

Carbohydrates

US04CCHE21

For S. Y. B. Sc.

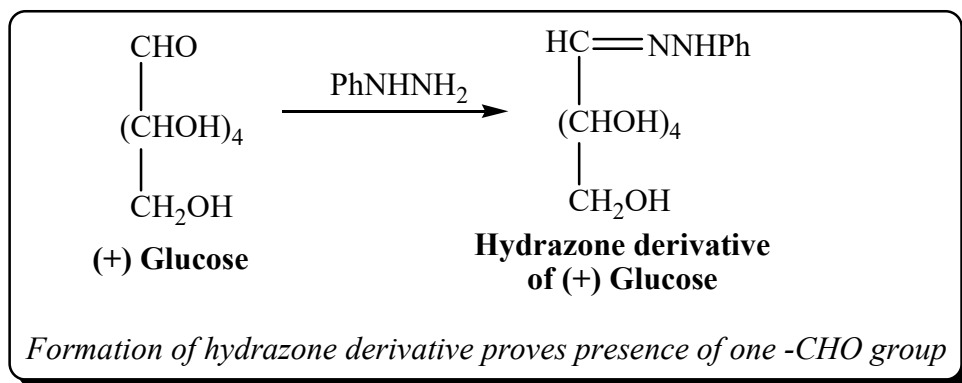
By: Dr Vipul Kataria

❖ Introduction

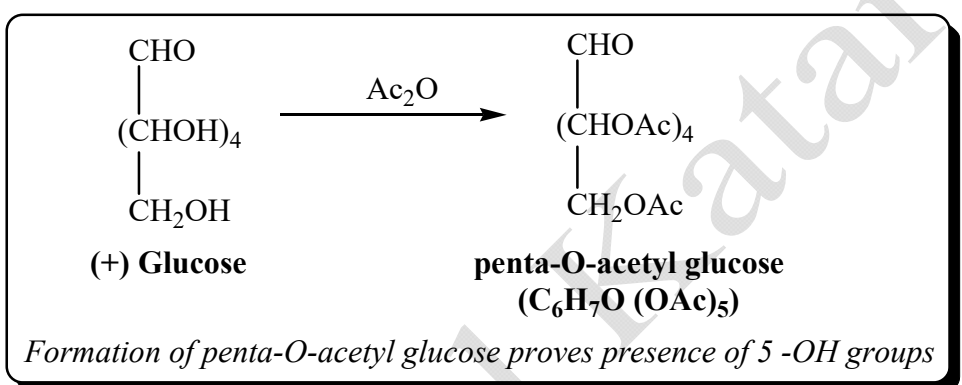
- **Carbohydrates are polyhydroxy aldehydes, polyhydroxy ketones or compounds that can be hydrolysed to them.**
- Carbohydrates are vital compounds distributed in nature at great extent, those are important for living.
- A carbohydrate that **can't be hydrolysed** to simpler compound is known as **monosaccharide**.
- A carbohydrate that can be **hydrolysed to two monosaccharide molecules** is known as **disaccharide**.
- A carbohydrate that can be **hydrolysed to many monosaccharide molecules** is known as **polysaccharide**.
- If monosaccharide contains **–CHO (aldehyde) group** then it is referred as **aldose**.
- If monosaccharide contains **–CO (ketone) group** then it is referred as **ketose**.
- Monosaccharide can be named according to number of carbons.
 - 3 carbons – Triose
 - 4 carbons – Tetrose
 - 5 carbons – Pentose
 - 6 carbons – Hexose
- Carbohydrates that reduce **Fehling's (or Benedicts's) or Tollen's reagent** are known as **reducing sugars**. All the monosaccharides (whether aldose or ketose) are reducing sugars. Most of the disaccharides except **Sucrose** (sucrose is also known as table sugar) are reducing sugars.

❖ Prove structure of Glucose or Evidences for Glucose structure

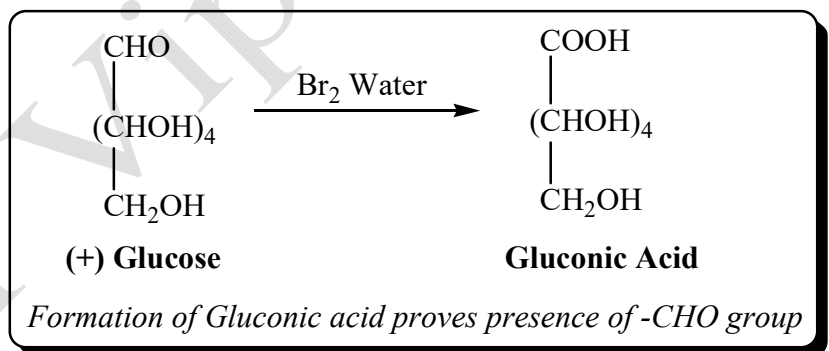
- (+) Glucose has molecular formula $C_6H_{12}O_6$ and empirical formula CH_2O that has been proved by **elemental analysis**.
- (+) Glucose reacts with **phenyl hydrazine** and produce **hydrazone derivative** that proves presence of **one –CHO group**.



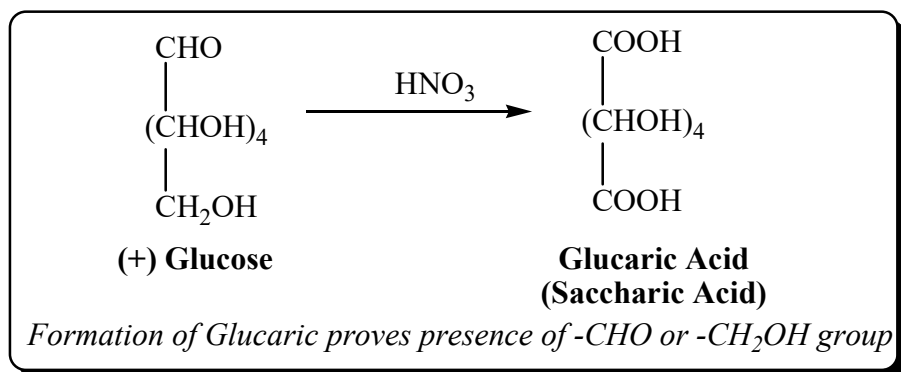
- (+) Glucose reacts with **Ac₂O (Acetic Anhydride)** to give **penta-O-acetyl glucose** that confirms presence of **5 -OH groups**.



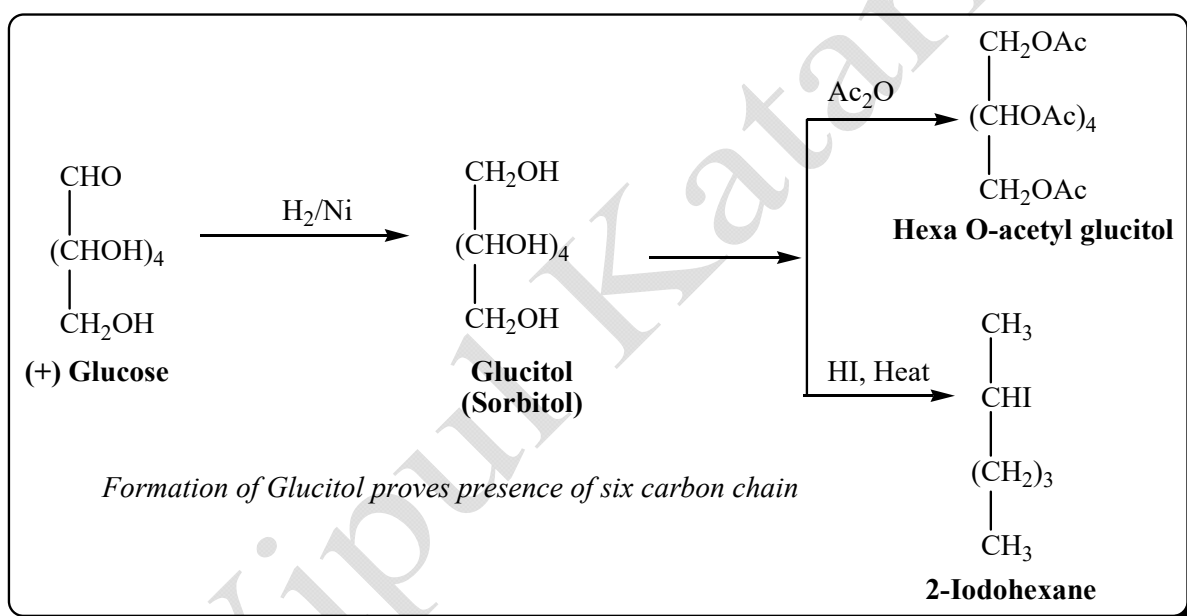
- (+) Glucose reacts with **Br₂ water** to give **Gluconic acid** that proves presence of – **CHO group**.



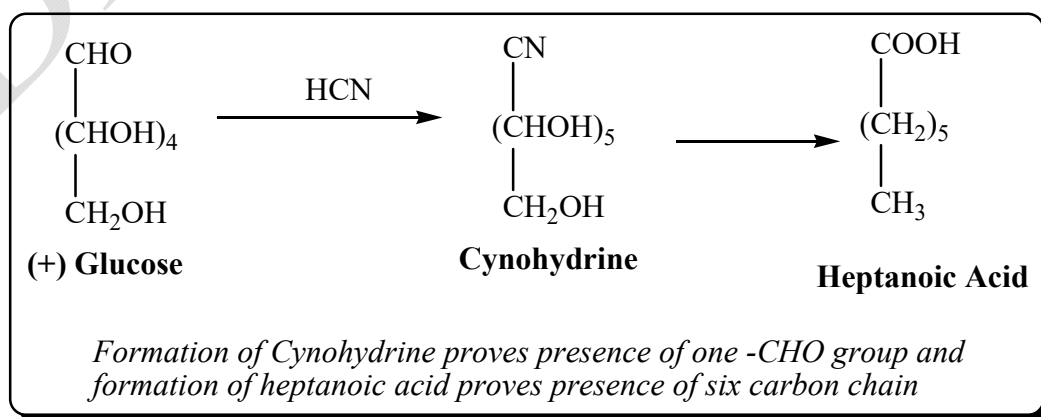
- (+) Glucose reacts with **HNO₃ (nitric acid)**. Nitric acid is strong acid that works as strong oxidizing agent. It oxidise (+) Glucose to **dicarboxylic acid** known as **Glucaric acid (Saccharic acid)**. It oxidise –CHO group as well as CH₂OH group.



- (+) Glucose reacts with **H₂/Ni (Raney Nickle)** to give **Glucitol** that proves presence of **six carbon chain**.

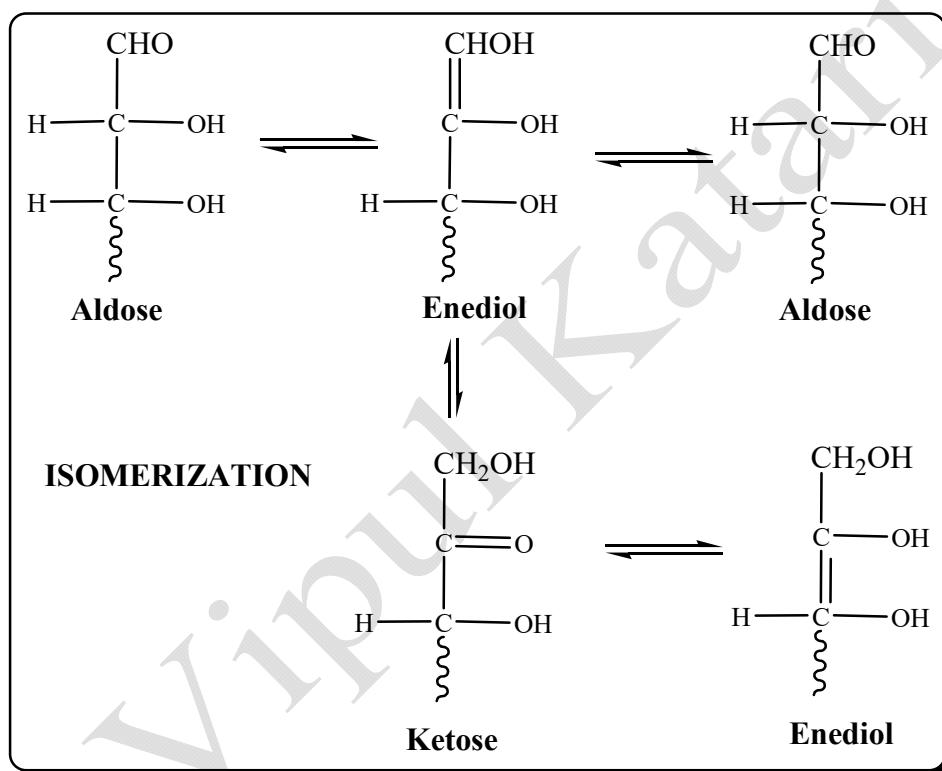


- (+) Glucose reacts with **HCN** to give **cynohydrine** that proves presence of **-CHO group** which on further hydrolysis gives **heptanoic acid** that proves presence of **six carbon chain**.



