03 Biomolecules

Biomolecules are biological molecules, which are produced by cells and living organisms. Biomolecules have a wide range of sizes and structures.

Biomolecules perform many of biological functions within the cell and organisms which are important for life.
For example: Proteins and Carbohydrates.

Biomolecules :

1. Amino acids & Proteins
2. Carbohydrates
3. Vitamins
4. Nucleic Acids

Amino Acids & Proteins :

Proteins : Proteins are the most abundant-biomolecules of the living beings. The chief sources of proteins are milk, cheese, pulses, peanuts, fish, meat etc. These are high molecular mass complex, biopolymers of amino acids.

Amino Acids : Each living cell is made up of thousands of different proteins. All-natural proteins are polymers of α-(L) amino acids and on partial hydrolysis give peptides of varying molecular masses which upon complete hydrolysis give α-amino acids.

$$\text{Natural proteins} \xrightarrow{\text{Hydrolysis}} \text{Peptides} \xrightarrow{\text{Hydrolysis}} \alpha\text{-amino acids}$$

Proteins have number of important biological roles, both structural and functional.

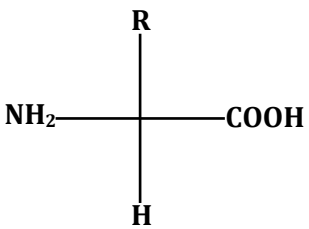
$$\text{Proteins} \longrightarrow \text{Proteios (Greek)} \longrightarrow \text{Primary or prime importance}$$

The word protein is derived from the Greek word proteios which means primary or off prime importance.

Proteins are polymer of α-amino acids. α-amino acids are the compound that have one or more amino group and carboxylic groups in a molecule. α-Amino acids are the back bone of protein.

α-Amino acids

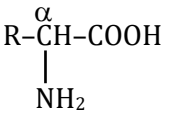
- The standard amino acids are 20 common α-amino acids that are found in nearly all proteins. The standard amino acids differ from each other in the structure of the side chains bonded to their a carbon atoms.
- All the standard amino acids are L-amino acids except glycene.
- Glycene ($NH_2–CH_2–COOH$) does not show optical activity.



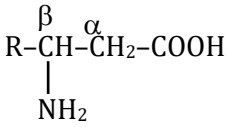
α-Amino acid

1. Classification of amino acids on the basis of relative position of NH_2 group

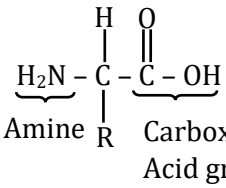
The amino acids contain amino as well as carboxylic acid group. On the basis of position of amino group in the chain, these are named as α, β, γ etc. amino acid.



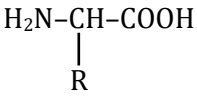
α-amino acid



β-amino acid



or



where, R = alkyl, aryl, or any other group, but never contain unstable, strained cycles or functional groups.

2. Classification of α-amino acids on the basis of requirement

(i) Non-essential Amino Acids

Can be synthesized in the body

Eg.

1. Alanine (Ala)	2. Asparagine (Asn)	3. Aspartic acid (Asp)	4. Cysteine (Cys)
5. Glutamic acid (Glu)	6. Glutamine (Gln)	7. Glycine (Gly)	8. Proline (Pro)
9. Serine (Ser)	10. Tyrosine (Tyr)		

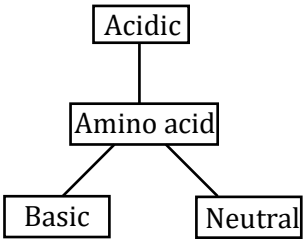
(ii) Essential Amino Acids

Cannot be synthesized in the body supplied in the diet.

Eg.

1. Phenylalanine (Phe)	2. Threonine (Thr)	3. Tryptothan (Try)	4. Methionine (Met)
5. Leucine (Leu)	6. Isoleucine (Ile)	7. Lysine (Lys)	8. Histidine (His)
9. Valine (Val)	10. Arginine (Arg)		

3. Classification of Amino Acids on the basis of chemical structures

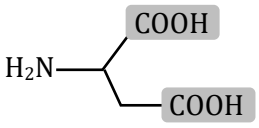


Acidic Amino Acids :

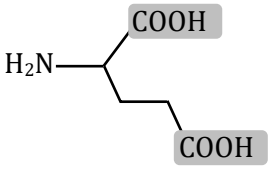
Acidic ⟶ Carboxylic groups > Amino group

eg.

Aspartic acid



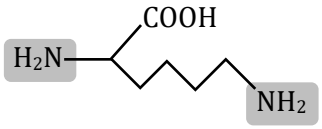
Glutamic acid



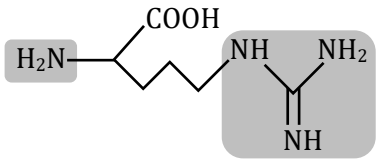
Basic Amino Acid :

Basic ⟶ Basic groups > Carboxylic groups

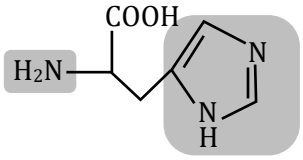
Lysine



Arginine

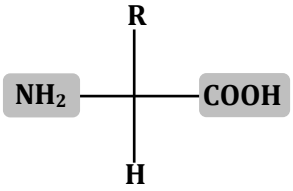


Histadine



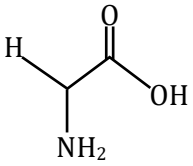
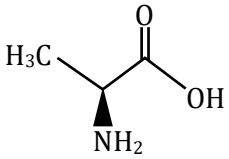
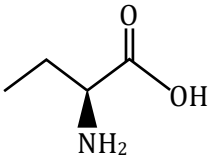
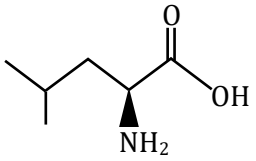
Neutral Amino Acids :

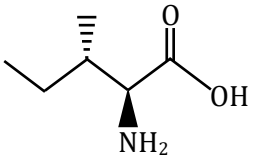
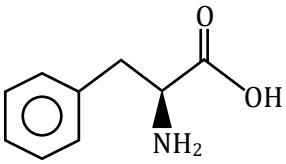
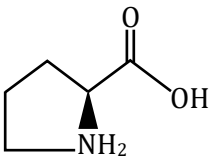
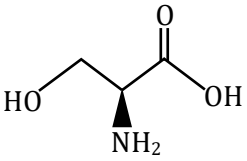
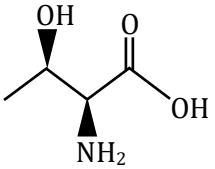
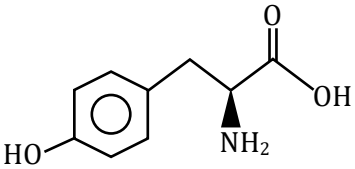
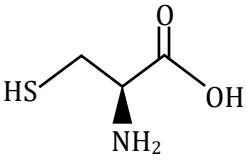
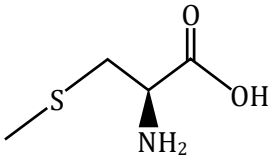
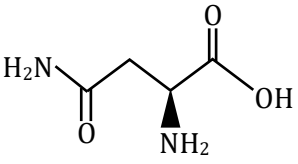
Neutral ⟶ Amino groups = Carboxylic groups

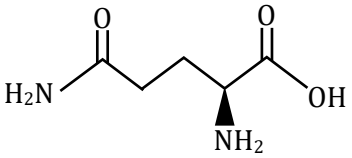
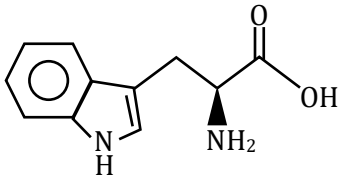
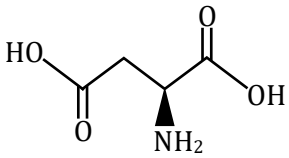
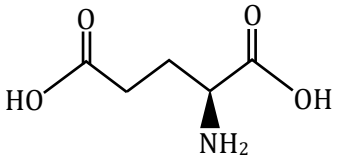


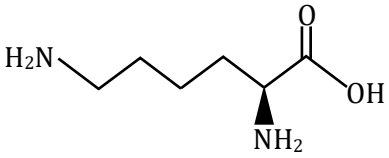
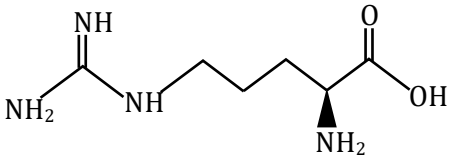
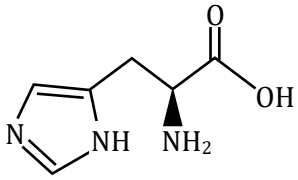
α-Amino acid

Table of Amino Acid :

Name	Three letter symbol	One letter symbol	Structure	Isoelectric Point
Glycine	Gly	G		5.97
Alanine	Ala	A		6.0
Valine	Val	V		5.96
Leucine	Leu	L		5.98

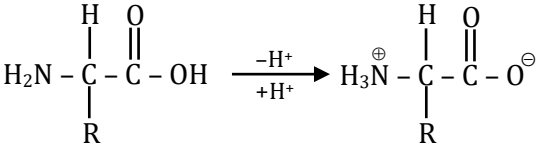
Name	Three letter symbol	One letter symbol	Structure	Isoelectric Point
Isoleucine	Ile	I		6.02
Phenylalanine	Ile	I		5.48
Proline	Pro	P		6.30
Serine	Ser	S		5.68
Threonine	Thr	T		5.60
Tyrosine	Tyr	Y		5.66
Cysteine	Cys	C		5.07
Methionine	Met	M		5.74
Asparagine	Asn	N		5.41

Glutamine	**Gln**	**Q**		**5.65**
Tryptophan	**Trp**	**W**		**5.89**
Side chain is acidic				
Aspartic acid	**Asp**	**D**		**2.8**
Glutamic acid	**Glu**	**E**		**3.2**

Side chain Basic				
Lysine	**Lys**	**K**		**9.74**
Arginine	**Arg**	**R**		**10.8**
Histidine	**His**	**H**		**7.6**

Zwitterion (Dipolar Nature of Amino acids) :

In a neutral amino acid solution, the –COOH loses a proton and the $-\ddot{N}H_2$ of the same molecule picks up one. The resulting ion is dipolar, charged but overall electrically neutral. This is called Zwitterion (German, "two ions"). Therefore, amino acids are amphoteric.



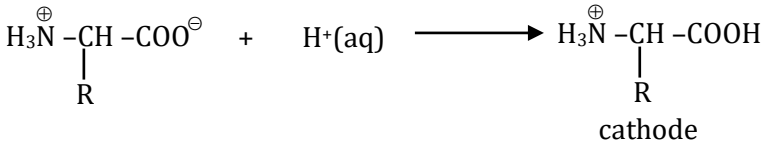
Zwitterion

There are around 20 amino acids in the living system.

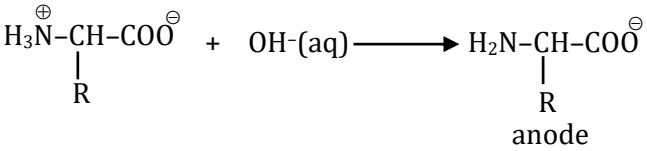
Isoelectric point of α-amino acids :

Isoelectric Point (PI) : The pH at which the amino acid shows no tendency to migrate when placed in an electric field is known as its isoelectric point.
Because of amphoteric nature in acidic solution it exist as the +ve ion. Hence it migrate towards cathode while in basic solution it exist as –ve ion and migrates towards anode.

In strongly acidic medium



In strongly basic medium



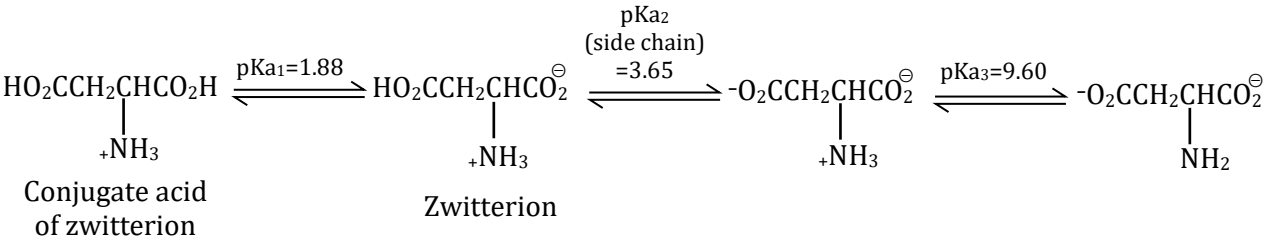
At some intermediate pH amino acids exist as a neutral dipolar ion i.e. the concentration of the cation and anions are equal and it does not migrate towards either electrode, this pH is called iso electric point of amino acid which is different for different amino acids.

For example :

(i) For neutral amino acid : pH of isoelectric point varies between 5.1 to 6.5. E.g. Glycine has pH value 6.0 PI for neutral amino acid is calculated as $\frac{pK_{a_1}+pK_{a_2}}{2}$

(ii) For acidic amino acid : Where there are two COOH groups and one NH_2 group then isoelectric pH is around 3. E.g. Aspartic acid.

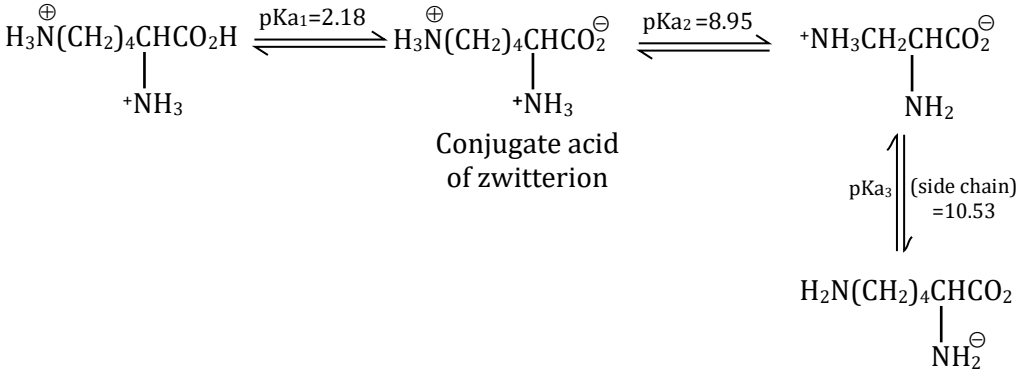
Aspartic acid :



PI for basic amino acid is calculated as $\frac{pK_{a_2}+pK_{a_3}}{2}$

The PI of Lysine is the average of $\mathbf{pK_{a_2}}$ (8.95) and the pK_a of the side chain (10.53) or 9.74.

Lysine :



Note: **(i)** Amino acid has minimum aqueous solubilities at their isoelectric points.

(ii) For acidic amino acid pI < 7

(iii) For basic amino acid pI > 7

Electrophoresis :

Q. Write the structure of alanine at pH 2.5, 10.5 and 6.

The movement of charged molecules (like amino acid) under the influence of an electric field is called electrophoresis. Electrophoresis separates amino acids on the basis of their pI values.

Amino acid is positively charged (moves towards cathode) if pH of the solution < pI

Amino acid is negatively charged (moves towards anode) if pH of the solution > pI

Q. How will you separate a ternary mixture of arginine, alanine & aspartic acid?

Ans. A few drops of a solution of an amino acid mixture are applied to the middle of a piece of filter paper. When the paper is placed in a buffer solution (pH = 5) between the two electrodes and an electric field is applied then arginine & alanine with pI > pH move towards the cathode and aspartic acid with pI < pH moves towards the anode. Out of arginine & alanine, alanine will move slowly towards the cathode due to lesser positive charge.

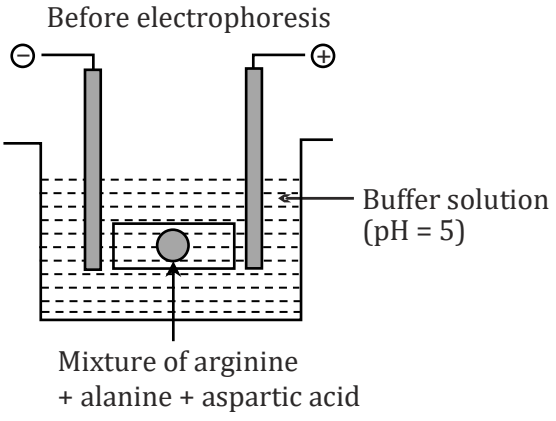
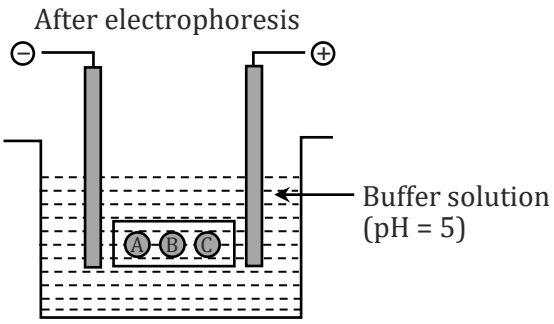


Figure (i)



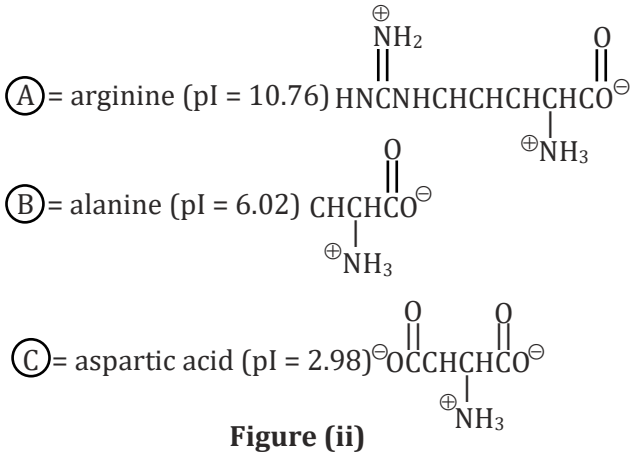


Figure (ii)

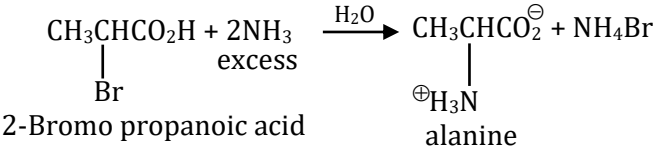
When different amino acids are involved in pepetide formation.
Then total number of polypeptide possible = X^n
[X = type of amino acid interacting,
n = number of amino acid molecule are interacting.]

Q. Glycine can form how many Dipeptide ? [Ans. One]
Q. Glycine can form how many Tripeptide ? [Ans. One]
Q. Glycine and Ala can form how many Dipeptide ? [Ans. Four]
Q. Gly, Ala, and Phenyl Ala can form how many Dipeptide ? [Ans. Nine]
Q. Gly, Ala, can form how many Tripeptide ? [Ans. Eight]

A polypeptide with more than hundred amino acid residues, having molecular mass higher than 10,000 is called a protein. However, the distinction between a polypeptide and a protein is not very sharp. Polypeptides with fewer amino acids are likely to be called proteins they ordinarily have a well defined conformation of a protein such as insulin which contains 51 amino acids.

(a) General methods of preparation

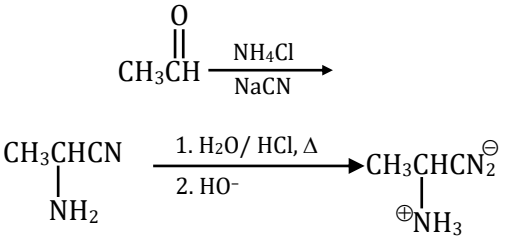
1. Aminolysis of α-halocarboxylic acid



2-Bromo propanoic acid alanine

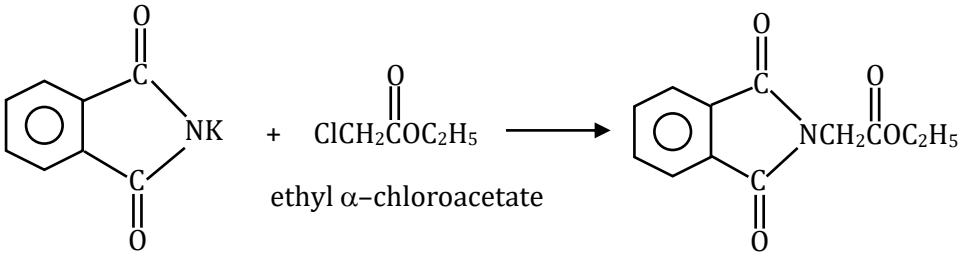
2. By strecker synthesis :

Aldehyde reacts with a mixture of NH_4Cl and NaCN to form α-aminonitrile (as an intermediate) which on hydrolysis gives an amino carboxylic acid.

