

МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ РЕСПУБЛИКИ БЕЛАРУСЬ
БЕЛОРУССКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ
КАФЕДРА БИООРГАНИЧЕСКОЙ ХИМИИ

О. Н. РИНЕЙСКАЯ, К. Г. ПРОКОПЧИК

БИООРГАНИЧЕСКАЯ ХИМИЯ ПРАКТИЧЕСКИЙ КУРС

BIOORGANIC CHEMISTRY PRACTICAL COURSE

Практикум



Минск БГМУ 2008

УДК 577.1 (811.111) (076.5)
ББК 28.072 (81.2 Англ–923)
Р 51

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Перевод с рус. яз. О. Н. Ринейской, К. Г. Прокопчик

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Издание содержит рекомендации ко всем лабораторным занятиям по биоорганической химии. Каж-
дая тема включает цель занятия, литературу для подготовки, вопросы для обсуждения, стандартную
форму протокола лабораторной работы.

Предназначено для студентов 1-го курса, обучающихся на английском языке.

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Plan Of Laboratory Classes

1. Introduction to practical course. Classification and nomenclature of organic compounds.
2. Spatial structure of organic molecules. Configuration and conformations. tereoisomerism, its role for biological activity demonstration.
3. Chemical bond structure and atom effects in the organic molecules.
4. Acid-base properties of organic compounds. Oxidation reactions.
5. Classification and mechanisms of reactions in organic chemistry. Reactions of radical substitution. Electrophilic addition reactions (A_E).
6. Electrophilic substitution reaction of aromatic compounds. Nucleophilic substitution at sp^3 -hybridized carbon atom. Competitive elimination reactions.
7. Carbonyl compounds and their biologically important reactions.
8. Concluding session on the theme: "Theoretical fundamentals of basic classes of organic compound structure and reactivity".
9. Heterofunctional compounds of aliphatic series. The structure and reactivity of oxy-, oxo- and amino acids.
10. Biologically active heterofunctional compounds of benzene and heterocyclic series, metabolites and bioregulators.
11. Lipids. Structure, properties. Lipid peroxidation.
12. Carbohydrates. Monosaccharides.
13. Oligo- and polysaccharides.
14. Structure and reactivity of amino acids acting as heterofunctional compounds and their qualitative reactions.
15. Peptides. Their structure, reactivity and importance. the levels of protein organization.
16. Purine and pyrimidine bases. Nucleosides. Nucleotides. Nucleic acids.
17. Concluding session on the theme: "Biopolimers and their structural components. Lipids".
18. Low-molecular bioregulators. Steroids. Alkaloids.

Thematic Plan Of Lectures

1. Spatial structure of organic molecules and stereoisomerism.
2. Atom effects in the organic molecules.
3. Acid-base properties of organic compounds.
4. Common rules of organic compound reactivity as chemical base of a biological functioning.
5. Biologically important heterofunctional compounds.
6. Lipids, classification. Representatives of simple and complex lipids, their properties and biological role. Lipid peroxidation.
7. Monosaccharides. Structure, tautomeric forms, reactivity, biological role.
8. Polysaccharides. Classification, structure, properties, role in the processes of vital activity.
9. Amino acids. Classification, stereoisomerism, properties. Representatives of amino acids, their biological role.
10. Peptides, proteins. The levels of protein organization.
11. Nucleotides and nucleic acids.
12. Some natural and synthetic biologically important compounds.

Safety Rules During The Work In The Chemical Laboratory

The students are to attend classes in chemical laboratory in cotton gowns. The students are fixed to the constant workplaces, which they should keep in order. When the experiment is carried out, each student should be on his workplace. In the process of work time it is necessary to keep silence, order and cleanliness in the laboratory, avoid haste.

It is strictly forbidden:

- to carry out the experiments, not connected with the indicated practical course;
- to work with reagents in the absence of the teacher;
- to have a meal in the laboratory;
- to taste chemicals.

After the end of the experiment each student should submit an account of the work that have been done, then to wash up chemical crockery, clean a workplace and ask the student on duty to check it.

Responsibilities of the student on duty:

- to get all the necessary equipment from the laboratory assistant;
- to keep an order in the laboratory;
- the student on duty should leave the laboratory the last, after receiving the sanction from the laboratory assistant.

Work with alcohol lamps. Precautions

Careless work with an alcohol lamp can result in a fire, that is why it is necessary to follow the below requirements:

- the wick of an alcohol lamp should tightly enter the aperture of a metal bush; the topping should be put forward for 1 cm and fluffed up;
- the bush should close the aperture of a alcohol lamp tightly; the alcohol lamp should be filled with alcohol no more than $\frac{2}{3}$ of the volume;
- the lighting of an alcohol lamp should be carried only by matches, it is strictly forbidden to light an alcohol lamp from another alcohol lamp, because the bush can stoop and coming out steams of alcohol can be fired;
- to blow out an alcohol lamp only by covering it with bell-glass;
- when heating up substances in chemical glassware it is necessary to heat them at the top or mid-range flame, not touching a wick, because a wick is always cool, and when hot glass contacts with it, glass may burst.

WORK WITH CHEMICAL GLASSWARE. PRECAUTIONS

Heating substances in glassware should be performed gradually, slightly rotating it and cautiously shaking from time to time. When heating a test tube with a liquid on the open fire, ejaculation of a liquid is possible. Because of this fact, the aperture of a test tube should be directed aside from you and from your neighbours. Especially it is necessary to avoid injuring the eyes with hot splashes, that it is why it is forbidden to bend forward to the test tube and look inside. When heating the test tube, it should be kept at the angle of inclined position (45°), so that splashes will hit walls of a glassware and will not be thrown outside. When working with an exhaust tube it is necessary to keep an eye on the end of an exhaust tube in the liquid, through which gas passes. You can remove an alcohol lamp from under a test tube with a reaction mixture only when the bottom end of an exhaust tube is removed out of a liquid. If the liquid starts to rise in an exhaust tube, it is necessary to let down a test tube immediately, so that the fluid level in it will become lower than the end of an exhaust tube, and to continue heating it up until the gas coming out pushes the liquid out of an exhaust tube.

WORK WITH CHEMICAL REAGENTS. PRECAUTIONS

Reagents necessary for work except for easily inflammable liquids and strong and toxic substances, should be on a working table, placed in supports with the numbered jacks. The little bottle with the corresponding reagent has the same number. Little bottles with liquids are closed by rubber corks with pipettes in them.

It is not recommended to take out little bottles from jacks of a support. If you want to take the substance, it is necessary to press the little bottle to a bottom of a jack by your left hand, and cautiously take out a cork with a pipette by your right hand. To take the necessary quantity of a reagent with a pipette and to close the little bottle with the same cork. The spatula (a little glass shovel) is built-in in a cork for taking crystal reagents.

WORK WITH INFLAMMABLE LIQUIDS (IL). PRECAUTIONS

IL (diethyl ether, alcohol, toluene, acetone, acetoacetic ether) are kept in small quantities in an exhaust cupboard. Experiments with these substances are carried out under draught, far from open fire and the turned on small stoves. If an ignition of the IL happened in a vessel, it is necessary to cover it quickly with a fire-prevention blanket. If the burning liquid has been spilt, it must be extinguished by sand. If the clothes begin to fire, it is necessary to wrap up quickly and densely in a fire-prevention blanket.

WORK WITH ACIDS AND ALKALIS. PRECAUTIONS

Concentrated solutions of nitric, sulphuric, hydrochloric acids, nitrosulphuric acid are kept in the exhaust cupboard. All experiments with concentrated acids and alkalis are carried out only in the exhaust cupboard. The dilution of concentrated acids is possible only by pouring **acids to water**, not the other way. It is necessary to cover carelessly spilt on the floor acids and alkalis by sand and after that to clean up.

WORK WITH TOXICANTS. PRECAUTIONS

Toxic organic substances — aniline, methyl amine, toluene, picric acid are kept in an exhaust cupboard. It is necessary to be cautious with these substances, not to inhale their steams, to avoid injuring the hands as they can penetrate through the skin. In case of emergency when these substances got on hands, it is necessary to wash up quickly the hands with warm water and soap. If inhaled the steams — immediately to go out in the fresh air.

First-aid treatment in case of accidents:

- in case of hands are cut with glass first of all it is necessary to remove all the splinters out of the wound, then to treat the wound with an alcohol solution of iodine and to put a bandage;
- in case of thermal burns happen it is necessary to treat the burnt place with the 70 % solution of ethanol;
- in case of burns are caused by solutions of acids or alkalis it is necessary to wash up the burnt site with water quickly and to put an aseptic bandage;
- in case of acids or alkalis hit the eyes it is necessary wash them with water carefully and to refer the victim to the outpatient clinic;
- in case of skin burns caused by bromine it is necessary quickly to wash the injured place off with ethanol and to put anti-burn emulsion;
- in case of burns caused by hot organic liquids it is necessary to wash out the injured place with ethanol;
- in case of burns caused by liquid phenol it is necessary to massage the emerged sites of white skin with a glycerine until normal skin color is restored then to wash with water and to put the gauze bandage moistened with a glycerine solution;
- after providing the first-aid treatment it necessary to address to the health center of the university or to the outpatient clinic.

Labwork № 1

Theme: Classification and Nomenclature of Organic Compounds. Spatial Structure of Organic Molecules. Configuration and Conformations

Objective: to study composition unity, configuration and conformation concept for organic molecules.

Recommended literature:

1. **Zurabyan, S. E. *Fundamentals of Bioorganic Chemistry. Textbook for medical students* / S. E. Zurabyan. 2006. P. 5–32, 74–81.**

2. **Solomons, T. W. *Fundamentals of Organic Chemistry* / T. W. Solomons. John Willey&sons, 1994. P. 116–127, 501–504, 643–644, 715–723.**

Problems for discussion:

1. Introduction into bioorganic chemistry: the definition of subject, objects learned by bioorganic chemistry.

2. Classification of organic compounds:

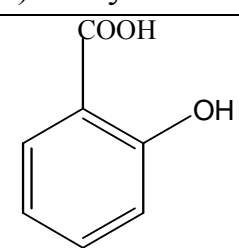
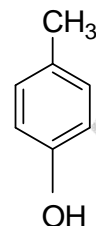
- according to the carbon chain structure;
- according to the functional groups.

3. Nomenclature of organic compounds:

- trivial (or common) nomenclature;
- systematic nomenclature IUPAC.

Exercises

1. Give the IUPAC names for the following compounds:

a) lactic acid $\begin{array}{c} \text{H}_3\text{C} - \text{CH} - \text{COOH} \\ \\ \text{OH} \end{array}$	b) <u>oxaloacetic acid</u> $\text{HOOC} - \text{CH}_2 - \text{C}(=\text{O}) - \text{COOH}$	c) serine $\begin{array}{c} \text{H}_2\text{C} - \text{CH} - \text{COOH} \\ \quad \\ \text{OH} \quad \text{NH}_2 \end{array}$	d) salicylic acid 
e) 	f) isoprene $\text{H}_2\text{C}=\text{C}(\text{CH}_3)-\text{CH}=\text{CH}_2$	g) $\begin{array}{c} \text{O} - \text{CH}_3 \\ \\ \text{H}_3\text{C} - \text{CH} - \text{OH} \end{array}$	h) cysteine $\begin{array}{c} \text{H}_2\text{N} - \text{CH} - \text{COOH} \\ \\ \text{CH}_2 \\ \\ \text{SH} \end{array}$

2. Write a structural formula for each of the following compounds:

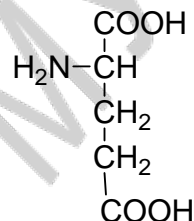
- 3-hydroxy-3-carboxypentanedioic acid (citric acid);
- 1,1,2-trichloroethene;
- 2-amino-4-methylthiobutanoic acid;

- d) 4-methylpentanol-2;
- e) 4-aminobenzoic acid;
- f) dimethoxymethane;
- g) 2,6-diaminohexanoic acid;
- h) 2,2-dimethylpropanal.

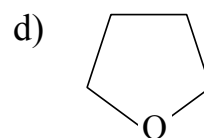
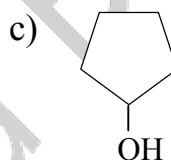
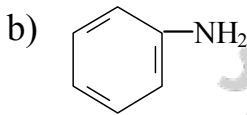
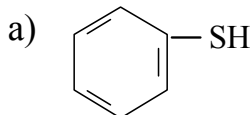
Test control

1. Give the IUPAC name for the following amino acid:

- a) 2-aminobutanedioic acid;
- b) 2-aminopropanoic acid;
- c) 2-aminopentanoic acid;
- d) 2-aminopentanedioic acid.



2. Indicate a heterocyclic compound:



3. Give the IUPAC name for the following compound $\text{CH}_3\text{-CHCl-CH}_2\text{-CHBr-CH}_3$:

- a) 2-chloro-4-bromobutane;
- b) 2-bromo-4-chlorobutane;
- c) 2-chloro-4-bromopentane;
- d) 2-bromo-4-chloropentane.

4. Select compounds containing hydroxyl group:

- a) ethanol;
- b) phenol;
- c) ethanal;
- d) 2-hydroxypropanoic acid.

5. Indicate methylpropylether:

- a) $\text{CH}_3\text{-O-CH}_2\text{-CH}_3$;
- b) $\text{CH}_3\text{-CH}_2\text{-CH}_2\text{-O-CH}_3$;
- c) $\text{CH}_3\text{-CH}_2\text{-O-CH}_2\text{-CH}_3$;
- d) $\text{CH}_3\text{-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-CH}_3$.

6. Select the unsaturated compounds:

- a) cyclohexane;
- b) propenal;
- c) pentane;
- d) butadiene-1,3.

