Analysis of an Unknown Sugar

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Introduction

By weight carbohydrates are the most abundant class of organic chemical compounds on Earth, making up to 50% of its mass. Carbohydrates are categorized as bioorganic compounds because they are part of biological systems. The name carbohydrates evolved from its empirical formula: $C_nH_{2n}O_n = C_n(H_2O)_n$. It was believed that carbohydrates were hydrates (molecules which contain water) of carbon. It is now known that this is not true, but the name persists.

The terms sugar and carbohydrate are sometimes used interchangeably. Sugar usually refers to a single carbohydrate unit or short linked groups of carbohydrates that are soluble in water. Simple carbohydrates are classified as monosaccharides, and contain a single sugar unit. Complex carbohydrates are: Disaccharides (two linked sugar units), oligosaccharides (three to ten linked sugar units) and polysaccharides (more than ten sugar subunits).

Analyzing and elucidating the complete structure of sugars is not a simple task, because of their complex structures and the presence of a combination of functional groups. However, some qualitative tests have been developed to obtain information about the compounds and to classify carbohydrates according to their structural type.

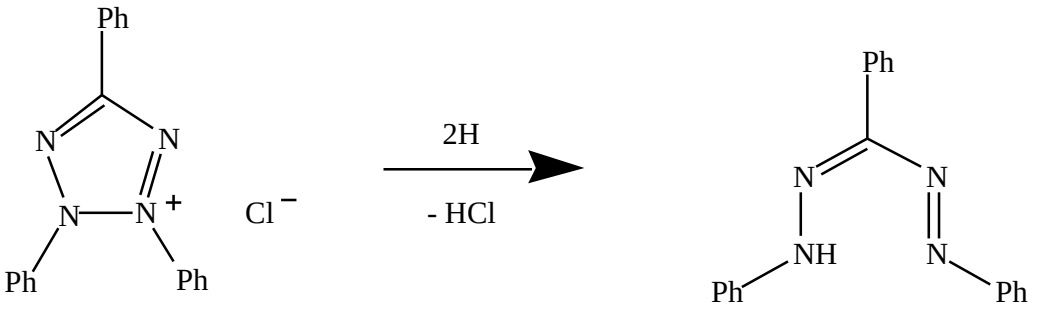
Sugars usually do not have sharp melting points. They sometimes decompose during heating, thus, their melting points are not used as primary physical constants for identification. Sugar's osazone derivatives are easily prepared, and the melting points of these derivatives, found in the *Handbook of Tables for Organic Compound Identification*, are characteristic for each compound, and therefore used for sugar identification.

Similar to experiment 7 concerning the identification of an unknown alcohol, aldehyde or ketone, you will be given one of the unknown samples listed in Table 1. You will run the tests on a series of standards along with your unknown sugar (listed a - f in the procedure section). Before the lab session, prepare a chart listing each test and the meaning of all expected results. Find the corresponding structures for each of the sugars listed on Table 1. Make a table, that lists the tests that will be performed in this experiment and the expected results for each sugar. Comparable tables (Tables 3 and 4) were provided for you in experiment 7.

Red Tetrazolium Test

Several tests have been developed over the years to determine whether a sugar is reducing or non-reducing. Fehling's and Benedict's tests are classic tests that use cupric ions. These cupric ions are easily reduced in hot basic solution to cuprous oxide, which precipitates out of solution as a yellow to brick red solid. The Tollens test is another example. The Tollens reagent ($Ag(NH_3)_2^+OH^-$) oxidizes the aldehyde group of the sugar to a carboxylic acid. The reagent is reduced to silver metal, which precipitates on the wall of the test tube forming a mirror. These tests all have a limited efficiency and some of the reagents are rather toxic. A new compound now available for reducing/non-reducing sugar test is "red tetrazolium" or 2,3,5-triphenyl-2*H*-tetrazolium chloride. It is more sensitive, not quite as hazardous, and easier to prepare than the above mentioned reagents. The red tetrazolium reagent is fairly colorless and water-soluble. It is easily reduced in hot basic solution, and converted into an insoluble,

