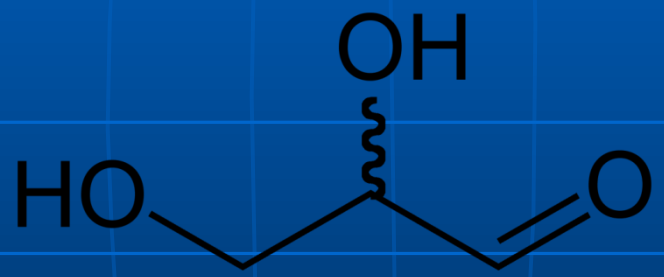
Carbohydrates

Aim of practicals

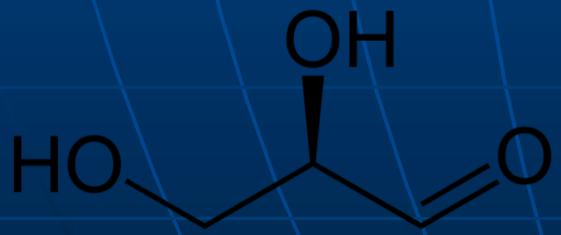
- Become familiar with properties of carbohydrates based on the results of the determinations of known carbohydrate sample
- Detection of an unknown sugar

The configuration of monosaccharides

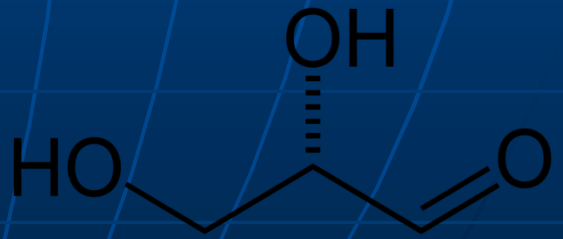
Monosaccharides as optically active compounds are present in two rows D and L configuration, which can be derived from the glyceraldehyde standard:



glyceraldehyde

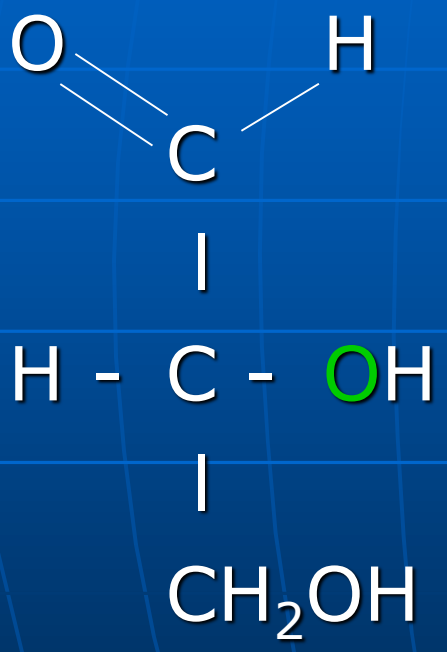


D-glycerin aldehyde

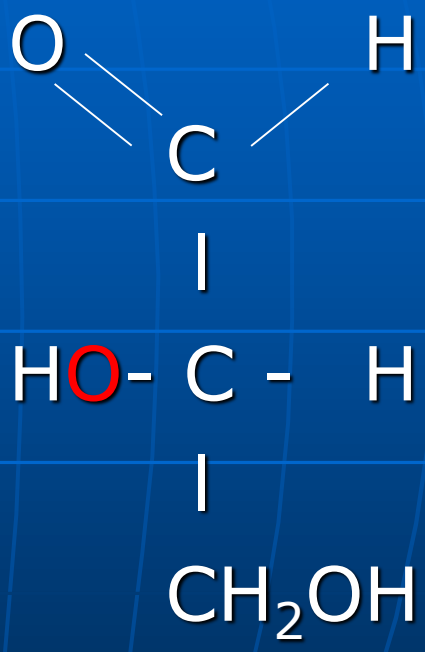


L-glycerin aldehyde

The stereoisomerism of carbohydrates



D-glycerin aldehyde



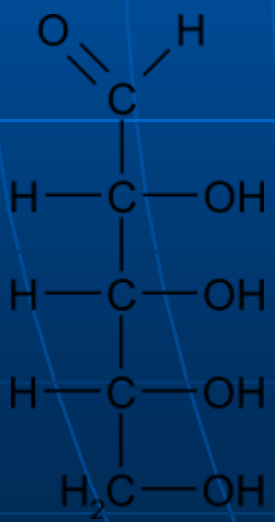
L-glycerin aldehyde

The configuration of monosaccharides

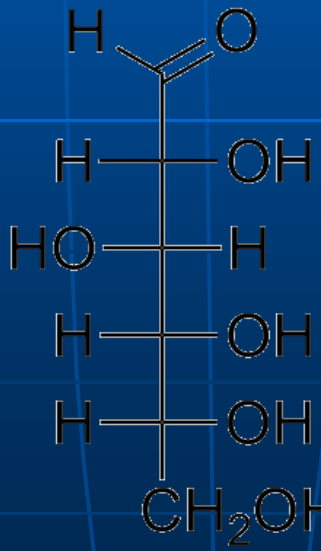
In monosaccharides with more than one asymmetric carbon atom the position of the -OH group on the last asymmetric carbon atom on the right side shows that sugar belongs to a series of D isomers.

An enantiomer that rotates light as a clockwise direction is determined (+) or right-handed, and in the opposite direction - Left-hand (-).

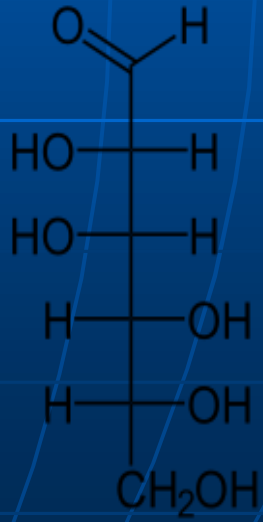
Biological significance have only D-monosaccharides.



