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Qualitative Testing for Carbohydrates

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Purpose of the Experiment

Study the reactions of various carbohydrates with reagents used to classify and identify these compounds.

Background Information

The body gets its energy from three main classes of food: carbohydrates, proteins, and fats. Of these classes, **carbohydrates** are the most important source of energy. When digesting food, the body begins to digest carbohydrates first. This process is relatively efficient, producing waste products that are innocuous (water) or readily removed (carbon dioxide).

Carbohydrates are widely distributed in plant tissues and are even found in certain animal tissues, such as liver and muscle. Water-soluble carbohydrates often have a sweet taste and therefore are called **sugars**. Another term for carbohydrate is saccharide.

Carbohydrates are actually polyhydroxyaldehydes and polyhydroxyketones, often but not always with the general formula $(CH_2O)_n$, where n equals 3 or more. We refer to polyhydroxyaldehydes as **aldoses** and polyhydroxyketones as **ketoses**.

Carbohydrates are divided into three general classes, depending on the number of carbohydrate molecules they contain. Individual carbohydrate molecules are classified as **monosaccharides**. Monosaccharides are further classified by the number of carbon atoms they contain. For example, we call monosaccharides containing 5 carbons, pentoses, and those containing 6 carbon atoms, hexoses. Common examples of monosaccharides include ribose, a five-carbon aldose known as aldopentose; glucose and galactose, six-carbon aldoses known as aldohexoses; and fructose, a ketohexose.

Carbohydrates containing a few monosaccharide units are classified as **oligosaccharides**. Oligosaccharides can be further subdivided into disaccharides (two-monosaccharide units), trisaccharides (three-monosaccharide units), and so forth. Common examples of disaccharides are sucrose (glucose–fructose), table sugar; maltose (glucose–glucose), corn syrup; and lactose (galactose–glucose), milk sugar.

Carbohydrates containing a large number of monosaccharide units are classified as **polysaccharides**. Of the many known polysaccharides, starch, cellulose, and glycogen are the most important. All three are made up of hundreds of thousands of glucose units connected in various patterns. One common characteristic of all oligo- and polysaccharides is that they can be hydrolyzed to their monosaccharide units by heating in slightly acidic solution.

98 97 96 95 94 — 12 11 10 9 8 7 6 5 4 3 2 1

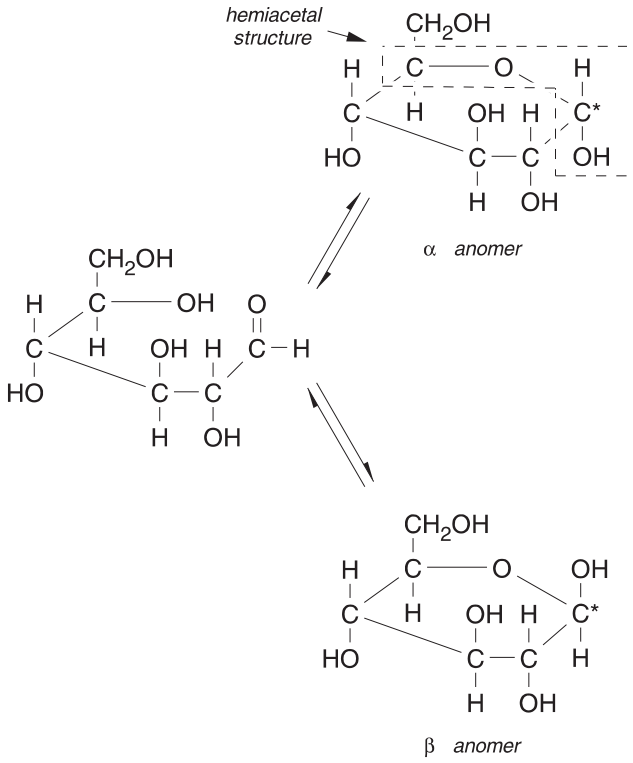


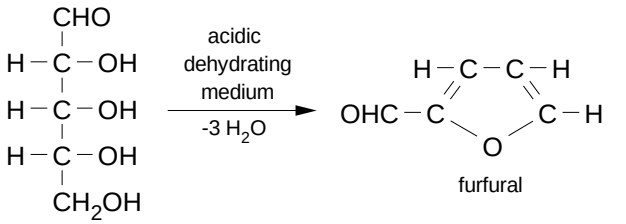
Figure 1 *Hemiacetal formation, showing the hemiacetal structure in the dotted box and the two anomers*

