Experiment 20
Identification of Some Carbohydrates

Carbohydrates are the direct product of the photosynthetic combination of carbon dioxide and water. By weight, they are the most common organic compounds on earth. Since most have the empirical formula $C_nH_{2n}O_n = C_n(H_2O)_n$ it was initially believed that they were hydrates of carbon. Hence the name. In actuality they are polyhydroxylaldehydes and ketones and exist as cyclic hemi- and full acetals. The cyclic forms may be five-membered rings (furanose) or six-membered rings (pyranose). They are classified as mono-, di-, and polysaccharides. The term sugar applies to mono-, di-, and oligosaccharides, which are all soluble in water and thereby distinguished from polysaccharides, which are not soluble in water.

The most commonly encountered carbohydrates are starch, glycogen, inulin, cellulose, sucrose, fructose, arabinose, mannose, glucose, maltose, galactose, and lactose. The method of analysis used in this experiment is one of elimination. The assumption is made that the compound is a single carbohydrate and tests are applied sequentially until a positive result is obtained. The carbohydrate giving the positive test is thus identified.

The sugars that we will identify are shown below.

Figure 20.1 Monosaccharides

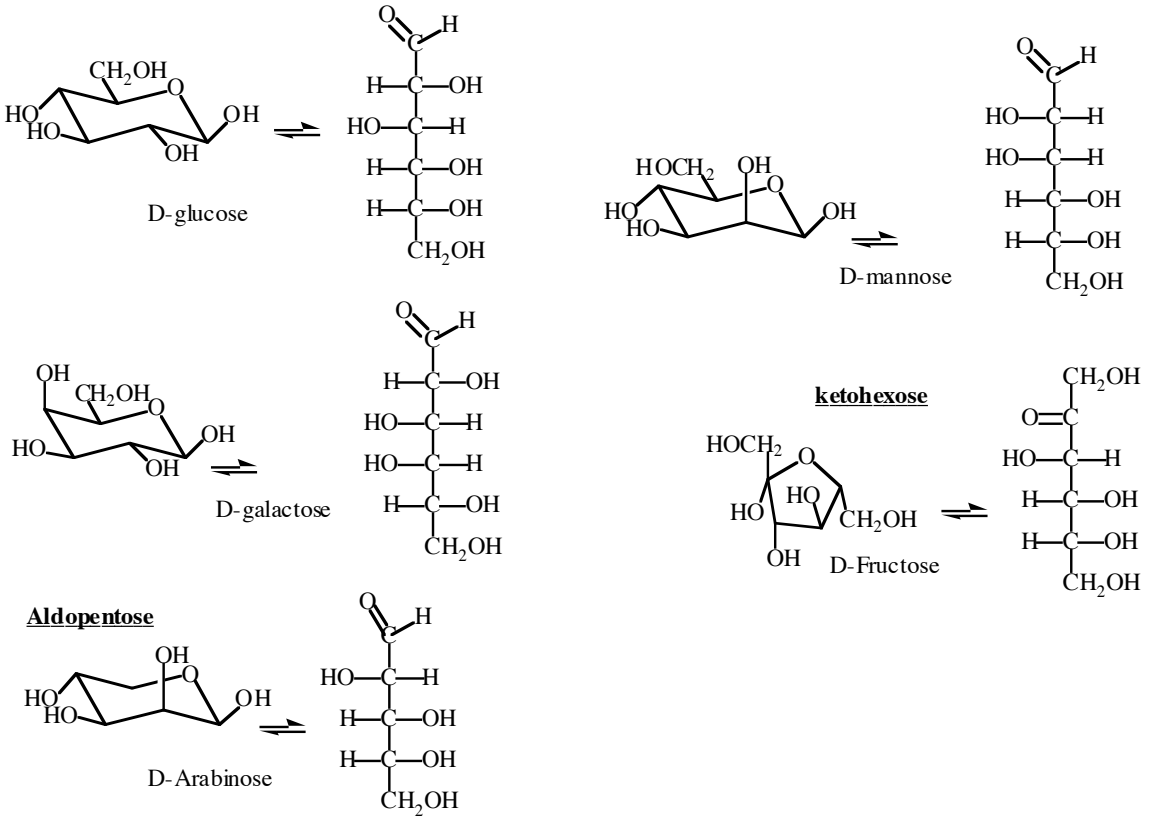
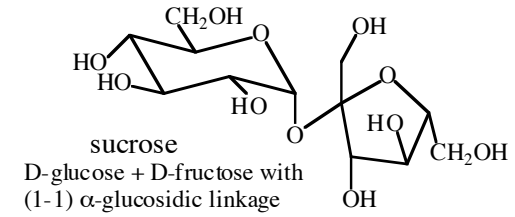
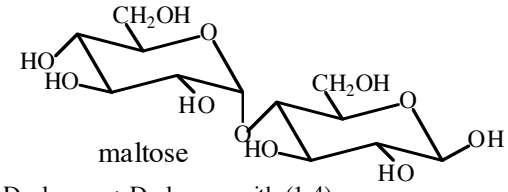


