BIOMOLECULES (CARBOHYDRATES)

Carbohydrates (hydrates of carbon) are naturally occuring 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 Starch $(C_6H_{10}O_5)_n$

Sucrose (Cane sugar) - $C_{12}H_{22}O_{11}$, 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

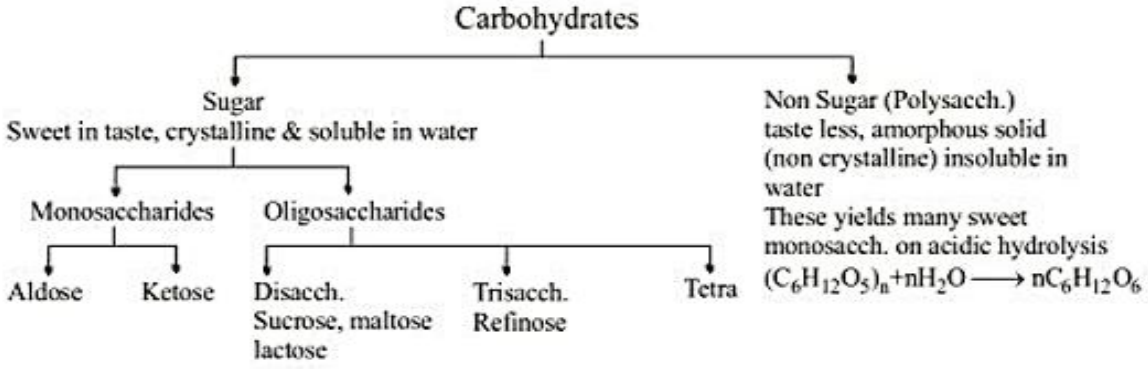
There are many compounds, which shows chemical behaviour of carbohydrate but do not confirm the general formula $C_x(H_2O)_y$ such as - $C_5H_{10}O_4$ (2–deoxyribose), $C_6H_{12}O_5$ (Rahmnose)
$C_7H_{14}O_6$ (Rahmnohexose)

Modern definition of carbohydrate: Carbohydrates are polyhydroxy aldehyde or ketone
or
Substances which yield these (polyhydroxy aldehyde or ketone) on hydrolysis

Carbohydrates $\xrightarrow{H^+/H_2O}$ Poly hydroxy aldehyde or ketone

Carbohydrates are also known as Saccharides.

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

Oligosaccharides :
These are the sugars which yields 2–10 monosaccharides units on hydrolysis. Such as.

(a) Disaccharides : Two monosaccharide unit on hydrolysis (may or may not be same).
Ex. - Sucrose, Maltose

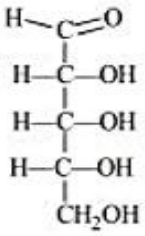
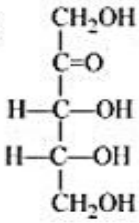
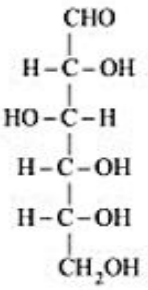
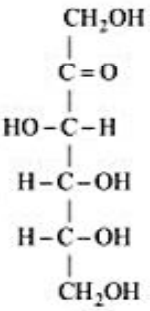
(b) Trisaccharides : Three monosaccharide unit on hydrolysis.

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

Note :- A group of polysaccharides which are not so widely used in nature is pentosans $(C_5H_8O_4)_n$ 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.

If –CHO group is present in monosaccharide, then it is known as aldose.

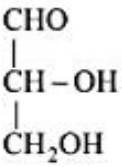
If $-\overset{\overset{O}{\|}}{C}-$ group is present in monosaccharide , then it is known as ketose.

		Aldoses	**Ketoses**
3C	Tropose or Triose	Aldotriose	Ketotriose
4C	Tetrose	Aldotetrose	Ketotetrose
5C	Pentose	Aldopentose	Ketopentose
5C	including –CHO	Aldopentose (Ribose) 	
5C	including $-\overset{\overset{O}{\|}}{C}-$	Ketopentose  (Ributose)	
6C	Hexose	Aldohexose	Ketohexose
6C	including –CHO	Aldohexose (Glucose)  D – glucose	
6C	including $-\overset{\overset{O}{\|}}{C}-$	Ketohexose (Fructose)  D – fructose	

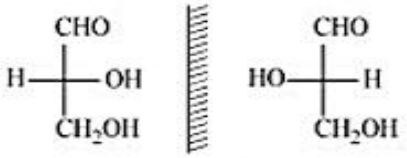
STEREOCHEMISTRY OF CARBOHYDRATES :

D & L-Sugars : The series of aldoses or ketoses in which the configuration of the penultimate C-atom (C-next to CH_2–OH group) is described as D-sugars if –OH is towards RHS & L-sugars if it is towards LHS.

Smallest carbohydrate — Aldotriose — Glyceraldehyde

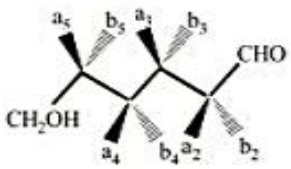


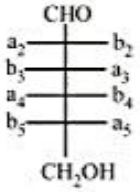
Fischer projection



D-Glyceraldehyde (+) L-Glyceraldehyde (–)

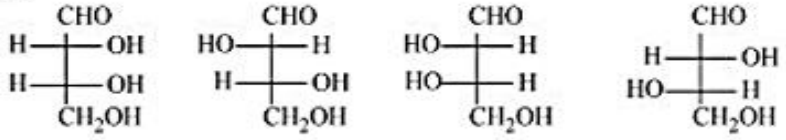
Fisher formula of compounds containing many asymmetric carbon.





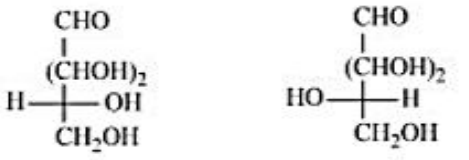
Classification of Aldotetros : (i) Erythrose
(ii) Threoese

C-4



D-Erythrose D-Threose L-Erythrose L-Threose

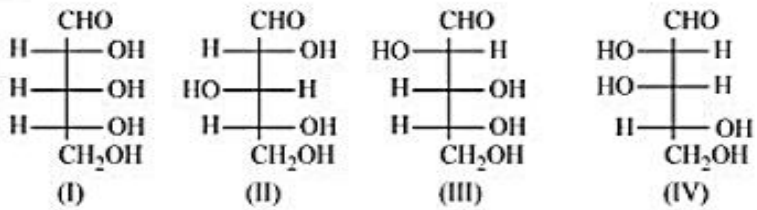
C-5



D-Aldopentose L-Aldopentose

No. of C* = 3 (in Aldopentose)
No. of optical isomers $2^3 = 8$
No. of D Sugars 4
No. of L Sugars 4

D-Aldopentose :

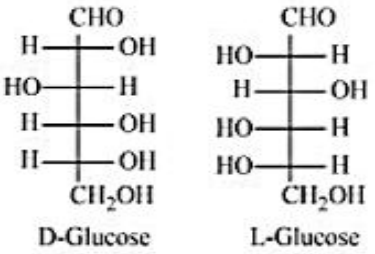


(I) (II) (III) (IV)

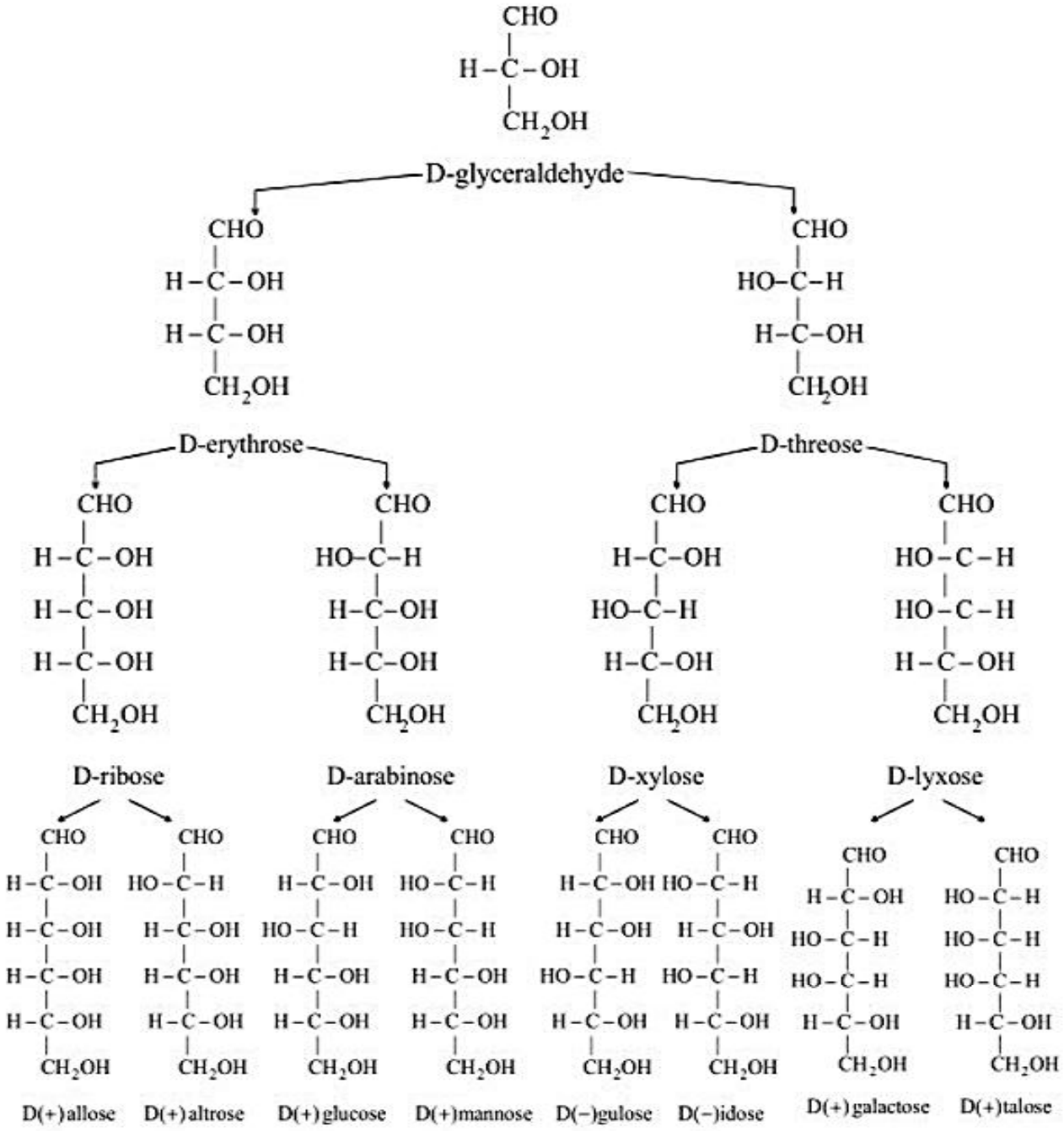
All Isomeric D-sugars are diastereomers.

Aldohexose :

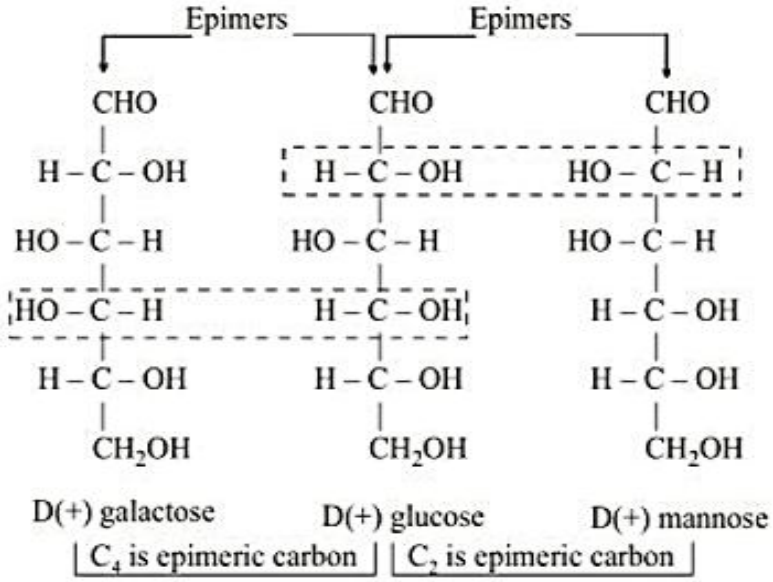
No. of C* = 4
No. of stereoisomers = $2^4 = 16$
No. of D-sugars = 8
No. of L-sugars = 8



The D–family aldoses



Epimers: A pair of diastereomers that differ only in the configuration about of a single carbon atom are said to be epimers. D(+)-glucose is epimeric with D(+)- mannose and D(+)-galactose as shown below.



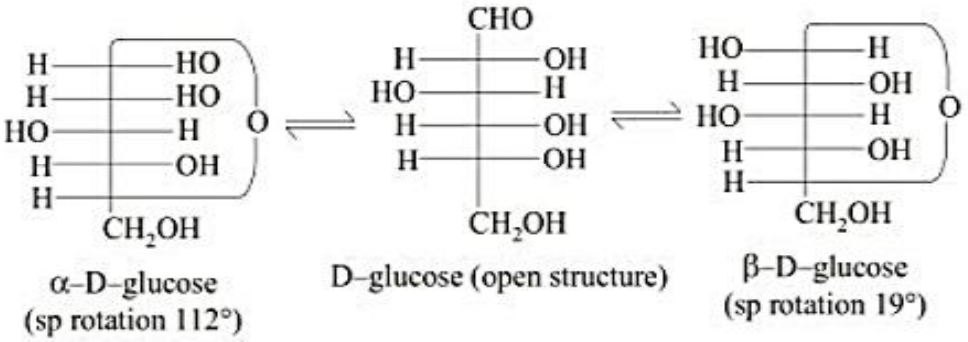
Another example with C_2 epimeric carbon is

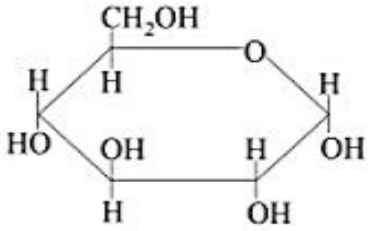
Anomers: Anomers are the stereoisomers which differs at a single chiral centre out of many & are ring chain tautomer of the same open chain compound.