D-ribose

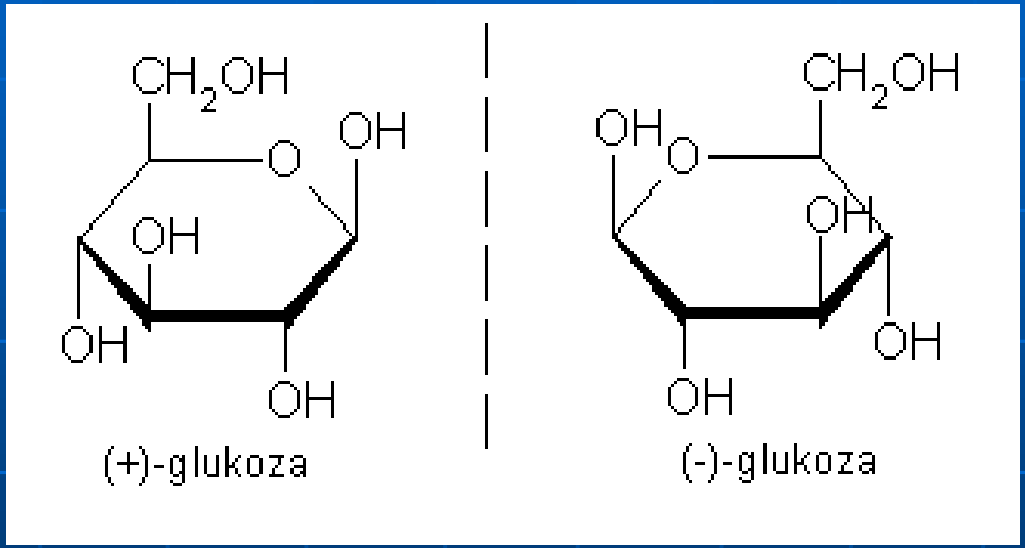


D-glucose



D-mannose

The racemic mixture - equimolar mixture of right- and left-handed enantiomer



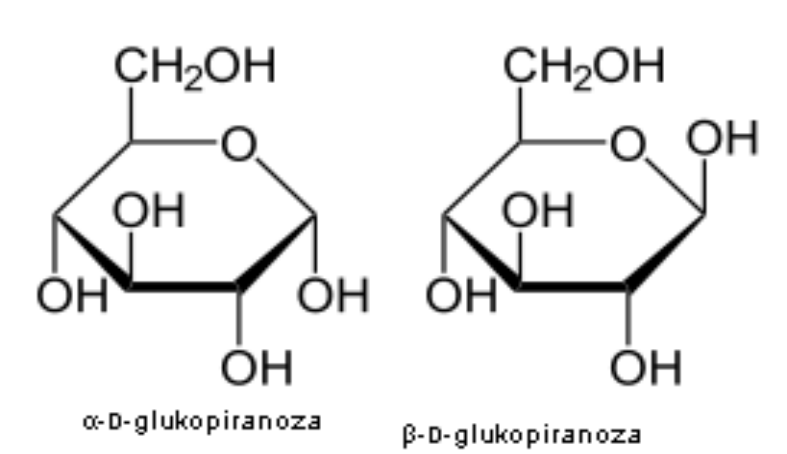
The anomers of carbohydrates

An anomer is a type of stereoisomer and epimer found in carbohydrate chemistry. While an epimer is a stereoisomer that differs in configuration at any single stereogenic center, an anomer is a cyclic saccharide and an epimer that differs in configuration, specifically at the hemiacetal/acetal carbon, also called the anomeric carbon.

The anomeric carbon is the carbon derived from the carbonyl carbon (the ketone or aldehyde functional group) of the open-chain form of the carbohydrate molecule.

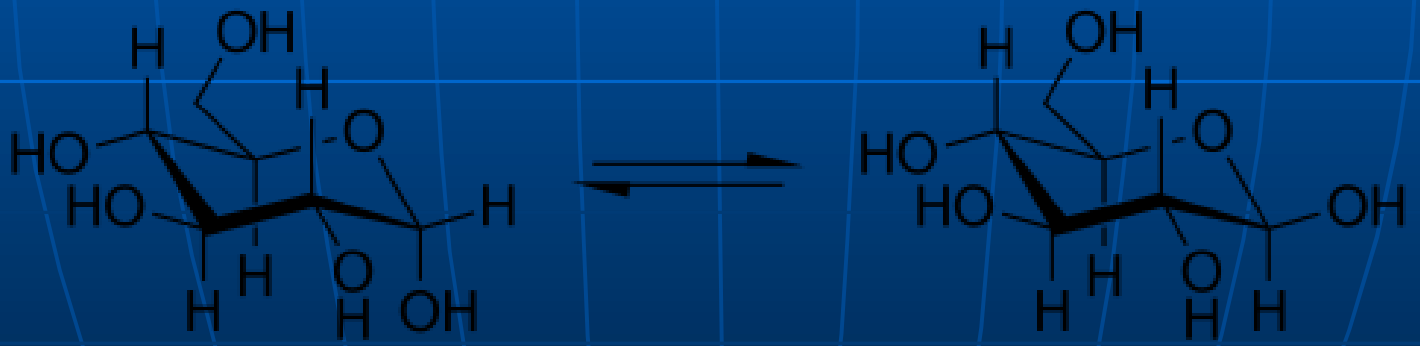
Anomerization is the process of conversion of one anomer to the other.

Anomer α means isomer where OH group at anomeric carbon atom islocated under the plane of the ring while anomer β - above the plane, respectively.



Mutarotation

Mutarotation is the change in the optical rotation because of the change in the equilibrium between two anomers, when the corresponding stereocenters interconvert. Cyclic sugars show mutarotation as α and β anomeric forms interconvert. The optical rotation of the solution depends on the optical rotation of each anomer and their ratio in the solution.