Labwork № 2
Theme: Stereoisomerism, its Role for Biological
Activity Demonstration

Objective: to study the dimensional organization and discuss a stereoisomerism role for interaction specificity on a molecular scale understanding.

Recommended literature:

1. *Zurabyan, S. E.* Fundamentals of Bioorganic Chemistry. Textbook for medical students / S. E. Zurabyan. 2006. P. 61–81.
2. *Solomons, T. W.* Fundamentals of Organic Chemistry / T. W. Solomons. John Willey&sons, 1994. P. 164–198.

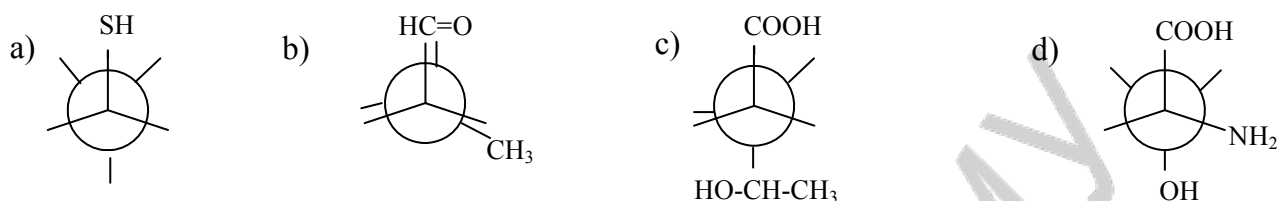
Problems for discussion:

1. Stereoisomerism. Classification of stereoisomers.
2. A spatial structure of a sp^3 -hybridized carbon atom. Configuration. Stereochemical formulas. Molecular models.
3. Ethane configuration and conformations, torsion strain. Newman projections.
4. Butane conformations. Van der Waals strain. Long-chain compound conformations. Energy diagram of conformations.
5. Carbocyclic compound conformations, angle strain. Cyclohexane conformations. A cyclohexane ring in the biologically important compounds.
6. Chiral and achiral molecules. Chiral centers. Optical activity is the property inherent chiral molecules.
7. Fisher's projective formulas. Enantiomers.
8. Relative D-, L-nomenclature of stereoisomers. Glyceraldehyde as the configuration standard. R, S-system of a configuration designation.
9. Racemic mixtures. Methods of racemic substance division.
10. Diastereoisomerism. Stereoisomers of tartaric acid.
11. Cis-, trans-isomerism. Stereoisomers of butenedioic and oleic acids.

Exercises

1. Write all possible conformations by means of Newman projections for the following compounds.
 - a) ethanethiol;
 - b) ethanol;
 - c) propane;
 - d) butanedioic acid;

- e) 2-aminoethanol;
 f) ethanediol-1,2;
 g) 2-amino-3-hydroxypropanoic acid (along C₂-C₃). Write the structural formulas for the following Newman projections:



2. Draw the possible chair conformations of the following compounds:

- a) cyclohexanol;
 b) cyclohexylamine.

3. Draw the preferred conformation for the following compounds:

- a) 2-methylcyclohexanol;
 b) 1,4-diisopropylcyclohexane;
 c) 1,3-dichlorocyclohexane.

4. Draw the conformation of 1,3-dimethylcyclohexane with 1,3-diaxial interaction.

5. Write D- and L-isomers by means of Fisher projections for the following compounds:

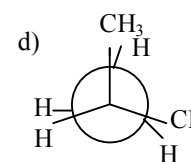
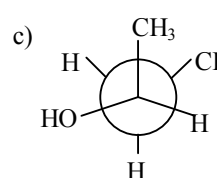
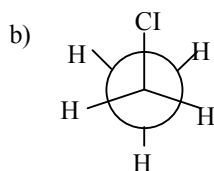
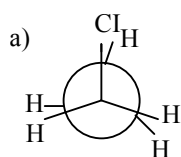
- a) 2-aminopropanoic acid;
 b) tartaric acid (2 chiral centers);
 c) 2-hydroxypropanal;
 d) 2-hydroxybutanedioic acid;
 e) 2-amino-3-hydroxybutanoic acid (2 chiral centers).

6. Write R- and S-isomers for the following compounds:

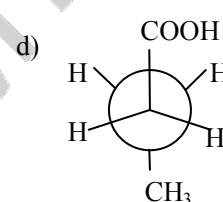
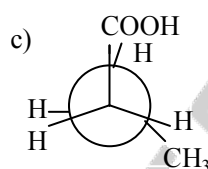
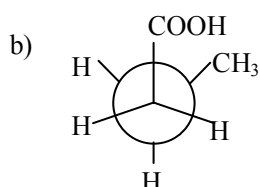
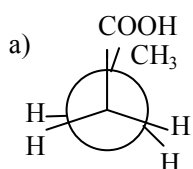
- a) 2-hydroxypropanoic acid;
 b) 2-amino-3-methylbutanoic acid.

Test control

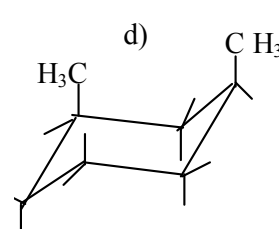
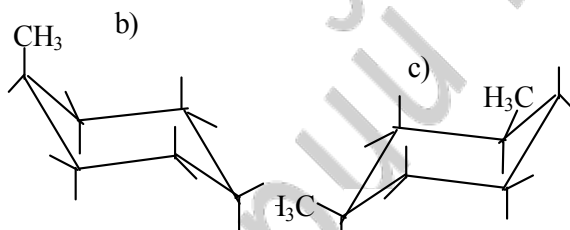
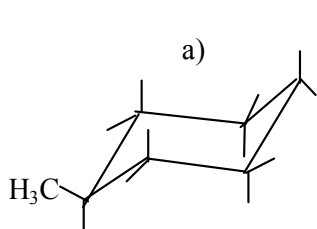
1. Select the most stable chloroethane conformation. Name it.



2. Indicate the most stable butanoic acid conformation:



3. Select the most stable methylcyclohexane conformation:



4. Chiral molecules are:

- α -hydroxybutyric acid;
- α -hydroxybutyric acid;
- γ -hydroxybutyric acid;
- γ -hydroxyvaleric acid.

5. Enantiomers are:

- pairs of stereoisomers which concern to each other as a subject and its display in an ideal plane mirror, possess in achiral surroundings identical chemical and physical properties, except for a sign on optical rotation;
- pairs of stereoisomers which at mixing in equimolar ratio form a racemic substance;
- pairs of stereoisomers of the same substances not being a mirror image of one another and possessing various chemical and physical properties;
- pairs of stereoisomers, capable to pass each other due to rotation of atoms or groups of atoms on a line σ -bond.

6. Which of the following statements are correct:

a) 2-hydroxybutanedioic acid is the participant of a metabolism (in the Krebs's cycle);

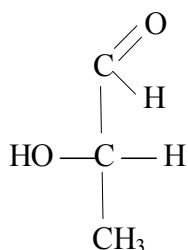
b) hydroxybutanedioic acid is monohydroxycarboxylic acid;

c) natural hydroxybutanedioic acid is a D-isomer;

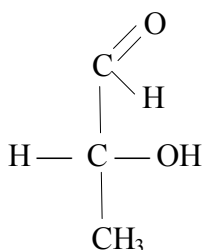
d) salts of hydroxybutanedioic acid are called malates.

7. Select stereoisomers of the "configuration standard":

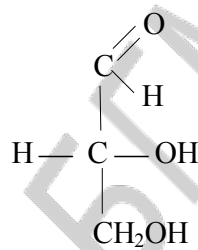
a)



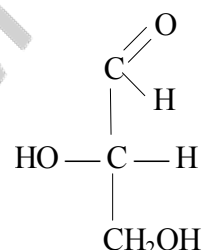
b)



c)



d)



8. What statements concerning racemic mixtures are true:

a) at mixture equimolar quantities D- and L-stereoisomers the inactive mixture named racemic is formed optically;

b) racemic substances are formed at chemical synthesis without observance of special conditions;

c) racemic substances can be divided on optically active enantiomers by means of only physical methods;

d) from racemic substances can be evolved enantiomers by means of microbiological (biochemical), chemical methods and by an affinity chromatography.

9. Select the compounds containing two chiral centers:

a)	b)	c)	d)
$\begin{array}{c} \text{COOH} \\ \\ \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{H}-\text{C}-\text{OH} \\ \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{COOH} \\ \\ \text{H}-\text{C}-\text{NH}_2 \\ \\ \text{CH}_2 \\ \\ \text{H}-\text{C}-\text{CH}_3 \\ \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{COOH} \\ \\ \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2\text{SH} \end{array}$	$\begin{array}{c} \text{COOH} \\ \\ \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{H}-\text{C}-\text{CH}_3 \\ \\ \text{CH}_2 \\ \\ \text{CH}_3 \end{array}$

Labwork № 3
Theme: Chemical Bond Structure and Atom effects
in the Organic Molecules

Objective: to develop knowledge about chemical bond structure, dimensional and electronic effects of substituents.

Recommended literature:

1. *Zurabyan, S. E.* Fundamentals of Bioorganic Chemistry. Textbook for medical students / S. E. Zurabyan. 2006. P. 33–44.
2. *Solomons, T. W.* Fundamentals of Organic Chemistry / T. W. Solomons. John Willey&sons, 1994. P. 27–52.

Problems for discussion:

1. An electronic and dimensional structure of sp^2 -hybridized carbon atom.
2. Conjugated systems. Conjugation energy.
3. Cyclic conjugated systems. Aromaticity. Huckel's rule. Aromaticity of benzoic and non-benzoic systems.
4. Aromaticity of heterocyclic systems (pyrrole, pyridine, pyrimidine, imidazole, purine, porphine).
5. Inductive effect.
6. Mesomeric (or resonance) effect. Electron donating and electron withdrawing substituents.

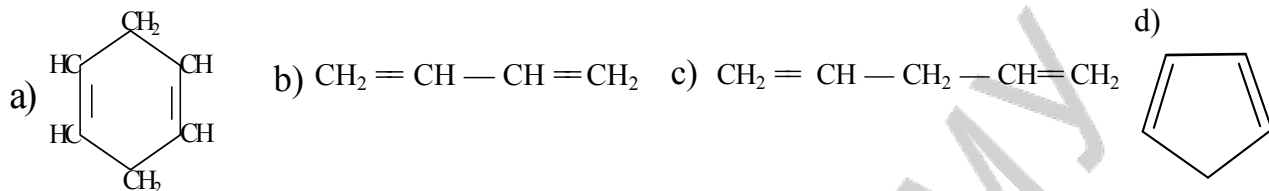
Exercises

1. What kind of the conjugated system exists in the following compounds?
 - a) vinyl amine;
 - b) butadiene-1,3;
 - c) 2-methylbutadiene-1,3;
 - d) propenal;
 - e) propenoic acid;
 - f) pyrrole;
 - g) pyridine.
2. Define aromaticity by the means of Huckel's rule for the compounds:
 - a) benzene;
 - b) anthracene;
 - c) phenanthrene;
 - d) cyclopentadienyl anion;
 - e) cycloheptatrienyl cation;
 - f) pyrrole (draw the electronic structure of pyrrole nitrogen);
 - g) pyridine (draw the electronic structure of pyridine nitrogen);
 - h) pyrimidine;
 - i) imidazole;
 - j) purine.
3. Show the electron density distribution in the molecules with inductive and mesomeric effects:
 - a) 1-chlorbutane;
 - d) propanal;
 - g) 4-aminobenzoic acid;

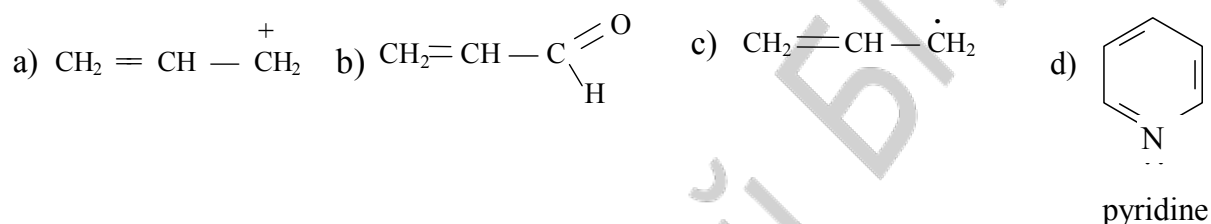
- b) vinylamine; e) benzylamine; h) phenol.
 c) benzoic acid; f) toluene;

Test control

1. Indicate formulas of compounds with the conjugated double bonds:



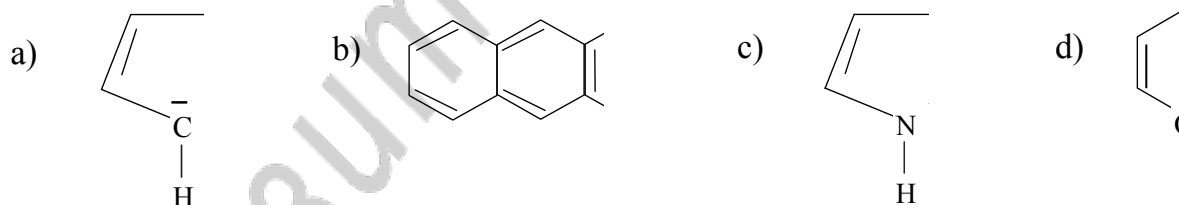
2. Select the compounds with π - π -conjugation:



3. Which of the following statements are correct for aromatic compounds:

- a) low thermodynamic stability;
 b) addition reactions are more feature than substitution reactions;
 c) substitution reactions are more feature than addition reactions;
 d) high stability of compounds to oxidant and temperature action.

