postmenopausal women. These are the estrogens, of which estradiol is the most importent. They maintain the female reproductive tissues in a fully functional condition, promote the estrous state of preparedness for mating, and stimulate development of the mammary glands and of other feminine characteristics. Estrogenic steroids have been isolated from urines of pregnant female mammals of many species, including humans, from placental and adrenal tissues, and, unexpectedly, from the testes and urines of stallions.

(I) Molecular Formula: $C_{18}H_{22}O_2$

(II) Molecular Structure:

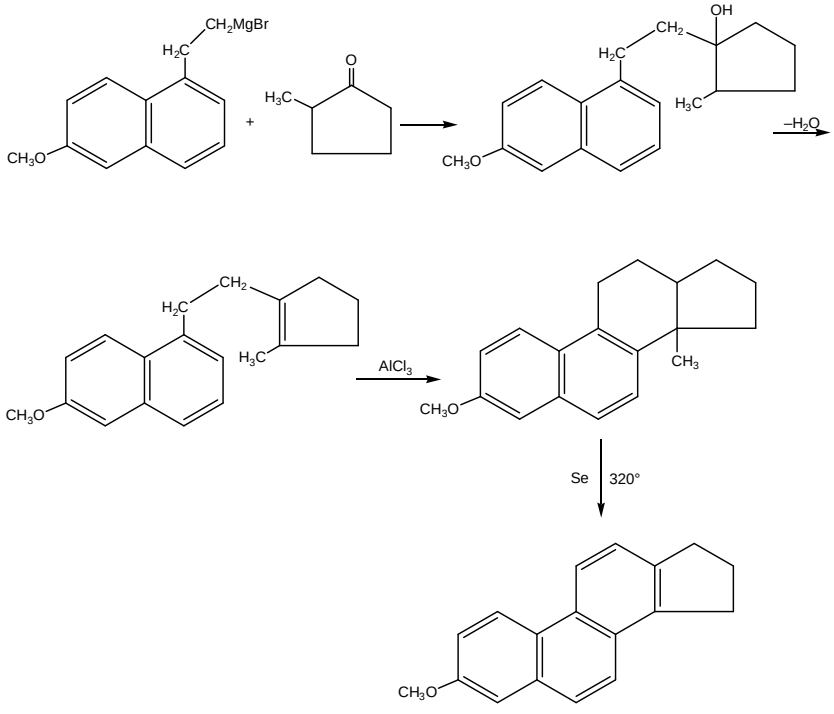


3-hydroxyestra-1,3,5(10)-triene-17-one

(III) Presence of keto group: When the estrone react with the one equivalent of hydroxyl amine and semicarbazide then yields monooxime and monosemicarbazone, it reveals that estrone have only one ketonic group.

(IV) Presence and position of the phenolic group: **(a)** Presence of phenolic group: Estrone forms moester derivative with the acid. Estrone also soluble in alkali to form phenoxide ion, give colouration with $FeCl_3$, it suggest that hydroxyl group is phenolic in nature.

(b) Position of phenolic group: When the monomethyl ether derivative of Grignard reagent with 2-methyl cyclopentanone which further undergoes to distillation then results 7-methoxy-1,2-cyclopentophenenthrene, its formation refers that phenolic group present at C-3 position in benzene ring.

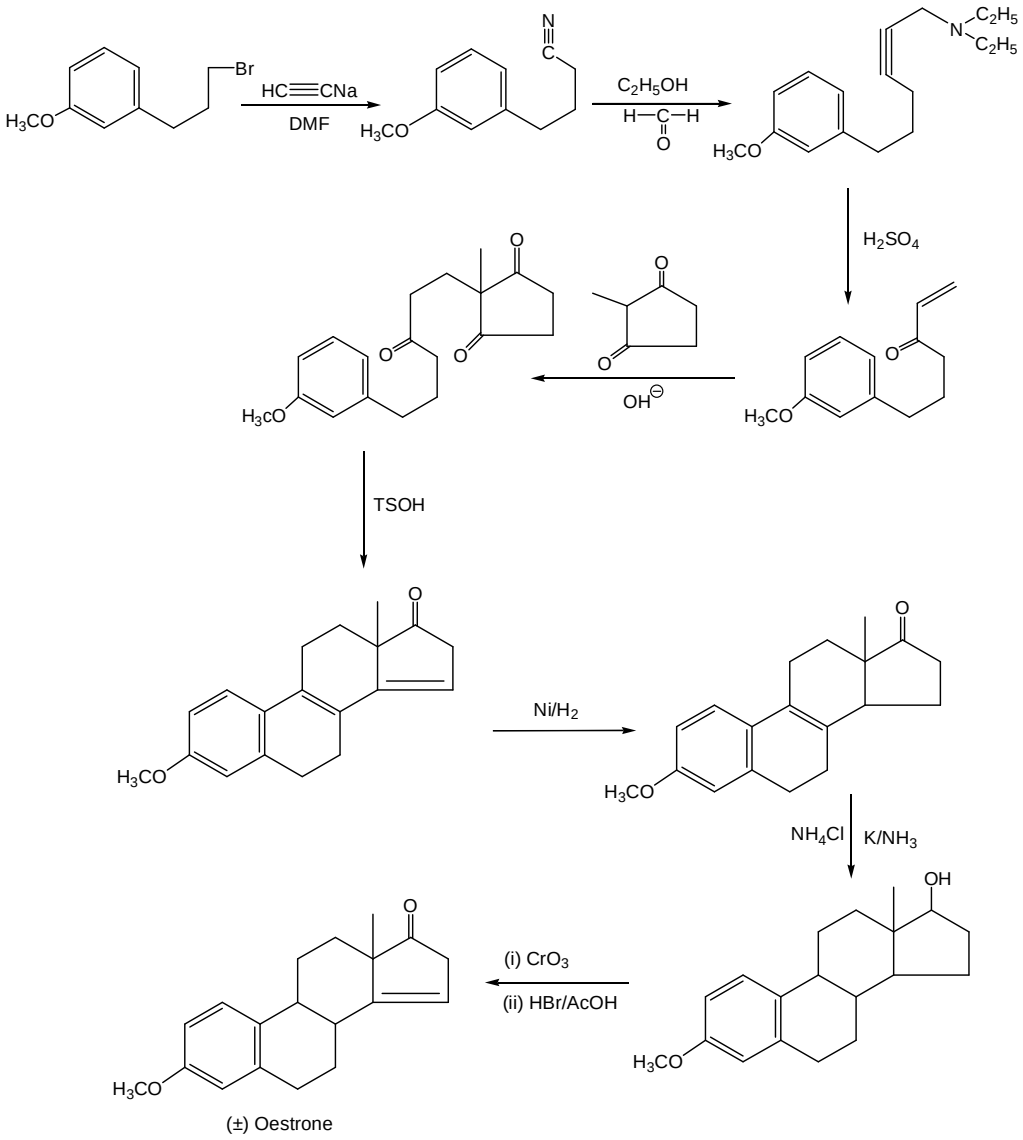


7-methoxy-1,2-cyclopentophenenthrene

(V) Presence of the steroid nucleus: The X-ray study of the oestrone suggest

that oestrone possess steroid nucleus. When steroids distilled with the Zinc then yields chrysene (i.e. hydrocarbon).

(VI) Synthesis of Oestrone:



(±) Oestrone

2.9 Summary

Steroids are obtained from animals and human beings. The isolation of steroids from animals and humas beings involve many chemical process, so it is a time consuming process. When the steroids extract is obtained then it will be purified by many chemical process. The steroids may affect many body activities like growth, male and female characteristics and reproductive process. This chapter covers the

study of steroids and sex hormones e.g. cholesterol, testosterone and oestrone under molecular formula, structure, separation, chemical reaction and synthesis.

2.10 Glossary

- Steroids are obtained from the animals and human beings.
- Steroids are optically active compounds which posses physiological action.
- Nomenclature of steroids can be done on the basis of their general skeleton (1,2-cyclopentophenanthrene nucleus) and substituents.
- Many physiological action are carried out in human body by the sex hormones.
- Steroids are obtained as a mixture which are further purified by many chemical and physical methods.
- Steroids possess mainly oxygen as heteroatoms in form of functional groups (hydroxyl and ketonic group).
- Spectroscopic and X-ray techniques are also useful in the structure elucidation of steroid.

  This chapter introduces with the whole study of cholesterol, testosterone and oestrone.

2.11 Review questions / comprehensive questions

1. Give the introduction of steroids including their occurrence and isolation ?
2. Write short note on following points
   (i) Nomenclature of steroids
   (ii) Stereochemistry of steroids
3. Give the details about the structure of cholesterol.
4. Explain the following in cholesterol:
   (a) Presence and nature of hydroxyl group
   (b) Presence and position of double bond

(c) Presence of cyclopentophenanthrene nucleus

(d) Synthesis of cholesterol

5. Explain the detailed structure of the testosterone.
6. Give the evidences of the presence of following in oestrone –

(a) Presence and position of hydroxyl (phenolic) group

(b) Presence of steroid nucleus

(c) Synthesis of oestrone

2.12 References and suggested readings

- Organic chemistry, Volume 2 : Stereochemistry and the chemistry of natural products, fifth edition – I.L. Finar.
- Organic chemistry – J. Calyden, Greeve, S. Warren and Others (Oxford University Press) 2001.
- Biogenesis of natural products, Pergamon (1967, 2nd edn.)
- Steroids reaction mechanism, Elsevier (1968).

Unit - 3

Prostaglandins

Structure of Unit:

3.0 Objectives

This chapter is based on the synthesis of types of prostaglandins and their different analogues, this short review will concentrate only on the synthesis of the E-series prostaglandins and some of their analogues. Specifically, this chapter will present an up to date review of the synthetic methods involving the conjugate addition

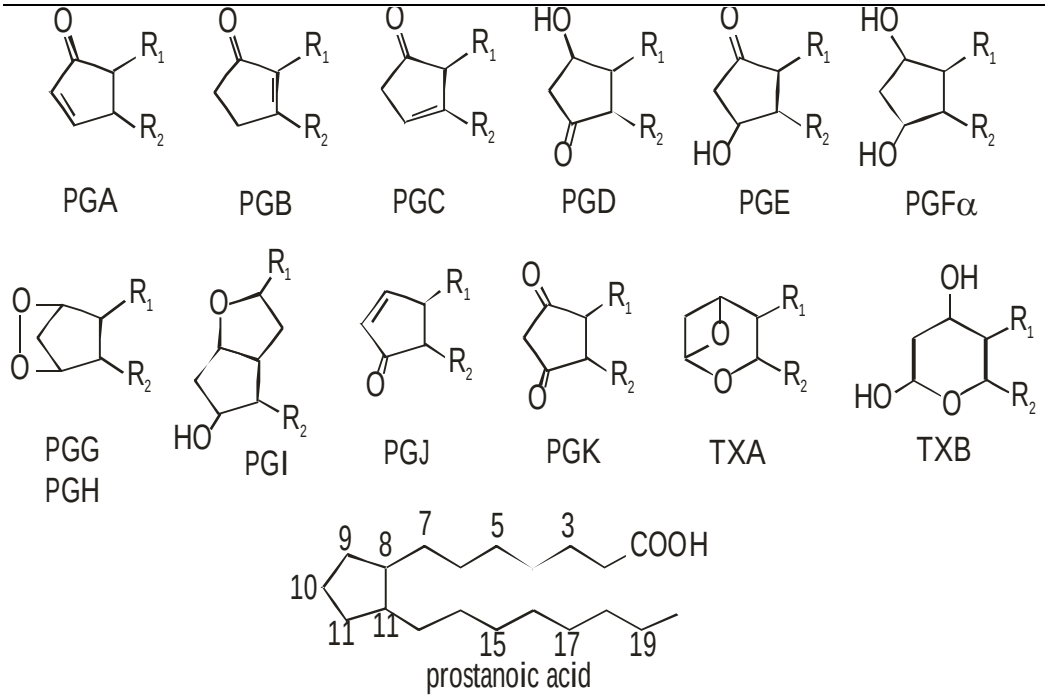
approach that is used to synthesize the E-series prostaglandins and their analogues with modified side chains only. The E-series PGs are the most widely studied.

3.1 Introduction

Prostaglandins (PGs) were discovered by Swedish physiologist (Nobel laureate), Ulf von Euler in 1935 and other investigators were given the term "Prostaglandin" anticipating the active material could be the origin from the prostate gland. PGs were first isolated and characterized by K. Bergstrom from Karolinska Institute in 1957. 2 In 1971, it was determined that aspirin-like drugs could inhibit the synthesis of PGs. PGs are classified among the family of eicosanoids along with leukotrienes (LT), thromboxanes (TX) and Lipoxins (LX). The PGs and TXs are collectively identified as prostanoids. PGs exist and are synthesized in virtually every tissues and organs of the living body. These are like hormones in that they act as chemical messengers, but they are not transported from one place to another in the body rather they are synthesized within the cells when required. They play important regulatory roles in many normal cellular functions, especially in relation to inflammatory responses, regulating fat metabolism, hormones, pain, fever as well as the cardiovascular, immune, and central nervous systems.

3.1.1 Prostaglandin Structure

Prostaglandins are unsaturated carboxylic acids, consisting of a 20 carbon skeleton that also contains a five member ring and are based upon the fatty acid, arachidonic acid. There are a variety of structures one, two, or three double bonds. On the five member ring there may also be double bonds, a ketone, or alcohol groups.



3.2 Occurrence

Prostaglandin any of a group of naturally occurring, chemically related fatty acids that stimulate contractility of the uterine and other smooth muscleand have the ability to lower blood pressure, regulate acid secretion of the stomach, regulate body temperature and platelet aggregation, and control inflammation and vascular permeability; they also affect the action of certain hormones. Nine primary types are labeled A through I, the degree of saturation of the side chain of each being designated by subscripts 1, 2, and 3. The types of prostaglandins are abbreviated PGE2, PGF2α, and so on.

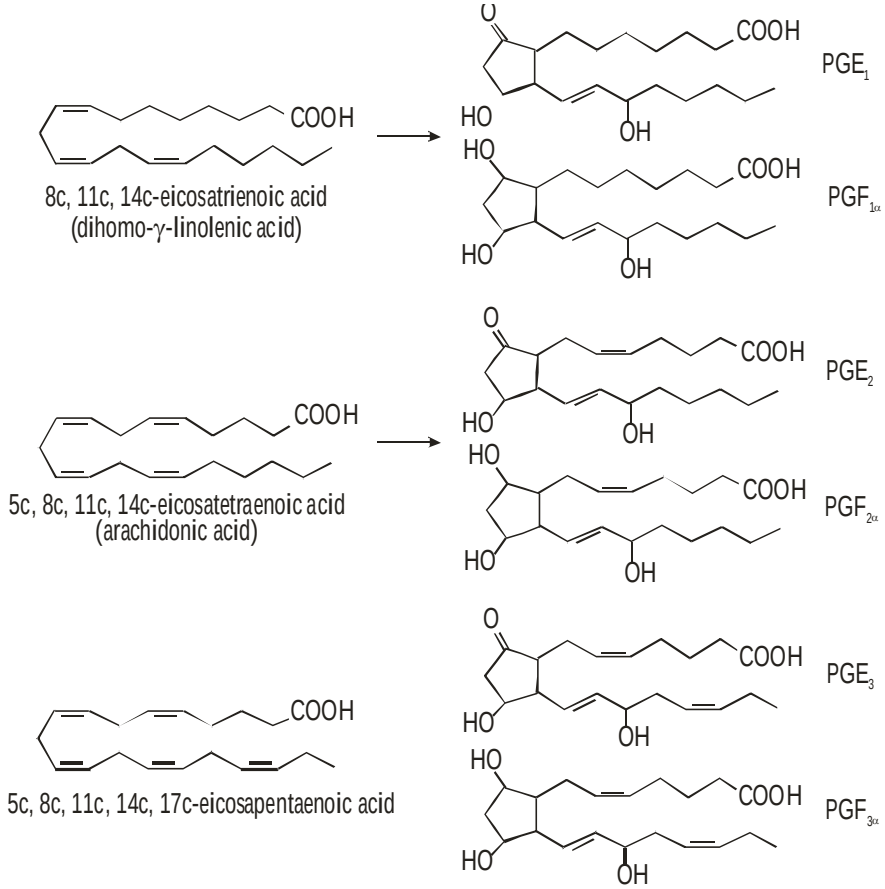
3.3 Nomenclature

In the approved nomenclature, each prostaglandin is named using the prefix 'PG' followed by a letter A to K depending on the nature and position of the substituents on the ring. Thus PGA to PGE and PGJ have a keto group in various positions on the ring, and are further distinguished by the presence or absence of double bonds or hydroxyl groups in various positions in the ring. PGF has two hydroxyl groups while PGK has two keto substituents on the ring. PGG and PGH are bicyclic endoperoxides. An oxygen bridge between carbons 6 and 9 distinguishes *prostacyclin (PGI). Thromboxane A (TXA)* contains an unstable bicyclic oxygenated ring structure, while thromboxane B (TXB) has a stable oxane

ring. In addition, all prostaglandins have a hydroxyl group on carbon 15 and a *trans*-double bond at carbon 13 of the alkyl substituent (R_2).

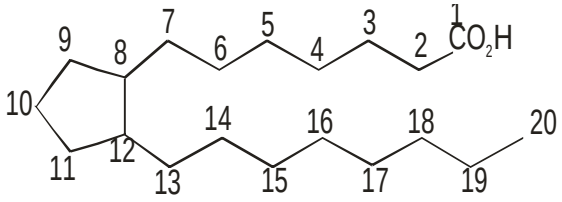
Further, a numerical subscript (1 to 3) is used to denote the total number of double bonds in the alkyl substituents, and a Greek subscript (α or β) is used with prostaglandins of the PGF series to describe the stereochemistry of the hydroxyl group on carbon 9. This is illustrated for prostaglandins PGE and PGF_α of the 1, 2 and 3 series below, as examples.

The number of double bonds depends on the nature of the fatty acid precursor. Thus, the prostaglandins PGE_1, PGE_2 and PGE_3 are derived from 8*c*,11*c*,14*c*-eicosatrienoic (dihomo-γ-linolenic), 5*c*,8*c*,11*c*,14*c*-eicosatetraenoic (arachidonic) and 5*c*,8*c*,11*c*,14*c*,17*c*-eicosapentaenoic acids, respectively. Of these, PGE_2 is the most common and is involved in many physiological processes. Dihomo-prostaglandins derived from adrenic acid (22:4(n-6) have also been detected in cell preparations, but no such compounds are produced from docosahexaenoic acid .



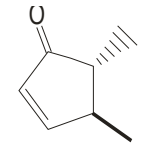
Numbering always starts at the carboxylic acid (1-position) and continues around

the molecule for the entire 20-carbon atoms. They are named as derivatives of prostanoic acid. Thus the PG designates the compound as a derivative of prostanoic acid.

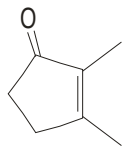


Prostanoic Acid

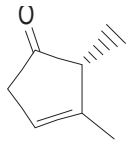
The next letter refers to the type of functionality present at the 8-12 and 15-positions. The origin of these letters refers back to the methods used in the original isolation of these compounds. Thus PGE was partitioned into an Ether layer, while PGF partitioned into phosphate buffer. This prostaglandin was isolated in Sweden where phosphate is (Fosfat). PGA and PGB were so named because of their stability to Acids and Bases. After that letters of the alphabet were just filled in - there is no significance to the letters.



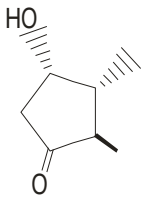
PGA



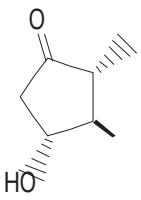
PGB



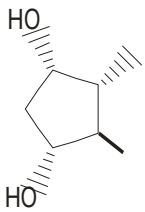
PGC



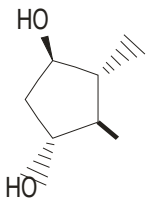
PGD



PGE



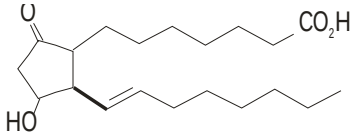
PGFα



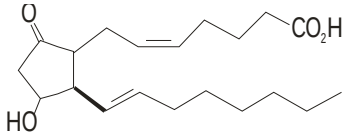
PGFβ

The subscripted number refers to the number of double bonds in the prostaglandin. Thus PGE_2 has 2 double bonds, one originating from the 5-position and the second

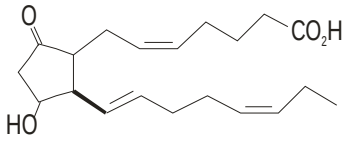
from the 13-position, wherease PGE_1 has only one double bond (originating from the 13-position) and PGE_3 has 3 double bonds (originating at the 5,13, and 17-positions).



PGE_1



PGE_2



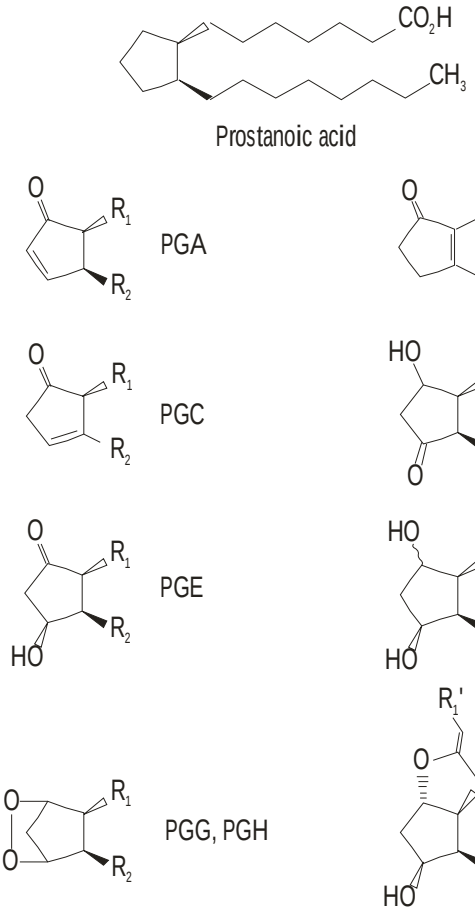
PGE_3

The subscripted Greek letter, as in $PGF_{2\alpha}$ refers to the orientation of the extra hydroxyl group on the ring. Note that in most prostaglandins there is a single hydroxyl group in the ring and another on the side chain at the 15-position and that they are always oriented a (behind the plane). $PGF_{2\alpha}$ has an extra hydroxyl on the ring and so its orientation must be specified.

3.4 Classification

The structure of PGs comprises of an oxygenated cyclopentane ring with a heptanoic acid side chain (α-side chain) and an octene side chain (ω-side chain) on adjacent positions of cyclopentane and such a basic structural unit is referred to as a prostanoic acid. PGs differ from the other eicosanoids in the substitution model on the cyclopentane ring and the side-chains, and these differences are accountable for the various biological activities of the members of the prostaglandin family. PGs are generally classified as PGA, PGB, PGC, PGD, PGE, PGF, PGG, and PGH referring to the different oxygen functionalities in the cyclopentane ring substitution patterns. For each general PG class is sub-classified based on the degree of unsaturation (i.e., PGE1, PGE2, and PGF2). The letters and numbers that follow the initial PG abbreviation indicate the nature of the unsaturation and substitution. For example, the subscript 1 in PGE1 indicates one double bond in the side chains, while the 2 in PGE2 indicates two double bonds in the side chains.

Classification of Prostaglandins



3.5 Biogenesis and Physiological Effects

The prostaglandins are lipid mediators with physiological effects, such as regulation of the contraction and relaxation of smooth muscle tissue. They are synthesized in the cell from the essential fatty acids (EFAs).they have been implicated in inflammation, pain, pyrexia, cardiovascular disease, cancer, glaucoma, allergic rhinitis, asthma preterm labor, male sexual dysfunction and osteoporosis. Physiological action of the prostaglandins 1. Once a call responds to a prostaglandin by changing the intracellular concentrations of some key substance, those changes then trigger a sequence of reactions that produce a "physiological response" (i.e. a contraction, secretion, excitation, etc.). 2. Since the number or prostaglandins and the target cells is large, the effects of these hormones is very variable. 3. An interesting example of opposing action of a pair of prostaglandins is seen in blood coagulation. PGI2, produced by endothelial cells, acts on the local muscle to relax them, decreasing blood pressure and also acts with

a receptor on the platelet to inhibit aggregation. It has been suggested PGI2 synthesis prevent platelets from aggregating and/or sticking to vessel walls. When a vessel is injured, the platelets produce TXA2 which blocks PGI2 binding, thus permitting platelet aggregation. Furthermore, TXA2 either inhibits PGI2 synthesis, or it has a direct action on the smooth muscle cell opposite to that of PGI2, thus increasing the muscle contraction (and the low blood pressure). Inhibition of prostaglandin synthesis. 1. A major mechanism to explain the anti-inflammatory action of certain steroids has been traced to their inhibition of phospholipase A2. This inhibition caused a decrease of arachidonic acid (also the other polyenoic acids which are the precursors of the PG1 and PG3 series), and hence an inhibition of prostaglandin synthesis. 2. Cyclo-oxygenase is inhibited by non-steroidal anti-inflammatory agents such as aspirin, indomethacin and phenylbutazone. In fact the only known biochemical affect of aspirin is prostaglandin synthesis suppression via cyclooxygenase inhibition. Leukotrienes 1. Blood cells (Polymorphonuclear leukocytes, mast cells, etc.) appears to synthesize leukotrienes rather than prostaglandins from arachidonic acid. Again the story is complex. Each cell type specializes in which leukotrienes they synthesize or will responded to. 2. The regulatory first step involves a lipoxygenase enzyme. Lipoxygenase is not inhibited by aspirin, thus leukotriene production is reduced only by the anti-inflmmatory steroids. 3. Leukotriene C is synthesized by Mast cells and has been extremely potent muscle constractant that severely constricts the small airways of the lungs during an asthma attack. 4. Neutrophils synthesize another leukotriene, which alters cell motility and chemotaxis in the immune reponse. Cox is one of two isoenzymes. There are two primary cyclooxygenase enzymes: Cox1 and Cox2. Cox1 help maintain platelet and kidney function and are much needed to maintain homeostasis. Cox2 lead to the production of substances that cause acute or chronic discomfort in joints. Cox2 inhibitors stop the creation of cox2. Go to the Cox2 link on this site to read more about cox2. Aspirin and other traditional nonsteroidal antiinflammatory drugs (NSAIDs) inhibit the enzyme cyclooxygenase (COX), which is involved in the production of prostaglandins. Prostaglandins are intercellular messengers that are found in high concentrations at sites of chronic inflammation. They are capable of causing vasodilatation, increasing vascular permeability and sensitizing pain receptors. Although many NSAIDs were developed that block the action of COX, all produced gastritis in many patients especially the elderly, those patients with a prior history of peptic ulcer disease and

patients on corticosteroids. It is now known that there are two COX enzymes cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX- 2). The traditional NSAIDs bind to the active sites of both COX-1 and COX-2. Gastritis is caused by the inhibition of COX-1, which is a gastric COX that regulates mucosal cell production of mucous. (The mucous acts as a barrier to the acid and pepsin present in gastric secretions.) Cox2 inhibitors Cox is an abbreviation for "cyclooxygenase." There are two primary cyclooxygenase enzymes: Cox-1 and cox-2. Cox1 helps maintain platelet and kidney function and are integral to maintaining homeostasis. Cox2 is one of several enzymes that lead to the formation of substances that can cause joint and connective tissue problems. Researchers discovered that cox2 enzyme is involved in several major diseases including: · Alzheimers · Rheumatic and Osteo-Arthritis · Cancer · Kidney disease · Osteoporosis A new class of cox2 inhibiting pain relief medications are beginning to enter the market. These new medications are reportedly safer alternatives to the current NSAIDs (Non-steroidal Anti-Inflammatory Drugs). The older medications can be problematic because of serious side effects: diarrhea, nausea, vomiting, kidney problems, liver problems, bleeding ulcers Therefore, drug companies are marketing safer pain medications, primarily for use on arthritis and related aches and pains.

PG Receptor	Endogenous Ligand	Signaling Pathway
EP_1	PGE_2	Increased Ca++ via PLC stimulation
EP_2	PGE_2	Increased cAMP via AC stimulation
EP_3	PGE_2	Decreased cAMP via AC inhibition
EP_4	PGE_2	Increased cAMP via AC stimulation
FP	$PGF_{2\alpha}$	Increased Ca++ via PLC stimulation

DP	PGD_2	Increased Ca++ via PLC stimulation
IP	PGI_2	Increased Ca++ via PLC stimulation
TP	TxA_2	Increased Ca++ via PLC stimulation

Some diuretics, such as furosemide, may act in part by releasing prostaglandins in the kidney. Prostaglandins inhibit the action of vasepressin on the kidney tubules, resulting in enhanced urinary excretion of water. The resultant tendency to dehydration from this enhanced excretion of water leads to local secretion of another kidney prostaglandin that stimulates the secretion of renin. Renin stimulates the production of aldosterone, which has the affect of conserving sodium and water, thus combating the dehydration and elevating the depressed blood pressure. Although prostaglandins were first detected in semen, no biologic role for them has been defined in the male reproductive system. This is not true, however, for females. It has been shown that prostaglandins mediate the control of GnRH over LH secretion, modulate ovulation, and stimulate uterine muscle contraction. Discovery of this last property has led to the successful treatment of menstrual cramps (dysmenorrhea) through the use of NSAIDs as inhibitors of prostaglandin synthesis. Prostaglandins also play a role in inducing labor in pregnant women at term or in inducing therapeutic abortions.

Summary of the Physiologic Actions of the Eicosanoids

Eicosanoid	**Biochemical and Physiologic Action**
PGD_2	• Weak inhibitor of platelet aggregation
PGE_1	• Bronchial Vasodilation • Inhibitor of lipolysis • Inhibitor of platelet aggregation • Contraction of GI smooth muscle

PGE_2	• Stimulates hyperalgesic response (sensitize to pain) • Renal and bronchial vasodilation • Inhibitor of platelet aggregation • Stimulates uterine smooth muscle relaxation • Cytoprotection: Protects GI epithelial cells from acid degradation • Reduces gastric acid secretion • Elevates thermoregulatory set-point in anterior hypothalamus (fever) • Promotes inflammation
PGF_2	• Stimulates breakdown on corpus luteum (luteolysis): Animals • Stimulates uterine smooth muscle contraction • Bronchial constrictor
PGI_2	• Potent inhibitor of platelet aggregation • Potent transient CV vasodilator, then vasodilator • Bronchial dilator • Uterine relaxant • Sensitize/amplify nerve pain response
TXA_2	• Potent inducer of platelet aggregation • Potent vasconstrictor (bronchioles, renal) • Decreases cAMP levels in platelets • Stimulates the release of ADP and 5-HT from platelets
LTB_4	• Increases leukocyte chemotaxis and aggregation
LTC/D_4	• Slow-reacting substance of anaphylaxis • Potent and prolonged contraction of ileal smooth muscle (Animals)

	• Contraction of lung parenchymal strips (Animals) • Bronchoconstriction in humans • Increased vascular permeability in skin (Animals)
5- or 12-HPETE	• Vasodilation of gastric cirulation (Animals)
5- or 12-PETE	• Aggregates human leukoctyes • Promotes leukocyte chemotaxis

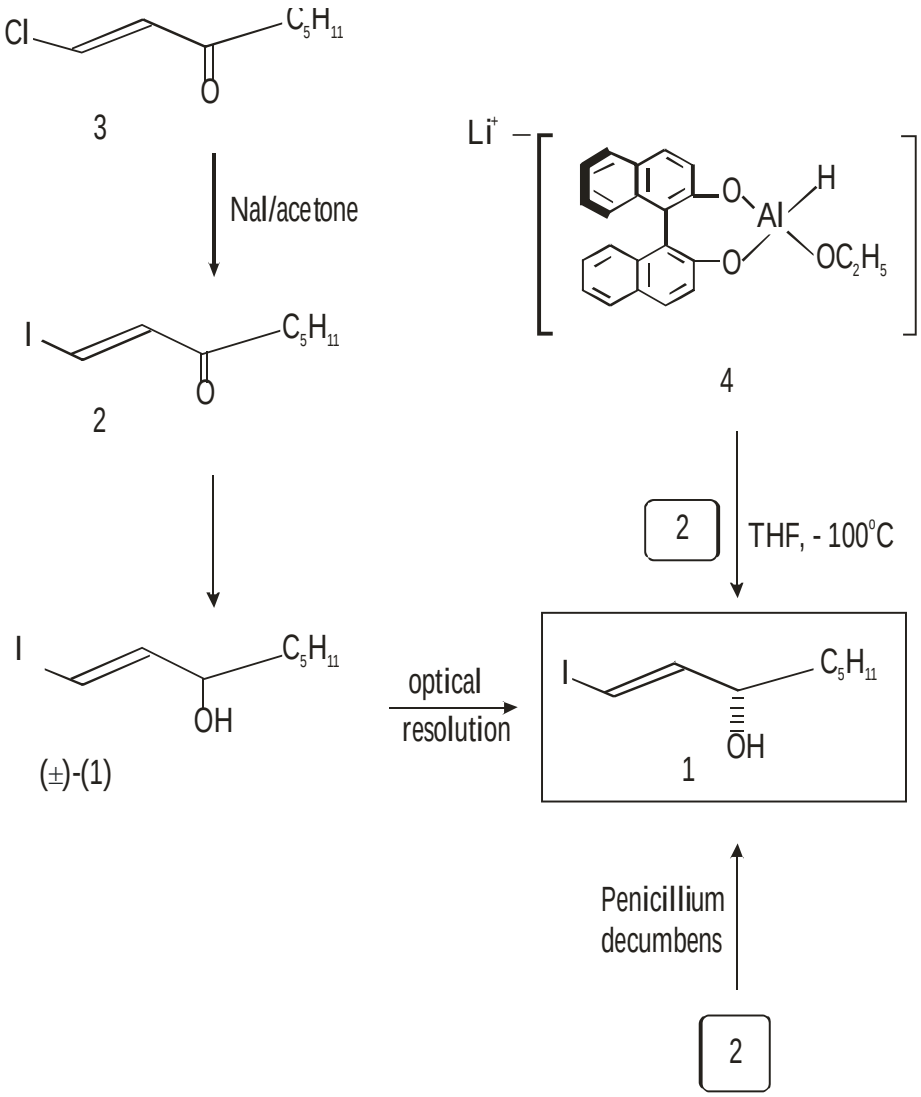
3.6 THE SYNTHESIS OF NATURAL E-SERIES PROSTAGLANDINS.

Because of the increasing demand for prostaglandins, biosynthesis which used to be the only source of these compounds became inadequate to meet this demand. Therefore, the development of an efficient PG chemical synthesis has been necessary as the only way to provide sufficient quantities of these compounds. A simplified retrosynthetic analysis of $PGF_{2\alpha}$, PGE_1, and PGE_2 reveals the synthons shown in scheme 1. The Corey synthesis which consists of a two-fold Wittig type chain extension of the chiral dialdehyde synthon shown in scheme 1 will not be discussed here since it is developed through the use of cyclic systems. The two other methods suggested by the retrosynthetic analysis both involve the development of the synthesis *via* conjugate addition type reactions and hence they are the subject of this review.

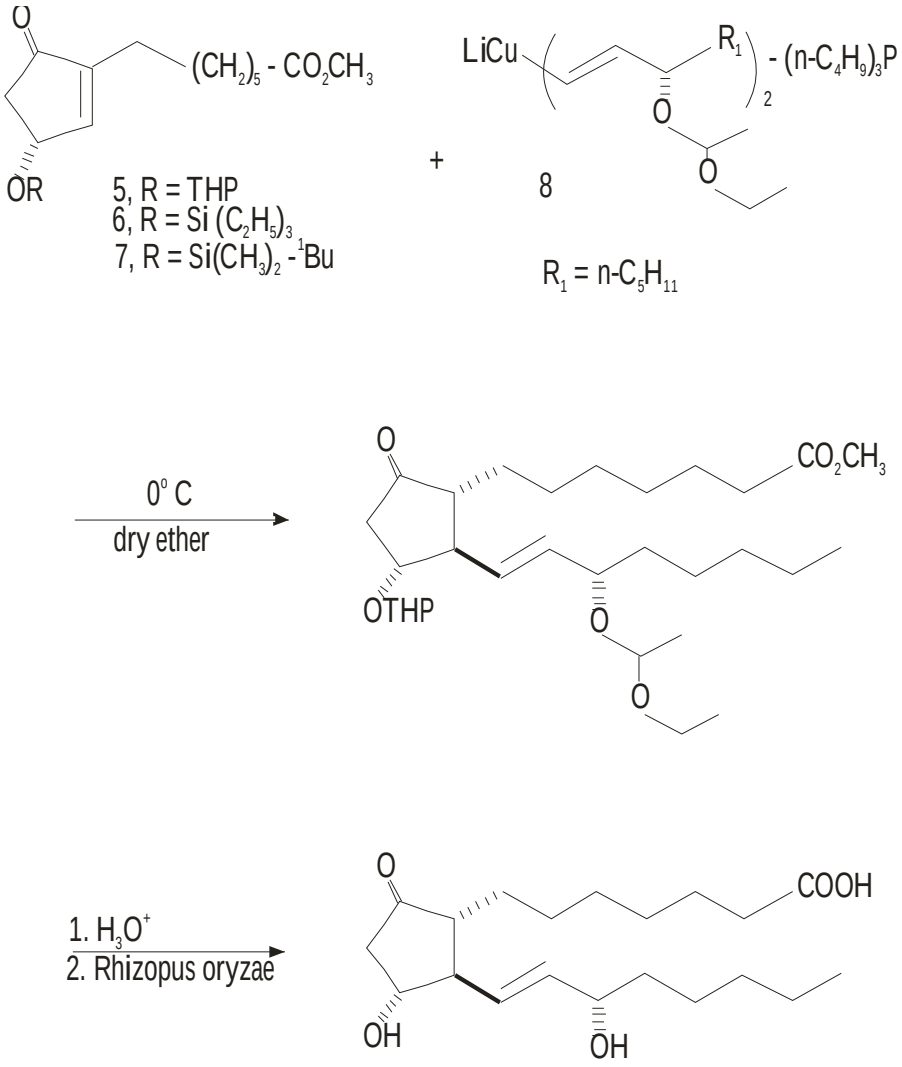
3.6.1(I) Synthesis of $PGE_{1\alpha}$, $PGE_{2\alpha}$, $PGE_{3\alpha}$

Organometallic reagents have played an important role in developing many synthetic methodologies to prostaglandins *via* the conjugate addition approach. The construction of PGs *via* the 1,4 addition of organocuprate reagents to a, f3-unsaturated ketones was initially developed by Sih and Fried in 1972. They found that the chiral organocuprate reagent **8** underwent conjugate addition to 2-cyclopenten-1-one to give exclusively the 1,4-addition adduct. Sih and his group later 4b developed this approach for the synthesis of natural PGE_1. They reported that addition of the chiral cuprate complex **8**, prepared from the organolithium derivative of the co side-chain, to the optically active cyclopentenone derivative **7** afforded, after deprotection, natural PGE_1 in good yield (scheme 2). This type of conjugate addition has been modified and improved extensively in the past ten

years as the search for more selective reagents and catalysts continued. Weiss and co-workers also developed a novel method for effecting the 1,4-conjugate additions to cyclopentenones using trialkyl-trans-1-alkenylalanates reagents. They reported the synthesis of PGE_1 and congeners via conjugate addition of alanate reagents, carrying the co side-chain of prostaglandin, to the cyclopentenone derivative (±)-1.



Scheme 1 : Synthetic routes to optically active trans-1-(S)-hydroxy-1-iodo-octene, 1



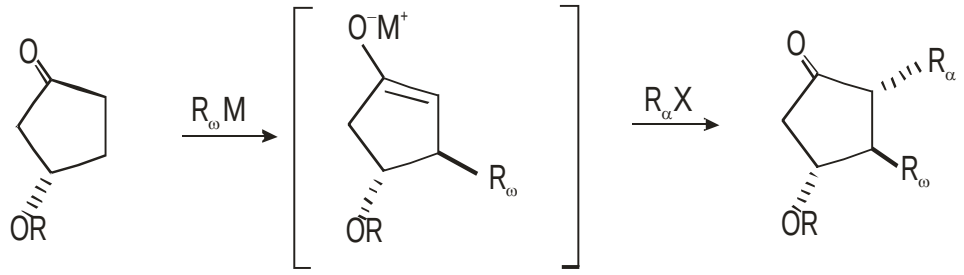
Scheme 2 : Synthesis of PGE1 by conjugate cuprate aaddition

II. **The three-component conjugate addition method.**

For directness and high flexibility, the three-component coupling process has been developed recently as the shortest and most convenient synthetic route for the preparation of PGs. As the

name suggests, the three-component coupling process is a one-pot combination of the cyclopentenone ring and the two side chains to construct the PG skeleton. The synthesis is initiated by a nucleophilic transfer of the m side-chain unit to a protected 4-(R)-hydroxy-2-cyclopentenone **9** followed by an electrophilic trapping of the enolate intermediate **10** with a side-chain equivalent (RαX) leading to the required prostaglandin skeleton 11 (scheme 3).

Since organocuprate reagents have been used to deliver organic groups to the position of a, unsaturated ketones, one might expect that conjugate addition of the $\bar{\omega}$ side-chain unit to the 4-hydroxy-2-cyclopentenone followed by alkylation of the resulting enolate with alkyl halides carrying the side-chain could lead directly to PGs derivatives. However, in reality, such a process is not easy to achieve (see below).



9 10 11

R is a suitable protecting group ,

$R_\omega M$ is the organometallic complex carrying the lower side chain of PG,

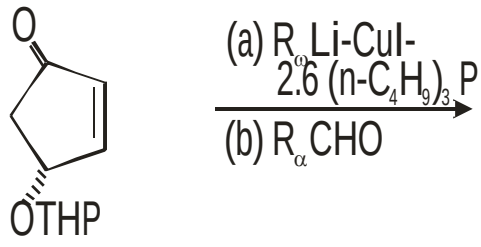
$R_\omega M$ = Li , Cu, etc.

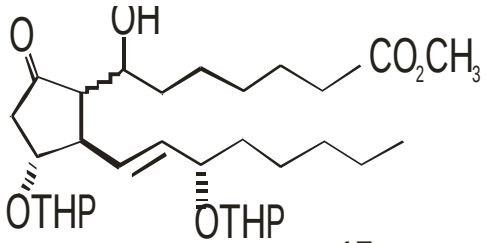
$R_\alpha x$ is the halide carrying the upper side chain of PG, Rex. X = I or Br.

Scheme 3 : Basic strategy of the three-component coupling process.

The second objective was to develop a method for the conjugate addition that allows clean enolate trapping which has been the troublesome step in the three-component coupling process. Organometallic reagents have been employed extensively to facilitate such coupling; however, they have to be used in excess in order to ensure the conjugate addition. When Noyori and his co-workers reinvestigated the use of excess of the $\bar{\omega}$ side-chain equivalent, they found that the excess organometallic compound only made the reaction system more complicated. This was rationalized by the fact that if the nucleophile carying the m side-chain is not used in one equivalent, the resulting enolate would not be the only strong nucleophile present in the reaction system. Thus, this would disturb the reaction between the enolate species and the $\bar{\omega}$. side-chain electrophile. In this context, they prepared the organocopper reagent from equimolar amounts of copper(I) iodide and the organo lithium compound (carrying the $\bar{\omega}$ side-chain) and 2-3 equivalents of tri-nbutylphosphine. When this reagent was allowed to react with 2-cyclopentenone, the conjugate addition product was obtained in high yield.

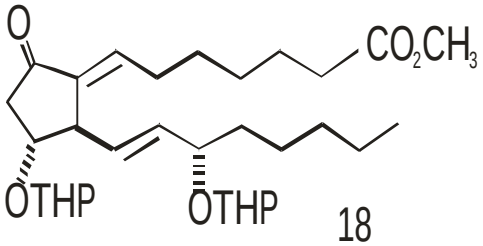
More importantly, the enolate was trapped efficiently with one equivalent of an aldehyde to give the aldol adduct On these bases, they synthesized PGE_1 in five steps 7, 9 via the three-component coupling process which recently10 came to be known as the Aldol route.





17

12, R = THP
13, R = $Si(C_2H_5)_3$
14, R = $Si(CH_3)_2$ - ^{1}Bu



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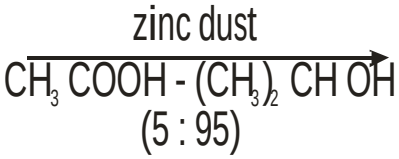

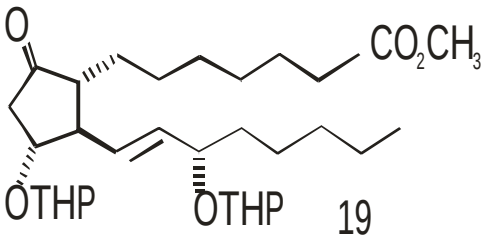

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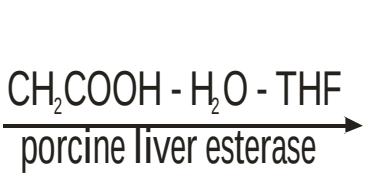
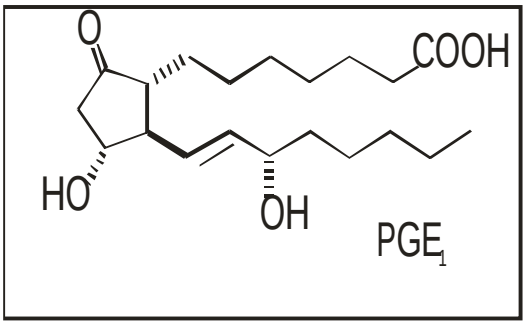
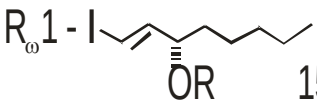




$R_\alpha CHO$ - OHC-$(CH_2)_5$ - $COOCH_3$



15, R = THP
16, R = $Si(CH_3)_2$ - ^{1}Bu

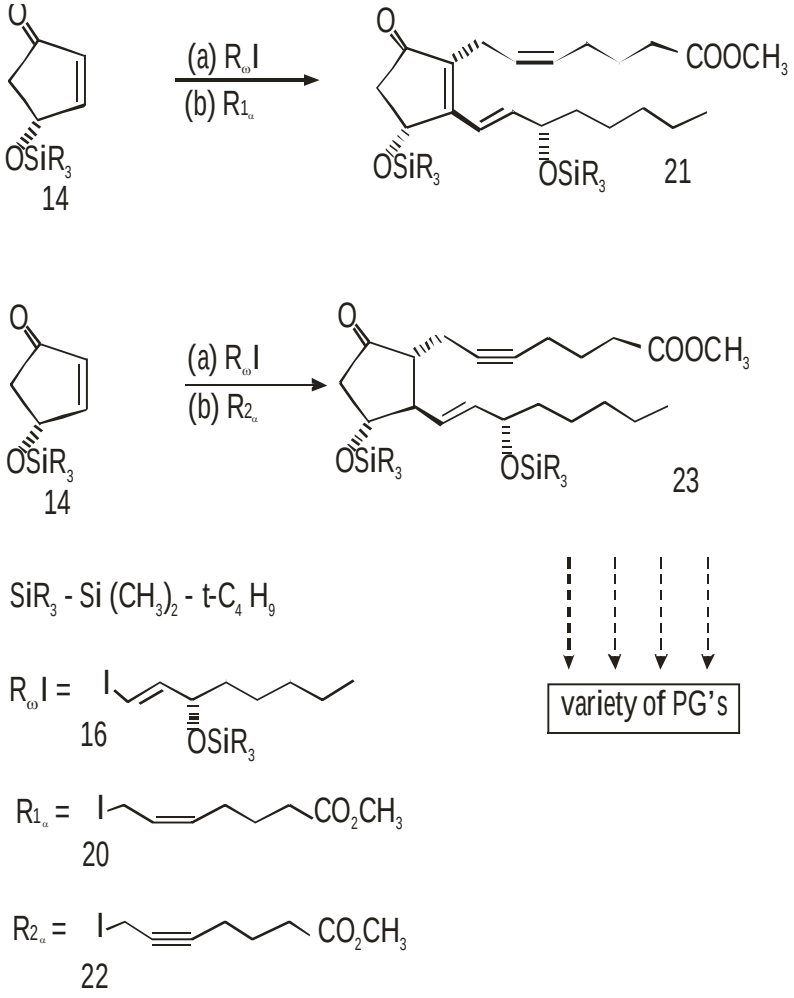
Scheme 4 . Convergent synthesis of PGE1 (aldol route).

The synthesis is illustrated in scheme 4. When the organocopper reagent formed from iodide **15** was coupled with the enone **12,** and the resulting enolate was trapped with methyl 6 formylhexanoate, the desired aldol **17** was obtained in 83% yield. Removal of the C-7 hydroxyl group by methanesulfonyl chloride and 4-(dimethylamino) pyridine then gave **18** in 92 % yield. Exposure of **18** to zinc dust in 2-propanol / acetic acid (95 : 5) gave **19** in 84% yield. The yield of **19** was improved to 90% when tributyl tin hydride and di-t-butylperoxide were used. Removal of the tetrahydropyranyl protective groups and subsequent enzymatic hydrolysis of the ester functional group afforded natural PGE1 in 56% overall yield.

Another, even shorter, synthesis of natural PGE1 using this three-component coupling process has been reported • The method involved the tandem conjugate addition of the lower side chain equivalent to enone **14** followed by Michael addition of the generated enolate across a nitroolefin carrying the $\bar{\omega}$-side chain to give the expected conjugate addition adduct which was easily transformed to PGE1. As illustrated in Scheme 4 the four chiral centres of natural PGE1 are constructed in an efficient way. The absolute configuration at C-11 and C-15 is established at the stage of preparation of starting enone and the $\bar{\omega}$ side-chain components. The trans relationship of the three ring-substituents is effected by the conjugate addition of the organo copper reagent and subsequent operations. The trans relationship is favoured kinetically because of steric interactions with the C-11 functionality. At this stage, it is important to emphasize the point that the synthesis of (-)-PGE1 presented above is still considered to be an indirect route for construction of the PGE1 skeleton. That is to say that although the synthesis employs the three-component coupling process mentioned above, subsequent operations had to be done in order to construct the required PGE1 derivative 19.

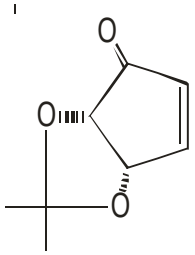
Recently, a three-step route to PGE2 was established through the tandem conjugate addition/alkylation sequence (scheme5). The treatment of the enone **14** with the organocopper reagent, prepared from **16** in the presence of hexamethyl phosphoric triamide (HMPA) and triphenyltin chloride, followed by addition of Zallylic iodide **20** afforded the PGE2 derivative **21** in 78% yield. This was a single-pot

preparation. Natural PGE2 is then obtained by removal of the protecting groups. When this three-component coupling is done with the proparyglic iodide **22,** the 5,6-dehydro-PGE2 derivative 23 is produced. Compound **23** serves as a common intermediate for the synthesis of some naturally occuring PGs. For example, partial hydrogenation of the 5,6 triple bond could be done over 5% Pd/BaSO4 catalyst to give PGE2. Unfortunately the above direct approaches do not work for the construction of PGE1 skeleton because a reactive a side-chain equivalents such as **20 or 22** would be needed to achieve efficient enolate trapping . Recently, the discovery that dimethyl zinc was found to enhance the alkylation of lithium enolates improved this triply convergent synthesis.



Scheme 5 : Direct three component coupling process for the synthesis of the PGE_2 derivative 21 and the 5, 6-dihydro-PGE_2 derivative 23.

Johnson and Penning succeeded in eliminating the equilibration of the enolate resulting from the conjugate addition of the $\bar{\omega}$ side-chain to the enone in the three component coupling process. They postulated that enolate equilibration would be suppressed in the presence of a-oxygen functionality constrained in the five membered ring. This requirement was satisfied by the enone 24 which was prepared in six steps in 40% yield from cyclopentadiene. When the conjugate addition was carried out, the trapping of enolate and subsequent alkylation went cleanly to afford the expected conjugate addition/ enolate trapping adduct in excellent yield.

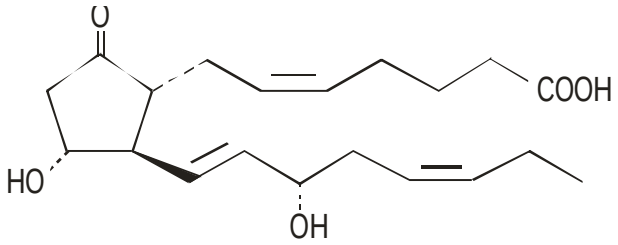


All the discussion above has dealt with PGE1 and PGE2 and nothing has been said concerning PGE3· This was done intentionally because most of the literature work has been done on PGE1 and PGE2. PGE3 is actually a PGE2 analogue in which there is additional

double bond between C-17 and C-18. Therefore most of the chemistry presented above should apply for PGE3. The difficulty in synthesizing PGE3 lies in the synthesis of the lower $\bar{\omega}$ side-chain with the extra double bond. Recently, Okamoto and coworkers developed a highly efficient synthesis of natural PGE3.

PGE_3 :

Prostaglandin E_3 (PGE_3) is formed *via* the cyclooxygenase (COX) metabolism of eicosapentaenoic acid.[1] In human ocular tissue, it comprises 2.4% of the COX products formed.[1] When applied to the eyes of a rabbit, a 1 µg dose of PGE_3 decreases intraocular pressure from 21 mmHg to about 17 mmHg.[2]



3.7 Summary

The many actions of the prostaglandins in reproductive physiology are truly remarkable, as is our rapidly expanding knowledge of these effects. Our knowledge remains far from complete, however, and much more research is needed to fully elucidate the role(s) of prostaglandins in many physiologic processes, particularly in areas such as luteolysis, where the hope is that these biologically active lipids may provide a method for regulating menstruation and fertility. Other important fields of clinical significance that deserve further attention are parturition and ductus arteriosus function. Further developments in prostaglandin research are likely to include the clinical application of more selective inhibitors, antagonists, and long-acting superpotent agonist analogues of prostaglandins.

3.8 Review Question

1. What molecule is the source of arachidonic acid and where is it found?
2. What role does arachidonic acid play in prostaglandin production.
3. What is the action of prostaglandins synthesis on diuretics ?
4. Outline the physiological roles of prostaglandins in the body.
5. Identify the prostaglandins that belong to the omega-3 and omega-6 prostaglandin.
6. Give the brief account of the synthesis of prostanoids highlight the clinical significance if any of this pathway.
7. How are prostaglandins catabolized in the body?
8. Discus the synthesis of PGE_1, PGE_2.

3.9 Reference and Suggested reading

- Textbook of organic chemistry, Vol II by I L Finar
- Chemistry of natural products, Vol 1- 12, by Atta-Ur-Rahman
- An introduction to the chemistry of terpenoids and steroids, by William Templeton
- Systematic identification of flavonoid compounds by Mabry & Markham

Unit - 4

Rotenoids and Porphyrins

Structure of the Unit:

4.1 Objectives

After study of this unit students will be able to understand

- Structure and synthesis of rotenoids
- Structure and synthesis of hemoglobin
- Structure and synthesis of chlorophyll

4.2 Introduction: Rotionoids

The organic compounds which are having Rotexen ring system are called rotenoids. Rotenoids are present in several tropical and subtropical plants. These are derived from isoflavone and found in same plants containing isoflavone. Rotanoids are isoflavnons that have been modified with one extra carbon atom.

Rotenone is an odourless, colourless, crystalline ketonic chemical compound used as an insecticide, piscicide, and pesticide. It is classified by world health organization as moderately hazardous and mildly toxic to humans and other mammels but it is extremely toxic to insects, pest and fishes. It occurs naturally in the seeds and stems of several plants, such as the jicama vine plant; and the roots of several members of Fabaceae.However, rotenone has a serious limitation to its widespread usage due to its susceptibility towards ultraviolet rays or solar irradiation. Owing to rotenone's high photolability, either breaking down or isomerizing in the presence of sunlight will decrease its bioactivities under field conditions, resulting in poor persistence in the environment and inadequate field performance.

4.2.1 Structure: Rotenone

The molecular formula of rotenone is $C_{23}H_{22}O_6$. It is a colourless crystalline compound having melting point 165-166ºC.

Rotenone (fig.1) was first isolated by Geoffroy in 1892 from *Lonchocarpus nicou*. Several other related compounds are also known and all of them possess the same fused tetracyclic skeletal structure named rotexen (fig.2). Rotenone is 6a,12a,4', 5'-tetrahydro-2,3-dimethoxy-5'-isopropenylfurano-(3',2',8,9) 6H-rotexen-12-one. Rotenone can be readily dehydrogenated using oxidizing agents such as potassium permanganate in acetone, potassium ferricyanide in methanol, perbenzoic acid in chloroform, manganese dioxide in acetone or iodine-sodium acetate in ethanol to obtain 6a, 12a-dehydrorotenone (fig.3). On treatment with alcoholic potash dehydrorotenone gives an unstable intermediate (fig.4) which undergoes further

hydrolytic cleavage to yield derrisic acid (fig.5). Derrisic acid, on oxidation with alkaline hydrogen peroxide, breaks down to derric acid (fig.6) which on further oxidation gives rissic acid (fig.7). When rotenone is subjected to a vigorous treatment with hot alkali, it undergoes extensive degradation to yield tubaic acid (fig.8).

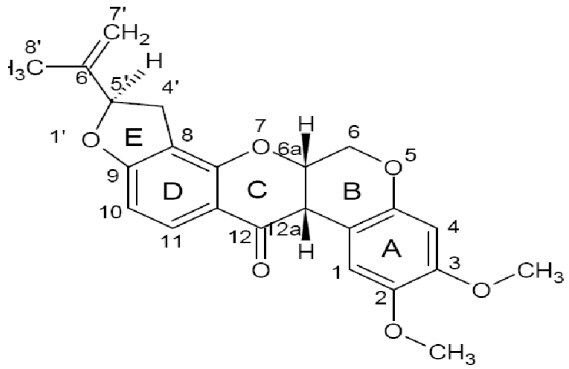


Fig.1

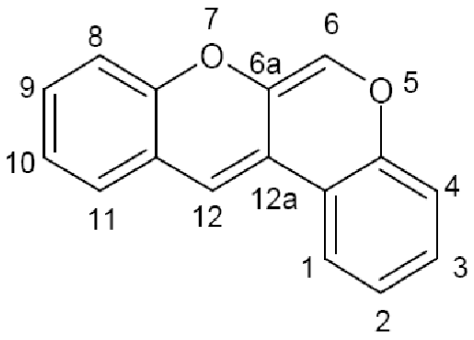


Fig. 2

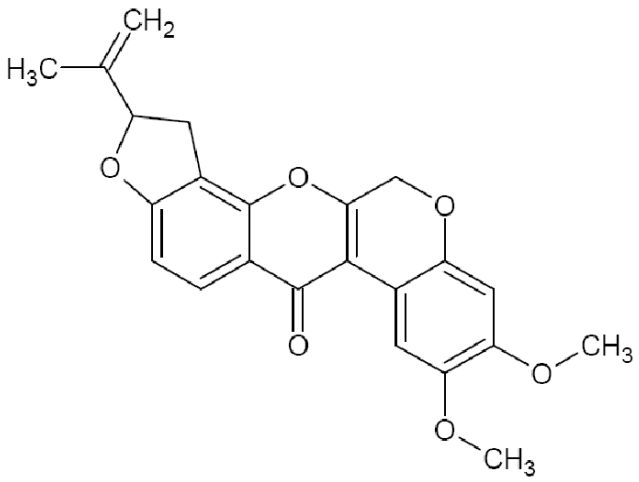


Fig.3

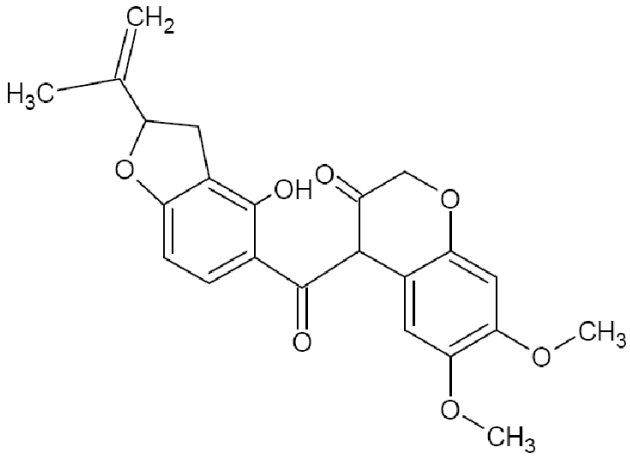


Fig.4

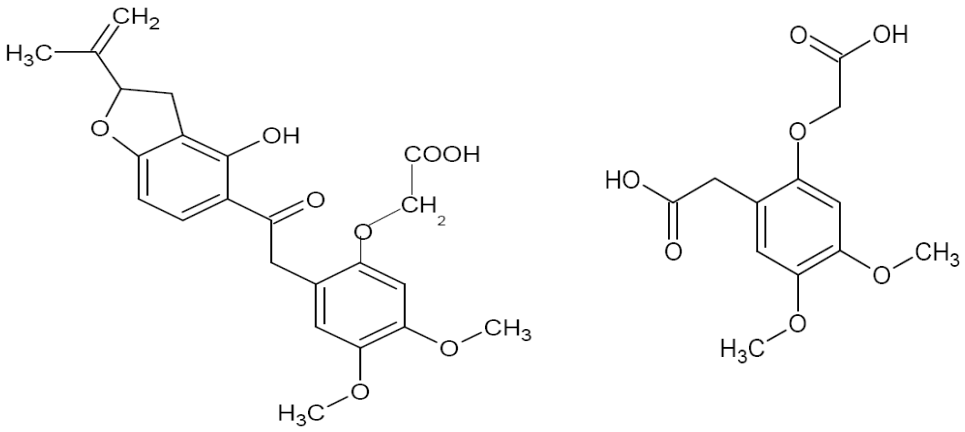
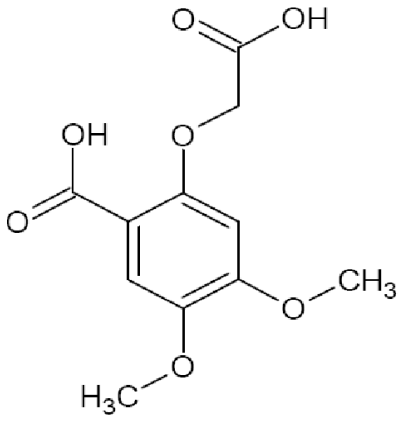


Fig.5

Fig. 6



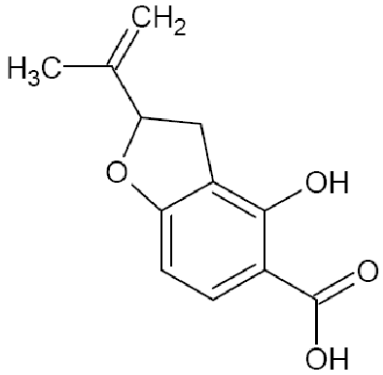
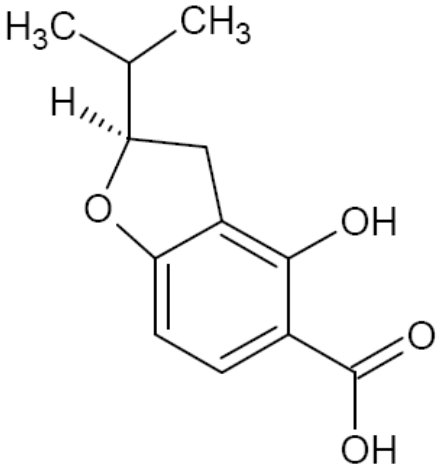


Fig.7

Fig.8

This salicylic acid derivative (it gives a violet colour with alcoholic ferric chloride) gives a dihydro derivative (Fig.9) on catalytic hydrogenation.It indicate presence of phenolic –OH group. Both (Fig.8) and (Fig.9) on prolonged hydrogenation yield the optically inactive tatrahydrotubaic aciod (Fig.10). The latter on decarboxylation, by heating to its melting point, gives 2-isoamylresorcinol (tetrahydrotubanol) (Fig.11).



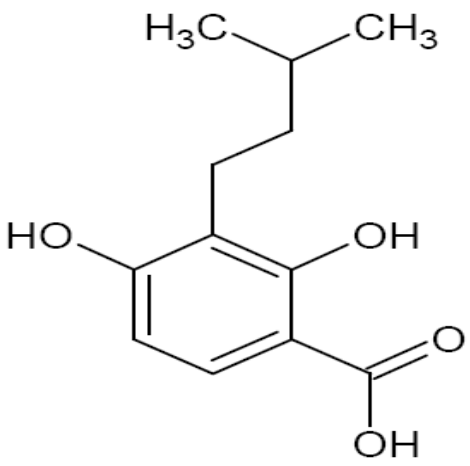


Fig.9 Fig.10

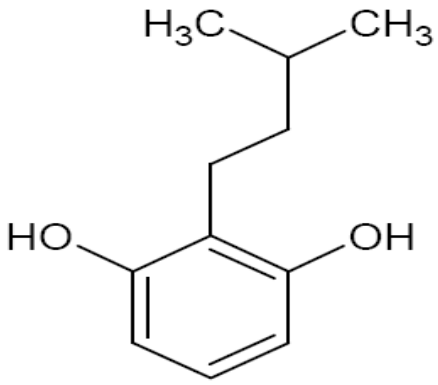


Fig.11

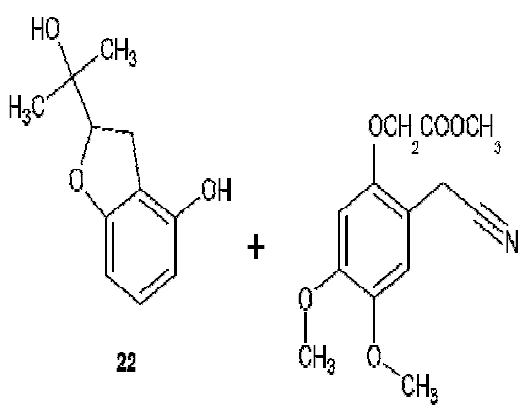
The reactions described above thus revealed the two parts of rotenone structure. Combining these data, the complete pentacyclic structure of rotenone was deduced.

Besides this derrisic acid forms a mono oxime derivative with NH_2OH. It gives negative test of aldehyde which indicate the presence of ketonic group. Zeisel method indicate the presence of two $–OCH_3$ group. On estrification with diazomethane derrisic acid produces a mono methyl ester, which indicate the presence of one –COOH group. The presence of carbonyl group in hydrogenation products points out that Tubanol is attached to derrisic acid through –CO- group in derrisic acid.

4.2.2 Synthesis of rotenone

The conversion of derrisic acid (Fig.**5**) to dehydrorotenone (Fig.**3**) was readily achieved but the selective hydrogenation of the latter to rotenone proved difficult.

Ultimately, in 1958, Miyano andMatsui succeeded in synthesising rotenone from dehydrorotenone by a two-step process involving reduction with sodium borohydride followed by Oppenauer oxidation of the resulting secondary carbinol. The first step is a Hoesch condensation between tubanol hydrate (Fig.12) and methyl derric acid nitrile (Fig.13). The resulting compound (Fig.14) on treatment with phosphorus tribromide in pyridine underwent dehydration to yield racemic methyl derrisate (Fig.15). On reaction with sodium acetate in acetic anhydride, racemic methyl derrisate give racemic dehydrorotenone (Fig.3). The meta rotenone obtained from (Fig.3) by the two-step reduction/oxidation mentioned above could be converted into (-)-rotenone by refluxing with carbon tetrachloride when the natural rotenone-CCl_4 separated out.



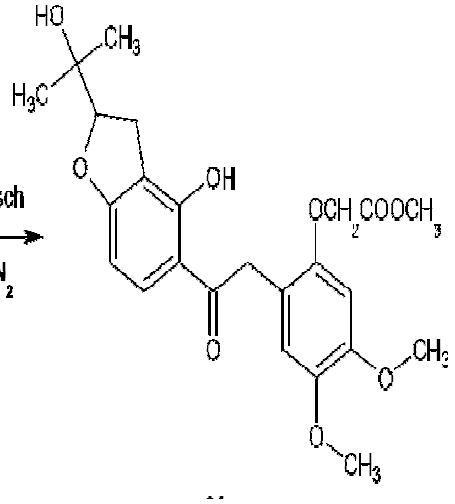
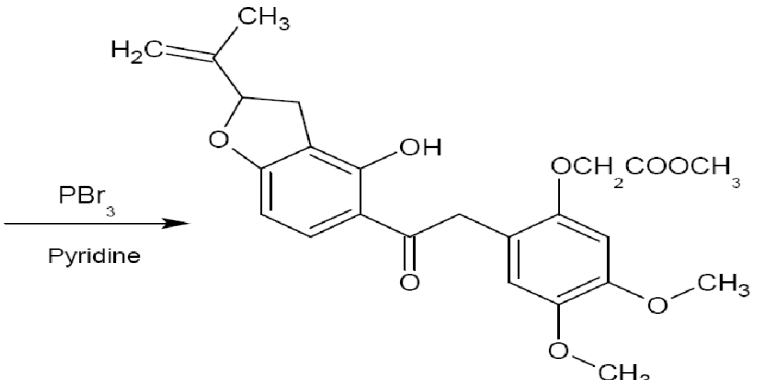


Fig.12 Fig.13 Fig.14



14

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Fig.15

Rotenone is difficult to handle in the pure state, as it is sensitive to light and oxygen, quickly decomposing to less toxic products.

Crombie and co-workers reported a novel and elegant synthesis of racemic isorotenone (Fig16 & 17).

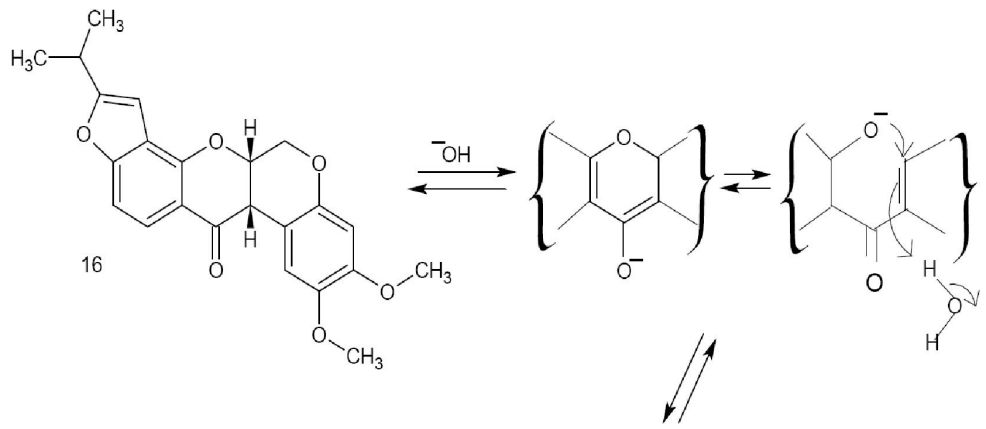


Fig.16

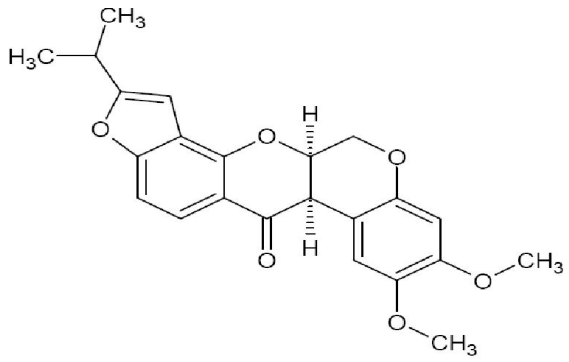


Fig.17

He used isoflavone (Fig.18) as the starting material. This compound, on treatment with dimethylsulphoxonium methylide (**Fig.19**) gave the vinyl coumaranone. On

treatment with pyridine, (**35**) underwent a rearrangement to yield the target compound. Using this method, other rotenoids, including rotenone,have been synthesized.

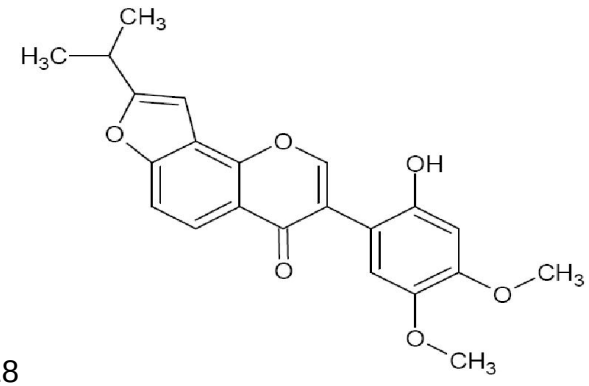


Fig.18

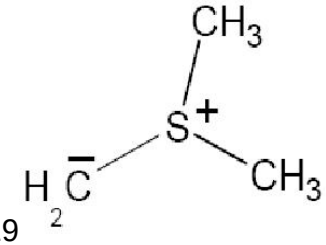
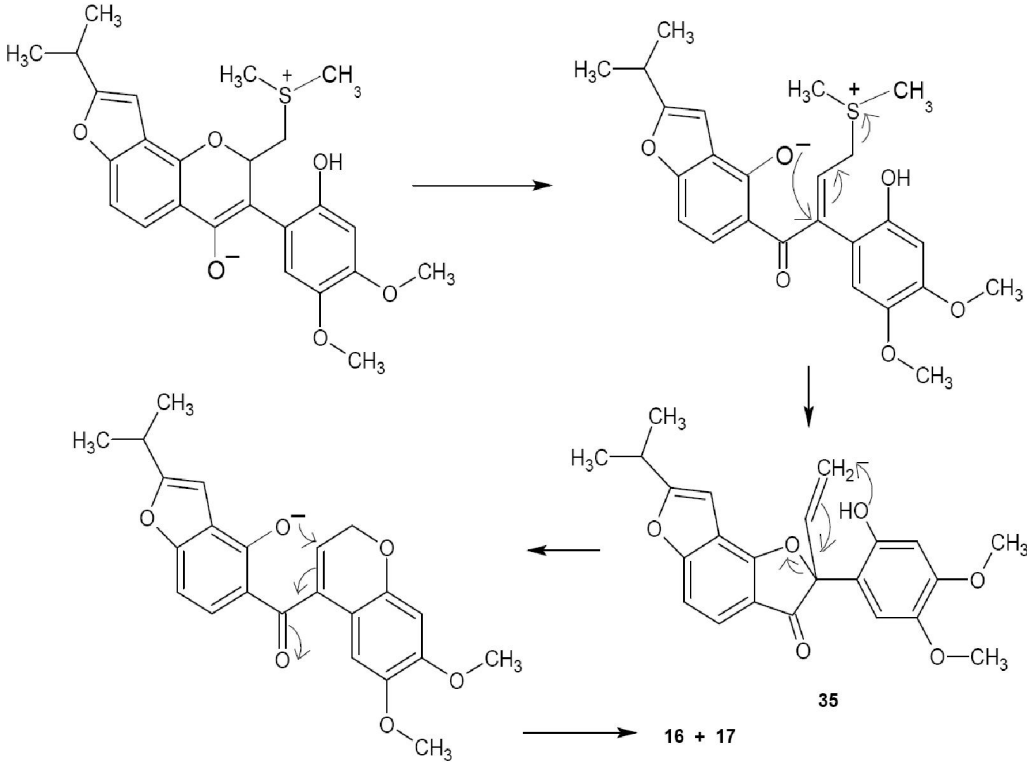


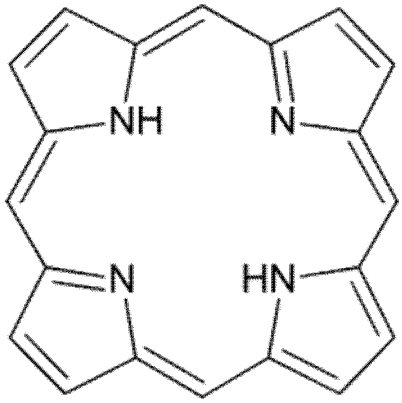
Fig.19



4.3 Introduction: Porphyrine

The natural porphyrine pigment contains a complex cyclic structure composed of four pyrrole subunits interconnected at their α carbon atoms via methine bridges (=CH–). Four pyrrole rings combine through methane bridges and resultant structure is known as porphins. Substituted porphines are called porphyrins.The porphyrin macrocycle has 26 (delocalized) pi electrons. Porphyrin macrocycles are highly conjugated systems and consequently they typically have very intense absorption bands in the visible region.

Two most important derivatives of porphyrrine are chlorophyll and hemoprotein(porphyrins combined with metals and protein).These compounds play vital role in biological activities.



Structure of porphine(Parent porphyrine)

4.3.1 Hemoglobin

Approximately one third of the mass of a mammalian red blodd cell is hemoglobin. Its major function is to carry oxygen from lunges through the arteries to the tissues and help to carry carbon dioxide through the veins back to lungs. The ability of hemoglobin to bind oxygen depends on the presence of a bound prosthetic group called heme. The heme group gives blood to its distinctive red color.

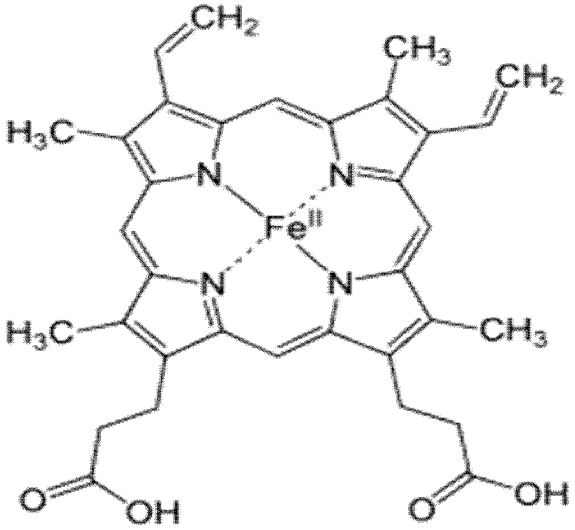
4.3.1.1 Structure of hemoglobin

Hemoglobin is mainly divided into two parts viz heme part and globin part.

(I) Heme structure:

Heme consists of an organic component and a central iron atom. The organic component called protoporphyrin, is made up of four pyrrol rings linked by methyl bridges to form a tetrapyrrole ring. Four methyl groups, two venyl groups, and two propionate side chains are attached.

The iron atom lies in the center of the protoporphyrin, bonded to the four pyrrol nitrogen atom. Under normal conditions, the iron is in the ferrous(Fe^{+2}) oxidation state. The iron ion can form two additional bonds, one on each side of the heme plane.These binding sites are called the 5^{th} and 6^{th} co-ordination sites. The iron ion lies approxmetly 0.4 Å outside the porphyrin plane because an iron ion, in this form, is slightly too large to fit into the well-defined hole within the porphyrin ring.The binding of O_2 molecule at 6^{th} co-ordination site of the iron ion substantially rearranges the electrons within the iron so that iron becomes effectively smaller, allowing it to move into plane of porphyrin.

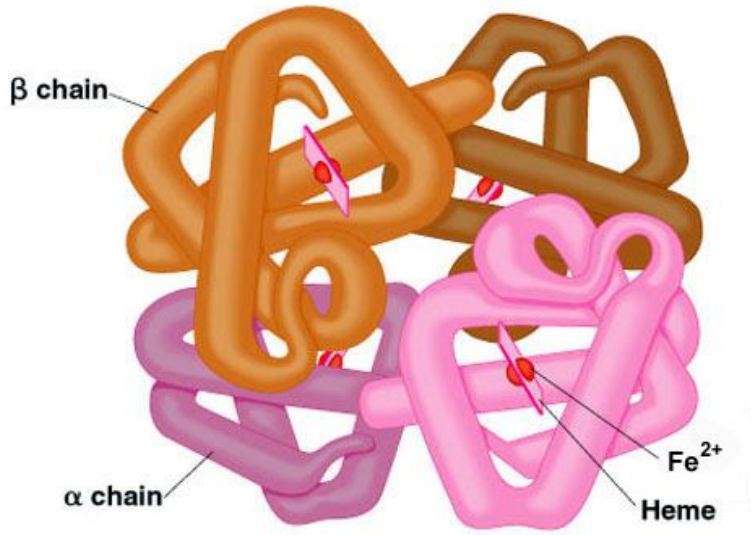


Structure of heme

(II) Protein Structure:

The hemoglobin molecule is made up of four polypeptide chains: two alpha chains of 141 amino acid residues each and two beta chains of 146 amino acid residues each. The alpha and beta chains have different sequences of amino acids,

but fold up to form similar three-dimensional structures. The four chains are held together by noncovalent interactions. There are four binding sites for oxygen on the hemoglobin molecule, because each chain contains one heme group. In the alpha chain, the 87th residue is histidine F8 and in the beta chain the 92nd residue is histidine F8. A heme group is attached to each of the four histidines. The hemoglobin molecule is nearly spherical, with a diameter of 55 Å. The four chains are packed together to form a tetramer. The heme groups are located in crevices near the exterior of the molecule, one in each subunit. Each alpha chain is in contact with both beta chains. However, there are few interactions between the two alpha chains or between the two beta chains.



Hemoglobin molecule

Each polypeptide chain is made up of eight or nine alpha-helical segments and an equal number of nonhelical ones placed at the corners between them and at the ends of the chain. The helices are named A-H, starting from the amino acid terminus, and the nonhelical segments that lie between the helices are named AB, BC, CD, etc. The nonhelical segments at the ends of the chain are called NA at the amino terminus and HC at the carboxyl terminus.

To form the tetramer, each of the subunits is joined to its partner around a twofold symmetry axis, so that a rotation of 180 degrees brings one subunit into congruence with its partner. One pair of chains is then inverted and placed on top

of the other pair so that the four chains lie at the corners of a tetrahedron. The four subunits are held together mainly by nonpolar interactions and hydrogen bonds. There are no covalent bonds between subunits. The twofold symmetry axis that relates the pairs of alpha and beta chains runs through a water-filled cavity at the center of the molecule. This cavity widens upon transition form the R structure to the T structure to form a receptor site for the allosteric effector DPG (2,3 diphosphoglycerate) between the two beta chains. The heme group is wedged into a pocket of the globin with its hydrocarbon side chains interior and its polar propionate side chains exterior.

4.3.1.2 Synthesis of hemoglobin

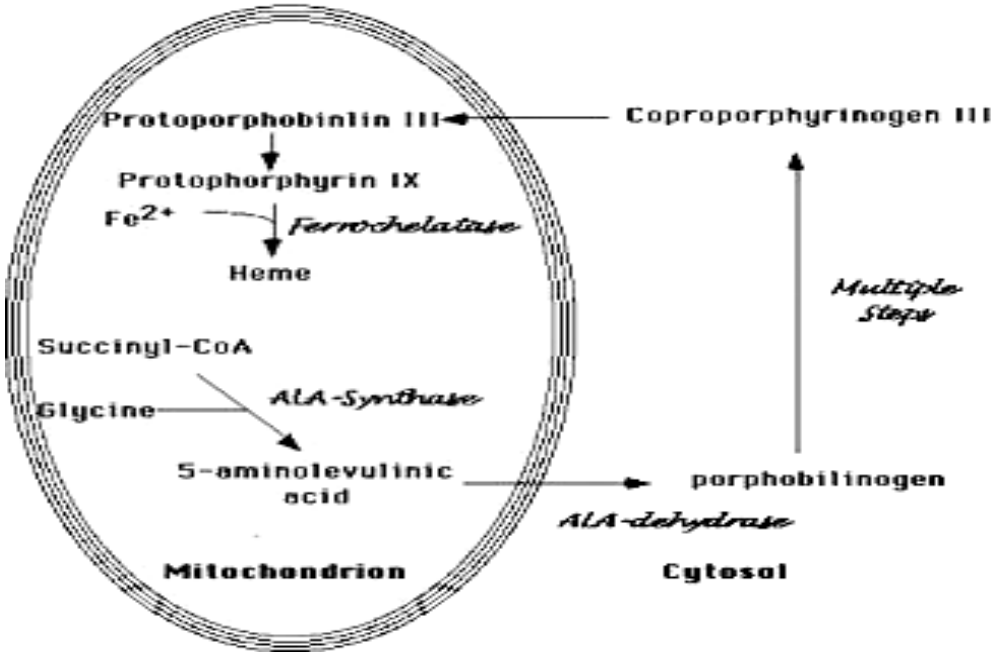
Hemoglobin synthesis requires the coordinated production of heme and globin. Heme is the prosthetic group that mediates reversible binding of oxygen by hemoglobin. Globin is the protein that surrounds and protects the heme molecule. Hemoblobin is synthesized inside developing red blood cells(In immature RBC's Cytosol) during Intermediate normoblast stages- It begins in the proerythroblasts and continues slightly even into the reticulocyte stage. Chemical steps in the formation of hemoglobin are as:

1. 2 alpha ketoglutonic acid(from Creb's cycle) + 2 glycine $\longrightarrow$ pyrrole
2. 4 pyrrole $\longrightarrow$ protoporphyrine
3. porphyrine + Fe^{+} $\longrightarrow$ heme
4. 4 heme + 4 polypeptide chain(2 alpha + 2 beta) $\longrightarrow$ 1 hemoglobin molecules

(i) Heme Synthesis:

Heme is synthesized in a complex series of steps involving enzymes in the mitochondrion and in the cytosol of the cell. The first step in heme synthesis takes place in the mitochondrion, with the condensation of succinyl CoA and glycine by aminolevulic acid synthase to form 5-aminolevulic acid (ALA). This molecule is transported to the cytosol where a series of reactions produce a ring structure called coproporphyrinogen III. This molecule returns to the mitochondrion where an addition reaction produces protoporhyrin IX.

The enzyme ferrochelatase inserts iron into the ring structure of protoporphyrin IX to produce heme. Deranged production of heme produces a variety of anemias. Iron deficiency, the world's most common cause of anemia, impairs heme synthesis thereby producing anemia. A number of drugs and toxins directly inhibit heme production by interfering with enzymes involved in heme biosynthesis.



Heme synthesis

(ii) Globin Synthesis:

Two distinct globin chains (each with its individual heme molecule) combine to form hemoglobin. One of the chains is designated alpha. The second chain is called "non-alpha". With the exception of the very first weeks of embryogenesis, one of the globin chains is always alpha. A number of variables influence the nature of the non-alpha chain in the hemoglobin molecule. The fetus has a distinct non-alpha

chain called gamma. After birth, a different non-alpha globin chain, called beta, pairs with the alpha chain. The combination of two alpha chains and two non-alpha chains produces a complete hemoglobin molecule (a total of four chains per molecule).

The combination of two alpha chains and two gamma chains form "fetal" hemoglobin, termed "hemoglobin F". With the exception of the first 10 to 12 weeks after conception, fetal hemoglobin is the primary hemoglobin in the developing fetus. The combination of two alpha chains and two beta chains form "adult" hemoglobin, also called "hemoglobin A". Although hemoglobin A is called "adult", it becomes the predominate hemoglobin within about 18 to 24 weeks of birth.

The pairing of one alpha chain and one non-alpha chain produces a hemoglobin dimer (two chains). Two dimers combine to form a hemoglobin tetramer, which is the functional form of hemoglobin.

4.3.2 Chlorophyll

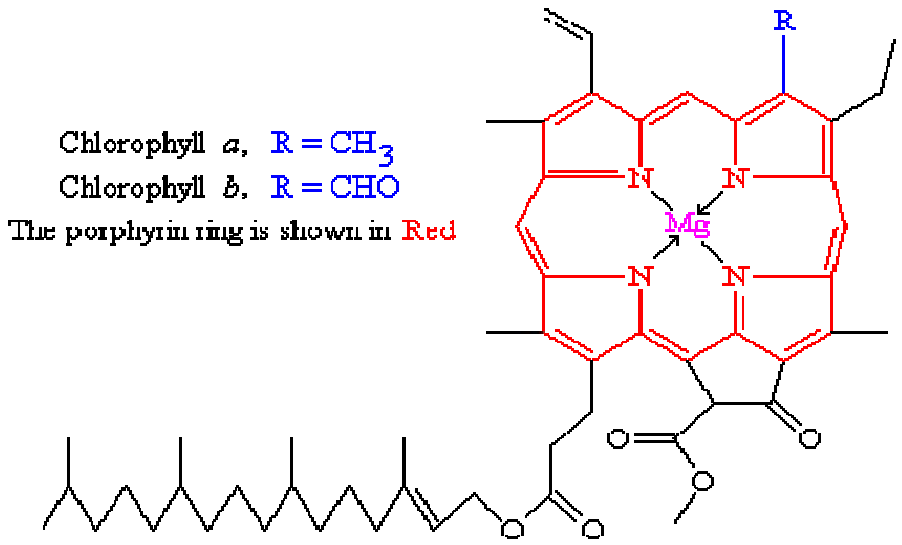
The trapping of light energy is the key to photosynthesis. The first event is the absorption of light by a photoreceptor molecule. The principal photoreceptor in the chloroplasts of most green plant is chlorophyll-a, a substituted tetrapyrrole. The four nitrogen atoms of the pyrroles are coordinated to a magnesium ion.

There are actually 2 main types of chlorophyll, named *a* and *b*. They differ only slightly, in the composition of a side chain (in *a* it is $-CH_3$, in *b* it is CHO). Both chlorophylls absorb light most strongly in the red and violet parts of the spectrum. Green light is absorbed poorly. Thus when white light shines on chlorophyll-containing structures like leaves, green light is transmitted and reflected and the structures appear green.

4.5.2.1 Structure of chlorophyll

In chlorophyll nitrogen atoms of four pyrrole rings coordinated to magnesium ion in a square planar arrangement. Thus in chlorophyll the central ion is magnesium, and the large organic molecule is a porphyrin. Unlike a porphyrin such as heme, chlorophyll has a reduced pyrrol ring and an additional 5-carbon ring fused to one of the pyrrol rings. Another distinctive feature of chlorophyll is the presence of phytol, a highly hydrophobic 20-carbon alcohol, estrified to an acid side chain.

There are several forms of chlorophyll. The structure of one form, chlorophyll *a*, is shown below-



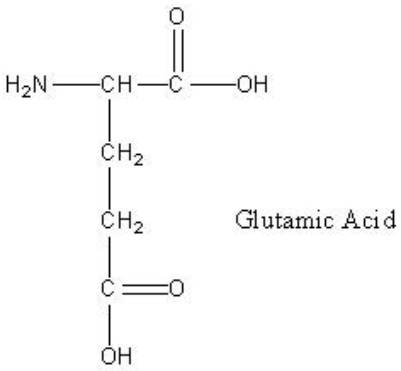
Note the system of alternating single and double bonds that run around the porphyrin ring. Although single and double bonds are drawn in fixed positions, but actually the "extra" electrons responsible for the double bonds are not fixed between any particular pair of carbon atoms but instead are free to migrate around the ring. This property enables these molecules to absorb light and make them effective photoreceptor. Chlorophyll a's peak molar extinction coefficient (a measure of a compound's ability to absorb light) is higher than 10^5 $M^{-1}cm^{-1}$, among the highest observed for organic compound.

4.5.2.2 Synthesis of chlorophyll

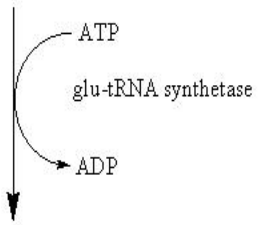
In higher plants, Chlorophyll is synthesized in chloroplasts through the cooperative activity of many enzymes. In the first phase of chlorophyll biosynthesis, the glutamic acid is converted to 5-aminolevulinic acid (ALA). This reaction is unusual in that it involves a covalent intermediate in which the glutamic acid is attached to a transfer RNA molecule. This is one of a very small number of examples in biochemistry in which a tRNA is utilized in a process other than protein synthesis. Two molecules of ALA are then condensed to form porphobilinogen (PBG), which ultimately form the pyrrole rings in chlorophyll. The next phase is the assembly of a porphyrin structure from four molecules of

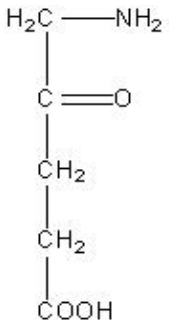
PBG. This phase consists of six distinct enzymatic steps, ending with the product protoporphyrin.

All the biosynthesis steps up to this point are the same for the synthesis of both chlorophyll and heme. But here the pathway branches and the fate of the molecule depend on which metal is inserted into the center of the porphyrin. If magnesium is inserted by an enzyme called magnesium chelatase, then the additional steps needed to convert the molecule into chlorophyll take place; if iron is inserted, the species ultimately becomes heme.