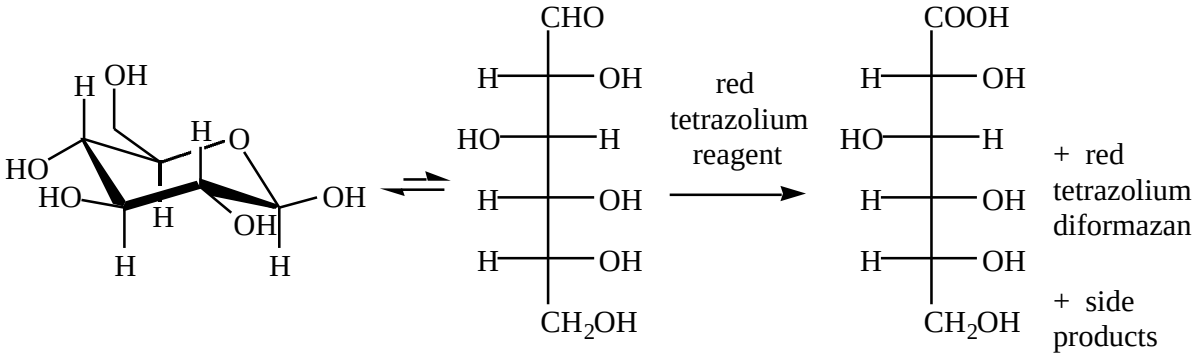
intensely colored product. Due to the high sensitivity of this test, it is extremely important to work with very clean test tubes.



Red tetrazolium

Red tetrazolium diformazan

Reducing sugars exist in the hemiacetal or hemiketal form, and can easily equilibrate in solution with a small but significant amount of the open aldehyde or hydroxyketone form. The aldehyde group can then be directly oxidized to the corresponding carboxylic acid.

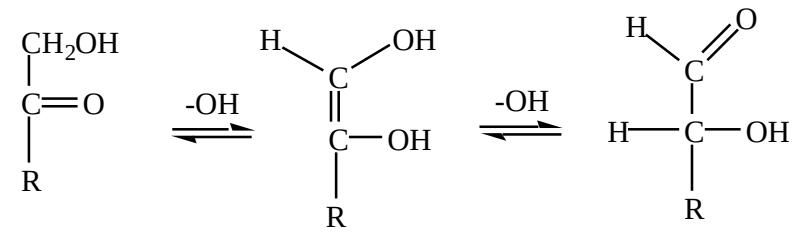


hemiacetal

open-chain form

acid

This test cannot differentiate between aldoses and ketose because the basic conditions promote endiol rearrangements of ketones. The open hydroxyketose can easily isomerize under basic conditions to an aldose, which subsequently oxidizes to an acid also giving a positive test result in the red tetrazolium test.



