

## Experiment 27

Fig. 27-1



# CARBOHYDRATES

### Text Topics and New Techniques

Carbohydrate chemistry,  
qualitative tests for carbohydrates.

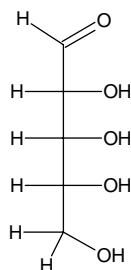
Emil Fischer (1852 - 1919) received the Nobel Prize in chemistry in 1902 for his elegant work on the structure of sugars. He also was responsible for developing many important reactions including Fischer esterification.  
<http://nobelprize.org/chemistry/laureates/1902/fischer-bio.html>

### Discussion

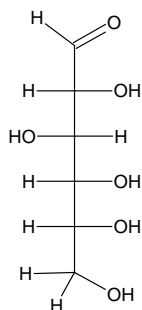
Unraveling the chemistry of sugars was one of the most challenging problems that confronted chemists in the 19th century. Although H. Fehling had quantitatively analyzed sugars as early as 1849 (1) and Alexander Butlerov had succeeded in synthesizing sugars in 1861 by polymerizing formaldehyde, (2, 3) all that was definitely known about sugars prior to the 1870s was that their empirical formulas were approximately  $\text{CH}_2\text{O}$ . For example, the formulas of glucose and sucrose are  $\text{C}_6\text{H}_{12}\text{O}_6$  and  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$  respectively. Please note that these formulas could also be written as  $\text{C}_6(\text{H}_2\text{O})_6$  and  $\text{C}_{12}(\text{H}_2\text{O})_{11}$ . Originally, these formulas led to the misconception that sugars contained hydrated carbons. The term carbohydrate was then coined to describe this class of compounds. Despite the fact that later structure determinations revealed that “hydrated carbons” is an incorrect description of the bonding in sugars, the name has stuck. The molecular masses of sugars were first measured in 1888 by Bernhard Tollens using a freezing point depression method developed in 1880 by Francois-Marie Raoult (4).

One of the principal hurdles in the development of carbohydrate (or sugar) chemistry was finding reactions of sugars that would yield crystallizable addition products (derivatives). Before the development of spectroscopy in the middle to late 20th century, derivatives played an extremely important role in the analysis and identification of organic compounds. In 1884 Emil Fischer succeeded in making phenylhydrazine derivatives of sugars (known as osazones) (5). The understanding of carbohydrate chemistry proceeded rapidly thereafter. As knowledge about carbohydrates increased, tests that are still used today were developed to distinguish between the different types of sugars. You will use some of those tests in this experiment to determine the identity of an unknown carbohydrate.

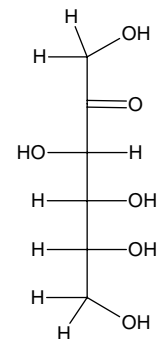
Carbohydrates may differ from one another in a variety of ways. Simple sugars (monosaccharides) may differ in the number of carbons in the carbon chain. The most common simple carbohydrates are hexoses (with 6 carbons) such as glucose and fructose, and pentoses (with 5 carbons) such as ribose. Simple carbohydrates with as few as 3 carbons and as many as 9 carbons are found in nature. [Please note that the figures below are called Fisher Projections. At every junction of lines, there is a carbon.]



D-ribose  
5 carbon aldehyde



D-glucose  
6 carbon aldehyde

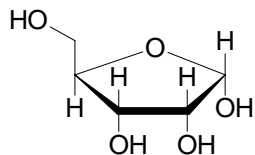


D-fructose  
6 carbon ketose

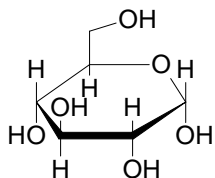
open forms of:

Another way in which the simple sugars differ is in the positions of the carbonyl groups. If the carbonyl group is at the end of the chain (an aldehyde), the sugar is an aldose. If the carbonyl is not at the end of the chain (a ketone), the sugar is a ketose. The structures shown above for glucose and fructose illustrate this difference. In some simple sugars, such as 2-deoxyribose (found in DNA), an OH group is replaced by a hydrogen. These sugars are known as desoxy sugars.