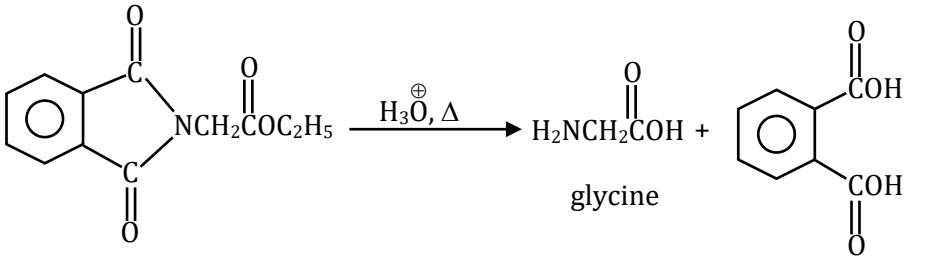


2-aminopropanertirile alanine

3. By Gabriel Synthesis :



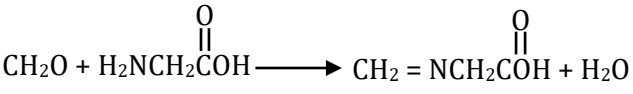
N–potassium phthalimide



phthalic acid

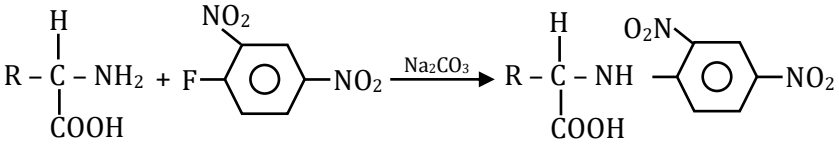
(b) Chemical reactions :

1. Formaldehyde reacts with amino acids to form N-methylene amino acids. In this reaction basic character is lost and thus, free acid can be determined by titration - Sorenson titration method for amino acids.

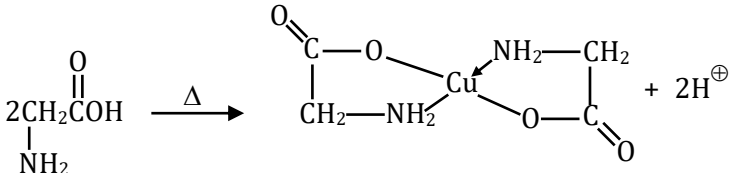


N–methylene glycine

2. DNFB **(2, 4-dinitrofluorobenzene)** also called **Sanger's reagent** reacts with the free amino group of terminal amino acid in a peptide or protein to form yellow coloured dinitro phenyl amino acid. This is thus, used to determine N-terminal amino acid.



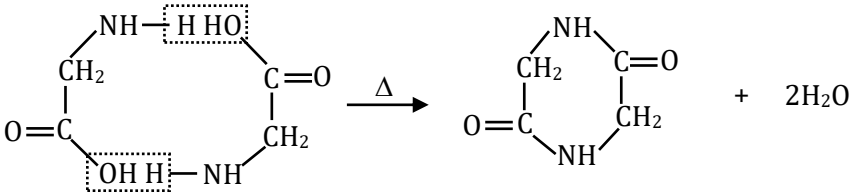
3. $Cu^{2\oplus}$ salts form blue coloured complex with amino acids.



Cupric glycinate
(deep blue)

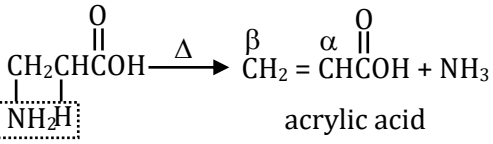
4. Effect of Heat :

α-amino acids undergo intermolecular dehydration on heating at about 200°C to give diketopiperazines.



glycine 2,5-diketopiperazine

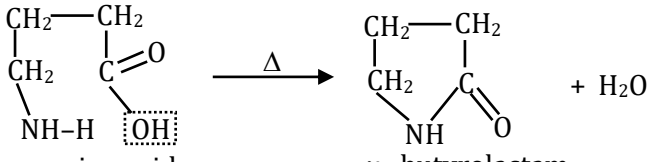
β-amino acids undergo intramolecular deamination on heating to form α, β-unsaturated acids.



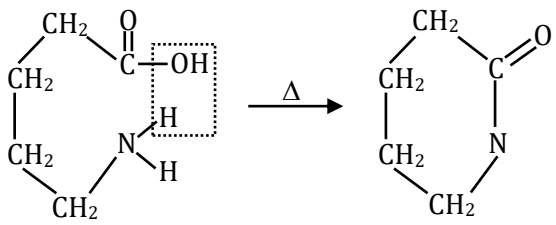
acrylic acid

γ-amino acids and δ-amino acid undergo intramolecular dehydration to form cyclic amides called.

Lactams.

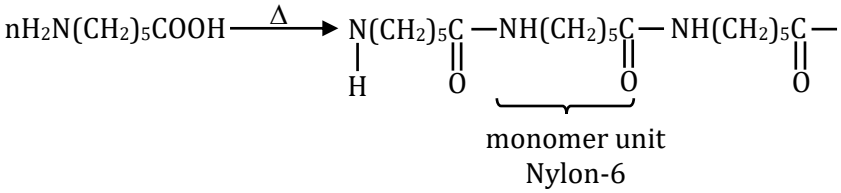


γ amino acid γ –butyrolactam

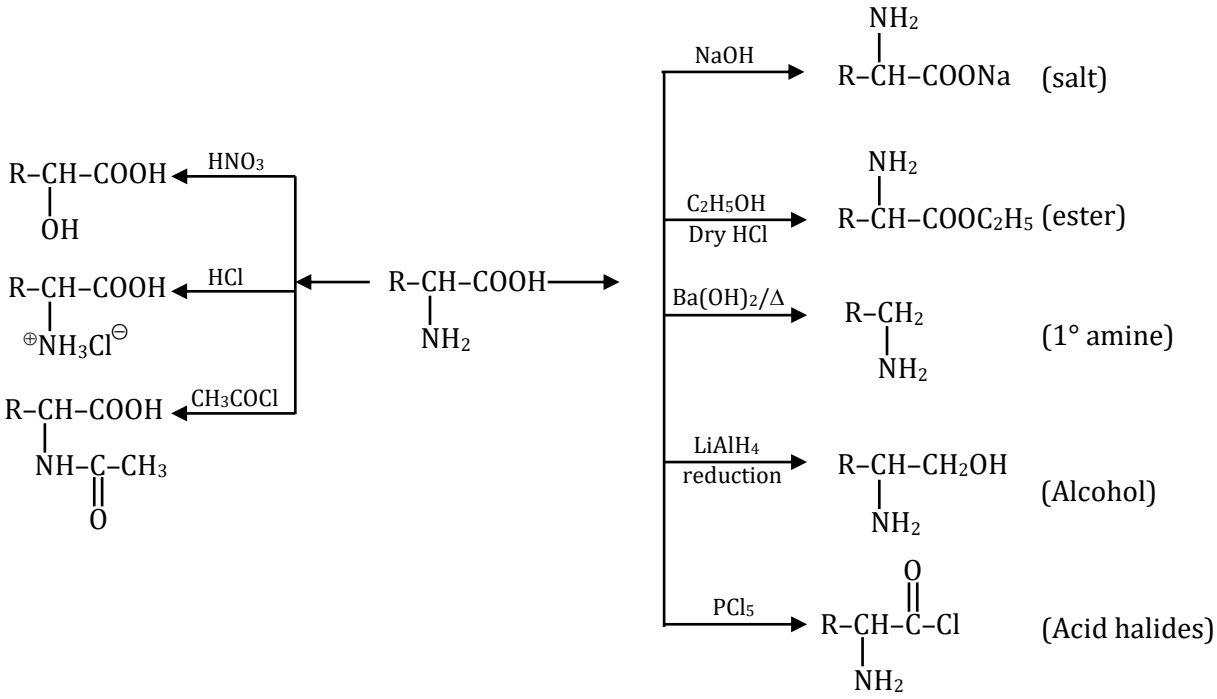


δ – amino acid δ – lactam

In case of ε-amino acid, intramolecular cyclisation would given a seven-membered ring, which is formed with difficulty. Hence, there is intermolecular polymerisation forming nylon-6.



(c) Other Reactions of α-Amino Acid :

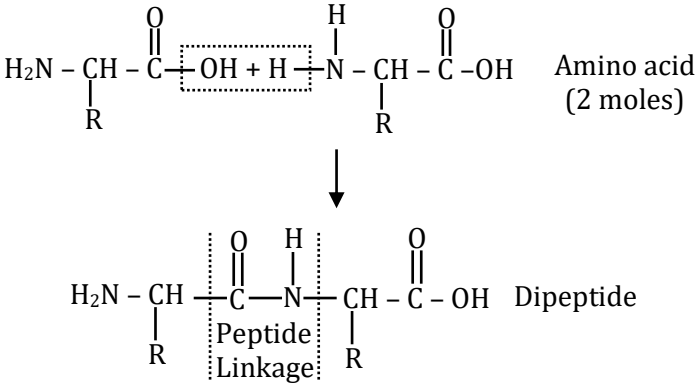


Peptides bonds and Proteins :

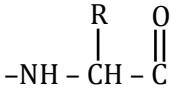
Peptides (Proteins) : Peptides are condensation polymers of α-amino acids formed by condensation of amino group of one α-amino acid with the carboxyl group of same or different α-amino acid by elimination of water. They are classified as di, tri, tetra, pentapeptides etc. according to two, three, four, five etc molecules of the same or different amino acid combining together. It determines their specific physiological functions in the living organism

Structure of Proteins (Peptides)

Amino acids are bifunctional molecules with $-NH_2$ group at one end and –COOH at the other. Therefore, –COOH of one molecule and $-NH_2$ of another molecule interact by elimination of H_2O to form an amide-like linkage.



Peptide Linkage : The amino acid unit having free – NH_2 groups is called N-terminal end whereas the amino acid unit with free – COOH group is called C-terminal end. The structure is written with N-terminal end to the left and C-terminal end to the right. At N-terminal or C-terminal further bond formation take place and tri, tetra, pentapeptide are formed.



unit repeated in polypeptides.

Polypeptides : More than 10 amino acids join together is called polypeptides which is a linear chain natural polymer.

Naming of polypeptides : Naming of polypeptides starts from –N–terminal residue, and suffix -ine of amino acids is replaced by -yl for all except amino acid of C-terminal residue.

Example : Glycine → Glycyl, Alanine → Alanyl, Lysine → Lysyl

Alanylglycylphenylalanine means Ala-Gly-Phe or A-G-F.

Proteins :

A polypeptide with more than 100 amino acid residues (mol. mass > 10,000) is called a protein but, a few polypeptide with lesser number of amino acid is also known.

Example: Insulin have 51 amino acids.

Classification of Proteins :

(I) On the basis of molecular structure : Proteins have been classified into two parts.

(i) Fibrous proteins (ii) Globular proteins

(i) Fibrous Proteins : When polypeptide chain run parallel and fibre like structure then it is called fibrous protein. In Fibrous protein chain are held together by hydrogen and disulphide bond. These are insoluble in water.

Ex. Keratin, myosin.

(ii) Globular proteins : When polypeptide chain is folded to form spheroidal shape it is called globular protein. Such folding is because of a folding of polypeptides in such a way that lipophilic (fat soluble) part are pushed inward and hydrophilic part is pushed outward. These are soluble in water and sensitive to small change in temperature and pH.

Ex. Albumins in egg, enzyme and some hormones, etc.

(II) On the basis of chemical composition :

(i) Simple proteins : Simple proteins on hydrolysis give only α-amino acids. For example albumin in the white portion of eggs, glutenin in wheat, oxygenin in rice, keratin in hair, nails horns etc.

(ii) Conjugated Proteins : In conjugated proteins, protein part. is combined with non-protein part. On hydrolysis these give a non-protein part in addition to the α-amino acids. This non-protein portion is called PROSTHETIC GROUP. It function is to control the biological function of the protein. Prothetic groups may be carbohydrate, phophate, lipids (ester of higher fatty acids) and so on.

Ex. Casein of milk, haemoglobin of blood are example of conjugated proteins.

Structure of Proteins :

Structure and shape of proteins can be studied at four different levels i.e. primary, secondary, tertiary and quarternary, each level being more complex than the previous one.

(i) Primary structure of Proteins :

Each polypeptide in a protein has amino acids linked with each other in a specific sequence and it is this sequence of amino acids that is said to be the primary structure of that protein. Any change in this primary structure i.e. sequence of amino acids creates a different protein.

Ex. Normal Haemoglobin: –Val – His – Leu – Thr – Pro – Glu –Lys–

Sickle cell anemia Haemoglobin: –Val – His – Leu – Thr – Pro – Val – Lys–

(ii) Secondary structure of Proteins :

The shape in which a long polypeptide chain can exist is called secondary structure of proteins. The following two different secondary structure are possible.

(a) α-Helix structure

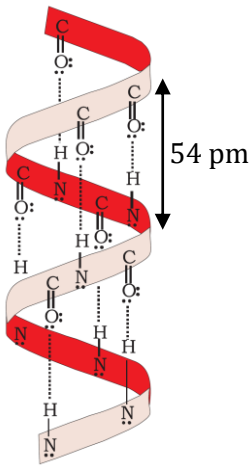
(b) β-pleated sheet structure

(a) α-Helix structure :

In α Helix a polypeptide chain forms all possible H–bonds by twisting into a right-handed screw (helix) –NH group of each amino acid residue H–bond to the C=O of an adjacent turn of helix.

(a)

(a) A right-handed α-Helix



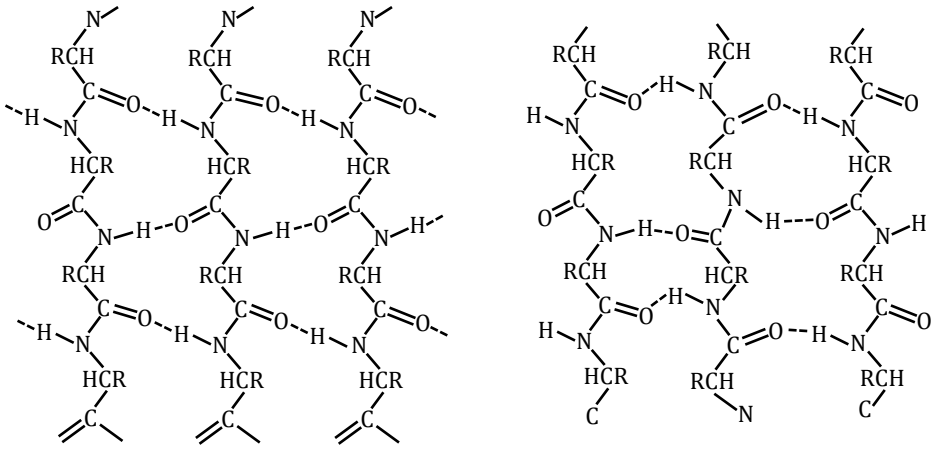
(b)

(b) Hydrogen bonding in α-Helix

(b) β-pleated sheet structure or simply β-structure :

In β structure all peptide chains are streteched out to a nearly maximum extension and then laid side by side which are held together by inter molecular H– bond.

The poly peptide chains can link together in parallel and antiparallel sequence. These are represented as follows :



(a) (PARALLEL) (b) (ANTIPARALLEL)

β-Conformation of Proteins (a) Parallel (b) Anti-parallel

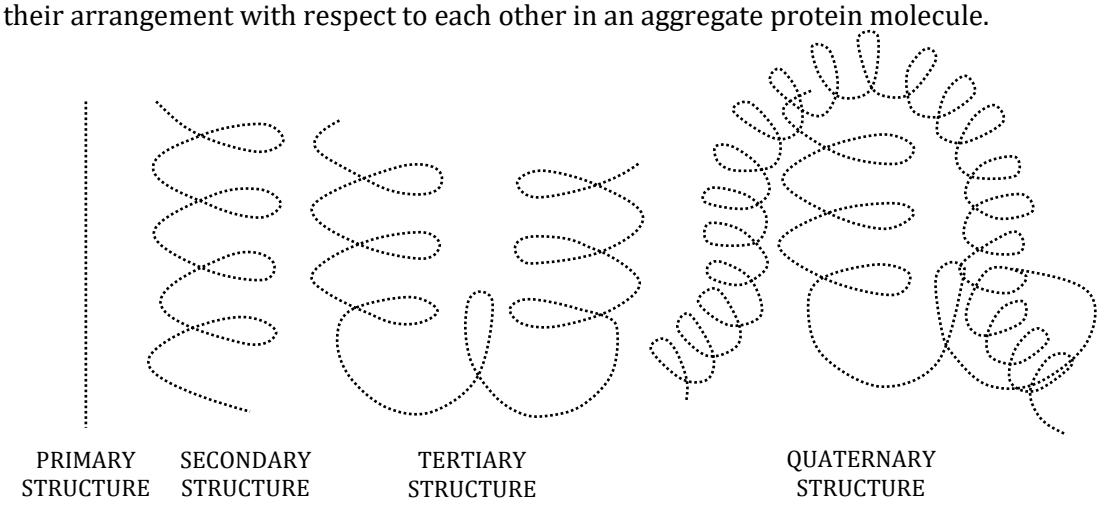
(iii)Tertiary structure of proteins :

The tertiary structure of protein represent overall folding of the polypeptide chains i.e. further folding of the secondary structure producing a 3D structure.

It gives rise to two major molecular shapes fibrous and globular.

(iv) Quarternary structure of proteins :

There are certain proteins which are composed of two or more polypeptide chains referred to as sub-units or protomoss. The quarternary structure refers to the determination of the number of sub-units and their arrangement with respect to each other in an aggregate protein molecule.



Diagrammatic representation of four levels of protein structure
(two subunits of two types in quaternary structure)

Denaturation of proteins:

When protein in native form is subjected to a physical change like temperature or pH, the H–bonds are disturbed. As a result, globules get unfold and helices get uncoiled therefore proteins loses its activity. During denaturation 2° and 3° structures get destroyed but 1° structure remain the same.

Ex: Coagulation of egg while on boiling and curdling of milk caused by bacteria present in milk.

Test of Amino acids and Proteins

1. Biurate Test **2. Nin hydrin Test** **3. Xanthoproteic test**

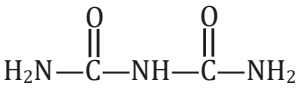
4. Sakaguchi test **5. Millon's Test**

1. Biurate Test

Reagent : (i) Hydrated $CuSO_4$ + KOH solution + Sod. Pot. Tartarate

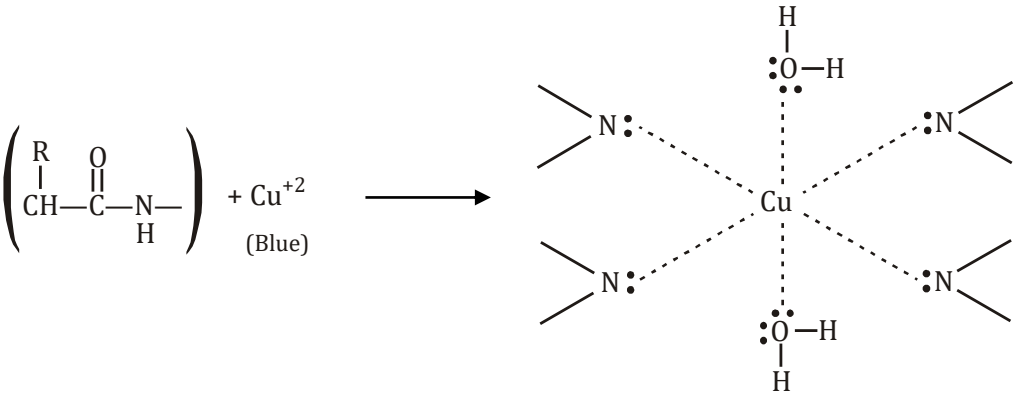
Biuret is not present in this reagent.