Aldoses undergo an intramolecular reaction between a hydroxyl group and the carbonyl group. The result is a cyclic **hemiacetal**, shown in Figure 1. We refer to the carbonyl carbon involved in the reaction as the **anomeric** carbon (C* in Figure 1). At this carbon atom there are two stereochemical configurations possible, alpha (α) and beta (β). The two molecules containing these configurations are called **anomers**.

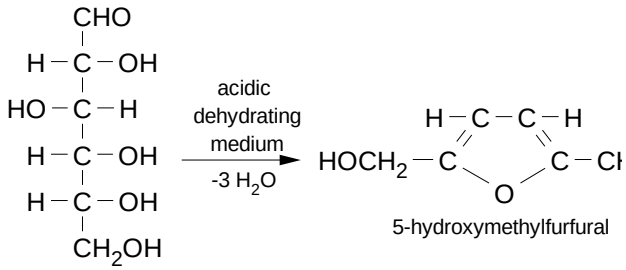
Cyclic hemiacetal anomers are more stable than the open-chain. Therefore, solutions of aldoses always have a higher concentration of anomers than of open-chain aldoses. A similar intramolecular reaction occurs between a hydroxyl group and the carbonyl group of a ketose to form a hemiketal.

Carbohydrate test reagents can be divided into three general classes based on the type of reaction involved. One class, **dehydrating acids**, causes carbohydrates to undergo dehydration, and form either furfural (from a pentose) as shown in Equation 1 or 5-hydroxymethylfurfural (from a hexose) as shown in Equation 2.

The presence of furfural or 5-hydroxymethylfurfural can be detected by the addition of one of the second class of test reagents, called **condensation reagents**. These are phenolic compounds that react



(Eq. 1)



(Eq. 2)

with furfural or 5-hydroxymethylfurfural to yield highly colored products. Reagents included in this class are Molisch reagent, Bial's reagent, and Seliwanoff's reagent. These two classes of reagents are frequently used together as part of a two-step analysis.

The third class of test reagents consists of solutions containing copper(II) ions. These reagents oxidize certain carbohydrates. Two common reagents in this class are Benedict's and Barfoed's reagents. We refer to the carbohydrates that reduce the copper(II) ions in these solutions to copper(I) oxide as **reducing sugars**. Reducing sugars are all aldoses containing either a free aldehyde group or a cyclic hemiacetal.

Note that under the reaction conditions used in tests with Benedict's and Barfoed's reagents, α-hydroxyketoses will also reduce copper(II) ions, giving a positive (but incorrect) test result. This is due to the slow conversion of the α-hydroxyketone group to an α-hydroxyaldehyde group, as shown in Figure 2. The *R* attached to the groups in Figure 2 represents the part of the molecule not involved in the reaction.

Disaccharides are oligosaccharides containing two monosaccharide units joined by an acetal or ketal linkage. The linkage results from the reaction of the hemiacetal or hemiketal form of one monosaccharide

