EXPERIMENT #12 – CARBOHYDRATES

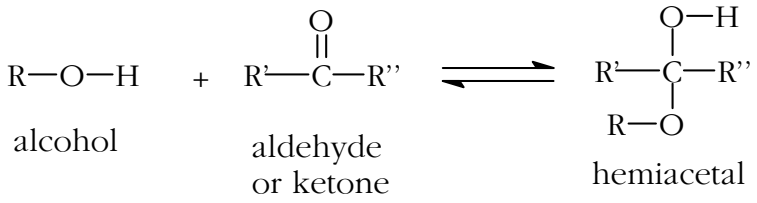
Introduction –

The name "carbohydrate" suggests that this class of compounds somehow consists of hydrated carbon. This idea gains some credence from the facts that (1) the molecular formulas of many carbohydrates are of the form $C_x(H_2O)_y$ and (2) when certain dehydrating agents are added to certain carbohydrates (concentrated sulfuric acid to sucrose, for example) a black carbonaceous mass is formed. In fact, carbohydrates are polyhydroxy aldehydes or ketones, or compounds that can be hydrolyzed to give polyhydroxy aldehydes or ketones.

The simplest carbohydrates are called *monosaccharides;* they are simple sugars and, as the name suggests, contain one sugar unit. When two sugar units are covalently bonded together, the carbohydrate is a *disaccharide*. If a few sugar units are joined together, the compound is called an *oligosaccharide*, and if many sugar units are joined together, the carbohydrate is called a *polysaccharide*. Glucose (grape or blood sugar) and fructose (found in many fruits) are monosaccharides; sucrose (table sugar) is a disaccharide; starch and cellulose are polysaccharides.

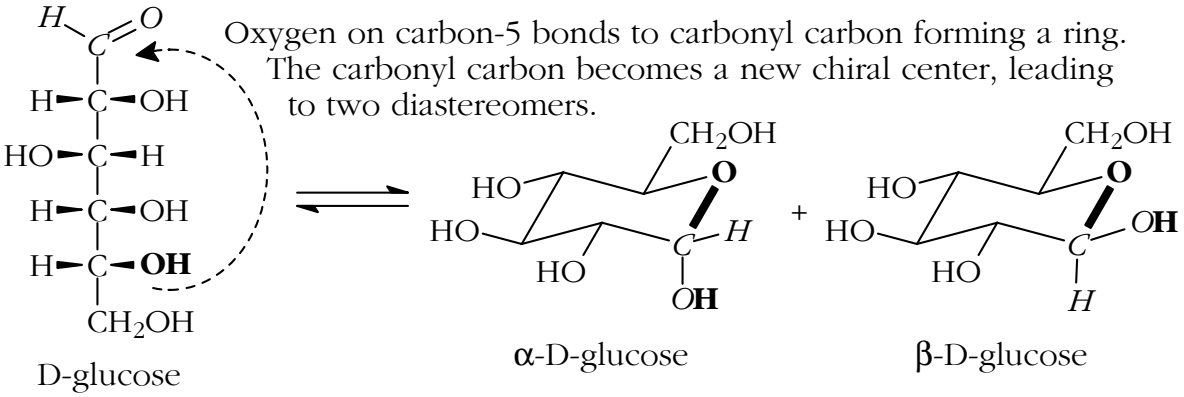
Monosaccharides that are aldehydes can be called aldoses and those that are ketones can be called ketoses.

Alcohols can react with aldehydes and unhindered ketones to produce compounds known as hemiacetals. (The hemiacetal functional group has a carbon attached to an -OH and an -OR group; it is an alcohol and an ether.) In aqueous solution this is a reversible reaction; an equilibrium is established in which molecules of the alcohol and carbonyl components react to form molecules of the hemiacetal at the same time that other molecules of the hemiacetal are breaking apart to form molecules of alcohol and carbonyl compound.