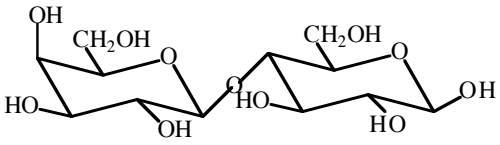
Figure 20.2 Disaccharides



sucrose
D-glucose + D-fructose with
(1-1) α-glucosidic linkage

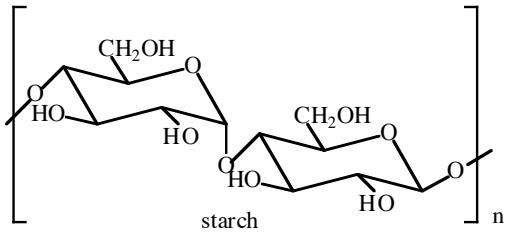


maltose
D-glucose + D-glucose with (1-4)
α-glucosidic linkage

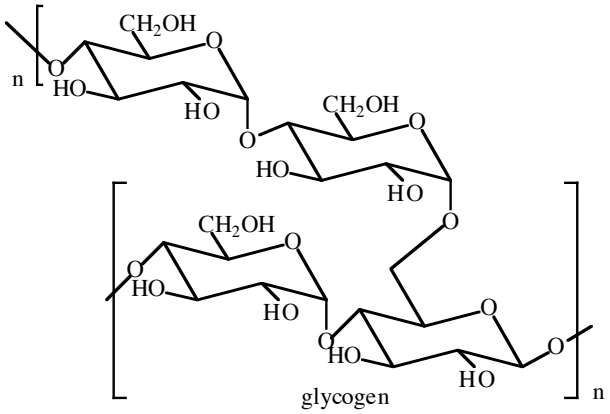


lactose D-galactose + D-glucose with (1-4)
β-glucosidic linkage

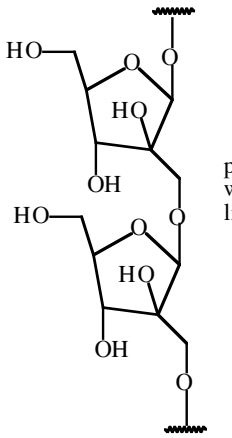
Figure 20.3 Polysaccharides



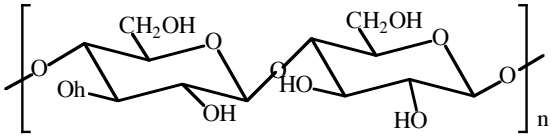
starch
polymer of D-glucose with (1-4) α-glucosidic linkage



glycogen
polymer of D-glucose with (1-4)
α-glucosidic linkages and branch at α (1–6)



