

CARBOHYDRATES

Test

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General Information:

- Carbohydrates are the most abundant class of organic compounds found in living organisms.
- They originate as products of photosynthesis, an endothermic reductive condensation of carbon dioxide requiring light energy and the pigment chlorophyll.

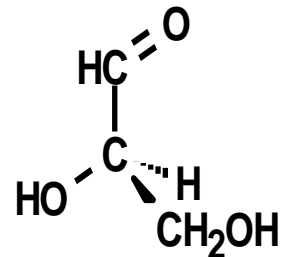
General Information:



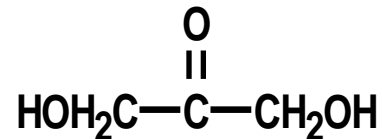
- The formulas of many carbohydrates can be written as carbon hydrates, $\text{C}_n(\text{H}_2\text{O})_n$, hence their name.
- The carbohydrates are a major source of metabolic energy, both for plants and for animals that depend on plants for food.
- Aside from the sugars and starches that meet this vital nutritional role, carbohydrates also serve as a structural material (cellulose), a component of the energy transport compound ATP, recognition sites on cell surfaces, and one of three essential components of DNA and RNA.
- Carbohydrates are called saccharides or, if they are relatively small, sugars.

A- Simple Sugars

- Contain the elements carbon, hydrogen, and oxygen.
- The name carbohydrate literally means water compounds of carbon.
- The general formula for simple sugars is $C_n(H_2O)_n$.
- This class of compounds is better described as Polyhydroxy aldehydes and ketones.
- The simplest carbohydrates are glyceraldehyde and dihydroxyacetone.



glyceraldehyde



dihydroxyacetone

A - Methods of Classification:

Several methods are used to classify carbohydrates.

1-One method of classification is based on whether the carbohydrate can be broken down into smaller units.

- Monosaccharides

cannot be broken down into smaller units by hydrolysis.
Sometimes called simple sugars.

- Disaccharides

can be broken down (hydrolyzed) into two monosaccharide units.

- Oligosaccharides

can be broken into three to six monosaccharide units.

- Polysaccharides

composed of 7 or more mono-saccharide units.

A - Methods of Classification:

Several methods are used to classify carbohydrates.

2-Another method is based on the number of carbons found in a simple sugar.

- If it has 3 carbons it is called a triose.
- If it has 4 carbons it is called a tetrose.
- If it has 5 carbons it is called a pentose.
- If it has 6 carbons it is called a hexose.

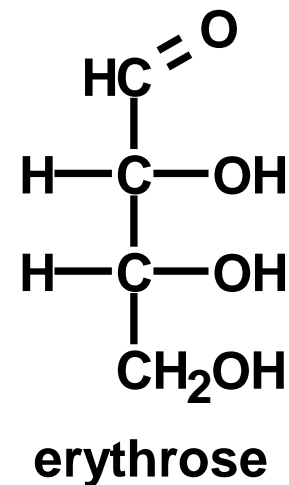
A - Methods of Classification:

Several methods are used to classify carbohydrates.

3-Another method uses the kind of carbonyl group.

A- Aldose:

A monosaccharide with an aldehyde group.

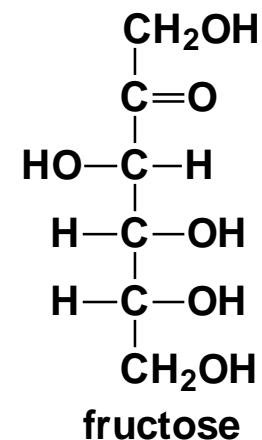


A - Methods of Classification:

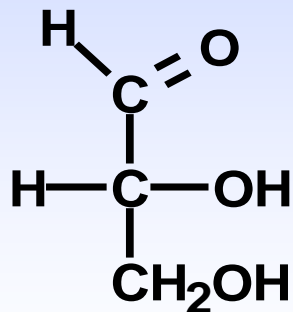
Several methods are used to classify carbohydrates.

B- Ketose

A monosaccharide with a ketone group..

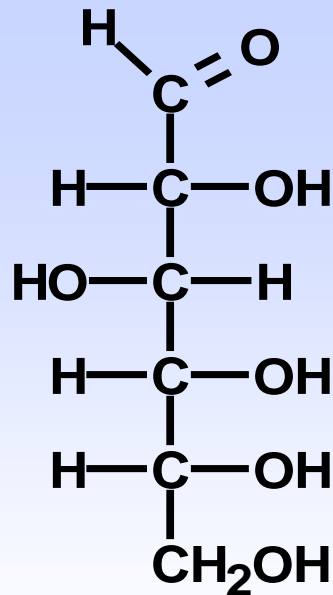


Usually combine the carbonyl classification and the number classification together.



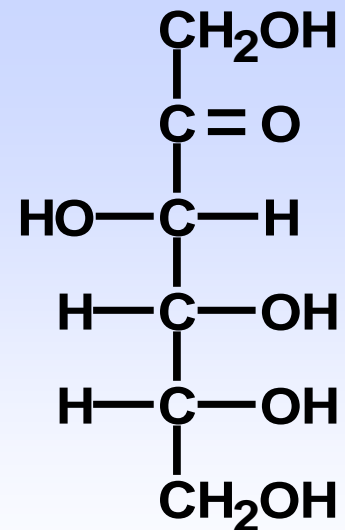
glyceraldehyde

aldotriose



glucose

aldohexose



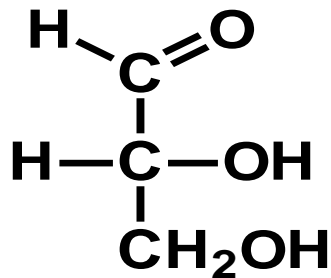
fructose

ketohexose

B- Stereoconfigurations of simple sugars.

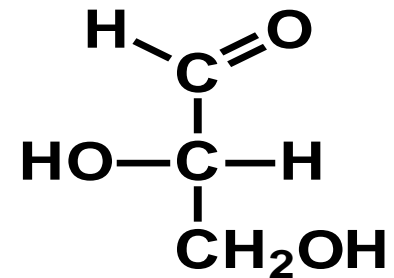
Carbohydrates contain many stereocenters.

- 1- If the OH group is found on the right side of the carbon chain, the sugar is designated as a D sugar.
- 2- If the OH group is found on the left side of the chain of carbons, the sugar is designated as an L sugar.



