CARBOHYDRATES (UNIT- 2)

A carbohydrate is an organic compound with the general formula **$C_n(H_2O)_m$** which consists only of carbon, hydrogen and oxygen. Carbohydrates include sugar, glucose, starch, cellulose etc. They are important component of genetic molecule such as DNA and RNA.The carbohydrates (saccharides) are divided into three chemical groups: monosaccharides, oligosaccharides and polysaccharides. carbohydrates serve for the storage of energy (e.g., starch in plants and glycogen in animals).

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + \text{Energy}$$

Carbohydrates contain mainly two functional group Carbonyl (C=O) and hydroxyl (OH).

Definition -Carbohydrates are polyhydroxy aldehydes or ketones, or compounds that give these product on hydrolysis and contain at least one asymmetric carbon atom.

Classification of Carbohydrates: Carbohydrates are broadly divided into following classes-

- **Monosaccharides**
  - **Aldoses**: Aldotetroses, Aldopentoses, Aldohexoses
  - **Ketoses**: Ketotetroses, Ketopentoses, Ketohexoses
- **Oligosaccharides**
  - Disaccharides, Trisaccharides, Tetrasaccharides
- **Polysaccharides**
  - Heteropolysaccharides, Homopolysaccharides

1. **Monosaccharides:** These are the simplest carbohydrate which generally contain 4-10 carbon atoms.that cannot be hydrolyzed into simpler compounds. They may be Aldoses and Ketoses whether they contain an aldehyde (CHO) group or Keto (C=O) group.

Aldotetroses: Erythreose, Threose

$$CHO\text{-}CHOH\text{-}CHOH\text{-}CH_2OH$$

Ketotetroses: Erythrulose

$$CH_2OH\text{-}\overset{\overset{O}{\|}}{C}\text{-}CHOH\text{-}CHOH\text{-}CH_2OH$$

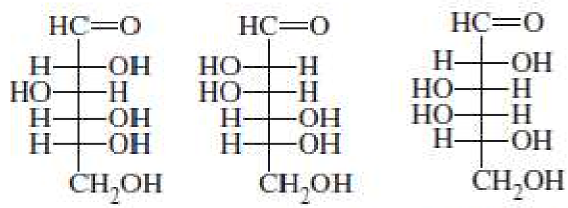
Aldopentoses: Arabinose, Xylose, Ribose

CHO-CHOH-CHOH-CHOH-CH2OH

Ketopentoses: Xylulose, Ribulose

Aldohexoses: Glucose, Mannose, Galactose

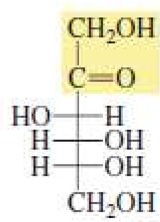
$$CHO\text{-}CHOH\text{-}CHOH\text{-}CHOH\text{-}CHOH\text{-}CH_2OH$$



D-glucose D-mannose D-galactose

Ketohexoses: Fructose, Sarbose

$$CH_2OH\text{-}\overset{\overset{O}{\|}}{C}\text{-}CHOH\text{-}CHOH\text{-}CHOH\text{-}CH_2OH$$



D-fructose

2. **Oligosaccharides:** (Oligo- few): These are the carbophydrates which can be hydrolysed into few monosaccharides.They are further classified into following classes.

   Disaccharides ($C_{12}H_{22}O_{11}$): Sucrose, Maltose, Lactose

   Trisaccharides ($C_{18}H_{32}O_{16}$): Raffinose

   Tetrasaccharides ($C_{24}H_{42}O_{21}$): Stachyose

3. **Polysaccharides:** These are the carbohydrates which can be hydrolysed into hundereds or even thousand monosaccharides.

   Homopolysaccharides: They contain only type of monosaccharides. Ex. Starch, Cellulose

   Hetropolysaccharides: They contain different type of monosaccharides, eg- Inulin

- On the basis of taste carbohydrates are further classified as:

  Sugar: All monosaccharides and disaccharides

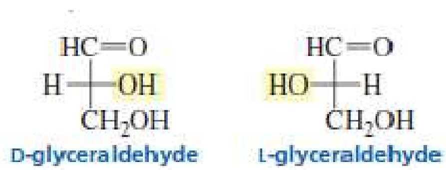
  Non sugar: Polysaccharides

- On thre basis of reducing property:

Reducing Sugar: All carbohydrates which reduce tollen reagent and fehling reagentare knows as reducing sugar. : All monosaccharide and most of the disaccharides except sucrose are reducing sugar. ex. Sucrose

Nonreducing sugar: carbohydrates which reduce tollen reagent and fehling reagentare knows as non reducing sugar.eg. Polysaccharides, sucrose.

D-Sugar: Carbohydrates which follow the configuration D-glyceraldehyde are known as D-sugar.



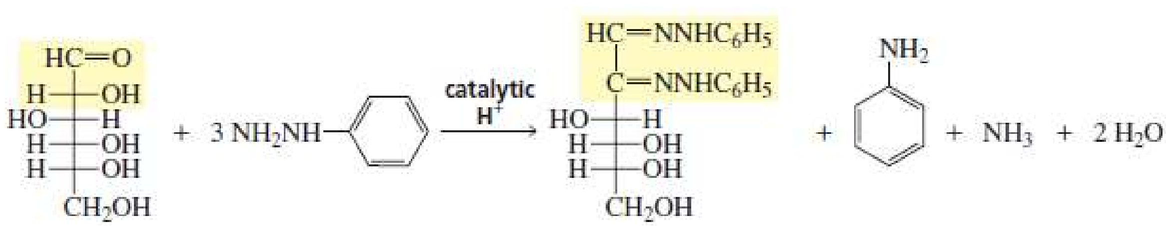
MONOSACCHARIDES

- Glucose (dextrose)
- Fructose (Laevulose, Fruit sugar)

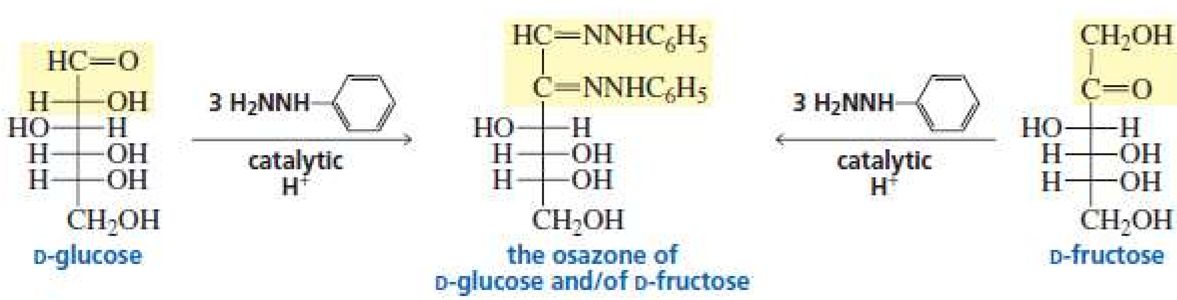
Reactions of monosaccharides:

Question: Write a note on glucosazone.

1. **Reaction with phenyl hydrazine (formation of glucosazone/osazone):** When glucose react with excess of phenylhydrazine it form glucosazone which contain two phenylhydrazine residues per molecule; a third molecule of the reagent is turned into aniline and ammonia. Osazone is **yellow coloured** crystalline solid which is insoluble in water. Formation of osazone is characterstic reaction of **α-hydroxyketone.**