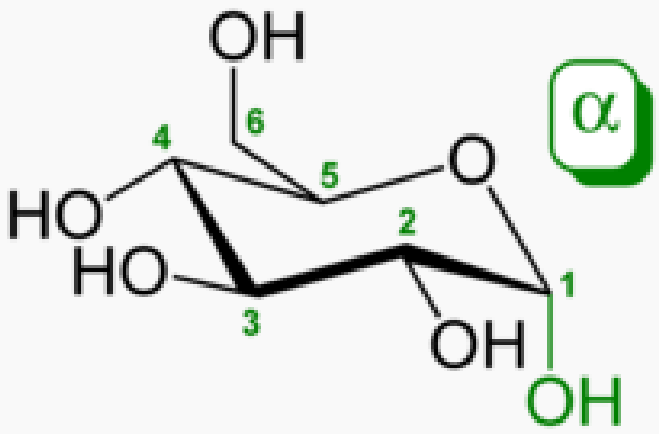
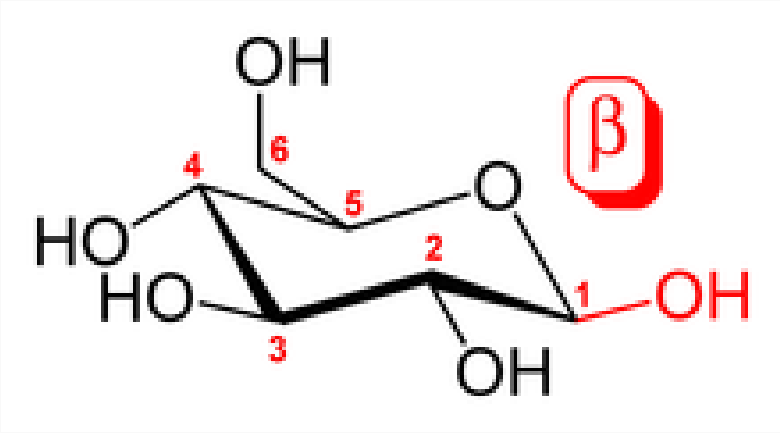


The epimers of carbohydrates

The epimers are these diastereomers which differ in the position of only one -OH group, but other than the C-1 in C-aldoses or 2 in C-ketoses and other than the last asymmetric carbon atom.

The reactions which change the position of the substituents on a single asymmetric carbon atom in carbohydrates are called epimerization reactions. In this way sugars may be converted to some other hexoses.

The epimers of carbohydrates

	
α-D-glucopyranose	β-D-glucopyranose

Characteristic reactions of sugars

Oxidation of sugars

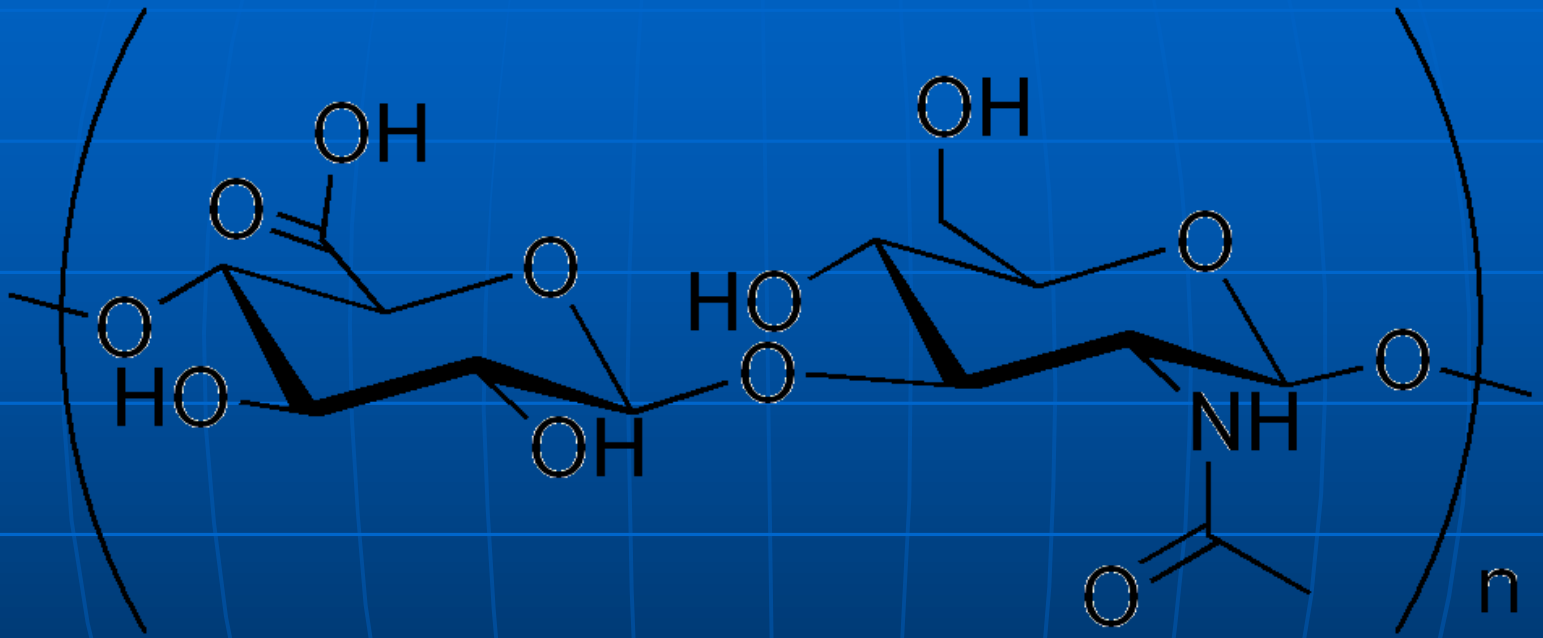
Oxidation of the hydroxyl group at C6 leads to the uronic acids:

Glucose (before oxidization)

The β-D form of glucuronic acid (after oxidization).

Uronic acids - are components of heteroglycans, participate in detoxification reactions (coupling reactions of steroid hormones, bilirubin, drugs, xenobiotics)

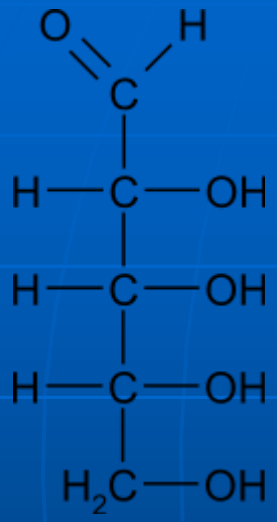
Hyaluronic acid



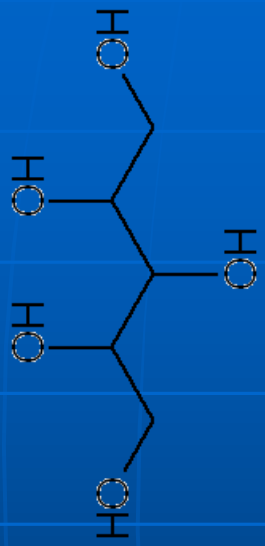
Chemically it is contrary to its name no acid, but the biopolymer, where the disaccharide repeating units are amide formed from D-glucuronic acid and D-N-acetylglucosamine.