D-glyceraldehyde

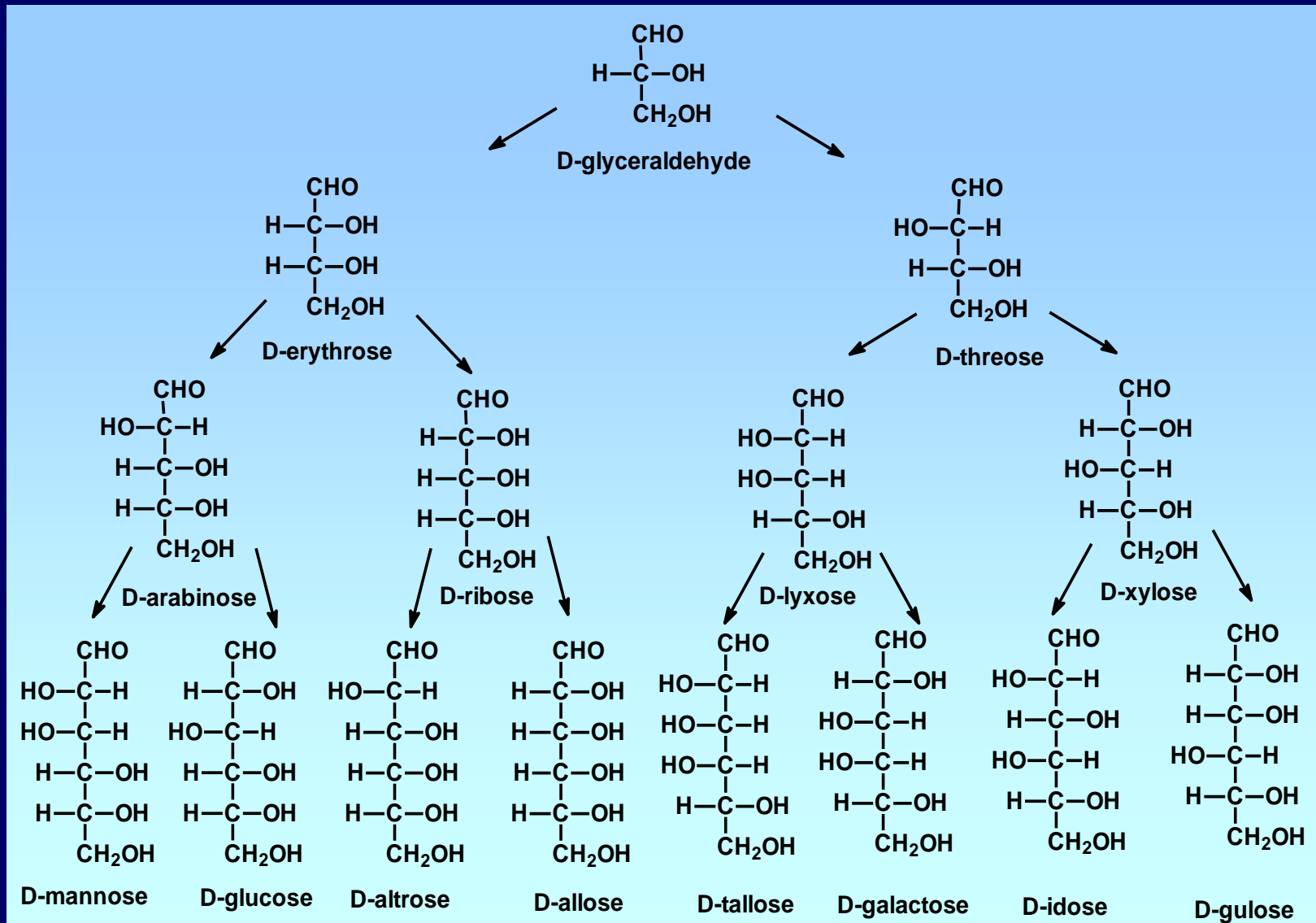
D-aldotriose



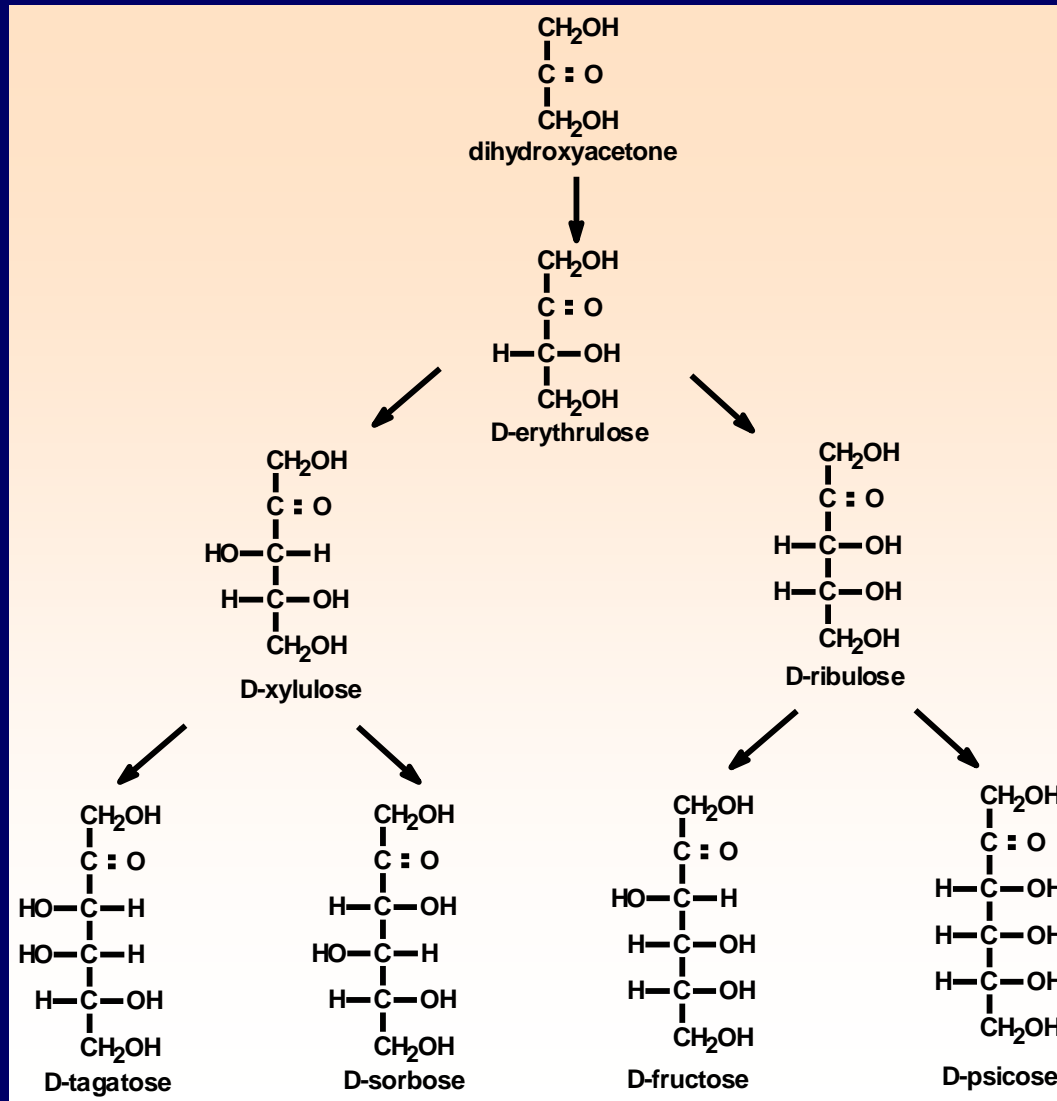
L-glyceraldehyde

L-aldotriose

B- Stereoconfigurations of simple sugars.

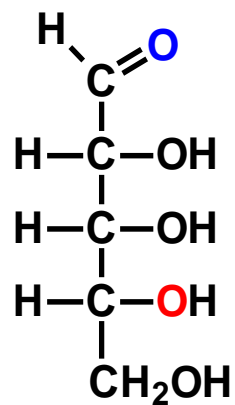


B- Stereoconfigurations of simple sugars.