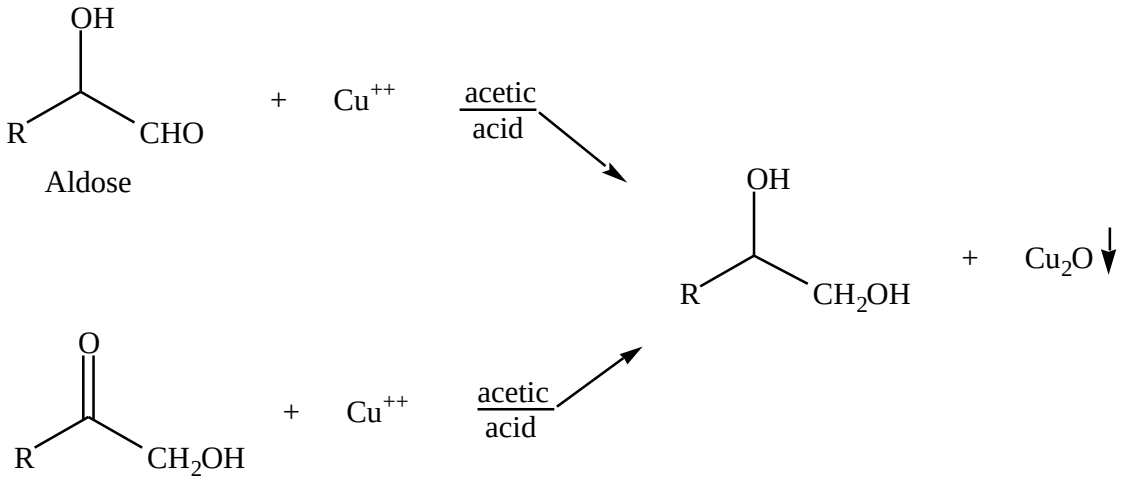
a ketose

endiol intermediate

an aldose

Barfoed's Test

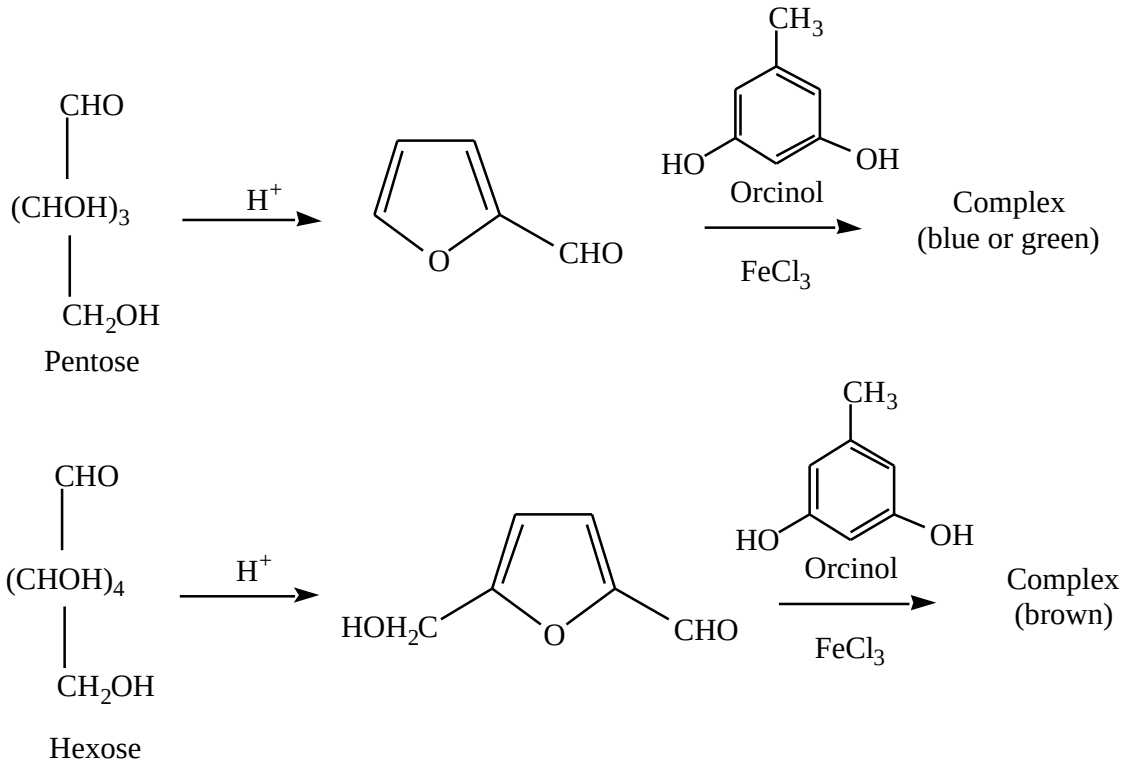
Barfoed's test is used to differentiate between reducing mono- and more complex saccharides (disaccharides, etc). The free aldehyde or ketone of reducing sugars quickly reduces copper (II) to copper (I). The reddish precipitate of cuprous oxide appears as evidence of the reaction. Disaccharides and trisaccharides oxidize at a much slower rate than monossacharides. Therefore the rate of reaction can be used to identify whether the reducing sugar is a monosaccharide or not. Monosaccharides will react within 3 minutes and more complex sugars will take more than 5 minutes.