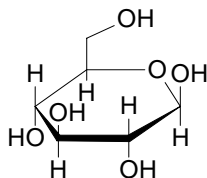
While the open forms of the sugars illustrated above can account for many of the properties of sugars, some properties of sugars are inconsistent with open form structures. It is possible for sugars to form cyclic hemiacetal (or hemiketal) structures such as those drawn below.



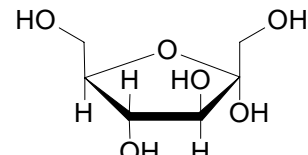
$\alpha$ -D-ribofuranose



$\alpha$ -D-glucopyranose



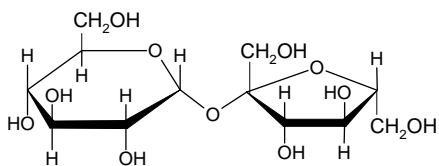
$\beta$ -D-glucopyranose



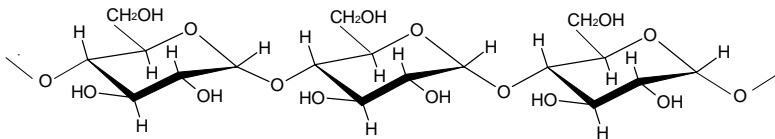
$\alpha$ -D-fructofuranose

Sugars exist predominately in the ring forms but the ring opens and closes in solution resulting in the interconversion (isomerization) of structures such as  $\alpha$ -D-glucopyranose to  $\beta$ -D-glucopyranose. The discovery of two D-glucoses was part of the evidence that led chemists to realize that the open form does not account for some of the observed properties of the sugars. The open form is still important as many of the reactions of sugars are the result of reactions of the open form.

The variety of carbohydrates does not end here. It was discovered that many sugars could be broken down into simpler sugars. The sugars that cannot be broken down into simpler sugars such as ribose, glucose and fructose are called monosaccharides. Simple sugars can combine with each other to form disaccharides (made of 2 simple sugar units) such as sucrose, oligosaccharides (made of a few simple sugar units), and polysaccharides (made of many simple sugar units) such as starch.



sucrose  
 $\alpha$ -D-glucose and  $\beta$ -D-fructose linked



section of unbranched amylose (starch)  
 $\alpha$ -D-glucose polymer

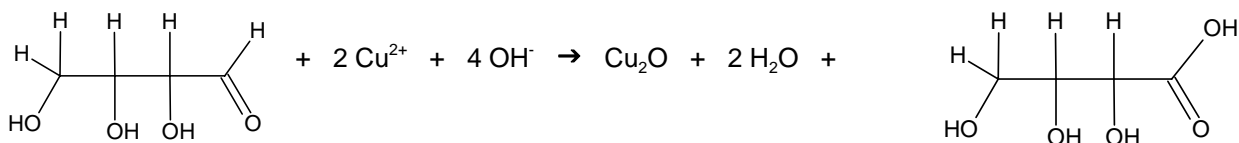
Disaccharides can be distinguished from each other by various chemical tests. Some reduce  $\text{Cu}^{+2}$  in alkaline solution (reducing sugars), some undergo fermentation and some form insoluble oxidation products (mucic acid test). Disaccharides (and monosaccharides) can be distinguished from starch by the unique reaction starch gives with iodine.

### Procedure:

**Caution:** Beakers of hot water should be manipulated using beaker tongs. Hot test tubes should be handled using a test tube clamp.

1% aqueous solutions of fructose, glucose, lactose, sucrose, starch and xylose will be available as test solutions. Each student will be provided with a 1% solution of one of the sugars in a container labeled with a code. The objective is to use the tests below to determine the identity of your unknown.

**A. The reducing sugar test.** The method Fehling used in 1849 to analyze sugars was to oxidize them to aldonic acids with  $\text{Cu}^{+2}$  ion in alkaline solution. As the sugar is oxidized, the copper is reduced to orange-brown  $\text{Cu}_2\text{O}$ .