The name of test is derived from a specific compound, biuret, which gives a positive test with this reagent



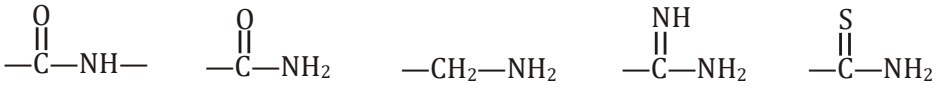
biuret

When a protein reacts with copper (II) sulphate (blue), the positive test is the formation of a violet colored complex.



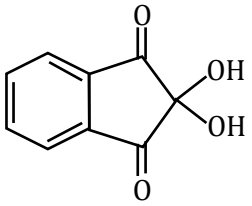
(Violet)

The biuret test works for any compound containing two or more of the following groups.



2. Nin hydrin Test

Reagent :

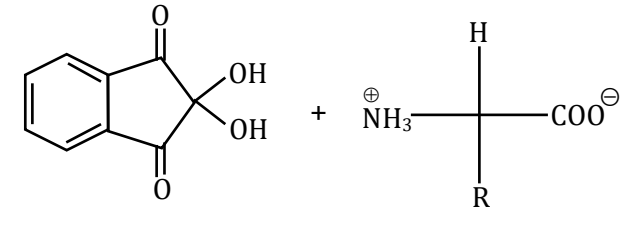


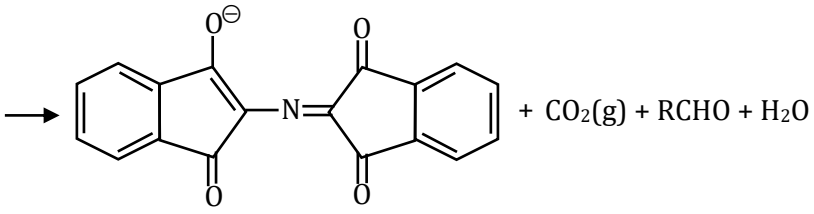
Ninhydrin hydrate

IUPAC name : 2, 2-Dihydroxy in dane-1,3-dione

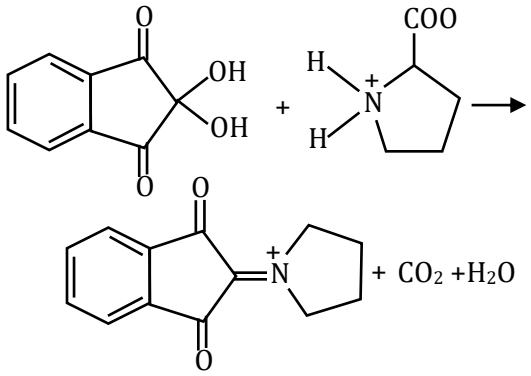
The ninhydrin test is a test for amino acids and proteins with a free $-NH_2$ group.

α-amino acids with primary amino group gives blue/purple solution





α-amino acids with secondary amino group "proline" gives yellow solution



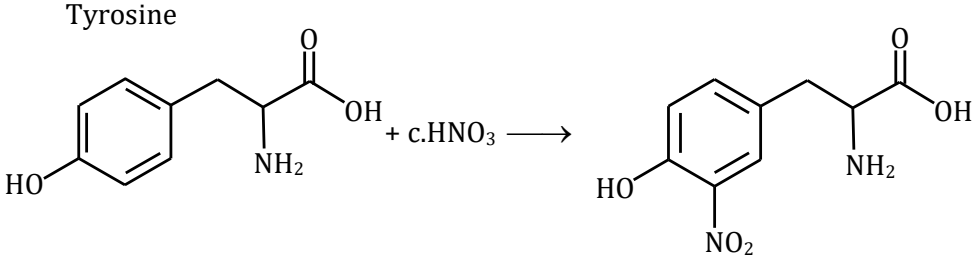
3. Xanthoproteic test

Reagent : Nitrating mixture

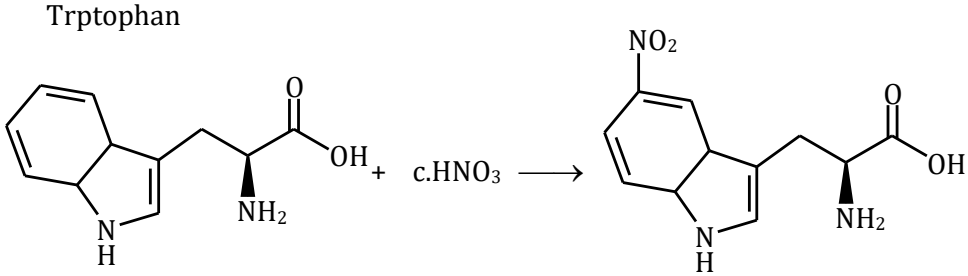
This test is used for aromatic amino acids which give positive result from other amino acids. Such as tyrosine, and tryptophan gives Xanthoproteic test, phenyl alanine does not respond with this test.

Principle : Xanthoproteic test is used to detect amino acids containing an aromatic nucleus (tyrosine, tryptophan and phenylalanine) in a protein solution which gives yellow color nitro derivatives on heating with conc. HNO_3. The aromatic benzene ring undergoes nitration to give yellow colored product. Phenylalanine gives negative or weakly positive reaction though this amino acid contains aromatic nucleus because it is difficult to nitrate under normal condition. On adding alkali to these nitro derivative salts, the color change for yellow to orange.

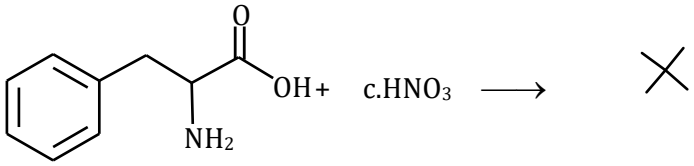
Tyrosine



Trptophan



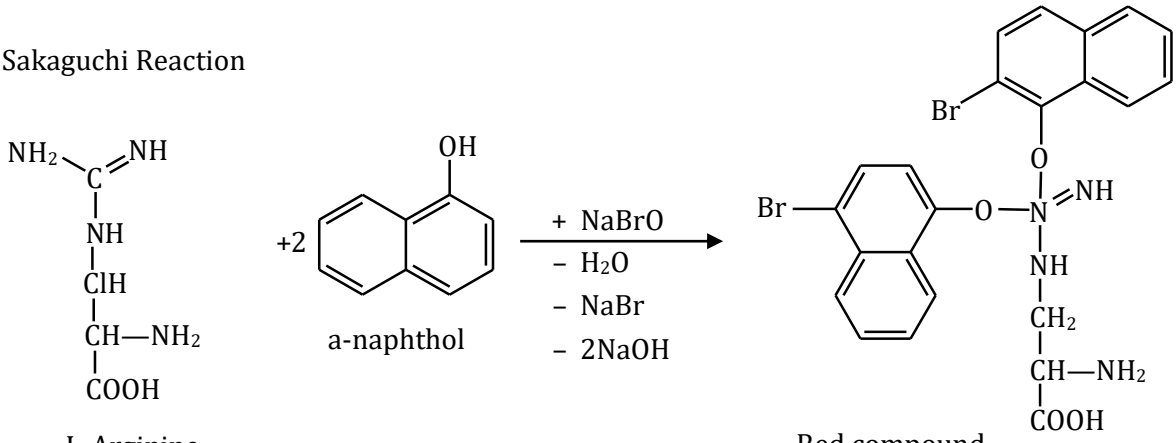
Phenylalamine



4. Sakaguchi test

The Sakaguchi test is a chemical test used for detecting the presence of arginine in proteins Sakaguchi reagent consists of α-Naphthol and a drop of sodium hypobromite. The guanidine group in arginine reacts with Sakaguchi reagent to form a red-coloured complex.

Sakaguchi Reaction

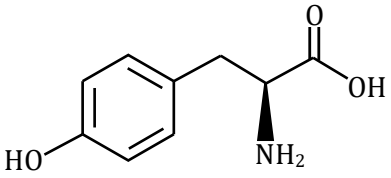


L-Arginine

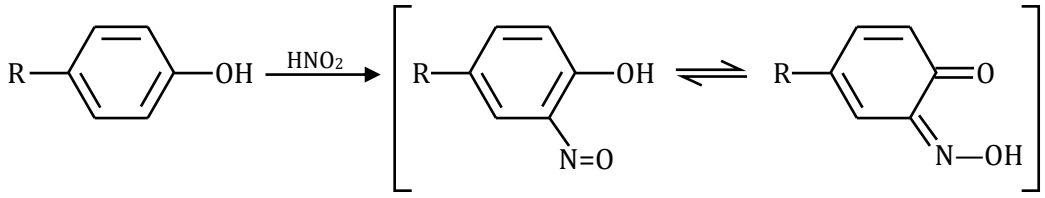
Red compound

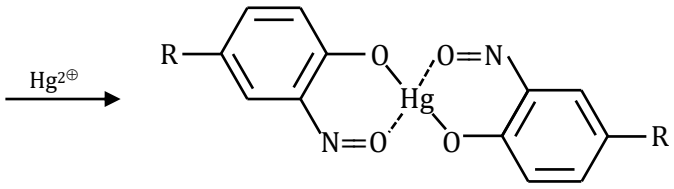
5. Millon's Test

Millon's reagent is an analytical reagent used to detect the presence of soluble proteins. A few drops of the reagent are added to the test solution, which is then heated gently. A reddish-brown coloration or precipitate indicates the presence of tyrosine residue which occur in nearly all proteins



Tyrosine Here R = $-CH_2CH(NH_2)COOH$.





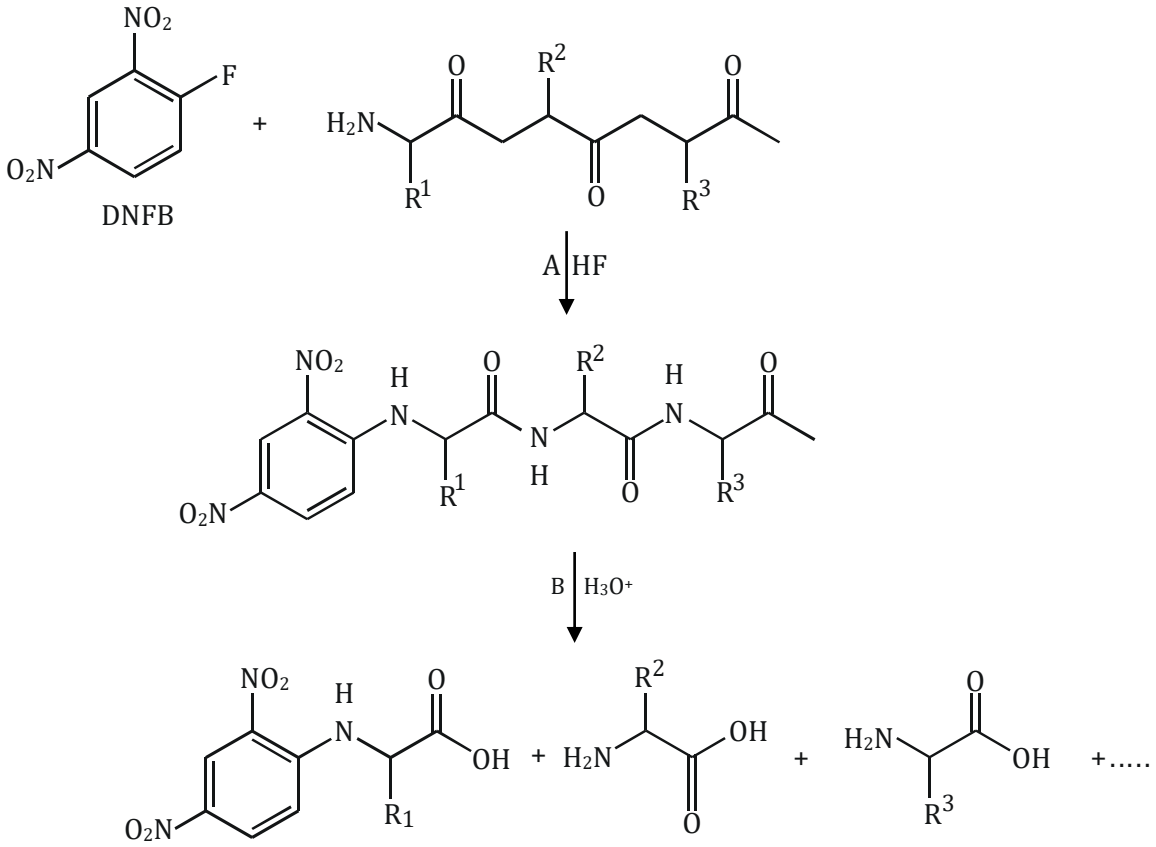
reddish-brown colored complex

<u>Sanger's Method of sequencing of Amino acids of polypeptide chain</u>

It is N-terminal amino acid analysis

Sanger's reagent (1-fluoro-2,4-dinitrobenzene)

1. React the peptide with a reagent that will selectively label the N-terminal amino acid.
2. Hydrolyse the protein.
3. Determine the amino acid by chromatography and comparison with standards.



CARBOHYDRATES :

Carbohydrates (hydrates of carbon) are naturally occurring compounds having general formula $C_x(H_2O)_y$, which are constantly produced in nature & participate in many important bio-chemical reactions.

Ex.	Glucose	$C_6H_{12}O_6$	$C_6(H_2O)_6$
	Fructose	$C_6H_{12}O_6$	$C_6(H_2O)_6$
	Cellulose and Satrch	$(C_6H_{10}O_5)_n$	$(C_6(H_2O)_5)_n$

- Sucrose (Cane suger) – $C_{12}H_{22}O_{11}$, and
- Maltose (Malt Suger) $C_{12}(H_2O)_{11}$

But some compounds which have formula according to $C_x(H_2O)_y$ are not known as carbohydrate

Ex.	CH_2O	Formaldehyde
	$C_2(H_2O)_2$	Acetic acid
	$C_3(H_2O)_3$	lactic acid

There are many compounds, which shows chemical behaviour of carbohydrate but do not confirm the general formula $C_x(H_2O)_y$ such as -

$C_5H_{10}O_4$ (2–deoxyribose), $C_6H_{12}O_5$ (Rahmnose) , $C_7H_{14}O_6$ (Rahmnohexose)

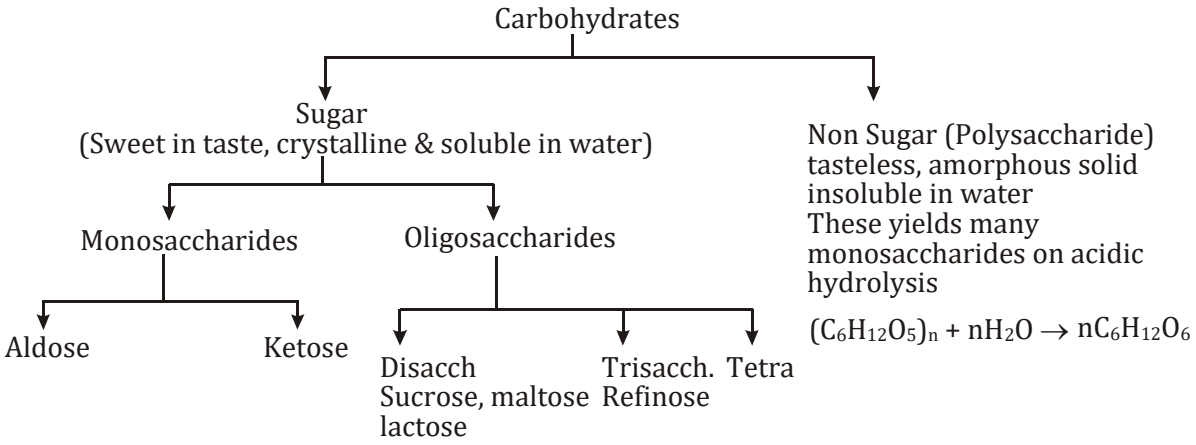
Modern Concept : Carbohydrates are polyhydroxy aldehyde or ketone

or

Substances which yield these (polyhydroxy aldehyde or ketone) on hydrolysis

- Carbohydrates $\xrightarrow{H_2O/H^+}$ Polyhydroxy aldehyde or ketone
- Carbohydrates are also known as Saccharides.
- In plants carbohydrates are synthesised by photosynthesis

Classification of carbohydrates :



Monosaccharides : (simple sugars)

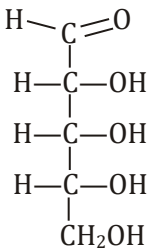
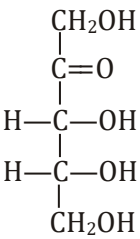
These are the sugars which cannot be hydrolysed into smaller molecules.

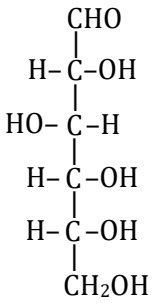
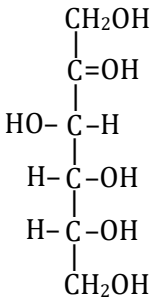
General formula is $C_nH_{2n}O_n$

Ex. Glucose, Fructose, Ribose, Deoxynbose

- If aldehyde group is present in monosaccharide, then it is known as aldose.
- If ketone group is present in monosaccharide , then it is known as ketose.

On the basis of C-atom monosaccharides can be farther classified as, Triose, Tetrose, Pentose, Hexoses.

		Aldoses	**Ketoses**
3C	Tropose or Triose	Aldotriose	Ketotriose
4C	Tetrose	Aldotetrose	Ketotetrose
5C	Pentose	Aldopentose	Ketopentose
5C Including –CHO	Aldopentose (Ribose)		
5C Including 	Ketopentose		(Ributose)
6C	Hexose	Aldohexose	Ketohexose

6C	Including –CHO	Aldohexose (Glucose)  D– glucose
6C	Including 	Ketohexose (Fructose)  D– fructose

Oligosaccharides :-

These are the sugars which yeilds 2–10 monosaccharides units on hydrolysis.

These are of following types

(a) Disaccharides :- Gives two monosaccharide unit on hydrolysis (may or may not be same).

Ex. Sucrose, Maltose, Lactose

(b) Trisaccharides :- Gives Three monosaccharide unit on hydrolysis.

Polysaccharides :- These are the non sugars which yeild a large no of monosaccharide units on hydrolysis General formula - $(C_6H_{10}O_5)_n$.

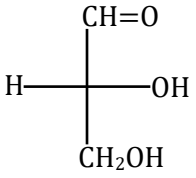
Ex. Starch, Cellulose, Glycogen

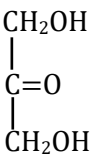
Note :- A group of polysaccharides which are not so widely used in nature is pentosans $(C_6H_{10}O_5)_n$ Monosaccharides, General formula $C_x(H_2O)_y$ x = 3 – 8. Nomenclature of monosaccharides are given according to the no. of carbons present in them.

Some facts:

(i) Number of carbons in monosaccharides are generally 3 to 7.

(ii) Simplest aldose is Glyceraldehyde and simplest Ketose is Dihydroxyacetone.

