Reading material for 2nd year LS2102 Biochemistry practical prepared by Dr. Rituparna Sinha Roy. Distribution of this teaching material is not permitted

The reading material is developed based these books, wikepedia and several internet links:

1. Laboratory Handbook on Biochemistry (Authors: S. Shanmugan, T. Sathish Kumar, K. PanneerSelvam)
2. An Introduction to Practical Biochemistry (Author: David T Plummer)
3. Introductory Practical Biochemistry (Authors:S. K. Sawhney, R. Singh)
4. Internet sources and wikipedia

Qualitative analysis of Carbohydrates

Introduction

A carbohydrate is an organic compound that consists only of carbon, hydrogen, and oxygen, usually with a hydrogen:oxygen atom ratio of 2:1 (as in water); in other words, with the empirical formula $C_m(H_2O)_n$.

Carbohydrates are one of the most important components in many foods. Some carbohydrates are digestible by humans and therefore provide an important source of energy, whereas others are indigestible and therefore do not provide energy. However indigestible carbohydrates are still useful as *dietary fiber*. Consumption of significant quantities of dietary fiber is beneficial to human nutrition, helping reduce the risk of certain types of cancer, coronary heart disease, diabetes and constipation. It is, therefore, important to determine the type and concentration of carbohydrates in foods for a number of reasons which include

- *Standards of Identity* - foods must have compositions which conform to government regulations
- *Nutritional Labeling* - to inform consumers of the nutritional content of foods
- *Detection of Adulteration* - each food type has a carbohydrate "fingerprint"
- *Food Quality* - physicochemical properties of foods such as sweetness, appearance, stability and texture depend on the type and concentration of carbohydrates present.

Carbohydrates can be classified into three following groups:

Monosaccharides

Monosaccharides are water-soluble crystalline compounds. They are aliphatic aldehydes or ketones which contain one carbonyl group and one or more hydroxyl groups. Most natural monosachharides have either five (pentoses) or six (hexoses) carbon atoms. Commonly occurring hexoses in foods are glucose, fructose and galactose, whilst commonly occurring pentoses are arabinose and xylose. The reactive centers of monosaccharides are the carbonyl and hydroxyl groups.

Disaccharides

A disaccharide is the carbohydrate formed when two monosaccharides undergo a condensation reaction which involves the elimination of a small molecule, such as water, from the functional groups only. Like monosaccharides, disaccharides form an aqueous solution when dissolved in water. Three common examples are sucrose, lactose and maltose. There are two different types of disaccharides: reducing disaccharides, in which one monosaccharide, the reducing sugar, still has a free hemiacetal unit; and non-reducing disaccharides, in which the components bond through an acetal linkage between their anomeric centers and neither monosaccharide has a free hemiacetal unit. Cellobiose and maltose are examples of reducing disaccharides. Sucrose and trehalose are examples of non-reducing disaccharides.

Polysaccharides

The majority of carbohydrates found in nature are present as polysaccharides. Polysaccharides are high molecular weight polymers of monosaccharide (> 20). Polysaccharides containing all the same monosaccharides are called homopolysaccharides (*e.g.*, starch, cellulose and glycogen are formed from only glucose), whereas those which contain more than one type of monomer are known as heteropolysaccharides (*e.g.*, pectin, hemicellulose and gums).

In solution the open-chain form of monosaccharide stays in equilibrium with the two *anomeric* ring forms of the cyclic derivative as shown by the following figure:

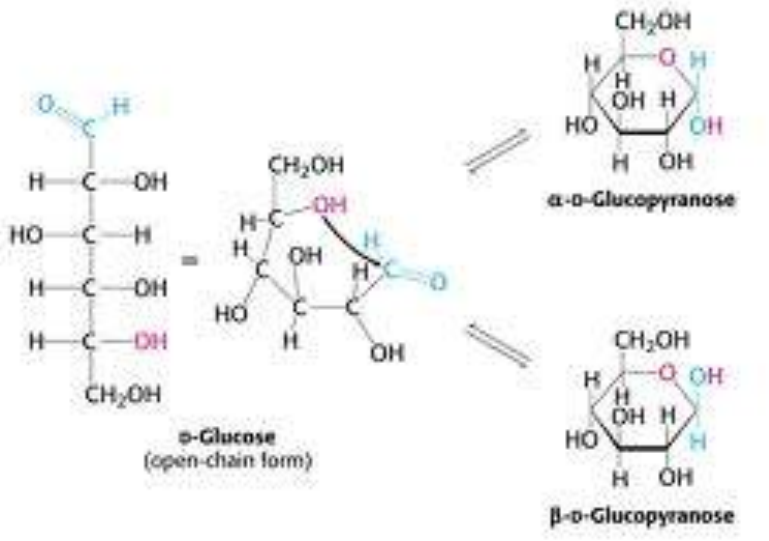


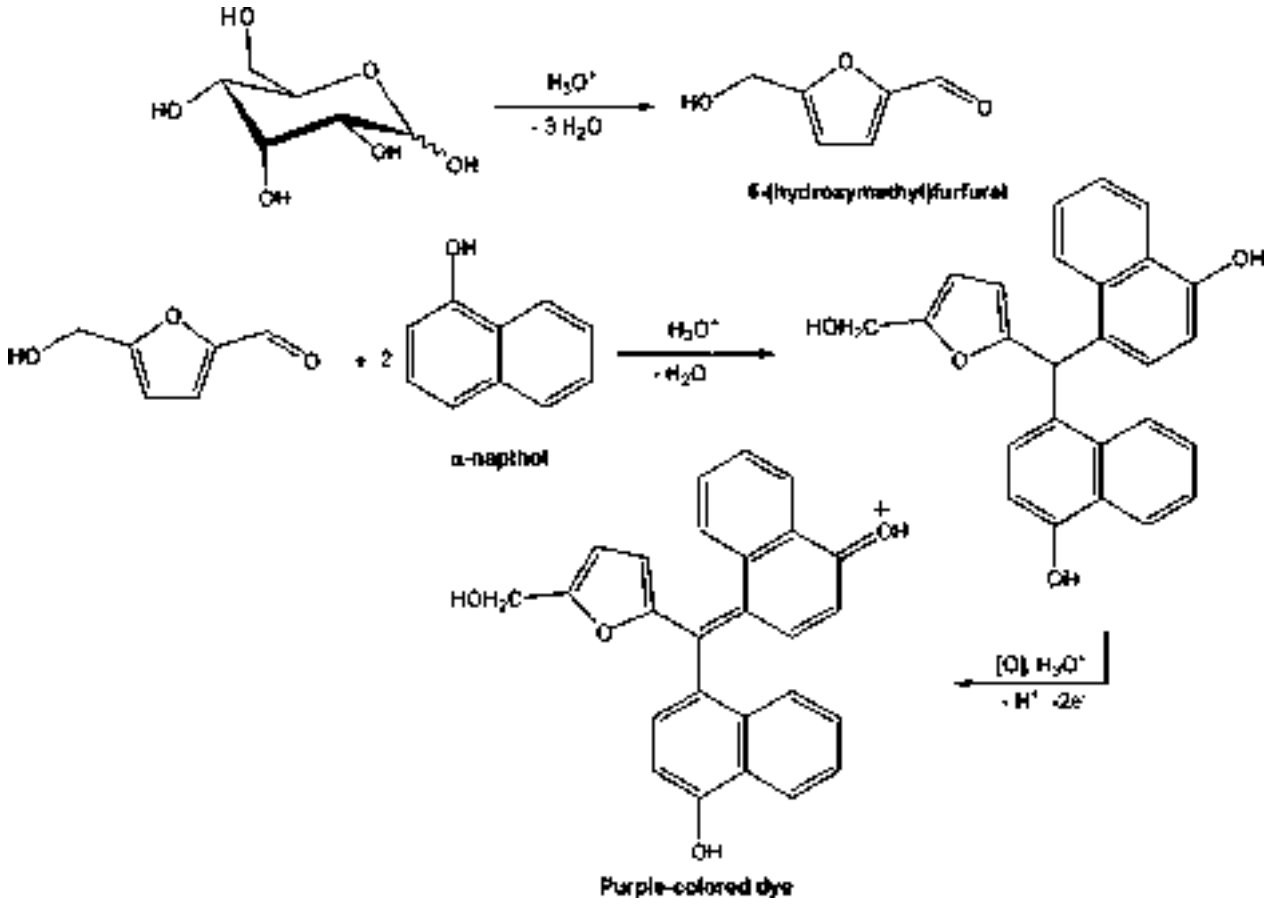
Figure 1. Open-chain and cyclic form of D-glucose.