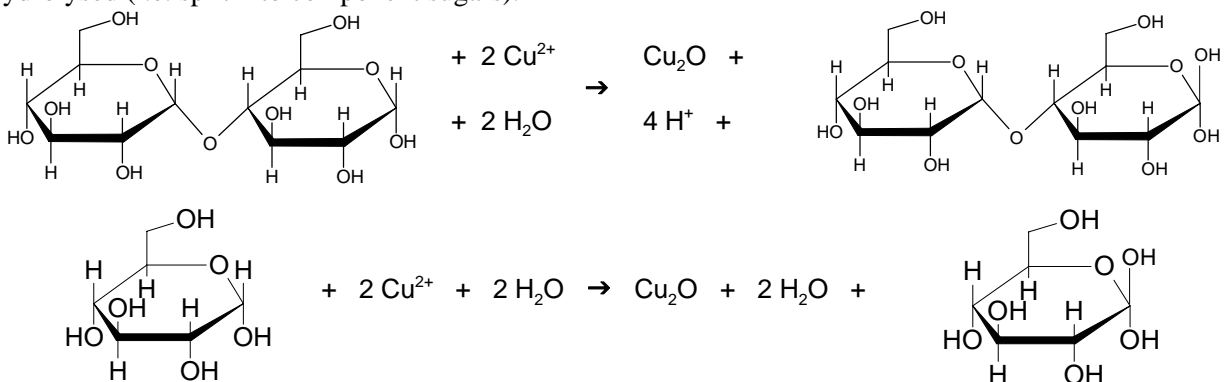


The appearance of this colored precipitate identifies the carbohydrate as a reducing sugar. However, not all carbohydrates give positive tests. To test positive, the carbohydrate must have an oxidizable aldehyde group, or be easily isomerized to (changed into) a sugar with an oxidizable aldehyde group.

Fehling's procedure was improved upon in 1908 by S.R. Benedict (6). The Benedict test is essentially the same as Fehling's except that only one reagent is required instead of two.

1. Put 250 mL of deionized water into a 600 mL beaker, put the beaker on a hot plate and heat the water to boiling.
2. Put 1 mL of each of the 1% carbohydrate solutions to be tested (fructose, glucose, lactose, sucrose, starch, xylose) in labeled test tubes (one solution per tube). Put 1 mL of your 1% solution of your unknown in a seventh tube. Add 2 mL of Benedict's solution to each of the tubes and place the tubes in the boiling water for 3 minutes. Record your results in the chart on page E27-8.

**B. Barfoed's test.** The test to distinguish monosaccharides from disaccharides was developed before Fischer had prepared sugar derivatives and before the molecular masses of sugars were known. Chemists speculated that sugars could link up with each other because it had been observed that some sugars possess close to twice the molecular mass of other sugars. This suggested that the heavier sugars were composed of two simple sugar units. Another clue that led to the belief that sugars could link up with each other was that some sugars that reduced Fehling's solution would reduce additional Fehling's solution after they were hydrolysed (i.e. split into component sugars).



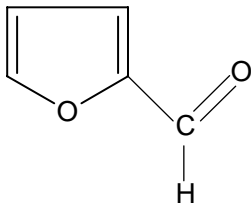
In 1873, C.T. Barfoed (7, 8) developed a simple test to distinguish monosaccharides from disaccharides. As with Benedict's and Fehling's tests, this test also depends on the reduction of  $\text{Cu}^{2+}$  to  $\text{Cu}_2\text{O}$ . However this test is done in slightly acidic solution. Under these conditions it takes longer for a reducing disaccharide to reduce Barfoed's reagent than it takes a monosaccharide.

1. Clean out the test tubes from the previous tests and transfer 1 mL of each of the carbohydrate solutions to be tested in the 7 labeled tubes.
2. Add 2 mL of Barfoed's reagent to each of the tubes and put them in the boiling water bath for 4 minutes. Record your results in the chart on page E27-8.

