University of Tikrit

College of Pharmacy

Department of Clinical Laboratory Sciences

Title of the course: Practical Biochemistry 1

Level: 3rd Class, 1st Semester

General Reactions of Carbohydrates

Objective: To identify different types of carbohydrates using simple chemical tests:

Introduction

Carbohydrates are a broad class of compounds with diverse chemical structures and biological functions. They are defined as organic compounds that include carbon, hydrogen and oxygen. They are also defined as polyhydroxy aldehydes or ketones, or substances that yield these upon hydrolysis. In most organisms, carbohydrates, primarily in the form of glucose, serve as the main source of energy and provide carbon for the synthesis of proteins, nucleic acids, lipids, and other carbohydrates. Some carbohydrates have structural roles, such as cellulose in plants, while others, like starch in plants and glycogen in animals and bacteria, serve as carbon and energy storage molecules. Carbohydrates can be classified based on their chemical structure and complexity. The main categories include:

1. Monosaccharides (Simple Sugars)

These are the most basic units of carbohydrates and cannot be broken down further. They typically contain 3-7 carbon atoms. They include:

a. Trioses; composed of three carbon atoms (e.g: glyceraldehyde).

b. Tetroses: composed of four carbon atoms (e.g: erythrose).

c. Pentoses: composed of five atoms (e.g: ribose, xylose, ribulose and arabinose).

d. Hexoses: composed of six carbon atoms.

1-Aldohexoses: contain aldehde group (e.g: glucose, galactose, manoss).

2- Ketohexoses: contain ketone group (e.g: fructose).

Examples: Glucose, Fructose, Galactose

Key Characteristics: Soluble in water, sweet-tasting

2. Disaccharides (Two Simple Sugars)

These are formed by the combination of two monosaccharide molecules through a glycosidic bond.

Examples: Sucrose (Glucose + Fructose), Lactose (Glucose + Galactose), Maltose (Glucose + Glucose)

Key Characteristics: Soluble in water, sweet-tasting, need to be broken down into monosaccharides for absorption

a- Reducing sugars: they contain free aldehyde or ketone groups that are capable of reducing an oxidizing agent. Examples are maltose that is composed of two molecules of glucose, and lactose, that is composed of glucose and galactose.

b-Non-reducing sugars: they lack the free aldehyde or ketone groups. An example iss is sucrose which is composed of one molecule of (-glucose) and one molecule of (-fructose) linked together by (-1-2 glycosidic linkage).

3. Oligosaccharides (3 to 10 Monosaccharides)

These carbohydrates consist of a small chain of 3-10 monosaccharide units. They are not easily digestible by human enzymes but play a role in digestion, especially as prebiotics.

Examples: Raffinose, Stachyose

Key Characteristics: Not sweet-tasting, found in foods like legumes, act as prebiotics

4. Polysaccharides (Complex Carbohydrates)

Polysaccharides are long chains of monosaccharide units, often consisting of hundreds or thousands of sugar molecules. They serve as energy storage or structural components in cells.

Examples:

Starch: is a strong form of glucose in plants. It is composed of both amylase and amylopectin. Amylose is alinear polymer of glucose unites; wherease amylopectin is a branched chain of glucose unites. Glycogen (animal energy storage, stored in liver and muscles)

Cellulose (structural component in plants, dietary fiber for humans)

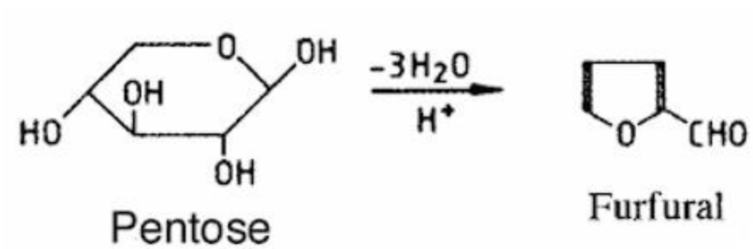
Key Characteristics: Insoluble in water, not sweet-tasting, complex and slowly broken down for energy