inulin
polymer of D-fructose
with (2-1) B-D-fructose
linkages

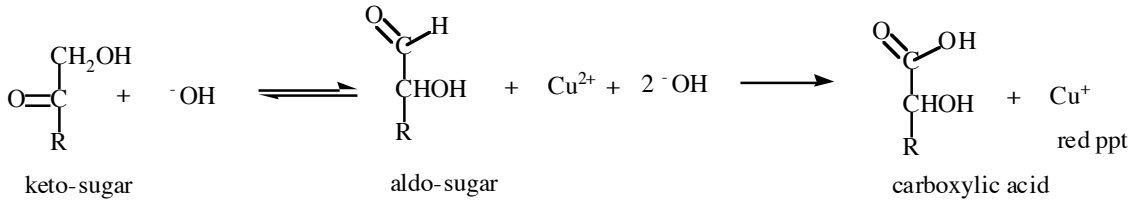


cellulose
polymer of D-glucose with (1-4)
β-glucosidic linkage

Sugars that have a free aldehyde group (or can from one under the reaction conditions) are oxidized by cupric ion (Cu^{2+}) to produce the carboxylic acid and cuprous ion (Cu^{1+}). These are called reducing sugars. All monosaccharides are reducing sugars since keto-sugars such as fructose can isomerize to aldo-sugars. In our experiment we will use Fehling's solution as the source of our copper II ion. This is prepared by mixing together, at the moment it is to be used, equal volumes of a solution of copper sulfate

(Fehling's solution A) and an alkaline solution of sodium potassium tartrate (Fehling's solution B)