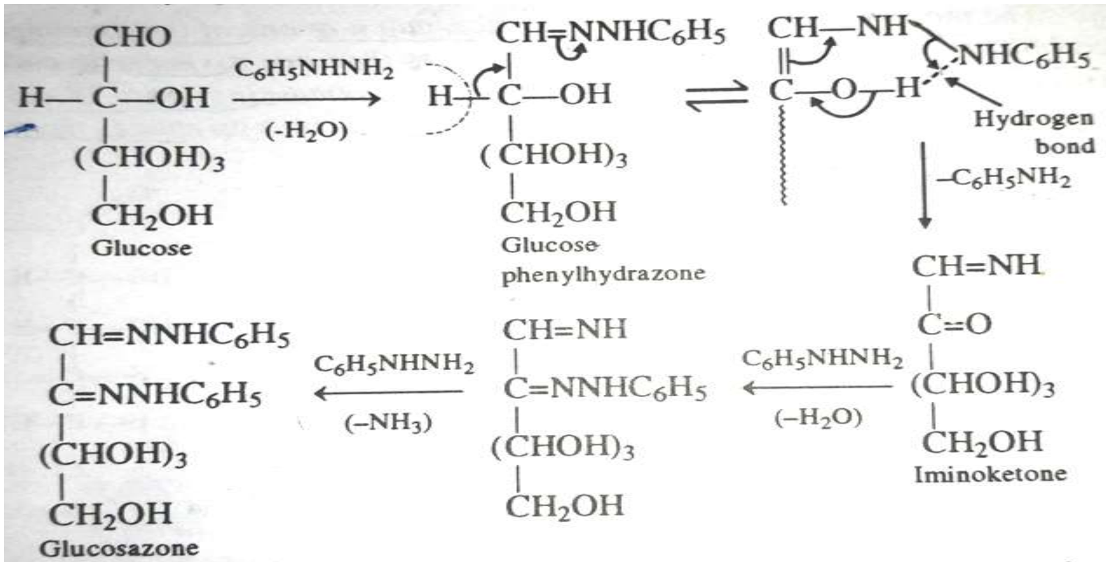


Glucose Phenylhydrazine Glucosazone



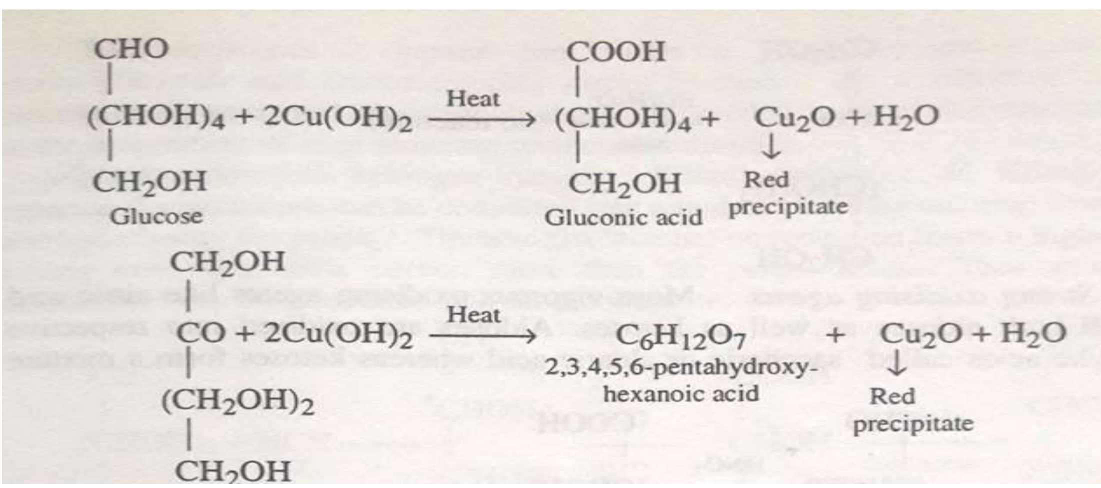
Fructose also form same Osazone

<u>Mechanism of formation of Glucosazone</u>



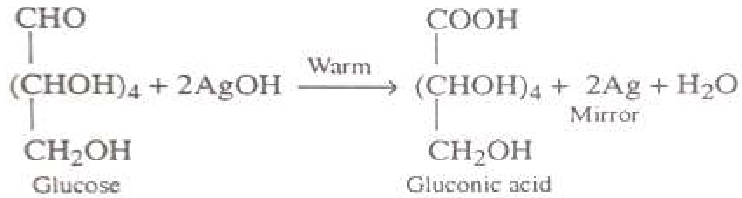
2. **Oxidation reaction:**

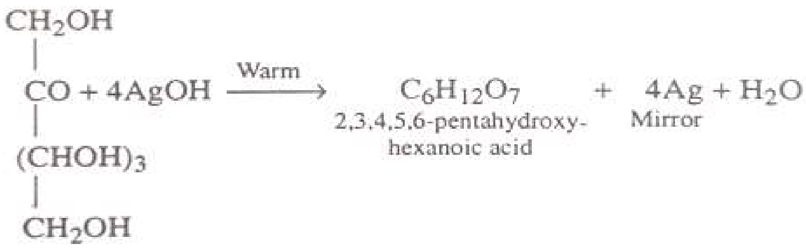
Fehling Test: When glucose is treated with mixture of Fehling reagent A and Fehling reagent B, they form brownish-red precipitate of of Cuprous oxide.



Fructose

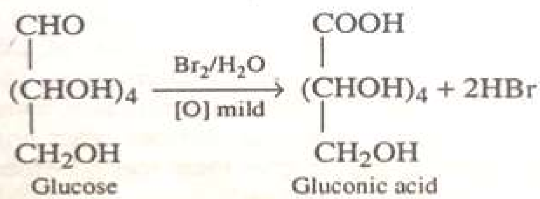
Tollen's test: When glucose and fructose are treated with tollen reagent (Ammonical solution of silver nitrate) they form Silver mirror on the inner wall of the test tube.





[Silver nitrate on treatment of ammonia gets converted into AgOH purpose of this reaction].

(c) Bromine water : A dilute solution of bromine in water functions a oxidising agent. Bromine attacks comparatively more labile aldehydic fun glucose and has no effect on keto group of fructose. Thus, glucose deco bromine water and fructose does not.



All aldoses can similarly be oxidised into respective aldonic acids. Ket not oxidised with bromine water.

<u>Structure elucidation of Glucose</u>

Structural elucidation is the process of determining the chemical structure of compound.Structue of glucose is determined under following heads.

A. Open chain structure of glucose
B. Stereochemistry
C. Cyclic structure
D. Determination of bring size
E. Representation of glucose

A. Open chain structure of glucose

1. **Molecular formula:** by elemental analysis and molecular weight the molecular formula of glucose was found to be $C_6H_{12}O_6$.

2. **Straight chain form:** Reduction of glucose in the presence of HI/P give n – hexane which indicate that glucose are arranged in the form of straight chain.

3. **Presence of carbonyl group:** Glucose reacts with hydroxylamine (NH_2OH) and form and form oxime of glucose. Hydroxylamine is carbonyl group reacting reagent which indicate that glucose contain one C=O group.

4. **Presence of aldehyde group:** Gentle oxidation of glucose with Br-water give gluconic acid which contain one COOH group which indicate that COOH gp must be obtained from oxidation of CHO. So carbonyl group (C=O) of glucose must be in the form of CHO.

5. **Presence of five hydroxyl group:** Glucose react with Acetic anhydride and form Gluco-pentaacetate. Which indicate glucose contain 5-OH group.

6. **Presence of primary alcohol (CH_2OH):** Strong oxidation of glucose with nitric acid (HNO_3) give glucaric acid, which contain two COOH group and second COOH group must be obtained from oxidation of primary alcohol (CH_2OH).

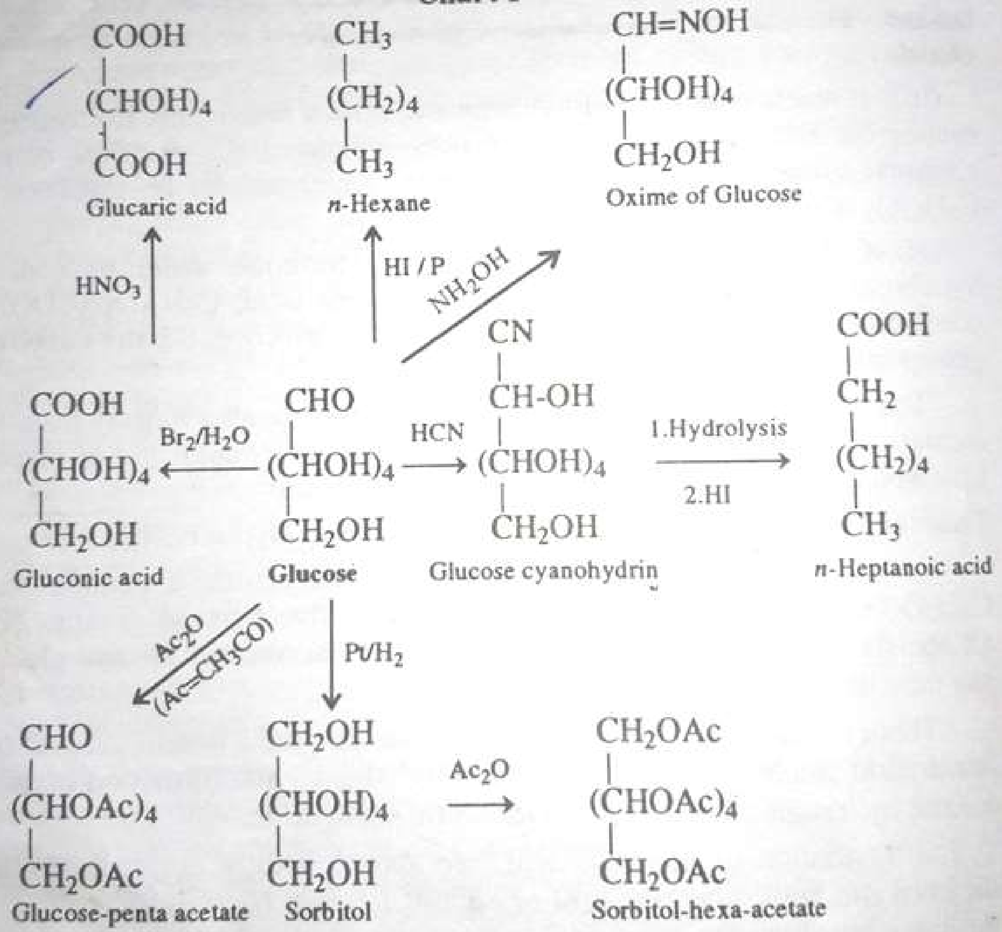
All these reaction indicate that in open chain form CHO group & CH_2OH must be present on terminal position hence glucose can be represented as structure I (Bayer's formula)

$$\overset{6}{HOCH_2}-\overset{5}{CHOH}-\overset{4}{CHOH}-\overset{3}{CHOH}-\overset{2}{CHOH}-\overset{1}{CHO}$$

I (Baeyer's formula)

This structure explains satisfactorily all the foregoing facts (see Chart I).

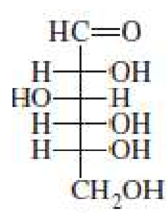
Chart I



This open-chain structure of glucose as an aldohexose is further confirmed by the fact that glucose consumes *five* moles of periodic acid forming formaldehyde (1 mole) and formic acid (5 moles).

$$C_6H_{12}O_6 + 5\ HIO_4 \longrightarrow CH_2O + 5\ HCOOH + 5\ HIO_3$$

Stereochemistry: in bayer's formula there are 4 asymmetric carbon atom, n=4, thus total no. of stereosomers $2^n = 2^4 = 16$, so there is total 16 possible stereoisomers (8 pair of enantiomers) are available for glucose. Oot of 16 structure of glucose, Fisher assign following formula for D-glucose:



D-glucose

B. **Cyclic structure of D glucose:** The open chain structure has following limitation-

1. Glucose does not give some characteristic reaction of glucose such as:

   a. Glucose + Shiff'reagent ⟶ No reaction

   b. Glucose + sodium bisulphite ⟶ No reaction

2. **Mutarotation:** D(+) glucose exist in two isomeric form α-D(+) glucose and β-D(+) glucose. The specific rotation of an aqueous solution of -D(+) glucose gradually decreases from +112° to +52.7° and specific rotation of β- D(+) glucose increases from +19° to +52.7° to

   $$\underset{[\alpha]_D=+112°}{\alpha\text{-D(+) glucose}} \rightleftharpoons \underset{[\alpha]_D=+52.7°}{\text{Equlibrium mixture}} \rightleftharpoons \underset{[\alpha]_D=+19°}{\beta\text{-D(+) glucose}}$$

   Such a change in value of specific rotation to give an equilibrium value is called mutarotation.