This experiment aims to introduce you with the identification of unknown carbohydrates.

Principle of the method

Molisch's test :

All carbohydrates – monosaccharides, disaccharides, and polysaccharides – should give a positive reaction, and nucleic acids and glycoproteins also give a positive reaction, as all these compounds are eventually hydrolyzed to monosaccharides by strong mineral acids. Pentoses are then dehydrated to furfural, while hexoses are dehydrated to 5-hydroxymethylfurfural. Either of these aldehydes, if present, will condense with two molecules of naphthol to form a **purple**-colored product, as illustrated below by the example of glucose.



Scheme 1. Reaction of α-napthol with furfural derivative.

Iodine Test:

Iodine test is an indicator for the presence of starch. Iodine solution (iodine dissolved in an aqueous solution of potassium iodide) reacts with starch producing **a blue-black color.**

The appearance of the deep blue color is due to the formation of starch-iodine complex. Generally, the starch molecule assumes a helical bundle shape exposing hydroxyl groups and here, the electronegative iodide ion interacts with the electropositive hydrogen belongs to the –OH group. This results a polyiodide starch complex.

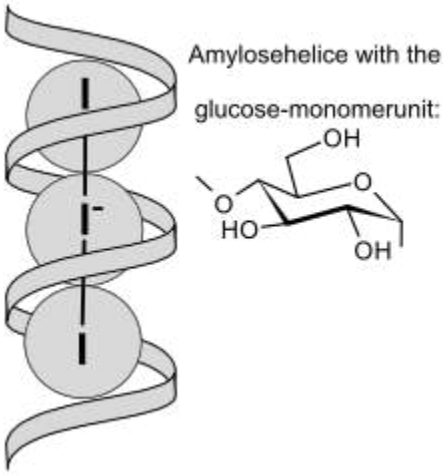


Figure 2. Schematic view of in amylosehelice embedded I_3^- ion.

Fehling's Test:

Fehling's Solution (deep blue colored) is used to determine the presence of reducing sugars and aldehydes. Perform this test with **fructose, glucose, maltose,Xylose, Sucrose.**

Fehling's test differentiates between aldehydes and ketones. Aldehydes can be oxidized by Cu^{2+} in the presence of a **strong base** (**alkaline**) to form carbonic acids. Ketones cannot be oxidized by this reaction. When the Cu^{2+} oxidizes the aldehydes it is reduced to Cu^{+}, and forms the compound Cu_2O, which is a **reddish** precipitate. That is how you know you have a reducing sugar (sugar molecule with an aldehyde as functional group).

$$RCHO + 2Cu^{2+}\ (blue) + 4OH^{-} \rightarrow RCOOH + Cu_2O\downarrow\ (red) + 2\ H_2O$$

Scheme 2. Chemical reaction of Fehling's solution with aldehyde.

Why is sodium potassium tartarate used in Fehling's solution?

Fehlings soluition is alkaline. Mixing cupric ions with an alkaline solution would result in formation of cupric hydroxide of very low water solubility. By adding tartrate ions, the cupric ions will form a complex with the cupric ions which will keep them dissolved in the alkaline solution. The type of cation of the tartaric acid is of no importance other than for solubility reasons.

Barfoed's Test:

Barfoed's reagent, cupric acetate in acetic acid (**acidic media**) , is slightly acidic and is balanced so that is can only be reduced by monosaccharides but not less powerful reducing sugars. Disaccharides may also react with this reagent, but the reaction is much slower when compared to monosaccharides. Perform this test with **glucose, maltose and sucrose**.

The is due to the reducing action of the free functional group present in the sugar in the acidic medium. Reducing monosaccharides may form red cuprous oxide (Cu_2O) within 2-3 min and that for disaccharide takes about 10 min.

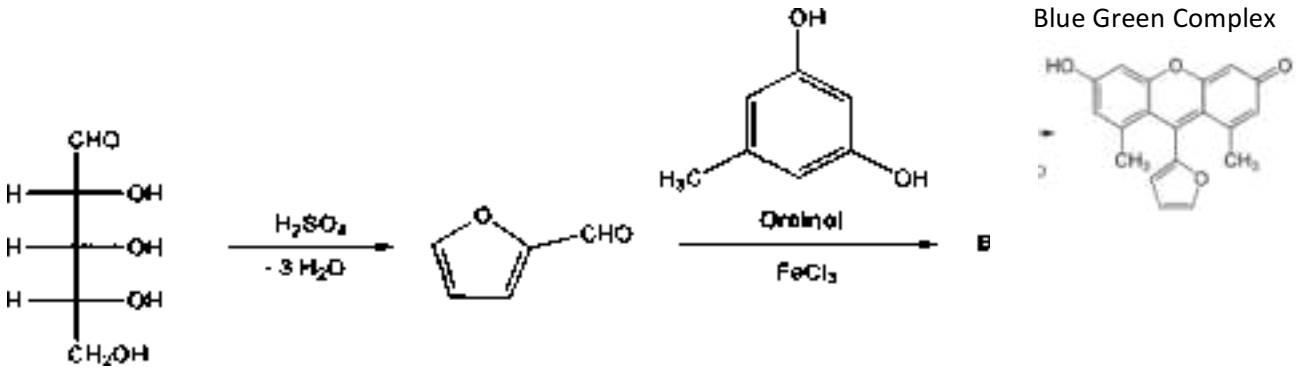
$$RCHO + 2Cu^{2+} + 2H_2O \rightarrow RCOOH + Cu_2O\downarrow (red) + 4H^+$$

Scheme 3. Chemical reaction for the Barfoed's test.