Characteristic reactions of sugars

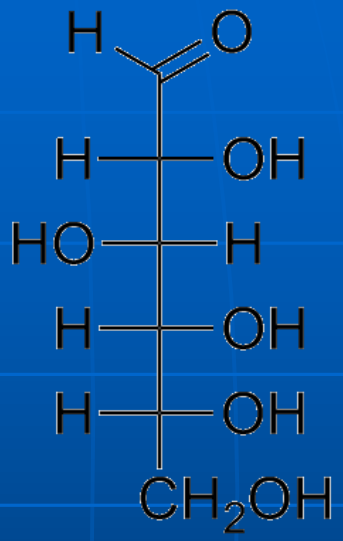
Reducing sugars leads to the formation of polyhydric alcohol.



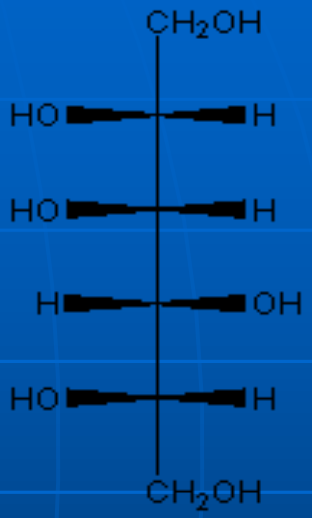
D-ryboza



rybitol



D-glukoza



sorbitol

Ribitol is a component of vitamin B2 (riboflavin), and FAD (flavin adenine dinucleotide).

D-sorbitol causes swelling and nerve damage. May cause cataract.

Galactitol accumulated in the lens of the eye may contribute to the development of cataracts

Characteristic reactions of sugars

Phosphate esters of sugars formed by reactions catalyzed by kinases - phosphate donor is ATP and energy.

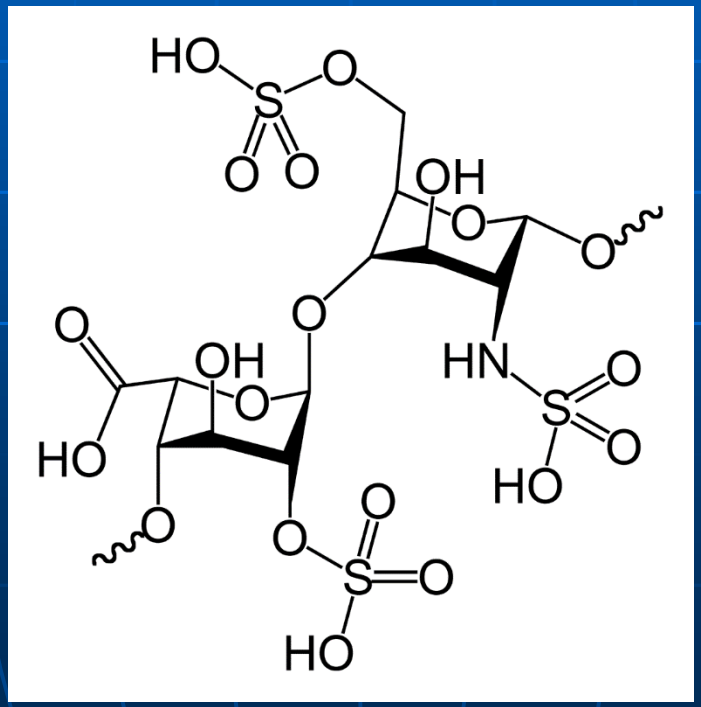
In living organisms, D-glucose-1-phosphate is produced by phosphorolysis of glycogen. phosphosugars are intermediates in a number of reactions - glyceraldehyde 3-phosphate.

5-phosphoribosyl-1-pyrophosphate is used in the synthesis of purine and pyrimidine nucleotides.

Phosphorylated sugars are in the form of divalent anions, and are stronger acids than phosphoric acid. This charge prevents them overcome bilayer membrane barriers. Thus, phosphate esters are forms of sugars, which are retained inside the cell.

Characteristic reactions of sugars

Sulfate esters of sugars carry negative charges. They are components of biologically important heteroglycans (heparin).