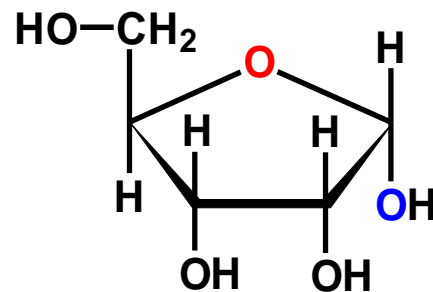


Cyclic Structures:

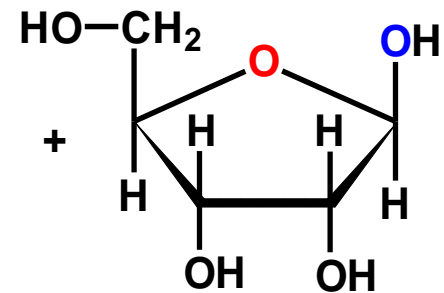
- Five membered sugar rings are known as **furanose rings**.



D-ribose



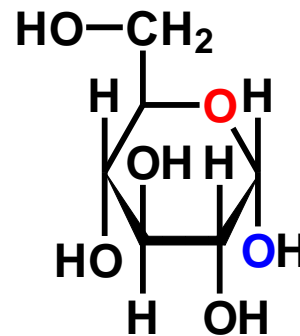
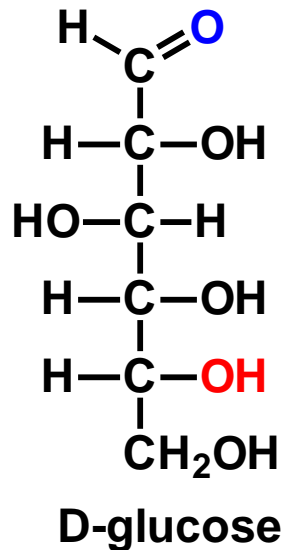
α -D-ribofuranose



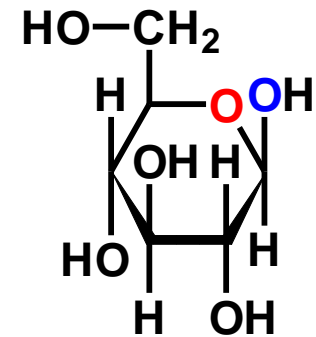
β -D-ribofuranose

Cyclic Structures:

- Six membered sugar rings are known as **pyranose rings**.



α-D-glucopyranose



β-D-glucopyranose

Carbohydrate Anomers:

- Formation of either of the cyclic form has created a new stereocenter.
- These stereoisomeric ring forms of carbohydrates are called **Anomers**.
- **Anomers:**
are carbohydrates that differ by the stereo-configuration of the carbon involved in ring formation.
- The greek letters α and β are used to describe the configuration about the ring forming carbon.
- **The α anomer** always has the OH group oriented in a downward fashion on the anomeric carbon of a D-sugar.
- **The β anomer** always has the OH group oriented in an upward fashion on the anomeric carbon of a D-sugar.

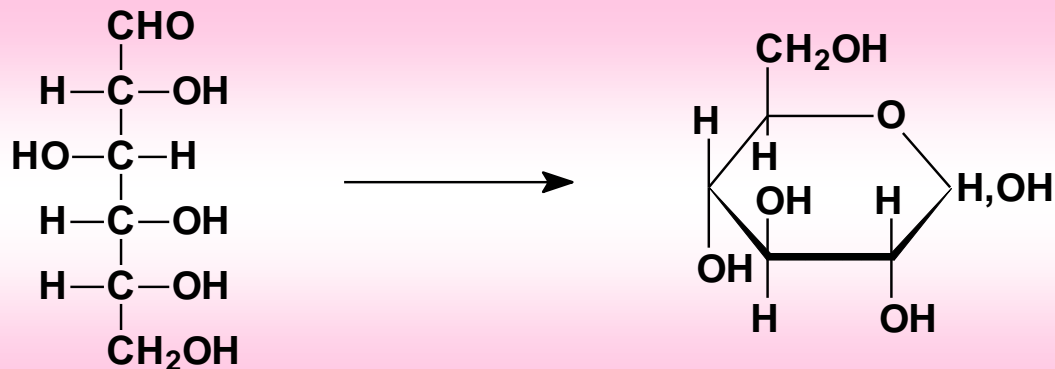
Important Carbohydrates

* Monosaccharides

composed of three to seven carbon atoms.

1- Glucose

- The most abundant hexose in our diet.
- The building block of complex carbohydrates.
- Component of the disaccharides: sucrose, maltose and lactose.
- Found in the polysaccharides: starch, cellulose and glycogen.



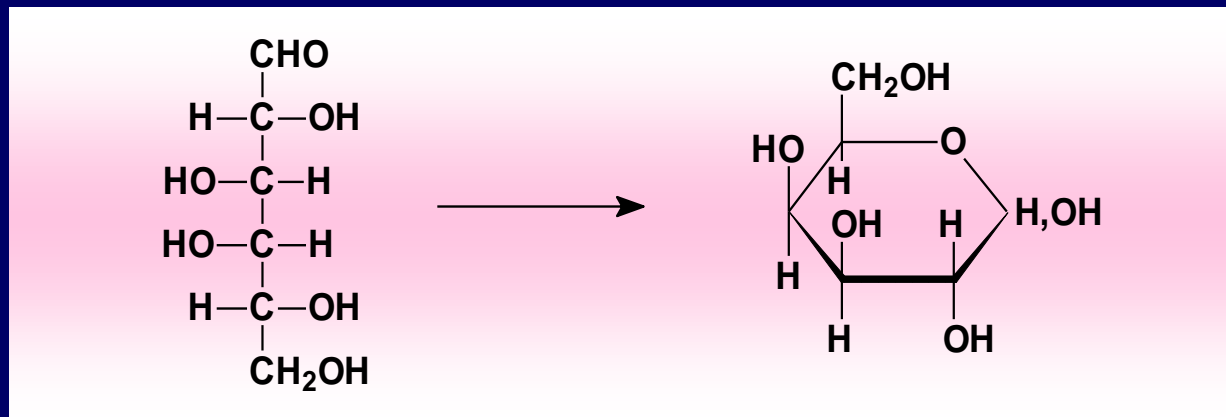
Important Carbohydrates

* Monosaccharides

composed of three to seven carbon atoms.

2. Galactose

- Found in the disaccharide, lactose.
- Found in the cellular membranes of the brain and nervous system.
- Galactose is the C-4 epimer of glucose.



Important Carbohydrates

* *Monosaccharides*

composed of three to seven carbon atoms.

3. Fructose

- Sweetest of the carbohydrates.
- Component of the disaccharide sucrose.
- Fructose is a keto sugar.