The test is similar to the reaction of Fehling's solution to aldehydes.

Bial's Test:

Bial's Test is to determine the presence of pentoses (5C sugars). The components of this reagent are orcinol, HCl and ferric chloride. In this test, the pentose is dehydrated to form furfural and the solution turns **bluish** and a precipitate may form. Perform this test with **ribose, xylose and glucose.**

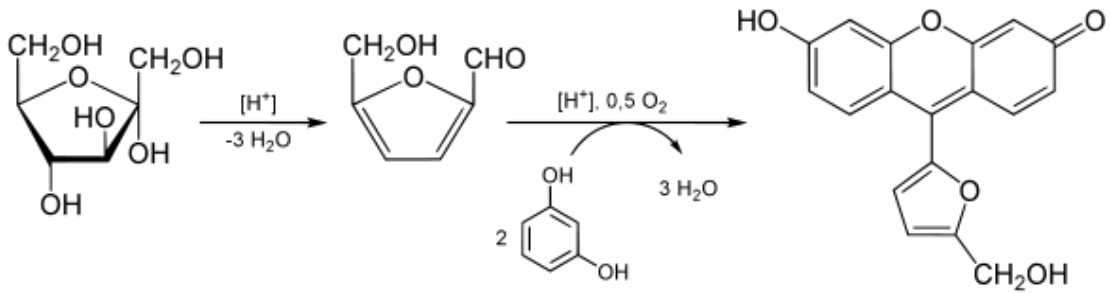


Scheme 4. Chemical reaction for the Bial's test.

Five membered Furan rings contain four carbons but sugars with furan rings can contain more carbons outside the ring, and all sugars with a furan ring will react in Bial's test. The pentose furanoses (for example, Xylose) will react with Bial's reagent to form green solution, as the hexose furanose will react to form olive/brown solution. Bial's contains orcinol (5-methyl- 1,3 dihydroxybenzene), the parent compound of the litmus dyes in concentrated HCl.

Seliwanoff's Test:

Seliwanoff's Test distinguishes between aldose and ketose sugars. Ketoses are distinguished from aldoses via their ketone/aldehyde functionality. If the sugar contains a ketone group, it is a ketose and if it contains an aldehyde group, it is an aldose. This test is based on the fact that, when heated, ketoses are more rapidly dehydrated than aldoses. Perform this test with **glucose, fructose, maltose** and **sucrose.**

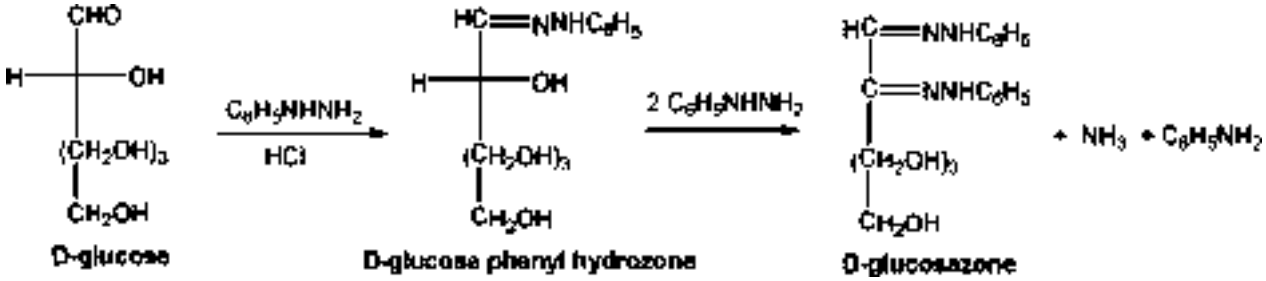


Red

Scheme 5. Seliwanoff's test reaction.

Phenyl Hydrazine test (not in the syllabus):

The keto or aldehyde groups of simple carbohydrates will react with phenylhydrazine. But instead of yielding typical phenylhydrazones they make what is termed osazones where the OH group immediately adjacent to the keto group is oxidized and it too adds phenylhydrazine to form the yellow to pale orange osazones that have definite melting points. So assignment of presumptive identity can be done my taking the melting point of a PURIFIED osazone from a purified carbohydrate.



Scheme 6. Osazone formation by phenylhydrazine.

Experimental procedure

Experimental procedure

a) Molisch's test for carbohydrates

Test: To about 1.0 mL of sugar solution add few drops of molisch's reagent and shake well.

To this reaction mixture, add a few drops of concentrated sulphuric acid along the sides of the tubes and observe the colour at the interface between the two liquids.

Obs1. Formation of red/ violet colour at the liquid interface: confirms the presence of carbohydrates.

Obs2: No characteristic change in colour: it confirms the absence of carbohydrates.

b) Iodine test for polysaccharide

Test: To about 1.0 mL of sugar solution add few drops of iodine solution slowly along the side of the test tube.

Obs1: Formation of deep blue colour: Deep blue colour due the formation of starch iodine complex. This confirms the presence of polysaccharides.

Obs2: No characteristic change in colour: it confirms the absence of polysaccharides.

c) Fehling's test for reducing sugar

Test: To 1 mL of Fehling's solution A, add 1 mL of Fehling's solution B and mix with a few drops of sugar solution. Boil the reaction mixture for 5 minutes in a water bath.

Obs1: Appearance of redish brown precipitate: confirms the presence of reducing sugar.

Obs2: No characteristic change in colour: it confirms the absence of reducing sugars.

c) Barfoed's test for monosaccharide

Test: .To about 1.0 mL of sugar solution mix 1.0 mL of Barfoed's reagents. Boil the reaction mixture in a water bath for few minutes.

Obs1: Red precipitate within 2-3 min: presence of reducing mono saccharides.

Obs2: Red precipitate in 7-10 min: presence of reducing disaccharides, lactose, maltose.

Obs3: No characteristic change in colour: it confirms the absence of monosaccharides.

e) Bial's test for pentoses

Test: Mix 1.0 mL of sugar solution with 1.0 mL of bial's reagent and boil the contents in a boiling water bath for 3 min.

Obs1: Formation of green colour: confirms presence of pentose sugars.

