AL-RASHEED PHARMACY
Dpt.

3rd year 1st semester

Biochemistry

Lab 2

Seliwanoff's Test and Bial's Test

By

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Seliwanoff's Test

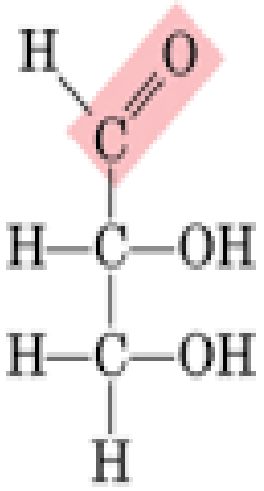
Introduction

Ketose and aldose are monosaccharides which can be differentiated based on the group they contain. An aldose is defined as a monosaccharide whose carbon skeleton has an aldehyde group. They are primarily found in plants.

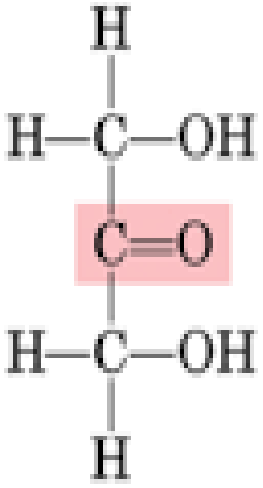
- Ketose is a monosaccharide whose carbon skeleton has a ketone group. They are used in processed food. Examples of ketose are ribulose, fructose, etc.

Difference Between Aldose And Ketose

Characteristics	Aldose	Ketose
Group	Aldehyde	Ketone
Found	Primarily in plants	In processed food
Isomerization	isomerize into ketoses	isomerize into aldoses only in the presence of reducing sugar’
Seliwanoff’s Test color	Light pink	Deep cherry red
Example	Glucose, ribose, and galactose	Fructose, erythrulose, and ribulose



Aldose



Ketose

Seliwanoff's test definition

Seliwanoff's test is used to differentiate between sugars that have a ketone group (ketose) and sugars that have an aldehyde group (aldoses). This test is a timed color reaction specific to ketohexoses.

Objectives of Seliwanoff's test

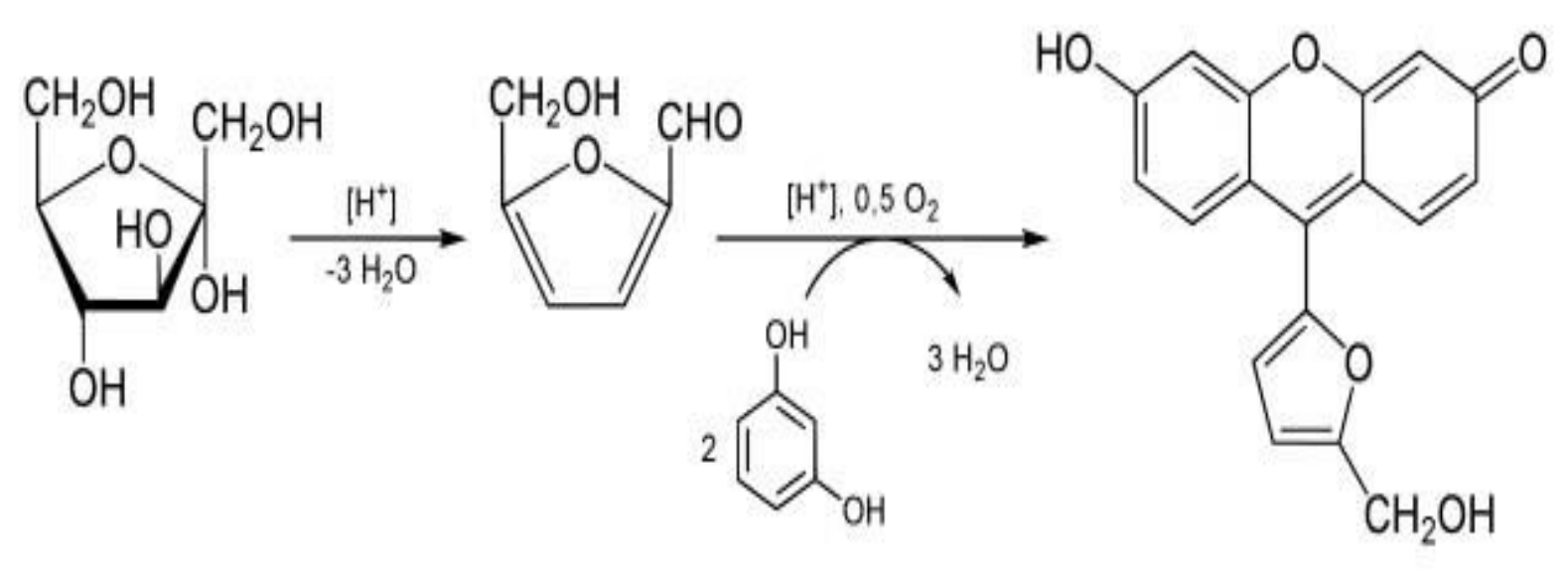
- To detect the presence of ketohexoses in a given sample.
- To distinguish ketoses from aldoses.

