Федеральное государственное бюджетное образовательное учреждение
высшего образования
**«Рязанский государственный медицинский университет
имени академика И.П.Павлова»
Министерства здравоохранения Российской Федерации**
(ФГБОУ ВО РязГМУ Минздрава России)

Кафедра общей и фармацевтической химии

Л.В.Кубасова

ПРАКТИКУМ
ПО БИООРГАНИЧЕСКОЙ ХИМИИ

для студентов 1 курса лечебного факультета, обучающихся по специальности 31.05.01 Лечебное дело

Рязань, 2018

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Chair of general and pharmaceutical chemistry

L.V. Kubasova

LABORATORY MANUAL OF BIIOORGANIC CHEMISTRY FOR FOREGN STUDENTS

Ryazan, 2018

Introduction

This book is a sum of laboratory works, which are necessary to comprehend theoretical rules of bioorganical chemistry in-depth and also to receive practical skills.

This laboratory works include theoretical basis and characteristics of the most important bioactive compounds.

The practical training permits to strengthen knowledge of the properties of the most important classes of organic compounds and of their metabolites-peculiarities, to study a set of modern methods in experimental chemistry. It lays the foundation of the further studies in general and clinical biochemistry. This book is helpful to special branch of science in which such modern methods are used. It is intended for foreign students.

Laboratory safety

A chemical laboratory can potentially be a dangerous place to work. There are flammable liquids, poisonous and corrosive chemicals and fragile glassware in the laboratory. They can be a course of serious injuries. But this risk can be minimized if you know the rules of safety and if they are followed.

There is danger of fire in organic chemistry laboratory, because flammable liquids are often used. Therefore you must avoid using flaints in the laboratory. You can heat most organic liquids with a steam bath or a hot plate.

If you carry out some experiments with a few drops of organic compounds in a test tube you can use a bunner.

If you should have afire, get away from it and don't panic. If the fire is small and it is in a container you can place an asbestos pad on top of the container. Usually it is enough for extinguishing the fire.

If a fining liquid has been spilt on the table you can use an asbestos veil or sand.

Almost all chemicals in organic chemistry laboratory are poisonous and haraful ever: in small amounts. So, you must observe the following precaution and than you can protect yourself from this danger.

Never let any chemicals come in contact with your skin, because some liquids (such as aniline, nitrobensene. phenol and others) may be

absorbed through the skin. This is dangerous. If any chemical has been spilled on the skin, wash the area immediately with soap and water.

Any noxious, volatile, chemicals should be used in a fume hood.

If you need to determine an odor of some organic compounds, never hold your nose over the container. You must not inhale deeply either. You should hold the substance about 15 centimeters of your nose and use your hand to gently fan the vapors toward you.

Never place your fingers in your mouth and always wash your hands when leaving the laboratory.

Never bring food into the laboratory.

Never heat a flask or a test tube that is not open to the atmosphere.

Never add solids or boiling chips to any boiling liquids. The hot liquid can escape from the reaction vessel and possibly burn you.

When you heat *a* substance in a test tube, don't point it at yourself or anyone else. The hot liquid may bump and be thrown from the tube.

The laboratory glassware may be broken under strain or excessive pressure and you may have cuts. If you need to insert a glass tube or thermometer into the hole of a stopper, you should carefully do it by lubricating the glass with glycerine.

You must be especially careful while working with concentrated acid and alkalies. If these compounds get to the akin, they may cause burns.

In case of chemical burns you should wash your skin with water. Acid burns may then be treated with the solution of sodium bicarbonate and alkali bums - with the solution of boric-acid.

The result of your laboratory work must b e written in the font of a table:

Names of the laboratory investigations	Schemes of reaction	Observations	Conclusions

Saturated Hydrocarbons

Experiment 1. Preparation of methane

Put into a dry test-tube some amount of mixture, consisting of anhydrous sodium acetate and sodium hydroxide. Close the test-tube with the help of a stopper with gas tube. At first heat equally the test tube, then strongly heat the part of the test tube, where the mixture is.

Put the gas tube into a test glass with a solution of potassium

$$CH_3\text{-}COONa + NaOH \xrightarrow{t^0} \underset{\text{methane}}{CH_4\uparrow} + Na_2CO_3$$

permanganate and let the methane bubbles during 1 minute. Then pass the methane through bromine water (an aqueouse solution of bromine). What do you see? Violet colour of potassium permanganate and yellow - brown colour of bromine will not disappear.

Experiment 2.Bromination of saturated hydrocarbons

The basic components of liquid paraffin are the saturated hydrocarbons. Halogenation of alkanes goes on radical mechanism and consequently proceeds under action of light more effectively. Light generated radical particles. As a result of reaction the admixture of bromoalkanes is formed. The interaction of brominated pruducts depends on structure of organic substances and on conditions of reaction.

$$\underset{\text{dodecane}}{CH_3\text{-}(CH_2)_9\text{-}CH_2\text{-}CH_3} + Br_2 \xrightarrow{h\nu} \underset{\text{2-bromododecane}}{CH_3\text{-}(CH_2)_9\text{-}CH(Br)\text{-}CH_3} + HBr$$

Take two dry test-tubes. Add 5 drops of a liquid paraffin into two test-tubes and 1drop of bromine solution in tetrachloromethane. One test-tube leave on light, another - in a dark place. A bit later (3-4 minutes) compare intensity of colouring of solutions. Explain observable differences.

Write down the results of experiment in your note-book.

The recommendation of writing a protocol: write steps of bromination and discuss its orientation.

Unsaturated Hydrocarbons.

Experiment 3. Preparation and chemical properties of ethylene (ethene)

Pour into a dry test-tube 4-5 ml of the mixture, consisting of 96% ethyl alcohol and concentrated sulphuric acid in proportion 1:2. Close the test-tube by means of a stopper with gas tube. Carefully heat the mixture:

$$\underset{\text{ethyl alcohol}}{CH_3\text{-}CH_2\text{-}OH} \xrightarrow{t,\ H_2SO_4} \underset{\text{ethene}}{CH_2{=}CH_2} + H_2O$$

The formed ethylene pass into the tube containing bromine water. The reddish brown colour of bromine quickly disappears:

$$CH_2{=}CH_2 + Br_2 \rightarrow \underset{\text{1,2- dibromoethane}}{\overset{\quad Br \quad Br}{CH_2\text{-}CH_2}}$$

Pass the ethylene into a solution of potassium permanganate, containing a few drops of 10% solution of sodium carbonate (Na_2CO_3). The violet colour of the solution quickly disappears and brown precipitate is formed:

$$3\ CH_2{=}CH_2 + 2\ KMnO_4 + 4\ H_2O \longrightarrow 3\ \overset{\quad OH \quad OH}{CH_2\text{-}CH_2} + 2\ MnO_2\downarrow + 2\ KOH$$

Write down the results of experiment in your note-book.