Other easily oxidizable substances such as hydroxyaldehydes and ketones will give a positive result as well. To avoid false positive, make sure that the glassware used is very clean.

Bial's Test

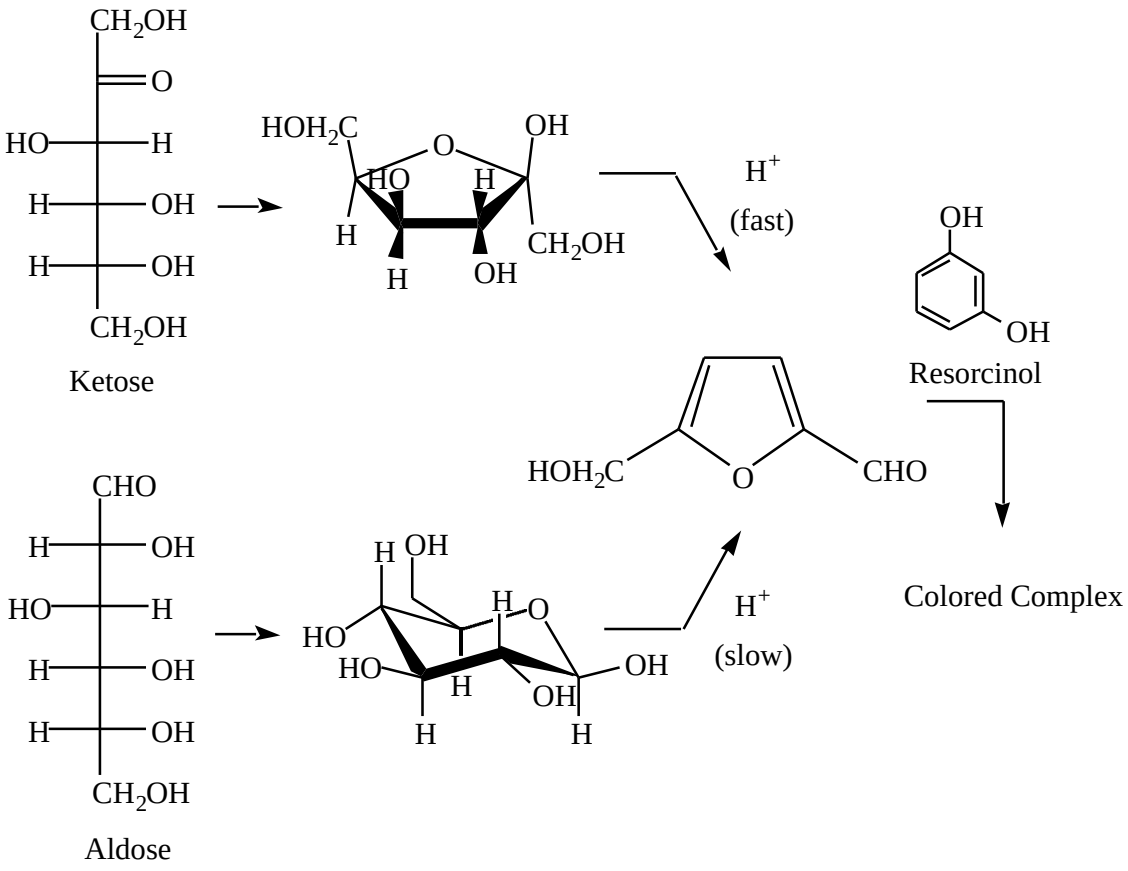
Bial's test is used to differentiate between pentoses and hexoses. In the presence of concentrated hydrochloric acid, pentoses react to give furfural. Furfural then reacts with orcinol and ferric chloride, both present in the Bial's reagent, to give a blue or green color complex. Hexoses, on the other hand, dehydrate to form 5-hydroxymethyl furfural, which in turn reacts with ferric chloride and orcinol to give a brownish color compound. Di- and polysaccharides give the same results but at a much slower rate, because monosaccharides dehydrate faster.