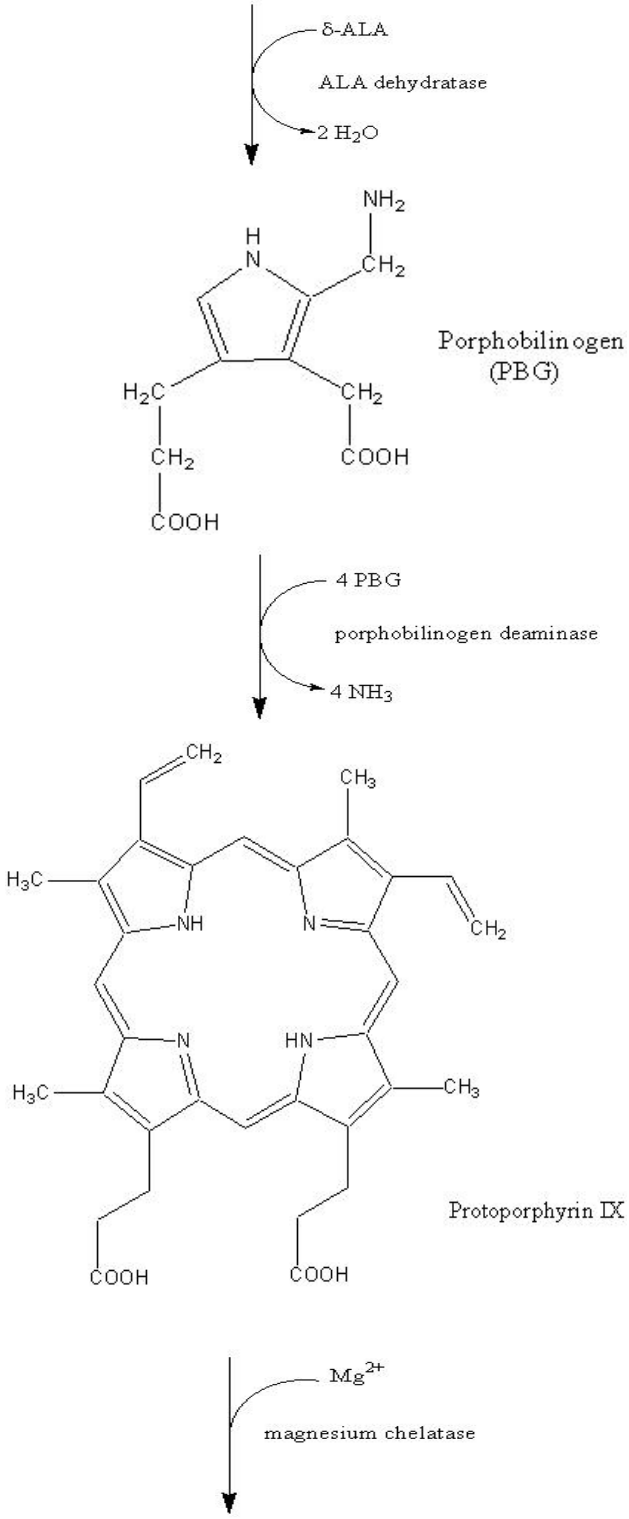


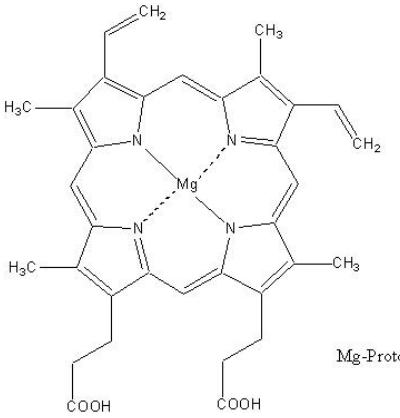
Glutamic Acid



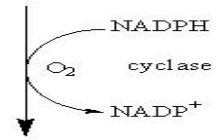


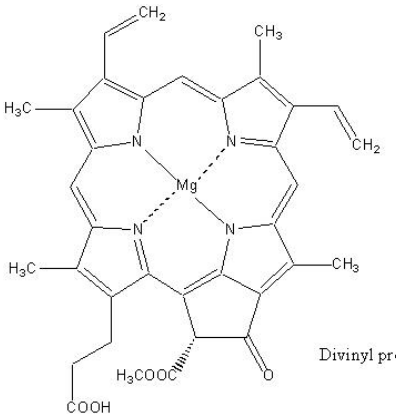
δ-Aminolevulinic acid
(δ-ALA)


δ-ALA
ALA dehydratase
2 H2O
NH2
H
N
CH2
H2C
CH2
CH2
COOH
COOH
Porphobilinogen
(PBG)
4 PBG
porphobilinogen deaminase
4 NH3
CH2
CH3
H3C
NH
N
CH2
N
HN
H3C
CH3
COOH
COOH
Protoporphyrin IX
Mg2+
magnesium chelatase

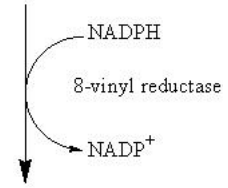


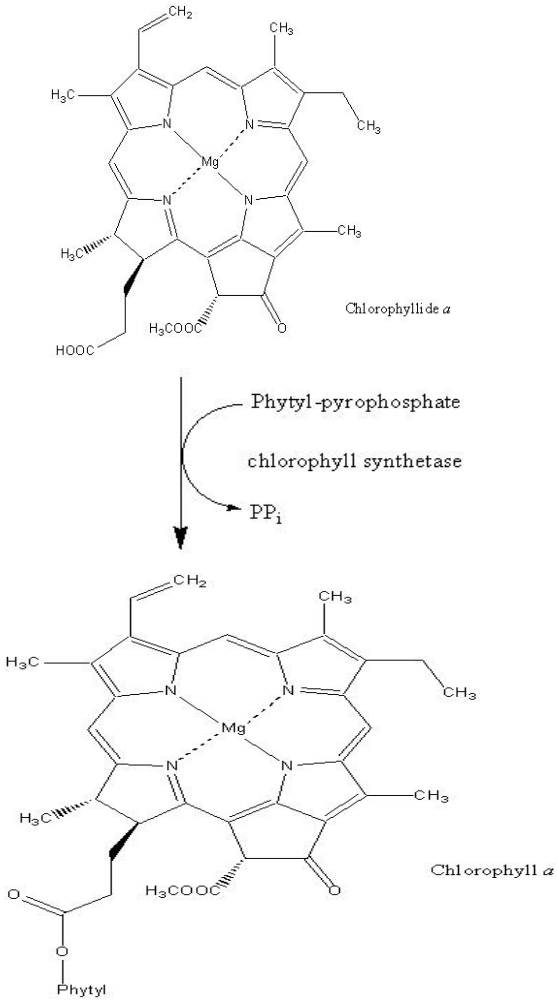
Mg-Protoporphyrin





Divinyl protochlorophyllide *a*





The next phase of the chlorophyll biosynthetic pathway is the formation of the fifth ring by cyclization of one of the propionic acid side chains to form protochlorophyllide. The pathway involves the reduction of one of the double bonds in ring, using NADPH. This process is driven by light in angiosperms and is carried out by an enzyme called protochlorophyllide oxidoreductase (POR). Seedlings of angiosperms grown in complete darkness lack chlorophyll, because the POR enzyme requires light. These *etiolated* plants very rapidly turn green when exposed to light. The final step in the chlorophyll biosynthetic pathway is the attachment of the phytol tail, which is catalyzed by an enzyme called chlorophyll synthetase.

4.4 Summary

The organic compounds which are having Rotexen ring system are called rotenoids. Rotenone is an odourless, colourless, crystalline ketonic chemical

compound used as an insecticide, piscicide, and pesticide. Roatnone can be synthesed from dehydrorotenone by a two-step process involving reduction with sodium borohydride followed by Oppenauer oxidation of the resulting secondary carbinol.

The natural porphyrine pigment contains a complex cyclic structure composed of four pyrrole subunits. Porphyrin macrocycles are highly conjugated systems and consequently they typically have very intense absorption bands in the visible region. Two most important derivatives of porphyrrine are chlorophyll and hemoprotein (porphyrins combined with metals and protein).These compounds play vital role in biological activities.

Approximately one third of the mass of a mammalian red blodd cell is hemoglobin. It consists of two parts: heme part and globin part(a protein). Heme consists of an organic component and a central iron atom. The hemoglobin molecule is made up of four polypeptide chains. Hemoblobin is synthesized inside developing red blood cells during Intermediate normoblast stages.

In chlorophyll nitrogen atoms of four pyrrole rings coordinated to magnesium ion in a square planar arrangement. Thus in chlorophyll the central ion is magnesium, and the large organic molecule is a porphyrin. Chlorophyll is synthesized by glutamic acid in chloroplasts through the cooperative activity of many enzymes.

Unit -5

Vitamins

Structure of unit

5.1 Introduction

Vitamins are organic substances which are necessory for the proper functioning of our body or an organic chemical compound is called a vitamin when the organism cannot synthesize the compound in sufficient quantities, and must be supplied through the diet. For example, ascorbic acid (vitamin C) is a vitamin for humans, but not for most other animal organisms. Vitamins are important nutrients found to be essential for life. Unlike other classes of nutrients, vitamins serve no structural function nor provide significant energy. Common food forms of most vitamins require some metabolic activation into a functional form. Vitamines have closely related chemical or functional similarities. Some vitamins function as coenzymes, others function as antioxidants, although some vitamins (A and D), act as hormones. In total, you need 13 vitamins for good health, and they were initially named in the alphabetical order that they were discovered. Since their initial discovery, this order has gone through some revisions, and the vitamins got somewhat shuffled around and classified into two main groups. So, the purpose of this lesson is to sort through the alphabet soup of vitamins and provide some help to remember how they are classified.

The term vitamin neither includes the essential nutrients, such as dietary minerals, essential fatty acids, or essential amino acids (which are needed in greater amounts than vitamins) nor the great number of other nutrients that promote health, and are required less often to maintain the health of the organism. Universally thirteen vitamins are recognized at present. Vitamins are classified on the basis of their biological and chemical properties, not their structure. Thus, each "vitamin" refers to a number of vitamer compounds that all show the biological activity associated with a particular vitamin. So vitamins are grouped under an alphabetized vitamin "generic descriptor" title, such as "vitamin A", which includes the compounds retinal, retinol, and four known carotenoids. Some of the vitamines are listed below with their discovery dates sources:

Year of discovery	**Vitamin**	**Food source**
1913	Vitamin A (Retinol)	Cod liver oil
1913	Vitamin B_1 (Thiamine)	Rice bran
1920	Vitamin C (Ascorbic acid)	Citrus, most fresh foods

Year of discovery	Vitamin	Food source
1920	Vitamin D (Calciferol)	Cod liver oil
1920	Vitamin B_2 (Riboflavin)	Meat, dairy products, eggs
1922	(Vitamin E) (Tocopherol)	Wheat germ oil, unrefined vegetable oils
1926	Vitamin B_{12} (Cobalamins)	Liver, eggs, animal products
1929	Vitamin K_1 (Phylloquinone)	Leafy green vegetables
1931	Vitamin B_5 (Pantothenic acid)	Meat, whole grains, in many foods
1931	Vitamin B_7 (Biotin)	Meat, dairy products, eggs
1934	Vitamin B_6 (Pyridoxine)	Meat, dairy products
1936	Vitamin B_3 (Niacin)	Meat, grains
1941	Vitamin B_9 (Folic acid)	Leafy green vegetables

5.2 General functions of vitamines

Vitamins perform many diverse physiochemical and biochemical functions. For eg. vitamin D, have hormone-like functions which act as regulators of mineral metabolism, or regulators of cell and tissues growth. Vitamin E and sometimes vitamin C used as antioxidants. The largest number of vitamins, the B complex vitamins, function as precursors for enzyme cofactors, that help enzymes in their work as catalysts in metabolism. Vitamins may be tightly bound to enzymes as part of prosthetic groups, For eg. biotin is part of enzymes involved in making fatty acids. Vitamines may be less tightly bound to enzyme catalysts as coenzymes,easily detachable molecules that function to carry chemical groups or electrons between molecules. For example, folic acid may carry methyl, formyl, and methylene groups in the cell. Vitamines also role in the assisting of enzyme-substrate reactions.

Vitamins are essential for the normal growth and development of a multicellular organism. For eg. a fetus begins to develop, at the moment of conception, from the nutrients it absorbs. It requires certain vitamins and minerals for growth. These nutrients facilitate the chemical reactions that produce among other body parts, skin, bones and muscle. If there is serious deficiency in one or more of these nutrients, a child may develop a deficiency disease. Even minor deficiencies may cause permanent damage of the organs. Once growth and development are completed, vitamins remain essential nutrients for the healthy maintenance of the cells, tissues, and organs that make up a multicellular organism. Dietary supplements contain vitamins, but may also include other ingredients, such as minerals, herbs. Scientific evidence supports the benefits of dietary supplements for persons with certain health conditions. In some cases, vitamin supplements may have unwanted effects, especially if taken before surgery, with other dietary supplements or medicines, or if the person taking them has certain health conditions. They may also contain levels of vitamins many times higher, and in different forms, than one may ingest through food.

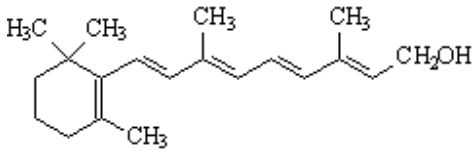
Naming of some vitamins:

Previous name	**Chemical name**	**Cause of name changing**
Vitamin B_4	Adenine	DNA metabolite; synthesized in body
Vitamin B_8	Adenylic acid	DNA metabolite; synthesized in body
Vitamin F	Essential fatty acids	Needed in large quantities (does not fit the definition of a vitamin).
Vitamin G	Riboflavin	Reclassified as Vitamin B_2
Vitamin H	Biotin	Reclassified as Vitamin B_7
Vitamin J	Catechol, Flavin	Catechol nonessential; flavin reclassified as Vitamin B_2
Vitamin L_1	Anthranilic acid	Non essential

Previous name	Chemical name	Cause of name changing
Vitamin L_2	Adenylthiomethylpentose	RNA metabolite; synthesized in body
Vitamin M	Folic acid	Reclassified as Vitamin B_9
Vitamin O	Carnitine	Synthesized in body
Vitamin P	Flavonoids	No longer classified as a vitamin
Vitamin PP	Niacin	Reclassified as Vitamin B_3
Vitamin S	Salicylic acid	Proposed inclusion of salicylate as an essential micronutrient
Vitamin U	S-Methylmethionine	Protein metabolite; synthesized in body

5.3 General properties of vitamins

Vitamin A is a thermally stable in oxygen-free environment and can tolrate the heat of 60, 100 and 120°C. But, at the air at higher temperatures (about 60°C) it decomposes rapidly, especially under acidic conditions. Sunlight also promotes vitamin A decomposition.



Vitamin A

This chapter introduce with the fundamental chemistry of the vitamins i.e. water soluble and fat soluble e.g. tocopherols and tocotrienols relevant to their antioxidant action. The general agreement that α-tocopherol is the most efficient antioxidant and vitamin E homologue *in vivo*, there was always a considerable discrepancy in its absolute and relative antioxidant effectiveness *in vitro*, especially when compared to γ-tocopherol. Many chemical, physical, biochemical, physicochemical, and other factors seem responsible for the observed discrepancy between the relative antioxidant potencies of the tocopherols *in vivo* and *in vitro*.

This paper aims at highlighting some possible reasons for the observed differences between the tocopherols (α-, β-, γ-, and δ-) in relation to their interactions with the important chemical species involved in lipid peroxidation, specifically trace metal ions, singlet oxygen, nitrogen oxides, and antioxidant synergists. Although literature reports related to the chemistry of the tocotrienols are quite known, they also were included in the discussion in virtue of their structural and functional resemblance to the tocopherols.

Solubilities of eight different species of the fat-soluble vitamins A, D, E, and K in supercritical carbon dioxide were measured atdifferent range of temperature and pressure. Solubilities have been determined by an analytical method using the direct coupling of an equilibrium cell to a supercritical fluid chromatographic system with UV detection.

5.4 Classification of vitamins

Fourteen substances are in nature now generally recognized as vitamins. Vitamins are described according to their solubility, they may be fat or water soluble. This method of classification and their discovery as labeled by McCollum as "fat-soluble A" and "water-soluble B." Our body needs to consume 14 different vitamins to maintain normal health.The important vitamines are the vitamin B complex (folate, B_{12}, B_6, biotin, pantothenic acid, niacin, riboflavin and thiamine) as well as vitamins A, C, D, E and K. The each vitamin is essential for different functions of the animal and human body. In most cases, people are able to get sufficient vitamins simply from consuming a diet that is well-balanced. The main classification for vitamins is based on solubility as some are soluble in water while others are soluble in fat. The vitamins which are soluble in fat are stored by the body and therefore can accumulate. On the other hand, the kidneys flush out water soluble vitamins. Another way that some people classify vitamins is based on how they were obtained: either from food or naturally from food. This method, however, can become complicated because many of the foods we consume on a daily basis are vitamin fortified. On the basis of their soloubilities vitamines can be classified in to two categories.

5.4.1 Fat soluble vitamines

Vitamins are classified on the basis of their solubility or in other words, the vitamin's ability to dissolve into another substance (solvents). For eg. fat-soluble

vitamins are the vitamins which dissolve in fat. Because fat is easily stored in our body, fat-soluble vitamins can be stored within your fat. This means they can accumulate and be saved for later use. Because the body stores fat-soluble vitamins in its cells, they are not flushed out as simply as the water-soluble vitamins. This means that they do not require as frequent ingestion as water-soluble vitamins but you still need sufficient amounts. It is important to remember that consuming too much of fat-soluble vitamins can cause toxicity. We are particularly sensitive to high levels of vitamin D as well as high levels of vitamin A specifically from animal sources. Simply consuming a balanced diet should provide sufficient fat-soluble vitamins.

The fat-soluble vitamins are A, D, E and K. Now these four letters represent four different vitamins, but if you try to pronounce them like they spell a word, then you might pronounce them as 'attic.' So, a great little memory jogger for recalling the fat-soluble vitamins is 'The fat cat is in the ADEK (attic).' These vitamins are important for the normal functioning of your body. For example, carrots are so important for eyes, so you could see better at night. This is because carrots contain vitamin A, which helps with vision. Playing in sunshine may be helpful for skin. This was also good advice because exposure to the sun helps your body make vitamin D, which is a vitamin that helps calcium absorption for healthy bones. Vitamin E helps with your 'immunity-E' because it works as an antioxidant protecting your cells from free radicals. And vitamin K is needed for blood clotting, or would it help you recall this fact if you spelled clotting with a 'K' and thought of vitamin K as the blood 'K-lotting' vitamin.

Vitamin Name	**Function**	**Dietary Sources**
Vitamin A	Vitamin A helps with healthy mucous membranes and skin, vision, tooth and bone growth and the health of the immune system.	**From retinol (animal sources)**: liver, eggs, fortified margarine, butter, cream, cheese, fortified milk. **From plant sources (beta-carotene)**: dark orange vegetables (pumpkin, sweet potatoes, winter squash, carrots) and fruits (cantaloupe, apricots); dark green leafy vegetables.

Vitamin K	Vitamin K is required for correct blood clotting.	Vegetables from the cabbage family, leafy green vegetables, milk; it is also produced in the intestinal tract by the bacteria.
Vitamin E	Vitamin E is an antioxidant and helps protect the cell walls.	Nuts and seeds, egg yolks, liver, whole-grain products, wheat germ, leafy green vegetables and polyunsaturated plant oils (safflower, cottonseed, corn, soybean).
Vitamin D	Vitamin D is stored in the bones and is required to properly absorb calcium.	Fortified margarine, fortified milk, fatty fish, liver, egg yolks; the skin can also produce vitamin D when it is exposed to sunlight.

Vitamin A. The active forms of vitamin A participate in three essential functions: visual perception, cellular differentiation, and immune function. A number of food sources are available for vitamin A. Preformed vitamin A is abundant in animal foods and provitamin A carotenoids are abundant in dark-colored fruits and vegetables.

Dietary forms of vitamin A and provitamin A carotenoids		
Consumed	**Absorbed**	**Bioconverted**
Dietary or supplemental Vitamin A	Retinol	Retinol
Supplemental beta-carotene	beta-carotene	Retinol
Dietary beta-carotene	beta-carotene	Retinol
Dietary alpha-carotene or beta-cryptoxanthin	alpha-carotene or beta-cryptoxanthin	Retinol

Relative carotene concentration increases when consumed with oil or associated with plant matrix material. That is part of the plant vitamin source, not separated out as a supplement. The presence of dietary fat stimulates the secretion of bile acids and improves the absorption of carotenoids.

The richest sources of vitamin A are fish oil, liver, and other organ meats. Whole milk, butter, and fortified margarine and low-fat milks are also rich in the vitamin.

In the United States carrots, fortified spreads, and dairy products are the leading sources of vitamin A to the diet.

Vitamin D. Vitamin D is essential for life in human beings and animals. It is one of the most important regulators of calcium homeostasis and was historically considered the "anitrachitic" factor. The biological effects of vitamin D are achieved only by its hormonal metabolites, including two key kidney-produced metabolites: 1,25$(OH)_2$ vitamin D and 24,25(OH) vitamin D. In addition to its role in calcium metabolism, research has identified that vitamin D plays an important role in cell differentiation and growth of keratinocytes and cancer cells and has shown that it participates in the process of parathyroid hormone and insulin secretion (Bouillon et al. 1995).

Vitamin D_3, the naturally occurring form of the vitamin, is produced from the provitamin, 7-dehydrocholesterol, found in the skin under the stimulation of ultraviolet (UV) irradiation or UV light. Vitamin D_2 is a synthetic form of vitamin D that is produced by irradiation of the plant steroid ergosterol. A requirement for vitamin D has never been precisely defined because vitamin D is produced in the skin after exposure to sunlight. Therefore, humans do not have a requirement for vitamin D when sufficient sunlight is available. The fact that humans wear clothes, live in cities where tall buildings block the sunlight, use synthetic sunscreens that block UV rays, and live in geographical regions of the world that do not receive adequate sunlight contributes to the inability of the skin to synthesize sufficient vitamin D.

A substantial proportion of the U.S. population is exposed to suboptimal levels of sunlight during the winter months. Under these conditions, vitamin D becomes a true vitamin and must be supplied regularly in the diet.

The use of 1,25 $(OH)_2$ vitamin D for treatment of hypoparathyroidism, vitamin D–resistant rickets, renal osteodystrophy, osteoporosis, and psoriasis opens the door for potential toxicity because this form of the vitamin is much more toxic and the body's metabolic controls are bypassed. When this medication is being used, careful monitoring of plasma calcium concentrations is required.

Salt-water fish are good unfortified sources of vitamin D. Small quantities are derived from eggs, beef, butter, and vegetable oils. Fortification of milk, butter, margarine, cereals, and chocolate mixes help in meeting the dietary requirements. Excessive amounts of vitamin D are not available in usual dietary sources.

However, excessive amounts can be obtained through supplements that result in high plasma levels of 25(OH) vitamin D.

Vitamin E. Vitamin E (also called tocopherol) is found in cell membranes and fat depots. Because of their chemical structure, there are eight stereoisomers of each of the tocopherols. In addition to each of the stereoisomers, each occur in alpha, beta, gamma, and delta forms.

The various forms of vitamin E have different biological activity, with the natural source isomer—*R*,*R*,*R*,-alpha-tocopherol—being the most active. In supplements you may see this isomer called by its former name, *d* -alpha-tocopherol. Synthetic vitamin E is called *ll -rac* -alpha-tocopherol or *dl* -alpha-tocopherol in supplements. Because of the many forms of vitamin E in plants available synthetically, the relative activities of each form is complex. Current evidence indicates that vitamin E from natural sources has approximately twice the bioactivity in humans that the *ll-rac* (synthetic) vitamin does.

The tocopherol content of foods varies widely depending on storage, processing, and preparation. The best sources of vitamin E are the common vegetable oils and products made from them. However, most of the tocopherols may be removed in processing. Wheat germ and walnuts also have high amounts of tocopherols.

Vitamin K. Vitamin K was named after the first letter of the German word *Koagulation*. For many years blood coagulation was assumed to be the sole physiological role for vitamin K. We now know that vitamin K plays an essential role in the synthesis of proteins including prothrombin and the bone-forming protein, osteocalcin. Dietary vitamin K absorption is enhanced by dietary fat and is dependent on bile and pancreatic enzymes. The human gut contains large amount of bacterially produced vitamin K, but its contribution to the maintenance of vitamin K status has been difficult to assess. The vitamin K produced by bacteria in the gut is less biologically active even though it is stored in the liver and present in blood. Current understanding supports the view that vitamin K source may partially satisfy the human requirement but the contribution is much less than previously thought.

The drug warfarin, widely prescribed as an anticoagulant, functions through inhibition of vitamin K. As a result, alterations in vitamin K intake can influence the efficacy of warfarin. The effective dose of warfarin varies from individual to individual, as does the dietary intake of vitamin K. The best solution appears to be

to establish the necessary dose of warfarin and urge patients to maintain a constant intake of foods high in vitamin K in their diets. Only a small number of food items contribute substantially to the dietary vitamin K.

Collards, spinach, and salad greens are high in vitamin K. Broccoli, Brussels sprouts, cabbage, and Bib lettuce contain about two-thirds as much, and other green vegetables contain even less. Vitamin K is also found in plant oils and margarine, with soybean and canola oils having the highest amounts. U.S. food intake surveys indicate that spinach, collards, broccoli, and iceberg lettuce are the major contributors of vitamin K in the diet.

Vitamines and their deficiency diseases

Vitamin A:

Properties :

- Soluble in fat and insoluble in water
- Viscous, colorless oil or pale yellowish substance
- Heat stable in absence of air

Source of Vitamin A

Liver, heart, kidney, milk, codliver oils, fishliver-oils, butter, eggs, carrots, cabbage, vegetables, green leaves, mangoes, potatoes tomatoes, spinach, papaya etc.

Functions

- Effect on reproductive processes, differentiation, and immune system
- Essential for growth and night vision
- Required for bone and teeth formation, influence genetic expression, reproduction to manufacture R.B.C etc.
- Maintain the health and activity of epithelial tissues, and glands prevent infection, maintains nutrition and function of the nervous tissue.
- Controls the action of bone cells and formation, helps in normal fertility and glucose synthesis.
- Acts as antioxidant.
- Helps in RNA and protein metabolism.

Vitamin A Deficiency Diseases

- Night-blindness, Xerophthalmia, Keratinisation of skin and mucous membrane.
- Retardation of growth in children, defective growth of bone and teeth, skin lesions, Bitot's, sports etc.
- Abnormalities in respiratory, GU and GI epithelium, Diarrhoea, Kidney stone, bladder disorders, infections of vagina, depression of immune reactions, anaemia, injury to brain and nerve causes paralysis, stunted skull and spine.

Vitamins D (Cholecalciferol)

Properties

- Soluble in fat solvents but insoluble in water
- Heat stable
- White crystalline material
- Ordinary boiling does not destroy it.

Source

Fish liver oils e.g., cod liver oil, halibut - liver oil etc. Butter, milk, eggs, liver. In sub coetaneous tissue, 7 dihydrocholesterol is conveted to vitamin D by UV light.

Functions

- Control calcium and phosphorus absorption from the small intestine, concerned with calcium metabolism, helps in the bone and teeth formation.
- Minimize the losses of calcium and increases phoshate excretion by the kidneys, affects insulin secretion in pancreases.

Deficiency Disease

Causes Rickets (directive bone growth) in childless, osteomalacia in adults, disturbs calcium and phosphorous absorption.

A knowledge of the minerals, vitamins and other substances needed for the human body to function at optimum levels is very useful and worthy of deep study. It has been shown that, probably due to modern intensive farming methods, the levels of vitamins and minerals found in fresh vegetables etc is significantly lower now than they were in the past. These initial lower levels along with over cooking,

processing, microwave cooking etc means that it is a challenge in the modern age to eat healthily. Residual pesticides and chemical addictives that increase shelf life or "improve" flavor etc further reduce the nutritional value of the food generally available. For this reason the wise person, avoiding morbid obbsession, takes an interest in the quality of food that they consume.

Nutritional supplements, whilst largely unnecessary in past ages as a general rule, now have a place in the modern diet. Apart from any physical symptoms a diet lacking essential vitamins can effect the mental/psychological and emotional well being as well leading to depression, laziness, agression and poor memory etc etc. On this page I will collect a list of properties for the wide range of minerals and vitamins that are relevent to human wellbeing.

5.4.2 Water-soluble Vitamins

If a vitamin is not fat-soluble, then it is classified as water-soluble vitamins. Because our body is a watery environment, these vitamins can move through our body very easily, and they can also be flushed out in urine. So, our body does not store water-soluble vitamins. Water-soluble vitamins are able to freely travel throughout the body and any unnecessory quantities are usually flushed out by the kidneys. Small doses of water-soluble vitamins are required by the body and this type of vitamin is not as likely to approach toxic levels as fat-soluble vitamins. In addition, vitamin C, choline, folate, vitamin B_6 and niacin have higher consumption limits. Consumption of high levels of vitamin B_6 during long periods of time can cause irreversible nerve damage.

Vitamins B-complex and vitamin C both are water-soluble vitamins. The B vitamins were initially thought to be just one vitamin, but later it was discovered that they were a group of vitamins with different characteristics, this is why B vitamins have numbers and different names. There are eight B vitamins, including vitamin B_1, B_2, B_3, B_5, B_6, B_7, B_9 and B_{12}. Some of the vitamins, such as vitamin B_6 and B_{12}, are usually referenced by their numbers, but all eight of these B vitamins have a corresponding name. In order, their names are thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folate and cobalamin. Now, remembering all of the names of the B vitamins can seem a bit like trying to figure out a riddle, so it might help you to recall the B vitamins by using the mnemonic, 'These Riddles Need Practice, Practice Builds Future Character.'

The B-complex vitamins are important for energy, so it might help you to think of the B vitamins as 'Busy bees that are full of energy.' More specifically the B vitamins convert energy from the nutrients you eat into ATP, which is the energy your body runs on. The majority of B vitamins are generally found in foods from all of the food groups; however, getting enough B_{12} can be a bit tricky. Vitamin B_{12} is lacking in grains, fruits and vegetables but found in meats and dairy products. Because of this, strict vegetarians sometimes have to plan their diets carefully to ensure that they are getting enough B_{12}.

Most people are able to consume sufficient quantities simply by consuming a balanced diet. However some vegetarians as well as those over 50 years of age may require supplements for sufficient B_{12} intake.

Vitamin Name	**Benefits**	**Dietary Sources**
Ascorbic acid (vitamin C)	Ascorbic acid is an antioxidant and it is a portion of an enzyme that is required for protein metabolism. It also helps with iron absorption and is important for the health of the immune system.	Only found in vegetables and fruits, especially: kiwifruit, mangoes, papayas, lettuce, potatoes, tomatoes, peppers, strawberries, cantaloupe and vegetables that are part of the cabbage family
Thiamine (vitamin B_1)	Thiamine is a portion of an enzyme that is required for energy metabolism and it is important for nerve function.	Found in moderate amounts in all of the nutritious foods: nuts and seeds, legumes, whole-grain/enriched cereals and breads, pork
Riboflavin (vitamin B_2)	Riboflavin is a portion of an enzyme that is required for energy metabolism. It is also important for skin health and normal vision.	Enriched, whole-grain cereals and breads, leafy green vegetables, milk products
Niacin (vitamin B_3)	Niacin is a portion of an enzyme that is required for energy metabolism. It is also important for skin health as well as the digestive and nervous systems.	Peanut butter, vegetables (particularly leafy green vegetables, asparagus and mushrooms), enriched or whole-grain cereals and breads, fish, poultry and meat

Pantothenic Acid (vitamin B_5)	Pantothenic acid is a portion of an enzyme that is required for energy metabolism	It is widespread in foods.
Pyridoxine (vitamin B_6)	Pyridoxine is a portion of an enzyme that is required for protein metabolism. It also helps with the production of red blood cells.	Fruits, vegetables, poultry, fish, meat
Folic Acid (vitamin B_9)	Folic acid is a portion of an enzyme that is required for creating new cells (particularly red blood cells) and DNA.	Liver, orange juice, seeds, legumes, leafy green vegetables. It is now added to many refined grains.
Cobalamin (vitamin B_{12})	Cobalamin is a portion of an enzyme required for the production of new cells and it is important to the function of nerves.	Milk, milk products, eggs, seafood, fish, poultry, meat. It is not present in plant foods.
Biotin	Biotin is a portion of any enzyme that is required for energy metabolism.	It is widespread in foods and can be produced by bacteria in the intestinal tract.

Properties of some water soluble vitamines are discussed below:

Thiamin. Thiamin was the first vitamin to be identified. In modern times, thiamin deficiency is seen most commonly in association with chronic alcoholism. Only a small percentage of large doses are absorbed, and elevated serum levels result in its active urinary excretion. After an oral dose of the vitamin, peak excretion occurs in about two hours.

There are no reports of adverse effects from the consumption of excess thiamin consumed in food or supplements. No upper level can be set due to the lack of reported findings associated with adverse effects.

Food sources from which most of thiamin is derived include enriched, fortified, or whole-grain products, such as bread, bread products, mixed foods that contain grain, and ready-to-eat cereals. Foods that are especially rich in thiamin include

yeast, lean pork, and legumes. Thiamin is absent from fats, oils, and refined sugars while Milk, milk products, seafood, fruits, and vegetables are not good sources for thiamin.

Riboflavin. The second vitamin was named vitamin B_2 or riboflavin. Most dietary riboflavin is consumed as a complex of food protein. Signs of riboflavin deficiency are sore throat, redness, and edema of the throat and oral mucous membranes, cheilosis (cracking of the skin around the mouth), and glossitis (red tongue). Vitamin B_2 deficiency most often occurs in combination with other nutrient deficiencies. The B vitamins are quite interrelated; for example, niacin requires riboflavin for its formation from the amino acid tryptophan, and vitamin B_6 requires riboflavin for conversion to the active coenzyme form.

When riboflavin is absorbed in excess amount, then its little amount is stored in the body tissues. No adverse effects associated with riboflavin consumption from food or supplements have been reported. The greatest contribution of riboflavin from the diet comes from milk and milk drinks, followed by bread products and fortified cereals. Especially good food sources of riboflavin are eggs, lean meats, milk, broccoli, and enriched breads and cereals.

Niacin. The term "niacin" refers to nicotinamide and nicotinic acid. The coenzymes, the active form of niacin in the body, are synthesized in all tissues of the body. The amount of niacin in the body is the result of absorbed nicotinic acid and nicotinamide, as well as conversion of the amino acid tryptophan. Excess niacin is excreted through the urine.

Pellagra is the classical manifestation of niacin deficiency. Pellagra has been seen in areas where corn (low in niacin and tryptophan) is the dietary staple.

Niacin, given as nicotinic acid in doses from 4 to 6 g/day, is one of the oldest drugs used in the treatment of hyperlipidemia, which consists of elevated blood levels of triglycerides and cholesterol. Niacin lowers low-density lipoprotein (LDL) cholesterol and triglyceride concentration. This therapeutic effect is not seen with nicotinamide. Nicotinic acid in therapeutic doses can cause flushing and headache in some people. These side effects are not harmful.

Dietary intake of niacin comes mainly from mixed dishes containing meat, poultry, or fish, followed by enriched and whole-grain breads, and fortified cereals. Significant amounts of niacin are found in red meat, liver, legumes, milk, eggs, alfalfa, cereal grains, yeast, and fish.

Vitamin B_6. Vitamin B_6 is a coenzyme for more than 100 enzymes involved in the metabolism of amino acids, glycogen, and nerve tissues. Microcytic anemia, reflecting decreased hemoglobin synthesis, can be seen in deficiency states. The interaction of vitamin B_6 and folate has been shown to reduce the plasma concentrations of homocysteine and decrease the incidence of cardiovascular disease.

This was probably an artifact of hormonal stimulation of tryptophan catabolism rather than vitamin B_6 deficiency. At the time these studies were conducted, estrogen concentrations were three to five times higher in contraceptive agents than they are today.

No adverse effects have been associated with intakes of vitamin B_6 from food. However, large doses of pyridoxine used to treat carpal tunnel syndrome and premenstrual syndrome have been associated with sensory neuropathy. It appears that the risk of developing sensory neuropathy decreases quite rapidly at dosages below 1 g/day. Food sources of vitamin B_6 include fortified, ready-to-eat cereals; mixed foods with meat, fish, or poultry as the main ingredient: white potatoes, starchy vegetables, and noncitrus fruits. Vitamin B_6 is widely distributed in foods; good sources are meats, whole-grain products, vegetables, and nuts.

Folate. Folate is a vitamin B that exists in many chemical forms. Folic acid, the most stable form of folate, occurs rarely in food, but is the form used in supplements and fortified food products. Folate coenzymes are involved in numerous reactions that involve DNA synthesis, purine synthesis, and amino acid metabolism. The most well known is the conversion of homocysteine to methionine. It is this reaction that reduces the concentration of homocysteine in the plasma, and may lower the risk of cardiovascular disease.

The metabolic interrelationship between folate and vitamin B_{12} may explain why a single deficiency of either vitamin leads to the same hematological changes. In either folate or vitamin B_{12} deficiency, megaloblastic changes occur in the bone marrow and other replicating cells.

Pregnant women are at risk for developing folate deficiency because of the heightened demands imposed by increased synthesis of DNA. Low folate status is associated with poor pregnancy outcome, low birth weight, and fetal growth retardation because of the possible incidence of neural tube defects during the preconception period (that is, just before and during the first 28 days of

conception), the Food and Nutrition Board recommends that women who are capable of becoming pregnant should consume 400 <g/day of synthetic folic acid, derived from dietary supplements or fortified food.

Recommendations for intake of folate are dependent on variation in bioavailability. Supplemental folate is nearly 100 percent absorbed, while absorption of folate found in foods is only about 50 percent. Fortified foods approach the level of bioavailability of folate found in supplements. No adverse effects have been associated with the consumption of normal folate-fortified foods.

Folates are found in nearly all natural foods. Protracted cooking or processing may destroy folate. Foods with the highest folate content include yeast, liver, other organ meats, fresh green vegetables, and some fruits.

Vitamin $B_{12.}$ Vitamin B_{12} can be called as cyanocobalamin. This is the only vitamin B_{12} preparation used in supplements. An adequate supply of vitamin B_{12} is essential for normal blood formation and neurological function. The absorption of vitamin B_{12} is dependent on several physiological steps. In the stomach, food-bound vitamin B_{12} is dissociated from proteins in the presence of stomach acid. Vitamin B_{12} then binds with protein and in the intestine the vitamin B_{12} binds with intrinsic factor for absorption. If there is a lack of sufficient acid in the stomach or intrinsic factor in the intestine, malabsorption occurs and the resulting condition caused is pernicious anemia.

The anemia of vitamin B_{12} deficiency (completely reversed by addition of B_{12}) is indistinguishable from that seen with folate deficiency. Because up to 30 percent of people older than fifty are estimated to have atrophic gastritis with low stomach acid secretion, older adults may have decreased absorption of B_{12} from foods. Thus, it is recommended that most of the vitamin B_{12} consumed by adults greater than fifty-one years of age be obtained from fortified foods or supplements.

Vitamin B_{12} is present in all forms of animal tissues. It is not present in plants and thus does not occur in fruits or vegetables. Because a generous intake of animal foods is customary in the United States, B_{12} intake from foods is usually adequate. People who avoid eating animal products may obtain most of their requirement through fortified foods.

Vitamin C. Ascorbic acid (the chemical name for vitamin C) is a potent antioxidant in animals and plants. Vitamin C is important in the synthesis of collagen. Some evidence indicates that vitamin C reduces virus activity by

inhibiting viral replication. Many anecdotal reports support a role for vitamin C supplementation to reduce the severity of cold symptoms.

Some epidemiological evidence indicates that supplemental vitamin C protects against risk for myocardial infarction. However, large-scale epidemiological studies do not suggest a benefit of vitamin C supplementation on cardiovascular health risks.

Non-heme iron absorption from food is enhanced two-to threefold in the presence of 25 to 75 mg of vitamin C, presumably because of the ascorbate-induced reduction of ferric iron to ferrous iron, which is less likely to form insoluble complexes in the intestine. However, vitamin C has no effect on increasing iron absorption from heme iron. Unlike most animal species, humans lack the ability to synthesize ascorbic acid; thus, the diet is the sole source for this vitamin.

The current requirement of vitamin C is 90 mg/day for adult men and 75 mg/day for adult women. During pregnancy the RDA is 85 mg/day, and 120 mg/day during lactation. This level was set as a guideline for people using dietary supplements and was based on reports of gastrointestinal symptoms reported when too much vitamin C was taken.

Almost 90 percent of vitamin C in the diet comes from fruits and vegetables, with citrus fruits, tomatoes, tomato juice, and potatoes being the major contributors. It is also added to some processed foods as an antioxidant.

Pantothenic acid. Pantothenic acid was named after the Greek, meaning "from everywhere," because it is so widespread in foods. Pantothenic acid is essential in the diet because of the inability of animals and humans to synthesize the pantoic acid moiety of the vitamin. Pantothenic acid plays a primary role in many metabolic processes, such as oxidative metabolism, cell membrane formation, cholesterol and bile salt production, energy storage, and activation of some hormones.

Pantothenic acid deficiency in humans is rare because of its ubiquitous distribution in foods. Many health claims are made regarding the role of pantothenic acid in ameliorating rheumatoid arthritis, lowering cholesterol, enhancing athletic performance, and preventing graying of hair. However, sufficient information is lacking at this time and so firm recommendations may not be made. No reports of adverse effects of oral pantothenic acid in humans have been reported.

As mentioned above, pantothenic acid is found in a wide variety of both plant and animal foods. Because of its thermal lability and susceptibility to oxidation, significant amounts are lost during processing. Rich food sources include chicken, beef, liver, and other organ meats, whole grains, potatoes, and tomato products.

Biotin. In mammals, biotin serves as a coenzyme for reactions that control such important functions as fatty acid metabolism and gluconeogenesis. Biotin is recycled upon degradation of enzymes to which it is bound. Biotin from pharmaceutical sources is 100 percent bioavailable. Deficiency is rare but has been seen in patients on parenteral nutrition without biotin supplementation. Lipoic acid and biotin have structural similarities, thus competition potentially exists for intestinal or cellular uptake. This may be of concern in settings where large doses of lipoic acid are administered or taken as supplements.

Biotin is distributed widely in natural foods. Those rich in biotin include egg yolk, liver, and some vegetables.

Choline. Choline has been considered a nonessential nutrient because humans can synthesize sufficient quantities. However, when hepatic function is compromised, hepatic choline synthesis is decreased and thus choline is now considered "conditionally" essential. Nutrition Board study suggested that graded doses of choline intake be studied regarding their effects on organ function, plasma cholesterol, and homocysteine levels.

Choline functions as a precursor for phospholipids and acetylcholine, and betaine. Choline and choline-containing lipids, mainly phosphatidylcholine, are abundant in foods of both plant and animal origin. Rich sources include muscle and organ meats and eggs. To date there are no nationally representative estimates of choline intake from food or supplements.

Vitamin C:

Properties

- White crystals, soluble in water, heat lavish
- Good reducing agents
- Early oxidized at 1000C in presence of oxygen
- Cannot stand cooking or canning

Sources: Guava, amla, green chilli, amaranth leaves, citrus fruits, green vegetables, potatoes, tomatoes, cheese, milk etc.

Functions :

Acts as antioxidant.

- Essential for formation of collagen present between cells.
- Necessary for the formation of osteoblasts and red blood cells.
- Helps to reduce the ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) and is absorbed only in this form.
- Essential for the utilization of folic acid
- Takes part in oxidation and reduction reactions in the tissues.
- Helps in bone formation.
- Helps in wound healing.
- Prevents formation of free radical in the body.

Deficiency Diseases

- Scurvy, a disease characterized by sore, spongy gums, loose teeth, fragile blood vessels, swollen joints, and anemia.
- Delay in wound healing.
- Pain in bones.
- Skin becomes rough and dry.
- Pyrexia, rapid pulse and susceptibility to infection.

Vitamin B_1 (Thiamine):

Properties

- White, crystalline substance
- Water-soluble
- Heat labile
- Unstable at high temperature and in alkaline medium
- Stable in acid medium
- On oxidation it gives a yellowish dye called thiochrome.

Sources :Rice polishing, dried yeast and wheat germ are rich sources of vit. B1. Whole cereals like wheat, oats, legumes, oil seeds and nuts are good sources. Milled cereals, vegetables, fruits, meat and fish are poor sources. On milling, vit. B1 is lost from cereals.

Functions

- Acts as a co-enzyme in carbohydrate metabolism
- Require for the synthesis of glycine
- It has a specific action on nerve tissue
- Requires for the maintenance of normal gastro-intestinal tone and motility
- Maintains normal appetite.

Deficiency Diseases

- Beriberi - nervous, system affected, muscles become weak and painful paralysis can occur.
- Heart failure, wet beriberi, dry beriberi, infantile beriberi, oedemia, children's growth is impaired, keto acids accumulate in the blood, wernicke's-korsakoff's syndrome etc.
- Loss of appetite, fatigue, irritability, depression and constipation occur.

Vitamin B_2 (Riboflavin)

Properties

- Yellow crystals
- Soluble in water
- Heat soluble in neutral and acid media
- Destroy by light.

Sources

Milk, liver, kidney, muscle, butter, chicken, fish, yeast, cheese, raw egg, white grains, green vegetable such as spinach, peanuts, fruits such as apple, orange etc. Functions

- Essential for growth, essential for tissue oxidation related to carbohydrate, fat and protein metabolism.
- Maintain mucosal, epithelial and ocular tissues.

- Essential for normal vision.

Deficiency Diseases

Symptoms

- Tongue sore at the corner of the mouth.
- Loss of hair, skin becomes dry and scaly.
- Arrest of growth.
- Dermatitis around nose and lips, inflammation of tongue, angular stomatitis and cheilosis, photophobia, cataract etc.
- Scrotal or vulval dermatitis, intense itching etc.
- Disturb carbohydrate metabolism.

5.5 Summary

Such organic substances which are important for the proper functioning of our body or an organic chemical compound is called a vitamin when the organism cannot synthesize the compound in sufficient quantities, and must be supplied through the diet. For example, ascorbic acid (vitamin C) is a vitamin for humans, but not for most other animal organisms. Vitamins are important nutrients which are necessory for life. Unlike other classes of nutrients, vitamins serve no structural function nor provide significant energy. Mostly vitamins needs metabolic activation into a functional form. Some vitamins function as coenzymes, antioxidants, although some vitamins (A and D), act as hormones. Vitamins are classified in to two categories on the basis of their soluble properties:

(i) Fat soluble vitamins: Vitamins-A, D, E and K are considered as fat soluble vitamins.

(ii) Water soluble vitamins: Vitamins B and C are considered as water soluble vitamins.

5.6 Glossary

- The term vitamin neither includes the essential nutrients, such as dietary minerals, essential fatty acids, or essential amino acids (which are needed in greater amounts than vitamins).

- Vitramins are required to maintain the health of the organism in minute amount. Universally thirteen vitamins are recognized at present.
- Vitamins are classified on the basis of their biological and chemical properties, not their structure.
- Thus, each "vitamin" refers to a number of vitamer compounds that all show the biological activity associated with a particular vitamin.
- So vitamins are grouped under an alphabetized vitamin "generic descriptor" title, such as "vitamin A", which includes the compounds retinal, retinol, and four known carotenoids.
- Vitamins are essential for the normal growth and development of a multicellular organism. For eg. a fetus begins to develop, at the moment of conception, from the nutrients it absorbs. It requires certain vitamins and minerals for growth.
- These nutrients facilitate the chemical reactions that produce among other body parts, skin, bones and muscle.
- Deficiency of one or more of these nutrients may cause permanent damage of the organs.
- Once growth and development are completed, vitamins remain essential nutrients for the healthy maintenance of the cells, tissues, and organs.
- Dietary supplements contain vitamins, but may also include other ingredients, such as minerals, herbs etc.
- Scientific evidence supports the benefits of dietary supplements for persons with certain health conditions.
- In some cases, vitamin supplements may have unwanted effects, especially if taken before surgery, with other dietary supplements or medicines, or if the person taking them has certain health conditions.
- They may also contain levels of vitamins many times higher, and in different forms, than one may ingest through food.
- Vitamins are classified according to their solubility, they may be fat or water soluble.

- This method of classification and their discovery as labeled by McCollum as "fat-soluble A" and "water-soluble B." Our body needs to consume 13 different vitamins to maintain normal health.
- The important vitamines are the vitamin B complex (folate, B_{12}, B_6, biotin, pantothenic acid, niacin, riboflavin and thiamine) as well as vitamins A, C, D, E and K. The each vitamin is essential for different functions of the animal and human body.

5.7 Review questions / comprehensive questions

1. What are vitamins? Give the details of general properties of the vitamins.
2. Give the basis of classification of vitamins and their classification in two categories.
3. Explain the following points in detail:

   (i) Fat soluble vitamins

   (ii) Water soluble vitamins
4. Give the details of following vitamins and also discuss the deficiency diseases:

   Vitamins: A, D, E.
5. Explain the following points in detail:

   (a) Vitamin C, its source and deficiency diseases

   (b) Vitamin B, its source and deficiency diseases

5.8 References and suggested readings

- Organic chemistry, Volume 2 : Stereochemistry and the chemistry of natural products, fifth edition – I.L. Finar.
- Janos Zempleni, John W. Suttie, Jesse F. Gregory III - 2013 - Medical
- Simic, M.G., and Karel, M. (1980)*Autoxidation in Food and Biological Systems*, Plenum Press, New York

- Machlin, L.J. (1984) Vitamin E, in*Handbook of Vitamins: Nutritional Biochemical & Clinical Aspects* (Machlin, L.J., ed.), 99–145, Marcel Dekker, New York and Basel
- IUPAC-IUB Joint Commission on Biochemical Nomenclature (1982) Nomenclature of Tocopherols and Related Compounds: Recommendations 1981,*Eur. J. Biochem. 123*, 473–475.

Unit - 6

Small Ring Heterocycles

Structure of Unit:

6.0 Objective

The study of hetrocyclic compounds is great interest in the theoretical as well as practical point of view. Hetrocyclic compounds occur widely in nature as well as non-naturally occurring compounds. Knowledge of hetrocyclic chemistry is useful in biosynthesis and metabolism of drug.

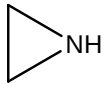
6.1 Introduction

The properties of three-membered heterocycles are mostly a result of the great bond angle strain (BAEYER strain). The resultant ring strain imparts on the compounds high chemical reactivity. Ring opening leading to acyclic products is typical to acyclic products is typical.

6.2 Three membered heterocycles

Simple examples of this type are aziridine, oxirane and thirane containing respectively nitrogen, oxygen and sulphur as the hetero atom.

6.3 Aziridines

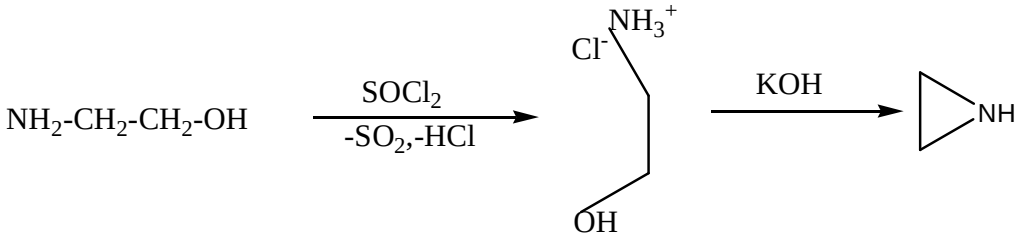


Aziridine is also known as ethylene imine. The plane in which the N-atom, its nonbonding electron pair and the N-H bond are situated is perpendicular to the plane of the aziridine ring.

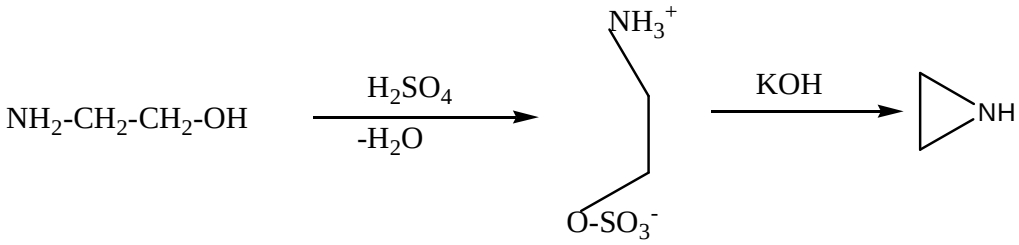
6.3.1 Methods of Preparation

(i) Cyclization of ß-substituted amines (**Gabriel Method)**

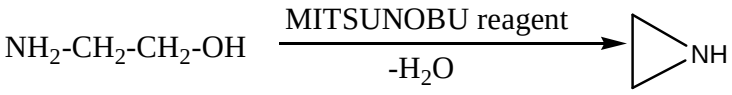
- β-Amino alcohols react with thionyl chloride to give chloramines, which can be cyclized to aziridines by alkali hydroxide.



- Sulfate esters (obtained from amino alcohols and sulfuric acid) when treated with alkali also form aziridines.

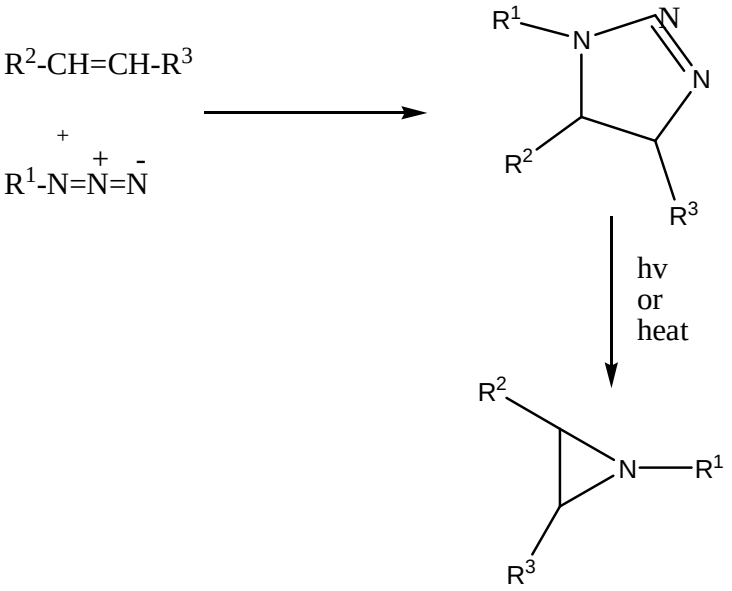


- In the presence of MITSUNOBU reagent (triphenylphosphane/diethyl azodicarboxylate) β-amino alcohols directly cyclodehydrated to aziridines.



(ii) Thermal or photochemical reaction of azides with alkenes

Phenyl azide reacts with alkenes to give 4,5-dihydro-l,2,3-triazoles (1,3-dipolar cycloaddition) which are thermally or photochemically converted into aziridines through loss of nitrogen:

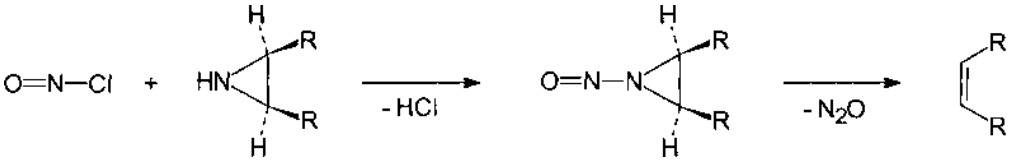


6.3.2 Physical Properties

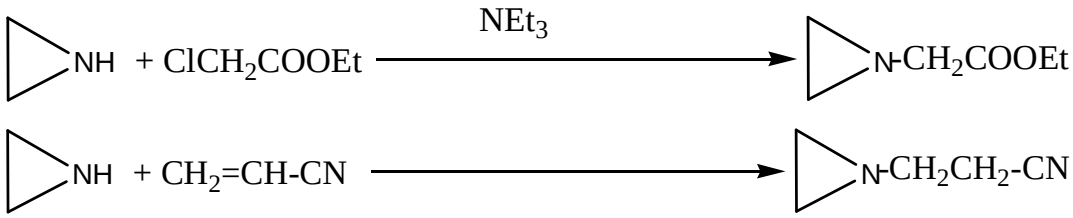
Aziridine is a colourless**,** water**-**soluble, poisonous liquid which have 57°C boiling point.

6.3.3 Chemical Reactions

(i) Deamination to alkenes

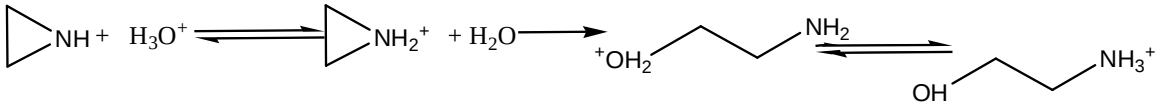


(ii)Reactions with electrophilic reagents



(iii) Ring opening reaction of aziridines

The ring-opening of the aziridines is catalyzed especially effectively by acids. The acid-catalysed hydrolysis to give amino alcohols for example:

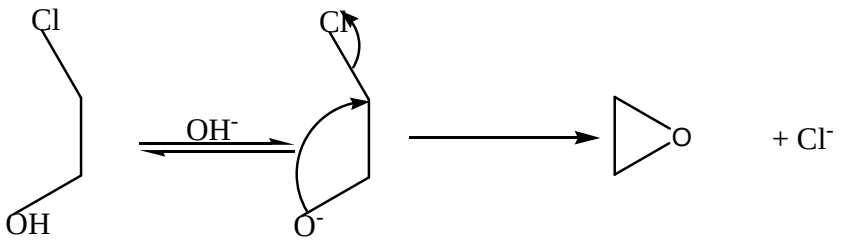


6.4 Oxiranes

Oxiranes are also known as epoxides.

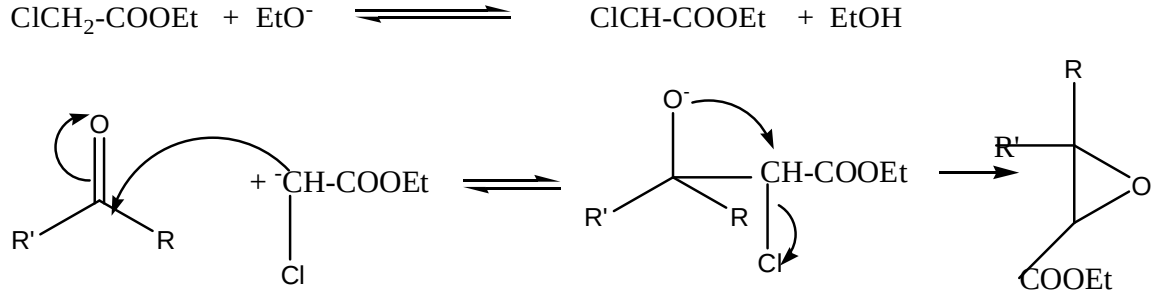
6.4.1 Methods of Preparation

(i) Cyclodehydrohalogenation of β-halo alcohols



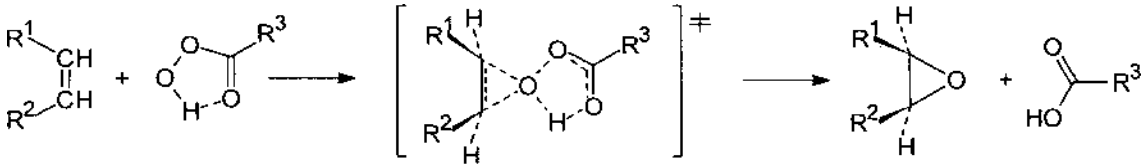
(ii) Darzens reaction

The reaction of a-halo esters with carbonyl compounds in the presence of sodium ethoxide leads to 2-(ethoxycarbonyl) oxiranes.



(iii) Epoxidation of alkenes

Peroxyacids (peroxybenzoic acid, *m*-chloro peroxybenzoic acid or monoperoxyphthalic) react with alkenes to give oxiranes. This reaction is a stereospecific reaction i.e. cis-alkene give cis- oxiranes and trans-alkene give trans-oxiranes.



6.4.2 Physical Properties

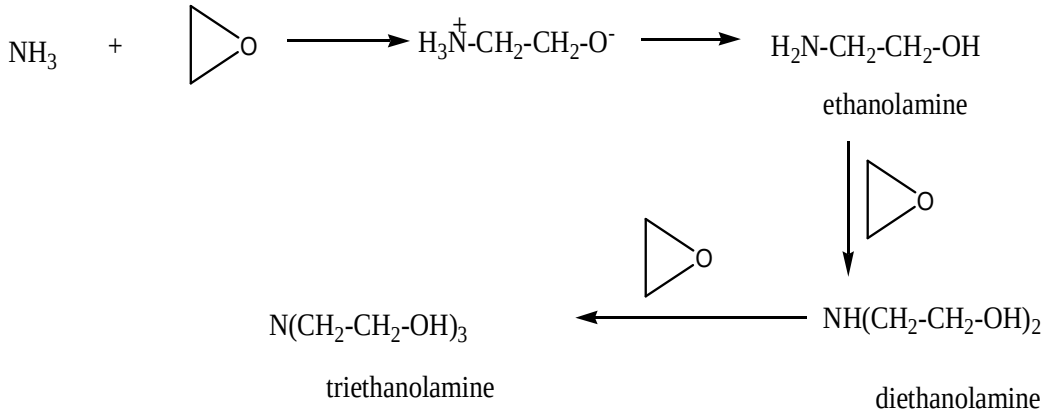
Oxirane

- colourless,
- water-soluble,
- b.p 10.5°C,
- extremely poisonous gas
- is made on an industrial scale by direct air oxidation of ethene in the presence of a silver catalyst.
- is important as an intermediate in the petrochemical industry.

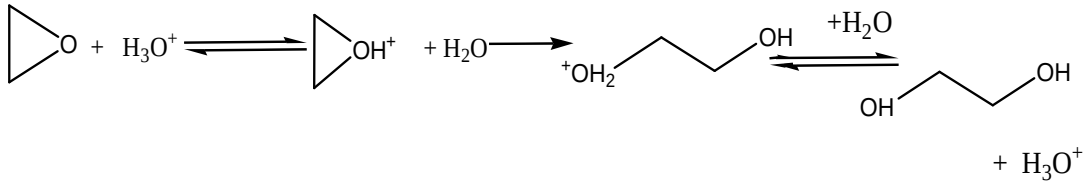
6.4.3 Chemical Reactions

(i) Ring-opening by nucleophiles

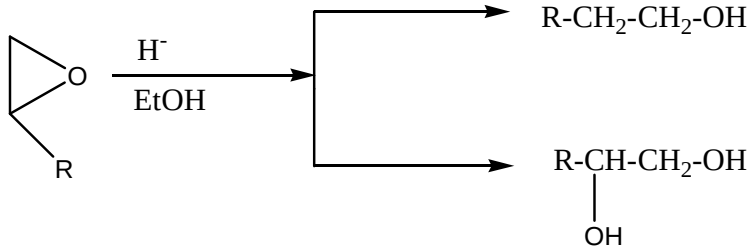
(a) When nucleophiles like ammonia or amines react with oxiranes, ring of oxiranes open to give amino alcohols.



(b) The ring-opening of the oxiranes is also catalyzed by acids. The acid-catalysed hydrolysis to give 1,2-diols for example:

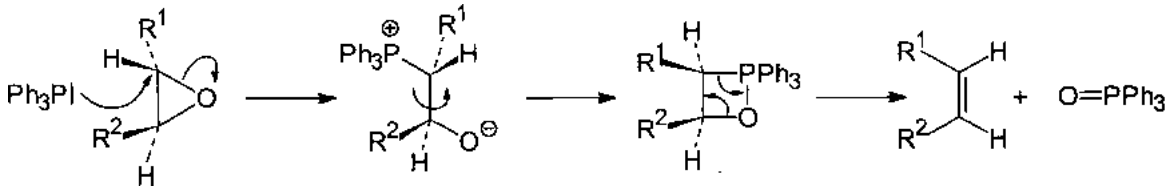


(c) Ring opening by hydride ion: Oxiranes are reduced by sodium borohydride to give alcohols. This reaction can be viewed as a ring-opening by the nucleophilic hydride ion:



(ii) **Deoxygenation to olefins**

A number of reagents deoxygenate oxiranes to give olefins. For instance, a trans-oxirane yields a (Z)-olefin on treatment with triphenylphosphane at 200°C.

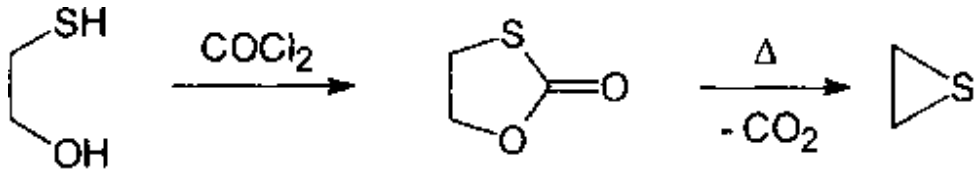


6.5 Thiiranes

Sulfur containing three membered saturated heterocycles are called as thiiranes. These are also known as thiacyclopropanes of episulfides.

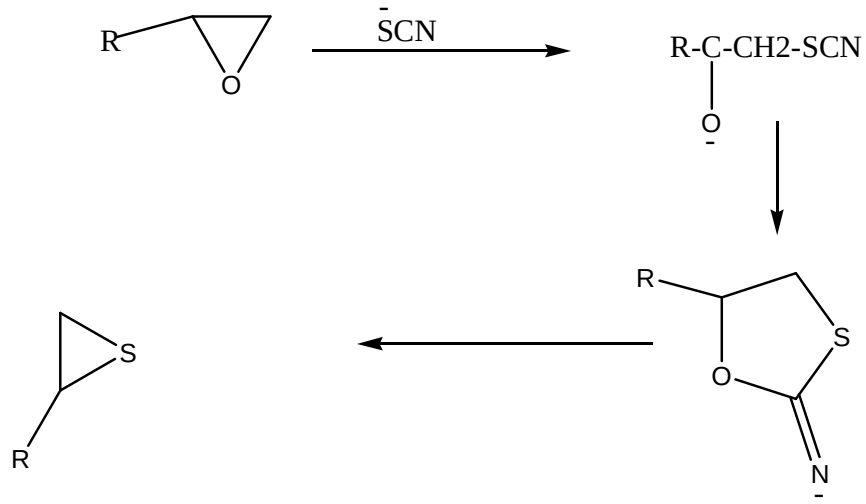
6.5.1 Methods of Preparation

(i) cyclization of β substituted thioles



(ii) From Oxiranes

This is the most widely used method which involves the reaction of oxiranes with the thiocyanate ions.



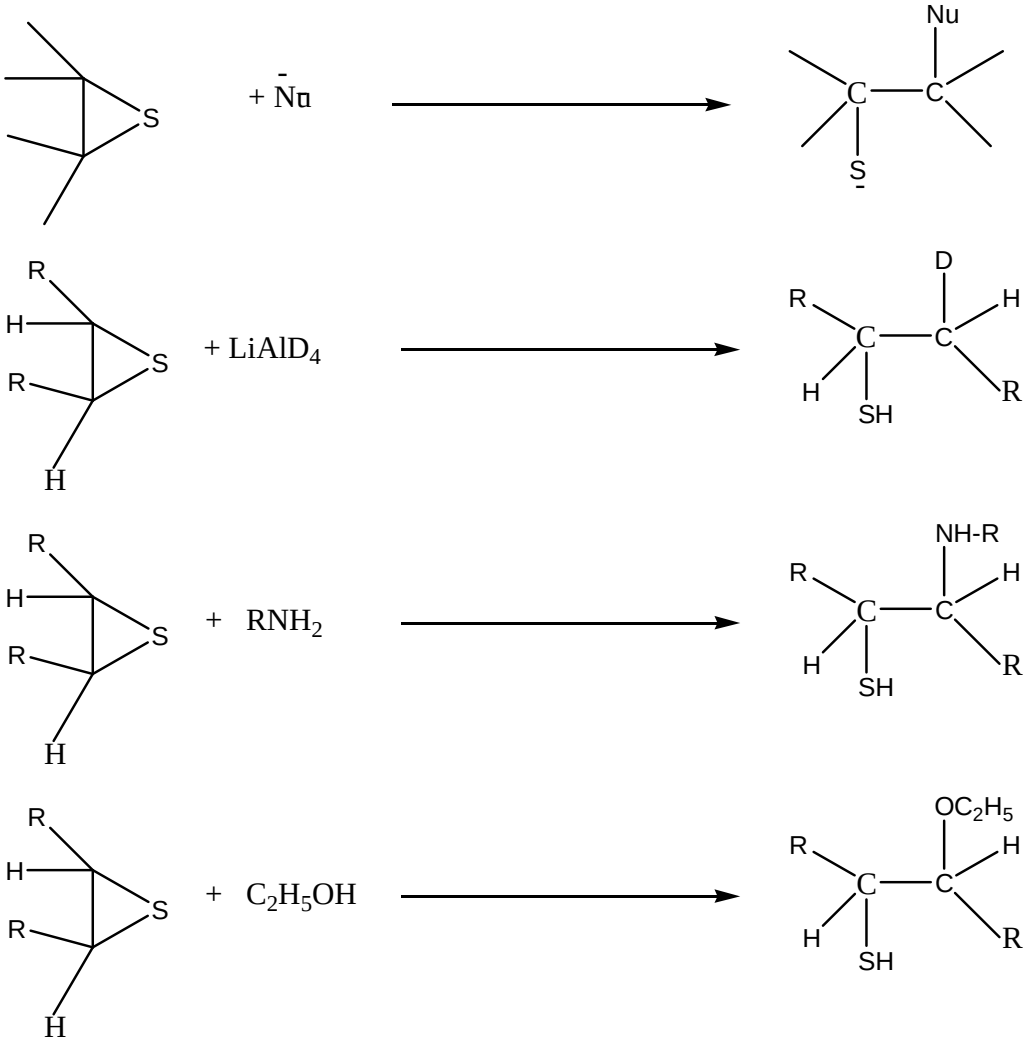
6.5.2 Physical Properties

- b.p.=55^0 C
- Colourless liquid
- Springly soluble in water

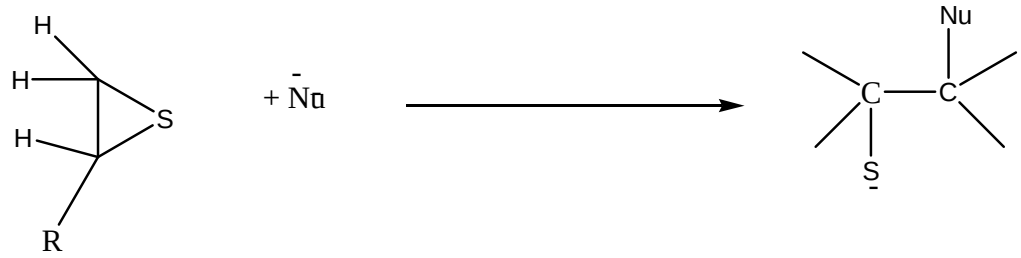
6.5.3 Chemical Reactions

(i) Nucleophilic Ring Opening Reactions

These reactions proceed through stereospcifically with the inversion of configuration at the site of attack i.e. incoming nucleophiles attack opposite to the heteroatom (sulfur atom).

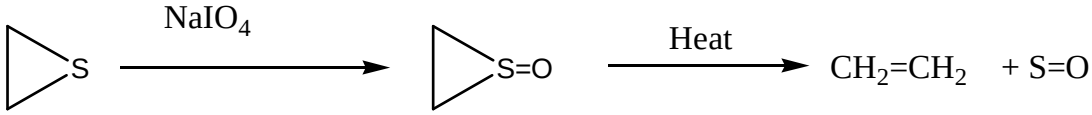


In asymmetrically substituted thiiranes, the nucleophile attacks at the less substituted carbon atom from the opposite side of the sulfur atom.



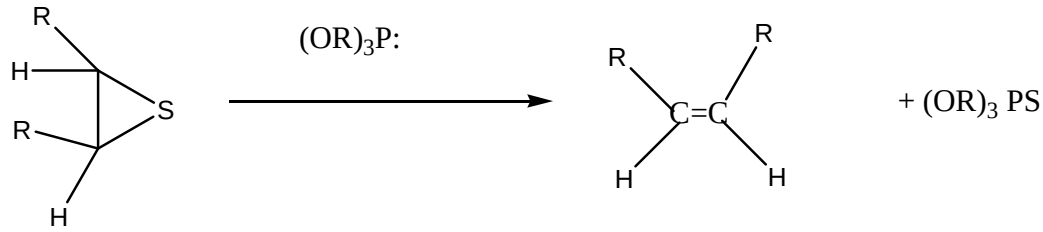
(ii) Oxidation

Thiiranes are oxidized by sodium periodate or peroxy acids to give thiirane oxides. They decompose at higher temperature into alkenes and sulfur monoxide.



(iii) Desulfurization to alkenes

The reaction of thiiranes with tertiary phosphines, desulferise the thiiranes gives the corresponding alkenes.

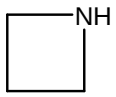


6.6 Four membered heterocycles

Four membered heterocycles are the heterocyclic analogs of cyclobutane and are considered

to be derived by replacing a $-CH_2$ group by a heteroatom. The four membered saturated heterocycles containing nitrogen,oxygen and sulphur are known as azitidines, oxetanes, and thietanes respectively.

6.7 Azetidines

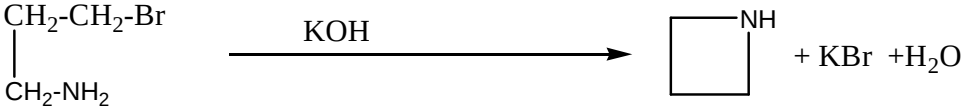


Azetidines are thermally stable and less reactive than aziridines. They behave in their reactions almost like secondary alkylamines. The *pKa* value of azetidine is 11.29 and so it is more basic than aziridine *(pKa* = 7.98) and even dimethylamine *(pKa* = 10.73).

6.7.1 Methods of Preparation

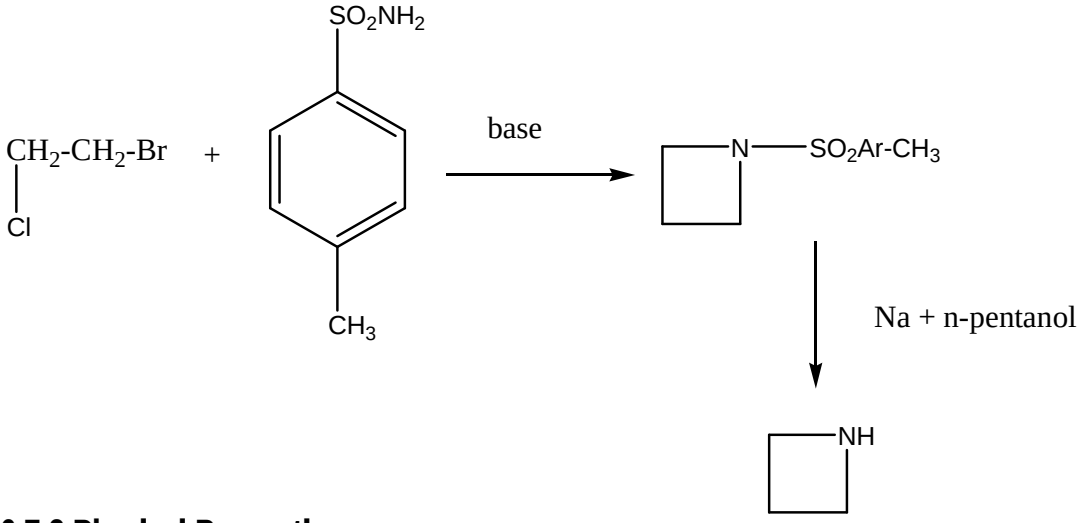
(i) Intramolecular Cyclization

γ-Halogen substituted amines are dehydrohalogenated by bases and gives azetidines.



(ii) Action of p-toluenesulfonamide and bases on 1,3-dihaloalkanes

Azetidine is prepared by the cycloaddition of trimethylene chlorobromide with *p*-toluenesulfonamide followed by the reduction with sodium and n-pentanol.



6.7.2 Physical Properties

Azetidine is a

- water-miscible,
- colourless liquid of bp 61.5°C

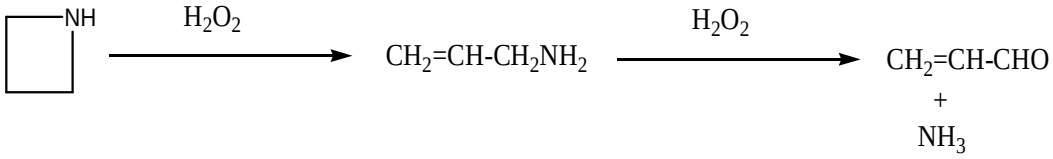
- smells like ammonia

6.7.3 Chemical Reactions

(i) Nucleophilic ring opening

Azitidines show lesser degree of reactivity towards nucleophilic ring opening reactions than the three membered aziridines.

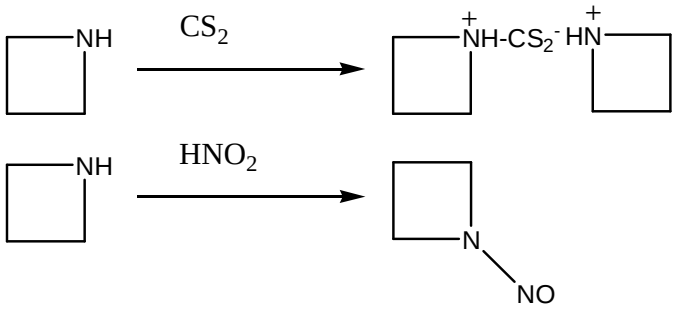
Azitidines are quite resistant towards bases and nucleophiles. However hydrogen peroxides cleaves the ring and gives acrolein and ammonia.



(ii) Fictionalization at nitrogen

Azitidine behave like secondary aliphatic amines and undergo similar reactions.

The reaction with carbon disulfide forms a salt and with nitrous acid gives N-nitrosoazetidine.



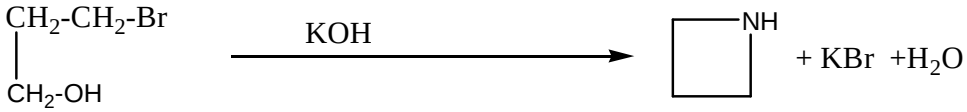
6.8 Oxetanes

This is also oxacyclobutanes. The oxetane ring represents a slightly distorted square because the bond angle at the O-atom is 92°.The strain enthalpy has been determined thermochemically to be 106.3 kJ moH and so only 7.7 kJ mol"1 less than that of oxirane, although the bond angles are 30° larger.

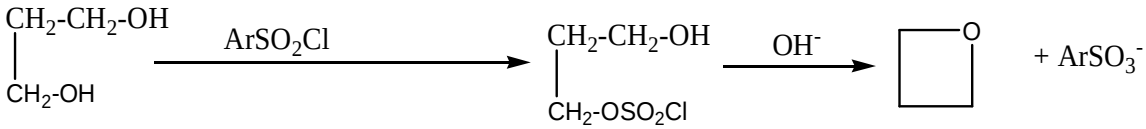
6.8.1 Methods of Preparation

(i) Intramolecular cyclization reaction

(a) Oxetanes are prepared by the Intramolecular cyclization of 1,3-halohydrines in the presence of a base.

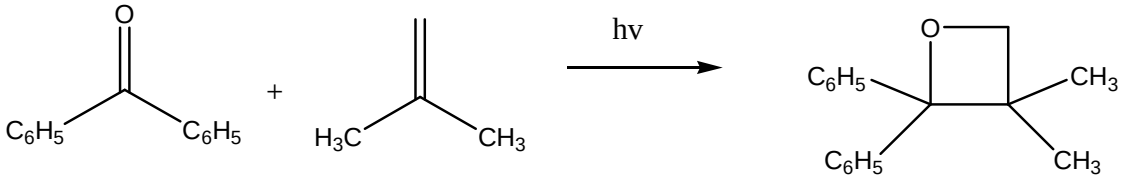


(b) Oxetanes can be prepared from 1,3-diols via monoarene sulfonates.



(ii) Paterno-Büchi reaction

Photochemical [2+2] cycloaddition of carbonyl compounds to olefins gives oxetanes.This reaction is known as Paterno-Buchi reaction.



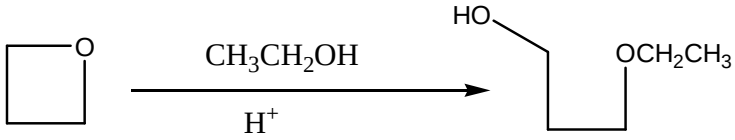
6.8.2 Physical Properties

Oxetane is a colourless, water-miscible liquid of bp 48°C.It is obtained in 40% yield by heating (3- chloropropyl)acetate with coned KOH solution .

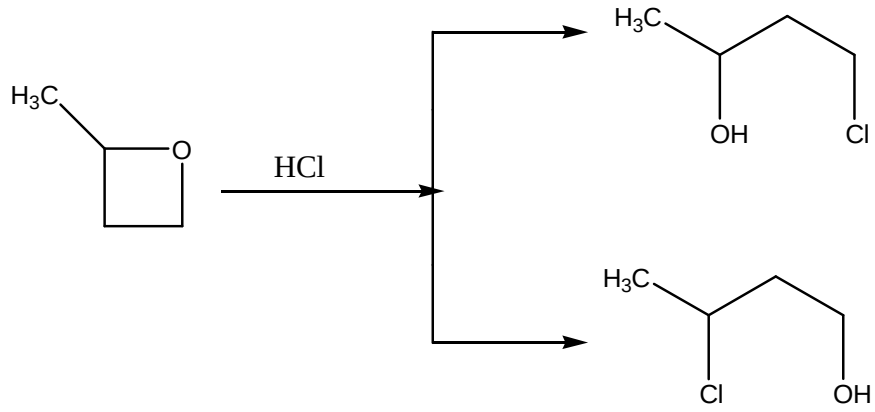
6.8.3 Chemical Reactions

(i) Electrophilic ring opening reaction

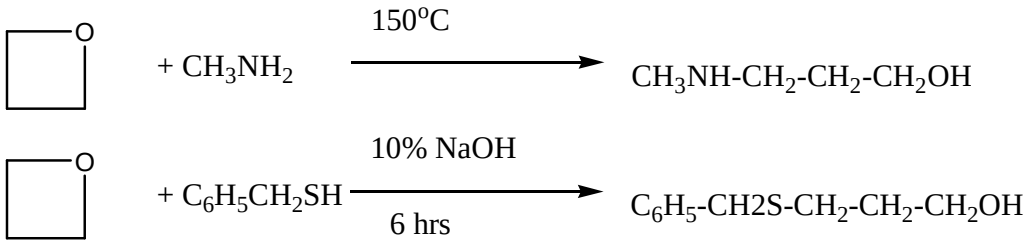
Oxetane readily undergo ring opening reaction under acidic conditions.



In unsymmetrically substituted oxetane, the direction of ring cleavage depends on the "push-pull" mechanism and generally two products are formed.



(i) Nucleophilic ring opening reaction

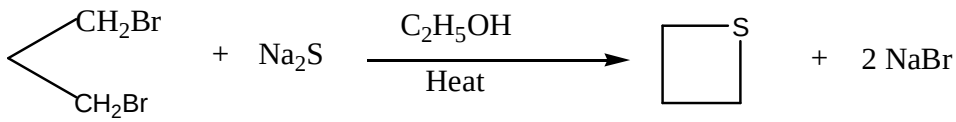


6.9 Thietanes

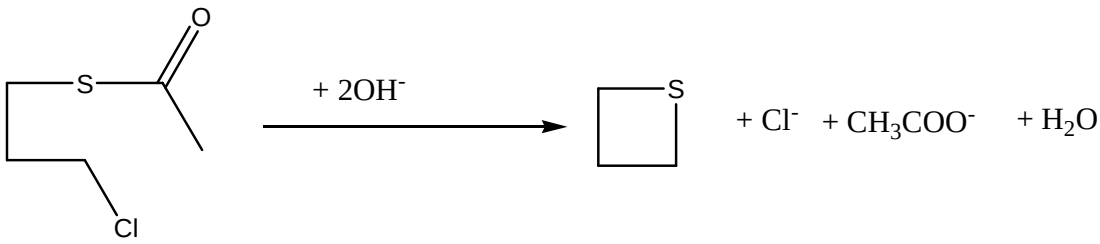
The thermochemically determined strain enthalpy of thietane is only 80 kJ mol^{-1}. The activation energy for the ring inversion was found spectroscopically to be 3.28 kJ mol^{-1} and lies above the four lowest vibration levels.

6.9.1 Methods of Preparation

(i) From 1, 3-Dihaloalkanes



(ii) Cyclization of y-halo thiols or their acetyl derivatives by bases



6.9.2 Physical Properties

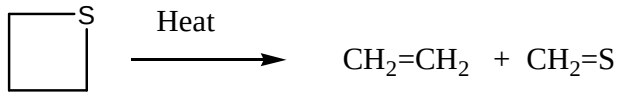
Thietane is a

- colourless
- water-insoluble liquid
- b.p 94°C,

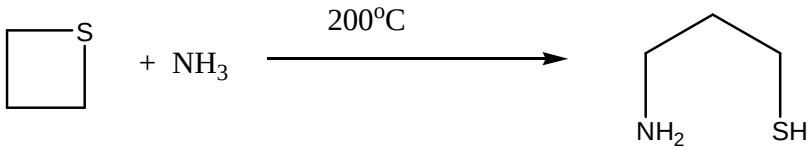
6.9.3 Chemical Reactions

(i) Ring opening reactions

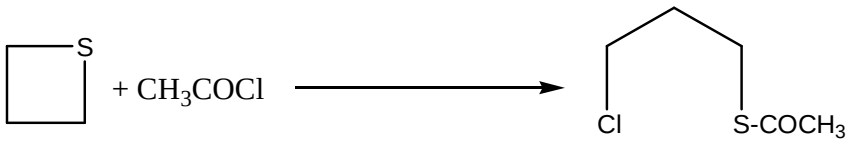
(a) Thietane is cleaved on heating in gas phase to give ethylene and thioformaldehyde.



(b) Reaction with ammonia

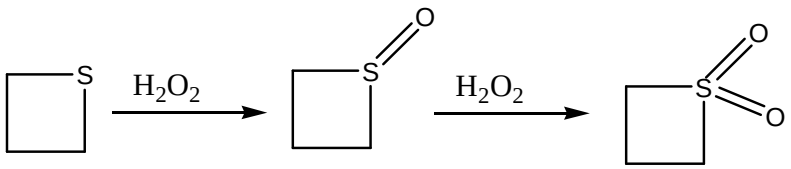


(c) Reaction with Acetyl Chloride



(d) Oxidation

Hydrogen peroxide or peroxy acids oxidize thietanes to 1,1-dioxides (cyclic sulfones) via 1-oxides.



6.10 Summery

- The reactivity of the compounds is determined mainly by the ring strain, but also by the nature of the heteroatom or heteroatoms.
- A typical reaction of three-membered heterocycles is nucleophilic ring-opening resulting in the formation of 1,2-disubstituted aliphatic compounds.
- A consequence of three-membered heterocycles possessing nonbonding electron pairs is that they behave as BRÖNSTED bases as well as LEWIS bases. Accordingly, they react with BRÖNSTED acids and with electrophiles.
- Appropriate reagents remove the heteroatoms to form alkenes (deoxygenation, desulfonation, deamination).
- Oxygen-containing heterocycles can be synthesized by the action of peroxy compounds on alkenes, ketones or imines.
- Ring-opening by nucleophiles proceeds more slowly than with three-membered heterocycles and is catalysed by acids.

6.11 Review Question

1 Give the methods of preparation and chemical reactions of Aziridines.

2 Give the methods of preparation and chemical reactions of Oxiranes.

3 Give the methods of preparation and chemical reactions of Thiiranes.

4 Give the methods of preparation and chemical reactions of Azetidines.

5 Give the methods of preparation and chemical reactions of Oxetanes.

6 Give the methods of preparation and chemical reactions of Thietanes.

6.12 Reference and Suggested reading

- Hetrocyclic Chemistry-II, R.R.Gupta, M.Kumar,V.Gupta, Springer,1998
- Hetrocyclic Chemistry , Raj K.Bansal,New Age International Publishers,2010(Fifth Edition)
- The Chemistry of Hetrocycles, Theopil Eicher , Siegfried Hauptmann, Wiley-VCH,II Edition

Unit-7

SIX MEMBERED HETROCYCLES WITH ONE HETROATOM : Synthesis and reactions of pyrylium salt and pyrones and their comparison with pyridinium & thiopyrylium salts with pyridones. Synthesis and reaction of Quinolizinium and benzopyrylium salts, coumarins and chromones

Structure of Unit

7.0 Objective

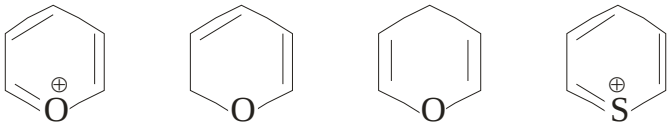
Heterocyclic compounds are a major class of organic compounds characterized by the fact that the some in their molecules are joined into rings containing atleast one atom of an element other than carbon these compounds are of great important in pharmaceuticals, pesticides, and herbicides, as well as dyes and plastics.

7.1 Introduction

Six membered heterocyclic compounds have a wide range of applications. They are used as optical brightening agents, as antioxidants, as corrosion inhibitors and as additives with a variety of other functions. Many dyestuff and pigments have heterocyclic structures.

7.2 Six Membered heterocyclic with one heteroatom

The simple examples of this class are pyyrlium salt (1) pyrones (2,3) and thiopyryilium salt (4)

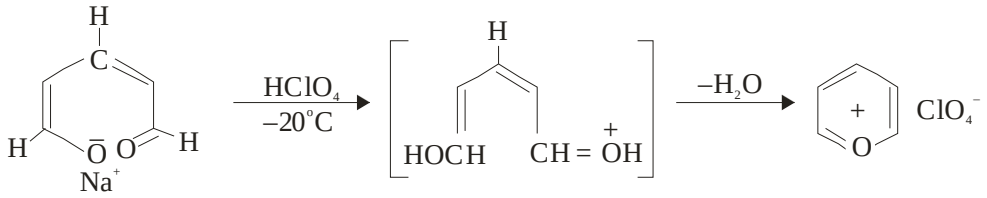


(1) (2) (3) (4)

7.3 Pyrylium salt

7.3.1 Methods of Preparations

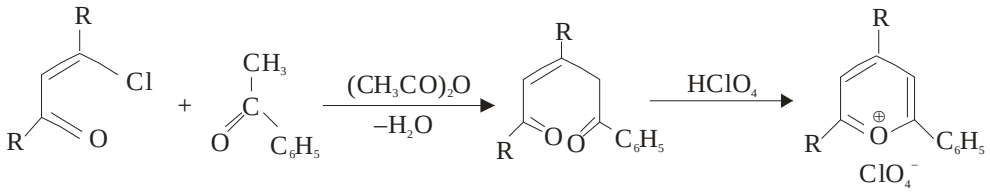
(i) From 1,5 – dicarbonyl derivations :



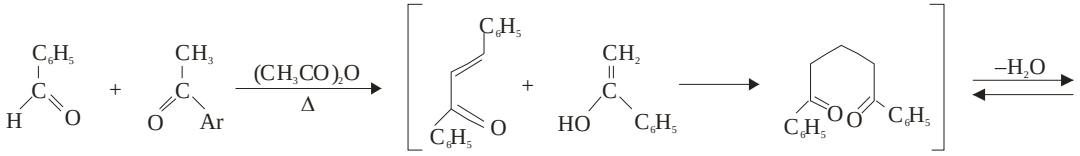
Sodium salt of glutanaldehyde

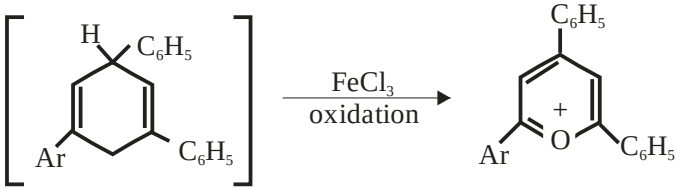
Pyrylium Perchlorate

(ii) From Chlorovinyl ketones :

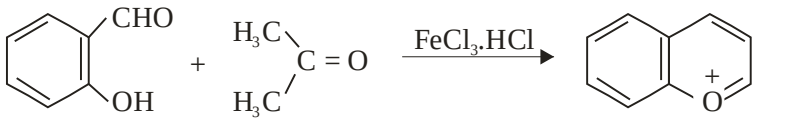


(iii) **Hantzsch Type Synthesis :** This involves the reaction of an aromatic aldehyde with two moles of aryl methyl ketone in acetic anhydride with the resultant formation of a pyran. Which on oxidation yields the pyrylium system.





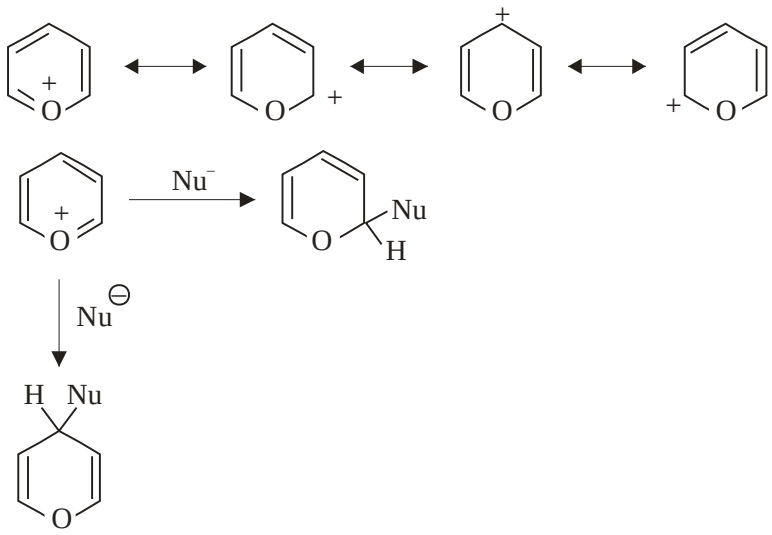
(iv) From Acetone :



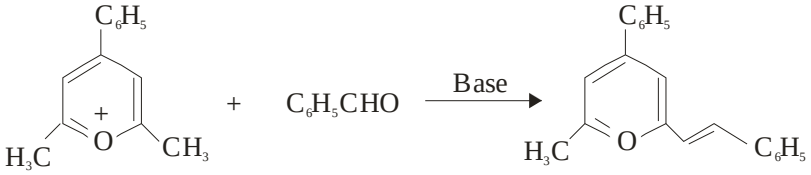
1-Benzopyrylium cation

7.3.2 Chemical Reactions

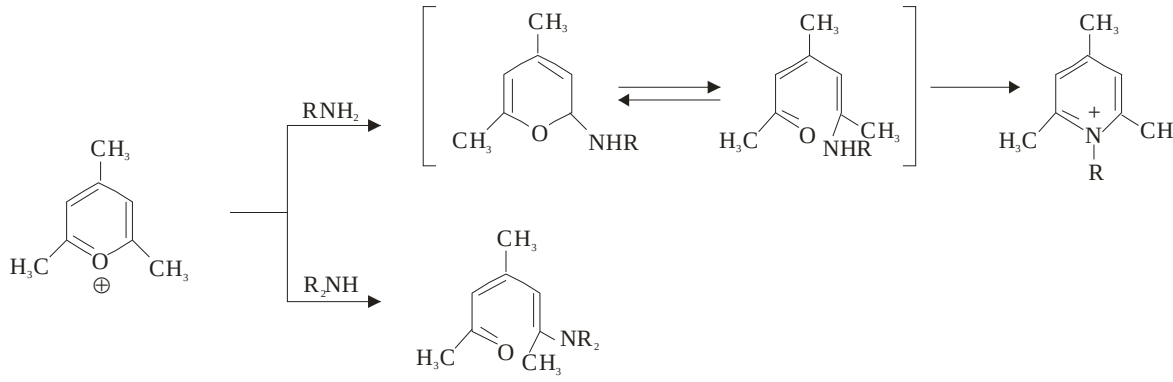
(i) **Attack by Nucleophilic Reagents :** A nucleophilic attack takes place at C-2, C-4 or C-6 positions as is evident from the various resonance structures of the pyrylium ion.