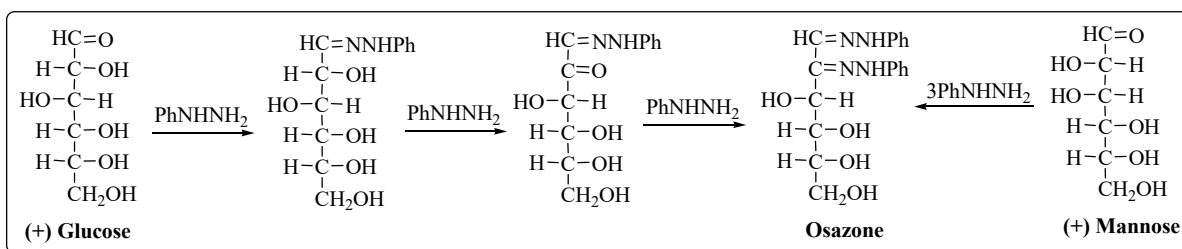
❖ Oxidation of Aldose

- Aldoses can be hydrolysed in four important ways, (1) **By Fehling or Tollen's reagent**, (2) **By Bromine water** (3) **By Nitric Acid** (4) **By Per-iodic acid**.
- **Oxidation by Fehling or Tollen's Reagent:** **Fehling** (Fehling A is aqueous solution of copper(II) sulphate, Fehling B is aqueous potassium sodium tartrate) and **Tollen's reagent** (Ammonical Silver Nitrate) both are alkaline reagent and treatment of them with sugars lead to **isomerization or decomposition of the carbon chain**, an equilibrium established between monosaccharide and an enediol structure.



- **Oxidation by Bromine water and nitric acid:** Bromine water oxidizes **aldoses**, but not **ketose**. It is useful to **differentiate** between aldose and ketose. Aldose on oxidation with bromine water produces **Gluconic acid (monocarboxylic acid)**. Glucose upon oxidation with nitric acid produces **Glucaric acid (dicarboxylic acid)**, $-\text{CHO}$ and $-\text{CH}_2\text{OH}$ both group oxidize to $-\text{COOH}$.

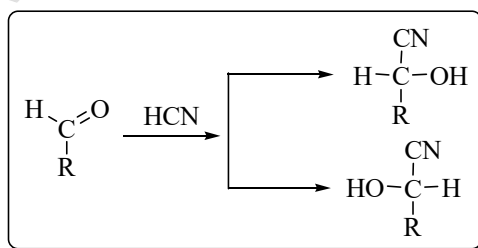
- In 1884, Fischer synthesized **osazone** starting from **glucose and mannose** and found some interesting observations.



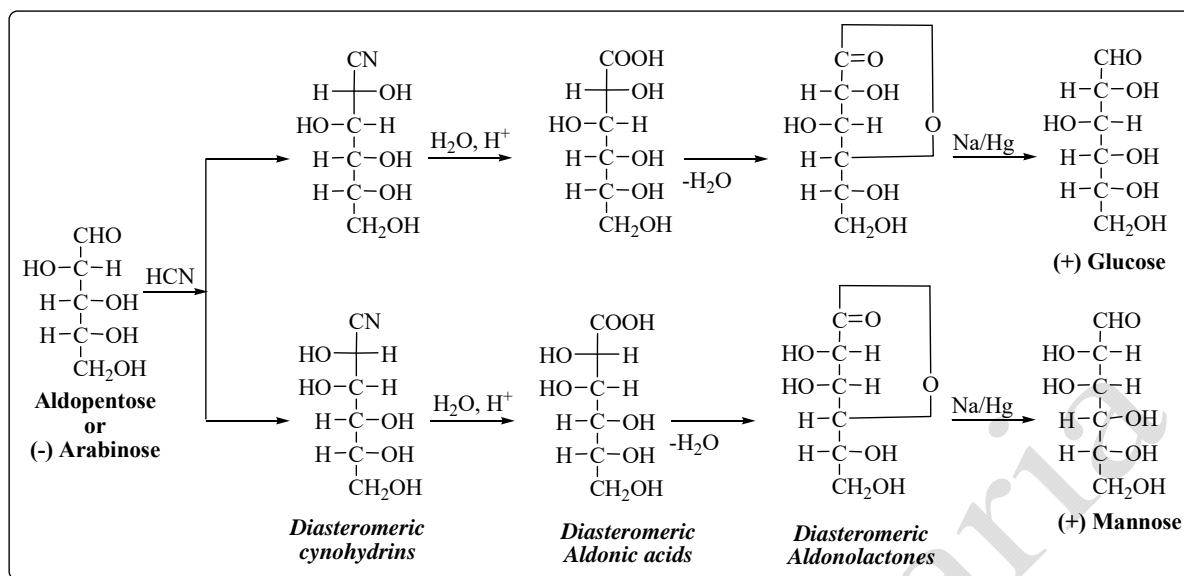
- The osazone formation destroyed configuration at **C-2 in aldose**.
- It does not affect other carbon atoms **except C-1 and C-2**.
- The **glucose and mannose** give same **osazone** hence we can say that glucose and mannose have **different configuration at C-2 carbon** and other structure is same.
- **Pair of diastereomers of aldoses that differ only in configuration at C-2 are called epimers and the process of converting one epimer to another is called epimerization.**

❖ Lengthening of carbon chain of Aldose (Kiliani-Fischer Synthesis)

- The lengthening of carbon chain is **not only useful to synthesize aldoses** but also useful for **determining their configuration**.
- Killiani-Fischer synthesis is one of the **most useful methods** for conversion of an aldose into another **aldose having one more carbon atom**.
- Let us understand what happens when a cyano group (-CN) is added to aldehyde.



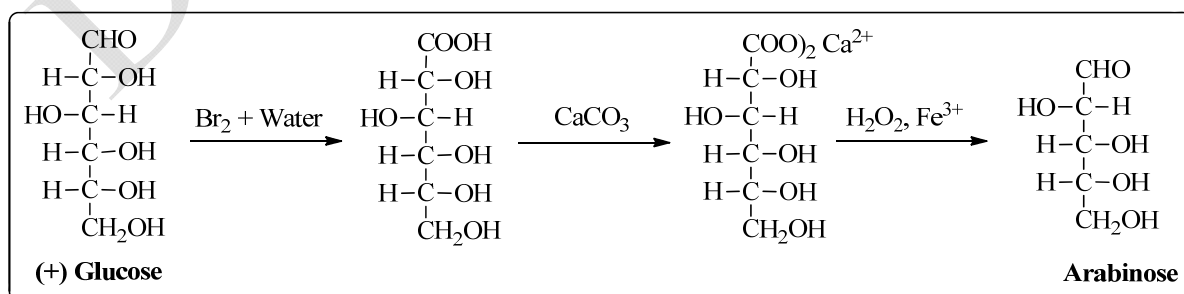
- When **HCN** is added to aldehyde it produces a **pair of cynohydrine** (enantiomers).
- **Hydrolysis of cynohydrin** produces corresponding **carboxylic acid**.
- The **-COOH and -OH** group present in the molecule tend to produce **γ-lactone**.
- The γ-lactone upon **reduction** with **Na/Hg** produces aldose having one more carbon atom than starting material.
- Now let us see the complete Killiani-Fischer reaction.



- The starting material is **aldopentose** or **(+) arabinose** which upon reaction with **HCN** gives **diastereomeric cynohydrin** which upon **hydrolysis** produces **aldonic acids** which **dehydrated** to **aldonolactone** that upon reaction **Na/Hg** gives **(+) Glucose** and **(+) Mannose**.
- **(+) Glucose** and **(+) Mannose** are epimers of each other means differ in configuration at only C-2.

❖ Shortening of carbon chain of aldose (Ruff Degradation)

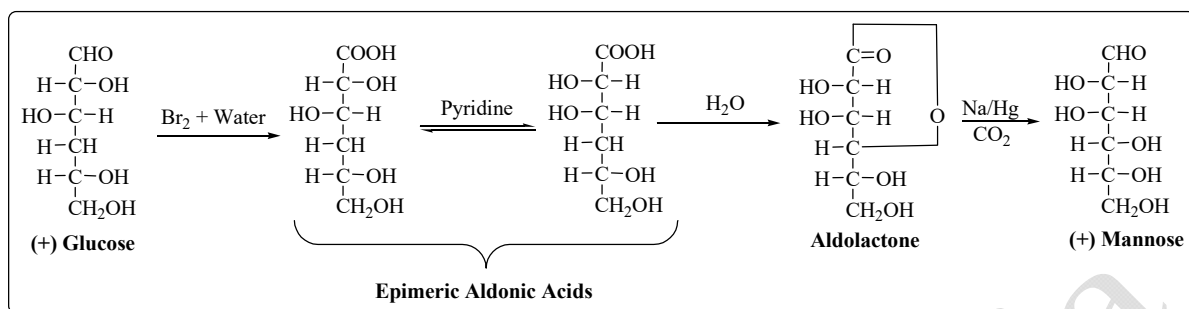
- There are many ways to convert an aldose into another aldose **having one less carbon atom**.
- One of these methods for **shortening the carbon chain** is **Ruff Degradation**.
- An **aldose** is oxidized by **bromine water** to the **aldonic acid** and further reaction with **CaCO_3** leads to **calcium aldonate** which upon reaction with **hydrogen peroxide** in presence of **ferric salts** yields carbonate ion and an **aldose of one less carbon atom**.



❖ Conversion of an aldose to its epimers ((+) Glucose to (+) Mannose)

- An **aldose** is oxidized to **aldonic acid** by **bromine water**.

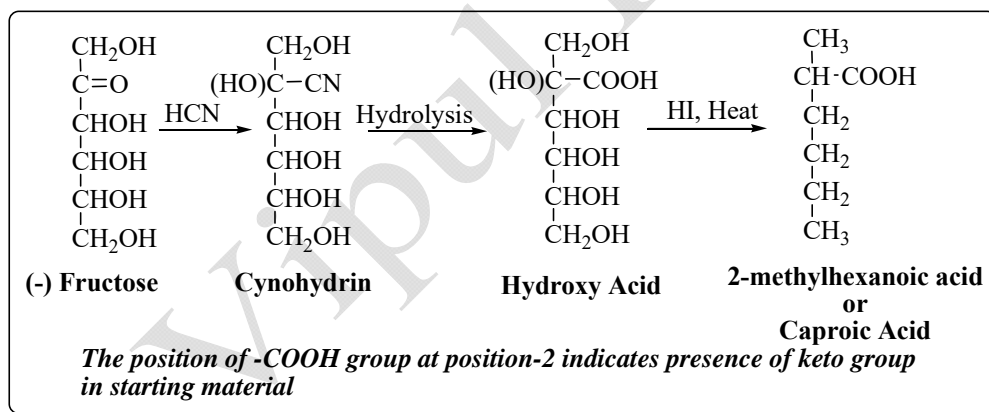
- An **aldonic acid** is in equilibrium with its **epimer** in presence of **pyridine** (tertiary amine).



- From the equilibrium mixture, **epimeric aldonic acid** is separated.
- The separated epimeric aldonic acid is reduced to **aldose** via its **lactone**.

❖ Prove that Fructose is 2-ketohexose

- The most important ketose is (-) Fructose.
- From the following reaction sequence we can establish that Fructose is ketose rather than aldose.

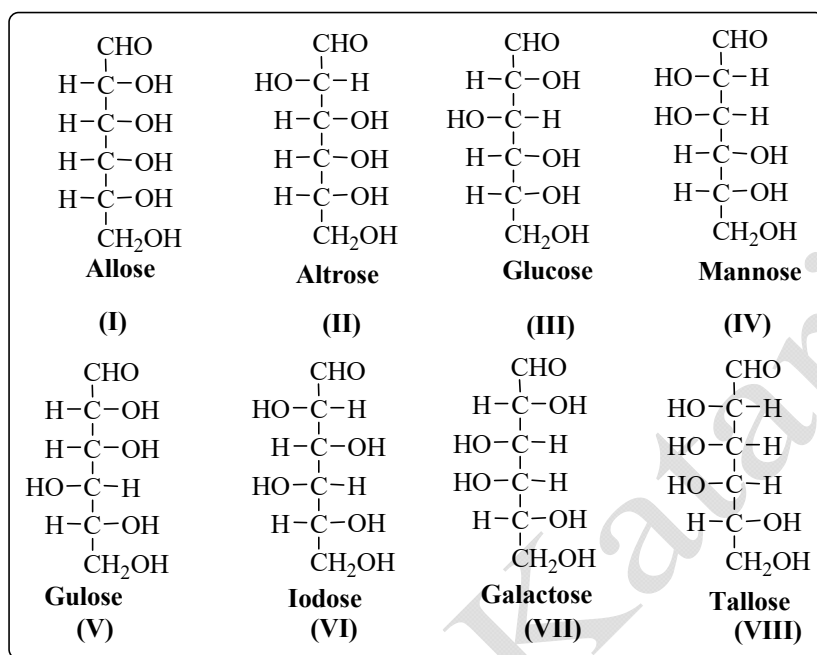


- The fructose upon reaction with HCN followed by HI gives 2-methyl hexanoic acid which indicates presence of keto group at position-2 in starting material.
- Hence, it is proved that fructose is 2-keto hexose.