Sometimes the color of the product is not visible or weak. In such case the product can be concentrated by first diluting the solution with water, and then extracting the product with a small volume of cyclohexanol. The product is then concentrated in the alcohol layer and its color should be visible.

Seliwanov's Test

Seliwanov's test distinguishes between aldoses and ketoses based on their ability to form furfurals. Ketoses form furanoses more rapidly than do aldoses, and these furanoses dehydrate rapidly to form furfurals. Furfurals immediately react with resorcinol, present in Seliwanov's reagent to give a colored complex. Aldoses are more likely to form pyranoses, and these dehydrate to furfural at a much slower rate. They will give a positive test result only after prolonged heating.



Preparation of the Osazone Derivative

As we learned in experiment 7, one of the best methods for derivatizing aldehydes and ketones consists in their conversion into hydrazones either with phenylhydrazine or with 2,4-dinitrophenylhydrazine. Emil Fischer found that the reaction of an aldose or a ketose with phenylhydrazine yielded a yellow crystalline solid insoluble in water. He called these *osazones*. Unlike simple aldehydes and ketones, sugars do not form simple phenylhydrazones. They react with three equivalents of phenylhydrazine at both the C1 and C2 carbons to give an osazone. The first equivalent forms an imine with the aldehyde or ketone. The second equivalent oxidixes the alcohol adjacent to the first imine to a carbonyl group. The third equivalent then reacts with the newly formed carbonyl group. Since the reaction is at C1 and C2, a ketose will give the same osazone as the related aldose. These derivatives usually have sharp melting points, and can be used to identify the corresponding monosaccharides.

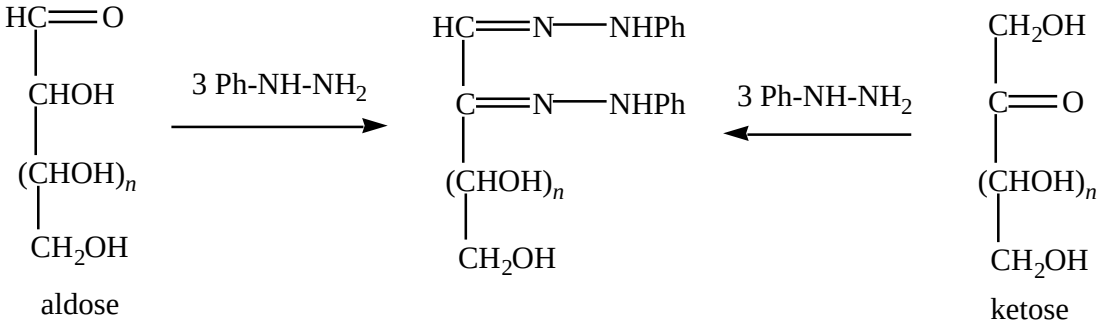


Table 1

Saccharide	**Melting Point** (°C) **Sugar**	**Melting Point (°C) Osazone Derivative**
L-Arabinose	160	166
D-Cellobiose	225	198
D-Fructose	104	210
D-Galactose	167	201
L-Glucose	143	208
D-Gentobiose	195	164
D-Lactose monohydrate	201	200
D-Lyxose	106	164
D-Maltose monohydrate	102	206
D-Mannose	132	210
L-Rhamnose monohydrate	94	182
D-Ribose	87	164
L-Sorbose	165	156
Sucrose	185	205
D-Xylose	145	164

(a) Data taken from the *Handbook of Tables for Organic Compound Identification, 3rd Edition* (Rappaport, Z., Ed.; CRC Press: Boca Raton, Fla, 1967), pp. 329-333.