(ii) Deprotonation of substituted pyrylium salt :



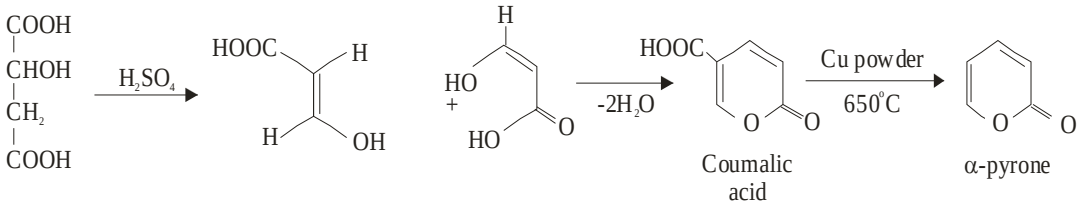
(iii) Reaction with primary amine and secondary amine :



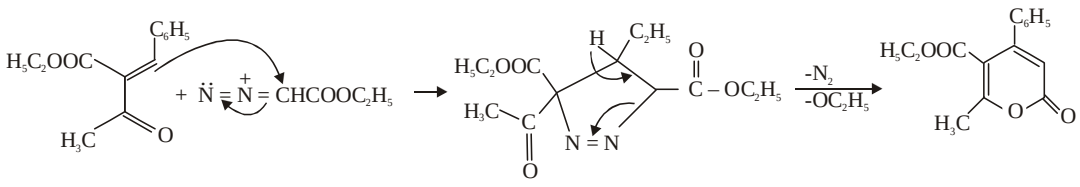
7.4 Pyrones

7.4.1 Methods of Preparation

(i) From Malic acid :

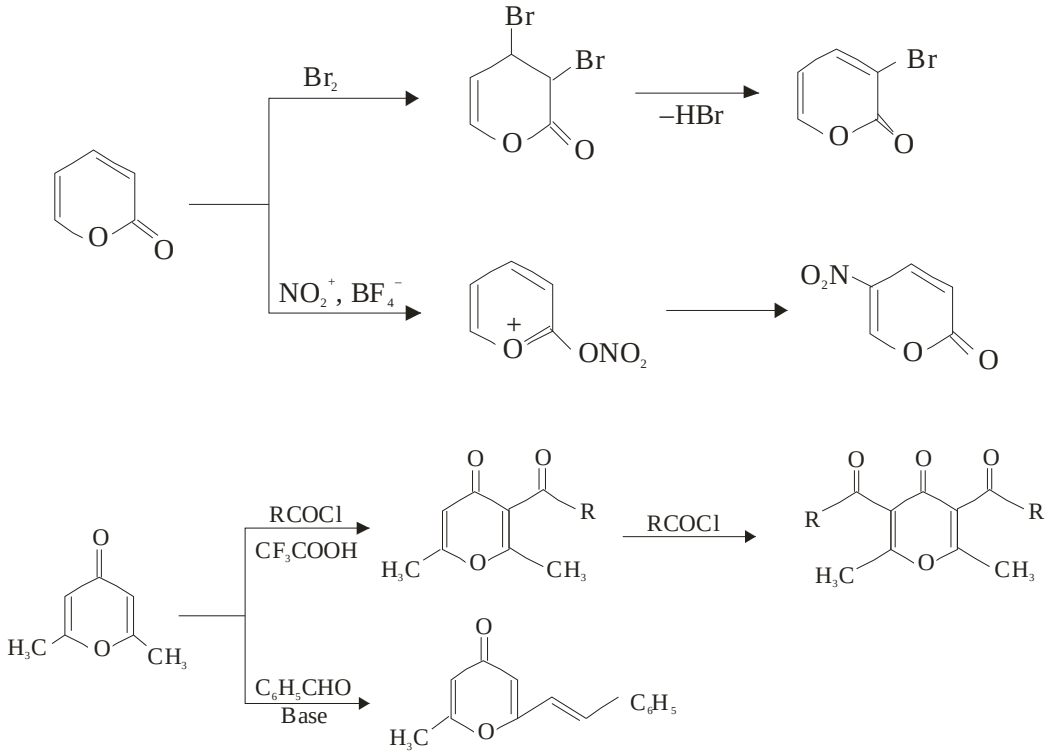


(ii) Reaction of ethyl diazoacetate with keto ester :

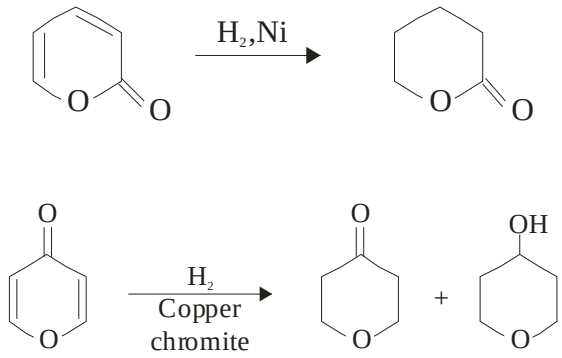


7.4.2 Chemical Reactions

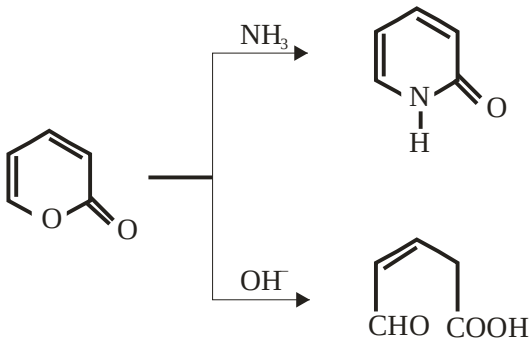
(i) Electrophilic reactions :

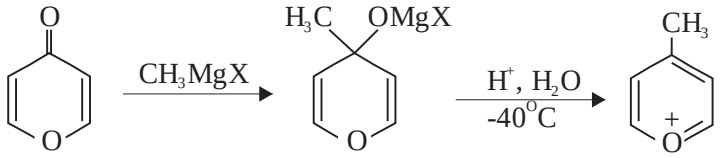


(ii) Reaction with Reducing agent :

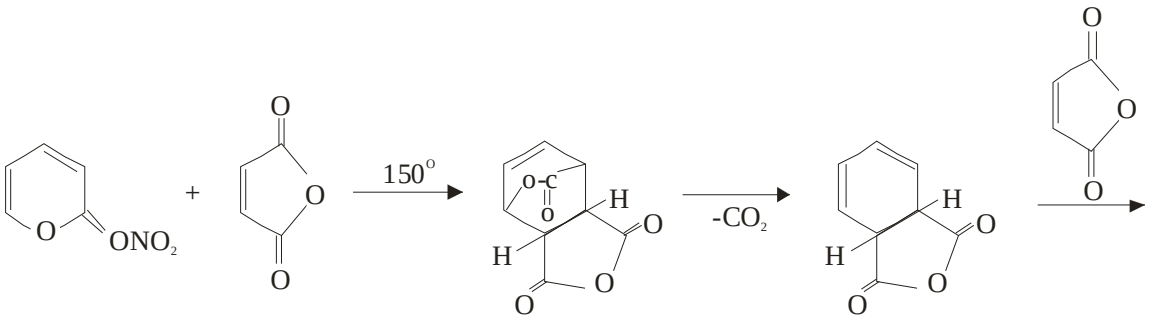


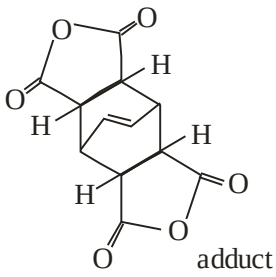
(iii) Nucleophilic Reactions :



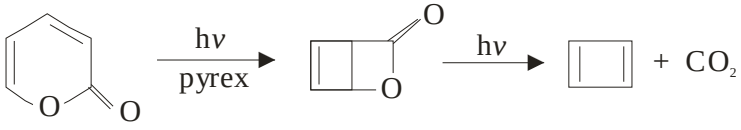


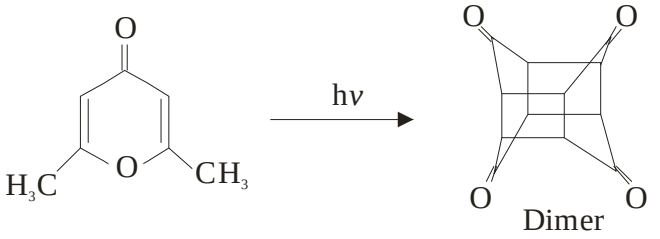
(iv) Diels-Alder Reaction :





(v) Photochemical Reaction :





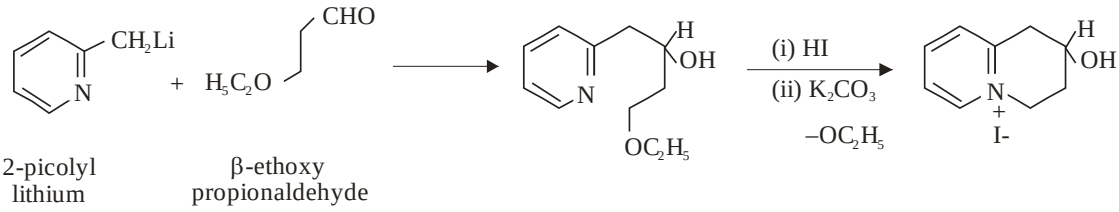
7.4.3 Comparison of pyrylium salt and pyrones with pyridinium & thiopyrylium salts with pyridones:

Pyrylium salt which are not very stable through they have some aromatic character and the lone pair of electron on oxygen involved in bonding, then oxygen gets positive charge .but □ and □ pyrones are the six membered heterocycles which do not possess aromatic sextet. Pyrylium salts are formed by the reaction of acid or electron acceptor metallic ion with pyridine. Due to the availability of electrons on nitrogen atom, pyridine forms crystalline salts. These salts are not usually isolated and they act as acylating and sulphonating agents.Thiopyrylium salts are stable but pyridon-2-one is nonbenzenoid and nonaromatic structure.

7.5 Quinolizinium salt

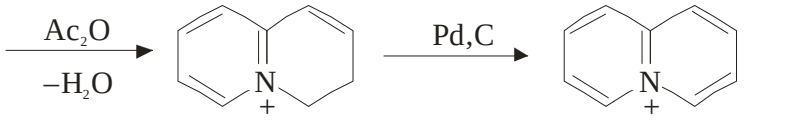
7.5.1 Methods of Preparations

(i) Condensation Reaction :



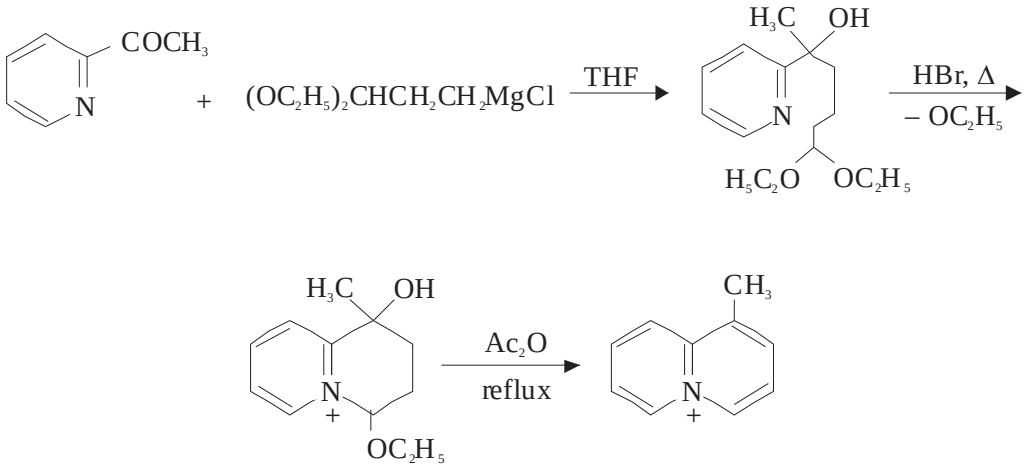
2-picolyl lithium

β-ethoxy propionaldehyde

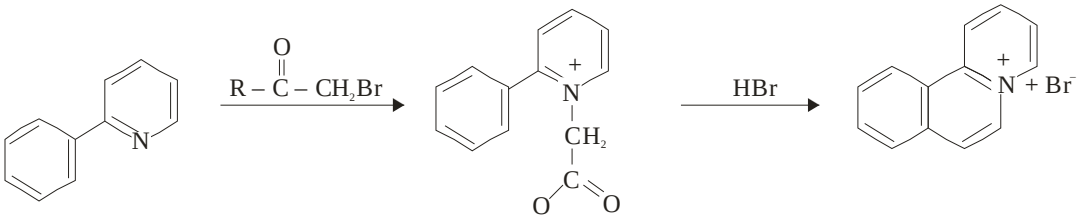


Quinolizinium ion

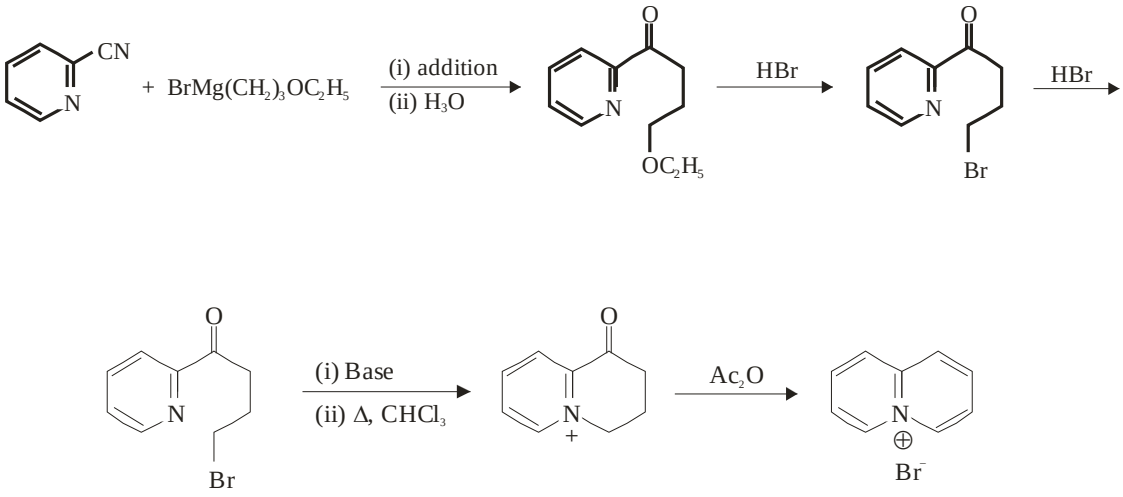
(ii) Glover and Jones method :



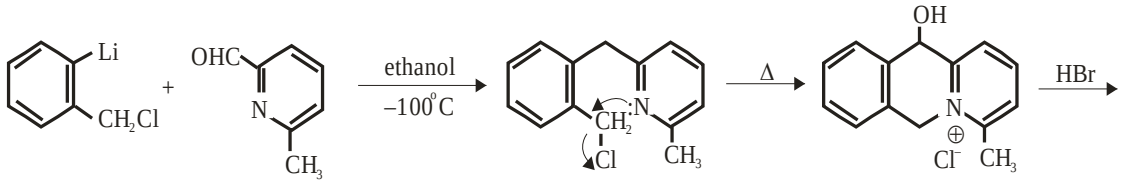
(iii) From 1-phenylpyridine :

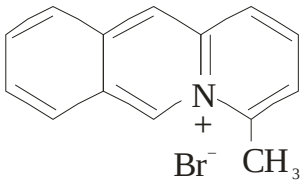


(iv) From 2-Cyanopyridine :



(v) From *o*-lithiobenzyl chloride :

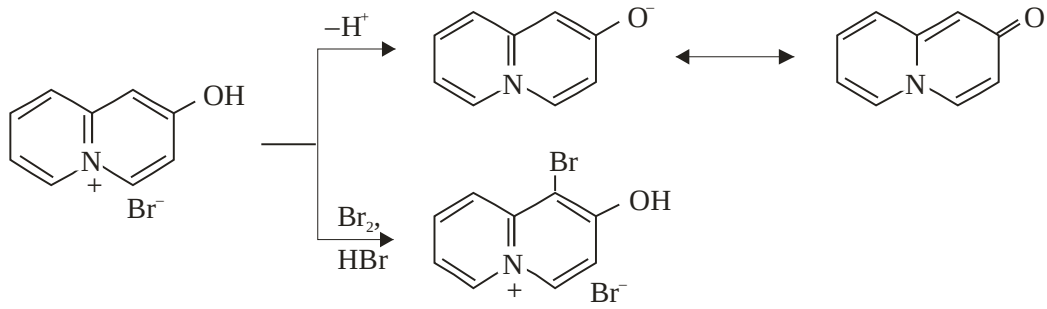




4- Methylacridizinium Bromide

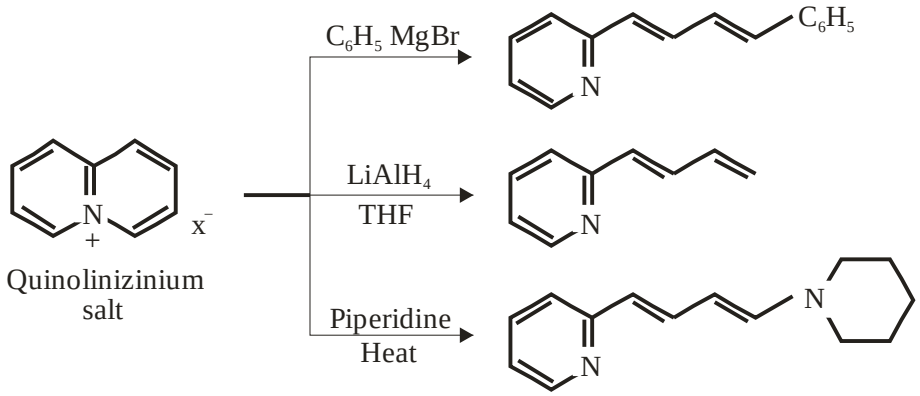
7.5.2 Chemical Reactions

(i) Electrophilic Substitution :

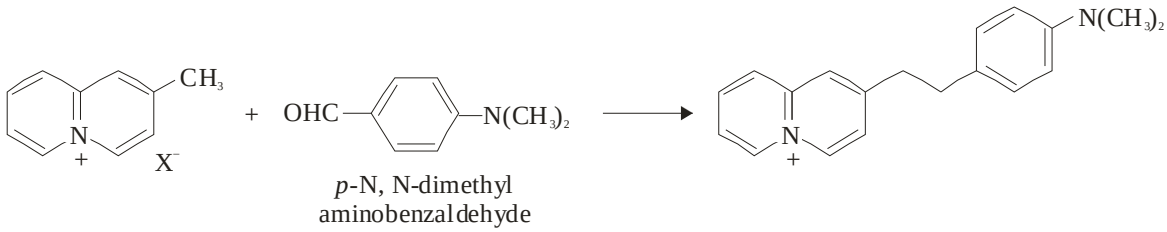


2-bromo -1- hydroxy quinolizinium bromide

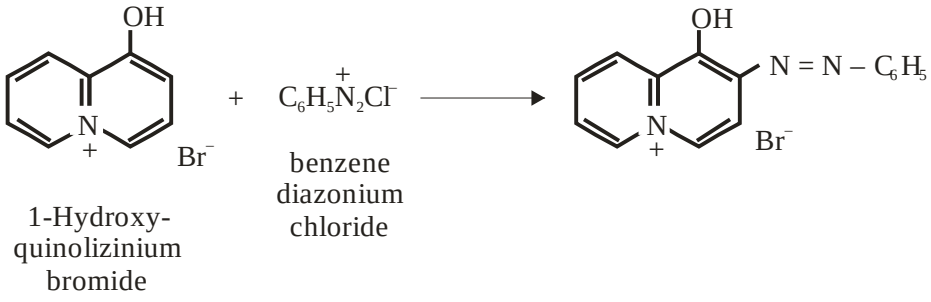
(ii) Nucleophilic substitution :



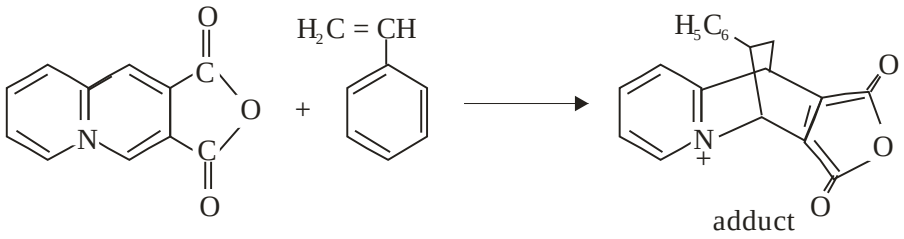
(iii) Condensation Reaction :



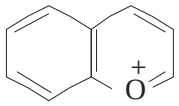
(iv) Coupling Reaction :



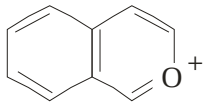
(v) Cycloaddition Reaction :



7.6 Benzoprylium Salt



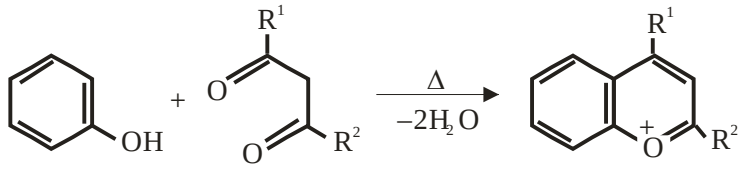
1-benzopyrylium cation



2-benzopyrylium cation

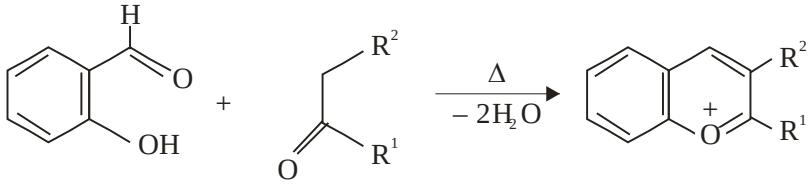
7.6.1 Methods of Preparations

(j) From phenol :

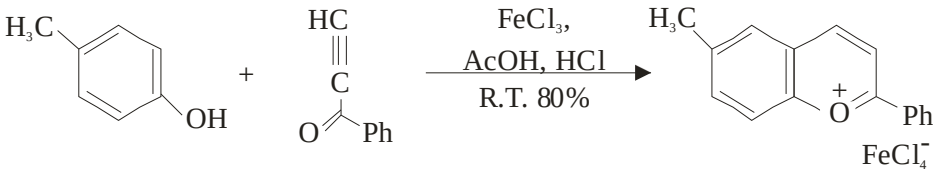


Phenol

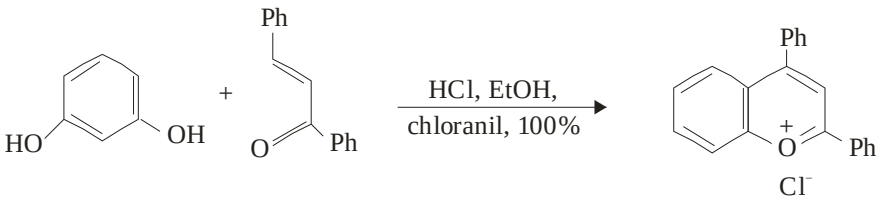
(ii) From *o*-hydroxy benzaldehyde :



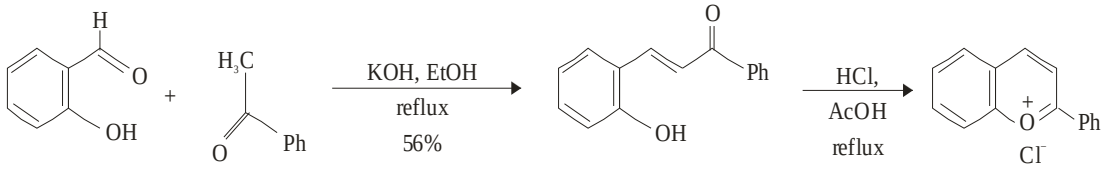
(iii) From Acetylenic Ketone :



(iv) From Chalcones :



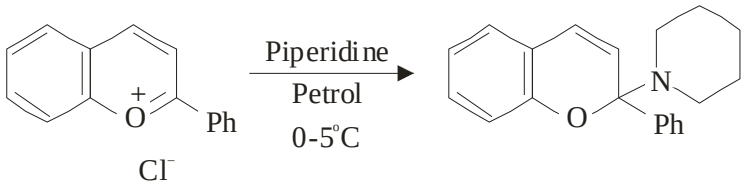
(v) From Acetophenone :

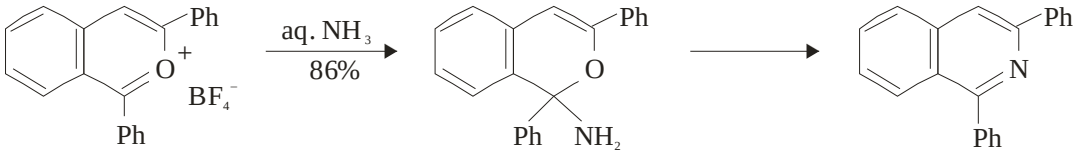


7.6.2 Chemical Reactions

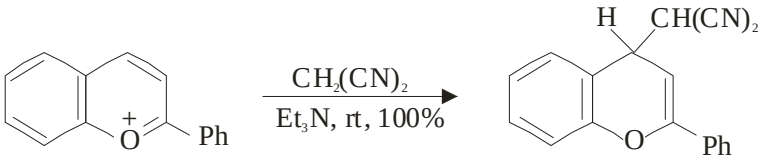
(i) Reaction with Nucleophilic Reagents :

(a) Reaction with Ammonia and amines :

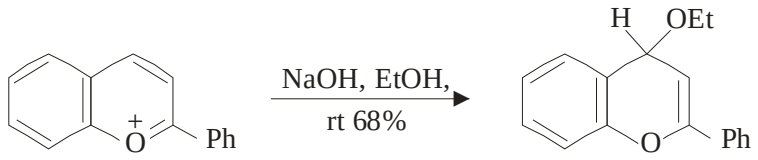




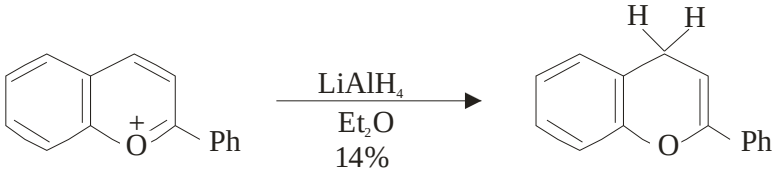
(b) Reaction with carbon nucleophiles :



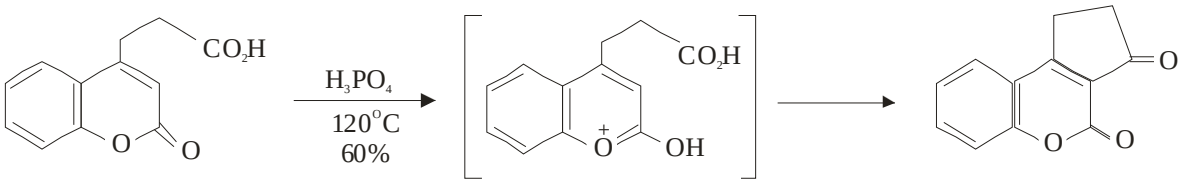
(c) Reaction with alcohol :



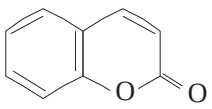
(ii) Reaction with Reducing agent :



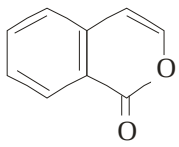
(iii) Reaction with Electrophilic reagent :



7.7 Coumarins



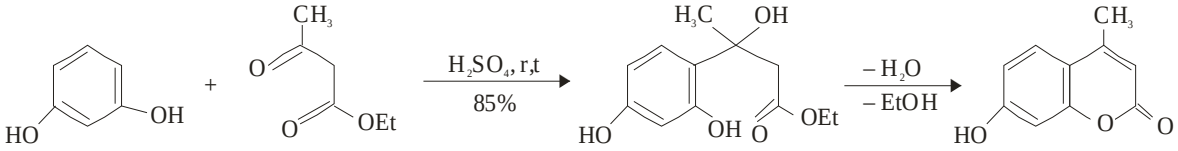
Coumarin



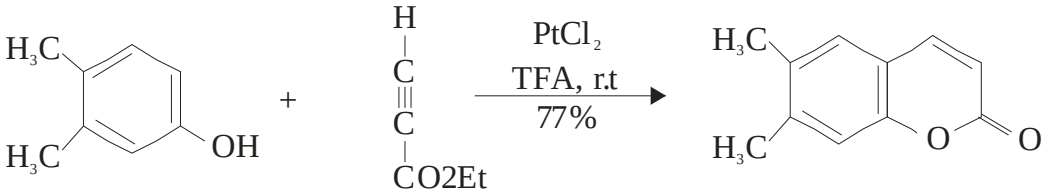
isocoumarin

7.7.1 Methods of Preparations

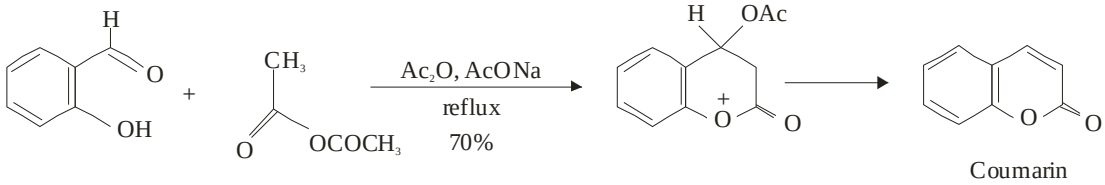
(i) Pechmann Synthesis : From phenol



(ii) From the reaction of phenol and propiolate :

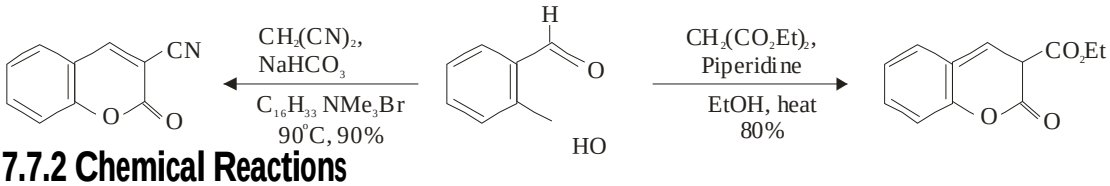


(iii) From *o*-hydroxy benzaldehyde :



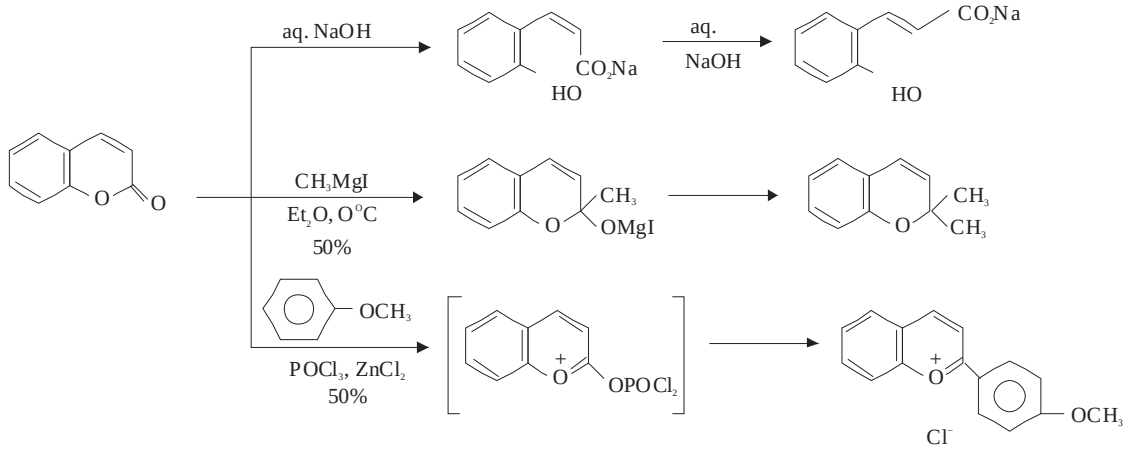
Coumarin

(iv)

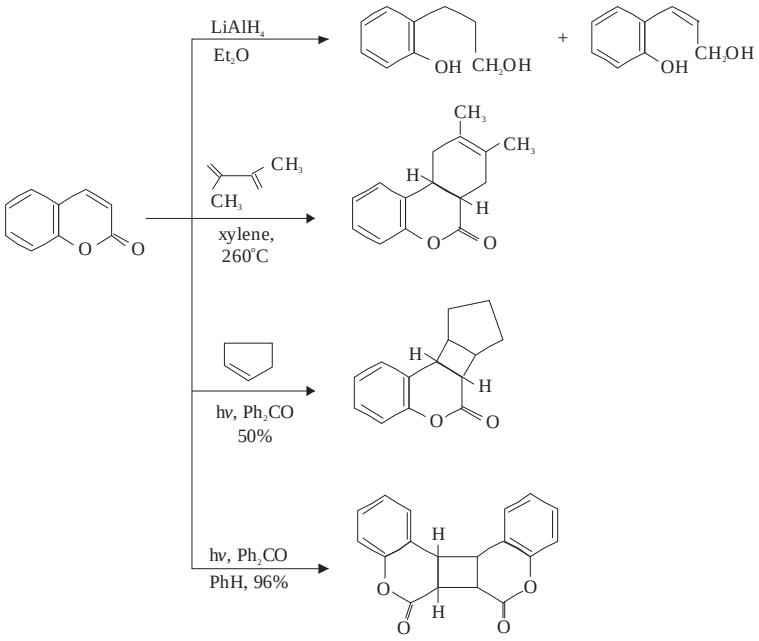


7.7.2 Chemical Reactions

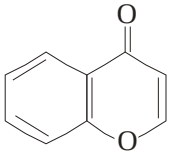
(i) Reaction with Nucleophilic reagents:



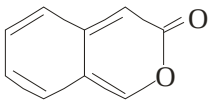
(ii) Reaction with reducing agent & cycloaddtion reaction :



7.8 Chromones



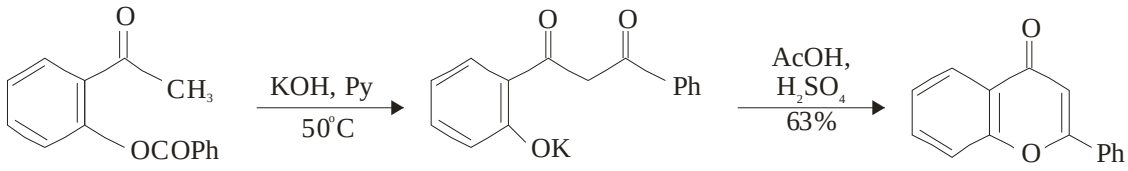
Chromone
[4H-1-benzopyran -4-one]



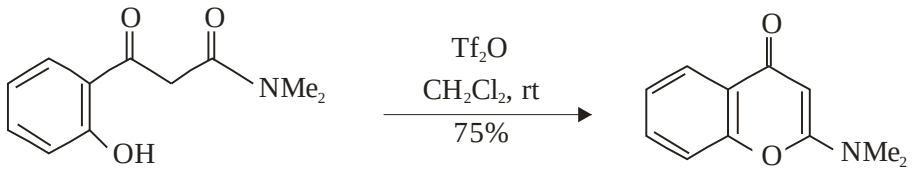
[2H-2-benzopyran -3- one]

7.8.1 Methods of Preparations :

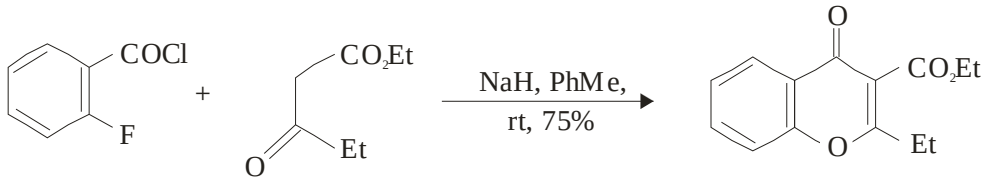
(i) From o-hydroxyacyl – arenes with ester :



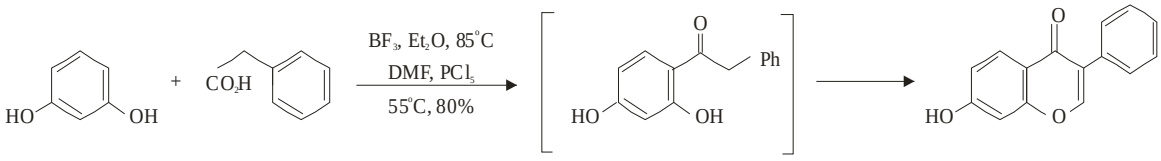
(ii) From ring closure of 1-(ortho-hydroxyaryl) -1,3-Ketoamides



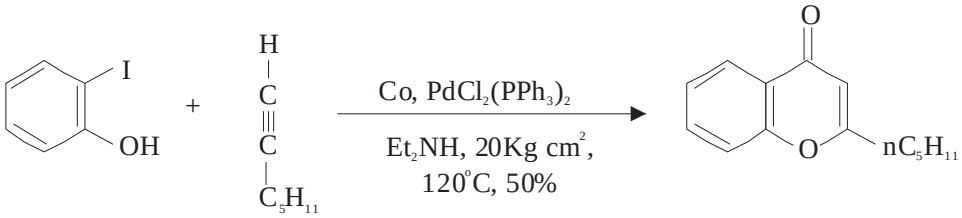
(iii) From ortho-fluorobenzoyl chloride :



(iv) From phenol :

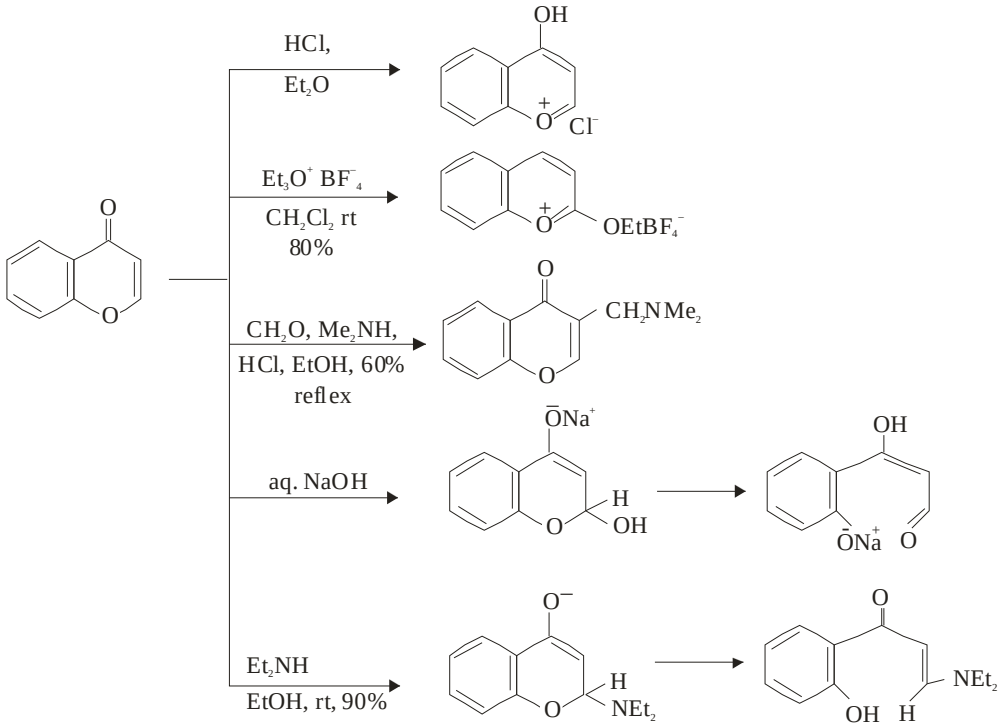


(v) From ortho-hydroxy aryl alkynyl ketones :



7.8.2 Chemical Reactions

(i)



7.9 Summary

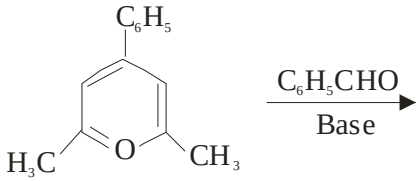
Pyrylium cations are unusaually stable oxonium ions with aromatic character and undergo some reactions analogous to those of pyridinium cations. The 2-,4- and 6-positions are strongly activated to nucleophilic attack and these reactions often result in ring opening unless the reaction medium is strongly acidic.

2H- Pyran -2- ones are nonaromatic unsaturated lactons which can be cleaved by nucleophiles and undergo cyclization and cycloaddition reactions typical of conjugated dienes. The 4H-pyran-4- are system is also nonaromatic and shows the properties expected of a vinylogous lactone ; it can however, be protonated by mineral acids on the exocyclic oxygen atom to give a pyrylium cation.

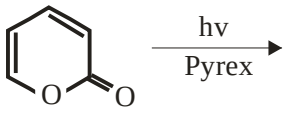
7.10 Review Questions

1 Predict the major product of the following reactions :

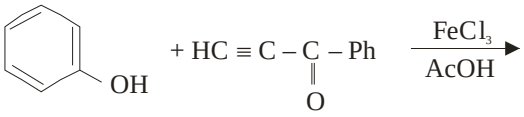
(a)



(b)



(c)



2 Give the structures of Benzopyrylium salt.

3 How could one construct a 1-benzopyrylium salt from a phenol ?

4 How can ortho hydroxyaryl aldehydes be used to prepare coumarins ?

5 How can ortho-hydroxy-ketones be used to prepare chromones ?

7.11 Reference and suggested readings

- Organic chemistry Bhupinder Mehta & Manju Mehta, PHI leaving PVT. Ltd New Delhi (2010).
- Heterocyclic chemistry III[rd] Ed. Thomas L. Gilchrist, Pearson education 2008.
- Heterocyclic chemistry V [th] Ed. Raj K. Bansal New Age International Publisher (2014).
- Heterocyclic chemistry 5[th] Ed. J.A. Joule and K. Mills John Wiley (2010).

Unit – 8

SIX MEMBERED HETEROCYCLES WITH TWO OR MORE HETEROATOMS

Synthesis and reactions of diazines, triazines, tetrazines and thiazines

Structure of unit

8.0 Objectives

Heterocyclic compounds have a wide range of applications: they are predominant among the type of compounds used as pharmaceuticals, as agrochemicals and as veterinary products. They are used as optical brightening agents, as antioxidants, as corrosion inhibitors and as additives with a variety of other functions. Many dyestuffs and pigments have heterocyclic structures.

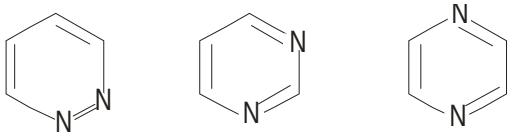
8.1 Introduction

Heterocyclic compounds are widely distributed in nature. Many are of fundamental importance to living systems. Nucleic acid bases which are the derivatives of the pyrimidine and purine ring system. Some diamino pyrimidine including pyrimethamine and trimethoprim are antimalarial agents.

8.2 Six membered Heterocycles with two or more heteroatoms

Aromatic six-membered heterocycles that contain two, three and four ring nitrogen atoms are named systematically as diazines, triazines and tetrazines.

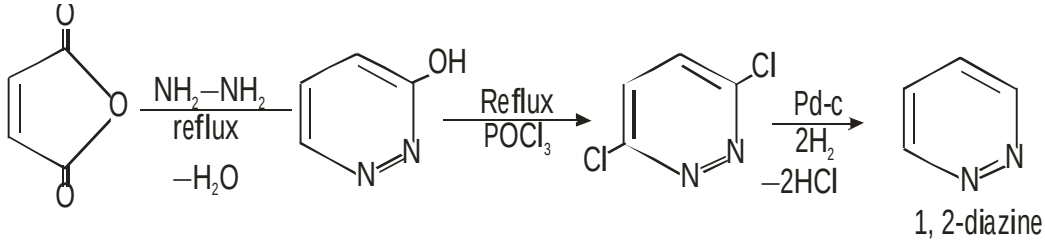
8.3 Diazines



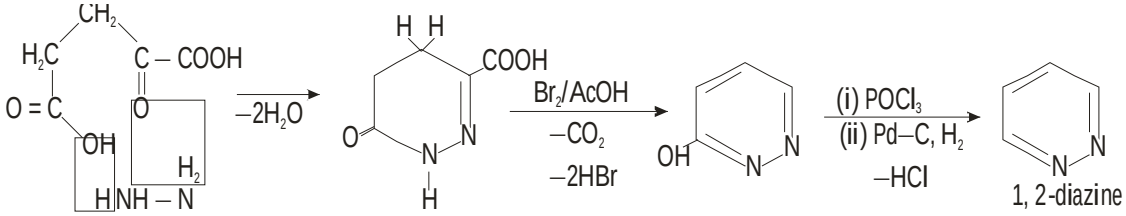
1, 2-Diazine or pyridazine | 1, 3-diazine or pyrimidine | 1, 4-diazine or pyrazine

8.3.1 Methods of preparations

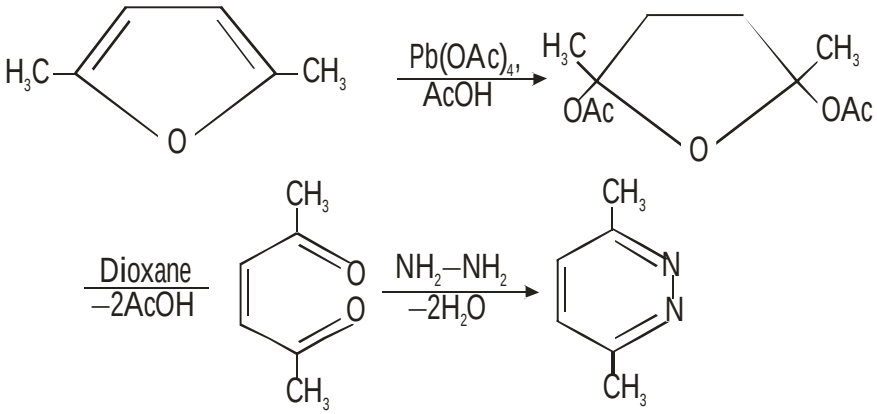
(i) From maleic anhydride :



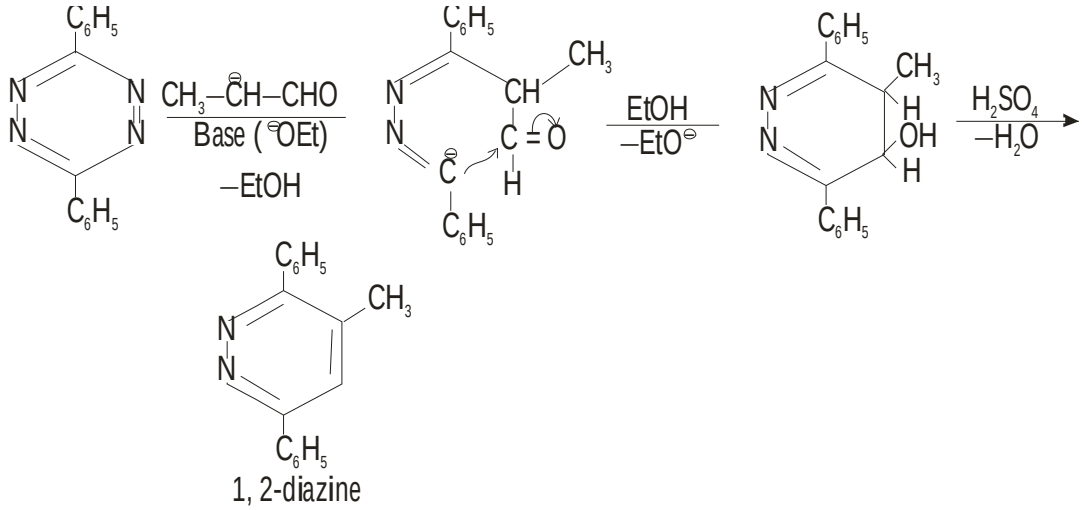
(ii) From □-keto acids :



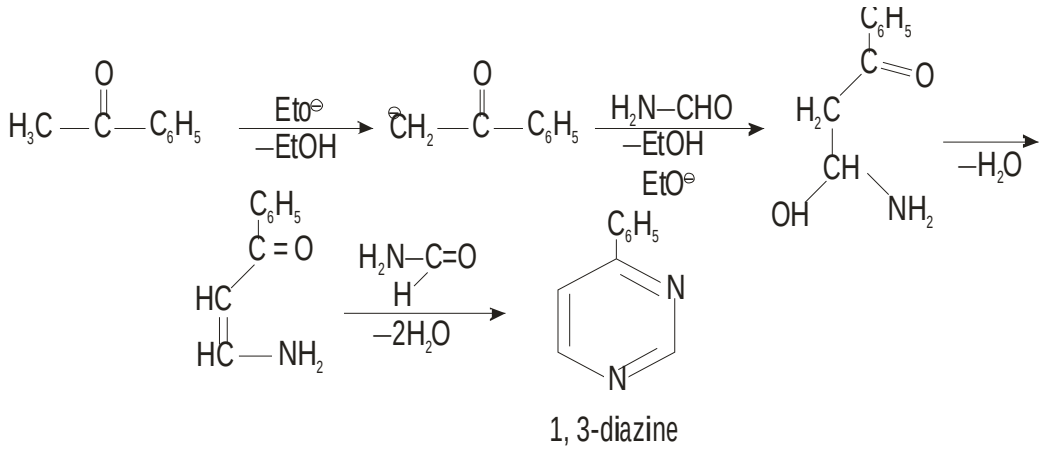
(iii) From Furan derivatives :



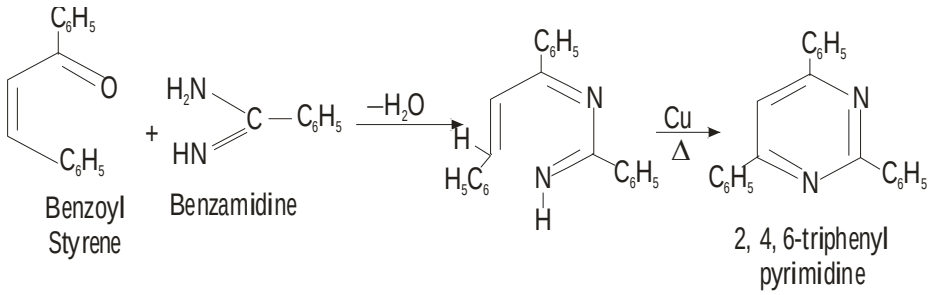
(iv) From Tetrazine



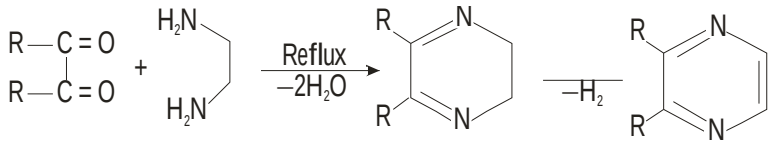
(v) From acetophenone :



(vi) From Benzamidine:

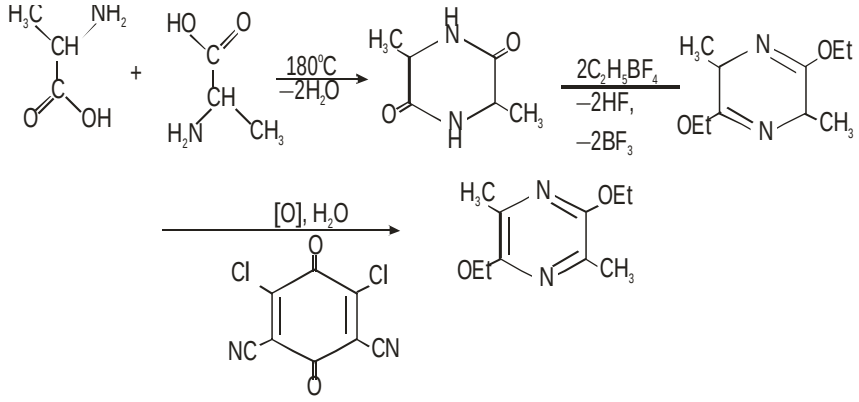


(vii) From 1, 2-diketone :

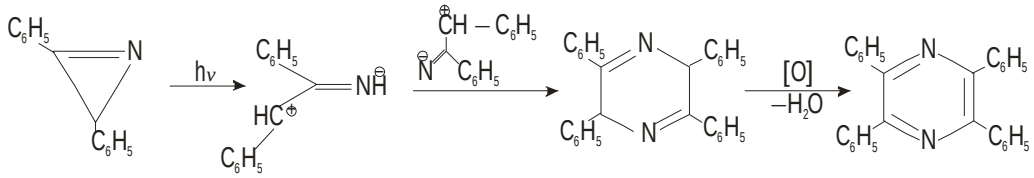


2, 3-dialkyphrazine

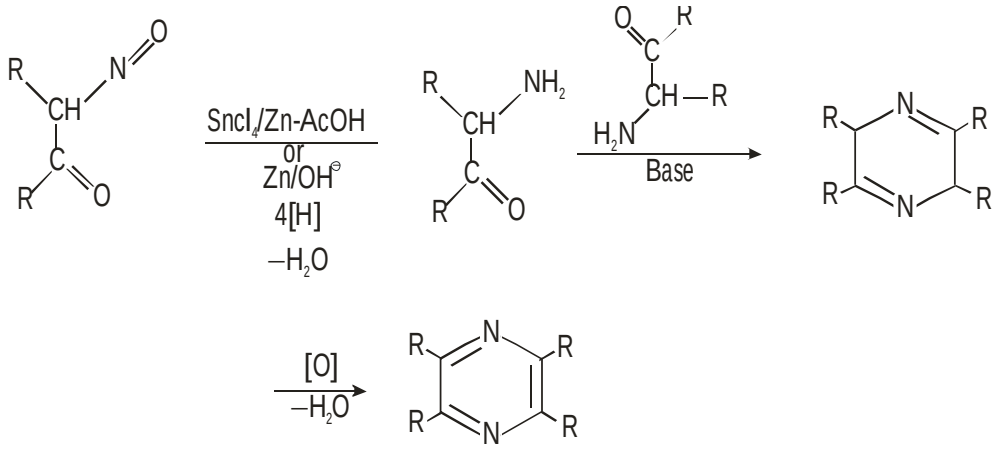
(viii) From acid:



(ix) From Azirine derivative :



(x) From nitroso ketone :

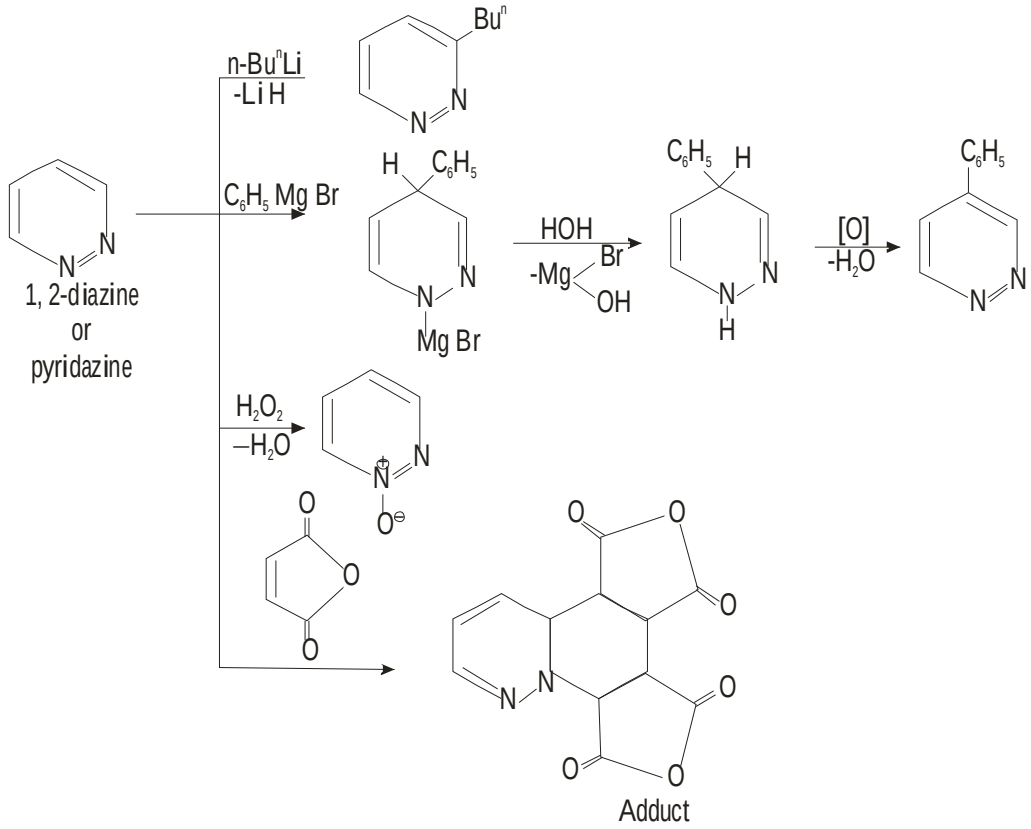


8.3.2 Physical Properties

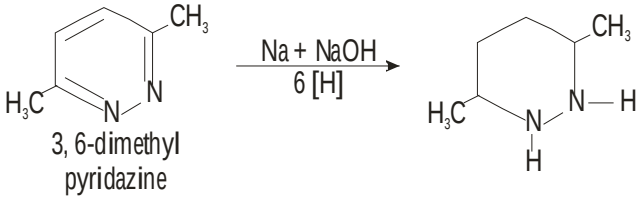
1, 4-diazine is colourless solid, it has m.p. 54°C. It is weaker base (p_{ka} = 0.6). 1, 2-diazine is colourless liquid having boiling point 207°C. It is miscible with water and benzene. It's dipole moment is 4D. Pyrimidines is a colourless compound, m.p. 225°C, b.p. 124°C. It is weakly basic as compared to pyridine

8.3.3 Chemical Properties

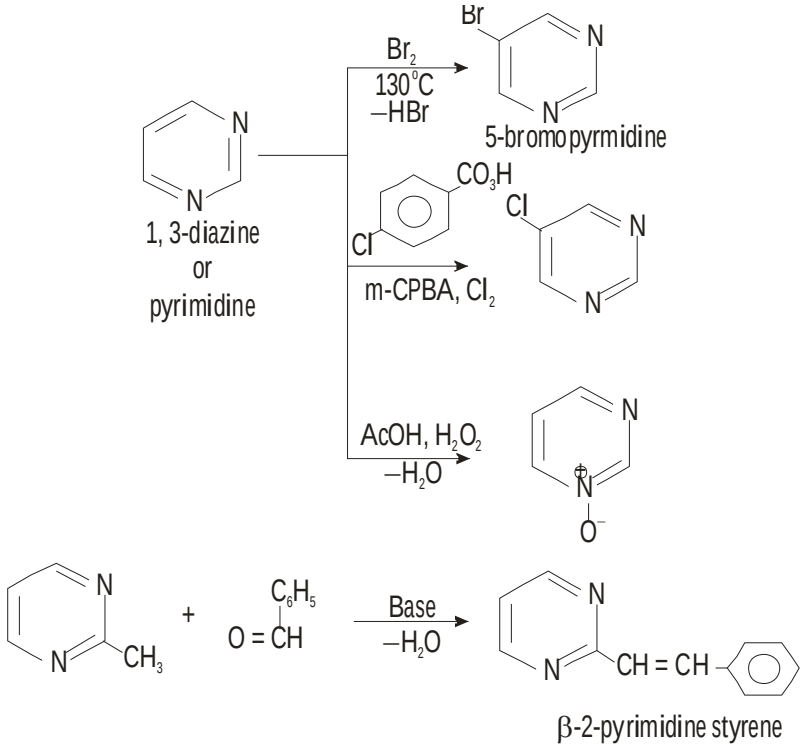
(i)



(ii) Reduction :



(iii)



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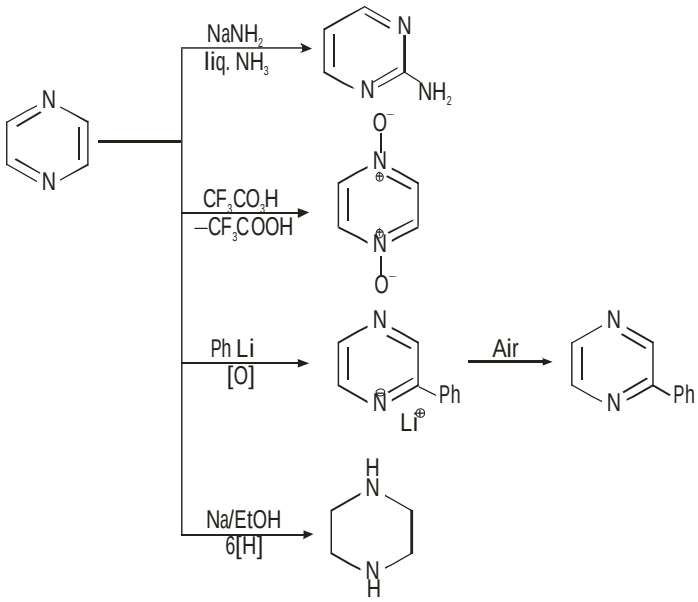
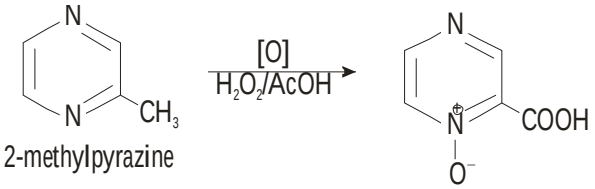
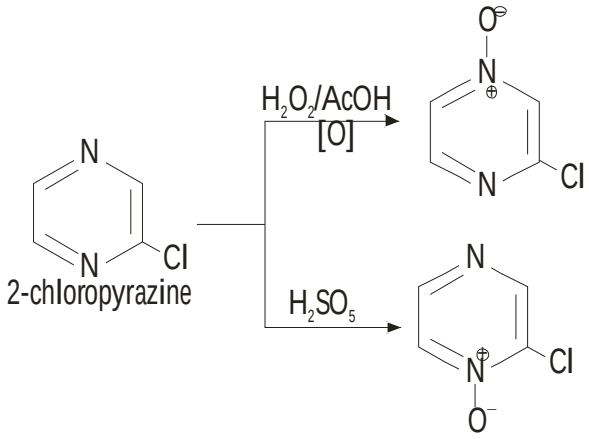


(v)



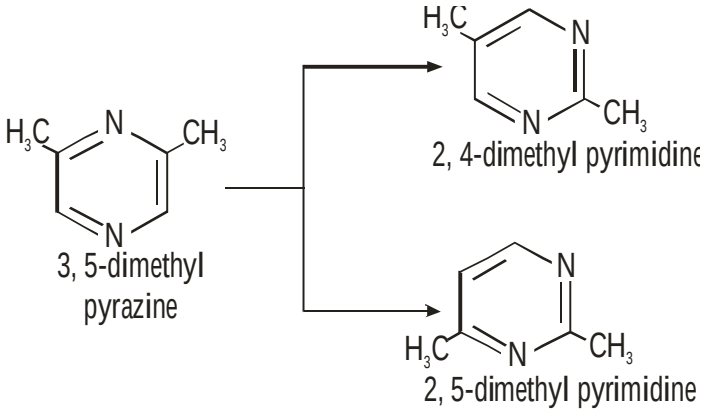
2-methylpyrazine

(vi)



2-chloropyrazine

(vii) Photochemical reaction :

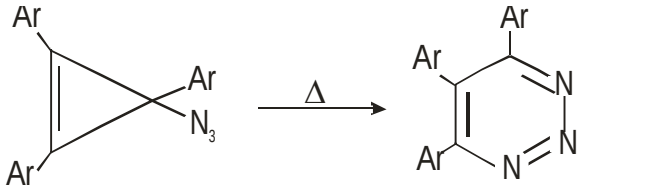


8.4 Triazine

Heterocyclic ring contain three nitrogen atoms are known as Triazine.

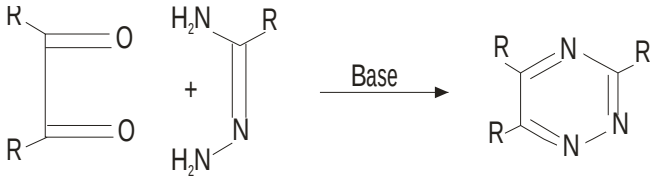
8.4.1 Methods of Preparations

(i) From thermal rearrangement of 3-azido cyclopropene:

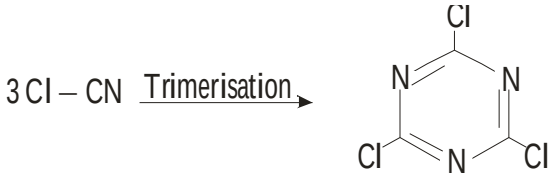


4, 5, 6-triaryl-1, 2, 3-triazine

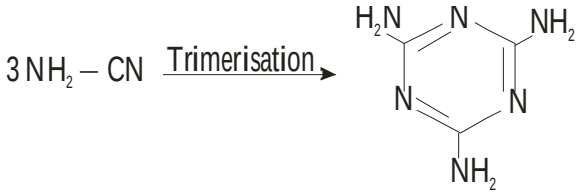
(ii) From 1, 2-dicarbonyl compounds :



(iii) From trimerisation of cyanogen chloride :

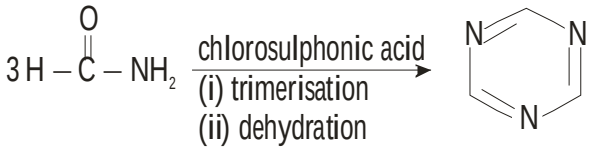


(iv) From trimerisation of cynamide



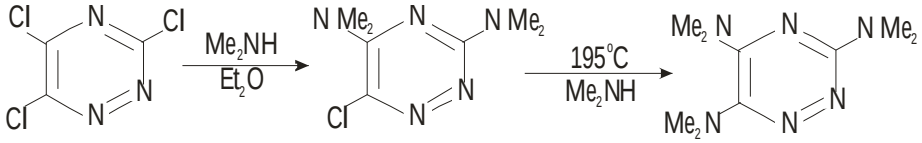
Melamine

(v) From trimerisation of farmamide :

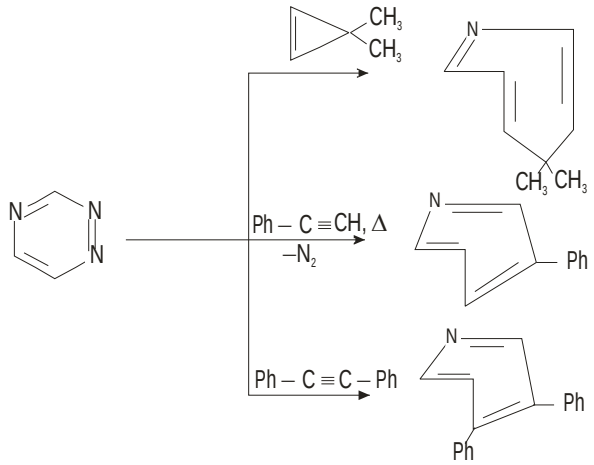


8.4.2 Chemical Reactions

(i) Aromatic nucleophilic substitution reaction :

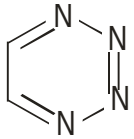


(ii) Diels-Alder reactions:

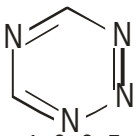


8.5 Tetrazine

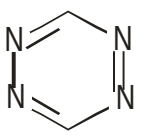
These are six membered heterocyclic compound containing four nitrogen and two carbon atoms.



1, 2, 3, 4-tetrazine



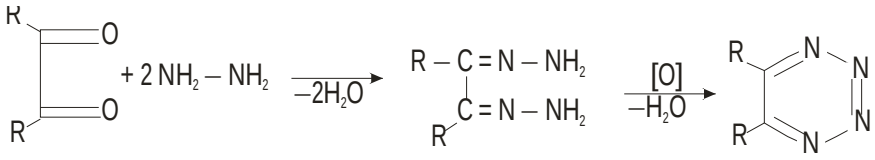
1, 2, 3, 5-tetrazine



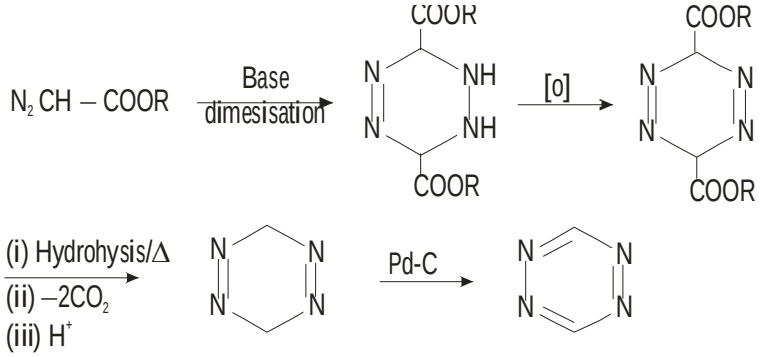
1, 2, 4, 5-tetrazine

8.5.1 Method of Preparations

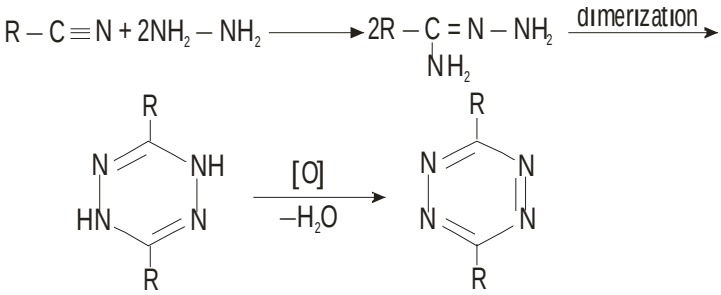
(i) From □-diketone :



(ii) From dimerization of diazoester :

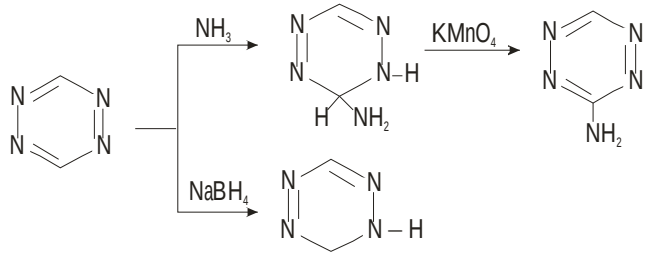


(iii) From alkyl cyanide:

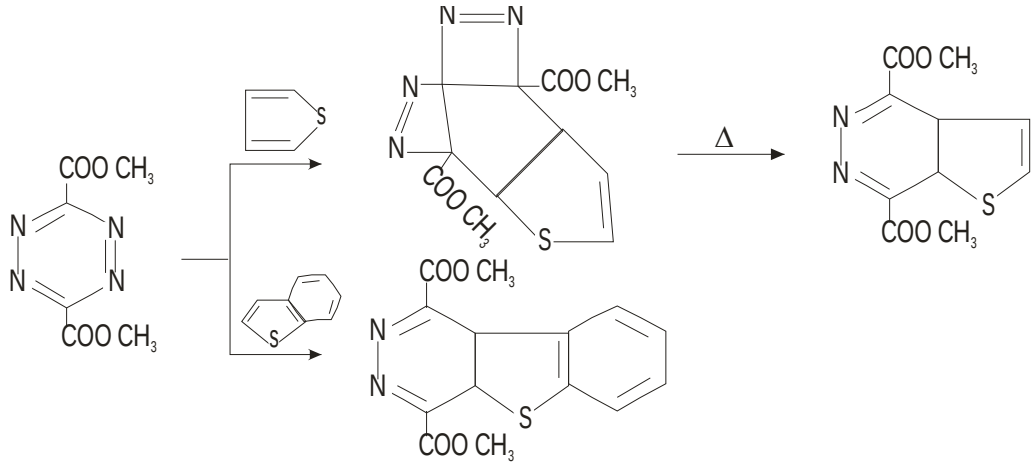


8.5.2 Chemical Reactions

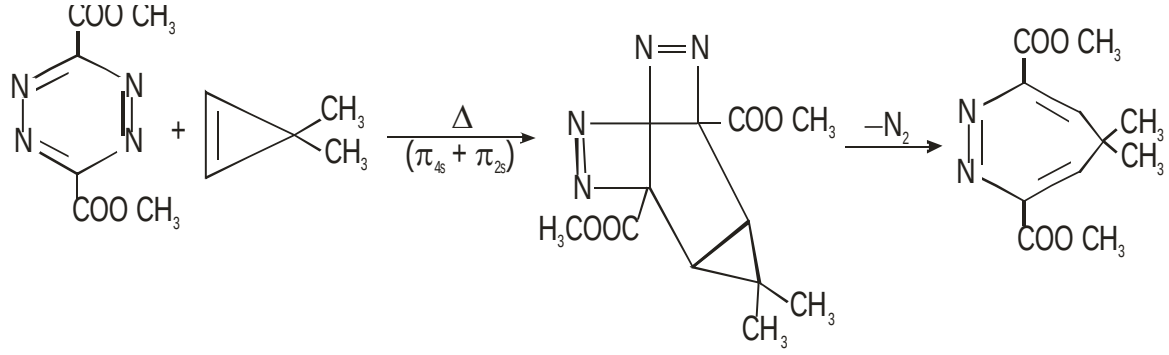
(i) Nucleophilic addition reactions :



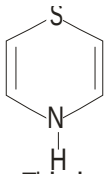
(ii) Diels-Alder reactions:



(iii) Reaction with cyclopropene :



8.6 Thiazines

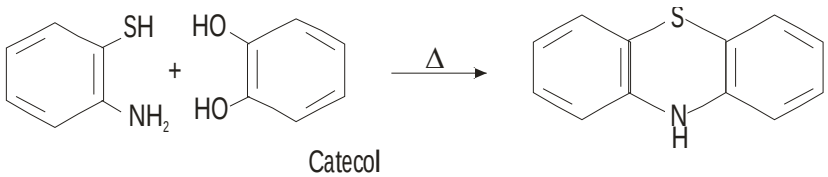


Thiazine

It is act as insecticide. It is also a member of dyes eg : methylene blue which is used as a biological oxidizing agent, reducing agent and also used as the indicator.

8.6.1 Methods of Preparations

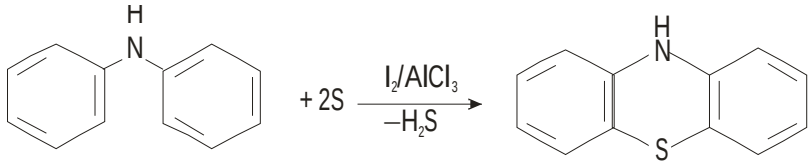
(i) From o-amino thiophenol :



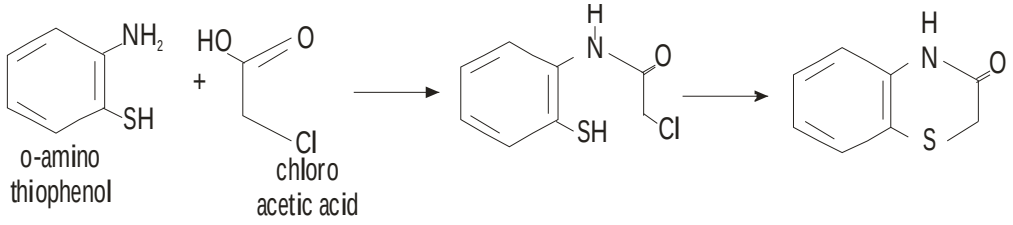
Catecol

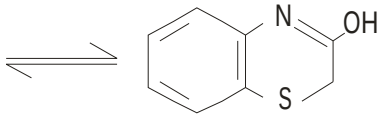
Phenothiazine

(ii) It can be prepared by the fusion of diphenylamine with sulphur in the presence of iodine and $AlCl_3$.

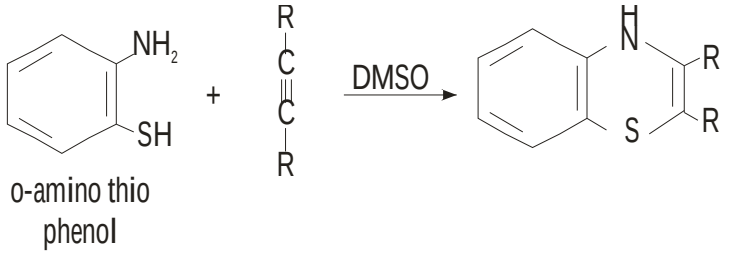


(iii) Reaction of *o*-aminothiophenol with chloroacetic acid:





(iv) From oxidative cyclization reaction:

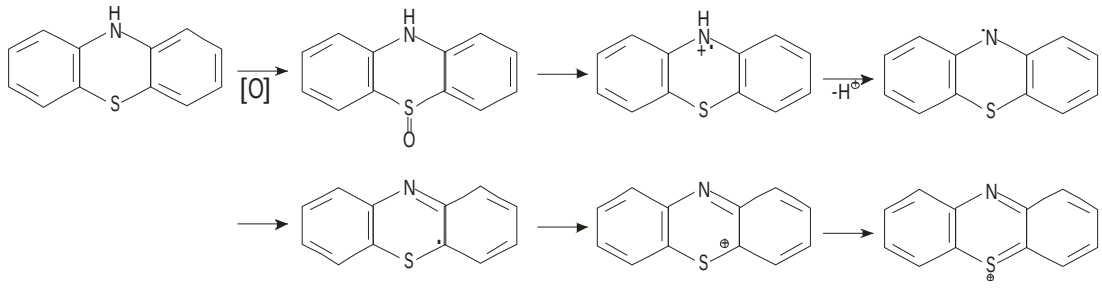


8.6.2 Physical Properties

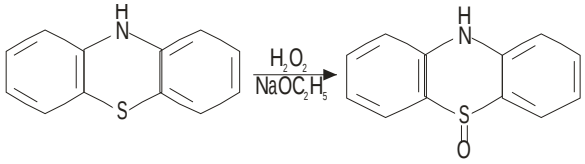
It is solid which has m.p. 185°C. It is basic in nature. When it react with acid to from salt.

8.6.3 Chemical Properties

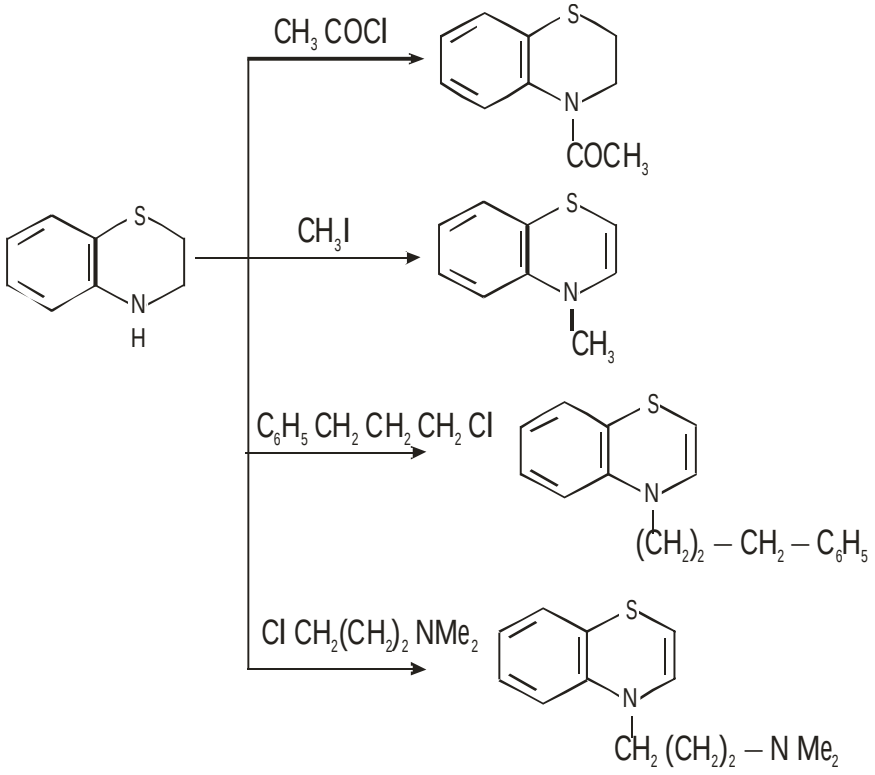
(i) Oxidation:



(ii) Reaction with H_2O_2:



(iii) N-Substitution reactions:



8.7 Summary

(i) The diazines pyridazine, pyrimidine and pyrazine are electron deficient aromatic heterocycles. All are weaker bases than pyridine. Electrophilic

substitution take place only in derivatives with strongly electron releasing group, the 5-position of pyrimidine being the least deactivated.

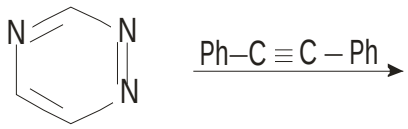
(ii) Nucleophile attack the diazines, triazines and tetrazines readily. The rate of displacement of leaving groups increases with the number of nitrogen atoms in the ring. In a particular ring system the rate is greatest at the carbon atoms which are best activated by ring nitrogen atoms.

(iii) 1, 2, 4, triazines and 1, 2, 4, 5-tetrazines can act as electron deficient dienes in Diels Alder reactions.

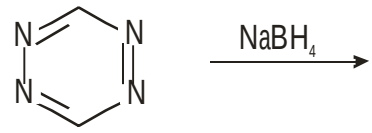
8.8 Review Question

1. Which is the only chlorodiazine that does not undergo easy nucleophilic displacement, and why ?
2. What is the product from hydrazine and a 1, 4-keto ester ? How would it be converted into a pyridazinone ?
3. What is the difference between pyridazine and pyrazine ?
4. What compounds are produced at each stage is the following sequences :
   (a) pyridazin-3-one reacted with $POCl_3$
   (b) Chloropyrazine with $BuNH_2$/120°C
5. What substitution pattern is easiest to achieve in the ring synthesis of pyrazines ?
6. Give any two methods of preparations of tetrazines ?
7. What is diazine ? Give its various structures.
8. How we can prepare pyridazine from maleic anhydride.
9. Complete the following reactions :

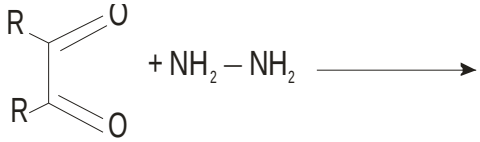
   (a)



   (b)



(c)



10. Give the trimerisation of cyanogen chloride.

8.9 Reference an suggested readings

- Heterocyclic chemistry Vth Ed. J.A. Joule and K. Mills, John Wiley 2011
- Heterocyclic chemistry IIIrd Ed. Thomas L. Gilchrist, Pearson Education 2008.
- Heterocyclic chemistry IInd Ed. R.R. Gupta, M. Kumar, V. Gupta, Springer 1998
- Organic chemistry, O.P. Agrawal, Goel Publishing House, Meerut.

Unit - 9

SEVEN AND LARGE MEMBERED HETEROCYCLES : Synthesis and Reactions of Azepines, Oxepines, Thiepines, Diazepines, Azocines, Diazocines, Dioxocines, Dithiocines And Thiazepines.

Structure of Unit

9.0 Objectives

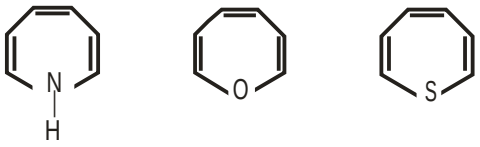
The study of heterocyclic compounds is a vast and expanding area of chemistry because of their applications in medicine, agriculture, photodiodes and other fields. Many heterocyclic compounds occur naturally and are actively involved in biology e.g. nucleic acid (purine and pyrimidine bases), vitamines (Thiamine B_1 Riboflavin B_2, Pyridoxol B_6 and Ascorbic acid C), heme and chlorophyll, penicillins, cephalosporins, alkaloids etc, various heterocycles are promising structural moiety for drug designing.

9.1 Introduction

Seven or larger ring heterocycles exist in natural Products; the porphyrines are widely distributed as pigments such as chlorophylls and hemoglobins. Although nitrogen, oxygen and sulfur are the common atoms found together with carbon in heterocyclic rings. Heterocyclic compounds are potential bioactive molecules and exhibit various bioactivities such as anti-inflammatory, anti-tubercular, antioxidant etc.

9.2 Seven membered Heterocycles

The seven-membered heterocyclic rings containing one hetroatom are the heterocyclic analogues of 1, 3, 5 – cycloheptatrien – these are azepine (1), oxepine (2) and theipine (3)

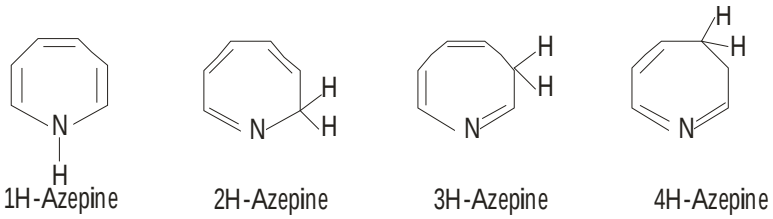


(1) (2) (3)

X-Ray crystallography has shown that these systems are not planar and hence behave as polyenes. They do not complete with Huckel's rule of $(4n+2)\square$ electrons and are thus non-aromatic and possess a high reactivity.

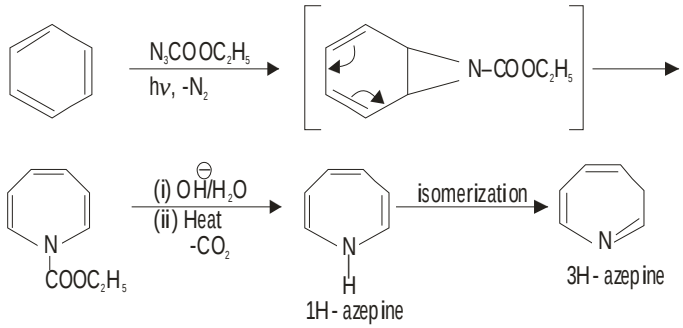
9.3 Azepines

Four tautomeric forms designates as 1H, 2H-, 3H- and 4H- azepine may be drawn for azacyclo heptatriene. The numbering commences from the nitrogen atom Except 3H- azepine none of the others have been isolated.

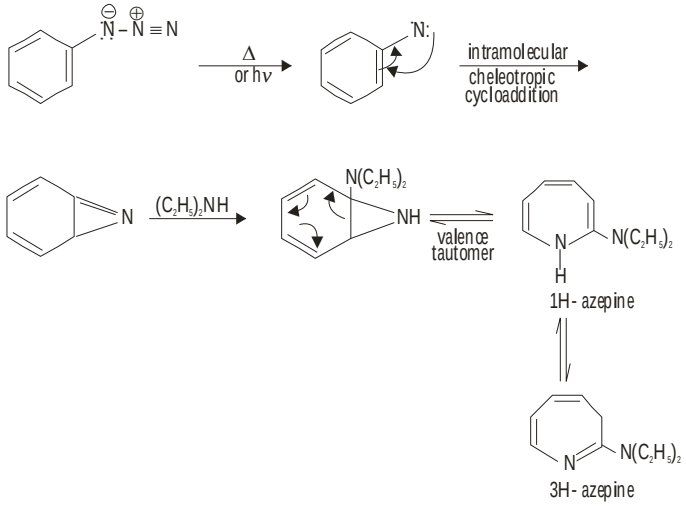


9.3.1 Methods of Preparations

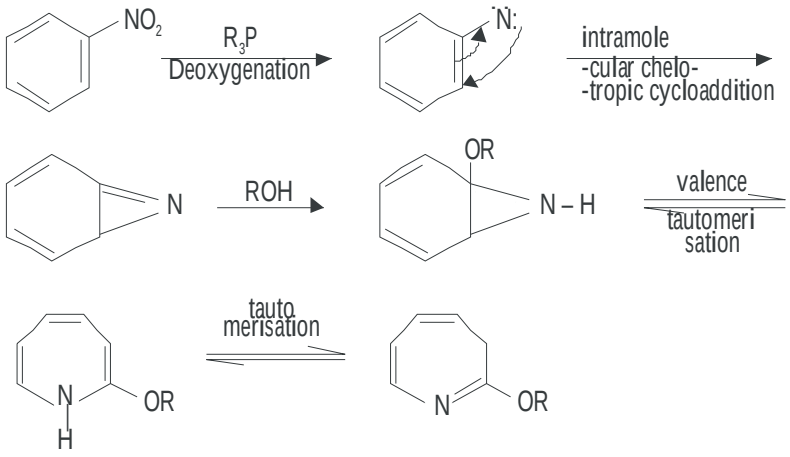
(i) Valence Bond isomerisations: This reaction involves a reorganization of the σ and π electrons and does not involve a migration of any atom or group. Such organizations which are accompanied by corresponding changes in atomic distances and bond angles are known as valence – bond isomerization. For example ; preparation of N-ethoxycarbonyl-1H-azepine(I) from benzene and ethoxy carbonylnitrene (:$NCOOC_2H_5$). The compound (I) on hydrolysis gave 1H azepine which on isomerizes in 3H- azepine.



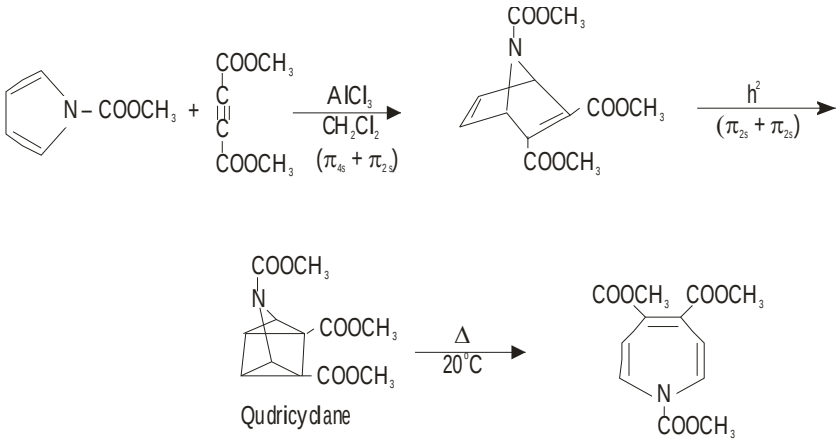
(ii) From phenyl azide :



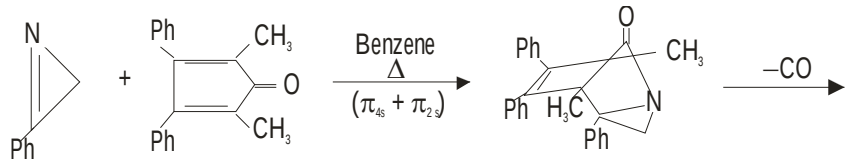
(iii) From nitrobenzene :

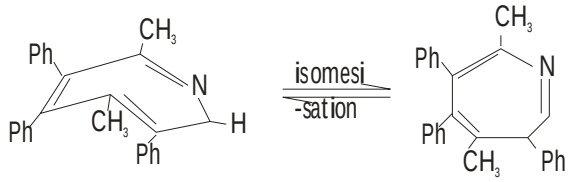


(iv) From N-Substituted pyrole :

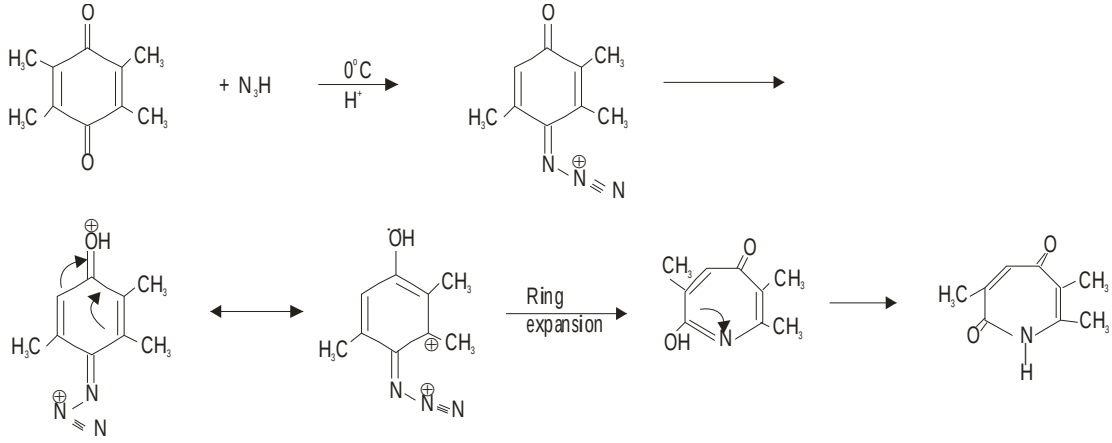


(v) From Azirine derivatives :

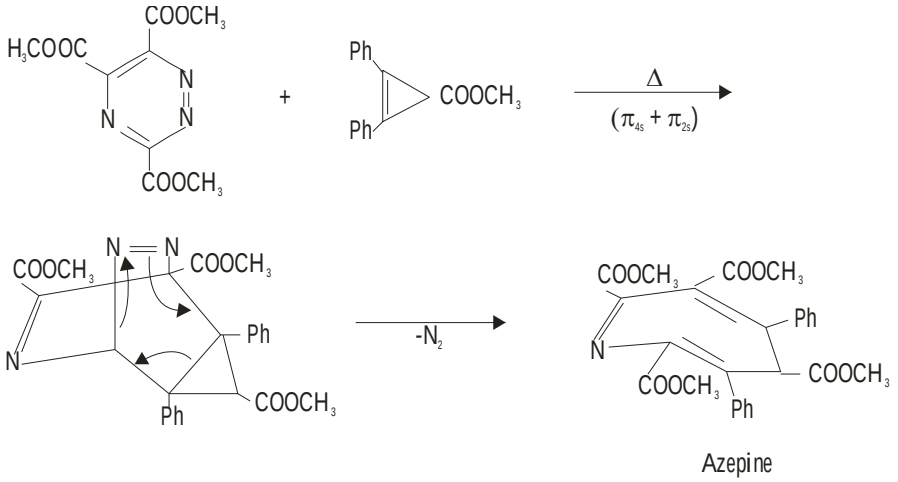




(vi) From *p*-benzoquinone :



(vii) Cycloaddition reaction of cyclopropene with 1,2,4- triazine :



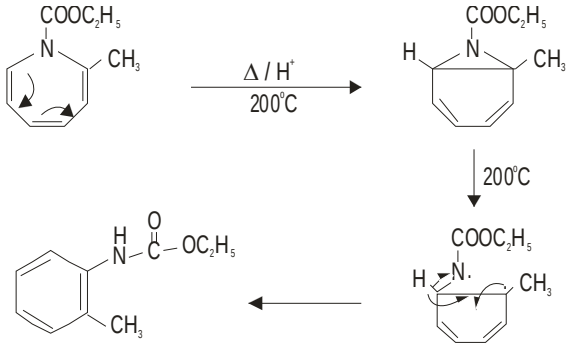
Azepine

9.3.2 Physical Properties

1H- azepine and N- substituted-1H-azepines are very unstable and readily rearrange to the 3H- azepine tautomer. In the basic solution the 4H isomer can be isolated and stored but isomerizes to 3H azepine, thus the stabilities of azepine tautomers decreases in the order 3H>4H>1H.

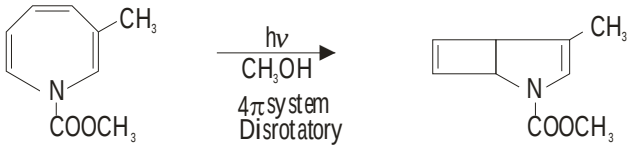
9.3.3 Chemical Properties

(1) Thermal Reaction : N-carboethoxy azepines on heating undergoes first electrocyclic reaction followed by aromatization and gives N- phenylcarbamates.