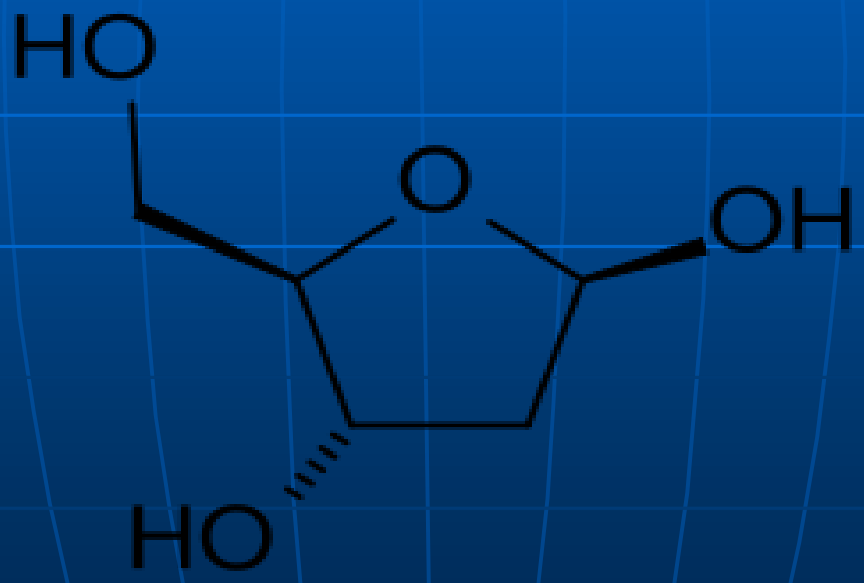


heparin

Characteristic reactions of sugars

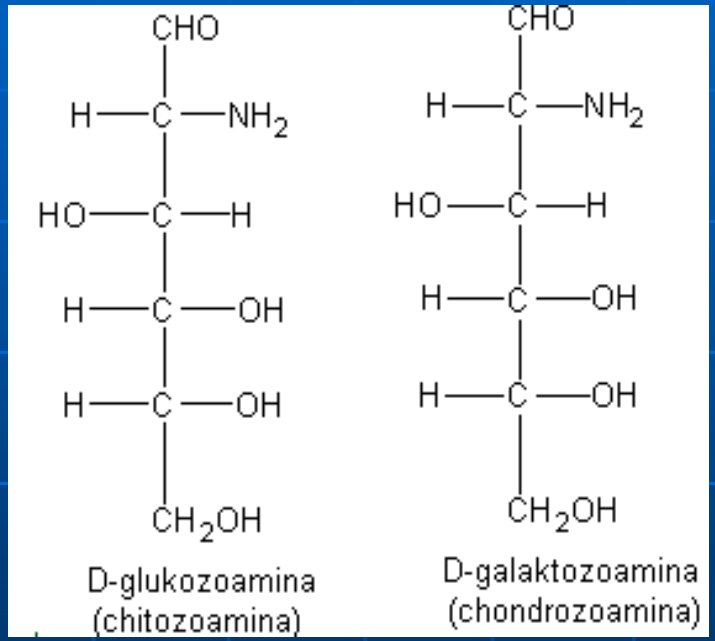
Deoxysugars devoid of a hydroxyl group

2-deoxy-β-D-ribofuranose is a constituent of deoxyribonucleic acid.



Characteristic reactions of sugars

Amino sugars are derived from monosaccharides in which the hydroxyl group at the 2-position is replaced by an amino group.



In living organisms are further acetylated and are constituents of heteroglycans (sialic acids).

Characteristic reactions of sugars

Glycosides are produced during the reaction of the OH group on the carbon with a hemiacetal hydroxyl group of alcohols and phenols to form acetals called glycosides.

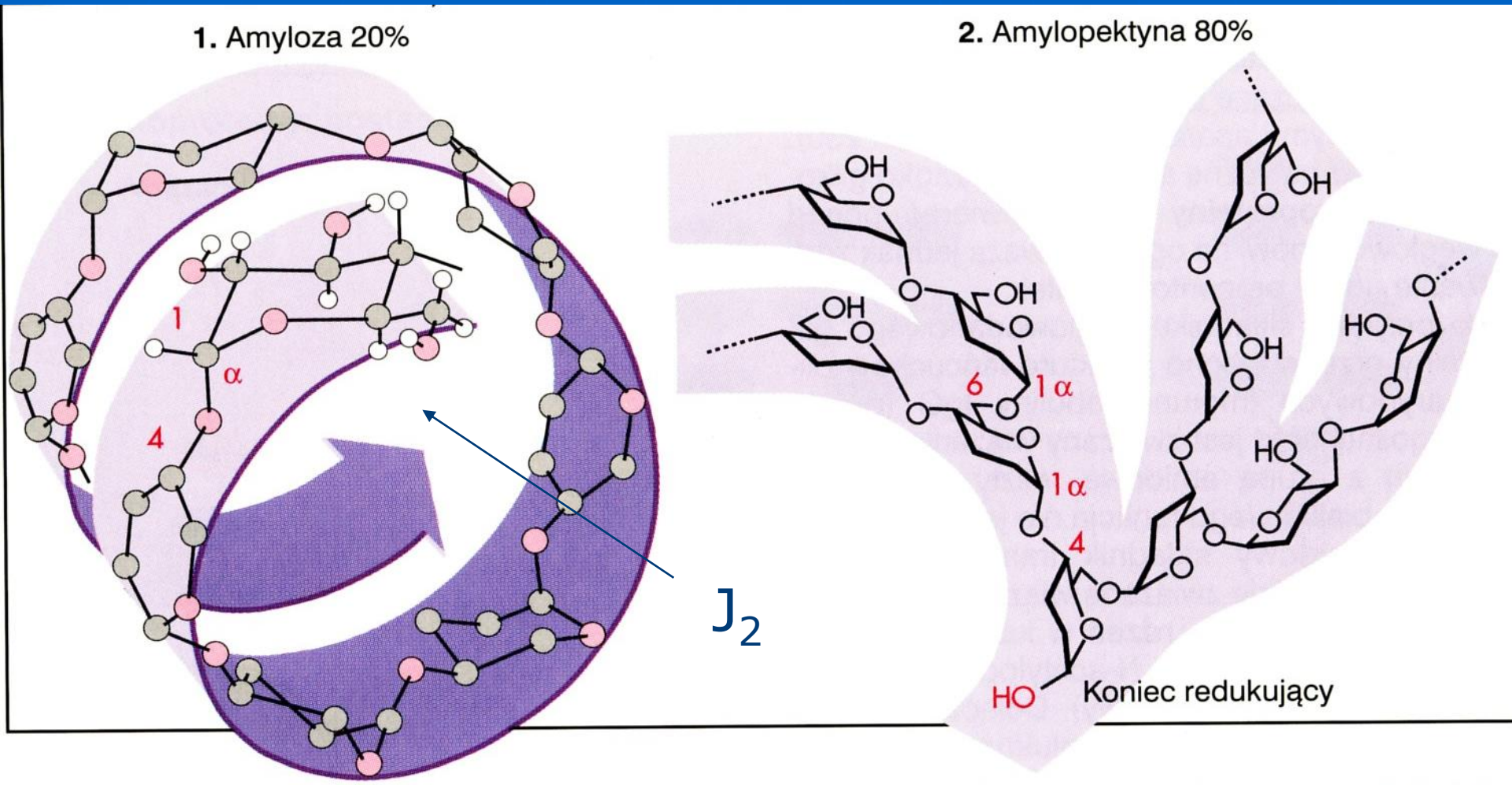
O-glycosides, N-glycosides, S-glycosides - are readily soluble in water, are involved in the detoxification.