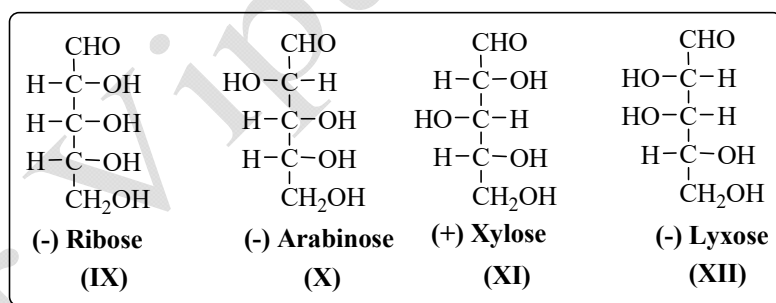
❖ Fischer's proof for configuration of (+) Glucose

- (+) Glucose was known to be an **aldohexose** but as an aldohexose it could have any one of **16 possible structures**.
- Glucose contains **4 asymmetric carbon** atoms so 16 stereoisomers.
- **Emil Fischer** tried to find out exact which structure is (+) Glucose and for that he received **noble prize in 1902**.

- It was very well known that glucose is present in nature in D form so Fischer rejected **eight possible structures that contains last –OH group on left side.**
- Now only eight structures were remained that can be drawn as following.

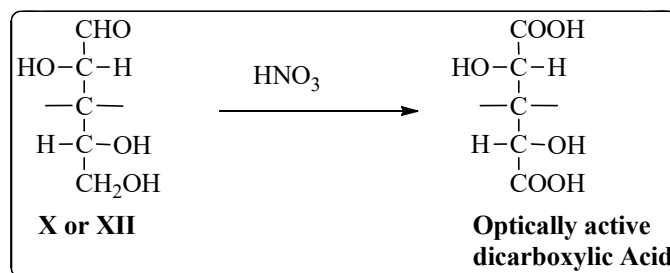


- Since Fischer's arguments were dependent upon relationship between (+) **Glucose** and (-) **Arabinose** he also had to consider eight possible structures of aldopentose. Again he rejected four structures having L configuration.

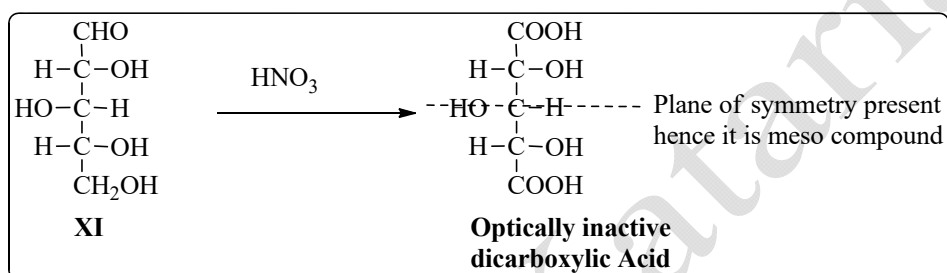
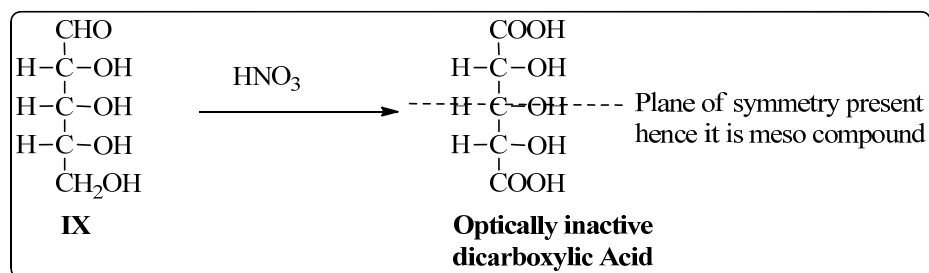


Arguments:

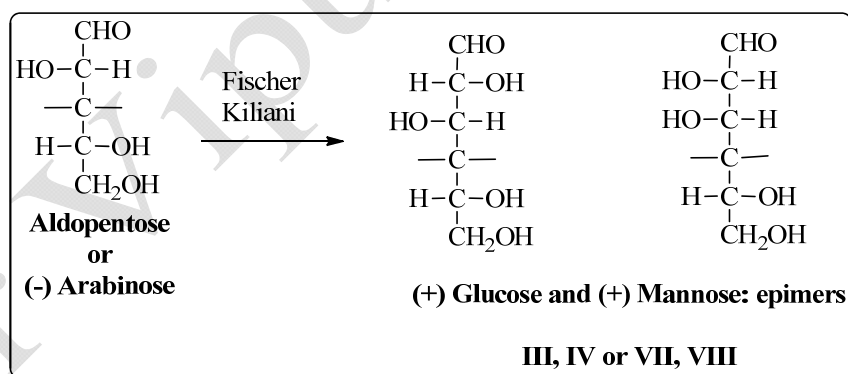
- **Argument 1:** Upon oxidation by nitric acid, (-) arabinose yields an optically active compound that is dicarboxylic acid.



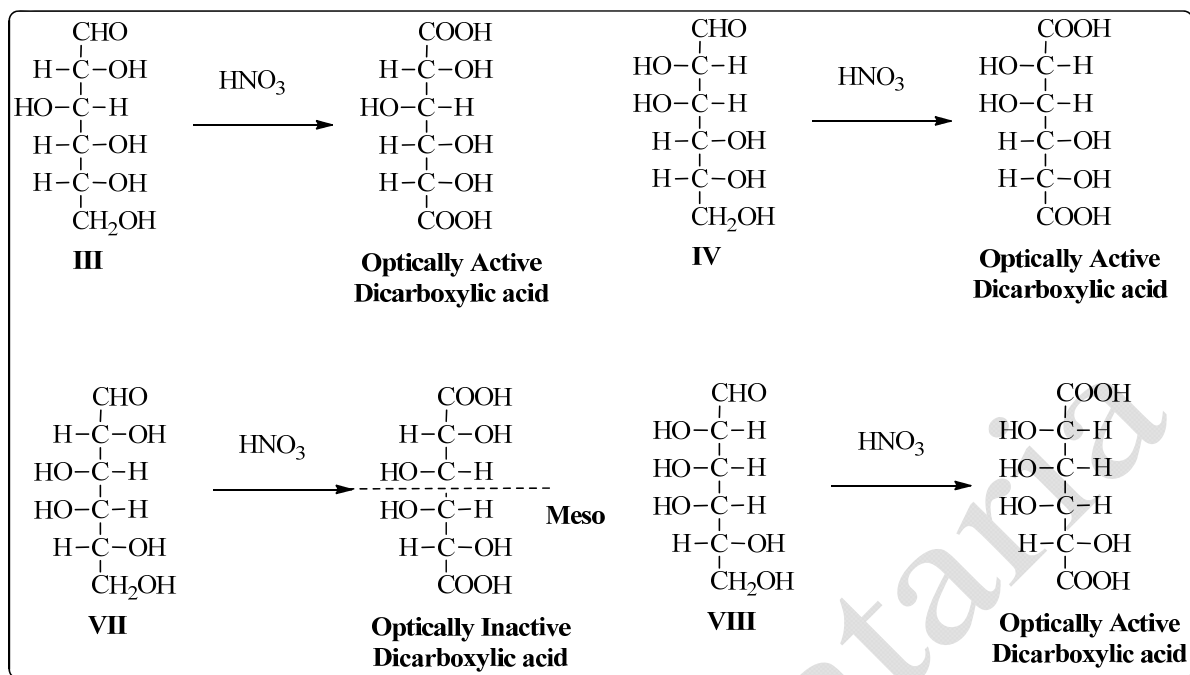
- If we carry out reaction of structure number IX or XI with HNO_3 then we will get optically inactive compound.



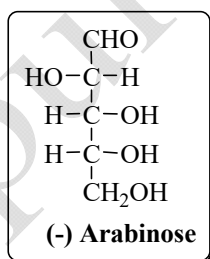
- **Argument 2:** (-) Arabinose is converted by the Fischer-Kiliani synthesis into (+) Glucose and (+) Mannose. (+) Glucose and (+) Mannose are epimers (C-2 Epimer) differing only in configuration about C-2 only. C-3 in (+) glucose having OH on left side therefore structure of glucose can be from structure number III, IV, VII or VIII.



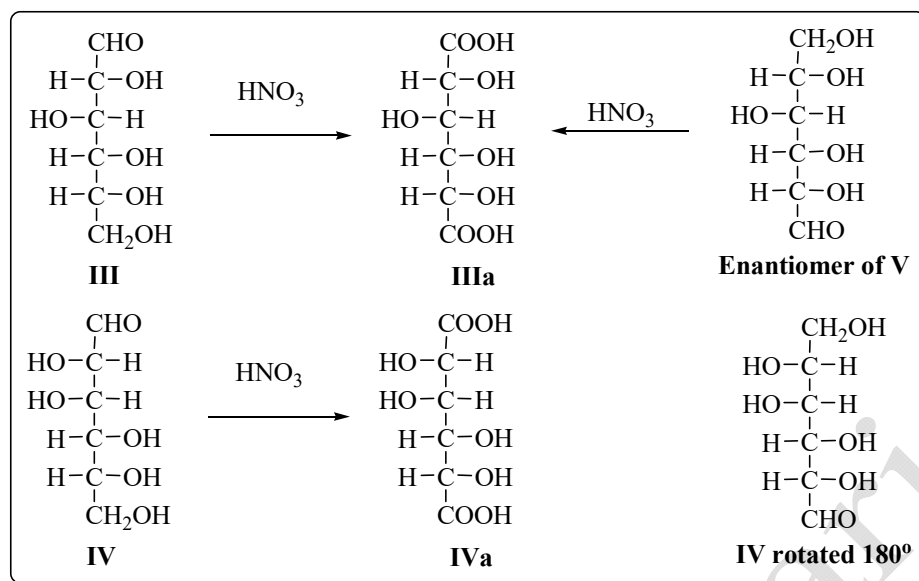
- **Argument 3:** (+) Glucose and (+) Mannose upon oxidation with conc. HNO_3 yield optically active dicarboxylic acid. This means that $-\text{OH}$ on C-4 must be on the right side as in III, IV and VIII. Therefore structure number VII can't be glucose.



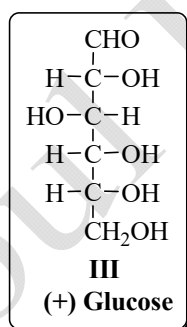
- Arabinose must have that same -OH group on the right side and hence has configuration X. Fischer-Kiliani synthesis of (-) Arabinose gives mixture of Glucose and Mannose.



- (+) Glucose and (+) Mannose have configurations III and IV, but one question remains: which compound has which configuration? One more step is needed – the most elegant step in this elegant sequence.
- **Argument 4:** Oxidation of another hexose, (+) Gulose (remember it is Gulose not Glucose) yields the same dicarboxylic acid (Glucaric Acid).
- If we examine two possible configuration for (+) Glucaric acid are IIIa and Iva.
- We can see that only IIIa can be derived from two different hexoses; from III and enantiomer of V.

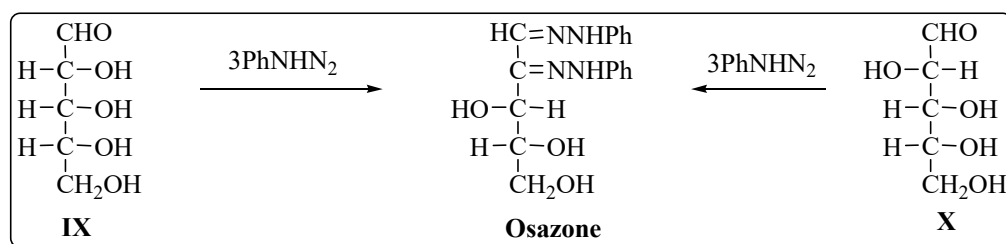


- The acid IVa can be derived from just one hexose: from IV
- It follows that (+) Glucaric acid has configuration IIIa and therefore that (+) Glucose has configuration III.

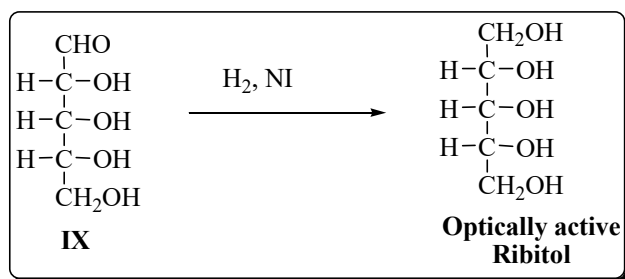


❖ Configuration of Aldoses

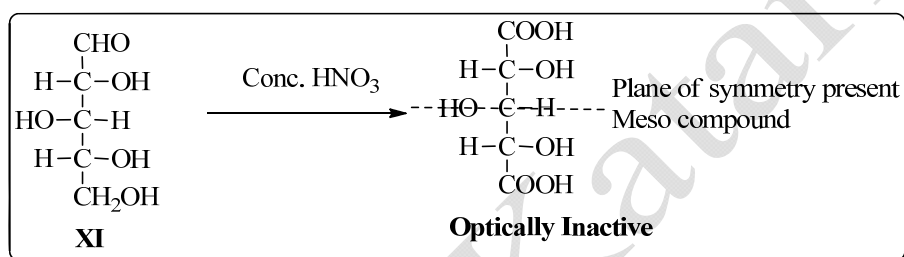
- As we have successfully derived the **structure of glucose and arabinose**.
- Let us see, **assignment of configuration of some other monosaccharides**.
- The aldopentose, (-) **ribose and (-) arabinose** both yield same **osazone**.
- As the structure for (-) **arabinose is X** then (-) **ribose must be IX**.