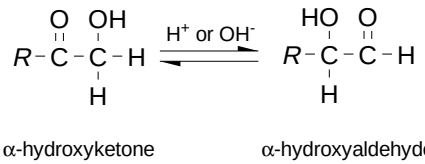


Figure 2 *Conversion of an α-hydroxyketone to an α-hydroxyaldehyde*

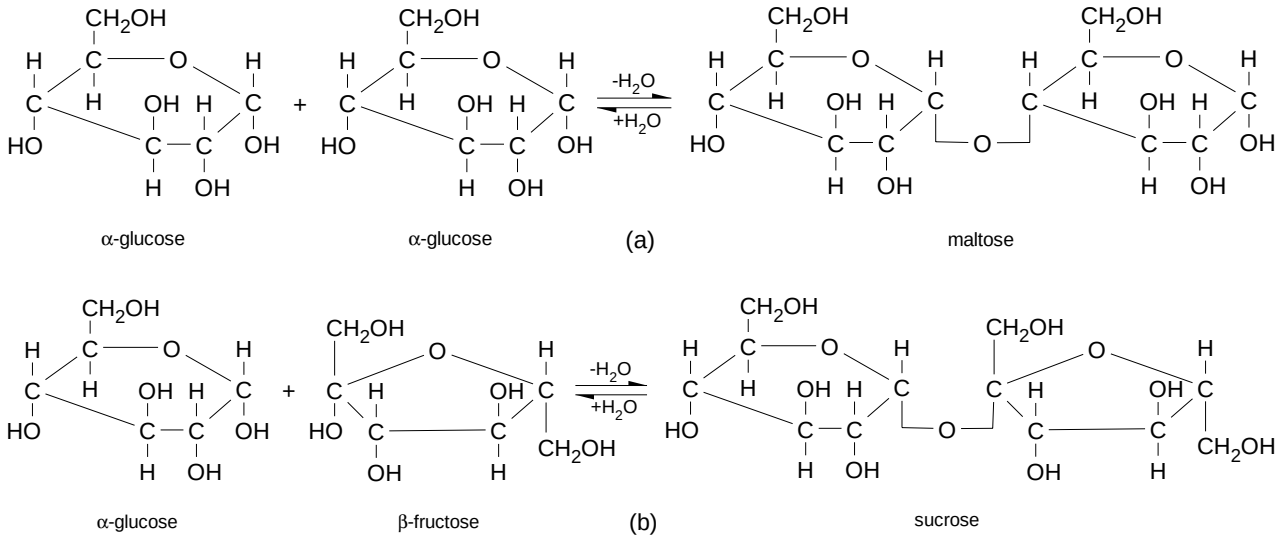


Figure 3 (a) *Formation of a reducing disaccharide*; (b) *formation of a nonreducing disaccharide*

with one of the hydroxyl groups on another monosaccharide. Disaccharides containing a free hemiacetal or hemiketal, such as maltose in Figure 3(a), are reducing sugars. Disaccharides containing no free hemiacetals or hemiketals, such as sucrose in Figure 3(b), are **nonreducing sugars**. As shown in Figure 3, disaccharide linkages can be broken by the addition of water. This reaction occurs slowly, but it can be catalyzed by acidic media.

Identifying Products of Carbohydrate Condensation Reactions

Many carbohydrates can be identified using condensation reagents, which react with the carbohydrates to produce highly colored products. The precise nature of the condensation reaction varies with the carbohydrate under investigation. Often the carbohydrate is initially dehydrated into smaller saccharide units, using a dehydrating acid. The smaller units, or molecules, formed will produce a more highly colored complex with the condensation reagent, as in Molisch's, Bial's, and Seliwanoff's tests. In other cases the carbohydrate must possess certain structural features that allow it to form a condensation product, such as in the iodine and potassium iodide test (I_2/KI). The following section describes two types of condensation reactions: those used to generally classify carbohydrates, and those used to identify specific carbohydrates.

Molisch test

The Molisch test uses concentrated sulfuric acid as the dehydrating acid. This acid dehydrates all carbohydrates, so the test is used to distinguish between carbohydrates and non-carbohydrates. The dehydration products of carbohydrates, furfural or 5-hydroxymethylfurfural, result from the reaction of the sulfuric acid with pentoses and/or hexoses (Eq. 1 and 2). These products condense with α-naphthol to yield a purple condensation product. The two-step process is represented by Equation 3.

carbohydrate → dehydration product → purple product (Eq. 3)

Iodine and Potassium Iodide Test

Starches form deeply colored blue-black complexes with iodine. Starches contain α-amylose, a helical saccharide polymer, and amylopectin. Iodine forms a large complex polysaccharide with the α-amylose helix, producing the blue-black color. Simpler oligosaccharides and monosaccharides do not form this complex with iodine. Thus, the I_2/KI test can be used to distinguish starches from other carbohydrates.