Obs2: No characteristic change in colour: it confirms the absence of pentose sugars.

d) Seliwanoff's test for keto sugar

Test: To about 1.0 mL of Seliwanoff's reagent add 2-3 drops of sugar solution and heat the mixture in a straight flame to just boil for about 15 seconds.

Obs1: Formation of cherry or deep red colour: presence of keto-sugars.

Obs2: No characteristic change in colour: it confirms the absence of keto-sugar.

g) Phenyl Hydrazine test for osazone (glucosazone/fructosazone, lactosazone, maltosazone)

Test: To about 2 parts of phenyl hydrazine add 3 parts of sodium acetate to prepare the phenyl-hydrazine mixture. Take 2 mL of this mixture add equal volume of sugar solution and heat in a boiling water bath for about 30 min. Allow the content to cool at room temperature or gently under tap water for few seconds. View the formed crystal under microscope.

Obs1: Yellow corn sheaves shaped crystals: presence of Glucosazone and fructosazone.

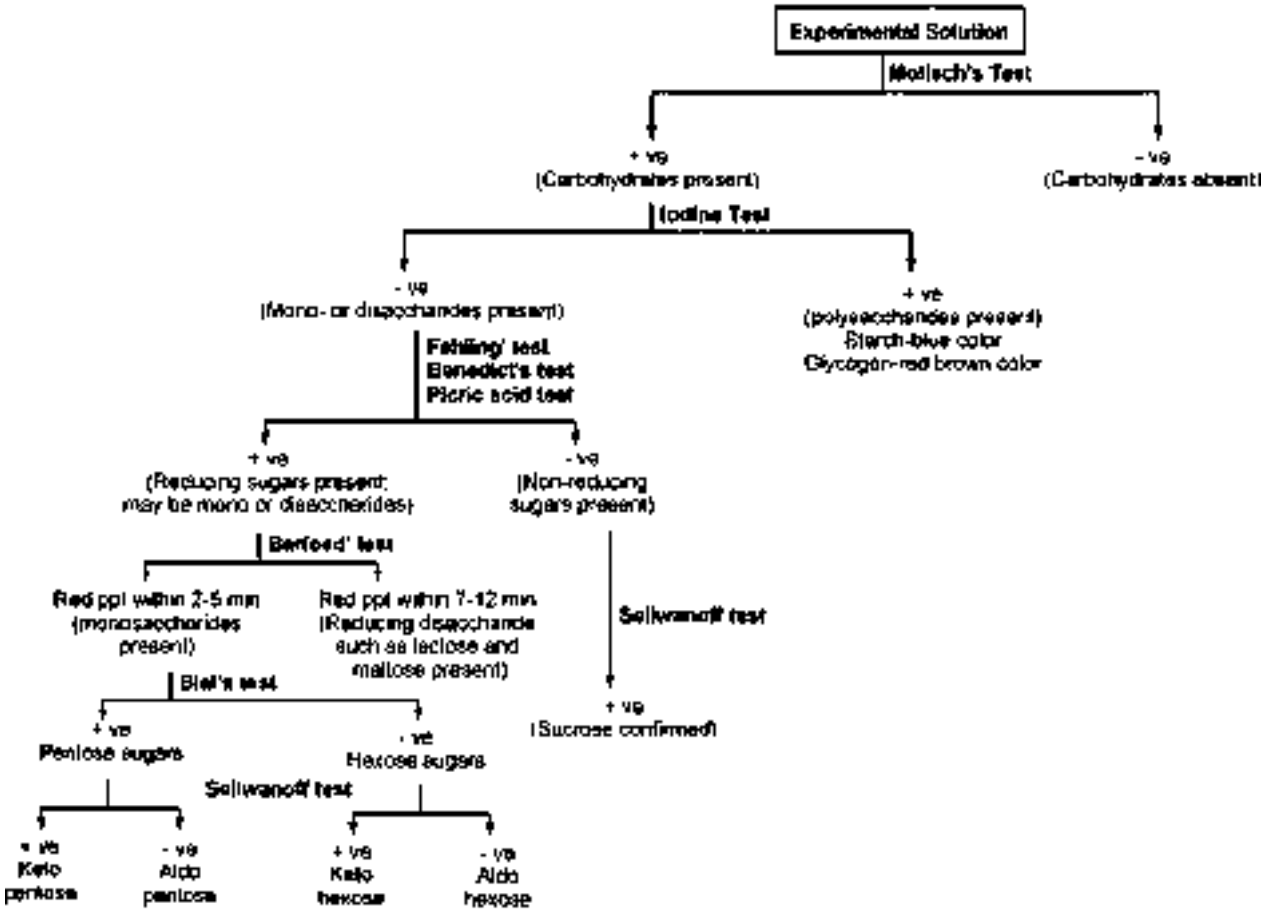
Obs2: Large flower petal shaped crystals: presence of galactosazone.

Obs3: Cotton ball or powder puff shaped crystals: Presence of lactosazone.

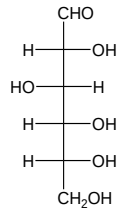
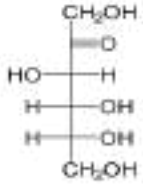
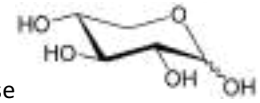
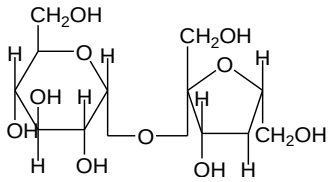
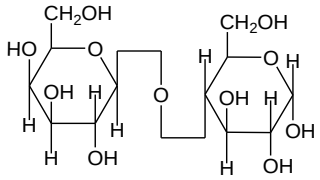
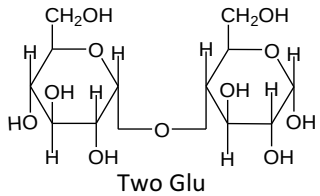
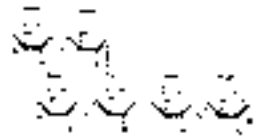
Obs4: Rod shaped crystals: presence of pentosazone.