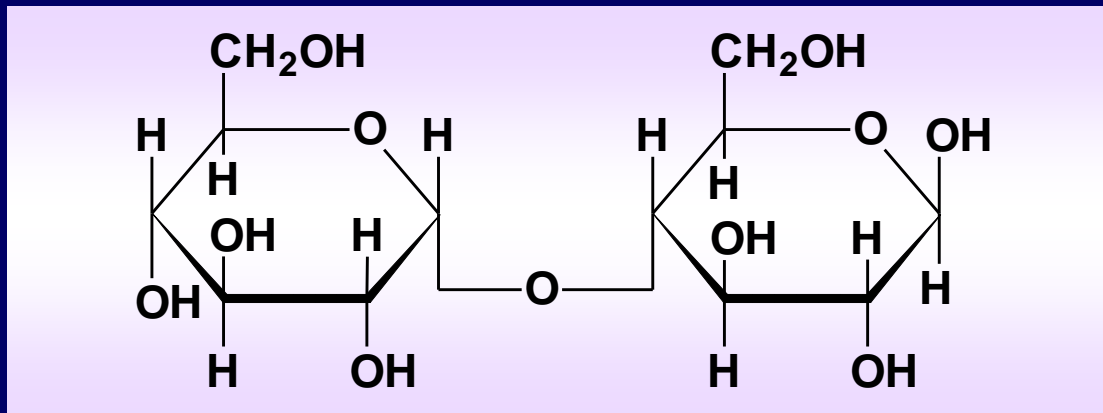
Important Carbohydrates

* Disaccharides

composed of 2 monosaccharide units.

1. Maltose - malt sugar.

- Used in cereals, candies and the brewing of beverages.
- Composed of two D-glucose sugars joined by an α -1,4 linkage.



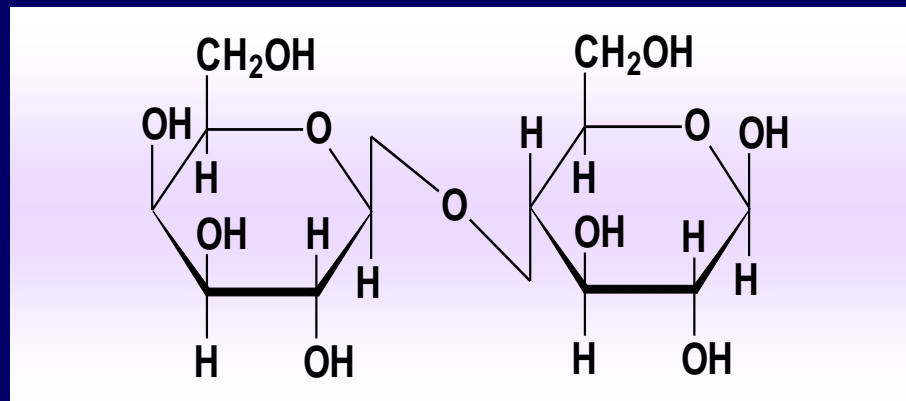
Important Carbohydrates

* Disaccharides

composed of 2 monosaccharide units.

2. Lactose - milk sugar.

- Found in milk and milk products.
- Composed of one galactose and one glucose unit joined by a β -1,4 linkage.



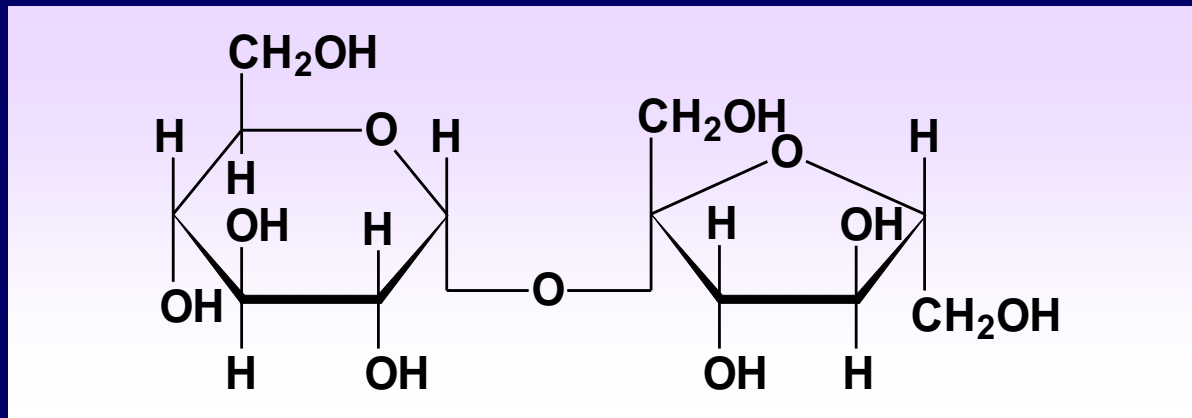
Important Carbohydrates

* Disaccharides

composed of 2 monosaccharide units.

3. Sucrose - table sugar.

- Product of sugar cane and sugar beets.
- Composed of one glucose and one fructose unit.
- Linkage is at both anomeric carbons.



Important Carbohydrates

* Polysaccharides

composed of many (more than 10) monosaccharide units.

- 1- Cellulose:
- Major structural material of plant cells.
- Consists of many glucose units joined by β -1,4 linkages.

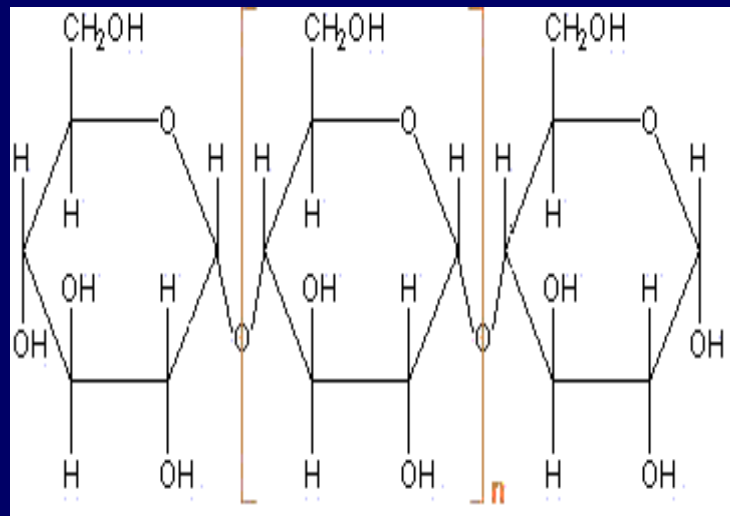
Important Carbohydrates

* Polysaccharides

composed of many (more than 10) monosaccharide units.

2. Starch:

- Storage form of glucose found in rice wheat, potatoes, grains and cereals.
- Consists of many glucose units joined by α -1,4 linkages.
- Maltose is the disaccharide starting material.



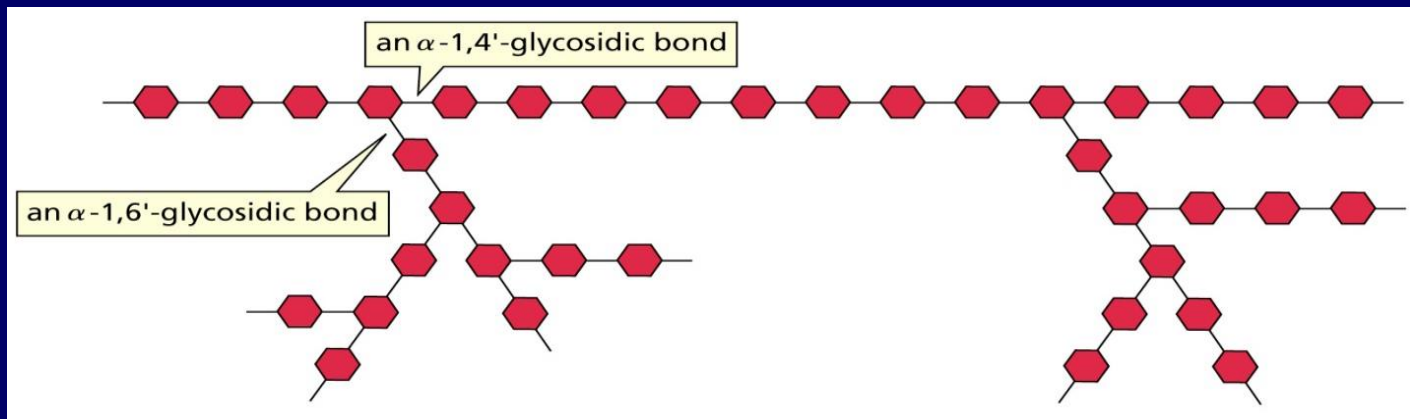
Important Carbohydrates

* Polysaccharides

composed of many (more than 10) monosaccharide units.