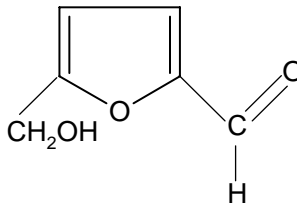
**C. Seliwanoff's test.** In 1887, T. Seliwanoff developed a test to distinguish ketoses from aldoses (9). Ketoses react rapidly with Seliwanoff's reagent to produce a red or orange color. Aldoses take longer to react. The color is produced by the reaction between resorcinol and the dehydration products of the sugars. Although sucrose isn't technically a ketose, it also gives a positive test because it hydrolyzes in the reagent to produce glucose and fructose (a ketose).

1. Clean out the test tubes from the previous tests and put 1 mL of Seliwanoff's reagent into each of them (**Caution, this reagent is somewhat corrosive**).
2. Put 3 drops of each of the carbohydrate solutions to be tested into the appropriate test tubes and put the tubes into the boiling water bath for 1 minute. Record your results in the chart.

**D. Bial's Orcinol test.** A test to distinguish between pentoses and hexoses was developed by M. Bial in 1902 (10). Pentoses dehydrate under acid conditions to produce furfural while hexoses dehydrate to hydroxymethyl furfural.



furfural



hydroxymethyl furfural

These compounds can react with a mixture of iron (III) chloride and orcinol (Bial's orcinol reagent). Furfural (from pentoses) gives a blue (at first green) color with the reagent. The reaction product of  $\text{Fe}^{+3}$ , orcinol and hydroxymethylfurfural (from hexoses) is yellow or brown without any blue color.

1. Clean out the test tubes from the previous tests and put 1 mL of Bial's orcinol reagent into each of them (**Caution: This reagent is corrosive and its vapors are irritating**).
2. Put 3 drops of the carbohydrate solutions to be tested in the appropriate test tubes and put the tubes in the boiling water bath until there has been a color change in all of them. This may happen very quickly if the reagent is fresh. Record your results in the chart on page E27-8.

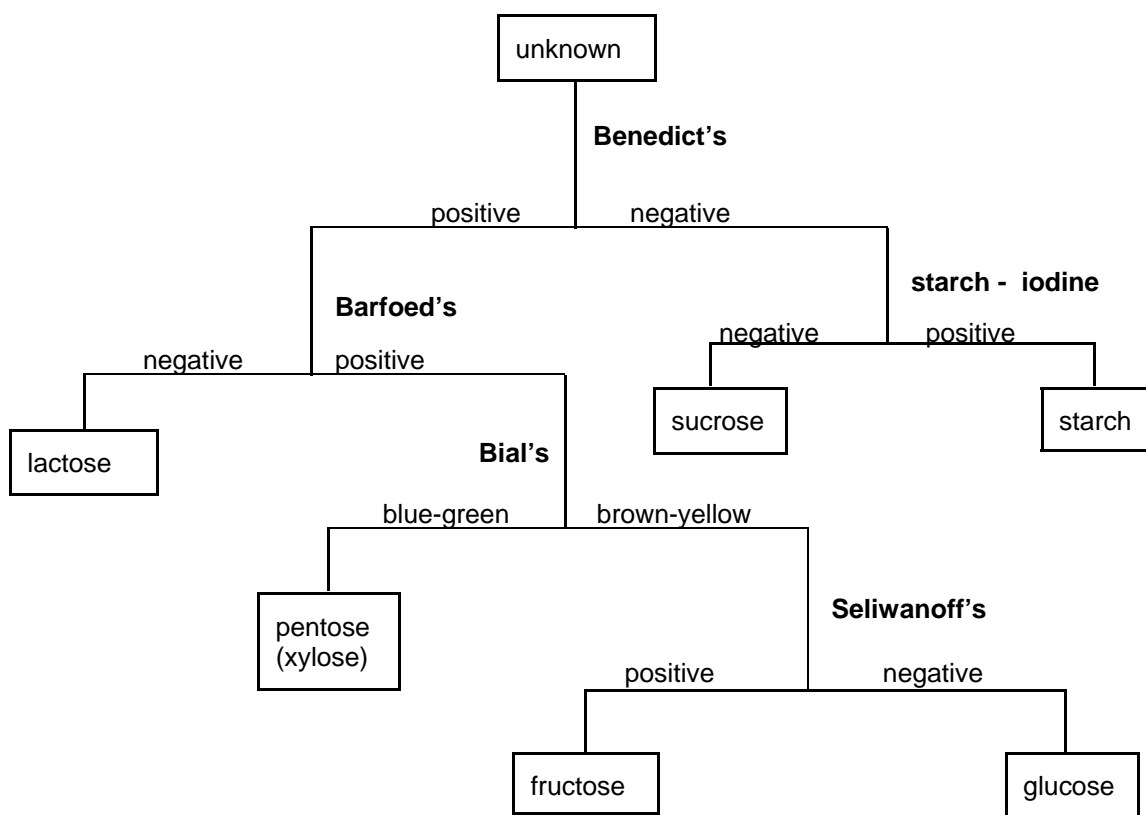
**E. The Starch-iodine test.** The reaction of iodine with starch has long been known. Clean out the test tubes from the previous tests and test 1 mL of each carbohydrate with 1 mL of aqueous iodine. You do not need to use the hot water bath for this test. Record your results in the chart on page E27-8.