Obs5: Small petal shaped crystals: presence of maltosazone

Complete analysis of carbohydrate sample

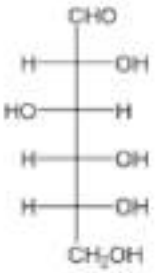


Reactions in qualitative test for carbohydrates

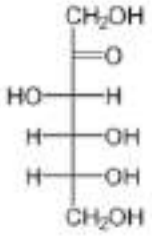
Sample	Molisch's Test	Fehling's Test	Barfoed's Test	Seliwanoff's Test	Osazone Test	Iodine test	Bial's Test
Glucose 	+	+	+ Within 2-3 mins	–	Yellow corn sheaves shaped crystals	–	–
 Fructose	+	+	+	+	Yellow corn sheaves shaped crystals	–	–
 Xylose	+	+	+ (within 2-3 mins)	–		–	+ (green)
Sucrose  Glu + Fruc	+	–	–	+	–	–	–
Lactose  Gla + Glu	+	+	+ After 7-10 mins	–	Cotton ball or powder shaped crystals	–	–
Maltose  Two Glu	+	+	+ After 7-10 mins	–	Small petal shaped crystal	–	–
Starch 	+	–	–	–	–	+	–

Qualitative analysis of Carbohydrates

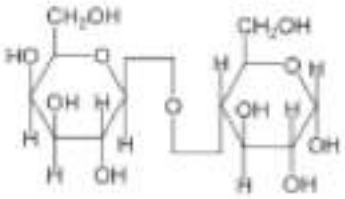
A carbohydrate is an organic compound that consists only of carbon, hydrogen, and oxygen, usually with a hydrogen:oxygen atom ratio of 2:1 (as in water); in other words, with the empirical formula $C_m(H_2O)_n$.



Glucose

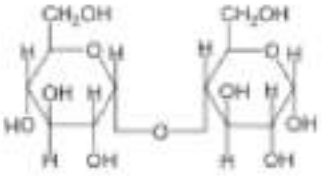


Fructose

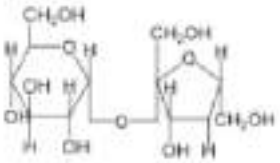


Lactose

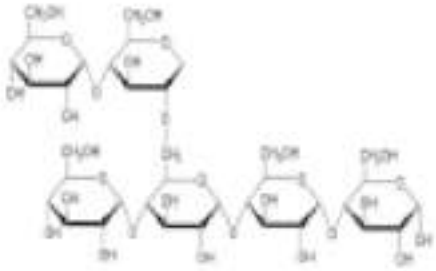
Galactose +Glucose



Maltose (Glucose+Glucose)



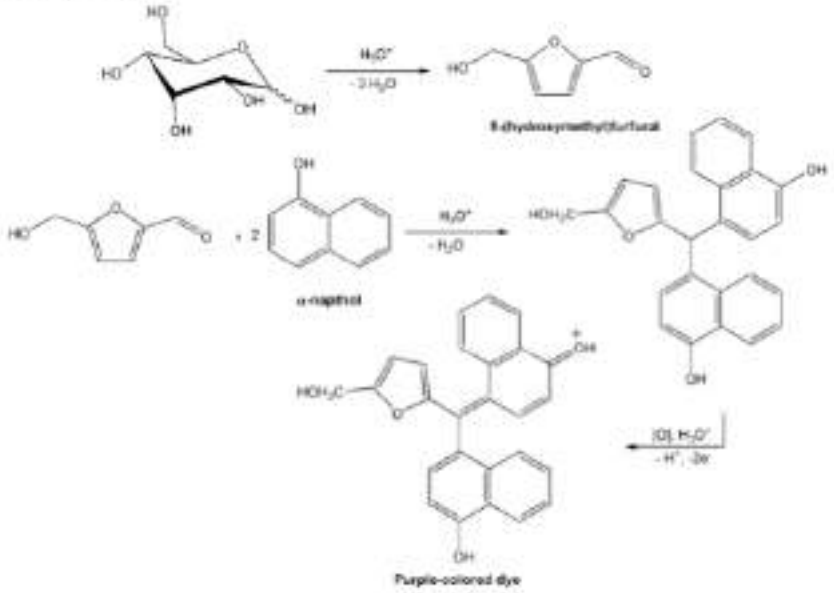
Sucrose (Glucose+Fructose)



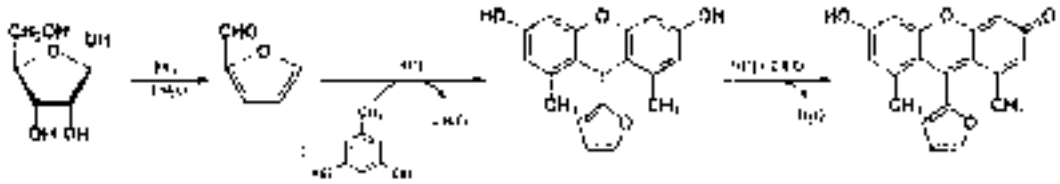
Starch

Molisch's test (Austrian botanist Hans Molisch)

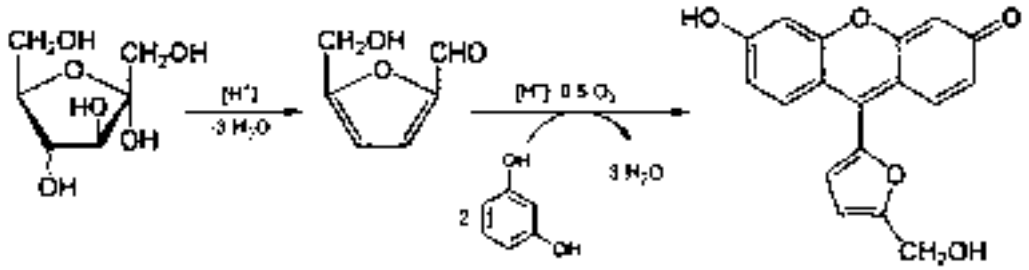
All carbohydrates – monosaccharides, disaccharides, and polysaccharides – should give a positive reaction and glycoproteins also give a positive reaction, as all these compounds are eventually hydrolyzed to monosaccharides by strong mineral acids. Pentoses are then dehydrated to furfural, while hexoses are dehydrated to 5-hydroxymethylfurfural. Either of these aldehydes, if present, will condense with two molecules of naphthol to form a **purple**-colored product, as illustrated below by the example of glucose.



Bial's Test



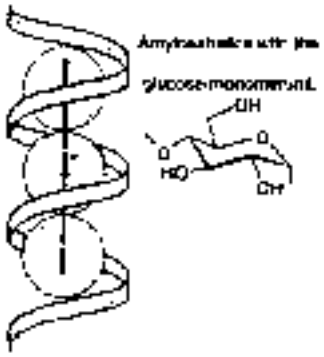
Seliwanoff's Test



Iodine Test:

Iodine test is an indicator for the presence of starch. Iodine solution (iodine dissolved in an aqueous solution of potassium iodide) reacts with starch producing a **blue-black color.** Apply this test to all the polysaccharides provided Urea reacts directly with diacetyl monoxime under strong acidic conditions to give a yellow condensation product. The reaction is intensified by the presence of ferric ions and thiosemicarbazide. The intense red colour formed is measured at 540nm.

The appearance of the deep blue color is due to the formation of starch-iodine complex. Generally, the starch molecule assumes a helical bundle shape exposing hydroxyl groups and here, the electronegative iodide ion interacts with the electropositive hydrogen belongs to the –OH group. This results a polyiodide starch complex.