Cardiac glycosides (steroid)

Identification of an unknown sugar

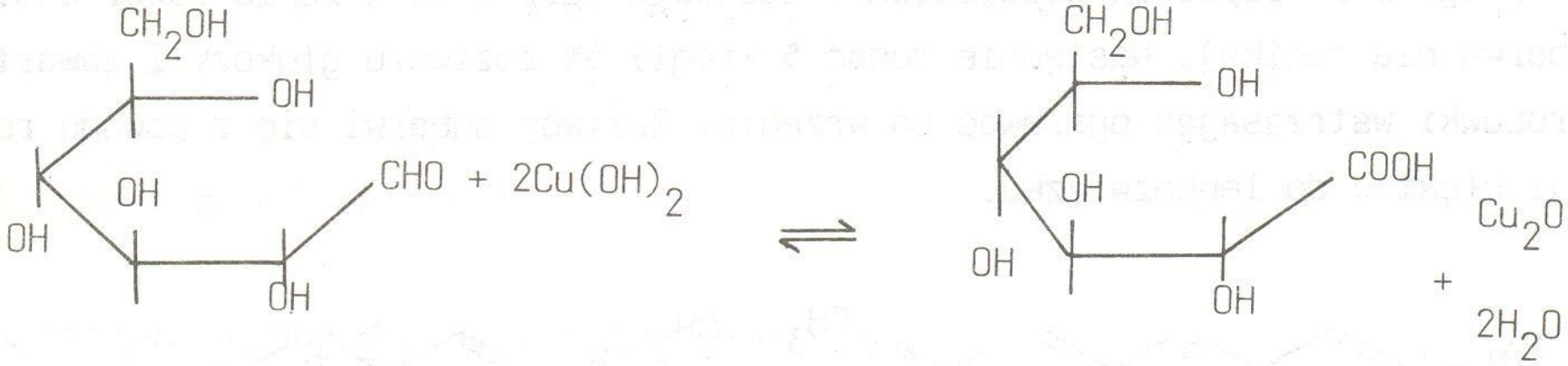
1. Perform the test of Molisch
2. Perform the iodine test
3. Perform other attempts

The iodine test



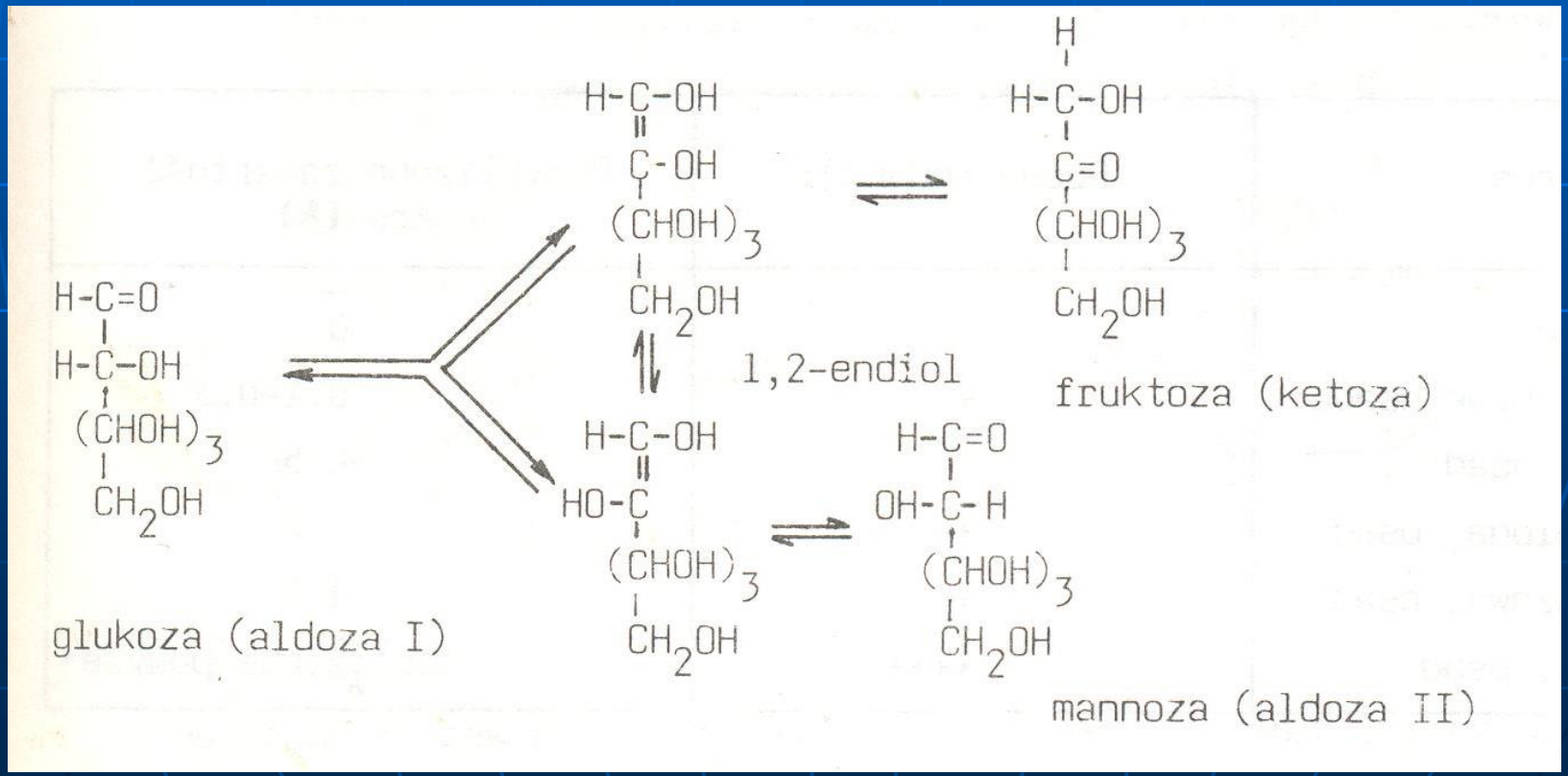
Test of Benedict

It is based on reducing properties of aldehydes by the reaction of Cu 2^{+} ions.



Test of Benedict

The principle of determination is the same as in the tests of Fehling, Trommer, Barfoed. All monosugars including ketoses give positive results due to epimerisation as well as reducing disaccharides such as maltose and lactose.