Experiment 12

A series of quick test-tube qualitative chemical tests will be used to identify an unknown sugar. The identity of the unknown will be verified by preparing its osazone derivative and determining its melting point.

Safety: Remember to wear appropriate eye protection. Bial's reagent is prepared with concentrated hydrochloric acid and Seliwanov's reagent is very acidic. Phenyldrazine is toxic and is absorbed through the skin. Glacial acetic acid is corrosive. Handle with care, wear gloves and avoid contact with skin when handling all of these compound. Dispose all waste in the appropriate labeled containers provided.

All the qualitative tests will be performed on the following 1% carbohydrate solutions:

a. Glucose (an aldohexose).
b. Fructose (a ketohexose).
c. Lactose (a reducing disaccharide).
d. Sucrose (a non-reducing disaccharide).
e. Galactose (an aldohexose).
f. Arabinose (an aldopentose).
g. An unknown.

Obtain a sample of a 1% aqueous solution of your unknown and record the identification number.

First you will use **red tetrazolium** to determine whether your unknown is a reducing or non-reducing sugar. Place one drop of your unknown solution in a clean labeled test tube. Add 1 ml of 0.5% aqueous solution of red tetrazolium, and 1 drop of 3 M NaOH. Mix and place the test tube in a beaker of hot water. Record the time it takes to develop an intense color. Run the test simultaneously on drops of each of the six standard solutions listed above.

To determine if you have a monosaccharide or a disaccharide you will perform **Barfoed's test**. Place 1 ml of your unknown and 2 ml Barfoed's reagent in a labeled test tube. Mix and place the test tube in a hot water bath for 15 minutes. Record the time it takes for a precipitate to form. Monosaccharides will react within 3 minutes; more complex sugars will take longer than 5 minutes. Run the test simultaneously on 1 ml of each of the six standard solutions listed above.

Bial's test will indicate if the unknown is a pentose or hexose. Place 1 ml of your unknown solution in a labeled test tube. Add 1ml of Bial's reagent, a boiling chip, and place the test tube in a hot water bath. Bring to a boil and record the color change. Run the test simultaneously on 1 ml of distilled water as control, as well as on 1 ml of each standard solution listed above.

Aldoses can be distinguished from ketoses based on their ability to form furfurals using **Seliwanov's test**. Place 1 ml of your unknown solution in a test tube. Add 1 ml Seliwanov's reagent and mix well. Heat the test tube in a boiling water bath for 2 minutes. Record any color change. Run the test simultaneously on 1 ml of each of the six standard solutions listed above.

To prepare the **osazone derivative**, place 3 ml of your unknown solution in a clean test tube. Add 1 ml of phenylhydrazine reagent and place the test tube in a hot water bath. Heat in boiling water for 30 minutes. Shake and flick test tube occasionally to relieve supersaturation. After 30 minutes, stop heating and cool the test tube in an ice-water bath. Filter your product using vacuum filtration. Scrape off your sample onto a clean piece of filter paper, and dry rapidly between pieces of filter paper (air-dried osazones tend to melt lower than their literature values). Determine the melting point with a rapid heating rate of 30-40 ^{o}C per minute (osazones melt with decomposition, so you don't want to take too long). NOTE: If no precipitate is observed after 30 minutes of heating, cool and scratch the test tube to induce crystallization.

Post-laboratory Questions

1. After you have identified your unknown, prepare a report that summarizes the results of your tests and that argues how your suggested structure is supported by each of the test results.

2. Show the chemical reactions of your unknown in the tests that you performed. If there was no reaction, explain why.

3. Name two aldopentoses that would give osazones with the same melting point.

4. Suggest a reason to explain why air-dried sugars have a lower melting point than the literature values.