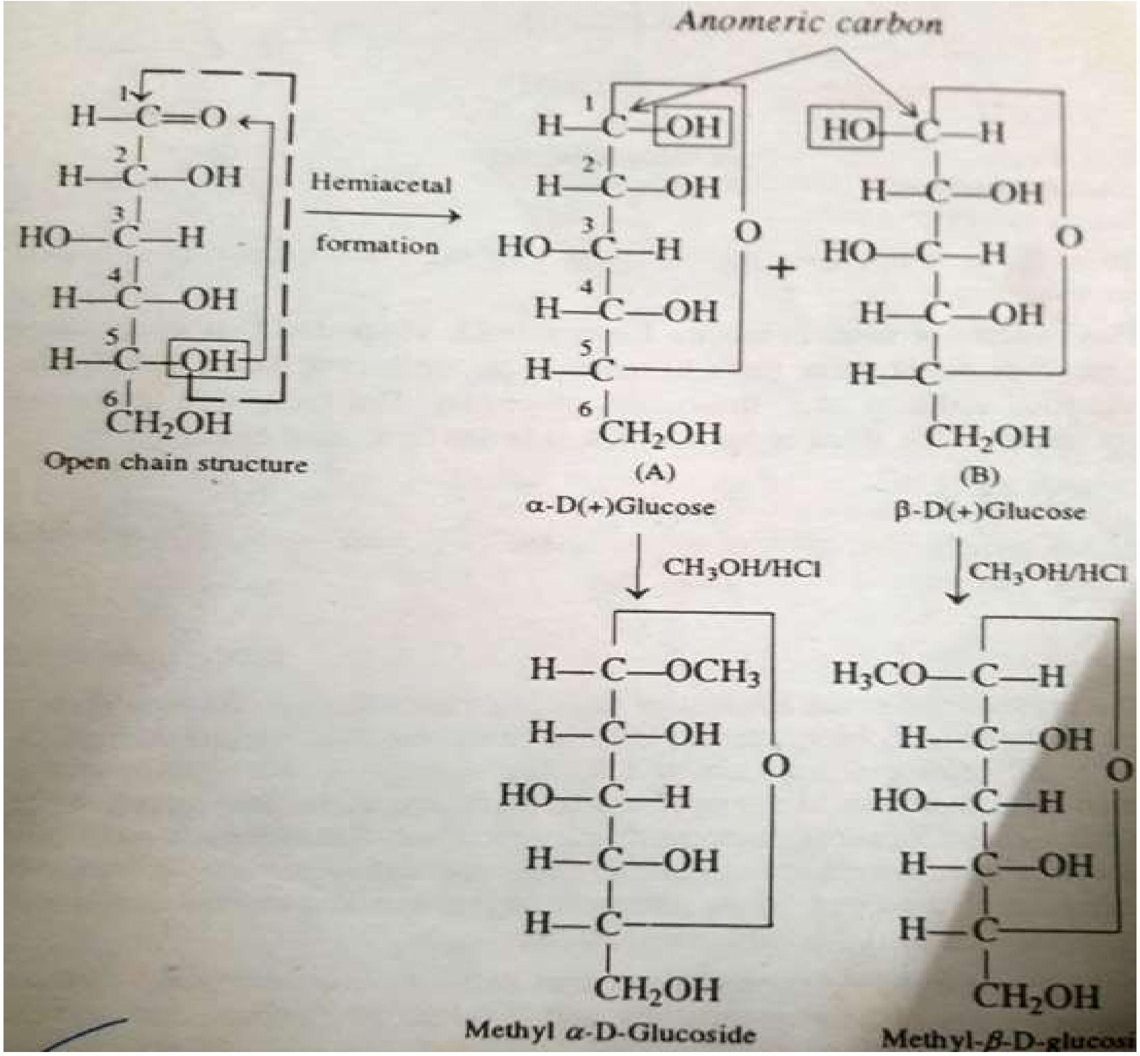
3. Treatment of glucose with methanol & dry HCl give methyl D-glucoside.

   Glucose + CH_3OH/HCl ⟶ Methyl α-D - glucoside + Methyl β-D- glucoside

   Methyl-D-glucoside is hemiacetal. To explain this, cyclic structure of glucose involves formation of an intramolecular hemiacetal reaction between CHO and OH of C-5.

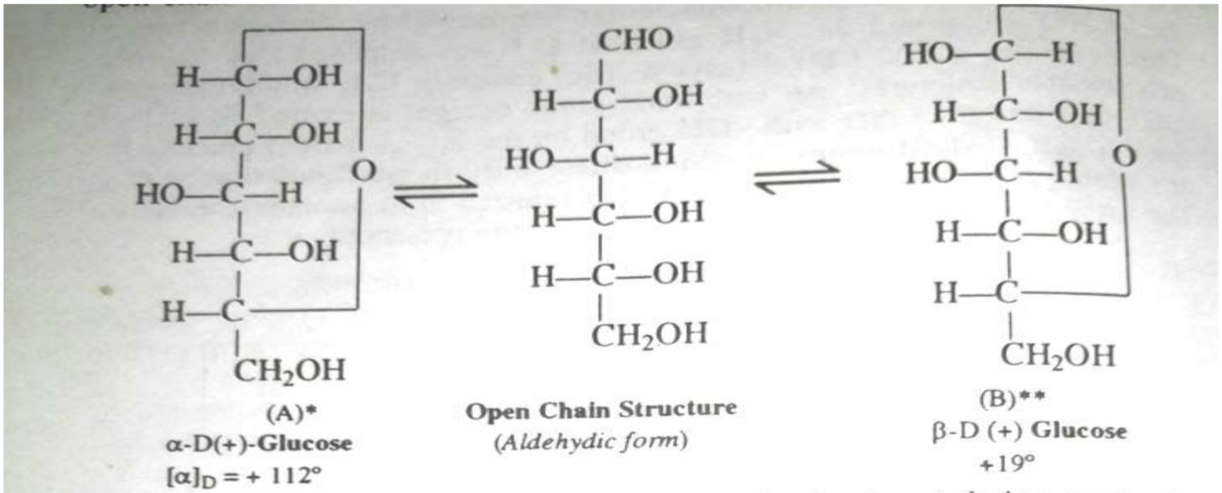
   So two structure of glucose is possible.

   Anomer: Glucose exist in two isomeric form one with OH group on left side and one with OH on right side of C-1 atom, such a pair of diastereomers which differ in configuration only at C-1 is reffered as Anomer and C1 carbon atom is known as anomeric carbon.



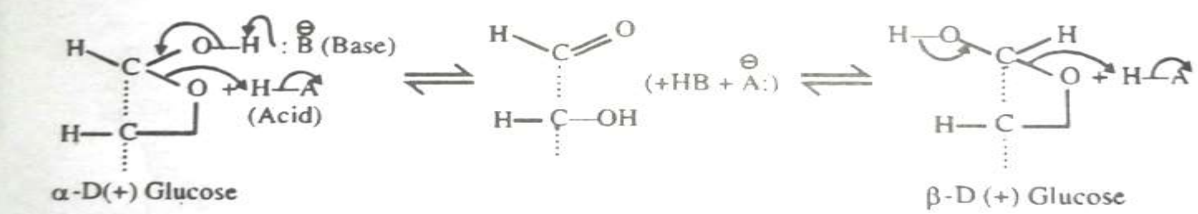
Anomeric carbon
H—C=O
H—C—OH
HO—C—H
H—C—OH
H—C—OH
CH₂OH
Open chain structure
Hemiacetal
formation
H—C—OH
H—C—OH
HO—C—H
H—C—OH
H—C
CH₂OH
(A)
α-D(+)Glucose
+
HO—C—H
H—C—OH
HO—C—H
H—C—OH
H—C
CH₂OH
(B)
β-D(+)Glucose
CH₃OH/HCl
CH₃OH/HCl
H—C—OCH₃
H—C—OH
HO—C—H
H—C—OH
H—C
CH₂OH
Methyl α-D-Glucoside
H₃CO—C—H
H—C—OH
HO—C—H
H—C—OH
H—C
CH₂OH

In **Mutarotation** α -D(+) glucose and β-D(+) glucose are converted through open chain form into equilibrium mixture.



Thus, *the easy opening and the closing of the hemiacetal ring accounts for mutarotation.*

Mechanism of mutarotation. Lowry 1925, suggested that since water is an amphiprotic solvent (acts as an acid as well as a base), if helps in simultaneous addition and elimination of proton. The following concerted mechanism showing third order kinetics is believed to take place.



D: Determination of ring size:

Strong oxidation of 2,3,4,6- tetra o- methyl D glucose give give trimethoxy glutaric acid and dimethoxy succinic acid which indicate that C-5 atom of glucose invole in ring formation.