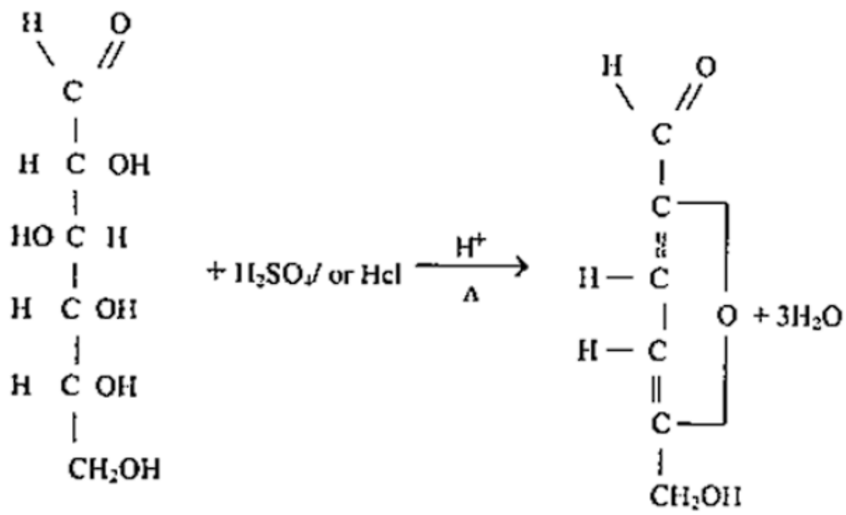
I. Effect of acids on carbohydrates

The treatment of carbohydrates with strongly acidic solutions hydrolyses the glycosidic bonds in disaccharides and polysaccharides, yielding monosaccharides. These monosaccharides are then subjected to various color reactions, which are more or less characteristic of these sugars. There is a common feature in these reactions (e.g., Molisch, Anthrone, Bial's, and Seliwanoff's tests):

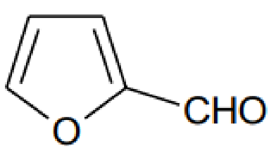
a. **Heating with strong acids** causes the hydrolysis of disaccharides and polysaccharides, yielding monosaccharides (simple sugars).

b. **Ring formation due to the loss of water molecules** (i.e., dehydration) from monosaccharides occurs. In these processes, pentoses lose three molecules of water, forming a cyclic compound called furfural, while hexoses yield hydroxymethylfurfural. With prolonged heating, other decomposition products, such as levulinic acid, may form.

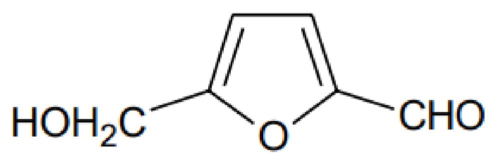




D - Glucose 5 – Hydroxy

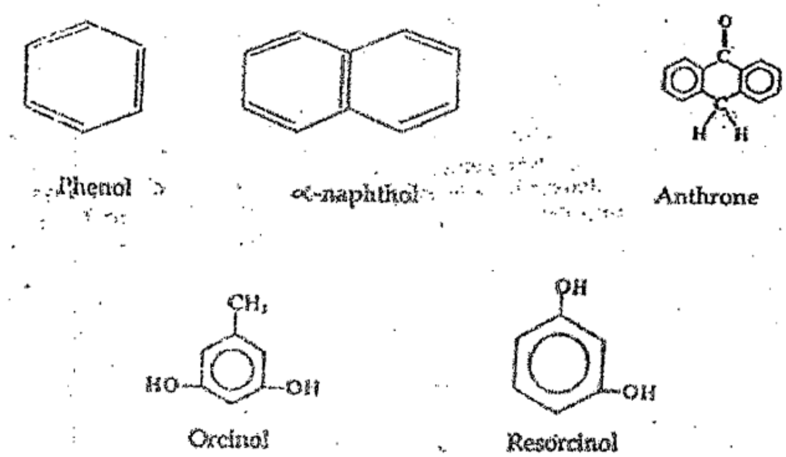


Furfural



Hydroxy methyl furfural

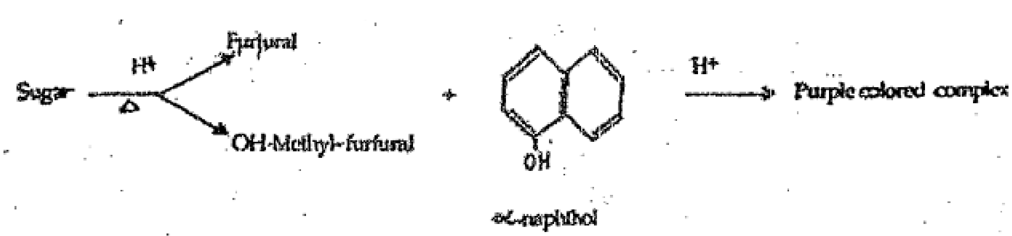
c. These furfurals are colorless compounds which undergo condensation reactions with phenolic substances this is the second thing common with these four tests. The phenolic substance is different for each test. These phenolic substances are all derivatives of phenol and they have the same basic structure. The following are examples of these compounds.



The condensation reactions yield colored complexes. The actual nature of these complexes has not been established for the majority of these condensation reactions.

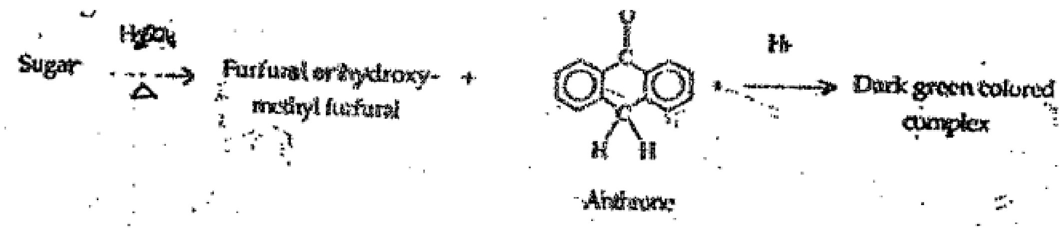
A – Molisch's Test

Molisch's test is a very sensitive general test for all carbohydrates because they all give furfural or hydroxylmethyl furfural upon heating with strong hot acidic solutions. It is performed by mixing a few drops of an alcoholic solution of a-naphthol (molisch's reagent) with the sugar solution in the presence of concentrated sulfuric acid to yield a purple condensation colored complex. The intensity of the color is proportional to the amount of sugar present in the solution.