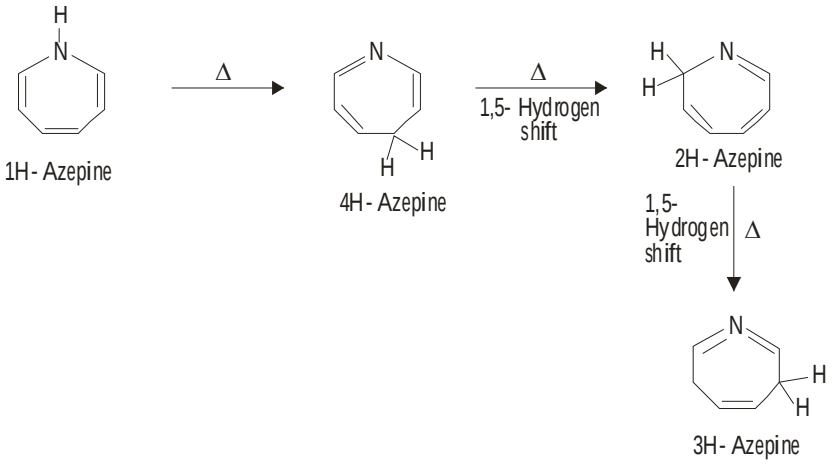


N- phenyl carbamates

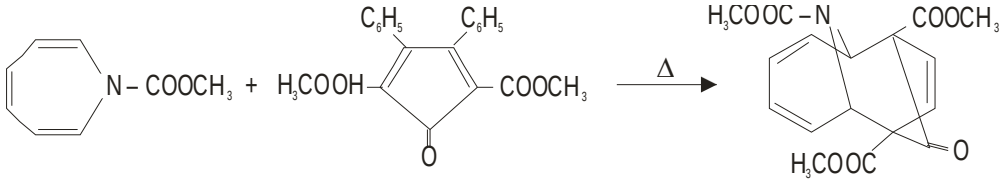
(2) Photochemical Reaction : When azepine is treated with photochemically, it undergoes electrocyclic reaction. It is also called ring contraction reaction.



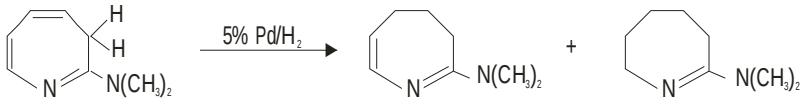
(3) Hydrogen Shift :



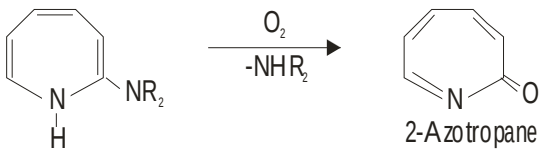
(4) Diels-Alder Reaction :



(5) Reduction :



(6) Oxidation :



9.4 Oxepins

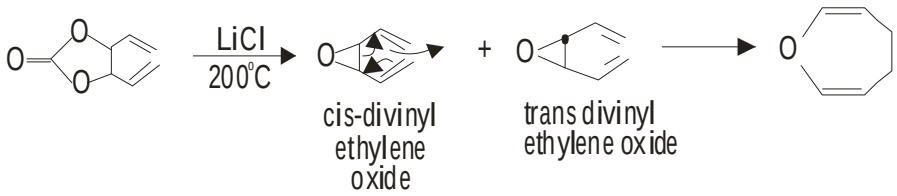
oxepin (1) containing one oxygen atom in the ring was first synthesized by vogel and coworkers. Subsequently it was demonstrated that benzene oxide(2)

interconverts to its valence isomer oxepin by a thermally allowed disrotatory electrocyclic process, Benzene oxide is obtained by the epoxidation of planar benzene.

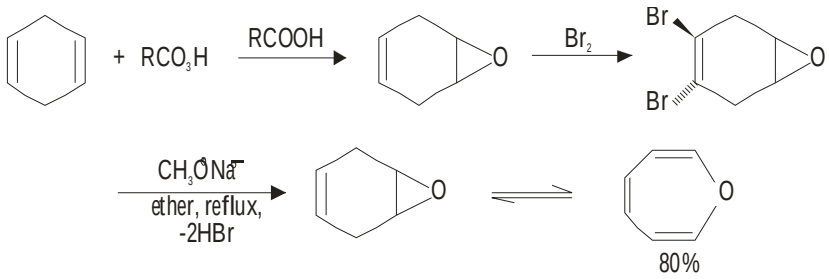
(1) (2)

9.4.1 Methods of Formation

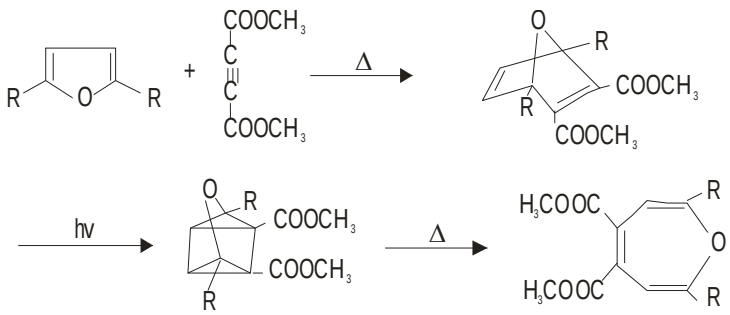
(i) Valence Bond isomerization :



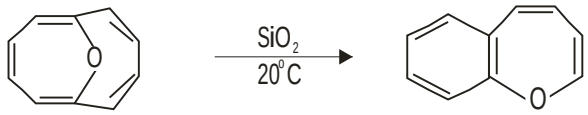
(ii) From cyclohexa -1,4- diene :



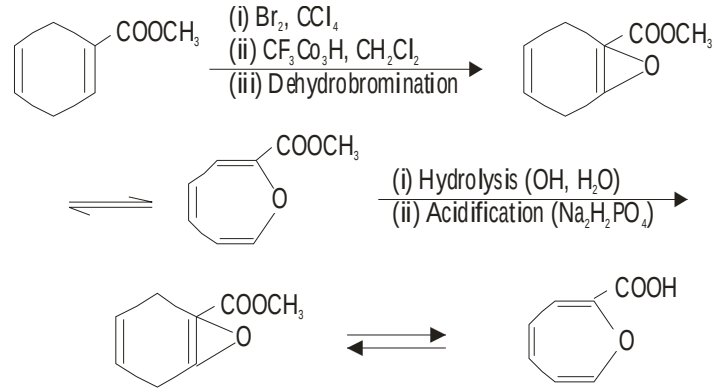
(iii) From furan derivatives :



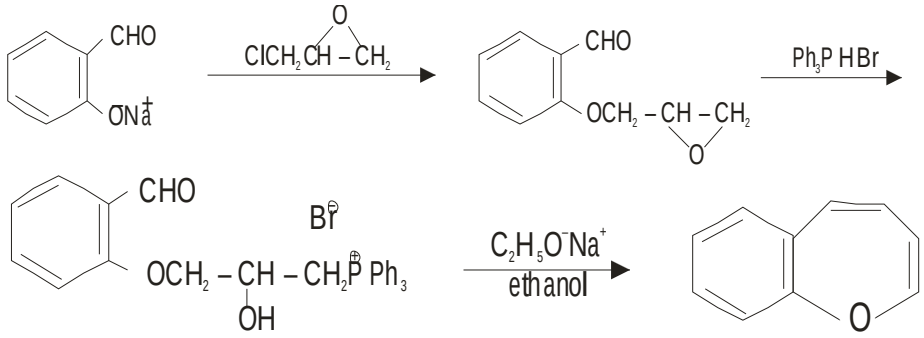
(iv) From 1,6- oxido [10] annulene :



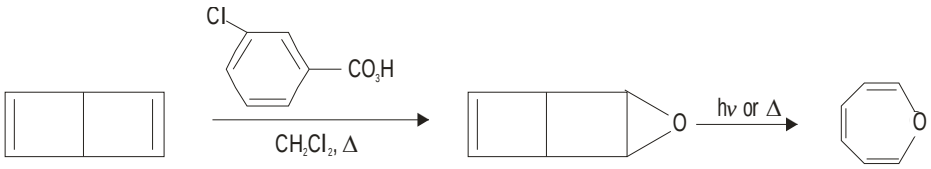
(v) From 1- Carbomethoxy cyclohexa -1,4- diene :



(vi) From Sodium Salt of Salicyaldehyde :



(vii) From Dewar benzene :

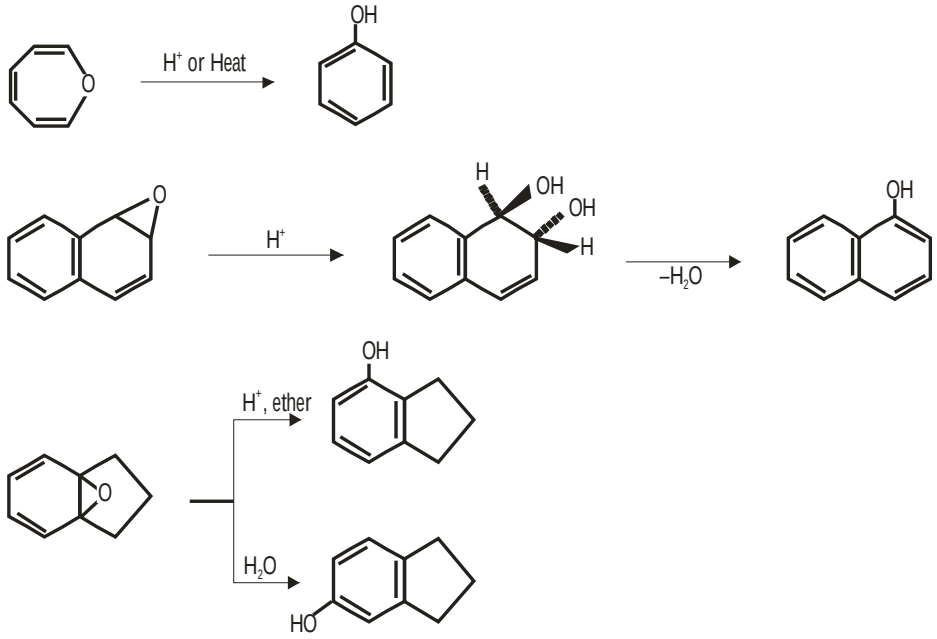


9.4.2 Physical Properties

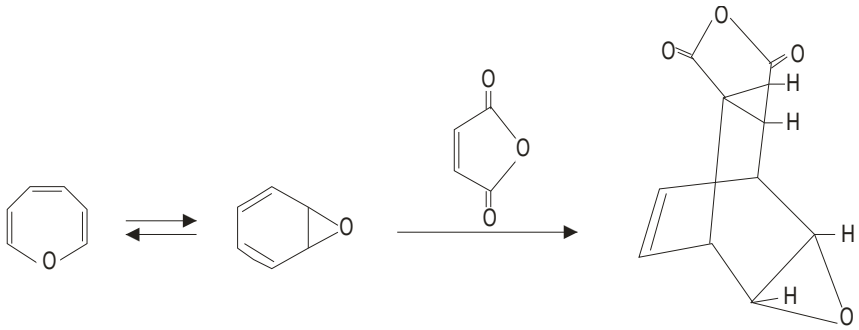
Oxepin is an orange liquid, b.p., 380°C/30mm. The U.V. spectrum of the orange compound in isooctane solution show's λ_{max} at 305 nm assigned to oxepin with a shoulder at 271nm due to oxepin.

9.4.3 Chemical Reaction

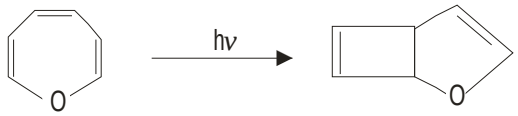
(i) Reaction with Acids:



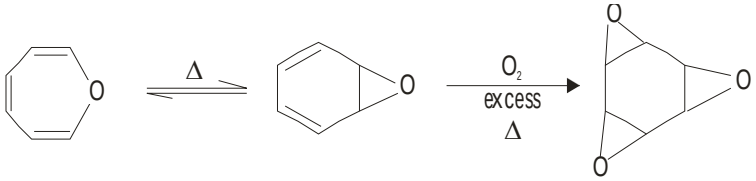
(ii) Diels-Alder Reaction :



(iii) Photochemical Reaction :

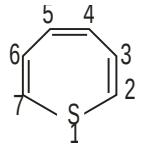


(iv) Reaction with O_2 :



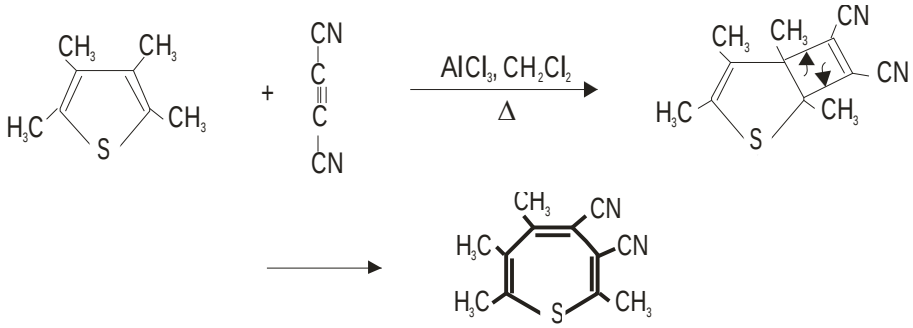
9.5 Thiepins

Thiepin (1) contains one sulfur atom in a seven membered ring. It contains 8 π electrons, is nonplanar and antiaromatic.

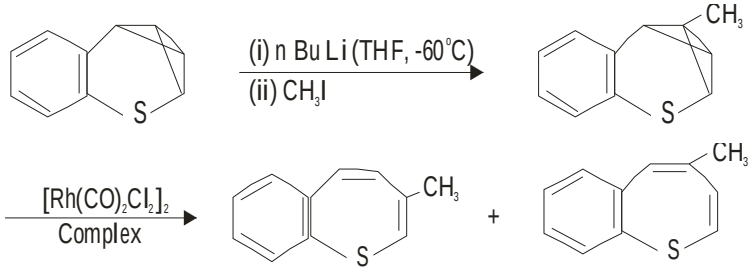


9.5.1 Methods of Preparations

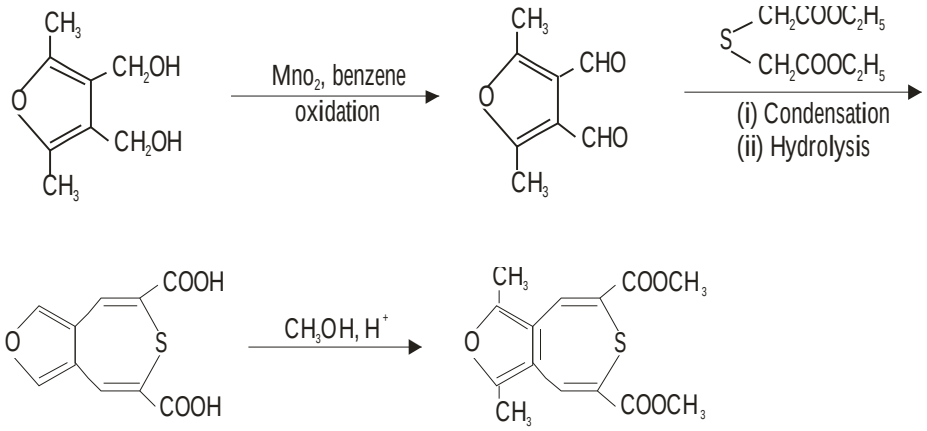
(i) From 2,3,4,5- tetramethyl thiophene (wynberg and Helder) :



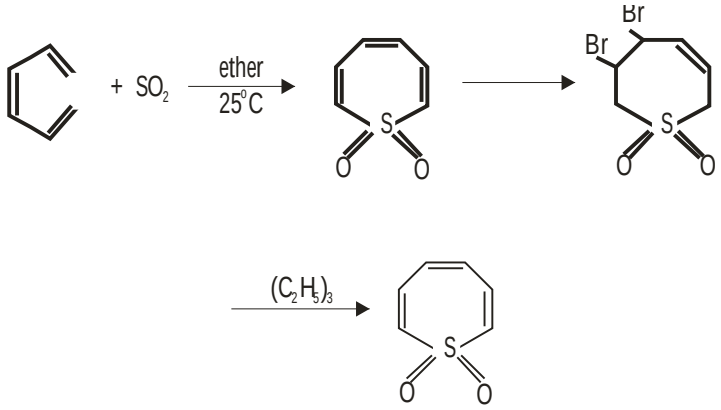
(ii) From isomerisation of 4,5-benzo -3- thiotricyclo [4,1,0,0] heptane (Murata et.al) :



(iii) From furan derivative :

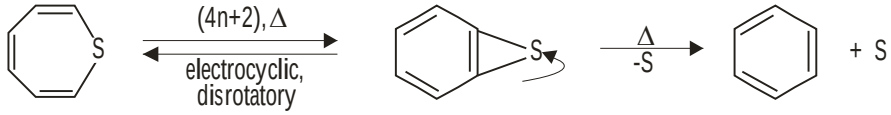


(iv) From cis hexatriene :

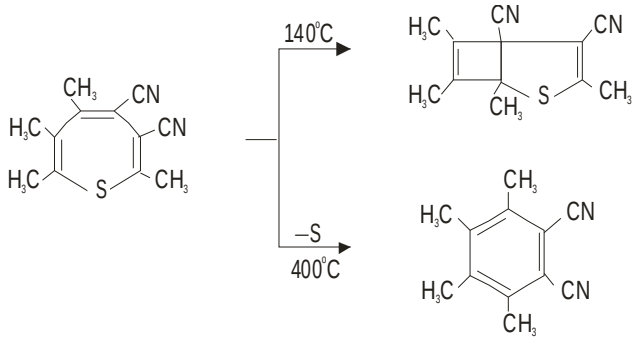


9.5.2 Chemical Reactions

(i) Valence tautomerism

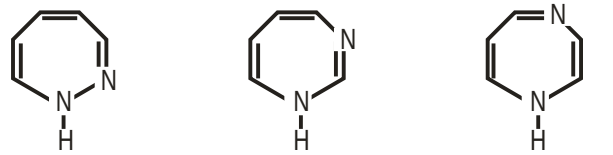


(ii)



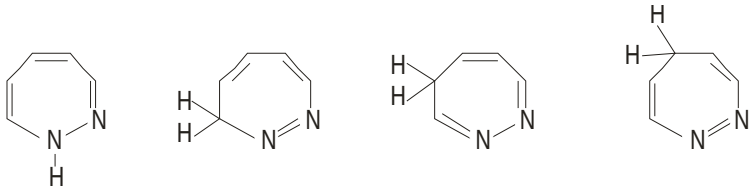
9.6 Diazepines

Diazeine are of the following types :



1,2- diazepine 1,3- diazepine 1,4- diazepine

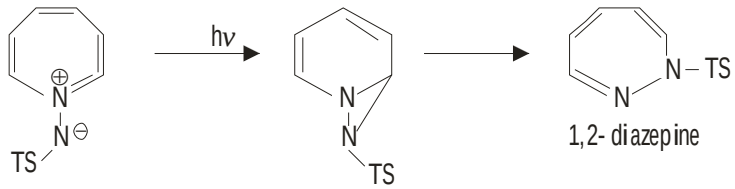
1,2- diazepine exist as the following tautomeric forms:



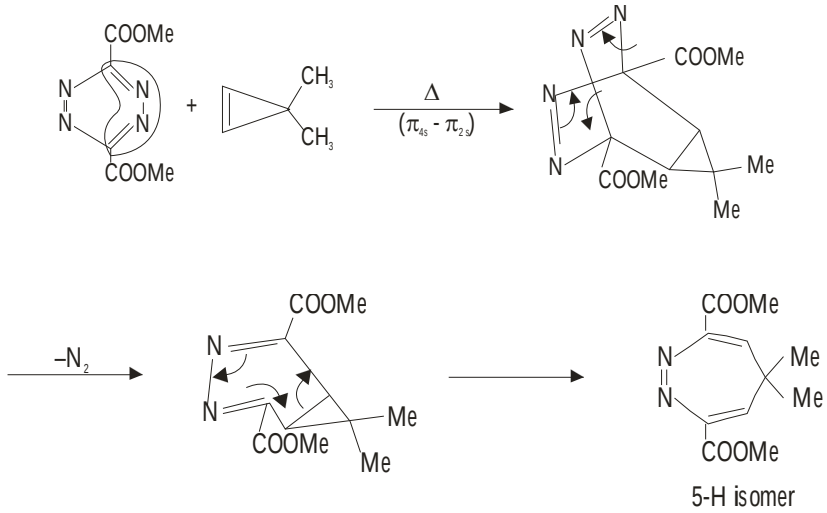
1-H isomer 3H- isomer 4H- isomer 5-H- isomer

9.6.1 Methods of Preparations

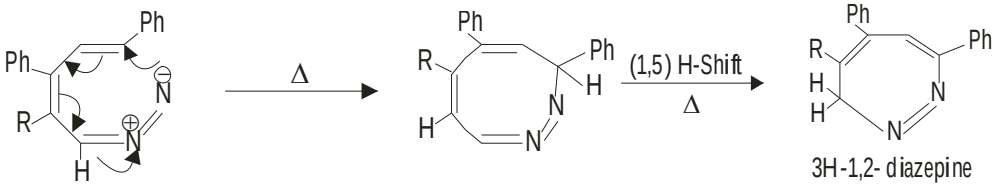
(1) From pyridine –N- ylide :



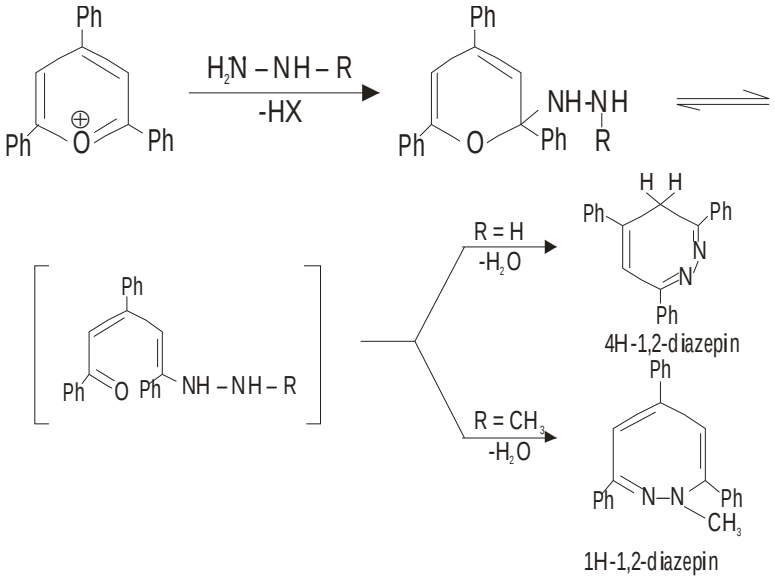
(ii) From cycloaddition reaction :



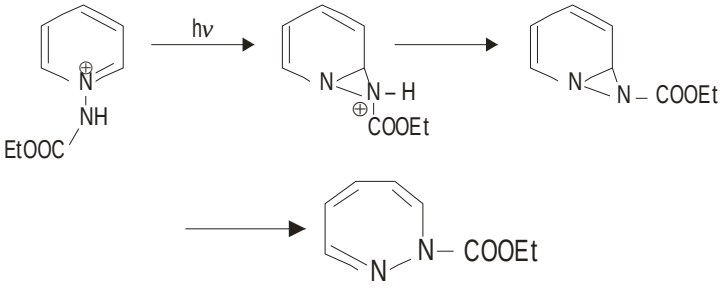
(iii) By electrocyclization reaction :



(iv) From pyrylium salt :

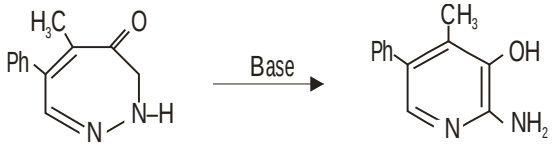


(v) From pyridinium salt :

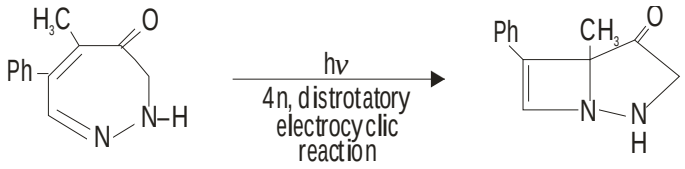


9.6.2 Chemical Properties

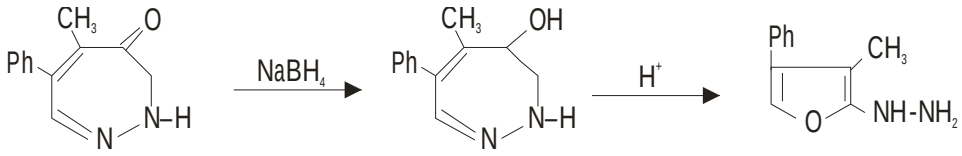
(i) Reaction with base :



(ii) Photochemical reaction :

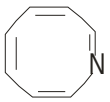


(iii) Reduction:



9.7 Azocines

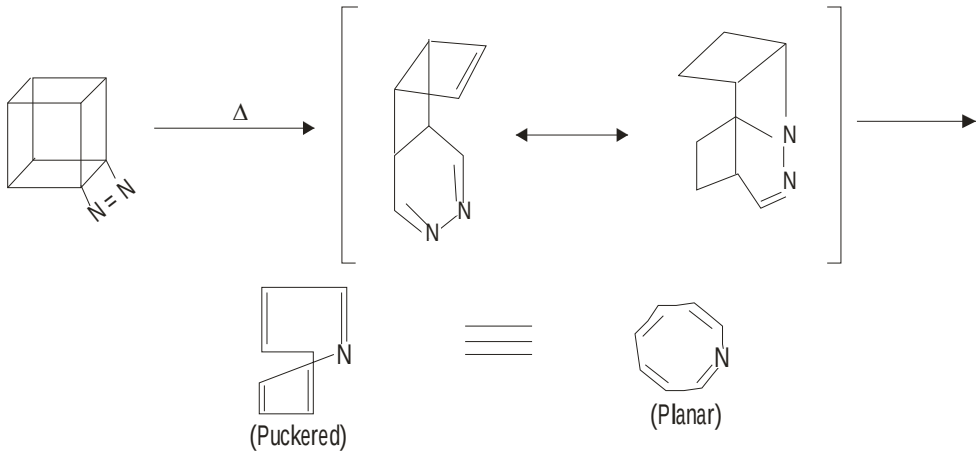
Azocines (1) is the nitrogen analog of cycloctatetraene – It is thermally labile. It decomposed at 50°C with polymerization. It is non-aromatic and non planar.



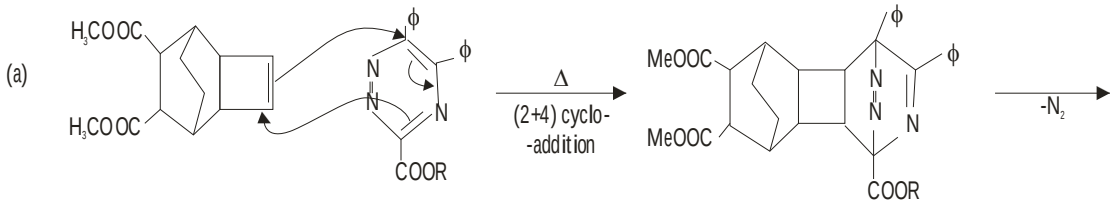
(i)

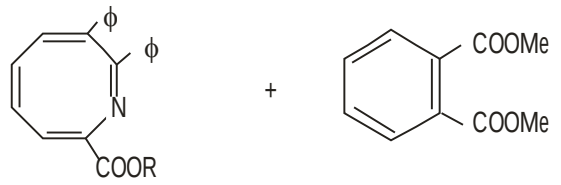
9.7.1 Methods of Preparations

(i) Flash vaccum Pyrolysis : (From diazabasketen)

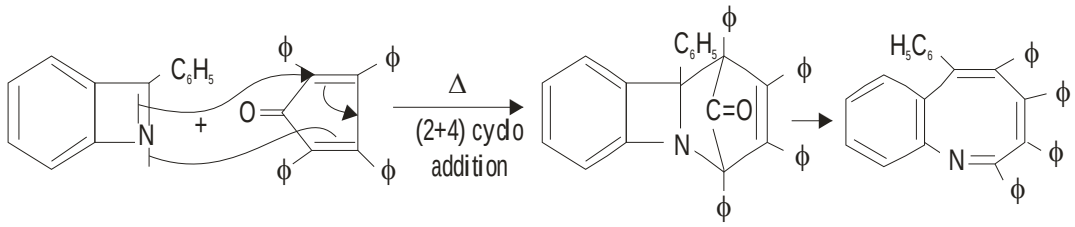


(ii) Diels Alder Reaction :

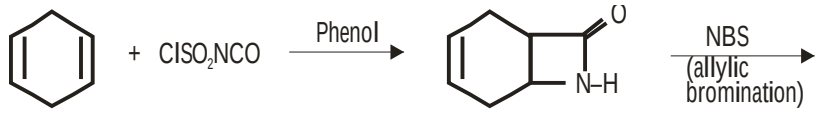


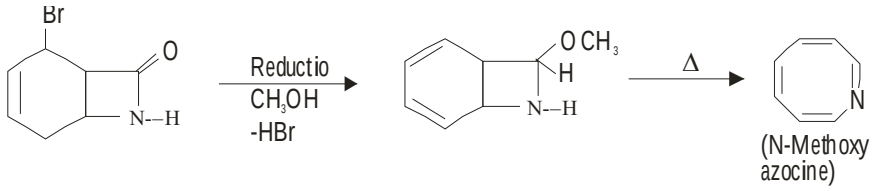


(b)

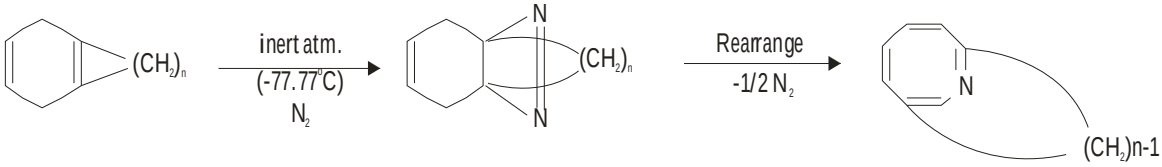


(iii) Baquett Synthesis :





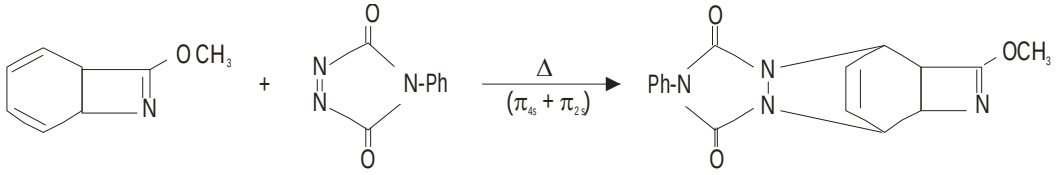
(iv) From azamethylene derivatives :



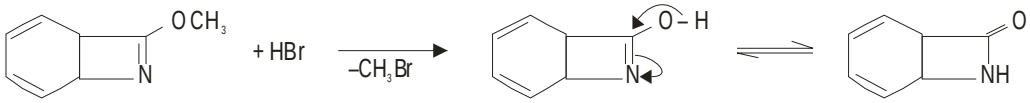
9.7.2 Chemical Reactions

(i) Valance isomerisation :

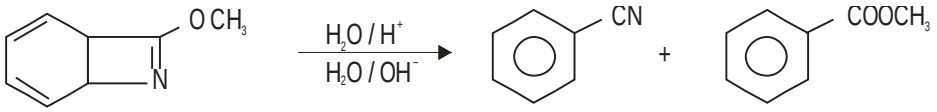
(ii) Reaction with N-phenyldihydrotriazolidione :



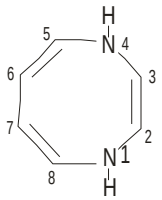
(iii) Reaction with HBr :



(iv) Hydrolysis in the presence of acidic or basic medium :



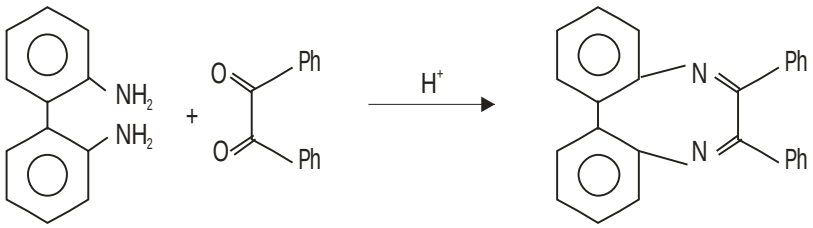
9.8 Diazocine



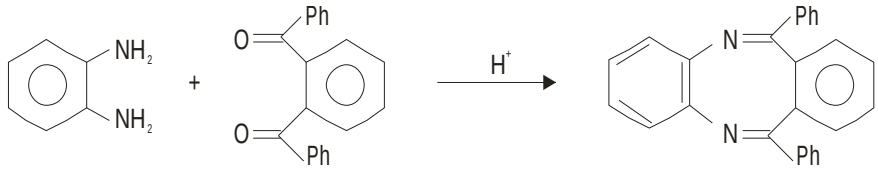
1,4- diazocine

9.8.1 Methods of Preparations

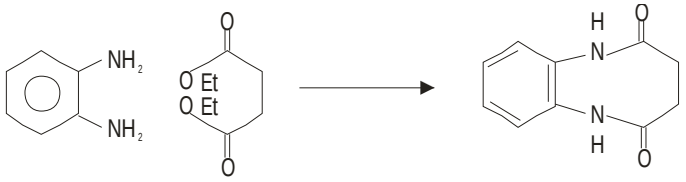
(i) From 1,2-diketones :



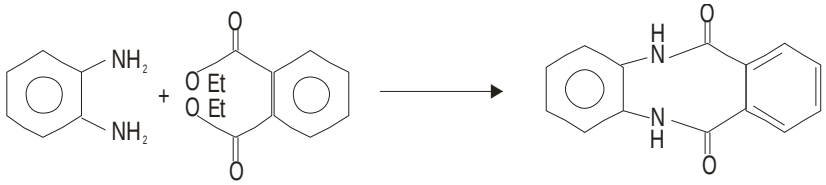
(ii) From O-phenylene diamine :



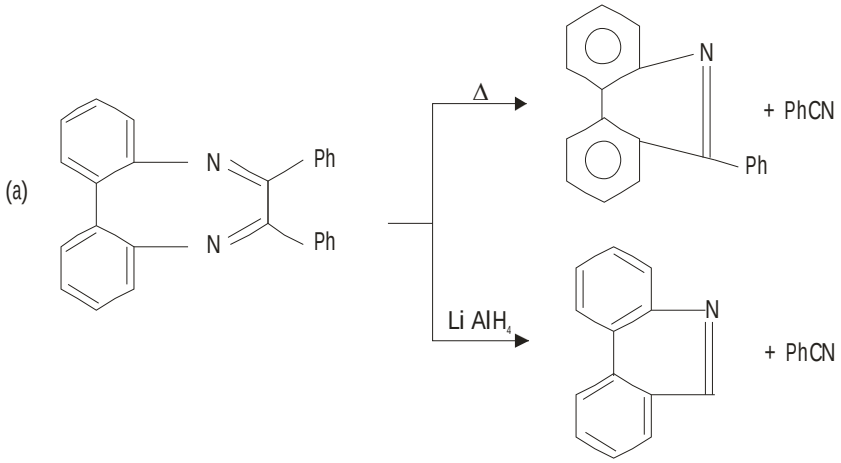
(iii) From *o*-phenylenediamine and diethyl succinate :

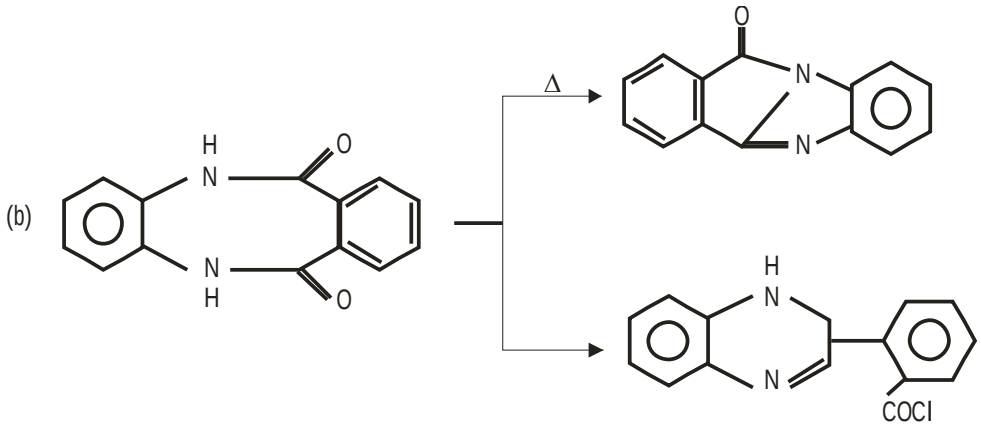


(iv) From *o*-phenylene diamine & diethylphthalate :

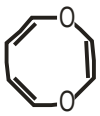


9.8.2 Chemical Reactions





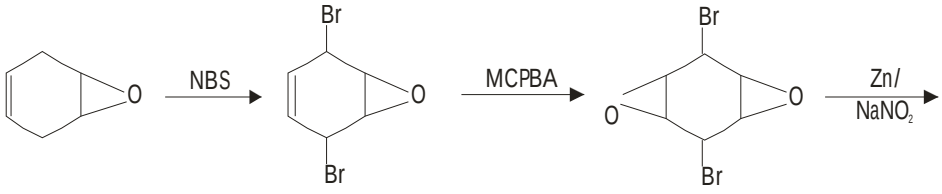
9.9 Dioxocine



1,4- Dioxocine

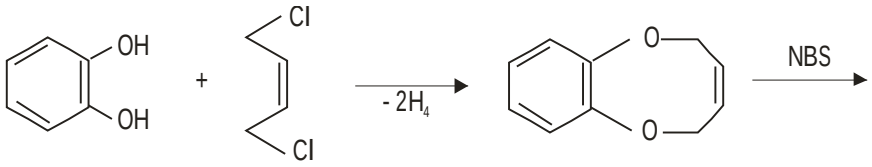
9.9.1 Methods of Preparations

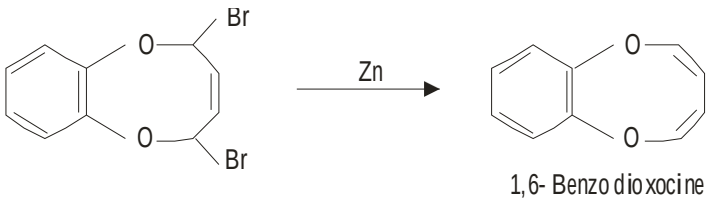
(i) From Epoxicyclohexene :



1,4- dioxocine

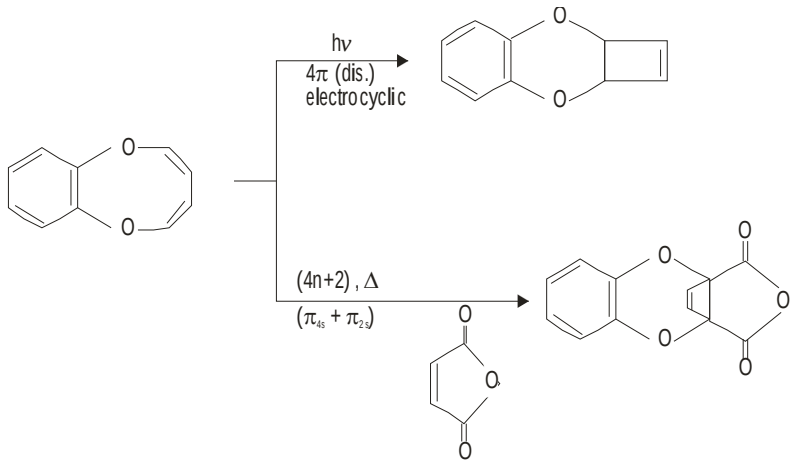
(ii) From 1 Catechol :



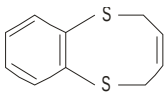


1,6- Benzo dioxocine

9.9.2 Chemical Reactions



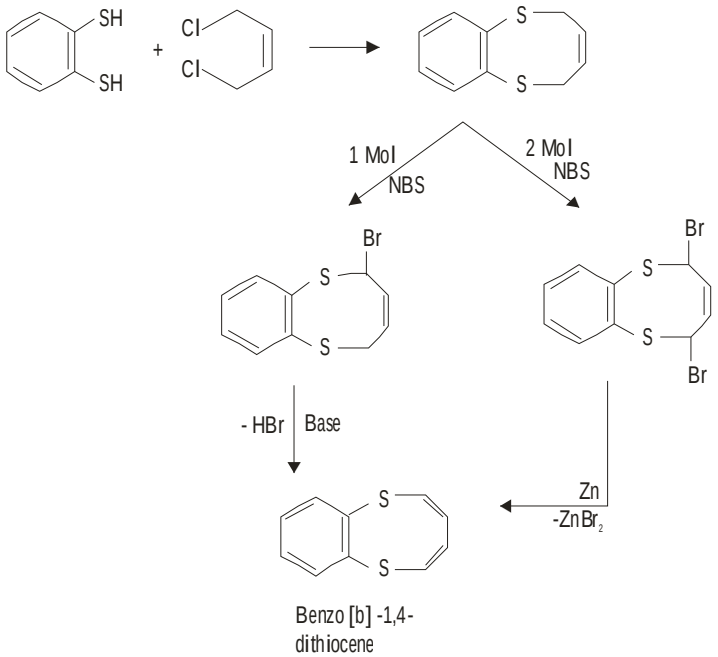
9.10 Dithiocine



1,4- Dithiocine

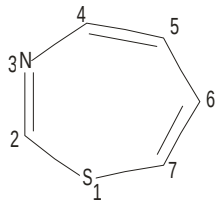
9.10.1 Methods of Preparations

(i) From 1,4- dichlorobut -2-ene



Benzo [b] -1,4-dithiocene

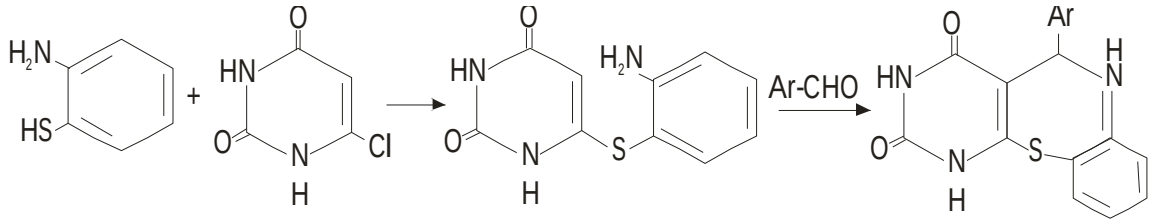
9.11 Thiazepine



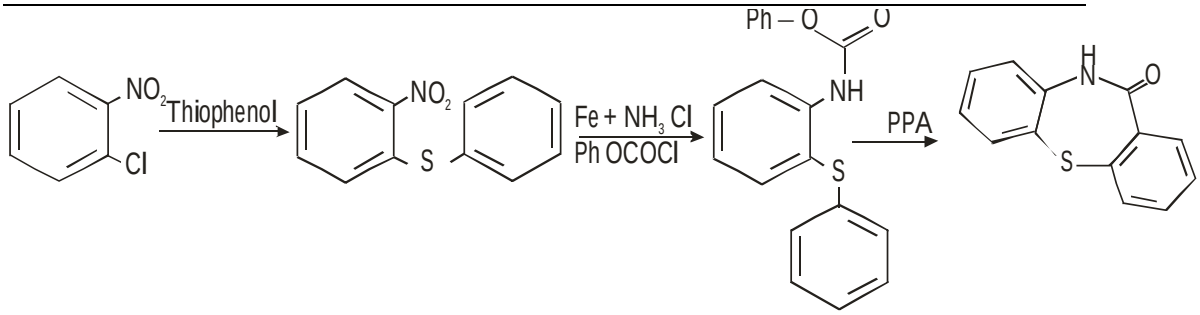
1,3 - Thiazepine

9.11.1 Methods of preparations

(i) From 2-mercaptoaniline :



(ii) From *o*-chloronitrobenzene :



9.12 Summary

(i) Unsaturated seven-membered heterocycles are nonpolar polyenes which show no evidence of cyclic delocalization.

(ii) Oxepins exit in equilibrium with bicyclic valence tautomers (benzeneoxide), the position of the equilibrium being dependent upon the nature and position of substituents.Thiepins are unstable because sulfur is easily extruded from the bicyclic tautomers. 1H-Azepines show little tendency to tautomerise in this way. 1H- Azepines unsubstituted on nitrogen usually rearrange to the more stable 3H-

isomer. Azepine and benzene oxide undergo cycloaddition reaction of cis –diene.

9.13 Review Questions

1 Describe different methods for preparation of azepines.

2 Write a short note on valence bond isomerisation.

3 Write two methods for the preparation of 3H- azepines from reagents involving nitrene intermediates.

4 What are the structures of thiazepin and azocine ?

9.14 Reference and suggested readings

- Heterocyclic chemistry Vth Ed. Raj K. Bansal,New Age International Publisher 2013
- Heterocyclic chemistry IIIrd Ed. Thomas L. Gilchrist, Pearson Education 2008.
- Heterocyclic chemistry IInd Ed. R.R. Gupta, M. Kumar, V. Gupta, Springer 1998
- Organic chemistry, O.P. Agarwal, Goel Publishing House, Meerut.
- Heterocyclic chemistry V.K. Aluwalia, Narosa Publishing House, 2012

Unit-10

HETEROCYCLIC SYSTEM CONTAINING PHOSPHORUS :

Heterocyclic Ring Containing Phosphorus : Introduction Nomenclature, Sythesis And Characteristic of 5- and 6- Membered Ring Systems – Phosphorinanes. Phosphorines, Phospholanes and Phospholes.

Structure of Unit

10.0 Objective

Organophosphorus compounds are degradable organic compounds containing carbon - phosphorus bonds, used primarily in pest control. These compounds are highly effective insecticides, though some are also lethal to humans at minuscule doses and include some of the most toxic substance ever created by man.

10.1 Introduction

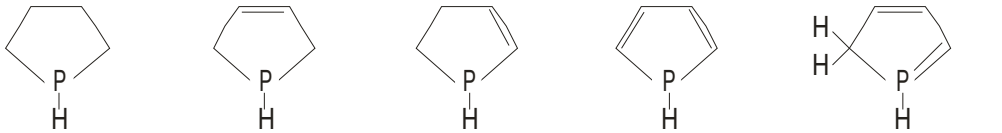
Heterocyclic chemistry has contributed significantly to medicinal, pharmacological chemistry and biochemistry. Phosphorus containing heterocycles are promising structural moiety for drugs. Phosphorus heterocycles are potential bioactive compounds and exhibit antibacterial, antiinflammatory, antitumoral, antihypertensive and insecticidal activities.

10.2 Nomenclature

5- Membered phosphorus heterocycles are analogous of pyrrole and 6-membered phosphorus containing heterocycles are pyridine analogs.

10.3 5 and 6- Membered ring systems

(a) 5- Membered ring system :



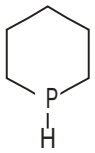
Phospholene 3-phospholene 2-phospholene 1H-phosphole 2H-phosphole

Tertiary phosphine incorporated in the saturated five membered ring are quite stable and exhibit all of the usual properties of this function. This is also true of the two isomeric systems that contain one double bond (phospholene).

(b) 6- Membered ring system :

phosphorinane or phosphinane | 1,2,5,6-tetrehydro-phosphinine | 1,2-dihydro phosphinine | phosphorine or phosphinine

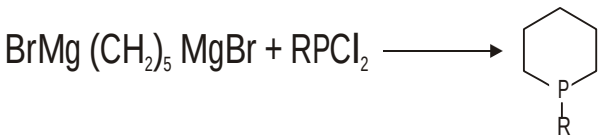
10.4 Phosphorinanes



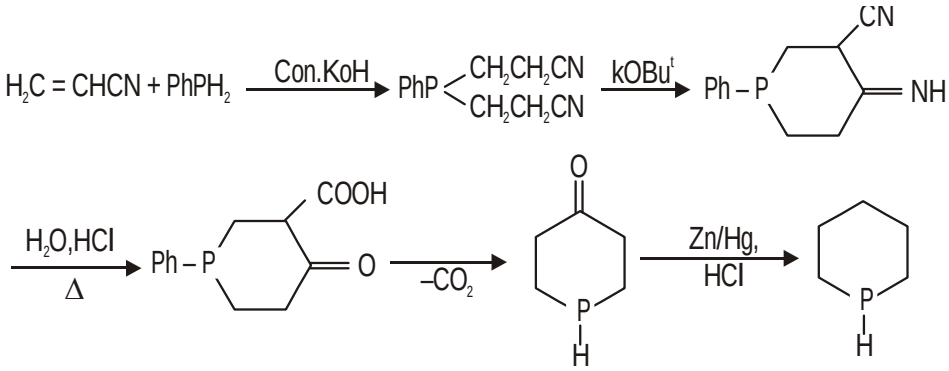
The saturated phosphorinanes are phosphinanes.

10.4.1 Methods of Preparations

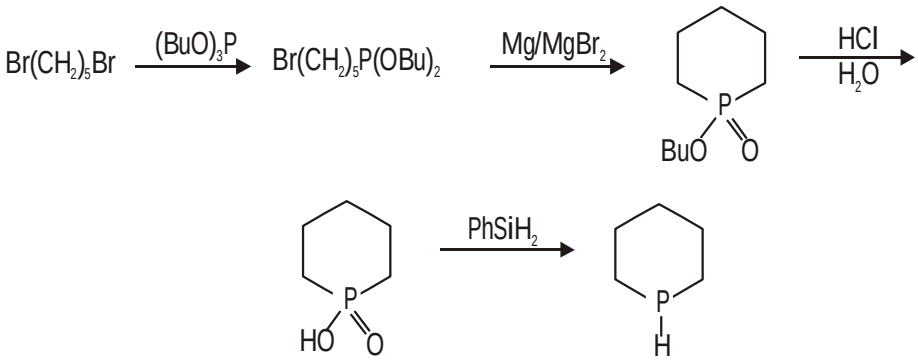
(i) From 1,5- pentane derivatives :



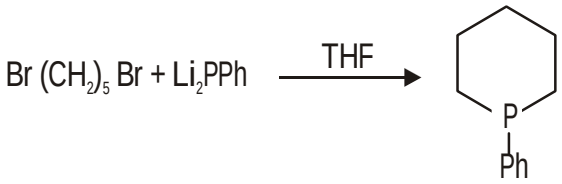
(ii) From cyanoalkene :



(iii) From 1,5- dibromopentane :



(iv) From pentane derivatives :



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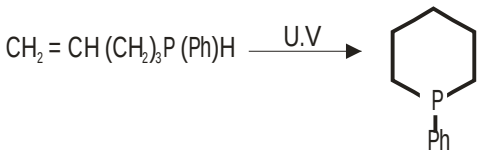

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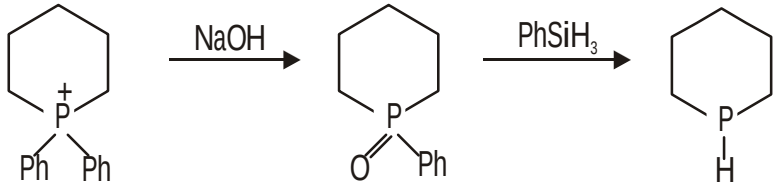
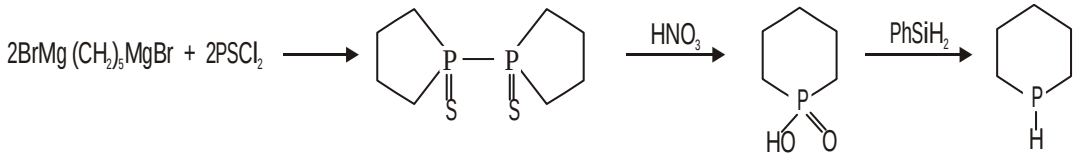
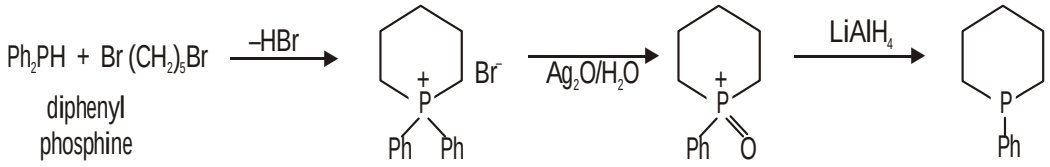


(vii) From dibromopentane magnesium bromide :



(viii) From diphenylphosphine :

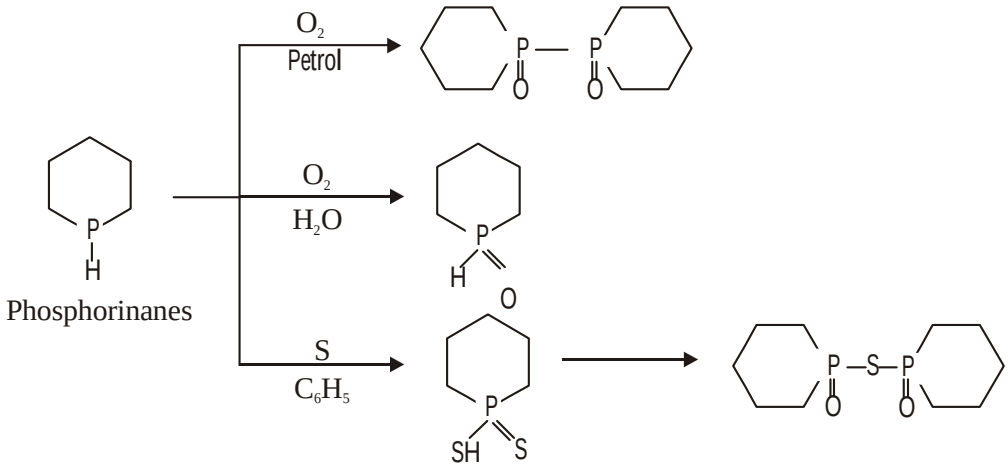


10.4.2 Physical Properties :

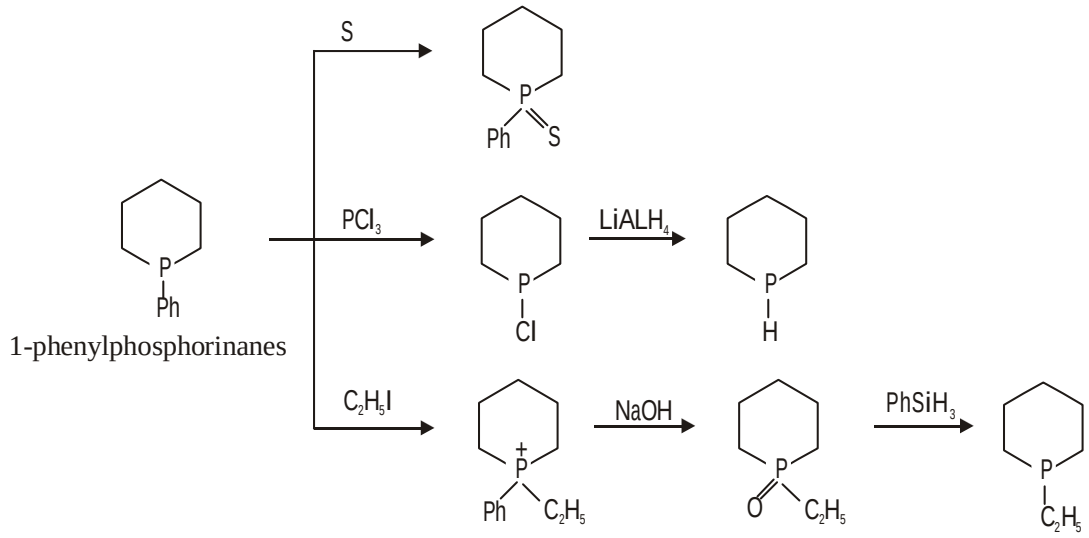
Phosphorinanes is a liquid and has b.P. = 118 – 121°C. It has characteristic phosphine odour.

10.4.3 Chemical Properties

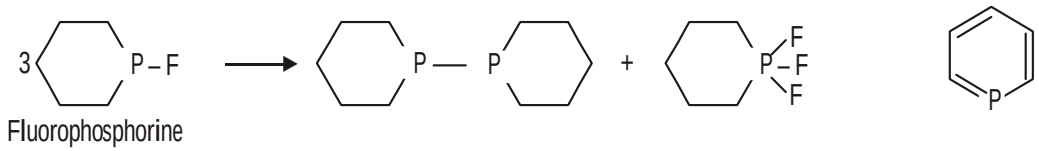
(i)



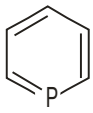
(ii)



(iii) Fluorophosphorinane undergoes disproportionation :



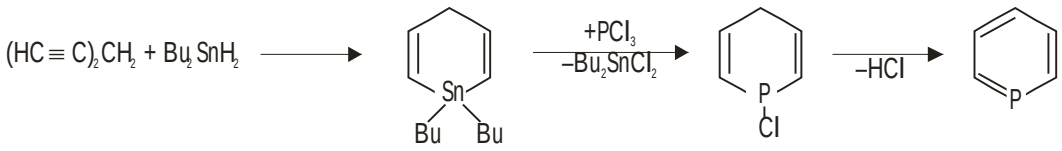
10.5 Phosphorines



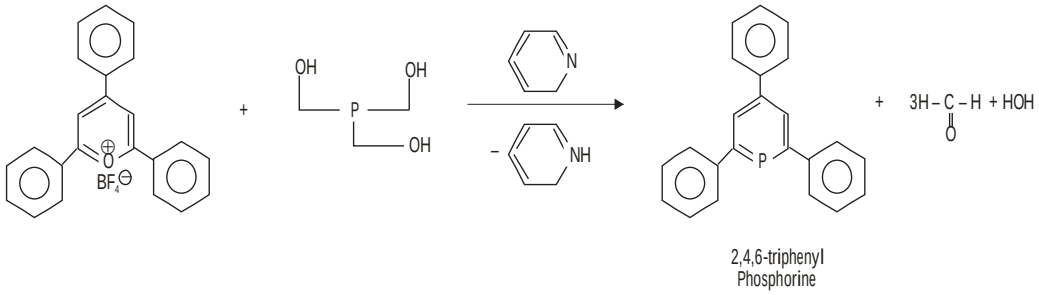
Phosphorine (IUPAC name: phosphinine) is a heavier element analog of pyridine, containing a phosphorus atom instead of aza moiety. It is also called phosphabenzene and belongs to the phosphaalkene class. Phophorine is generally stable against air and moisture and can be handled without special inert atmosphere equipment, so it is different from silabenzene , which is usually not only air and moisture – sensitive but also thermally unstable without extensive steric protection . This stability of phosphorine comes from the close electronegativities of phosphorus (2.1) and carbon (2.5).

10.5.1 Methods of Preparations

(i) From alkyne derivatives:



(ii) From pyrylium salt :



2,4,6-triphenyl Phosphorine

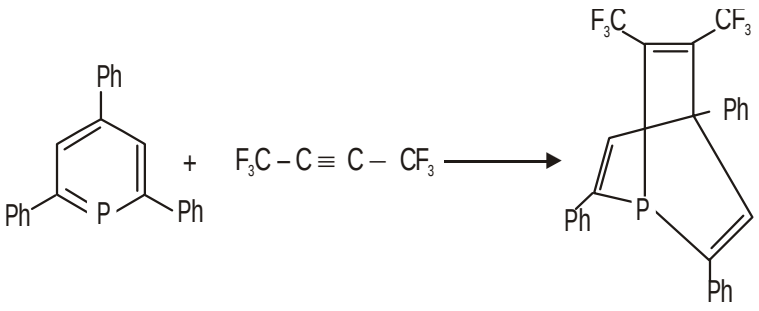
10.5.2 Physical Properties

Phosphorine is a colourless volatile liquid which is very reactive, air sensitive and characteristic odour.

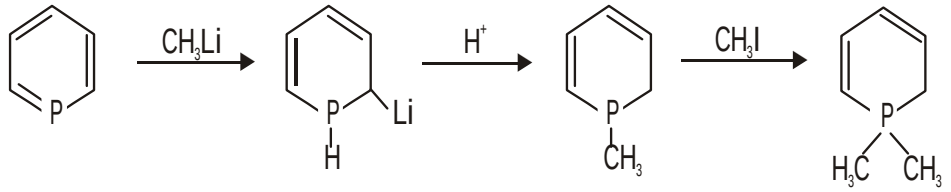
10.5.3 Chemical Properties

Phosphorine is a planar aromatic compound with 88% of the aromaticity of that of benzene.

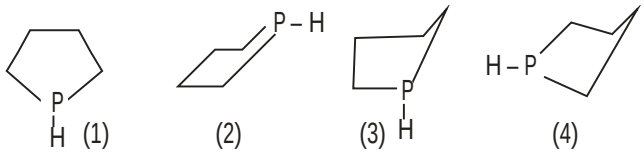
(i)



(ii)



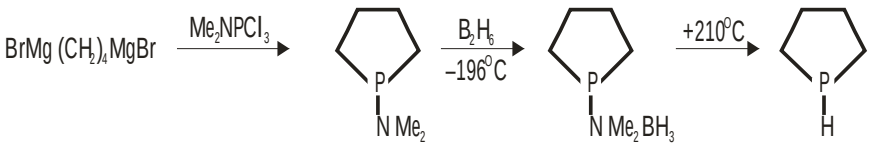
10.6 Phospholanes



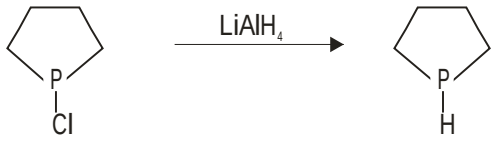
Phospholanes usually adopt a folded evelope configuration with which there are three possible alternative arrangements (2,3,4).

10.6.1 Methods of Preparations

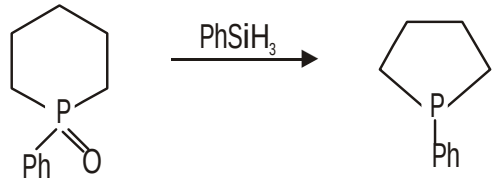
(i) Phospholane can be prepared via the dimethylamine borane adduct :



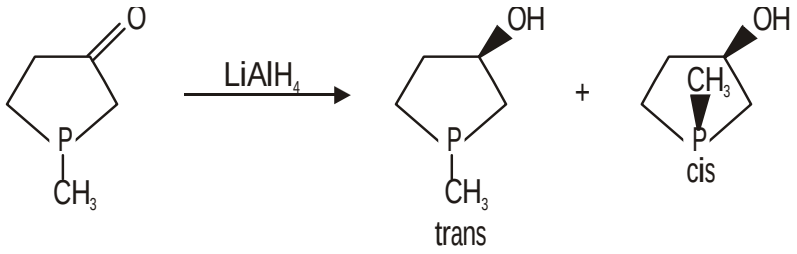
(ii) From chloro derivative :



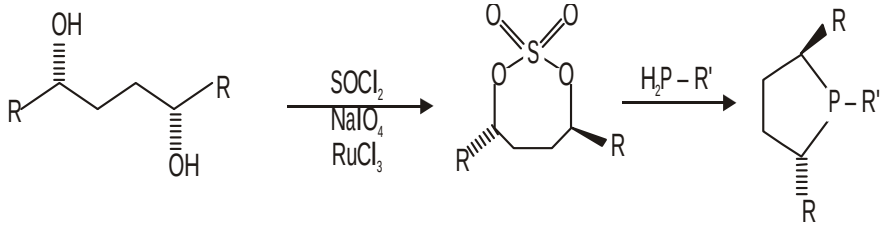
(iii) From phenyl phospholane -1- oxide :



(iv)



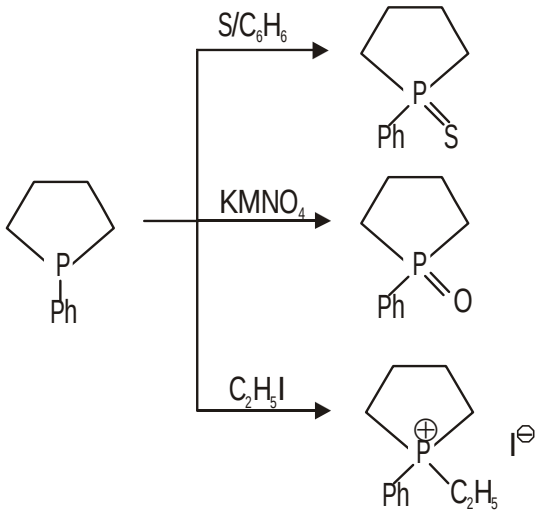
(v)



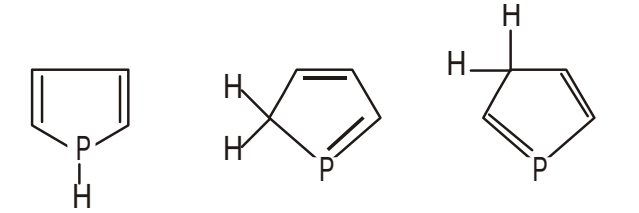
10.6.2 Physical Properties

It is saturated five membered phorphorus heterocycles. It 's b.p. 103^{o}C.

10.6.3 Chemical Properties



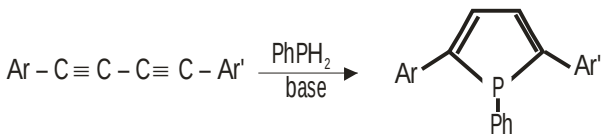
10.7 Phospholes



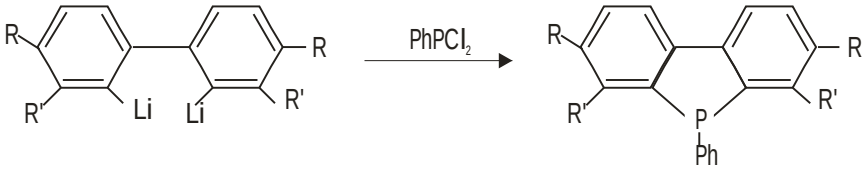
It is a phosphorus analog of pyrrole.

10.7.1 Methods of Preparations

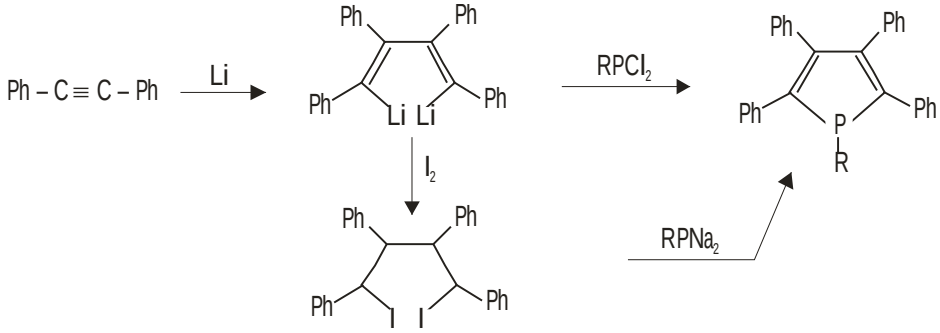
(i) From aryl alkyne derivative:



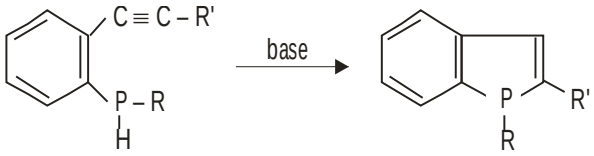
(ii) From aryl lithium compound :



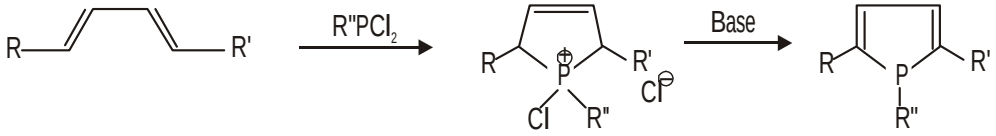
(iii) From substituted alkyne :



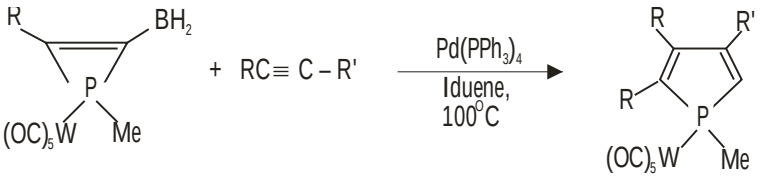
(iv) From aryl phosphine :



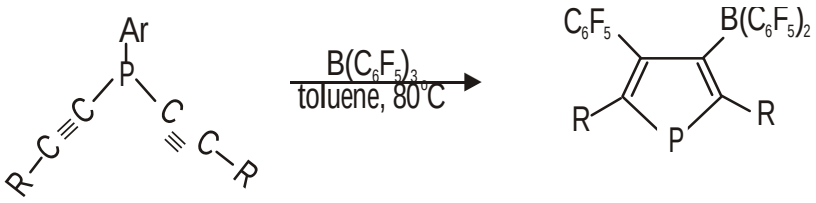
(v) from substituted diene :



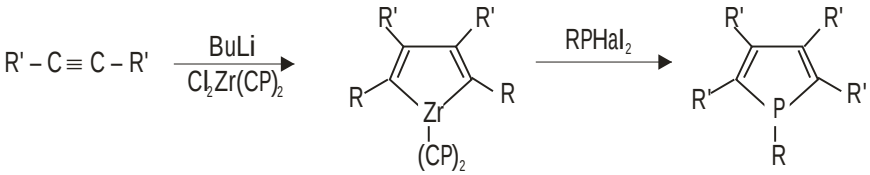
(vi)



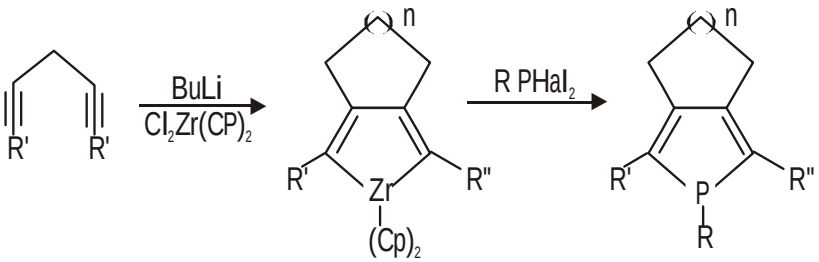
(vii)


Ar
P
C
C
R–C≡C
C≡C–R
B(C6F5)3
toluene, 80°C
C6F5
B(C6F5)2
R
P
R

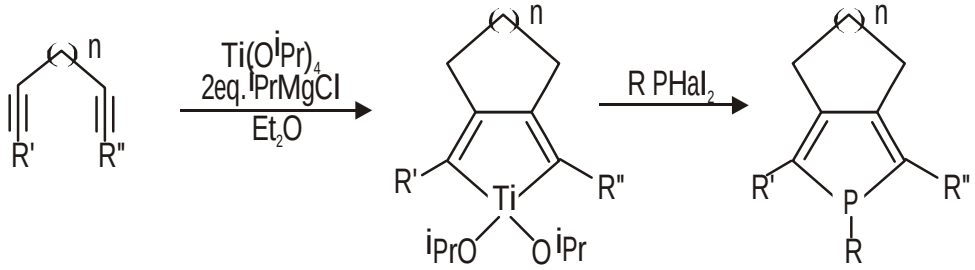
(viii)


R' – C ≡ C – R'
BuLi
Cl2Zr(CP)2
R'
R'
R
R
Zr
(CP)2
RPHal2
R'
R'
R'
R'
P
R

(ix)

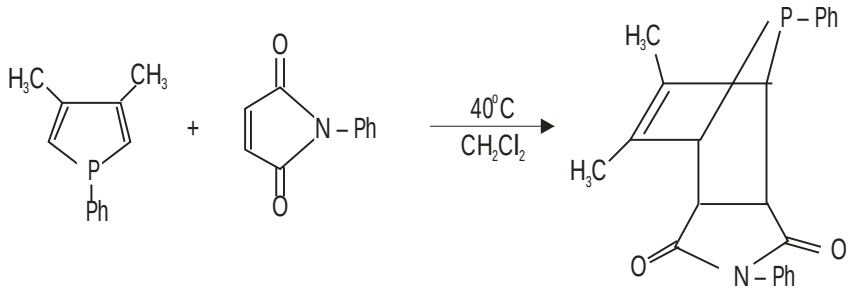

R'
R'
BuLi
Cl2Zr(CP)2
n
R'
R"
Zr
(Cp)2
R PHal2
n
R'
R"
P
R

(x)

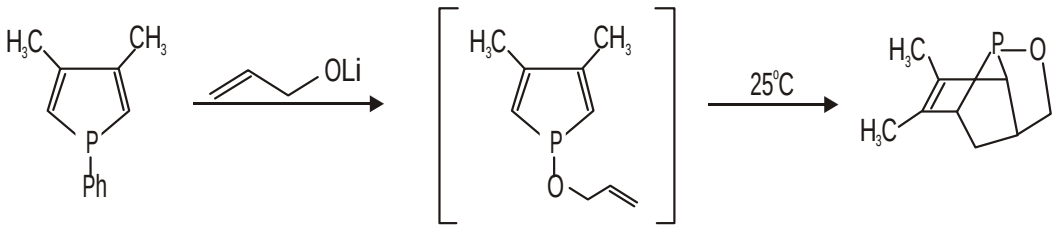

n
R'
R"
Ti(OiPr)4
2eq. iPrMgCl
Et2O
n
R'
R"
Ti
iPrO
OiPr
R PHal2
n
R'
R"
P
R

10.7.2 Chemical Reactions

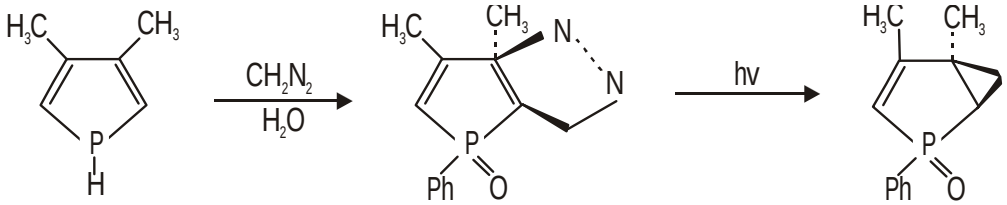
(i)



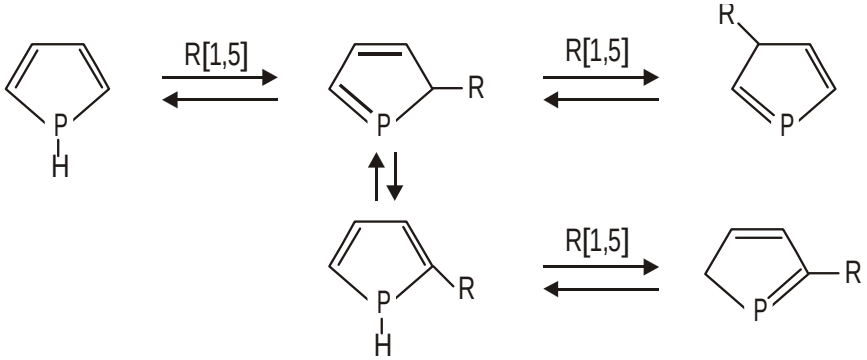
(ii)



(iii)



(iv)



10.8 Summary

Pyridine has good σ donating ability than the phosphorine. The lone pair of electron on pyridine is its HOMO. On the other hand the HOMO and LUMO of phosphorine are its π and π^* orbitals and the lone pair is located at the lower energy level. This phosphorines are much better π - acceptor ligands. but less good σ donors compare to pyridines. phosphorines undergoes electrophilic substitution reactions like aromatic compounds eg. bromination, acylation etc.

10.9 Review Questions

1 What is phosphorinanes? Give any two methods of preparation of phosphorinanes.

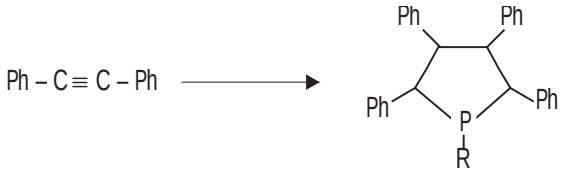
2 What are the difference between phospholes and phosphorines.

3 Predict the mechanism for the following reactions.

(a)

(b)

(c)



4 Explain phosphorines act as π -accepton ligand ?

10.10 Reference and suggested readings

- Phosphorus chemistry biochemistry and Technology VIth Ed. D.E.C Corbrige 2013.
- The Chemistry of Heterocycles, T. Eicher and S. Hauptmann, Thieme.
- An introduction to the Heterocyclic compounds, A.M. Acheson, John Wiley.
- Phosphorous Heterocycles IInd Raj K. Bansal (2010) springer.
- Modern Heterocyclic chemistry Vol-I Julio- Alvarez- Builla, Juan Jose aquero Jose Barluenga (2011)

Unit – 11

Bio-organic Chemistry: Introduction, Basic considerations. Proximity effects and molecular adaptation.

Structure of Unit

11.0 Objective

Bio-organic chemistry is the tools of organic chemistry to the understanding of biological processes. Bio-organic chemistry deals with reaction which occurs in the living system and explains biological form and function in chemical terms. Bio-organic chemistry related with biomolecules and biomolecules may be regarded as derivatives of hydro carbon, i.e. the compounds of carbon with different functional group.

11.1 Introduction

Organic chemistry deals with chemistry of the carbon compound. Bio-organic chemistry constitutes the discipline at the interface of the more areas of organic chemistry and biology.

All of the organic compounds from which living organisms are constructed are products of biological activity. These molecules were selected during the course of biological evolution for their fitness in performing specific biochemical and cellular function. Biomolecules can be characterized and understood in the same to terms that apply to molecules of inanimate matter.

Bio-organic chemistry involves various organic processes like addition, elimination, rearrangement, reduction, isomerization, oxidation and transport phenomenon across membranes by diffusion, osmoses etc. to biological systems of animals and plants. Bioorganic chemistry is of utmost utility in our sophisticated and advanced life style too, as our different food stuff, our medicinal syrups and our various drinks are products of bio-organic reactions. All living processes require energy, which is obtained by performing chemical reactions inside living cells. These biochemical processes involve oxidations and reductions.

Biomolecules are compounds of carbon with different functional group. Bioorganic chemistry is biochemical processes with the application of the tools of organic chemistry.

Bioorganic chemistry is, a young and rapidly growing science arising from the overlap of biochemistry and organic chemistry. The organic chemistry is related to the development of methodology to synthesize organic molecules of biological importance. Knowledge of biochemistry gives the idea of what would be useful to synthesis for a fruitful response which can only be possible via organic chemistry. So knowledge of organic chemistry give rise to the concept of building models chemically synthesized in the laboratory to study the complex biological processes.

Bio-organic chemistry is the branch of chemistry which utilized the principle, technique and tool of organic chemistry to understand of biochemical and biophysical process and its various origin like protein sequencing, production of proteins through genetic engineering, of human insulin, molecular recognition, biogenesis of natural product, recognize the factors essential in the human diet and enzyme involving melalloenzyme and cofactors, the contiguous areas of bioorganic chemistry.

11.2 Basic consideration

Bio-organic chemistry is the branch of chemistry which is explains the biochemical process by using the tool of chemistry. Therefore, the need for the multidisciplinary approach become obvious and there must have to two laboratories- one for synthesis, and another for the biological study. Thus knowledge of organic chemistry give rise to the concept of building of organic models chemically synthesized in the laboratory to study the complex biological processes.

Chemists develop a various method to understand the organic mechanism and develop new compound. A new dimension results from this combination of chemical and biological sciences; that is the concept of model building to study. By means of simple organic models, many biological reactions as well as the specificity and efficiency of the enzyme involved have been reproduce in the test tube. A large portion of organic chemistry has been classically devoted to natural products. Many of those results have turned out to be wonderful tools for the discovery and characterization of specific molecular events in living systems. All living processes require energy, which is obtained by performing chemical reactions inside cells. Biological oxidations are thus the main source of energy to drive a number of endergonic biological transformations. Biological oxidations are main sources of energy. Combustion of food like as lipids and sugars to produce energy is used for a various functions such as replication, growth, maintenance, muscular work and heat production. By using breathing bio-chemical process molecular oxygen is reduced to water. Energy is stored in the form of ATP.

Biological combustions give some heat, but a large part of energy is used in molecular engine. Only large macromolecules can carry enough molecular information from the points of view of substrate, recognition and thermodynamic efficiency of transformation.

A new dimension result from the biological and chemical sciences is concept of model building to study and sort out the various parameter of a complex.

A-large number of organic structures can depicts infinite number of functional and structural properties of proteins. We use chemically based biotechnology and continue to add other techniques that are not only applicable.

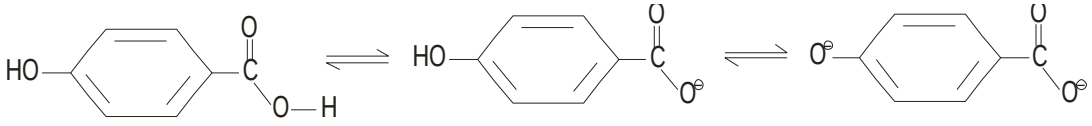
11.3 Proximity Effect

Proximity effects arising from the influence of substituents that are physically close to the acid or base function under consideration and due to the presence of groups in proximity to each other.

Proximity effect in organic chemistry involve the proximity of reactive groups in a chemical transformation which leads to an acceleration in the rate of the reaction some examples revealing to these facts, are discussed as :

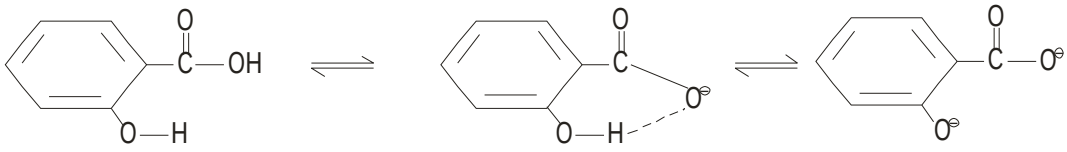
(a)Intramolecular hydrogen bonding:

Such hydrogen bonding are explained in scheme-1, The stabilization of the carboxylate anion by the hydroxyl hydrogen in ortho-hydroxy benzoic acid leads to a much lower pK_{a1} value and to a much higher pK_{a2} value compared with p-hydroxybenzoic acid, in which no intra molecular hydrogen bonding is possible.



pK_{a1} = 4.48 pK_{a2} = 9.32

p-Hydroxy benzoic acid



pK_{a1} = 2.97 pK_{a2} = 13.40

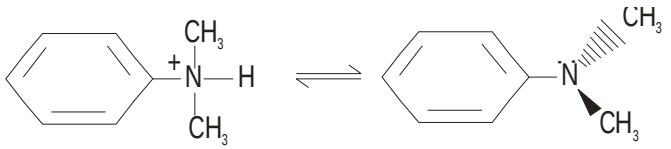
Scheme -1: Proximity effect on acidity constant by H-bonding

(b) Steric effects

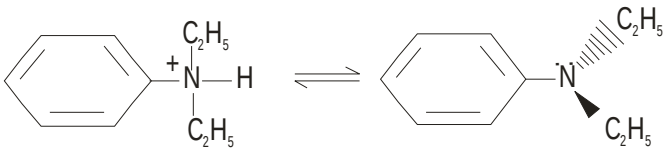
Steric effect may have a measurable impact on the pKa of a given acid or base. This involves steric constraints that inhibit optimum solvation of the ionic species by the water molecules, or hinder the resonance of the electrons of a given acid or base group with other parts of the molecule by causing these groups to twist with respect to one another and to avoid coplanarity.

Example: The large difference found between the pKa of N, N dimethyl aniline and of N, N diethyl aniline (scheme-2) is partially due to the large ethyl

substituents that limit free rotation and thus, the orientation of the free electrons of the nitrogen atom.



pKa = 5.20



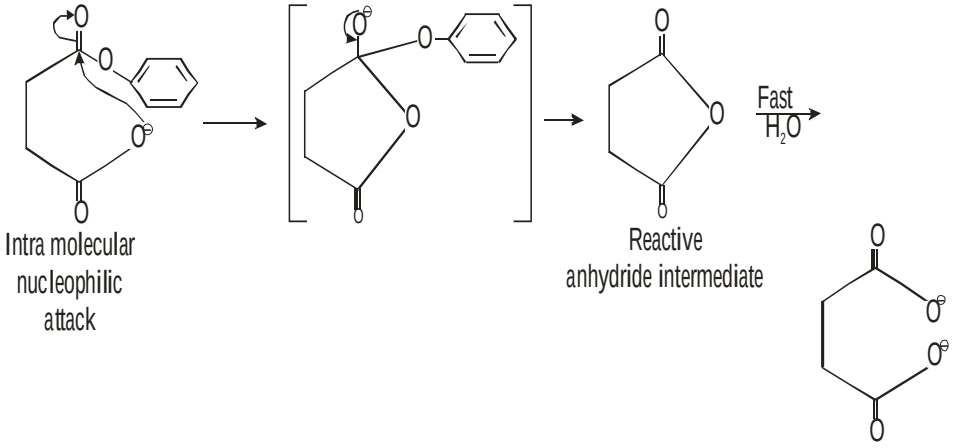
pKa = 6.20

Scheme-2: Proximity effect on acidity constant by steric effect

(c) There are many examples of organic reactions that are intramolecular, that is, they involve two or more functional group with in the same molecule, rather than functional group in different molecule. Intramolecular reactions generally proceed much more rapidly and under much milder reaction conditions than their intermolecular counterparts, which makes sense since the two reacting groups are already "in close proximity" to one another.

A useful concept in proximity effect is that of effective concentration. In order to explain the effective concentration of participating group, we compare the rate of the intra molecular reaction with the rate of the corresponding intermolecular reaction, where the reagent and the participating group are present in separate molecules.

We illustrate the hydrolysis of a series of phenyl esters in aqueous solution at pH-7.The reference reaction in this case is the hydrolysis of phenyl acetate catalyzed by sodium acetate at the pH-7. Introductions of a carboxylate group into the same molecule as the ester leads to an enhancement of the rate of ester hydrolysis, which for phenyl succinate is 23000 times faster than phenyl acetate. This rate acceleration is due to the neighboring carboxylate group can attack the ester to form cyclic anhydride intermediate shown in scheme-3. This intermediate is more reactive than the original ester group.

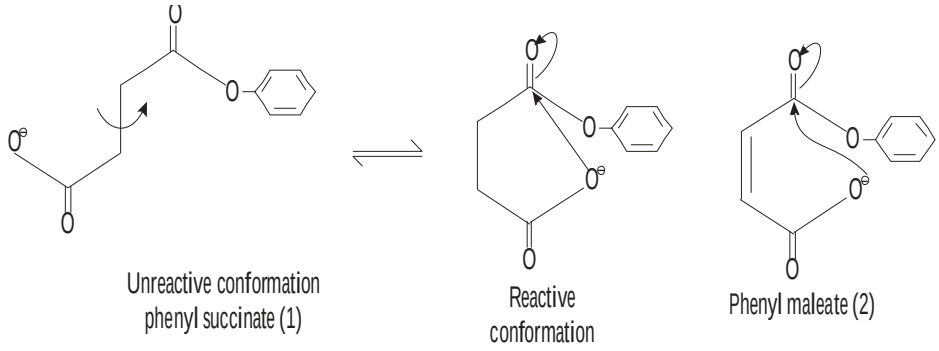


Scheme-3: Mechanism for intramolecular hydrolysis of phenyl succinate

Phenyl phthalate has an effective concentration of acetate ions of 2×10^5 M. while phenyl Maleate has an effective concentration of 10^{10} M. let the same molecule containing a trans-double bond has no rate acceleration at all. So it is clear that by holding the reactive groups rigidly in close proximity to one another remarkable rate acceleration can be achieved.

Why is the hydrolysis of (2) in which a five membered anhydride is formed, so much faster than the hydrolysis of (1), in which an apparently similar five membered anhydride is formed?

The answer is that in (2) the reactive group is held in the right orientation to react, as shown in shceme-4.



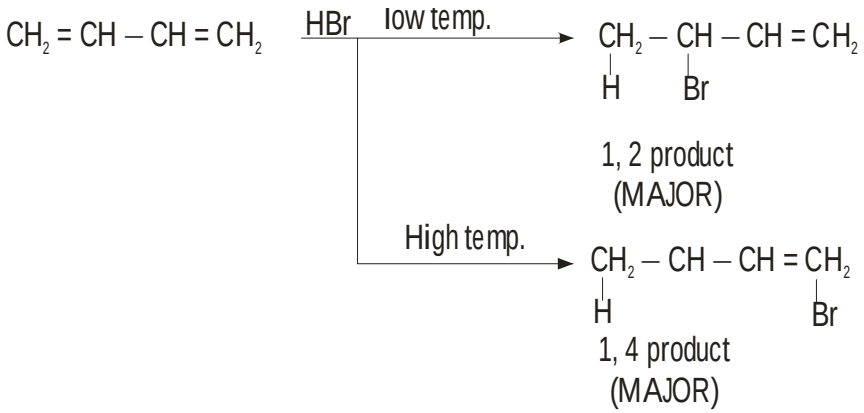
Scheme – 4:

Intramolecular hydrolysis of phenyl succinate (1) versus phenyl maleate (2)

The same effect operates in enzyme active sites, and is major factor in enzyme catalysis. The binding of substrates and cofactors at an enzyme active site of defined three dimensional structures brings the regents in to

close proximity to one another and to the enzyme active site functional groups. This increase the probability of correct positioning for reaction to take place, so it speed there action.

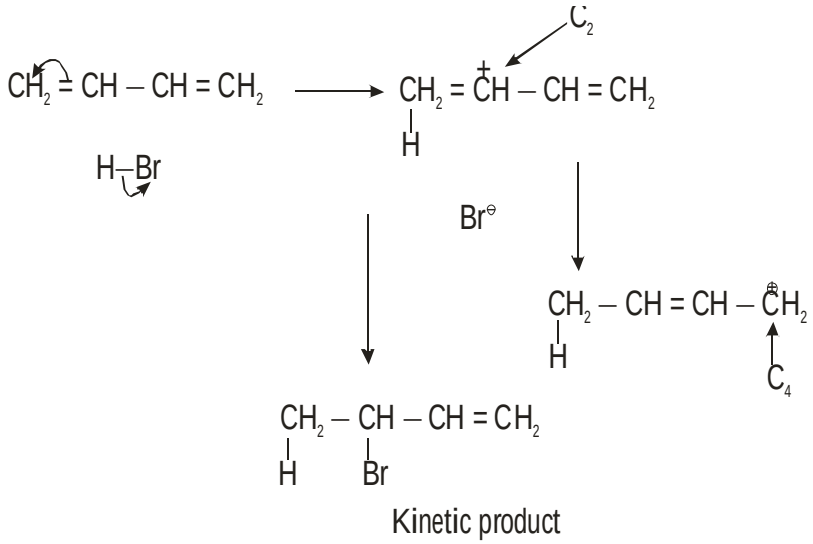
(d) The reaction 1, 3 butadiene with HBr at low temperature and higher temperature than 1, 2 product (3-bromo-1-butene) and 1, 4 product (1-bromo-2-butene) are formed respectively.



At low temperature 1, 2 products is major while at higher temperature 1, 4 product is major

In the addition of HBr to 1, 3 butadine is the 1, 2 product formed faster but the 1, 4 product more stable. The 1, 4 products are more stable because it has two alkyl groups bonded to the carbon-carbon double bond. Where the 1, 2 product has only one methyl.

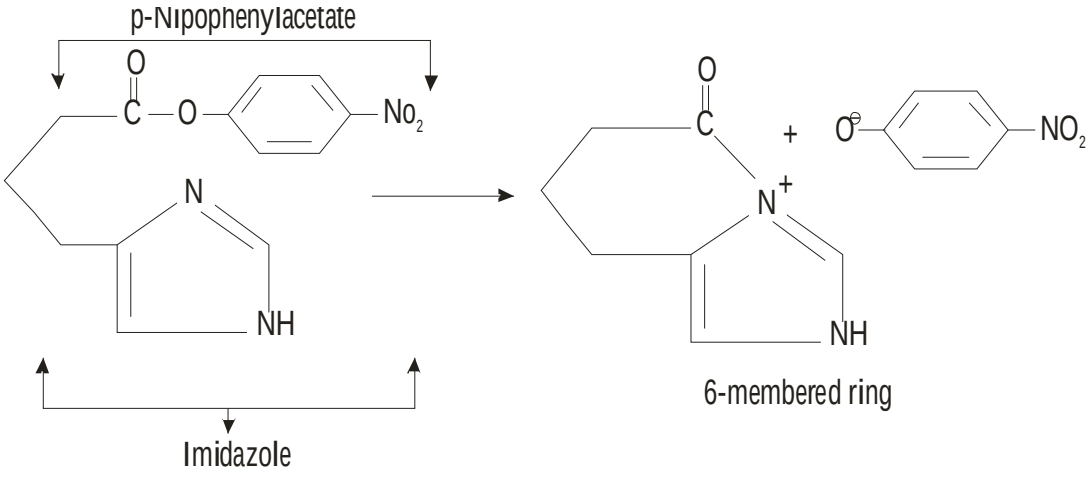
The 1, 2-product is the kinetic product due to proximity effect. When H^+ adds to the double bond, Br^- is closer to the adjacent carbon (2) than it is to C_4. Even through the resonance stabilized carbocation bears a partial positive charge on the C_2 and C_4, attack at C_2 is taster than because of Br^- is closer to this carbon. (Scheme-5).