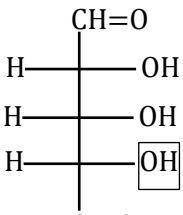

(D-Glyceraldehyde)


(Dihydroxyacetone)

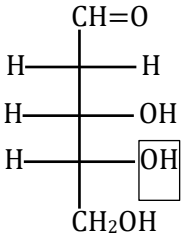
(iii) Most naturally occurring monosaccharides are :

(a) Pentoses E.g. D-Ribose (present in RNA) and 2-Deoxyribose (present in DNA)

(b) Hexoses E.g. D-Glucose, D-Mannose, D-Allose, D-Galactose and D-Fructose



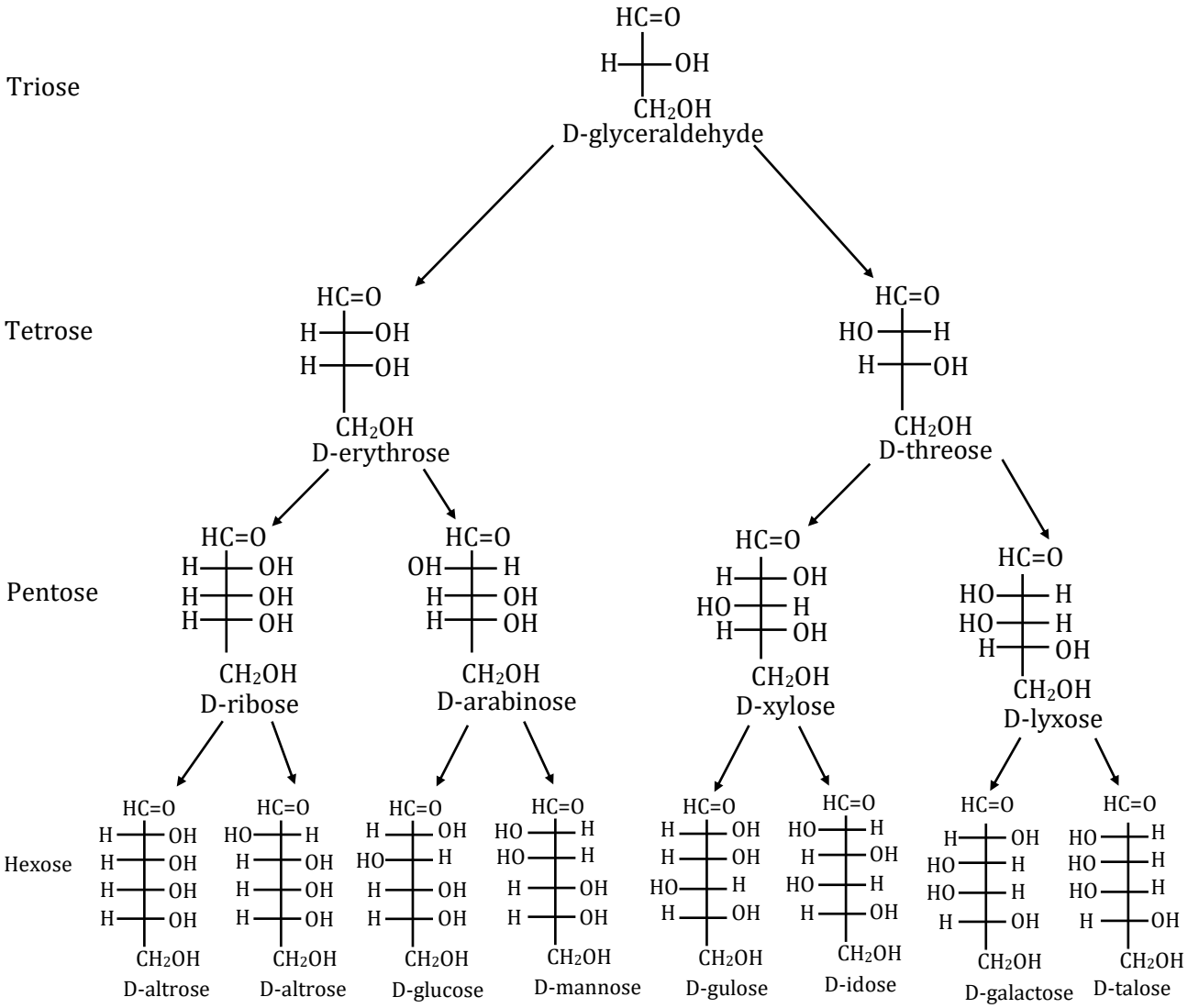
(D–Ribose)



(D–2–Deoxyribose

3.Stereochemistry of Aldoses:

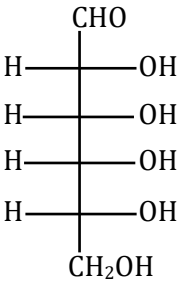
The monosaccharides are chiral and may have D or L configuration. A simple illustration of all 'D' forms is given below.



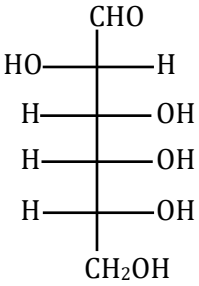
Optical isomers of Aldohexoses :

Aldohexoses have four asymmetric carbon atoms, therefore they have sixteen optical isomers out of which 8 are D and 8 are L variety (overall Eight pairs of enantiomers).

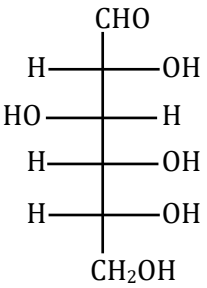
D-variety of them are as follows



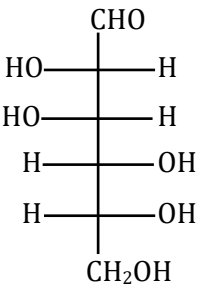
D(+)-allose
(I)



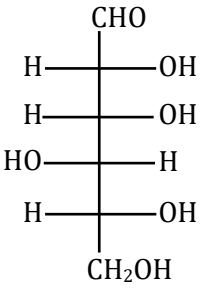
D(+)-altrose
(II)



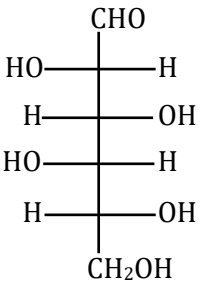
D(+)-glucose
(III)



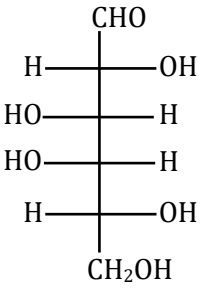
D(+)-mannose
(IV)



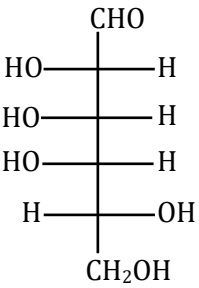
D(–)-gulose
(V)



D(–)-idose
(VI)



D(+)-galactose
(VII)



D(–)-talose
(VIII)

Note :

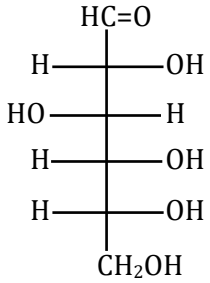
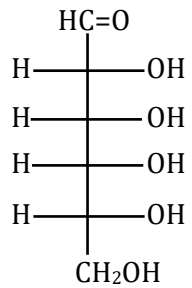
1. D-aldohexoses shown above have diastereomeric relationship with each other

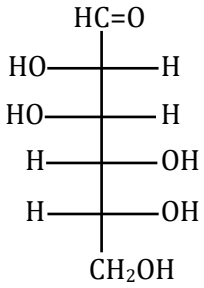
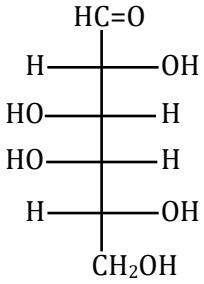
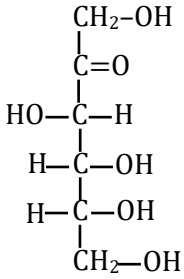
2. D-aldohexoses can be either dextro (+) or laevo (-).

4. Structure of aldohexoses :

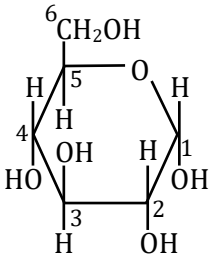
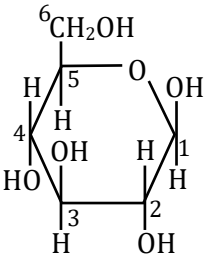
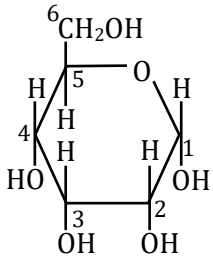
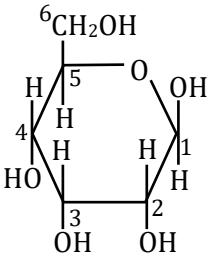
All form of Aldose or ketose may exist in open chain form as well as in cyclic pyranose or furanose form.

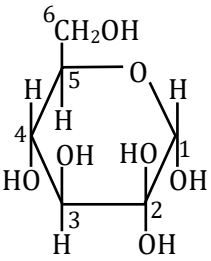
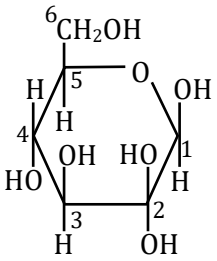
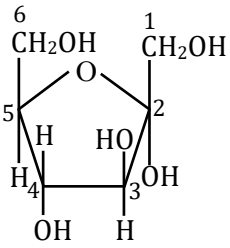
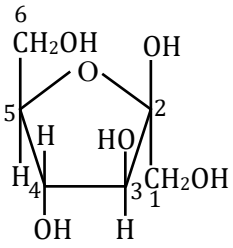
(i) Open chain structure of mono saccharides

Carbohydrate	Structure	Functional Group	Typical nature
D-Glucose		aldehyde	3rd (L)
D-Allose		aldehyde	No (L)

D-Mannose		aldehyde	2,3 (L)
D-Galactose		aldehyde	3, 4 (L)
D-Fructose		Ketone	3(L)

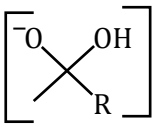
(ii) Cyclic structure of monosaccharides

Carbohydrate	Cyclic structure	
Glucose	 α-D-glucopyranose	 β-D-glucopyranose
Allose	 α-D- Allopyranose	 β-D-Allopyranose

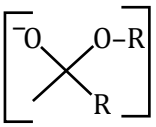
Mannose	 α-D- Mannopyranose	 β-D- Mannopyranose
Fructose	 α-fructofuranose	 β-D- fructofuranose

5. Anomers :

Anomers are diastereomers that differ in the configuration at the acetal or hemiacetal C atom of a sugar in its cyclic form (Anomeric carbon: A carbon bonded with two 'O' atoms). For example, α D(+) and β -D(+) glucose are anomers. α-D(–) and β-D(–) fructose are anomers.



Hemiacetal functional group

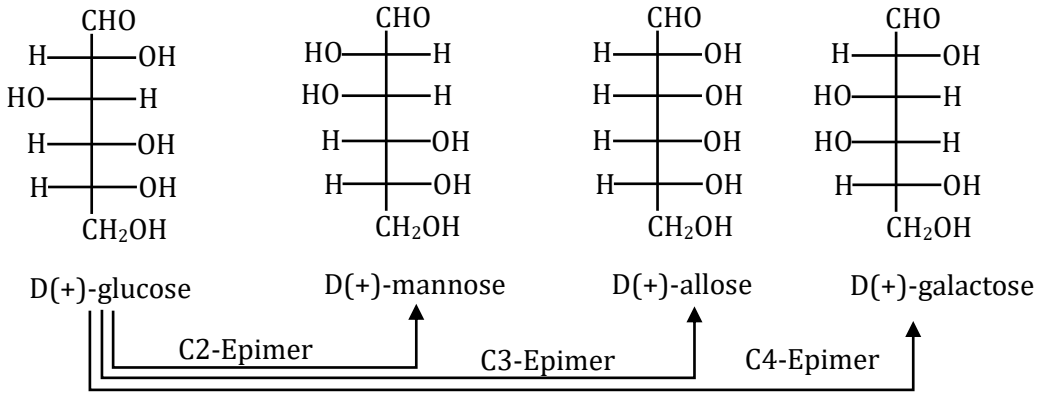


Acetal functional group

6. Epimers :

Diastereomers with more than one stereocentre that differ in the configuration about only one stereocentre (other than anomeric carbon) are called epimers.

i. D-Erythrose and L-threose are epimers.
ii. D-glucose and D-galactose are C-4 epimers and
iii. D-idose and D-talose are C-3 epimers.
iv. D-glucose and D-mannose are C-2 epimers.
v. Epimerisation of glucose at C-2 gives mannose.
vi. Epimerisation of glucose at C-3 gives allose.
vii. Epimerisation of glucose at C-4 gives galactose.



Reducing and non-Reducing properties of Sugars :

	(I) Reducing sugars	(II) Non Reducing sugars
1.	Reduces Tollen's reagent, Fehling's solution & Benedicts's solution.	Don't reduce Tollen's, Fehling's & Benedict's solution.
2.	Should have atleast one hemiacetal or hemiketal functional group.	Should have acetal linkage.
Ex.	All Mono and Oligosaccharides except Sucrose	Ex. All Polysaccharides and few Oligosaccharides (Ex. Sucrose)

MONOSACCHARIDES

GLUCOSE

Glucose is the most common monosaccharide. It is known as Dextrose because it occurs in nature principally as the optically active dextrorotatory isomers. It is act as a reducing agent (reduces both Fehling's solution and ammonical silver nitrate solution **; Tollen's reagent**). It is known as **dextrose** and found as grapes, honey, cane sugar, starch and cellulose.

Preparation of Glucose :

(i) By acid hydrolysis of canes sugar (a disaccharide) :
If sucrose is boiled with dil. HCl or H_2SO_4 in alcoholic solution. Glucose & fructose are obtained in equal amount.

$$\underset{\text{Disaccharides}\\\text{sucrose}}{C_{12}H_{22}O_{11}} \xrightarrow{H_2O/H^+} \underset{\alpha\text{-glucose}}{C_6H_{12}O_6} + \underset{\beta\text{-fructose}}{C_6H_{12}O_6}$$

(ii) By enzymatic action over starch : Glucose is obtained by hydrolysis of starch by boiling it with dil. H_2SO_4 at 393 K under pressure.

$$\underset{\text{Polysaccharides}\\(C_6H_{10}O_5)_n}{\text{Starch}} \xrightarrow{\text{Diastase}} \underset{\text{Disaccharides}\\(C_{12}H_{22}O_{11})}{\text{Maltose}} \xrightarrow{\text{Maltose}} \underset{\text{Monosaccharides}\\(C_6H_{12}O_6)}{\text{Glucose}}$$

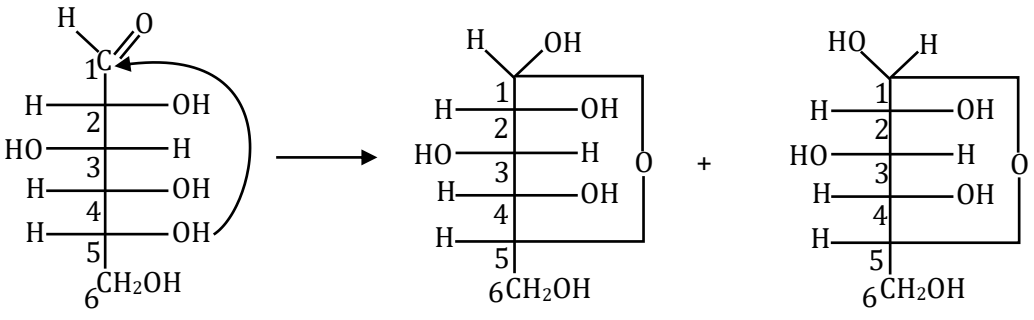
Structure of Monosaccharides :

Open chain structure (Fisher projection) and Cyclic structure (Haworth projection) :

(1) Despite having aldehyde group, glucose does not give schiff's test & it does not form the hydrogen sulphite (bisulphite) addition product with $NaHSO_3$.

(2) The pentaacetate of glucose does not react with hydroxylamine indicating the absense of free –CHO group. This behaviour could not be explained by open chain structure. It was proposed that one of –OH group add to CHO group, forms a cyclic structure.

These two cyclic hemiacetal forms of glucose differs only in configuration of the hydroxyl group at C_1, called anomeric carbon. Such isomers i.e. α-form & β-form, are called anomers.



D-Glucose
Specific rotation (+52.7°)

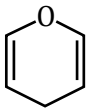
α-D-Glucose
Specific rotation (+112°)

β-D-Glucose
Specific rotation (+19°)

Note : β-form of D-glucose is more stable than α-D-Glucose.

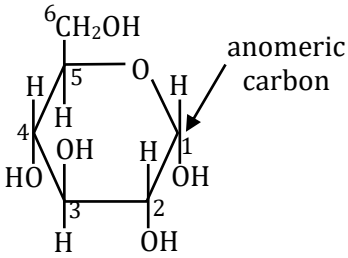
Haworth projection

The six membered cyclic structure of glucose is called pyranose structure (α- or β-), in analogy with pyran and five membered cyclic structure of monosaccharides is called furanose structure (α or β) in analogy with furan.

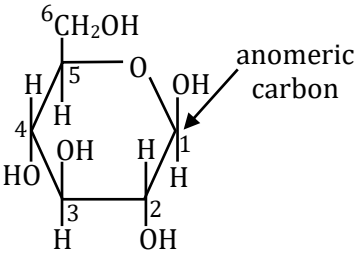


Pyran

Furan

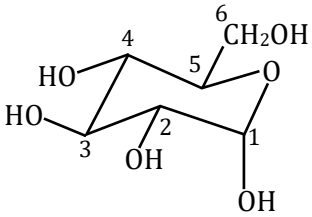


Specific rotation (112°)
α-D-glucose
α-D-glucopyronose

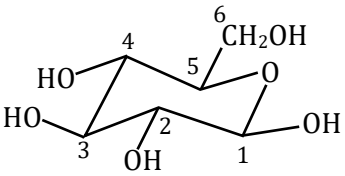


Specific rotation (19°)
β-D-glucose
β-D-glucopyronose

α and β-Glucose are anomer's of each other (i.e. change in configuration of 1st carbon atom only)



α-D-Glucose

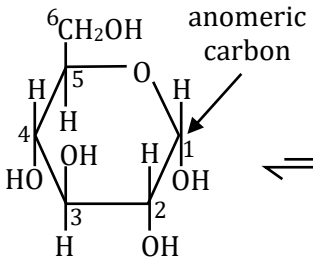


β-D-Glucose

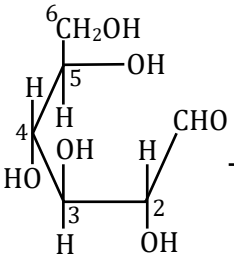
Properties of Anomers : Mutarotation

When one of the pure glucose anomers dissolve in water, an intersting change in the specific rotation is observed. When the α-anomer dissolves, its specific rotation gradually decreases from an initial value of +112° to +52.7°. When the pure β anomer dissolves, its specific rotation gradually increases from +19° to the same value of +52.7°. **This change (mutation) in the specific rotation is called mutarotation**. What is happening to each solution ?