The two sugars that differs in configuration only on the carbon that was the carbonyl carbon in the open chain form is called as anomers α glucose and β glucose are known as anomers their equilibrium mixture contains 36 % α–D–glucose , 63.8 % β-D-glucose and 0.2 % open chain form.

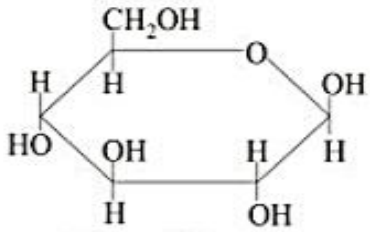
C_1 Carbon is known as anomeric carbon.

Haworth suggested to write α glucose and β glucose in pyran structure 





Haworth formula
α–D–glucopyranose



Haworth formula
β–D–glucopyranose

Anomers are epimers but epimers may not be anomers.

CYCLIC STRUCTURES OF MONOSACCHARIDES

Many five membered and six membered monosaccharides occur in cyclic form. Cyclic structures of monosaccharides are established by many experiments. The cyclic structure is due to intramolecular hemiacetal formation between aldo / keto group and OH of any one carbon. The ring formed are generally six membered (pyranose) or five membered (furanose). Each cyclization results in creation of a new asymmetric centre apart from the existing ones. The isomers resulting from cyclizations are called anomers. Example, when D-glucose (open structure) cyclise, it gives α-D-glucose and β-D-glucose.

Haworth projection :

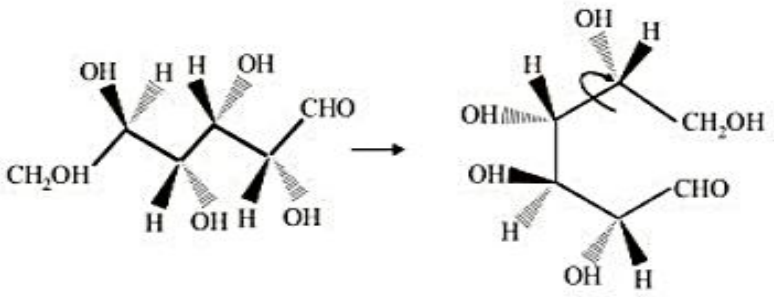
Many of monosaccharides form cyclic structures. The actual structure is almost planer and be represented by Haworth projection, which is a way of depicting three - dimensional cyclic structure.

Rule -1 : In a Haworth projection draw a fisher projection in which ring oxygen is in a down position.

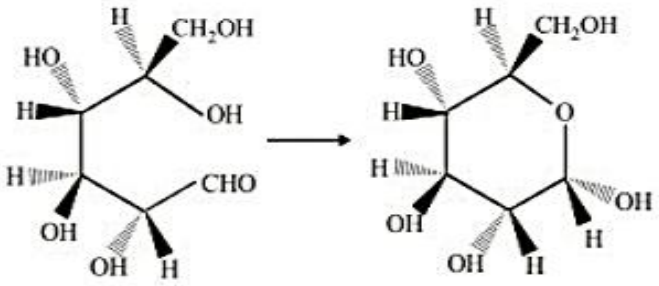
Rule -2 : Imagine that carbon chain of fisher projection is folded around a barrel or drum, which provide a ring lies in a plane ⊥ to the page.

Rule -3 : Now plane of ring is turned 90° so that anomeric carbon is on the right and the ring oxygen is in the rear. Obtained projection is a Haworth projection.

Example : (D-glucose)

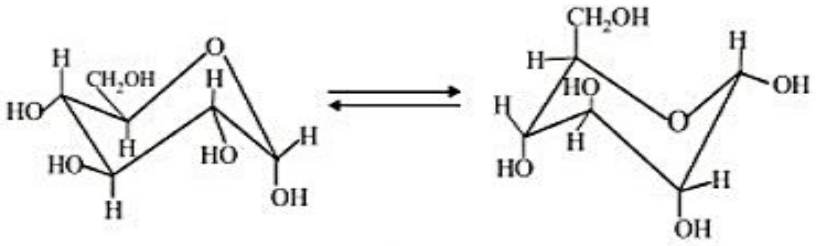


Projection :

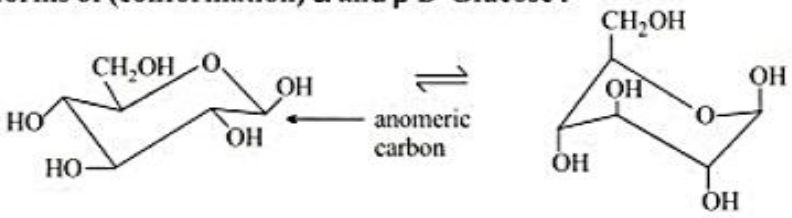


Hawarth projection

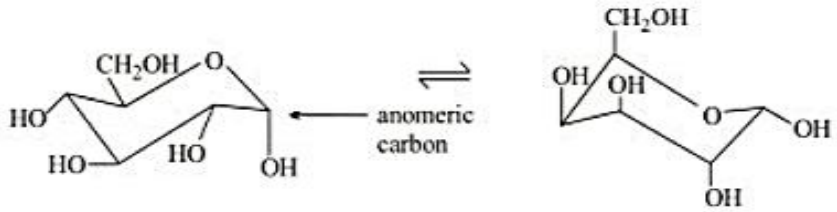
⇒ Chair conformation of D–glucose



Chair forms of (conformation) α and β D-Glucose :

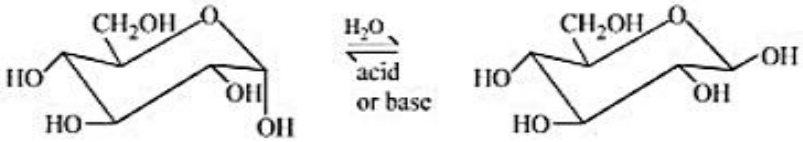


β-D-Glucose (most stable glucose form) all groups are equatorial.



α-D-Glucose –OH group at anomeric carbon is axial.

Mutarotation



α-anomer β-anomer

Specific rotation of α glucose + 112° Specific rotation of β glucose + 19°

Equilibrium mixture $[\alpha]_D = 52.5$ degree mL g^{-1} dm^{-1}

Fresh α-glucose ⟶ 52.5 ⟵ **Fresh β-glucose**

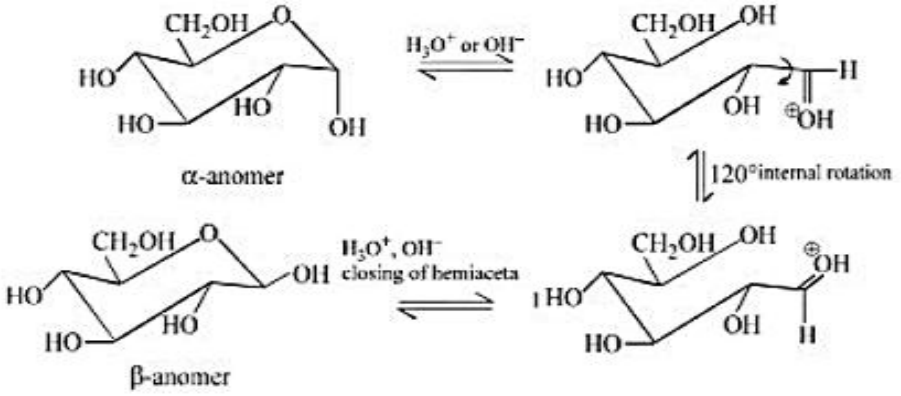
112° 36 % α glucose 19°

63.8 % β glucose

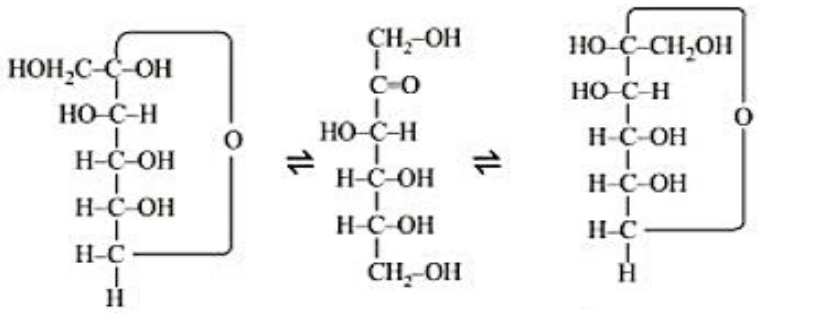
When pure α-D glucose is dissolved in water its specific rotation is found to be +112° with time, however the specific rotation of the solution decreases ultimately reaches stable value of +52.5°. When β D-glucose is dissolved in water, it has a specific rotation of 19°. The specific rotation of this solution increases with time also to + 52.5 °.

This change of optical rotation with time is called mutarotation. It is caused by the conversion of α and β glucopyranose anomers into an equilibrium mixture of both. Mutarotation is catalyzed by both acid and base, but also occurs is even in pure water. Mutarotation is characteristic of the cyclic hemiacetal form of glucose.

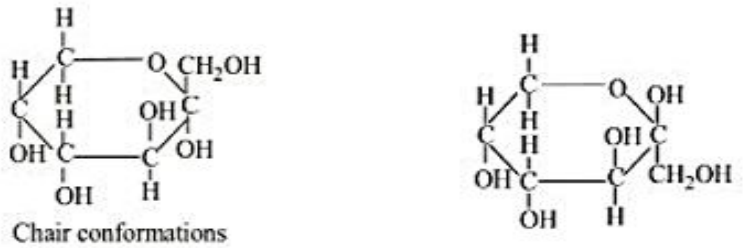
Mutarotation occurs first by opening of the pyranose ring to the free aldehyde form.



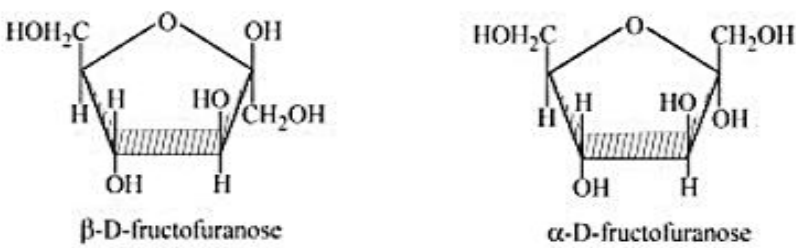
Structure of fructose



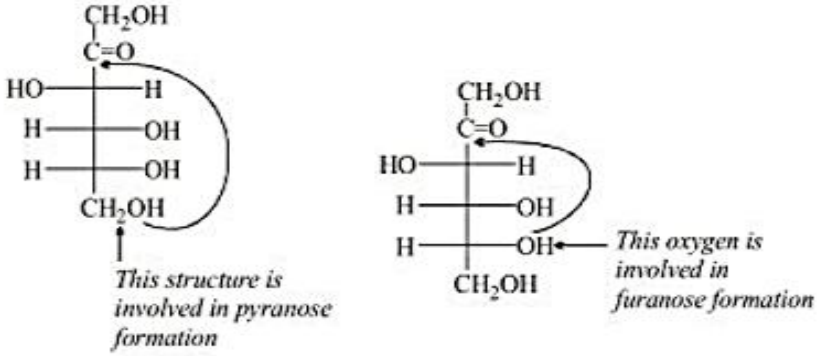
Ring structure of fructose C, Pyranose structures 6 membered ring, $C_2 - C_6$ linkage



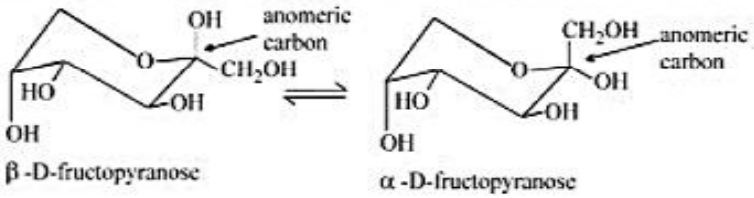
Furanose structure (5 membered ring)

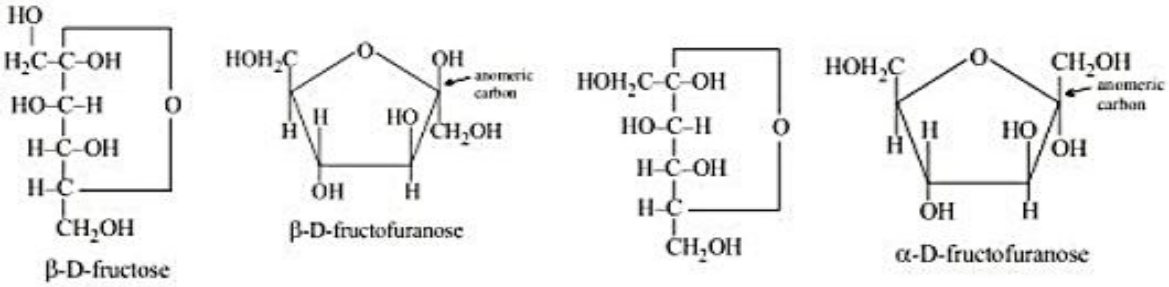


Mutarotation: Fructose undergo complex mutarotation. The structure of the cyclic hemiacetal form of d-fructose can be derived from it's carbonyl (Ketone) form using the methods described as follows.