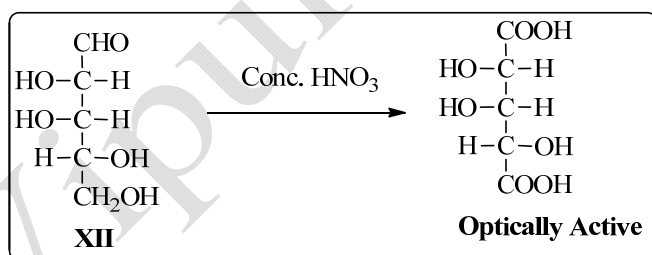
- This configuration of Ribose is also confirmed by reduction of **IX to ribitol**.



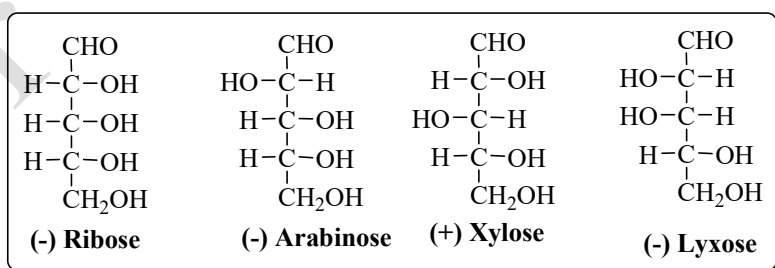
- The two remaining aldopentose (+) **xylose** and (-) **lyxose** must have configuration XI and XII.
- (+) Xylose upon oxidation by conc. HNO_3 gives **optically inactive dicarboxylic acid** therefore it must be configuration XI.



- (-) Lyxose upon oxidation gives **optically active dicarboxylic acid** hence it must be configuration XII