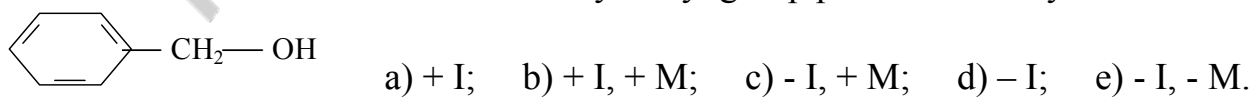
4. Which of the following compounds are aromatic?



5. Which of the following substituents possesses positive inductive effect?



6. What electronic effects does hydroxyl group possess in benzyl alcohol?



Labwork № 4
Theme: Acid-base Properties of Organic Compounds.
Oxidation Reactions

Objective: to develop knowledge about acidity and basicity of organic compounds, about the factors influencing their expressiveness; to generate skills for qualitative determination of organic compound acidity and basicity.

Recommended literature:

1. *Zurabyan, S. E.* Fundamentals of Bioorganic Chemistry. Textbook for medical students / S. E. Zurabyan. 2006. P. 47–59.
2. *Solomons, T. W.* Fundamentals of Organic Chemistry / T. W. Solomons. John Wiley&sons, 1994. P. 86–102.

Problems for discussion:

1. The Brensted theory of organic compound acidity and basicity. The Lewis electronic theory of organic compound acidity and basicity. Classification of organic acids.
2. The quantitative and qualitative characteristics of acidity. The factors influencing on the acidic properties of organic compounds.
3. Oxidation reactions of alcohols, thiols and phenols. Antioxidants and their role in processes of vital activity.
4. Basicity. The factors influencing on the basic properties of organic compounds.
5. Amphoteric properties of organic compounds. Hydrogen bonds.

Exercises

1. Write the conjugate base of the following acids:

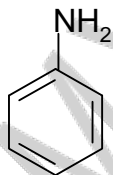
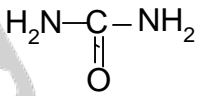
a) CH_3COOH	b) $\text{H}_3\text{C}-\text{CH}_2-\overset{+}{\text{O}}\begin{matrix} \text{H} \\ \text{H} \end{matrix}$	c) H_2O	d) $\text{C}_2\text{H}_5-\overset{\oplus}{\text{N}}\text{H}_3$	e) $\text{C}_6\text{H}_5\text{OH}$
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2. Compare acidity of compound in the following groups:

- a) ethanol and ethanthiol;
- b) propanol-1 and propantriol-1,2,3 (glycerine);
- c) acetic and monochloroacetic acids;
- d) ethanoic and ethanedioic acids;
- e) phenol and 4-aminophenol;
- f) benzoic acid and 4-hydroxy benzoic acid;

- g) propanoic and 2-oxopropanoic acids;
- h) propanoic and propenoic acid;
- i) pyrrole and imidazole.

3. Write the conjugate acid of the following bases:

a) $\text{CH}_3\text{-NH}_2$	b) H_2O	c) OH^-	d) CH_3COO^-	e) 	f) 
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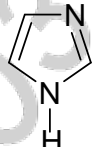
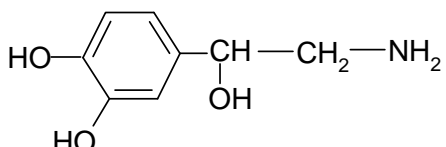
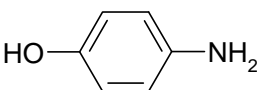
4. Compare basicity of compound in the following groups:

- a) dimethyl ether, dimethylamine, dimethylsulfide;
- b) ethylamine and aniline;
- c) ethylamine and diethylamine;
- d) 2-aminoethanol and ethylamine;
- e) pyrrole and pyridine;
- f) pyridine and pyrimidine.

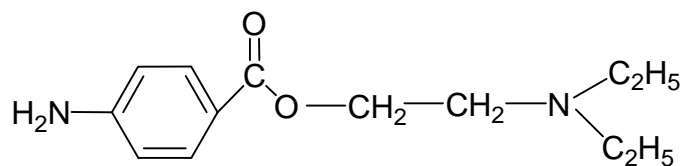
5. Write the ethanol oxidation reaction in vivo and in vitro.

6. Write the 2-amino-3-mercaptopropionic acid oxidation reaction scheme.

7. Write down the salt formation reaction of the following compounds with HCl:

a) $\text{CH}_3\text{-NH}_2$	b) 	c) 	d) 
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8. Designate all basic sites in the novocaine structure and define the most strong basic site:



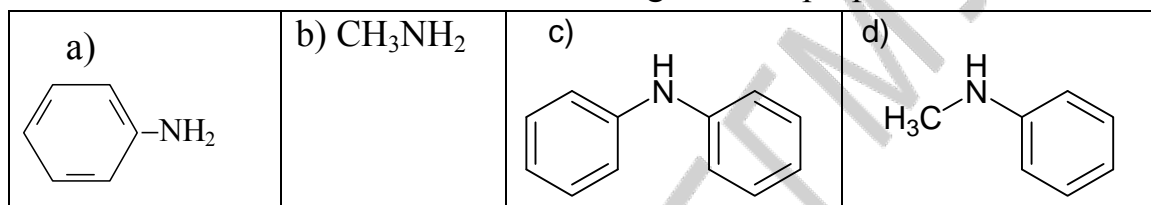
Write the reaction of novocaine with hydrochloric acid.

Test control

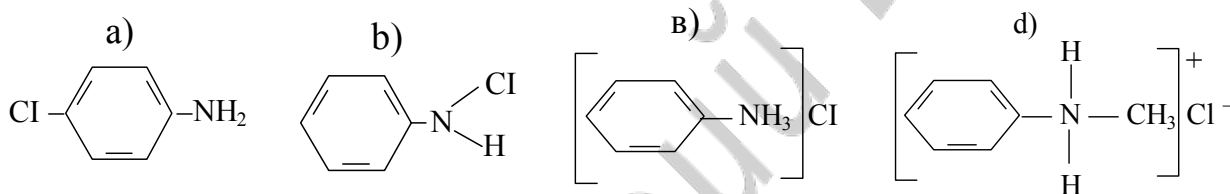
1. With regard to anion stability indicate which of the following acids are stronger than acetic acid:

- a) 2-chloroacetic acid;
- b) trichloroacetic acid;
- c) carbonic acid;
- d) formic acid.

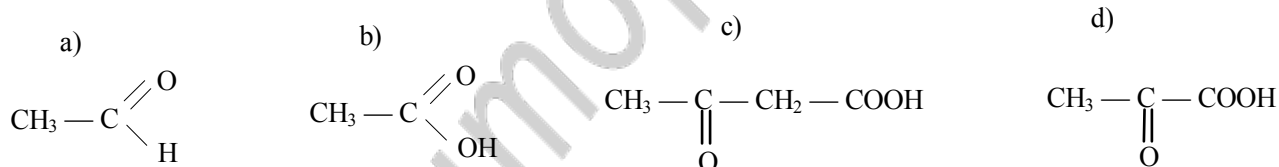
2. Select the formula of amine with the strongest basic properties:



3. Select the methylphenylamine chloride:



4. Indicate the strongest CH-acid:



5. Indicate the correct statements:

- a) the dimethylamine basic properties are stronger than ammonia has;
- b) the trimethylamine basic properties are stronger than dimethylamine has;
- c) the methylamine basic properties are stronger than aniline has;
- d) the ammonia basic properties are stronger than aniline has.

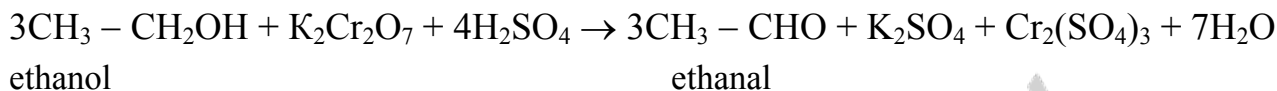
6. With regard to anion stability indicate the strongest acid:

- a) 4-aminobenzoic acid;
- b) benzoic acid;
- c) 4-nitrobenzoic acid;
- d) phenol.

Practical part

1. Oxidation of primary alcohols.

Alcohol oxidation reaction is carried out in narrow term. Primary alcohols are oxidized to aldehydes.



Accomplishment: add 5 drops of H_2SO_4 (23) dilute solution and 3 drops of $\text{C}_2\text{H}_5\text{OH}^*$ to 5 drops of $\text{K}_2\text{Cr}_2\text{O}_7$ (24). Carefully mix and heat.

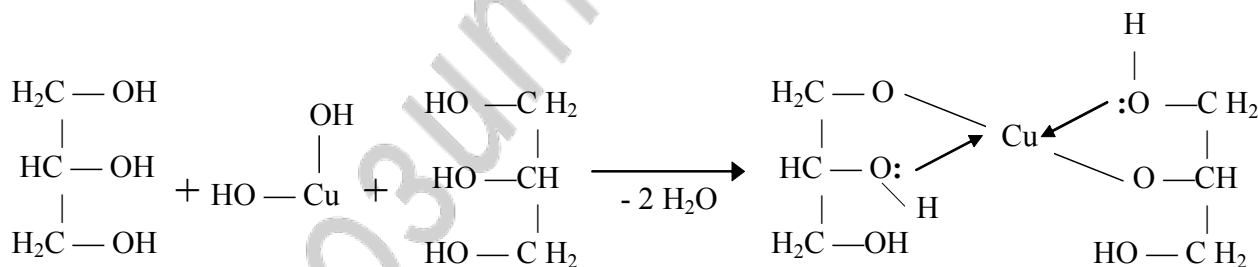
Notice: reagents marked with asterisk () are in the draft.

Observed changes: _____

Conclusion: _____

2. Qualitative test on polyols.

Unlike primary alcohols polyols react not only with alkali metals but with some metal hydroxides. In reaction of glycerine with copper (II) hydroxide complex compound is formed:



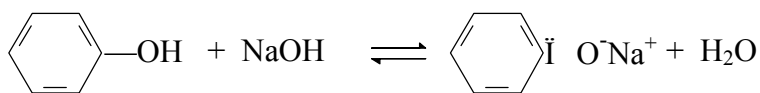
Accomplishment: to 5 drops of NaOH (21) solution add 1–2 drops of solution CuSO_4 (26), shake, add 2 drops of glycerine (4), shake.

Observed changes: _____

Conclusion: _____

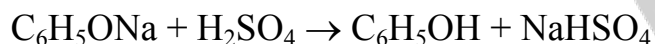
3. Sodium phenoxide production and its decomposition.

Phenols possess more strong acidic properties than alcohols because stability of phenoxide anion raises according to negative charge delocalization along bond conjugate system. Phenols unlike alcohols are capable to react with alkalis.



Water-soluble sodium phenoxide is formed.

Mineral acids replace phenol from phenoxides.



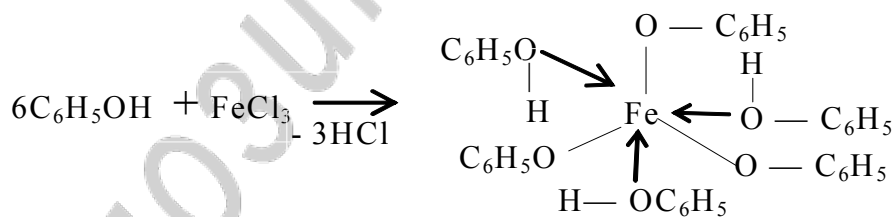
Accomplishment: to 10 drops of phenol water emulsia (27) add on drops solution of NaOH (21) until transparent solution has been obtained. Add on drops dilute solution of H₂SO₄ (23), and again emulsia is formed.

Observed changes: _____

Conclusion: _____

4. Qualitative test on phenol.

This is a qualitative test on the hydroxyl group bound with unsaturated carbon atom. Phenol as an acid reacts with ion Fe³⁺ forming the complex compound.



Accomplishment: to 10 drops of phenol water emulsia (27) add 1–2 drops of solution of FeCl₃ (8), shake.

Observed changes: _____

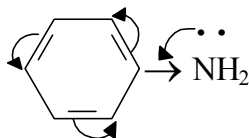
Conclusion: _____

5. Comparison of the methyl amine and aniline basic properties.

Aliphatic radicals possessing positive inductive effect +I increase electronic density on the nitrogen atom therefore aliphatic amines are stronger bases than ammonia NH₃.



In aromatic amines nitrogen atom unshared electronic pair participates in the aromatic ring π -electronic system therefore aniline is weaker base than methyl amine.



Accomplishment: one litmus band is moistened with water solution of methylamine* and another is with water solution of aniline*.

Observed changes: _____

Conclusion: _____

Labwork № 5

Theme: Classification and Mechanisms of Reactions in Organic Chemistry. Reactions of Radical Substitution (sr). Electrophilic Addition Reactions (ae)

Objective: to study classification and mechanisms of organic reactions and to develop skills of carrying out of qualitative tests for double bond detection in organic compounds.

Recommended literature:

1. *Zurabyan, S. E.* Fundamentals of Bioorganic Chemistry. Textbook for medical students / S. E. Zurabyan. 2006. P. 92–110.
2. *Solomons, T. W.* Fundamentals of Organic Chemistry / T. W. Solomons. John Willey&sons, 1994. P. 267–273, 297–314, 323–328, 340–354, 371–372.

Problems for discussion:

1. Organic reaction mechanism definition. Homolytic and heterolytic mechanisms of bond cleavage.
2. Intermediates in organic chemistry. Free radicals, carbocations and carbanions.
3. Organic reactions classification according to the direction and result of reaction.
4. Classification of reagents in organic reactions
5. Reactions of radical substitution (S_R). Alkanes and cycloalkanes.
6. Electrophilic addition (A_E) to alkenes: hydrogenation, halogenation, hydrohalogenation and hydration reactions. The Markovnikov's rule.
7. Addition to conjugated alkenes.