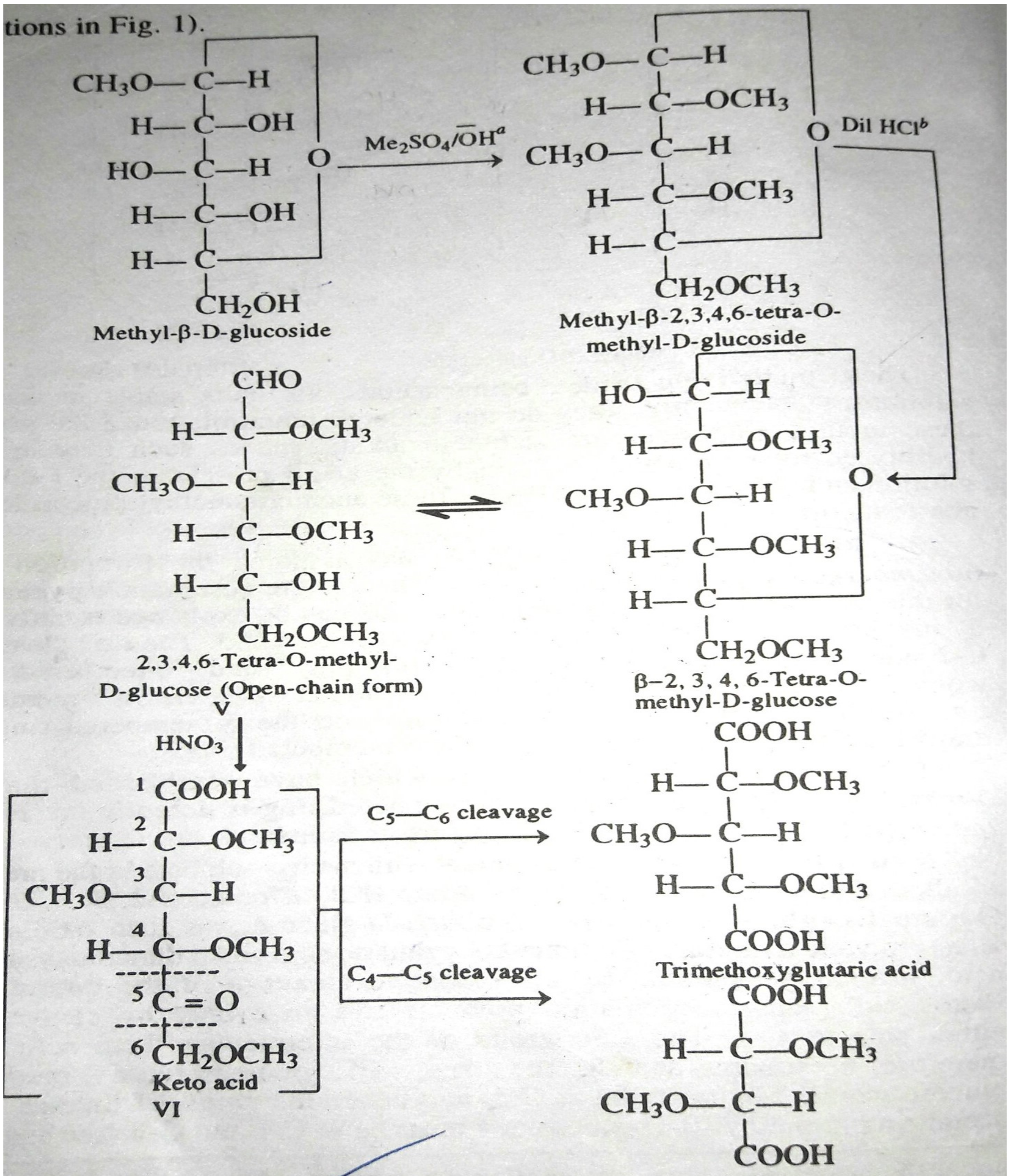


Fig 1. Ring size in Glucose

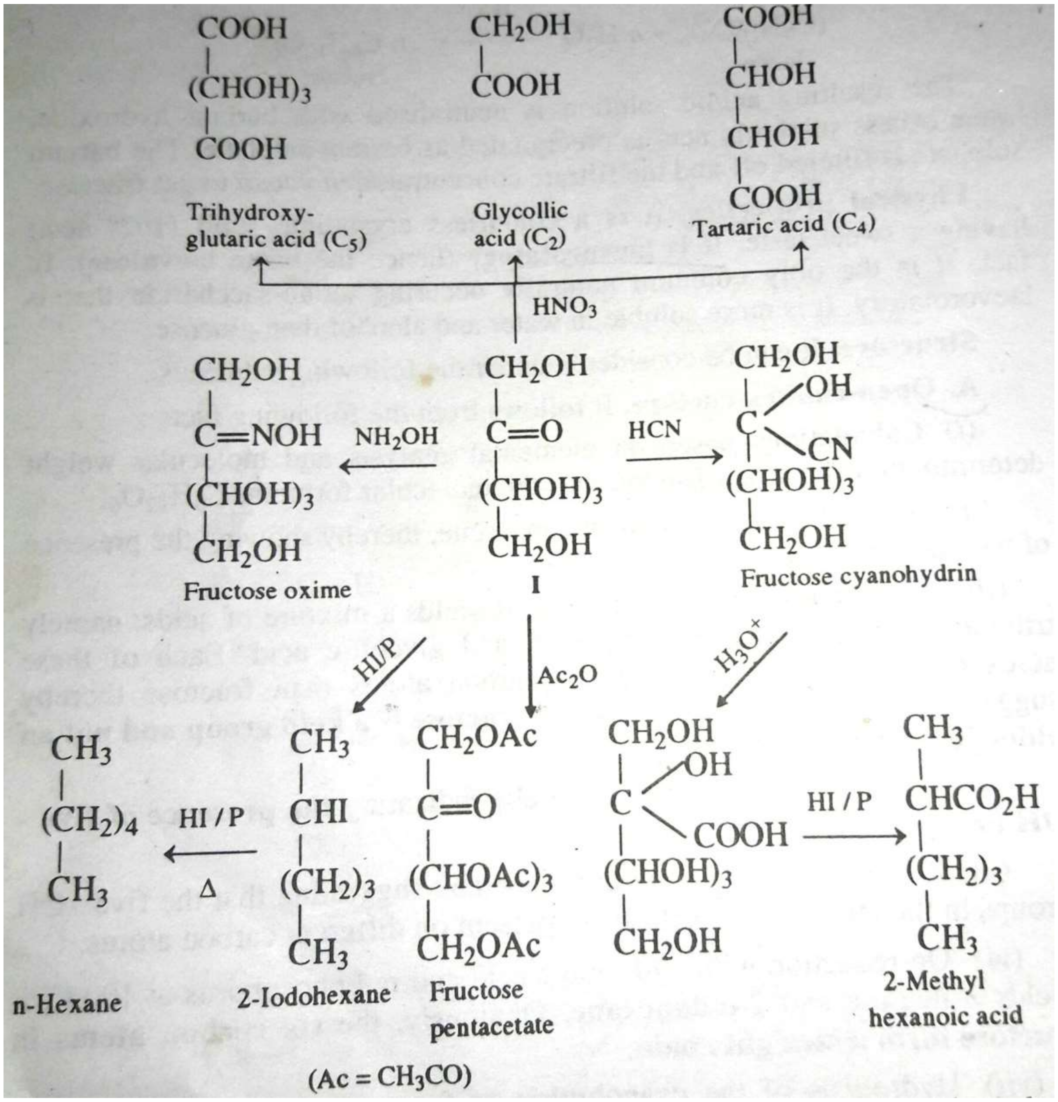
E: Representation of Glucose:

Fructose

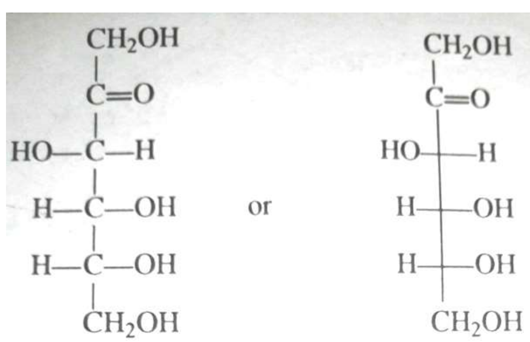
Open chain structure of Fructose

7. **Molecular formula:** by elemental analysis and molecular weight the molecular formula of Fructose was found to be $C_6H_{12}O_6$.
8. **Straight chain form:** Reduction of Fructose in the presence of HI/P give n – hexane which indicate that Fructose are arranged in the form of straight chain.
9. **Presence of carbonyl group:** Fructose reacts with hydroxylamine (NH_2OH) and form and form oxime of glucose. Hydroxylamine is carbonyl group reacting reagent which indicate that Fructose contain one C=O group.
10. **Presence of five hydroxyl group:** Fructose react with Acetic anhydride and form Gluco-pentaacetate. Which indicate glucose contain 5-OH group.
11. **Presence of Keto group:** Strong oxidation of Fructose with nitric acid (HNO_3) give trihydroxyglutaric acid, tartaric acid and glycolic acid, which shows that carbonyl group of fructose is keto group not aldehyde.

- All the reaction of fructose is shown in **chart II**

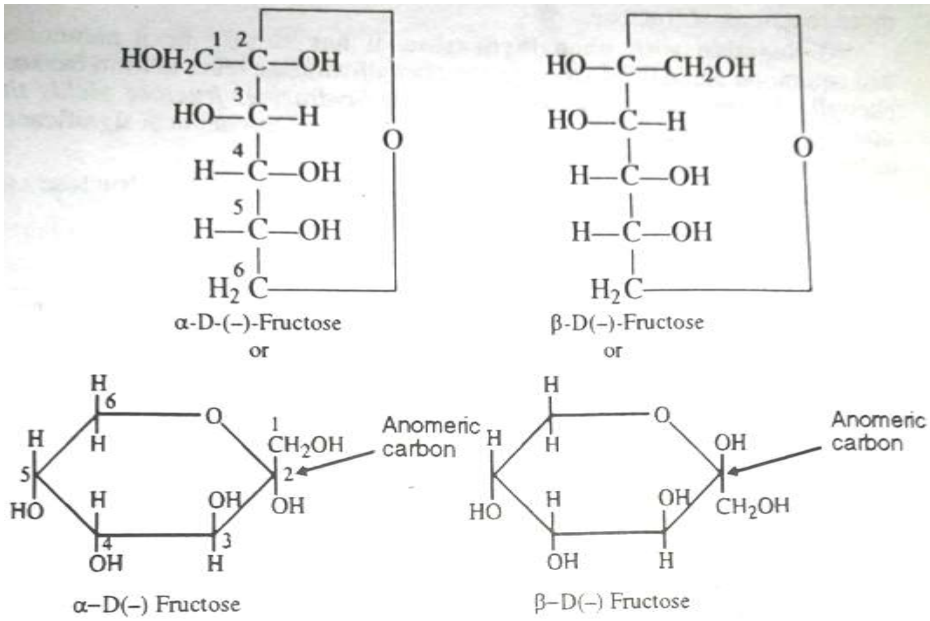


So open chain structure of fructose can be represented as:



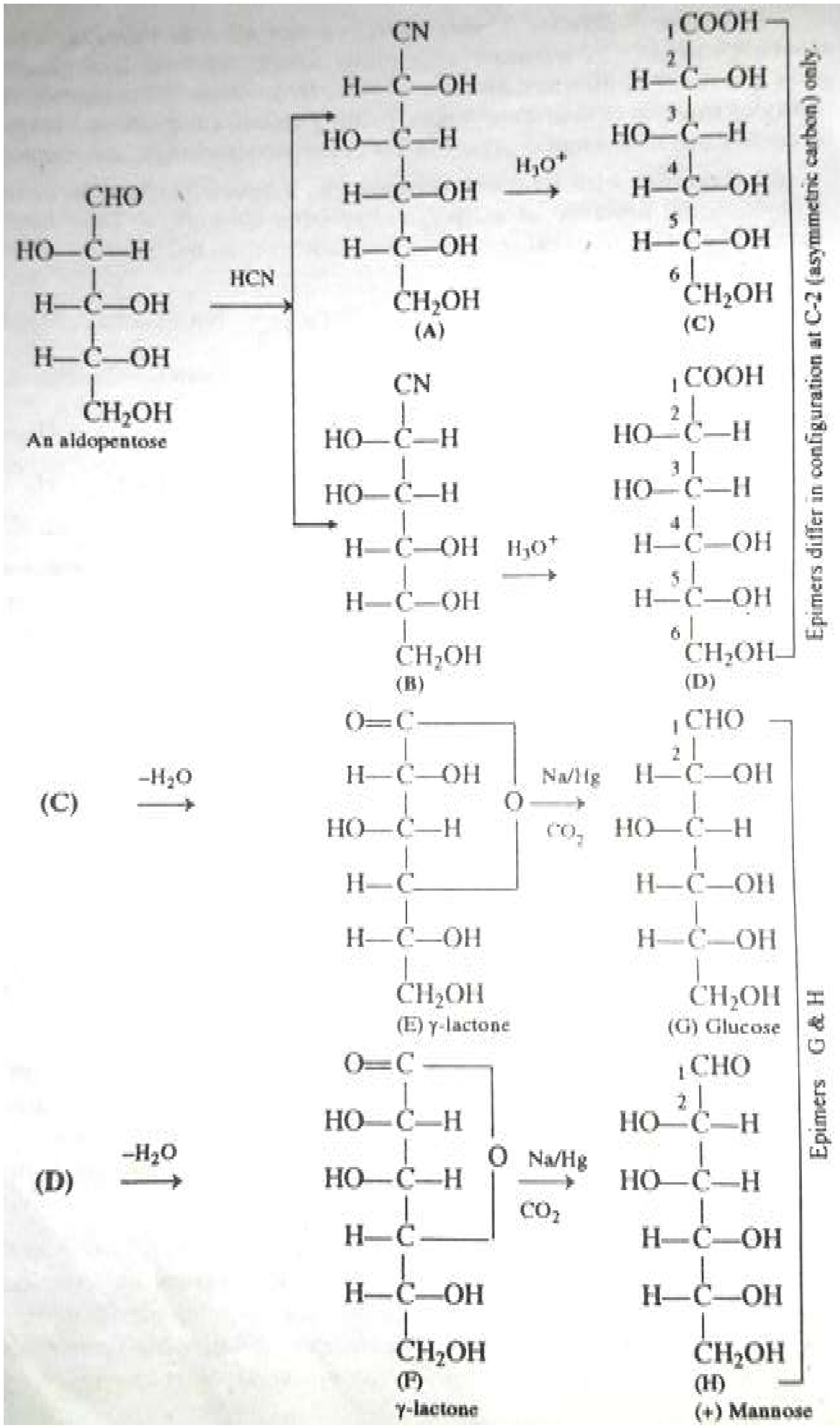
Cyclic structure of Fructose: Fructose undergo mutarotation like glucose fructose also exist as

Cyclic structure.



Question: Write a note on Ascending Series of aldoses

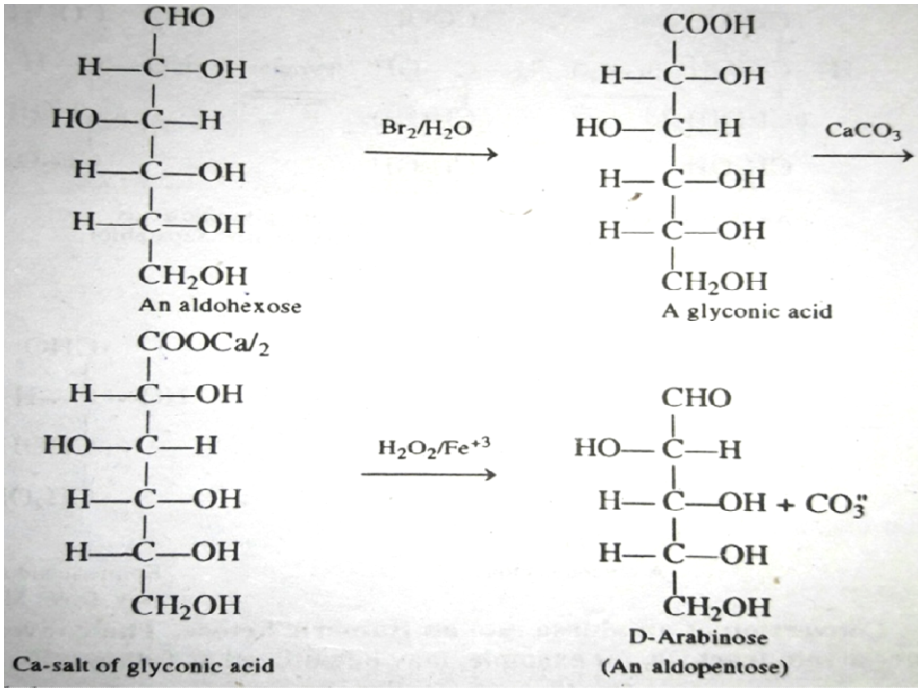
Killani Fisher synthesis ***(Ascending Series of aldoses):*** In this method aldopentose is converted into aldohexose. In this method a pair of diastereomeres are generated which are epimers.(epimers have different configuration at C-2 atom like glucose and mannose)



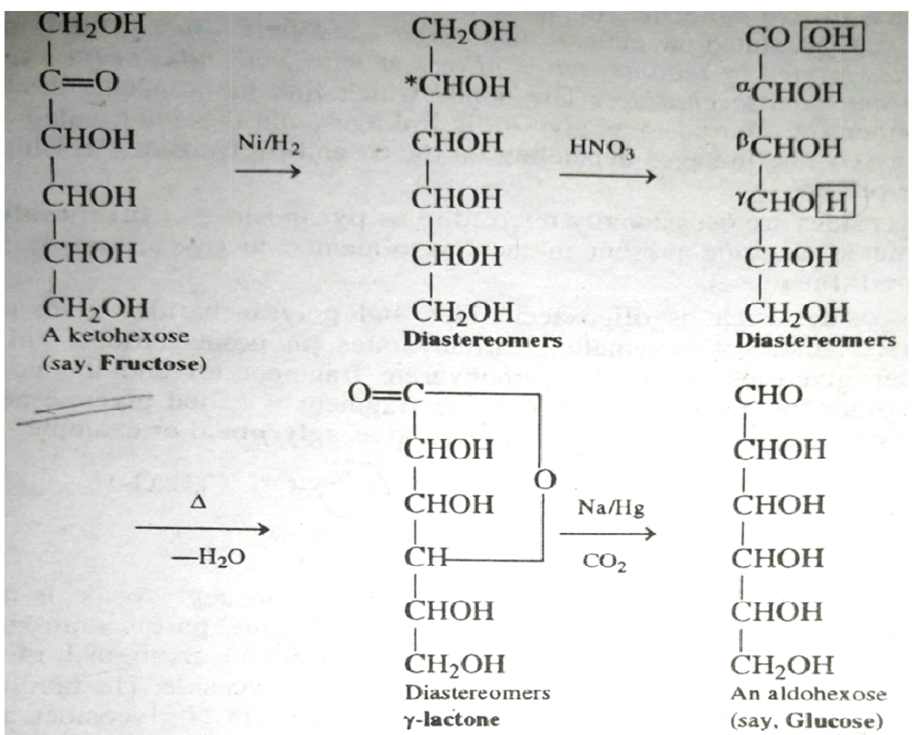
An aldopentose
HCN
H3O+
(A)
(B)
(C)
(D)
Epimers differ in configuration at C-2 (asymmetric carbon) only.
-H2O
Na/Hg
CO2
(E) γ-lactone
(F)
γ-lactone
(G) Glucose
(H)
(+) Mannose
Epimers G & H

Question: Write a note on **descending** Series of aldoses

Ruff degradation: ***(Descending Series of aldoses):*** In this method aldohexose is converted into aldopentose