Experiment 4. Preparation and chemical properties of acetylene

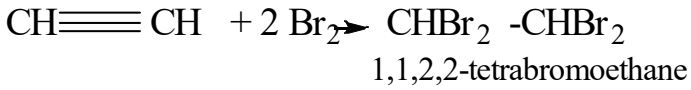
Put into a test-tube a small piece of calcium carbide (CaC_2) and add 1 ml of water. Close the test-tube by means of a stopper with gas tube:

$$\underset{\text{calcium carbide}}{CaC_2} + 2\ H_2O \rightarrow \underset{\text{acetylene}}{CH{\equiv}CH}\uparrow + Ca(OH)_2$$

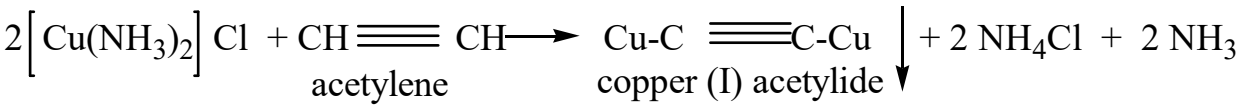
Pass the formed gas into a solution of potassium permanganate containing some drops of 10% sodium carbonate (Na_2CO_3). Violet colour of solution quickly disappears, brown precipitate is formed:

$$3CH{\equiv}CH + 8\ KMnO_4 + 4\ H_2O \rightarrow \underset{\text{oxalic acid}}{3\ HOOC\text{-}COOH} + 8\ MnO_2\downarrow + 8\ KOH$$

Pass the acetylene into bromine water, the solution becomes colourless:



Pour into a test-tube 2-3 ml of ammonia solution of copper (I) chloride and pass acetylene into it. Colourless solution at first becomes red and then red-brown precipitate of copper (I) acetylide is formed:



This reaction can be carried out in other way. Wet a piece of filter paper by ammonia solution of copper (I) chloride and hold it in acetylene stream. A red-brown spot is appeared.

This reaction is very sensitive and is used for finding of acetylene traces.

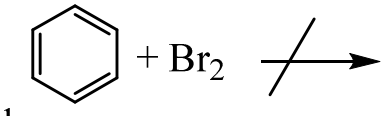
Write down the results of experiment in your note-book.

Aromatic Compounds.

Experiment 5. Benzene

Benzene is very stable aromatic system containing cyclic clouds of delocalized π-electrons. These π-electrons are more involved in holding together carbon nuclei than are the π-electrons of carbon-carbon double bond. Benzene does not discolour bromine water and potassium permanganate solution at usual conditions.

Into a test-tube with bromine water add 3 drops of benzene. Shake the mixture. As density of benzene is less than that water benzene rises to surface. Gradually lower layer of bromine water is discoloured and upper layer becomes yellow because of better solubility of bromine in benzene that water.



benzene

Into a test-tube with 5 drops of water add 1 drop of potassium permanganate solution. Add 3drops of benzene, shake. No change.

Write down the results of experiment in your note-book.

Experiment 6. Bromination of aromatic hydrocarbons

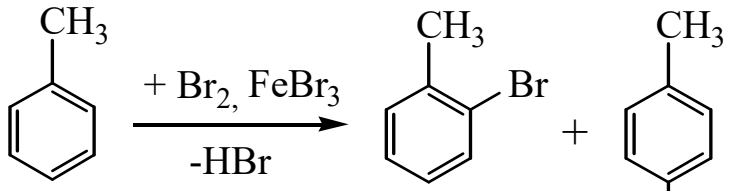
The action of chlorine and bromine on benzene depends on the conditions. Bromination on heating without a catalyst occurs according to free radical substitution in side chain. Lewis acids as catalysts of electrophilic substitution reaction direct reagent into ring.

$C_6H_5CH_3 + Br_2 \longrightarrow C_6H_5CH_2Br + HBr$

toluene — benzyl bromide

$$C_6H_6 + Br_2 \xrightarrow{FeBr_3} C_6H_5Br + HBr$$

benzene — bromobenzene



toluene — *o*-bromotoluene — *n*- bromotoluene

a) Place 10 drops of toluene into a dry test-tube, add 1-2 drops of 1% bromine solution in tetrachloromethane. Reflux the mixture. Gradually the colour disappears. Liberation of hydrogen bromide is proved by the colour change of pH-paper. Pour the reaction product into a cup and heat on water bath. Unreacted toluene is evaporated and sharp odor of benzyl bromide is appeared.

b) Into two dry test-tube place: in the first - 10 drops of benzene, in the second - 10 drops of toluene. Add to each 1-2 drops bromine solution in the tetrachloromethane and add 2 small spoons of ferrum filings. Reflux the mixture. The solution is discoloured.

Write down the results of experiment in your note-book.

Experiment 7. Oxidation of alkylbenzene.

The benzene ring renders an aliphatic side chain quite susceptible to oxidation. The side chain is oxidized doun to the the ring only carboxyl groups remaining to indicate the position of original side chan. Potassium permanganate is generally used for this purpose. Such oxidations take place in biological systems. So toluene

is converted to benzoic acid and taken out of organism. That is why toluene is less toxic than benzene.

$$C_6H_5CH_3 + 3[O] \xrightarrow{t} C_6H_5COOH + H_2O$$

toluene — benzoic acid

Place into test-tube 5 drops of water, 1 drop of hotassium permanganate solution and 1 drop of sulfuric acid solution, add 1 drop of toluene. Vigorously shaking heat the test-tube till the solution brown colour is formed.

Write down the results of experiment in your note-book.

Alcohols, Phenols, Ethers, Amines, and Organohalogens.

Experiment 8. Preparation of chloroethane from ethanol

Alcohols react readily with hydrogen halides to yelled alkyl halides and water. The reaction is carried out by heating the alcohol with the concentrated acid. В этом случае гидроксильная группа спиртов замещается на галоген.

$$NaCl + H_2SO_4 \rightarrow HCl + NaHSO_4$$

$$C_2H_5OH + HCl \rightarrow C_2H_5Cl + H_2O$$

ethanol — chloroethane

Place dried sodium chloride into a test-tube (the height of the low must be about 3 mm). Add 3 or 4 drops of ethanol to moisten sodium chloride. Then add? 3 or 4 drops concentrated sulfuric acid (BE CAREFUL: CONCENTRATED SUFURIC ACID SEVERE BURNS!) and heat. In some seconds place the hole of tube into the fier. Gassing chloroethane gives a green flame.

Write down the results of experiment in your note-book.

Experiment 9. Beilshtein's reaction

The Beilshtein's test is the easiest, simplest, and fastest method for detecting the presence of a halogen in the organic compounds.? Clorine-, bromine- and iodine-containing compounds all give a green flame when burned with copper wire. A volatile copper halide is responsible for the color.

Form a flat coil on the end of the copper wire. Heat the copper coil in a burner flame until the wire produces no green color or other color change in the flame. Allow the copper coil to cool partially, then dip it into the liquid researched. Place the copper coil coated with the sample into the flame. A flash of green is positive test for chlorine, bromine or iodine. Record your observation and your conclusion in your notebook.

Experiment 10. Preparation of sodium ethylate and there hydrolysis

Alcohols possess extremely weak acidic properties (more weakly, than water) therefore for experiment the absolute alcohol may be utilized only. Active metals (K, Na) liberate hydrogen from alcohols to form alkoxides, e.g., sodium reacts with ethanol to form sodium ethoxide:

$$\underset{\text{ethanol}}{2\ C_2H_5OH} + 2\ Na \rightarrow \underset{\substack{\text{sodium ethoxide}\\ \text{(sodium ethylate)}}}{2\ C_2H_5ONa} + H_2\uparrow$$

Alkoxides are white solids, readily soluble in water with hydrolysis:

$$C_2H_5ONa + H_2O \rightarrow C_2H_5OH + NaOH$$

In the test-tube put some anhydrous copper (П) sulphate (height of layer is about of 2 mm) and 2-3 ml of ethyl alcohol. Carefully shake up. The precipitate of copper П) sulphate (is painted in ligth-blue color. Solution filter in the dry test-tube. Put there a small piece of metal sodium. Close the test-tube by a stopper. Начинается выделение водорода.(hydrogen is coming out). After the terminal of reaction bring the tube (having discovered stopper) to a flame of the burner . Hydrogen ignites with characteristic note.