Exercises

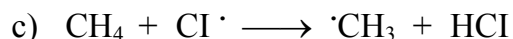
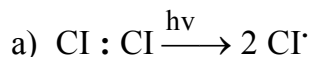
1. Describe the mechanism of the following free radical reactions:
 - a) bromination of propane;
 - b) chlorination of 2-methyl butane.
2. Write down the scheme of chlorination reaction of:
 - a) cyclohexane;
 - b) cyclopropane;
 - c) methylcyclohexane.
3. Write the oxidation reaction scheme of 1,4-dihydroxy benzene. What is the antioxidant?
4. Describe the reaction mechanism of interaction of the following compounds:
 - a) ethene and HCl;
 - b) propene and HCl;
 - c) ethene and H_2O ;
 - d) propenoic acid and HCl;
 - e) butene-2-oic acid and H_2O ;
 - f) butadiene-1,3 and HBr;
 - g) 2-methyl butadiene-1,3 and Br_2 ;
 - h) 3-carboxypentene-2-dioic acid (aconitic acid) and H_2O .

Test control

1. What particles are formed as a result of covalent bond heterolytic cleavage:
 - a) two radicals;
 - b) electrophile and nucleophile;
 - c) two electroneutral particles;

d) positively and negatively charged ions.

2. Select chain termination steps in the chlorination reactions of alkanes:



3. Which of the following reagents react with ethylene according to the A_E mechanism:

a) bromine water;

b) oxygen;

c) KMnO_4 weak solution;

d) water at concentrated solution of H_2SO_4 presence.

4. Which of the following compounds is mainly formed as a result of acrylic acid hydrochlorination reaction:

a) 2-chloropropanoic acid;

c) 3-chloropropanoic acid;

b) chloranhydride of propanoic acid;

d) α -chloropropanoic acid.

5. Select the nucleophilic reagents:

a) H^+ ;

b) H_2O ;

c) $\text{C}_2\text{H}_5\text{OH}$;

d) OH^- .

6. Select the electrophilic reagents:

a) $\text{CH}_3\text{-NH}_2$;

b) H^+ ;

c) $\text{CH}_3\text{-OH}$;

d) $^+\text{CH}_3$.

7. The following factors may result in homolysis:

a) acid catalysis;

d) heating;

b) base catalysis;

e) using of peroxides.

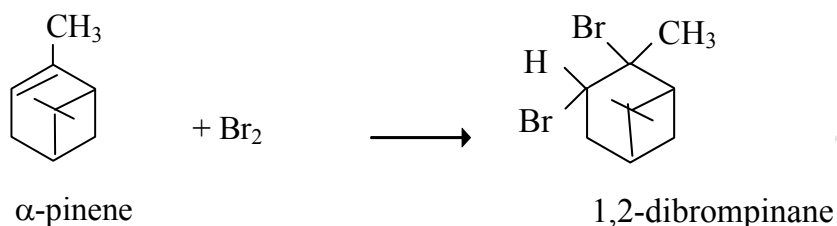
c) ultraviolet radiation;

8. Find the accordance between substrate (column I) and typical reaction mechanism (column II):

Column I	Column II
a) butane	1) A_E
b) cyclohexane	2) A_R
c) 2-methyl propene	3) S_R
d) cyclopropane	
e) chloroethene	

Practical part

1. Qualitative test on the alkenes with bromine water.



Accomplishment: to 4 drops of bromine water* add 2 drops of α -pinene* and shake.

Observed changes: _____

Conclusion: _____

2. Qualitative test on the alkenes with potassium permanganate.



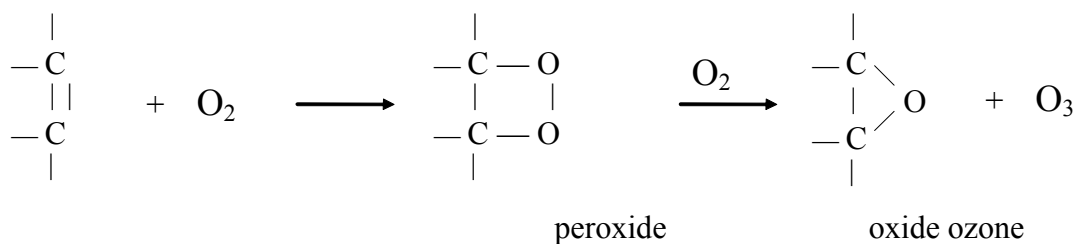
Accomplishment: to 6 drops of KMnO₄ (14) solution add 1 drop of α -pinene and shake.

Observed changes: _____

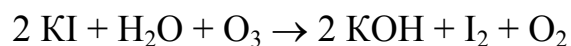
Conclusion: _____

3. Air oxygen activation with unsaturated compounds.

Terpene oxidation by oxygen of air results to peroxide compound formation. Peroxide compounds easily are decayed activating oxygen and transform it into ozone:



The obtained ozone oxides potassium iodide to free I₂. To detect iodine starch is added.



Accomplishment: to 1 drop of 0,5 % gelatinized starch* add 1 drop of 0,5 N solution of KI (20), shake, add 1 drop of α-pinene*, shake 20–30 seconds.

Observed changes: _____

Conclusion: _____

Labwork № 6

heme: electrophilic substitution reaction of aromatic compounds.

Nucleophilic substitution at sp^3 -hybridized carbon atom.

Competitive elimination reactions

Objective: to develop knowledge about reactivity of aromatic compounds and study reactivity halogen derivatives of hydrocarbons and alcohols.

Recommended literature:

1. *Zurabyan, S. E.* Fundamentals of Bioorganic Chemistry. Textbook for medical students / S. E. Zurabyan. 2006. P. 92–110.
2. *Solomons, T. W.* Fundamentals of Organic Chemistry / T. W. Solomons. John Willey&sons, 1994. P. 599–612, 614–629, 636–637, 519–522, 525–534.

Problems for discussion:

1. Mechanism of electrophilic substitution reactions (S_E) in aromatic compounds. I and II sort directing substituents.
2. Features of S_E reactions in a heterocyclic aromatic compound raw.
3. The mechanisms of nucleophilic substitution reaction (S_{N1} and S_{N2}) at sp^3 -hybridized carbon atom in the alkane halogen derivatives.
4. Competitive elimination reactions in alkyl halides and alcohol raw.

Exercises

1. Write the mechanism of the following reactions:
 - a) chlorination of benzene ($AlCl_3$ as catalyst);
 - b) nitration of benzene;
 - c) sulfonation of benzene;
 - d) alkylation of toluene with CH_3Cl ($AlCl_3$ as catalyst);
 - e) alkylation of benzene with propene (H^+);
 - f) alkylation of benzene with ethanol;
 - g) bromination of toluene ($AlCl_3$ as catalyst);
 - h) chlorination of benzoic acid ($AlCl_3$ as catalyst);
 - i) sulfonation of pyridine;
 - j) sulfonation of pyrrole.
2. Describe the mechanism of the reactions:
 - a) propanol-1 + $HCl \rightarrow$;
 - b) ethylchloride + $NaOH \rightarrow$;
 - c) tert-butylchloride + $NaOH \rightarrow$;
 - d) 2-chloropropanoic acid + $NaOH \rightarrow$.
3. Write down the elimination reaction mechanism:
 - a) propanol-2 + $H_2SO_4 \rightarrow$ (when heated);

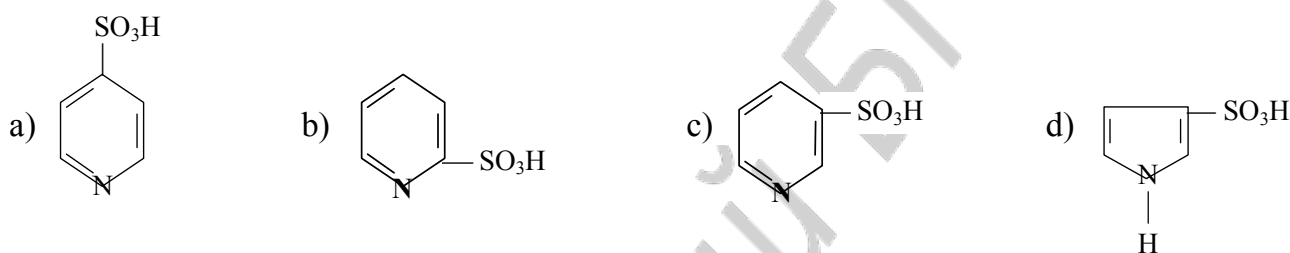
- b) tert-butanol + $\text{H}_2\text{SO}_4 \rightarrow$ (when heated);
 c) propyl chloride + NaOH (alcohol solution) \rightarrow .

Test control

1. Which of the following compounds are formed as a result of phenol nitration reaction:

- a) p-nitrophenol; c) m-nitrophenol;
 b) o-nitrophenol; d) o-, m-dinitrophenol.

2. Which of the following compounds is mainly formed as a result of pyridine sulfonation reaction:



3. Select the substituents of the I sort:

- a) $-\text{C}_2\text{H}_5$; c) $-\text{COH}$;
 b) $-\text{OCH}_3$; d) $-\text{CH}(\text{CH}_3)_2$.

4. The alkylation reaction of benzene can be carried out with:

- a) Br_2 ; c) $\text{C}_2\text{H}_5\text{OH}$;
 b) HNO_3 ; d) $\text{CH}_2=\text{CH}-\text{CH}_3$.

5. The following product is mainly formed as a result of interaction of propanol-2 and H_2SO_4 :

- a) ethane; c) propene;
 b) propane; d) 2-sulfo propane.

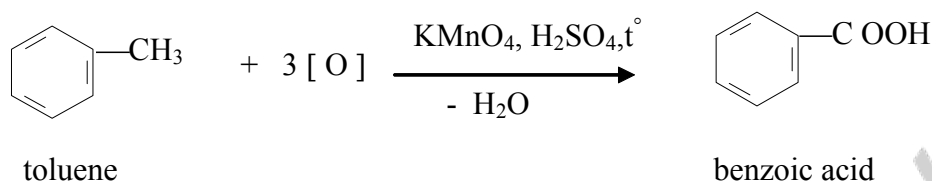
6. The reaction of 2-methylpropanol-1 with HCl is mainly carried out according to the following mechanism:

- a) E_1 ; c) $\text{S}_{\text{N}}1$;
 b) E_2 ; d) $\text{S}_{\text{N}}2$.

Practical part

1. Toluene oxidation.

Oxidation stability is one of the main properties of aromatic systems. Toluene oxidation reaction goes in a side chain but the aromatic kernel is stored:



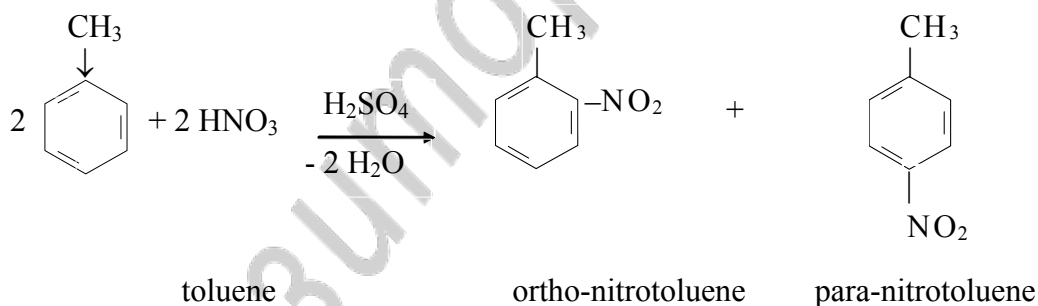
Accomplishment: to 10 drops of KMnO_4 water solution (14) add 10 drops of dilute solution of H_2SO_4 (23) and 5 drops of toluene*, carefully heat and shake.

Observed changes: _____

Conclusion: _____

2. Toluene nitration.

Toluene is aromatic compound and is characterized with an ability to enter in the electrophilic substitution reactions. Methyl group is the substituent of the I sort and directs other substituents into ortho- and para-positions.



Accomplishment: to 5 drops of toluene* add 10 drops of nitric mixture*, carefully shake 2–3 minutes, pour into beaker with water and fix smell.

Observed changes: _____

Conclusion: _____

Labwork № 7
Theme: Carbonyl Compounds And Their Biologically
Important Reactions

Objective: to study features of aldehydes, ketones, carboxylic acids reactivity and develop skills to carrying out of qualitative reactions on aldehydes, ketones and carboxylic acids.

Recommended literature:

1. **Zurabyan, S. E.** *Fundamentals of Bioorganic Chemistry. Textbook for medical students* / S. E. Zurabyan. 2006. P. 133–147.

2. **Solomons, T. W.** *Fundamentals of Organic Chemistry* / T. W. Solomons. John Willey&sons, 1994. P. 642–661, 674–675, 686–689, 692–696, 714–723, 726–735, 740–745, 752–761.

Problems for discussion:

1. An electronic structure of a carbonyl group. The reactionary centers in aldehydes and ketones.

2. Mechanism of nucleophilic addition reaction (A_N). Addition of water and alcohols. Reactions of aldehydes and ketones with amines. Reduction reactions.

3. Reaction of CH-acidic center. Aldol condensation reactions. Haloform reactions.

4. Oxidation reactions are qualitative tests on aldehyde group. Oxidation reactions of ketones. Disproportionation reactions.