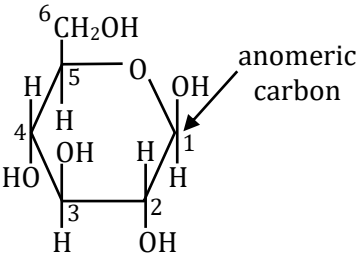
Initially solution with only one anomeric form, undergoes equilibrium to the same mixture of α-and β-forms. The open chain form is in intermediate in the process of equilibrium. For mutarotation at least one hemiacetal group must be present in the sugar therefore **all reducing sugars will mutarotate.**



Specific rotation (112°)
α-D-glucose
α-D-glucopyranose



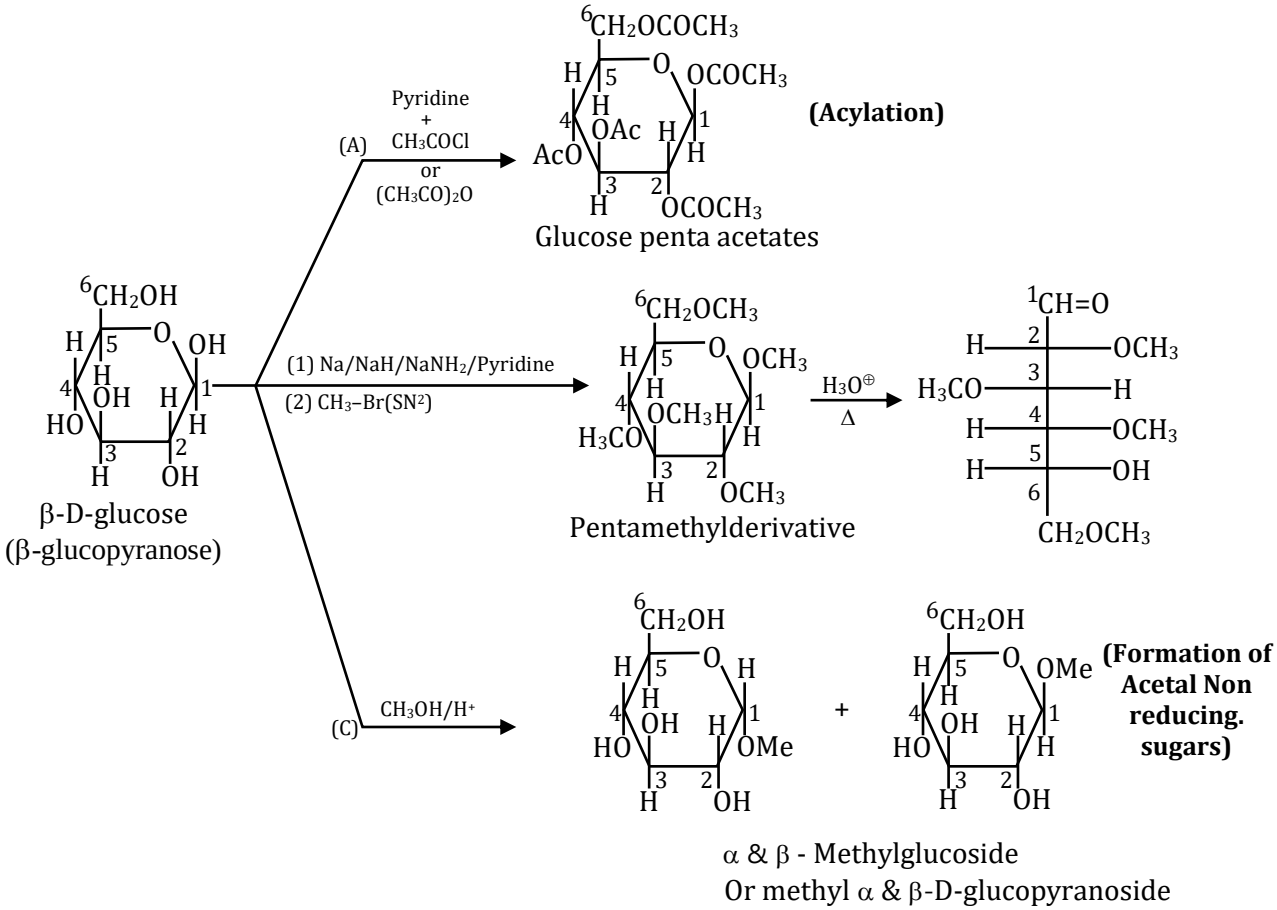
Open chain form of
-D-glucose
α-specific (+52.7°)



Specific rotation (19°)
β-D-glucose
β-D-glucopyranose

Chemical Reactions of Anomers (Glucose) :

(1) Reaction due to OH group:



Note:

(A) Acylation with acid halide or acetic anhydride gives pentaacetates which confirms the presence of five –OH groups.

(B) After Hydrolysis product of pentamethyl derivatives, aldehyde group and hydroxy of C_5 regenerated hence hydroxy of C_5 is involved in the hemiacetal formation.

(C) (i) Sugars in the form of **acetals** are called **glycosides**. (glucose → glucoside, mannose →mannoside, ribose → riboside, fructose →fructoside etc).

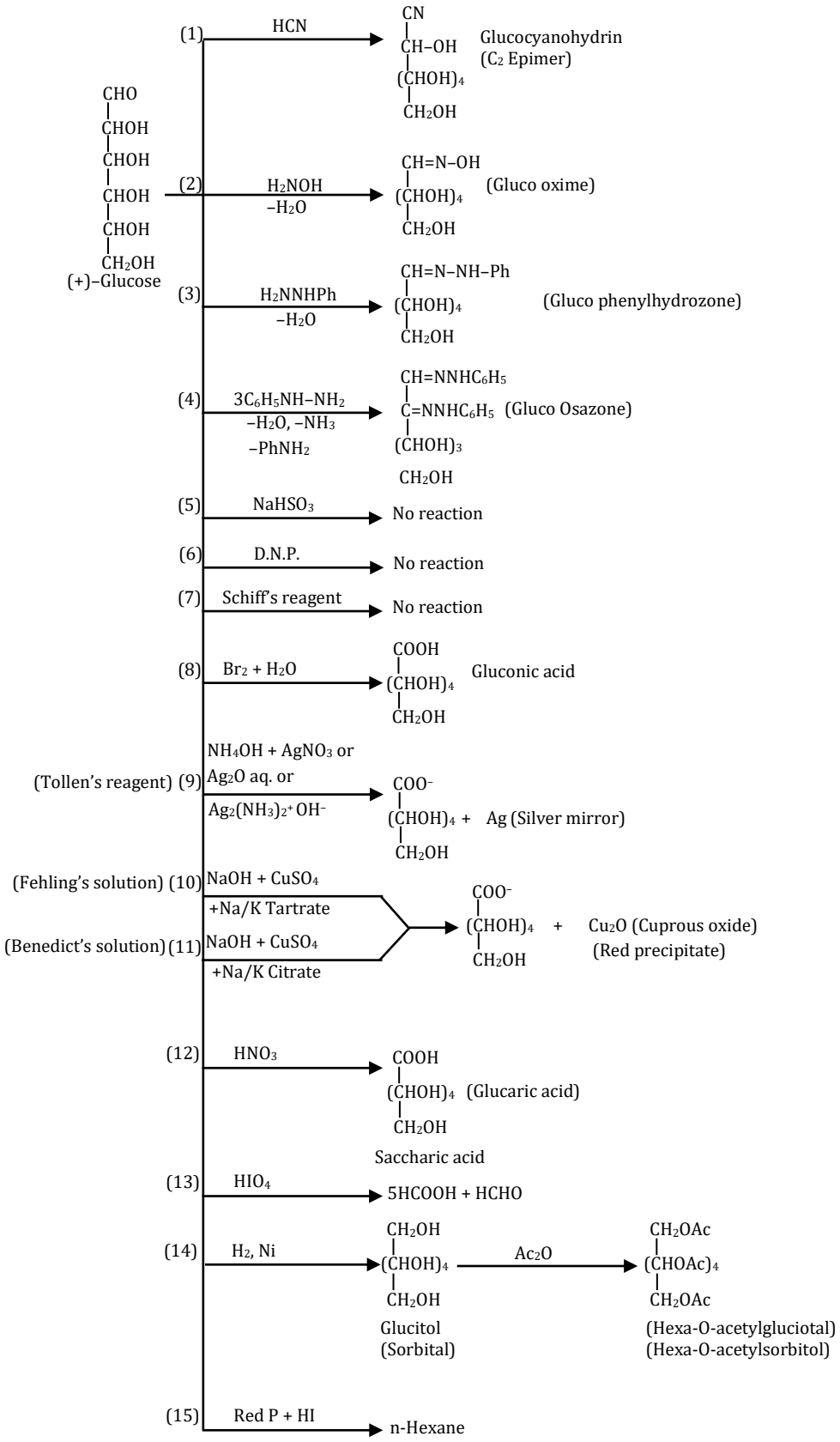
(ii) In the formation of glycosides only one mole of alcohol is required so monosacchardies are already present in the hemiacetal form with one of the hydroxyl group and carbonyl group.

(iii) Glycosides are non-reducing and will not show mutarotation because in neutral and basic condition glycosides are stable (cyclic form cannot open to the free carbonyl compound).

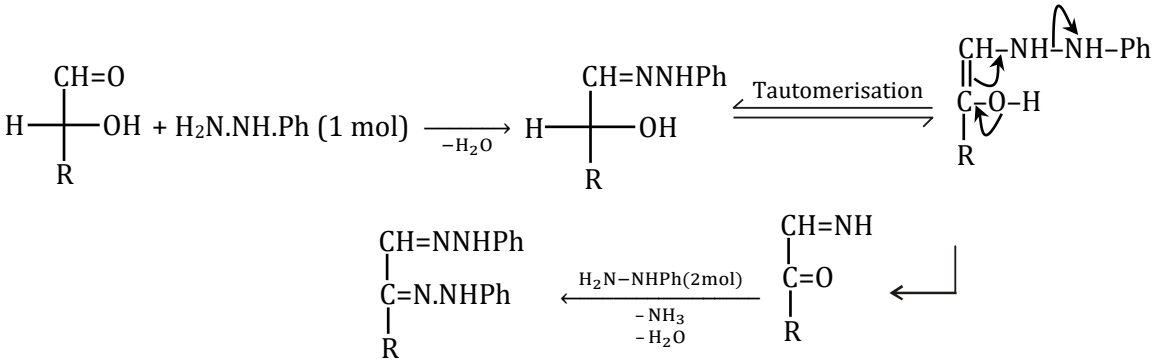
(iv) After acidic hydrolysis of glycosides, product form will have reducing property and also show mutarotation.

(2) Reaction due to aldehyde :

In aqueous solution, α-Anomer or β-Anomer remains in the equilibrium with each other by small amount of open chain forms (0.02%), in which carbonyl group is regenerated and gives various reactions.

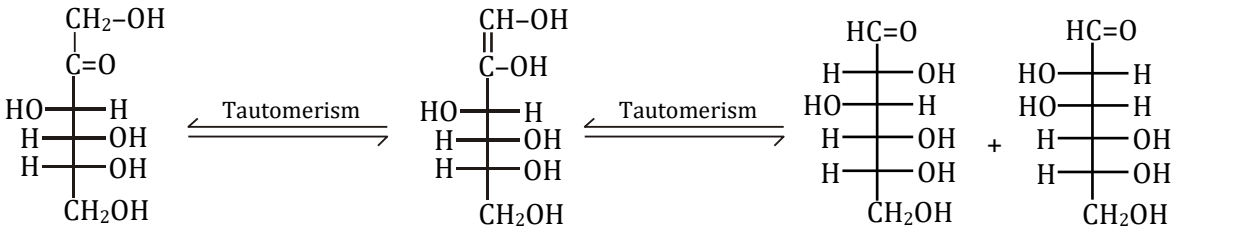

CHO
CHOH
CHOH
CHOH
CHOH
CH2OH
(+)–Glucose
(1) HCN
CN
CH–OH
(CHOH)4
CH2OH
Glucocyanohydrin
(C2 Epimer)
(2) H2NOH
–H2O
CH=N–OH
(CHOH)4
CH2OH
(Gluco oxime)
(3) H2NNHPh
–H2O
CH=N–NH–Ph
(CHOH)4
CH2OH
(Gluco phenylhydrozone)
(4) 3C6H5NH–NH2
–H2O, –NH3
–PhNH2
CH=NNHC6H5
C=NNHC6H5
(CHOH)3
CH2OH
(Gluco Osazone)
(5) NaHSO3
No reaction
(6) D.N.P.
No reaction
(7) Schiff's reagent
No reaction
(8) Br2 + H2O
COOH
(CHOH)4
CH2OH
Gluconic acid
(Tollen's reagent) (9) NH4OH + AgNO3 or
Ag2O aq. or
Ag2(NH3)2+ OH–
COO–
(CHOH)4
CH2OH
+ Ag (Silver mirror)
(Fehling's solution) (10) NaOH + CuSO4
+Na/K Tartrate
(Benedict's solution) (11) NaOH + CuSO4
+Na/K Citrate
COO–
(CHOH)4
CH2OH
+ Cu2O (Cuprous oxide)
(Red precipitate)
(12) HNO3
COOH
(CHOH)4
CH2OH
(Glucaric acid)
Saccharic acid
(13) HIO4
5HCOOH + HCHO
(14) H2, Ni
CH2OH
(CHOH)4
CH2OH
Glucitol
(Sorbital)
Ac2O
CH2OAc
(CHOAc)4
CH2OAc
(Hexa-O-acetylgluciotal)
(Hexa-O-acetylsorbitol)
(15) Red P + HI
n-Hexane

1. Since glucose & fructose (Aldoses/Ketoses) reacts with HCN, H_2NOH, H_2NNHPh which indicates the presence of carbonyl group but they don't react with DNP, $NaHSO_3$& Schiff's reagent (weak reagents) therefore we can conclude that carbonyl group is not free, but remains in the form of cyclic structures.
2. In the formation of osazone, C_1&C_2 are only involved so glucose, fructose and C-2 epimers (Glucose & Mannose, Threose and Erythrose) give same osazone.
3. Osazone are crystalline solid having sharp melting point so used for identifying the carbohydrates. In the osazone formation three molecules of NH_2NHPh is overall consumed out of which two molecule react with nucleophilic addition/elimination reaction forming hydrazone whereas one molecule undergoes redox reaction.



(Osazone)

4. Both glucose and fructose gives test with Tollen's reagent, Fehling's solution and Benedict's solution because in basic medium, ketoses remains in the form of dynamic equilibrium with Aldoses (C-2 epimers) by the process of **tautomerisation/enediol rearrangement as below**.



D-fructose

[Enediol of D-fructose
Enediol of D-Glucose
Enediol of D-Mannose]

Intermediate

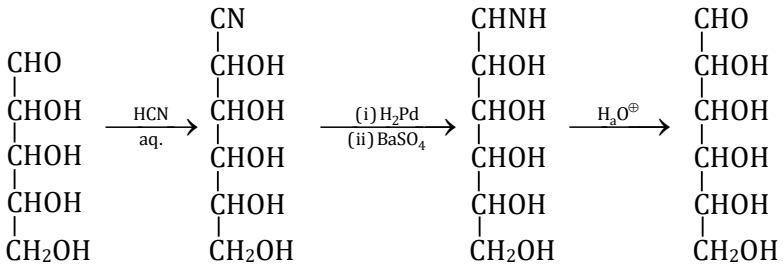
D-Glucose D-Mannose

5. Only Br_2/H_2O is used for the identification of Aldoses & Ketoses. (Mild oxidising agent like bromine water (Neutral) Oxidises only aldehydic group).
6. Oxidation with HNO_3, gives information that one primary alcohol is present in aldoses and two primary alcohols are present in ketoses.
7. Reduction product with Na/Hg and H_2O gives only one alcohol with aldoses and two alcohols with ketoses (C-2 epimers)
8. Reduction product with Red P & HI, gives n-Hexane which indicates that all the six carbon atoms are linearly arranged.

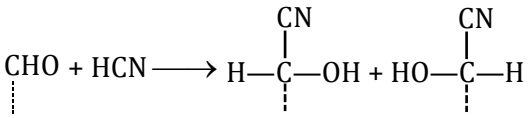
(A) Method of ascending the sugar series:

An aldose may be converted into its next higher aldose e.g. an aldopentose into an aldohexose.

(i) By Kiliani Fischer upgradation:

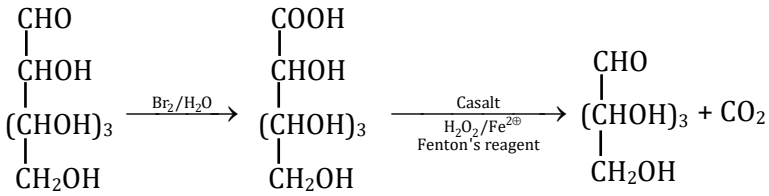


Theoretically two lactones are possible, since two cyanohydrin may be formed when hydrogen cyanide adds on to the aldopentose (a new asymmetrical carbon is produced)

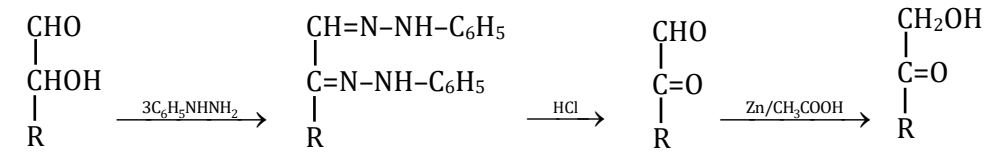


(B) Method of descending the sugar series :

(ii) Ruff's method :



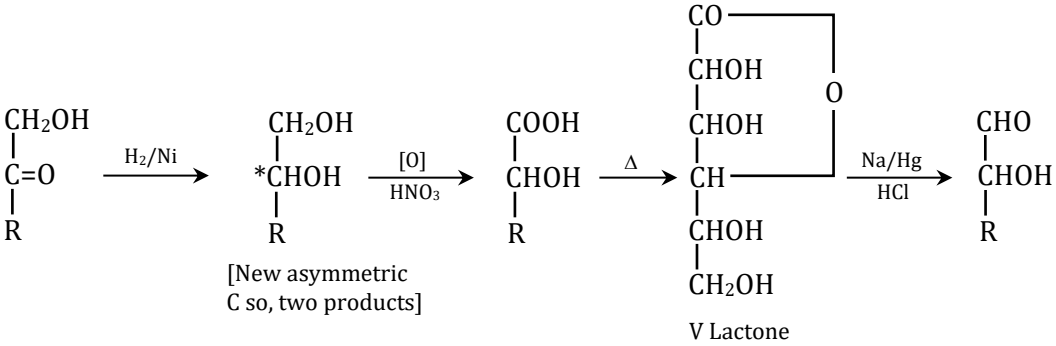
(C) Conversion of an aldose into a ketose :



Glucose — Osazone — Osone — Fructose

An aldehyde group is reduced more readily than a ketonic group.

(D) Conversion of a Ketose into an aldose:



[New asymmetric C so, two products]

V Lactone

FRUCTOSE :

Fructose preparation:

(1) By acid hydrolysis of cane sugar.

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} \xrightarrow{H_2O/H^+} \underset{\alpha\text{-glucose}}{C_{12}H_{22}O_{11}} + \underset{\beta\text{-fructose}}{C_6H_{12}O_6}$$

Disaccharides

(2) By enzymatic action of sucrose.

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} \xrightarrow{\text{invertase}} \text{Glucose} + \text{Fructose}$$

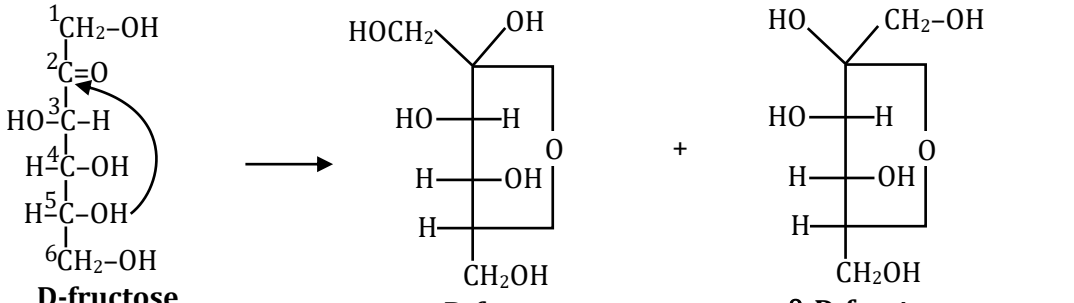
Note :

(i) Glucose & fructose obtained by acid hydrolysis of sucrose can be separated by treating with $Ca(OH)_2$ which forms calcium glucosate & calcium fructosate. Calcium fructosate, being water insoluble, is seperated out easily.

(ii) Fructose is the sweetest monosaccharide.

Structure of fructose:

It also exist in two cyclic forms which are obtained by the addition of –OH at C5 to the $\left(\diagup C{=}O\right)$ group.



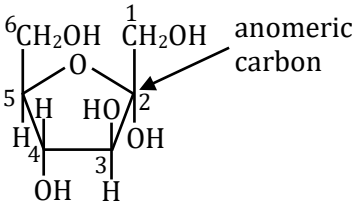
D-fructose
Specific Rotation (– 92°)

α-D-fructose
Specific Rotation (– 21°)

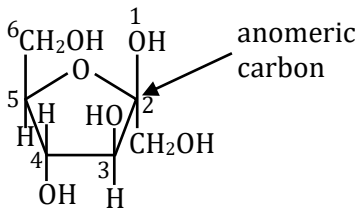
β-D-fructose
Specific Rotation (– 133°)

Haworth projection :Fructofuranose

The five membered ring & is named as furanose with analogy to the compound furan.