In the tubes there is a white precipitate of sodium ethylate or its solution. To addition 2-3 drops of water the precipitate is dissolved. In addition of 1 drop of alcohol solution of phenolphthaleinum there is a crimson staining.

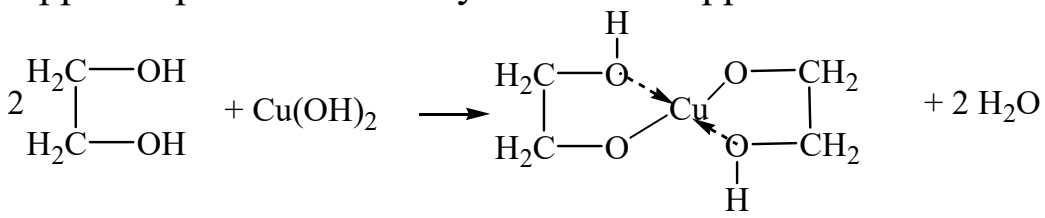
Write down the results of experiment in your note-book.

Experiment 11. Reaction of glycerol with copper (II) hydroxide

This reaction is useful for detection of polyalcohols, or same other organic compounds having two hydroxyl groups on adjacent carbons.

$CuSO_4 + 2NaOH \rightarrow Cu(OH)_2\downarrow + Na_2SO_4$

copper sulphate sodium hydroxide copper hidroxide



1,2-ethanediol

Add 2 drops of copper (II) sulphate solution and 2 drops of sodium hydroxide solution in a test tube. The blue precipitate of copper hydroxide is formed. Add to this precipitate 2 drops of 1,2-ethanediol. The blue precipitate is dissolved and the dark-blue solution is formed.

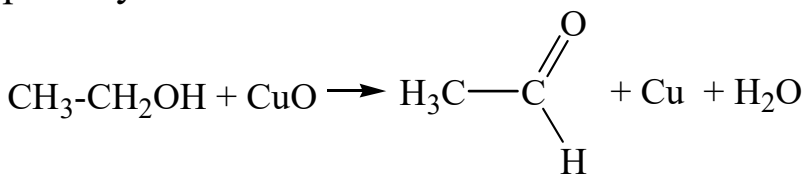
The references to drawing up of protocol:

What properties shows the ethylene glycol in this reaction? Compare an acidity of ethylene glycol and ethanol. Specify the cause of this difference.

Write down the results of experiment in your note-book.

Experiment 12. Oxydation of ethanol.

A primary alcohol can be oxydized first to an aldehyde and then to a carboxylic acid. Ethanol is oxidized by copper (II) oxide to acetic aldehyde and reduce the copper (II) oxide to metallic copper. This reaction is used for detecting the presence of ethanol and other primary alcohols.:



ethanol ethanal (acetaldehyde)

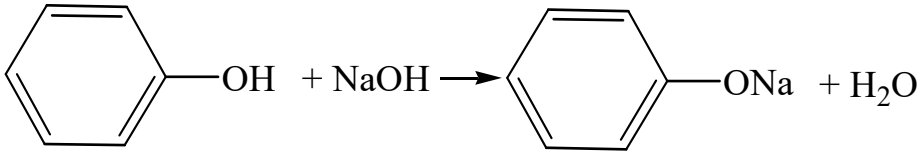
Place 5 drops of ethanol into a test-tube. Heat the copper coil in the burner flame. Медная проволока покрывается черным слоем оксида меди (II).(copper wire is being covered with black copper

oxide). Place the hot copper coil into the ehtanol. And the characteristic odor of acetic aldehyde appears.

Write down the results of experiment in your note-book.

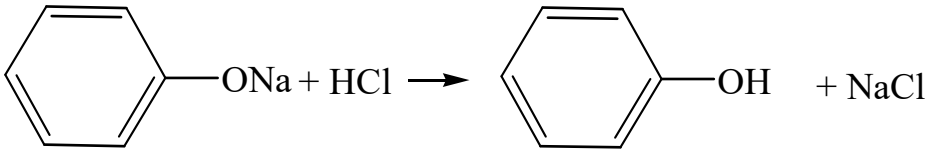
Experiment 13. Formation and decomposition of sodium phenoxide.

The functional group of phenols is a hydroxyl group (OH) bonded to a benzene ring, so they are weak acids. Phenols react with strong bases such as sodium hydroxyde (NaOH) to form water-soluble salts. This reaction confirms acidic properties of phenol:



phenol sodium phenoxide

Phenols are stronger acids than water and alcochols. ?But phenols have more weak acidic properties, than inorganic acids therefore sodium phenoxide (sodium
phenylate) is destroy? by a hydrochloric acid, and water emulsion of phenol is formed:



sodium phenoxide (transparent solution)

phenol (non transparent solution-emulsion)?

Place 3 drops of water and some crystals of the phenol into the test-tube, shake up. The emulsion of phenol in the water is formed, because phenol is only slightly soluble in water. Add to this emulsion some drops of sodium hydroxyde solution. The emulsion is converted into transparent solution. Add to this solution some drops of 10% hydrochloric acid solution. The emulsion is formed again.

Write down the results of experiment in your note-book.

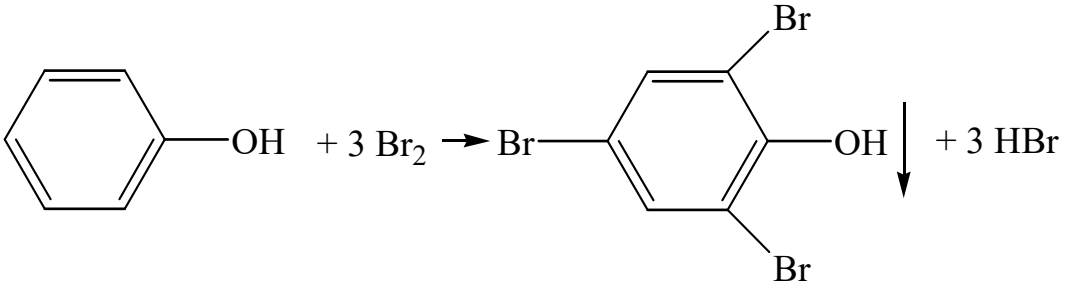
Experiment 14. Qualitative determination of phenol

a) Ferric chloride test.

Phenol reacts with ferric chloride to give a blue or violet complex. Dissolve a small grain of phenol in water. Add 3-5 drops of 3% aqueous ferric chloride solution. The blue-violet color appears. The equation of this reaction we do not consider.

b) Bromine water test.

The phenolic hydroxyl group activates aromatic ring toward electrophilic substitution and phenol reacts with bromine in water to give substitution product. A positive test is both the loss of color by aqueous bromine solution and precipitation of substitution product. Hydroxyl group is ortho, para director.? Phenols are often identified through bromination products.



phenol 2,4,6- tribromophenol

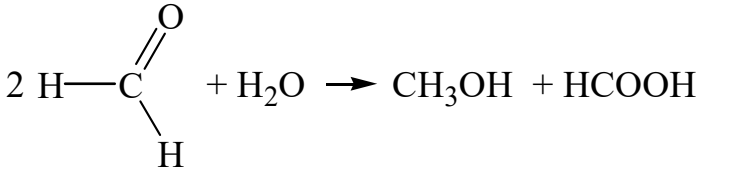
Dissolve a small grain of phenol in water. Add a saturated solution of bromine in water, drop by drop. Phenol обесцвечивает бромную воду и gives white precipitate of bromination product.