5. Formaldehyde. Application in medicine. Toxicity.

6. Reactions sites of carboxylic acids and derivatives.

7. Acidic properties of carboxylic acids.

8. Decarboxylation reaction. Biogenic amines. Cyclic anhydrides.

9. Nucleophilic substitution reactions. Esterification reaction. Amides, acyl chlorides, anhydrides. Their hydrolysis.

Exercises

1. Write reaction schemas of ethanal reduction in vivo and in vitro.

2. Write down the reduction reaction of 2-oxopropanoic acid with $NADH \cdot H^+$.

3. Describe the mechanism of acetalization reaction:

a) ethanal with methanol;

b) methanal with propanol-2;

c) 2-methylpropanal with methanol.

4. Describe the mechanism of intramolecular acetalization reaction to form cyclic hemiacetal:

- a) 5-hydroxyhexanol;
- b) 4-hydroxypentanal;
- c) 4-hydroxybutanal;
- d) 5-hydroxy-5-methylhexanal;
- e) 3,4-dihydroxypentanal.

5. Write the reaction of hydration of the following compounds:

- a) ethanal;
- b) 2-hydroxyethanal;
- c) 2,2,2-trichloroethanal.

6. Describe the mechanism of interaction of:

- a) ethanal and methylamine;
- b) methanal and 2-amino-3-methylpropanoic acid;
- c) ethanal and aniline (aminobenzene);
- d) butanone-2 and ethylamine.

7. Write the schema of aldol condensation reaction on an example of 2-methylpropanal, describe the mechanism.

8. Describe the mechanism of disproportionation reaction for the following compounds:

- a) formaldehyde;
- b) benzaldehyde.

9. Compare the acidity of ethanoic and ethanedioic acids. Write the reaction of salt formation of the stronger acid with $\text{Ca}(\text{OH})_2$.

10. Write down the decarboxylation reaction of the following compounds:

- a) malonic acid;
- b) 2-aminopentanedioic acid.

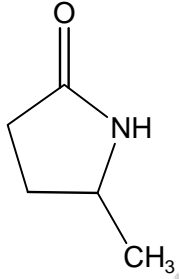
11. Show schemas of anhydride formation for butanedioic and pentanedioic acids.

12. Write the esterification reactions of the methanoic acid with propanol-2.

13. Write down the hydrolysis reaction of methyl benzoate:

- a) acidic hydrolysis;
- b) alkaline hydrolysis.

14. What products would be obtained from the hydrolysis of each of the following amides:

a)	$\text{CH}_3 - \text{C} \begin{array}{l} \text{=O} \\ \text{NH}_2 \end{array}$	c)	$\text{H}_2\text{N} - \text{CH} \begin{array}{l} \\ \text{CH}_2 \\ \\ \text{OH} \end{array} - \text{C} \begin{array}{l} \text{=O} \\ \\ \text{NH} \end{array} - \text{CH} \begin{array}{l} \\ \text{CH} - \text{CH}_3 \\ \\ \text{CH}_3 \end{array} - \text{COOH}$
b)	$\text{CH}_3 - \text{C} \begin{array}{l} \text{=O} \\ \text{NH} - \text{CH}_3 \end{array}$	d)	

Test control

1. Select reagents by means of which it is possible to find out presence of aldehydic group:

- a) Schiff's reagent; c) Cu (II) hydroxide at room temperature;
b) Cu (II) hydroxide when heated; d) I₂ + NaOH.

2. As a result of reaction of reduction in vivo acetic aldehyde are formed:

- a) ethanol and NADH + H⁺; c) ethanal and NAD⁺;
b) ethanol and NAD⁺; d) methanol and NAD⁺.

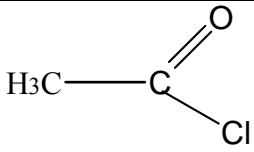
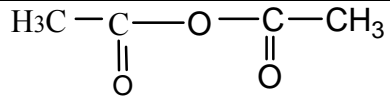
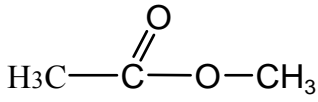
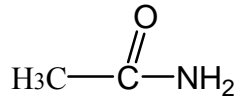
3. Which compounds are formed as a result of secondary alcohol oxidation:

a)	$\text{C}_3\text{H}_7 - \text{C} \begin{array}{l} \text{=O} \\ \text{CH}_3 \end{array}$	c)	$\text{CH}_3 - \text{CH}_2 - \text{C} \begin{array}{l} \text{=O} \\ \\ \text{O} \end{array} - \text{CH} - \text{CH}_3 \\ \\ \text{CH}_3$
b)	$\text{CH}_3 - \text{C} \begin{array}{l} \\ \text{CH}_3 \\ \\ \text{CH}_3 \end{array} - \text{C} \begin{array}{l} \text{=O} \\ \text{H} \end{array}$	d)	$\text{CH}_3 - \text{CH} \begin{array}{l} \\ \text{CH}_3 \end{array} - \text{C} \begin{array}{l} \text{=O} \\ \text{H} \end{array}$

4. What reaction leads to acetal formation:

- a) 1 mol of methanal + 1 mol of propanal;
b) 1 mol of propanal + 1 mol of methanol;
c) 1 mol of ethanal + 2 mol of methanol;
d) 1 mol of propanal + 2 mol of ethanol.

5. Arrange in a number on decrease of reactivity in S_N reactions of the following compounds:

1)		3)	
2)		4)	

- a) 1>3>2>4; c) 3>1>2>4;
 b) 2>3>4>1; d) 4>3>1>2.

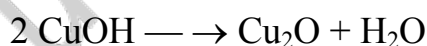
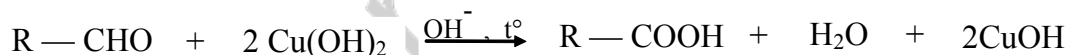
6. Decarboxylation reaction of 2-amino-3-hydroxypropanoic acid leads to the formation:

- a) 3-hydroxypropanoic acid; c) 2-aminopropanol;
 b) 2-aminoethanal; d) 2-aminoethanol.

Practical part

1. Formaldehyde oxidation with $\text{Cu}(\text{OH})_2$ in alkaline medium.

Qualitative tests on aldehydes are connected with easy oxidizability of aldehydic group with oxides or hydroxides of metals in medium at heating, thus aldehydes turn into carboxylic acids with the same number of carbon atoms and the ion of metal is reduced. As an oxidizer the Trommer's reagent (fresh obtained copper (II) hydroxide) is used.



Accomplishment: to 1–3 drops of formaline (32) add 10 drops of NaOH solution (21) and 1–2 drops of CuSO_4 (26). Mixture is heated to boiling point.

Observed changes: _____

Conclusion: _____

2. Reaction of formaldehyde with Schiff's reagent.

Reaction goes according to the A_N mechanism with the Schiff's reagent without heating.

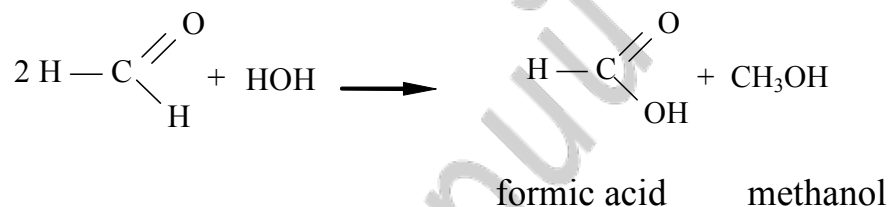
Accomplishment: to 3–4 drops of the Schiff's reagent* add 1 drop of formaldehyde solution (32).

Observed changes: _____

Conclusion: _____

3. Disproportionation reaction of formaldehyde.

Disproportionation reaction is interaction of two aldehyde molecules when one aldehyde molecule is reduced to alcohol due to another aldehyde molecule is oxidized to a carboxylic acid. Water formaldehyde solution has acidic medium of reaction.



Accomplishment: to 3–4 drops of formaline (32) add 1 drop of methyl red indicator*.

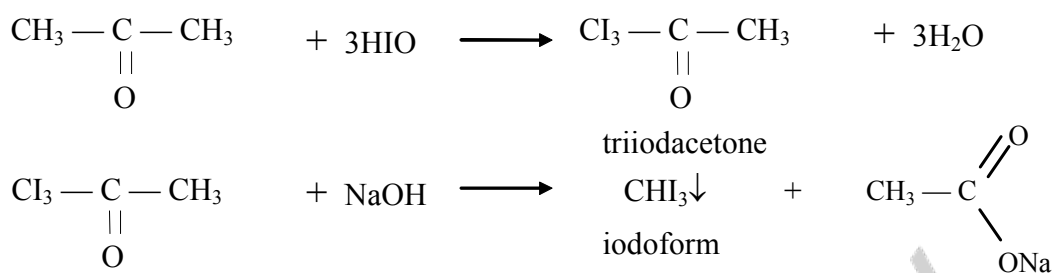
Observed changes: _____

Conclusion: _____

4. Acetone detection by transformation to iodoform (iodoform reaction).

Iodoform reaction is connected with ability of carbonyl containing compounds to substitute hydrogen atom at α -carbon atom on halogen and the following cleavage of carbon-carbon bond with iodoform (CHI_3) formation.





Accomplishment: to 6–8 drops of Lugol (47) solution (I_2 in KI solution) add NaOH solution (21) to disappearing of color, then pour 1–2 acetone drops*.

Observed changes: _____

Conclusion: _____

5. Colored reaction on the acetone with sodium nitroprusside.

Reaction with sodium nitroprussiate $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$ is used in a clinical practice to discovery of acetone in urine at a diabetes. Aromatic carbonyl compounds do not yield this reaction.

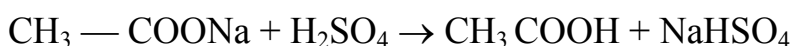
Accomplishment: to 1 acetone drop add 5 drops of sodium nitroprussiate $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$ (35) and 3–4 drops of NaOH (21) solution. In 2–3 minutes add 3 drops of acetic acid (36).

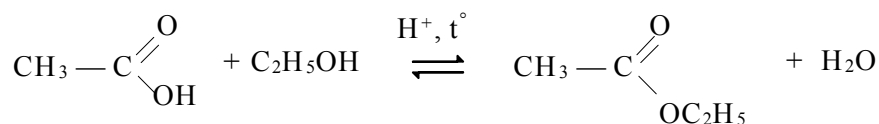
Observed changes: _____

Conclusion: _____

6. Ethyl acetate formation.

To detect the carboxylic acids the esters production reaction can be used if esters have specific smell. The reaction is carried out according to the nucleophilic substitution mechanism (S_N).





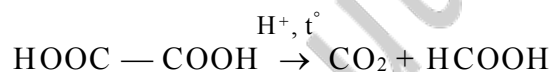
Accomplishment: to 5 drops of ethanol add 5 drops of H₂SO₄ concentrated solution* and waterless CH₃COONa (42), heat. Pour solution to another test-tube with water.

Observed changes: _____

Conclusion: _____

7. Oxalic acid decarboxylation.

Result of the oxalic acid decarboxylation is carbon dioxide which forms CaCO₃ when mixed with the lime water (solution of Ca(OH)₂).



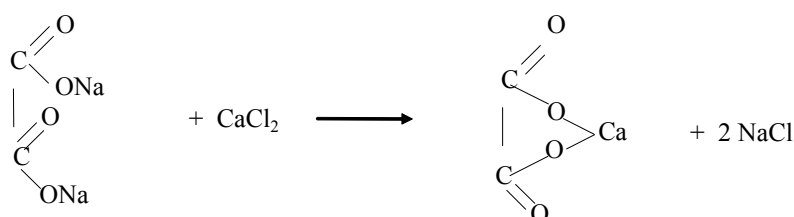
Accomplishment: in dry test-tube add crystal oxalic acid (mass ≈ 0,5 g). Test-tube is closed by flatus tube and heat. The end of flatus tube put into test-tube with 15 drops of lime water (2).

Observed changes: _____

Conclusion: _____

8. Detecting of oxalate ion.

To detect oxalic acid and its salts (oxalates) the insoluble calcium salt formation reaction is used:



The formed calcium oxalate crystals aren't soluble in water and acetic acid, but rather well soluble in inorganic acids such as HCl, HNO₃. Calcium oxalate crystals appear in urine during some pathologic states and often form renal calculus (nephrolithes).

Accomplishment: to 5 drops of the sodium oxalate (40) add 1 drop of calcium chloride (41). The test-tube contents divide into two portions, in one add 5 drops of HCl solution (9), in other add 5 drops of acetic acid (36).

Observed changes: _____

Conclusion: _____

Labwork № 8

Concluding Test “Theoretical Fundamentals of Basic Classes of Organic Compound Structure and Reactivity”

Remind the program material from the theme № 1 to № 7.

Recommended literature:

Study the literature from the theme № 1 to № 7.

Labwork № 9

Theme: Heterofunctional Compounds Of Aliphatic Series.

The structure And Reactivity Oxy-, Oxo-And Amino Acids

Objective: to develop skills to predict chemical properties biologically important heterofunctional compounds taking into account a structure and interference of various functional groups.

Recommended literature:

1. *Zurabyan, S. E.* Fundamentals of Bioorganic Chemistry. Textbook for medical students / S. E. Zurabyan. 2006. P. 161–171.

2. *Solomons, T. W.* Fundamentals of Organic Chemistry / T. W. Solomons. John Willey&sons, 1994. P. 827–837.

Problems for discussion:

1. Polyfunctional compounds: classification, chemical properties.

2. Heterofunctional compounds: classification, a role in biological processes.
3. Aminoalcohols: their biological role.
4. Hydroxyacids. A structure, typical and specific properties of α -, β -, γ -hydroxy and amino acids.
5. A citric acid: a structure, properties. Citrates.
6. Oxoacids. Acid properties and reactivity. Ketone bodies.
7. Keto-enol tautomerism. Decarboxylation reactions of oxoacids.
8. Amides of carbonic acid. Urea.