It happens that the crystalline form of D-fructose is β-D-Fructopyranose. When crystals of this form are dissolved in water, it equilibrates to both pyranose & furanose forms.

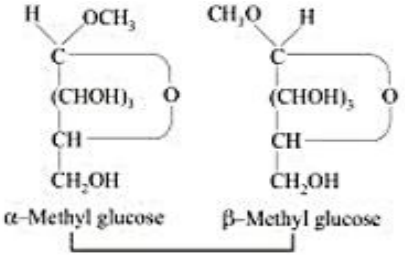




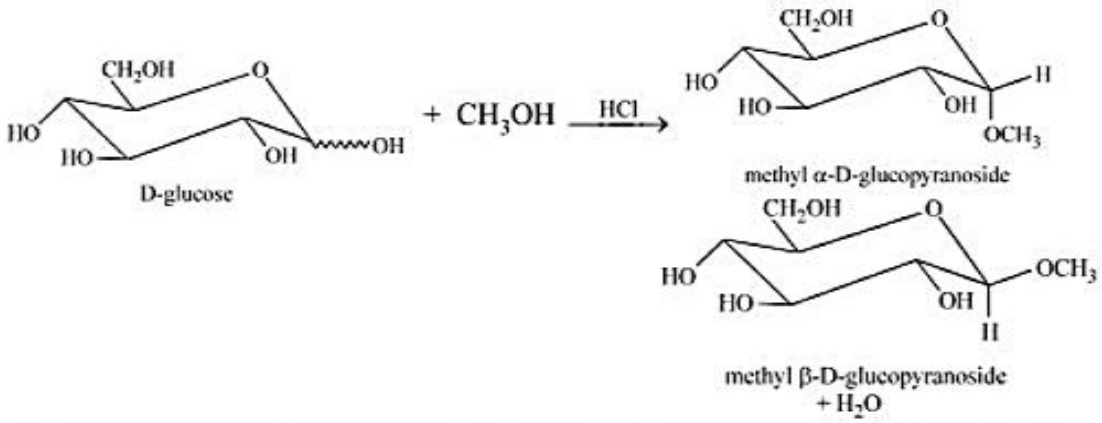
* All monosaccharides are reducing sugars, hence they show mutarotation.
* Starch, cellulose are Polymers of Glucose
* Lactose and sucrose are disaccharides
* Sucrose is a non reducing sugar, gives negative test for Benedict and tollen's reagent, they do not form osazone and do not show mutarotation.
* Acetals of carbohydrates are called as GLYCOSIDE

FORMATION OF GLYCOSIDES

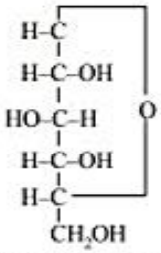
Glucose reacts with methyl alcohol in presence of dry HCl to form α and β-methyl glycoside of glucose. The reaction takes place only on OH of hemi-acetylic carbon. Other hydroxyl groups are unreactive.



To methylate all the OH groups, methylating agent used is dimethyl sulphate.



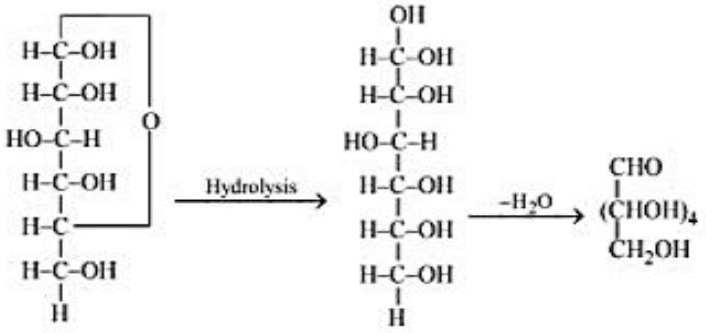
Such compounds are called glucoside (cyclic acetals). They are special type of acetals in which one of the oxygen of the acetal linkage is the ring oxygen of the pyranose or furanose.



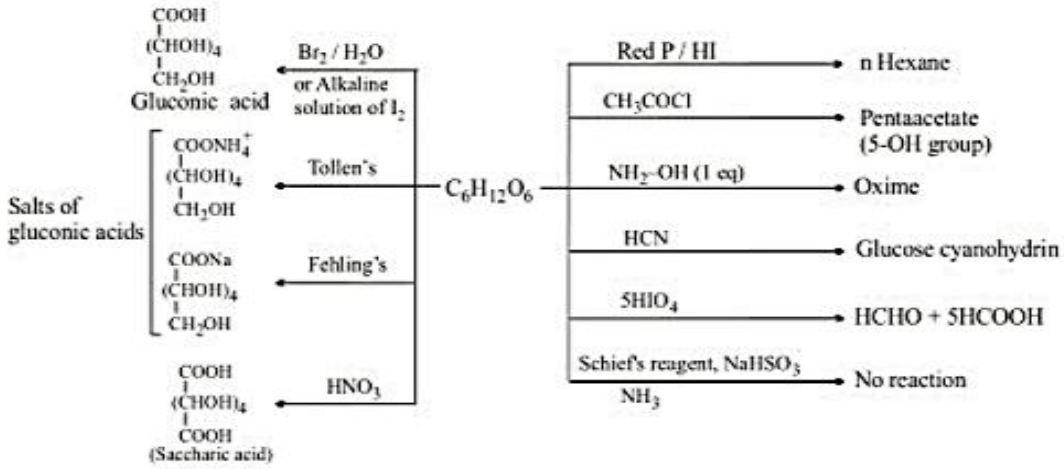
Ring structure of glucose :

(i) Glucose does not give pink colour with schief reagent.
(ii) Does not form adduct with $NaHSO_3$, NH_3
(iii) Glucose exist in two isomeric form
(iv) It show mutarotation

Since there is no free aldehyde group, so it does not react with weak reagent (NH_3, $NaHSO_3$) but strong reagent (HCN, NH_2OH, $C_6H_5NH-NH_2$) break up ring

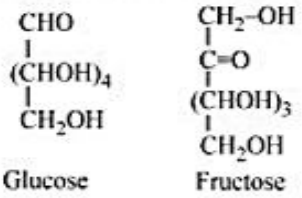


REACTIONS OF GLUCOSE



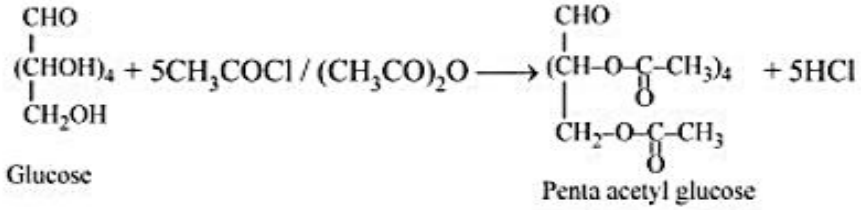
These reactions indicate that glucose has 6-C straight chain with one –CHO group & 5-OH group.

GENERAL REACTIONS OF MONOSACCHARIDES



Glucose Fructose

1. Acetylation :

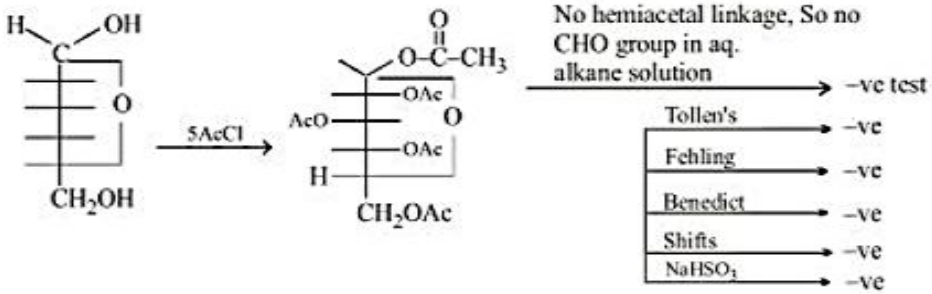


Glucose Penta acetyl glucose

This reaction suggests presence of 5(–OH) group.

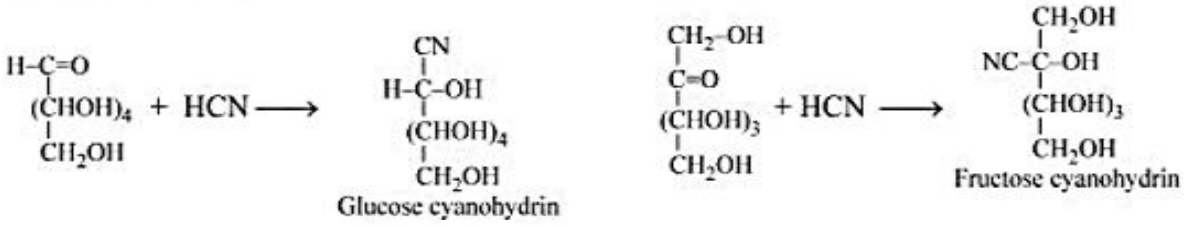
Q. The penta acetate of glucose give –ve test with Tollen's reagent & Fehling solution, explain?

Sol.



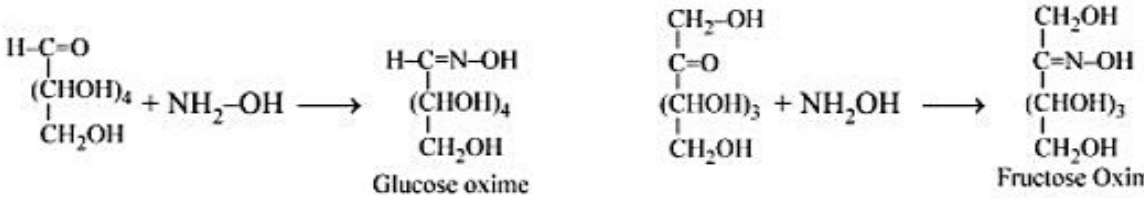
2. Red by HI / Red P:

3. Reaction with HCN:



Glucose cyanohydrin Fructose cyanohydrin

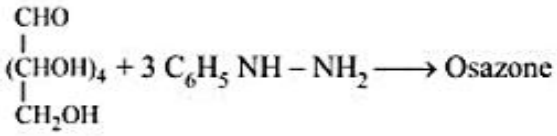
4. Reaction with NH_2–OH (hydroxyl amine):



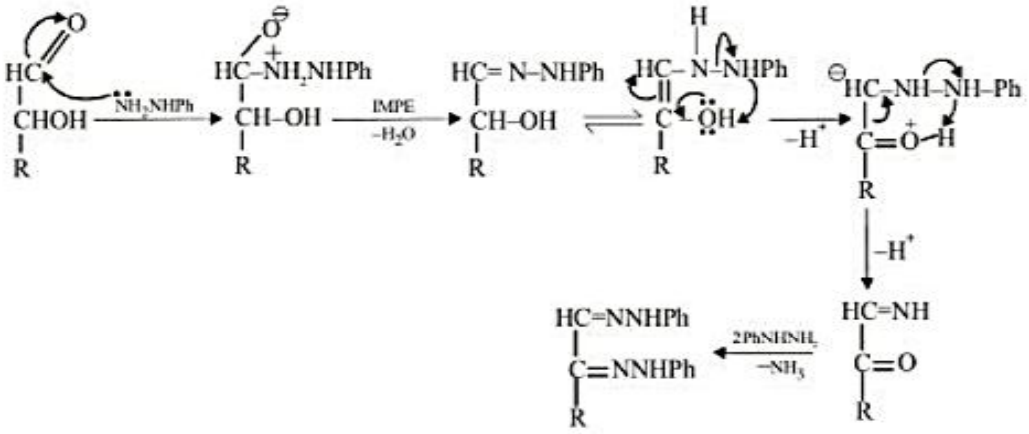
Glucose oxime Fructose Oxime

5. Reaction with phenyl hydrazine: Both glucose and fructose give "osazone".

Reaction with glucose :



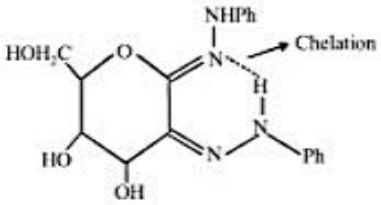
Mechanism :

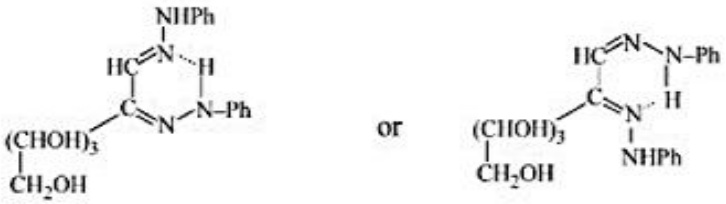


Both compounds give same product because structure of last four C is same in both (glucose & fructose)

Only C-1 and C-2 in glucose and fructose are involved in osazone formation addition reaction do not run through out the chain. The failure to undergo further reaction has been explained by stabilization of the osazone by chelation.

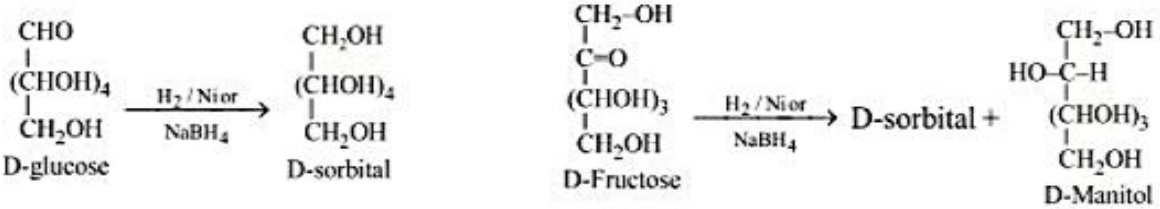
Osazone :



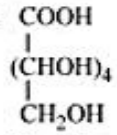


So we do not get hexaphenyl hydrazone.