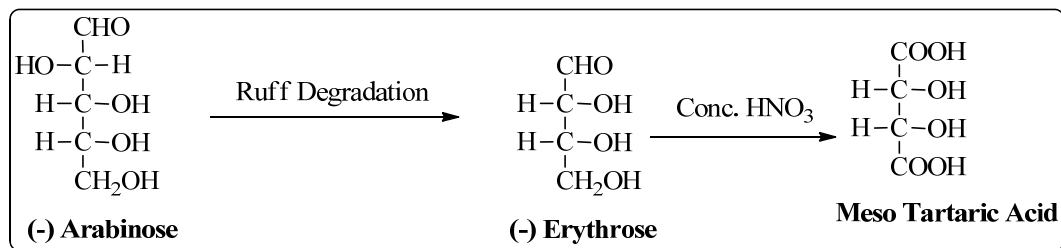


Therefore, exact configuration of aldopentose is as under

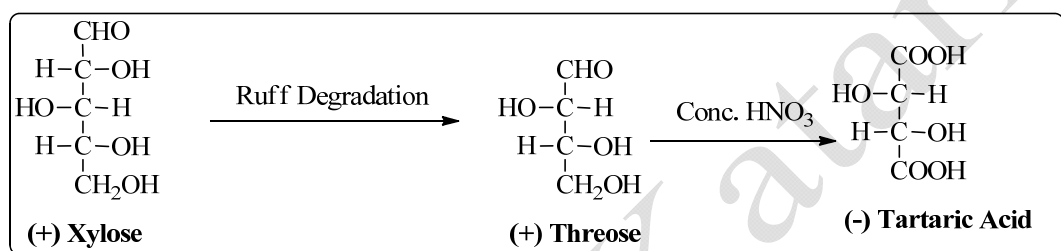


❖ Some useful conversations

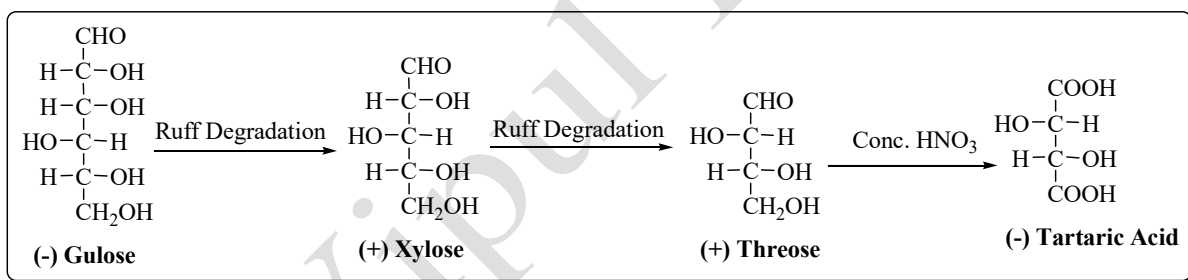
➤ (-) Arabinose to meso tartaric acid



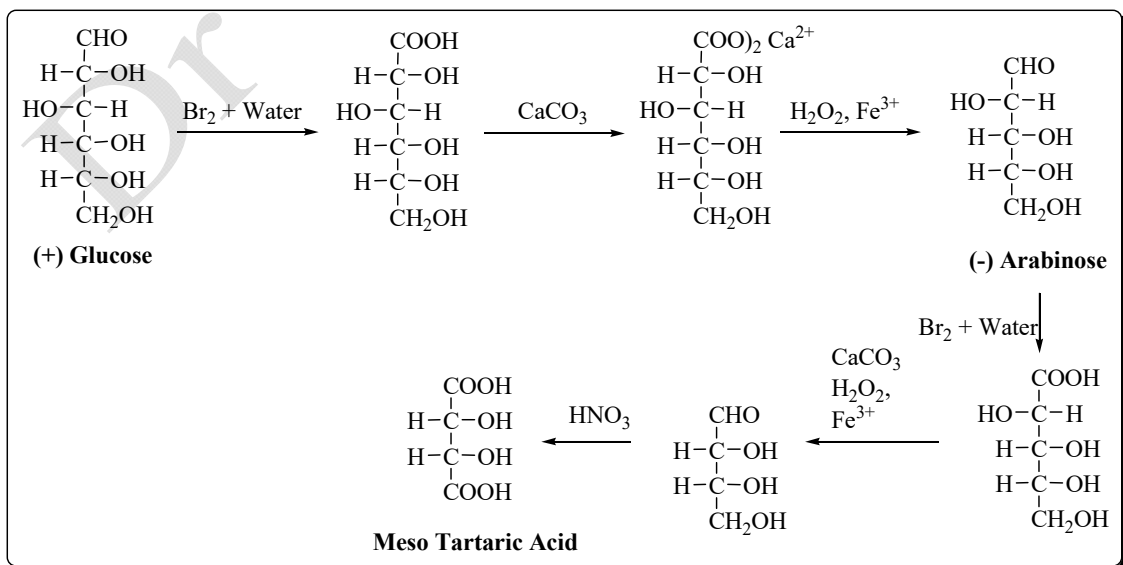
➤ (+) Xylose to Tartaric Acid



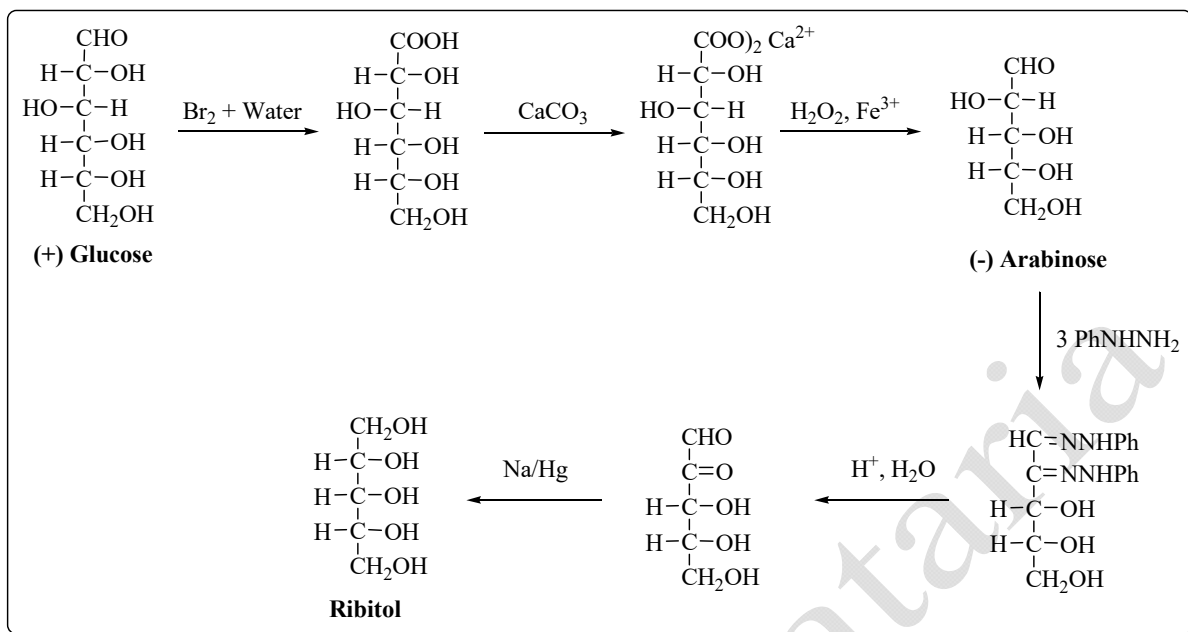
➤ (-) Gulose to (-) Tartaric Acid



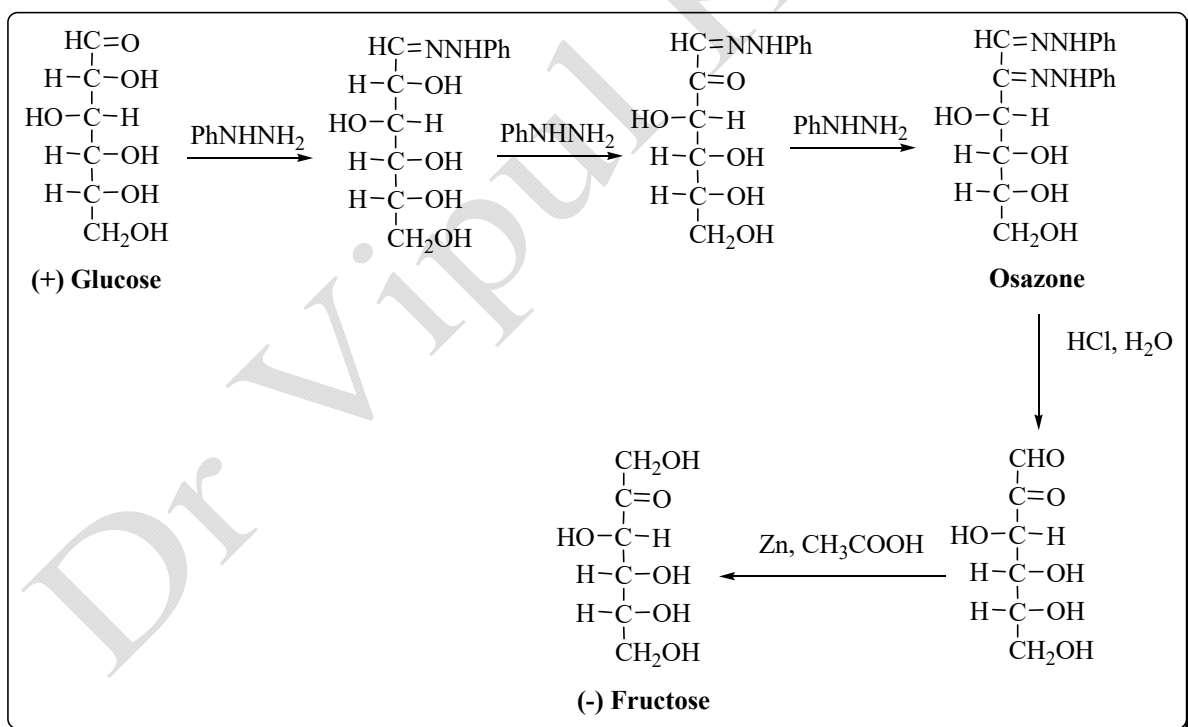
➤ (+) Glucose to Meso Tartaric Acid



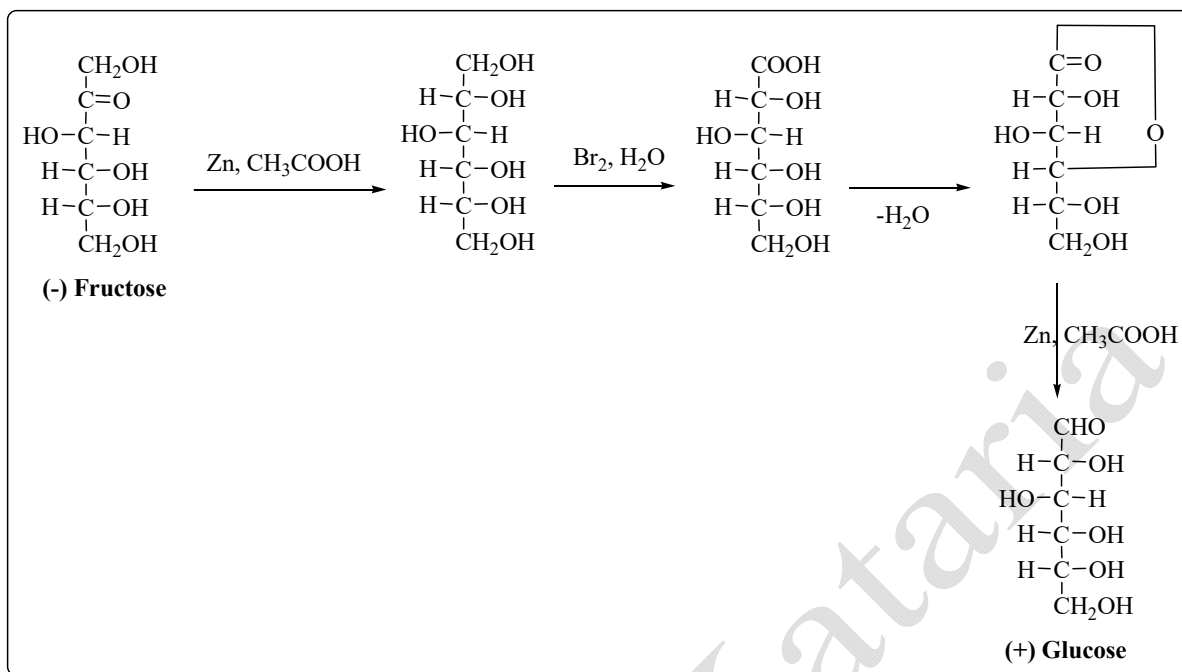
➤ (+) Glucose to Ribitol



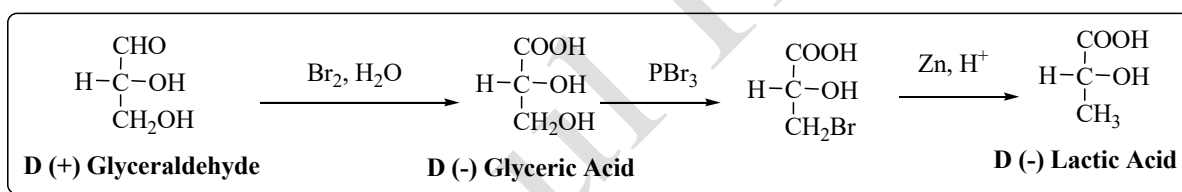
➤ (+) Glucose to (-) Fructose



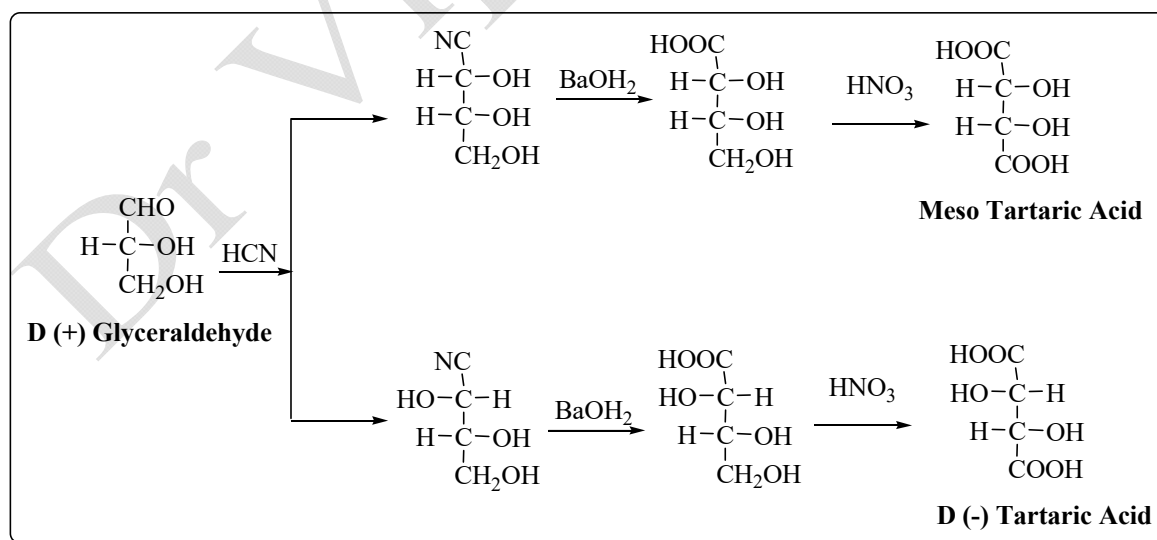
➤ (-) Fructose to (+) Glucose



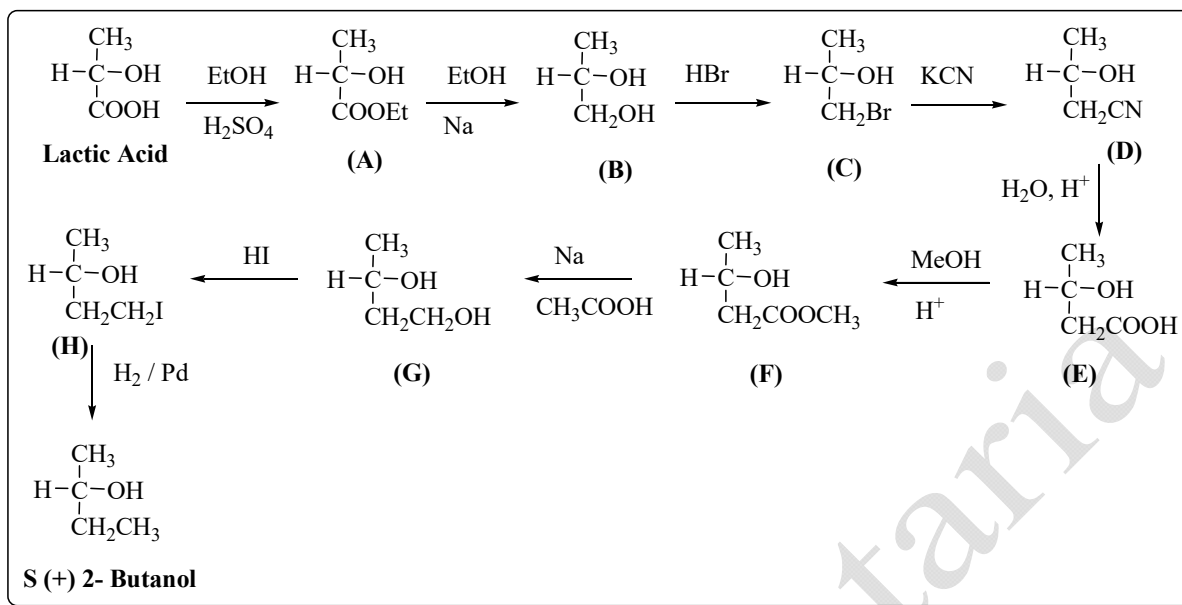
➤ D (+) Glyceraldehyde to D (-) Lactic Acid



➤ D (+) Glyceraldehyde to D (-) Tartaric acid and Meso Tartaric Acid

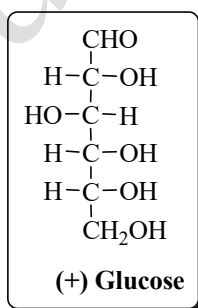


➤ **S (+) 2-Butanol from Lactic Acid**

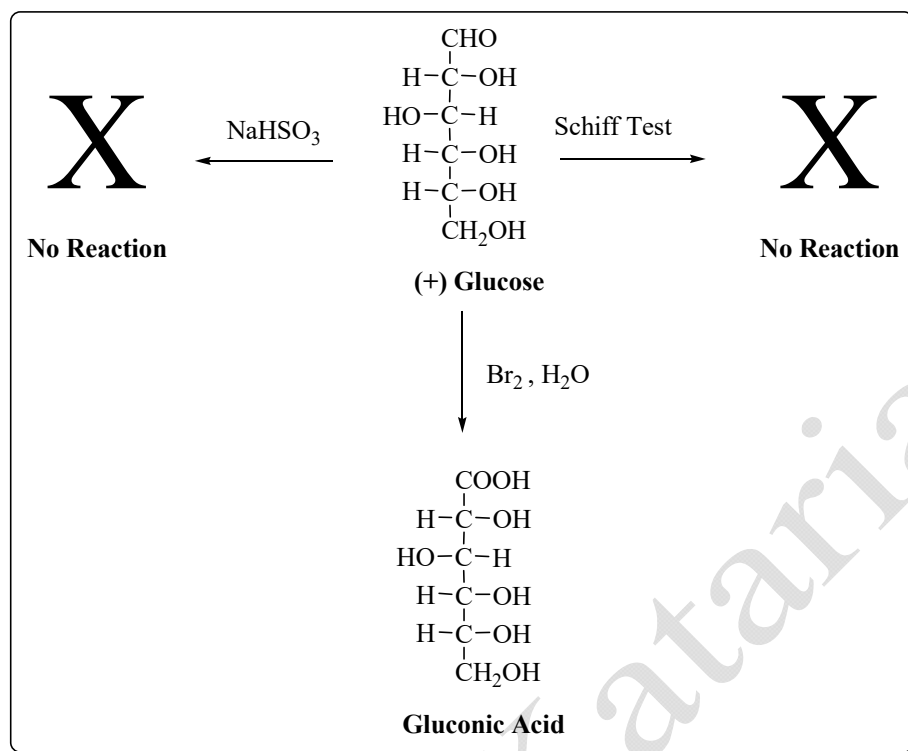


❖ **List the facts that are inconsistent with penta-hydroxy aldehyde structure (Linear) of Glucose**

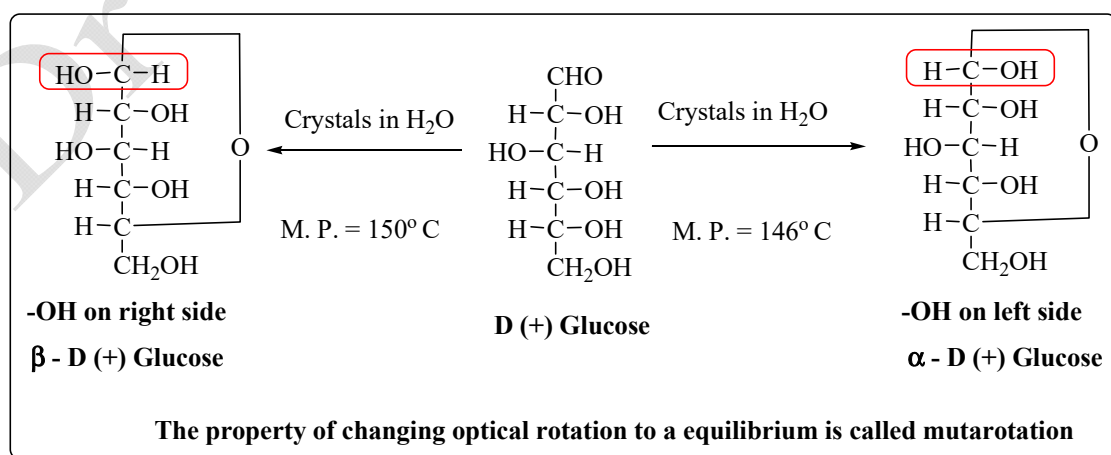
- We have seen that Emil Fischer proved the linear structure of Glucose that is following:



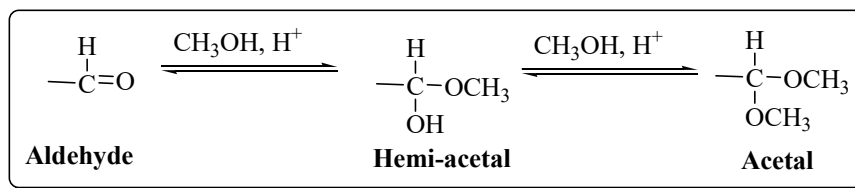
- Later on, it was observed that glucose might have some other arrangement as it doesn't give many tests according to functional groups present in it.
- Following are the arguments that prove that Glucose does not have the linear structure.
- (a) D (+) Glucose does not give some reactions those are typical for any aldehyde compound. Glucose readily oxidised to corresponding acid however it gives negative Schiff test and does not form bisulphite addition product.