Principle of Seliwanoff's test

- The reagent of this test consists of resorcinol and concentrated HCl.
- The acid hydrolysis of polysaccharides and oligosaccharides yields simpler sugars.
- Ketoses are more rapidly dehydrated than aldoses.
- Ketoses undergo dehydration in the presence of concentrated acid to yield 5-hydroxymethyl furfural.

- The dehydrated ketose reacts with two equivalents of resorcinol in a series of condensation reactions to produce a complex (not a precipitate), termed xanthenoid, with deep cherry red color.
- Aldoses may react slightly to produce a faint pink to cherry red color if the test is prolonged.
- Other carbohydrates like sucrose and inulin also give a positive result for this test as these are hydrolyzed by acid to give fructose.

Reaction


CH2OH
CH2OH
O
HO
OH
OH
[H+]
-3 H2O
CH2OH
CHO
O
[H+], 0,5 O2
OH
2
OH
3 H2O
HO
O
O
O
CH2OH

Requirements

- **Reagent**
- **Seliwanoff's reagent:** Dissolve 50 mg resorcinol in 33 ml concentrated HCl and make it 100 ml with water.
- Test sample
- Distilled water

- **Materials required**
- Test tubes
- Test tube stand
- Pipettes
- **Equipment**
- Water bath

Procedure of Seliwanoff's test

- Take two clean, dry test tubes and add 1 ml of the test sample in one test tube and 1 ml of distilled water in another as blank.
- Add 2 ml of Seliwanoffs' reagent to both the test tubes.
- Keep both the test tubes in a water bath for 1 min.
- Observe the formation of color and note it down.

Seliwanoff's Test- Definition, Principle, Procedure, Result, Uses

Negative
Seliwanoff's Test

Ketoses Absent

Positive
Seliwanoff's Test

Ketoses Present

cherry red-colored
complex formed

- The formation of the cherry red-colored complex indicates a positive result which means that the given sample contains ketoses.
- The absence of such color or the appearance of the color after a prolonged period of time indicates a negative result which means that the test sample doesn't have ketoses.

Uses of Seliwanoff's test

- Seliwanoff's color reaction is used in the method for the colorimetric determination of fructose in fermentation media.
- A modified version of this test can be used for the determination of the concentration of ketoses in a given sample.