3. Glycogen:

- Animal starch. Storage form of glucose found in the liver and muscle of animals.
- Contains many highly branched glucose units.
- Joined by α -1,4 linkages and branched by α -1,6 linkages.



Important Carbohydrates

* Polysaccharides

composed of many (more than 10) monosaccharide units.

4. Dextrin:

- Mixture of branched and un-branched soluble polysaccharides produced by partial hydrolysis of starch by acids or amylases.

Reducing sugars

- Any sugar that contains either:
 - 1- A free aldehyde group.
 - 2- An α -hydroxy ketone group.
 - 3- A hemiacetal linkage
- The presence of any of these groups allows the carbohydrate to undergo easy oxidation.
- If the sugar gets oxidized it causes reduction.
- Thus the name “reducing sugar”.

QUALITATIVE TESTS FOR CARBOHYDRATES

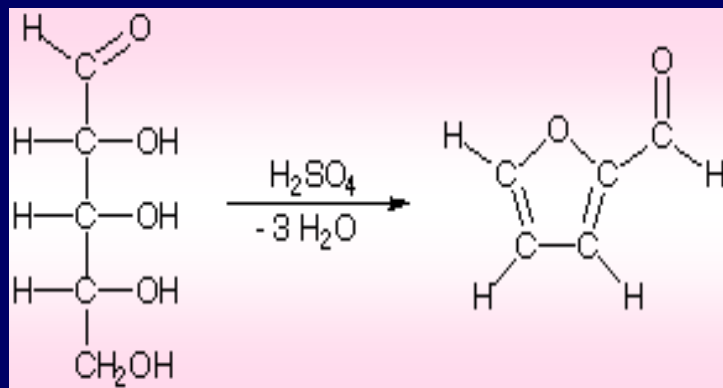
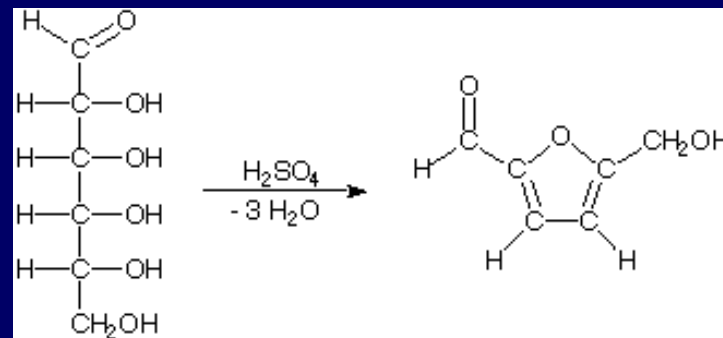
Exp. 1 Molisch Test

- It is the general test for all carbohydrates.
- Monosaccharides give a rapid positive test. Disaccharides and polysaccharides react slower.

Exp. 1

Molisch Test

- The Molisch reagent dehydrates pentoses to form furfural.
- dehydrates hexoses to form 5-hydroxymethyl furfural.
- The furfurals further react with - naphthol present in the test reagent to produce a purple product.

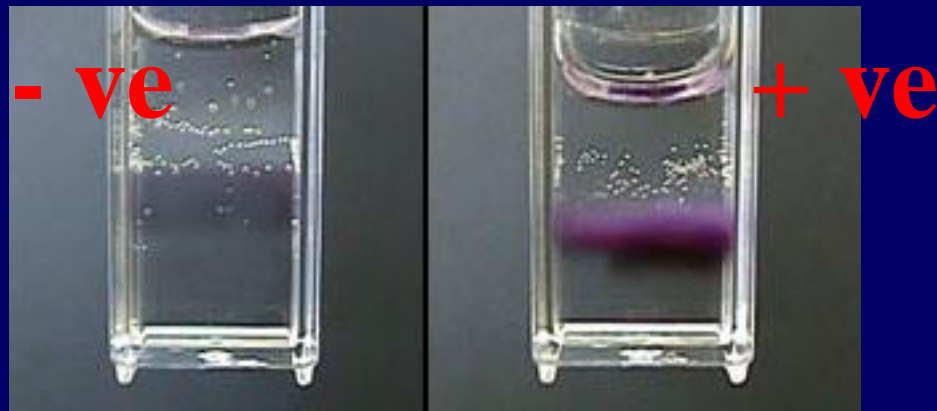


Exp. 1

Molisch Test

■ Method:

- 1ml test solution + 2 drops of α -naphthol
- mix well
- add conc. H_2SO_4 down the side of the tube to form the ring at the interface of the two layers.