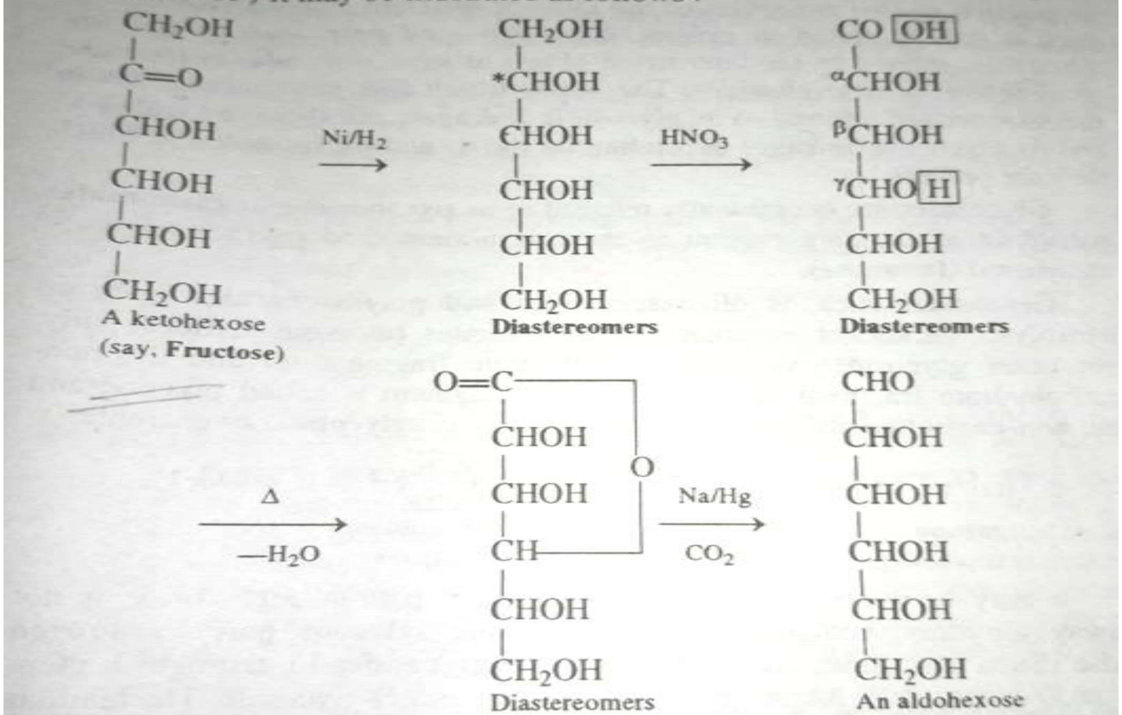


<u>**Conversion of Glucose (Aldose) into Fructose (Ketose):**</u>

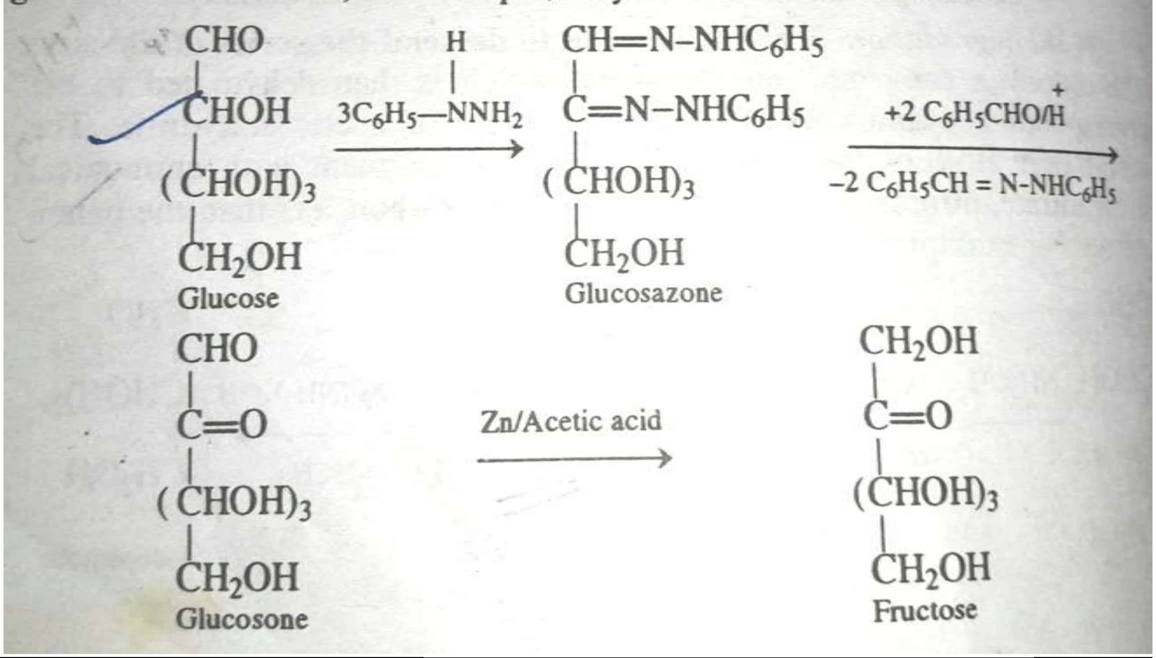


Conversion of Fructose (Ketose) into Glucose (Aldose):

Conversion of a ketose into an aldose. (say, conversion of fructose into glucose) It may be illustrated as follows :



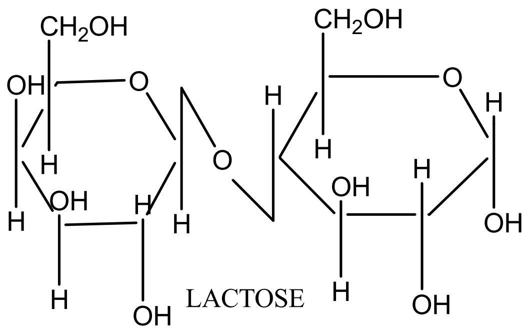
Conversion of an aldose into an isomeric ketose. The conversion of glucose into fructose, for example, may be outlined as follows :

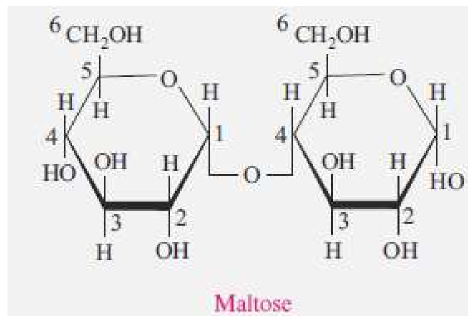


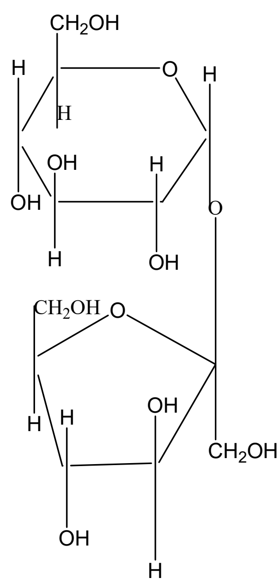
<u>**Disaccharides**</u>

- Disaccharides are the carbohydrates which are made up of two monosaccharide unit.
- After hydrolysis they give two molecule of monosaccharides.
- Disaccharides $\xrightarrow{H_2O}$ Monosaccharide. + Monosaccharide.
- Ex. Maltose $\xrightarrow[H^+/OH^-]{\text{Acidic hyrolysis}}$ Glucose + Glucose
- Both monosaccharide are linked with each other by glycosidic linkage.

Examples: Maltose , Lactose and Sucrose







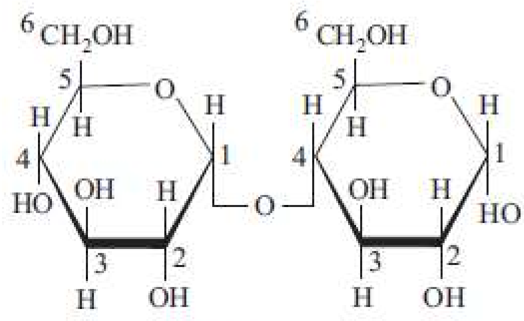
Sucrose

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

Structure:

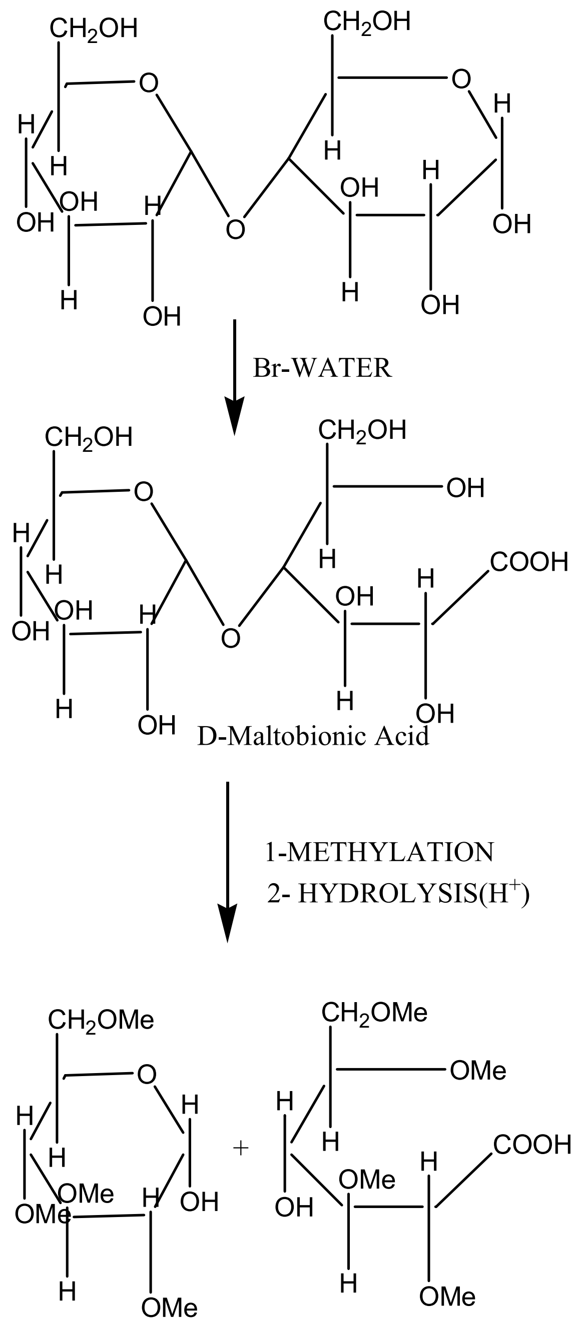
- By elemental analysis and molecular weight the molecular formula of Maltose was found to be $C_6H_{12}O_6$..
- Maltose exist in α-and β-form.
- Maltose undergo mutarotation