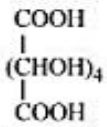
6. Catalytic reduction:



7. Oxidation:

Glucose $\xrightarrow[Br_2 \text{ water}]{\text{Tollen's,Fehling}}$ 

Gluconic acid

Glucose $\xrightarrow{\text{dil}HNO_3}$ 

Saccharic acid

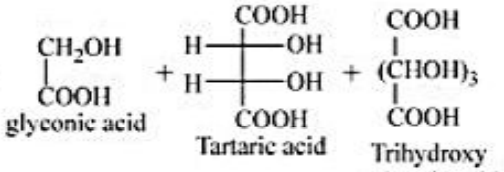
Glucose $\xrightarrow{HIO_4}$ $5HCOOH + HCHO + 5HIO_3$

Oxidation of fructose :

Fructose $\xrightarrow{\text{Tollen's,Fehling}}$

Fructose reduces tollen's & fehling reagent because in basic medium fructose isomerises to glucose.

Fructose $\xrightarrow{Br_2\text{water}}$ No reaction

Fructose $\xrightarrow{HNO_3}$ 

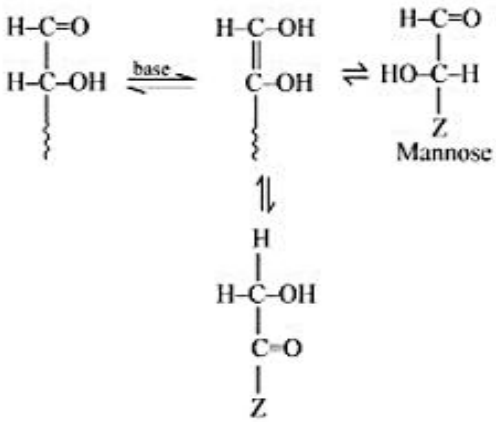
glyceonic acid — Tartaric acid — Trihydroxy glutaric acid

8. Reaction with enzyme:

Glucose or Fructose $\xrightarrow[\text{Zymase enzyme}]{\text{Yeast}}$ $2C_2H_5OH + 2CO_2$

9. Reaction with dil NaOH / $Ca(OH)_2$

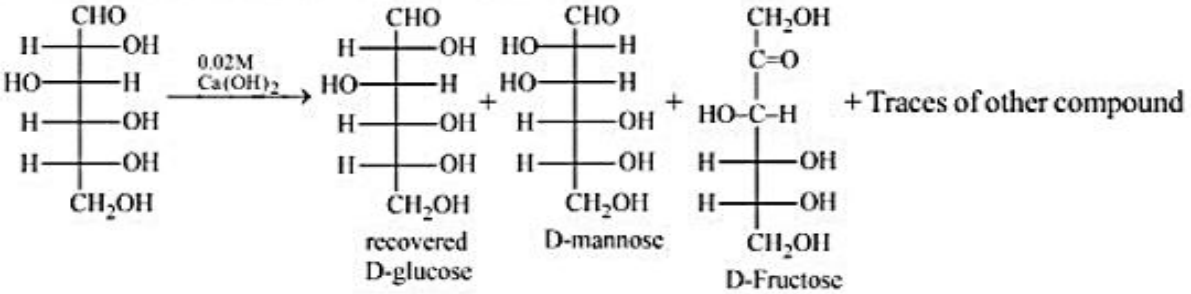
Glucose or fructose $\xleftarrow{\text{dil NaOH}}$ Glucose + Fructose + Mannose



Mannose

Base-catalyzed isomerisation of aldoses and Ketoses:

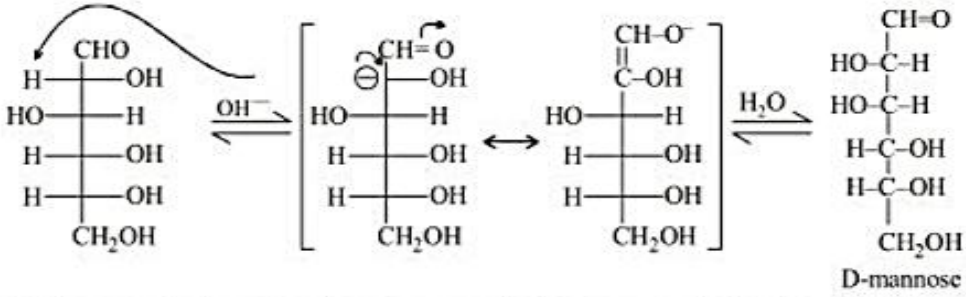
Although glucose in solution exists mostly in its cyclic hemiacetal forms it is also in equilibrium with a small amount of it's acyclic aldehyde form.



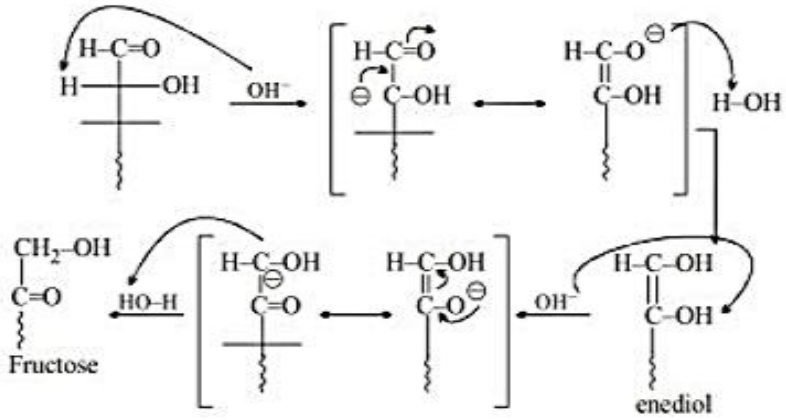
recovered D-glucose — D-mannose — D-Fructose

+ Traces of other compound

Mechanism : Like other aldehyde with α-hydrogen, glucose ionise to give small amount of its inolate ion in base. Protonation of this enolate ion at one face of the double bond gives back glucose & protonation at the other face gives mannose.



The enolate ion can also be protonated on oxygen to give a new enol called enediol this enediol converts to fructose as follows.



Method of ascending the sugar series : An aldose may be converted into it's next higher aldose eg. an aldopentose into an aldohexose.

SOME IMPORTANT CARBOHYDRATES

1. **Sucrose ($C_{12}H_{22}O_{11}$) :** → It is white, crystalline & sweet substance soluble in water obtained from the sugar cane. When heated above its melting point, it forms a brown substance known as caramel. It's aqueous solution is dextrorotatory $[\alpha]_D = 66.5°$

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{H^+} \underset{\text{D-Glucose}\ \alpha\,[D] = 52.7°}{\overset{\text{dextro}}{C_6H_{12}O_6}} + \underset{\text{D-Frucose}\ [\alpha]_D = -92.4°}{\overset{\text{laevo}}{C_6H_{12}O_6}}$$

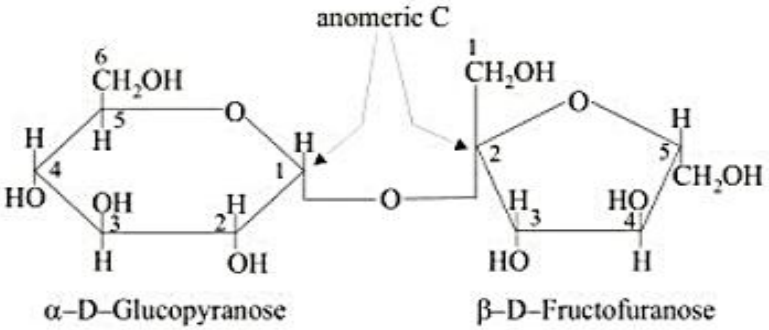
mixture is laevorotatory (–)

Thus hydrolysis of sucrose brings about a change in the sign of rotation, from dextrol (+) to leavo (–) & such a change is known as inversion of cane sugar and the mixture is known as *invert sugar*.

The inversion of cane-sugar may also be effected by the enzyme invertase which is found in yeast.

Sucrose is non-reducing sugar because it has stable acetal linkage & in aq. solution it can not give free carbonyl group and so it does not reduces Tollen's & Fehling's solution.

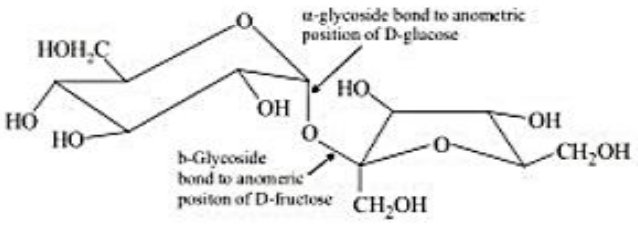
This indicates that neither the aldehyde group of glucose nor the ketonic group of fructose is free in sucrose.

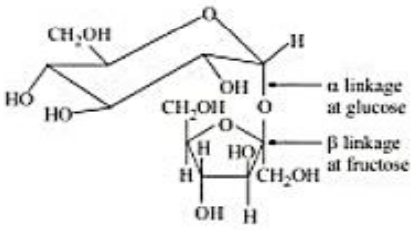


Structure of sucrose
(α–D–glucopyranosyl–β–D–fructofuranoside)

All non reducing sugars do not show mutarotation .

There is no free carbonyl group so it is non reducing sugar





2. Maltose: It is obtained by partial hydrolysis of starch by the enzyme diastase present in malt i.e., sprouted barely seeds.

$$2\,(C_6H_{10}O_5)_n + n\,H_2O \xrightarrow{\text{Diastase}} n\,C_{12}H_{22}O_{11}$$

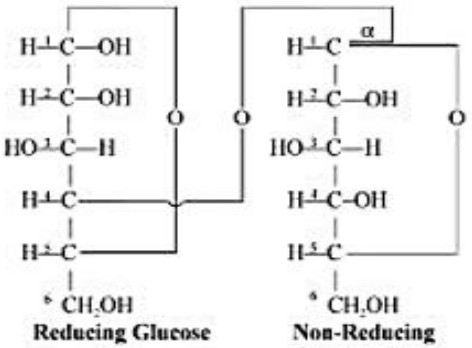
Starch → Maltose

As stated above, hydrolysis of one mole of maltose yields two moles of D-glucose. Maltose is a reducing sugar since it forms an osazone, undergoes mutarotation and also reduces Tollen's and Fehling's solutions, Methylation studies have revealed that

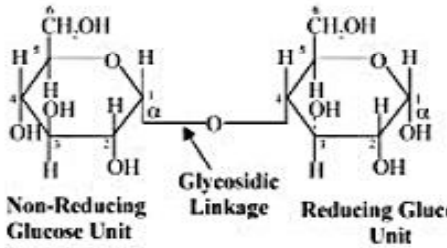
(i) both glucose units are present in the pyranose form.

(ii) C_1 of one glucose unit is linked to C_4 of the other

Further since maltose is hydrolysed by the enzyme maltose which specifically hydrolyses α-glycosidic linkage, therefore, the non-reducing glucose unit in maltose must be present in the α-form. In other words, C_1 – α of non-reducing glucose unit is attached to C_4 of the reducing glucose unit as shown in the figure on next page.



FISCHER



HAWORTH PROJECTION FORMULAE OF α-D-MALTOSE

3. Lactose (Milk sugar) $C_{12}H_{22}O_{11}$

Lactose occurs in milk and that is why it is called milk sugar.

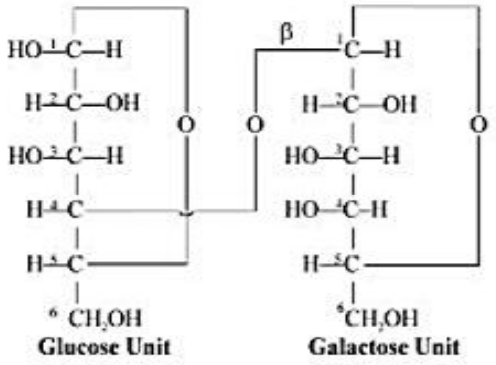
Lactose on hydrolysis with dilute acid or by enzyme lactase, yields an equimolar mixture of D-glucose and D-galactose. It is a reducing sugar since it forms an osazone, undergoes mutarotation and also reduces Tollen's or Fehling's solution. Methylation studies have revealed that

(i) both glucose and galactose are present in the pyranose form.

(ii) glucose is the reducing half while β-galactose is the non-reducing half.

(iii) C_1 of galactose unit is connected to C_4 of glucose unit.

Further since emulsin, as, enzyme which specifically hydrolyses β-glycosidic linkages also hydrolyses lactose, therefore, galactose must be present in the β-form. In other words, in lactose, C_1 – β of galactose is attached to C_4 of glucose as shown in figure.