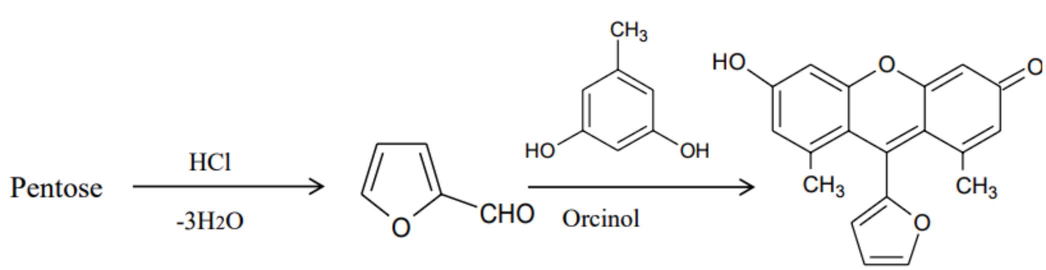
B-Anthrone Test:

It is also a general qualitative test for all carbohydrates. It can also be used for quantitative determination of carbohydrates because the intensity of the color is proportional to the amount of carbohydrate tested, the more sugar you add, the darker the color, and this can be determined spectrophotometry. It is performed by having acidic anthrone reagent (contains H_2SO_4) with the sugar solution to yield a dark green complex.



C-Bial's Orcinol Test:

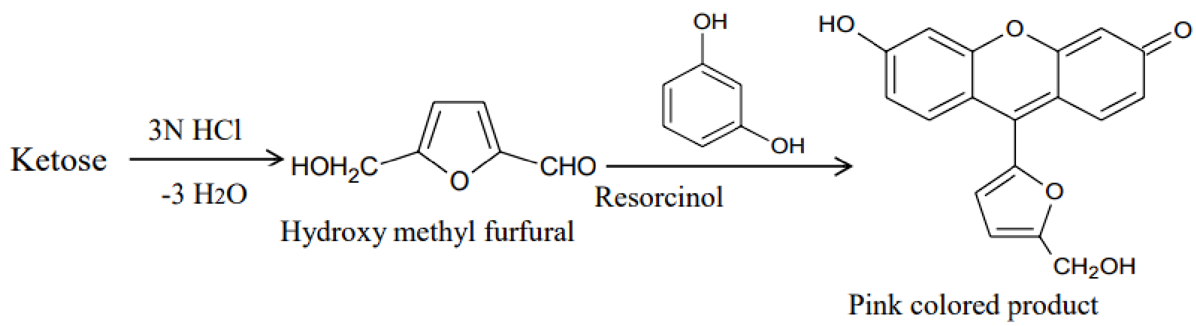
It is a sensitive specific test for the detection of pentoses, and other biochemical molecules containing these pentoses in their structure, such as ribose in RNA (ribonucleic acid) and deoxyribose in DNA (deoxyribonucleic acid). Thus, this test can be to distinguish pentoses from other sugars (hexoses). It is performed by mixing few milliliters of Bial's orcinol reagent with the sugar to yield a blue-green colored complex in the presence of pentoses, within a short time. Hexoses also react with this phenolic substance, but the product is yellow to brown in color.



Blue green colored product

D- Seliwanoff's (Resorcinol) Test:

This test is given by ketoses. Thus it is used to distinguish ketoses (fructose), from aldoses (glucose and mannose). Seliwanoff's is a solution of resorcinol in hydrochloric acid. Fructose gives a deep red color in a short time compared to a light pink color produced by glucose. When dehydrated (removal of water molecules) in concentrated acid, both ketohexoses and aldohexoses yield hydroxymethyl furfural and other decomposition products. They both yield the same products except that the relative amounts of the products vary depending on wether one works with aldoses or ketoses. Only small amounts of hydroxymethyl furfural are formed from aldohexoses. For this reason Seliwanoff's test can be used to distinguish between ketoses and aldose.



II. Effect of concentrated nitric acid on carbohydrates:

Mucic Acid Test:

The oxidation of some carbohydrates with concentrated nitric acid, $HNO3$, produces disaccharic acid or called dicarboxylic acid which differs in solubility. The dicarboxylic acid produced by galactose is insoluble (white crystals). Thus galactose may be distinguished from other carbohydrates. The nitric acid oxidizes both the aldehyde and the primary alcohol groups of monosaccharides to yield dicarboxylic acid.