Bial's Test

Bial's test uses concentrated hydrochloric acid as the dehydrating acid and orcinol with a trace of iron(III) chloride as the condensation reagent. Bial's test is used to distinguish between pentoses and hexoses. Pentoses subjected to the test yield a blue or green condensation product, while hexoses yield a muddy brown-to-gray condensation product, as shown in Equations 4 and 5.

pentose → dehydration product: furfural → blue or green condensation product (Eq. 4)

hexose $\rightarrow$ dehydration product: $\rightarrow$ (5-hydroxymethylfurfural)
muddy brown-gray condensation product (Eq. 5)

Seliwanoff's Test

Seliwanoff's test uses 6*M* hydrochloric acid as the dehydrating acid and resorcinol as the condensation reagent. Seliwanoff's test is used to distinguish between aldoses and ketoses. When mixed with Seliwanoff's reagent, ketopentoses and ketohexoses react within 2 minutes to form a cherry-red condensation product, as shown in Equation 6.

ketose $\rightarrow$ dehydration product $\rightarrow$ cherry-red product (within 2 min) (Eq. 6)

Aldopentoses react after 2 minutes to form a blue-green condensation product, which may further change to a peach color.

Identifying Reducing Sugars

Reducing sugars are oxidized by copper(II) ions in two other saccharide test solutions: Benedict's reagent, a mildly basic solution, and Barfoed's reagent, a mildly acidic solution. The presence of red copper(I) oxide precipitate indicates that the saccharide has reduced the copper(II) ions.

Benedict's Test

Benedict's test uses a mixture of copper(II) sulfate, sodium citrate, and sodium carbonate in a mildly basic solution. This reagent is used as a general test for detecting reducing sugars. If the saccharide is a reducing sugar, it will reduce the copper(II) ions to copper(I) oxide, a red precipitate, as shown in Equation 7.

$$\underset{\text{reducing carbohydrates}}{R-\text{CHO}} + 2\,Cu^{2+} + 5\,OH^- \rightarrow \underset{\text{carbohydrate ion}}{R-CO_2^-} + Cu_2O\,(s, red) + 3\,H_2O \quad \text{(Eq. 7)}$$

Barfoed's Test

Barfoed's test uses copper(II) ions in a slightly acidic medium. If the reaction time is carefully monitored, this test can be used to distinguish reducing monosaccharides from reducing disaccharides. Reducing monosaccharides cause the formation of copper(I) oxide within 2–3 minutes. Reducing disaccharides cause the formation of copper(I) oxide after approximately 10 minutes.

$$\underset{\text{reducing saccharide}}{R-\text{CHO}} + 2\,Cu^{2+} + 2\,H_2O \rightarrow \underset{\text{carboxylic acid}}{R-\text{COOH}} + Cu_2O\,(s, red) + 4\,H^+ \quad \text{(Eq. 8)}$$

In this experiment, you will use the various reagents to test and classify several carbohydrate solutions. You will classify the carbohydrates according to the number of molecules contained in the structure and the number of carbon atoms in the carbohydrate unit. You will also determine whether the carbohydrate is an aldose or ketose, and whether it is reducing or nonreducing. A classification flow chart used for carbohydrate identification in this experiment is shown in Figure 4 on the next page. You must make careful observations of both positive and negative results for each classification test. Using these observations, you will qualitatively identify an unknown carbohydrate solution.

Procedure

Chemical Alert

Molisch reagent—toxic and corrosive
iodine–potassium iodide reagent—highly toxic, corrosive, and irritant
Barfoed's reagent—corrosive and irritant
Bial's reagent—toxic and corrosive
Benedict's reagent—toxic and irritant
Seliwanoff's reagent—toxic and corrosive
concentrated sulfuric acid—toxic and corrosive

Caution: Wear departmentally approved eye protection while doing this experiment.

I. Reacting Known Carbohydrate Solutions

Note: Because test tubes are placed in a boiling-water bath, you will need to label them by marking the etched glass portion on the tube using either a pencil or marking pen. Do not use paper labels, which can become loose in the hot water.

1. Label three sets of 7 clean, dry 18 $\times$ 150-mm test tubes with the names of each of the seven carbohydrate solutions you are testing.