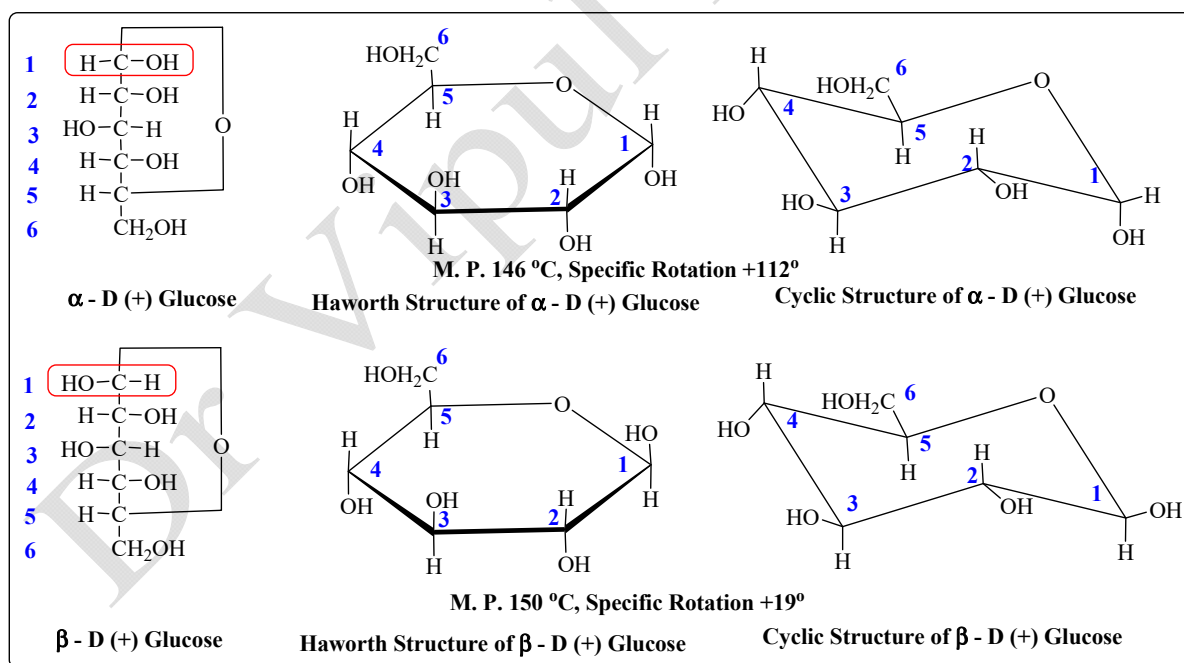
- (b) D (+) Glucose exists in two isomeric forms which undergo Mutarotation. When crystals of D (+) Glucose having melting point 146°C is dissolved in water, its specific rotation gradually drops from $+112^\circ$ to $+52.7^\circ$. When crystals of D (+) Glucose having melting point 150°C is dissolved in water, its specific rotation gradually rises from $+19^\circ$ to $+52.7^\circ$. This was much extraordinary behaviour of Glucose. It comes to a conclusion that D (+) Glucose exists in two isomeric forms, one with higher optical rotation is called α -D (+) Glucose and one with lower optical rotation is called β -D (+) Glucose. The property of changing optical rotation to a equilibrium is called mutarotation.



- (c) D (+) Glucose forms two isomeric methyl D-Glucosides. We know that aldehyde upon reaction with alcohol in presence of acid gives Acetal.

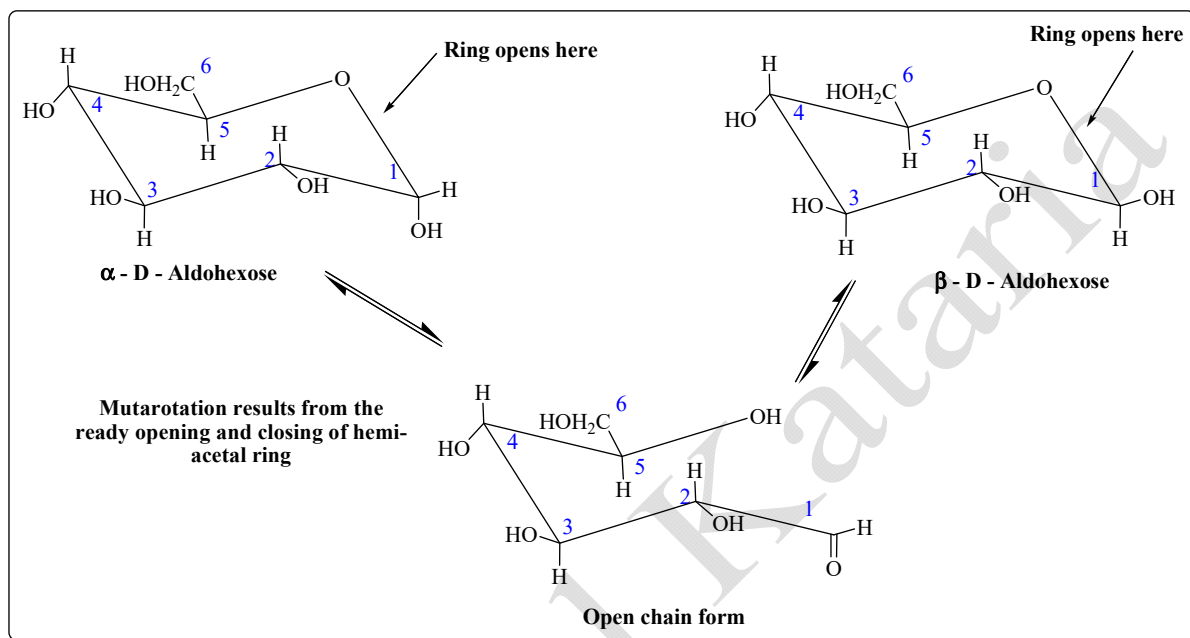


- When D (+) Glucose is reacted with methanol in presence of HCl, it forms hemiacetal means presence of only one methyl group still it has properties of full acetal. It does not spontaneously reverse back to aldehyde and alcohol on contact with water.
- Furthermore, two such mono methyl derivatives of D (+) Glucose are known, one having melting point of 165 °C with specific rotation + 158° and another having melting point of 107 °C with specific rotation -33°.
- The isomer with higher specific rotation is known as methyl α-D-glucoside and isomer with lower specific rotation is known as methyl β-D-glucoside.
- These glucosides does not undergo mutarotation and do not reduce tollen or fehling reagent.

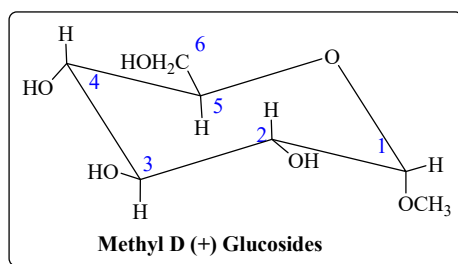


- **Hemi-Acetal undergoes mutarotation:** Hemi-acetal structure of D (+) glucose exists due to presence of aldehyde and alcohol in same structure.
- There are two isomeric forms of D (+) Glucose because this cyclic structure has one more chiral centre than linear structure of Glucose.

- α -D (+) Glucose and β -D (+) Glucose are diastereomers of each other differing in carbon number 1 (C-1) hence called anomers.
- As α -D (+) Glucose and β -D (+) Glucose readily hydrolysed by water. In aqueous solution either anomer is converted to another anomer via open chain compound. This mutarotation results from the ready opening and closing of hemi-acetal ring.



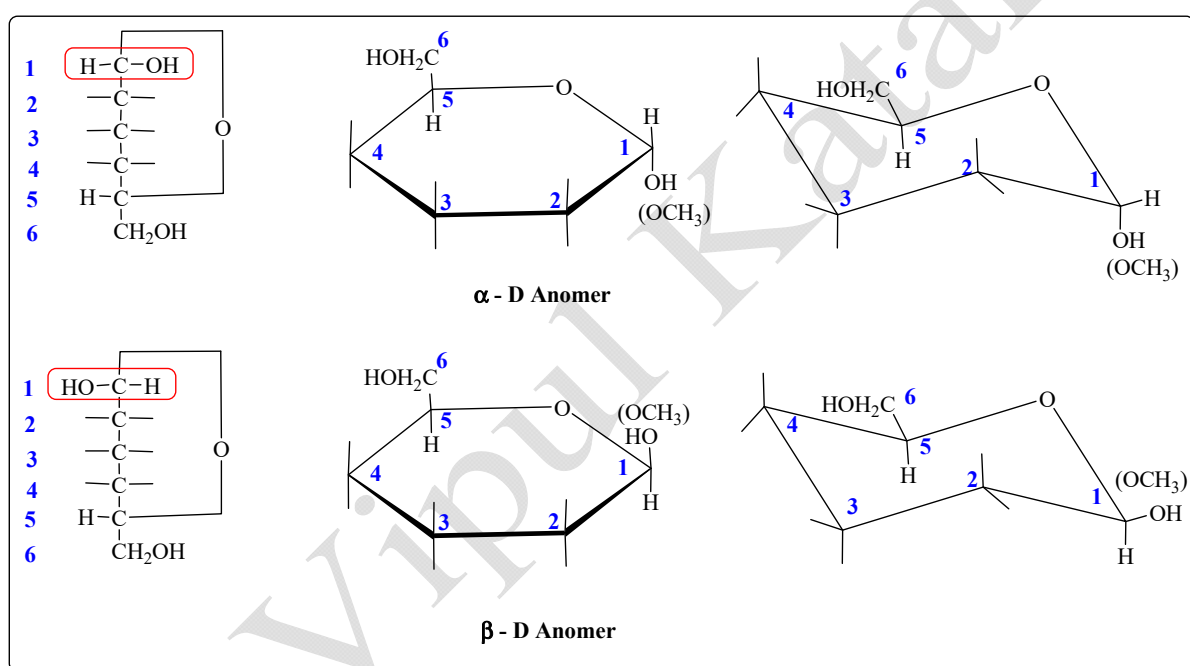
- The concentration of open chain form is less than 0.5% that is responsible for reduction of tollen's reagent and fehling reagent. However this concentration is too low for test like Schiff or bisulphite addition.
- **Methyl glucosides are non-reducing sugars.** When D (+) glucose is treated with methanol in presence of HCl, it results into methyl glucosides that contain only one methyl group (Hemi-acetal) however it behaves like full acetal and cannot undergo mutarotation.
- It does not contain Aldehyde group so it cannot reduce tollen or fehling reagent. It does not undergo osazone formation. They are stable and not readily hydrolysed to open chain structure.



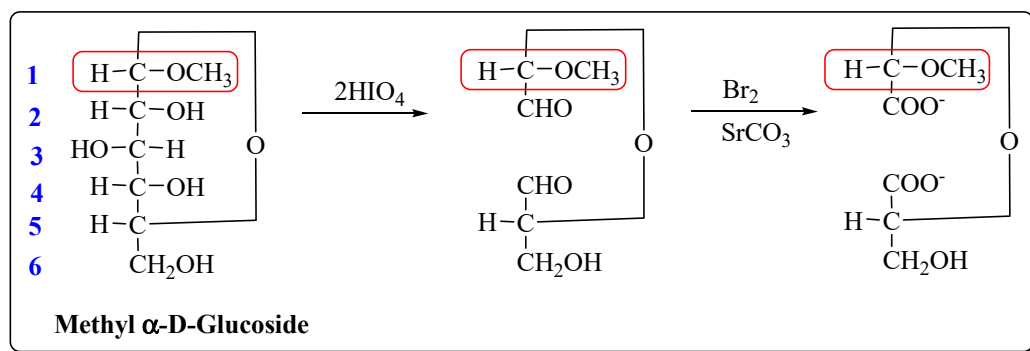
- So they are non-reducing sugar.

❖ The configuration about C-1

- For now, It was been proved that glucose and its glycosides have cyclic structure, certainly raised a question. What is the configuration about C-1 in each of the isomeric structures?
- In 1909, Hudson made the argument “*In D series the more dextrorotatory member of an α, β pair of anomer is to be named α -D and other one is β -D. These both are diastereomers of each other*”.
- If $-\text{OH}$ or $-\text{OCH}_3$ group is on right side (down side in case of cyclic structure) then the structure is α -D and if $-\text{OH}$ or $-\text{OCH}_3$ is on left side (upside in case of cyclic structure) then the structure is β -D.

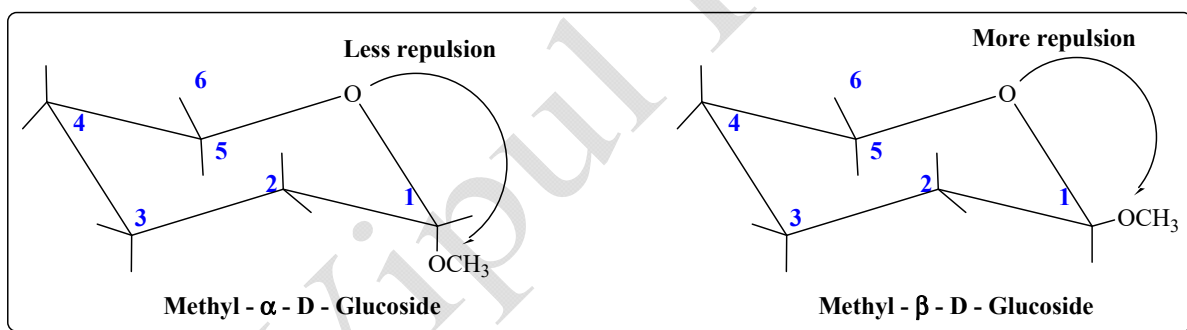


- Oxidation by HIO_4 destroys all the chiral centres at C-2, C-3, and C-4 but configuration is preserved about C- and C-5.
- The configuration about C-5 is same for all members of D family.
- The same products can be obtained from all these glycosides only if they also have the same configuration about C-1.
- The C-1 OH is on the right side in α -D series and on the left side in β -D series.