Exercises

1. Write the structural formulas of the following compounds:

a) glycerol;	g) maleic acid;	m) oxaloacetic acid;
b) ethylene glycol;	h) malic acid;	n) pyruvic acid;
c) inositol;	i) lactic acid;	o) α -oxoglutaric acid;
d) choline;	j) oxalic acid;	p) glyoxyl acid;
e) colamine;	k) malonic acid;	q) tartaric acid.
f) fumaric acid;	l) citric acid;	
2. Write schema of oxidation reaction in vivo of:

a) lactic acid;	b) malic acid.
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3. Write the equations of interaction of lactic acid and:

a) NaOH;	b) C_2H_5OH ;	c) CH_3COCl .
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4. Show the reactions proceeded at heating:

a) 2-hydroxybutanoic acid;	d) 3-aminobutanedioic acid;
b) 2-aminopropanoic acid;	e) 4-hydroxypentanoic acid;
c) 3-hydroxybutanoic acid;	f) 5-aminohexanoic acid.
5. Write down schema of the formation reaction of citric acid in vivo.
6. Write schemas of decomposition reactions of lactic and citric acids at heating at H_2SO_4 presence.
7. Show the schema of the formation reaction of choline from 2-amino-3-hydroxypropanoic acid.
8. Describe the acid-catalyzed hydrolysis reaction of acetylcholine.

9. Write down the tautomeric forms of pyruvic acid and show the schema of reaction with:

- a) Br_2 (enol form); c) H_3PO_4 (enol form);
b) $\text{C}_2\text{H}_5\text{OH}$ (keto form); d) in vivo $\text{NADH}\cdot\text{H}$ (keto form).

10. Write down the formulas of ketone bodies.

11. Write the salt formation reaction of urea with HCl .

Test control

1. What substances does lactic acid react with:

- a) acetylchloride; c) ammonia;
b) ethanol; d) potassium hydroxide.

2. Which of the following hydroxyacids at oxidation in vivo with coenzyme NAD^+ participation turns to an oxalacetic acid:

- a) lactic acid; c) citric acid;
b) malic acid; d) pyruvic acid.

3. Which heterofunctional compounds is intermolecular interaction reaction typical for:

- a) β -hydroxyacids; c) α -hydroxyacids;
b) β -amino acids; d) α -amino acids.

4. Which of the following compounds are ketone bodies:

- a) α -hydroxybutaric acid; c) β -hydroxybutaric acid;
b) acetoacetic acid; d) acetone.

5. Which of the following oxoacids participate in a cycle of tricarboxylic acids:

- a) pyruvic acid; c) oxalacetic acid;
b) acetoacetic acid; d) α -ketoglutaric acid.

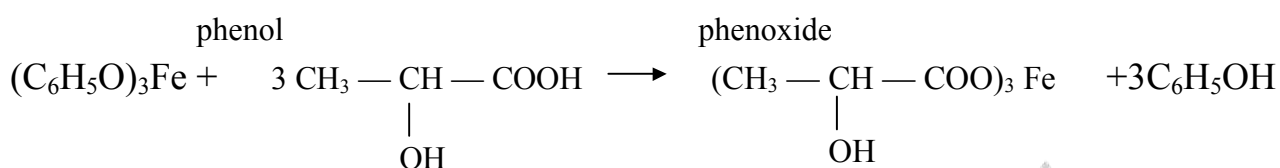
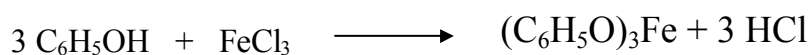
6. Which reagents does enol form of acetoacetate react with:

- a) bromine water; c) NaOH ;
b) FeCl_3 ; d) HCN .

Practical part

1. Qualitative test on lactic acid.

Lactic acid at Fe (III) phenoxide presence forms Fe (III) lactate:



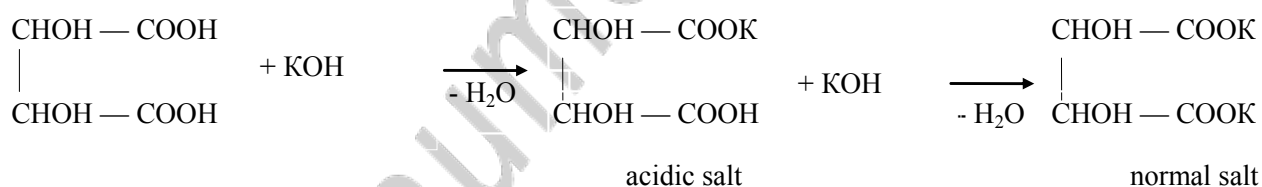
Accomplishment: pour in a test tube 5 drops of phenol (27) solution and add 1 drop of FeCl₃ (8) solution. Add several drops of lactic acid (49).

Observed changes: _____

Conclusion: _____

2. Evidence of two carboxyl groups in tartaric acid structure.

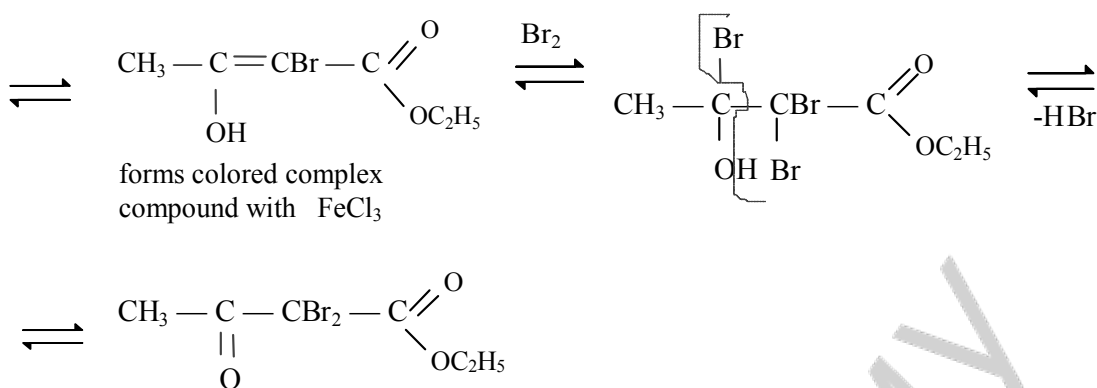
Tartaric acid as dibasic forms two salts — acid salt and neutral [normal] salt which differ with water solubility.



Accomplishment: to 8–10 drops of tartaric acid (50) add 2–3 drops of KOH solution (51), intensively intermix rubbing with glass rod against walls of a test tube. There is a crystal deposit. Add KOH solution into a test tube.

Observed changes: _____

Conclusion: _____



Accomplishment: add 1 drop of FeCl₃ (8) solution to 1 drop of acetoacetic ester* then add 2–3 drops of bromine water*. Fix at once after mixing of reagents and in some minutes.

Observed changes: _____

Conclusion: _____

Labwork № 10

Theme: Biologically Active Heterofunctional Compounds Of Benzene And Heterocyclic Series, Metabolites And Bioregulators

Objective: to develop knowledge about a structure and reactivity of some physiologically active derivatives of benzene and intercommunication of structure, properties and biological meaning of metabolites and antimetabolites.

Recommended literature:

1. *Zurabyan, S. E.* Fundamentals of Bioorganic Chemistry. Textbook for medical students / S. E. Zurabyan. 2006. P. 168–169, 236, 175–177, 178–179.

2. *Solomons, T. W.* Fundamentals of Organic Chemistry / T. W. Solomons. John Willey&sons, 1994. P. 805–807, 808, 869–870, 782–783, 849–850, 782–783.

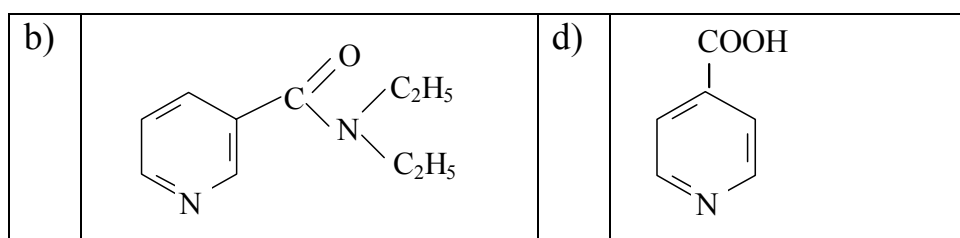
Problems for discussion:

1. Para-aminophenol. Paracetamol.
2. Salicylic acid, its derivatives.
3. Para-aminobenzoic acid, its derivatives.

4. Sulfanilic acid, sulfa drugs, their mechanism of action as antibacterial drugs. Antimetabolites.
5. Catecholamine. Biological role.
6. Nicotinic acid, nicotinamide. NAD^+ , NADH . Their biological importance.
7. Isonicotinic acid. Tuberculostatic drugs.
8. Barbituric acid, its derivatives.
9. Uric acid and urates.
10. Alkaloids.

Exercises

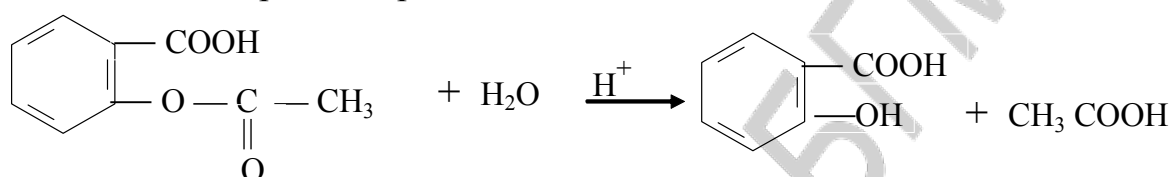
1. Show the reaction ($\text{S}_{\text{N}}2$) of paracetamol with $\text{C}_2\text{H}_5\text{OH}$ to phenacetin formation.
2. Write the schemas of formation reaction:
 - a) sodium salicylate;
 - b) methyl salicylate;
 - c) phenyl salicylate;
 - d) acetylsalicylic acid.
3. Write down the hydrolysis reaction of acetylsalicylic acid.
4. Write the structures of:
 - a) para-aminobenzoic acid;
 - b) anestesine;
 - c) novocaine.
5. Describe the acid-catalysed hydrolysis reaction of novocaine.
6. Write down the salt formation reaction of HCl with:
 - a) anestesine;
 - b) novocaine;
 - c) ultracaine;
 - d) lidocaine.
7. Write the salt formation reactions of sulfanilic acid with NaOH and H_2SO_4 .
8. Write down the amide of sulfanilic acid. Explain the mechanism of antibacterial action of sulfa drugs.
9. Write down adrenaline, noradrenaline, dopamine structures.
10. Write the addition reaction of hydride anion with pyridinium ion.
11. Write down tubazid and phthivazid.
12. Show the tautomeric forms of barbituric acid and phenobarbital.



Practical part

1. Test on the high quality of aspirin.

At hydrolysis of aspirin ortho-hydroxybenzoic acid is formed which with Fe (III) chloride forms complex compound.

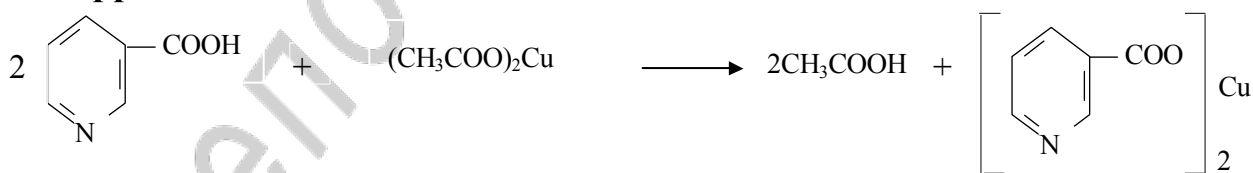


Accomplishment: place some grains of aspirin* and 5–6 drops of water in a test tube, shake it. Divide the test tube contents into 2 parts. To one part add 1 drop of FeCl₃ (8), another part boil for half a minute and then add 1 drop of FeCl₃.

Observed changes: _____

Conclusion: _____

2. Copper salt of nicotinic acid formation.



Accomplishment: place 1 spatula of nicotinic acid* in a test tube, add 10–15 drops of water, heat up to boiling. To a hot solution add 1–2 drops of acetic acid (36) and 3–4 drops of CuSO₄ (26).

Observed changes: _____

Conclusion: _____

3. Reactions to discover alkaloids.

Accomplishment: on an object-plate add 3 drops of investigated solution* at distance of 2 cm from each other. To the first drop add 1 drop of the Lugol's solution (47), to the second add 1 drop of 1 % picric acid solution*, to the third add 1 drop of a phosphomolybdic acid.

Observed changes: _____

Conclusion: _____

Labwork № 11

Theme: Lipids. Structure, Properties. Lipid Peroxidation

Objective: to develop knowledge about the saponifiable lipids.

Recommended literature:

1. *Zurabyan, S. E.* Fundamentals of Bioorganic Chemistry. Textbook for medical students / S. E. Zurabyan. 2006. P. 238–247.

2. *Solomons, T. W.* Fundamentals of Organic Chemistry / T. W. Solomons. John Willey&sons, 1994. P. 938–947.

Problems for discussion:

1. Classification of lipids, their biological role.
2. Fatty acids, their structure, properties and nomenclature. Alcohols which form fats and lipids.
3. Waxes, their composition and role.
4. Triacylglycerols, their structure, nomenclature, properties.
5. Phospholipids, their structure, nomenclature, physicochemical properties.
6. Sphingolipids, biological role.
7. The lipid peroxidation of cell membranes. Antioxidants.

Exercises

1. Write a structural formulas of the following triglycerides:

- 1-linoleoyl 2-palmitoyl 3-stearoylglycerol;
- 1,3-dioleoyl-2-linoleoylglycerol.