- Maltose is a reducing sugar
- Maltose reduce Tollen and fehling reagent.
- Maltose + Phenyl hydrazine ⟶ Matosazone
- Maltose + Br- water ⟶ Matobionic Acid
- After Acidic hydrolysis and enzymatic hydrolysis matose give two molecule of glucose
- Maltose $\xrightarrow[H^+/OH^-]{\text{Acidic hyrolysis}}$ Glucose + Glucose
- Maltose $\xrightarrow{\text{Enzymatic hyrolysis}}$ α-D(+) glucose + β-D(+) glucose. ‘



Maltose

- Since maltase enzyme only hydrolyse α-D(+) glucoses, it is conclded that maltose contain α-D(+) glucose. And maltose is disaccharide so both glucose units are joined by α-Glycosidic linkage.
- Maltose react with Br-water and form maltobionic acic, and and after methylation and hydrolysis it give 2,3,4,6- tetra- o- methyl glucose and 2,3,5,6- tetra –o- methyl glucose Which indicate that in maltose both glucose unit are joined by **C_1-C_4 - glycosidic linkage.**



2,3,4,6 tetra-o-methyl glucose

2,3,5,6 tetra-o-methyl gluconic acid

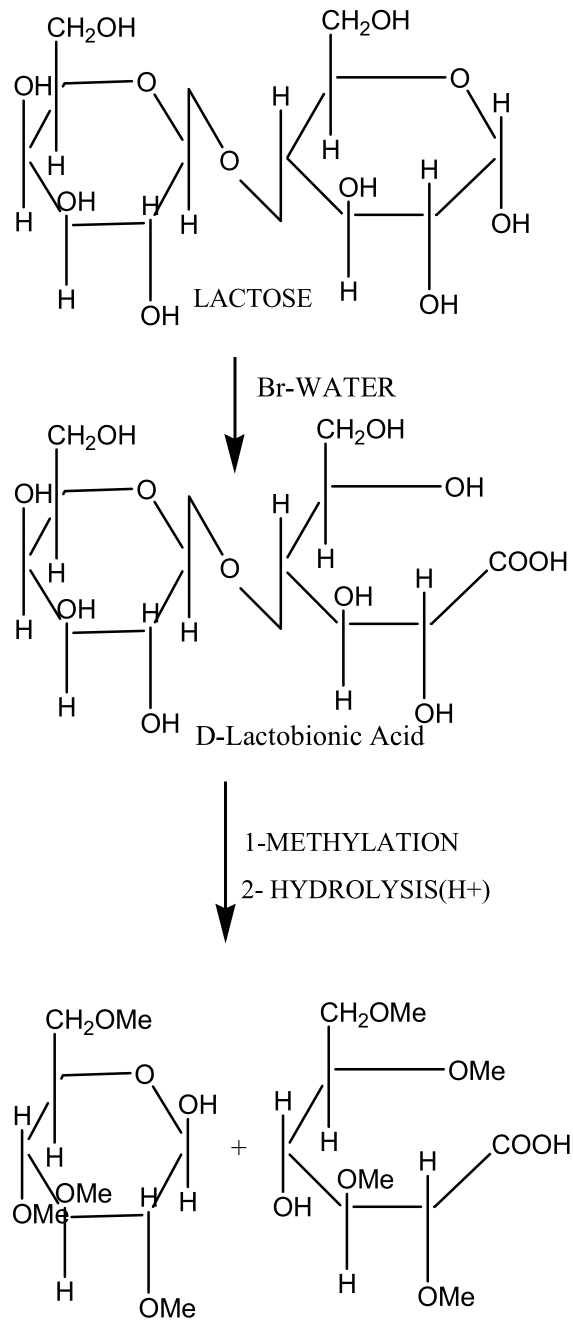
Lactose ($C_{12}H_{22}O_{11}$), Milk Sugar

Structure:

- By elemental analysis and molecular weight the molecular formula of Lactose was found to be $C_6H_{12}O_6$..
- Lactose exist in α-and β-form.
- Lactose undergo mutarotation
- Lactose is a reducing sugar

- Lactose reduce Tollen and fehling reagent.
- Lactose Phenyl hydrazine ⟶ lactosazone
- Lactose + Br- water ⟶ Lactotobionic Acid
- After Acidic hydrolysis and enzymatic hydrolysis Lactose give two molecule of glucose
- $$\text{Lactose} \xrightarrow[H^+/OH^-]{\text{Acidic hyrolysis}} \text{Glucose + Galactose}$$
- $$\text{Lactose} \xrightarrow[\text{Emulsin}]{\text{Enzymatic hyrolysis}} \alpha\text{-D(+) glucose} + \beta\text{-D(+) galactose}$$

Since Emulsin enzyme only hydrolyse β- glucosidic linkage so it is concluded that Lactose contain β-D(+) galactose. Lactose react with Br-water and form Lactobionic acic, and and after methylation and hydrolysis give 2,3,4,6- tetra- o- methyl glalactose and 2,3,5,6- tetra –o- methyl glucose acid. Which indicate that in maltose both glucose unit are joined by **C_1-C_4- glycosidic linkage.**



- 2,3,4,6 tetra-o-methyl- B-Galactose   2,3,5,6 tetra-o-methyl gluconic acid

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

Structure:

- By elemental analysis and molecular weight the molecular formula of Sucrose was found to be $C_6H_{12}O_6$..
- Sucrose does not exist in α-and β-form.
- Sucrose do not undergo mutarotation
- Sucrose is a **Non reducing** sugar

- Sucrose **do not reduce** Tollen and Fehling reagent.
- Sucrose +Phenyl hydrazine ⟶ no osazone formation

All these reaction indicate that sucrose **do not contain free C=O,** (carbonyl group)

- Inver sugar: Sucrose is dextrorotatory but is hydrolytic mixture is laevorotatory, due to this inversion of configuration sucrose is called invert sugar (honey)
- Sucrose ⟶ (+) Glucose + (-)Fructose

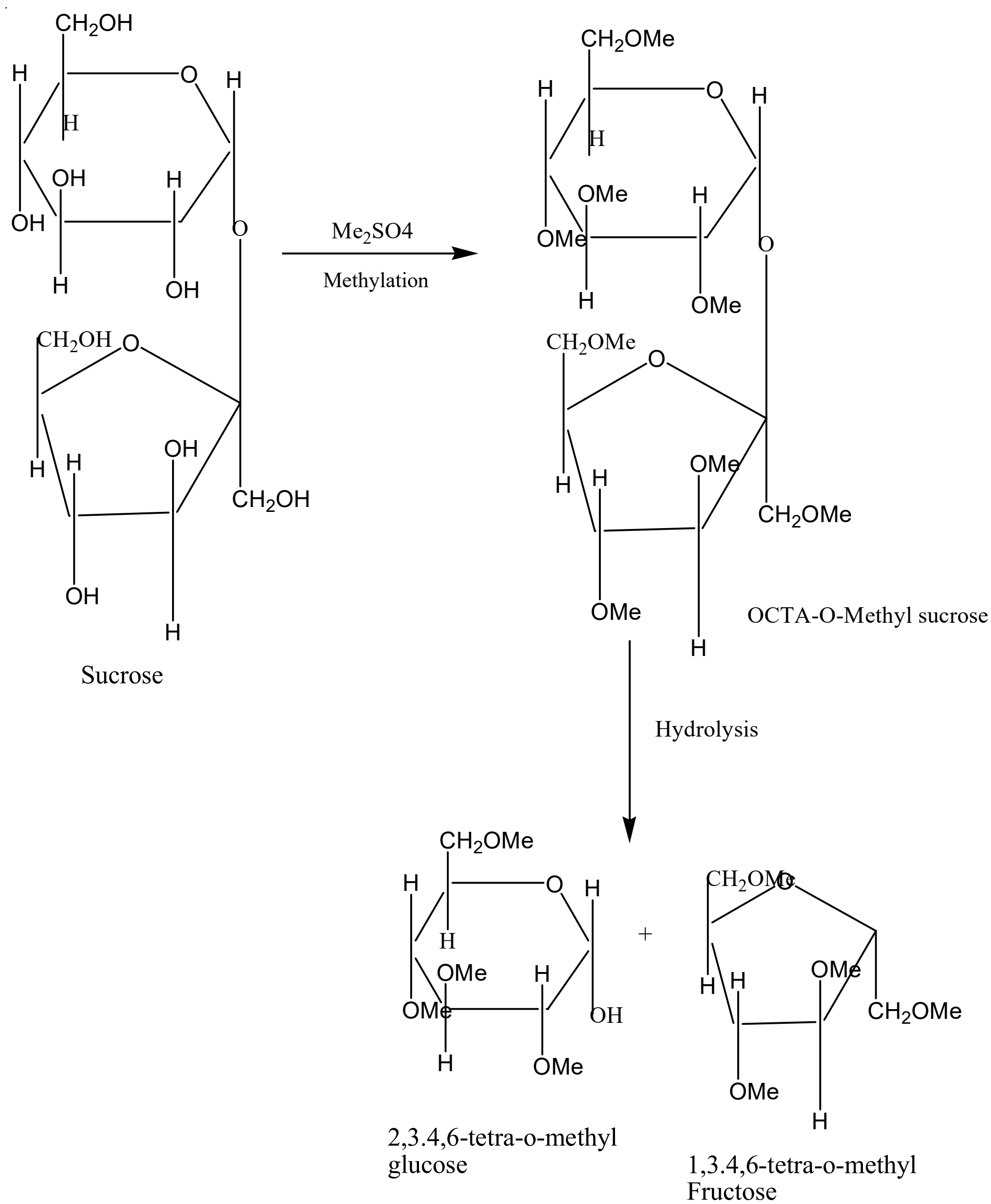
  +66.5º + 52.7º -94.4º
- After Acidic hydrolysis and enzymatic hydrolysis Sucrose give glucose and fructose.