❖ Anomeric Effect

- In methyl glucoside structure, there are optimum chances of repulsion between oxygen of the ring and oxygen of the OCH_3 group. The repulsion between dipole associated with C-1 oxygen and oxygen of the ring is referred as anomeric effect. Higher the repulsion higher the anomeric effect lower the stability.
- Upon Examination of both the structure it is clear that repulsion is more in β -isomer which makes it less stable. In α -isomer the repulsion is less.



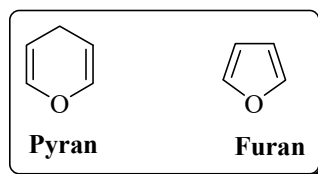
❖ Prove that D (+) Glucose contains six membered ring system.

OR

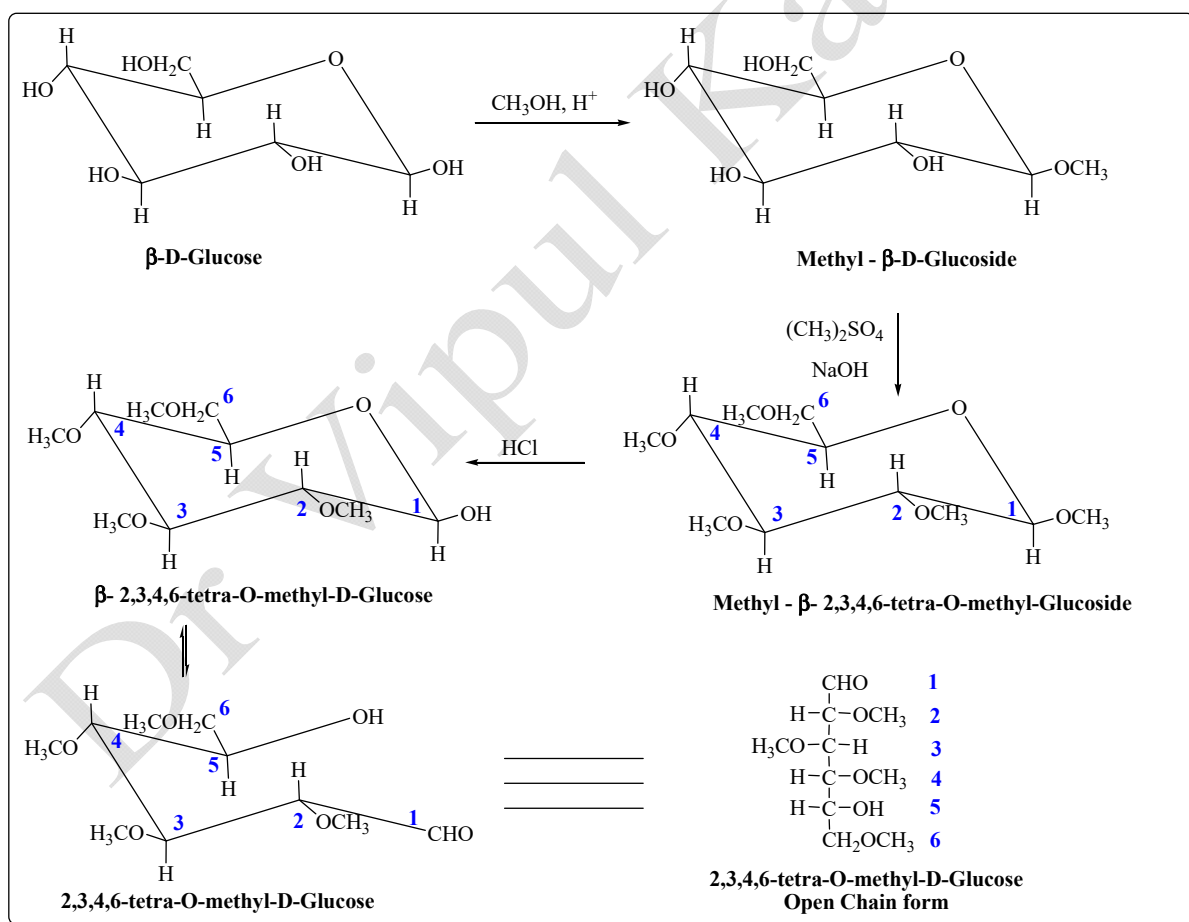
D (+) Glucose is a pyranose and not a furanose

- So far, we had represented six membered ring structure for α and β - D Glucose and glucosides.
- It means joining of C-1 and C-5 is there.
- However, other ring system (five membered) is also possible.
- Noble prize winner Sir E. N. Howarth proved the six membered structures for Glucose and glucosides.

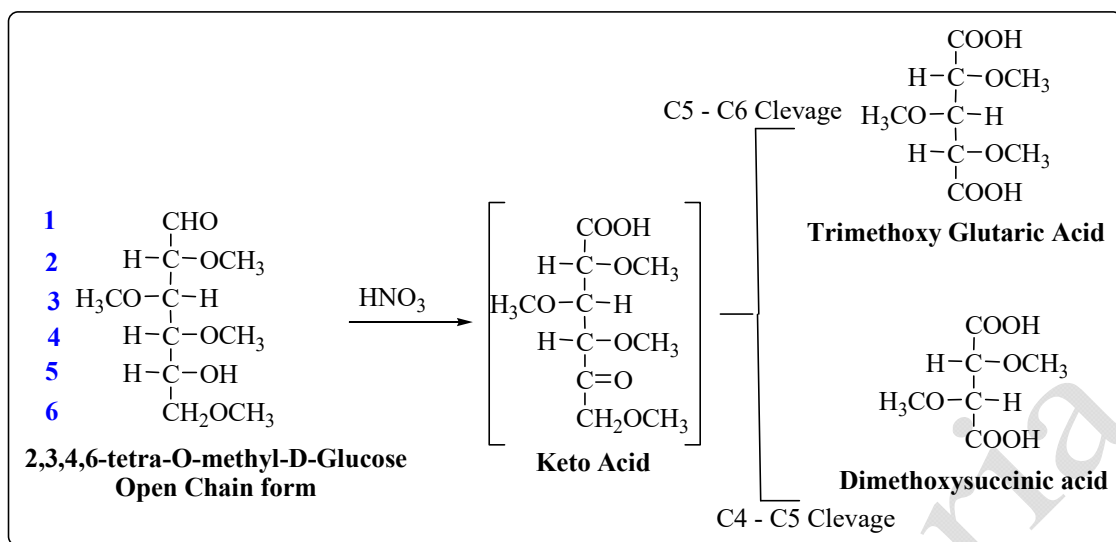
- The six membered ring system is known as pyranose and five membered is known as furanose.



- In Glucose, if joining is between C-1 and C-5 then the structure will be pyranose and if joining is between C-1 and C-4 then the structure will be furanose.
- When methyl β - D - Glucoside is reacted with methyl sulphate and NaOH, it will produce Methyl β - 2,3,4,6 - tetra-O-methyl-D-Glucoside.
- This product is reacted with dil. HCl then it will produce tetra-O-methyl glucose.
- This compound is a cyclic hemi-acetal which in presence of water also exists in open chain form.



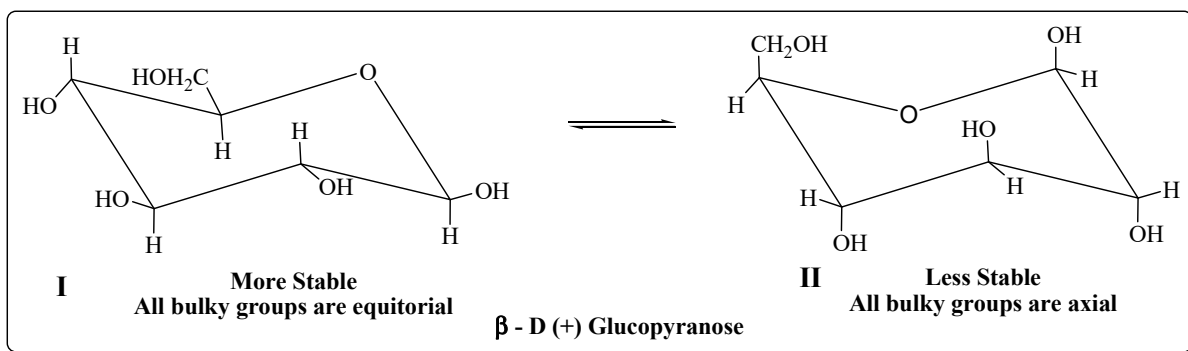
- This open chain form contains one aldehyde and one hydroxyl group along with four $-\text{OCH}_3$ groups.
- If we oxidise this open chain compound with HNO_3 , it will produce keto acid which upon cleavage can produce trimethoxy glutaric acid or dimethoxy succinic acid.



- Upon examination, it was found that trimethoxy glutaric acid was major product which confirms the presence of ketone group at C-5.
- Ketone is on C-5 hence we can say that original structure would have -OH group on C-5 that confirms the furanose ring structure in methyl glucoside and glucose structures.

❖ Conformation

- Although the linear and cyclic structures were proved up to early nineteenth's however the concept of conformation was understood in case of glucose in 1950.
- The different orientation of a compound that can be achieved by simple rotation about a single bond is called conformations and such isomers are known as conformers.
- D (+) Glucose contains six membered pyranose ring.
- Since C-O-C bond angle is 111° that is quite nearer to tetrahedral angle (109.5°).
- Then D (+) Glucose must be like cyclohexane ring (Chair conformation) to exclude angle, torsional and van der waals strain.
- There are two types of D (+) Glucose, α- D (+) Glucose and β-D (+) Glucose.
- For β - D (+) Glucose two chair conformations are possible.



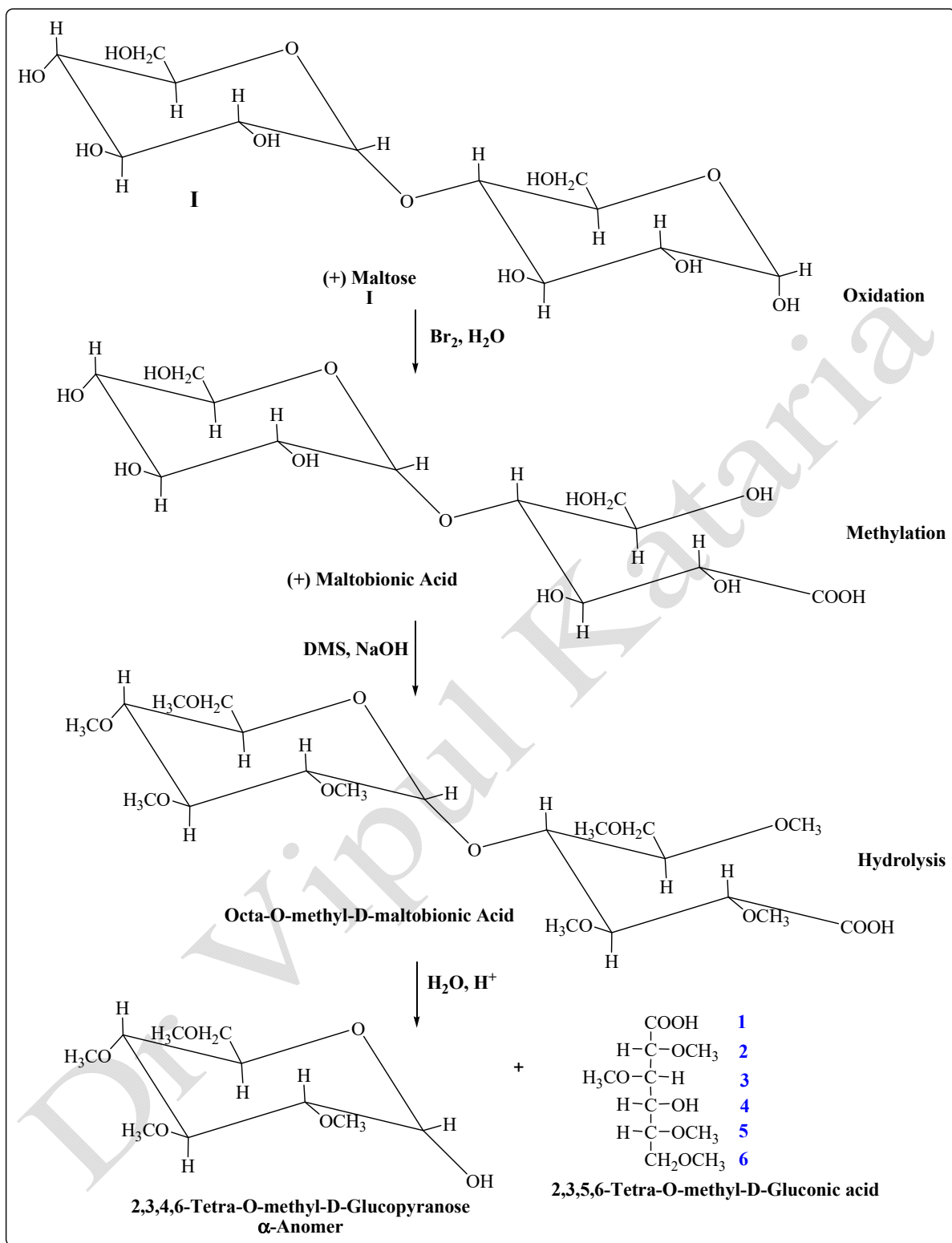
- In Structure I, all the bulky groups are on equatorial position that makes it more stable than structure II.