In the above example the hydroxy group was part of one molecule (the alcohol) and the carbonyl was part of another (aldehyde or ketone). In the case of monosaccharides the monosaccharide molecule contains both the hydroxy group and the carbonyl group. Hence, these two groups can react with each other within the same molecule. As a result of the new bond that is formed between the oxygen of the hydroxy group and the carbonyl carbon, the monosaccharide becomes a cyclic hemiacetal. This is shown below for the monosaccharide glucose. It is the **OH group in bold face** that reacts with the *aldehyde group, shown in italics*. The new bond that is formed between the hydroxy oxygen and carbonyl carbon to make the ring is shown bold.

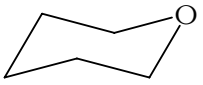
[We should mention, in passing, that glucose, like most carbohydrates, is a chiral molecule. The enantiomer shown is the D-enantiomer, and it is this enantiomer that almost always shows up in nature, in free or combined form. The other enantiomer, found only rarely in nature, is the L-enantiomer and it is, of course, the mirror image of D-glucose.]



Glucose in its open chain (polyhydroxyaldehyde) form.

Glucose in its hemiacetal (pyranose) forms.

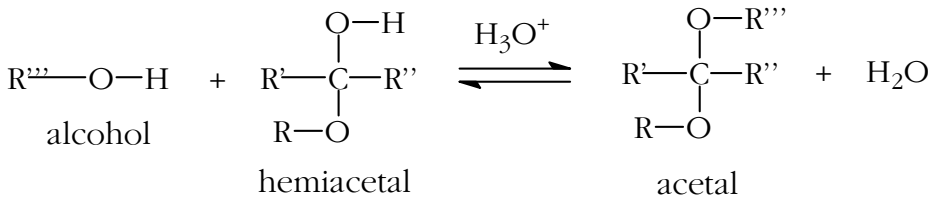
Two diastereomers of the hemiacetal (or pyranose) form of D-glucose are formed in the reaction as the result of the fact that the reaction generates a new chiral center in the molecule. When a monosaccharide contains a six-member ring including one oxygen, it can be called a pyranose, being named after pyran, the six-member cyclic ether that contains one oxygen.



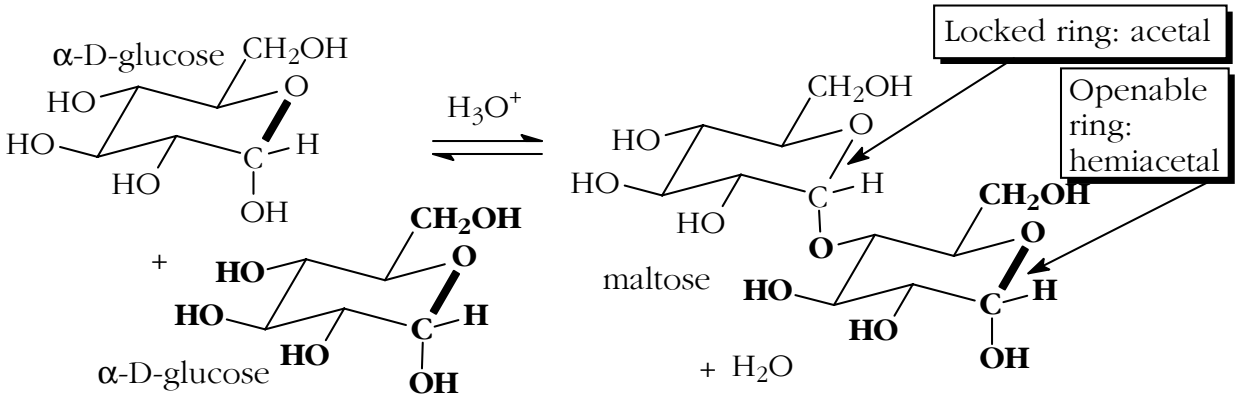
Pyran, a cyclic ether. Monosaccharides incorporating this structure are called pyranoses.