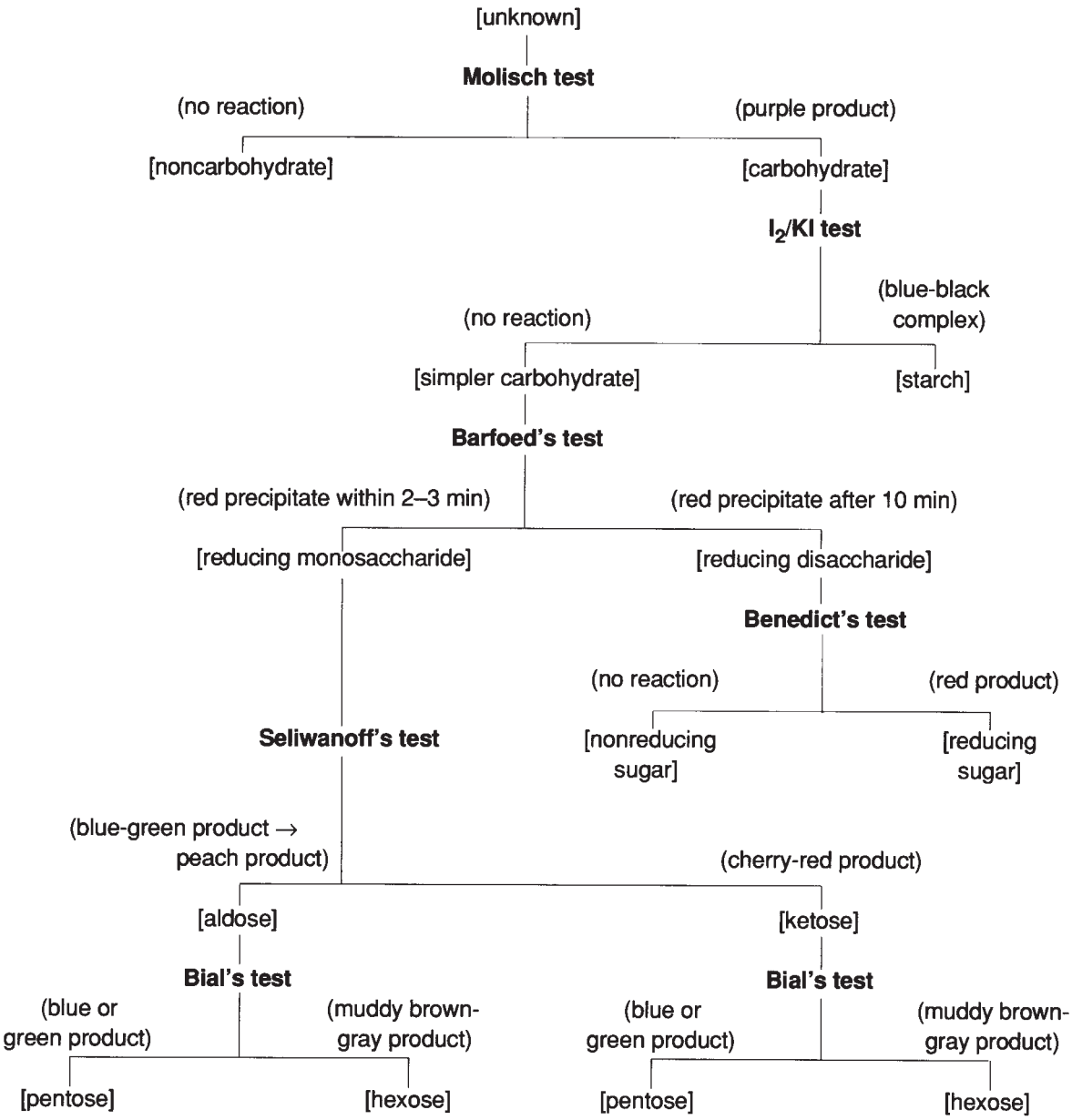


Figure 4 *Flow chart for classifying an unknown carbohydrate*

2. Obtain from your laboratory instructor, and transfer into one set of seven labeled test tubes, 10 mL of each of the following 1% carbohydrate solutions: xylose, fructose, glucose, lactose, sucrose, maltose, and starch.