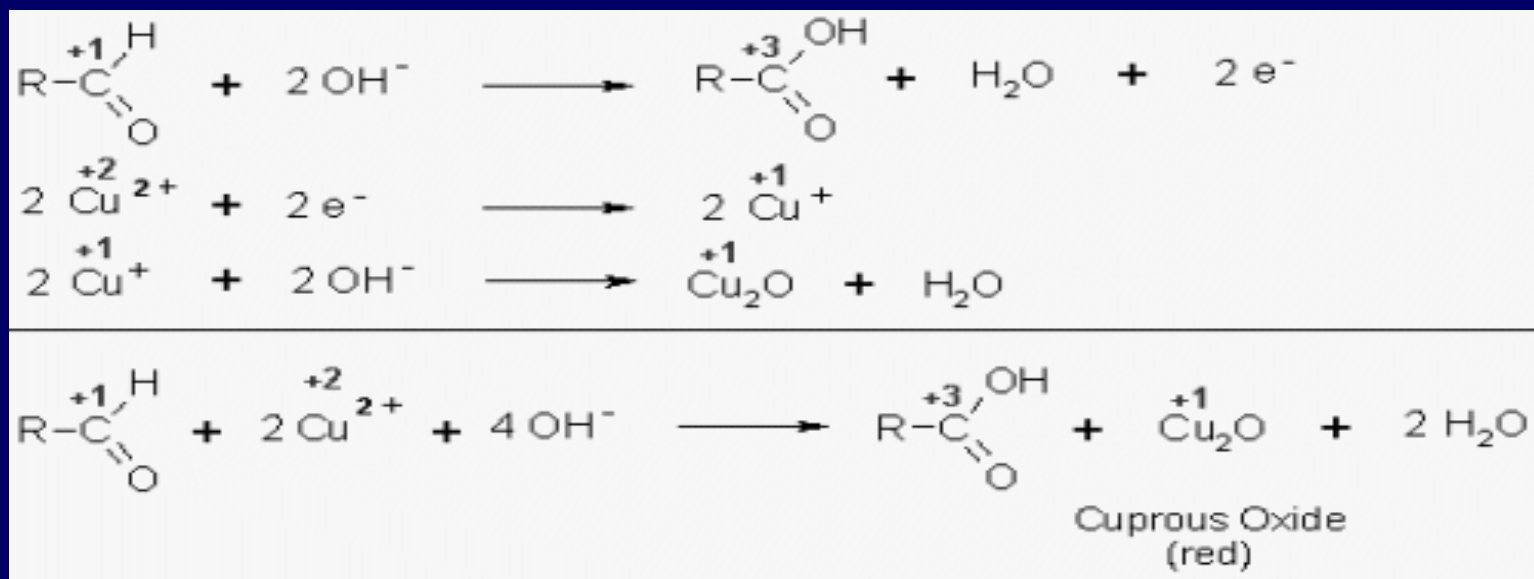


Exp. 2 Fehling's Test

- This test is used to differentiate between reducing and non reducing sugars.
- A reducing sugar reacts with Fehling's reagent in alkaline medium to form an orange to red precipitate.
- Fehling's reagent is commonly used for reducing sugars but is known to be not specific for aldehydes.
- Positive result is detected by reduction of the deep blue solution of cupric (II) to a red precipitate of insoluble cuprous oxide (Cu_2O).

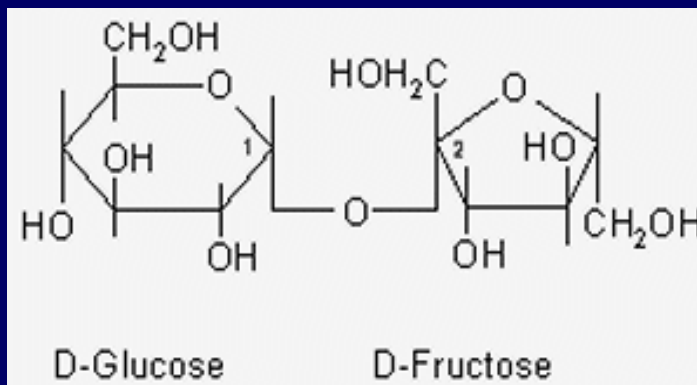
Exp. 2 Fehling's Test

- Positive result is detected by reduction of the deep blue solution of cupric (II) to a red precipitate of insoluble cuprous oxide (Cu_2O).



Exp. 2 Fehling's Test

- The sucrose does not react with Fehling's reagent. Sucrose is a disaccharide of glucose and fructose. Most disaccharides are reducing sugars (e.g. lactose and maltose)
- Sucrose is non-reducing sugar because the anomeric carbon of glucose is involved in the glucose- fructose bond and hence is not free to form the aldehyde in solution.



Exp. 2 Fehling's Test

- *Fehling's Reagent:* 2 solutions are required:

*Fehling's "A"

uses 7 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ dissolved in distilled water containing 2 drops of dilute sulfuric acid.

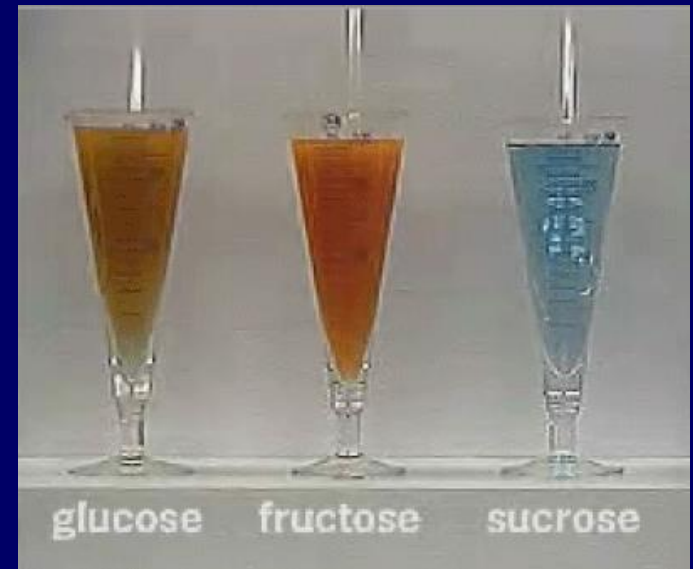
*Fehling's "B"

uses 35g of potassium tartrate and 12g of NaOH in 100 ml of distilled water.

Exp. 2 Fehling's Test

■ Method:

- 1ml test solution + 1ml Fehling's reagent
- heat the mixture in BWB (5min)
- Reddish brown ppt



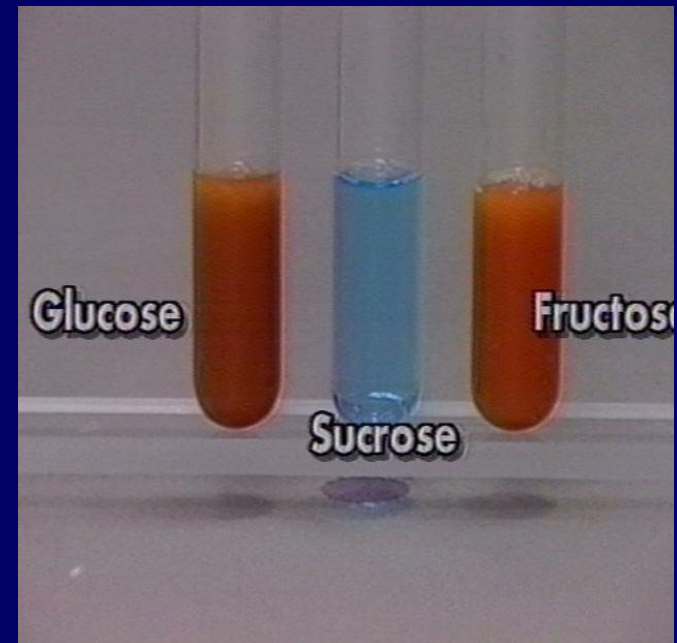
Exp. 3 Benedict's Test

- This test is used also to differentiate between reducing and non reducing sugars.
- It works on the same principle but Benedict is more stable than Fehling's reagent.
- Benedict's reagent contains blue copper(II) sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) which is reduced to red copper(I) oxide by aldehydes, thus oxidizing the aldehydes to carboxylic acids.
- The copper oxide is insoluble in water and so precipitates. The colour of the final solution ranges from green to brick red depending on how many of the copper (II) ions are present.

Exp. 3 Benedict's Test

■ Method:

- 1ml test solution +
1ml Benedict's reagent
- heat the mixture in BWB
(5min)
- Reddish brown ppt



Exp. 4 Barfoed Test

- It is a test used to differentiate between monosaccharides and disaccharides. This reaction will detect reducing monosaccharides in the presence of disaccharides. This reagent uses copper ions to detect reducing sugars in an acidic solution. Barfoed's reagent is copper acetate in dilute acetic acid (pH 4.6)
- Reducing monosaccharides are oxidized by the copper ions in a weak acidic medium to form a carboxylic acid and a reddish ppt of Cu_2O (cuprous oxide).
- Reducing disaccharides (lactose but not sucrose) undergo the same reaction but at slower rate.