Write down the results of experiment in your note-book.

Aldehydes and Ketones

Experiment 15. Disproportionation of formaldehyde

Formaldehyde as any aldehyde without hydrogen attached to α-carbon atom and can be disproportionated. Usually this reaction takes place in alkali solution but formaldehyde undergoes this reaction in aqueous solution.



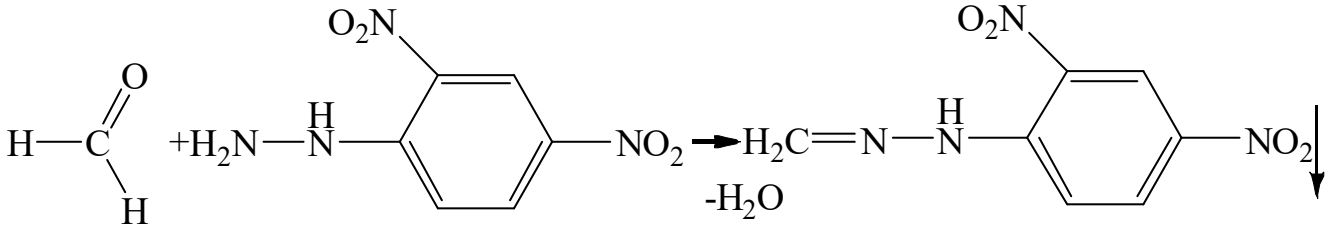
formaldehyde methanol formic acid

Pour into a test tube 2-3 drops of formalin (40% formaldahyde solution), add 1 drop of 0,2% methyl red solution. The methyl red turns red (shows acid reaction).

Write down the results of experiment in your note-book.

Experiment 16. Formation of 2,4- dinitrophenylhydrozone

Product of the reaction is a good cryctallizable substance with the dictinct melting point. This reaction is used for separation and identification of aldehydes and ketones.



formaldehyde 2,4-dinitrophenylhydrazine 2,4-dinitrophenylhydrazone of formaldehyde

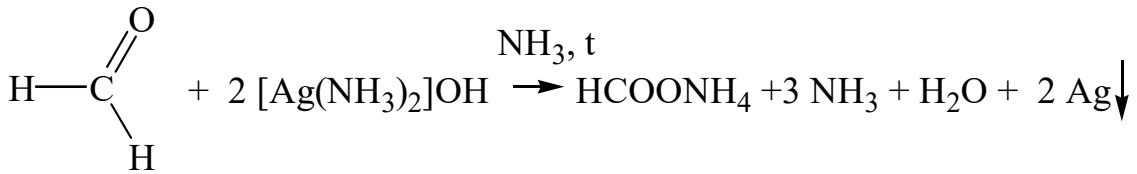
Pour into a test-tube 5 drops of 2,4-dinitrophenilhydrazine, add 1-2 drops of formaline (40% formaldahyde solution) yellow crystals are formed.

Write down the results of experiment in your note-book.

Write down the results of experiment in your note-book.

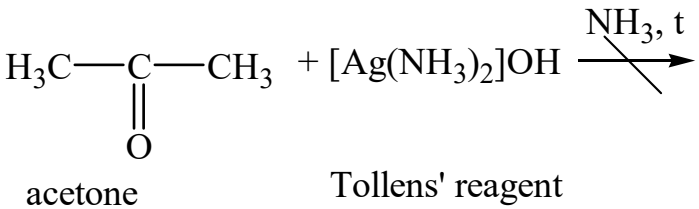
Experiment 17. Oxidation

a) Aldehydes are easily oxidized by variety of mild oxidizing agents.



formaldehyde Tollens' reagent formiate ammonia

Ketones are oxidized by strong oxidizing agent. This reaction is accompanied by destruction of carbon-carbon bonds.



acetone Tollens' reagent

In excess of Tollens' reagent formiate is oxidizid further:

$$HCOONH_4 + 2\,[Ag(NH_3)_2]OH \xrightarrow{NH_3,\ t} (NH_4)_2CO_3 + 3\,NH_3 + H_2O + 2\,Ag\downarrow$$

Pour into two test-tubes some drops of Tollens' reagent. (Tollens' reagent contains silver nitrate and ammounia in water). Add 2 drops of formaline to the first test-tube, 2 drops of acetone – to the second one. In the first test-tube a black precipitate is formed. If carefully heated the silver metal precipitates as a smooth, mirror-like deposit, hence the name silver mirror test. In the second tube there is no formation of black precipitate.

b) Formaldehyde as one of the most active aldehydes can reduce copper hydroxide to copper:

$$CuSO_4 + 2\,NaOH \longrightarrow Cu(OH)_2 + Na_2SO_4$$

$$HCOOH + 2\,Cu(OH)_2 \xrightarrow{t} HCOOH + Cu_2O + 2\,H_2O$$

formaldehyde — formic acid

$$Cu(OH)_2 \rightarrow CuOH \rightarrow Cu_2O \rightarrow Cu$$

blue — yellow — red — copper mirrow

$$CH_3\text{-}CO\text{-}CH_3 + Cu(OH)_2 \not\longrightarrow$$

acetone

Pour into two test-tubes 5 drops of sodium hydroxide and 5drops of water, add 1 drop of copper sulphate. Add to the first tube 3 drops of formalin, to the second – 3 drops of acetone. Heat carefully till boiling. In the first test-tube the precipitate at the first has yellow color, then red and later copper is formed.

Write down the results of experiment in your note-book.

Carbonic acids

Experiment18 Identification of acetic acid.

Ferrum acetate possesses yellow-red color. When heated ferrum acetate is hydrolised to form insoluble in water $(CH_3COO)_2FeOH$ (red-brown color):

$$3\,CH_3COONa + FeCl_3 \longrightarrow (CH_3COO)_3Fe + NaCl$$

sodium acetate — ferrum acetate

$$(CH_3COO)_3Fe + H_2O \rightarrow (CH_3COO)_2FeOH + CH_3COOH$$

Add some sodium acetate to a test-tube, add 3 drops of water and 2 drops of ferrum cloride solution yellow-red color is formed. Heat the solution to boiling. Red-brown precipitate is formed.

Write down the results of experiment in your note-book.

Experiment 19. Formation of ethyl acetate.

Acetic acid is converted directly into an ester when heated with an alcohol in the presence of catalyst usually concentrated sulfuric acid. The reaction is an example of nucleophilic substitution on sp^2-hybrid carbon atom. The product of the reaction possesses a pleasant odor that is used for identification of ethyl alcohol.

In dry test-tube put some anhydrous sodium acetate (height of layer is about of 2 mm) and 3 drops of ethyl alcohol. Add 2 drops of concentrated sulfuric acid and carefully heat. In a few seconds a characteristic freshen odor will be produced.

$$\underset{\text{sodium acetate}}{CH_3COONa} + H_2SO_4 \rightarrow \underset{\text{acetic acid}}{CH_3COOH} + NaHSO_4$$

$$CH_3COOH + C_2H_5OH \rightarrow \underset{\text{ethyl acetate}}{CH_3COOC_2H_5} + H_2O$$

Write down the results of experiment in your note-book.