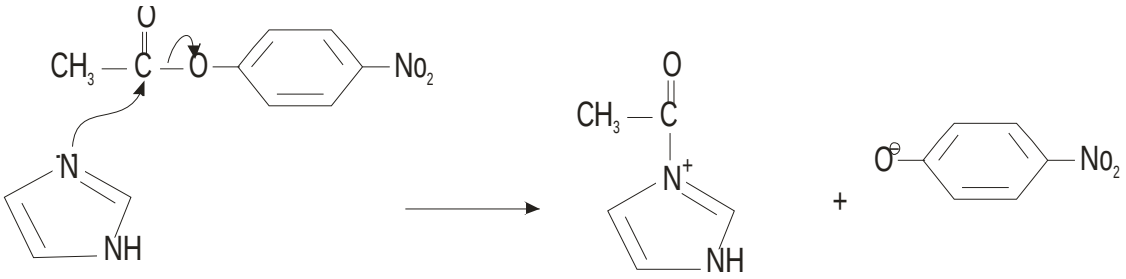
Scheme-5: The 1, 2 product is form taster due to proximity of Br^- to C_2

(e) Intramolecular reactions will occur faster than intermolecular reactions due to proximity effect which are explain as following example:

(i) Intramolecular bimolecular reaction of imidazole with p-nitrophenylacetate

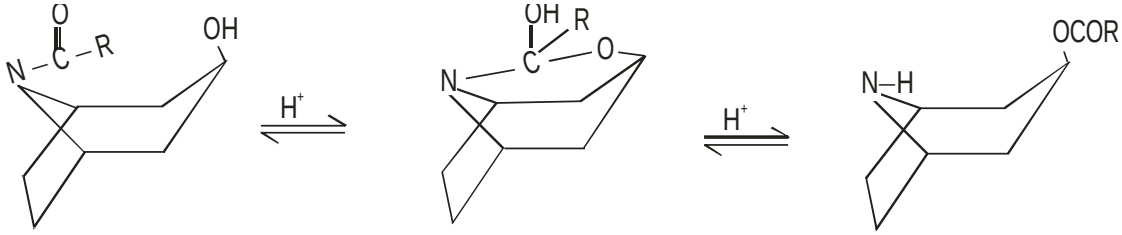


(ii) Intermolecular bimolecular reaction of imidazole with p-nitrophenylacetate.



N-Acetyl imidazolium

(f) Sterochemistry of tropone alkaloids are explained by proximity effects. It has been shown that N-acetyl nor tropine undergoes N-O acyl-migration via cyclic intermediate.



N-Acetyl-nor tropine (I)

11.4 Molecular Adaptation

Molecular adaptation useful in designing new drug because compound with similar structures may compete for the same biological target.

Following examples are explaining the molecular adaptation facts.

(i) Carbachol is an example of a molecular adaptation as for as the cholinergic receptor is concerned.

(ii) 5-flurocytosine is a common antibiotic used against bacterial in fections, which is an analog of natural base cytosine, 5 fluro cytosine a small peptide is attached containing D-amino acids. This peptide containing drug thus can sneak into the pacterial cell.

(iii) Cortisone derivatives are useful in the diagnosis and treatment of disorder of adrenal functioning and as anti in flammatory drugs. It is also important to molecular adaptation at conformational level. Steric, substitution, inductive and resonance factor are also important during molecular adaptation.

11.5 Summary

- Bio-organic chemistry contain various organic reaction like addition, elimination, reduction.
- In Bio-organic chemistry, organic molecules are synthesize for biological importance.
- Proximity effect acceleration the rate of chemical reaction.
- Intra molecular reaction are more faster than intermolecular reaction due to proximity effects.

11.6 Review Questions

1. What is proximity effect explain with suitable example ?
2. Explain the Bio-organic term.
3. Intramolecular reaction are faster than intermolecular reaction, why ?
4. Write a short note on molecular adaptation.

11.7 Suggested Reading and references

- Bio-organic chemistry – H. Dugas, C. penney, springer
- Bio-organic, Bioinorganic and supramolecular chemistry – P.S. Kalsi, J.P. Kalsi, Newage interurational
- Organic chemistry – G. Solomons, John wiley and sons.
- Organic chemistry – Vol. II, I.L. Finar.

Unit-12

Enzymes

Structure of Unit :

12.0 Objective

The study of enzymes has immense practical importance. In some diseases, especially inheritable genetic disorders, there may be a deficiency or even a total absence of one or more enzymes. For other disease conditions, excessive activity of an enzyme may be the cause. Measurements of the activities of enzymes in blood plasma, erythrocytes, or tissue samples are important in diagnosing certain illnesses. Many drugs exert their biological effects through interactions with enzymes. And enzymes are important practical tools, not only in medicine but in the chemical industry, food processing, and agriculture. We begin with descriptions of the properties of enzymes and the principles underlying their catalytic power, a discipline that provides much of the framework for any discussion of enzymes. We end with a discussion of how enzyme activity is regulated.

12.1 Introduction and historical perspective

Enzymes are biological catalysts which bring about chemical reaction in living cells. They are produced by the living organism, and are usually present in only very small amounts in the various cells (about 0.01per cent). They can also exhibit their activity even when they have been extracted from their source. All enzymes are globular proteins, many have been identified and a large number have been obtained in crystalline form.

Louis Pasteur was among the first to study enzyme action. He incorrectly hypothesized that the conversion of sugar into alcohol by yeast was catalyzed by "ferments" that could not be separated from living cells. In 1897 the German biochemist Eduard Buchner (1860-1917) isolated the enzymes which catalyze alcoholic fermentation from living yeast cells, represented in the equation:

The early twentieth century saw dramatic advancement in enzyme studies. Emil Fischer (1852-1919) recognized the importance of substrate shape for binding by enzymes. Leonor Michaelis (1875-1949) and Maud Menten introduced a mathematical approach for quantifying enzyme-catalyzed reactions. James Sumner (1887-1955) and John Northrop (1891-1987) were among the first to produce highly ordered enzyme crystals and firmly establish the protein nature of these biological catalysts. In 1937 Hans Krebs (1900-1981) postulated how a series of enzymatic reactions were coordinated in the **citric acid** cycle for the production of ATP from glucose metabolites. Today, enzymology is a central part of biochemical study.

12.2 Chemical and Biological catalysis

Chemical Catalysis

Catalysis, in chemistry, the modification of the rate of a chemical reaction, usually an acceleration, by addition of a substance not consumed during the reaction. The rates of chemical reactions that is, the velocities at which they occur depend upon a number of factors, including the chemical nature of the reacting species and the external conditions to which they are exposed. Catalysts are chemical substances which alter the rate of reaction and the process is known as chemical catalysis. The term catalysis is generally used for the rate enhancement is presence of a foreign substance which itself does not change during the course of reaction & can be recovered as such at the completion of reaction. In certain cases, one of the

reaction products itself acts as catalyst. For example, in the oxidation of oxalic acid by acidified potassium permanganate velocity increases as the reaction progresses. Here rate acceleration is due to the presence of Mn^{2+} ions which are formed during the reaction. This type of phenomenon is known as auto-catalysis.

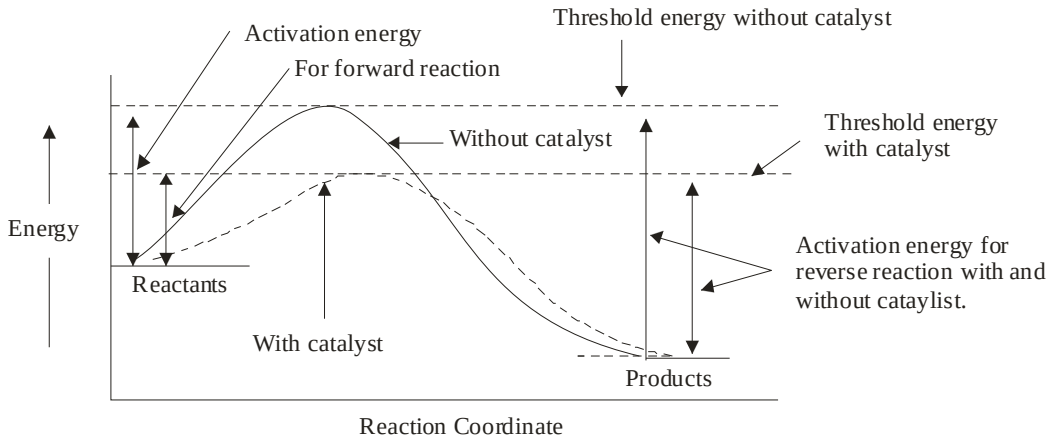


Fig. 12.2 : Effect of catalyst on the rate of reaction

Exact mechanism by which catalyst enhances rate of reaction is not understood. However, it is believed that catalytic rate acceleration is due to the fact that it provides new alternate low energy pathway for the reaction by lowering energy of transition state.

A particular phenomenon associated with the rates of chemical reactions that is of great theoretical and practical interest is catalysis, the acceleration of chemical reactions by substances not consumed in the reactions themselves substances known as catalysts. The study of catalysis is of interest theoretically because of what it reveals about the fundamental nature of chemical reactions; in practice, the study of catalysis is important because many industrial processes depend upon catalysts for their success. Fundamentally, the peculiar phenomenon of life would hardly be possible without the biological catalysts termed enzymes. In a catalyzed reaction, the catalyst generally enters into chemical combination with the reactants but is ultimately regenerated, so the amount of catalyst remains unchanged. Since the catalyst is not consumed, each catalyst molecule may induce the transformation of many molecules of reactant. For an active catalyst, the number of molecules transformed per minute by one molecule of catalyst may be as large as several million.

Where a given substance or a combination of substances undergoes two or more simultaneous reactions that yield different products, the distribution of products

may be influenced by the use of a catalyst that selectively accelerates one reaction relative to the others. By choosing the appropriate catalyst, a particular reaction can be made to occur to the extent of practically excluding another. Many important applications of catalysis are based on selectivity of this kind.

Since a reverse chemical reaction may proceed by reversal of the steps constituting the mechanism of the forward reaction, the catalyst for a given reaction accelerates the reaction in both directions equally. Therefore, a catalyst does not affect the position of equilibrium of a chemical reaction; it affects only the rate at which equilibrium is attained. Apparent exceptions to this generalization are those reactions in which one of the products is also a catalyst for the reaction. Such reactions are termed autocatalytic.

Cases are also known in which the addition of a foreign substance, called an inhibitor, decreases the rate of a chemical reaction. This phenomenon, properly termed inhibition or retardation, is sometimes called negative catalysis. Concentrations of the inhibitor may in some cases be much lower than those of the reactants. Inhibition may result from (1) a decrease in the concentration of one of the reactants because of complex formation between the reactant and the inhibitor, (2) a decrease in the concentration of an active catalyst ("poisoning" of the catalyst) because of complex formation between the catalyst and the inhibitor, or (3) a termination of a chain reaction because of destruction of the chain carriers by the inhibitor.

Characteristics of catalysis: chemical catalysis is characterized by following aspects:

1. It remains unchanged in regard to amount and composition at the end of reaction. However, it may undergo some physical change.
2. Only a small amount of catalyst is required. For example, one mole of colloidal platinum can catalyse decomposition of 10^8 litres of hydrogen peroxide. However, in some homogenous catalytic reactions, the rate of reaction is proportional to the concentration of the catalyst. For example, rate of inversion of cane sugar catalysed by hydrogen ions present in the solution.
3. Rate of heterogeneously catalysed reaction increases with the surface area of catalyst; this is why solid catalyst is more effective in finely divided state.

The catalyst does not alter the position of equilibrium in reversible reaction; it only hastens the attainment of equilibrium. For instance, use of platinum asbestos as catalyst in formation of sulphur trioxide.

$$2SO_2 + O_2 \xrightarrow{\text{Pt Asbestos}} 2SO_3$$

Causes considerable change in the rate of reaction; but does not alter the composition of reactants and product at the equilibrium at the given condition of temperature & pressure.

Catalyst does not initiate the reaction it only alters reaction rate.

4. Catalyst is specific. For a particular reaction a particular catalyst is required. For instance, manganese dioxide can catalyse the decomposition of potassium chlorate but not that of potassium perchlorate or potassium nitrate. Thus, manganese dioxide is specific in its action. Almost all catalysts are specific in action. Substances catalyzing biological processes are known as enzymes. Thus, all enzymes are catalysts but all catalysts are not enzymes.

$$2KClO_3 \xrightarrow[\text{Catalyst}]{MnO_2} 2KCl + 3O_2$$

$$C_6H_{12}O_6 \xrightarrow[\text{Enzyme}]{\text{Zymase}} 2CH_3OH + 2CO_2$$

5. Catalysts only alter rates of reaction, they do not initiate them. For examples; $KClO_3$ upon heating will always decompose to give potassium chloride and oxygen irrespective to absence or presence of catalyst. However, manganese dioxide will enhance rate of decomposition. Nitrogen & hydrogen always combine to produce ammonia, but presence of catalyst accelerates rate of formation.

   But, there are some exceptions. Carbon dioxide and hydrogen give different products in presence of different catalysts as given below:

$$CO + 2\,H_2 \xrightarrow{Cu} \underset{\text{Formaldehyde}}{HCHO}$$

$$CO + 2\,H_2 \xrightarrow{Zno + Cr_2O_3} \underset{\text{Methanol}}{CH_3OH}$$

$$CO + 3H_2 \xrightarrow{Ni} \underset{\text{Methane}}{CH_4} + H_2O$$

6. Catalysts work most effectively at certain specific temperature which is known as optimum temperature for its working or effectiveness.
7. Certain substances mar effectiveness of catalysts; they are known as catalytic poisons. For example, in contact process for the formation of sulphuric acid sulphur trioxide is needed, which is prepared by the combination of sulphur dioxide and oxygen. Rate of its formation is slowed down by the presence of arsenic compounds even if they are present in traces.

Biological Catalysis:

Enzymes are "biological catalysts." "Biological" means the substance in question is produced or is derived from some living organism. "Catalyst" denotes a substance that has the ability to increase the rate of a chemical reaction, and is not changed or destroyed by the chemical reaction that it accelerates.

Generally speaking, catalysts are specific in nature as to the type of reaction they can catalyze. Enzymes, as a subclass of catalysts, are very specific in nature. Each enzyme can act to catalyze only very select chemical reactions and only with very select substances. An enzyme has been described as a "key" which can "unlock" complex compounds. An enzyme, as the key, must have a certain structure or multi-dimensional shape that matches a specific section of the "substrate" (a substrate is the compound or substance which undergoes the change). Once these two components come together, certain chemical bonds within the substrate molecule change much as a lock is released, and just like the key in this illustration, the enzyme is free to execute its duty once again.

Many chemical reactions do proceed but at such a slow rate that their progress would seem to be imperceptible at normally encountered environmental temperature. Consider for example, the oxidation of glucose or other sugars to useable energy by animals and plants. For a living organism to derive heat and other energy from sugar, the sugar must be oxidized (combined with oxygen) or metabolically "burned"

However, in a living system, the oxidation of sugar must meet an additional condition; that oxidation of sugar must proceed essentially at normal body temperature. Obviously, sugar surrounded by sufficient oxygen would not oxidize very rapidly at this temperature. In conjunction with a series of enzymes created by the living organism, however, this reaction does proceed quite rapidly at temperatures up to 100°F (38°C). Therefore, enzymes allow the living organism to make use of the potential energy contained in sugar and other food substances.

Enzymes or biological catalysts allow reactions that are necessary to sustain life proceed relatively quickly at the normal environmental temperatures. Enzymes often
increase the rate of a chemical reaction between 10 and 20 million times what the speed of reaction would be when left uncatalyzed (at a given temperature).

Nutrients locked in certain organics are complex macromolecules, or in hard-to-digest matrices may be released or predigested by a high degree of heat or concentrated acid treatment. In an alternative manner, specific enzymes can promote the pre-digestion of certain complex nutrients and facilitate the release of highly digestible nutrients in organics during processing without the need of excessive heat or rigorous chemical treatment.

12.3 Remarkable properties of Enzymes:

1. Catalytic power: The catalytic power of enzymes facilitates life processes in essentially all life-forms from viruses to man. Many enzymes retain their catalytic potential after extraction from the living organism, and it did not take long for mankind to recognize and exploit the catalytic power of enzyme for commercial purposes. In fact, the earliest known references to enzymes are from ancient texts dealing with the manufacture of cheeses, breads, and alcoholic beverages, and for the tenderizing of meats. Today enzymes continue to play key roles in many food and beverage manufacturing processes and are ingredients in numerous consumer

products, such as laundry detergents (which dissolve protein-based stains with the help of proteolytic enzymes). Enzymes are also of fundamental interest in the health sciences, since many disease processes can be linked to the aberrant activities of one or a few enzymes. Hence, much of modern pharmaceutical research is based on the search for potent and specific inhibitors of these enzymes .

Both catalysts and enzymes lower the activation energy of a reaction thereby increasing its rate .A catalyst can be positive (increasing reaction rate) or negative (decreasing reaction rate) in nature. They react with reactants in a chemical reaction to give rise to intermediates that eventually release the product and regenerate the catalyst. Consider a reaction where **C** is a Catalyst, **A** and **B** are reactants and **P** is the Product.

A **typical catalytic chemical reaction** would be:

$$A + C \longrightarrow AC$$
$$B + AC \longrightarrow ABC$$
$$ABC \longrightarrow PC$$
$$PC \longrightarrow P + C$$

The catalyst is regenerated in the last step even though in the intermediate steps it had integrated with reactants. Enzymes rates of biological processes at extremely faster rates. A chemical reaction in presence of enzymes proceeds hundred to one million times faster.

1. **Specific:** one **of** the most characteristics properties of enzymes is their specificity of action. This specificity may be manifested in one of three ways:

   a) An enzymes may catalyse a particular type of reaction, e.g., esterase hydrolse only esters. Such enzymes are said to be reaction specific. On the other hand, an enzyme may be specific for a particular compounds or class of compounds. These enzymes are substrate specific, e.g., urease hydrolyses only urea; phosphatases hydrolyses only phosphate esters.

   b) Many enzymes exhibit a kinetic specificity, e.g., esterases, although hydrolyzing all esters, hydrolyse the various esters at different rates; pepsin hydrolyses the peptide link, but is most active for those links in which, among other things, the amino group belongs to an aromatic

amino-acid and the carboxyl group is one of a dicarboxylic amino-acid.

c) Many enzymes are stereospecific, e.g., maltase hydrolyses alpha-glycosides but not beta-glycosides, whereas emulsion hydrolyses the latter but not former.

It should be noted, however, that a given enzyme can exhibit more than one of the specificities, e.g., esterases, while hydrolyzing only esters, may also hydrolyse one enantiomer (of an optically active ester) more rapidly than the other.

2.Regulation:

The reaction velocity decreases as function of time in enzyme-substrate reaction the wane in velocity may be depend to following reason:

i) The enzyme may undergo some progressive inactivation at the temperature.

ii) The enzymes may undergo some progressive inactivation at the P^H of the reaction due to instability.

iii) The eviscration of substrate level due to the occupancy of active sites.

iv) The product of reaction may inhibit the enzyme if the medium is not properly buffered.

v) Enzyme activity can be reduced or inhibited by the presence of various compounds, first competitive and second irreversible. In competitive inhibition, the inhibitor is a compound whose structure and geometry closely resemble that of the normal substrate.

Competition occurs between the two (at the active site), but inhibition can be reversed by increasing the concentration of the normal substrate. Therefore follows that the enzyme inhibitor complex readily regenerates the two molecules in competitive inhibition. On the other hand, in irreversible inhibition the inhibitor forms a highly stable enzyme-inhibitor complex, and if sufficient inhibitor is present, the catalytic effect of the enzyme towards its normal substrate is completely lost.

vi) The approach to an equilibrium and associated influence of the reverse reaction. These reason, enzymes are studied within their velocity. The initial velocity may be determined from slope of tangent to the curve at the zero.

vii) Enzyme- activity : concentration of enzyme also alters enzymatic activity. Increase in enzyme concentration increases enzyme activity, because increase active centres of enzymes, and reaction velocity becomes double with doubling the concentration of enzyme.

Several contributing factors have been suggested to account for the high efficiency of enzyme catalysed reactions.

a) Proximity effect- Binding of the reactant molecules to the enzyme result in an 'increased concentration' of the reactant molecules.

b) Binding causes the reactant molecules to be correctly oriented and consequently the transition state is reached more readily.

c) Binding produces a strain effect in the reactant molecules and consequently the bonds to be broken are 'deformed', thereby being brought to a state close to those existing in the transition state. Thus energy of activation of the reaction is lowered. For most of the enzymatic reactions, if the substrate concentration is initially very high, the rate will first follow a zero-order course and later on will fall down to first-order kinetics.

The entire reaction is thus a mixture which cannot be described by a single reaction order. The rate is equal to [product]/time and hence

[Enzyme] time = [product]

12.4 Nomenclature and classification

A common method of naming enzymes is to add the suffix ase to the name of the substrate, i.e., the substance being acted upon, e.g., esterase act on esters, amylase

on starch (amylum), protease on proteins, urease on urea, etc. Names are also used for particular enzymes, e.g., urease, amylases, or as general names for groups of enzymes, e.g., esterases, proteases, etc.

The above nomenclature is still widely used, but it has led to difficulties as more and more enzymes have been isolated. Because of this, the international commission on enzymes(1961) has recommended a systematic method of nomenclature and classification. According to this system, enzymes are divided into six main groups according to the nature of the reaction that is catalysed, and each main group is given a code number. The main groups are :

1. **Oxidoreductases.** These enzymes catalysed oxidation-reduction reactions, and include oxidases (direct oxidation with molecular oxygen), dehydrogenases(removal of hydrogen from substrates), etc.
2. **Transferases.** This group of enzymes catalyses the transfer of various functional groups, e.g., transaminase.
3. **Hydrolases.** This catalyse hydrolytic reactions, e.g., proteases (protiens), esterases(esters), etc.
4. **Lyases.** There are two types of lysases, one which catalyses addition to double bonds and the other which catalyses removal of groups and leaves double bonds.
5. **Isomerases.** These catalyse various types of isomerisation, e.g., epimerases, etc.
6. **Ligases.** These enzymes catalyse the formation of a bond between two molecules and is a companied by the breaking of pyrophosphate bond of ATP or similar triphosphate.

Each of these main groups is divided into sub groups which take the number of their main group followed by another number which specifies the type of group in the substrate that undergoes reaction. The sub groups are also divided in to sub-subgroups, these are indicated by a third figure which gives more detailed information on the groups involved in the reaction. Finally a fourth figure indicates the serial number of the enzyme in its sub-subgroup. Thus, an enzyme is specified by four numbers(separated by points), e.g., 1.1.1.1 is the oxidoreductase which is involved in hydrogen transfer from a **CHOH** group to $\mathbf{NAD^+}$ or $\mathbf{NADP^+}$ as acceptor. The trivial name of this enzyme is alcohol dehydrogenase.

The systematic names of enzymes consist of two parts, the first part specifying the substrate (or substrates) and the second part, which ends in 'ase', indicates the nature of the reaction that is catalysed. For example, let us consider the reaction:

L-alanine + 2-oxoglutarate →pyruvate + L-glutamate

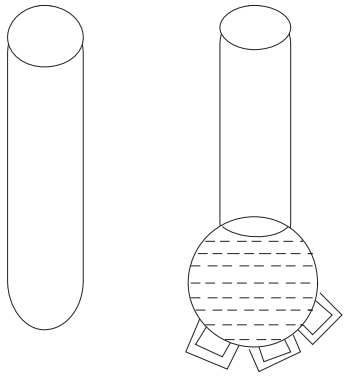
This reaction is catalysed by the enzymes,the transaminase. Since this is a subgroup of the main group of enzymes, the transferases, the common name transaminase has been changed to the more systematic name aminotransferase. Thus, this enzyme is named as L-alanine: 2-oxoglutarate aminotransferase; its Enzyme Commission number is 2.6.1.2. The trivial name of this enzyme is alanine aminotransferase, and was formerly called glutamic- pyruvic transaminase.

12.5 Extraction and Purification

In the extraction & purification of enzymes availability and cost of starting material is of prime importance. Concentration of a single enzyme may vary in different tissues. It is important to choose tissue in which enzyme is in high concentration. For this-reason yeast, bacteria & fungi have certain advantages as source materials. Advantage is that these cells may be cultivated under conditions favourable for the production of enzyme in question. There is, however, one disadvantage of difficulty in obtaining large quantities of microbial cells other than yeast.

Once starting material has been selected, a series of steps can be performed over that to effect extraction & isolation of enzyme. A few specific examples of certain methods are discussed below:

1. **Sedimentation:** If liver tissue is homogenized, as in Potter-Elvenhjem apparatus rather than common blending devices, many of mitochondrial & other particulate cell bodies remain intact. They are easily sedimented out of solution, and with them goes a repertoire of enzymes. Physical separation by sedimentation is of practical utility only in initial phase of separation.



Potter - Elevenhijem
'homogenizer

Fig. 12.5 : Potter-Elvenhjem homogenizer

2. **Extraction:** Earlier classification of enzymes was in two main classes (i) soluble or lyoenzyme and (ii) bound or desmoenzyme. This is a poor classification; since desmoenzymes are probably those enzymes for which proper methods of solution have yet not been discovered.

   The acetone-powder (from which enzymes can be removed by extraction with buffer) is, by the virtue of its fat-free nature, often the easiest material from which enzymes can be extracted. In any event, a fine-grinding is the first-step. Methods for removing enzymes from micro-organisms include autolysis, lysozyme digestion, grinding, freezing & thawing, sonic disintegration, shaking with solvents, shaking with fine-glass beads and, finally, explosion by sudden release by pressure.

3. **Salt Fractionation:** Ammonium sulphate is most useful salt in enzyme fractionation. Its advantages are high solubility in water (760 g/lt) & a roughly neutral reaction (pH 5 to 6) in concentrated solution. Dixon developed a monogram (chart) for the preparation of ammonium sulphate solutions and Kunits gave an equation for calculating the ammonium sulphate to be added to a solution to give the desired final concentration. One disadvantage in use of ammonium sulphate in slightly alkaline solution is that, even at pH 9.3; 50% of ammonium ions are converted into ammonia. The pH of ammonium sulphate solution should be controlled by means of a buffer. However, for crystallization of beef-liver glutamic dehydrogenase sodium sulphate has been used extensively.

4. **Solvent Fractionation:** Water-miscible solvents like acetone, ethanol, methanol and dioxane help in the isolation of enzymes.

In acetone extraction one should start below 0°C & then proceed towards higher temperatures, Fractionation is done upto highest temperature that will not cause much yield-loss. Because, acetone absorbs strongly in U.V. region, it must be completely removed by dialysis or by distillation under reduced pressure before the product is subjected to spectral-analysis.

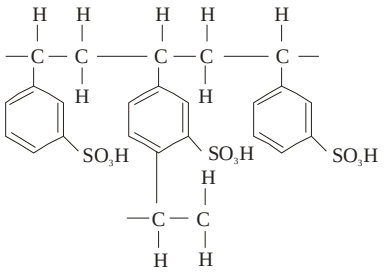
Ethanol has found increasing application in the isolation of enzymes. It has been used to obtain crystalline **lactic dehydrogenase** from rat-liver.

5. **Solvent-Metal ion fractionation:** An important method for that separation of blood-proteins is by the combination of metal-ions & solvents particularly Zn^{2+} and ethanol. The zinc-salts of proteins are often more soluble than the sodium and potassium-salts and separate out more easily from that solution. From these, metal ions may be removed by the treatment with citrate, ethylene diamine tetraacetate, or ion-exchange resin.

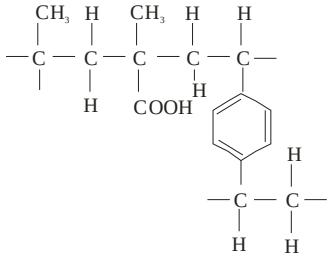
6. **Adsorption:** A variety of substances have been used as protein adsorbents. One of the earliest preparations is hydrated aluminium oxide Calcium phosphate gel has also proved very useful & bentonite has been employed in the isolation of lysozyme.

7. **Adsorption chromatography:** Column chromatography on adsorbents is very effective for the separation of proteins & hence for enzymes also. Anger prepared calcium phosphate gel. This same adsorbent has been critically examined by Swingle & Tiselius for general protein chromatography. Zechmeister has published a review on general subject of enzyme chromatography.

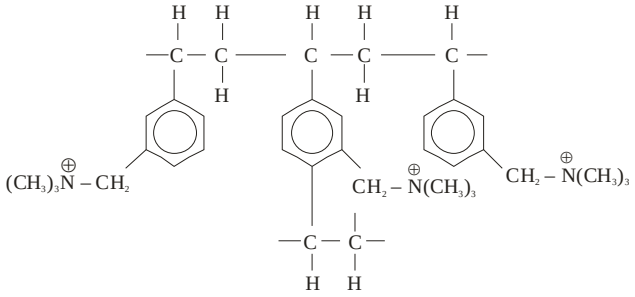
In another approach, using biochemically specific adsorbent, isolation of enzyme depends upon its catalytic specificity rather than on its general properties as protein. For instance, in the isolation of mushroom tyrosinase various adsorbents containing p-azophenol & related groups were prepared from aromatic ethers of cellulose.



Sulphonic acid cation exchanger



Carboxylic acid cation exchanger



Quaternary amine anion exchanger

Cytochrome **C** was purified in 1950, by the passage through a column of amberite IRC-50 & same method was useful in isolating cytochrome from Ustilago. Ribonuclease & lysozyme have been purified in a similar manner Ion-exchange chromatography is essentially an electrophoretic separation in' which the resin serves as an electrode and gravity as the other. It may yet develop into valuable tool as the chemical industry places more new resins in hands of enzymologists. Structures of some cation-exchangers & anion-exchangers are shown.

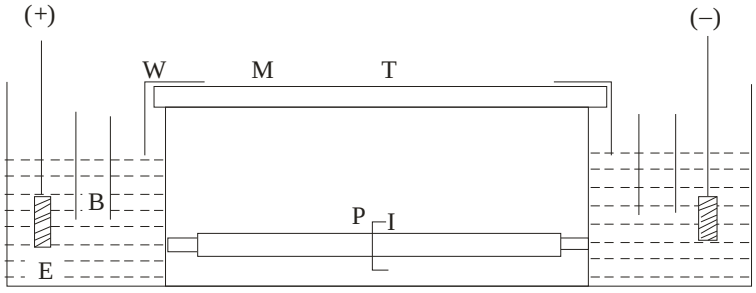
9. Complex Formation: In this procedure **protamine** has been used to a lesser extent as complexing agent. Some enzymes, e.g., **muscle lactic dehydrogenase,** were isolated as the inactive mercury salts. Reactivation was achived by dialysis against cysteine or potassium cyanide. Basic lead acetate has been employed to throw down undesired proteins

10. Denaturation Reactions : Brief heating from 50°C-70°C, is one of the early steps. This method is successful in the crystallization of alcohol dehydrogenase from yeast. Denaturation with trichloroacetic acid is used in the isolation of cytochrome C. Shaking with chloroform is a very effective way remove contamination proteins.

11. Dialysis : Theorell & Akeson designed electrophoretic cell for separating proteins from salts. A current of air blown over a dialysis bag containing a protein solution is an exceedingly gentle method to effect concentration. As long as evaporation continues, the solution will remain at low temperature.

12. Preparative electrophoresis : Large cells containing upto 100 mL, may be used with special electrodes in the electrophoretic apparatus. Method in which components are completely separated, has been designated as "Zone electrophoresis."

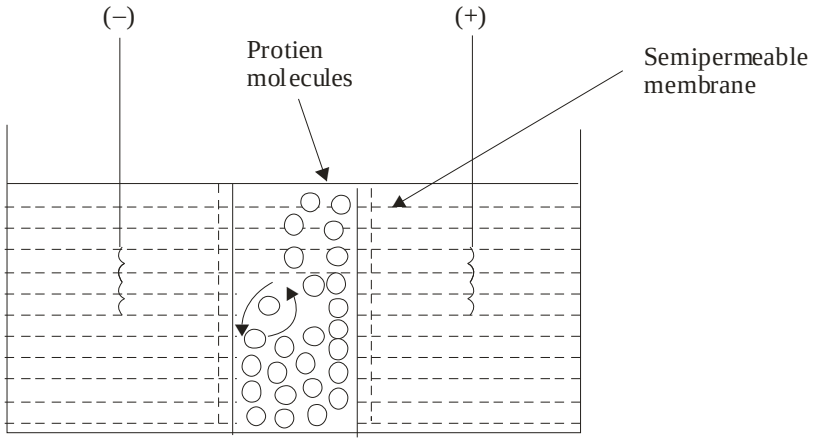
Zone electrophoresis on starch bed or on blocks of sponge rubber has become a very popular tool for protein isolation. A schematic diagram for preparative zone, electrophoresis cell is given below :



B = Buffer Compartment With Baffles
E = Platinum electrodes
W = Filter paper wick
P = Pressure equalizing tube
M = Medium, such as starch, sponge
T = Trench for sample

This novel technique developed by **Kolin** is worth mentioning. Here buffer solutions of unequal density are layered over each other to give pH gradient in vertical tube. The sample is introduced & when current is turned on, the various proteins migrate to their isoelectric points & stop. The method is capable of very rapid resolution, since further the proteins from pI (isoelectric point) the more rapidly they will migrate to isoelectric zone.

The **"electrophoresis-convection"** apparatus of Kirkwood may be used for separating less complex mixtures. Electrophoresis convection may be regarded as a refinement of the process of Electrodecantation as illustrated below :



A schematic diagram of the Electrophoresis convection Apparatus

The pH throughout the apparatus is alkaline to the isoelectric-point of the protein, and the protein has migrated to the anode. This will cause a higher density next to the right side of the membrane, and the protein will settle down on that side, thus giving rise to convection and eventually to a collection of the component at the bottom of the cell.

"Purification (Criteria of Purity)"

The majority of the past and present industrial uses of enzymes have been with crude preparations. However, many of the uses for immobilized enzymes require highly purified forms. Techniques for the large-scale initial isolation and partial purification of enzymes from microbial, plant & animal sources make use mainly of traditional processing steps, scaled-up in some instances directly from the art of the research laboratory. In other cases, newer equipment has been developed, especially for cell disruption and centrifugation. The use of semipermeable membranes has proved very useful in the initial isolation steps. However, major advances in membrane development are needed for the fractionation of enzyme mixtures by this technique.

Final purification of enzymes is a tedious task, be it in the laboratory or in industrial plants, with chromatographic methods in preponderance. Gel permeation chromatography & affinity chromatography hold very high promise for simplifying the purification of enzymes. The later method consists of contacting a crude enzyme preparation with a solid support to which is attached a reversible inhibitor or some other types of molecule which will

selectively & reversibly bind with the enzyme of interest. With the enzymes thus bound to immoblized inhititors, the support-inhibitor-enzyme complex is separated from the initial crude feed and the purified enzyme eluted form the support-inhibitor portion. The methods like gel filteration & affinity chromatography as well as other chromatographic methods are appropriate because of reduction in cost of purification of enzymes as well as due to larger supply & varieties of enzymes. However, development needs to be done in scaling up these methods. In purification anaphylactic or precipitation reactions are also useful. In a method antigen-antibody reaction is carried out in a gel such as agar. The compounds are visible as precipitated zone in gel.

12.6 Summary

- **Classification of enzymes by function**
- **Oxidoreductases** which are involved in oxidation, reduction, and electron or proton transfer reactions
- **Transferases**, catalysing reactions in which groups are transferred
- **Hydrolases** which cleave various covalent bonds by hydrolysis
- **Lyases** catalyse reactions forming or breaking double bonds
- **Isomerases** catalyse isomerisation reactions
- **Ligases** join substituents together covalently
- Velocity of enzymes change according to specificity and regulation.

12.7 Review Question

1. What is meant by substrates of enzymatic reactions?
2. How does the formation of the enzyme-substrate complex explain the reduction of the activation energy of chemical reactions?
3. Why can it be said that the enzymatic action is highly specific?
4. What is the chemical basis of enzyme catalysis?
5. What are the "active" and "regulatory" sites on an enzyme?
6. For each of the reactions below, provide the name of the appropriate enzyme type.

a. Joining of two molecules
b. Transfer of functional groups
c. Oxidation-reduction reactions
d. Intramolecular rearrangements
e. Hydrolysis reactions
f. Addition to double bonds

7 Do classify of enzymes.

12.8 Reference suggested

- Bennett, T. P., and Frieden, E.: *Modern Topics in Biochemistry,* pg. 43-45, Macmillan, London (1969).
- Holum, J.: *Elements of General and Biological Chemistry*, 2nd ed., 377, Wiley, NY (1968).
- Martinek, R.: Practical Clinical Enzymology: *J. Am. Med. Tech., 31,* 162 (1969).
- Harrow, B., and Mazur, A.: *Textbook of Biochemistry,* 109, Saunders, Philadelphia (1958).
- Pfeiffer, J.: *Enzymes, the Physics and Chemistry of Life*, pg 171-173, Simon and Schuster, NY (1954)

Unit - 13

Theory and Kinetics of Enzymes

Structure of Unit:

13.0 Objective

Enzyme kinetics is the study of the chemical reaction that are catalysed by enzymes. In enzyme kinetics, the reaction rates is measured and the effects of varying the conditions of the reaction are investigated. Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of this enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme.

13.1 Fisher's lock and key and Koshland's induced fit hypothesis

The interaction of substrate and enzyme was visualized in term of a lock and key model (also known as template model), proposed by Emil Fisher in 1898.

According to this model, the union between the substrate and the enzyme take place at the active site more or less in manner in which a key fits a lock and results in the formation of an enzyme substrate complex.

Substrates are the substances which enzymes act on. The enzyme active is the region where the substrate binds and catalyzes the chemical reaction.

The Fischer's lock and key hypothesis and Koshland's induced fit hypothesis are two hypotheses suggested to explain catalyses and specificity of enzymes.

The lock and key hypothesis use where the fit between the substrate and active site is very specific like that of a lock and key. A temporary enzyme substrate complex is formed and the products with a different shape from the substrate once formed escapes from the active site leaving it free to attach to another substrate molecule. This explains enzyme specificity and loss of activity when the enzymes. However it is too rigid.

Koshland's induced fit hypothesis suggests in the presence of the substrate the active site may change in order to fit the substrates change. The enzyme is flexible and molds to fit the substrate molecule like gloves fitting one's hand or clothing on a person. The enzymes initially have a binding configuration which attracts the substrate. On binding to the enzyme the substrate disturbs the shape and causes it to assume a new configuration. The active site is then molded into a precise conformation.

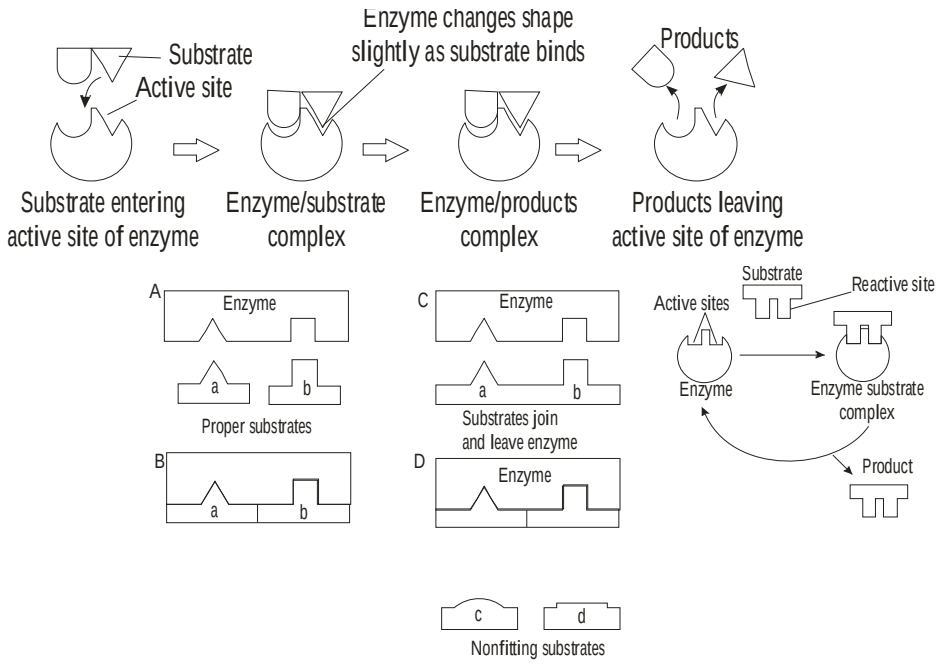


Fig. 13.1 Fischer's Lock and Key and Koshland's Catalyses and Specificity of enzyme

13.2 Concept and identification of active sites by the use of inhibitors

Active sites:

As the substrate molecules are comparatively much smaller than the enzyme molecules, there should be some specific regions or sites on the enzyme for binding with the substrate. Such sites of attachment are variously called as 'active sites' or 'catalytic sites' or 'substrate sites'. Although the enzymes differ widely in their properties, the active site present in their molecule possesses some common features

1. The active site occupies a relatively small portion of the enzyme molecule.
2. The active site is neither a point nor a line or even a plane but is a 3-dimensional entity. It is made up of groups that come from different parts of the linear amino acid sequence. The amino acid residues located at the active site are 35, 52, 59, 62, 63 and 107.
3. Usually the arrangement of atoms in the active site is well defined, resulting in a marked specificity of the enzymes. Although cases are known where the active site changes its configuration in order to bind a substance which is only slightly different in structure from its own substrate.
4. The active site binds the substrate molecule by relatively weak forces.
5. The active sites in the enzyme molecules are grooves or crevices from which water is largely excluded. It contains amino acids such as aspartic acid, glutamic acid, lysine serine etc. The side chain groups like -COOH, -NH_2, -CH_2OH etc., serve as catalytic groups in the active site. Besides, the crevice creates a micro-environment in which certain polar residues acquire special properties which are essential for catalysis.

It is believed that when the substrate molecule binds to the active site, its parts are held together in such a way as to cause distortion of chemical bonds, i.e., the bonds are weakened. This distortion of chemical bonds of substrate increases its reactivity, and thus speeds up the rate of reaction. The products of reaction are released because they are less firmly bound, this mechanism is called strain model of catalysis.

Inhibitors:

Inhibitors are compounds which decrease the rate of an enzyme catalysed reaction. Inhibition is shown below:

Concept and identification of active site by the use of inhibitors.

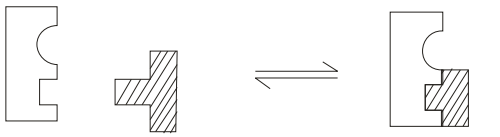


Fig. 13.2 Inhibition

Inhibitors are of two types:

i) Irreversible inhibitors
ii) Reversible inhibitors

i) Irreversible inhibitors: Irreversible inhibitor help in the identification of active sites. Irreversible inhibition involves the covalent bonding of inhibitors to a functional group at the active site or elsewhere on enzyme .Irreversible inhibitors include some of the pesticides, e.g., parathion is an active inhibitor of insect acetylcholine stearase upon which normal propagation of nervous impulses relies. Since inhibition is irreversible in which the substrate in the catalytic cycle is converted into a chemically reactive product which remains bound to active site through covalent bonding, the enzyme is rendered permanently inactive and the identification of active site is done on the basis of its reaction with inhibitors.

ii) Reversible inhibitors: A reversible inhibitor dissociates very rapidly from its target enzyme because it becomes very loosely bound with the enzyme.

13.3 Affinity labelling and enzyme modification by site directed mutagenesis

Affinity labeling can be applied to catalytic-regularity and antibody active sites, as it does not depend on having a uniquely active group within this site.

Affinity labeling can be represented as follows :

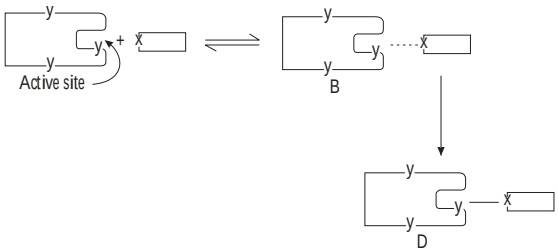
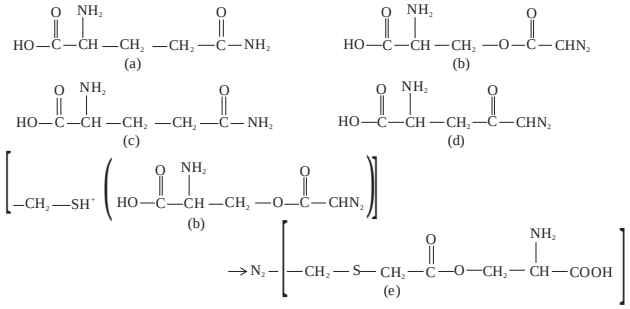


Fig. 13.3 Schematic representation of affinity labelling: After the reversible complex B is formed, the irreversible product) D is produced by reaction between functional group X of the labelling reagent and group *Y* of active site.

In this method labelling reagent first combines specifically & reversibly with the site to form complex B by virtue of a suitably small & reactive group X on the reagent, it can then react with one or more amino acid residues *Y* in the site to form irreversible covalent bond. Formation of irreversible complex so increases the local concentration of the labelling reagent in the site, as compared to that in concentration in free solution, that reaction with the group *Y* in the site is markedly favoured over reaction with any similar group *Y* outside the site. The group *Y* need not to be an unusually reactive residue of its kind for this to occur. One thing important to mention is that group *X* of reagent should be small so as to be encompassed within the active site of enzyme. Needless to say that this interaction modifies the enzyme by bringing about mutagenesis of its active site.

During affinity labelling enzymes modification related chemical principles are apparently involved. An interesting example is catalysis of transfer of amide group of L-glutamine (a) to formylglycinamide ribonucleotide to form formylglycinamidine nucleotide & glutamic acid.

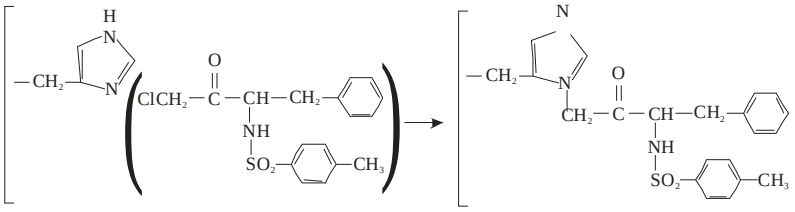


Buchanan and his co-workers found that this enzyme was specifically & irreversibly inhibited by the glutamine antagonists O-diazoacetyl-L-serine IL-azaserine (b) or 6-diazo-5-oxo-L-norleucine (C) but not by their D-analogs or by the closely similar compound 5-diazo-4-oxo-L-norvaline (d). These striking results suggest that the active compounds first form a specific & reversible complex in the enzyme binding site for L-glutamine, following which the diazoacetyl group (group *X)* reacts with some suitably positioned residue *Y* in the active site. The stereochemical analogy between the diazoacetyl group of these reagents and the amide group of L-glutamine that is transferred in the normal functioning of the enzyme active site strongly suggests that the group *Y* is within the site. Subsequent work by French *et al.* & David *et. al* showed that this group is a cysteinyl residue (e). This cysteinyl residue is apparently uniquely reactive, since azaserine does not react with free cysteine, with the highly reactive(—SH) group in activated papain or with (-SH) group of serum mercaptaalbumin.

The general idea that active site binding can be used to direct a specific chemical modification was realized and developed independently in a number of laboratories. The studies of Baker *et al.* were designed to inactivate certain enzymes specifically & irreversibly primarily for chemotherapeutic purposes, rather than to obtain structural information about active sites.

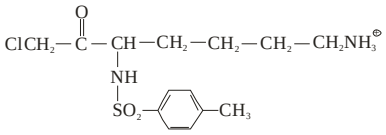
Another important example of the use of affinity labelling (Scheme-I) is the reaction of chymotrypsin with the chloromethylketone derived from N-tosyl-L-phenylalanine (TPCK) (a). Here the chloromethylketone is the group *X* attached to substrate-like N-tosylphenylalanyl residue. Specific mechanism as depicted in the general example (Fig. 13.3) was followed since the native enzyme was modified to the extent of 1 mol per mole of protein, but was

not modified in presence of 8 M urea. In other words, an initial reversible binding to active site of native enzyme was implicated. The labelled enzyme was completely inactivated (b). Trypsin, having a different substrate specificity, was not affected by TPCK, but has been shown to be similarly inactivated by its own specific-affinity labelling reagent, the chloromethylketone derived from N-tosyl-L-Lysine (TLCK) (c).



TPCK
(a)

(b)



TLCK
(c)

Scheme-I

The chloromethylketones in each case alkylate histidyl residues that were long inferred to be present in these active sites.

13.4 Kinetics of Enzyme Action

Enzyme kinetics can be studied in two parts:

(i) Energy of activation

(ii) Steady state enzyme kinetics

(i) Energy of activation: Enzymes catalyse the rate of reaction through the alternate pathway incorporating low energy of activation and thus make the reactions generally occurring under drastic condition possible at normal physiological temperature. Energy of activation is the energy required to reach the reactants to transition state at which new bonds are partially formed & the old bonds are partially broken & is the state of highest energy during the course of reaction, therefore *is* highly unstable & breaks down to give the lower energy products. Out of all the molecules of reactants only a small fraction contains enough energy to react, *i.e.* the threshold energy. Increase

in temperature makes more molecules to aquire energy equivalent to threshold energy (Fig. 13.3(a). But in living systems the limitation is that reactions take place under almost isothermal conditions as there is very little temperature variation. Infact, enzymes help in the formation of enzyme-substrate complex by activating the reactant molecules. Enzyme substrate complex has high energy & makes the reactants with strained bonds, therefore, they react at faster rate. In addition, in enzyme-substrate complex reactant molecules are so oriented that their reaction becomes a certainity and not a matter of chance, that is why reaction rate becomes thousands of time faster.

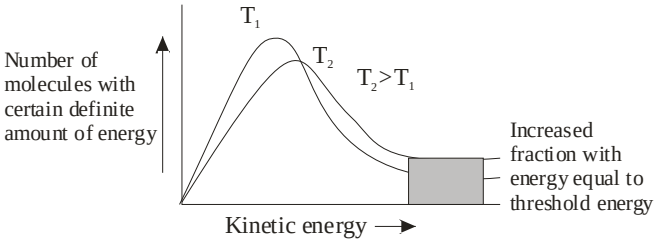


Fig. 13.4 (a) Effect of temperature increase on fraction with threshold energy

Alternatively, it may be said that in presence of enzyme, energy of activation to reach transition state (T.S.) is lowered (Fig.13.4(b)). However, all over free energy change, under standard conditions of temperature and pressure ($\Delta G°$) of reaction, remains unaltered.

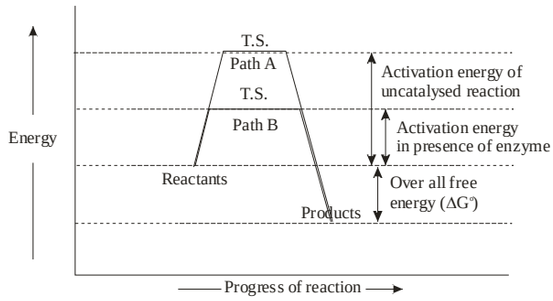


Fig.I3.4(b)Effect of enzyme on Transition state of a reaction.

The enzyme catalyses both forward as well as backward reaction of equilibrium to the same extent. Since $\Delta G°$ is not changed, equilibrium constant also remains unchanged, but equilibrium is achieved quickly. Proportion of reactant & product of catalysed as well as uncatalysed reaction also remains same.

(B) Steady-state enzyme kinetics: This theory, proposed by Michaelis and Menten in 1913, is based upon following assumptions:

(i) Enzyme-substrate complex (ES) is in equilibrium with enzyme & substrate $E + S \underset{K_{-1}}{\overset{K_1\ fast}{\rightleftharpoons}} ES$; E = Enzyme, S = Substrate

(ii) Product-formation is possible only through enzyme-substrate complex:

$$E + S \xrightarrow[K_2]{slow} P + E \quad [P = product]$$

Based on these assumptions Michaelis-Menten equation can be derived as given below:

Let us consider formation of enzyme substrate complex ES.

$$E + S \rightleftharpoons ES \qquad \ldots(1)$$

$$\frac{d[ES]}{dt} = K_1[E][S]$$

It is clear from assumptions (i) and (ii) that clear equilibrium is not achieved in the fast process, as Enzyme-substrate complex [ES] is constantly being removed in the slow process. Concentration of enzyme is very much less than concentration of substrates. Therefore, [E]<<[S]. Hence, [ES] << [S]. The rate of reaction is given as:

$$r = \frac{d[S]}{dt} = \frac{+d[P]}{dt} = K_2[ES] \qquad \ldots (2)$$

Using steady state approximation for the formation of

ES:

$$\frac{d[ES]}{dt} = K_1[E][S] - K_{-1}[ES] - K_2[ES] = 0 \quad \ldots (3)$$

Concentration of free enzymes *[E]* is not measurable in living process. But total enzyme concentration $[E]_0$ is measurable and can be given by Equation (4).

$$[E]_0 = [E] + [ES] \quad \ldots (4)$$

Here [ES] is bound enzyme concentration, therefore [E] can be given as

$$[E] = [E]_0 - [ES] \quad \ldots (5)$$

Putting the value of eq. (3), we get

$$\frac{d[ES]}{dt} = K_1\{[E]_0 - [ES]\}[S] - K_{-1}[ES] = -K_2[ES] = 0 \quad \ldots (6)$$

Upon simplification of above equation & grouping the constants

$$K_1[E]_0[S] = [K_{-1} + K_2 + K_1[S]\}[ES] \quad \ldots (7)$$

$$[ES] = \frac{K_1[E_0][S]}{K_{-1} + K_2 + K_1[S]} \quad \ldots (8)$$

Upon putting this value of [ES] in eq. (2)

$$r = \frac{K_1K_2[E_0][S]}{K_{-1} + K_2 + K_1[S]} \quad \ldots (9)$$

$$r = \frac{\frac{K_2[E]_0[S]}{(K_{-1} + K_2)}}{K_1 + [S]} = \frac{K_2[E]_0[S]}{K_m + [S]} \quad \ldots (10)$$

[Upon dividing both numerator and denominator of eq. (9) by K_1]

Here K_m is Michaelis constant & equation (10) is Michaelis-Menten equation value of K_m is given by the expression :

$$K_m = \frac{(K_{-1} + K_2)}{K_1}$$

This equation correlates the components of enzyme reaction, [S] & [E], to initial & maximum velocity through rate constant K_m.

$$K_m = \frac{\text{Rate of breakdown of ES}}{\text{Rate of Formation of ES}}$$

13.5 Leonor Michaelis Maud L. Menten (1913) and Lineweaver-Burk plots

Leonor Michaelis and Maud L. Menten (1913), while studying the hydrolysis of sucrose catalyzed by the enzyme invertase, proposed this theory. Their theory is, however, based on the following assumptions :

1. Only a single substrate and a single product are involved.
2. The process proceeds essentially to completion.
3. The concentration of the substrate is much greater than that of the enzyme in the system.
4. An intermediate enzyme-substrate complex is formed.
5. The rate of decomposition of the substrate is proportional to the concentration of the enzyme-substrate complex.

The theory postulates that the enzyme (E) forms a weakly-bonded complex (ES) with the substrate(S). This enzyme-substrate complex, on hydrolysis, decomposes to yield the reaction product (P) and the free enzyme (E). These reactions may be symbolically represented as follows:

$$E + S \rightleftharpoons ES \rightarrow E + P$$

Although one may not feel any difficulty (at least theoretical) in describing the kinetics of these reactions, yet the difficulty is encountered when one starts to determine the concentration of ES or even S practically. The same difficulty was experienced by Michaelis and Menten, who devised an equation where these immeasurable quantities were replaced by those which could be easily measured experimentally.

Following symbols may be used for deriving Michaelis-Menten equation :

(E_t) = total concentration of enzyme

(S) = total concentration of substrate

(ES) = concentration of enzyme-substrate complex

(E_t) – (ES) = concentration of free enzyme

The rate of appearance of products (i.e., velocity, V) is proportional to the concentration of the enzyme-substrate complex.

$$V = k \neq (ES) \qquad \ldots(11)$$

The maximum reaction rate, V_m will occur at a point where the total enzyme E_t is bound to the substrate. Then the maximum concentration of ES will be equal to the total enzyme concentration, E_t.

Thus :

$$V_m = k \times E_t \qquad \ldots.(12)$$

Dividing equation (11) by (12) we get :

$$\frac{V}{V_m} = \frac{(ES)}{(E_t)} \qquad \ldots(13)$$

With the help of this equation, one can easily measure the immeasurable quantities, (ES) and (E_t), in terms of the reaction rates experimentally.

Now coming back to the reversible reaction, $E + S \rightleftharpoons ES$, one can write the equilibrium constant for dissociation of ES as K_m which is equal to :

$$K_m = \frac{(E_t) - (ES) \times (S)}{ES}$$

Or $$(ES) \times K_m = (E_t) \times (S) - (ES) \times (S) \qquad ...(14)$$

Or $$(ES) \times K_m + (ES) \times (S) = (E_t) \times (S)$$

Or $$(ES) \times [K_m + (S)] = (E_t) \times (S)$$

$$(ES)/(E_t) = \frac{(S)}{K_m + (S)} \qquad \ldots.(15)$$

Substituting the value of (ES)/E_t) from equation (13) to equation (15), we get :

$$\frac{V}{V_m} = \frac{S}{K_m + (S)}$$

Or $$V = V_m \times (S) / K_m + (S) \qquad \ldots(16)$$

$$K_m = (S)[V_m / V - 1] \quad \text{....(17)}$$

Equation (16) is called as Michaelis-Menten equation. This can be used to calculate K_m after experimentally determining the reaction rates at various substrate concentrations. This equilibrium constant, K_m, is usually called Michaelis constant. It is a measure of the affinity of an enzyme for its substrate. Referring to the equation (14), the greater the concentration of ES complex, the lower is the concentration of free enzyme and consequently the lower is the value of K_m. For experimental determination of K_m, the velocity of the reaction (relative activity of the enzyme) is measured as a function of substrate concentration. When $V = \frac{1}{2}V_m$, it will be seen from equation (16) that K_m is numerically equal to the substrate concentration or in other words K_m is equal to the concentration of the substrate which gives half the numerical maximal velocity, V_m. Thus, it is possible to determine K_m. The K_m, shown in Fig. 13.5(a), is indicated to be 0.017 M. It is noteworthy that for any enzyme-substrate system, K_m has a characteristic value which is independent of the enzyme concentration.

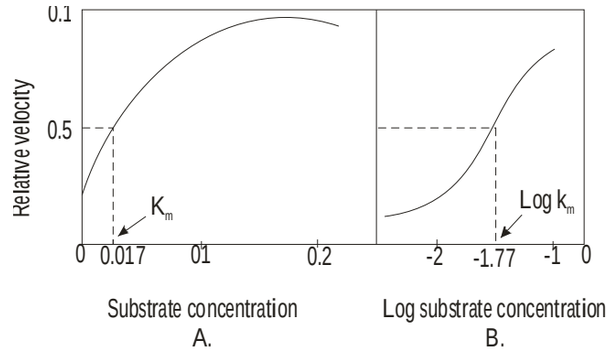


Figure 13.4(a) relative initial velocity as a function of substrate concentrating (A) and as a function of the logarithm of the substrate concentration (B) for the action of yeast invertase on sucrose.

Lineweaver-Burk Plots :

The method described above for the determination of K_m is somewhat complex and, therefore, simpler methods have been divised. Two such methods (Fig. 13.5(b)) are given below :

(i) **First method :** A convenient means of evaluating K_m and V_m is to plot kinetic data as the reciprocals of V and [S]. Such a double reciprocal plot was proposed by Hans Line weaver and Dean Burk in 1934. If one takes the reciprocal of Michaelis-Mental equation, the following equation is obtained:

$$\frac{1}{V} = \frac{K_m + (S)}{V_m \times (S)}$$

Or

$$\frac{1}{V} = \frac{K_m}{V_m} \times \frac{1}{(S)} + \frac{1}{V_m} \quad \text{...(18)}$$

This is known as Line weaves-Burk equation. This equation is of the form y= mx + b, if one considers the variables to be $\frac{1}{V}$ and $\frac{1}{(S)}$. When one plots the graph against these two variables, a straight line is obtained. The slope of this corresponds to $\frac{K_m}{V_m}$ and the $\frac{1}{V}$ intercept

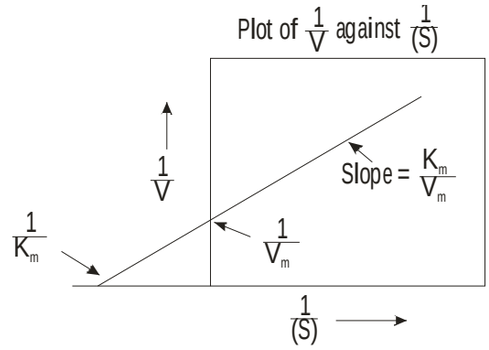
corresponds to $\frac{1}{V_m}$. Since V_m can be determined from the intercept, the K_m may also be calculated.

(ii) Second method : Another graphical method for the measurement of K_m from experimental data on V as a measure of (S) makes use of the above Line weaver-Burk equation. Multiplication of both sides of this equation by (S) gives :

$$\frac{(S)}{V} = \frac{K_m}{V_m} + \frac{(S)}{V_m} \qquad \ldots(19)$$

A plot of $\frac{(S)}{V}$ versus (S) gives a straight line. The intercept of the line on $\frac{(S)}{V}$ axis is $\frac{K_m}{V_m}$ and the slope is $\frac{1}{V_m}$.

Plot of $\frac{1}{V}$ against $\frac{1}{(S)}$



(First Method)

Plot of $\frac{(S)}{V}$ against (S)

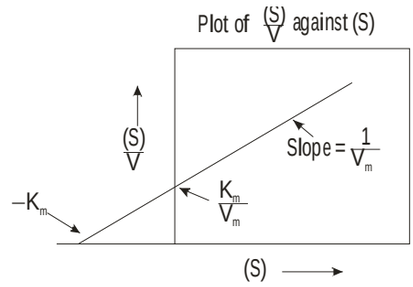


Fig. 13.5(b) Two methods of obtaining liner plots involving measurement of initial velocity, V and Substrate concentration, (S)

V_m and K_m be obtained from the intercept on the slope and the abscissa.

A Lineweaver-Burk plot provides a quick test for adherence to Michaelis-Menten Kinetic and allow easy evaluation of the critical constant.

As we shall see, it also allows discrimination between different kinds of enzyme inhibition and regulation. A disadvantage of lineweaver-Burk plot is that a long extra-polation is often required to determine K_m, with corresponding uncertainity in the result. Consequently, other ways of plotting the data are sometime used. One such alternative is to rearrange equation (16) into the form,

$$V = V_m - K_m \frac{V}{[S]} \qquad \ldots(20)$$

And graph V versus V/[S]. This yields what is called an Eadie-Hofstee plot. [Fig. 13.4(c)]

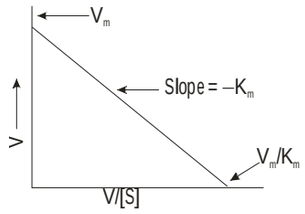


Fig. 13.4(c) An Eadie-Hofstee Plot

13.6 Enzyme Inhibitors: reversible and irreversible inhibition

Compounds which convert the enzymes into inactive substances and thus adversely affect the rate of enzymatically-catalyzed reaction are called as enzyme inhibitors. Such a process is known as enzyme inhibition. Two broad classes of enzyme inhibitors are generally recognized: reversible and irreversible, depending on whether the enzyme-inhibitor (EI) complex dissociates rapidly or very slowly.

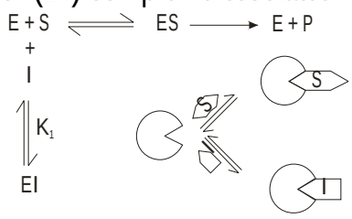


Fig. 13.6.1 : Competitive inhibition

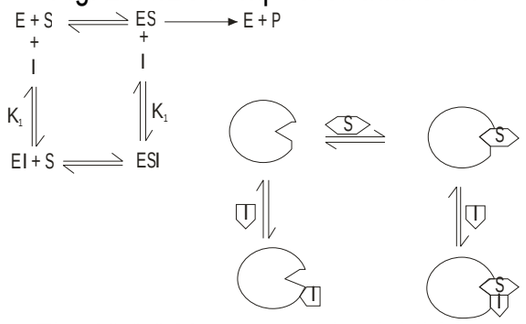


Fig. 13.6.2 Noncompetitive inhibition

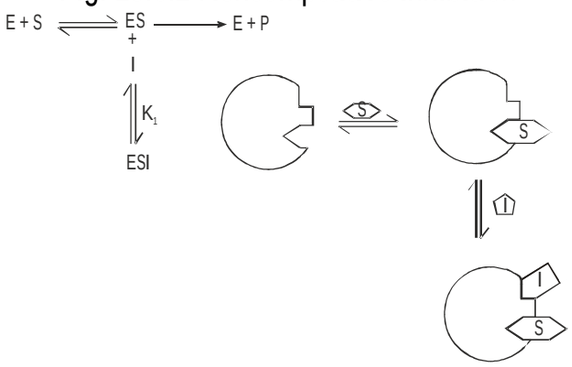


Fig. 13.6.3 Uncompetitive inhibition

1 Reversible Enzyme Inhibition :

A reversible inhibitor dissociates very rapidly from its target enzyme because it becomes very loosely bound with the enzyme. Three general types of reversible inhibition Fig. 13.6(1, 2, 3) are distinguished: competitive, noncompetitive and uncompetitive, depending on three factors:

a. whether the inhibition is or is not overcome by increasing the concentration of the substrate,

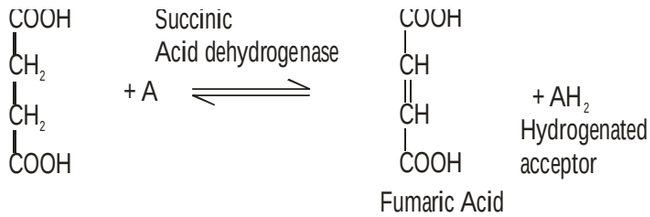
b. whether the inhibitor binds at the active site or at allosteric site, and

c. whether the inhibitor binds either with the free enzyme only, or with the enzyme substrate complex only or with either of the two.

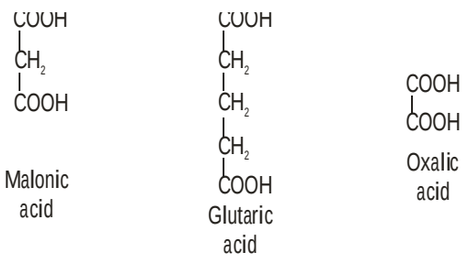
13.6.1 (A) Competitive or Substrate analogue inhibition.

This type of competition occurs at the active site. Here the structure of the inhibitor (I) closely resembles with that of the substrate (S). It may, thus, combine with the enzyme (E), forming an enzyme-inhibitor (EI) complex rather than an ES complex. The inhibitor, thus, competes with the substrate to combine with the enzyme. "The degree of inhibition depends upon the relative concentrations of the substrate and the inhibitor". Thus, by increasing the substrate concentration and keeping the inhibitor concentration constant, the amount of inhibition decreases and conversely a decrease in substrate concentration results in an increased inhibition. It may, however, be noted that in competitive inhibition, the enzyme can bind substrate (forming an ES complex) or inhibitor (EI), but not both (ESI). Thus, we see that a competitive inhibitor diminishes the rate of the reaction by reducing the proportion of the enzyme molecules bound to a substrate. Competitive inhibition can be analyzed quantitatively by steady-state kinetics. Because the inhibitor binds reversibly to the enzyme, the competition can be biased to favour the substrate only simply by adding more substrate. When more substrate is present, the probability that an inhibitor molecule will bind is minimized, and the reaction exhibits a normal V_{max}. However, the [S] at which $V_0 = 1/2\ V_{max}$, the K_m will increase in the presence of inhibitor. Some well-known examples of competitive inhibition are given below:

(i) An enzyme, succinic acid dehydrogenase (succinode- hydrogenase) catalyzes the conversion of succinic acid to fumaric acid.

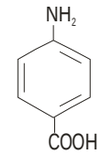


Many organic compounds, which are structurally related to succinic acid, combine with the enzyme, thus inhibiting the reaction. A few inhibitors of this reaction are : Malonic acid is most efficient of all these inhibitors. When the inhibitor : enzyme ratio is 1 :50,the enzyme is inhibited 50%. Malonic acid differs from succinic acid in having one rather than two methylene groups.

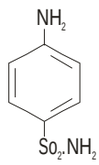


(ii) Many microorganisms, like bacteria, synthesize the vitamin folic acid from para-aminobenzoic acid (PABA). Sulfanilamide and other sulfa drugs are structural analogues of PABA. Hence, Sulfa drugs act as enzyme inhibitor and occupy the active site of some bacterial enzymes catalyzing this reaction. Failure of PABA to combine with the bacterial enzymes at the active site results in blocking off the folic acid synthesis.

The resulting deficiency of this vitamin is fatal to these microorganisms. Since man lacks the enzymes necessary for folic acid synthesis from PABA, folic acid is needed as a vitamin in the diet. Thus, the sulfa drugs inhibit growth of the bacteria in man by competing with PABA for the active centres of the bacterial enzymes.



p-amino benzoic acid
PABA
(Substrate)



p-amino benzene sulfonamide or
sulfanilamide
(inhibitor)

(iii) Competitive inhibition is used therapeutically to treat patients who have ingested methanol ($CH_3.OH$), a solvent found in gas-line antifreeze. Methanol is converted to formaldehyde by the action of the enzyme alcohol dehydrogenase (E.C. No. 1.1.1.1) . Formaldehyde damages many tissues esp., the optic ones, causing blindness. Ethanol competes effectively with methanol as a substrate for alcohol dehydrogenase. Ethanol, thus, acts as an inhibitor for the substrate methanol and competes with it to occupy the active site of the enzyme. Methanol-poisoning may, thus, be cured by an intravenous infusion of ethanol to the patients so that formaldehyde formation is considerably lowered. Most of the methanol can be extracted harmlessly in the urine.

(iv) A physiologically important example of competitive inhibition is found in the formation 2,3- bisphosphoglycerate (BPG) from 1,3-bisphosphoglycerate. Bis-phosphoglycerate mutase, the enzyme catalyzing this isomerization reaction, is completely inhibited by even low levels of 2,3-bisphosphoglycerate. In fact, it is not uncommon for an enzyme to be completely inhibited by its own product because of the product's structural resemblance to the substrate. However, increasing the concentration of substrate checks the inhibitory effect. Michaelis-Menten equation may also be applied to the competitive inhibition of enzymes. Here, besides the normal equation:

$$E + S \rightleftharpoons ES \rightarrow E + P$$

one must also consider the equilibrium state between the enzyme and the inhibitor, I as follows :

$$E + I \rightleftharpoons EI$$

In the presence of the competitive inhibitor, henceforth, the concentration of the free enzyme would be expressed as :

$$[(E) - (ES) - (EI)]$$

The dissociation of the enzyme-inhibitor compound, K_i would then be defined as :

$$K_i = [(E)- (ES)- (EI)]- (1)/ (EI)$$

Deriving the Michaelis-Menten equation for this case of inhibition, one obtains as follows :

$$V = V_m \times (S)\, K_i / K_m K_i + K_m (I) + K_i (S) \quad \ldots(21)$$

Reversal of this equation gives the modified form of Lineweaver-Burk equation :

$$\frac{I}{V} = \frac{K_m K_i + K_m(I) + K_i(S)}{V_m \times (S) K_i}$$

or

$$\frac{I}{V} = \frac{K_m}{V_m}(1 + (I)/K_i) \times 1/(S) + 1/V_m \quad \ldots(22)$$

When 1/V is plotted against 1/(S), the intercept $1/V_m$ remains the same as in the case of non-inhibited reaction but the slope, which is now $\frac{K_m}{V_m}\left(1 + \frac{(I)}{K_i}\right)$, is increased by the.

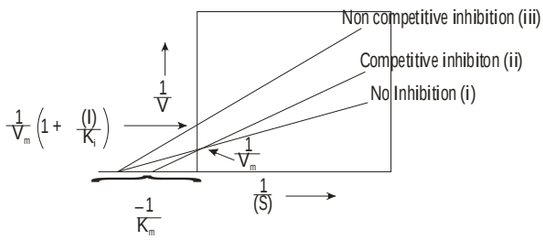


Fig. 13.6(1, 2, 3) [Double reciprocal graph (Lineweaver-Burk Plots)]

A plot of $\frac{1}{V_m}$ versus $\frac{1}{(S)}$ for an enzyme reaction with (i) no inhibitor (ii) a competitive inhibitor and (iii) a noncompetitive inhibitor.

Factor, $1 + \frac{(I)}{K_i}$, thus, if the substrate concentration is large enough, the effect of the competitive inhibitor can be overcome and V_m may be reached. The phenomenon of competitive inhibition is of practical value. It may be employed to prevent the growth of one organism in the presence of other, for example : (a) bacteria growth inhibited in the presence of an animal (b) growth of the insect inhibited on the fruit tree.

13.6.2 (B) Noncompetitive inhibition :

Here no competition occurs between the substrate, S and their inhibitor, I (Fig. 13.6.2). The inhibitor has little or no structural resemblance with the substrate and it binds with the enzyme at a place other than the active site (i.e., at the allosteric site).

Since I and S may combine at different sites, , formation of both EI and ESI complexes takes place. Both ES and ESI may break down to produce the reaction product (P). It may, however, be noted that in noncompetitive inhibition, the inhibitor and substrate can bind simultaneously to an enzyme molecule since there binding sites are different and hence do not overlap. The enzyme is inactivated when inhibitor is bound, whether or not substrate is also present. Thus, it is apparent that a noncompetitive inhibitor acts by lowering the turnover number rather than by decreasing the proportion of enzyme molecules that ate bound to the substrate. Non-competitive inhibition, in contrast to competitive inhibition, cannot be overcome by increasing substrate concentration. The inhibitor effectively lowers the concentration of active enzyme and hence lower V_{max}. There is almost no effect on K_m, however. Certain noteworthy examples of noncompetitive inhibition are as follows :

1. Various heavy metals ions (Ag^+, Hg^{2+}, Pb^{2+}) inhibit the activity of a variety of enzymes.

Urease, for example, is highly sensitive to any of these ions in traces. Heavy metals for M mercaptides with sulfhydryl (-SH) groups of enzymes :

$$E_{nz} - SH + Ag^+ \rightleftharpoons E_{nz} - S - Ag + H^{\oplus}$$

The established equilibrium inactivates enzymes that require a (-SH) for activity. Because of the reversibility of mercaptide formation, the inhibition can be relieved by removal of the heavy metal ion. In the treatment of lead poisoning, advantage is taken of the metal's affinity for (-SH) groups. Therefore, the sulfhydryl compounds are administered to interact with the metal in the circulatory system and form mercaptides, which are then excreted.

2. Similarly, cyanide and hydrogen sulfide strongly inhibit the action of iron-containing enzymes like catalase and peroxidase.

The Lineweaver-Burk equation for this type of inhibition would be :

$$\frac{1}{V} = \left[1 + \frac{(I)}{K_i}\right]\left[\frac{1}{V_m} + \left(\frac{K_m}{V_m}\right) \times \frac{1}{(S)}\right]$$

Thus, here both the slope and the intercept are altered, rather increased by the factor $1 + \frac{(I)}{K_i}$ in contrast to competitive inhibition where only the slope is changed. Moreover, the maximum velocity attained is less than that found in noninhibited case.

13.6.3 (C) Uncompetitive inhibition :

An uncompetitive inhibitor also bind at an alosteric (like the noncompetitive inhibitors) but the binding takes place only with the enzyme-substrate (ES) complex, and not the free enzyme molecule.

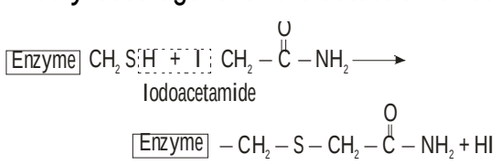
This type of inhibition is rare in one-substrate reaction, but causes a type of product-inhibition in reactions with multiple substrate and products.

2.Irreversible Enzyme Inhibition:

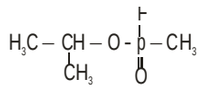
Although irreversible inhibition was once categorized and tested as noncompetitive inhibition, it

is now recognized as a distinct type of inhibition. Irreversible inhibitors are those that combine with or destroy a functional group on the enzyme that is essential for its activity. In fact, an irreversible inhibitor dissociates very slowly from its target enzyme because it becomes very tightly bound to its active site, thus inactivating the enzyme molecule. The bonding between the inhibitor and enzyme may be covalent or noncovalent. Two common examples of irreversible inhibition are discussed below:

i). Alkylating reagents, such as iodoacetamide, irreversibly inhibit the catalytic activity of some enzymes by modifying cysteine and other side chains. Iodoacetamide is a widely-used agent for the detection of sulfhydryl group.

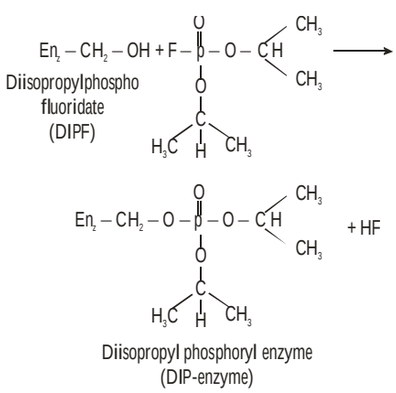


ii).Organophosphorus compounds, such as diisopropyl-phosphofluoridate, DIPF, are potent irreversible inhibitors of enzymes that have active seryl residues at their catalytic sites. DIPF is closely



sarin, a nerve gas

related chemically to nerve gas, whose lethality is due to the inactivation of *acetylcholinesterase*, an enzyme critical for the transmission of nerve impulses. Acetylcholinesterase cleaves the neurotransmitter acetylcholine, an essential step in normal functioning of nervous system.



Inactivation of an enzyme (with a critical cysteine residue) by iodoacetamide.

A special class of irreversible inhibitors are the **suicide inhibitors.** These compounds are relatively unreactive until they bind to the active site of a specific enzyme. A suicide inhibitor is designed to carry out the first few chemical steps of the normal enzyme reaction. Instead of being transformed into the normal product, however, the inhibitor is converted to a very reactive compound that combines irreversibly with the enzyme. These are also called **mechanism-based inactivators**, because they utilize the normal enzyme reaction mechanism to inactivate the enzyme.

13.7 Summary

Most enzymes have certain kinetic properties in common. When substrate is added to an enzyme, the reaction rapidly achieves a steady state in which the rate at which the ES complex forms balances the rate at which it reacts. As [S] increases, the steady-state activity of a fixed concentration of enzyme increases in a hyperbolic fashion to approach a characteristic maximum rate, V_{max}, at which essentially all the enzyme has formed a complex with substrate. The substrate concentration that results in a reaction rate equal to one-half V_{max} is the Michaelis constant K_m, which is characteristic for each enzyme acting on a given substrate.

The Michaelis-Menten equation

$$V_0=V_{max}\,(S)/K_m + (S)$$

relates initial velocity to [S] and V_{max} through the constant K_m. Michaelis-Menten kinetics is also called steady-state kinetics. K_m and V_{max} have different meanings for different enzymes. The limiting rate of an enzyme-catalyzed reaction at saturation is described by the constant k_{cat}, the turnover number. The ratio k_{cat}/K_m provides a good measure of catalytic efficiency. The Michaelis-Menten equation is also applicable to bisubstrate reactions, which occur by ternary-complex or Ping-Pong (double-displacement) pathways.

13.8 Review question:

1 Explain Fisher's lock and key model of enzyme action.

2 Write short notes on: (i) Affinity labeling (ii) Enzyme modification by side directed mutagenesis

3 Explain the Michaelis-Menten and Lineweaver-Burk plots.

4 Describe the reversible and irreversible inhibition.

13.9 Reference and suggested reading :

- Abelson JN, Simon MI (editors) : Methods in Enzymology. *Academic Press Inc., New York. 1992.*
- Annual Review : Advances in Enzymology. *Interscience Publishers, Inc., New York. 1941- Current.*
- Atkinson DE : Regulation of Enzyme Action. *Ann Rev. Biochem.* **35 :** *85-124, 1966.*
- Bell JE, Bell ET : Proteins and Enzymes. *Prentice-Hall Inc., New Jersey. 1988.*
- Bernhard S : The Structure and Functions of Enzymes. *W.A. Benzamin Inc., New York 1968.*
- Boyer PD, Lardy H, Myrbäck K (editors) : The Enzymes. *3rd ed. 16 vols. Academic Press, Inc., New York. 1970-1983.*

Unit -14

Mechanism of Enzyme Action: Transition-state theory, orientation and steric effect, acid-base catalysis, covalent catalysis. strain or distortion. Examples of some typical enzyme mechanisms for chymotrypsin. ribonuclease, lysozyme and carboxypeptidase

Structure of Unit

14.0 Objective

Enzymes are proteins that catalyze chemical reactions. In these reactions, the molecules at the beginning of the process are called substrates, and the enzyme converts them into products. Enzymes are usually very specific as to which reactions they catalyze and the substrates that are involved in these reactions. In this unit we study the enzyme mechanisms for chymotrypsin. ribonuclease, lysozyme and carboxypeptidase and chemical reaction which is catalyzed by acid –base.

14.1 Introduction

Enzymes are biocatalysts which increase the rate of biochemical reaction in a living system. Enzymes are proteins and cause the reaction to occur even under thermodynamically unfavorable conditions. Enzyme permit the body reaction's to take place rapidly and efficiently at body temperature with relatively low reagent concentration in water.

The catalytic activity of enzyme depends on integrity of their native protein conformation. It and enzyme is broken down into its components amino acid, its catalytic activity is always destroyed.

Enzyme, like other protein has high molecular weight. Some enzyme requires no chemical groups for activity other than amino acid residues. Other require an additional chemical component called a cofactor- either one or move inorganic ions such as $Fe^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, K^{+}, Ni^{2+}$ or a complex or metal-organic molecule called coenzyme (Biocytic, Coenzyme-A). A coenzyme or metal ion that is very tightly or even covalently bonded to the enzyme protein is called a prosthetic group.

The enzymatic catalysis of reaction is essential to living systems. All enzyme reaction occurs within the confines of packet on the enzyme is called the active site. The molecule that is bound in the active site and acted upon by the enzyme is called the substrate.

Enzyme is derived from the yeast and was coined by Frederick W. Kuhne. First enzyme recognized as protein was Jack bean urease crystallized in 1926 by James B. Summer of Cornell University.

Enzymes differ from catalyst in following criteria:-

(i) Mild reaction condition

(ii) Higher reaction rates

(iii) Capacity for regulation

(iv) Greater reaction specificity.

A-Simple enzyme reaction shown as following way:

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightarrow E + P$$

Here E = Enzyme

S = Substrate

ES = Transient complex of the enzyme with substrate

EP = Transient complex of the enzyme with product.

Enzyme Kinetics:

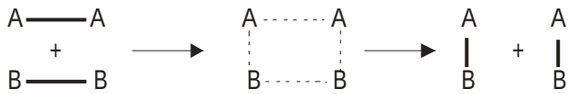
To studying the mechanism of an enzyme-catalyzed reaction is to determine the rate of the chemical reaction is known as enzyme kinetics.

14.2Transition state theory:

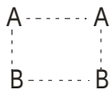
Transition state theory gives a deeper understanding of the reaction process.

Transition starter was developed by Henry Erying, Polanyi and Evans in 1935.Enzyme catalyzed chemical reaction is understood by transition state theory.

Consider a bimolecular reaction between two molecules A_2 and B_2, progresses through the formation of activated complex, which decomposes to yield the product AB.



In this reaction A_2 and B_2 diatomic molecule approach to each other, at some point in chemical reaction, there exist a high state represented as



The high free energy state is called the transition of the system. In this high energy state A-A bond and B-B bond is in the process of breaking while the A----B is in the process of forming.

Reactants generally approach one another along the path of minimum free energy, so they called reaction co-ordinate .A plot of free energy versus the reaction co-ordinate is known as transition state diagram. (Figure-1).

The reactant and product are state of minimum free energy and transition state corresponds to the highest point of the diagram.

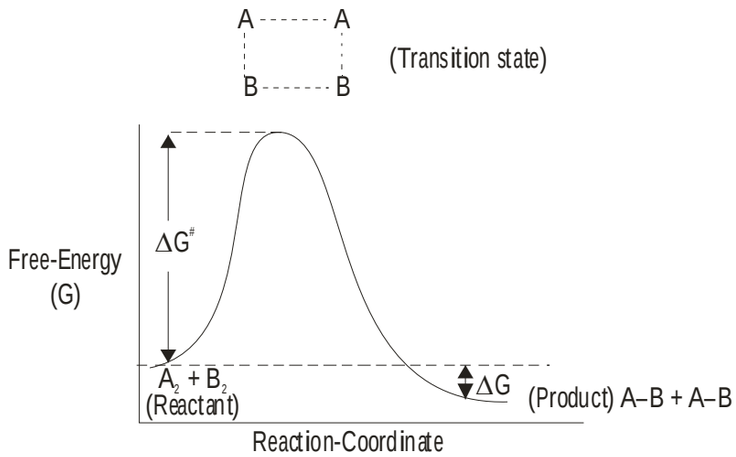


Figure-1 Transition state diagram

The free energy difference between the reactant and the transition state is free energy of the activation ($\Delta G^{\#}$) and free energy difference between the reactant and the product is overall free energy charge (ΔG).

The concentration of the transitions state reaching system is small, so the decomposition of the transition state to product is postulated to be the transition state to process of the overall reaction. The reaction rate of chemical depends on the $\Delta G^{\#}$, thus the lower the valve of $\Delta G^{\#}$, the faster the reaction rate, this is because the lower the $\Delta G^{\#}$, the larger the number of reactant molecules that have sufficient energy to achieve easily the transition state.

Transition state: The transition state is not a chemical species with any significant stability and should not be confused with a reaction intermediate. It is simply an instant molecular moment in which events such as bond breakage, bond formation, and charge development have proceeded to the precise point at which decomposition to either substrate or product is equally.

Effect of catalyst: The function of catalyst is to increase the rate of reaction. Catalyst does not affect reaction equilibrium. Catalysts lowering the transition state free energy for the reaction. The difference between the values of $\Delta G^{\#}$ for uncatalyzed reaction and catalyzed reaction ($\Delta G^{\#}$cat) indicates the efficiency of catalyst. Catalyst lowers the free energy barrier by the same amount for both the forward and backward reaction. Catalyst can accelerate the conversion of reactant to products.

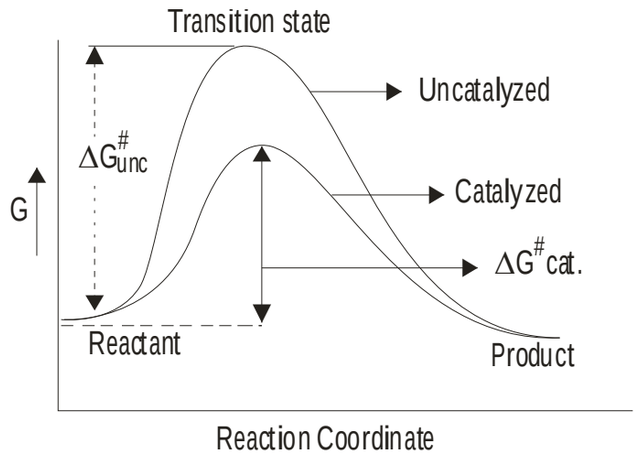


Figure 2: Effect of catalyst on the transition state diagram of reaction

The enzymatic catalysis, first proposed by Haldane (1930), was elaborated by Linvs Pauling (1946). An enzyme must be complementary to the reaction transition state. The weak interaction between Enzyme and substrate occur only in the transition state. The free energy released by the formation of these interactions partially offsets the energy required to reach the top of the energy hill. The enzyme catalyzed reaction is much faster than the uncatalyzed reaction. The weak bonding interactions between the enzyme and the substrate provide a major driving force for enzymatic catalysis. (Figure-3)

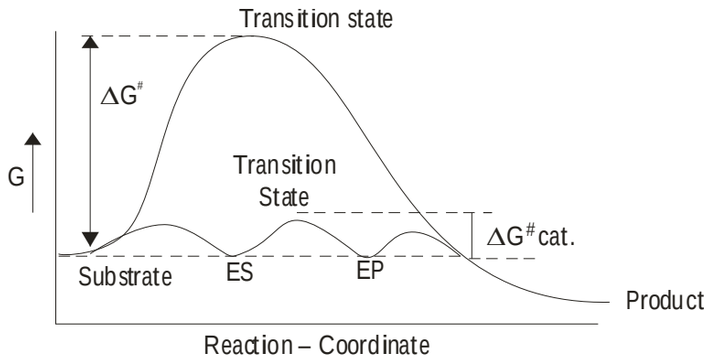


Figure 3: Role of Enzyme in Catalysis

14.3 Catalytic mechanism

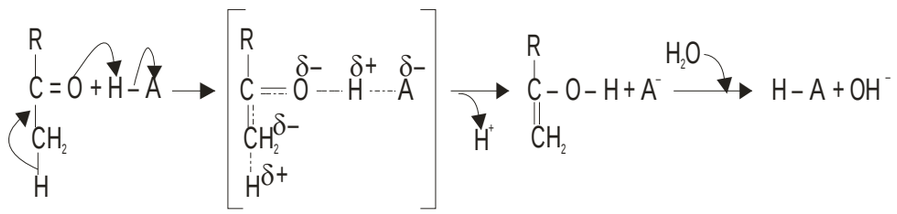
Bond cleavage and formation by a variety of catalytic mechanism that enzyme employ, including acid-base catalysis, covalent catalysis and metal catalysis.

14.3.1 Acid-base catalysis:

General acid-base catalysis is a process in which acid and base may donate and accept protons in order to stabilize developing charges in the transition state.

Acid-base catalysis are classified as

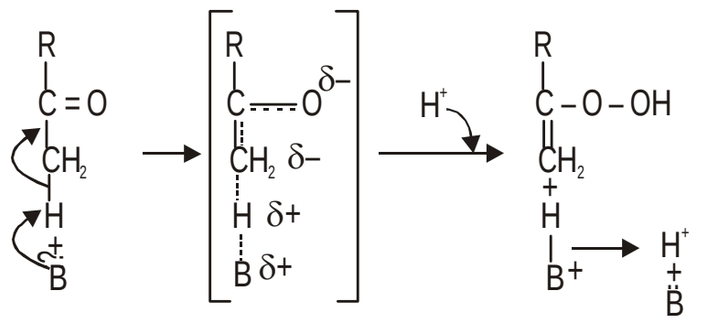
(a) General acid-catalysis: General acid catalysis is a process in which proton transfer from an acid to reactant species to lower free energy of transition state. For example acid-catalyzed keto-enol tautomerization reaction occurs fastly as result of lower free energy of its transition state. (Scheme-1)



Scheme 1: General acid-catalyzed keto-enol tautomerization

(b) General-base catalysis:

General-base catalysis is a process in which proton abstract by a base (**Scheme** -2)

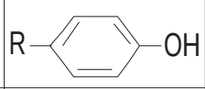
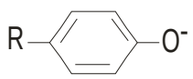
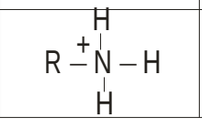


Scheme -2**: General-base catalysis keto-enol tautomerization**

A variety of weak organic acids can supplement water as proton donors or weak organic base can serve as proton acceptors.

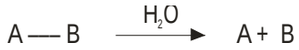
A number of amino acid side chains can act as proton donors and acceptors (Table-1). Theses group can allow the proton transfer, providing increase the rate of reaction.

Table 1: Amino-acid in general acid-base catalysis

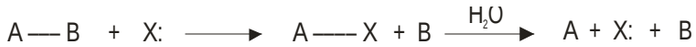
S.No.	Amino Acid residues	General acid form	General base form
1.	Ser	R–OH	$R–O^-$
2.	Tyr		
3.	Cys	R–SH	$R – S^-$
4.	Glu, Asp	R–COOH	$R–COO^-$
5.	Lys, Arg		$R–\ddot{N}H_2$

14.3.2 Covalent catalysis:

Covalent catalysis is a process in which a transient covalent bond is formed between the enzyme and the substrate. Consider the hydrolysis of a bond between groups A and B.

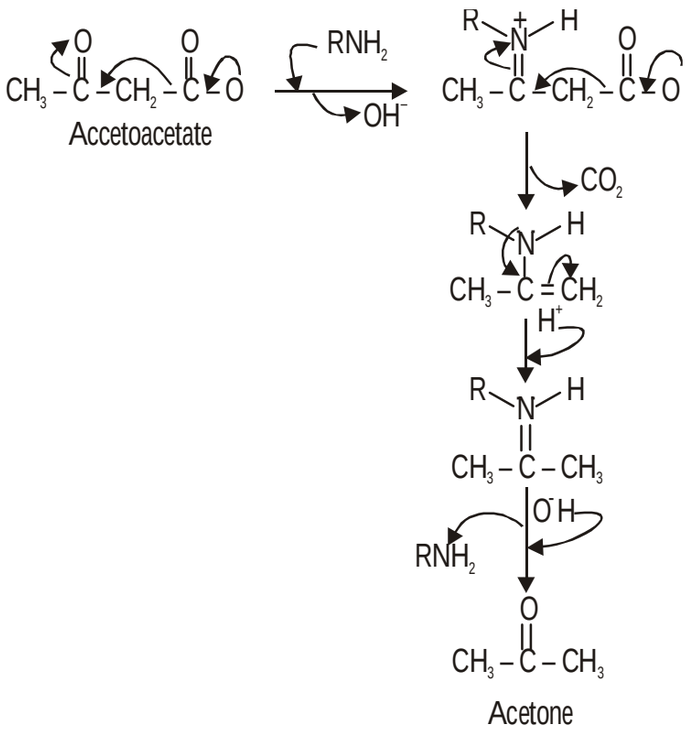


In the presence of a covalent catalyst the following reaction become faster than uncatalyzed reaction.



Covalent catalysis increases the rate of reaction through transition state formation by a covalent bond between catalyst and substrate.

Such type of catalysis is also known as nucleophilic catalysis because covalent bond is formed by the reaction of a nucleophilic group on the catalyst with an electrophilic group on the substrate. The decarboxylation of acetoacetate is an example of covalent catalysis process, which is shown as following way:-



Generally covalent catalysis can be divided into following steps:

(i) A covalent bond is form between the catalyst and the substrate.

(ii) The withdrawal of electron from reaction centre.

(iii) The elimination of the catalyst.

An important feature of covalent catalysis is that the more stable the covalent bond formed, the less easily it can converted into final steps of a reaction.

A good covalent catalyst has high nucleophilicity, the ability to form a good leaving group and with high polarizability. Such as imidazole and thiol are good covalent catalysts. Enzyme commonly employs covalent catalytic mechanism as is indicated by the large variety of covalent linked enzyme substrate reaction. The covalent bond formed between the enzyme and the substrate can active a substrate for further reaction in manner that is usually specific to the particular group.

14.4 Strain or distortion

When a substrate binds to a catalyst that is more complementary in structure of electronic characteristics to the transition state, the substrate may distort in order to optimize binding iteration. That is because the catalyst is designed to optimally bind the transition state, it necessarily is not optimal for the most stable structure of the ground state.

If the substrate distorts in order to compensate for this, we can speak of such distortion as a strain on the substrate. The strain pushes the structure of the substrate towards a form closer to the transition state, we state that the substrate has activated.

The strain raises the energy of the substrate and this can be thought of as an alternative way to diminish. Changes in the electronic structure of a substrate can also be considering activation. In order for a strain placed in a substrate to facilitate a chemical reaction that strain must be along the reaction coordinate. The strain must push the reactant towards the transition on the energy surface, either structurally or electronically. The strain must be party fully relived upon achieving the transition state. A strain put into a reactant that remains in the transition state will not having an effect of the rate of the reaction.