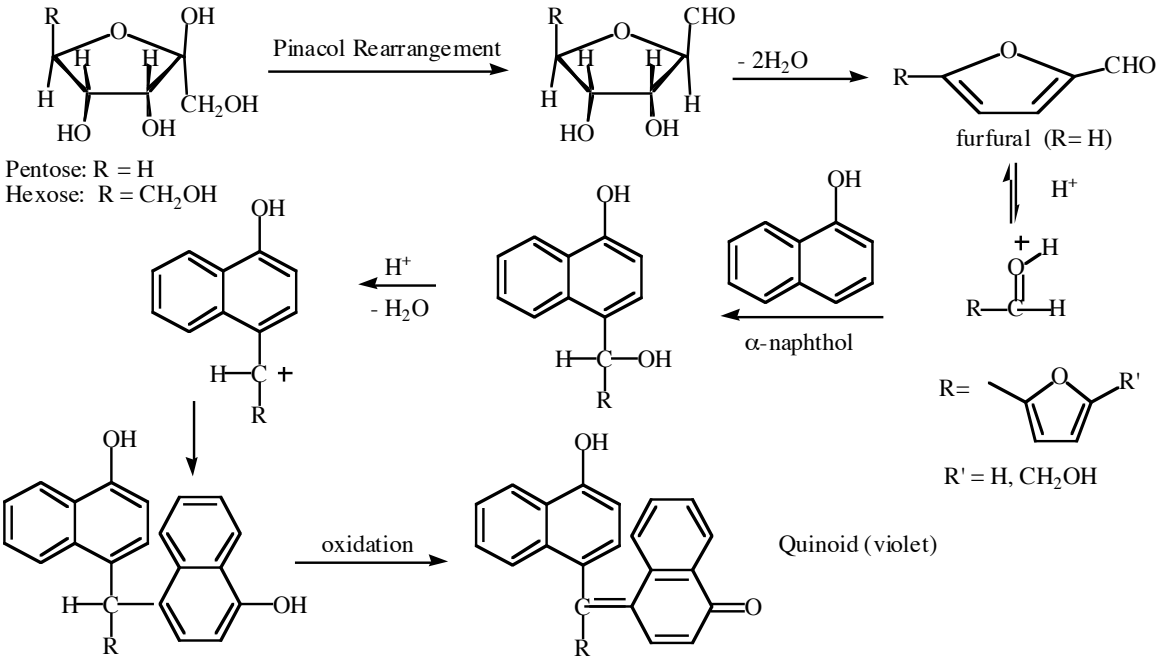
Figure 20.4 Reducing Sugars



Test 1 Molisch Test

The Molisch test is a general test for carbohydrates. Under strongly acidic conditions, monosaccharides undergo dehydration to provide furfural (R = H) from pentoses sugars or 5-hydroxymethylfurfual (R = CH_2OH) from hexose sugars as shown in Figure 20.4. Furfural or its derivative can then react with two moles of α-naphthol to provide the violet-colored quinoid. These reactions with α-naphthol are examples of electrophilic aromatic substitution which occurs at the *para* position of the ring that is activated by the phenolic hydroxy group. Polysaccharides undergo at least partial hydrolysis under the conditions of the test, and the resulting monosaccharides, as well as any other monosaccharides present, are dehydrated to provide furfural or 5-hydroxymethyl furfural. Concentrated sulfuric acid is used as the acid catalyst and dehydrating agent.

Figure 20.5 Molisch Test: Dehydration and Quinoid Formation



Procedure

Place about 5 mg of any carbohydrate in 1 mL of water in a small test tube and mix with two drops of a 10% solution of α-naphthol in water. Using a medicine dropper, add 1.0 mLconcentrated sulfuric acid down the side of the test tube so that it forms a layer underneath the water layer (tilt the test tube). Be careful not to mix the layers. A purple color between the two layers indicates a carbohydrate.

Test II Solubility

Add about 50 mg of the carbohydrate to 1.0 mL of cold water. If the substance is insoluble it is a polysaccharide. The mono- and disaccharides are all soluble in cold water.

Note 1: Make sure that the substance is given sufficient time to dissolve.

Note 2: Glycogen forms a colloidal suspension so that the mixture is milky. This test alone is "almost" sufficient to prove that the compound is glycogen.

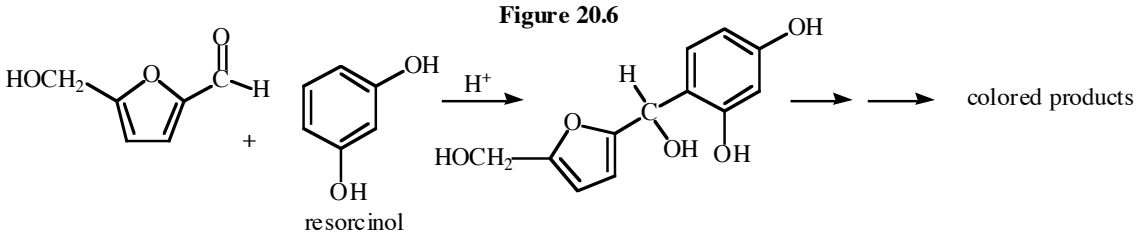
Test III Identification of the Polysaccharides

(A) Starch: Treat a suspension of the carbohydrate with a drop of 10% Iodine-Potassium Iodide solution. If a blue-black color results, the substance is starch.

(B) Glycogen: Iodine-Potassium Iodide gives a faint red color with glycogen. However, if it is not observed the existence of a colloidal suspension in the solubility test should be taken as confirmation of glycogen.

(C) Inulin: The polysaccharide inulin is a polyfructose. The reagent used causes hydrolysis. Seliwanoff's reagent (0.5 g resorcinol in a liter of 10% HCL) is used. When heated in this reagent hexose sugars undergo dehydration to form hydroxymethyl furfural. This then condenses with resorincol (1,3-dihydroxybenzene) to give a red product. The exact structure of the red colored product is not known but it is believed to be the result of multiple electrophilic aromatic substitutions just as in the Molisch Test. The reaction is shown in Figure 20.6.

Figure 20.6



To 5.0 mL of the reagent, add a spatula tip of the carbohydrate and boil for 30 seconds (Do not boil longer than 30 seconds). The development of a red color confirms the inulin.