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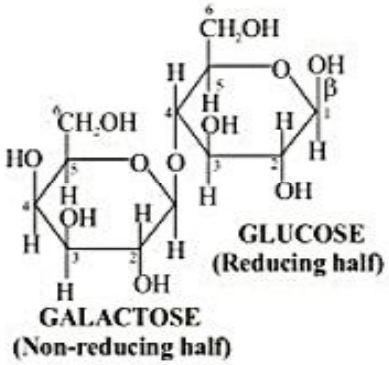
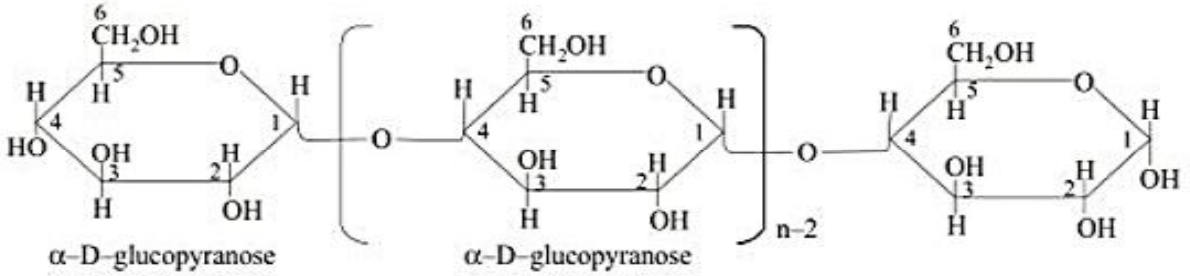


4. Starch Amylum, $(C_6H_{10}O_5)_n$

Occurrence : The value of n (100 – 3000) varies from source to source. It is the chief food reserve material or storage polysaccharide of plants and is found mainly in seeds, roots tubers etc. Wheat, maize, rice, potatoes, barley, bananas and sorghum are the main sources of starch. Starch occurs in the form of granules, which vary in shape and size depending upon their plant source.

Occurs in all green plants. Starch consists of two fractions, one being known as α–amylose, which gives blue colour with iodine. This blue colour is believed to be due to the formation of an inclusion complex. An aqueous solution of α–amylose slowly forms a precipitate, since α-amylose has a strong tendency to 'revert' to the insoluble state in solution. Amylopectin is insoluble in water and is stable towards both hydrolysis to maltose by the enzyme diastase and to D(+)-glucose by dilute acids (amylopectin gives about 50 percent of maltose).



Structure of Starch (α–D–glucoamylose)

α–amylose consists of an unbranched chain, with a molecular weight varying between 10,000(n ≈ 60) and 10,00,000(n ≈ 6,000), The value of n depends on the source and treatment of α–amylose.

Properties : (i) Starch is a white amorphous powder sparingly soluble in water. Its aqueous solution gives a blue colour with iodine solution due to the formation of an inclusion complex. The blue pears on cooling.

(ii) On hydrolysis with dilute mineral acids or enzymes, starch beaks down first to smaller molecules (n > n'), then to maltose and finally to D-glucose.

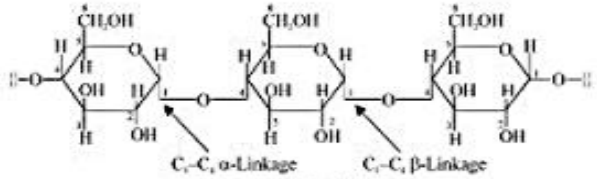
$$(C_6H_{10}O_5)n \xrightarrow{H^+/H_2O} (C_6H_{10}O_5)_{n'} \xrightarrow{H^+/H_2O} C_{12}H_{22}O_{11} \xrightarrow[\text{or Maltase}]{H^+/H_2O} C_6H_{12}O_6$$

Starch — Maltose — D-Glucose

(iii) Starch is a non-reducing saccharide. It neither reduces Tollen's reagent or Fehling's solution nor forms an osazone. This suggests that all hemiacetal OH groups of glucose units at C_1 are not free but are involved in glycosidic linkages.

Composition : Starch is not a single compound but is a mixture of two components–a water soluble component called amylose (10-20%) and a water insoluble component called amylopectin (80-90%). Both amylose and amylopectin are polymers of α-D-glucose.

Structure of amylose : Amylose is water soluble and gives blue colour with iodine solution. It may have 100-3000 glucose units, i.e., its molecular mass can vary from 10,000 to 500,000. It is a linear polymer of α-D-glucose in which C_1 of one glucose unit is attached to C_4 of the other through α-glycosidic linkage as shown in figure.



STRUCTURE OF AMYLOSE

Pectins

Pectins are found in plant and fruit juices. Their characteristic property is the ability of their solutions to gelate, i.e. form jellies. They have a high molecular weight and are polygalacturonic acid (linked 1,4) with the carboxyl groups partially esterified with methanol.

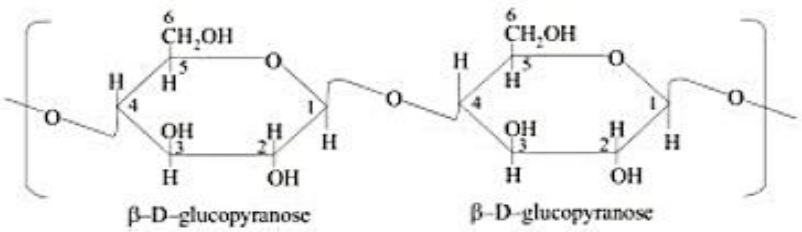
Glycogen $(C_6H_{10}O_5)_n$:

Glycogen is found in nearly all animals cells, occurring mainly in liver. It is the reserve carbohydrate of animals and so is often known as 'animal starch'. It has also been isolated from plant sources.

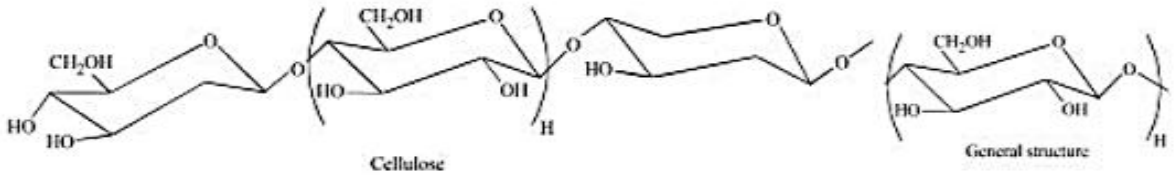
Glycogen is a white powder, soluble in water, the solution giving a purplish-red colour with iodine. On hydrolysis with dilute acid, glycogen gives D(+)-glucose. The molecular weight of glycogen has been given as 10,00,000 to 50,00,000 and glycogen contains highly branched chains. Glycogen has a structure similar to amylopectin, except that it has more cross-linking.

5. Cellulose:

Cellulose is colourless, solid which is insoluble in water & organic solvents. But it is soluble in ammonical cupric hydroxide (Schweizer's reagent) or in conc. HCl cellulose is a regular polymer of d-glucopyranose residues connected by β-1,4 glycosidic linkages. It is straight chain polymer.

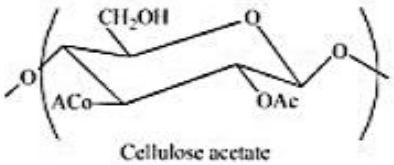


Structure of Cellulose



Some points about cellulose :

1. General empirical formula $(C_6H_{10}O_5)$
2. Cellulose + $H_2O \xrightarrow{H^+}$ 96% of crystalline D-glucose
3. No. of monomer units in cellulose are 1000 – 1500 in one molecule.
4. Cellulose doesn't show mutarotation (like starch)
5. It is non reducing sugar because there is no hemiacetal linkage.
6. Acetylations nitration & methylation of cellulose give trisubstituted cellulose which suggest that only three – OH groups are free.



Tests for carbohydrates:

(i) When heated in a dry test tube, it melts, turns brown and finally black, giving a characteristic smell of burning sugar.

(ii) When warmed with a little concentrated H_2SO_4, it leaves a charred reside of carbon.

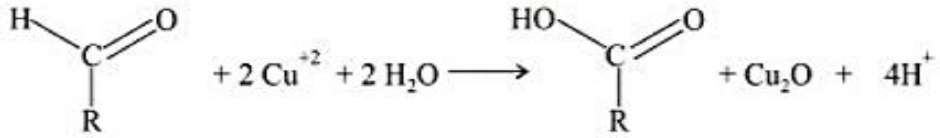
(iii) **Molisch's Test** (named after Austrian botanist Hans Molisch) is a sensitive chemical test for the presence of carbohydrates, based on the dehydration of the carbohydrate by sulfuric acid to produce an aldehyde, which condenses with two molecules of phenol (usually α-naphthol, though other phenols (e.g. resorcinol, thymol) also give colored products) resulting in a red- or purple-colored compound.

The test solution is combined with a small amount of Molisch's reagent (α-naphthol dissolved in ethanol) in a test tube. After mixing, a small amount of concentrated sulfuric acid is slowly added down the sides of the sloping test-tube, without mixing, to form a bottom layer. A positive reaction is indicated by appearance of a purple ring at the interface between the acid and test layers.

All carbohydrates — monosaccharides, disaccharides, and polysaccharides — should give a positive reaction, and nucleic acids and glycoproteins also give a positive reaction, as all these compounds are eventually hydrolyzed to monosaccharides by strong mineral acids. Pentoses are then dehydrated to furfural, while hexoses are dehydrated to 5-hydroxymethylfurfural. Either of these aldehydes, if present, will condense with two molecules of naphthol to form a purple-colored product, as illustrated below by the example of glucose:

BARFOED'S TEST

Barfoed's Test is a chemical test used for detecting the presence of monosaccharides. It is based on the reduction of copper (II) acetate to copper (I) oxide (Cu_2O), which forms a brick-red precipitate. Barfoed's reagent consists of a 0.33 molar solution of neutral copper acetate in 1% acetic acid solution. The reagent does not keep well and it is therefore advisable to make it up when it is actually required. Reducing monosaccharides are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper (I) oxide within three minutes. Reducing disaccharides undergo the same reaction, but do so at a slower rate and ppt. will come after 10 min. For Non reducing saccharides ppt. will not form.



The aldehyde group of the monosaccharide which normally forms a cyclic hemiacetal is oxidized to the carboxylate. A number of other substances, including sodium chloride may interfere.

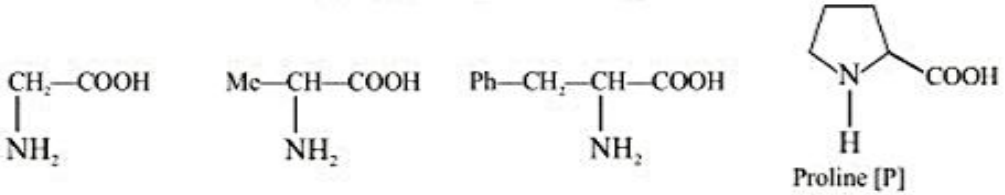
STARCH

Plants store glucose as the polysaccharide starch. The cereal grains (wheat, rice, corn, oats, barley) as well as tubers such as potatoes are rich in starch.
Starch can be separated into two fractions--amylose and amylopectin. Natural starches are mixtures of amylose (10-20%) and amylopectin (80-90%).

Iodine - KI Reagent: Iodine is not very soluble in water, therefore the iodine reagent is made by dissolving iodine in water in the presence of potassium iodide. This makes a linear triodide ion complex with is soluble. The triodide ion slips into the coil of the starch causing an intense blue-black color.

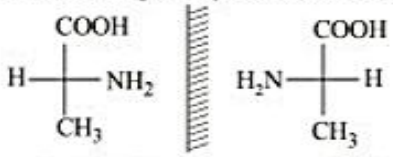
AMINO ACIDS AND PROTEINS

Bifunctional compounds $R-\underset{\displaystyle NH_2}{\underset{|}{CH}}-COOH$ having an acidic corboxylic group & a basic amino group. There are 20 amino acids commonly found is proteins and are standard amino acids. All are α amino acids. Most of them have 1° amino group.($-NH_2$). However proline is a 2° amino

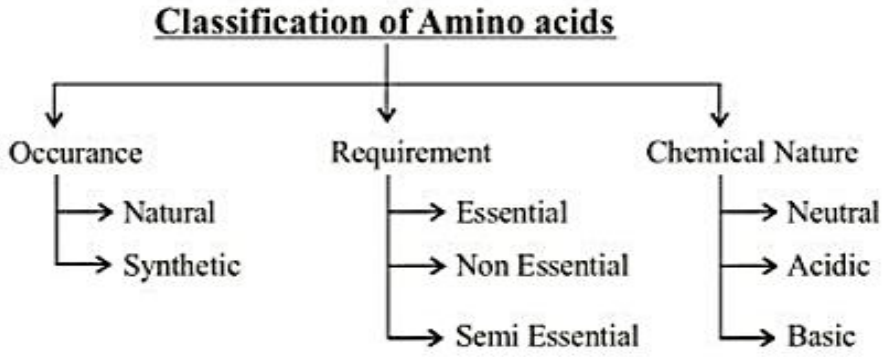


(Gly) Glycine [G] Alanine [A] Phenyl alanine [F] Proline [P] (α-imino acid)

All amino acids are chiral molecules with atleast one chiral carbon except glycine, $H_3N^{\oplus}CH_2COO^{-}$. Except Glycine all other amino acids are optically active & can be assigned D & L configuration.



D-Alanine L-Alanine



Based on requirement

1. Essential amino acids can not be synthesized in human body so dietary intake is required. For any human being 1 gm a day is required.
2. Semi essential amino acids can be synthesized in human body but dietary intake is required during growing stages (when more of cell division is required).

   For example : Early childhood, pregnancy and lactating mother.
3. Non essential amino acid - Body can synthesize them.

Chemical classification

Neutral - Amino acid having equal number of NH_2 and COOH.

Neutral amino acids are further classified as polar and nonpolar depending on whether their side chains have polar substituents (for example, asparagine with an NH_2CO group) or are completely hydrocarbon in nature (for example alanine, valine etc.).

Acidic - Amino acid having more COOH than NH_2

Aspartic acid and glutamic acids, each with a second CO_2H in their side chain are acidic amino acids.