Limitations of Seliwanoff's test

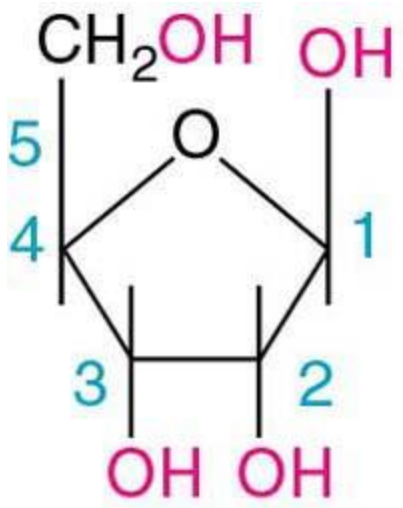
- The high concentration of glucose or other sugar may interfere by producing similar colored compounds with Seliwanoff's reagent.
- Prolonged boiling can transform glucose to fructose by the catalytic action of acid and form cherry red-complex giving a false-positive result.
- This test is a generalized test and doesn't distinguish between specific ketoses, and a separate test is required for the particular ketose sugar identification.

Bial's Test

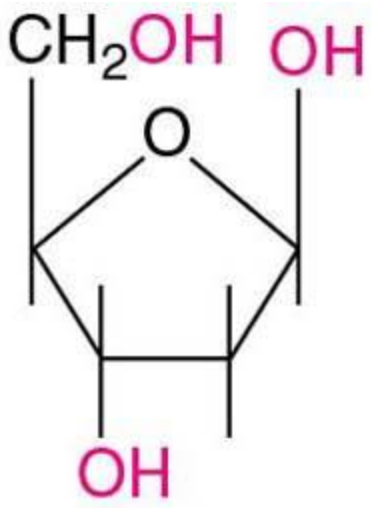
Introduction

a pentose is a monosaccharide (simple sugar) with five carbon atoms.The chemical formula of all pentoses is C5 H10 O5

Pentoses are very important in biochemistry. Ribose is a constituent of RNA, and the related molecule, deoxyribose, is a constituent of DNA.



Ribose



2-deoxyribose

Bial's Test Definition

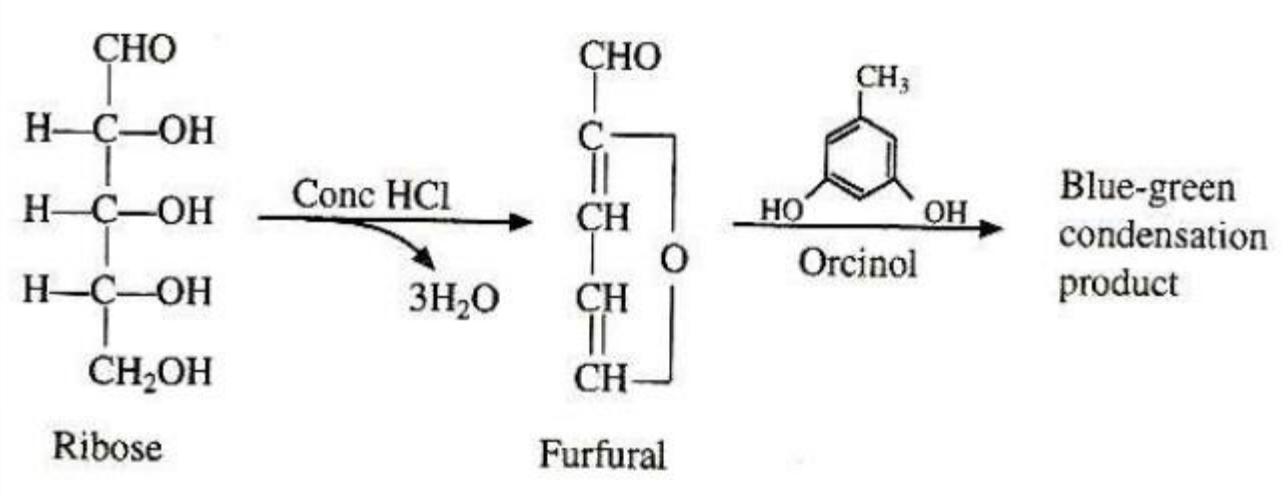
It is a sensitive specific test for the detection of pentoses,and other biochemical molecules containing these pentoses in their structure,such as ribose in RNA and deoxyribose in DNA.