(D) Cellulose: Cellulose is identified by the fact that it gives a negative response to all of the above tests.

Test IV Identification of the Water Soluble Carbohydrates

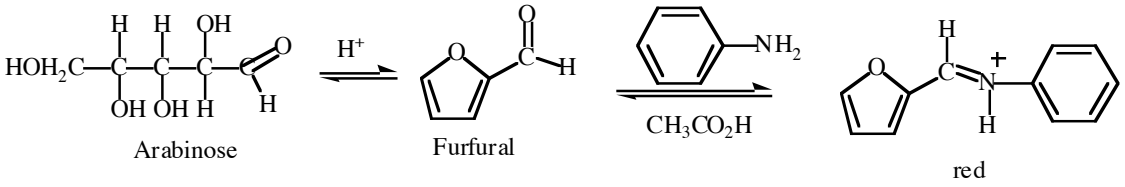
(A) Sucrose: Add about 10 g. of the carbohydrate to a mixture containing 1.0 mL of Fehling's A and 1.0 mL of Fehling's B solutions and heat to boiling. A negative test, failure to reduce the blue Cu (II) ion to red Cu (I), confirms sucrose.

(B) Fructose: The test for fructose is identical with that for inulin. If a small amount of the unknown added to 5.0 mL Seliwanoff reagent gives a cherry red color on heating for 30 seconds or less, fructose is confirmed.

(C) Arabinose: Add 0.5 g of arabinose to 5 mL water in a medium test tube and stir to dissolve. Add 5 drops of Fehling's solutions A and B and boil the test tube for one minute. Formation of a colored precipitate is confirmation of arabinose. Go to the Bial's test for additional confirmation.

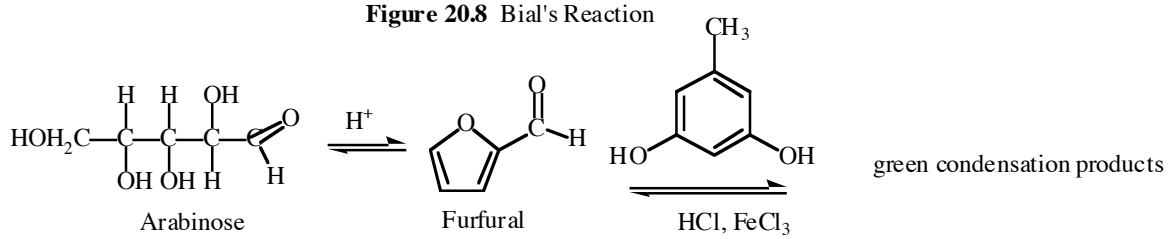
(D) Aniline Acetate Test: Add about 0.3 g of the unknown to 5 mL of 3.0 M HCl in a small test tube. Boil the test tube in a water bath for about one minute and then insert a cylindrical roll of freshly prepared aniline acetate paper (prepared by dipping a piece of filter paper in an equimolar solution of aniline and glacial acetic acid). Continue boiling for a minute longer. Arabinose will color the paper cherry red (see Figure 20.7).

Figure 20.7 Aniline Acetate Reaction



(E) Bial's reaction: An alternate for pentoses like arabinose involves Bial's reagent (1.5 g of orcinol dissolved in 500 mL of concentrated HCl to which 20 drops of ferric chloride are added). To 5 mL of the reagent, add about 25 mg of the unknown and heat gently to the boiling point and then cool see Figure 20.8). If the solution turns green, the test is positive. In some cases a green flocculent precipitate may form.