Disaccharides

Carbohydrates that can be hydrolysed to two monosaccharide units are known as disaccharides.

❖ (+) Maltose

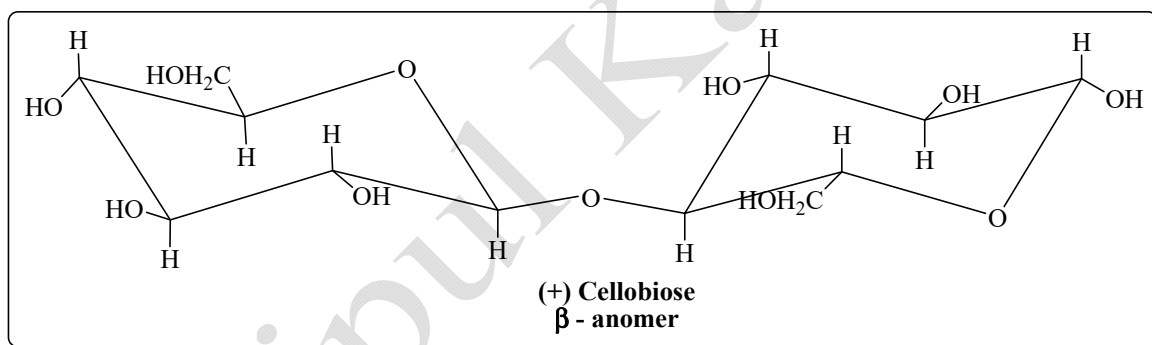
- (+) Maltose can be obtained by partial hydrolysis of starch in aqueous acid.
- (+) Maltose has the molecular formula $C_{12}H_{22}O_{11}$.
- It reduces Tollen's reagent and Fehling solution and hence it is reducing sugar.
- It reacts with Phenyl hydrazine to form an osazone.
- It can be oxidised with Br_2 and water to a monocarboxylic acid known as Maltobionic acid.
- (+) Maltose exists in α and β isomer with specific rotation of $+168^\circ$ and $+112^\circ$ respectively. It undergoes mutarotation in solution and equilibrium specific rotation is 136° .
- Upon acidic hydrolysis it gives two molecules of glucose, hence it is glucoside.
- Upon enzymatic hydrolysis it gives two molecules of D (+) Glucose. There is 1-4 glucoside linkage, therefore it α -glucoside linkage between carbonyl group of on D (+) Glucose unit and $-OH$ group of the other.
- (+) Maltose contains six membered ring: Maltose upon reaction with Br_2 gives oxidised product that is Maltobionic acid.
- This acid upon methylation gives octa-O-methyl-D-maltobionic acid which upon further hydrolysis gives 2,3,4,6-tetra-O-methyl-D- glucopyranose and 2,3,4,5-tetra-O-methyl-D-Glucose.



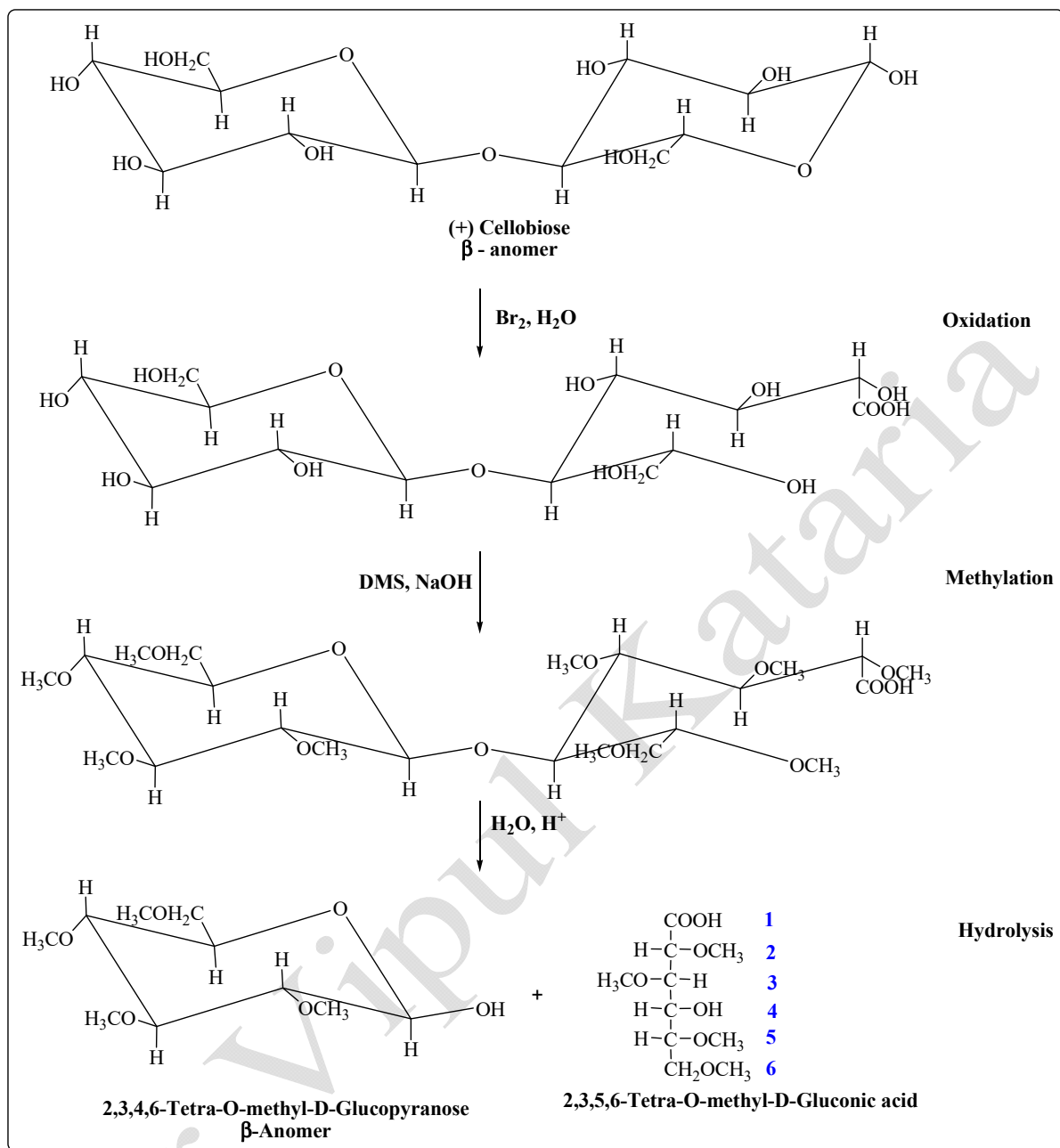
- These facts indicate that (+) Maltose has structure I. The $-\text{OH}$ group on C-4 that serves as the alcohol in the glucoside formation. Both the part has six membered ring: Pyranose ring.

❖ (+) Cellobiose

- When cellulose is treated with sulphuric acid and acetic anhydride for several days, a combination of acetylation and hydrolysis takes place and yield octaacetate of (+) cellobiose.
- Alkaline hydrolysis of octaacetate of (+) cellobiose yields (+) cellobiose.
- (+) Cellobiose has the molecular formula $C_{12}H_{22}O_{11}$.
- It is reducing sugar.
- It can form osazone with phenyl hydrazine.
- It exists in alpha and beta isomeric forms that can undergo mutarotation.
- It can be hydrolysed to two molecules of D (+) Glucose.
- The sequence of oxidation, methylation and hydrolysis is same as described for maltose.
- It contains two pyranose rings and a glucoside linkage to an -OH group and C-4.



- It differs from maltose in linkage, maltose has alpha linkage whereas cellobiose has beta linkage.
- The reaction sequence is same as we seen in case of maltose.
- The oxidation is followed by methylation and finally hydrolysed to glucopyranose and open chain gluconic acid derivative having free -OH group at C-4.

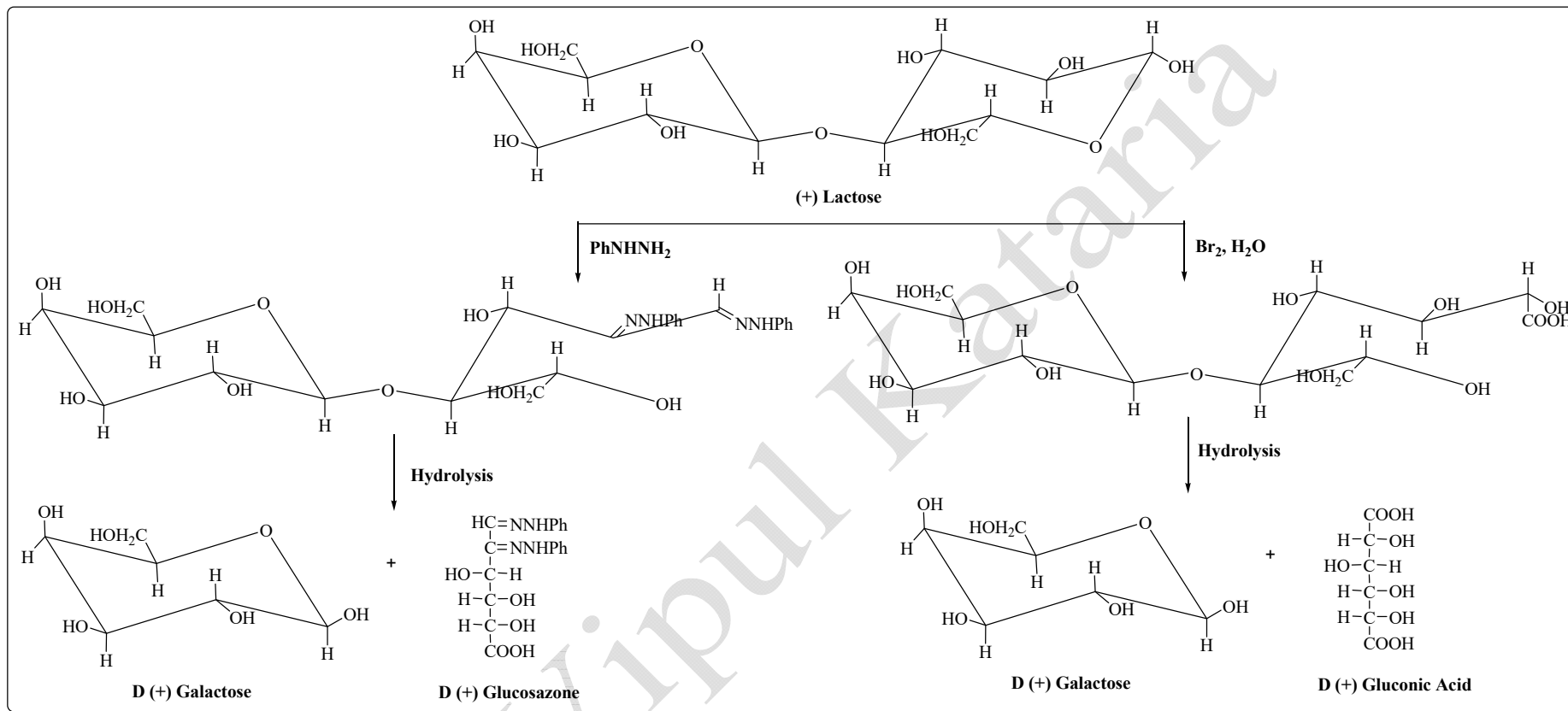


- It has been proved that (+) Cellobiose is 4-O-(β -D-glucopyranosyl)-D-glucopyranose.

(+) Lactose

- (+) Lactose is present in human and cow milk in concentration of 5%.
- It is also present in Whey.
- (+) Lactose has molecular formula of $\text{C}_{12}\text{H}_{22}\text{O}_{11}$, is reducing sugar and forms Osazone, exists in alpha and beta isomers which undergo mutarotation.
- Acidic hydrolysis of emulsion converts (+) Lactose into equal amount of d (+) Glucose and D (+) Galactose.

- Oxidation by Br_2 and water leads to lactobionic acid.
- It has 1-4 glucoside linkage.
- (+) Lactose treated with Br_2 and water to give lactobionic acid which upon hydrolysis gives D (-) Gluconic acid and D (+) Galactose.
- (+) Lactose upon reaction with excess phenyl hydrazine to give Osazone which upon hydrolysis gives D (+) Galactose and D (-) Glucosazone.
- Hydrolysis of lactose derivative shows that glucose is the reducing unit and lactose is 4-O-(β -D-galactopyranosyl)-D-Glucopyranose.
- The reaction is on next page.



I have work hard preparing this material.....

You also work hard preparing from this material!!!!

Best Luck...