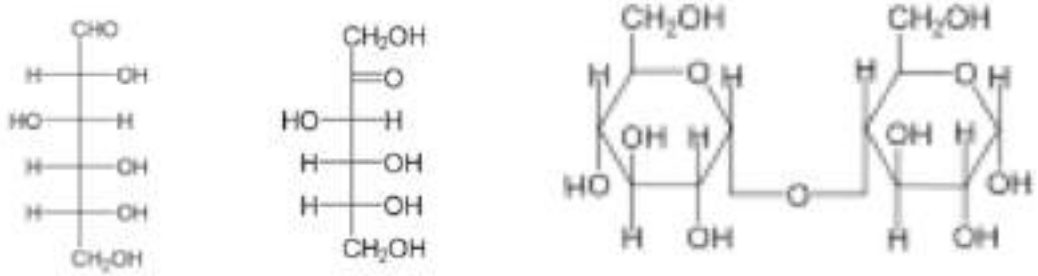


Fehling's Test (German chemist Hermann von Fehling in 1849)

Fehling's Solution (deep blue colored) is used to determine the presence of reducing sugars and aldehydes. Perform this test with **fructose, glucose, maltose.**

Fehling's test differentiates between aldehydes and ketones. Aldehydes can be oxidized by Cu^{2+} in the presence of a **strong base (alkaline)** to form carbonic acids. Ketones cannot be oxidized by this reaction. When the Cu^{2+} oxidizes the aldehydes it is reduced to Cu^{+}, and forms the compound Cu_2O, which is a **reddish** precipitate. That is how you know you have a reducing sugar (sugar molecule with an aldehyde as functional group).

$$RCHO + 2Cu^{2+}\ (blue) + 4OH^{-} \rightarrow RCOOH + Cu_2O\downarrow\ (red) + 2\ H_2O$$

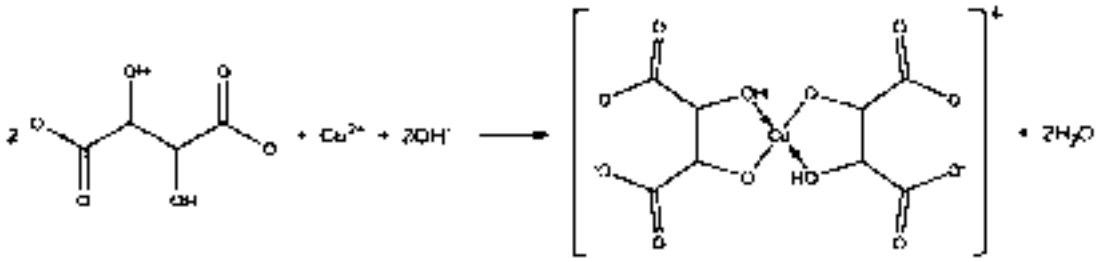


Glucose **Fructose** **Maltose**

Why is sodium potassium tartarate used in Fehling's solution?

Fehlings soluition is alkaline. Mixing cupric ions with an alkaline solution would result in formation of cupric hydroxide of very low water solubility. By adding tartrate ions, the cupric ions will form a complex with the cupric ions which will keep them dissolved in the alkaline solution. The type of cation of the tartaric acid is of no importance other than for solubility reasons.

Fehling's solution



Fehling A: $CuSO_4$ (blue coloured)

Fehling B: aqueous sodium potasium tartarate (Rochelle's salt) + NaOH

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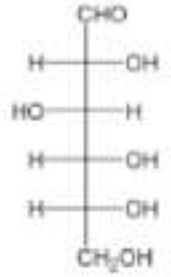
Barfoed's Test (Danish chemist Christen Thomsen Barfoed, 1873)

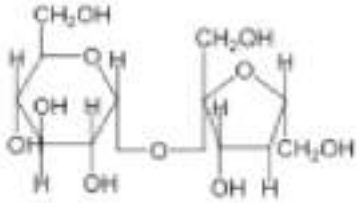
Barfoed's reagent, cupric acetate in acetic acid **(acidic media)** , is slightly acidic and is balanced so that is can only be reduced by monosaccharides but not less powerful reducing sugars. Disaccharides may also react with this reagent, but the reaction is much slower when compared to monosaccharides. Perform this test with **glucose, maltose and sucrose**.

The is due to the reducing action of the free functional group present in the sugar in the acidic medium. Reducing monosaccharides may form red cuprous oxide (Cu_2O) within 2-3 min and that for disaccharide takes about 10 min.

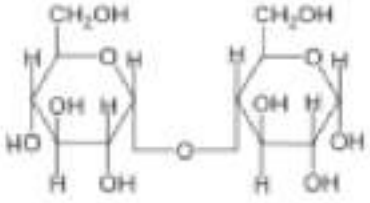
$$RCHO + 2Cu^{2+} + 2H_2O \rightarrow RCOOH + Cu_2O\downarrow (red) + 4H^+$$



Glucose



Sucrose (Glucose+Fructose)



Maltose (Glucose+Glucose)

Barfoed's reagent consists of a 0.33 molar solution of neutral copper acetate in 1% acetic acid solution.

Why sulfuric acid is used for dehydration, not hydrochloric or nitric acid?

In case of HCl elimination reaction will compete with nucleophilic reaction due to Cl^- ions which is a good nucleophile . HCl and HNO_3 are not good dehydrating agents wheras H_2SO_4 is. For example, elimination reaction in alcohols takes place in the presence of sulphuric acid leading to the formation of alkenes.

Hydrochloric and nitric acids are not very concentrated in their "concentrated" forms, about 37% and 55% by weight. The rest is water.

Sulfuric and phosphoric acids are essentially pure in their "concentrated" forms, about 98%. The remainder is water.

Mostly for dehydration we use concentrated acids which remove water from given substances and they become less concentrated.

Firstly we need to take such a acid it can not react with the substance.

Here if we take HCl it will dehydrated the alcohol but it react with alcohol to form alkyl chloride.

HCl + ROH ——— RCl + H2O.

Similarly if we take HNO3 it will dehydrated but it will form nitro ester with alcohol.

HNO3 + ROH ——— RNO2 + H2O.

But if we use H2SO4 it will dehydrated and it will form some by products depending on temperatures.

H2SO4 + ROH ———- ROSO3 + H2O. At temperature below 413 k.

If we increase the temperature ROSO3 react with acid to form ester

H2SO4 + ROH ——— ROR + H2O at temperature 413 k.