Basic - Amino acid having more NH_2 than COOH

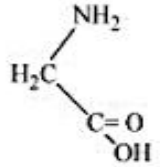
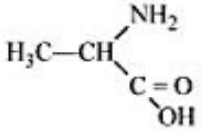
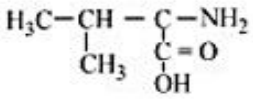
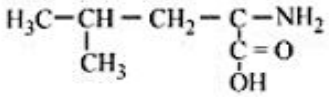
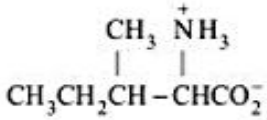
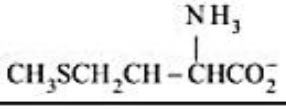
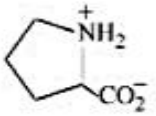
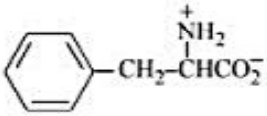
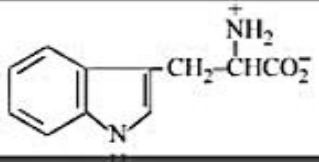
Lysine, arginine and histidine, each with a basic site in their side chain are basic amino acids.

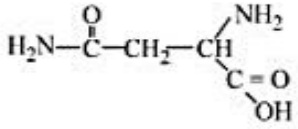
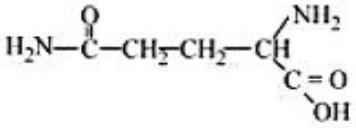
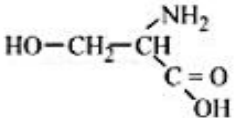
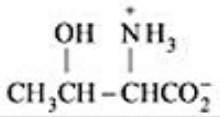
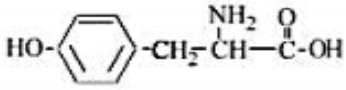
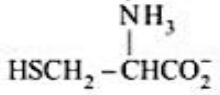
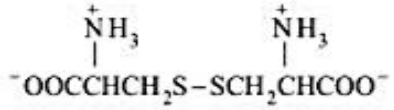
Proteins: The name protein is taken from the Greek word "proteios", which means "first". Of all chemical compounds, proteins must almost certainly be ranked first, for they are the substance of life.

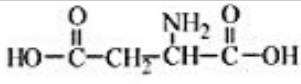
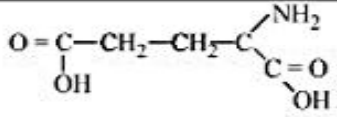
Proteins make up a large part of the animal body, they hold it together and they run it. They are found in all living cells.

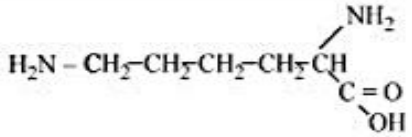
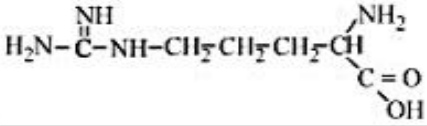
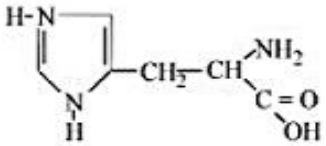
Chemically, proteins are high polymers. They are polyamides and the monomers from which they are derived are the α - amino carboxylic acids. A single protein molecule contains hundreds or even thousands of amino acid units. These units can be of twenty-odd different kinds. The number of different combinations, i.e., the number of different protein molecules that are possible, is almost infinite.

I. Neutral amino acids (with nonpolar side chains)

NAME	ABBREVIATIONS	STRUCTURAL FORMULAE	ISOELECTRIC POINT[pI]
@Glycine	Gly(G)		6.0
Alanine	Ala(A)		6.0
Valine*	Val(V)		6.0
Leucine*	Leu(L)		6.0
Isoleucine*	Ile(I)		6.0
Methionine*	Met(M)		5.7
@@Proline	Pro(P)		6.3
Phenylalanine*	Phe(F)		5.5
Tryptophan*	Trp(W)		5.9

2. Neutral amino acids (with polar, but nonionized side chains)			
NAME	**ABBREVIATIONS**	**STRUCTURAL FORMULAE**	**ISOELECTRIC POINT[pI]**
Asparagine	Asn(N)		5.4
Glutamine	Gln(Q)		5.7
Serine	Ser(S)		5.7
Threonine*	Thr		5.6
Tyrosine	Tyr(Y)		5.7
Cysteine	Cys		5.1
‡ Cystine	Cys–Cys		

3. Acidic amino acids (side chain with carboxylic acid group)			
NAME	**ABBREVIATIONS**	**STRUCTURAL FORMULAE**	**ISOELECTRIC POINT[pI]**
Aspartic acid	Asp(D)		2.8
Glutamic Acid	Glu(E)		3.2

4. Basic amino acids (side chain with nitrogenous basic group)			
NAME	**ABBREVIATIONS**	**STRUCTURAL FORMULAE**	**ISOELECTRIC POINT[pI]**
Lystine*	Lys(K)		9.7
Arginine*	Arg(R)		10.8
Histidine*	His(H)		7.6

Note :

* Amino acids with an asterisk are essential amino acids, that must be supplemented through diet.

† At pH = 7, Asp and Glu have a net negative charge and exist as anions. At pH = 7, Lys and Arg have a net positive charge and exist as cations. Rest of the amino acids at this pH exist in the neutral form.

‡ Structurally, in cystine, the two cysteine molecules are joined through sulfur (disulfide linkage).

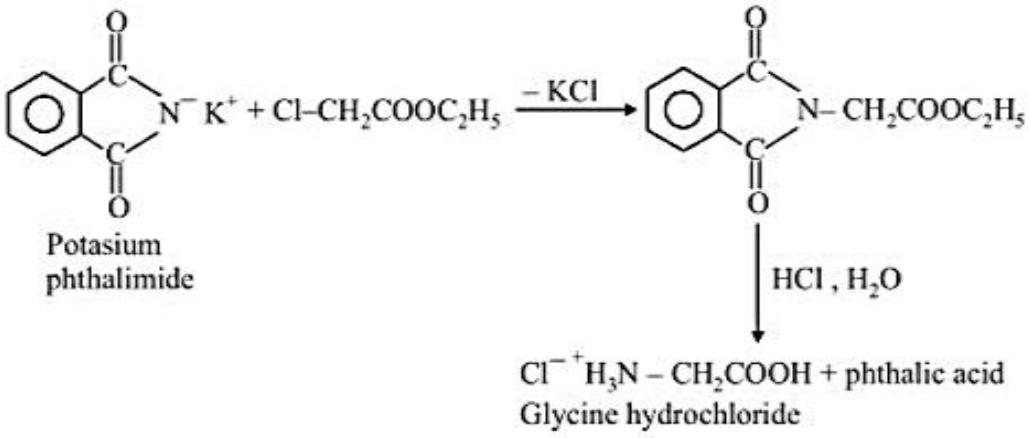
@@ Proline is an α-imino acid, all amino acids are primary amines except proline and 4-hydroxyproline, which are 2° amines.

@ Except Glycine all other amino acids are optically active.

Preparation of amino acids

(a) **Gabriel Phthalimide synthesis**

Better yields are generally obtained by the gabriel phthalimide synthesis ; the α - halo esters are used instead of α - halo acids .



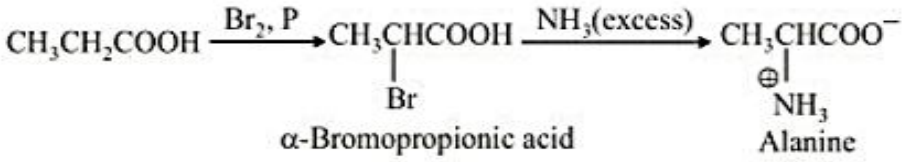
Potasium phthalimide

$Cl^{-}\ {}^{+}H_3N - CH_2COOH$ + phthalic acid

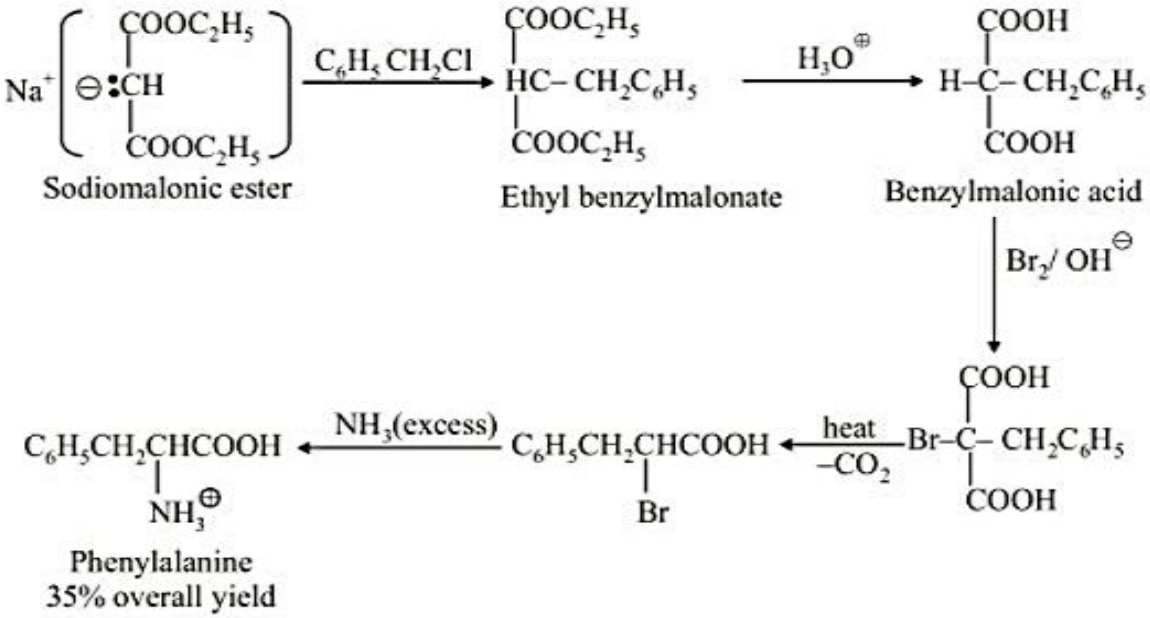
Glycine hydrochloride

(b) **Amination of α - Halo acids**

Sometimes an α - chloro or α - bromo acid is subjected to direct ammonolysis with a large excess of concentrated ammonia. For example.

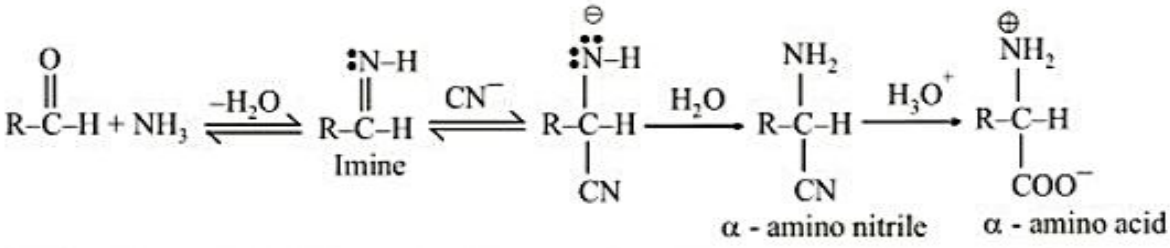


(c) **From diethyl malonate**



(d) **Strecker's synthesis**

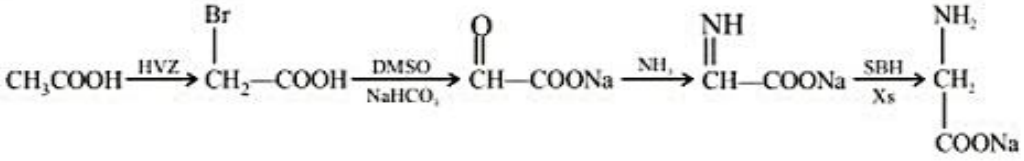
Strecker's synthesis is also used for preparing α - amino acids



In this reaction general aldehyde is treated with mixture of ammonium chloride and KCN in aqueous solution which forms NH_3 and HCN, $NH_4Cl + KCN \xrightarrow{\text{aqueous}} NH_4CN + KCl$,

$NH_4CN \xrightarrow{\text{aqueous}} NH_3 + HCN$

(e) **Using KOOP synthesis**



Properties of Amino acids

Although the amino acids are commonly shown as containing an amino group and a carboxyl group, $H_2NCHRCOOH$, certain properties, (both physical and chemical) are not consistent with this structure

I. Physical properties

In contrast to amines and carboxylic acids, the amino acids are nonvolatile crystalline solids, which melt with decomposition at fairly high temperatures.

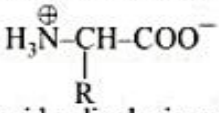
They are insoluble in non-polar solvents like petroleum ether, benzene or ether and are appreciably soluble in water.

Their aqueous solutions behave like solutions of substances of high dipole moment.

Amino acids as dipolar ions

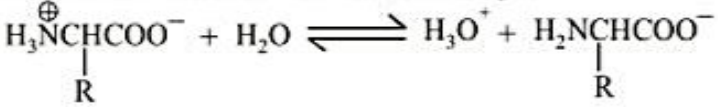
Acidity and basicity constant are ridiculously low for –COOH and $–NH_2$ groups. Glycine, for example, has $K_a = 1.6 \times 10^{-10}$ and $K_b = 2.5 \times 10^{-12}$, whereas most carboxylic acids have K_a values of about 10^{-5} and most aliphatic amines have K_b values of about 10^{-4}.