Figure 20.8 Bial's Reaction

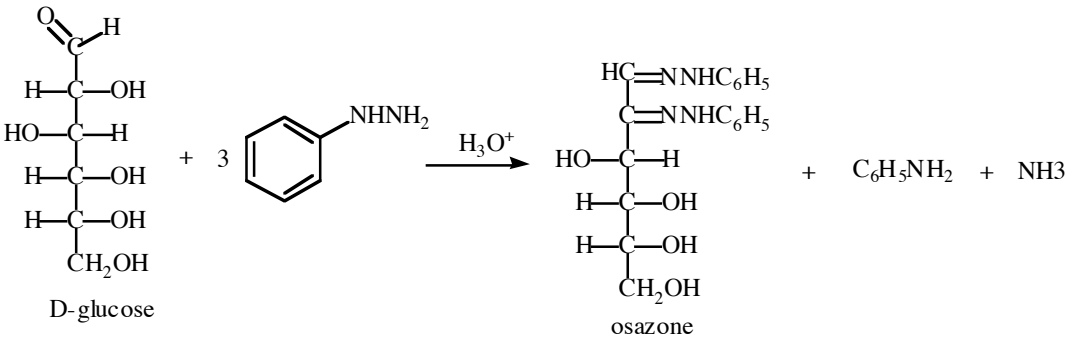


Test V Osazone Test

Carbohydrates that have an aldehyde or ketone carbonyl group, either free or in equilibrium with a hemiacetal, will react with phenylhydrazine to form bright yellow,

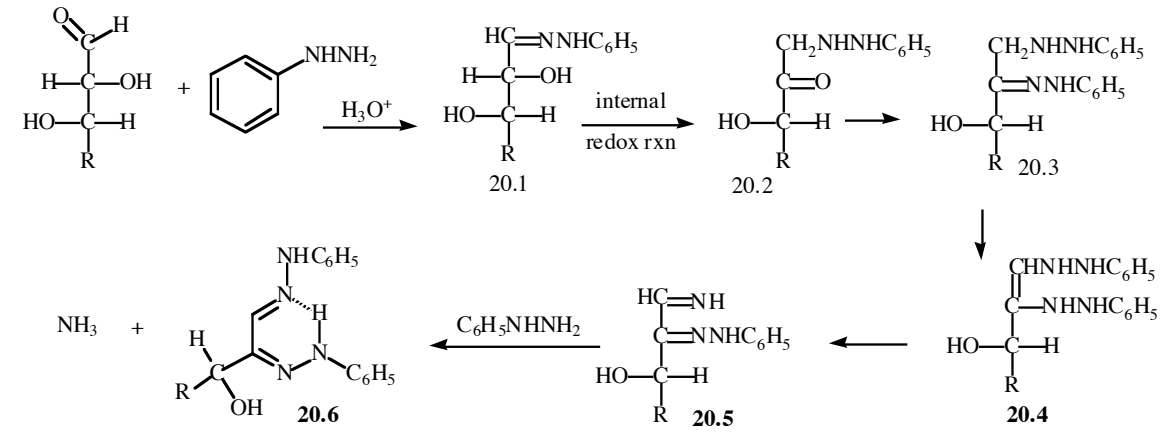
crystalline derivatives called osazones. These derivatives may usually be identified readily both by their melting points and by their crystalline forms. The reactions involved are shown in Figure 20.9.

Figure 20.9 Osazone Formation



The accepted mechanism for the reaction is shown in figure 20.10. Following the formation of the phenylhydrazone **20.1**, there is an internal oxidation-reduction reaction that involves the tautomeric migration of two hydrogens from C-2 to the hydrazone moiety to give the carbonyl product **20.2**. The newly formed carbonyl group condenses with a second equivalent of phenylhydrazine to give **20.3** which undergoes subsequent tautomerization to **20.4**. Following a 1,4-elimination of aniline, which produces **20.5**, a third equivalent of phenylhydrazine condenses with the imine group to give the osazone **20.6** and ammonia. The formation of the intramolecular hydrogen bond in **20.6** prevents another internal oxidation-reduction sequence between the secondary alcohol at C-3 and the hydrazone group at C-2 and the introduction of a third phenylhydrazine unit.