  Sucrose $\xrightarrow{\text{Acidic hydrolysis}}$ Glucose + (-)Fructose

  Sucrose $\xrightarrow{\text{Enzymatic hydrolysis}}$ α-D (+) glucose +β-D (-) Fructose

Since Sucrase enzyme only hydrolyse β- glycosidic linkage so it is concluded that Sucrose contain β- -Glycosidic linkage.

Sucrose and after methylation and hydrolysis give 2,3,4,6- tetra- o- methyl glucose and 1,3,4,6- tetra –o- methyl Fructose. Which indicate that in msucrose both glucose and fructose unit are joined by **C_1-C_2- glycosidic linkage.**



Carbonyl group of sucrose is not free (involve in linkage) due to this reason sucrose –

- Sucrose do not reduce tollen and fehling reagent
- Does not react with phenyl hydrazine thus not form osazone.
- Does not undergo mutarotation.

Polysaccharides

Starch

- Molecular formula: $(C_6H_{10}O_5)n$
- Starch is high molecular wt compound
- Starch consist of two component :

(a) Water soluble (Amylose- 20%)

(b) Water insoluble (Amylopectin- 80%)

Hydrolysis of starch:

Starch $(C_6H_{10}O_5)n$ ⟶ Soluble starch $(C_6H_{10}O_5)n$ ⟶ Dextrin $(C_6H_{10}O_5)n$ ⟶ Maltose $(C_{12}H_{22}O_{11})$ ⟶ Glucose $(C_6H_{12}O_6)$ + Glucose $(C_6H_{12}O_6)$

1. Amylose:

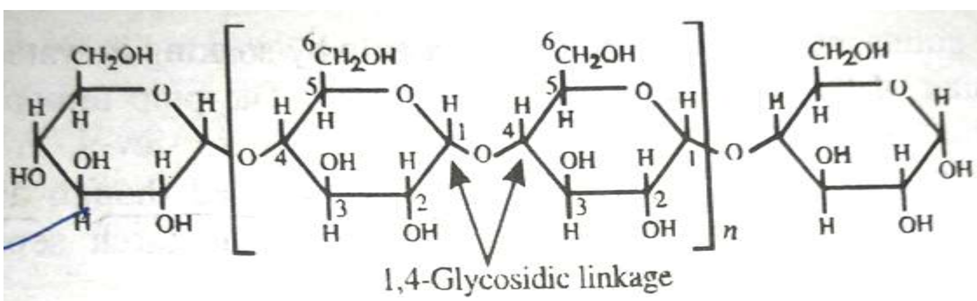
Amylose after hydrolysis gives maltose. And after hydrolysis give two molecule of glucose.

Amylose ⟶ Maltose ⟶ Glucose + Glucose

$(C_6H_{10}O_5)$ $(C_6H_{10}O_5)$

Amylose $\xrightarrow[\text{Hydrolysis}]{Me_2SO_4}$ 2,3,6- Tri- o- methyl glucose + 2,3,4,6- Tetra- o- methyl glucose

So formation these degradation product indicate that maltose contain 1- 4 glycosidic linkage and structure of amylose can be represented as follows:



Structure of amylose (*Haworth projection formula*)

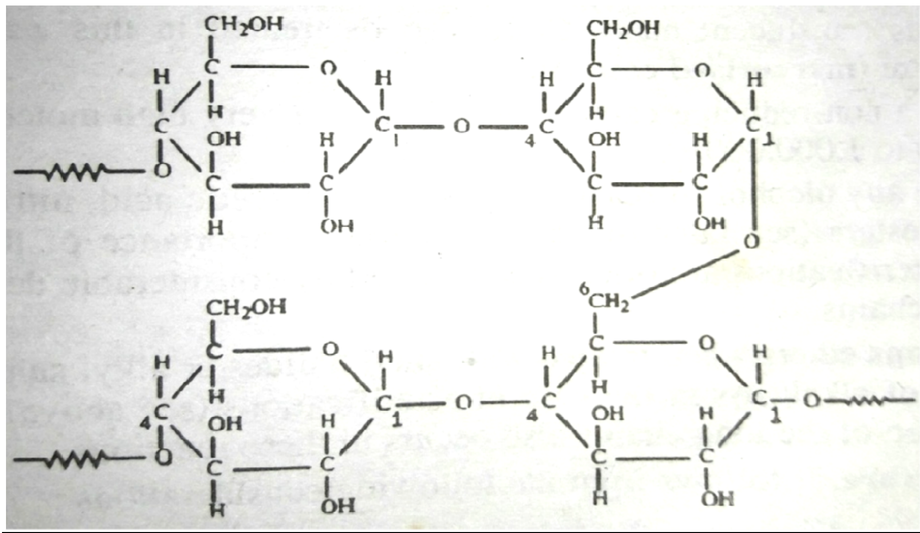
Amylose contain 100 glucose unit which are joined together by 1,4 glycosidic linkage and amylose is present in straight chain form.

Amylopectin:

Amylopectin ⟶ Maltose ⟶ Glucose + Glucose

Amylose $\xrightarrow[\text{Hydrolysis}]{Me_2SO_4}$ 2,3,6- Tri- O- methyl glucose (90%) + 2,3,4,6- Tetra- O- methyl glucose (5%) + 2,3- Di- O- methyl glucose (5%)

So formation these degradation product indicate that maltose contain 1- 6 glycosidic linkage and structure of amylopectin can be represented as follows



Amylopectin

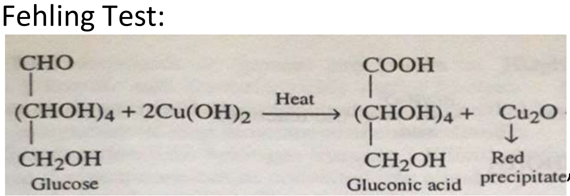
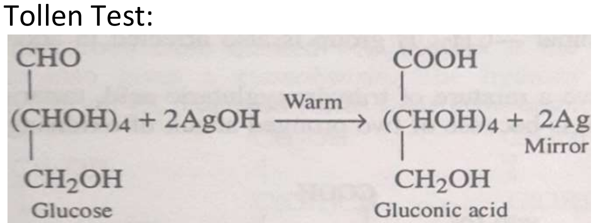
- Amylopectin contain 1000 glucose unit.
- Amylopectin is highly branched structure and consist of several short chin of about 20 glucose unit each one end of these chain are joined to C1 to a c6 of next chain.

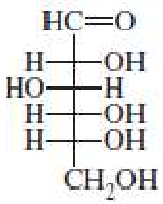
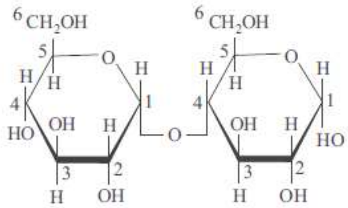
Identification test of Carbohydrates

S.No.	Name of test	Procedure	Observation
1	Molisch's Test	Take 2 ml of the test solution of carbohydrate in a test tube and 2 drops of α-naphthol solution (Molish reagent). Incline the tube and dropwise add approx 1 ml of conc. H_2SO_4 along the sides of the tube. Do not mix the content.	Formation of violet colour at the junction of the two liquids indicates the presence of carbohydrates.
2	Fehling's Test	Take 2 ml of the test solution of carbohydrate in a test tube and add equal volumes of Fehling A & Fehling B (1:1) reagent and boil the solution on boiling water bath, mix the content.	Formation of brownish-red precipitate of cuprous oxide indicate the presence of reducing sugar
3	Tollen's test	Take 2 ml of the test solution of carbohydrate in a test tube and add Tollen' s reagent boil the solution on boiling water bath	Formation of silver mirror on the inner wall of test tube indicate the presence of reducing sugar

4	Benedict's Test	Take 2 ml of Benedict's reagent in a test tube, add 5-6 drops of the test solution of carbohydrate and mix well. Place the test tube in a boiling water bath for 5 minutes	Formation of red brown precipitate indicate the presence of carbohydrated
5	Barfoed's Test	Take 2 ml of the test solution of carbohydrate in a test tube add about 2-3 mL of Barfoed's reagent. Mix it well and boil it for one minute in the water bath and allow to stand for a few minutes.	Formation of a red colour of cuprous oxide in 5 minute indicate the presence of monosaccharides and in 17 to 12 minute indicate the presence of disaccharides.
6	Iodine Test	Take 2 ml of the test solution of carbohydrate in a test tube and add 2 drops of iodine solution	Formation of blue-black colour indicate the presence of polysaccharides (starch)
7	Sulphuric acid test	Take 2 ml of the test solution of carbohydrate in a test tube and add 5 drops of sulphuric acid heat the solution	Formation of black colour indicates the presence of carbohydrates.

Difference between reducing and non reducing sugar

SNo.	**Reducing Sugar**	**Non Reducing Sugar**
1.	All carbohydrates which have capability to oxidize other reagent but get itself reduce are known as reducing sugar	All carbohydrates which have no capability to oxidize other reagent are known as non reducing sugar
2.	Reducing sugar contain free CHO or C=O group	Non reducing sugar do not contain free CHO or C=O group
3.	All carbohydrates which reduce tollen reagent, benedict reagent and fehling reagent, are knows as reducing sugar. Fehling Test:  Tollen Test:  	Carbohydrates which do not reduce tollen reagent, benedict reagent and fehling reagent are knows as non reducing sugar.

4.	Ex. All monosaccharide and most of the disaccharides except sucrose are reducing sugar	Ex. Disaccharide such as Sucrose and Polysaccharides
5.	Ex. Glucose, Fructose, Maltose, Lactose  D-glucose , Maltose	Ex. Sucrose