Specific rotation (–21°)
α-fructose
α-fructofuranose

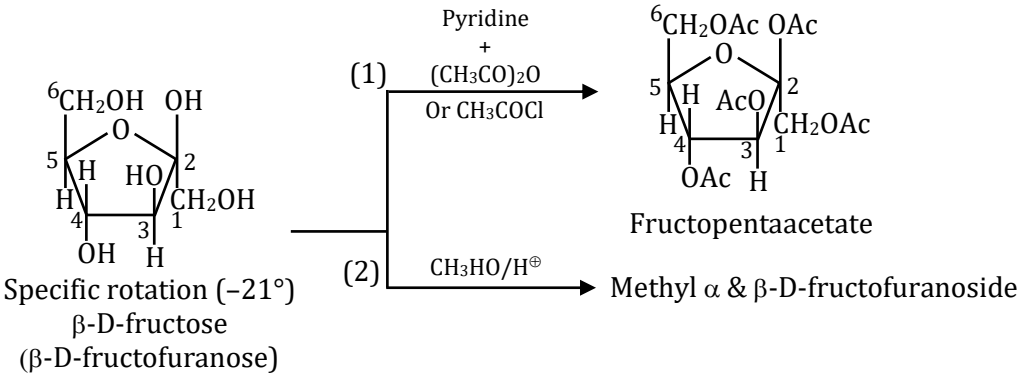


Specific rotation (–133°)
β-fructose
β-fructofuranose

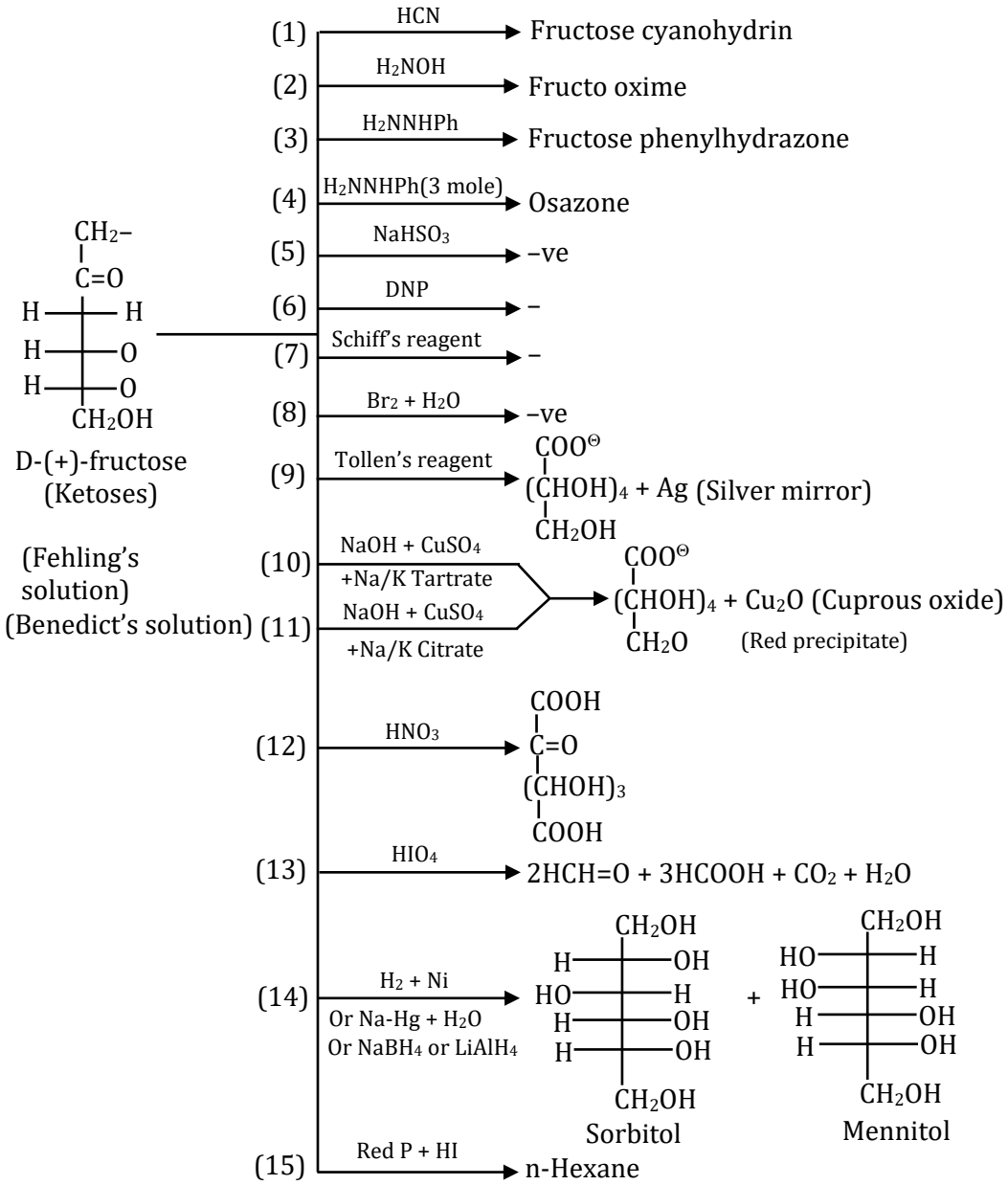
Chemical Reactions of Fructose :

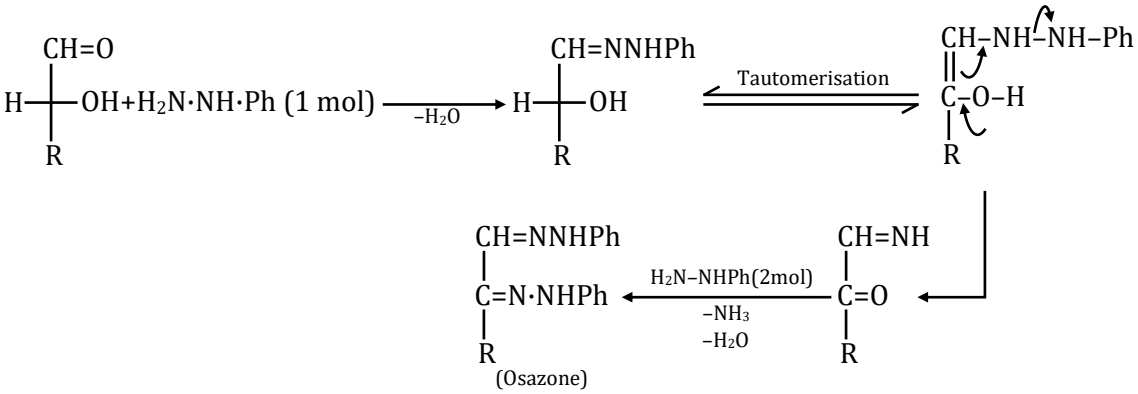
Reaction due to OH group at 2nd carbon :

(1) It forms fructose pentaacetate with acetyl chloride :



(2) Reaction due to keto group :





3. Both glucose and fructose gives test with Tollen's reagent, Fehling's solution and Benedict's solution because in basic medium, ketoses remains in the form of dynamic equilibrium with Aldoses (C-2 epimers) by the process of **tautomerisation/enediol rearrangement as below**.

DISACCHARIDES

Condensation of two monosacharides after loss of water molecule (Glycosidic bond), gives disacharides. Common examples are sucrose, maltose, lactose, cellubiose.

(A) Sucrose : (Cane sugar)

(i) Sucrose is a white crystalline solid, soluble in water.

(ii) When heated above its melting point, it forms a brown substance known as caramel.

(iii) Sucrose is dextrorotatory, its specific rotation being +66.5°.

(iv)On hydrolysis with dilute acids sucrose yields an equimolecular mixture of D(+)-glucose andD(–)-fructose :

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{HCl} \underset{\text{D - glucose}}{C_6H_{12}O_6} + \underset{\text{D - fructose}}{C_6H_{12}O_6}$$

$[\alpha] = +66.6°$ $[\alpha] = +52.7°$ $[\alpha] = -92.2°$

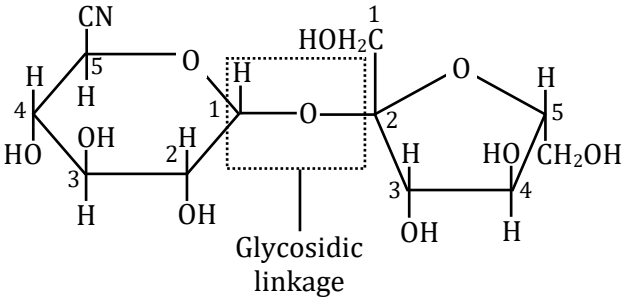
Since D(–)-fructose has a greater specific rotation than D(+)-glucose, the resulting mixture is laevorotatory. Since the hydrolysis of cane-sugar (sucrose) gives laevorotatory solution in place of original dextrorotatory solution therefore hydrolysis of cane-sugar is also known as the **inversion of cane-sugar or Inversion of sucrose** and the mixture of sugars are known as **invert sugar Ex. D - Glucose & D-Fructose.**

The inversion (i.e., hydrolysis) of cane-sugar may also be effected by the enzyme invertase which is found in yeast.

(v) Sucrose is not a reducing sugar, e.g., it will not reduce Fehling's solution or Tollen'sreagnet. It does not form an oxime or an osazone, and does not undergo mutarotation. This indicates that hemiacetal group is not present in the rings.

Sucrose (Cane sugar) $\xrightarrow{H_3O^{\oplus}}$ **α-glucose + β-fructose**

In sucrose two monosaccharides are joined together by an oxide linkage formed by loss of water molecule. Such linkage through oxygen atom is called glycosidic linkage. In sucrose linkage in between C1 of α-glucose and C2 of β-fructose. Since the reducing group of glucose & fructose are involved in glycosidic bond formation, sucrose is non-reducing sugar.



$C_1\,\alpha$–D–glucose + $C_2\,\beta$–D–fructose

α–D–Glucose

β–D–Fructose

(B) Maltose : (Malt sugar)

Maltose($C_{12}H_{22}O_{11}$) is produced by the action of malt (which contains the enzyme diastase) on starch:

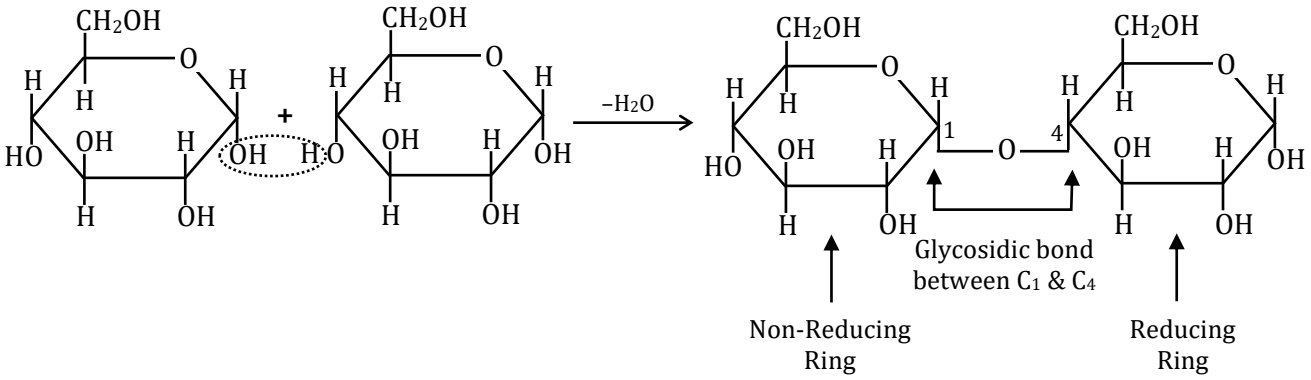
$$C_{12}H_{22}O_{11} \xrightarrow{H_3O^{\oplus}} 2C_2H_{12}O_6 \alpha\text{-D-Glucose}$$

When it is hydrolysed with dilute acids or by the enzyme maltase, maltose yields two molecules of D(+)-glucose. Maltose is a reducing sugar, e.g., it reduces Fehling's solution or Tollen's reagent; it forms an oxime and an osazone, and undergoes mutarotation. This indicates that at least one hemiacetal group (of the two glucose molecules) is free in maltose.

Maltose (Malt sugar) $\xrightarrow{H_3O^{\oplus}}$ **α-glucose + α-glucose**

Formation of Maltose ($C_{12}H_{22}O_{11}$)

$C_1\,\alpha$–D-Glucose + $C_4\,\alpha$-D-Glucose

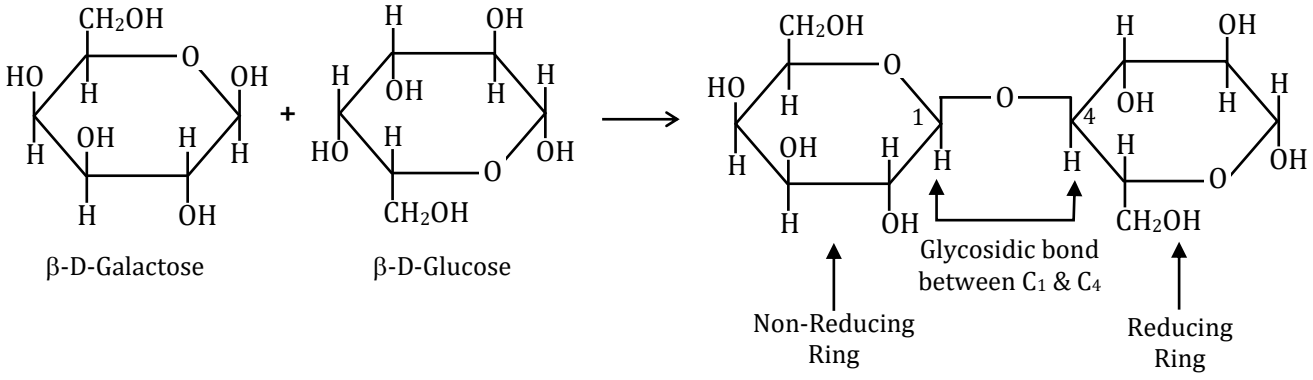


(C) Lactose : (Milk Sugar)

Lactose occurs in the milk of all animals and is dextrorotatory. It is hydrolysed by dilute acids or by the enzyme lactase, to an equimolecular mixture of D(+)-glucose and D(+)-galactose. Lactose is a reducing sugar.

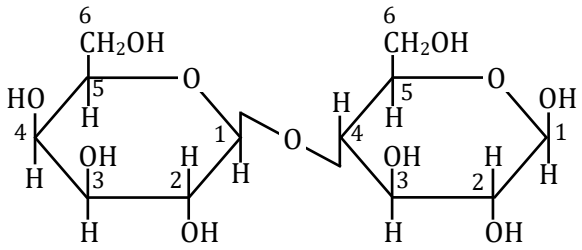
Lactose (Milk sugar) $\xrightarrow{H_3O^{\oplus}}$ β-galactose + β-glucose

Formation of Lactose ($C_{12}H_{22}O_{11}$)



β-D-Galactose

β-D-Glucose



$C_1\beta$–D–Galactose + $C_2\beta$–D–Glucose

The linkage is between C-1 of Galactose and C-4 of Glucose.

(D) Cellubiose :

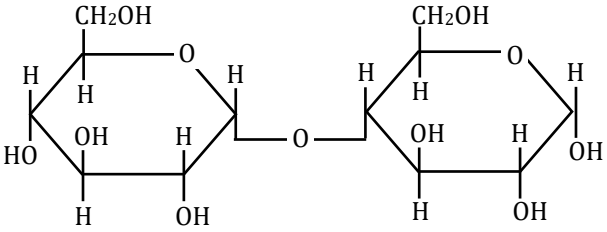
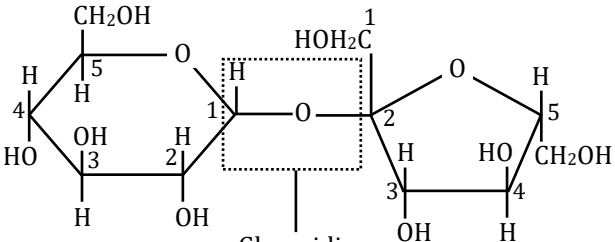
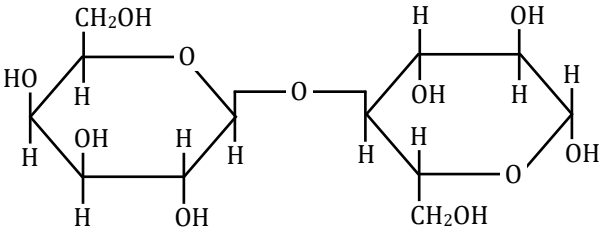
Cellubiose $\xrightarrow{H_3O^{\oplus}}$ β-glucose + β-glucose

β–D-Glucose + β-D-Glucose

C_1 C_4

Pyranose form Pyranose form

DISACCHARIDE :

Disaccharide	Structure	Monomeric unit linkage	Properties
Maltose	 Maltose (α-1,4-glycosidic linkage)	D (+) Glucose + D (+) Glucose (α-1, 4-glycosidic linkage)	Produced by action of malt on starch. Undergoes mutarotation.
Sucrose	 Glycosidic linkage	α-D-glucose+ β-D-Fructose (α-1,β-2 glycosidic linkage)	White crystalline solid, soluble in water. extrorotatory specific rotation + 66.5°
Lactose	 Lactose (β-1,4-glycosidic linkage)	D(+)Glucose+D(+) galactose (β-1,4- glycosidic linkage)	dextrorotatory

POLYSACCHARIDES

Polysaccharides : It contains large number of monosaccharide units joined together by glycosidic linkage (acetal bond). They are food storage or structural material.

(A) Starch, $(C_6H_{10}O_5)_n$

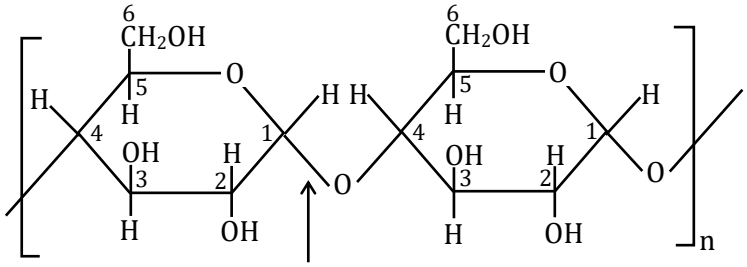
(i) Starch is the main contributor of carbohydrates in our diet. It exists exclusively in plants, stored in the seeds, roots, and fibres as food reserve. Example rice, potato.

(ii) Starch is actually a mixture of two structurally different polysaccharides, Amylose (15-20%) and Amylopectin (80-85%).

(iii) When starch is heated with hot water, it can be separated into its components. The part that is soluble in water is amylose and remaining fraction is amylopectin.

(iv) Both amylose and amylopectin are composed of D-glucose units.

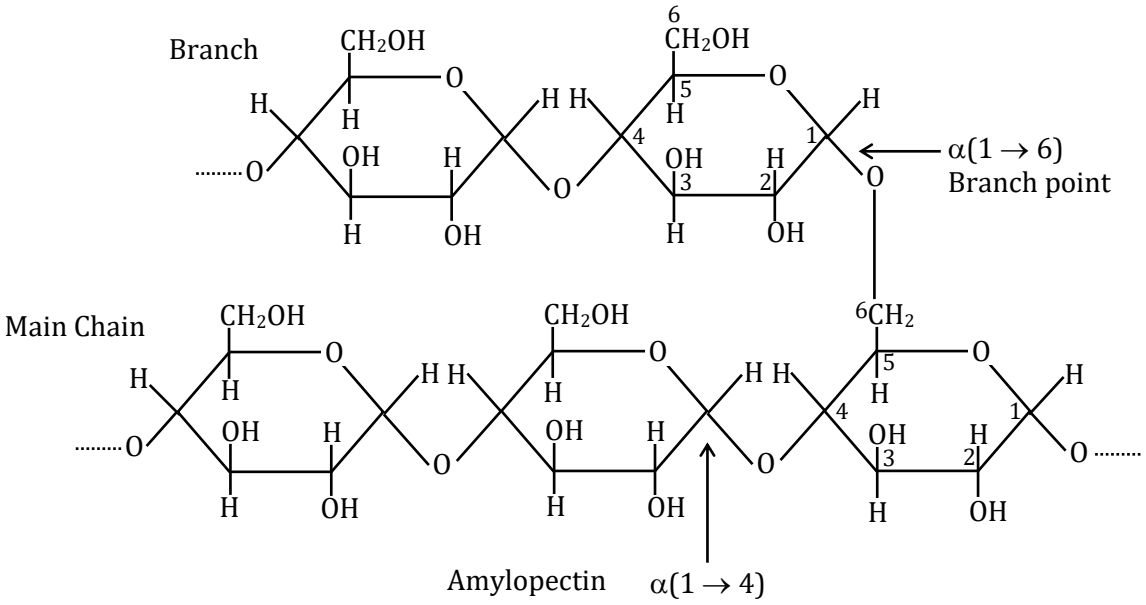
(v) The **amylose** molecule is made up of D-glucose unit joined by α-glycosidic linkages between C-1 of one glucose unit and C-4 of the next glucose unit. The number of D-glucose units in amylose range from 60-300.