All these properties are quite consistent with a dipolar ion structure for the amino acids (I)



Amino acids : dipolar ions (Zwitter ion)

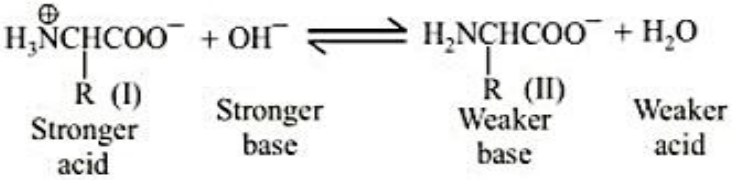
Physical properties - melting point, solubility, high dipole moment - are just what would be expected of such a salt. The acid-base properties also become understandable when it is realized that the measured K_a actually refers to the acidity of an ammonium ion, RNH_3^+,



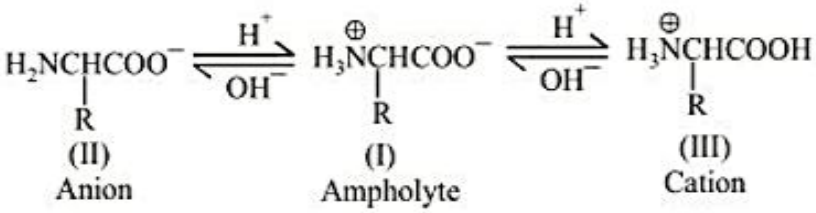
Acid

$$K_a = \frac{[H_3O^{\oplus}][H_2NCHRCOO^{\ominus}]}{[H_3\overset{\oplus}{N}CHRCOO^{\ominus}]}$$

When the solution of an amino acid is made alkaline, the dipolar ion (I) is converted into the anion (II). The stronger base, hydroxide ion, removes a proton from the ammonium ion and displaces the weaker base, the amine.



Stronger acid — Stronger base — Weaker base — Weaker acid

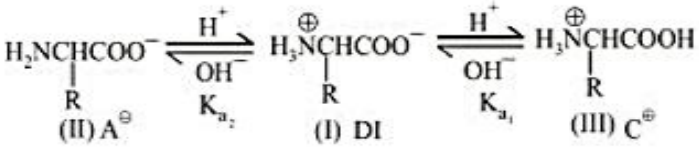


(II) Anion — (I) Ampholyte — (III) Cation

Wherever feasible, we can speed up a desired reaction by adjusting the acidity or basicity of the solution in such a way as to increase the concentration of the reactive species.

Isoelectric point of amino acids

What happens when a solution of an amino acid is placed in an electric field depends upon the acidity or basicity of the solution.



In quite alkaline solution, anions (II) exceed cations (III), and there is a net migration of amino acid toward the anode. In quite acidic solution, cations (III) are in excess, and there is a net migration of amino acid toward the cathode. If (II) and (III) are exactly balanced, there is no net migration ; under such conditions any one molecule exists as a positive ion and as a negative ion for exactly the same amount of time, and any small movement in the direction of one electrode is subsequently cancelled by an equal movement back towards the other electrode. **The hydrogen ion concentration of the solution in which a particular amino acid does not migrate under the influence of an electric field is called the isoelectric point (pI) of that amino acid. The isoelectric point (pI) is the pH at which the amino acid exists only as a dipolar ion with net charge zero.**

For glycine, for example, the isoelectric point is at pH 6.1.

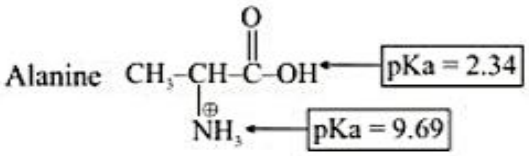
An amino acid usually shows its lower solubility in a solution at the isoelectric point, since here there is the highest concentration of the dipolar ion. As the solution is made more alkaline or more acidic, the concentration of one of the more soluble ions, (II) or (III) increases.

$$Ka_1 = \frac{[DI][H^{\oplus}]}{[C^{\oplus}]} \qquad Ka_2 = \frac{[A^{-}][H^{\oplus}]}{[DI]} \text{ at pI } [A^{\ominus}] = [C^{\oplus}]$$

$$\frac{[DI][H^{\oplus}]}{Ka_1} = \frac{Ka_2[DI]}{[H^{\oplus}]} \qquad [H^{\oplus}]^2 = Ka_1 \times Ka_2$$

$$\text{on taking antilog pI} = \frac{P^{Ka_1} + P^{Ka_2}}{2}$$

The PI of an amino acid that does not have an ionizable side chain. (e.g. alanine), is midway between its two pKa values.

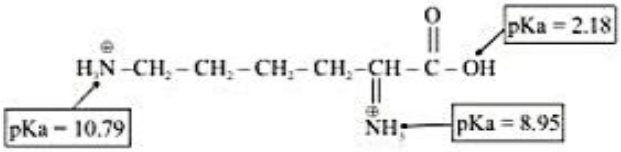


$$pI = \frac{2.34 + 9.69}{2} = \frac{12.03}{2} = 6.02$$

If an amino acid has an ionizable side chain, its PI is the average of the pKa values of the similarly ionizing groups (positive ionizing to uncharged or uncharged ionizing to negative.)

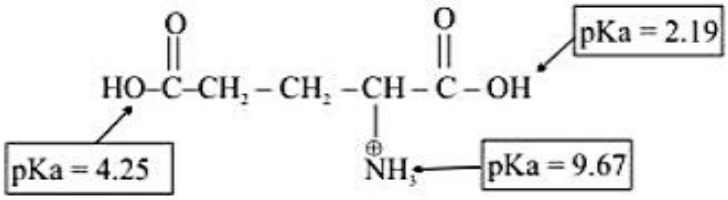
For example :

(i) Lysine



$$pI = \frac{10.79 + 8.95}{2} = \frac{19.74}{2} = 9.87$$

(ii) Glutamic acid



$$pI = \frac{21.19 + 4.25}{2} = \frac{6.44}{2} = 3.22$$

An amino acid having more COOH than NH_2 or more acidic COOH will have pI less than 7.
An amino acid having more $-NH_2$ than COOH or more basic $-NH_2$ will have pI more than 7.

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

Electrophoresis

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.

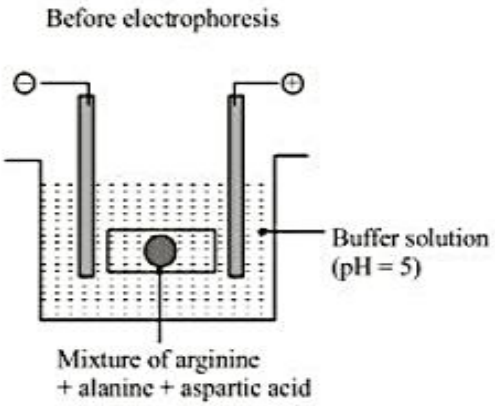


Figure (i)

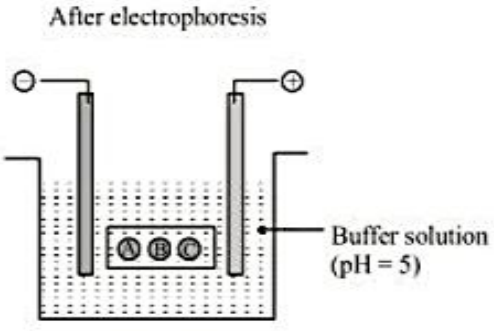
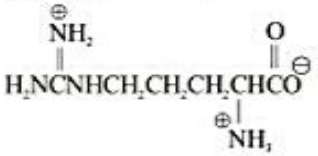
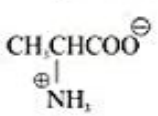


Figure (ii)

Ⓐ = arginine (pI = 10.76)



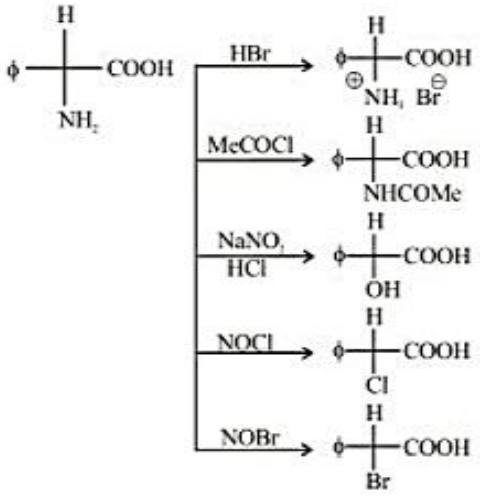
Ⓑ = alanine (pI = 6.02)

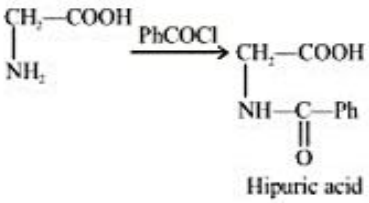


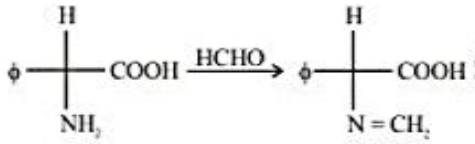
Ⓒ = aspartic acid (pI = 2.98)

General reactions of amino acids

(1) Reactions due to – NH_2 group

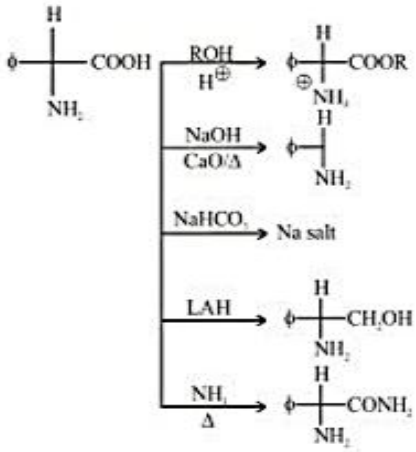






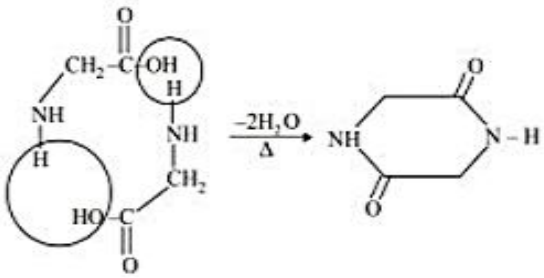
Reactions is used to block – NH_2 group during volumetric analysis in.

(2) Reactions due to – COOH group.

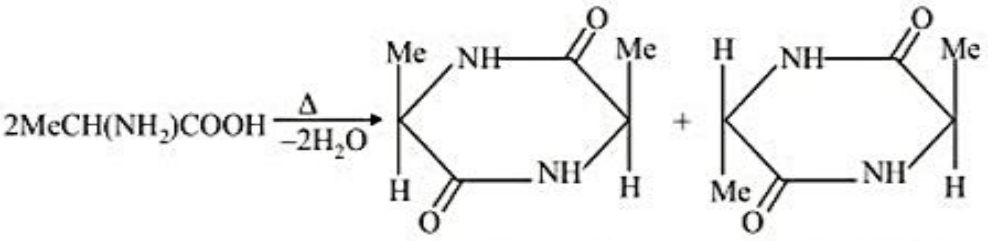


(3) Heating Effect

(i) Heating of amino acids leads to intermolecular dehydration to form cyclic diamides.

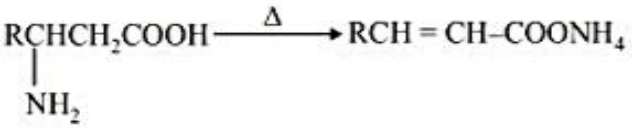


(ii) When alanine is heated, then two diastereomers are obtained. One of them (trans) is not resolvable.

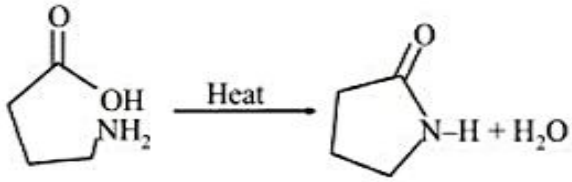


Cis (Racemic) Trans (Meso)

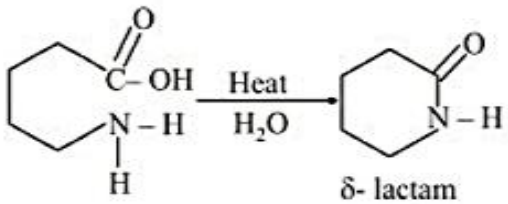
(iii) When β - amino acids are heated, α, β - unsaturated salt are formed.



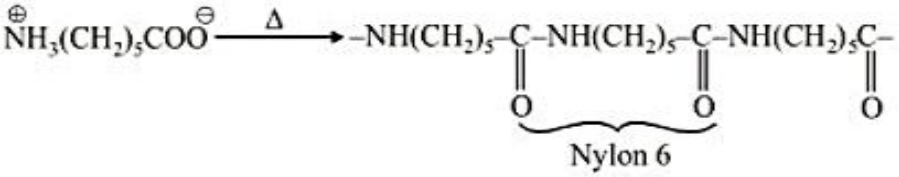
(iv) γ,δ,ε - amino acids when heated alone gives γ, δ - lactam and polymer respectively. The reason for the formation of polymer is that when ε - amino cyclises intramolecularly, it leads to large angle strain within the compound



γ- lactam



δ- lactam

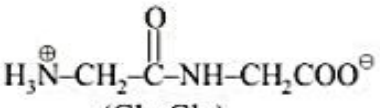


Nylon 6

(4) Peptide

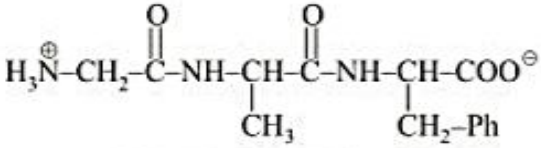
Peptides are amides formed by interaction between amino groups and carbonyl groups of amino acids.