Experiment 20. Acidity. Salt formation

a) The acid is acted upon the strongly electropositive metals with the liberation of hydrogen and formation of salt.

$$2\ CH_3COOH + Mg \rightarrow \underset{\text{magnasium acetate}}{(CH_3COO)_2Mg} + H_2\uparrow$$

Pour into a test- tube 1-2 ml of acetic acid solution, add a piece of magnasium, liberation of hydrogen is observed.

b) Acetic acid displaces weaker and more volatile acids, forming acetates. When limestone is treated with an acetic acid solution, carbon dioxide is formed.

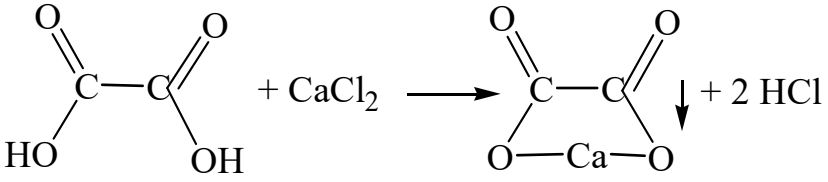
$$CaCO_3 + 2\ CH_3COOH \rightarrow \underset{\text{calcium acetate}}{(CH_3COO)_2Ca} + CO_2\uparrow + H_2O$$

Put into a test-tube a few pieces of limestone and add 1 ml of acetic acid solution. This is accompanied by a “hissing” because of liberation of carbon dioxide.

Write down the results of experiment in your note-book.

Experiment 21.Reaction of oxalic acid with calcium chloride

Calcium salt of oxalic acid (calcium oxalate) is white crystalline substance with characteristic form of crystals в виде почтовых конвертов (looks like envelopes). Calcium oxalate is insoluble in acetic acid and soluble in hydrochloric acid.



oxalic acid calcium oxalate

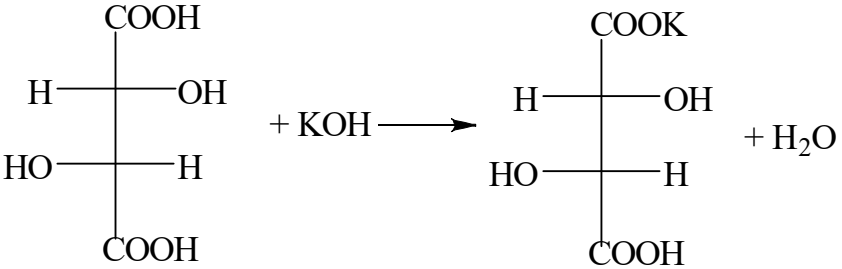
Put some crystals of oxalic acid in the test-tube. Add 4-5 drops of water to dissolve the acid. Then add 5 drops of 5% calcium chloride. The white crystals of calcium salt of oxalic acid are obtained. Add to the precipitate 1 drop of acetic acid solution - precipitate is insoluble. Add to the precipitate 2-3 drops of hydrochloric solution – precipitate are soluble.

Write down the results of experiment in your note-book.

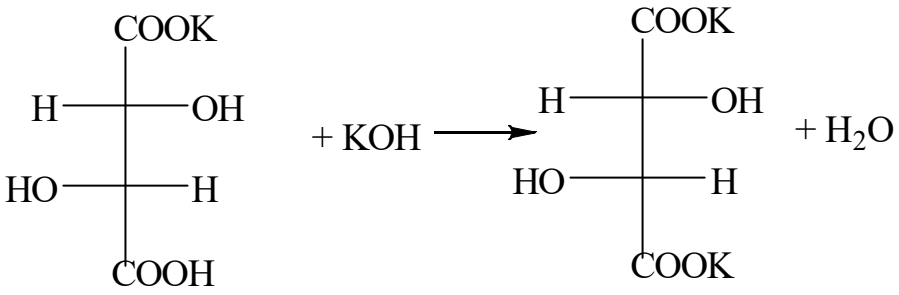
Heterofunctional derivatives of aliphatic hydrocarbons

Experiment 22. Confirmation of two carboxyl groups in D-tartaric acid.

Tartaric acid forms two different in physical properties potassium salts- normal and acid.



D-tartaric acid potassium hydrogen tartrate (acid salt- insoluble in water)



potassium tartrate
(normal-soluble in water)

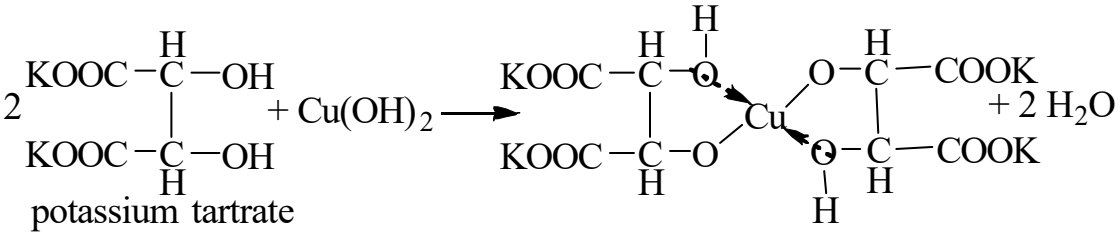
Add 1 drop of tartaric acid solution to a test tube, add 2 drops of potassium hydroxide, vigorously shake, a precipitate of acid potassium salt is gradually forming. Add to test tube 4-5 drops of potassium hydroxide. The precipitate dissolves, so normal soluble in water salt forms. Keep the solution till the next experiment.

Write down the results of experiment in your note-book.

Experiment 23. Confirmation of two hydroxyl groups in D-tartaric acid

Diol groups of D-tartaric acid form complex salt with $Cu(OH)_2$ in alkali solution:

$$\underset{\text{copper sulphate}}{CuSO_4} + \underset{\text{sodium hydroxide}}{2NaOH} \rightarrow \underset{\text{copper hydroxide}}{Cu(OH)_2\downarrow} + Na_2SO_4$$



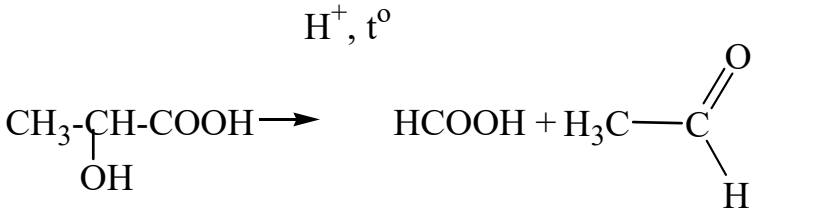
potassium tartrate

Add 2 drops of copper sulphate solution and 2 drops of sodium hydroxide solution to a test tube. A blue precipitate forms. Add to this precipitate the solution prepared in the previous experiment. The blue precipitate dissolves forming transparent dark blue solution of complex salt. Heat the solution till boiling.

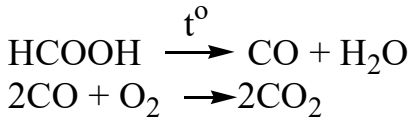
Write down the results of experiment in your note-book.