Objectives of Bial's Test

- To detect the presence of **carbohydrates**.
- To distinguish the pentoses from other derivatives of carbohydrates like the hexoses.

Principle of Bial's Test

This test is based on the principle that under hydrolysis pentosans are hydrolyzed into pentoses. Further, pentoses are dehydrated to yield furfural, which in turn condense with orcinol to form a blue-green precipitate. In the presence of hexoses, hydroxyfurfural is formed instead of furfural which upon condensation with orcinol forms a muddy brown colored precipitate. The intensity of the precipitation is directly proportional to the concentration of the pentoses in the sample.


CHO
H—C—OH
H—C—OH
H—C—OH
CH2OH
Ribose
Conc HCl
3H2O
CHO
C
CH
CH
CH
O
Furfural
CH3
HO
OH
Orcinol
Blue-green
condensation
product

Requirements

A. Materials required

Equipment:

Water Bath.

Chemicals/Reagents:

Bial's Reagent

Ribose sugar sample

Glasswares and other equipment:

Test tubes, Test tube stand, Pipettes, Beaker, Wash bottle.

B. Reagents

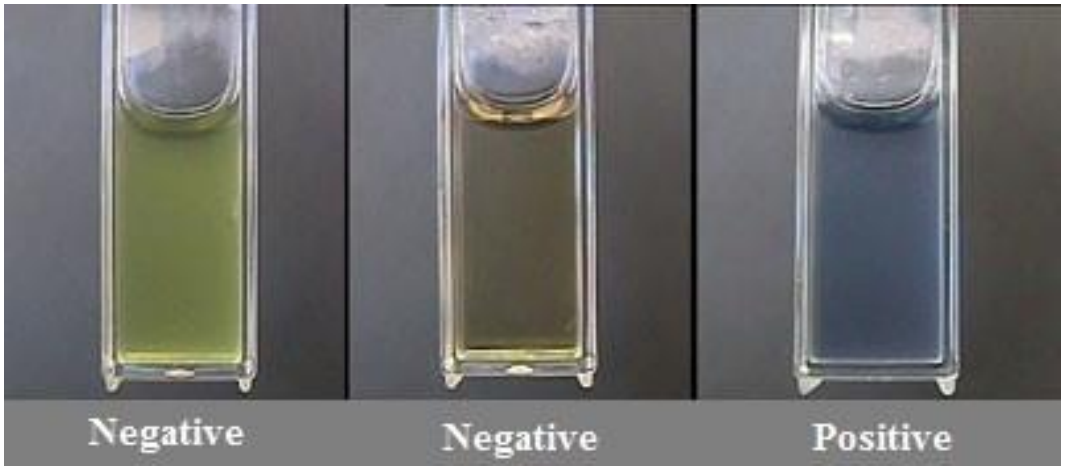
- Bial's Reagent: 300 mg of orcinol is dissolved in 5 ml ethanol. Add 3.5 ml of this mixture to 100ml of 0.1% solution of ferric chloride.
- Ribose stock solution: 200µg ribose per mL distilled water stock solution of ribose is to be prepared from the stocked solution.

Procedure of Bial's Test

- Take 2ml of Bial's reagent in test tube.
- Add 4-5 drops of test solution to this reagent.
- Keep in water bath for 30 seconds.
- Look for the development of bluish green color.

Result interpretation for Bial's test:

- **Positive Bial's test:** formation of blue color (eg. Ribose sugar)
- **Negative Bial's test:** formation of any other color indicates negative test. Hexose sugar (glucose, fructose) generally gives green, red or brown color product.


Negative
Negative
Positive

Uses of Bial's Test

- This test is used to detect the presence of pentose and pentosans in different samples.
- This test can additionally be used for the quantification of RNA in a sample.

THANK YOU FOR LISTINING

Prepared by:

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