Molisch Test

> ***Caution:*** Molisch reagent contains concentrated sulfuric acid, which is toxic and corrosive. It can cause severe burns. Prevent eye, skin, clothing, and combustible material contact. Avoid ingesting the substance. If you spill any reagent or acid, immediately notify your laboratory instructor.

> ***Note:*** Do not place your thumb over the open end of a test tube when mixing its contents. Your laboratory instructor will suggest ways in which you can safely and thoroughly mix the contents of a test tube.

3. Transfer 2 mL of each of the carbohydrate solutions from the first set of labeled test tubes into the

second set. Add 2 drops of Molisch reagent to each tube in the second set, and mix well with a clean glass stirring rod.

Add 2 mL of concentrated sulfuric acid to each of the test tubes in the third set.

> ***Caution:*** In the next step, you will be adding aqueous solutions to concentrated sulfuric acid. You must be extremely careful while performing this step.

4. One at a time, hold the test tubes containing the concentrated sulfuric acid at an angle of about 30° from the vertical and slowly pour the carbohydrate–Molisch reagent solutions from the second set of test tubes into them. Do not shake or mix the resulting solutions. Two separate layers will form.

> ***Note:*** When testing your unknown in Step 5, if there is no color change at the interface between the two layers, the test result is negative. Do not continue the tests for the samples that test negative for the Molisch test.

5. Record your results on Data Sheet 1.

6. Discard the test solutions containing Molisch reagent into the container provided by your laboratory instructor and labeled "Discarded Molisch Reagent Solutions."

7. Wash the second and third sets of test tubes with soap or detergent solution. Rinse three times with tap water and once with distilled or deionized water. Allow the test tubes to drain in order to remove as much of the water as possible.

Iodine and Potassium Iodide Test

> ***Caution:*** The I_2/KI solution is toxic, corrosive, and an irritant. If you spill any of the solution on yourself or on the bench, immediately notify your laboratory instructor.

8. Transfer 2 mL of each of your carbohydrate solutions into one set of the washed, drained, labeled test tubes. Add 2 drops of the I_2/KI solution to each test tube.

9. Record on Data Sheet 1 the positive or negative results that you observe after you mix the solutions with the I_2/KI reagent.

10. Discard the test solutions containing the I_2/KI solution into the container provided by your laboratory instructor and labeled "Discarded I_2/KI Solutions."

11. Wash the test tubes with soap or detergent solution. Rinse three times with tap water and once with distilled water. Allow the test tubes to drain.

> ***Caution:*** In the next four tests you will place the test tubes containing the reaction solutions in a boiling-water bath. Be careful that you do not come in contact with the steam or the hot apparatus, and that you do not knock over the bath.

> ***Note:*** Before using the boiling-water bath, be sure to add two boiling chips to the beaker of water, in order to prevent the test tubes from bumping as the water boils.

12. To set up a boiling-water bath, place an 800-mL beaker through an iron support ring small enough that the lip of the beaker will not pass through it. Clamp the ring and beaker to a support stand. Place a ceramic-centered wire gauze on a tripod or another iron ring support under the beaker. If you use a ring, clamp it to the support stand. Add 350 mL of water to the beaker, along with two boiling chips. Heat the water to boiling, using a Bunsen burner flame placed under the beaker.

Barfoed's Test

> ***Caution:*** Barfoed's reagent is corrosive and an irritant. If you spill any of the solution on yourself or on the bench, immediately notify your laboratory instructor.

> ***Note:*** A red precipitate before 5 min have elapsed indicates a positive result for a reducing monosaccharide. If a red precipitate appears at about 10 min, the sample is a reducing disaccharide.

13. Transfer 1 mL of each of your carbohydrate solutions into one set of the washed, drained test tubes. Add 3 mL of Barfoed's reagent to each test tube. Using a test tube clamp, place the test tubes containing the

reaction mixtures in your boiling-water bath. Note the time on Data Sheet 1. Allow the test tubes to stand in the boiling-water bath for 5 min. After 5 min, use the test tube clamp to cautiously remove the hot test tubes and observe the contents. Replace in the boiling-water bath for 5 additional minutes any test tubes that have no red precipitate. Cool under running water any test tubes that contain a red precipitate. Place the cool test tubes from the bath in a test tube rack.