Amylose

1,4-Glycosidic linkage of α-D-Glucose

(vi) **Amylopectin** has a branched-chain structure. It is composed of chains of 25 to 30 D-glucose units joined by α-glycosidic linkages between C-1 to one glucose unit and C-4 of the next glucose unit. These chains are in turn connected to each other by 1, 6-linkages.



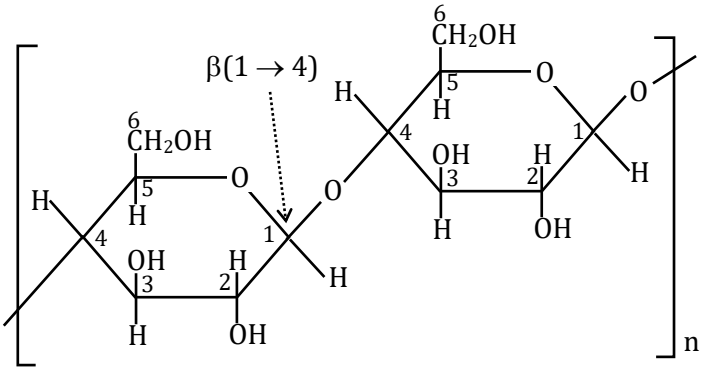
1,4-Glycosidic linkage of α-D-Glucose with Branching at 1,6

α-amylose soluble in water, and the solution gives a blue colour with iodine. Amylopectin is insoluble in water, is stable in contact with water, and gives a violet colour with iodine.

(B) Glycogen (Animal Storage)

It is also like amylopectin but branching will take place after every 5 to 6 glucose unit. (highly branched)

(C) Cellulose, $(C_6H_{10}O_5)_n$



Cellulose

1,4-Glycosidic linkage of β-D-Glucose

1. Cellulose is linear chain natural polymers of β-D glucose units joined by 1, 4-glycosidic linkage (Natural linear polymers).
2. Cellulose is the main structural material of tree and other plants. Wood is 50% cellulose, while cotton wool is almost pure cellulose.
3. Artificial silk, rayon, is used collectively to cover all synthetic or manufactured fibres from cellulose.
4. The nitrates are prepared by the reaction of cellulose with a mixture of nitric and sulphuric acids, and the degree of 'nitration' depends on the concentrations of the acids and the time of the reaction. Cellulose trinitrate (12.2 – 13.2%N) is known as **gun-cotton** and is used in the manufacture of blasting explosives and smokeless powders.

Starch : (Plant Storage, Polymer of α–D-glucose),

Glycogen : (Animal Storage, Polymer of α–D-glucose),

Cellulose : (Plant Skeleton, Polymer of β–D-glucose)

TEST OF CARBOHYDRATES :

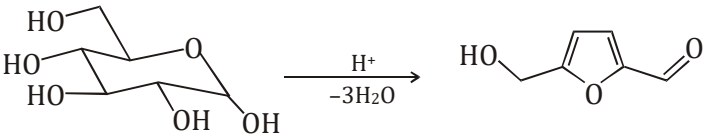
1. Molish Test	2. Barfoed Test	3. Salivanoff's Test.	4. Bial's Test
5. Osazone Test	6.Benedict Test	7. Fehling Test	8. Tollen's Test
9. Iodine Test			

1. Molish Test

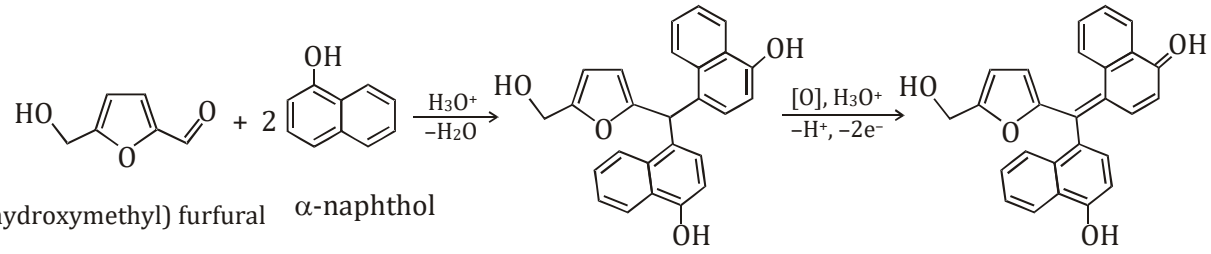
Reagent : 5% solution of α-Napthol in alcohol + Few drops of conc. H_2SO_4

Molish test is the general test for the identification of all carbohydrates (Monosaccharides, Disaccharides and Poly saccharides) and Glycoprotein, in this test, the carbohydrate under go dehydration upon the introduction of concentrated HCl or H_2SO_4 resulting in the formation of an aldehyde. This aldehyde under goes condensation along with two phenol type molecules (like α-naphthol, resorcinol and thymol) resulting in the formation of a purple or reddish-purple coloured complex.

Oligosaccharide or poly saccharides + H_2SO_4 $\xrightarrow{H_3O^+ \text{ (Hydrolysis)}}$ Monosaccharides



D-glucose (monosaccharide) 5-(hydroxymethyl) furfural



5-(hydroxymethyl) furfural α-naphthol

purple colored dye
or
violet colored complex

2. Barfoed Test

(i) Reagent Copper acetate solution in water

(ii) Buffered with few drops of Acetic acid

This test is used to differentiate reducing monosaccharide from a disaccharide sugar

It is done in mild acidic medium.

$$RCHO + 2Cu^{2\oplus} + 2H_2O \longrightarrow RCOOH + Cu_2O \text{ ppt} + 4H^{\oplus}$$

It is based on the reduction of copper(II) acetate to copper(I) oxide (Cu_2O), which forms a brick-red precipitate.

Reducing monosaccharides react with Barfoed's reagent much faster than disaccharides and produce red precipitate of copper (I) oxide within three minutes.

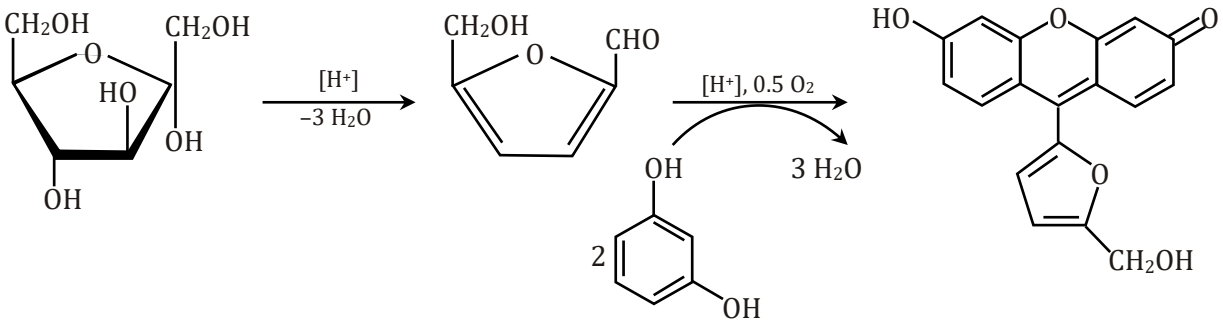
Disaccharide sugars as they are weaker reducing agents, react at a slower rate, so they do not form red precipitate even for ten minutes.

3. Selivanoff's Test

Reagent : 0.5% Resorcinol in conc. HCl and heat for 5 minutes

It is test of Ketose sugar (eg Fructose), it is used to differentiate Ketose sugar from aldose sugar.

This test relies on the principle that keto hexose are more rapidly dehydrated than aldoses to form 5-Hydroxy methyl furfural when heated in acidic medium, which on condensation with resorcinol, a cherry red (or Brown red) colored complex is formed rapidly indicating a positive test.



Fructose Resorcinol Cherry red ppt

Aldose sugars react with the Seliwanoff's reagent to give faint pink colour intensifies with time.

4. Bial's Test

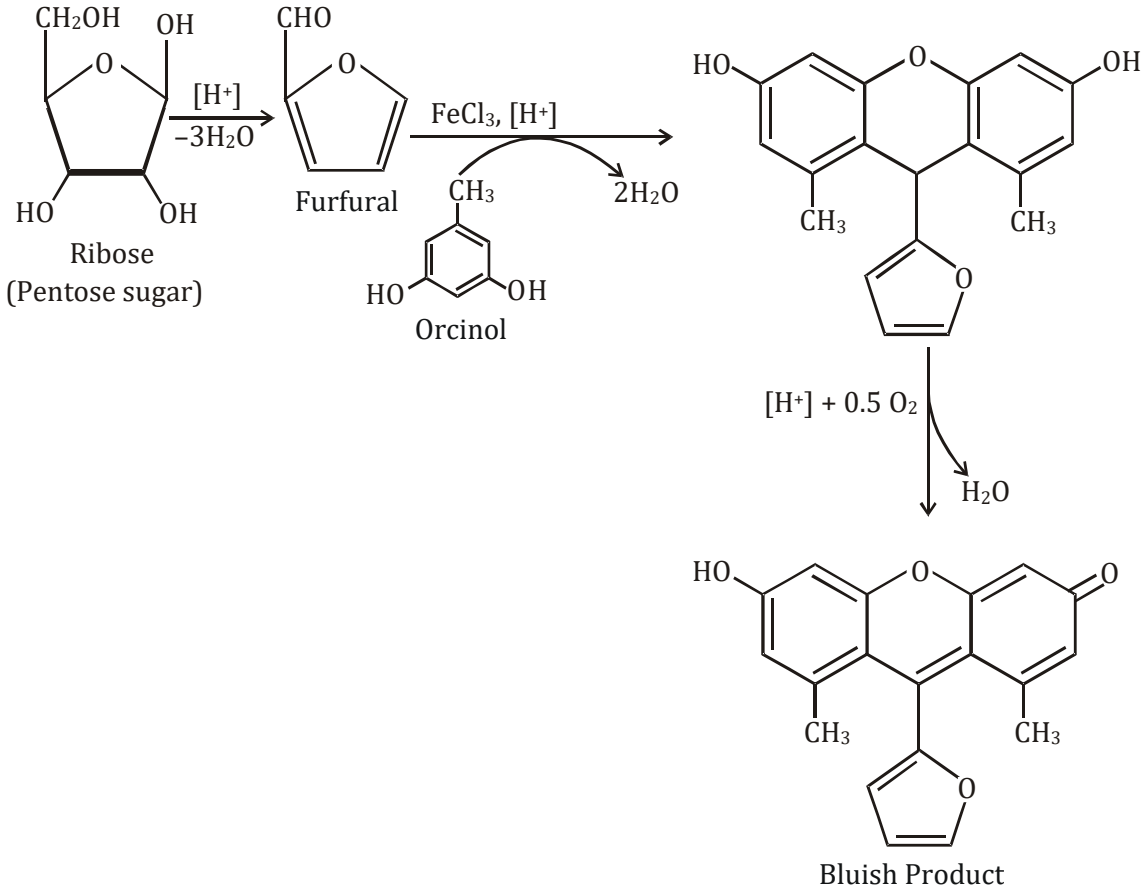
Reagent : Orcinol and a little $FeCl_3$ dissolved in ethanol

Bial's test is positive for Pentoses

This test is used to differentiate Pentose and Hexose sugar

The test reagent dehydrates pentoses to form furfural. Furfural further reacts with Bial's reagent (a solution of orcinol, HCl and ferric chloride). orcinol and the iron ion present in the test reagent to produce a bluish product .

Figure insert



Specifically Pentose sugar gives bluish colored complex.

All other colors indicate a negative result for pentoses.

Note: hexoses generally react to form green, red, or brown products.

5. Osazone Test

Reducing Sugars when heated with Phenyl Hydrazine,Characteristic yellow crystals of Osazone are formed with specific shape.

Glucose,Mannose & Fructose gives same Osazone crystals, like NEEDLE SHAPED.

Maltose gives Maltosazone crystals, like SUNFLOWER SHAPED.

Lactose gives lactosazone crystals,

like TIGHT BALL or POWDER PUFF SHAPED.

6. Benedict test

Carbohydrates which has Aldehyde functional group (Not Aromatic Aldehyde) or Having alpha hydroxy ketone gives positive Benedict Test

It is in mild Basic Medium.

$CuSO_4 \longrightarrow Cu^{++} + SO_4^{2-}$

$2Cu^{++}$ + Reducing Sugar $\longrightarrow Cu^{+}$

$Cu^{+} \longrightarrow Cu_2O$ ppt

Glucose (Blue Red Solution) ,Galactose (Orange Red Solution)
Maltose (Dark Brown with Brick Red Solution),Fructose (Dark Brown with Brick Red Solution)
Xylose (Brick Red solution)

7. Fehling's test

All reducing Carbohydrates give Positive Fehling's test with Fehling solution. Carbohydrates which has Aldehyde functional group (Not Aromatic Aldehyde) and alpha hydroxy carbonyl also gives positive Fehling's test (e.g. fuctose). While Ketones give negative fehling test. During this reaction the aldehyde group is oxidised to acid while the copper ions are reduced to red/brown precipitate of Cu_2O. This is a common test used to detect glucose in urine as positive indication of diabetes.

$CuSO_4 \longrightarrow Cu^{\oplus\oplus} + SO_4^{2\ominus}$

$2Cu^{\oplus\oplus}$ + Reducing Sugar $\longrightarrow Cu_2O$ (Red / Brown ppt)

8. Tollen's test

This test is also given by reducing sugars. Carbohydrates reacts with Tollens reagent forms a silver mirror on the inner walls of the test tube. This confirms the presence of reducing sugars. Silver ions are reduced to metallic silver.

$$RCHO \xrightarrow[(ii)H^+]{(i)\left[Ag(NH_3)_2\right]OH} RCOOH + Ag \text{ (Silver mirror)}$$

Note : Sucrose , poly sachharides also others non reducing sugar do not give Benedict test, Fehling test and Tollen's Test.

9. Iodine Test

The iodine test is used to test for the presence of starch. Starch turns into an intense 'Deep blue' colour upon addition of aqueous solutions of the triodide anion.

NUCLEIC ACIDS

Nucleic acids were first discovered by a Swiss biochemist, **Friedrich** Miescher (1869) who called them **nuclein** due to their acidic nature.

Chemical analysis of chromosomes shows presence of two nucleic acids-DNA (Deoxyribo nucleic acid) and RNA (Ribo nucleic acid).

Nucleic acid is a macromolecule & consists of many (polymer) monomeric units, called nucleotides. Each nucleotide is composed of a nucleoside and a phosphate group. Thus, nucleotide is a phosphoric ester of nucleoside.

Each nucleoside consists of sugar molecule and a nitrogenous base. The relationship can be shown as given below.

Nucleic acid = many nucleotides
Nucleotide = nucleosides + phosphate
Nucleoside = sugar + nitrogenous base
Thus nucleotide = phosphate + sugar+ nitrogenous base
Nucleic acids bear different components that are briefly discussed below.

1. Phosphoric acid :

The acidic nature of nucleic acids is due to the presence of phosphoric acid. Sugar of nucleoside combines with phosphoric acid by a phosphodiester bond formed at 5^{th} or 3^{rd} carbon of the sugar.

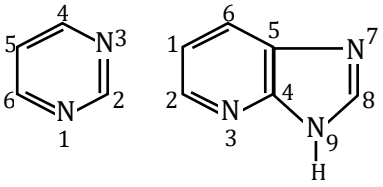
2. Sugar :

It is a five carbon (pentose) sugar. There are two types of pentose sugars-ribose and deoxyribose. Deoxyribose sugar has one oxygen atom less at second carbon. Ribose sugar is present in RNA while deoxyribose sugar occurs in DNA.

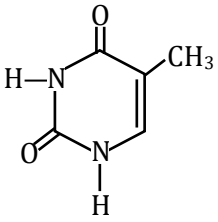
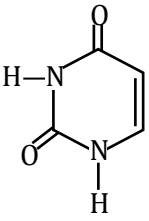
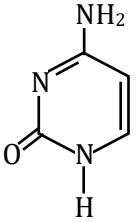
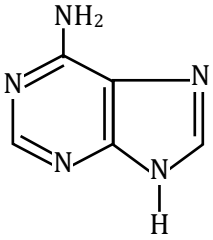
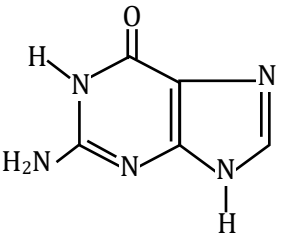
3. Nitrogenous bases : they have two catagories.

(a) Pyrimidine : It includes cytosine, thymine and uracil. Pyrimidine bases are made of only one ring of carbon.

(b) Purine : It includes Adenine and guanine. Purine bases are made of two ring of carbon and nitrogen bases of DNA contains adenine, guanine, cytosine and thymine. In RNA, **uracil** is present in place of **thymine.**



Pyrimidine Purine

Pyrimidines			Purines	
				
Thymine (T)	Uracil (U)	Cytosine (C)	Adenine (A)	Guanine (G)

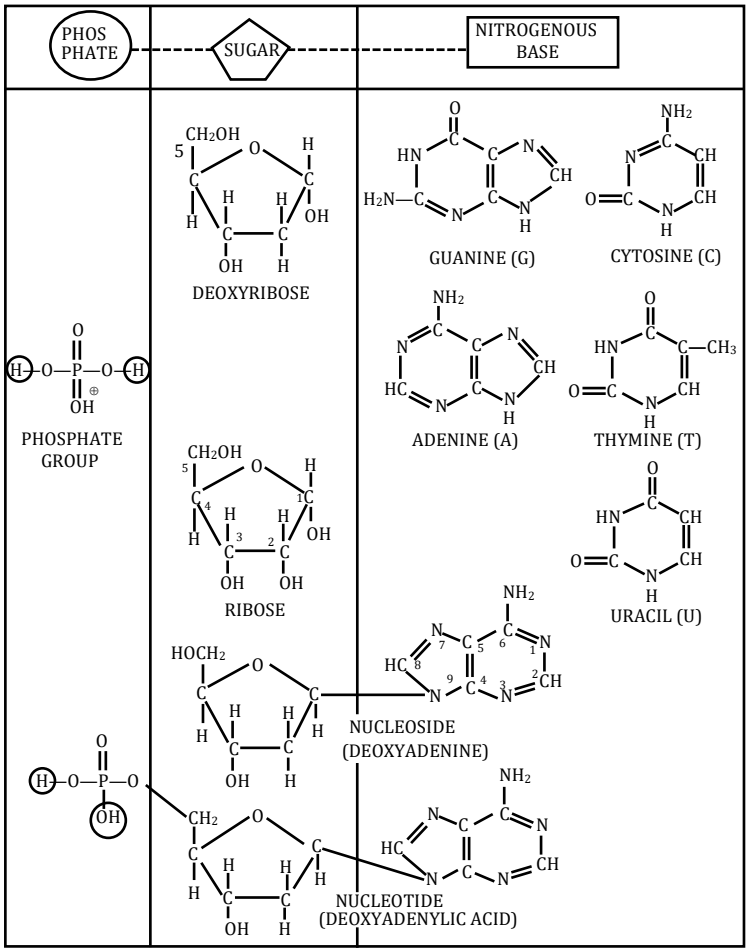


Fig. Building blocks of nuclectides

Many nucleotide monomer units join one another to give rise to polynucleotide chain.