Exp. 4

Barfoed Test

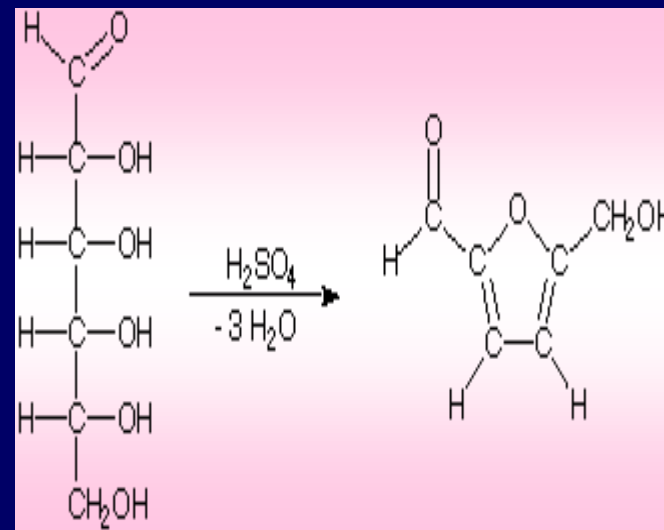
■ Method:

- 1 ml of the solution to be tested + 2 ml of freshly prepared Barfoed's reagent.
- Place test tubes into a boiling water bath and heat for 2 minutes.
- Remove the tubes from the bath and allow to cool.
- Formation of a green, red, or yellow precipitate is a positive test for reducing monosaccharides.
- Do not heat the tubes longer than 3 minutes, as a positive test can be obtained with disaccharides if they are heated long enough.

Exp. 5

Seliwanoff Test

- The test reagent dehydrates ketohexoses to form 5-hydroxymethylfurfural.
- 5-hydroxymethylfurfural further reacts with resorcinol present in the test reagent to produce a red product within two minutes.
- Aldohexoses react to form the same product, but do so more slowly.

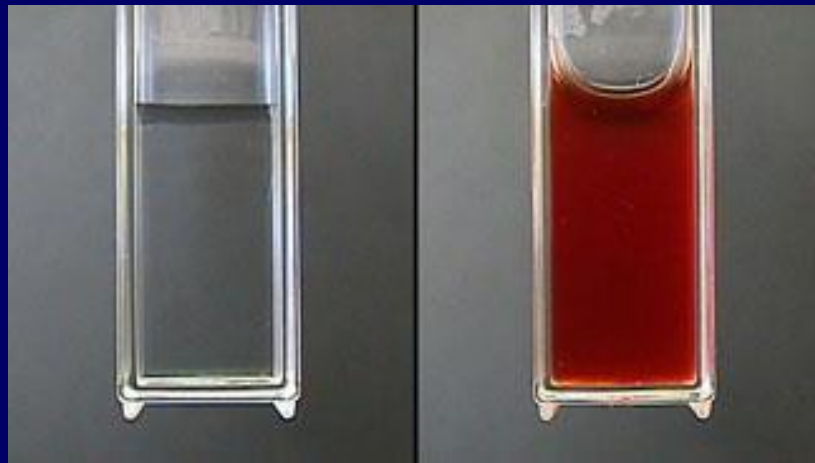


Exp. 5

Seliwanoff Test

■ Method:

- 1/2 ml of a sample + 2ml of Seliwanoff's reagent (a solution of resorcinol and HCl) is added.
- The solution is then heated in a boiling water bath for two minutes.
- A positive test is indicated by the formation of a red product.
- In case of sucrose, avoid over-boiling because sucrose may be hydrolyzed to its component (glucose and fructose) and gives false positive result.



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Exp. 6 Hydrolysis Test

- Sucrose is the only non-reducing sugar so it does not reduce the alkaline Cu solutions (Fehling and Benedict). Sucrose must first be hydrolyzed to its component and then test for.

Exp. 6 Hydrolysis Test

■ Method:

- 6 ml of 1% sucrose in a test tube + 2 drops of concentrated hydrochloric acid (HCl).
- Heat the tube in a boiling water bath for 15 minutes.
- Then test for Fehling, Benedict and all the previous tests.

Exp. 7

Iodine Test

(Test for Polysaccharides)



1- Starch:

- 1/2 mL of the fresh starch solution + 1 drop of the iodine solution.
- A dark blue color indicates a positive test for starch.
- If the yellow color of the iodine reagent simply becomes diluted, no starch is present.
- Record the observation as positive (blue) or negative (yellow).

Exp. 7

Iodine Test

(Test for Polysaccharides)

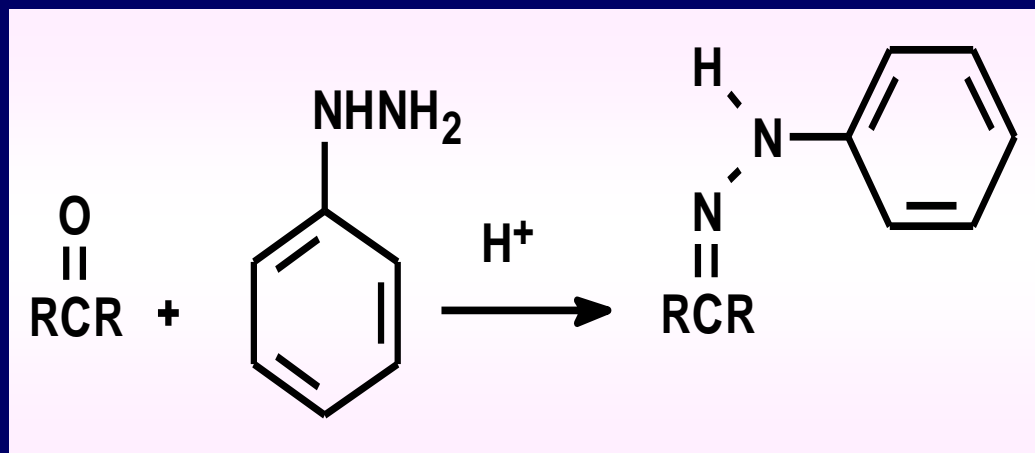


2- Dextrin:

- 1/2 mL of the fresh dextrin solution + 1 drop of the iodine solution.
- A **violet** color indicates a positive test for dextrin.
- If the **yellow** color of the iodine reagent simply becomes diluted, no dextrin is present.
- Record the observation as positive (violet) or negative (yellow).

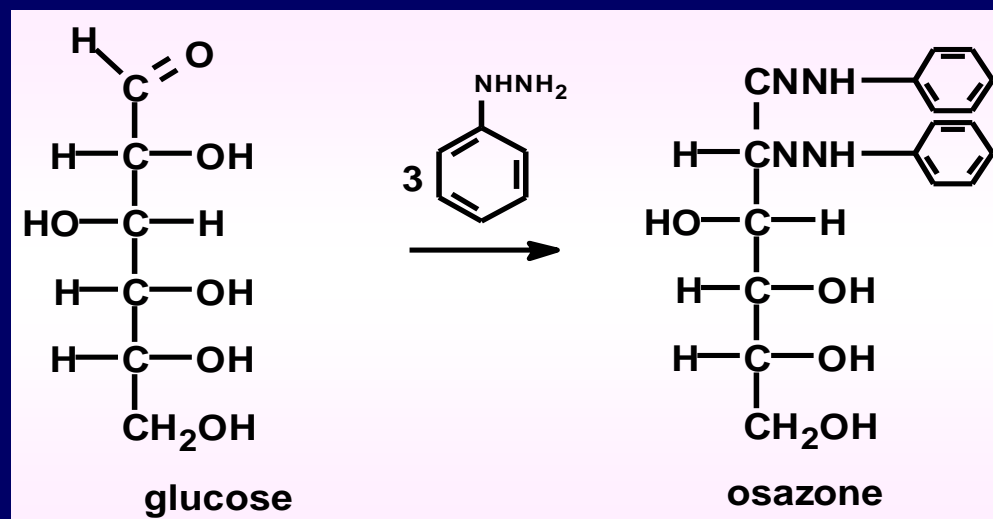
Exp. 8 The preparation of osazone

- Phenyl hydrazine reacts with normal carbonyls to produce phenyl hydrazones.