14.5 Example of some typical enzyme mechanism

We present the mechanism for enzymes like chymotrypsin, ribonuclease, lysozyme and carboxy peptidase. Such enzymes are the best understood or because they cover all possible classes of enzyme chemistry.

14.5.1 Chymotrypsin:

It is an endoprotolytic enzyme, i.e. and enzyme which catalyses the hydrolysis of a peptide bonds. This protease enzyme is specific for cleavage of peptide bonds adjacent to aromatic amino acid residues. Molecular weight of chymotrypsin is 25,000.

Structure: A chymotrypsin contains three polypeptide chains A, B and C respectively (figure-4). These polypeptides chains are linked by two inter-disulphide bonds and three intra- disulphide bonds.

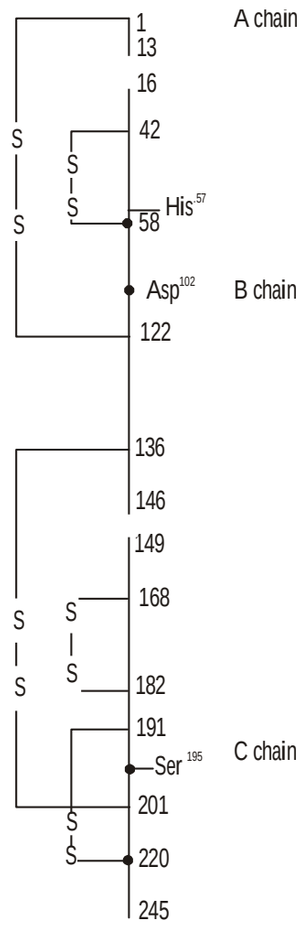


Figure-4: Structure of chymotrypsin

These two inter disulphide bonds are present in between 1 amino-acid residues to 122 amino acid residues and second are present in between 136 amino acid residues to 201 amino acid residues.

The three intra disulphide bonds are present in between (i) 42 amino acid residues to 58 amino acid residues, (ii) 168 amino acid residues to 182 amino acid residue (iii) 191 amino acid residues to 220 amino acid residues.

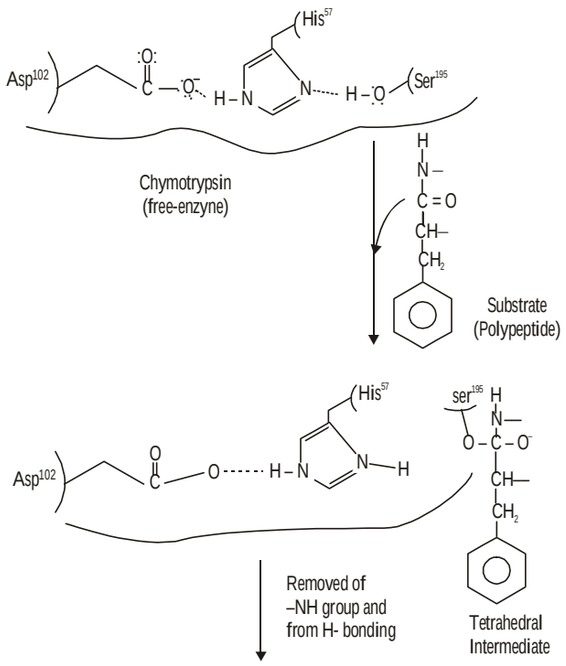
The important feature of the structure of chymotrypsin is presence of catalytic triod. It is made of His^{57}, $Asp^{102}$and ser195 amino acid.

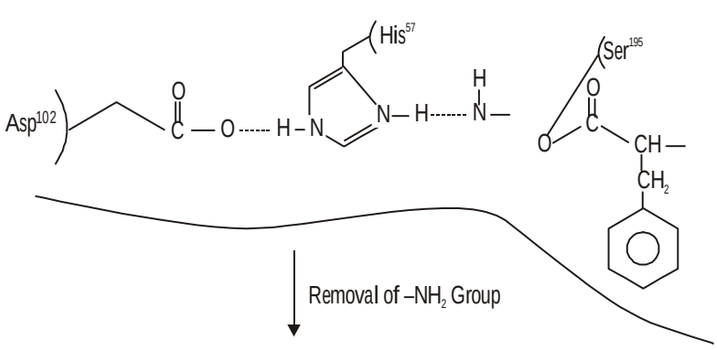
Mechanism: Chymotrypsin increases the rate of peptide bond hydrolysis. The enzyme does not catalyze a direct attack of water on the peptide bond is cleaved and an ester linkages is formed between the peptide carbonyl carbon and the enzyme.

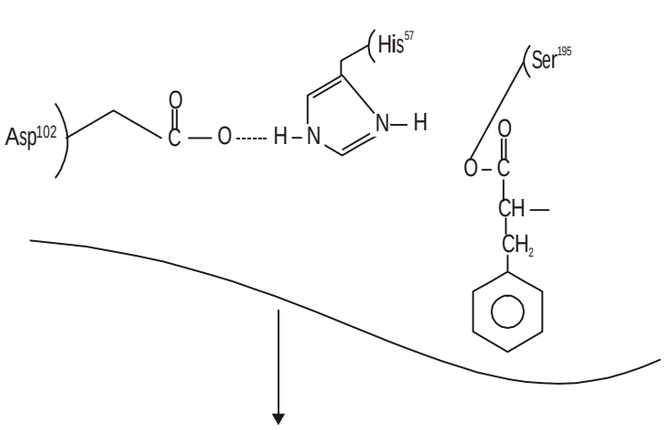
The nucleophile, -OH group of serine, attacks the >C=0 group of peptide bond to form a tetrahedral inter mediate, in which the carbonyl oxygen acquires a negative charge.

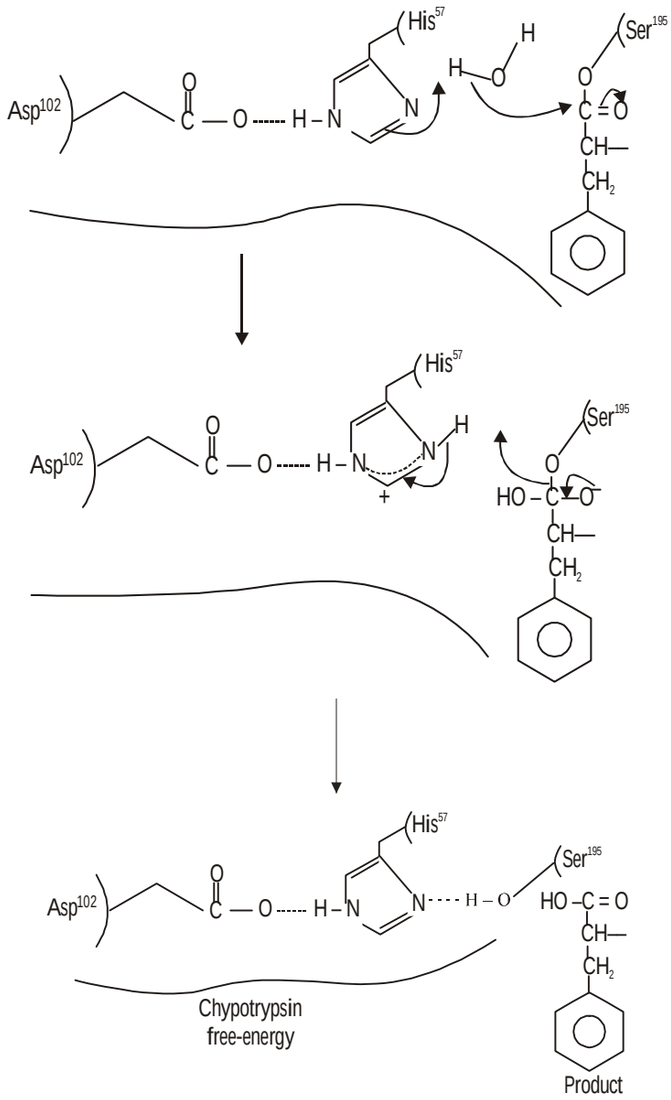
Serine is made more nucleophilic by transfer of proton from histidine which is able to accept that proton because it can transfer a proton to a carboxylate anion on a placed aspartic acid residue. The tetrahedral intermediate breaks up after transfer a proton from histidine to the amide nitrogen which has formed an amine as a leaving group. The histidine retains its proton from aspartic acid to form acylated serine residue.

The ester bond of serine groups is hydrolysis and the peptide fragments diffuse away from the active site due to this chymotrypsin enzyme regenerated and product are formed.







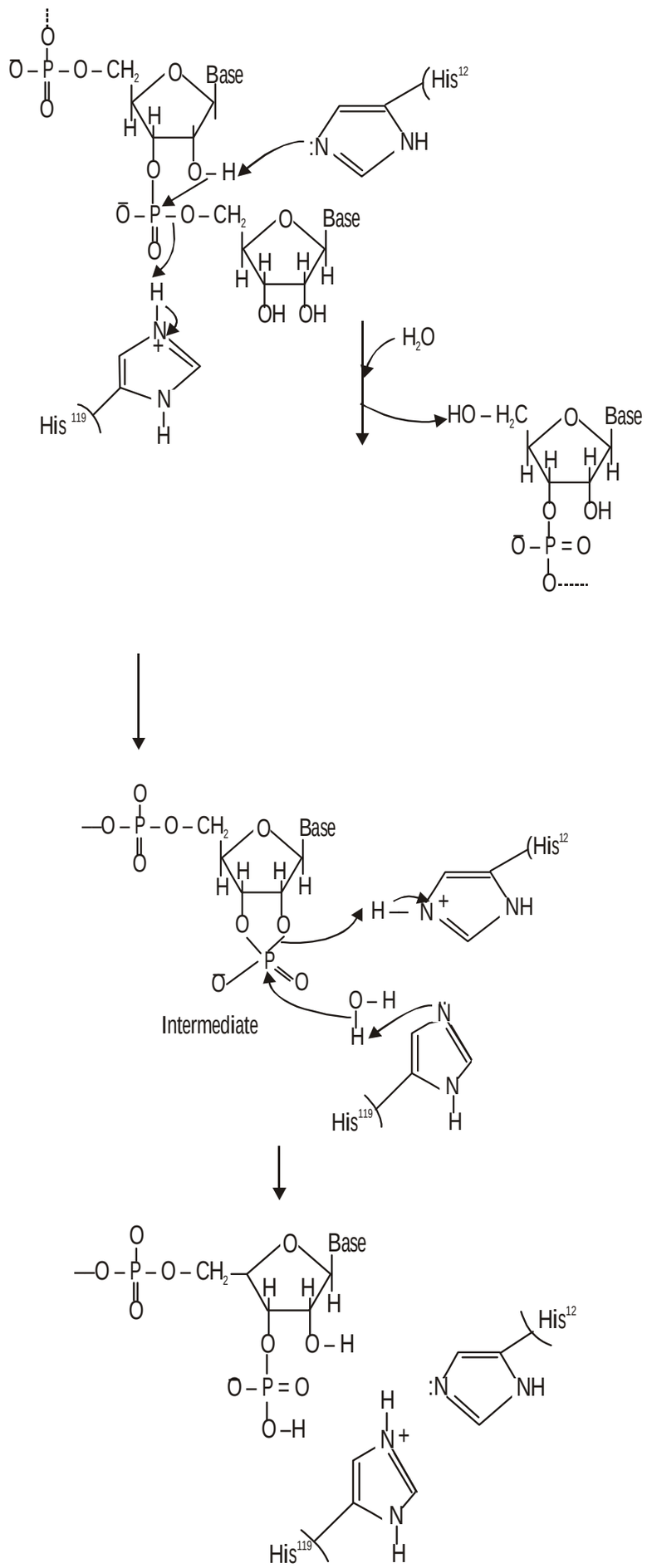


14.5.2 Ribonuclease (R Nase A)

Ribonuclease is digestive enzyme which is secreted by the pancreas in to the small intestine.

Ribonuclease provide on example of enzymatically acid-base catalysis on the basis of X-ray study, ribonuclease has two essential His residues like His12 and His119.

The mechanism of ribonuclease enzyme are illustrated below :



In this ribonuclease mechanism, His-12 acts as a general base catalyst and His-119 act as a general acid catalyst. So His-12 abstract a proton from an RNA2' –OH

group and His-119 to protonate the leaving group, His-119 to protonate the leaving group. His-119 attack of water by base catalysis.

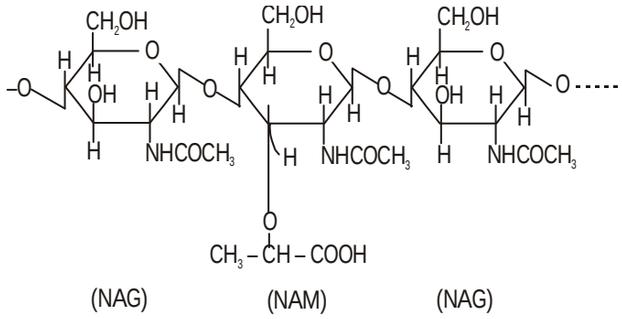
The cyclic intermediate is hydrolyzed in which water replaces the leaving group. So His-12 act as general acid and His-119 act as general base to form hydrolyzed RNA.

14.5.3 Lysozyme

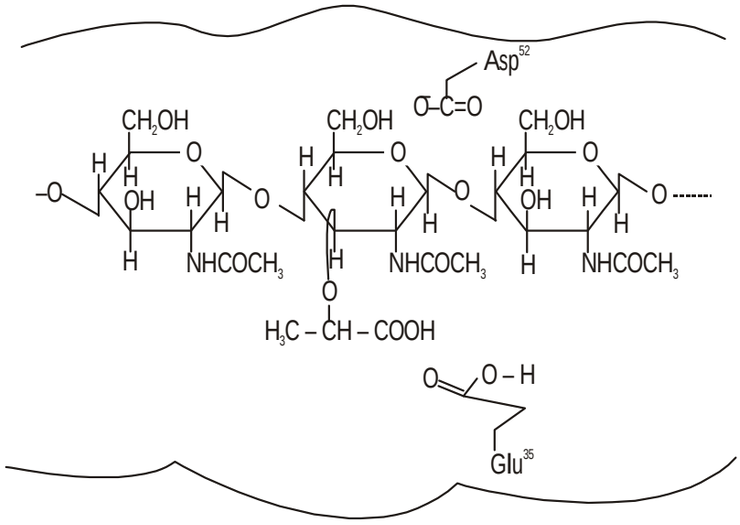
Lysozyme enzyme was discovered by A. flemming (1922) and was named as lysozyme, because it can lyse, or degrade bacterial cell wall of certain bacteria. So lysozyme is also known as a bactericidal agent.

Lysozyme is an enzyme occurs in egg white and human tears that catalyses the hydrolysis of polysaccharide in the protective cell walls of the some bacterial. The polysaccharide substrate of lysozyme is formed by □ (1-4) glycosidic linkages form N-acetylmuramic acid (NAM) to N-acetyl glucosamine (NAG) in cell wall as given as following way:

- NAG – NAM – NAG – NAM -

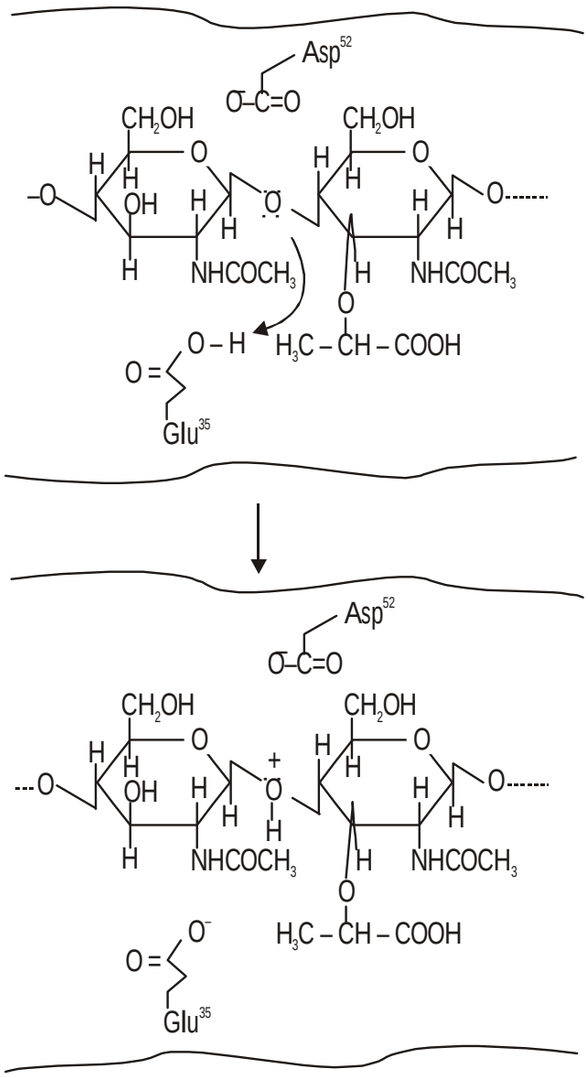


Lysozyme is a small protein whose single polypeptide chain consists of 129 amino acid residues. The active site of lysozyme has Glu 35 and Asp 52 which are two catalytic groups, shown as following way

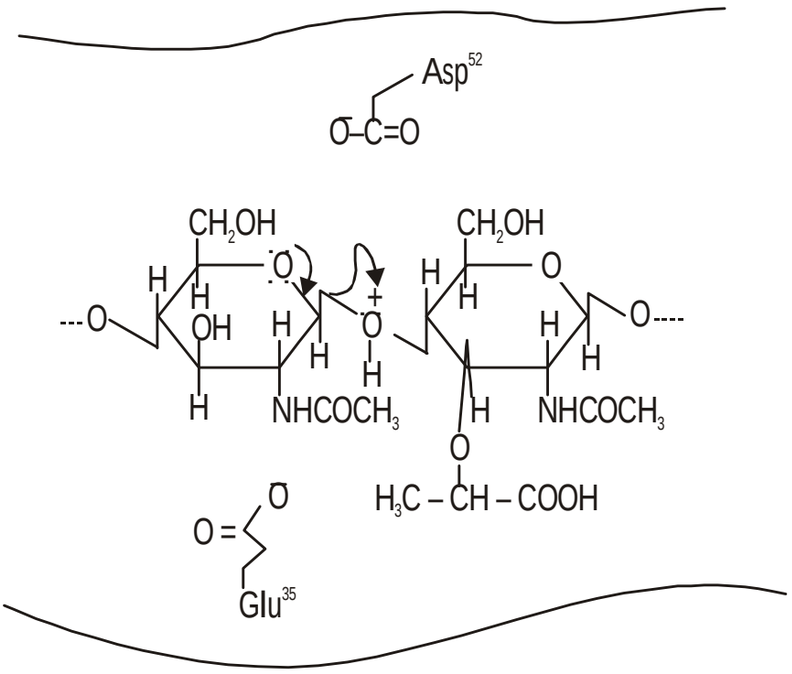


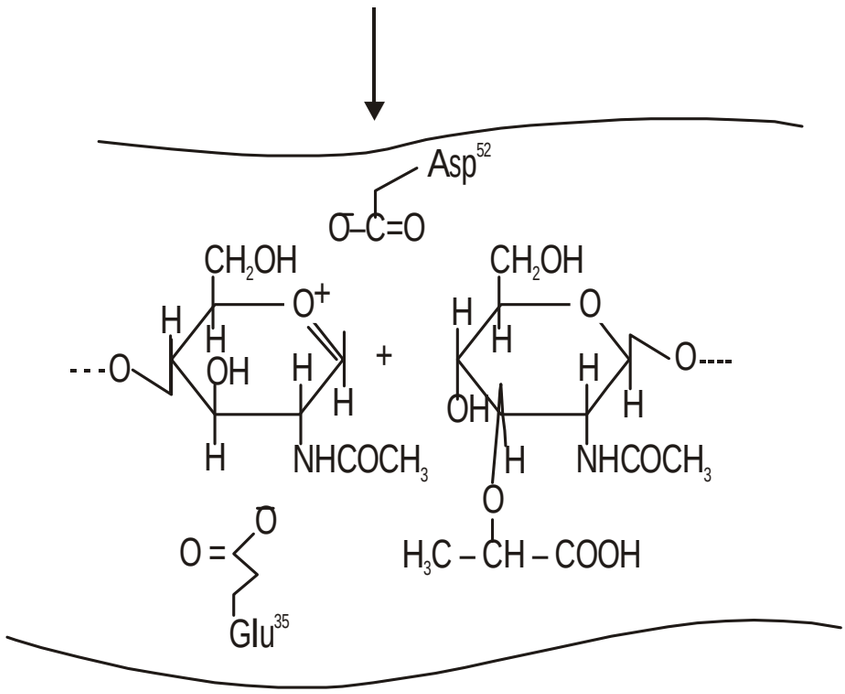
Mechanism: The enzyme lysozyme hydrolysis by following steps:-

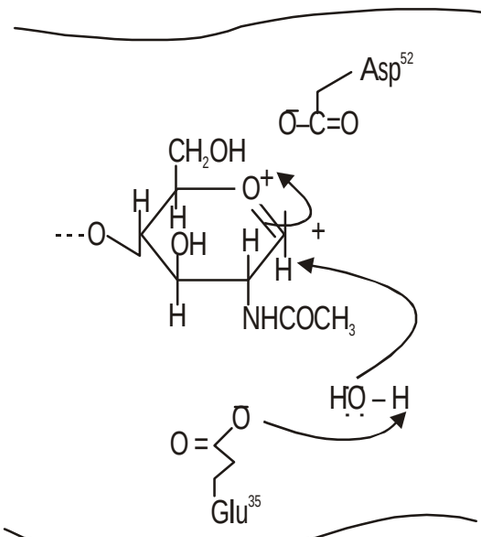
(I) Glu35 acting as a general acid catalyst which protonates the acetal oxygen atom.

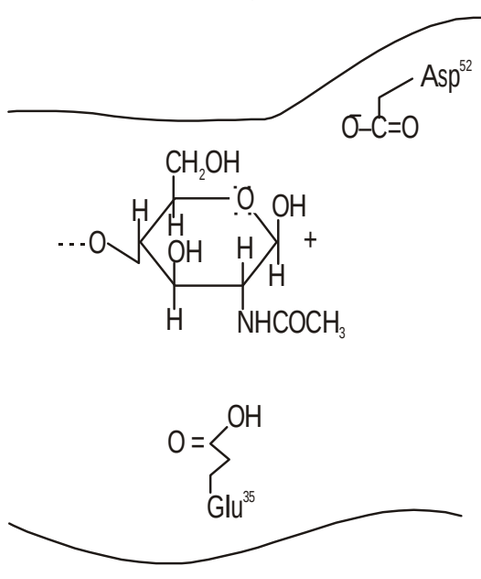


(II) A carbonation is formed by the displacement of an alcohol to right hand side of the molecule. This cation is stabilized by the oxygen atom in the carbohydrate ring and also by the residue (Asp52) present on the enzyme active site.




Asp52
O–C=O
CH2OH
O+
H
H
OH
H
H
H
NHCOCH3
---O
+
CH2OH
O
H
H
OH
H
H
NHCOCH3
O----
O
H3C – CH – COOH
O =
O
Glu35


Asp52
O–C=O
CH2OH
O+
H
H
OH
H
H
H
NHCOCH3
---O
+
HO – H
O =
O
Glu35


Asp52
O–C=O
CH2OH
O
OH
H
H
OH
H
H
H
NHCOCH3
---O
+
O =
OH
Glu35

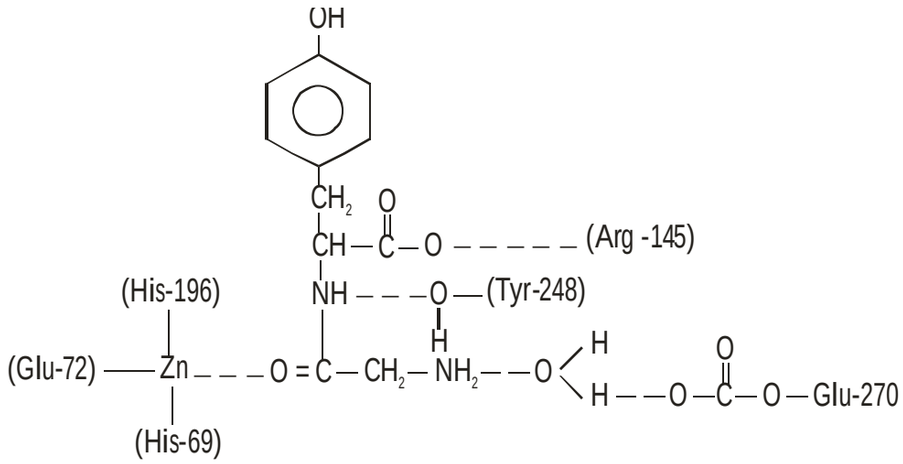
14.5.4 **Carboxy peptidases – A**

Carboxy peptidase-A is a metallo enzyme and Zn^{2+} bound to single polypeptide chain of 307 amino acids and relative molecular mass 34470. Carboxypeptidase-A synthesized and secreted by the exocrine cells of the pancreas.

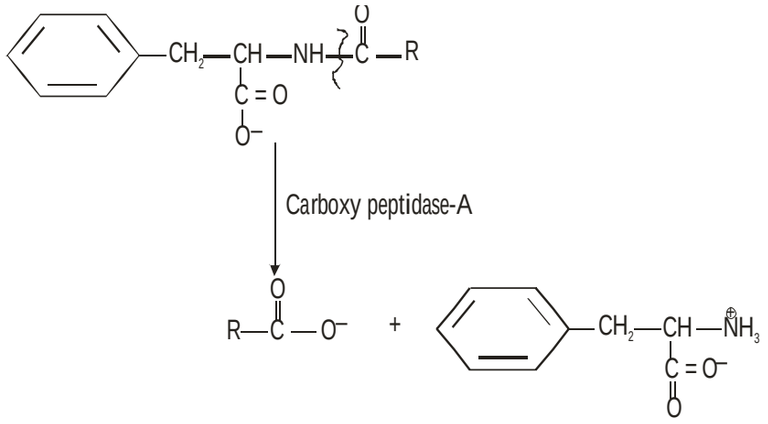
Carboxypeptidase catalysis the hydrolysis of C-terminal amino acids from polypeptide substrates and is specific for the hydrophobic amino acids.

Structure of carboxy peptidase:

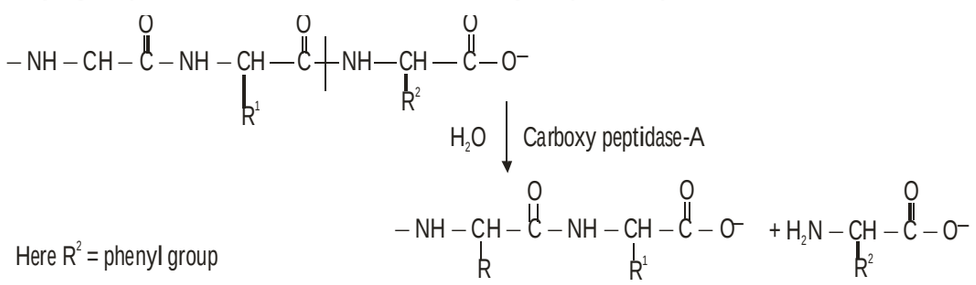
The Zn^{2+} ion is present at the active site of carboxy peptidase and Zn^{2+} is linked with Glu – 72, His-196 and His-69. A model was proposed for the structure enzyme substrate complex which is given as:



When carboxypeptidase enzyme catalyzes the hydrolysis of the C-terminal peptide bond of a protein to form C-terminal amino acid.



Carboxypeptidase-A provide the side chain of the C-terminal amino acid is a phenyl group due to this it can bend in the hydrophobic pocket in the active site.



14.6 Orientation and steric effect

Enzymes use a catalyst in organic reactions. Enzyme orient substrate and catalytic group for catalysis. Enzyme function similarly by placing catalytically functional

groups in the proper position for reaction. Enzymes are more catalytically efficient such efficiency arise from the specific physical condition at enzyme catalytic sites that promote the corresponding chemical reactions such effects are known as orientation.

Enzyme catalyzed reactions in three steps :

(i) The catalytic group of enzyme contact with substrates.

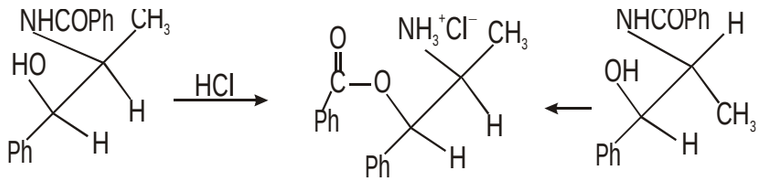
(ii) Enzyme binds their substrates in the proper orientations for reaction. Molecules react most readily if have the proper orientation.

Example: In bimolecular nucleophilic substitution reaction the incoming nucleophile attacks the opposite direction to that of the bond of the leaving group.

(iii) Enzyme freeze out the translational and rotational motions of their substrate and catalytic groups.

An enzyme may bind the transition state of the reaction it catalyzes with greater affinity than its substrates. The transition state proposed that enzymes strained their substrate towards the transition state geometry through binding sites into which undistorted substrates did not properly fit. Conformational analysis effects the reactivity and orientation on account steric effect.

Example: Rearrangement of N-benzoyl norephedrine; in this rearrangement N to O migration take place; but in the other not at all. When configuration in (I) methyl and phenyl group are anti to each other. Which is most favourable situation but in which is most favourable situation but in (II) Nitrogen gauche to oxygen; methyl and phenyl gauche to each other; which is unfavourable condition for reaction to proceeds.



14.7 Summary

- Enzymes are steroselectivity and stcrospecificity.
- Lysozyme brings about the cleavage of bacterial cell wall.
- Histidine acts as a general acid as well as a general base catalyst due to the presence of imidazole ring.
- Zinc form part of the active site of more than 100 enzymes.
- The digestive enzyme is secreted by the pancreas into small intestine
- Acid base, covalent, metal-ion and electrostatic catalysis are the types of catalytic mechanism.
- Transition state diagram is obtaine by plot versus the reaction coordinate and free energy.

14.8 Review questions

1. Explain acid-base catalyst with suitable example.
2. What is the specificity of carboxy peptidase-A?
3. How the enzyme isozyme destroy the cell wall.
4. What is proximity effect?
5. Explain transition state diagram.
6. Chymotrypsin is a serine protease which specifically hydrolyzes the peptide bond adjacent to aromatic amino acid residue. Explain

14.9 Reference and suggested readings

- Bio-organic, Bioinorganic and supramolecular chemistry :- P.S. Kalsi, J.Pl Kalsi.
- Bio-organic chemistry: A-chemical approach to enzyme action: H. Dugas, and C. penny.
- Biochemistry : Voet and Voet
- Lehinger principles of biochemistry; D.L Melson, M.M cox.

Unit-15

Types of Reactions Catalysed by Enzymes

Structure of Unit:

1.1 Objectives

In this unit the students will be able to understand about

- Enzyme catalysed reactions
- Enzymes involved in biochemical reactions
- Type of reactions catalysed by enzyme and their mechanism
- Intermediates formed during reaction
- Enzyme catalysed carboxylation and decarboxylaation

15.2 Introduction

Chemical reactions carried out in living organisms follow the same rules of reactivity as reactions carried out in the laboratory. The "solvent" is often different, the temperature is often different, and the catalyst is certainly different, but the reactions occur by the same fundamental mechanisms. That's not to say that *all* bioorganic reactions have obvious laboratory counterparts—some of the most chemically interesting biotransformations cannot be duplicated in the laboratory without an enzyme because too many side reactions would occur.

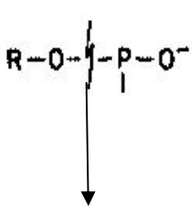
Enzymes are proteins that can accelerate rate of biochemical reactions. The functions of catalysis are specific, and they only catalyze certain reactions. Enzymes only increase the rate of the reaction but not change the reaction equilibrium. In other words, with the presence of the enzyme, the reaction proceed thousands times faster, but the amount of final product is the same as without the enzyme present.

The catalytic activity usually depends on the presence of cofactors, which are not proteins. If enzyme is not bound to cofactors then it is called apoenzyme and if it is bound to cofactors then it is called holoenzyme. Cofactors are divided into two groups: metal and coenzymes. Metal cofactors can catalyze in many ways. They can ease the formation of nucleophiles, stabilize intermediates, and link enzymes and substrates. Coenzymes are small organic molecules. They can be bound to enzymes tightly or loosely. They are called prosthetic groups if they are bound to enzyme tightly.

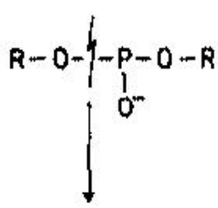
15.3 Nucleophilic displacement on phosphorus atom

The enzyme-catalyzed transfer of phosphoryl groups is ubiquitous in intermediary metabolism. Such reactions are central to the energy balance of all organisms, and are also involved in cellular control mechanisms at every level. Essentially every metabolic process that must be driven thermodynamically uphill-whether it be the maintenance of a transmembrane chemical potential, the coupling of chemical free energy to mechanical, electrical, or photochemical events, or the synthesis of a metabolite of higher free energy-involves a displacement at the phosphorus atom of a phosphoric monoester or anhydride. These reactions are coupled by the phosphokinases and ATPases to the free energy change that results from the cleavage of the phosphoric anhydride of a nucleoside triphosphate.

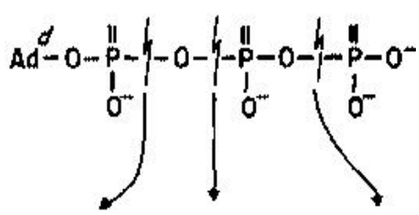
Figure given bellow are summarized the classes of enzyme that catalyze displacements at phosphorus. The enzymes that handle phosphoric monoesters' fall into three categories: the phosphatases, where water is the acceptor of the phosphoryl group (these include enzymes such as alkaline phosphatase that are merely hydrolytic, and enzymes such as the ATPases where the free energy available is coupled to some other metabolic function); the kinases, where a nucleoside triphosphate is the phosphoryl donor and some molecule other than water is the acceptor; and the mutases, for which the acceptor is another functional group on the donor molecule. The enzymes that handle phosphoric diesters are either hydrolytic (e.g. the nucleases) or nucleotidyl transfer catalysts.



Phosphokinases
Phosphatases
Nucleotidases
Phosphomutasesb



nucleases
phosphodiesterases
phopholipaaeac



nucleotldyl tronsferaaeae nucleotldyl cyclases

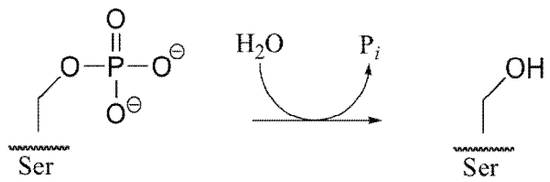
pyropphospho klnases

phosphokinases ATPases

While kinase enzymes catalyze the phosphorylation of organic compounds, enzymes called **phosphatases**. The reactions catalyzed by kinases and phosphatases are *not* the reverse of one another: kinases transfer phosphoryl groups from ATP (or sometimes other nucleoside triphosphates) to various organic

compounds, while *phosphatases transfer phosphoryl groups from organic compounds to water*, which is a hydrolysis reaction.

Look again the serine phosphatase reaction. Two very different things could be happening, given the products that result. The reaction could be a phosphoryl transfer reaction, in which a phosphorus-oxygen bond is broken. Alternatively, the water could be attacking the *carbon* of the serine side chain, breaking a carbon-oxygen bond and expelling the phosphate group in a nucleophilic substitution reaction.



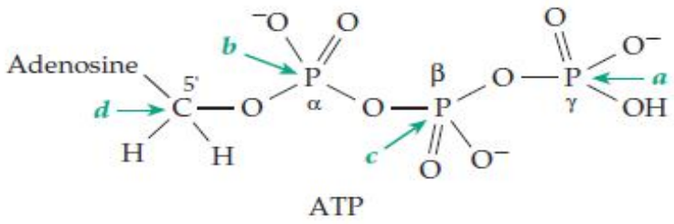
In order to find out which of these two mechanisms applies to phosphatases, scientists incubated a phosphatase enzyme with $H_2{}^{18}O$, then used mass spectrometry to determine that the ^{18}O ended up exclusively in the free phosphate. This result supports the idea that phosphatase reactions are phosphoryl transfer reactions not nucleophilic substitutions.

15.4 Multiple displacement reactions and the coupling of ATP cleavage to endergonic processess

A combination of successive displacement reactions of two types is required in many enzymatic reactions, including most of those by which the cleavage of ATP is coupled to biosynthesis. To harness the group transfer potential of ATP to drive an endergonic metabolic process there must be a mechanism of coupling. Otherwise, hydrolysis of ATP within a cell would simply generate heat. *An essential part of the coupling mechanism usually consists of a nucleophilic displacement on phosphorus followed by displacement on carbon.* Likewise, the synthesis of ATP and related compounds often begins with a displacement on carbon followed by one on phosphorus.

1. Transfer of Phospho, Pyrophospho, and Adenyl Groups from ATP

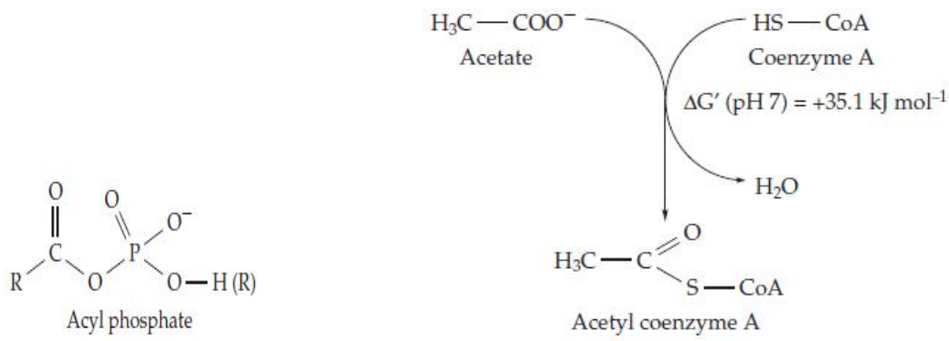
The first step in coupling ATP cleavage to any process is transfer of part of the ATP molecule to a nucleophile Y, usually by displacement on one of the three phosphorus atoms. The nucleophilic attack may be (*a*) on the terminal phosphorus with displacement of ADP or (*b*) on the internal phosphorus with displacement of inorganic pyrophosphate. In the first case, Y – PO_3H is formed; in the latter, Y-adenyl is formed. More rarely, displacement occurs (*c*) on the central phosphorus with transfer of a pyrophospho group to the nucleophile. Still less frequent (*d*) is a displacement on C-5'. If the nucleophile Y in any of these displacement reactions is H_2O, the resulting hydrolysis tends to go to completion, i.e., the phospho, adenylyl, and pyrophospho groups of ATP all have high group transfer potentials. If Y is an – OH group in an ordinary alcohol the transfer reaction also tends to go to completion because the group transfer potential of a simple phosphate ester is relatively low. Consequently, phosphorylation by ATP is often used as a means of introducing an essentially irreversible step in a metabolic pathway.



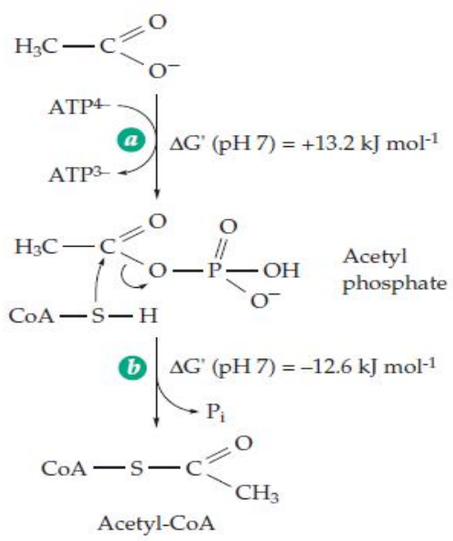
ATP

2. Acyl Phosphates

Transfer of a phospho or adenylyl group from ATP to the oxygen atom of a carboxylate group yields an acyl phosphate, a type of metabolic intermediate of special significance. Acyl phosphates are mixed anhydrides of carboxylic and phosphoric acids in which *both the acyl group and the phospho group have high group transfer potentials.* As a consequence, acyl phosphates can serve as metabolic intermediates through which the group transfer potential of ATP is transferred into other molecules and is harnessed to do chemical work. A typical example is the synthesis of acetyl coenzyme A. The complete structure of – SH group-containing coenzyme A is shown in fig.



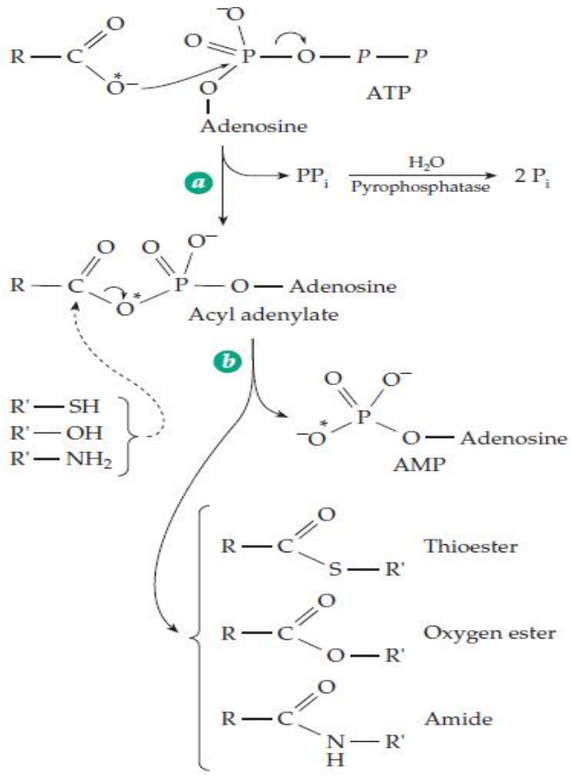
Coupling of the two reactions is accomplished by first letting an oxygen atom of the nucleophilic carboxylate group attack on P of ATP to form acetyl phosphate. In the second step (step *b*) the sulfur atom of the – SH group of coenzyme A (often abbreviated CoA– SH) attacks the carbon atom of the acetyl phosphate with displacement of the good leaving group Pi. While *G'* for step *a*, is moderately positive (meaning that a relatively low concentration of acetyl phosphate will accumulate unless the [ATP] / [ADP] ratio is high), the equilibrium in step *b*, favors the products.



The two reactions are catalyzed by acetate kinase and an *S*-acetyltransferase, respectively. Most eukaryotic cells make acetyl-CoA from acetate by coupling the synthesis to cleavage of ATP to AMP and Pi. A single enzyme acetyl-CoA synthetase (acetate thiokinase) catalyzes both steps in the reaction.

3. General Mechanism of Formation of Thioesters, Esters, and Amides

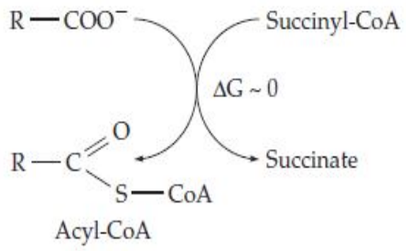
The sequences given bellow are general ones used by cells for linking carboxylic acids to – OH, – SH, and – NH2 groups to form oxygen esters, thioesters, or amides, respectively. ATP can be cleaved at either any one of two P.



An enzyme catalyzing a reaction similar to that of acetyl-CoA synthetase is succinyl-CoA synthetase. The first step is formation of a phosphoenzyme by transfer of the phospho group from ATP to N of histidine.The phospho group is then transferred to succinate to form succinyl phosphate, which reacts with coenzyme A, as in step *b*.

4. Coenzyme A Transferases

The following problem in energy transfer arises occasionally: A thioester, such as succinyl-CoA, is available to a cell and the energy available in its unstable linkage is needed for synthesis of a different thioester. It would be possible for a cell to first form ATP or GTP, using a synthetase reaction in reverse; then the ATP or GTP formed could be used to make the new linkage by the action of another acyl-CoA synthetase. However, special enzymes, the CoA transferases, function more directly. The mechanism is not obvious.

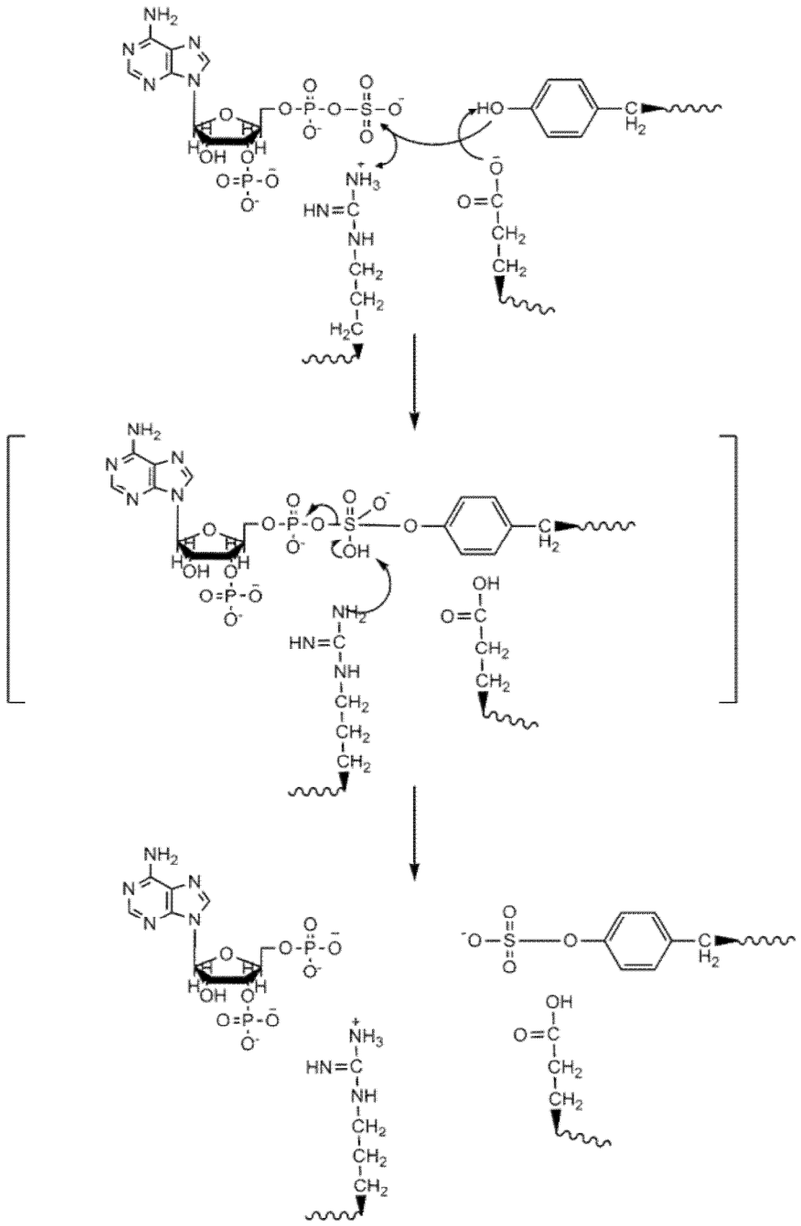


The transferred of CoA from one acyl group to another (while still retaining the high group transfer potential of the acyl group) can be explained by kinetic studies of succinyl-CoA–acetoacetate CoA transferase. The enzyme alternates between two distinct forms, one of which has been shown to contain bound CoA

15.5 Transfer of sulphate

Addition of sulfate to another molecule is called sulfation. It often refers to a phase II enzyme reaction. This biotransformation process uses its cosubstrate 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to transfer sulfate to a xenobiotic. Another example of a biological sulfation reaction is in the creation of sulfated glycosaminoglycans. Here, the sulfate group is being added either via oxygen (O-sulfation) or nitrogen (N-sulfation). Nucleophilic displacements on sulfur or on selenium atoms are steps in a variety of enzymatic reactions.

Sulfation is catalyzed by tyrosylprotein sulfotransferase (TPST) in Golgi apparatus. The reaction catalyzed by TPST is a transfer of sulfate from the universal sulfate donor 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to the side-chain hydroxyl group of a tyrosine residue. Based on crystal structure of TPST-2 with C4 complement and PAP, an SN2-like in-line displacement mechanism has been proposed. In this mechanism, both PAPS and the target tyrosine bind to the same active site in the enzyme and are orientated in a way such that a glutamic acid residue acts as a catalytic base on the tyrosine hydroxyl group, an arginine residue acts as a catalytic acid, and serine and lysine residues are used to stabilize the SN2-like intermediate. The deprotonated hydroxyl would attack the sulfonate group, and then displace the phosphate group and PAP would be released, along with the sulfotyrosine residue.

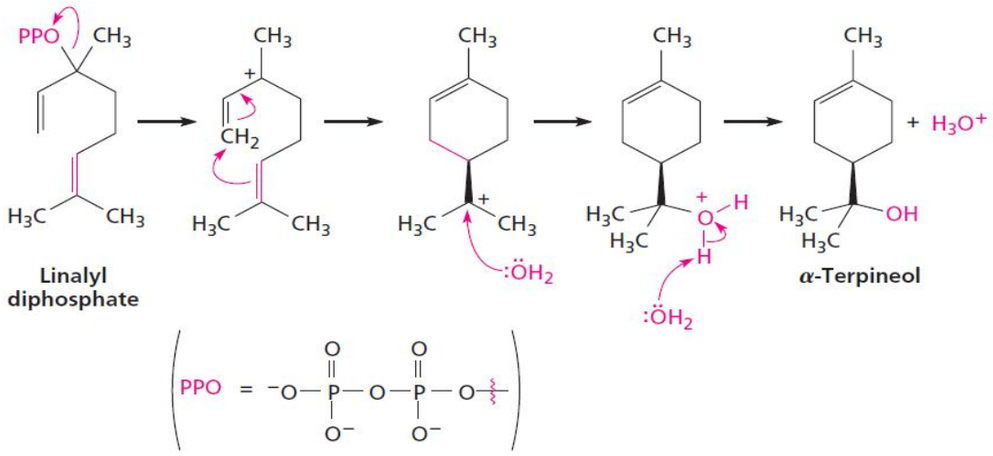


15.6 Addition and elimination reactions

i) Addition Reactions

An **electrophilic addition reaction** is initiated by addition of an electrophile to an unsaturated (electron-rich) partner, usually an alkene, and leads to formation of a saturated product.

Biological examples of electrophilic addition reactions occur frequently in the biosynthetic routes leading to steroids and other terpenoids, although they are less common elsewhere. The electrophile in such reactions is a positively charged or positively polarized carbon atom, which often adds to a CAC bond within the same molecule. As an example, a-terpineol, a substance found in pine oil and used in perfumery, is derived biosynthetically from linalyl diphosphate by an internal electrophilic addition reaction. Following formation of an allylic carbocation by dissociation of the diphosphate (here abbreviated PPO), electrophilic addition to the nucleophilic CAC bond at the other end of the molecule occurs, giving a second carbocation that then reacts with nucleophilic water. A proton transfer from the protonated alcohol to water yields a-terpineol.



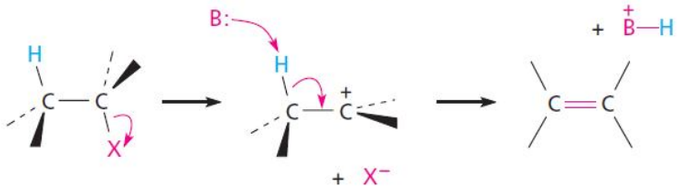
The biosynthesis of α-terpineol from linalyl diphosphate

ii) Elimination Reactions

The elimination of HX to yield an alkene appears to be the simple reverse of an electrophilic addition of HX. In fact, though, **elimination reactions** are a good deal more complex than additions and can occur by any of several mechanisms. In the laboratory, the three most common processes are the E1, E2, and E1cB reactions, which differ in the timing of COH and COX bond-breaking. In the E1 reaction, the COX bond breaks first to give a carbocation intermediate,

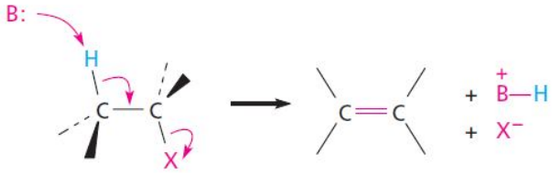
which then undergoes base abstraction of H1 to yield the alkene (the exact reverse of the electrophilic addition reaction). In the E2 reaction, base-induced COH bond cleavage is simultaneous with COX bond cleavage, giving the alkene in a single step. In the E1cB reaction (cB for "conjugate base"), base abstraction of the proton occurs first, giving a carbanion intermediate that undergoes loss of X2 in a subsequent step to give the alkene.

E1 Reaction: C–X bond breaks first to give a carbocation intermediate, followed by base removal of a proton to yield the alkene.

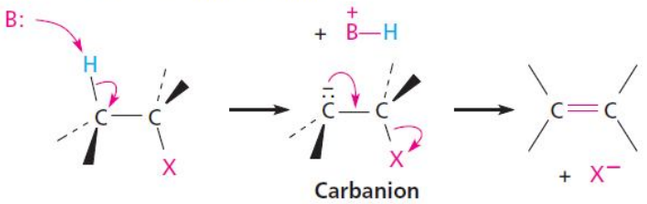


Carbocation

E2 Reaction: C–H and C–X bonds break simultaneously, giving the alkene in a single step without intermediates.

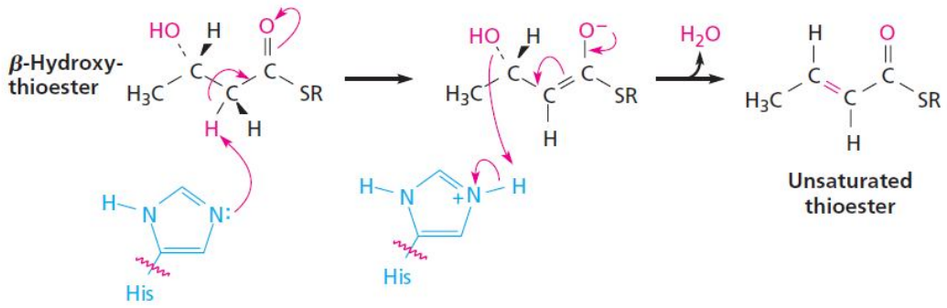


E1cB Reaction: C–H bond breaks first, giving a carbanion intermediate that loses X^- to form the alkene.



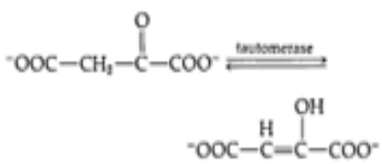
Carbanion

Examples of all three mechanisms occur in different biological pathways, but the E1cB mechanism is particularly common. The substrate is usually an alcohol (X 5 OH) or protonated alcohol (X 5 OH21), and the H atom that is removed is usually made acidic, particularly in E1cB reactions, by being adjacent to a carbonyl group. Thus, b-hydroxy carbonyl compounds (aldol reaction products) are frequently converted to a,b-unsaturated carbonyl compounds by elimination reactions. An example is the dehydration of a b-hydroxy thioester to the corresponding unsaturated thioester, a reaction that occurs in fatty-acid biosynthesis. The base in this reaction is a histidine residue in the enzyme, and the elimination is assisted by complexation of the OOH group to the protonated histidine as a Lewis acid. Note that the reaction occurs with *syn* stereochemistry, meaning that the OH and OOH groups in this example are eliminated from the same side of the molecule.



15.7 Enolic intermediates in isomerization reactions

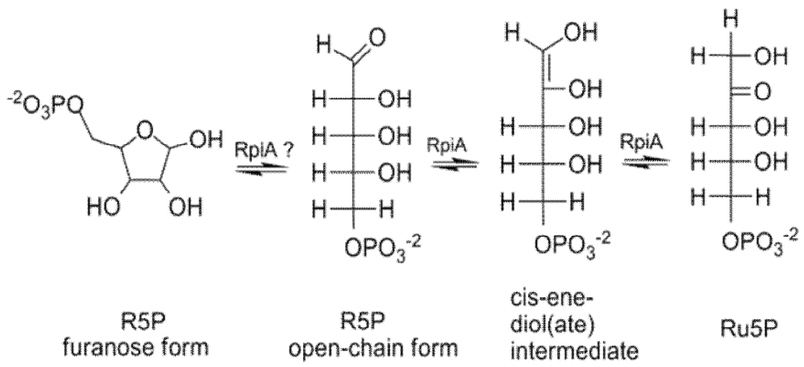
Enols (also known as alkenols) are alkenes with a hydroxyl group affixed to one of the carbon atoms composing the double bond. Enolate anions formed from carbonyl compounds play a central part in metabolism. They are intermediates in isomerization process and instrumental in creating reactive nucleophilic centers on carbon atoms. A group of tautomerases catalysze the keto-enol transformation itself. The activity of widely distributed oxoenolate keto-enol tautomeraase is especially high in animal tissues.



Ribose-5-phosphate isomerase (Rpi) is an enzyme that catalyzes the conversion between ribose-5-phosphate (R5P) and ribulose-5-phosphate (Ru5P). It is a member or a larger class of isomerases which catalyze the interconversion of chemical isomers (in this case structural isomers of pentose). It plays a vital role in biochemical metabolism in both the pentose phosphate pathway and the Calvin cycle. The systematic name of this enzyme class is D-ribose-5-phosphate aldose-ketose-isomerase.

In the reaction, the overall consequence is the movement of a carbonyl group from carbon number 1 to carbon number 2; this is achieved by the reaction going through an enediol intermediate. Through site-directed mutagenesis, Asp87 of

spinach RpiA was suggested to play the role of a general base in the interconversion of R5P to Ru5P.[5]



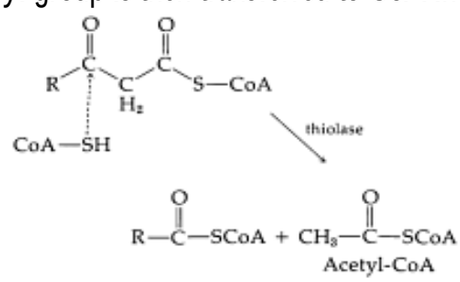
The first step in the catalysis is the docking of the pentose into the active site in the enzymatic cleft, followed by allosteric closing of the cleft. The enzyme is capable of bonding with the open-chain or ring form of the sugar-phosphate. If it does bind the furanose ring, it next opens the ring. Then the enzyme forms the eneldiol which is stabilized by an lysine or arginine residue. Calculations have demonstrated that this stabilization is the most significant contributor to the overall catalytic activity of this isomerase and a number of other like it.

15.8 β-Cleavage and condensation

In metabolic reactions carbonyl group facilitates cleavage of an adjacent C-H bond , so it can also assist the cleavage of a C-C bond. The best known reactions of this type are the aldol cleavage and the decarboxylation of beta-keto acids. The latter has been referred to as β decarboxylation. The term will be extended to include other reactions by which bonds between the α and β carbon atoms of a carbonyl compound are broken or formed, and these will be referred to as β cleavage and β condensation. The β condensation reactions consist of displacement or addition reactions in which an enzyme bound enolate anion acts as the nucleophile. We can group these condensation reactions into three categories.

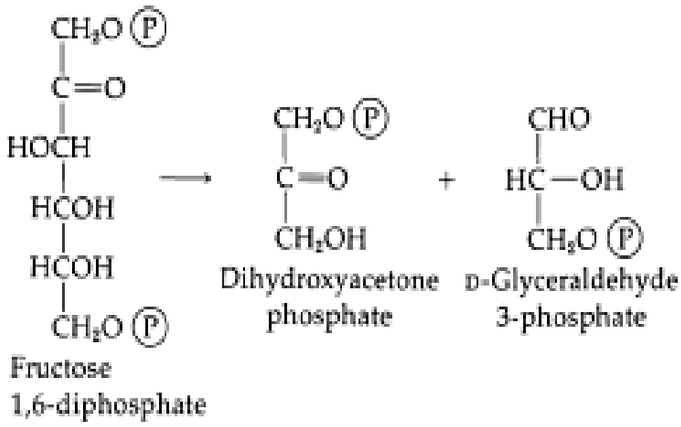
i) Displacement on carbonyl group:

A beta keto acid, with proper catalysis, is susceptible to hydrolysis by attack of water on the carbonyl group. The thiolase enzyme cleave β ketoacyl derivatives of Co A molecule. This is the chain cleavage step in the β oxidation sequence by which fatty acid chains are degraded. Thiol group in enzyme reacts initially with the β-carbonyl group to give an enzyme boun S-acyl intermediate. The acyl group is then transferred to CoA in a second step.



ii) Addition of an enolate anion to a carbonyl group or an imine

The aldol condensation is one of the commonest reactions by which C-C bonds are formed and cleaved in metabolism. The best known aldolase cleaves fructose diphosphate during glycolysis. The enzyme is found in all living tissues.an important enzyme in the pentose phosphate pathways of metabolism catalyzes aldol cleavage of one substrate, such as fructose 1,6-diphasphate, but instead of releasing free dihydroxyacetone phosphate transfers this 3-carbon unit to another aldose.

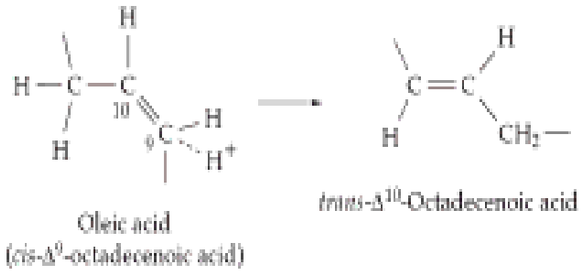


iii) Addition of an enolate ion to carbon dioxide

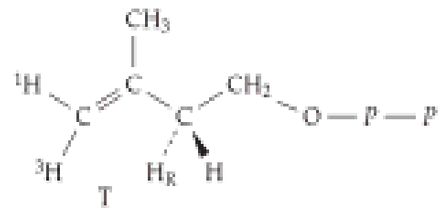
The addition of an enolate anion to CO_2 to form a beta keto acid represents one of the commonest means of incorporation of CO_2 into organic compounds. The reverse, decarboxylation, is a major mechanism of biochemical formation of CO_2.

15.9 Some isomerization and rearrangement reactions

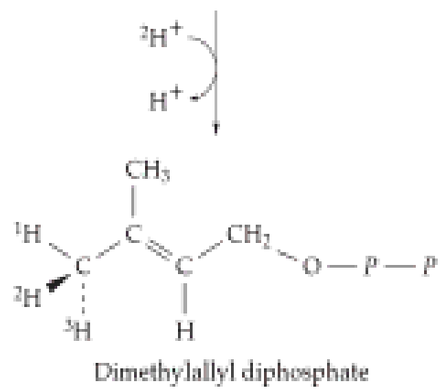
A few metabolic reactions do not fit into any of the categories discussed and do not depend upon coenzyme. Some of these involve transfer of alkyl groups or of hydrogen atoms from one carbon to another. The hydrogen atoms move by direct transfer without exchange with the medium. Oleac acid isomerizes to form trans octadecanoic acid catalysed by a soluble enzyme.



The second example is isomerization of isopentenyl diphosphate to dimethylallyl diphosphate. As shown in figure, the pro-R proton is lost from C-2 and a proton is added to the reface at C-4. When the reaction was carried out in H_2O a chiral methyl group was produced. A concerted proton addition and abstraction is also possible, the observed trnse stereochemistry being expected for such mechanism.

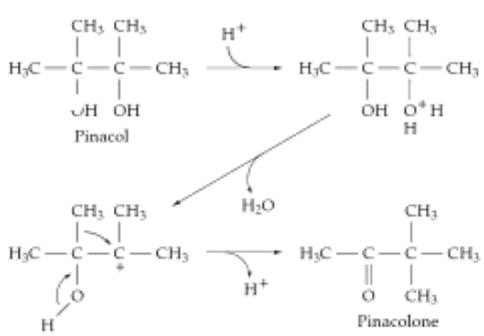


Isopentenyl diphosphate



Dimethylallyl diphosphate

Another reaction is biosynthesis of leucine and valine. The rearrangement is often compared with the nonenzymatic acid-catalyzed pinacol-pinacolone rearrangement in which a similar shift of an alkyl group takes place.



Pinacol

Pinacolone

15.10 Carboxylation and decarboxylation reactions

i) The metabolic context of carboxylation and decarboxylation:

Some of the most important carbon-carbon bond-forming and bond-breaking processes in biological chemistry involve the gain or loss, by an organic molecule, of a single carbon in the form of CO_2. You undoubtedly have seen this chemical equation before in an introductory biology or chemistry class:

$$6CO_2 + 6H_2O + \text{energy} \longrightarrow C_6H_{12}O_6 + 6O_2$$

This of course represents the photosynthetic process, by which plants (and some bacteria) harness energy from sunlight to build glucose from individual carbon dioxide molecules. The key chemical step in which carbon dioxide is 'fixed' (in other words, condensed with an existing organic molecule) is called a **carboxylation** reaction. It is catalyzed by the enzyme ribulose 1,5-bisphosphate carboxylase, commonly known as Rubisco, in the 'Calvin cycle' of carbon fixation.

The reverse chemical equation is also probably familiar :

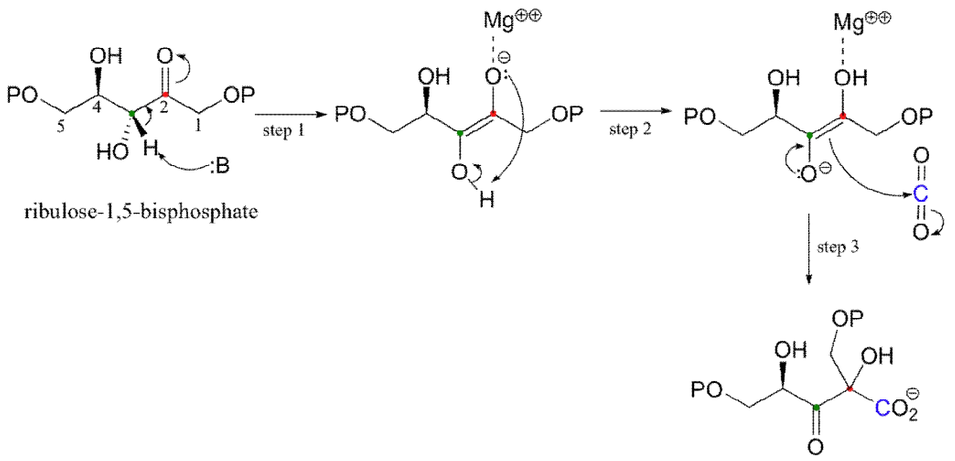
$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{energy}$$

This equation expresses what happens in respiration: the oxidative breakdown of glucose to form carbon dioxide, water, and energy. In the course of this

transformation, each of the carbon atoms of glucose is eventually converted to individual CO_2 molecules. The actual chemical step by which a carbon atom, in the form of carbon dioxide, breaks off from a larger organic molecule is called a **decarboxylation** reaction. The key decarboxylation steps in the conversion of glucose to carbon dioxide occur in the citric acid (Krebs) cycle and the pentose phosphate pathway. Let's now look at the organic mechanisms of some carboxylation and decarboxylation reactions.

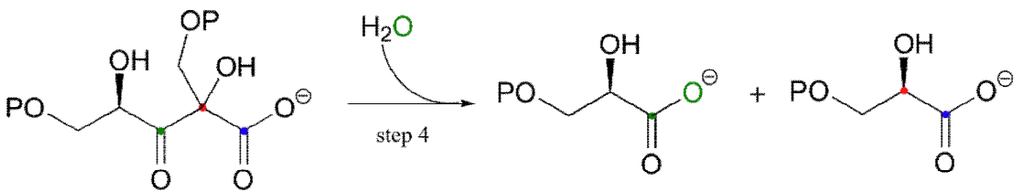
ii) The carboxylation mechanism of Rubisco, the carbon fixing enzyme

Carboxylation reactions are essentially just aldol condensations, except that the carbonyl electrophile is CO_2 rather than a ketone or aldehyde. The mechanism for Rubisco, the key carbon-fixing enzyme in plants and photosynthetic bacteria (and the most abundant enzyme on earth!), is shown below. Magnesium ion plays a key charge-stabilizing role throughout the reaction. Step 1, not surprisingly, is deprotonation of an alpha-carbon to form an enolate.



Step 2 is simply an intramolecular proton transfer, which has the effect of creating a different enolate intermediate and making C_2 into the nucleophile for an aldol-like attack on CO_2 (step 3). Carbon dioxide has now been 'fixed' into organic form - it has become a carboxylate group on a six-carbon sugar derivative.

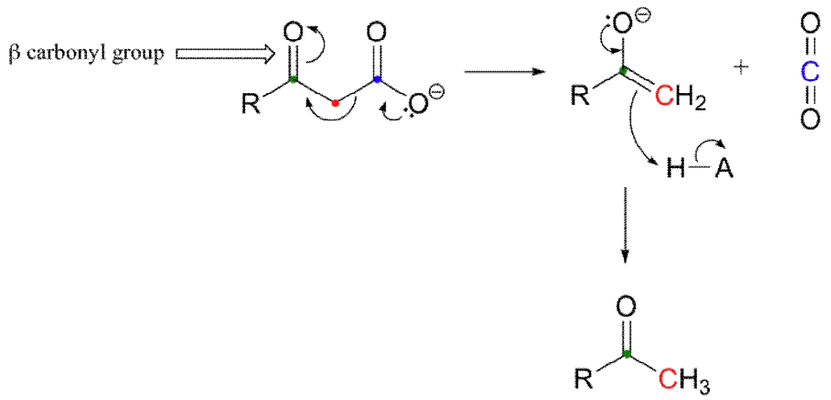
To follow the Rubisco mechanism through to its endpoint:



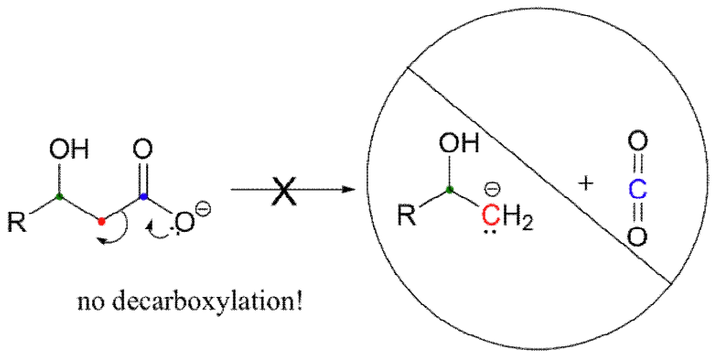
Step 4 is a retro-Claisen mechanism, with a water nucleophile and enolate leaving group. After protonation of this enolate, we are left with two molecules of 3-phosphoglycerate, which are incorporated into the 'gluconeogenesis' pathway of glucose synthesis.

iii) Decarboxylation

Mechanistically, a decarboxylation has parallels to retro-aldol cleavage reactions:

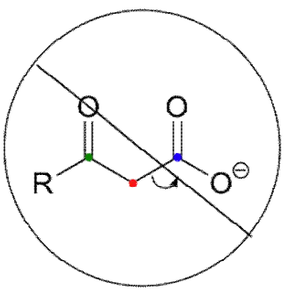


Just as in retro-aldol reactions, the electrons from the broken carbon-carbon bond have to have some place to go - they must, in other words, be stabilized - for the decarboxylation step to take place. Quite often, the electrons are stabilized by the formation of an enolate, as is the case in the general mechanism pictured above. This of course means that a carbonyl group must be positioned beta to (*i.e.* two carbons down from) the carboxylate carbon. If there is no stable place for the electrons in the carbon-carbon bond to go, then a decarboxylation is very unlikely.



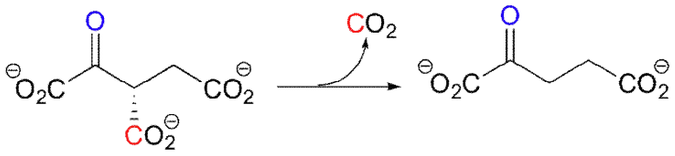
no decarboxylation!

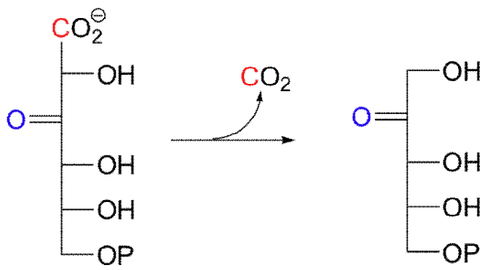
Be especially careful, when drawing decarboxylation mechanisms, to resist the temptation to treat the CO_2 molecule as the leaving group:



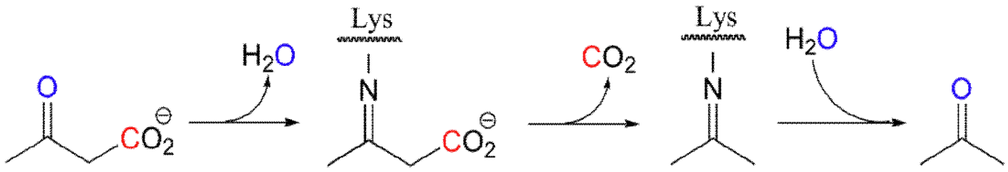
This is *not* what a decarboxylation looks like! In a decarboxylation step, it is the *organic* part of the molecule that is, in fact, the leaving group, 'pushed off' by the electrons on the carboxylate.

Below are two important key b-carbonyl decarboxylation steps in glucose metabolism, each representing a point at which a carbon derived from glucose is released as CO_2.

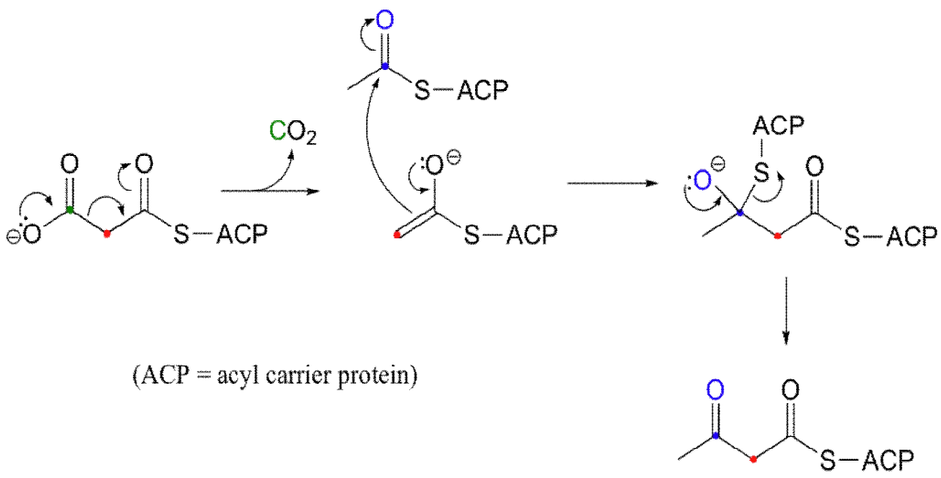




The reaction catalyzed by acetoacetate decarboxylase relies on an imine group, rather than a carbonyl, to stabilize the carbanion intermediate. This is a mechanistic parallel to a type II retro-aldolase reaction.



There are some examples of decarboxylation reactions in which the carbanion intermediate goes on to form a new carbon-carbon bond, rather than becoming protonated as in the example we have seen so far. This reaction in the fatty acid biosynthetic pathway is a decarboxylation followed by a Claisen condensation. A thiol group on an 'acyl carrier protein' is part of the thioester functional group in this reaction.



(ACP = acyl carrier protein)

15.11 Summery

Chemical reactions carried out in living organisms follow the same rules of reactivity as reactions carried out in the laboratory. The “solvent” is often different, the temperature is often different, and the catalyst is certainly different, but the reactions occur by the same fundamental mechanisms. Enzyme catalysis is the increase in the rate of a chemical reaction by the active site of a protein. The mechanism of enzyme catalysis is similar in principle to other types of chemical catalysis. By providing an alternative reaction route the enzyme reduces the energy required to reach the highest energy transition state of the reaction. The reduction of activation energy (Ea) increases the amount of reactant molecules that achieve a

sufficient level of energy, such that they reach the activation energy and form the product. As with other catalysts, the enzyme is not consumed during the reaction (as a substrate is) but is recycled such that a single enzyme performs many rounds of catalysis.

The enzyme catalysis followed by different mechenisms some of which are nucleophilic displacement, addition and ellimination. The enzyme-catalyzed transfer of phosphoryl groups is ubiquitous in intermediary metabolism. Such reactions are central to the energy balance of all organisms, and are also involved in cellular control mechanisms at every level. Enolate anions formed from carbonyl compounds play a central part in metabolism. They are intermediates in isomerization process and instrumental in creating reactive nucleophilic centers on carbon atoms.

15.12 Review questions /comprehensive questions

1 Explain nucleophilic displacement on phosphorous atom with suitable example.

2 Give general mechanism of formation of thioesters, esters, and amides.

3 What is sulfation? How is it catalyzed by tyrosylprotein sulfotransferase enzyme?

4 Explain addition reaction for the synthesis of terpineol.

5 How many biological pathways are possible for elimination reaction to takes place in cell. Explain each.

6 What do you understand by beta condensation? Explain any two categories of beta condensation.

7 What is carboxyaltion and decarboxylation? Explain importance of these reactions in biological system with suitable examples?

15.13 References and suggested readings

- Organic chemistry of enzyme-catalyzed reactions vol-2 by Richard B. Silverman, Elsvier.
- Bender, M. L. and L. J. Braubacher, Catalysis and Enzym Action, McGraw-Hill, New York, 1973.
- Boyer, P. D., Ed. The Enzymes, Academic Press, New York, 1970.
- Walsh, C., Enzymatic Reaction Mechanisms, W. H. Freeman, San Francisco, 1979.
- Silverman, R. B. (1999) *The Organic Chemistry of Enzyme-Catalyz Reactions*, Academic Press, San Diego, California

Unit-16

Carbon-13 NMR Spectroscopy I

General considerations: chemical shift (aliphatic, olefinic, alkyne, aromatic, hetcroaromatie and carbonyl carbon), coupling constants.

Structure of Unit

16.0 Objective

The main objective is to study the NMR spectra of those carbon nulei which is NMR active that is whose nuclear spin quantum number is non zero. such nuclei when placed in external magnetic field undergo precessional motion and if such nuclei are irradiated with radio wave frequency equal to the precessional frequency there shall be the inversion of the spin which generates the electric signal which when amplifies which give signals, the study of the spin decoupled (off resonance) and selective coupled 13CNMR spectra greatly helps in the structure elucidation of the compounds which can be synthesized in thel laboratories for the various pharmaceutical application.

16.1 Introduction

Lauterbur detected first ^{13}C-signal in 1957. The greater problems associated with ^{13}C-NMR is its much lower sensitivity of this nucleus compared with the proton, as the result the development of this spectroscopy was delayed. The advance development of ^{13}C-NMR came after the introduction of Fourier transform (FT) NMR made this technique time saving and highly accuracy in terms of the data and signal analysis.

16.2.1 Comparison Of ^{13}c And ^{1}h Nucleus

For NMR active spin quantum number of nucleus must have a quantum number other than zero ($I \neq 0$). Both ^{13}C and ^{1}H nuclei have a common spin quantum number = 1/2.According to the quantum mechanics the possible number of orientations of the nuclei which is NMR active is given by:

$$m = (2\,I + I)$$

Nuclear spin shall have two possible orientations in presence of external Magnetic field.

$$m1 = +1/2 \text{ and } m2 = -1/2$$

The factors which makes 13C more sensitive for NMR active Natural abundance of the ^{13}C and ^{1}H nucleus Relative abundance of Isotopes of carbon in nature is:^{12}C = 98.7%, ^{13}C= 1.108%

Spin quantum number of ^{12}C nuclei is I = 0, so they do not respond to the NMR spectroscopy as they have only one orientation in the presence of the external field. ^{13}C nucleus shows NMR activity. The small abundance of the 13C isotope is the reason of the slow development of this spectroscopy. The relative abundance of the

^{1}H and ^{13}C indicates that the 1H nucleus is approximately100 times more sensitive than ^{13}C.

16.2.2 Gyromagnetic constants for ^{13}C nuclei

In NMR the gyro magnetic constants are important parameter: the resonance condition equation:

$$\gamma H_0/2\prod$$

The resonance frequency of any given nucleus can be evaluated by the gyromagnetic constant; two different nuclei having different gyromagnetic constants will have different resonance frequencies.Let us compare the ^{13}C and ^{1}H nucleus:

$$\gamma^{1}H = 2.674 \text{ X } 10^{8}\ s^{-1}T^{-1}\ \gamma\ ^{13}C = 0.672 \text{ X } 10^{8}s^{-1}\ T^{-1}$$

The gyromagnetic constant is approximately 4:1.

According to the resonance condition equation ^{1}H nuclei requires an energy level of 60.00 MHz in a magnetic field of 14,100 G (1.4 T).

For bringing ^{13}C nuclei into resonance an energy level of 15.00 MHz is sufficient in the same magnetic field. ^{1}H and ^{13}C nuclei resonate at different regions in a given constant magnetic field. For resonating ^{1}H nuclei energy level of 400.00 MHz. is required Whilst, ^{13}C nuclei requires lower energy level (100.00 MHz) for the resonance in the same NMR instrument so in nuclei in a given uniform magnetic gyromagnetic constants determine the resonance frequencies .

Population difference between energy levels according to the Boltzmann distribution law depends on two parameters:

$N_\alpha/N_\beta = e^{-\Delta E/RT}$The parameters are temperature T and the energy difference ΔE between the energy levels. ΔE depends on gyromagnetic constant γ and uniform magnetic field. The energy difference will be four times more than that of carbon in a given magnetic field

The relaxation time of carbon nuclei is large than that of protons. Long spin-lattice relaxation time states that carbon nucleus has less efficient relaxation pathway and is easy to saturate.

The factors responsible for the less sensitivity of the 13C nuclei toward NMR activity are due to the following reasons:

(a) 13C nuclei natural abundance is very less

(b) Gyromagnetic constant of 13C is less.

(c) Relaxation times of 13C nuclei is very long

Sensitivity of 12C can be increased by applying high magnetic field, at high magnetic field the energy gap persisting between the two energy level is increased making the probability of the nuclei to be more in the ground state, hence more strong resonance signals can be obtained.

16.2.2 Origin of Signals when placed in External field

Interaction between magnetic moment of the dipole and the external magnetic field takes place when the sample is placed in the external feild. The magnetic moment will align with direction of the field. Torsional moment will force the alignment of the nuclei with the direction of the external magnetic field; as a result the nucleus shall start

Dynamic precessional motion like a spinning top, some nuclei shall precess along the direction of the field and some will precess opposite to the direction of the magnetic field, as shown in the fig given under

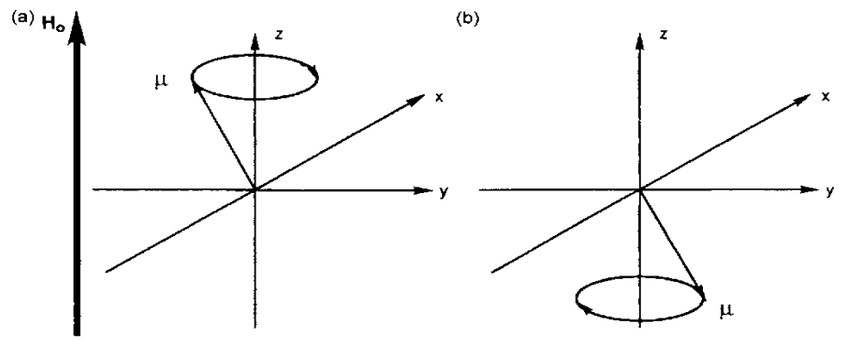


Figure 112 Precession of the nuclear moment μ of a (a) parallel and (b) antiparallel aligned nucleus in the external magnetic field H_0.

The nuclei having parallel orientation with the external magnetic field is slightly more in number than those of the nuclei which are anti parallel aligned with the external magnetic field.

The rotation of the nuclear moments of the nuclei around the applied external magnetic field, is called as the *Larmor frequency* .the factors which effects the Larmor Frequency are:

(A)Strength of the external magnetic field
(B) Gyromagnetic ratio, which reflects the intrinsic property of the nucleus.

When the sample placed in the external magnetic field and subjected to the radio waves, the nuclei comes under resonance when the radio waves frequency matches with the Larmor frequency, as result the nuclei with parallel spin with respect to the external magnetic field changes the alignment and have the anti parallel spin ,since the charge particle in motion develops electric signals as result the amplification of the signals can be recorded.

16.3 Chemical Shift

NMR spectra interpretation we requires the study of two important Parameters in detail:

(A) Chemical shift

(B) Coupling constant.

Electron density surrounding the nucleus determines the Chemical shift of 13C nuclei, circulation of electrons around the nuclei generates Induce magnetic fields

which can leads to either shielding or deshielding which will decide the chemical shift.

According to the Lenz law, the external magnetic field will be opposed by the induced magnetic field, as result local magnetic field strength the nucleus is reduced and is dependent upon the external magnetic field.

$$H_{sec} = \sigma H_0$$

Where σ represents the electron density around the nucleus.

Local field at the carbon nucleus due to diamagnetic shielding makes it smaller than the applied external field, as shown by the Lenz formula shown above. Carbon nuclei comes under the effect of external and the induced magnetic field, they are also influenced by the magnetic field of the groups in the vicinity which produces their own magnetic fields, hence the circulation of the electrons of the whole molecule has to be considered as a whole.

The strength of the diamagnetic shielding is given by Lamb equation:

$$\sigma \alpha r^{-1}$$

There is decrease in the diamagnetic quantity with the increase in the distance *r* between circulating electrons and nucleus.

Stronger diamagnetic shielding will be there by s-electrons than p-electrons as the distance for s electrons is less than the electrons in the p-orbital as there is spherical electron distribution, on hydrogen atom it leads to the dominant diamagnetic shielding effect, since the carbon nucleus lack spherical symmetry of electron density there is less diamagnetic shielding effect as compared to the proton.

Non spherical molecules have Paramagnetic shielding, large magnetic fields at the nucleus by the p-orbital electrons as they do not have the spherical symmetry. In carbon two p-electrons do not have spherically symmetric electronic distribution however the four s electrons of carbon are spherically symmetric in their distribution are in ground state, as a result the strength of the shielding, is reduced(Lenz law)

The paramagnetic shielding predominates for carbon nucleus, this not so significant in the case of hydrogen nuclei, because of the high excitation energy is required for the s-electrons. Hence effect of paramagnetic effect is more pronounced in case of the heavier nuclei. Where availability of energetic low-lying atomic orbitals is there.

The paramagnetic shielding is influenced by the average electronic excitation energy. Only σ bonds are present in Alkanes, having high energy gap in between highest occupied molecular orbitals (HOMO) and Lowest unoccupied molecular orbitals (LUMO) $\Delta E \approx 10ev$ making the role of paramagnetic shielding less significant.

16.2.3 Effect on chemical shift due to diamagnetic and paramagnetic sheilding

σ_{para} becomes an important factor when ΔE is smaller as can be seen in case of (a) alkenes wherein the energy of transition is $\Delta E \approx 8eV$ between $\prod$ molecular orbital

(HOMO) and the magnetically allowed $\prod \rightarrow \prod^*$ transition. (b) For carbonyl groups the energy gap ΔE ~ 7eV ($n \rightarrow \prod^*$ *transition*).as a result the resonating field for alkenes and aromatic compounds ($\delta c \approx 100\text{-}150$ppm), deshielding in case of carbonyl groups makes the resonating around($\delta co > 170$ppm). This makes different functional groups to have the chemical shift.

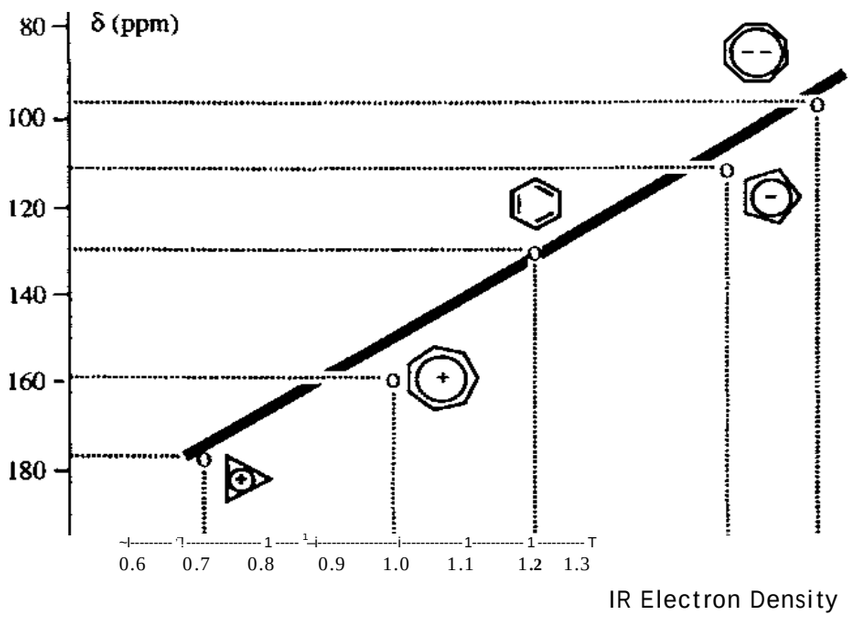
16.2.3 Comparison of The 13 C NMR Spectra of Acetone and Thioacetone

Comparison of the 13 C NMR spectra of acetone and thioacetone can be done as C=0 functional group of acetone resonates at 206.7 ppm, whilst for C=S of thioacetone is at 252.7 ppm, this difference in the chemical shift is mainly because of the difference in the excitation energy of the nonbonding electrons on sulphur and oxygen for the $n \rightarrow \prod^*$ transition.

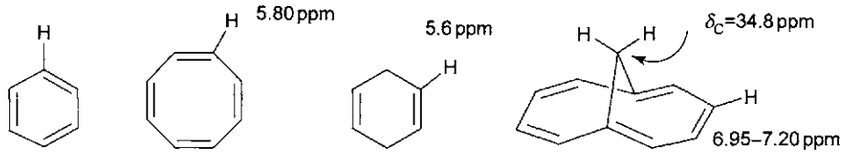
The important factor which also effects the chemical shift in 13C NMR is the distance of electron density of 2p orbital from the nucleus, repulsion is caused because of the high electron density on the nucleus which makes the bonding orbital to expands making the value of r to increase, which will reduce the paramagnetic shielding making the chemical shift to increase.

The resonance frequencies in the proton NMR, aromatic protons are shifted to the lower field (1-2 ppm) compared to olefines. This effect can be best explained in terms of an induced ring current, for aromaticity this paramagnetic shift (downfield shift) is the best criteria. The ring current effect is less important in case of 13C-NMR spectroscopy because it contributes very less to the total shielding as its contribution to the total shielding is ony2 ppm which is masked by other effects.

the effect of the electron density on the chemical shift is shown in the compounds shown below wherein the bridged and aromatic compounds have been shown



^{13}C chemical shifts of carbon atoms in some selected aromatic compounds versus IR-electron density.



<5_c=128.5ppm <5_c=131.5ppm <5_c=125.5and26.0ppm
5_C=128.7,126.1 and 114.6ppm

Carbons of cyclooctatetraene resonate at lower field. Methylene protons at the bridge are resonating at high field (-0.5 ppm), as they lies in the center of the diamagnetic ring current.

16.2.4 The influence of hybridization on the chemical shift

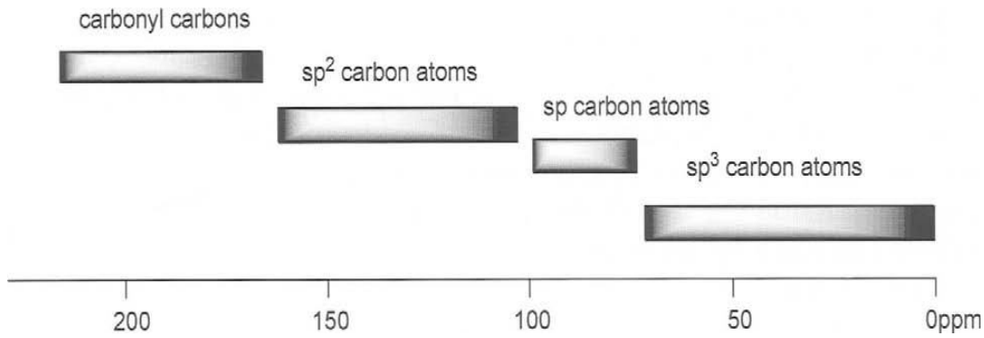
The resonance frequency of13C in the given compound depends on the hybridization of carbon atoms. The chemical shift trend for the molecules havinf different nature of hybridization can be given as

$$^{\delta}Sp2 > {}^{\delta}Sp > {}^{\delta}Sp3$$

Relative to TMS,Sp^3 carbon atoms resonate between - 10 and 80ppm .

- sp- hybridized carbon atom more shielded compared to olefinic carbons there resonating range is between 60 and 95 ppm.
- sp^2 carbons includes olefinic carbons and carbonyl carbons. Alkene resonating range is between 100 and 150 ppm whilst carbonyl carbons of aldehydes, ketones,and carboxylic acids, resonating range is between 160 and 220 ppm.

The relative position of the chemical shifts for the various hybridized carbons for 13C NMR is given below



16.2.5. (B) The study of the effect of Electronic effects on the chemical shift

Electronegativity (+1, - 1 effect) of the substituent that are attached at the carbon atom changes, the resonance frequencies. Through chemical bonds Inductive and mesomeric substituent effects are transmitted which is observed carbon atom.

The effect of halogen substituents on the chemical shifts in pentane derivatives (in ppm) is given the table.

X	CH_2	CH_2	CH_2	CH_2	CH_3
H	13.7	22.6	34.5	22.6	13.7

I	-7.4	10.5	-2.1	-1.1	-0.1
Br	19.3	10.1	-4.1	-0.7	0.0
CI	30.6	10.0	-5.3	-0.5	-0.1
F	70.1	8.0	-6.7	0.1	0.0

Electronegativity of the substituents reduce the shielding of the nucleus because of their - I effect, a relation between substituent electronegativities and 13 C chemical shifts and is seen for the α carbons.The bond polarization moves through the carbon chain due to electronegativity of the substituents attached to the carbon chain.

It is clear after the examination of the chemical shifts in Table given above that there is no correlation between the electronegativities of the substituent and the chemical shifts of β- and γ-carbon resonance frequencies. Increasing electronegativities of the substituent (F, CI, Br) increases the α-effect, exception to the pattern is with the substituent iodine atom.

16.2.4. (C) The study of the effect of Mesomeric effects on the chemical shift

Mesomeric effect

Interaction through the $\prod$-electrons, produces mesomeric effect (M),

when a conjugated system is attached with the substituent having nonbonding electrons or double bond it will bring change in the chemical shift.if the aromatic compounds are taken as the reference and the effect of the substituent on the ring is studied effect of mesomeric eefect can be easily understood. methoxyl group (+M-effect)The, increases the $\prod$-electron density being an electron-releasing substituent in the aromatic ring, while the nitro group (- M-effect), decreases $\prod$-electron density.

shielding in the *ortho* and *para* positions is seen by the electron-releasing substituents which makes carbon nucleus to resonate at high field i.e high chemical shift.electron-withdrawing substituents cause a decrease in the electron density on the carbon nucleus hence desheilding the nucleus making it to resonate at low field i.e low chemical shift.