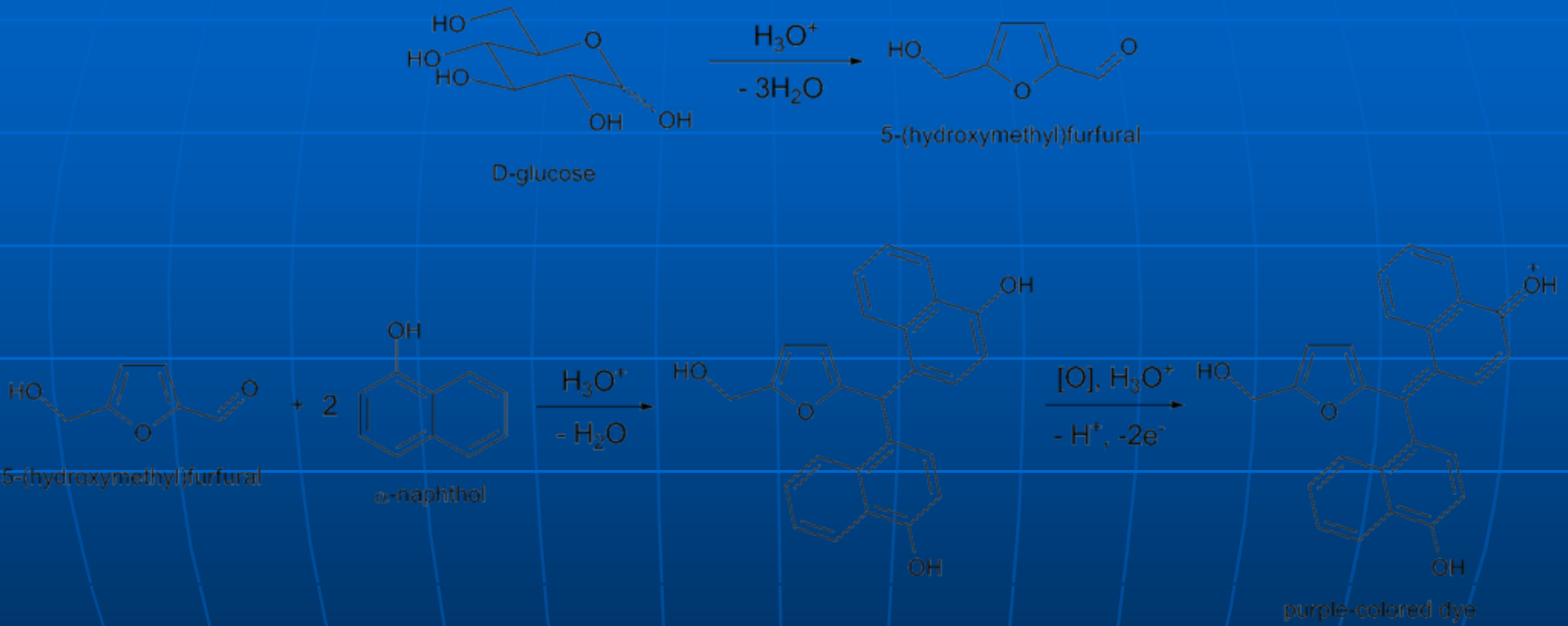


Test of Barfoed

Assay is used to distinguish monosugars from reducing disaccharides. It is performed in a water bath - to ensure stable conditions. Monosugars give a positive result after 3 min. of heating in a boiling water bath, while disaccharides after 8 - 15 min.

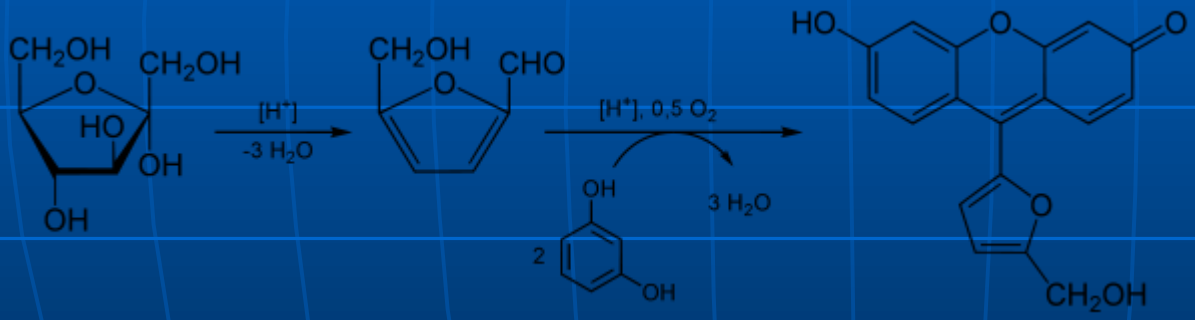
$$RCHO + 2Cu^{2+} + 2H_2O \rightarrow RCOOH + Cu_2O\downarrow + 4H^+$$

Test of Molisch with 1-naphthol



Test of Seliwanoff

- Test for ketoses (fructose) - Under the influence of hydrochloric acid aldose and ketose are converted to furfural derivatives, which react with resorcinol to produce a short-term heating cherry precipitate. Longer heating - over 30s - moves aldose in ketose, and the result will be a false positive.



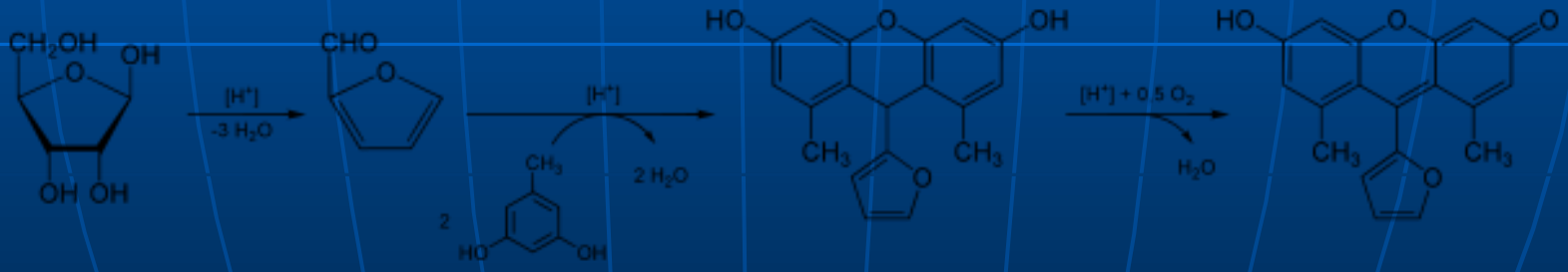
Test of Tollens

- Assay is used to detect pentoses
- Pentose under the influence of hydrochloric acid goes into furfural, which condenses with phloroglucinol giving cherry color.