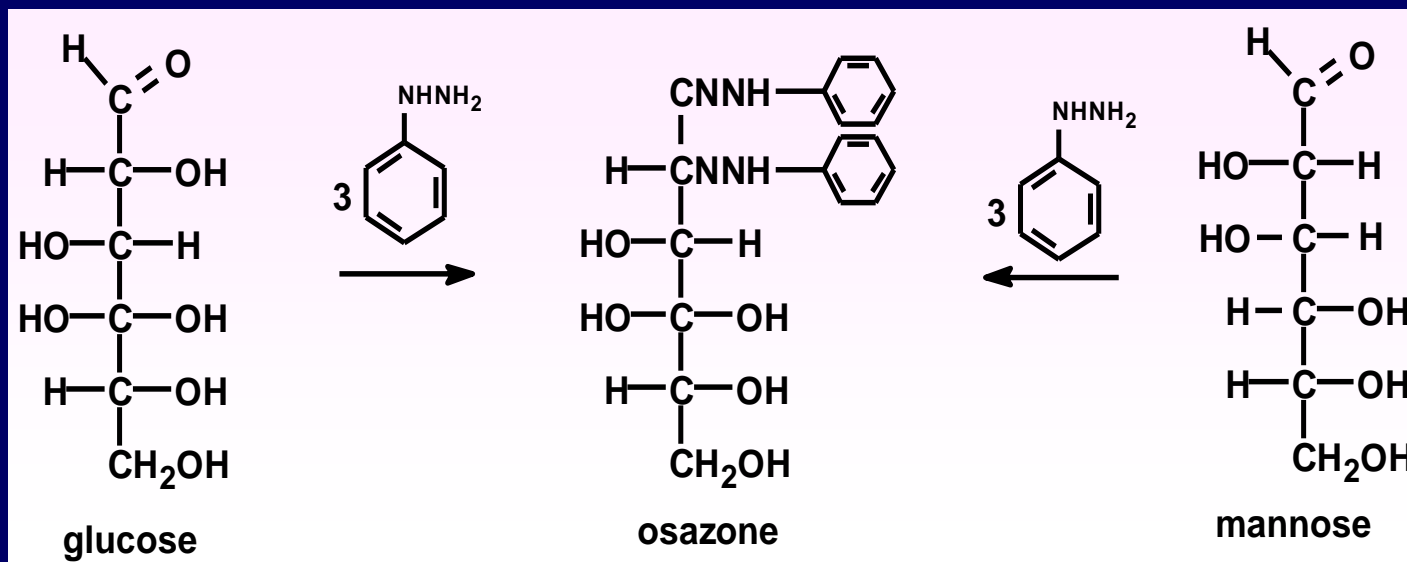
Exp. 8 The preparation of osazone

- 1- Sugars undergo a variation of this reaction in which 3 molecules of phenylhydrazine react with the sugar to produce a 1,2-diphenylhydrazone.
- These 1,2-diphenylhydrazones are known as osazones.



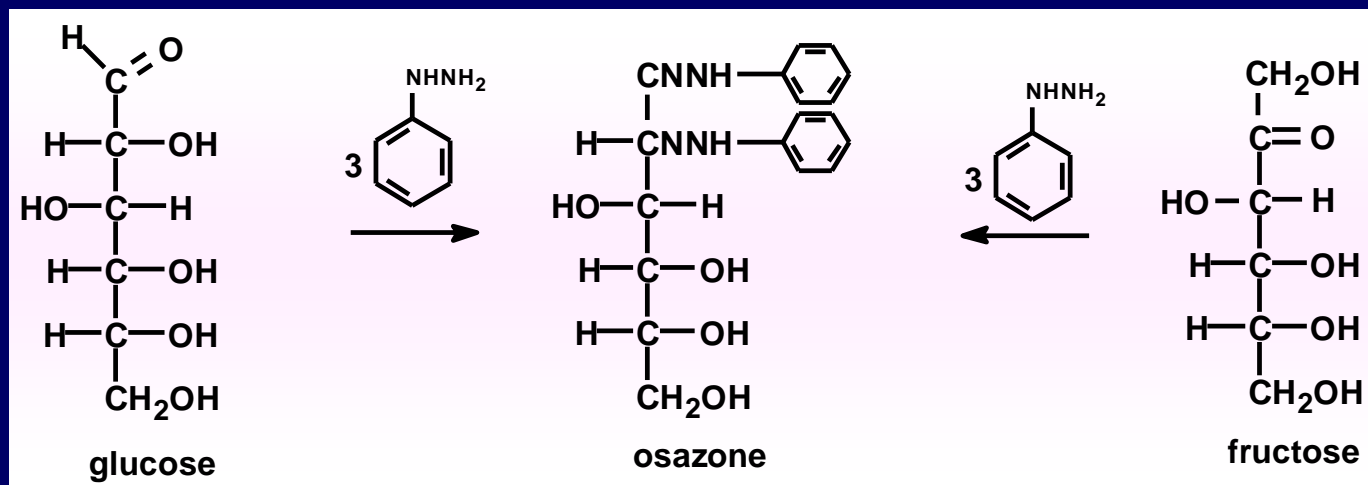
Exp. 8 The preparation of osazone

- Because both carbons 1 and 2 are involved in the reaction C-2 epimers produce the same osazone.



Exp. 8 The preparation of osazone

- Ketoses with configurations identical to aldoses below C-2 give the same osazones e.g. glucose and fructose.
- Explain glucose and fructose form the same osazone?



Exp. 8 The preparation of osazone

■ Characteristics of osazones:

- Have a characteristic shape.
- Have characteristic melting points.
- Specific time and whether the osazone is formed from hot solutions or only on cooling.

Exp. 8 The preparation of osazone

- Glucose + Phenyl hydrazine



Glucosazone (broomed shape)



- Fructose + Phenyl hydrazine



Fructosazone (broomed shape)

Exp. 8 The preparation of osazone

- Maltose + Phenylhydrazine



Maltosazone (spherical shape)



- Lactose + Phenyl hydrazine



Lactosazone



- Sucrose + Phenyl hydrazine
-ve (WHY?)

Exp. 8 The preparation of osazone

■ Method:

- 1/2 g of phenyl hydrazine +
1 spoon sodium acetate +
2ml of glucose solution
- BWB (45 min) until yellow crystals appear
- cold and examine a sample of crystals under microscope.
- Compare the crystal shapes with the supplied photographs.