The effect of the mesomeric effect on the chemical shift of 13CNMR can be estimated by the table given below.it is mandatory to mention here that ,the least affected position by the mesomeric effect , is the *Meta* position.

^{13}C-NMR chemical shift values in substituted benzene derivatives

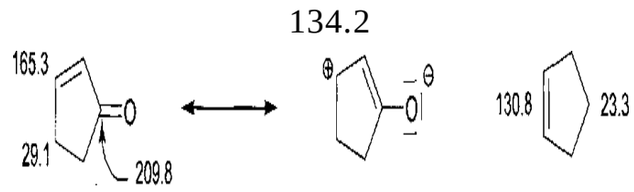
Substituent, X Chemical shifts of the carbon atoms

	C_1,	C_2,6	C_3,5	C_4	Substnt
-H	128.5	128.5	128.5	128.5	
-OCH_3	159.9	121.4	128.1	125.3	54.8

-NO_2	149.1	124.2	129.8	134.7	
-CHO	136.7	129.7	129.0	134.3	192.0

16.2.4 (D) The study of the effect of conjugation on the chemical shift

Conjugation also effects the chemical shift of ,αβ-unsaturated carbonyl compounds and are shielded as compared to the saturated carbonyls compounds. In case of cyclopentene the olefinic carbon resonates at 130.8 ppm whilst in cyclopent-2-enone β-olefinic carbon shows chemical shift at 165.3 ppm, α-olefinic carbon atom resonance shows chemical shift at 134.2 ppm.



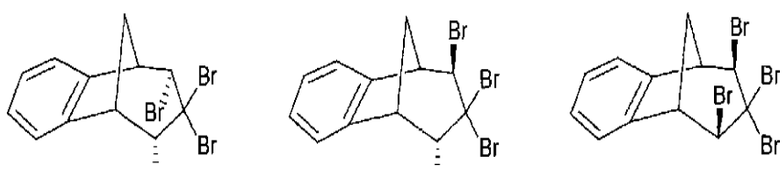
16.2.4 (D) The study of the effect of Steric effect *(γ-gauche effect)* on the chemical shift.

van der Waals radii of protons or the substituents which are closely spaced undergoes overlapping, γ-position of the substituents makes the carbon always shielded . Steric effect in acyclic and cyclic systems is pronounced if the system can have gauche conformation, as this on formation is essential, this interaction is called the γ-gauche steric effect.

steric interaction, makes the chemical shift of the γ-carbon atom to have chemical shift upfield.

The γ-effect arising from the bromine can be understood by taking bicyclic rigid systems as the reference, the oreintation of the halogen can effect the chemical shift of the bridged carbon atoms of the cyclic system can be seen from, the *exo* orientation of one bromine atom causes the bridge carbon to resonance to an upfield shift of about 4 ppm ,and the second exo-bromine atom leads to an additional upfield shift of 6 ppm. Furthermore, this examples hows the additivity of the γ-effect.

47.63 ppm 43.49 ppm 37.34 ppm



16.3.0 [13C] _Chemical Shifts of Organic Compounds

16.3.1 ALKANES

carbon nuclei in linear and branched saturated hydrocarbon shows chemical shifts in range of about 60 ppm. With the increase in the number of carbn atoms the chemical shift is lowered.

$$\delta c > \delta CH > \delta CH2 > \delta CH3$$

The effect of the substituent on the chemical shift of carbon, *k,* in alkanes is given by the equation.

$$\sum A_l K_{ln} + \sum S_{kl}$$

- δk is the chemical shift of the carbon nucleus of interest,
- n_{kl} is the number of the carbon atoms in position l with respect to C atom
- *k, A* are the additive shift parameters,
- S_{kl} are the steric correction parameters

^{I3}C-NMR chemical shift values of some hydrocarbons

Compound Chemical shifts of the carbon atoms

	c,	c_2	c_3	c_4	c_5
Methane	-2.3				
Ethane	5.7				
Propane	15.8	16.3			
n-Butane	13.4	25.2			
n-Pentane	13.9	22.8	34.7		
w-Hexane	14.1	23.1	32.2		
n-Heptane	14.1	23.2	32.6	29.7	
n-Octane	14.2	23.2	32.6	29.9	
n-Nonane	14.2	23.3	32.6	30.0	30.3
«-Decane	14.2	23.2	32.6	31.1	30.5
i-Butane	24.5	25.4			
2-Methylbutane	22.2	31.1	32.0	11.7	
2,2-Dimethylbutane	29.1	30.6	36.9	8.9	
2,3-Dimethylbutane	19.5	34.3			
2,2,3-Trimethylbutane	27.4	33.1	38.3	16.1	
2-Methylpentane	22.7	28.0	42.0	20.9	14.3

3-Methylpentane	11.5	29.5	36.9	18.8 (C_6)
3,3-Dimethylpentane	7.7	33.4	32.2	25.6 (Qs)

For evaluating the chemical shift parameters shift parameters, steric correction parameters are also required, for which the carbon atoms. Primary, secondary, tertiary, and quaternary carbon atoms are symbolized

Additive shift parameters .

Position of the carbon atom l	A_l **values (ppm)**
α	**9.1**
β	**9.4**
γ	**-2.5**
δ	**0.3**
ε	**0.1**

16.3.1 ALKANES Additive correction.

Additive correction parameters

Observed carbon atom	Neighbor carbon atom /			
	1°	2°	3°	4°
1° (primary)	0.0	0.0	-1.1	-3.4
2° (secondary)	0.0	0.0	-2.5	-7.5
3° (tertiary)	0.0	-3.7	-9.5	-15.0
4° (quaternary)	-1.5	-8.4	-15.0	-25.0

by using1^0, 2°, 3° and 4°, respectively. The shift and steric correction parameters given in Tables 13.2 can be used for evaluating the chemical shift of hydrocarbon can be evaluated.

For example, we calculate the 13C chemical shifts of n-hexane. Because of the Symmetry in the molecule, there are only three types of different carbon atoms. We have to calculate the chemical shifts of all three carbons one by one using eq. 60 and the given parameters in Tables 13.2 and 13.3.

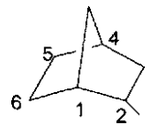
Table 12

16.3.1 ALKENES an Alkynes

In alkenes the 13c nmr ,chemical shift does includes the diamagnetic shielding as it is not so effective,since the energy gap between the high energy $\prod$ MO,the magnetically Aallowed $\prod \rightarrow \prod^*$ transition (ΔE=8eV) is considerably of less magnitude as result the paramagnetic shielding makes the chemical shift of the alkenes to go down field.the chemical shift range for the substituted alkene is from 75-175 ppm relative to TMS.

For determining the chemical shift of the alkene carbon alkyl group shift parameter has to be taken into consideration.the position pertaining to the exo and endo for the cyclic system is shown in the table given below

^{13}C-NMR chemical shift values in ***endo-*** and exo-2-substituted norbornanes Chemical shifts (ppm)



x =	Ci	c_2	c_3	c_4	c_5	c_6	c_7	X
H	36.8	30.1	30.1	36.8	30.1	30.1	38.7	
EXO-OH	44.5	74.2	42.4	35.8	28.8	24.9	26.6	-
endo-OH	43.1	72.2	39.6	37.7	30.3	20.4	37.8	-
EXO-NH_2	45.7	55.4	42.5	36.4	28.9	27.0	34.3	-
endo-NH_2	43.6	53.4	40.6	38.0	30.7	20.6	39.0	-
EXO-CN	42.3	31.1	36.4	36.5	28.6	28.5	37.4	123.4
endo-CN	40.2	30.2	35.6	37.0	29.4	25.2	38.7	122.6
	41.9	46.5	34.3	36.4	29.0	28.7	36.6	175.7
endo-$COOCH_3$	40.8	46.0	32.3	37.5	29.4	25.1	40.4	174.6
EXO-CH_3	43.5	36.8	40.2	37.3	30.3	29.0	35.0	22.3
endo-CH_3	42.2	34.6	40.7	38.2	30.6	25.1	38.9	17.4

$$—C_\gamma\text{-}C_\beta—C\alpha—C_k{=}C—C_\alpha\text{'-}C\beta'—C\gamma'—$$

For the evaluation of the chemical shift of the carbon atoms of alkenes ,the substituents attached to the carbon atoms of alkenes have to be taken in consideration and there substituent shift parameters have to be taken in account at the same time.

The formula for the evaluation of the chemical shift of the carbon atoms of the ethylene can be eavaluated from the given formula

$$\delta_k = 122.1 + \sum A_l n_{kl} + \sum S_{kl}$$

where

- S_k : chemical shift of the carbon nucleus under consideration,
- n_l is the number of the carbon atoms in position / with respect to C atom **k,**
- **A** is additive shift pa ameters,
- S_{kl} is the steric correction parameters.

The details of the chemical shift of the 13C NMR of the various unsaturated compound have been given below in the chart.

^{13}C-NMR chemical shift in some selected alkenes

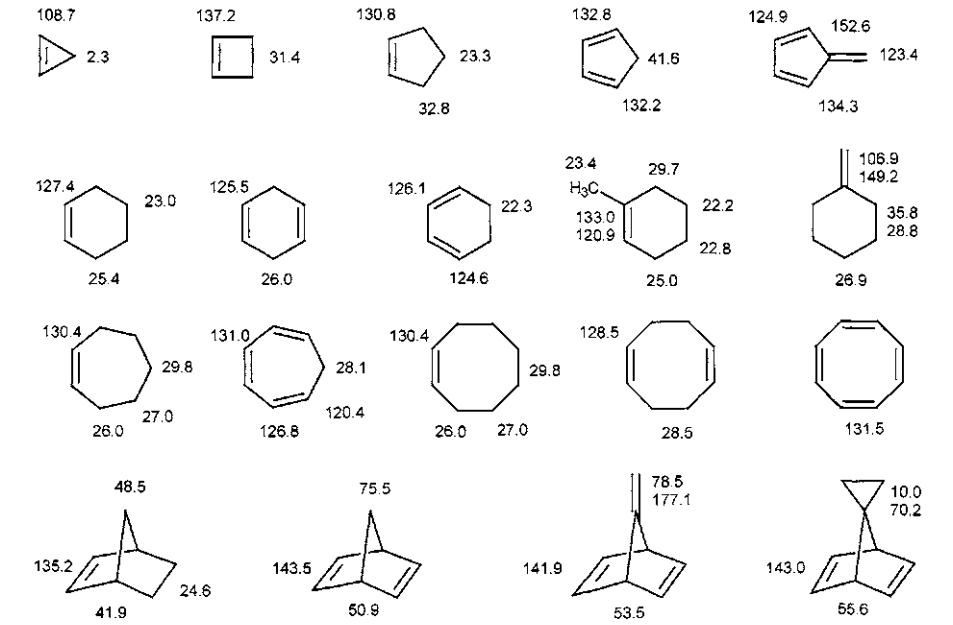
Compounds	Chemical shifts (ppm)				
	C_1	c_2	c_3	c_4	c_5
Ethene	123.5				
Propene	115.9	133.4	19.4		
1-Butene	113.5	140.5	27.4	13.4	
1-Pentene	114.5	139.0	36.2	22.4	13.6
1-Hexene	114.2	139.2	33.8	31.5	22.5
cw-2-Butene	11.4	124.2			
/rans-2-Butene	16.8	125.4			
fraw.s-2-Pentene	17.3	123.5	133.2	25.8	13.6
cw-3-Hexene	14.1	25.9	131.2		
1,3-Butadiene	116.6	137.2			
2,4-Hexadiene	12.9	124.9	125.3		

2,3-Dimethyl-1,3-butadiene	113.0	143.8

for alkynes the chemical shift in terms of delta is 71.9(for ethyne), for propyne the acetylinic carbon has chemical shift around 66.9 and 79.2 while propynol the shift is around 78.3 and carbon attached to hydroxyl group is 83.0 while for norbornene he alkene carbon has shift of 135.8.

Table 13.11

^{13}C-NMR chemical shift in some selected cyclic alkenes



16.3.1 Aromatic compounds

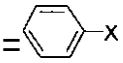
The effect of the ring current for the aromatic compound is less prone in 13 C NMR spectroscopy; as a result there is no shift in the low field region for the aromatic carbon as compare with those of the olefinic carbon. The region of resonance for the carbon of the aromatic compounds is similar with those of the oefinic carbon atoms. The resonating frequency of the aronmatic carbon is around100 -150 ppm by the attachment of the electrons withdrawing and donating groups the range can go between 90-180 ppm.

Correlation is there between the resonance of the aromatic carbon and the electron density on the ring. The electron density can be changed by the attachment of thy substituent consequently the chemical shift of the carbon of the ring can be increased or decreased, the substituent attached at not influence the chemical shift of the meta position carbon of the ring, so the mesomeric effect will be having the effect on the ortho and para position whilst the inductive effect shall be most operable on the ortho position, steric effect also effect the ortho position prominently

By using the empirical substituent parameters we can evaluate the chemical shift of the multisubstituted benzene by the equation given below:

$$^{\delta}c=128.5+\sum Z_i$$

Shift parameters in substituted benzenes

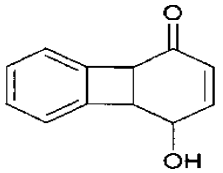
	Shift parameters (ppm)			
x = 	**ipso**	**ortho**	**meta**	**para**
-H	0.0	0.0	0.0	0.0
$-CH_3$	9.3	-0.1	0.7	-3.0
$-CH(CH_3)_2$	20.3	-0.2	-0.1	-2.6
$-CH=CH_2$	9.1	-2.4	-0.2	-0.9
-C=CH	-5.8	3.9	0.1	0.4
-Ph	13.0	-1.1	0.5	-1.0
-F	35.0	-14.4	0.9	-4.4
-Cl	6.4	0.2	1.0	-2.0
-Br	-5.9	3.0	1.5	-1.5
-I	-32.3	9.9	2.6	-0.4
-OH	26.6	-12.8	1.6	-7.1

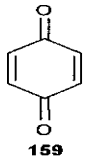
$-OCH_3$	31.4	-14.4	1.0	-7.8
-OCOCH3	23.0	-6.4	1.3	-2.3
-SH	2.0	0.6	0.2	-3.3
$-NH_2$	20.0	-14.1	0.6	-9.6
$-N(CH_3)_2$	22.2	-15.8	0.5	-11.8
$-N0_2$	20.6	-4.3	1.3	6.2
-COC1	4.8	2.9	0.6	6.9
-COOH	2.9	1.3	0.4	4.6
$-COOCH_3$	2.1	1.2	0.0	4.4
-CHO	8.2	0.5	0.5	5.8
$-COCH_3$	8.9	-0.1	-0.1	4.5
-CN	-15.5	1.4	1.4	5.0

16.3.2 Carbonyl compounds

There are two distinct region of frequencies where the carbonyls resonates.carboxylic carbonyls resonate in the range of 160-180 ppm,whilst aldehyde and ketones resonate in the range of 195-220 ppm.it is easy to distinguish the aldehydes from ketones as the proton coupled spectra of the 13C NMR for aldehydes are different from that of ketones where such coupled interaction is absent. The low field shift of the carbonyl carbon is due to the polarization of the carbonyl carbon.the saturated carbonyls shows low field chemical shift as

compared to the $\alpha\beta$ unsaturated carbonyl compounds where the electron density is increases due to the conjugation of the electrons. the relative position of the carbonyl carbon in the compounds are shown below.





159

16.3.2 Heterocyclic Compounds

The smallest member of the heterocyclic compound is oxirane prepared by the action of the peracids on alkene.They generally comes under the chemical shift of the range of 40-75ppm.the chemical shift of the heterocyclic compounds also depends on the nature of the substituent. aziridines and thiiranes shows the chemical shift at the higher field compared to the there analogues of the cyclopropane. The chemical shift of 13C NMR of the heterocyclic compounds is shown in the table give

16.3.2 [13C-NMR] shifts of saturated heterocyclic compounds

16.4 Coupling Constant: "J"

The spin quantum number of 13C-NMR, I = ½ just as the proton as the result the 13 c nuclei will also couple with the proton and give rise to the complex splitted spectra. The natural abundance of13C nuclei is only 1.1%. this causes the problem in sensitivity, there is an advantage in the carbon spectra, that probability of finding two ^{13}C nuclei in a fixed position is 10^{-4} so the coupling will be very weak (0.5% of the normal 13C sig nal intensity) , so the probability of finding the coupling is very less The 13 Carbon nuclei can couple with the proton as both the species are having the nuclear spin quantum number not equal to zero, as result the single signal of 13 C nucleus is further subjected to the internal magnetic field which further makes the carbon nucleus to resonate making the signal to split. The space between the splitted signals is called as the "Coupling Constant" denoted by "J".

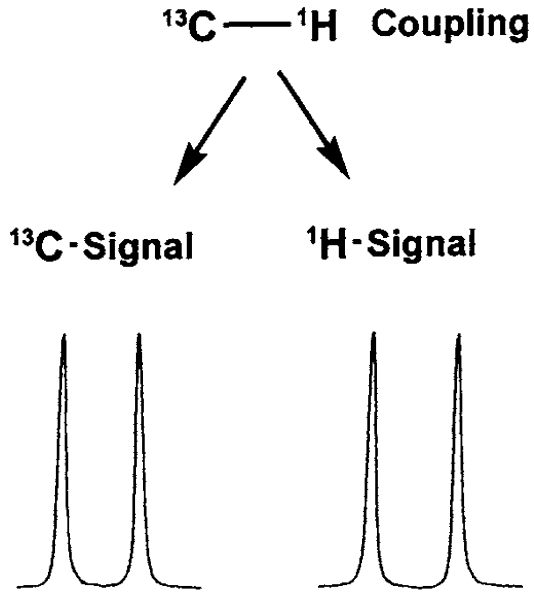


Figure 132 Appearance of $^{13}C-^{1}H$ couplings in the ^{1}H-NMR as well as in the ^{13}C-NMR spectra.

The coupling pattern also helps in the prediction of the near neighbor proton, this helps in the elucidation of the structure

16.4.1 Spin-spin coupling

There are three different ways or the mechanisms which contribute spin spin coupling. The dominant coupling scheme is “Fermi contact term” according to this spin spin coupling taking place between two nuclei A and B arising due to interaction of nuclear spin A and B.nuclei

16.4.2 The spin- spin interaction (Proton and carbon) through bonding electrons in the C-H bond

Magnetic moment of hydrogen atom 1H can align itself either in parallel or antiparallel to the external field the orientation of the hydrogen atom in ground state has parallel orientation. As per the energetic consideration the most preferred state is tha where nuclear magnetic moment of hydrogen atom1H and the magnetic moment of the nearest bonding electron are in the anti parallel orientations

According to the Pauli principle electrons present in the bond have their spin paired, so the magnetic moment of the electron (second) which is adjacent to 13 C have orientation parallel to the applied field

As a result each nucleus responds to the orientation of the other the antiparallel orientation of the H will make 13C to align itself in parallel orientation to the applied external applied field

Magnetic field around the nucleus which is in resonance will be affected by the orientation of the adjacent nucleus as a result nuclear magnetic moment of one nucleus makes the magnetic moment of the other nucleus in the opposite direction which occurs through the electrons in the bonds. In this case the coupling constant is defined to have the positive values. all 13 C-H coupling has the positive values.

16.4.3 The coupling constant over one bond (1JCH)

Factors affecting the coupling constant between carbon and the proton are :

(a) Hybridization of the carbon atom
(b) The electronic nature of the substituent attached to the carbon atom.
(c) Strain in the molecule

An empirical correlation between carbon coupling constants JCH and S character (denoted by S)is given by

$$JCH = 500S$$

Since organic compounds having only 1.1% 13C isotope, only 1.1% of the proton will be able to couple with carbon nuclei and will form satellite around the main peak in 1H NMR spectroscopy they are called as the satellite spectra.

Single bond C-H coupling constant JCH is between the ranges 100 to 200 Hz however the coupling for the proton –carbon for two and three bond is less than 20 Hz. Since 13 C-H coupled spectra create complex nature of the spectra so to simplify the spectra the decoupling is done by the broad band decoupling technique.

As the JCH=500S so as the S-Character of the hybrid orbital is increased the coupling constant is also increased

Hybridisation	S-Character	JCH
sp^3	0.25	125 HZ
sp^2	0.33	150-170 HZ`
sp	0.50	250 HZ

As it is clear from the table itself that is with the increase in the S character of the hybrid orbital the magnitude of the coupling constant also increases

As the substituent attached to the carbon atom shows the change in the magnitude of electro negativity the value of JCH also changes as shown in the table below.

1JCH coupling constants (HZ)for the methane halides

X	H	F	Cl	Br	I
CH4	125				
CH3X	125	149.1	150	151.5	151.1
CH2X2	125	184.5	178.0		

CHX3	125	239.1	209.0		

For the strained molecules a very large value of the coupling constant is observed

CYCLOHEXANE: JCH=127HZ

CYCLOPROPANE: JCH=161.0HZ

The change in the value of the coupling constant is attributed to the change in the hybridization in C-H bond with the increase in the ring strain carbon hybridi8sation changes in the way that exocyclic orbital (C-H bond) carbon hybridization also changes such that s character also changes

16.5 The factors that are affecting the coupling constants of the carbon- proton coupling are

1. Dihedral angle (Φ)
2. Bond length(R c-c)
3. Valence angle (θ)
4. Electro negativity effect of the substituent
5. Magnitude of the vicinal coupling approaches zero when the dihedral angle 90 degree while it is largest when Dihedral angle (Φ) is either a zero degree or 180 degree

Magnitude of the Trans coupling is more than cis coupling that J trans $>$ J cis Similar relationship between the vicinal carbon and proton coupling and vicinal proton proton coupling is observed the relationship is given by the formula

^{3}JCH=0.63 JHH.

16.6 Summary

- The magnetogyric ratio of 13 C is about ¼ that of 1H nucleus.
- The 13C-1H coupling constant are large(125-200 HZ)for the directly bonded protons thus the resulting multiplets tends to overlap and the spectrum becomes too complicated to study
- H1 NMR coupling constants and the gamma effect in 13 C NMR are highly useful in the deucing of the structure of the alkene.

- The lesser sensitivity of the 13C NMR spectra is mainly due to the less availability of the 13 C nucleus in nature around only 0.01%

16.7 Review Questions

1 How the 13C complex spectra can be simplified?

2 For the decoupled 13C NMR spectra of ,o,m,p xylene how many signals are there.

3 Why peak area measurement is not successful for the routine 13C-NMR spectra

4 Who studied the 13C NMR spectra ?

5 For neopentane the the 2-c which is coordinated to four carbon atoms there chemical shift is ?

16.7.0 Refrence Books

- Organic spectroscopy: P.S.Kalsi,new age publication
- Spectroscopy by : H.kaur,Pragati prakashan
- Molecular spectroscopy : Banwell,tata Mc Graw hill
- Organic spectroscopy: Kemp,Palgrave
- Spectroscopy by Jagmohan

Unit-17

Carbon -13 Nmr Spectroscopy II : Factors Affecting Chemical Shift, Internuclear Coupling and Homonuclear Coupling Of 13-C-Nmr Spectroscopy

Structure of Unit

17.0 Objective

The study of 13C-NMRspectroscopy is mainly helpful in the structural elucidation of the compounds occurring naturally as well as synthesized in the laboratories. The proton coupled and decouple 13-c NMR spectroscopy is of immense importance as the multiplicity is helpful as well as hampering the

spectra so as the conditions allow one can use the coupled and decoupled spectra of the 13-C-NMR for the structural elucidation of the compound

17.1 Introduction

13C-NMR spectroscopy is an important and versatile tool for solving various structural, stereochemical aspects of the complex organic molecules which in turn have an important impact on their pharmaceutical action. This help in drug designing and other synthesis of biochemical importance. In this topic the parameters which decides the chemical shift including the electronegativity, hybridization and the dihedral angles between the bonds are studied in detail and the decoupling of the protons to solve the complex spectra has been elucidated in simple way

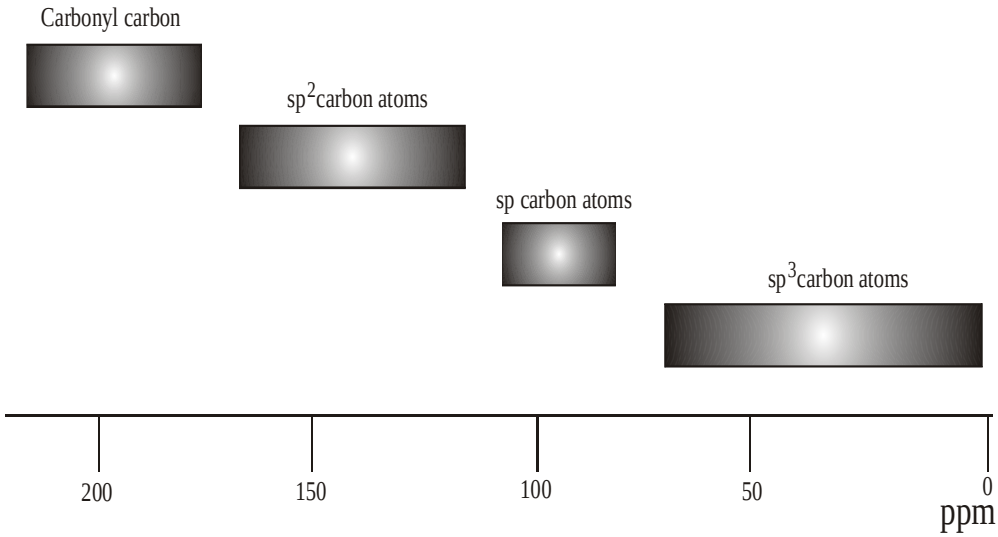
17.2 Factor Effects Chemical C-13 Shift

The various factors that effect the chemical shift of 13-C are :-(a) Carbon hybridisation (b) Electronic effect (c) steric effect

17.2.1(A) Effect of hybridisation on C-13 shift

Hybridisation effect the chemical shift which can be seen where sp^3 hybridised carbon. Show chemical shift around -10 and 80 ppm.relative to TMS, while sp^2 hybridised carbon of alkenes shows shift around 100-150ppm and carbonyl carbon around 160 and 220ppm.

The figure below shows the effect of the hybridisation on the chemcial shift.



17.2.2 (B) Electronic effect

There is the change in the resonance frequency, when the nature of substituent is changed wrt. inductive effect. The inductive effect extends upto γ - carbon atoms.

Table given below shows the effect of halogen in B-C-shift of pentane.

X	CH_2	CH_2	CH_2	CH_2	CH_3
H	13.7	22.6	34.5	22.6	13.7
I	-7.4	10.5	-2.1	-1.1	-0.1
Br	19.3	10.1	-4.1	-0.7	0
Cl	30.6	10.0	-5.3	-0.5	-0.1
F	70.1	8.0	-6.7	0.1	0

The effect of halogen substituent on the chemical shifts in pentane derivatives (in ppm)

-I effect of the substituent reduces the shielding and this effects propagates through bond. -I effect at carbon atoms with increase in the electro negativity of the atoms. The shielding decreases as in case of F, Cl, Br, I.

When substituent is Bromine and Iodine and up gradation in chemical shift is seen which is called "Heavy atom effect" which is prominently observed when there is multiple substitution. The main reason attributed to it is due to diamagnetic shielding occurring due the release of the electrons by the heavy atoms.

The diamagnetic shielding is given by

$$\sigma_{dia} = \frac{e^2 \mu_0}{4\pi 3 m_e} \sum Z r^{-1}$$

Z= atomic number, r = internuclear distance, me = electron mass, e = electron charge.

The effect on chemical shift by the substituent where in an increase in atom C number is correlated is given in the table.

	CH_3X	CH_2X_2	CHX_3	CX_4
X=I	-24.0	-53.8	-139.7	-292.5
X=Br	9.6	21.6	12.3	-28.5
X=Cl	25.6	54.4	77.7	96.7
X=F	75.0	109.0	116.4	118.6

^{13}C - NMR chemical shifts value in halo substituted methane derivatives

As the distance increases diamagnetic shielding decreases. β Carbon atoms do not show the heavy atom effect. Electro negativity does not have direct co-relation with diamagnetic deshielding.However there is correlation between the electro negativity and $\alpha-$ effect of halogen atoms which is violated by Iodine atom. It is seen for γ - carbon atoms, they are highly shielded and resonate at higher frequency this is called as - γ gauche effect. For cyclohexane system carbon atom 3, 4 and 5 resonate up field which is mainly due to γ - gauche steric compression.

17.2.3 Mesomeric Effect :

This effect is mainly through electron that may arise due to excess or the deficiency of $\pi-$electrons. These are a change in the chemical shift of ^{13}C - atoms when a substituent having non - bonding pair of electrons attached to the conjugated system.

As is clear from the substituted aromatic system where methoxyl group increases electron density (+ M) While the NO_2 group decreases the electron density from the aromatic system (- M effect).

From the table given below lone the electron releasing substituent increase electronic density at ortho and Para position making carbon to resonate at high field while electron withdrawing groups decreases the electron density at ortho and Para position making carbon to resonate at lower field.

Substituent, X	Chemical shifts of the carbon atoms				
	C_1	$C_{2,6}$	$C_{3,5}$	C_4	Substituent
- H	128.5	128.5	128.5	128.5	
- OCH_3	159.9	121.4	128.1	125.3	54.8
- NO_2	149.1	124.2	129.8	134.7	
- CHO	136.7	129.7	129.0	134.3	192.0

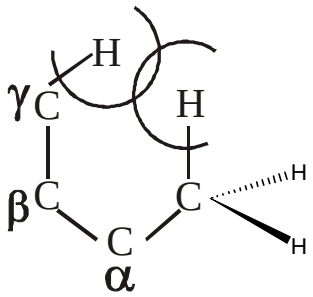
The carbonyl carbon in saturated system shows high resonating frequency while for unsaturated carbonyl compounds the resonating frequency is decreased. This mainly happens as oxygen atom due to its -M effect pulls the π electron making carbon dishielded and hence lower its chemical shift.

So it can be understood that any effect that affect the electron charge density of the carbon atom that may either be M effect or I effect. Will affect the chemical shift of 13-carbon atoms.

17.2.4 Steric Effect / - Gauche Effect

This effect mainly arises when there is overlapping of Vander-Waal radii of protons or substituent which are very close to each other, when substituent lies in γ-position the carbon atom gets shielded. This effect is not only shown by alkyl groups but by many other substituent also.

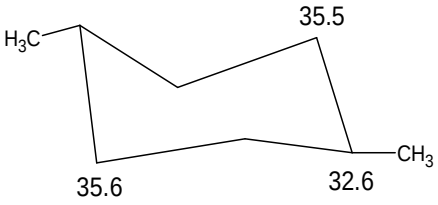
Gauche conformation is main criteria to observe the γ - effect for cyclic and acyclic systems.



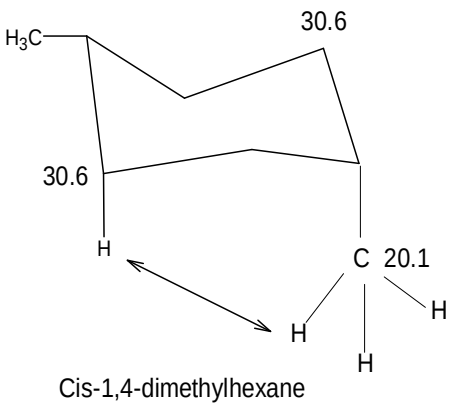
An upfield shift is noticed in such carbons due to this steric interaction. This effect not only causes the polarisation of C-H bond but also increases the electron density at both carbon atoms which farther leads to the diamagnetic shielding making carbon atoms to resonate at higher field causing up field shift.

For acyclic system γ -gauche effect can go up to -2ppm when the substituent at γ position is methyl. There is a correlation between -gauche effect and electro negativity as for halogens it can go up to -7ppm.

How the axial and equator position of methyl groups are responsible for γ - gauche effect can be seen in case of cis and Trans 1, 4- dimethyl cyclohexanes. As in case of cis isomer γ-gauche effect is seen which for Trans isomer it is not seen.

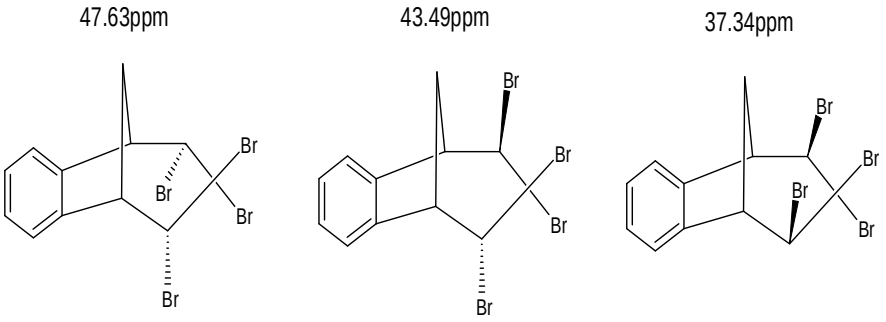


trans-1,4-dimethylhexane



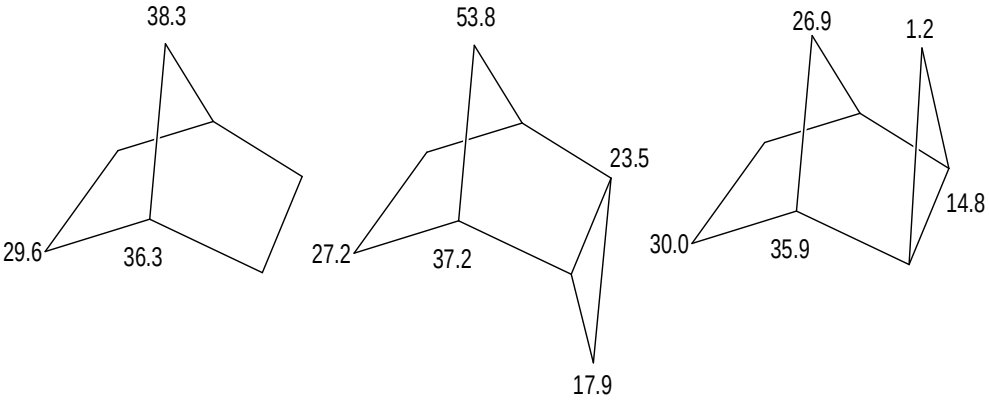
Cis-1,4-dimethylhexane

In bicyclic rigid system as is shown in figure given below for the Bromine substituted bicyclic rigid system when one Bromine atoms acquires exo position with reference to bridge carbon atom makes carbon to go downfield by 4 ppm and the second Bromine atoms makes bridge carbon atom to resonate downfield by 6ppm.

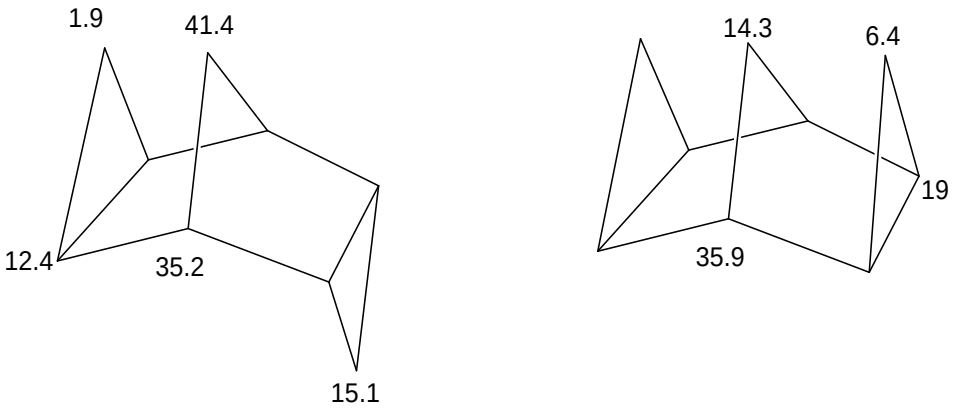


The bridged carton chemical shift is also effected by configuration of the fused cyclopropane ring i.e., whether it is exo or endo.

When the cyclopropane ring is exo with reference to the bridged carbon atom than the bridge atom is highly shielded while if the ring is endo than it is deshielded this up field and downfield shifts are attributed to the syn- γ -gauche effect (for exo) and anti-γ -gauche effect (for endo).



The synγ--gauche and anti -γ -gauche effects are additive in nature is demonstrated in figure below. Where the exo-exo isomer make bridge atom to get highly shielded to resonate it at 14.3ppm while for endo exo isomer the bridge atom resonate at 41.4 ppm.



So γ-gauche effect the 13-C chemical (endo isomer) shift effected as in syn-γ-gauche effect wherein the arrangement is sync planar makes the bridge atom shielded while for anit γ-gauche effect (exo isomer) wherein the arrangement is anti periplanar makes the bridge atom deshield making it to resonate low field.

17.2.5 Spin-spin coupling

Multiplicity of signals

If the non-equivalent atoms are decoupled the spectra is simple out if the atoms are coupled the spectra show splited pattern due to coupling of the non equivalent atoms.

17.3 Heteronuclear coupling

When 13-C NMR spectrum of acetone was obtained by doing decoupling of protons than two sharp signals are obtained for the two non-equivalent carbon atoms. However of proton decoupled spectra are studied for acetone molecule than spliting of singnals of 13-C nuclear is seen. where the carbon of carbonyl group shows septct pattern while the methyl carbon atoms shows quartet pattern as they are directly bonded to the three protons with I_H = 1/2.

For a molecular system having say (n) equivalent nuclei (P) with total spin quantum number I_P and *l* equivalent nuclei (Q) with I_Q and if the difference of the chemical shift between nuclei P and Q is large than number of lines in the NMR spectrum is given by formula:

$2nI_P+1$ for nucleus P $2lI_Q+1$ for nucleus Q

However as the natural abundance of C-13 is about 0.01% due to this low natural abundance of 13-C nucleus doublets occuring due to coupling of C-13 with protons is generally lost in the noise of ^{1}H - NMR spectrum.

Multiplet arising due to coupling of 13-C nucleus is having equidistant peaks. The distance between the splitted signals is termed in termed of coupling constants given by the symbol J.

For acetone coupling constant in carbon 13-C NMR is about 125.5Hz for methyl and 5.5Hz for carbonyl carbon. The magnitude of coupling constant decreases with increase in the numbers of bond between the non-equivalent nuclei.

Coupling constant magnitude remains unchanged with even changing the external magnetic field β_0 Nature of solvent, concentration and temperature changes the chemical shift but the coupling constant remains unchanged.

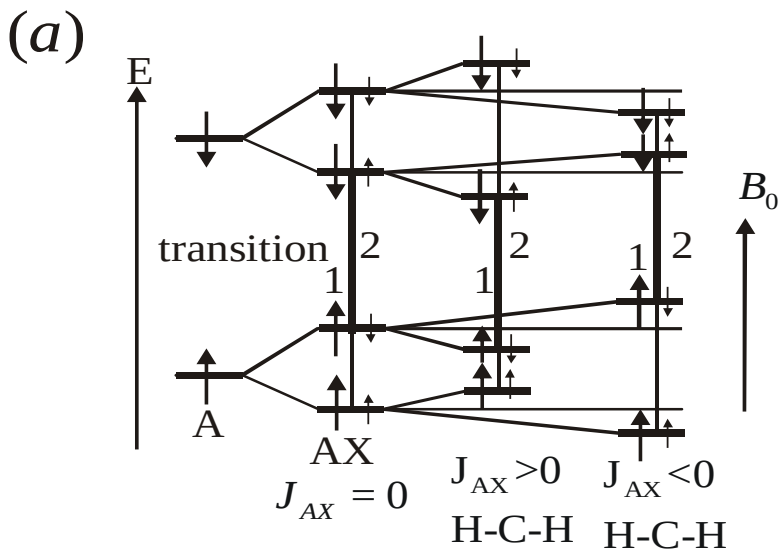
17.3.1 Origin of spin-spin heteronuclear coupling

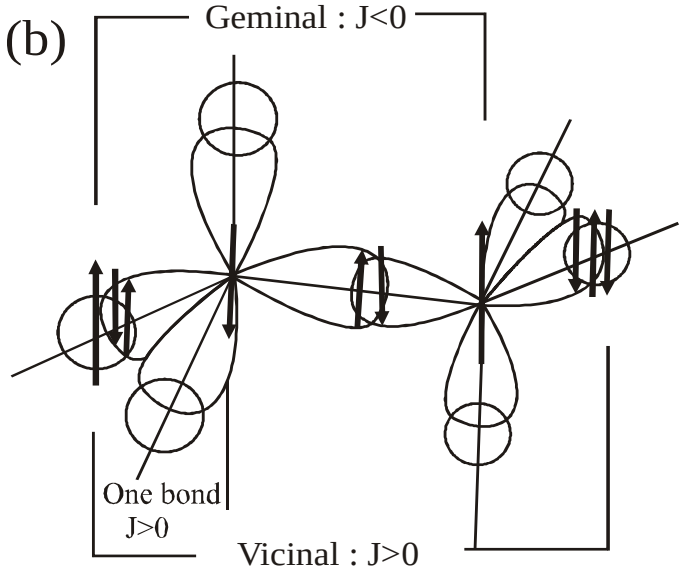
$C^{13} - H^1$ Coupling constant magnitude depends on the hybridization of carbon atom which is coupled to proton when nuclear energy levels splits. It gives rise to multiplets. If the nucleus says (A) can experience magnetic field caused by nucleus (X) which can be processing along or opposite to the magnetic field β_0 . As I_X = 1/2 for nucleus (A) the transition where $I_A \pm 1$ and $I_X = 0$ are allowed by the quantum mechanical rules. So only two transitions are possible for the nucleus A. Which requires the same energy?

$C^{13} - H^1$ When bonding electrons occupying molecular orbitals are having antiparallel orientations it represents the stable state according to the Pauli principle. For nuclei seperated by one and three bonds stabilisation is there for the antiparallel spins.As a result the J_{AX} is positive ($J_{AX} > 0$) The frequency corresponding to the stabilsation $\frac{1}{4}hJ_{AX}$ is .

Parallel precesion of A and X nuclei is destablised by $\frac{1}{4}hJ_{AX}$. This makes two transitions to take place with different frequency which makes A signal to eject splitted into doublet.

This is shown in the figure given below.

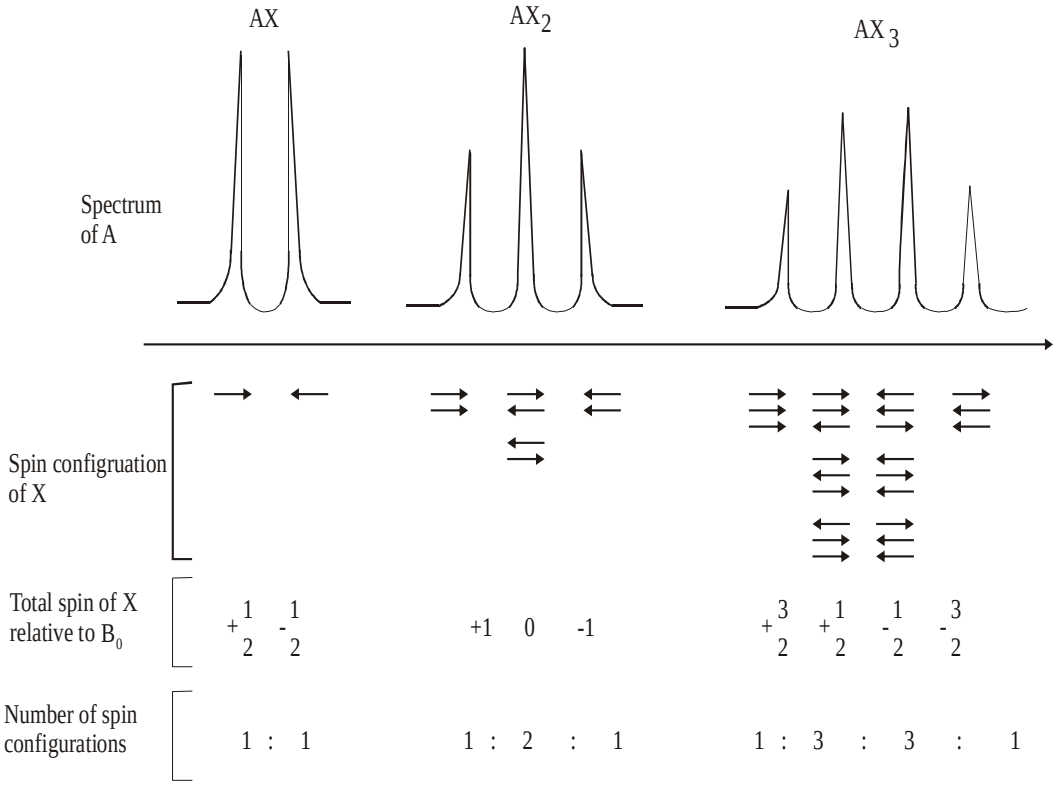




Energy levels of an AX spin system (a) and interaction of the nuclear spins A and X with I = 1/2, involving the bonding electrons (ethane molecular orbital model).

Parallel precesion is favoured for geminal nuclei A and X in such cases coupling constant is negative $J_{AX} < 0$ as shown in the figure (b). The splitting pattern of the peaks resulting due to coupling is given by the n^{th} binomial coefficients which gives the intensity of the splitted peaks. This is obeyed when the difference in the chemical shift of the nuclei A and X is large compared to their coupling constant J_{AX}. This is given by C-13 proton multplets. Becase the difference between carbon -13-nucleus. Larmor frequency

(22.6MHz at 2.13 Tesla) and proton (90MHz at 2.13 Tesla) is of several MHz. The coupling constant between C-13 and proton is from 0-300Hz.



Multiplicity and mutlplet line intensities of the A singnals in AX, AX_2 and AX_3 systems.

System	Multiplicity Multiplicity of signal A due to coupling with nuclei $X(I_X=\frac{1}{2})$
$AX(CH)$	$2=2.1.\frac{1}{2}+1$
$AX_2(CH_2)$	$3=2.2.\frac{1}{2}+1$
$AX_3(CH_3)$	$4=2.3.\frac{1}{2}+1$
AX_n	$2nI_X+1$

Multiplet line intensity Ratio of signal A due to coupling with n equivalent Nuclei $X(I_X=\frac{1}{2})$	
n=0	1
1	1 1
2	1 2 1
3	1 3 3 1
4	1 4 6 4 1
5	1 5 10 10 5 1

17.3.2 Mechanism of Heteronuclear coupling:-

According to "Fermi contact mechanism the nucleus say X and Y are magnetic and are connected by a single bond these heteronucleus are connected through electrons if say this electron spends some time close to the nucleus X than according to Pauli exclusion principle electron should have spin (-1/2). If I_z for nucleus is +1/2 as a result an electron with spin +1/2 shares orbital in X-Y bond

with another electron having +1/2 spin. This second electron with spin +1/2 spin spends more time with another nculeus Y having I_z = -1/2.

Thus within X have spin +1/2 of ^{13}C- ^{1}H coupling as shown in the figure below.

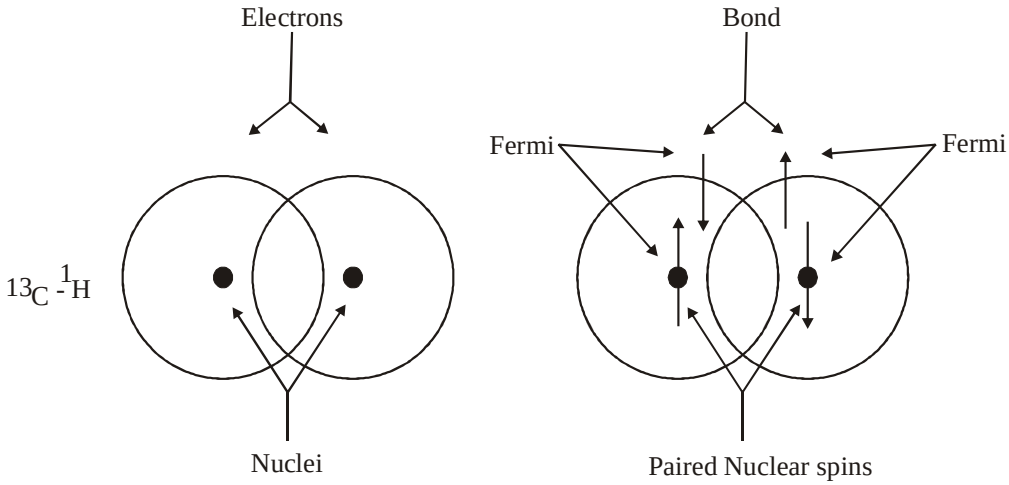


Diagram of the Fermi contact mechanism for the indirect coupling of two spins.

When the decoupling is off one bond coupling is noticed between C-13 and proton. The splitted signals helps in the easy pridiction of the structure.

The electrons involved in Fermi contact mechanism are occupied in s-orbitals only as the p-orbital has a node on nucleus. In case of proton electrons are in s-orbital so they contribute out for other nucleus those electrons participate which are in s-orbital only. So the magnitude of coupling constant depends on the s-character of the carbon with which it is attached.

Proton attached to sp^3 carbon atom (25% s-character) '$J_{^{13}C-H}$' is half in magnitude compared to that carbon which has sp hybridisation (50% s character). sp^2 hybrid carbon shows the coupling constant to have an intermediate value.The value for $J_{^{13}C-H}$ coupling for

Methane (sp^3) 125Hz Ethene (sp^2) 157Hz

Benzene (sp^2) 159Hz Ethyne (sp)249Hz

The correlation between the % s-character and magnitude of coupling constant J is given by the following equation $\%5(C-H)=0.2J\left(^{13}C-^{1}H\right)$.

In this equation if there is zero intercept, it means that there is no coupling when s- character is zero.

For cyclic system the contribution character by the orbitals can be ascertained by the value of J as shown below:

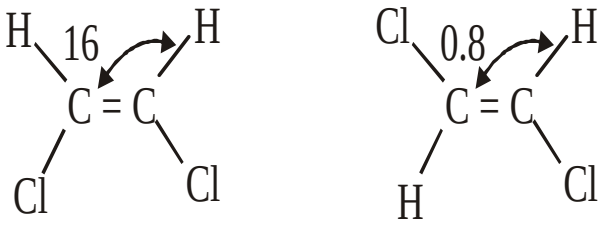
(a) Tricyclopentane : J = 144Hz : CH corresponds 29% s character

(b) Cubane : J = 160Hz : 32% s character(c) Quadricyclane : J = 179Hz : 36% s character

The relationship between J and s-character works in case of hydrocarbons but in case of polar molecules the variation in nuclear charge and effect of hybridisation changes the coupling constant.

Geminal proton-carbon coupling can include double bond (H-C=C). for these sp^2 carbon with coupling constant around -2.4Hz for ethene.

The stereochemistry of olefins can also be predicted by the coupling constant as is seen in case cis and trans dichloroethene for cis dichloroethene : 16Hz, Trans dichloroethene : 0.8Hz.



17.3.3 Homonuclear coupling

It deals whit the 13C-^{13}C correlation applying inadequate experiment (INADEQUATE) which means Incredible Natural Abundance Double Quantum Transfer Experiment which are mainly deals with the double quantum tranfer of those element whose abundance in nature is very low.

When such technique is applied to one dimensional and two dimensional they are called as 1D ADEQUATE AND 2D ADEQUATE respectively.

The coupling constants between the carbon atoms are evaluated in 1D-INADEQUATE experiment while correlation between 13C-^{13}C is done by 2D-INADEQUATE experiments.

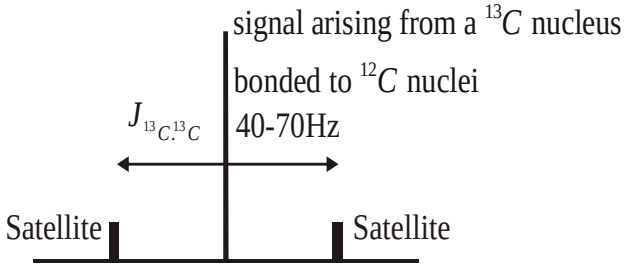
However inadequate detects only one in 10,000 of the molecules placed in the NMR sample tube.

The coupling of 13C - ^{1}H helps in the elucidation of the structure. However the situation for 13C - ^{13}C is quite different.

The natural abundance of ^{13}C is about 1% for the determination of coupling constants between neighbouring ^{13}C nuclei is required. The probability for the presence of 13C nuclei as the neighbour is 0.01 x 0.01 = 0.0001.

For performing such experiment some carbon atoms are labelled as ^{13}C than measurement of the satellite signals are done which are occuring on left and right side of the main signal but this technique suffers synthetic problem and cannot measure more than one coupling constant so a technique is required which can measure the coupling constant without labelling of carbon atoms.

In this technique the satellite signals belonging to carbon nucleus are observed left and right side of the main signal arising from 13C nucleus bonded to 12 C-nucleus. As shown in figure below.



View of the major and satellite signals in a proton-decoupled carbon NMR spectrum.

The satellite singnal has intensity of 1% with respect to main signal and as the satellite signal are resonating as a doublet, intensity decreasing to 0.5% of the main signal.

One bond coupling constant $^{1}J_{CC}$ is ranging between 40-70Hz.

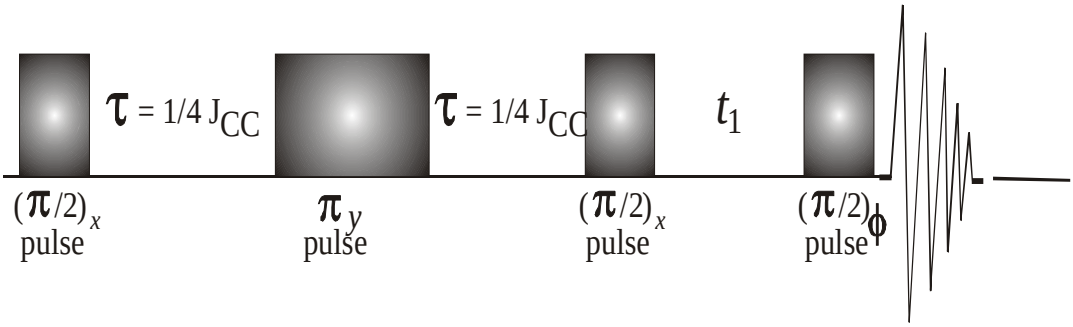
1ppm = 100Hz (100MHz instrument)

J_{CC} = 0.5ppm which is very small for carbon atoms. As the coupling constant value is less the satellite signals are located very close to each other.

As the intensity of main signal is 200 times more than satellite peaks as a result masking of satellite peaks are observed. However satellite signals are easily seen in proton-NMR where C^{13}-H coupling is large.

So in order to remove above cited problem if the main carbon signal is removed than 13C - ^{13}C coupling constants can be observed easily. This is done by 1D - INADEQUATE experiments.

1D-INADEQUATE technique pulse order is shown in the figure below.



Pulse sequence in a 1D-INADEQUATE experiment

In this technique proton channel is kept open and 90^0 pulse is sent to the system along X-direction and along Y-direction magnetic vector is rotated.

With frequency of J_{CC} at time unit $\tau = \frac{1}{4} J_{CC} / 2$ 13C - ^{13}C doblet signal will be diphase and as a result a phase difference will be there of 90^0 than after a pulse of 180^0 is applied along Y direction.

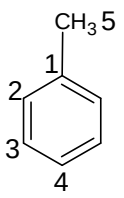
At , $\tau = \frac{1}{4} J_{CC}$ ie, second evolution of second period the magnetic vectors corresponding to the doublet satellite lines will have an allignment along +x or -x direction and corresponding to phase difference of 180^0. The significant stage in INADEQUATE is second pulse sent along X-direction. This pulse wills not parturb the magnetic vector of satellite however magnetic vector belonging to main signal gets rotated along -Z direction. At this stage NMR signal recorded along XY plane will occur due to magnetization and will mainly be due to satellite signals.By this technique 13C-^{13}C coupling constant can be evaluated.

17.4 Use of 13C-NMR

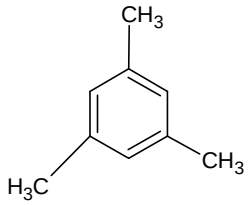
Data obtained from complete proton decoupling and selective decoupling of ^{13}C NMR can help in nucleidating of carbon skeleton.

(1) Sharp signals of ^{13}C peaks are seen for different carbon atom even when chemical environment is same

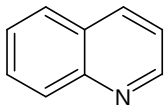
(a) for example proton decorpled spectra of toluene shown 5 peaks.



(b) Mesitylene shows three peaks



(c) Isoquinolene



9 peaks

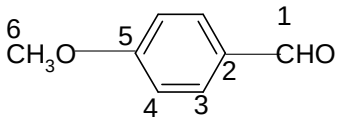
(d) Neopentane : 2 signals (singlet, quartet)

(e) Pentanol

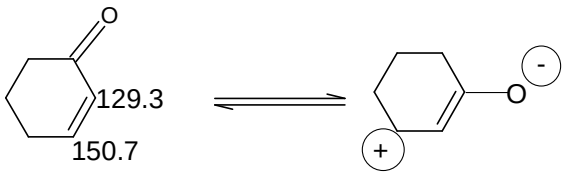
$$HO-\underset{62.1}{CH_2}-\underset{32.8}{CH_2}-\underset{28.5}{CH_2}-\underset{22.9}{CH_2}-\underset{14.1}{CH_3}$$

ppm

(f) p-methoxy benzaldehyde



(g) For α,β unsaturated carbonyl compounds the shielding and deshielding can be evaluated in terms of chemical shifts as shown below.



17.5 Summary

- The chemical shift of the carbon occurs as they are magnetically active as there nuclear spin quantum number is non zero
- The shift noit only depends on the electronegativity of the groupds ,mesomeric effect steric effectbut also depends on the dihedral angle of the carbon with carbon nearby and the proton also.
- The spectroscopy is greatly helpful in the structuiral determination of the compounds.

- The natural abundance being only 0.01% so the sensitivity is less than proton nmr spectroscopy

17.6 ReveiwProblems

1 why the 13 C-NMR spectroscopy is less sensitive than H-NMR spectroscopy?

2 how does the chemical shift of 13-C-NMR depends on the electronrgativity of the substituents?

3 discusss the effect of steric hinderenmce on the chemical shift.

4 show how does the chemical shift depends on the dihedral angle

5 writes the important uses of the 13-C-NMR spectroscopy

6. Predict number of 13-NMR signals in

   (a) Crystal of dimond (b) CO_2 gas

   (c) $Fe_2(CO)_9$ (d) O = C = S

7 Give expected order of . value for

   (i) sp^2 carbon atom of cyclobutane (ii) cyclopentane (iii) cyclohexane

8. Give ^{13}C - NMR of diethyl pthalate.

9. Give application of ^{13}C -NMR in organic and organometallic chemistry.

10. Compare ^{1}H - NMR and ^{13}C - NMR spectroscopy..

11. How 13C- NMR helpful distinguishing 1-hexene, 2-hexene, cis-3-hexene and Trans -3-hexene.

12. why 13C is NMR active while C12 not, explain.

13 why it is not possible to determine relative ratio of carbon atoms in a compound by integration of peak area in 13 CNMR as in PMR.

14 distinguish between cis and Trans 2 butene on the basis of 13CNMR spectroscopy.

17.7 References

- Organic spectroscopy: P.S.Kalsi,new age publication

- Spectroscopy by : H.kaur,Pragati prakashan
- Molecular spectroscopy : Banwell,tata Mc Graw hill
- Organic spectroscopy: Kemp,Palgrave
- Spectroscopy by Jagmohan

Unit - 18

Two Dimension NMR Spectroscopy

Structure of Unit

18.0 Objective

In conventional NMR spectroscopy we plot intensity against frequency, but the coupling being time variable and hence can be separated out in such a way as to establish which particular proton will couple with which carbon atom here comes two frequency axis and the technology becomes two dimensional NMR however the in formations are plotted in a pseudo three dimensional form

The art of presenting three dimensions which is a chapter to graphics and software in a cross section pattern by contours and peaks is the main objective of this unit

18.1 Introduction

NMR spectroscopy is a recognized powerful structural tool for the chemist the main difficulties involved are close chemical shift and higher order splitting. these are removed by using high magnetic field and new developments in pulse technology which eliminate uncertaininty in the interpretation of the spectra, a whole range of multipulse procedure are now available these includes COSY,INADEQUATE,APT,DEPT,NOESY and INAPT. in the present unit these all are discussed collectively.

18.2 INPET (Insensitive Nuclei Enhanced by Polarization Transfer)

Among the various nuclei having low occurrences in nature ^{13}C and ^{15}N are the two examples which have low sensitivity. During the coupling of these nuclei with other nucleus having high receptivity for example Proton. Much better observation can be taken with the help of pulse sequence. We should implement the Pulse in a certain way so that the desirable population of sensitive nucleus which is expressed in terms of 'S' is transferred to the Insensitive Nucleus which is abbreviated as 'I'. To serve this purpose a general sequence was given by Freeman known as Insensitive Nuclei Enhanced by Polarization Transfer i.e. INEPT, which is given as follows –

$^{1}H(S)\ 90°_{x}$ - 1/4J - $180°_{y}$– 1/4J - $90°_{x}$

$^{13}C(I)\ 180°_{y}$– 1/4J - $90°_{x}$- Acquire

Following figure exemplify the results of the Pulses for the two Spins. ^{1}H-^{13}C can be used as an example.

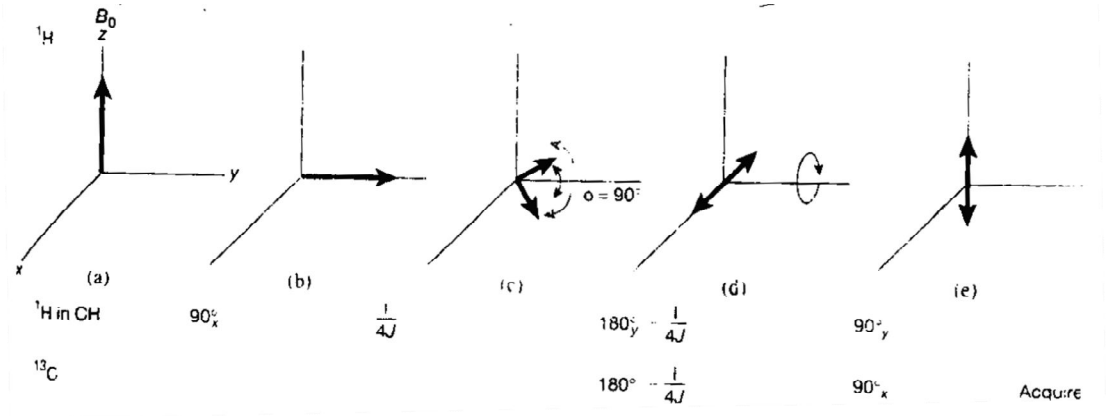


Figure 18-1: Pulse sequence for the INEPT experiment showing the effects on the ^{1}H spin vectors

Sensitive Nucleus (^{1}H)can be set into antiphase order by the application of Pulse of the first set. The Portionmagnetization will be shifted into the xy plane by applying the 1st 90^{o}pulse which is clearly visible in the fig 5-19(b).

The Pulse sequence given in the following figure shows the continuous divergence of Proton Vectors even on the application of simultaneous 180^{o}pulse on the Proton and the Carbon, which remove the effect of inhomogeneity.

The pulse sequence shown in figure 5-19(c) shows that the proton are separated by 90^o after $(4J)^{-1}$and it increase to 180^{o}after another $(4J)^{-1}$ interval, which is clear from the figure 5-19(d). The Proton Vector rotates onto the z-axis by the application of 90^{o}pulse in y-direction which is evident from the figure 5-19(e) while in the case-

a) The protons which points the +z direction are orbited to the two carbons whch are spin up 4 spin down carbon, but in.
b) Only the proton which ispointing in +z direction is rebited to the carbon spin + $\frac{1}{2}$ are also known as 'β' , whereas the proton related to the carbon spin - $\frac{1}{2}$ or 'α' used to point in the -z direction and this condition is called as **Antiphase**.

A comparative study between the spin energy of diagram after the application of Proton Pulse and the normal two spin system before applying the pulse can be carried out with the help of given

$$I_{max}(\text{INEPT}) = I_0\left|\frac{\gamma_{irr}}{\gamma_{obs}}\right|$$

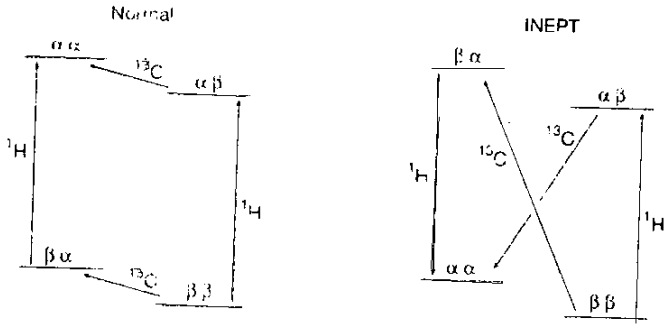


figure.

Figure 18-2: Spin states for a two-spin (13C – 1H) System, normally (left) and after the INEPT pulse sequence (right)

The ^{1}H resonance ($\beta\alpha \rightarrow \alpha\alpha$ and $\beta\beta \rightarrow \alpha\beta$) as compared to ^{13}C resonance ($\alpha\beta \rightarrow \alpha\alpha$ & $\beta\beta \rightarrow \beta\alpha$)

Mainly due to the Boltzman distribution which is represented by greater vertical displacement of the arrows for ^{1}H transition as compared to ^{13}C transition, the arrow represent to the absorption place in the given diagram.

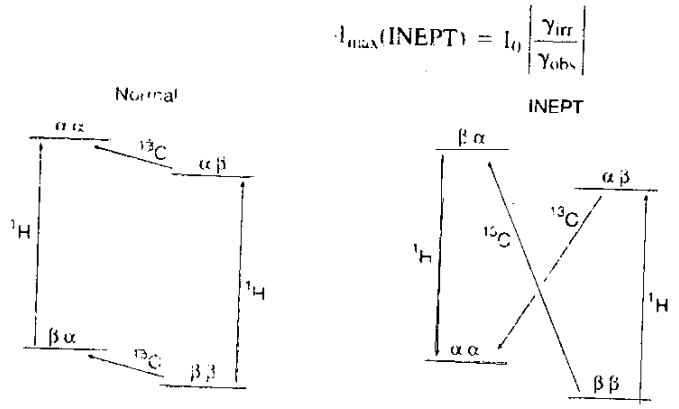


Figure 18-3 : Spin states for a two-spin (13C – 1H) System, normally (left) and after the INEPT pulse sequence (right)

For the insensitive nuclei, which can be expressed in terms of 'I' , Enhanced signals are obtained by the INEPT sequence following figure illustrate the example of Pyridine with the following of half positive and half negative peaks for a CH (Methine) group.

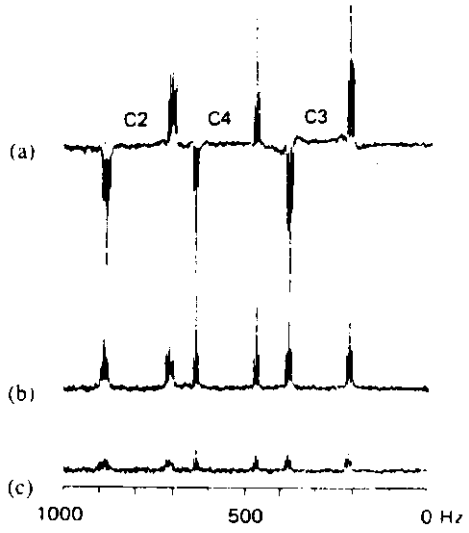


Figure 18-4 : The proton coupled ^{13}C spectrum of pyridine, (a) with INEPT, (b) with NOE only, and (c) unenhanced, on the same scale. (Reproduced with permission from G.A. Morris and R. Freeman, *J. Am. Chem. Soc.* , **101**, 760 (1979). Copyright 1979 American Chemical Society.)

The drawback in the INEPT experiment is the decoupling of -1 , 1 pattern for each CH resonance which leads to cancelling and making null signals. The INEPT intensifies of methylene triplet is -1, 0, 1 while for methyl quatrate

insensitive is -1 , 1-, 1, 1. On decoupling both will give null signals . To overcome this problem refocused INEPT pulse sequence was designed in such a way which could permit if decoupling by repeating the INEPT pulse second time in the following way.

Peak cancellation does not observed by the decoupling of protons during carbon acquisition. We will get a decoupled peaks with high intensifies in the spectrum. The comparative study for several experiments that have been performed for chloroform can be done with the help of following figure.

Fig 5-5

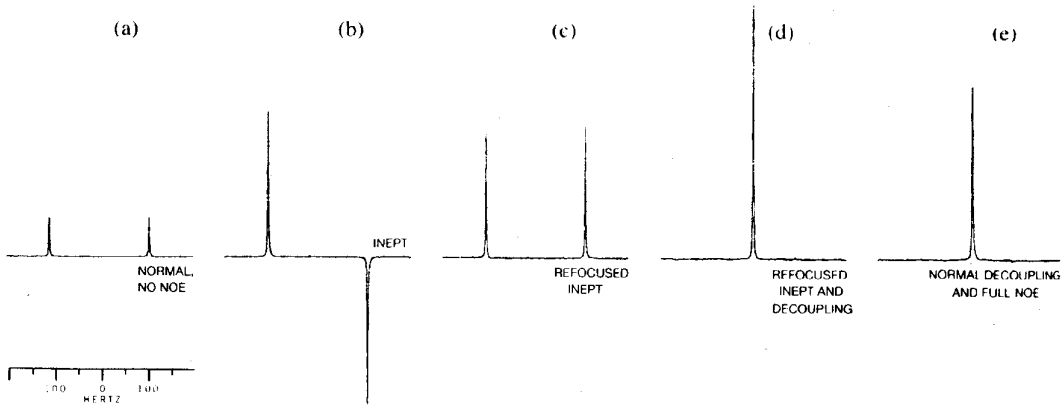


Figure 18-6 : The ^{13}C Spectrum of chloroform (a) without double irradiation, (b) with ^{1}H irradiation to achieve the INEPT enhancement, (c) with ^{1}H irradiation to achieve refocused INEPT enhancement, (d) with ^{1}H irradiation to achieve refocused INEPT enhancement and decoupling, and (e) with normal decoupling to achieve only the NOE. (Reproduced with permission from A. E. Derome, *Modern NMR Techniques for Chemistry Research,* Pergomon Press, Oxford, UK, 1987, p. 137.)

18.3 Inadequate

INADEQUATE stands for Incredible Natural Abundance Double Quantum Transfer Experiment. It was brought into limelight by Freeman. The pulse sequence was given to exhibit the double quantum resonance also known as Satellite resonance and to check the usual resonance termed as Single Quantum resonance.

The pulse sequence is given as - 90^{o}_{x} -T - 180^{o}_{y}–T - 90^{o}_{x}–Δ - 90^{o}_{ϕ} .The field inhomogeneity is refocused by the homonuclear 180°pulse . It permits the continuous divergence of the vector of various ^{13}C-^{13}C coupling setup. After application of first 90°pulse, the centre-band spin seems to stay on the y-axis if

synchronization takes place between the carrier frequency and the centre band of carbon resonance. We should fix the delay time to $(4J)^{-1}$which helps to diverge the vector from the spectrum of coupled ^{13}C-^{13}C by 180°after 2τ ($\phi = 2\lambda$ $(\Delta V)t = 2\lambda J$ ($^{2}/_{4J}$) = λ) and used to remain on the +x & -x axis respectively. When another 90° pulse is applied it helps in the rotation of centre band spin to the -z axis but the satellite spin remain as such *i.e.* on x-axis. So it can be concluded that the satellite spin can be detailed in the xy plane whereas the center band can meet.

The INADEQUATE spectrum obtained from Piperidine can be elucidated with the help of following figure.

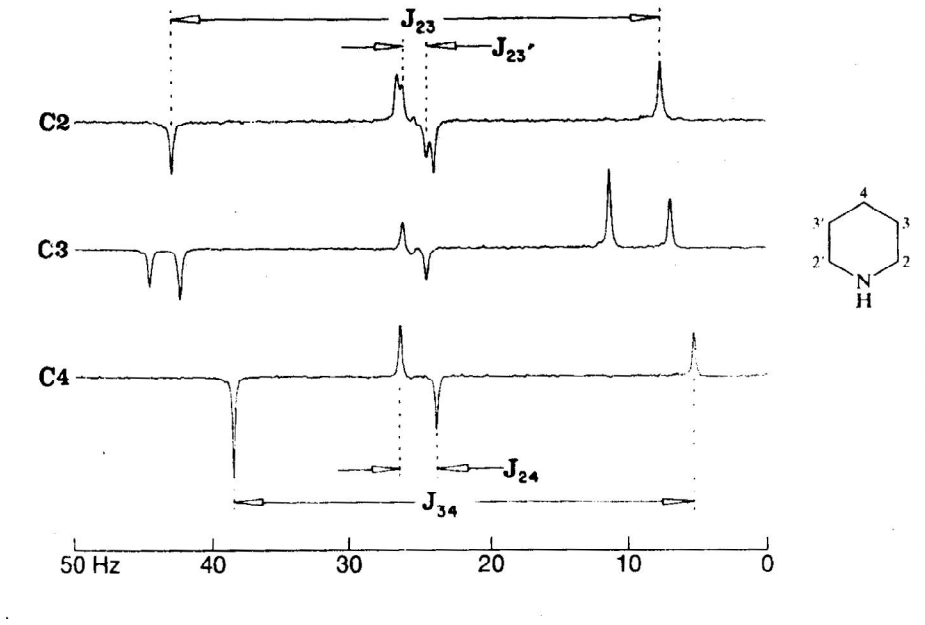


Figure 18-7: The one-dimension INADEQUATE spectrum for the carbons of piperdine. (Reproduced with permission from A. Bax, R. Freeman, and S.P. Kempsell, *J. Am. Chem. Soc.* , **102**, 4849 (1980). Copyright 1979 American Chemical Society.)

It is evident from the given spectra that the satellite (in double quantum) peak are in opposite phase. So with the help of a couple of peaks i.e. one up and one down which can also be expressed as (+1, -1) , ^{13}C-^{13}C coupling constant can be represented easily.

Two doublets can be observed in the spectrum of C-4 of piperidine in which the larger one indicate $^{1}J_{34}$ while the smaller one points to $^{2}J_{24}$. Two large sized doublets are obtained for C-3 because very little difference present in the one bond couplings $^{1}J_{34}$&$^{1}J_{34}$to the adjacent atom of carbon C-2 and the non-adjacent C-3 contains a small $^{3}J_{23}$in between $^{1}J_{23}$, $^{2}J_{24}$&$^{3}J_{23}$ can be seen in the spectrum of C-2.

18.4 APT (Attached Proton Test) Experiment

ATP stands for Attached Proton Test Experiments in which mode of four different types CH_n units is illustrated with the help of following pulse sequences. The behavior of Methine & Methyl group is shown as below :

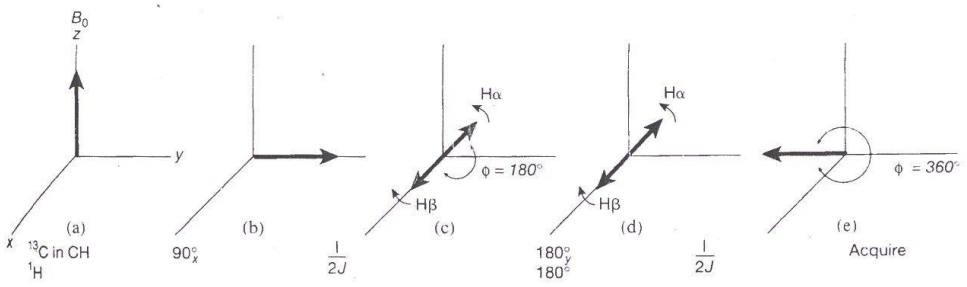


Figure 5-15 Pulse sequence for spectral editing of a methine (CH) resonance.

Figure 18-8: Pulse sequence for spectral editing of a methane (CH) resonance

And the Pulse sequence for n=0 (quaternary carbon) and methylene group can be seen in the following dia

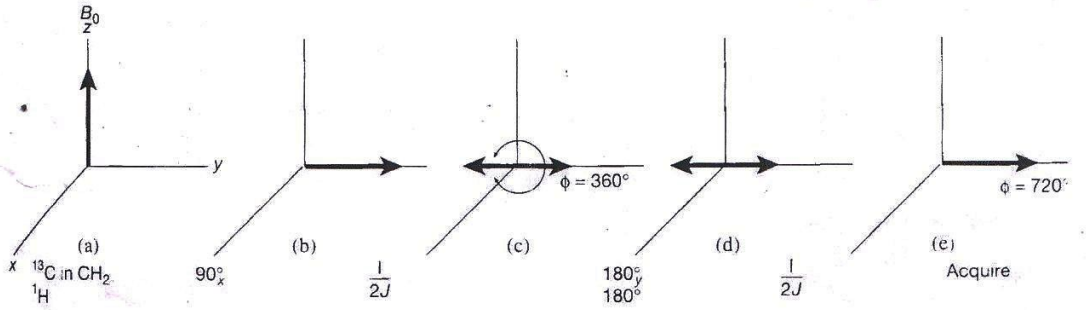


Figure 18-9: Pulse sequence for spectral editing of a methylene (CH_2) resonance

Among the various version of APT pulse sequence, one of the most accepted version is given in the following diagram :

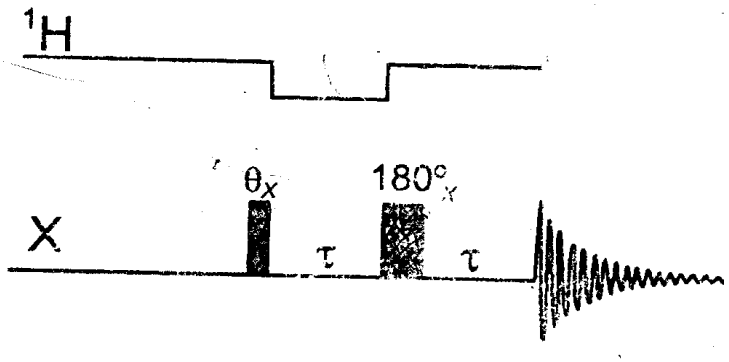


Figure 18-10 : An APT Pulse sequence

Two ways are generally adopted to carry out the APT experiment which shows resemblance with the standard ^{13}C NMR experiment. One of them includes the adjustments of the initial pulse (ɵ) to 90°, with relaxation delay (DT) of about

is continuous pulse, without a DT & setting $\alpha \neq 90°$ is the most effective method for collection NMR data.

If we want a rapid pulse, without a DT then the residual magnetization should be situated in the direction of +z axis. So it can be attain its equilibrium value by quick relaxation. This aim can be successfully bring about by adjusting $ɵ = \alpha + 90°$ (i.e. ,$90° < ɵ < 180°$). The magnitude of α is taken in such a way that it is having the same value in an ordinary C-13 NMR spectrum & then 90° is just added to that number. As a result residual magnetization commences along -z axis and is retrieved back to +z axis by 180° pulse.

Delay time 'τ' is choosen as the ultimate attribute to regulate the better focus of the carbon vectors. The factors which makes the matter a bit more complex are listed below :

i. The presence of variations in one bond C-H coupling seen in different molecules.
ii. The mode of the remnants of C-H depends on the hybridization of C-atom.

The former can be indemnify by setting the value of delay time 'τ' close to the mid point of the proposed range of one bond C-H coupling constants.

The next problem is caused by the value of sp^2 hybridized carbon atoms which dephase and refocus at comparatively faster rates then those shown by the sp^3 hybridized carbon. In case, sp^3 hybridization is present in all the carbon atoms of a compound the 'τ' should be fixed to $(J)^{-1}$ = & But if , sp^3 hybridization is uniformly present in all atoms of carbon then these values cab be changes to ^1J cn~ 160 Hz. & τ = 6ons. If the presence of sp^2 and sp^3 hybridizationboth have been detected then the value being preferred are ^1J c= 140 Hz & τ =7Hz .

Following figure shows the fully edited apt spectrum with $\tau = (J)^{-1}$. We can easily differentiate between methyl or methane group i.e. the carbon atom attached with odd number of Hydrogen atom and methylene or the quaternary carbon i.e. the carbon which are attached with the even number of Hydrogen atoms including zero & 4 even number

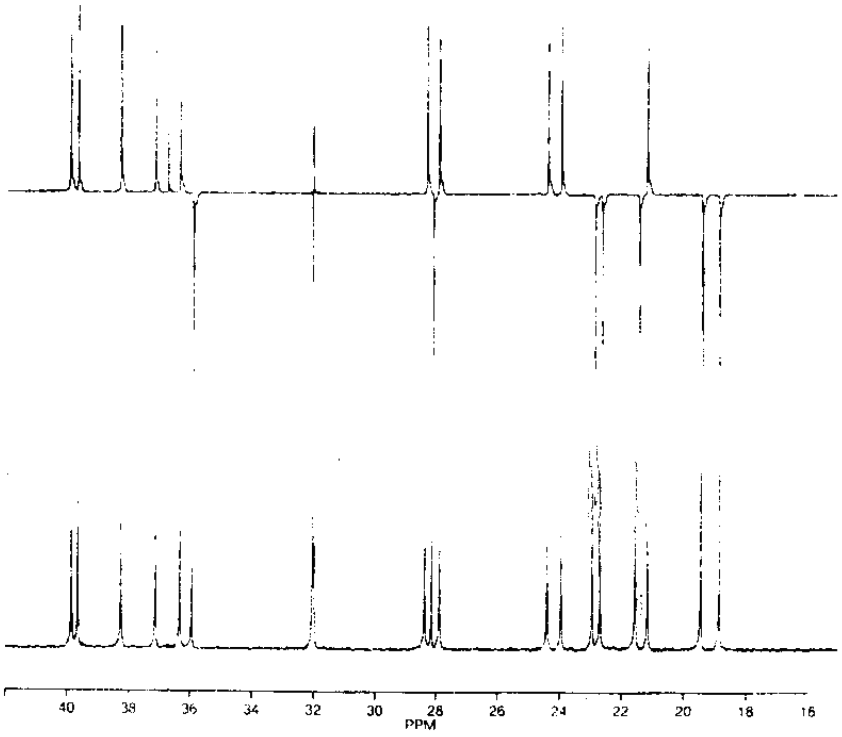


Figure 18-10: Lower: The normal proton-decoupled 13C spectrum of cholesteryle acetate.

Upper : The attached proton test (APT), phased do that CH2 and quaternary carbons are negative (Reproduced with permission from A. E. Derome, *Modern NMR Techniques for Chemistry Research,* Pergomon Press, Oxford, UK, 1987, p. 261.)

Relianceof APTon one-bond C-H coupling values &inadequancy to differentiate between the CH3 and CH carbons are the two major drawback of APT.

18.5 The Noesy Experiment

The basic COSY experiment has been further extended to NOESY experiment in which a third 90° pulse follows the two pulse sequence of basic COSY. To distinguish between the cross peaks obtained by positive NOE's (the cross peaks are positively phased) from those of COSY artifacts and EXSY (exchange spectroscopy) which occurs as negatively phased signal, if present in the spectrum, NOESY experiment is mandatory to carried out in phase sensitive mode. The diagonal peaks and cross peaks found as a result of negative NOE's will appear as negatively phased signals. Luckily COSY artifacts are generally not observed in the NOESY spectra. As they occurs in the opposite phase so can be easily differentiated from positively phased NOESY cross peaks. But they are harmful as they result in the cancellation of the positive cross peaks.

In the suspicion of signal cancellation because of COSY artifacts, it is advised to perform the selective ID NOE experiment as we have just described. This is useful to avoid the abashment caused by the misjudgment of evident NOE results. Lower temperature and lower field strength are the two conditions, which are advised to be used while running the NOESY experiment as it can help in the detection of COSY induced signal cancellation.

The two NOE's of molecules can be increased by decreasing the temperature, which results in the increment of dipole-dipole contribution to the overall relaxation, and decreasing the field strength, which reduces T_1's. Same measures are applied in case signal cancellation is detected due to EXSY artifacts. One more additional effect can seen, on lowering the temperature reduction on the exchange rate occur which reduces the EXSY cross peaks.

Mixing time (τ_m) and repetition times are the two factors which are responsible for the better detection of the NOESY correlation and their dependency is on $^1HT_1$'s in the molecule. RT's for small or moderate sized molecules is suggested to be around $2T_1$. So, for small molecules (with molecular weight approximately 400-450) it can be fixed to 1.2-1.8s for moderate sized molecules (with molecular weight ~ 500-750) can be 0.9-1.2s & if T_1's is unknown then it can be set to 2-3s.

NOESY cross peak are affected by mixing time also, if it is too short than NOE enhancement will not gain the desirable intensity which is required to detail and observe the NOESY cross peak. On the other hand if it is too long then due to relaxation the NOE enhancement will vanish causing absence of cross peak. So, it should be adjusted around the average T_1 value which can be 0.3 – 0.65 for the molecules (will around 400 - 750) and 1 – 2s for small molecules.

Suitable criteria for NOESY experiments are given below –

1) T_m for small or moderate size molecules should be 0.3 – 0.6s.
2) RT for small molecules (mole. will around 400 - 450) should be 1.2 – 1.8s and 0.9 – 1.2s for moderate size molecules or 2 – 3s for very small molecules.
3) Stead-state scan should be 8.
4) It should be in the multiple of 8.
5) It should be 256.
6) CP should be 768.

18.6 COSY (Correlation Spectroscopy) Experiment

Basic COSY

COSY is the most significant method providing the appropriate results even in the absence of proper calibration of 90°pulse. It is advised to perform the COSY experiment in absolute value instead to phase sensitive mode to avoid the mixed-phased line shape which usually occur in phase sensitive corilour plots. In COSY experiment different types of peak are obtained named as cross peak and diagonal peaks, if the former is phased to show the absorptive signals then the later will be anti-phase showing dispersion pattern.

The cross peaks which are situated near the diagonal remain hidden due to the presence of long tail like dispersion pattern shown by the more prominent diagonal signals.

Numerous disadvantages of phase-sensitive COSY over the other COSY experiments favors to avoid it.Gradients adaptation is a beneficial features of COSY experiments, its pulse sequence is shown in the given figure.

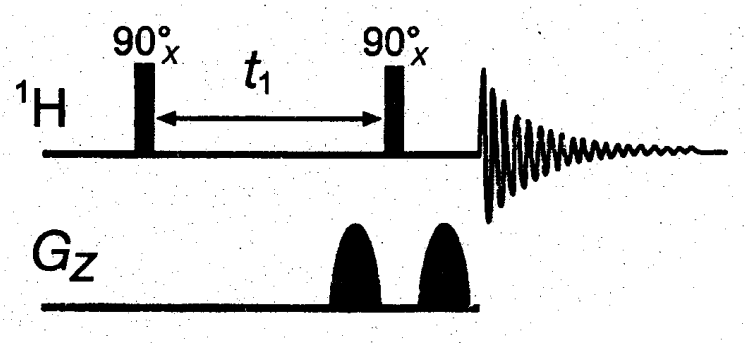


Figure 18-11: The gradient COSY pulse sequence.

The advantages of gradient COSY (gcosy) over non-gradient is the requirement of hardly two scan per time increment to set in possession of a spectrum by satisfying a phase cycle, which is not possible in the case of later. One more features gcosy proves its superiority over the non-gradient is the need of comparatively shorter relaxation delay times.

If RT is too short mainly when is less than optimum number required for phase cycle then Artifacts are generated . So, combined with LP method then not more than a minute is required to performed the gcosy experiment. Similarities in cosy pulse sequence &gcosy pulse sequence can be seen with the help of following diagram.

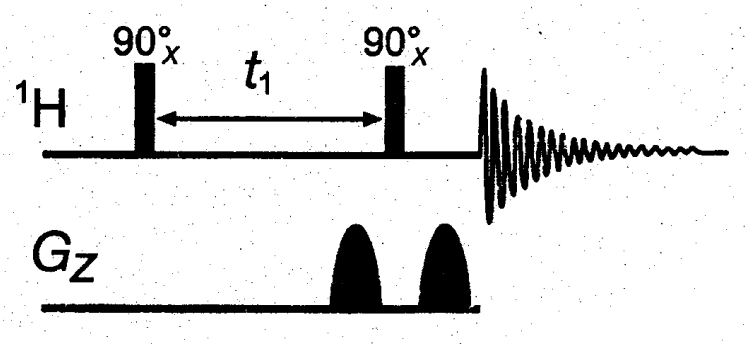


Figure 18-12 : The gradient COSY pulse sequence.

The second90°pulse is being flamed by a couple of 2 gradients. The suitable criteria for cosy experiment are given below –

1) Steady-state scan include 16 gradient scan and 8 non-gradient.
2) itshould be 1-2 (gradient) or 8 or 16 for non-gradeint.
3) should be 512.
4) LP should be 512.
5) It include pseudo-echo, sine bell or squared sine bell weighting.

18.7 Cosy 45

The COSY 45 experiment resemble the basic COSY experiment but the only difference between these two is of final pulse which is 45° in COSY 45 experiment instead of 90°. It is evident from the given figure.

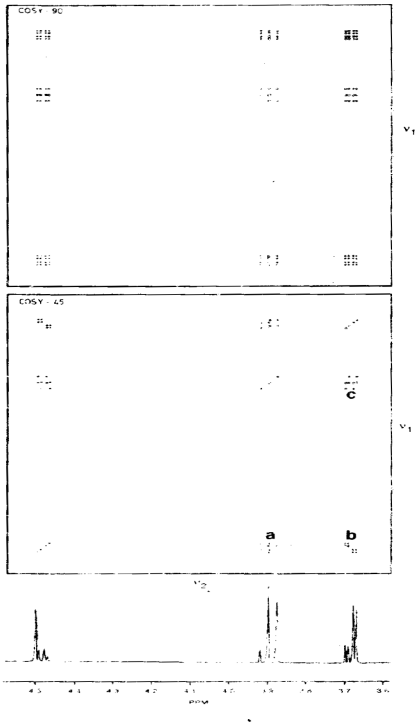


Figure 18-13 : The COSY90 (top) and COSY45 (bottom) spectra of 2,3-dibromopropionic acid. The ID spectrum at the bottom is a cross section through the COSY45 spectrum (Reproduced with permission from A. E. Derome, *Modern NMR Techniques for Chemistry Research,* Pergomon Press, Oxford, UK, 1987, p. 228.)

The COSY 45 experiment is more remarkable than the basic COSY experiment which can be justified by the following two reasons

1. 15% loss of sensitivity with respect to COSY is compensated by COSY 45 wherein suppression of both diagonal signals and cross peak which occurs from parallel transitions that is very close to the diagonal.
2. Cross peaks obtained by regressive and progressive transition can be observed by reducing the intensity of these signals leading to better results and these cross peaks are situated near the diagonal.

The comparative study of COSY 45 spectrum of T-2 toxin (7-1) with basic COSY spectrum can be performed with the help of following figure.

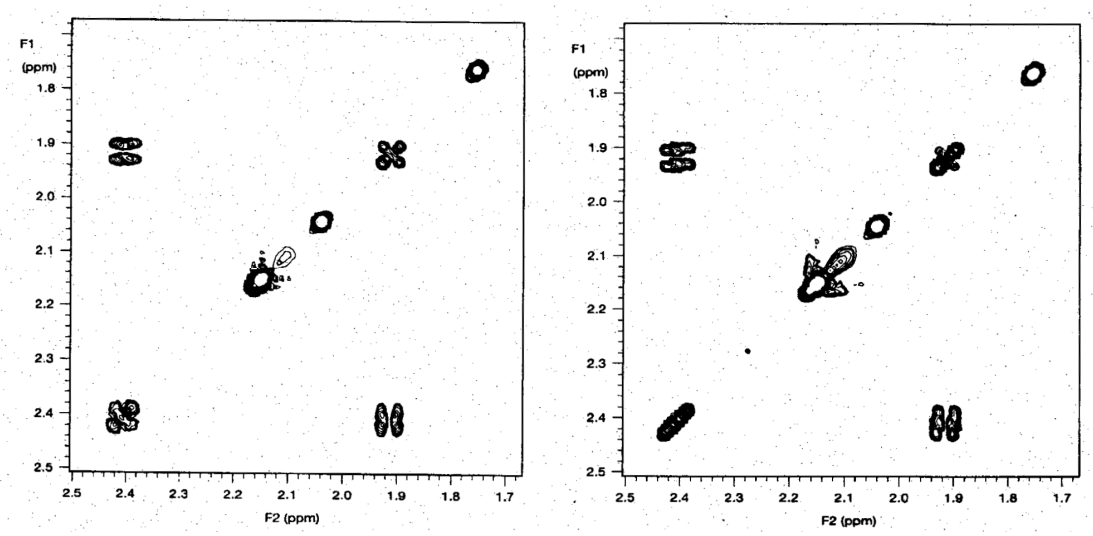


Figure 18-14 : Expansion COSY spectrum (left) and COSY45 spectrum (right) of T-2 Toxin.

The difference can be easily seen by observing the simplified diagonal signals and the cross peaks which are lifted in the COSY 45 spectrum.

18.7 Long range COSY

Cross peaks can be created by the germinal and vicinal (two or three bond) couplings leading to dominant magnetization which serves as a general conception of COSY experiment. Much significant information can be gathered by using long- range couplings. The magnetization transfer obtained by small couplings can be reinforced by installing a certain delay Δ during the evolution

along with detection periods [90° - T_1 – Δ - 90° - Δ – T_2 (acquire)]. Following figure offers a comparative study of COSY and LRCOSY experiment for naphthobiphenylenedianion.

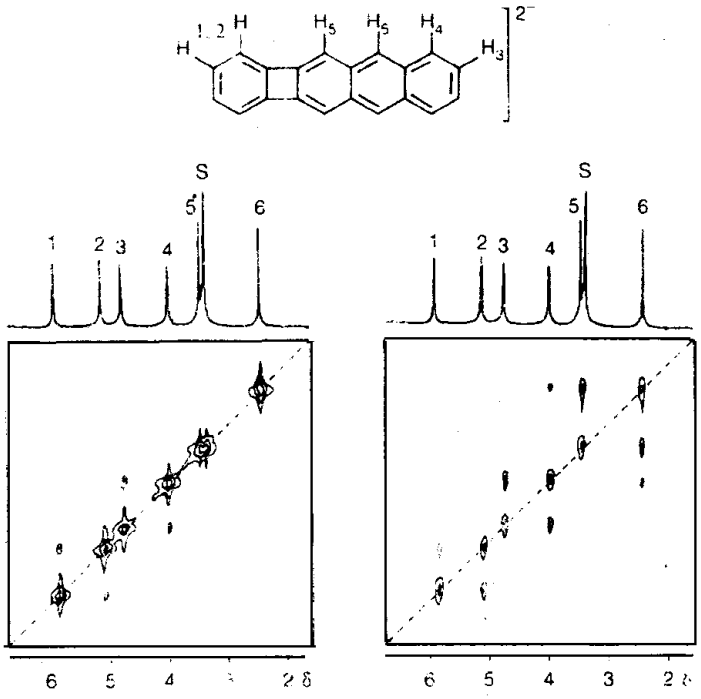


Figure 18-15 : The 400 MHz COSY (left) and LRCOSY (right) spectra of naphthobiphenylene dianion. (The singal S is from Solvent). (Reproduced with permission from H. Gunther, *NMR Spectroscopy*, 2d ed. John Wiley & Sons, Ltd., Chichester, UK, 1995, p. 300.)