**F. Optional additional test.**

**Fermentation.** Mix a little bakers yeast with 50 mL of a 1% carbohydrate solution. Put the mixture in a fermentation tube and put the tube in a warm place. The presence of gas (carbon dioxide) in the tube after several hours is a positive test. Note that this test requires large amounts of solution and a long time. Therefore it is better done as a demonstration.

## References

1. A significant portion of this experiment was developed and written by Dr. G. Webb-Kummer and Brian Stedjee at Modesto Junior College.
2. Morrow, E.; Sandstrom, W. *Biochemical Laboratory Methods*. second edition. John Wiley & Sons, **1935**, 148.
3. Leicester, H. *J. Chem Ed.*, **1940**, *17*, 203.
4. Gilman, H. *Organic Chemistry An Advanced Treatise*. 2nd ed., Volume II. John Wiley & Sons, **1943**, 1537.
5. *ibid.* p 1534.
6. *ibid.* p 1535.
7. Morrow, E.; Sandstrom, W. *Biochemistry Laboratory Methods*, 2nd ed., John Wiley & Sons, **1935**, p 149.
8. *ibid.* p 149.
9. Veibel, S. *J. Chem Ed.*, Sept., **1949**, *26*, 459.
10. Morrow, E.; Sandstrom, W. *Biochemical Laboratory Methods*, 2nd ed., John Wiley & Sons, **1935**, p 154.
11. *ibid.* p 152.



## Prelaboratory Preparation - *Experiment 27*

List all the goals of the experiment. Answer the questions below.

1. Should the flow diagram on the previous page work for the analysis of fructose, glucose, lactose, sucrose, starch and xylose? Explain your answer.
2. According to the flow diagram on the previous page, several of the tests in the chart on the next page are not necessary. Explain this statement and put the letters "NN" (not necessary) in each of the boxes on the next page that represents an unnecessary reaction for this analysis.
3. Which of the tests listed in the experiment would you use to distinguish between the following pairs of carbohydrates? Briefly explain each answer.
  - a. starch and maltose
  - b. ribose and glucose
  - c. fructose and glucose
  - d. galactose and lactose
  - e. galactose and glucose

## Observations and Conclusions

Record your observations in a table like the one below.

Carbohydrate	Fructose	Glucose	Lactose	Sucrose	Starch	Xylose	Unknown # _____
Benedict's test							
Barfoed's test							
Seliwanoff's test							
Bial's Orcinol test							
Iodine test							
Fermentation test							

2. What is the identity of your unknown (# = \_\_\_\_\_)? Justify your choice.