Are the structures of these lipids chiral?

2. Write the alkaline hydrolysis (saponification) reaction of 1-linolenoyl-2-arachidonoyl-3-stearoylglycerol. What is the soaps?

3. Draw the structural formulas of the following compounds:

- 1-stearoyl-2-oleoylphosphatidylserine;
- 1-stearoyl-2-linoleoylphosphatidylcholine;
- 1-palmitoyl-2-arachidonoylphosphatidylethanolamine;
- 1-palmitoyl-2-oleoylphosphatidylinositol.

Show hydrophobic and hydrophilic parts of these structures.

4. Write down the hydrolysis reactions of 1-stearoyl-2-linoleicphosphatidylserine.

- acidic hydrolysis;
- alkaline hydrolysis.

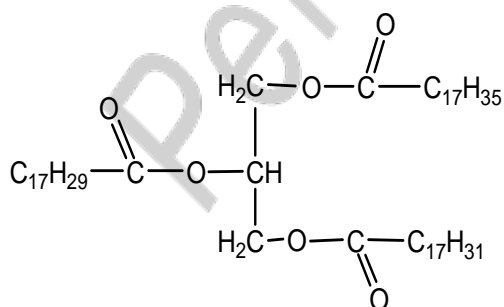
5. Describe the free-radical mechanism of lipid peroxidation reaction of linolenic acid.

6. What is the antioxidants? What antioxidants do you know? Write them.

Test control

1. Which of the following statements are correct for unsaturated fatty acids:

- they have a nonbranched structure;
- they contain one or a few π -bonds;
- they always have trans-configuration;
- they contain the even number of the carbon atoms.



2. Indicate the correct fat name having the following structure:

- 1-stearoyl-2-palmitoyl-3-linolenoylglycerol;
- 1-linolenoyl-2-palmitoyl-3-linoleoylglycerol;
- 1-oleoyl-2-stearoyl-3-arachidoyl glycerol;
- 1-stearoyl-2-linolenoyl-3-linoleoylglycerol.

3. What compounds belong to saponified lipids:

- a) cholesterol; c) glycolipids;
 b) phospholipids; d) waxes.

4. Which is the mechanism of fat hydrolysis in the acidic medium:

- a) A_E; b) A_N; c) A_R; d) S_N.

5. Which compounds can react with phosphatidylethylamine containing residues of palmitic and oleic acids:

- a) water;
 b) aqueous solution KOH;
 c) potassium permanganate solution;
 d) bromine water.

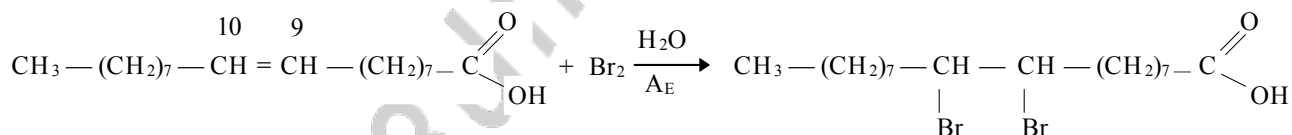
6. Which of the following compounds are formed as a result of the lipid peroxidation:

- a) malonic dialdehyde; d) amino acids;
 b) aldehydic acid; e) oxoacids.
 c) dienic conjugates;

Practical part

1. Qualitative reactions on the unsaturated acids which form fats.

A reaction with bromine water.



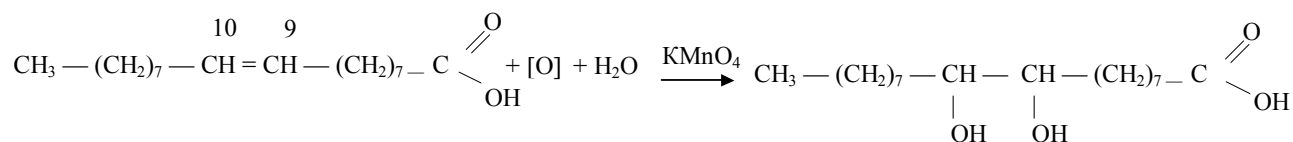
Accomplishment: to 1 drop of fat* add some drops of bromine water*. Shake the test-tube.

Observed changes: _____

Conclusion: _____

2. Oxidation reaction with potassium permanganate.

Oxidation occurs in the double bond location.



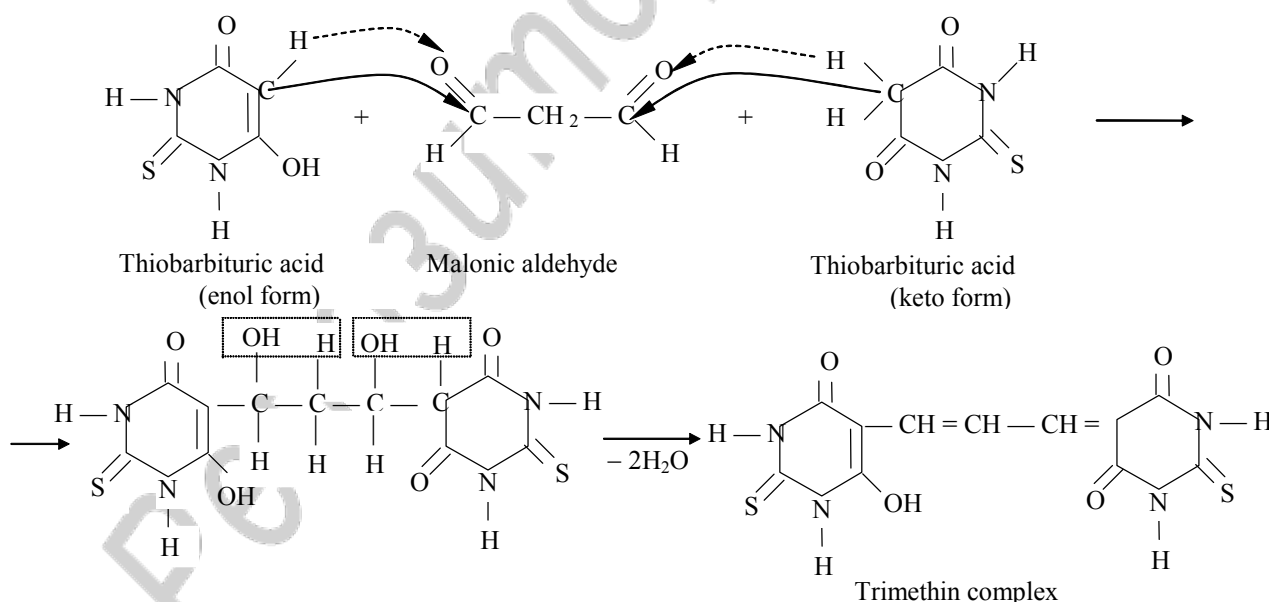
Accomplishment: to 1 drop of fat* pour 10 drops of KMnO_4 solution (14) and 2 drops of Na_2CO_3 (43). Shake the test-tube.

Observed changes: _____

Conclusion: _____

3. Malonic dialdehyde detection in the vegetable oil peroxidation products.

The model of lipid peroxidation is rancidification. One of the lipid peroxidation products is malonic dialdehyde which can be formed from ω -3 unsaturated fatty acid hydroperoxides. To detect the malonic dialdehyde the reaction with thiobarbituric acid is used which goes according to the nucleophilic addition mechanism.



Accomplishment: in a test-tube № 1 pour 10 drops of a fresh sunflower-seed oil* solution, in a test-tube № 2 pour 10 drops long time stored on the light (in conditions of oxygen access) sunflower-seed oil, in a test-tube № 3 pour 10 drops of margarine* solution (oils and margarine are dissolved in heptane-chloroform mixture in the vol-

ume ratio 1:1). Then in each of the test-tubes add on 10 drops of the TBA — reagent (0,8 % solution of thiobarbituric acid in an ice acetic acid)*. Test-tubes with a reaction mixture shake up, close with foil, place into boiling water bath. In 15 minutes take out the test-tubes and visually estimate color intensity of solutions.

Observed changes: _____

Conclusion: _____

Labwork № 12

Theme: Carbohydrates. Monosaccharides

Objective: to develop knowledge of a stereochemical structure, consider important properties of monosaccharides and gain skills to carry out qualitative reactions on monosaccharides.

Recommended literature:

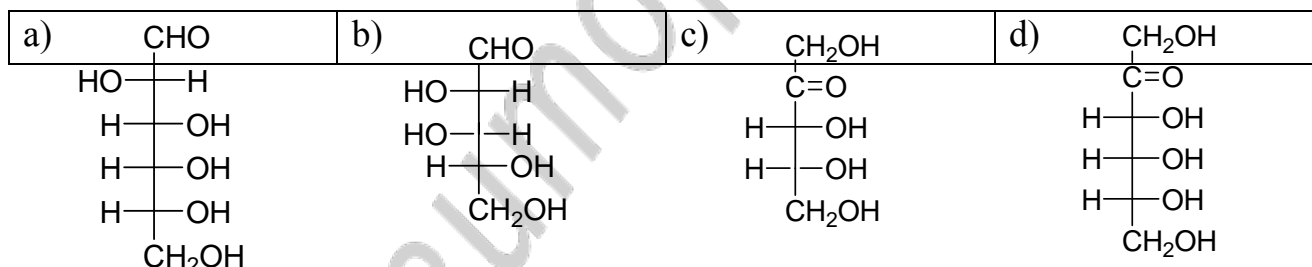
1. Zurabyan, S. E. Fundamentals of Bioorganic Chemistry. Textbook for medical students / S. E. Zurabyan. 2006. P. 189–199.
2. Solomons, T. W. Fundamentals of Organic Chemistry / T. W. Solomons. John Willey&sons, 1994. P. 891–920.

Problems for discussion:

1. Carbohydrates: definition, biological role.
2. Monosaccharides, definition, classification, stereoisomerism. Epimers.
3. Monosaccharide tautomerism. Anomers. Tautomeric forms of D-glucose, D-galactose, D-mannose, D-fructose, D-ribose, 2-deoxy-D-ribose. Fisher and Haworth formulas. Conformations of cyclic forms.
4. Chemical properties of monosaccharides. Glycosides (O- and N-glycosides). Ethers (alkyl oxides).
5. Monosaccharide esters. A biological role of monosaccharide phosphates.
6. Monosaccharide oxidation: aldonic, aldaric and uronic acids.
7. Monosaccharide reduction. Xylitol and sorbitol.
8. Epimerization reactions of monosaccharides. Mutual transformations of aldoses and ketoses.
9. Aminosugars. Their structure, properties and a biological role. Neuraminic acid.
10. Ascorbic acid (vitamin C) as water-soluble antioxidant.

Exercises

1. Classify the following monoses according to the type of carbonyl group and the number of carbon atoms. Show the chiral centers.



2. Write the all tautomeric forms of D-glucose, D-fructose, D-ribose, D-mannose (according to Fisher and Haworth).

3. Show the chair conformations of the following monoses:

- | | |
|--------------------------------|---------------------------------|
| a) α , D-glucopyranose; | c) β , D-galactopyranose; |
| b) β , D-glucopyranose; | d) α , D-mannopyranose. |

4. Write the interaction reaction between β , D-glucopyranose and ethanol at HCl presence.

5. Write the acid catalyzed hydrolysis reaction of:

- a) isopropyl- α ,D-galactopyranoside;
- b) methyl- β ,D-fructofuranoside.

6. Write the schemas of the following reactions:
 - a) oxidation of D-mannose with bromine water;
 - b) oxidation of D-glucose with HNO_3 ;
 - c) galacturonic acid formation;
 - d) sorbitol formation;
 - e) mannitol formation.

7. Write down the structures of glucose-6-phosphate, fructose-1,6-diphosphate.

8. Write down the enantiomer of the following monosaccharide:
What is the epimers? What epimers of glucose do you know?

9. Write the formulas of 2-deoxy-2-amino- β ,D-glucopyranose, 2-deoxy-2-amino- α , D-galactopyranose.

10. Write the interaction reaction between β , D-glucuronic acid and salicylic acid on hemiacetal hydroxyl group.

11. Give the formation reaction of neuraminic acid from pyruvic acid and 2-deoxy-2-aminomannose.

Test control

1. What monosaccharoses fall into to hexoses:

a) glucose;	b) ribose;	c) galactose;	d) fructose.
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2. Select differences between ribose and deoxyribose:
 - a) value of molar weight;
 - b) number of oxygen atoms in a molecule;
 - c) only ribose forms the cyclic form;
 - d) a number of hydroxyl groups.

3. What compound is formed as a result of monosaccharide with alcohol reaction at HCl presence:

a) ether;	c) ester;
b) acetal;	d) glycoside.

4. Which medium are glycosides hydrolyzed:

a) acidic;	b) neutral;	c) alkaline.
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5. Considering the stability of α - and β -anomeric forms of glucose in water solution:

- a) the α -form is steadier;
- b) the stability of α - and β -forms is identical;
- c) the β -form is steadier.

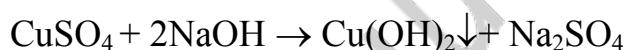
6. What esters of D-glucose and D-fructose are a part of vegetative and animal organisms?

- a) phosphates;
- b) nitrates;
- c) sulphates;
- d) acetates.

Practical part

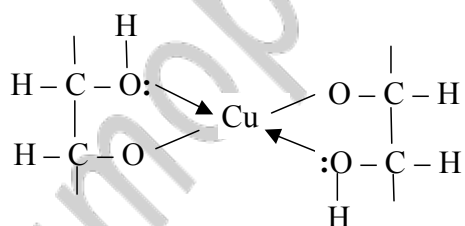
1. A qualitative test on the hydroxyl groups in the glucose molecule.