Experiment 24. Decomposition of lactic acid

When heated α-hydroxyacids eliminate formic acid forming carbonyl compound.



lactic acid formic acid acetic aldehyde

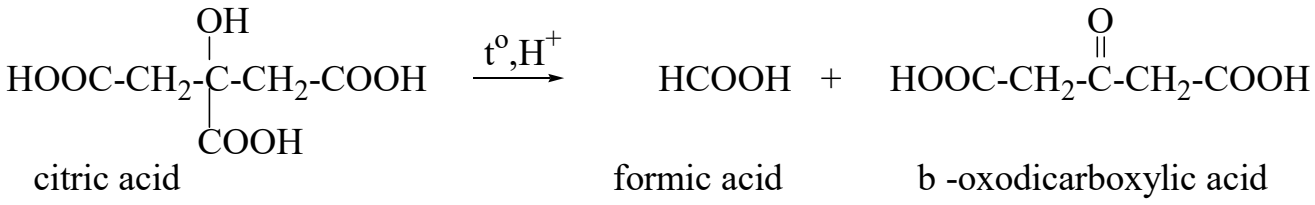


In a dry test tube add 1 drop of lactic acid, add 1 drop of concentrated sulphuric acid and heat. The liquid becomes dark, carbon monoxide is liberated. Bring the tube to a flame, fire carbon monoxide which burns with blue flame.

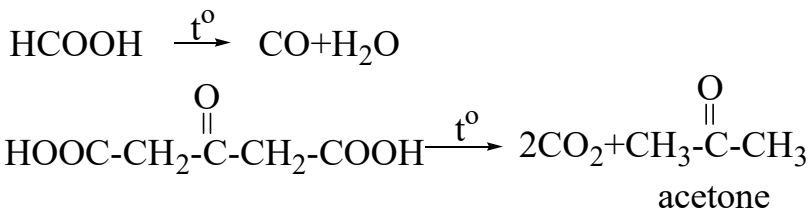
Write down the results of experiment in your note-book.

Experiment 25. Decomposition of citric acid by the action of concentrated sulphuric acid.

Citric acid eliminates formic acid forming β-oxodicarboxylic acid which is decarboxylated. The reactions are carried out in the presence of concentrated sulphuric acid:



citric acid formic acid b -oxodicarboxylic acid



acetone

The formation of CO, CO_2 and CH_3-CO-CH_3 are confirmed by the following reactions

$$2CO + O_2 \rightarrow 2CO_2$$

$$CO_2 + Ba(OH)_2 \rightarrow BaCO_3 + H_2O$$

$$CH_3\text{-}CO\text{-}CH_3 + 3I_2 \rightarrow CH_3\text{-}CO\text{-}CI_3 + 3HI$$

$$CH_3\text{-}CO\text{-}CI_3 + NaOH \rightarrow CH_3COONa + CHI_3\downarrow$$

sodium acetate iodoform

Put some citric acid into a dry test tube fitted with gas outlet tube, add a few drops of concentrated sulphuric acid. Heat the solution. Inmerse the end of the outlet tube (continue the heating) into a tube with solution of iodine in potassium iodide, almost collourless by the action of a few drops of sodium hydroxide. Iodoform forms – yellow precipitate with distinctive odour.

Write down the results of experiment in your note-book.

Experiment 26. Preparation of pyruvic acid and confirmation of its formation

Hydroxy group of lactic acid is easily oxidised forming oxoacid – pyruvic acid. The presence of carbonyl group in the product of reaction is confirmed by the interaction wich phenylhydrazine.

$$CH_3\text{-}\underset{\underset{OH}{|}}{CH}\text{-}COOH \xrightarrow{2[O]} CH_3\text{-}\underset{\underset{O}{||}}{C}\text{-}COOH$$

lactic acid piruvic acid

$$CH_3\text{-}\underset{\underset{O}{||}}{C}\text{-}COOH + NH_2\text{-}NH\text{-}C_6H_5 \longrightarrow CH_3\text{-}\underset{\underset{N\text{-}NH\text{-}C_6H_5}{||}}{C}\text{-}COOH\downarrow + H_2O$$

phenylhydrazine phenylhydrazone

Pour 1 ml of lactic acid into a test tube add 1 ml of potassium permanganate solution, add some phenylhydrazine hydrochloride and sodium acetate. Heat the mixture on water bath during 15-20 min. After cooling yellow precipitate of phenylhydrazone formes.

Write down the results of experiment in your note-book.

Carbohydrates

Experiment 27. Oxydation of monosaccharides

Carbohydrates which have a free aldehyde or a ketone group are easily oxydized, thereby acting as reducung agent. Oxydation by

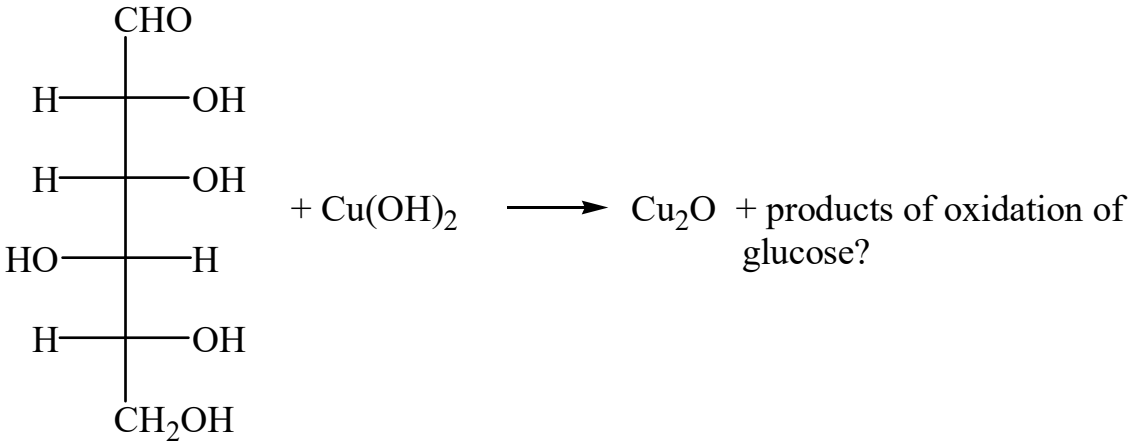
alkaline reagents (Tollens' reagent, Fehling's solution) decomposes the chain of sugars.

a)Monosaccharides reduce copper (П) hydroxide and give redish-orange precipitate of copper (I) oxide.

Place 2 drops of 2% copper sulphate solution and 2 drops of 10% sodium hydroxide solution. A blue precipitate of copper hydroxide is formed. Then add 2 drops of 1% glucose solution and heat the test-tube. A redish-orange precipitate of copper (I) oxide is formed.