Glucose is a reducing sugar. No, you won't lose weight on a glucose diet, but it will reduce even weak oxidizing agents like Fehling's solution ($Cu^{+2} \rightarrow Cu^{+1}$) or Tollen's reagent ($Ag^{+1} \rightarrow Ag^{0}$) This is because, owing to the equilibrium shown above, at least some of the glucose is in the polyhydroxyaldehyde form and aldehydes are very easily oxidized. Almost always, sugars that have a hemiacetal ring structure are in equilibrium with the open-chain aldose or ketose form. As a result, these are reducing sugars. This is reasonable for aldoses, because aldehydes are easily oxidized, but what about the ketones, which are not usually easily oxidized? Well, it turns out that in the basic test solutions (Fehling's or Tollen's) ketoses can isomerize to aldoses. Consequently, ketoses that form hemiacetals are reducing sugars (or, more exactly, are isomerized in the test solution to aldoses which are reducing sugars). In any event, the ketoses are called reducing sugars because the test reagent gives a positive test.

Let's consider the generic hemiacetal that was formed from an aldehyde or ketone and an alcohol, above. As shown below it can react with a second molecule of alcohol to form an acetal. The acetal is stable in neutral aqueous solutions; it will not revert to hemiacetal and alcohol unless a fairly strong acid catalyst is added. This is different from the hemiacetal which usually produces at least some alcohol and aldehyde or ketone when dissolved in neutral aqueous solutions.



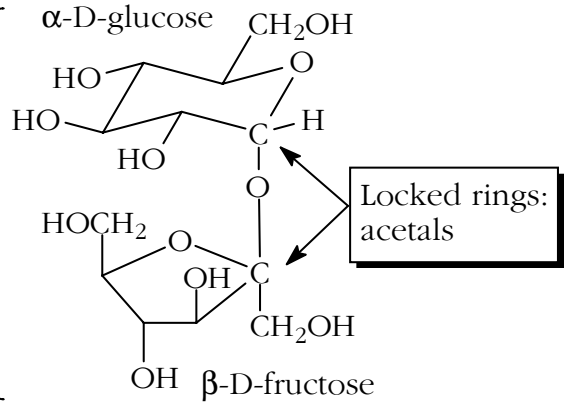
This same reaction can occur between the hemiacetal group of one monosaccharide molecule and a hydroxy group of another monosaccharide molecule. This leads to a disaccharide. For example, if the alcohol -OH on carbon-4 of an α-D-glucose molecule reacts with the hemiacetal -OH of a second α-D-glucose molecule the disaccharide maltose is formed.

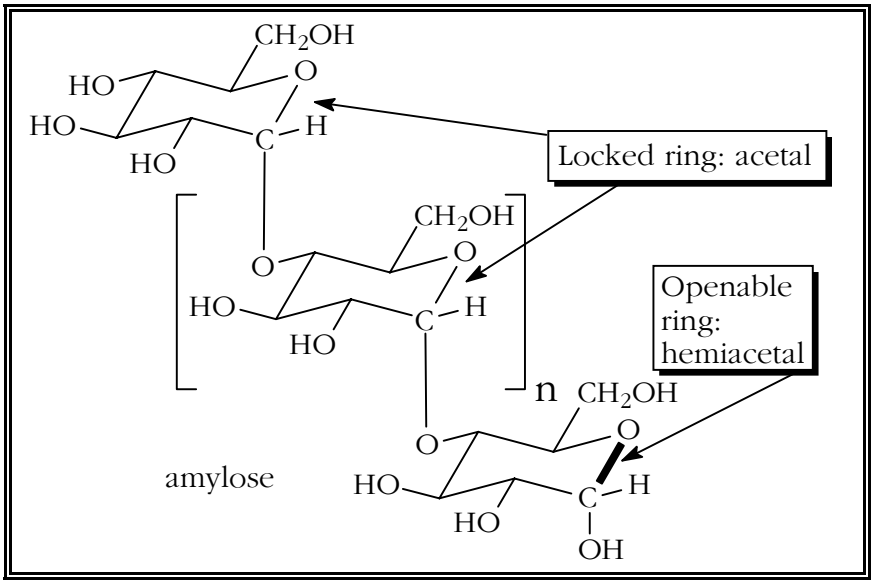


Maltose is a reducing sugar. The ring on the left contains the acetal group and will not open to give an aldehyde in neutral or basic aqueous solutions. However, the hemiacetal containing ring on the right will open in neutral or basic aqueous solutions to form an aldehyde. Therefore, maltose is a reducing sugar.