Figure 20.10 Mechanism for Osazone Formation



Although the time it takes for osazones to form may be used to make qualitative distinctions among the carbohydrates, the test will not be used in this way here. It will

rather be used to divide the remaining fine sugars in such a way so that they may be identified.

The reagent is prepared by dissolving 0.80 g of phenylhydrazine hydrochloride and 1.2 g of sodium acetate in 8 mL of water. To about 2 mL of this reagent, add about 0.1 g of the unknown in a small test tube. Immerse the test tube in a beaker of boiling water and note the time of immersion. Shake the test tube occasionally without removing it from the boiling water.

(A) The immediate formation of a dirty white precipitate confirms mannose. Mannose under these conditions does not form an osazone. The observed precipitate is the phenylhydrazone of the sugar. Mannose is the only one of the remaining carbohydrates that behaves this way.

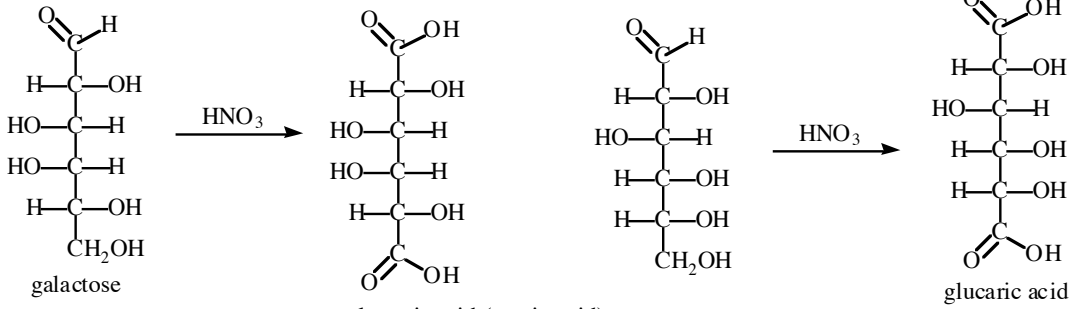
(B) The formation of an orange precipitate in less than 30 minutes indicates that the unknown is either glucose or galactose. If no precipitate forms in the allotted time then the unknown is either maltose or lactose.

(C) Glucose is oxidized by concentrated nitric acid to the water-soluble saccharic acid, whereas galactose is oxidized by the same reagent to the water insoluble mucic acid. This difference may be used to distinguish between the two hexoses.

Test VI Mucic Acid Test

This test is specific for galactose. Hot nitric acid oxidizes a sugar to the carboxylic acid (Figure 20.11). Aldoses are oxidized at both ends of the ring-opened form to provide dicarboxylic acids. Ketoses oxidize to give a mixture of dicarboxylic acids resulting from chain fragmentation. Galactaric acid (or mucic acid) from the oxidation of galactose tends to be much less soluble in the oxidizing medium (and water) than the saccharic acids obtained from other aldoses. This is partly due to the high molecular symmetry of galactraric acid (it is a meso compound).

Figure 20.11 Mucic Acid Test



In an evaporating dish, place about 2 mg of your unknown, 6.0 mL of water and 6.0 mL of concentrated nitric acid. Connect a large funnel to the aspirator with a rubber tube (Or a hood may be used instead). Evaporate over a small free flame stirring to avoid spattering a burning. When the contents become pasty, cool, dilute with cold water and

filter. Wash with cold water. The presence of a white precipitate on the filter confirms galactose. The absence confirms glucose.

We must now distinguish between maltose and lactose. Since maltose is a dimer of glucose and galactose, oxidation of the former with nitric acid gives a soluble saccharic acid, whereas the latter gives a mixture of saccharic and mucic acids. Therefore the mucic acid test serves also to distinguish between the two disaccharides. The melting point of mucic acid may be determined. With slow heating, it is 214 °C, while with rapid heating it will be found between 223-224 °C.