The amino bonds $\left(-NH-\underset{\underset{O}{\|}}{C}-\right)$ that link amino acid residues are called peptide bonds. So peptide bonds are the only covalent bonds that hold amino acid residues together in a peptide or protein. Depending upon the number of amino acid residues per molecule, they are known as dipeptides, tripeptides and so on and finally polypeptides. (By convention, peptides of molecular weight upto 1000 are known as polypeptides and above that as protein)



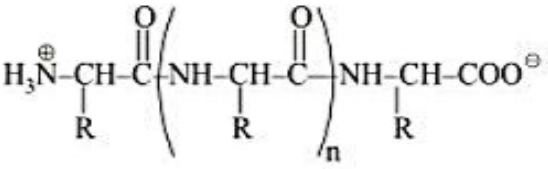
(Gly-Gly)

Glycine-Alaninedipeptide



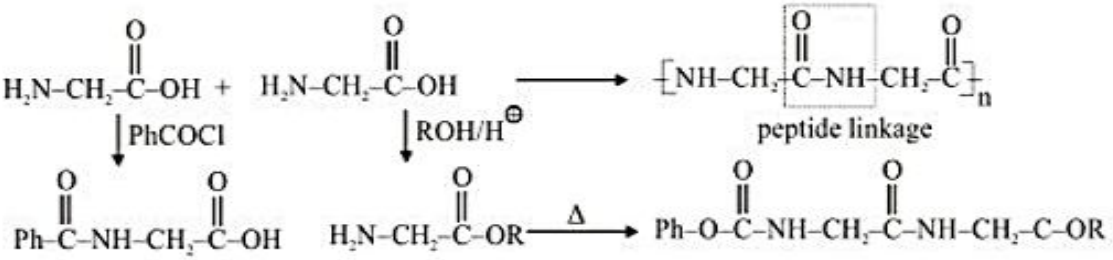
(Gly-Ala-Phen Tripeptide)

Glycine-Alanine-phenylalanine



Polypeptide

Dipeptides are made from two amino acids where as oligopeptides are made from 3 to 10 amino acids. If 11 to 100 amino acids are present together they called polypeptide and 100 onwards they are called as macropeptide.

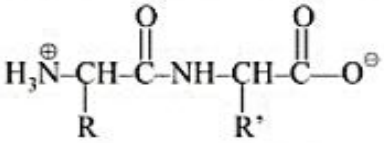


For the synthesis of polypeptides, the amino groups that are not to be linked in peptide bonds must be blocked so as to be unreactive. Then all other reactive functional groups must be protected to prevent their participation in the coupling produces. The coupling must be effected by a method that does not cause racemization or chemical alternation of the side chains.

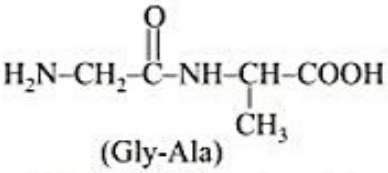
These type polyamide can be made only using part blocking technique.

Abbreviated name of amion acid with free NH_2 is written first.

By convention peptide are written with the free amino group (the –N–terminal amino acid) on the left and the free carbonyl group (the –C– terminal amino acid) on the right.

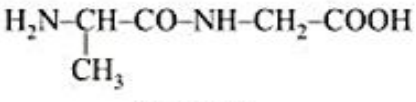


For the nomenclature of peptides abbreviated name of amino acid with free NH_2 group is written first. For example:



(Gly-Ala)

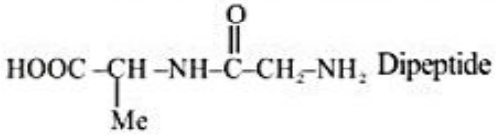
Glycine-Alanine-dipeptide



(Ala-Gly)

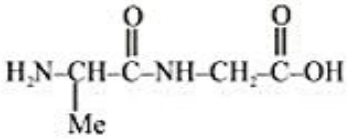
Alanine-Glycine-dipeptide

Polypeptide on hydrolysis give two amino acid are known as dipeptide



Dipeptide

Gly Ala [Glycine Alanine]



Ala - Gly [Alanine-Glycine]

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]

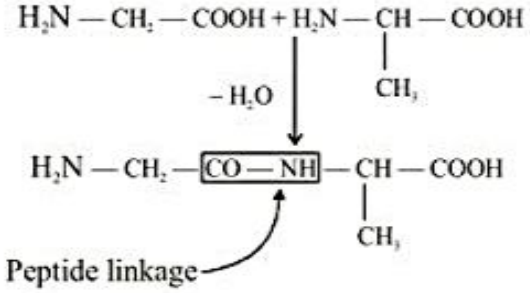
Q. Gly, Ala, and Phenyl Ala can form how many Dipeptide ? [Ans. Nine]

Q. Gly, Ala, can form how many Tripeptide ? [Ans. Eight]

When Macropeptide takes different shape due to intramolecular H - bonding between different layers is known as proteins

Proteins

You have already read that proteins are the polymers of α-amino acids and they are connected to each other by peptide bond or peptide linkage. Chemically, peptide linkage is an amide formed between — COOH group and — NH_2 group. The reaction between two molecules of similar or different amino acids, proceeds through the combination of the amino group of one molecule with the carboxyl group of the other. This results in the elimination of a water molecule and formation of a peptide bond — CO — NH —. The product of the reaction is called a dipeptide because it is made up of two amino acids. For example, when carboxyl group of glycine combines with the amino group of alanine we get a dipeptide, glycylalanine.



Glycylalanine (Gly-Ala)

If a third amino acid combines to a dipeptide, the product is called a tripeptide. A tripeptide contains three amino acids linked by two peptide linkages. Similarly when four, five or six amino acids are linked, the respective products are known as tetrapeptide, pentapeptide or hexapeptide, respectively. When the number of such amino acids is more than ten, then the products are called polypeptides. A polypeptide with more than hundred amino acid residues, having molecular mass higher than 10,000u 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.
Proteins can be classified into two types on the basis of their molecular shape.

(a) **Fibrous proteins :** When the polypeptide chains run parallel and are held together by hydrogen and disulphide bonds, then fibre-like structure is formed. Such proteins are generally insoluble in water. Some common examples are keratin (present in hair, wool, silk) and myosin (present in muscles), etc.

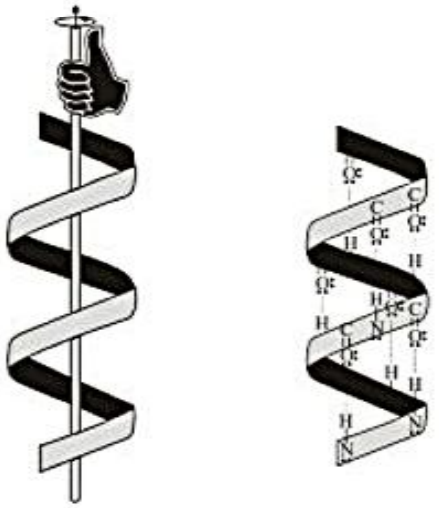
(b) **Globular proteins :** This structure results when the chains of polypeptides coil around to give a spherical shape. These are usually soluble in water. Insulin and albumins are the common examples of globular proteins.

Structure of Proteins

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

(i) **Primary structure of proteins :** Proteins may have one or more polypeptide chains. 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 the protein. Any change in this primary structure i.e., the sequence of amino acids creates a different protein.

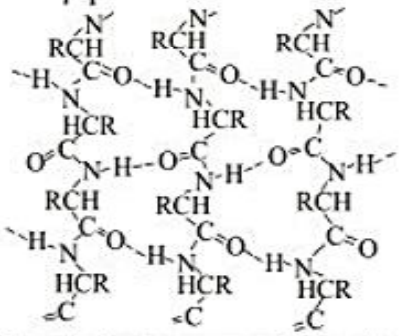
(ii) **Secondary structure of proteins :** The secondary structure of protein refers to the shape in which a long polypeptide chain can exist. They are found to exist in two different types of structures viz. α-helix and β-pleated sheet structure. These structures arise due to the regular folding of the backbone of the polypeptide chain due to hydrogen bonding between $-\overset{\overset{O}{\|}}{C}-$ and — NH — groups of the peptide bond.



α-Helix structure of proteins

α-Helix is one of the most common ways in which a polypeptide chain forms all possible hydrogen bonds by twisting into a right handed screw (helix) with the — NH group of each amino acid residue hydrogen bonded to the $\rangle C = O$ of an adjacent turn of the helix as shown in figure.

In β-structure all peptide chains are stretched out to nearly maximum extension and then laid side by side which are held together, by intermolecular hydrogen bonds. The structure resembles the pleated folds of drapery and therefore is known as β-pleated sheet.



β-Pleated sheet structure of proteins

(i) Ionic bonding : between COO^- and NH_3^+ at different sites.

(ii) H-bonding : mainly between side-chain NH_2 and COOH, also involving OH's (Of serine, for example) and the N–H of tryptophan.

(iii) Weakly hydrophobic Van der Waal's attractive forces engendered by side-chain R groups and

(iv) Disulfide cross linkages between loops of the polypeptide chain.

The same kind of attractive and repulsive forces responsible for the tertiary structure operate to hold together and stabilize the subunits of the quaternary structure.

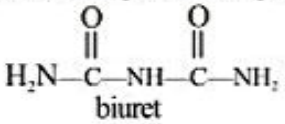
(iii) Tertiary structure of proteins : The tertiary structure of proteins represents overall folding of the polypeptide chains i.e., further folding of the secondary structure. It gives rise to two major molecular shapes viz. fibrous and globular. The main forces which stabilise the 2° and 3° structures of proteins are hydrogen bonds, disulphide linkages, van der walls and electrostatic forces of attraction.

(iv) Quaternary structure of proteins : Some of the proteins are composed of two or more polypeptide chains referred to as sub-units. The spatial arrangement of these subunits with respect to each other is known as quaternary structure.
According the their biological action, they are classified as enzymes, hormones, antibodies, etc.

Protein found in living system with definite configuration and biological activity is termed as native protein. If a native protein is subjected to physical or chemical treatment, which may disrupt its higher structures (conformations) without affecting its primary structure, the protein is said to be denatured. During denaturation, the protein molecule uncoils form an ordered and specific conformation into a more random conformation leading to precipitation. Thus denaturation leads to increase in entropy and loss of biological activity of the protein. The denaturation may be reversible or reversible. Thus, the coagulation of egg white on boiling of egg protein is an example of irreversible protein denaturation. However, in certain cases it is found that if the disruptive agent is removed the protein recovers its original physical and chemical properties and biological activity the reverse of denaturation is known as renaturation.

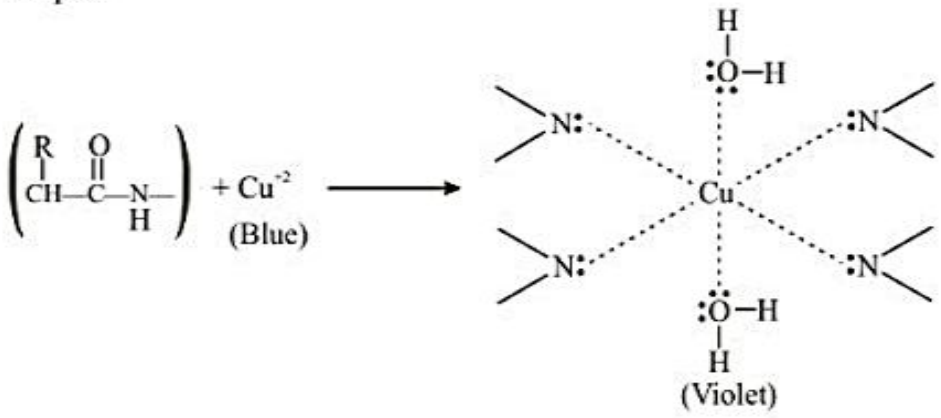
TESTS OF PROTEINS

Biuret test : Addition of a very dilute solution of $CuSO_4$ to an alkaline solution of a protein is done. A positive test is indicated by the formation of a pink violet to purple violet color.
The name of test is derived from a specific compound, biuret, which gives a positive test with this reagent

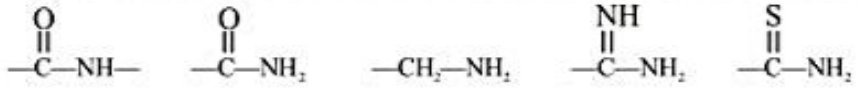


biuret

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

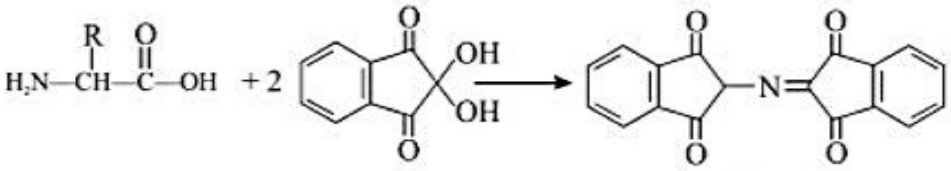


The biuret test works for any compound containing two or more of the following groups.



Ninhydrin Test : The ninhydrin test is a test for amino acids and proteins with a free $-NH_2$ group. Amino acids are detected by ninhydrin test. All amino acids give violet - coloured product with ninhydrin (triketo hydroindene hydrate) except proline and 4 - hydroxy proline, which gives yellow colour with it.

When such an $-NH_2$ group reacts with ninhydrin, a purple-blue complex is formed.



(Purple-Blue)

The same violet coloured dye forms from all α - AA's with 1° amino groups because only their nitrogen is incorporated into it. The 2° amines proline and 4 - hydroxyproline give different adducts that absorb light at a different and thus have a different yellow colour.