The two adjacent nucleotides are joined by formation of **phosphodiester bond** (a bond that involves two ester bonds). A polynucleotide chain is often written as 5'p 3'OH. This indicates that it is a dinucleotide with phosphate group (p) attached to the 5th carbon of terminal nucleotide and hydroxyl group (OH) is present at 3rd carbon of basal nucleotide.

(a) Structure of DNA (Deoxyribonucleic acid) :

J.D. Watson and F.H.C. Crick (1953) proposed double helical structure of DNA based on the results of **M.H.F. Wilkins and co-workers.** All these three persons were awarded Nobel Prize in 1962 for this work.

The following are some of the characteristic features of double helical structure of DNA.

(i) Each nucleotide consists of **sugar, phosphate and a nitrogenous base**. Many such nucleotides are linked by phosphodiester bonds to form a polynucleotide chain or strand.

(ii) Phospho diester bonds are formed between 5'carbon of sugar of one nucleotide and 3' carbon of sugar of the next nucleotide.

(iii) Nitrogenous base is attached to**1' carbon of sugar**. At this place **purine base** is attached by its **5' position** and **pyrimidine** by its **3' position**.

(iv) Polynucleotide strand is made of backbone of sugar and phosphate forming its long axis and bases at right angles to it.

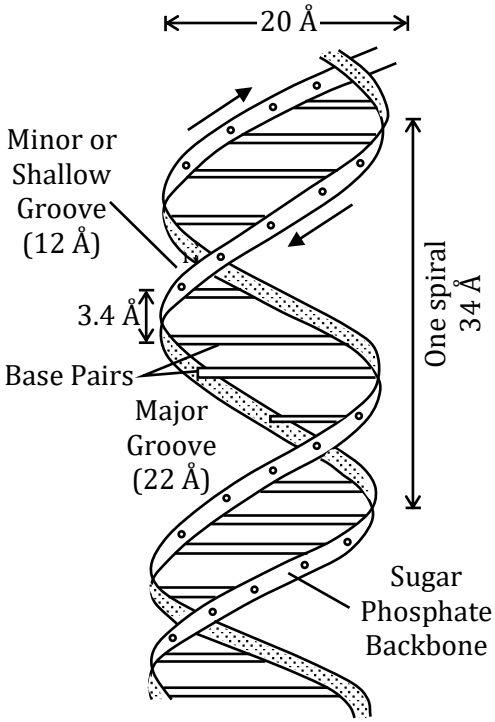


Fig. Structure of DNA- coiling in double helix of DNA

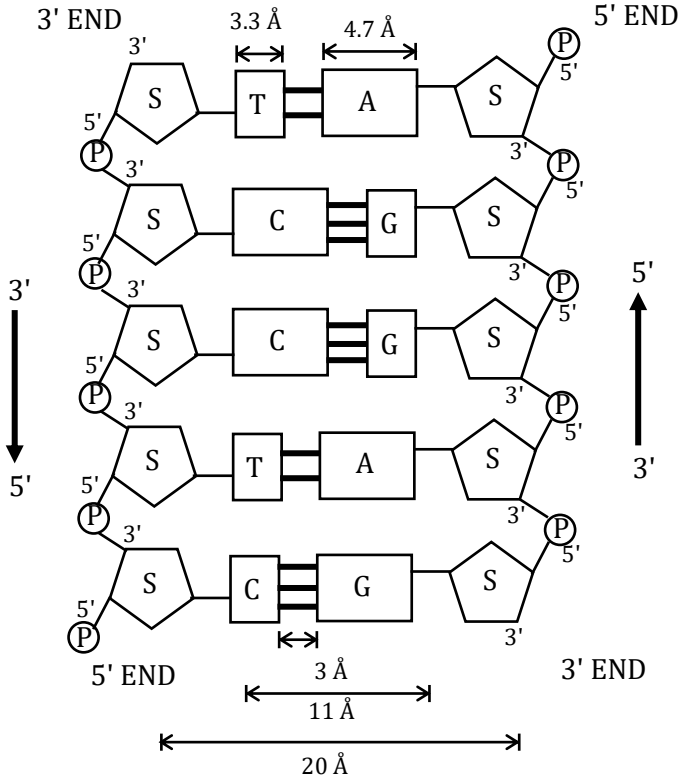


Fig. D. Arrangement of various constituents of DNA duplex.
(S = Sugar, P = Phosphate)

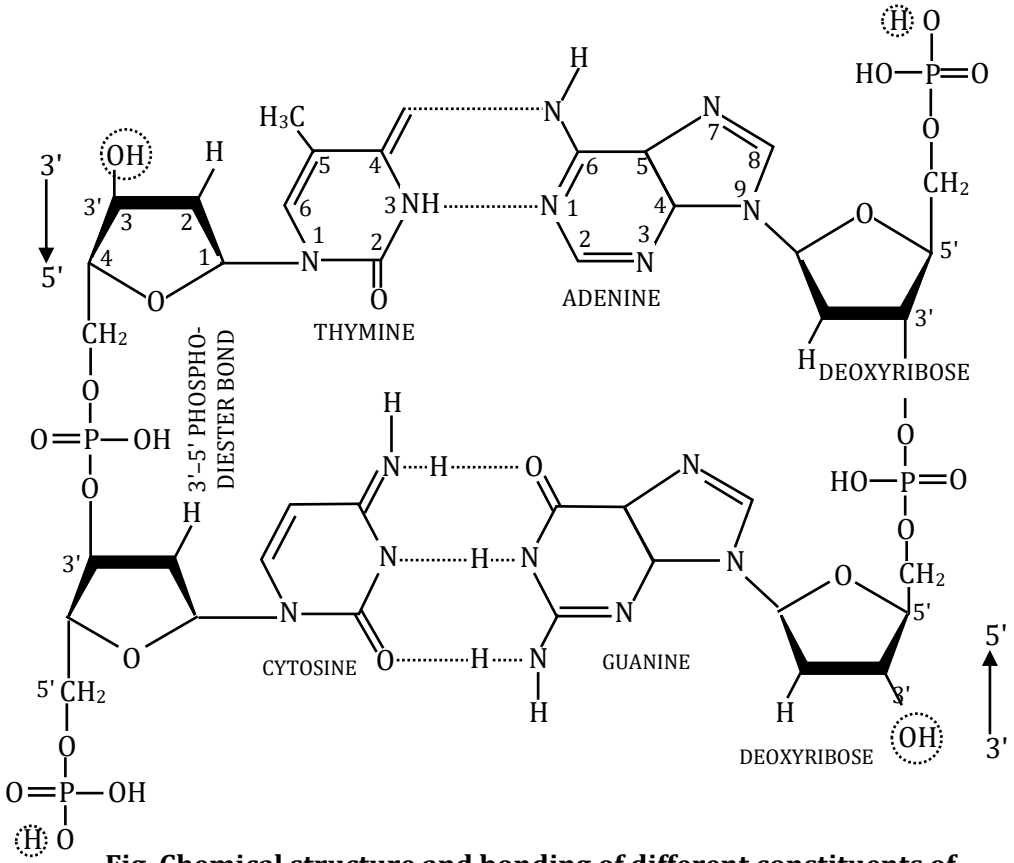


Fig. Chemical structure and bonding of different constituents of DNA. In the two chains.

Chargaffs rule.

(1) Purine and pyrimidine base pairs are in equal amount, that is, adenine + guanine =thymine + cytosine.

(2) Molar amount of purine-adenine is always equal to the molar amount of pyrimidine thymine. Similarly, guanine is equalled by cytosine.

(3) Sugar deoxyribose and phosphate occur in equimolar proportions.

(4) **The ratio of A + T/G + C is constant for a species.**

(5) Chargaff's rule states that in natural DNAs the base ratio **A/T** is always close to unity and the **G/C** ratio also to always close to unity. It indicate that **A** always pairs with **T** and **G** pairs with **C**. **A** and **T**, **G** and **C**, therefore, are complementary base pairs.

(6) Thus, if one DNA strand has **A**, the other would have **T** and if it has **G**, the other, would have **C**. Therefore, if the base sequence of one strand is **CAT TAG GAC,** the base sequence of other strand would be **GTA ATC CTG**. Hence, the two poly nucleotide strands are called complementary to one another.

(7) Two such complementary strands are joined with each another by hydrogen bonds between their complementary nitrogenous bases. There are three hydrogen bonds between cytosine and guanine and two hydrogen bonds between adenine and thymine.

(8) The two polynucleotide chains are helically coiled around the same axis in such a way that these can separate from one another only by uncoiling. Helical coiling is supposed to be right handed. Such a form of DNA is now called B-DNA

(9) The two chains or strands are antiparallel, i.e., they run in opposite directions in relation to their sugar molecules. Their 5'p 3' OH phosphodiester linkages are in opposite directions

(10) Double stranded DNA molecule has a diameter of 20A°.

(11) The helix makes one complete turn every 3.4 A° along its length.

(12) There are 10 nucleotides per turn of helix. Thus, the distance between two neighbouring base pairs is 3.4 A.

RNA : RNA is ribonucleic acid formed in the nucleus and is found in the cytoplasm of the cell.

(b) Types of RNA and their functions:

(1) r-RNA (Ribosomal – RNA): It is found in the ribosomes and it is usually associated with protein to form the ribosomes. It is synthesised in the nucleus by DNA. It is single stranded, comprising about 80% of the total RNA. It is metabolically stable.

Functions :

(i) This forms the site for protein synthesis.

(ii) It is also supposed to help the binding of m-RNA to the ribosomes during protein synthesis.

(2) m-RNA (Messenger RNA) : It is carries the genetic message code from the D.N.A. to ribosomes. It is produced by the DNA ; m-RNA is also single stranded and constitutes about 15% of total RNA.

Functions : It carries the genetic information from DNA to the ribosomes where protein is synthesised.

(3) t-RNA (Transfer–RNA) : It is synthesised in the nucleus by the DNA. It is also called soluble RNA. It is single stranded. There are 20 different kindes of t-RNA and each type has a specificity for a particular amino acid. It constitutes about 5% of total RNA. It has very short life.

Functions : It carries amino acids from different parts of cytoplasm to the ribosomes during protein synthesis.

Differences between DNA and RNA

DNA	RNA
1. It usually occurs inside nucleus and some cell.	1. Very little RNA occurs inside nucleus, most organelles of it is found in the cytoplasm.
2. DNA is the genetic material.	2. RNA is not the genetic material except in certain viruses, e.g. Reovirus.
3. It is double stranded with the exception of, some viruses (e.g. □ × 174).	3. RNA is single stranded except reovirus where is double stranded.
4. DNA contains over a million nucleotides.	4. Depending upon the type, RNA contains 70-1200 nucleotides.
5. DNA is of only two types, intra-nuclear and extra-nuclear.	5. There are at least three types of RNAs– mRNA, rRNA and tRNA.
6. It contains deoxyribose sugar.	6. It contains ribose sugar.
7. Nitrogen base thymine occurs in DNA along with three others– adenine, cytosine and guanine.	7. Thymine is replaced by uracil in RNA. The other three are similar– adenine, cytosine and guanine.
8. It replicates to form new DNA molecules.	8. It cannot normally replicate itself.
9. DNA transcribes genetic information to RNA.	9. RNA translates the transcribed message for forming polypeptides.
10. DNA controls metabolism and genetics including variations.	10. It only controls metabolism under instruction from DNA.
11. Purine and pyrimidine bases are in equal number.	11. There is no proportionality between number of purines and pyrimidine bases.

VITAMINS

Definition :

A group of biomolecules which are required in small amounts for normal metabolic process and for the life, growth and health of human beings and animal organisms.

Sources of Vitamins : Human body can synthesize vitamin e.g. some members of vitamin B complex synthesized by microorganisms present in the intestinal tract. Most of the vitamins cannot be synthesize by our body, therefore they must be supplied in the food. Plants can synthesize almost all vitamins. Vitamin D may either be supplied in the food or may be produced in the skin by the irradiation of ergosterol (a sterol present in our body) with ultraviolet light.

Classification of Vitamins : There is very little common to the various vitamins, therefore there are usually designated by alphabet letters A, B, C, D, E and K. Some of these are classified as sub groups e.g. B_1, B_2, B_3, B_4, B_6, B_{12} D_1, D_2 etc. About 25 vitamins are known to date. Vitamins are broadly classified into two categories.

(i) Water soluble vitamins : Vitamin B-complex and vitamin C are water soluble vitamins and must be supplied regularly in diet.

(ii) Fat soluble vitamins : These are oily substances and soluble in fats These are A,D,E and K. They are stored in liver and adipose (fat storing) tissues.

(iii) Biotin (Vitamin H) : It is neither soluble in water nor in fats. Lack of particular vitamin causes a specific deficiency disease. Some important vitamins Their characteristics, sources and deficiency diseases are summarised in the following table.

S. No.	Vitamins	Characteristics	Sources	Deficiency disease
1.	A (Retinal)	Soluble in oils and fat stable to heat. Promote growth and vision and increases resistance to diseases.	Milk, Butter, eggs, fish, liver oil, rice, kidney, green vegetables, mangoes sweet potatoes carrots, tomatoes etc.	**Xerophthalmia** (hardening of eye) **night blindness** and xerosis (drying of skin)
2.	B_1 Thiamine	Soluble in water destroyed by heat above 313 K.	Pulses, nuts, cereals (nice, wheat) rice polishings, yeast, egg yolk, milk, green vegetables and fruits.	Beriberi (paralysis of legs and general weakness) loss of appetite
3.	B_2 (Riboflavin or lactofilavin)	Soluble in water, sensitive to light but stable to heat.	Daily dosage 2-3 mg milk, yeast, green vegetables, meat, liver, kidney, egg white etc.	Retards general inflammation of tongue i.e. darkened tongue dermatitis and cheilosis (racking at corners of mouth and lips.
4.	B_6 (Pyridoxin or adermin)	Soluble in water	Rice, bran, yeast molasses, meat, fish, egg yolk etc.	Cause specific dermatitis and anaemia in man effects central nervous system.
5.	B_{12}(cyanocobalamin)	Water soluble, stable to heat.	Milk, egg, liver of animals	Anaemia (RBC deficient) inflammation of tongue and mouth.
6.	H (Biotin)	Neither soluble in water not in fats.	Yeast, liver, kidney and milk	Dermatitis, loss of hairs and paralysis.
7.	C (Ascorbic acid)	Soluble in water, destroyed by cooking.	**Citrus fruits,** amla tomatoes, green leafy vegetables. Daily dosage 75 mg.	Cause **scurvy** (bleeding of gums, pyorrhoea bleeding of teeth)
8.	D (Ergo calciferol)	Soluble in oil, fats stable to heat and resistant's to oxidation to controls calcium and Phosphorus meta-bolism.	Milk, butter, eggs, liver & meat. Daily dosage 0.025 mg.	**Rickets** (deformation of bones) osteomalacia (soft bones and joint pain)
9.	E (It is mixture of 4-vitamins a, b, g, d tocoferols)	Soluble in oils and fats, stable to heat and oxidation.	Vegetable oil like wheat germ oil, cotton seed oil peanut oil, soybean, eggs, milk, fish. Daily dosage 5 mg.	Loss of sexual power of reproduction (sterility) increased muscular weakness.
10.	K (phylloquinone mixture of two vitamin K_1 & K_2)	Soluble in oils and fats, stable to heat, sensible to light and alkali.	Vitamin K_1 , alfalfa leaf's vegetables, vit. K_1 occurs mainly in bacteria.	**Haemorrhage lengthens** the time of blood clotting. Excessive bleeding in injury (Vitamin K_1 & K_2) and alkali bacteria.

Enzymes Catalysis :

Enzymes are complex nitrogenous organic compounds which are produced by living plants and animals. They are actually protein molecules of high molecular mass and form colloidal solutions in water.

The enzymes are, also, termed as biochemical catalysts and the phenomenon is known as biochemical catalysis. Many enzymes have been obtained in pure crystalline state from living cells. However, the first enzyme was synthesised in the laboratory in 1969.

The following are some of the examples of enzyme-catalysed reactions:

(i) Inversion of cane sugar: The invertase enzyme converts cane sugar into glucose and fructose.

$$\underset{\text{Cane sugar}}{C_{12}H_{22}O_{11}\,(aq)} + H_2O(l) \xrightarrow{\text{Invertase}} \underset{\text{Glucose}}{C_6H_{12}O_6\,(aq)} + \underset{\text{Fructose}}{C_6H_{12}O_6\,(aq)}$$

(ii) Conversion of glucose into ethyl alcohol: The zymase enzyme converts glucose into ethyl alcohol and carbon dioxide.

$$\underset{\text{Glucose}}{C_6H_{12}O_6\,(aq)} \xrightarrow{\text{Zymase}} \underset{\text{Ethyl alcohol}}{2C_2H_5OH(aq)} + 2CO_2(g)$$

(iii) Conversion of starch into maltose: The diastase enzyme converts starch into maltose.

$$\underset{\text{Starch}}{2(C_6H_{10}O_5)_n(aq)} + nH_2O(l) \xrightarrow{\text{Diastase}} \underset{\text{Maltose}}{nC_{12}H_{22}O_{11}(aq)}$$

(iv) Conversion of maltose into glucose: The maltase enzyme converts maltose into glucose.

$$\underset{\text{Maltose}}{C_{12}H_{22}O_{11}\,(aq)} + H_2O(l) \xrightarrow{\text{Maltase}} \underset{\text{Glucose}}{2C_6H_{12}O_6\,(aq)}$$

(v) Decomposition of urea into ammonia and carbon dioxide: The enzyme urease catalyses this decomposition.

$$NH_2CONH_2(aq) + H_2O(l) \xrightarrow{\text{Urease}} 2NH_3(g) + CO_2(g)$$

(vi) In stomach, the pepsin enzyme converts proteins into peptides while in intestine, the pancreatic trypsin converts proteins into amino acids by hydrolysis.

(vii) Conversion of milk into curd: It is an enzymatic reaction brought about by lacto bacilli enzyme present in curd.

Table gives the summary of some important enzymatic reactions.

Zymase	Yeast	Glucose → Ethyl alcohol and carbon dioxide
Diastase	Malt	Starch → Maltose
Maltase	Yeast	Maltose → Glucose
Urease	Soyabean	Urea → Ammonia and carbon dioxide
Pepsin	Stomach	Proteins → Amino acids

Enzyme catalysis is unique in its efficiency and high degree of specificity.

The following characteristics are exhibited by enzyme catalysts:

(i) **Most highly efficient:** One molecule of an enzyme may transform one million molecules of the reactant per minute.

(ii) **Highly specific nature**: Each enzyme is specific for a given reaction, i.e., one catalyst cannot catalyse more than one reaction. For example, the enzyme urease catalyses the hydrolysis of urea only. It does not catalyse hydrolysis of any other amide.

(iii) **Highly active under optimum temperature**: The rate of an enzyme reaction becomes maximum at a definite temperature, called the optimum temperature. On either side of the optimum temperature, the enzyme activity decreases. The optimum temperature range for enzymatic activity is 298-310K. Human body temperature being 310 K is suited for enzyme-catalysed reactions.

(iv) **Highly active under optimum pH**: The rate of an enzyme-catalysed reaction is maximum at a particular pH called optimum pH, which is between pH values 5-7.

(v) **Increasing activity in presence of activators and co-enzymes**: The enzymatic activity is increased in the presence of certain substances, known as co-enzymes. It has been observed that when a small non-protein (vitamin) is present along with an enzyme, the catalytic activity is enhanced considerably.

Activators are generally metal ions such as Na^+, Mn^{2+}, Co^{2+}, Cu^{2+}, etc. These metal ions, when weakly bonded to enzyme molecules, increase their catalytic activity. Amylase in presence of sodium chloride i.e., Na^+ ions are catalytically very active.

(vi) **Influence of inhibitors and poisons**: Like ordinary catalysts, enzymes are also inhibited or poisoned by the presence of certain substances. The inhibitors or poisons interact with the active functional groups on the enzyme surface and often reduce or completely destroy the catalytic activity of the enzymes. The use of many drugs is related to their action as enzyme inhibitors in the body.