But if increase further it will form alkene

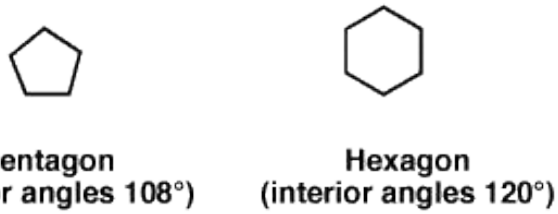
H2SO4 + ROH ——— RR+ H2O. At temperature 443 k.

But the products formed with HCl and HNO3 can not be reduces further with increasing temperatures . So for dehydration of alcohol we use only H2SO4 .

Ring Strain in Cyclopentane and Cyclohexane

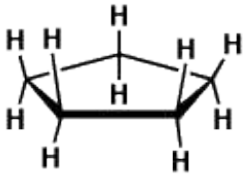
Ref: http://www.masterorganicchemistry.com/2014/04/18/ring-strain-in-cyclopentane-and-cyclohexane/

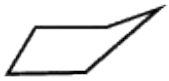
cyclopentane (ring strain: 6 kcal/mol) and cyclohexane (ring strain: 0 kcal/mol) is much happier.

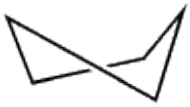


Pentagon (interior angles 108°) **Hexagon (interior angles 120°)**

For cyclopentane, the "flat" conformation has all of its C-C bonds eclipsed and has considerable torsional strain (about 10 kcal/mol). However, since there is some flexibility, what we in fact observe is two dominant conformations for cyclopentane (of comparable energy), the "envelope" and the "twist". While each of these conformations has some torsional strain (the C-C bonds are not perfectly staggered), both of these have considerably less torsional strain than flat cyclopentane.







"Flat" cyclopentane

Interior angle 108°

(no angle strain)

All C–C bonds eclipsed (torsional strain!!)

~10 kcal/mol of torsional strain!

"Envelope" cyclopentane **"Twist" cyclopentane**

both of these conformations have significantly less torsional strain than the "flat" conformation

not perfectly staggered C-C bonds

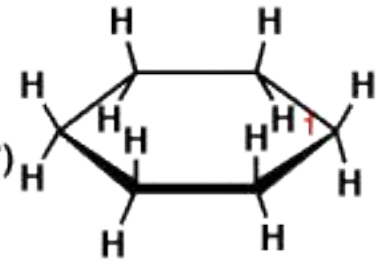
About 6 kcal/mol of torsional strain

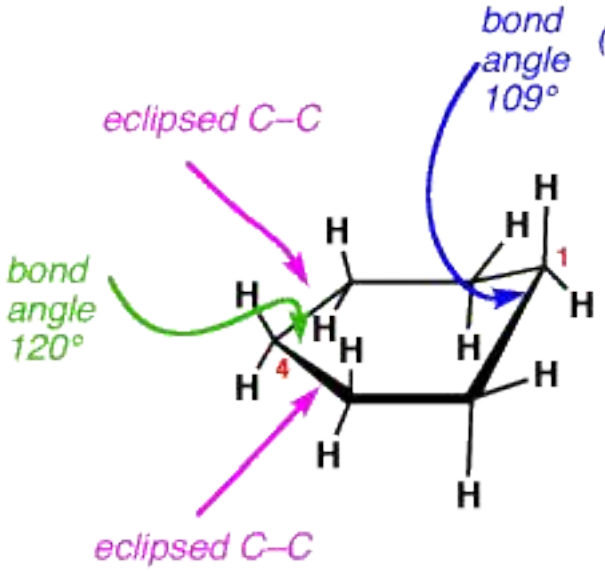
What about cyclohexane?

"Flat cyclohexane"

Ring strain > 20 kcal/mol

- angle strain
(all internal C-C bonds 120°)
- torsional strain
(all C–C bonds eclipsed)





"Half chair"

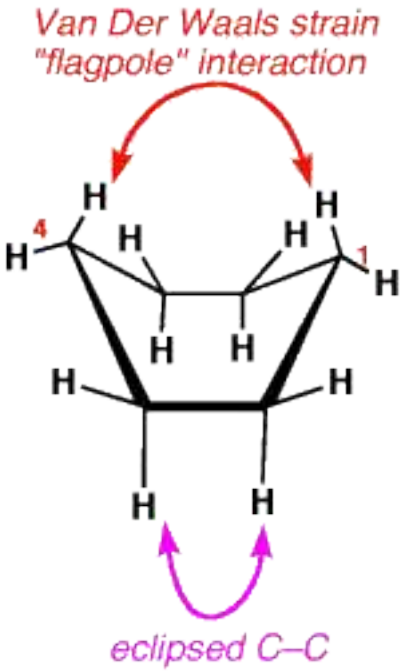
Ring strain 10.8 kcal/mol

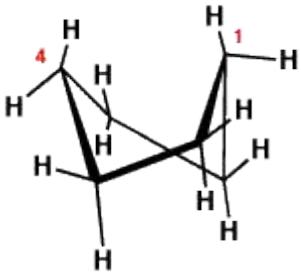
- **some angle strain (120°)**
- **torsional strain**

(two C–C bonds fully eclipsed, two more partially eclipsed)

"Boat" (aka hammock)

Ring strain 7.0 kcal/mol
·minimal angle strain
· torsional strain
(some C–C bonds eclipsed)
· Van Der Waals strain
("flagpole" interaction)





"Twist Boat"

Ring strain 5.5 kcal/mol

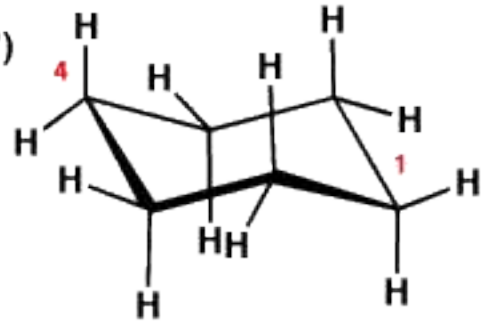
·relief of Van Der Waals strain through twisting
· minimal angle strain
· torsional strain
(some eclipsed C–C bonds)