Not all disaccharides are reducing sugars; not all disaccharides have a ring that contains a hemiacetal group. Sucrose (table sugar), for example, is composed of the monosaccharides α-D-glucose and β-D-fructose. In this case both rings contain the acetal group and neither ring will open to give an aldehyde or α-hydroxyketone in neutral or basic solution. Therefore, sucrose is not a reducing sugar.

Sucrose - a non-reducing disaccharide: both rings have acetal groups; there are no hemiacetals that would allow ring-opening to an aldehyde or hydroxy-ketone.





Starch is composed of two polysaccharides, amylose (*ca.* 20%) and amylopectin (*ca.* 80%). Both are polymers of α-D-glucose in its pyranose form. Amylose is a linear polymer, while amylopectin is branched. In other words, aside from branching which may occur, starch is the polysaccharide version of the disaccharide maltose. In each starch molecule, the pyranose glucose unit repeats itself hundreds or thousands of times. Each of these glucose units is held to the next through an acetal ether linkage. The last unit in the chain contains the hemiacetal group. However, since there is only one hemiacetal unit out of hundreds or thousands of acetal units, starch does not give a positive test with Tollen's or Fehling's test solutions.

If non-reducing disaccharides or the components of starch are hydrolyzed, the acetal functional groups will be converted to hemiacetals. Hydrolysis is catalyzed by strong acids and certain enzymes. For example, when sucrose, a non-reducing sugar, is hydrolyzed the reducing sugars glucose and fructose are formed. Likewise, when starch is hydrolyzed, the reducing sugar glucose is produced.

Fehling's solution contains copper(II) sulfate, sodium tartrate, and sodium hydroxide. The tartrate forms a blue complex with Cu^{+2}, which would otherwise be precipitated as $Cu(OH)_2$ in the basic solution. In the presence of a reducing sugar the Cu^{+2} is reduced to Cu^{+1}. Cu^{+1} is not complexed by tartrate; it precipitates from solution as brick-red copper (I) oxide, Cu_2O. The appearance of the brick-red color is a positive test as is a yellow-green color, which results from seeing the red precipitate through a blue solution.

When iodine is added to starch an intense dark blue color results. This is the result of the I_2 molecules being lined up head to tail surrounded by a helix of starch. Because of this color, aqueous starch can be used to detect iodine and aqueous iodine can be used to detect starch.

Objectives of the Experiment –

1. To examine the reducing and non-reducing properties of several carbohydrates.
2. To hydrolyze acetal groups in sucrose and starch and thereby form products that have the hemiacetal group.
3. To dehydrate a carbohydrate with sulfuric acid.

Procedure –

Caution: Aqueous sodium hydroxide and sulfuric acid are caustic. Wear goggles and in case of contact, wash with lots of water.

Set up a steam bath and a hot water bath that is large enough to hold two medium size test tubes and which is maintained at 45°C.

1. A reducing carbohydrate or not?

Label 5 medium size test tubes as follows: glucose, fructose, sucrose, lactose, starch. Place 20 drops of Fehling's A solution and 20 drops of Fehling's B solution in each of the test tubes. Add 10 drops of the corresponding 2% aqueous carbohydrate solution to each of the test tubes. Place the test tubes into the steam bath and steam them for about five minutes. Record the results on the report sheet.

2. Hydrolysis of sucrose (acid versus base catalysis).

Here you will determine if sucrose is hydrolyzed under acidic conditions, basic conditions, or both.

Label two medium size test tubes #1 and #2. To each of the test tubes add 3 ml of 2% aqueous sucrose.