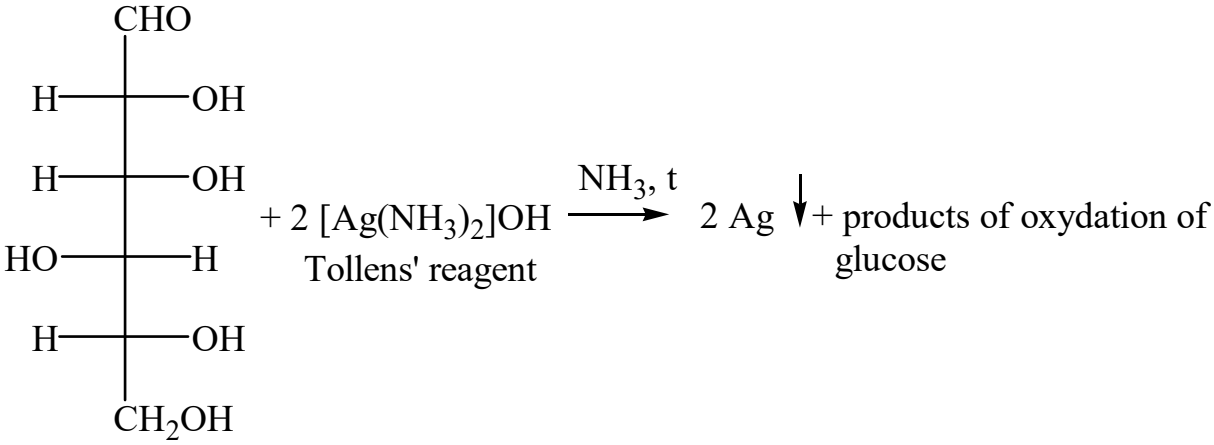
$CuSO_4$ + 2NaOH → $Cu(OH)_2\downarrow$ + Na_2SO_4

copper sulphate sodium hydroxide copper hidroxide



glucose

b) Monosaccharides are easy oxidized by Tollens' reagent. In this reaction the silver metal precipitates as a mirror-like deposit.



glucose

Pour into test-tube 10 drops of Tollens' reagent. (Tollens' reagent contains silver nitrate and ammounia in water). Add 5 drops of

1% glucose solution. Carefully heat obtained mixture and a black precipitate of silver or mirror is formed

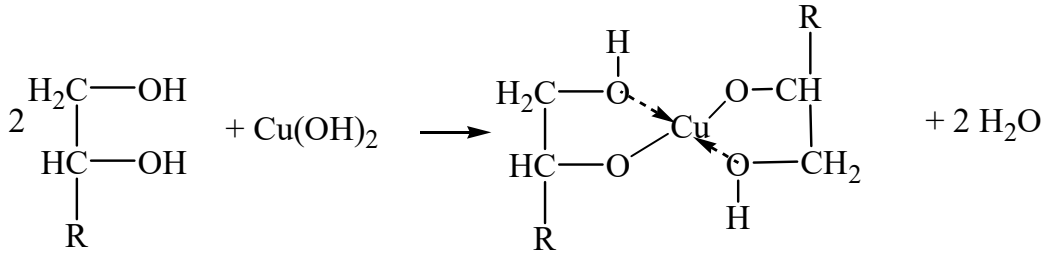
Write down the results of experiment in your note-book.

Experiment 28. Reaction of monosaccharides as polyalcohols

Monosaccharides contain diol fragment (two hydroxyl groups on adjacent carbons) in the structure and give reaction for polyalcohols.

$CuSO_4$ + 2NaOH → Cu $(OH)_2\downarrow$ + Na_2SO_4

copper sulphate sodium hydroxide copper hydroxide



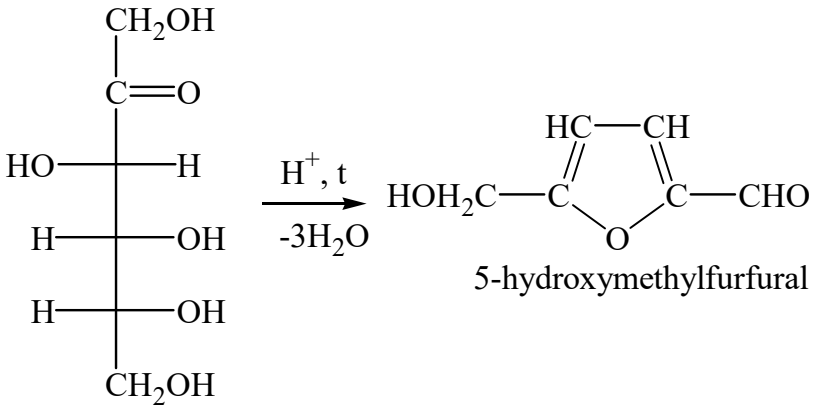
any monosaccharide

Add 2 drops of copper (II) sulphate solution and 2 drops of sodium hydroxide solution in a test tube. The blue precipitate of copper hydroxide is formed. Add to this precipitate 2 drops of 1% glucose solution. The blue precipitate is dissolved and the dark-blue solution is formed.

Write down the results of experiment in your note-book.

Experiment 29. Seliwanoff test

Seliwanoff test is the test for fructose. The Seliwanoff reaction is due to the conversion of fructose by hot hydrochloric acid into 5-hydroxymethylfurfural and condensation of the latter compound with resorcinol. The product of condensation has a deep red colour.



fructose

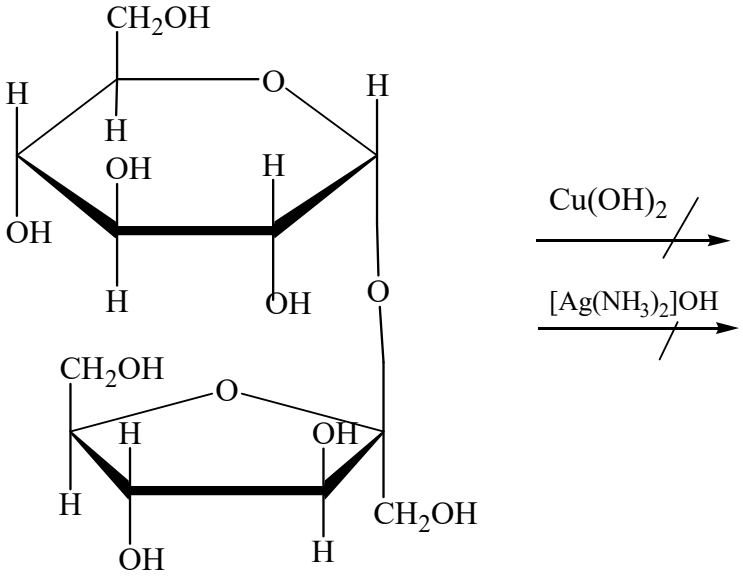
5-hydroxymethylfurfural

Place a graine of resorcinol and 2 drops of concentrated hydrochloric acid (BE CAREFUL: CONCENTRATED ACID SEVERE BURNS!) in a test tube. Add 2 drops of 1% fructose solution and heat.

Write down the results of experiment in your note-book.

Experiment 30. Non-reducing properties of sucrose

Sucrose is non-reducing disaccharide, therefore it can't be oxidized with copper (П) hydroxide (Fehling's solution) and ammonium solution of siver oxide. (Tollens' reagent)



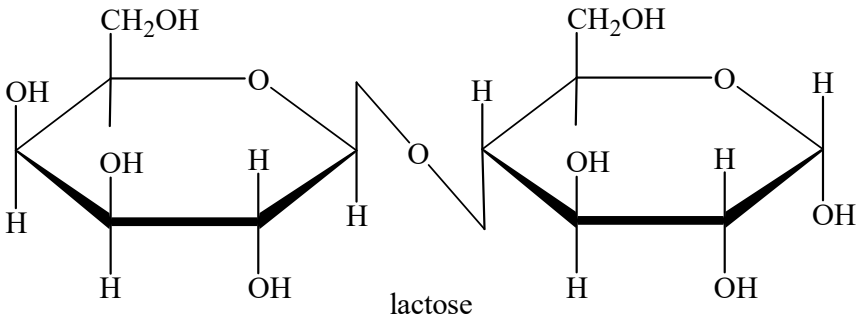
sucrose

Place 1 drop of 1% sucrose solution and 6 drops of 10% sodium hydroxide solution in a test-tube. Add 2 drop of 2% copper sulphate solution. A transparent blue solution is formed. Heat the test-tube. The color of the solution is constant.