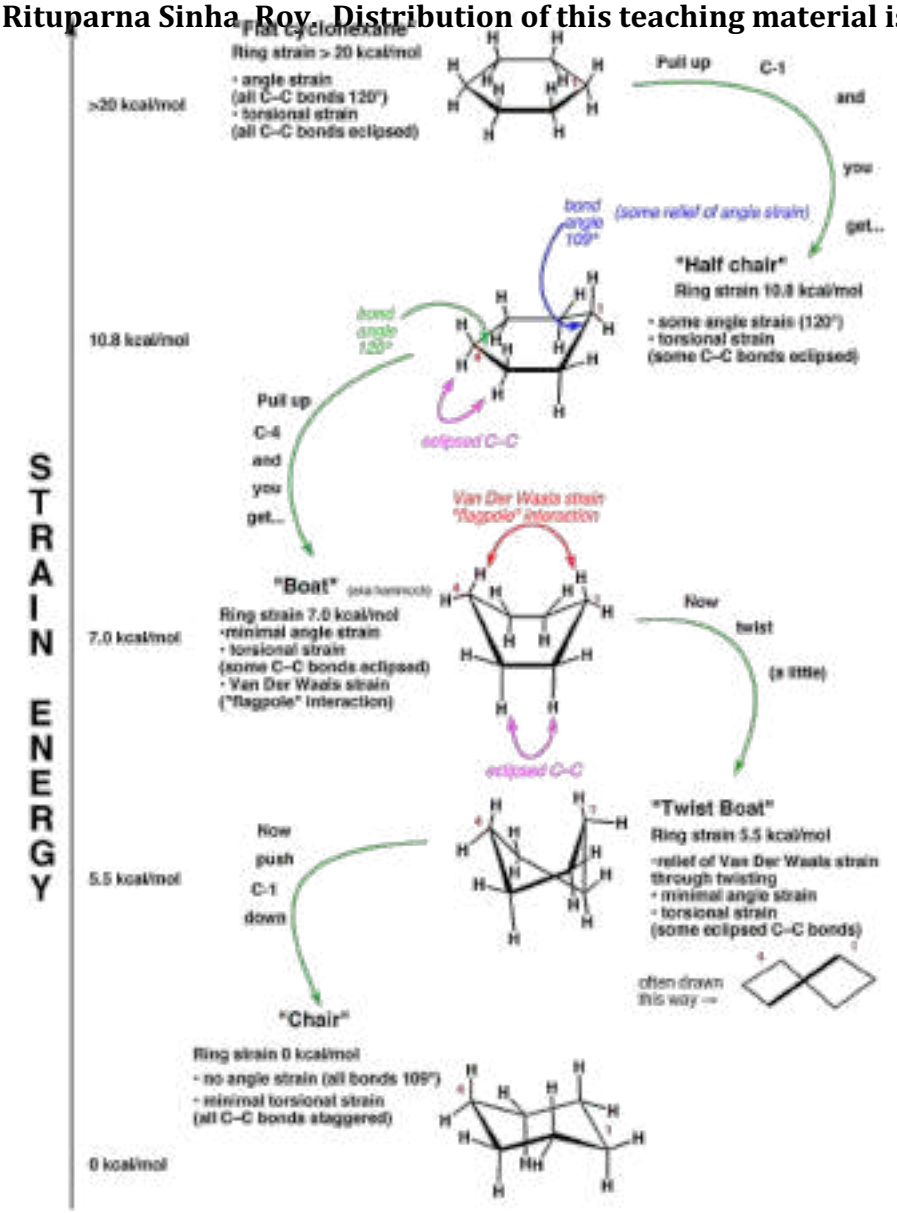
often drawn this way →

"Chair"

Ring strain 0 kcal/mol

- no angle strain (all bonds 109°)
- minimal torsional strain (all C–C bonds staggered)




The Many Shapes Of Cyclohexane
STRAIN ENERGY
"Flat" Cyclohexane
Ring strain > 20 kcal/mol
• angle strain
(all C–C bonds 120°)
• torsional strain
(all C–C bonds eclipsed)
>20 kcal/mol
Pull up C-1 and you get...
bond angle 109°
(some relief of angle strain)
"Half chair"
Ring strain 10.8 kcal/mol
• some angle strain (120°)
• torsional strain
(some C–C bonds eclipsed)
10.8 kcal/mol
bond angle 120°
eclipsed C–C
Pull up C-4 and you get...
Van Der Waals strain
"flagpole" interaction
"Boat" (aka hammock)
Ring strain 7.0 kcal/mol
•minimal angle strain
• torsional strain
(some C–C bonds eclipsed)
• Van Der Waals strain
("flagpole" interaction)
7.0 kcal/mol
Now twist (a little)
eclipsed C–C
"Twist Boat"
Ring strain 5.5 kcal/mol
•relief of Van Der Waals strain through twisting
• minimal angle strain
• torsional strain
(some eclipsed C–C bonds)
often drawn this way →
Now push C-1 down
5.5 kcal/mol
"Chair"
Ring strain 0 kcal/mol
• no angle strain (all bonds 109°)
• minimal torsional strain
(all C–C bonds staggered)
0 kcal/mol

LS2102 Biochemistry Practical, DBS, IISER Kolkata

Experimental procedure (Qualitative Analysis of Carbohydrates)

a) Molisch's test for carbohydrates:

Test: To about 1.0 mL of sugar solution add 6-8 drops of Molisch's reagent and shake well.
To this reaction mixture, add 8-10 drops of concentrated sulphuric acid along the sides of the tubes and observe the colour at the interface between the two liquids.

Obs1. Formation of red/violet colour at the liquid interface: confirms the presence of carbohydrates.

Obs2: No characteristic change in colour: it confirms the absence of carbohydrates.

N.B.: After adding sulphuric acid do not shake the tube

b) Iodine test for polysaccharide:

Test: To about 1.0 mL of sugar solutions add 2-3 drops of 1% iodine solution slowly along the side of the test tube.

Obs1: Formation of deep blue colour (Purple): Deep blue colour due the formation of starch iodine complex. This confirms the presence of polysaccharides.

Obs2: No characteristic change in colour: it confirms the absence of polysaccharides.

c) Fehling's test for reducing sugar:

Test: To 1 mL of Fehling's solution A, add 1 mL of Fehling’s solution B and mix with 10 drops of sugar solution. Boil the reaction mixture for 5 minutes in a water bath.

Obs1: Appearance of reddish brown precipitate: confirms the presence of reducing sugar.

Obs2: No characteristic change in colour: it confirms the absence of reducing sugars.

d) Barfoed's test for monosaccharide:

Test: To about 1.0 mL of sugar solution mix 1.0 mL of Barfoed's reagents. Boil the reaction mixture in a water bath for few minutes.

Obs1: Red precipitate within 5 mins: presence of reducing monosaccharides.

Obs2: Red precipitate in 20 mins: presence of reducing disaccharides, lactose and maltose.

e) Bial's test for pentoses:

Test: Mix 1.0 mL of sugar solution with 1.0 mL of Bial's reagent and boil the contents in a boiling water bath for 1-2 min.

Obs1: Formation of green colour: confirms presence of pentose sugars.

Obs2: No characteristic change in colour: it confirms the absence of pentose sugars.

f) Seliwanoff's test for keto sugar:

Test: To about 1.0 mL of Seliwanoff's reagent add 2-3 drops of sugar solution and heat the mixture in a water bath for about 15 seconds.

Obs1: Formation of cherry or deep red colour: presence of keto-sugar.

Obs2: No characteristic change in colour: it confirms the absence of keto-sugar.

Complete analysis of carbohydrate samples