14. Record the time for Barfoed's test on Data Sheet 1.

15. Discard the test solutions containing Barfoed's reagent into the container provided by your laboratory instructor and labeled "Discarded Barfoed's Reagent Solutions."

16. Wash the test tubes with soap or detergent solution. Rinse three times with tap water and once with distilled water. Allow the test tubes to drain.

Seliwanoff's Test

> ***Caution:*** Seliwanoff's reagent is toxic and corrosive. If you spill any of the solution on yourself or on the bench, immediately notify your laboratory instructor.

17. Transfer 10 drops of each of your carbohydrate solutions to one set of drained test tubes. Then add 15 drops of distilled water and 9 mL of Seliwanoff's reagent to each test tube. Using a test tube clamp, carefully place the test tubes containing the reaction mixtures in the boiling-water bath. Do not allow the test tubes to stand in the bath for more than 2.5 min. Use the test tube clamp to carefully remove the test tubes from the bath, place them in a test tube rack, and observe the solution color in each one.

18. Record your observations for Seliwanoff's test on Data Sheet 1.

19. Discard the test solutions containing Seliwanoff's reagent into the container provided by your laboratory instructor and labeled "Discarded Seliwanoff's Reagent Solutions."

20. Wash, rinse, and drain the test tubes as before.

Benedict's Test

> ***Caution:*** Benedict's reagent is toxic. If you spill any of the solution on yourself or on the bench, immediately notify your laboratory instructor.

21. Add 3 mL of Benedict's reagent to each of the test tubes in one set. Then transfer 10 drops of each of your carbohydrate solutions to the appropriately labeled tubes. Using a test tube clamp, place the test tubes containing the reaction mixtures in the boiling-water bath. After exactly 2 min, use the test tube clamp to remove the test tubes, and place them in a test tube rack.

22. Record all observations and the color of any precipitate on Data Sheet 1.

23. Discard the test solutions containing Benedict's reagent into the container provided by your laboratory instructor and labeled "Discarded Benedict's Reagent Solutions."

24. Wash, rinse, and drain the test tubes as before.

Bial's Test

> ***Caution:*** Bial's reagent is toxic and corrosive. Use a ***fume hood*** when working with Bial's reagent. If you spill any of the solution on yourself or on the bench, immediately notify your laboratory instructor.

25. Add 3 mL of Bial's reagent to each of the test tubes in one set. Then transfer 2 drops of each of your carbohydrate solutions to the appropriately labeled test tubes. Using a test tube clamp, place the test tubes containing the reaction mixtures in the boiling-water bath. Record the time on Data Sheet 1. Use the clamp to remove each test tube from the bath as soon as you see a color change. Place the tube in a test tube rack and record the time on Data Sheet 1. Do not allow the test tubes to stand in the bath for more than 5 min, even if no color change occurs.

26. Record the color change on Data Sheet 1, and calculate the time it took for the color to change.

27. Discard the test solutions containing Bial's reagent into the container provided by your laboratory instructor and labeled "Discarded Bial's Reagent Solutions."

28. Discard your carbohydrate test solutions into the drain, diluting with a large amount of running water. Wash, rinse, and drain all of the glassware as before.

> ***Note:*** Leave the boiling-water bath set up, so you can use it for the unknown carbohydrate solution in Part II.

II. Identifying an Unknown Carbohydrate Solution

Note: Each of the tests in Part I distinguishes between two classes of carbohydrates. It may not be necessary to perform all of the tests in order to identify your unknown sample in Part II.

29. Obtain 10 mL of a 1% unknown carbohydrate solution from your laboratory instructor in a washed and drained, 18 × 150-mm test tube. The unknown carbohydrate solution will be one of the solutions you tested in Part I. Label the test tube with the identification code for the unknown, and record the code on Data Sheet 2.

30. Repeat the tests outlined in Part I, Steps 3–28, using your unknown carbohydrate solution. You may stop as soon as you have identified your unknown solution. Record all observations on Data Sheet 2.

Note: If your laboratory instructor asks you to identify additional unknown carbohydrate solutions, prepare additional copies of Data Sheet 2.