Write down the results of experiment in your note-book.

Experiment 31. Reducing properties of lactose.

Lactose is reducing disaccharide, therefore it can be oxidized with copper (II) hydroxide (Fehling's solution) and ammonium solution of siver oxide. (Tollens' reagent).



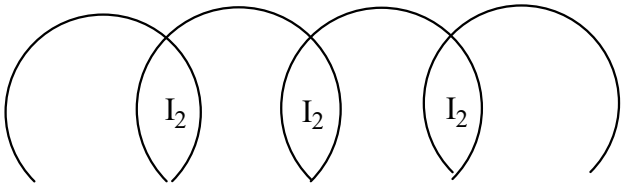
lactose

Place 1 drop of 1% lactose solution and 6 drops of 10% sodium hydroxide solution in a test-tube. Add 2 drop of 2% copper sulphate solution. A transparent blue solution is formed. Heat the test-tube. A redish-orange precipitate of copper(I) oxide is formed.

Write down the results of experiment in your note-book.

Experiment 32. Test for starch

Starch consists of two fractions. They are amylose and amylopectin. Amylose gives a blue color with iodine. This blue colour is considered to be due to the formation of an inclusion complex. The chains of amylose coiled in the form of helix inside which is founded an iodine molecule.



Place 5 drops of starch solution in a test-tube. Add 1 drop of dilute iodine. A blue color appears.

Write down the results of experiment in your note-book.

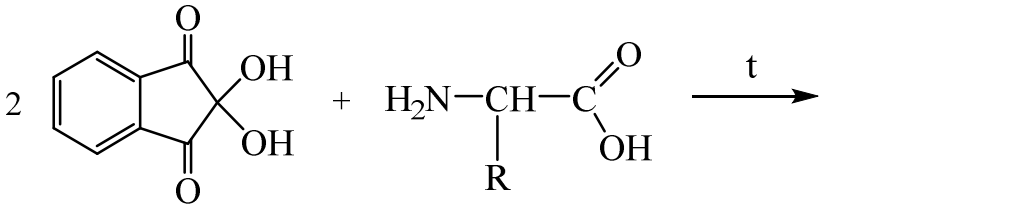
α-Amino acids and peptides

Experiment 33 Tests for α-amino acids and proteins.

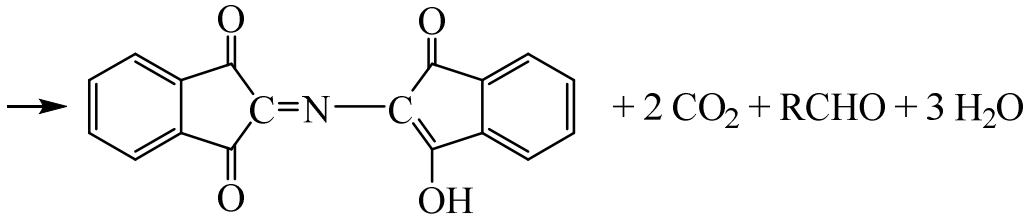
The amino acids undergo avariaty of colour characteristic reactions.

a) **Ninhydrin test**

Ninhydrin (idane-1,2,3-trion hydrate) reacts with α-amino acids to form a coloured product. All α-amino acids give the same blue product; proline (imino acids), howewever, gives a yellow product.



ninhydrin α–amino acid

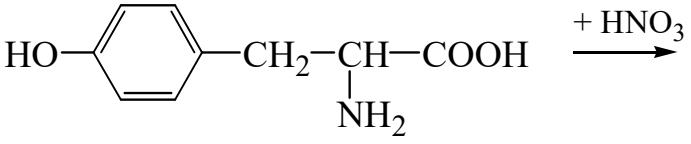


blue product (yellow with proline)

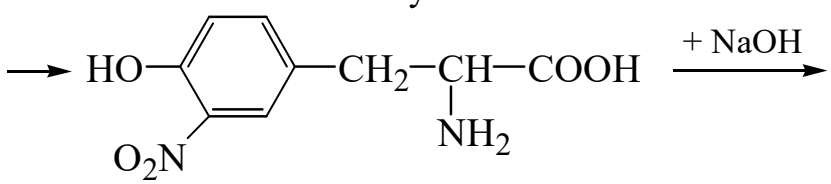
Place 4 drops of the egg protein solution in a test-tube. Add 2 drops of 0,1% ninhydrinn solution and heart to boiling. The redish-blue color appears.

b) Xanthoproteic test

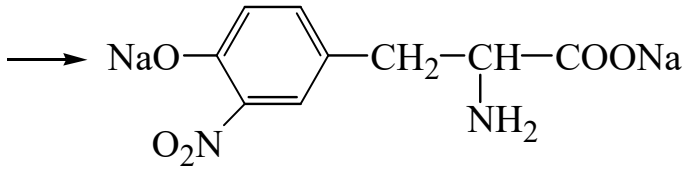
Some α-amino acids such as phenylalanine, tyrosine and tryptophan contain aromatic rings than can easily undergo nitration to yield yellow products. Any proteins containing these amino acids will therefore become yellow in the presence of nitric acid. After the nitration product is formed, the color may be intensified through the addition of a base.



tyrosine



yellow product

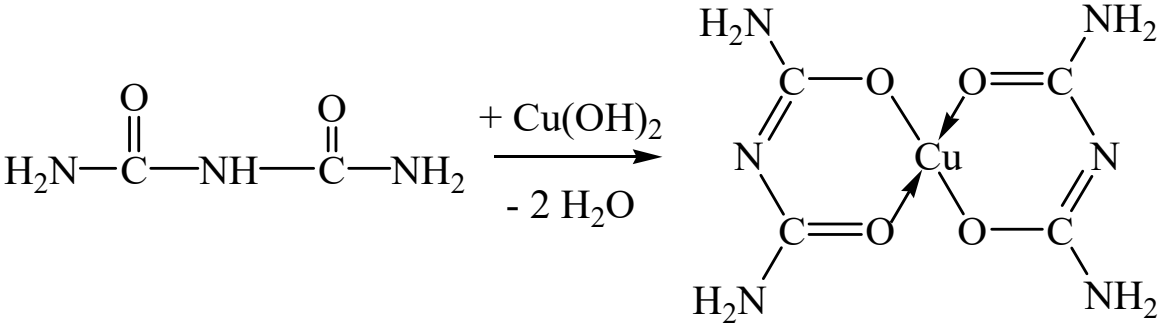


orange product

Place 10 drops of the egg protein solution and 2 drops of concentrated nitric acid in a test-tube. (CAUTION: CONCENTRATED NITRIC ACID SAUSES SEVERE BURNS. HANDLE WITH CARE!) Gently ware the mixture and observe any change in color. Cool the mixture and carefully add 10% sodium hydroxide solution.

c) Biuret test

The biuret test is general test for proteins containing two or more peptide bonds (-CONH-). Dipeptides do not give the test. Addition of a very dilute solution of copper sulfate to an alkaline solution of proteins produce a red or violet colour.



Place 5-6 drops of the egg protein solution and equal volume of 10% sodium hydroxide in a test-tube. After mixing this two reagents add 2 drops of 0,5% copper sulfate solution. A redish-violet colour appears.

Write down the results of experiment in your note-book.

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