Test of Bial

Test for the detection of pentoses. During heating in an acidic medium, pentoses are converted into furfural, which in the presence of $FeCl_3$ condenses with orcinol and forms a product of the blue-green color.

Hexose are converted under these conditions into hydroxymethylfurfural which reacts less well with orcinol that is why the product is of yellow-brown color.



Task 1

The aim of this task is to make selected tests to detect the presence of known sugars. The results of these tests will be useful in performing the second task. Therefore, note the results of performed tests.

Procedures

1. Molisch's test

Principles of determination

This is the general test for carbohydrate detection. Negative result of this test is more informative than a positive result. Negative result (lack of staining) informs that in a tested solution, there is no sugars, both in free form or attached to other compounds, e.g. glycoproteins and glycolipids. Positive result of this test can occur in presence of sugars and also other non-sugar substances, like acetone, formic acid, lactic acid, oxalic acid, citric acid. All these compounds can condense with alpha-naphthol and form colourful products (false positive results).

Procedure

Pipette 1 cm^3 of solution of chosen sugar to a tube and add 2 – 3 drops of Molisch reagent (solution of alpha-naphthol in an alcohol) and stir. Then, add **carefully** 1 cm^3 of concentrated sulphuric acid pouring it on the wall of tube set diagonally. As a result, acid layer on the bottom of the tube, under the water solution occurs. This kind of adding a reagent is called under layering. Wait a few minutes. On the border between two layers, red-purple ring is formed gradually.

2. Tollens's test for pentose detection

Principles of determination

Tollens reagent includes phloroglucinol {$C_6H_3(OH)_3$} dissolved in a concentrated chloric acid solution. Concentrated chloric acid solution works as dehydration factor. Pentose loses three molecules of water and is transformed to furfural. Phloroglucinol concentrates with furfural producing coloured product of reaction which has cherry colour.

Procedure

Measure 1 cm^3 of Tollens reagent to a tube and add 1 – 2 drops of arabinose solution. Heat it over the burner carefully and boil it over a dozen of seconds stirring briskly until cherry colour appears.

3. Seliwanoff's test

Principles of determination

This test detects the presence of ketoses. Seliwanoff's reagent includes resorcinol {$C_6H_4(OH)_2$} dissolved in 12% HCl. Under the influence of 12% HCl, only ketoses are transformed to furfurals (ketohexoses transform to 5-hydroxymethylfurfural), whereas aldoses do not transform in these conditions. Resorcinol concentrates with furfural producing cherry colour.

Procedure

Mix in a tube 1 cm^3 of Seliwanoff's reagent with 2 drops of fructose solution. Heat it carefully over the burner for 30 second or a little longer under cheery colour appears.

Attention: sucrose gives also the positive result of this test because sucrose includes fructose in its molecule, and sucrose disintegrates in 12% HCl solution.

4. Benedict's test

Principles of determination

The Benedict's reagent consists of $CuSO_4$, sodium citrate and Na_2CO_3, therefore, it is base. During reaction, cooper ions having +2 oxidation number are reduced to Cu^{+1} and precipitate in form of Cu_2O which has orange tint. Unfortunately, ketoses i.e. fructose gives also positive result of this probe. This phenomenon occurs because ketoses transforms in base solution to aldoses. This reaction is called epimerisation.

Procedure

Mix 1 cm^3 of Benedict's reagent with a few drops of examined sugar solution (glucose) in a tube and boil it over the burner. Observe the appearing of orange or red precipitate of cooper oxide.

5. Barfoed's test

Principles of determination

This test allows for the distinction of reducing disaccharides and monosaccharides.

Reducing reactions in organic chemistry occur easiest in base environment. As acidity of environment increases, the reducing properties of sugars decreases. This phenomenon is used to distinguish monosaccharides from reducing disaccharides. Barfoed's reagent includes Cu ions, similarly to Benedict's and Fehling's reagents but has higher pH than these two reagents.

Procedure

Measure 1 cm^3 of Barfoed's reagent to three tubes marked as G, L and S. Add 1 cm^3 of glucose solution to tube G, 1 cm^3 of lactose solution to tube L, and 1 cm^3 of sucrose to tube S. Put them into a boiling water bath for 3 minutes, get them out of the bath and observe the results. Then, put all three tubs into the boiling bath again for next 12 minutes. After this period of time, observe the results again.

Try to explain the results.

Task 2

The aim of task 2 is the identification of unknown, tested sugar. To do this, you need to make the tests described in task 1. Characteristic results of these tests are given in the table below.

Tests:	Arabinose	Glucose	Fructose	Lactose	Sacharose
Benedict's	+	+	+	+	-
Barfoed's	+	+	+	*	-
Tollens's	+	-	-	-	+/-
Seliwanoff's	-	-	+	-	+

+ positive result; - negative result; * the result is negative after 3 minutes of heating in boiling water bath, and positive after 15 minutes, however, you need to pour out the content of the tube to observe light precipitate on the bottom of the tube

Procedure

1. Take randomly selected tube with solution of unknown sugar. Note the number of your sample.
2. Make the Molisch's test to be sure that your sample includes sugar.
3. Make the test with iodine to confirm or exclude the presence of polysaccharides, like starch. Procedure of the test with iodine: mix 1 cm^3 of sugar solution with a few drops of iodine solution (aqueous solution of potassium iodide). In the presence of starch, navy blue colour appears. Iodine molecules (I_2) penetrate inside the starch molecule which has the form of spiral. Inside this spiral, molecules of iodine forms complex bounds, producing an intense blue/black colour.
4. Make all other tests described in task 1.

Note the results of your tests in the table below. Which sugar is in your sample? Explain your answer.

Tests:	your results:
Molisch's	
with iodine	
Benedict's	
Barfoed's	
Tollens's	
Seliwanoff's	