31. Dispose of your solutions in the appropriate containers. Wash, rinse, and drain any glassware you have used.

Caution: Wash your hands thoroughly with soap or detergent before leaving the laboratory.

Post-Laboratory Questions

(Use the spaces provided for the answers and additional paper if necessary.)

1. When an aldose reacts with Barfoed's reagent, what type of organic compound forms? What type of chemical reaction is this?

2. Explain why fructose, an α-hydroxyketose, reacts with Benedict's reagent. What structural rearrangement is necessary for this reaction to occur?

3. Explain what happened when you mixed sucrose with Seliwanoff's reagent. Was this the result you expected? Briefly comment.

4. An unknown carbohydrate solution tested positive with Molisch reagent, formed a red precipitate with Barfoed's reagent within 3 minutes, and turned blue-green when mixed with Bial's reagent. What carbohydrate is this?

5. Erythrose is a four-carbon aldose. Describe the results of testing it with the following reagents.

(1) Molisch

(2) Barfoed's

(3) Benedict's

(4) Seliwanoff's

name _____ section _____ date _____

name _____ section _____ date _____

Data Sheet 1

I. Reacting Known Carbohydrate Solutions

test reagent	*saccharide solution*						
	xylose	*fructose*	*glucose*	*lactose*	*sucrose*	*maltose*	*starch*
Molisch	_____	_____	_____	_____	_____	_____	_____
I_2/KI	_____	_____	_____	_____	_____	_____	_____
Barfoed's time:	_____	_____	_____	_____	_____	_____	_____
final	_____	_____	_____	_____	_____	_____	_____
initial	_____	_____	_____	_____	_____	_____	_____
elapsed	_____	_____	_____	_____	_____	_____	_____
Seliwanoff's time:	_____	_____	_____	_____	_____	_____	_____
final	_____	_____	_____	_____	_____	_____	_____
initial	_____	_____	_____	_____	_____	_____	_____
elapsed	_____	_____	_____	_____	_____	_____	_____
Benedict's time:	_____	_____	_____	_____	_____	_____	_____
final	_____	_____	_____	_____	_____	_____	_____
initial	_____	_____	_____	_____	_____	_____	_____
elapsed	_____	_____	_____	_____	_____	_____	_____
Bial's time:	_____	_____	_____	_____	_____	_____	_____
final	_____	_____	_____	_____	_____	_____	_____
initial	_____	_____	_____	_____	_____	_____	_____
elapsed	_____	_____	_____	_____	_____	_____	_____

Data Sheet 2

II. Identifying an Unknown Carbohydrate Solution

unknown identification code _____

test reagent	*observation*	*conclusion*
Molisch	_____	_____
I_2/KI	_____	_____
Barfoed's	_____	_____
time:		
final	_____	
initial	_____	
elapsed	_____	
Seliwanoff's	_____	_____
time:		
final	_____	
initial	_____	
elapsed	_____	
Benedict's	_____	_____
time:		
final	_____	
initial	_____	
elapsed	_____	
Bial's	_____	_____
time:		
final	_____	
initial	_____	
elapsed	_____	

name _____ section _____ date _____

Pre-Laboratory Assignment

1. Describe the hazards associated with the following test reagents.

(1) Molisch

(2) Bial's

(3) Seliwanoff's

2. Briefly explain the meanings of the following terms as they relate to this experiment. Include structural formulas if appropriate.

(1) aldohexose

(2) reducing sugar

(3) hemiacetal

3. Classify the following saccharides as either reducing or nonreducing sugars.

(1) ribose

(2) sucrose

(3) hydrolyzed starch

4. For each of the following test reagents, describe the appearance of the reaction mixture that indicates a positive result.

(1) iodine/potassium iodide solution

(2) Benedict's

(3) Seliwanoff's

5. For each of the following test reagents, describe the class and give a specific example of a carbohydrate that would yield a positive result.

(1) iodine/potassium iodide solution

(2) Benedict's reagent

(3) Seliwanoff's reagent

6. Which test could you use to distinguish between the following pairs of carbohydrates?

(1) fructose and galactose

(2) ribose and glucose

(3) glucose and maltose

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