To test tube #1 add 3 ml of deionized water and 3 drops of 3M sulfuric acid. To test tube #2 add 3 ml of deionized water and 3 drops of 3M aqueous sodium hydroxide.

Steam both test tubes in the steam bath for 5 minutes. Allow the tubes to cool to room temperature. Add 3M aqueous sodium hydroxide to tube #1 dropwise, with stirring, until the contents turn red litmus paper blue. You will now test the contents of each tube with Fehling's solution (as you did above in part 1) as follows –

Label medium size test tubes #1a and #2a. Place 20 drops of Fehling's A solution and 20 drops of Fehling's B solution in each of the test tubes. Add 20 drops of the liquid in test tube #1 to test tube #1a and 20 drops of the liquid in test tube #2 to test tube #2a. Place test tubes #1a and #2a into the steam bath and steam them for about five minutes. Record the results on the report sheet.

3. Hydrolysis of starch (acid versus enzyme catalysis).

Label two medium size test tubes #1 and #2. To tube #1 add 2 ml (40 drops) of 3M sulfuric acid. To tube #2 add about 2 ml of your saliva. Try not to hack up a "lungie" here; we're looking for saliva, produced by the salivary glands in your mouth, which allegedly contains an enzyme that hydrolyzes starch.

Add 2 ml of 2% aqueous starch solution to each of the test tubes. Stir the contents and place the tubes in the 45°C water bath for 30 minutes.

Label two small test tubes #1 and #2. Using two clean Pasteur pipettes transfer a few drops of liquid from each tube you have been heating into the correspondingly numbered small test tube. To each small test tube add 2 drops of 0.01M aqueous iodine solution. Record the colors of the materials in each test tube, comparing them with each other.

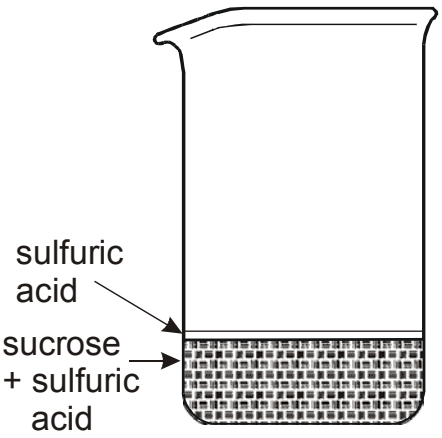
4. Kinetics of the acid catalyzed hydrolysis of starch.

Place 5 ml of starch solution in a medium size test tube and add 1 ml (20 drops) of 3M sulfuric acid. Stir the mixture and heat the tube in the steam bath for 5 minutes. Using a clean Pasteur pipette, transfer 3 or 4 drops from the heating test tube to a clean small test tube. Add 2 drops of 0.01M aqueous iodine solution to the small test tube. Observe the contents of the small test tube. If starch is still present, you will see a blue color. If you do see a blue color, continue heating the starch-acid mixture and reperform the iodine test using a clean Pasteur pipette and small test tube. Continue in this manner until you get a negative test for starch (no blue color). Record how long this took.

5. Dehydration of sucrose using concentrated sulfuric acid.

Warning! Concentrated sulfuric acid is very caustic – be careful and, as usual, wear goggles. If you get any on you wash it off immediately with lots of water. Since oxides of sulfur will be released into the air in this experiment, you should conduct it under a fume hood.

Place about 40 grams of sucrose in a 150 ml beaker. Place the beaker in a fume hood. While you are stirring the sucrose with a glass stirring rod add concentrated sulfuric acid at a moderate rate until the sucrose is thoroughly wetted with the acid but is not awash in it. When you are finished adding the sulfuric acid, there should be a thin layer of acid above the sucrose-acid mixture. Wait a few minutes. Record your observations.



When the contents of the beaker have cooled, hold the beaker under running water. After the solid contents of the beaker have been thoroughly washed with water you may deposit solids in a waste basket. Clean the beaker with soap and water.