The COSY spectrum contains the cross peaks which are found only between the [1,2 and 3,4] ortho neighbors while the LRCOSY spectrum is rich in additional cross peaks between [5,6 and 4,6] neighbors. So, the LRCOSY experiment is better to offer the useful information related the connectivity of fused aromatic rings.

18.8.0 DQF – COSY Experiment

Double Quantum Filtered experiment involves the addition of an extra 90° pulse and the multiple quantum coherences is converted into observable magnetization by phase cycling. Thus 2D spectrum is generated in which none of the singlet is obtained along the diagonal. We do not get any solvent peak in the spectrum of lysine, $^{+}NH_3CH(CH_2CH_2CH_2CH_2\ NH_2)\ CO_2^-$ as shown below

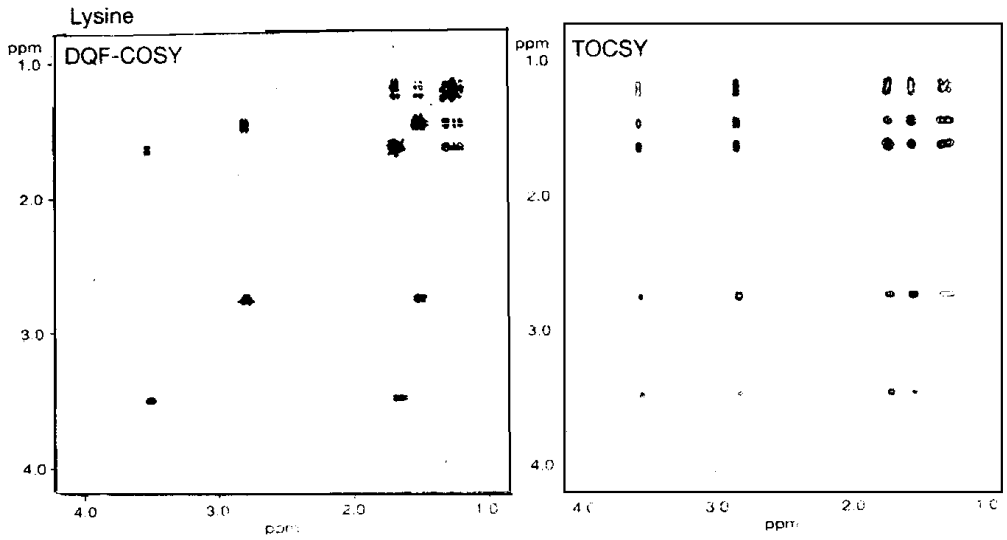


Figure 18-16 : The DOF-COSY & TOCSY spectra of lysine. (Reproduced with permission from J.N.S. Evan, Biomolecular *NMR Spectroscopy*, Oxford University Press. Oxford UK, 1995, p. 428.)

The prominent property of phase sensitive DQF-COSY is that the diagonals and cross peaks both are tuned into pure absorptions (at the same time) due to double quantum filtration.

Decrement in the size of diagonal signals takes place by this feature which is very helpful in the analysis of cross peaks near the diagonal. Sensitivity loss by a factor of 2 is suffered by the DQF-COSY which proves to be its one and only disadvantage. Further more simplified spectra can be obtained by using the Triple Quantum Filtered COSY (TQF - COSY) experiment which eliminates both singlet & AB or AX quartets, and is rarely used.

18.9.0 DEPT [Distortion less Enhancement by Polarization Transfer] Experiment

The DEPT experiment is in the habit of choosing as more desirable method in editing procedures. The pulse sequence of DEPT experiment is as shown below

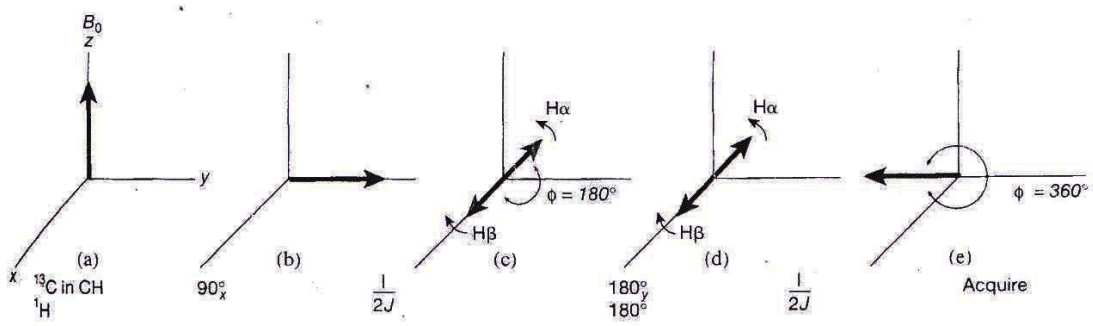


Figure 18-17 : Pulse sequence for spectral editing of a methane (CH) resonance

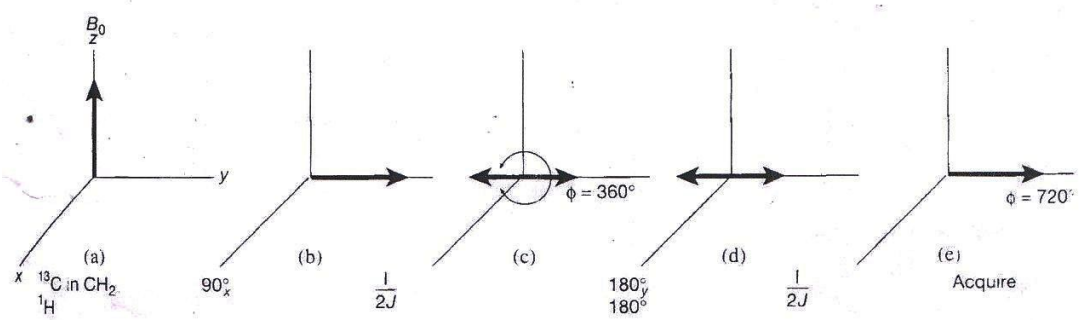


Figure 18-18 : Pulse sequence for spectral editing of a methylene (CH_2) resonance

It is very useful method to determine the spectra of carbon attached with hydrogen containing groups like methyl, methylene or methine carbons]. It is evident with the help of the following figure.

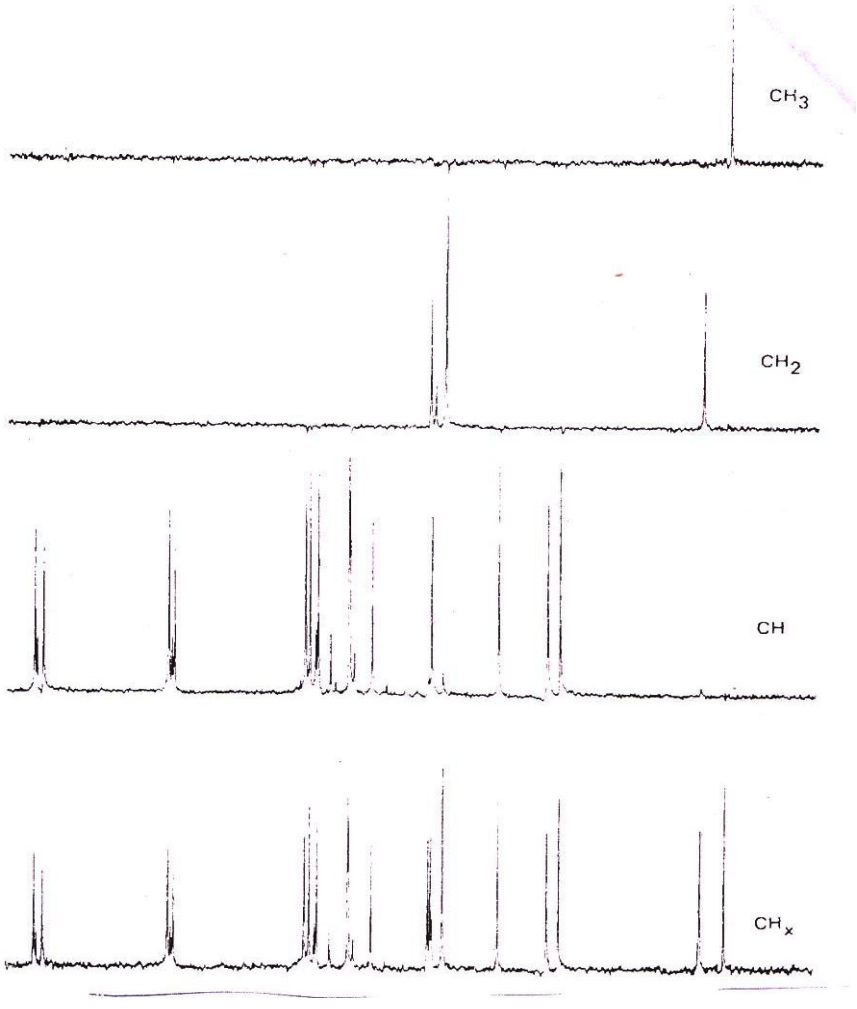


Figure 18-19 : Spectral editing of the 75.6 MHz 13C spectrum of the trisaccharide gentamycin by the DEPT sequence. The bottom spectrum contain resonance of all carbon attached protons, and the ascending spectra are respectively of the methane, methylene, and methyl carbons (Courtesy of Bruker Intruments, Inc.)

$(2J)^{-1}$ is the delay time which is expressed in terms of τ. As it is seen in the APT experiment that it is correlated with the hybridization of carbon atom [it should be 4ms for sp^3 hybridization, for sp^2 hybridization it should be 3ms and 3.6ms is applied to both].

The variable 0 pulse is the characteristic of the DEPT experiment which provide editing results showing resemblance with those produced in the APT experiment [by changing the delay time (τ)]. On comparing the DEPT and APT, DEPT is less sensitive to variation in $^1J_{CH}$ as it depends on an angle instead of delay time. Following results are obtained with different θ values

1. θ = 45° gives all carbons attached with protons/hydrogen atoms.
2. θ = 90° gives only methine carbons; finding some other signals.
3. θ = 45°, 90° and 135° gives fully edited spectra of CH and CH_3 with oppositely phrased CH_2 group and signals for quaternary carbons are absent.

The relaxation delay times in DEPT experiment is not correlated with the x-nucleus, T_1's but it is a function of the ^{1}H-, as it is a polarization transfer experiment.

Some more spectra criterion can be adopted as follows

1. DT should be 0.5-1.5s, which is 1-3 $^1HT_1$(max).
2. Steady state scans should be minimum of 0.8.
3. Number of scans should be multiple of 4 (serving the purpose of phase cycling)

It is also advised to add the collected information in between the 32 scans. It is generally for more stability purpose in longer experiments, carried out as follows –

For spectral editing despite of acquiring each spectra with different angles it is better to have 32 scans for θ = 45° and 32 scans for θ = 90°. This is done twice to get same S/N ratio and finally 32 scans for θ = 135°.

In order to accumulate sufficient scans for producing 4 scans whose S/N ratio can be approved, the above four-step cycle should be repeated. The sub-spectra

is produced by the addition and subtraction of these spectra in a proper way that we have already described in the figure.

DEPT is much more sensitive to some problems than the typical one-dimensional tech because the former is a subtraction experiment which is granted as one of its drawback. Steady state or dummy scans can be used to avoid the stability problems in many experiments.

The various difficulties pertaining to the poor signal cancellation are as under:

1. Lock stability – The lock power should be kept below saturation and the lock gain should be in the range of 30%.
2. Temperature of the sample should be constant during experiment.
3. Pulse calibration – It is critical to calibrate ^{13}C at 90° and 180°
4. Incomplete cancelation in spectral subtraction; steady state scans should be critical.

DEPT experiment has been modified to DEPTQ that seems to be much significant to determine the molecular structures of compounds.

DEPTQ is rich in editing features & polarization transfer sensitivity as in DEPT but its major advantage over DEPT is that it allows the detection & display of non-protonated nuclei in quaternary carbons.

18.10.0 Structured Elucidation – By DEPT experiment

DEPT experiment is the most significant method for the determination of number of H attached to each carbon atom present in any compound. The presence of 6 methyl, 4 methylene & 7 methine carbons in the 50mg sample of T-2 toxin is evident by the DEPT spectrum shown in the following figure.

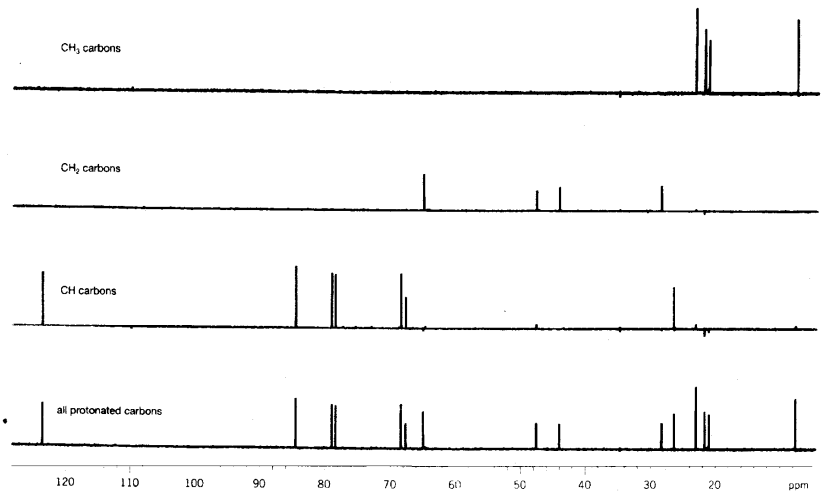
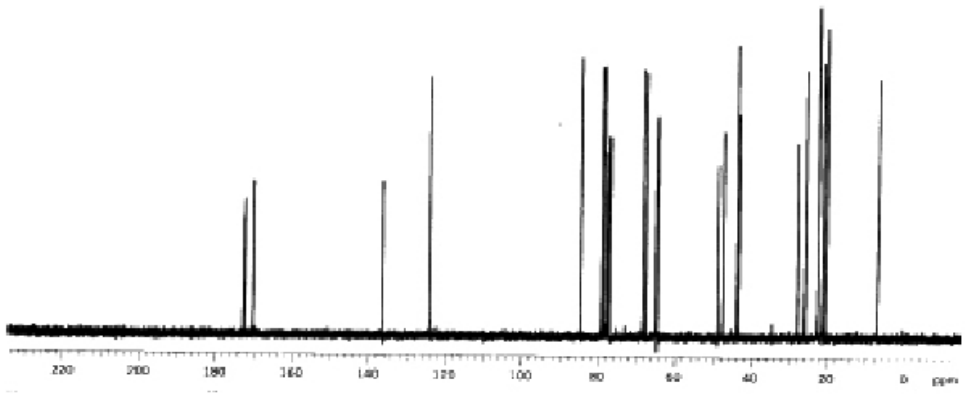


Figure 18-20 : DEPT subspectra of T-2 Toxin

It detects seven quaternary carbon by eliminating the 17 carbons bonded with hydrogen from the 24. The data of DEPT is shown in the 3rd column of following table.

Figure 18-21 : The 125MHz ^{13}C NMR Spectrum of T-2 toxin.



18.11 Summary

- 1H-1H connectivity comes from the coupling between hydrogen atoms present on adjacent atoms .
- Spin decoupling or double resonance technique falls under the category of 1D NMR
- COSY(Corelated spectroscopy) includes those techniques by which all 1H-1H connectivity can be evaluated without the intervention of spin decoupling.
- Both APT and DEPT work together to supplement the information obtained from off –resonance CMR spectra.
- the technique INADEQUATE is applied to detect the direct attachment between two 13 C atoms .this includes various mathematical operations.

18.12 Questions

1 Explain the COSY effect

2 What do you mean by NOESY

3 Write explainatory notes on

(a)APT

(b)INAPT

(c)INADEQUATE Techniques.

4 What are different reason for the close chemical shift,explain]

5 What are the advantages of using high magnetic field.

Unit-19

ORD and CD

Structire of the Unit :

19.1 Objective

Polarimetery is one of the oldest instrumental techniques which is used in the determination of the concentration of the substance it also find use to establish a

correlation between the structure and the optical rotation .the increasing importance of optically rotatory dispersion and circular dichorism measurement has been used in solving structural problems of organic and inorganic compounds. These two methods together provide a better understanding about the molecular structure and have been found useful in solving many chemical problems

19.1 Introduction

The first manifestation of optical activity, i.e property of rotating the plane of polarization of plane polarized light was pointed out by Arago in 1811. The increasing importance of optical rotator dispersion and circular dichroism in solving structural problems is evident by large number of research paper published every year involving organic as well as inorganic compounds. These two methods provide a better understanding about the molecular structure and can solve many chemical problems which either of these methods is unable to furnish individually.

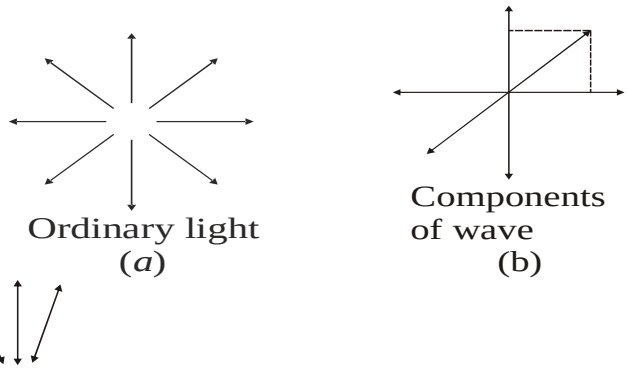
19.1.2 Theoretical considerations

19.1.3 Optical rotator dispersion (ORD) and circular dichroism (CD)

A beam of ordinary natural light can be thought of as a wave form (having an electric component and mutually perpendicular magnetic components) in which the vibrations occur in all plane perpendicular to the direction of propagation. If this natural light is passed through a component called polarizer, the emerging light beam vibrates only in the plane of the unabsorbed or unrefracted component. This is called as linearly polarized or plane polarized light.

Two linearly polarized light beams with equal amplitude but with their plane of polarization at right angles may be combined with a proper phase difference; they give rise to circularly polarized light. This is shown below.

Thus circularly polarized light represent a wave spiraling, around the direction of propagation of the ray either clockwise (right handed) or anticlockwise (left handed)



Polarisation of light

Different type polarisation

19.1.4 Optical Rotator Dispersion (Ord) And Circular Dichroism (Cd)

19.1.5 Optical Rotation And Circular Polarisation

When observed along the direction of propagation beam of plane polarized light appears to have its electric vector, which oscillates as a sine wave with the frequency of the light, confined to a perpendicular plane. The electric vector of the linearly plane. The electric vector of the linearly polarised light may be thought of as being made up of two components E_L and E_R which correspond to the left and right hand circularly polarised beams and have equal amplitudes. The right and the left hand circular vibrations meet in a line which determines the position of the plane of polarisation. In the following figure.

Thus circularly polarized light represent a wave spiraling, around the direction of propagation of the ray either clockwise (right handed) or anticlockwise (left handed)

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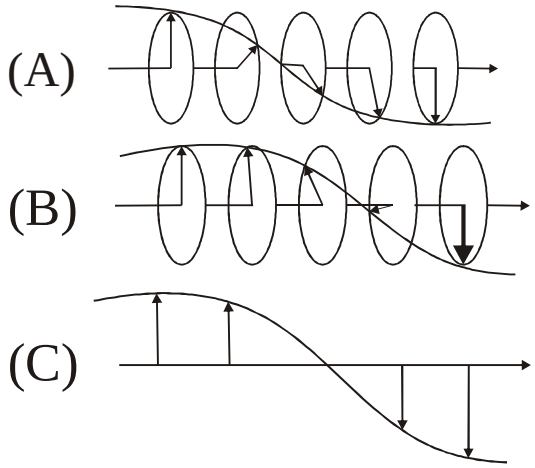


Left and right hand cirularly polarised light and their resultant plane polarised light

Shows how two such beams give a plane polarised resultant.

On passing the plane polarised light through an inactive material, the velocity of both the components are affected to the same extent and the plane of polarisation remains unchanged. On the other hand the interactions of two circularly polarised beams with an optically active material is found to be different thus during the passage through an optically active substance, one of the circularly polarised components is slowed down and thus have a different velocity than the other component. As a result of this phenomenon, the plane of polarisation is rotated through a definate angle due to the difference in the refraction of left and right circularly polarised light.

It is known that following relations exists between the refractive index (η) of medium and the velocity of light $\eta = \frac{C}{v}$ where η = refractive index of medium, C = velocity of light in vacuum, v = velocity of light in medium.

It was established by fresnal (1829) that's if a material shows different indices of refraction for the two components of a plane polarised light than one beam would be showed down on passage through the medium and plane of polarisation would be rotated. Thus we have

$$\eta_r = C/v_r;\ \eta_l = C/v_l \quad \text{or} \quad \frac{\eta_r}{\eta_l} = \frac{v_l}{v_r}$$

It was shown by fresnel that the angle of rotation per unit length (cm) of themedium is given by $\alpha = \frac{\pi}{\lambda}(\eta_l - \eta_r)$ Where n_l and n_r are the refractive indices of the light and right circularly polarised lights respectively and λ is the wavelength of the light. Thus it can be concluded that optical rotation is observed when $(\eta_l \neq \eta_r)$ and if $(\eta_l < \eta_r)$ it will be in opposite sense. Now because the differences in the index of refraction for right and left circulaly polarised light is small, an appreciable value of angle of rotation is observed. The quantity $(n_l - n_r)$ is called CIRCULAR BIREFRINGENCE.

19.1.6 Rotatory Dispersion

It is known that's refractive index (η) of the medium changes with the wave length λ of the light. It is given by the

$$n^2 = A + \frac{B}{\lambda^2} + \frac{C}{\lambda^4} + ...$$

Where A, B, C etc are constants. The variation of angle of rotation with the wave length of light is Known as ROTATORY DISPERSION.

It was Biot (1860) who pointed out that rotator dispersion is more interesting characteristic of a substance than a simple measurement of a given wavelength

Biot in 1817 suggested that the following inverse square relation $\alpha = \frac{A}{\lambda^2}$ exists between the angle of rotation and wavelength of light. In this relation A is a constant. Pvon lang (1863) and J.stephan (1864) derived an improved equation

using an additional constant B. This is given below $\alpha = A + \frac{B}{\lambda^2}$.

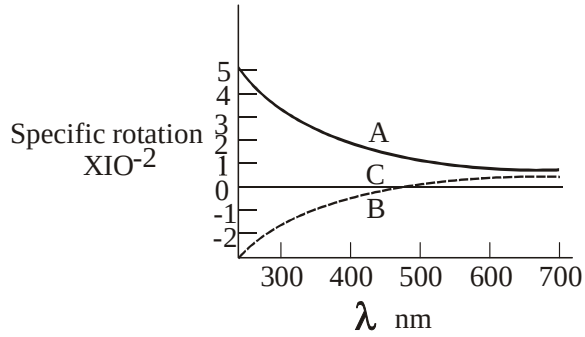
Lastly it was cotton (1896) who studied rotator dispersion detail and found that the curve showing the rotator power as a function of λ is not plane but possesses an S – form which, by its sign amplitude and appearance is characteristic of the chromophore taken and also of its asymmetry. He found that all the active chromophores contributes to the measured rotator power and out side the region of absorption this decreases with the increasing distance from the absorption band.

Another important contribution to the subject of rotator dispersion was made by P. Drude (1900) who found that the specific rotation can be expressed as a function of wavelength by the following equation $[\alpha] = \frac{k_1}{\lambda^2 - \lambda_1^2} + \frac{k_2}{\lambda^2 - \lambda_2^2} + \frac{k_3}{\lambda^2 - \lambda_3^2} + ...$ where k_1 , k_2 and k_3 etc are the rotation

constant corresponding to etc which are the wavelength corresponding to maximum absorption of the optically active absorption bands and is the wavelength of measurements. Substance which obey the above Drudes equation are said to exhibit "complex rotator dispersion". An example of such compound is ethyl tartarate.

Drudes equation in its simplest form can be written as $[\alpha] = \frac{k}{\lambda^2 - \lambda_0^2}$. In this equation λ is the dispersion constant , k is the rotation constant and these are characteristic of the substance under investigation. represent the wavelength of the nearest optically active absorption band in the ultraviolet or visible parts of spectrum.

A typical ORD curve is shown in the figure below



ORD Curves

In this curve A represents the plane positive ORD curve and B the plane negative curve. The word plane here means that there are no maximum or minimum in the curve.

In these equation λ and λ_0 are expressed in microns (μ) 10^{-4} om units, thus $4000A^0$ or 4000×10^{-8} om is 0.4 μ and so λ would be 0.4. The above Drude's equation is a one term equation and the substances obeying this equation are said to exhibit SIMPLE ROTATORY DISPERSION.

If the above single term equation is followed than it is evident that a plot of $\frac{1}{[\lambda]}$ against λ^2 should be a straight line but it is important to note that a straight line does not necessarily show simple dispersion.

If we use two terms drude's equation, it is evident that the first term is greater than second term (ie $k_1 > k_2$ or $\lambda_1 > \lambda_2$) thus $[\alpha]$ increases or decreases regularly with decreasing wavelength. Under these condition the dispersion is called as

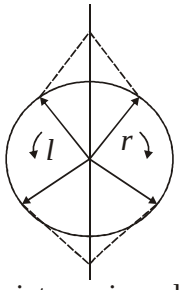
"normal dispersion" but when the two terms in the Drudes equation have different signs it when $k_1 > k_2 \,\&\, \lambda_1 < \lambda_2$ then $[\alpha]$ would first increase, reach a maximum and then decrease and the sign of the rotation will be reversed this type of behavior is called "anomalous" and is shown by substances like ethyltartarate Now in the Drude's equation $[\alpha] = \frac{k}{\lambda^2 - \lambda_0^2}$, $\lambda >> \lambda_0$ the equation is reduced to the following form $[\alpha] = \frac{k}{\lambda^2}$.

In the beginning the experimental determination of rotator power in ultra-violet region had been quite difficult but technological advancement in recent years has enabled the chemist to trace the dispersion curves of a large number of substances in relatively lattice time and with great accuracy. Now a days it is possible to combine a large number of results and then deduce rules of structural analysis.

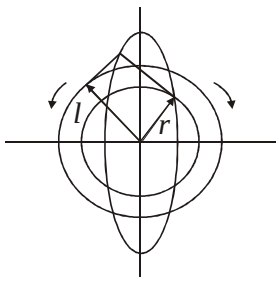
19.2 Circular Dispersion (Cd)

19.2.1 Cotton Effect

Aime cotton, a French physicist made pioneering studies on the phenomenon of circular dichroism in 1895. He made studied on optical activity of potassium chromium tartarate solution with in the absorption band and found that each optically active material absorbed left and right circularly polarized light to different extents, thus a linearly polarized ray of wavelength within an absorption band is converted into an elliptically polarized light on passing through the material as shown in the figure below



A picture view along the direction of propagation of the two vectors r and l of the circularly polarised beam and their resultant which lies in vertical plane.



Since the right and left handed components are different, the resultant vector describes a left handed ellipse.

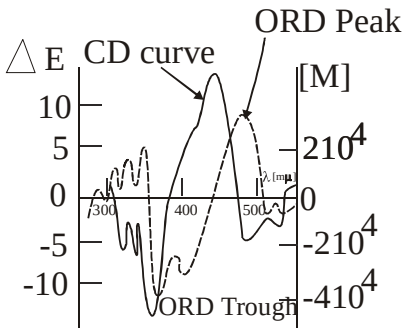
This phenomenon has been given that name CIRCULAR DICHROISM now if E_l and E_R are the molecular coefficient of absorption for the left and right

polarized light respectively, then the difference E_l-E_r is a measure of intensity of circular dichroism. This difference is represented by ΔE.

The magnitude of circular dichroism is expressed by the angle of elliptically and is given by the following equation $\phi = \frac{\pi}{\lambda}(k_l - k_r)$ where k_l and k_r are the absorption coefficient for the left and right circularly polarized light respectively. It is important to note that the sign of elliptically depends upon the relation values of k_l and k_r. On the long wavelength side of an optically active absorption band the sign of elliptically vibration is the same as that of rotation. Whereas on the short wavelength side the signs are reversed.

Now we can write the following relation for specific ellipticity $[\phi]$ as $[\phi] = \frac{\phi}{l \times C}$ where *l* is the length of path in om and C is the concentration of the compound under observation. In a similar manner the molecular ellipticity per unit length $[\theta]$ can be expressed as $[\theta] = \frac{[\phi]M}{100}$ where M is the molecular mass of the species. The above $[\theta] = 33 \times 10^2 [E_l - E_r]$ equation can also be written in terms of extinction coefficient E_l and E_r as .

It was observed by cotton that both the optically rotation and the circular dichroism were found to be dependent upon wavelength especially in the region of an electronic absorption band of the atom or ion lying at the centre of dissymmetry. The difference n_l - n_r (ie Δn) and E_l-E_r (ie ΔE) change with the wavelength and can be positive or negative. At a given wavelength Δn and ΔE for one enantiomorph are equal and opposite to those for the other eneantiomorph. He also found that optical rotation within the band is anomalous ie it increases to a maximum near the absorption band, then decreases to zero within the band and then finally increases in the other sense to a second maxima. The variation of n_l-n_r (ORD) and E_l -E_r(CD) with a given wavelength for a pair of enantiomorphs in the region of absorption band is depicted in the figure below.



Cotton Effect

Mathematically it has been established that ORD and CD are not independent the combination of the two is called as cotton effect".

The above figure also depicts the relations between ORD and CD. These are related in the way that the midpoints between peak and trough of ORD correspond to CD maxima and that of ORD trough coincides in wavelength within the absorption maxima. It is also clear from the above figure that the ORD curve is 'S' shaped whereas that of circular dichroism is bell shaped.

19.3 Application of ORD and CD

It is known that ORD and CD are related to refraction and absorption, thus they furnish useful informations about the structure of molecules. Both these techniques can give essential information about sterochemical feature of optically active substances. The technological advancement in the last two dedades has chemistry and biochemistry. The study of cotton effect of optically active coordination compounds has resulted in the elucidation of structure of inorganic complexes. Moreover these techniques have been found to be of immense use for the structural investigations, determination of absolute configuration and also in the field of conformational analysis.

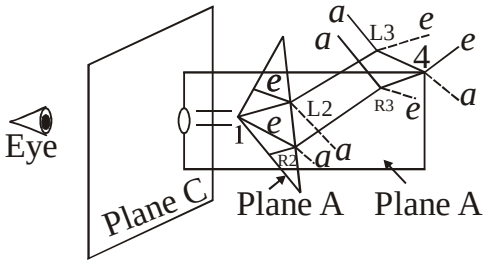
19.3.1 Octant Rules for Ketones

Carbonyl group is particularly suited for ORD studies because a keto group undergoes an optical transition of weak intensity at about $300m\mu$ which is sensitive to induced asymmetry and so this allows the measurements to be made without the inconversion of high absorption. It is fortunate that the carbonyl group is frequently present in many natural product (steroids, polycyclic torpenes etc). The postion of maxima for all simple ketones lie at about $300 \pm 5m\mu$. CD determination of simple ketones have two main qualities over other methods these are

(1) It is specific with respect to the nature of the ketonic position.

(2) Small structural transformation not affect the CD.

Octant rule is a very empirical rule in predicting the sign and magnitude of the cotton effects. Let us consider the compound cyclohexanone. This molecule is considered to have three orthogonal planes A, B and C. This is shown below in the figure.



The diagram showing the octant Rule

Since plane A passes through carbon atoms 1 and 4, the subsituent attached to carbon atom 4 lies in this plane carbon atom 1, L_2 and R_2 (L=left and R=right from observer's point of view) lie in plane B and the substituents at equatorial position at L_2 and R_2 are practically in this plane. The third plane C is perpendicular to the planes A and B and cuts the C = O bond at a point between carbon and oxygen atoms. These planes contain eight octants in space (planes A and B produce four octants and plane C produces four octants) which is the origin of the name of this rule. The octant rule is as follows

The octant rules state that atoms which are located in octant in front of and behind the plane located in C have a contribution in the lower left and far upper right octants make a negative contribution to the cotton effect and atoms in the far lower right and far upper left make a positive contribution. If the atoms lies on the axis of in any of the three, their contribution is zero.

Thus, the four octants in front of plane C are usually vacant and atoms are distributed into two different octants and the final sign will be the resultant of the individual contributions.

Compound 3-methylcyclohexanone shows a positive cotton effect which shows that the $>C=O$ group is present at the equatorial position with $-CH_3$ group on the upper left. If the axial representation of $>C=O$ group is done with $-CH_3$ group on the upper right, the molecule would have shown negative cotton effect.

19.3.2 Determination of Absolute Configuration

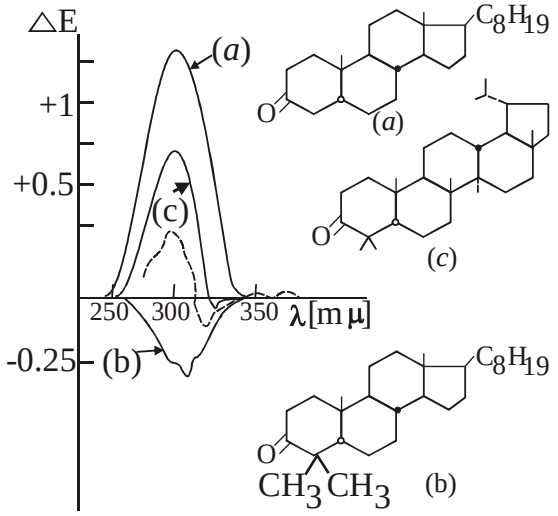
The determination of absolute configuration is of considerable in the chemistry of nature products.

Studies of the conformation in the torpenes have shown following three types of CD curves

(A) Positive circular dichroism similar to that of choleston-3-one .figure (a)

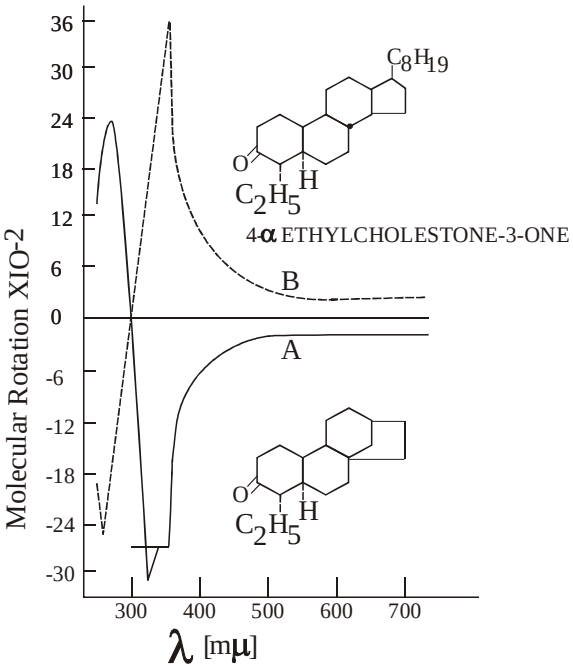
(B) Negative circular dichroism similar to that of 4,4-dimethylcholestan-3one figure (b)

(C) Complex curve with a small negative minimum on the long wavelength side and an important positive maximum at shorter wavelength as in Lupanone figure (c)



CD of some Terpenes

Determination of absolute configuration of organic compound by ORD method may be understood by taking the example of degradation of cafestol which occurs in coffee beans. This compound is degraded to the corresponding ketone and CD of the product is studied and is then compared with some similar compound. Following curve is obtained.



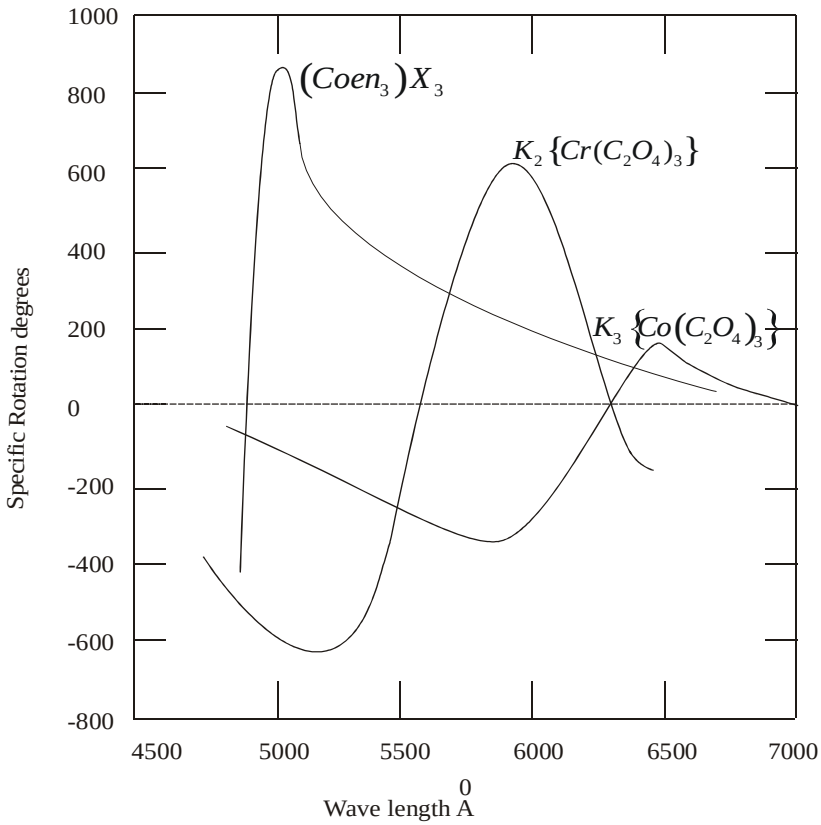
ORD Spectra for Absolute configuration

It has been found that one of the terpenes 4-α-ethylcyclostan-3-one , has exactly similar ORD spectra as that of the product but with opposite signs. Now because the absolute configuration of 4-α-ethylcyclostan-3-one is known, the configuration of the product can be deduced.

19.4 Assignment of Absolute Configuration of Optically Active Metal Chelates

It is known that rotator power of coordination compounds is markedly dependent upon the wavelength of the light used for their study. In many cases it may happen that rotation may even undergo change in sign as the wavelength is changed to that of an absorptions band.

The ORD curves of the optically active complexes like $\left[Co(en)_3\right]^{3+}$,$\left[Cr(OX)_3\right]^{3-}$ and $\left[Co(OX)_3\right]^{3-}$ have been studied. It is found from their study that these complex ions have the same absolute configuration . These curves are depicted



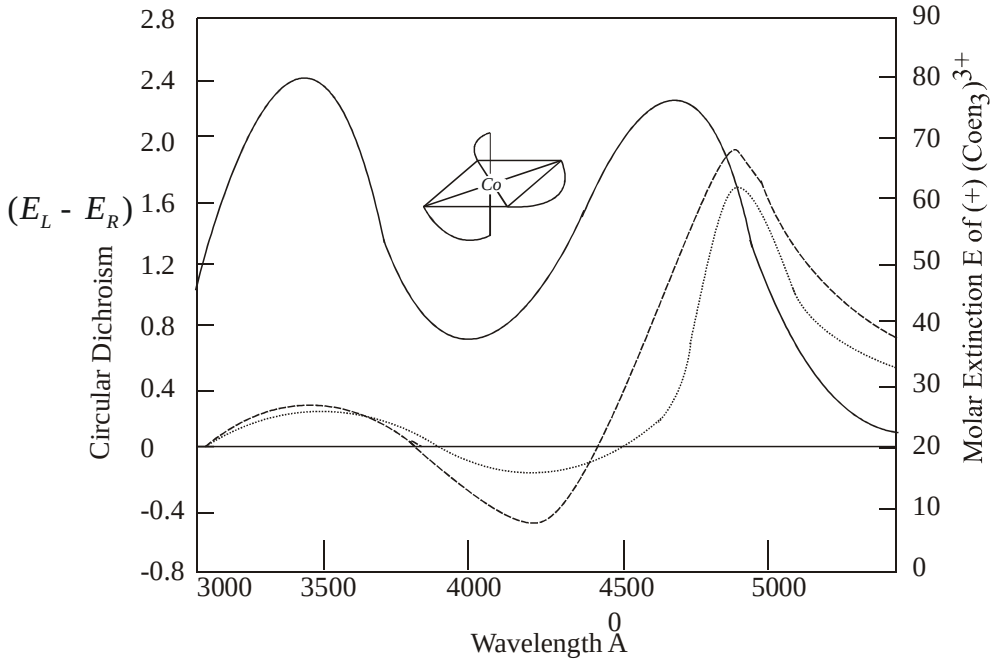
ORD and CD has been inoreasingly used in the studies of inorganic compounds specially the chelate complexes for the following purposes.

(1) correlation of the configuration of related dissymmetric molecules and

(2) To determine or confirm the electronic absorption bands.

The use of ORD and CD data have been mainly centred on the first aspect ie correlately the configuration of related dissymmetric molecules. A lot of work has been carried out in this direction but his problem is still in infancy.

As an example of determination of absolute configuration by the use of CD we may consider the case of following chelates. $(+)\left[Co(en)_3\right]^{3+}$ and $(+)\left[Co(l-pn)_3\right]^{3+}$ here sign (+) indicates that $[\alpha]$ is positive at the sodium D line. Absolute configuration of $(+)\left[Co(en)_3\right]^{3+}$ has also been determined by scattering of curves obtained for the above two compounds shown below in the figure.



The visible absorption of (+) $(Coen_3)^{3+}$ (solid line), CD of the same compound dotted line and CD of (+) $\{Co(l\text{-}\ pn_3)\}^{3+}$ (broken line).

It is found that the compound $(+)\left[Co(l-pn)_3\right]^{3+}$ must have the same absolute configuration as that of $(+)\left[Co(en)_3\right]^{3+}$ chelate.

The presence of an optically active substance in solution can greatly affect the equilibrium existing between d and l isomers of a complex. The optical rotation of $Zinc\alpha-camphor-\pi sulphonate$ phenanthroline is added to its solution to form $\left[Zn(phen)_3\right]^{2+}$, the optical rotation changes to +0.09. It has been observed that the rates of racemization of $d-\left[Ni(phen)_3\right]^{2+}$ and $l-\left[Ni(phen)_3\right]^{2+}$ differ in the presence of optically active cations and anions these studies indicate that the kinetics of the formation of these complexes in solution can be followed by measuring their optical rotation.

From the above discussion it can be concluded that ORD and CD curves are of immense use in assigning configuration of chelates. Similar complexes having similar configuration given similar ORD and CD curves.

19.5 Advantages of ORD And CD

Rotatory dispersion curves have following advantages

(1) A complex shows of cotton curves allows complete characterization of the asymmetry induced by the environment upon a given chromophore.

(2) When a unknown compound is to be compared with parent compound without chromophore and there is a difficulty in the preparation of parents substance rotator dispersion measurement under such situation constitutes are immersely more elegent tool for chemist over the ordinary polarimetric analysis.

19.6 Limitations

In spite of the fact that's rotator dispersion is advantageous over the classical polarimetric analysis, it has following limitations.

(1) Certain chromophorus absorb in far ultra violet region and produce a continuous background which super imposes the cotton effect of the chromophore.

(2) The resultant shape of the curve becomes tredious in presence of fine structure and so the study of each contribution becomes a delicate matter.

In spite of the fact that CD does not have the disadvantages of the ORD, until recently, it has been less developed than ORD, mainly because of the experiment and technical complexities. Thus

(a) CD adsorption curves can be more easily analysed than ORD curves because of their greater resolution and simple nature.

(b) When contribution background is strong as compared to cotton effect the ORD curves do not represent the effect of only the chromophore under examination.

(c) When the chromphore to be examined is present in a mixture of active substances having no absorption in the spectral region, CD is superior to ORD because no background of unknown magnitude is present in CD.

(d) The variables characterizing the optical activity are easily obtained with the help of CD.

Although CD is superior to ORD yet ORD has been valuable in solving problems involving asymmetric structure because ORD provide means of obtaining important informations about the absolute configuration of products in which chromophores are either absent or impossible to introduce chemically ORD in combination with CD and provide better

understanding about the molecular structure and can solve many problems which either of these methods is unable to furnish individually.

19.7.0 ORD and CD Measurements

19.7.1 ORD PHOTOMETERS

Spectropol arimeter, used for the measurement of optical rotation of a chiral molecule at different wavelength, differe form a polarimeter in that polarimeter we use only one wave length for example the sodium D line or mercury green line. A systeric line diagram of a spectropolarimeter is given below.

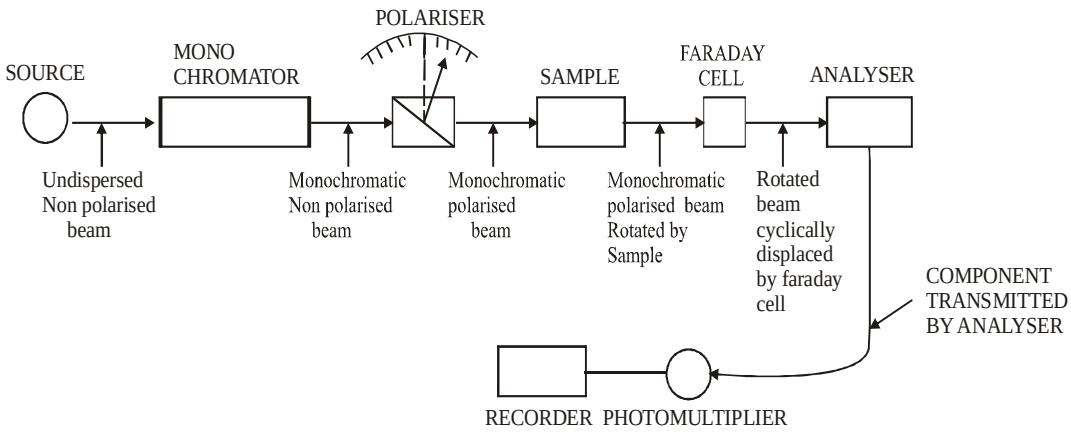
Schematic diagram of a spectropolarimeter

Here the light sourch and monochromatess are similar to those used in ultraviolet and visible spectrophotometers and polarizer and analyzer are made of Nicol prisms. Automatic recording spectropolarimeters work on following two methods (1) Null point method (2) Ratio method.

Most commonly used spectropolarimeters work on Null points principle.

In spectropolarimeter the beam of light from a conventional monochromator passes through a polarizer and enters the sample. The light emerging from the sample is deflected to a contain angle now enters the analyser. A motor driven device now modulates the emergent beam with respect to its state of polarisation. This causes the polarizer to oscillate through an angle of. The null point is as certained by the help of an imposed mechanical oscillator of the analyser which produces a modulation of the light beam striking the photomultiplier tube. In spectrophotometer the oscillation of the light beam is brought about by a magneto - optical effect for which a faraday cell is placed ahead of the analyser. The faraday cell is placed ahead of vitreous silica rod surrounded by a coil carry a 60Hz ac current. Now the emerging beam from the photomultiplier tube is separated into two singnals according to the left and right oscillations of the analyser. Now th difference of the two oscillations is fed into a null point seeking servo system. The polariser then moves to a position which multiplies the rotation caused by the sample under investigation.

In the following scheme a simplified diagram of cary spectropotentiometer is depicked



A simplified Schematic diagram of Cary spectropolarimeter

19.7.2 CD Measurement

The instrumental setup for CD measurements is almost similar to ORD measurements except that the detecting system is modified to measure the quantity E_t - E_r. When a ray of circularly polarised light passes through a dichroic material, it suffers no change in polarisation but is partially absorbed and degree of this absorption depends upon the nature of the circularly polarised light viz. left or right. There are two main considerations in the measurements of circular dichroism.

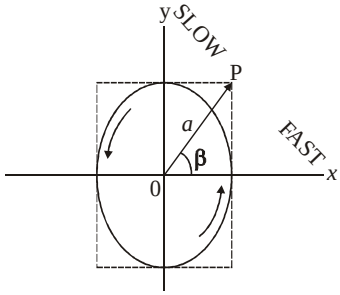
(a) The beam of light must be plane polarised and

(b) The plane polarised light must be resolved into right and left circularly polarised. Polarised beam and that of the components should be absorbed by one quarter wavelength of the other.

Experimentally the circulaly polarised light is produced by a polariser which is followed by a birefringent plate which actually, is a very possesses two axes, a slow and a rapid one. Two in phase through this plate with different velocities. Now energy with a difference of phase & which is proportional to the thickness d and the birefringence of the plate ie . $\delta = \frac{2\pi d\left(n_{slow} - n_{rapid}\right)}{\lambda}$

A quarter wave plate introduces a phase difference $\delta = \frac{\pi}{2}$.

Thus now if $\beta = 45^0$ we obtain right circularly polarised light and on the other hand if $\beta = -45^0$ we get a left circularly polarised light when $\beta = \pm 90^0$, plane polarised light is produced diagramtically it is shown below.



The devices used for this purpose is based on the total internal reflection such as fresnelrhomb and the pocket's electro-optical modulator. the Fresnelrhomb is a silica prism (made form fused quartz) in which relative log & between vibrations which are parallel to any perpendicular to the plane of incidence, is determined by the following Fresnel's formula viz $\tan\frac{\delta}{2} = \frac{\cos i}{\sin^2 i}\sqrt{\left(\sin^2 i - \frac{1}{n^2}\right)}$.

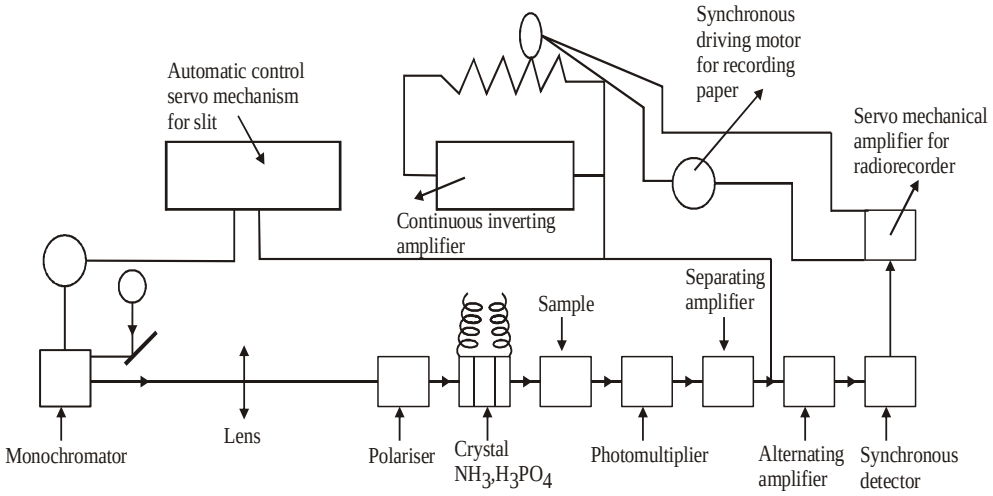
Where i = angle of incidence,

n = refractive index of the medium in which total internal reflection is produced, varies with wavelength.

The application of the above formula is, thus, subject to orificism. On the systems crystals (pocket's effect) a high electric field is applied with the help of a plate of potassium dihydrogen phosphate (KH_2PO_4) or similar piezoelectric crystals cut perpendiuclarly to optical axis. Under the influence of electric field, the crystal becomes biaxial.

Various measuing instruments are used in the measurement of CD. Some they are based on visual measurement (Kuhn and Brauns device) photoelectric measurement (Bradoz, Bilidon and mathien device and Mitchells device) and spectrophotometric measurement.

A schematic line diagram of a dichrograph used for CD measurement is shown below.



Schematic diagram of a Dichrograph

A beam of light illiminates the monochroator. The light emerging from monochromator is transferred to a quasi-parallel beam by a line and on passing through a polariser, two separate beam is eliminated by a diapharm and the other, the ordinary beam of polarised light now passes through a quarter wave system which is made of a plate of ammonium dihydrogen phosphate on two faces of which a light is applied alternatively. The plate is mounted in such away that in the course of one cycle of alternating voltage, the plane polarised light is changed first into left and then into right circularly polarised light.

The modulated beam now passes through teh sample containing an optical active dichroic material which absorbs right and left circularly polarised light to a different extant. The emerging beam is than analysed by various electronic systems and ultimately the spectrum is automatically recorded on a paper.

The precision of the measurement is affected by following factors

1. Rotation of sample 2.Imperfection in polarising system
3. Back ground noice 4. Defects in teh adjustment of the apparatus.

19.8 Summary

The technique ORDandCD is helpful in the assigning of the stereochemical aspect of the molecule as the whole which greatly helps in the synthesis of these molecules in the lab as the stereochemical aspect in the pharmaceuticals is

of great significance the actual structural ellucidiation is essential part of the synthesis in which it is of great use.

19.9 ReviewQuestions

1 what is mean b y the circularly polarised light?

2 what is octant Rule?

3 what is rotatory dispersion?

4 what is circular birefrengence?

5 what is cotton effect?

6 what are the advantages and the limitation of the ORD andCD?

7 ORD/CD are helpful in the structure ellucidation of the molecule,expalin with the example

19.10 References

- Chemical analysis an instrumental approach: A.K.shrivastav and P.c Jain,S Chand and Company
- Organic spectroscopy: P.S.Kalsi,New age publication
- Organic Synthesis: Michael B smith,Mc Graw Hill.

Unit - 20

19F and 13P NMR Spectroscopy

Structure of Unit

20.0 Objective

Basically the main objective of this unit is to study the structure and other parameters of fluorine and phosphorous containing compounds; it is observed these two elements are present in organic molecule which is of immense use in different field of chemistry, fluorine containg compounds exhibit long range coupling to an extent of maximum of five bonds whereas in case of phosphorous the study of multiplicity is of more of concerned various findings of spectra has been correlated with the structure and in turn of properties of the molecule the field is open for the

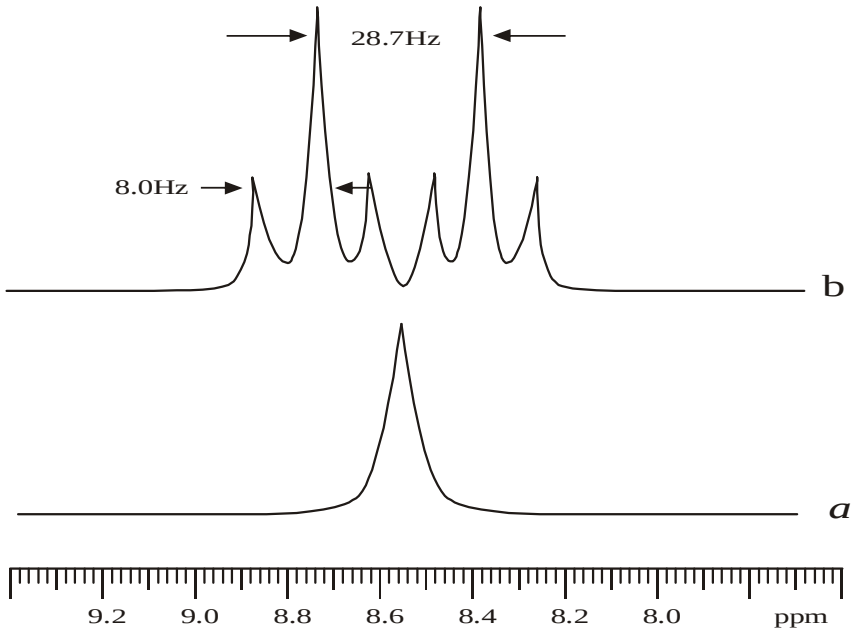
research and may include the study of thermodynamically and kinetic parameters of the various molecules

20.1 Introduction

Phosphorous is unusual element in the sense. It has only one isotope ie P-31 and as its spin is 1/2 so it is NMR active. Florine, Thorium and Yttrium are other those elements which have also one isotope which is also NMR active. As the sensitivity of P^{31} is very high so the measurement does not require sample concentration very high. The ratio of resonance frequencies for 14 and ^{31}P are 2.5:1.

20.2 Chemical shift and coupling constant

In the figure below part (a) represents a single one line at 8.5ppm of phosphorous atom this is extremely simple however for proton coupled spectrum of ^{31}P as shown in the figure (b).



Phosphorus - 31 spectra of compound 1.a protons decoupled; b proton-phosphorus coupling present

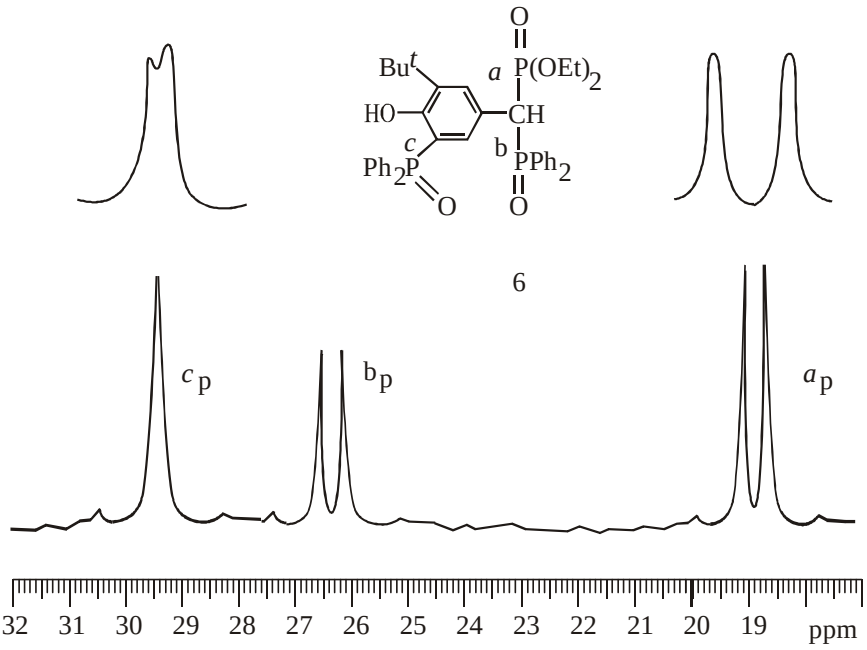
More information is provided by proton coupled spectrum.

By the above spectrum analysis one can get an idea of that proton which is showing measurable coupling with the phosphorous atom as the pattern shows the presence of two triplets separated by 28.7Hz. This coupling is mainly a two bond coupling between methene protons & phosphorous.

The triplet intensity of ratio 1:2:1 is because of three bonds coupling between phosphorous and methylene proton and coupling constant is 8Hz.

20.3 Coupled spectrum (p-p) coupling

There are many compounds that are having more than one phosphorous atom to illustrate above, the example can be taken of the compound given below where proton decoupled coupled phosphorous spectrum of the compound is shown.



Phosphorous-31 spectrum (202MHz) of compound 6,measurement time 2min.

This compound contains three chemically different phosphorous nuclei. The NMR spectrum of ^{31}P is same as ^{1}H-NMR so multiplet is expected arising from three signals.

The oxidation state of phosphrous is five and chemical shift range is 12ppm the methine carbon is attached two phosphorus atoms which are chemically non equivalent as they are phosphoate and phosphene oxide. So an expectation is there that coupling between ap to bp or cp is small as coupling is over five bonds coupling between ap and bp is to be large as the separator is of two bonds.

By the spectrum given above it is clear that low field signals with coupling constant value of 9.4Hz should be of cp. While the coupling constant value of 39Hz should be of the multiplets between ap and bp.

None the question arises that which signal corresponds to ap and which singnal corresponds to bp. The ans to this problem lies in the spectrum of the analogoues compound where phosphates such as ap absorbs to high field of 20ppm while phosphene oxides absorbs around 20ppm which corresponds to bp and cp.

20.4 Chemical shift

Chemical shift for p-31 is seen in the range of 1000ppm. As phosphorous possess lone pair, which makes the chemical shift to have widen range for organophosphorous compound ^{1}H, ^{13}C-NMR data is directly informed ^{31}p information the chemical shift for ^{31}p are relative to the phsophoric acid (85%) singlets.

Chemical shift of ^{31}p is found to be dependent on the concentration, sovlent and the presence of other compounds optical purity of the chiral phosphorous compounds can be provided by the chemical shift data of ^{31}p. Wherein when enantiomer forms addition product with enantiomer different diastereoisomeric products.

The main divisions for the 31-p NMR data have been made according to the number of atoms that are covalently bonded to the phosphorous. The table giving the data is assigned according to the preferences given below.

(1) If the atom attached to phosphorous is of highest group in periodic table than precedence is given to that group. Eg P-Halogen > P-chacogenides > etc.

(2) The next preference is given to the atoms of the highest atomic number (I>Br>Cl>F; Si>S>O; P>N; Si>C) this also extends to the atoms bonded to phosphorous. eg (PI_3 > PI_2 Br >PIBrCl> PIBrF).

(3) The third preference is taken up by the cyclic compounds where phosphorous atom is not part of the ring. Phosphorous heterocyclic compounds are placed in the order of increasing ring size.

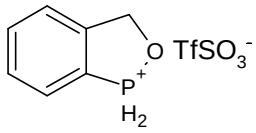
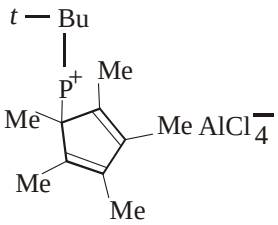
(4) The fourth precedence is given to these compounds which are having the smallest number of phosphorous carbon bonds. in the molecule. Some of the above cited precedence is shown in the given table.

Table : Phosphaethynes

Bonds to P	Compound	$\delta^{31}P(ppm)$	Ref.

C,P	$P \equiv CNH_2.HBr$	4.66	28
	$P \equiv CNHCOCH_2Cl$	0.53	28
	$P \equiv CNHBu.HBr$	2.60	28
	$P \equiv CNHPh.HBr$	4.60	28
	$P \equiv C-t-Bu$	-69.2	29

Table : Phosphenium compounds

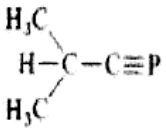
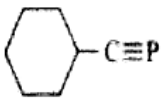
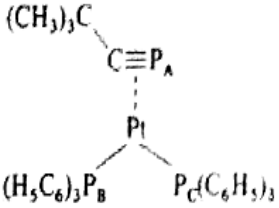
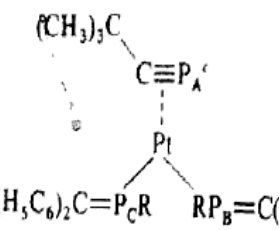
Bonds to P	Compound	$\delta^{31}P(ppm)$	Ref.
CIN,P	$ClP^+NMe_2AlCl_4^-$	325	30
OC,P		196.2	31
N_2P	$P^+(NMe_2)_2CF_3SO_3^-$	269.4	31
	$P^+(N-i-\mathrm{Pr}_2)_2AlCl_4^-$	313	30
	$P^+(NTms_2)_2AlCl_4^-$	450.3	32
NC,P	$t-BuP^+NMe_2AlCl_4^-$	513.2	32
	$PhP^+NMe_2CF_3SO_3^-$	183.4	31
	$PhP^+NMe_2CF_3SO_3^-$ in C_6H_{14}	170	31
	$PhP^+NMe_2CF_3SO_3^-$ in MeCN	192	31
C_2P		240	33

20.5 Chemical shift for 31-P with cordination number-1

The range of ^{31}P chemical shift of $RC \equiv P$ lies between +96ppm $\left[R = Si(CH_3)_3\right]$ and -207ppm(R=F) so we can see these compounds are displaced to higher field as compared to the chemical shift of ^{31}P compounds (C=P)Tabel given below gives NMR data of phosphalkynes.

In case of P(1) compounds that are stable at room temperature one can see the similarity between ^{13}C and ^{31}P chemical shifts. of (PC) uiits and also $^{1}J(PC)$, 2J(PC) and $^{3}J(PC)$ coupling constant. This may be because of the steric reason. Values of two $^{4}J(PH)$ coupling constants shows the difference in bond angle of C-C-H in rigid adamantane framework and the corresponding angle in t-butyl group.

^{31}P and ^{13}C chemical shifts (PPM) and coupling constants J of charcteristic

Compound	$\delta^{31}P$	$\delta^{13}C$	J(Hz)		Ref.
F—C≡P[a]	−207		$^{2}J(PF)$,	182.0	2
$(CH_3)_3C—C≡P$	−69.2	184.8	$^{1}J(PC)$,	38.5	3
			$^{2}J(PC)$,	18.2	3
			$^{3}J(PC)$,	6.0	3
			$^{4}J(PH)$,	0.9	3
Ad—C≡P[b]	−66.9	184.7	$^{1}J(PC)$,	39.0	32
			$^{2}J(PC)$,	18	32
			$^{3}J(PC)$,	6	32
			$^{4}J(PH)$,	0.3	32
	−66.3				32
	−62.0				32
$H_5C_6—C≡P$	−32.0	164.9	$^{1}J(PC)$,	48.3	26
H—C≡P	−32.0	154	$^{1}J(PC)$,	54.0	25
			$^{1}J(CH)$,	211	25
			$^{2}J(HCP)$,	43.9	25
$(CH_3)_3Si—C≡P$	+96	201.4	$^{1}J(PC)$,	13.9	19
	P_A, +83.1		$^{1}J(PtP_A)$,	62	27
	P_B, +25.3		$^{1}J(PtP_B)$,	3206	27
	P_C, +27.8		$^{1}J(PtP_C)$,	3587	27
	P_A, +38.1		$^{1}J(PtP_A)$,	115	28
	P_B, +201.1		$^{1}J(PtP_B)$,	3438	28
	P_C, +202.1		$^{1}J(PtP_C)$,	4048	28
$[Fe_2Pt(dppe)(CO)_6(C(CH_3)_3CP_A)]^d$	P_A, −52.8		$^{1}J(PtP_A)$,	128	30

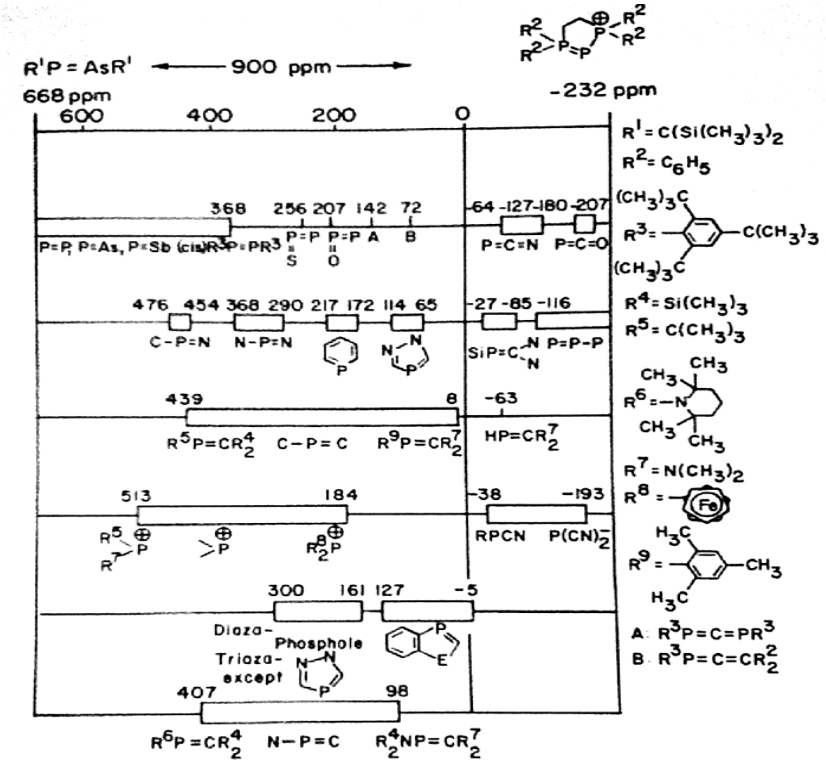
According to walsh rule as the electron eg activity of the substituents attached to the $RC \equiv P$ is increased, the phosphorous atom start using its hybrid orbital containing more of p- character for σ bond to carbon atom . So the lone pair at phosphorous atom occupies that orbital which has more of s-character. So if the shielding at phosphorous atom is increased. making atom to resonate at higher frequency. This can be seen by $\delta^{31}P$ for the groups Viz.

$R = Si(1.8), H(2.1), C(2.5) \& F(4.0)$ so by this it can be said that ^{1}J(PC) for $Me_3SiC \equiv P$ shows greater s-characterister on PC σ bond not explained to be at Maximum, however in this context we can say ^{1}J(PC) is also influenced by the d-orbitals of the neighbouring phosphorous atom but what shall be sign and the extent of this interaction is still unknown for phosphaalkynes.

20.5.1. Chemical shift for ^{31}P with cordination number-2

For various type of compounds of phosphorous with coordination number two P(2) δ^{31} chemical shift is shown in the table below

20 .5.2 Range of ^{31}P chemical shifts for P(2) compounds of various types



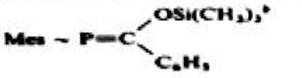
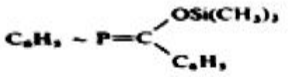
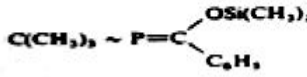
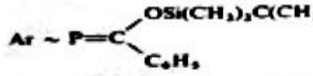
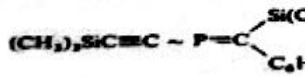
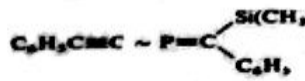
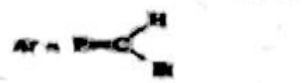
It can be seen from the table itself that $\delta^{31}P$ chemical shift difference between the cis and trans Ar-P=PAr is very striking.

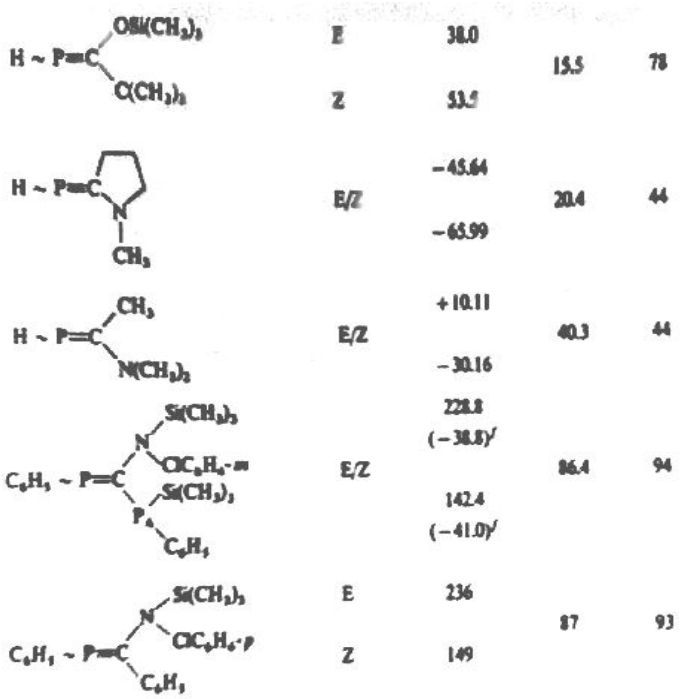
20.6 E/Z isomer of phosphaalkenes

^{31}P shift difference $(\Delta\delta)$ for the E/Z isomers of phosphaalkenes is upto 87ppm, the chemical shift for these pairs of isomers can be either positive or negative with respect to each other. So one can't correlate empirical by the individual isomer with there ^{31}P chemical shift. However the identification can be made through value of $^{u}\mathrm{J}(PE)(E=\text{element with spin 1/2, n}\geq 1/2)$.

The larger magnitude of coupling constant is assigned to that isomer where the nucleus E is coupled with the phosphorous atom, which is present to the same side of the P=C bond.

20.6.1 ^{31}P Chemical shift (PPM) of E/Z isomer pairs of phosphaalkenes

Compound	Isomer	δ^{31}P	$\Delta\delta$ (ppm)[a]	Ref.
	E	142.5	2	61
	Z	140.5		
	E	153.0	4	11
	Z	149.0		
	E	200.0	12	11
	Z	188.0		
	E	160.3	19	68
	Z	141.3		
	E/Z[a]	210.7 228.4	17.7	92
	E/Z	208.3 226.3	18	92
	E[a]	262.4	6.5	83
	Z	268.9		84



H ~ P=C(OSi(CH3)3)(C(CH3)3)	E	38.0		15.5	78
	Z	53.5			
H ~ P=C(N(CH3)CH2CH2CH2)	E/Z	−45.64		20.4	44
		−65.99			
H ~ P=C(CH3)(N(CH3)2)	E/Z	+10.11		40.3	44
		−30.16			
C6H5 ~ P=C(N(Si(CH3)3)(ClC6H4-m))(Si(CH3)3)	E/Z	228.8 (−38.8)[f]		86.4	94
		142.4 (−41.0)[f]			
C6H5 ~ P=C(N(Si(CH3)3)(ClC6H4-p))(C6H5)	E	236		87	93
	Z	149			

[a] Absolute value of the chemical shift difference of the isomers.
[b] Mes = 2,4,6-(CH3)3C6H2
[c] Ar = 2,4,6-[C(CH3)3]3C6H2
[d] E/Z pairs not assigned.
[e] See Section 2.1.1
[f] δ31P of Pa

However an exception to the above cited rule is seen in case of 2,4,6-$(CH_3)_3C_6H_2P=C(C_6H_5)\left[OSi(CH_3)_3\right]$ the $^3J\left(^{31}P^{21}Si\right)$ value of 7.1Hz for Z isomer is more corresponding to the J value for E isomer (6.4Hz) while the value of $^4J\left(^{31}P^{13}C\right)$ and $^5J\left(^{31}PH\right)$ are still consistent with this rule.

Trends in phosphorous-31. Chemical shift in phosphoalkenes For making an empirical statement for the influence of substituents at the phosphorous and carbon atoms of P=C units on $\delta^{31}P$ can be generated as :

A polarised (3p2p) π system is produced by phosphorous carbon double bond where sp^2 hybridised phosphorus atom interacts with π systems or p_z orbitals neighbouring atoms and so the atom is shielded and resonate at highfield.

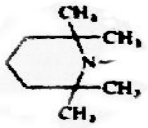
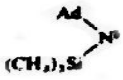
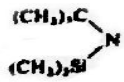
For the compounds $\left[N(CH_3)_2\right]\left[C(CH_3)_3P\right]^+$ and $(CH_3)CP=C\left[Si(CH_3)_3\right]_2$ the t-butyl group is incapable of conjugation which is shown by downfield position of $\delta^{31}P$

Influence of substituents on $\delta^{31}P$ (ppm) and coupling constants (Hz) of phosphaalkenes is shown in the table below.

A. $RP{=}C(R')_2$, $R = C(CH_3)_3$, C_6H_5

	$R = C(CH_3)_3$		$R = C_6H_5$			
R	$\delta^{31}P$	$^1J(PC)$	$\delta^{31}P$	$^1J(PC)$	$\Delta\delta$	Ref.
$Si(CH_3)_3$	438.7	94.3	375.9	85.0	62.8	108
$SSi(CH_3)_3$	322.3	73.0	282.0	71.0	60.3	100, 108
$Si(C_6H_5)$	285					110
C_6H_5			232			49
$N(C_6H_5)[Si(CH_3)_3]$	170		121		49	111
SCH_2-			44.9	51		105
$N(CH_3)_2$	91.94	72.66	28.37	67.1	63.6	44, 109
$OSi(CH_3)_3$	23.7		26.7	67.1	−3	98

B. $RP{=}C(R')_2$, $R' = Si(CH_3)_3$, $N(CH_3)_2$

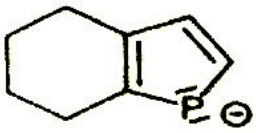
	$R' = Si(CH_3)_3$			$R' = N(CH_3)_2$		
R	$\delta^{31}P$	$^1J(PC)$	Ref.	$\delta^{31}P$	$^1J(PC)$	Ref.
$C(CH_3)_3$	438.7	94.3	108	91.94	72.66	44
$[(CH_3)_2Si]_2C$	422		96			
	406.8	75	113			
Ar'	393		96			
	392.2	94.6	116			
	391.3	93.7	113, 116			
$[(CH_3)_3Si]_2N$	383.4	98.0	112, 113	98		47
$2,4,6\text{-}(CH_3)_3C_6H_2$	381.0		91	8.53	68.4	109
C_6H_5	375.9	85	108	28.37*	67.1	109
Cl	343		115			
Br	342		115			
$(CH_3)_3SiC{\equiv}C$	288.3	78.7	92			
$C_6H_5C{\equiv}C$	284.9	78.4	92			
$(CH_3)_3SiC(H){=}C[OSi(CH_3)_3]$				30.0		69

The shift to higher field strength in case of phenyl compounds about 60ppm is mainly due to interaction of the system of benzene ring and P=C unit.

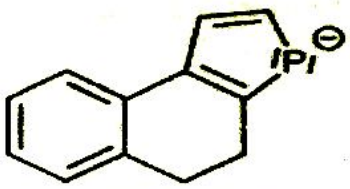
Chemical shift of cyclic compounds with doubly cordinated phosphorous

The ^{31}P chemical shift of cyclic planar compounds like phosphorous with delocalised six π electron system lies within the range of 50ppm. However the locallisation at higher field for $C_9H_{11}P = C(C_6H_5)_2$ around $\delta_{31}P = 233$ is due to extreme delocalisation.

For 1 and 2-phosphaazlenes $\delta_{31}P$ values are at high field. The coniicty for themulticycle potassium phosphlides is shown by low field position of ^{31}P signal.



$\delta^{31}P = 73.3$

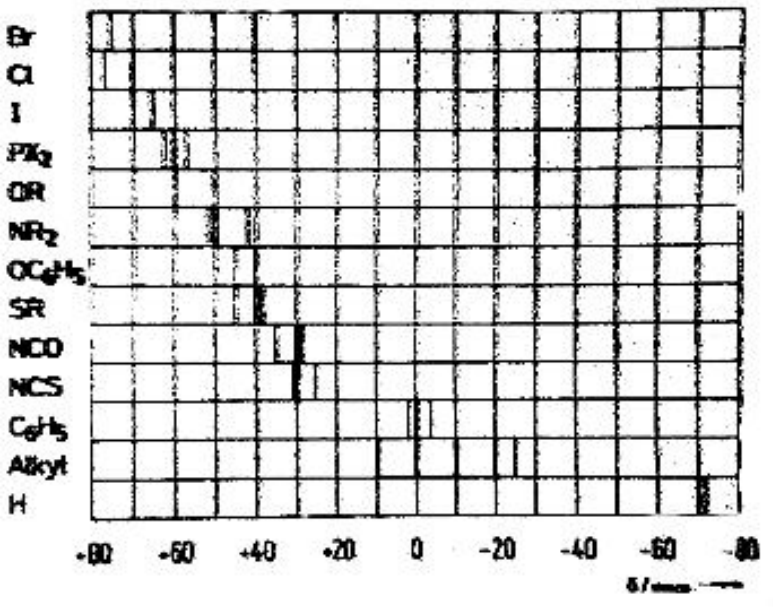


$\delta^{31}P = 81.7$

So C-P π bond contribution and the resonating structure where negative charge at different location of ring is criteria for stabilization.

Chemical shift for three coordinate phosphorous compounds

For the compounds of phosphorous with coordination number (III). Chemical shift can be evaluated by summation of the increment (approximation method) shown in figure.



Emperical equation were given by Grim and Mc Farlane, Maier, Fluck and Lornenz to evaluate the chemical shift.

$$\delta = -62 + \sum_{n=1}^{3} \sigma_n^P \left(\text{ for tertiary phosphines} \right)$$

$$\delta = -99 + 1.5 \sum_{n=1}^{2} \sigma_n^P \left(\text{ for secondary phosphines} \right)$$

$\delta = 163.5 + 2.5\sigma^P$ (For primary phosphines)

$$\delta = +21.5 + 0.26 \sum_{n=1}^{4} \sigma_n^P - 3.2m - 5.5l \left(\text{ for Quaternary amonium salt} \right)$$

Where σ^P are constants, which are the characteristic of the ligands.

m = No. of allyl, bezyl & cyclohexyl groups, *l* = No. of phenyl groups.

Chemical shift for 4 and 5 coordination number of phosphorous

Schmidpeter and Brecht demonstrated a linear correlation between $\sigma^{31}P$ and σ_{Para} the constant of Hammet equation (Substitiuent constant) in phosphinyl compounds. $\left(C_6H_5P^+YX\right)\left(Y=0^-, N\left(CH_3\right)_2\right)$ as the donor property of X increases shielding of phosphorous nucleus also increases. The chemical shift can be given by following equations.

$$\delta^{31}P\left(C_6H_5\right)_2 P(O)X = +39.6 + 22.7\sigma_X$$

$$\delta^{31}P\left(C_6H_5\right)_2 P^+\left[N\left(CH_3\right)_2\right]X = +66.7 + 36.7\sigma_X$$

20.7 Spin spin coupling

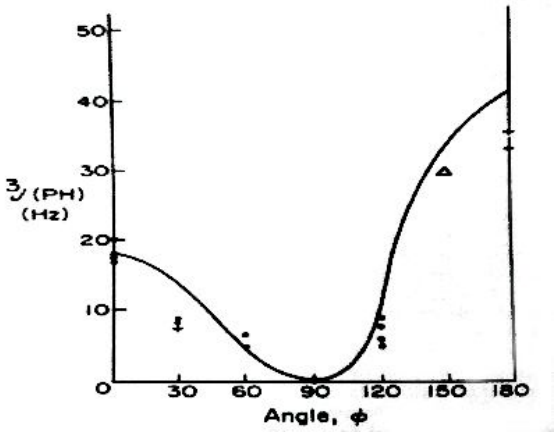
The coupling constant for phosphorous and proton through one, two, three and four bonds are dependent on the geometric disposition of atoms with respect to each other, here geometrical and stereochemical dependence of phosphorous-protons coupling is demonstrated.

Vicinal proton phosphorous coupling, ^{3}J(PH)

The magnitude of phosphorous proton coupling ^{3}J(PH) for system P-C-C-H, P-O-C-H^3, P-N-C-H^4, P-S-C-H^5 depends on the dihedral angular.

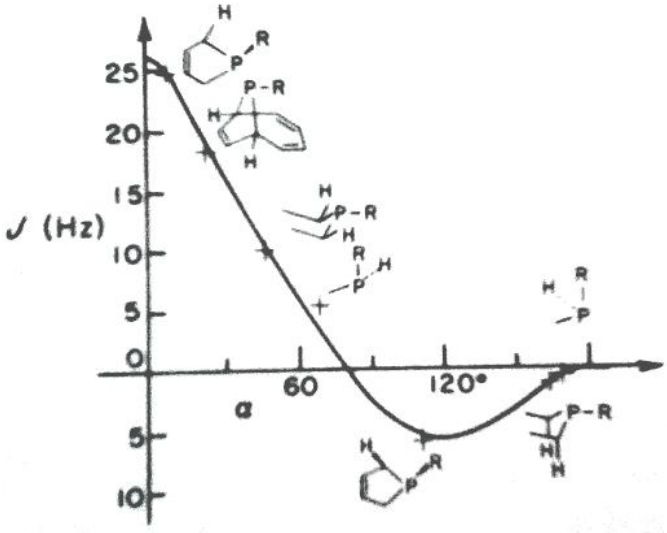
$^3J\left(PCCH\right)$ and $^3J\left(POCH\right)$ for phosphoryl (P=O) compounds dependence on the dihedral angle is shown by the Karplus curve. Which is based on the following equation. $^3J\left(PCCH\right) = 18\cos^2\phi PH; o^0 \le \phi \le 90^0$

$^3J\left(PCCH\right) = 41\cos^2\phi PH; 90^0 \le \phi \le 180^0$

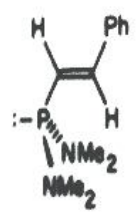


Geminal proton-phosphorous coupling $^{2}J(PH)$

For the geminal coupling between proton phosphorous coupling the J value depends o dihedral angle H-C-P which is shown in the figure given below

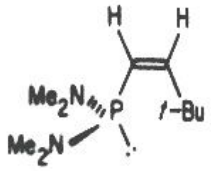


The syn pair proton relation and anti line pair - proton relation is expressed by the example given below. It is evident that 2J (PH) for tetracovalent compounds coupling constant are independent of dihedral angle.



35

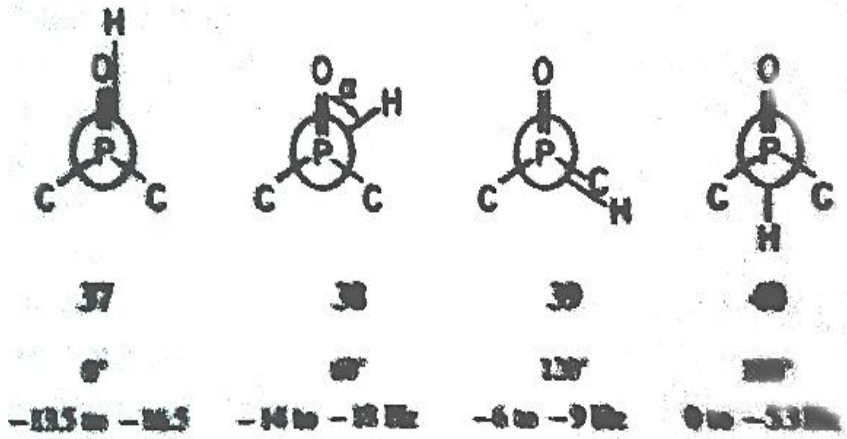
$^1J(HP) = 21.4$ Hz



36

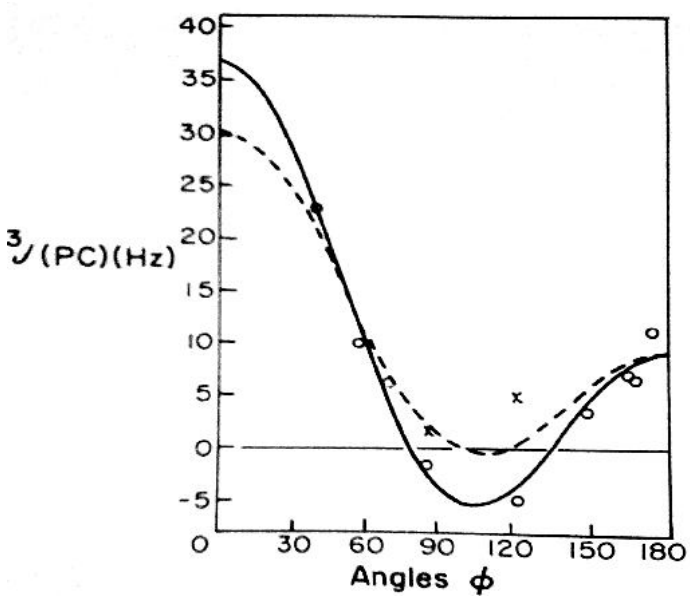
$^1J(HP) = 0.1$ Hz

The J value of compounds having P = O and hydrogen showing dependence on dihedral angle is shown in figure below.



^{31}P-^{13}Carbon coupling

It is not only depends on the number of bonds but also on dihedral angle also given by Karphis curve maximum when $\theta = 0^0$ and $\theta = 180^0$ and minimum when $\theta = 90^0$ .Figure below shows the dependence of $^3J(PC)$ with dihedral angle



20.8 F^{19}NMR spectroscopy

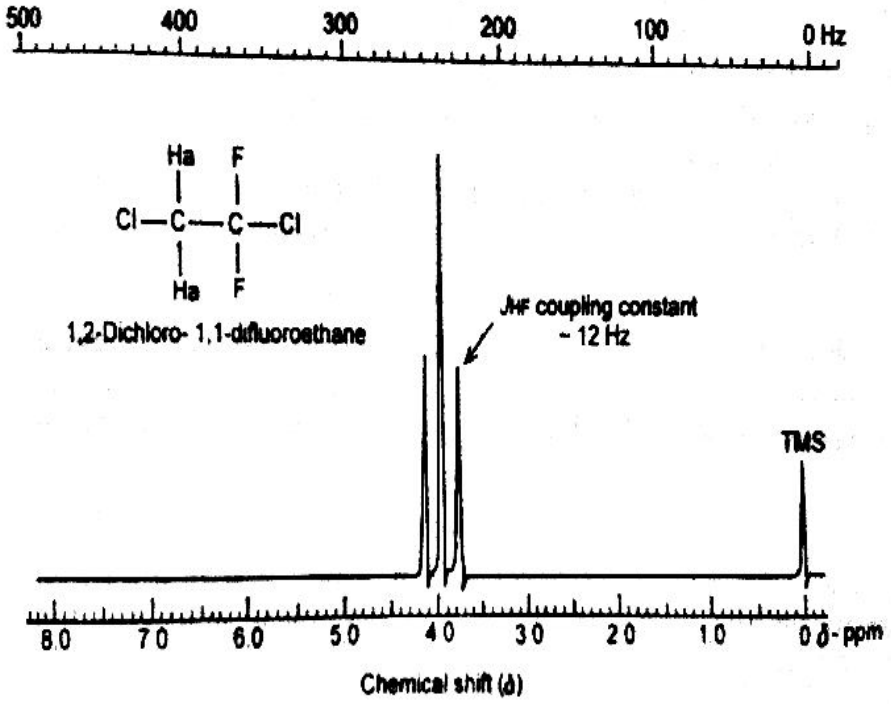
Nucleus whose nuclear spin quantum is non zero shows NMR activity. Proton can couple with Halogen mainly F not with Cl, Br and I.The reason for this is that due to large magnitude of electric quadrapole moment of halogen atom like Cl, Br, and I which decouples with the proton (Adjacent proton).

Since the spin Quantum number of F^{19} is 1/2 so the possible for orientation of the florine are two (+1/2, -1/2). At the same radiofrequency F^{19} nucleus does not absorbs at that magnetic field which is effective for protons. So the peaks which are seen for protons in ^{1}H-NMR is not observed for ^{19}F nucleus.

Eg for 1.2-dichloro-1,1-difluroethane

Two protons signals are split by the adjacent fluorine atoms coupling between ^{19}F and ^{1}H is strong geminal coupling for F^{19} and ^{1}H is H2 to 80Hz.

Vicinal coupling for F^{19} and ^{1}H is 1.2 to 30Hz



For compound CH_3CHFCl in ^{1}HNMR spectrum. CH_3CH should give doublet (3H) and a quartet ^{1}H but F atom split each line into doublet and forms double doublet, due to coupling of F atom with proton each line of quartet for -CH- is further split into two giving doublet of quartet. It can also seen for F nucleus also where F nucleus is appearing as doublet of quartet as it is under influence of -CH- group and $-CH_3$ protons. In compounds containing flourine this absorption range is 200ppm with high J value as a result in PMR spectrum where ranges from O to 10 ppm the signal of fluorine will be missing so for compound CH_3CHFCl. The following two signals will be seen

(i) Three proton double doublet Here $-CH_3$ groups is in form of double doublet (4 lines) here J_{HH} = 6Hz and J_{HF} = 22Hz.

(ii) One proton double quartet J_{HH}=6Hz, J_{HF}= 50Hz

The singals for fluorine are separated and do not appear in normal range from 0 to 10ppm.

Coupling constant (J) for HF is having long range than HH eg.

Vicinal F-F coupling J = 0 to 40Hz

Geminal F-F coupling J = 43 to 370Hz.

Trans flourine J = 106 - 148Hz

Cis flourine J = 0-58Hz

For Cis H x F, J = 0 to 22Hz

Trans H x F, J = 11 to 52Hz

In compounds of benzene having F attached couples with proton on the ring than

J_{ortho} = 7.5 - 11.8Hz J_{meta} = 4.3 - 8.0Hz J_{Para} = 0.2 -2.7 Hz

* For chemical shift in F^{11} it is difficult to predict as less than 1% of shielding in F^{19} nucleus takes place due to the effect of diamagnetic shielding.

* In CH_3COCH_2F proton decoupled spectrum singlet is observed for F atom which for proton coupled spectrum F atom couples with -CH_2- protons giving triplet geting splitting into three quarters via four bond coupling with methyl groups, ;CH_3 group gives doublet at $\delta = 2.2(J = 4.3Hz)$ due to long range, coupling by F nucleus the CH_2 proton shows doublet at $\delta = 4.75$ (J=48Hz) due to geminal coupling with F.

20.9Application

1. In PF_5 molecule there are two types of P-F bonds, three shorter P-F bonds (angle 120^0 coplanar) and two larger P-F bonds (angle 180^0) but F^{19} NMR shows single peak, it means that there is rapid exhange of flourine atoms at spatial positions.

   This berry pseudorotation is shown below which is studied by F^{19}NMR.

$$PF_5 \xrightarrow{Pseudorotation} Square \text{ pyramidal} \rightarrow \text{New TBP}(G.S.)$$

2. Me_2PF_3: This molecule shows free rotation but CH_3PF_4 shows pseudoratation.

3. SF_4 it contains two magnetically non-equivalent sites possessing flourine atoms but ^{19}F shows a single peak. It means that there is first exchange of ^{19}F atoms via Bimolecular T.S. involving bridged Species.

$SF_4 + SF_4 \Leftrightarrow F_3S-(F)_2-SF_3$ (Two S-F-S-bridges)

4. By ^{31}P-NMR specroscopy ortho, pyro and tri poly phosphates can be detected and estimated in presence of each other.Pyrophosphate gives single line at +5.57 ppm.

Tripolyphosphate gives five line spectrum, doublet, +4.81 ppm and relative amount of these species by there intansity ratios.

20.10 Questions

1. How many ^{19}F NMR absorpation for (a) PF_5 and (b) SF_4 (c) $(SF_6)^{2-}$
2. Explain following observation

   (1) Me_3CF ; $\delta_{ppm} = 1.30(d, 20Hz)$

   (ii) Me_2CHF; $\delta_{ppm} = 1.23(d-d, 23Hz \ \& \ 4\text{Hz})$
3. Write three isomeric state of CF_2BrCBr_2CN to explain follows

   (a) single ^{19}F peak at room temperature.

   (b) On lowering temperature AB quartet appears for two isomers while third rotamer shows single peak.

20.11 Summary

it is evedent tha there exist aproton –fluorine coupling in fluorine containing compound as fluorine has some typical frequency which is not far away 1H so the instrument needs slight modifications, in fluorine compounds the resonances tends to separate the various part of he spectrum,in aromatic compounds flourins is found to show coupling at ortho,meta and Para position in case of phosphorous compound the splitting is a special feature.

20.12 Refrence Books

- Organic Spectroscopy,William Kemp,palgrave publication
- Spectroscopy and molecular structure,T.N.Ojha,Shobhan Lal and company
- Application of Sectroscopy,John varkade