Definition of some hydroxyl groups in the monosaccharide composition is carried out with $\text{Cu}(\text{OH})_2$. This reaction is the same that on the polyatomic alcohols.



First forming sediment $\text{Cu}(\text{OH})_2$ is dissolved when polyatomic alcohol is added.

This is the evidence of some hydroxyl group presence in the compound.



Accomplishment: to 10–12 drops of glucose (54) solution add 4–5 drops of NaOH (21) and drop by drop of CuSO_4 (26).

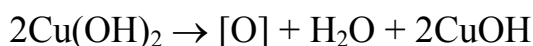
Observed changes: _____

Conclusion: _____

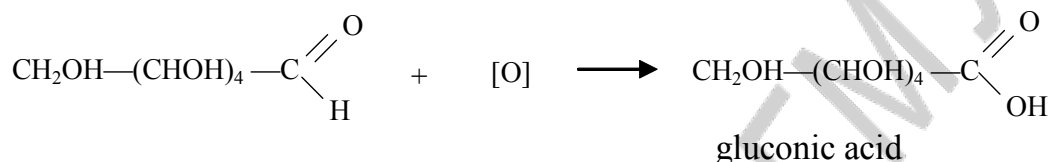
2. A qualitative test on the aldehyde group in the glucose molecule.

This reaction is carried out with the Fehling's reagent which is an alkaline solution of Cu^{2+} alcoholate with K^+ -, Na^+ -tartrates. Obtained chelate is stable and when heated the

color doesn't change. However if it is heated at the aldose presence alcoholate will be hydrolyzed. And obtained $\text{Cu}(\text{OH})_2$ oxidizes glucose.



Oxygen molecule oxidizes glucose and monosaccharide molecules are completely broken up into acids and oxoacids. The first intermediate of glucose oxidation is gluconic acid.



The Fehling's reaction is used to discover glucose in urine.

Accomplishment: pour 10–12 drops of glucose (54) solution in the test-tube and add 2 drops of the Fehling's reagent (55) and heat up.

Observed changes: _____

Conclusion: _____

3. Comparison of reactions of glucose and formalin with Schiff's reagent.

This qualitative test is negative for monosaccharides because of cyclic hemiacetal structure that hasn't aldehyde group.

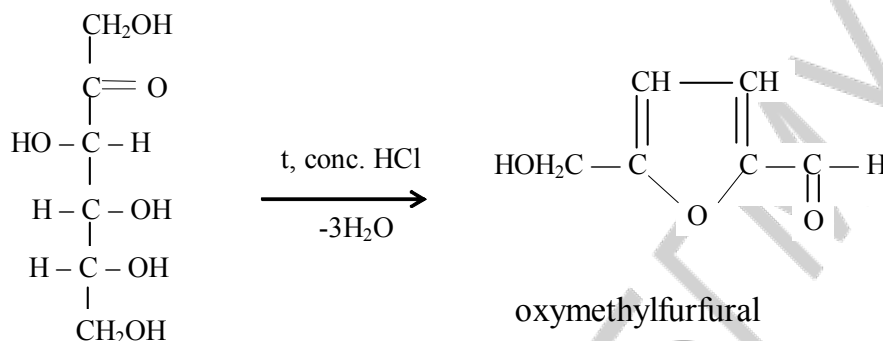
Accomplishment: in one test tube pour 5–7 drops of formalin (32), in another as much a solution of glucose (54) and add in everyone on 3 drops Schiff's reagent*. In a test tube with formalin — red violet color with glucose this reaction is negative.

Observed changes: _____

Conclusion: _____

4. The qualitative test on ketohexoses (the Selivanov's test).

The test is predicated on the oxymethylfurfural formation which is condensed with resorcinol forming complex compound of characteristic color.



Accomplishment: to 10 drops of fructose* solution add 10 drops of HCl* concentrated solution and 1 spatula of resorcinol* crystals. Heat up.

Observed changes: _____

Conclusion: _____

Labwork № 13

Theme: Oligo- And Polysaccharides

Objective: to develop knowledge of a structure, consider important chemical properties of homo- and heteropolysaccharides in view of their biological properties.

Recommended literature:

1. Zurabyan, S. E. *Fundamentals of Bioorganic Chemistry. Textbook for medical students* / S. E. Zurabyan. 2006. P. 199–208.

2. Solomons, T. W. *Fundamentals of Organic Chemistry* / T. W. Solomons. John Wiley&sons, 1994. P. 920–934.

Problems for discussion:

1. Classification of polysaccharides.

2. Disaccharides: maltose, cellobiose, lactose, lactulose, sucrose. Their structures and properties.

3. Starch: structure, biological role. Glycogen.

4. Cellulose: structure, biological role.

5. Dextrane as a source to obtain plasma substitutes.

6. Heteropolysaccharides.

Exercises

1. Write the oxidation reaction of following compounds with $\text{Cu}(\text{OH})_2$:

a) α -maltose; b) β -lactose.

2. Write down the acidic hydrolysis reaction of:

a) α -cellobiose; b) β -lactulose; c) sucrose.

3. Write the interaction reaction between lactose and $\text{C}_2\text{H}_5\text{OH}$ at HCl presence.

4. Show the structure of disaccharide, fragment of amilose and its conformation.

5. Show the amylopectin fragment containing $\alpha(1\rightarrow4)$ and $\alpha(1\rightarrow6)$ glycosidic bonds.

6. Explain why cellulose can provide structural function in the plant organisms? Show the fragment of cellulose structure.

7. Write down the fragment of dextrane. Designate the types of bonds.

8. Show the disaccharide fragment of heparin which consists of D-gluconate-2-sulfate and N-sulfo-D-glucosamine-6-sulfate. The bond type is $\alpha(1\rightarrow4)$.

Test control

1. Which disaccharide hasn't reducing properties?

a) maltose; b) sucrose; c) lactose; d) cellobiose.

2. Starch has the types of glycoside bond:

a) $\alpha(1\rightarrow4)$; b) $\alpha(1\rightarrow6)$; c) $\beta(1\rightarrow4)$; d) $\beta(1\rightarrow3)$.

3. Glycogen possesses the types of glycoside bond:

a) $\beta(1\rightarrow4)$; b) $\alpha(1\rightarrow6)$; c) $\alpha(1\rightarrow4)$; d) $\alpha(1\rightarrow3)$.

4. Select the homopolysaccharides:

a) heparin; c) dextrane;
b) cellulose; d) chondroitin sulfate.

5. Select the heteropolysaccharides:
- a) starch;
 - b) heparin;
 - c) glycogen;
 - d) hyaluronic acid.

6. Select the monosaccharides which are formed as a result of an acidic hydrolysis of hyaluronic acid:
- a) N-acetylglucosamine;
 - b) N-acetylgalactosamine;
 - c) D-glucuronic acid;
 - d) D-galacturonic acid.

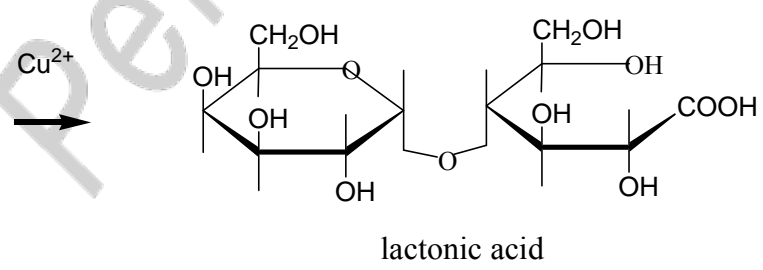
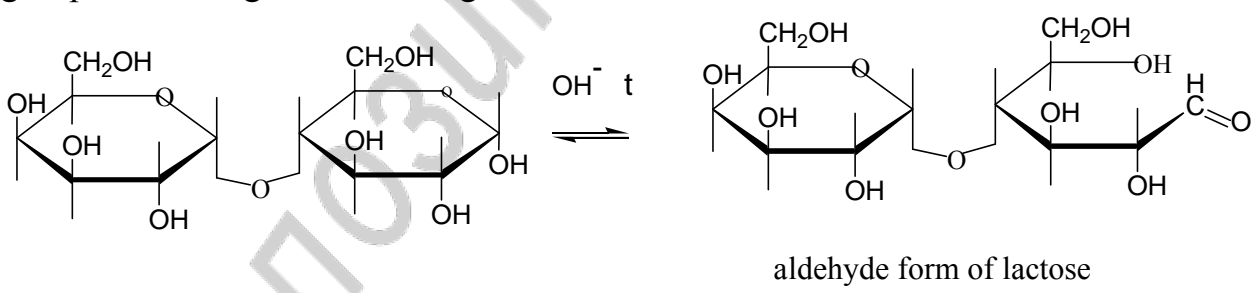
7. Select the monosaccharides which are formed as a result of an acidic hydrolysis of chondroitin sulfate:
- a) D-glucuronic acid;
 - b) D-galacturonic acid;
 - c) N-acetyl-D-mannosamine;
 - d) N-acetyl-D-galactosamine.

8. Cellulose has the following bond types:
- a) glycoside bonds;
 - b) disulfide bonds;
 - c) hydrogen bonds;
 - d) peptide bonds.

Practical part

1. The Fehling's reaction with sucrose and lactose.

Lactose has free hemiacetal hydroxyl groups and in alkaline medium when heated it can turn into tautomeric forms containing aldehyde groups that possess reducing properties. Unlike lactose (and maltose) sucrose hasn't free hemiacetal hydroxyl group and belong to unreducing disaccharides.



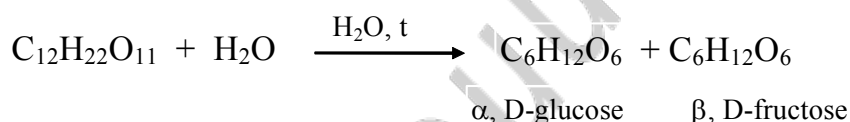
Accomplishment: take 2 test-tubes. In the one test-tube pour 10 drops of sucrose solution (57), in another pour the same quantity of the lactose solution (58), add to each test-tube 3–4 drops of the Fehling's reagent (55) and carefully heat up.

Observed changes: _____

Conclusion: _____

2. Sucrose hydrolysis.

When heated in an acidic medium sucrose is hydrolyzed into α ,D-glucopyranose and β , D-fructofuranose:



Glucose and fructose are discovered in acidic hydrolyzate after its neutralization. Glucose is discovered with the Fehling's test and fructose is discovered with the Seliwanov's test.

Accomplishment: pour 15–20 drops of sucrose solution (57) in the test-tube, add 7–8 drops of HCl* concentrated solution and a little of sand (25) (for uniform boiling). Boil the mixture.

a) pour the half of hydrolyzate in the clean test-tube add alkali (NaOH) (21). To neutralize the mixture add 1–2 drops of the Fehling's reagent (55) to appear coloration. In the case when solution is discolored it is necessary to add 2–3 drops of alkali (NaOH) (21). Then add 2 drops of Fehling's reagent (55) to solution and heat up.

b) to remainder of hydrolyzate add 5–6 drops of HCl* concentrated solution and 1 spatula of crystal resorcinol*, heat up.

Observed changes: _____

Conclusion: _____

3. The qualitative test on the starch.

Accomplishment: to 10–12 drops of gelatinized starch add 1 drop of the Lugol's solution (47). Fix the color change, heat up the solution and fix the changes.

Observed changes: _____

Conclusion: _____

4. Starch has no reducing properties.

Starch doesn't react with the Fehling's reagent and fresh obtained $\text{Cu}(\text{OH})_2$ (the Trommer's reagent).

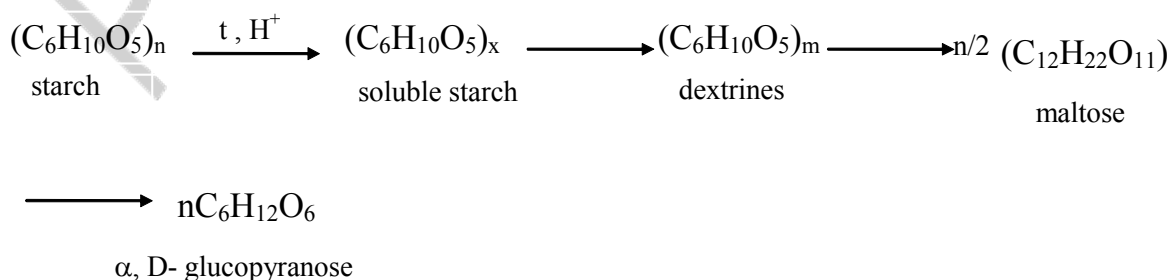
Accomplishment: carry out the Fehling's test (55) with 10–12 drops of gelatinized starch.

Observed changes: _____

Conclusion: _____

5. Acidic hydrolysis of the starch.

When heated starch hydrolysis is stepped in an acidic medium. As a result of hydrolysis the mixture of dextrans, maltose and glucose, which react with iodine solution and form different color. The end product of hydrolysis is α ,D-glucopyranose which is discovered with the Fehling's test.



Accomplishment: pour 2 of gelatinized starch to test-tube. Add 2 drops of H_2SO_4 (23). Warm the test-tube on the water bath during 20 minutes. Place 2 drops of this solution on the glass. Add 2 drop of the Lugol's solution (47). What is the result? Carry out the qualitative test on the glucose with the Fehling's solution (55).

Observed changes: _____

Conclusion: _____

Labwork № 14

Theme: Structure And Reactivity Of Amino Acids Acting As Heterofunctional Compounds

Objective: to discuss characteristics of amino acids as heterofunctional compounds acting as structural components of peptides and proteins; to form skills for carrying out qualitative reactions on the amino acids.

Recommended literature:

1. *Zurabyan, S. E.* Fundamentals of Bioorganic Chemistry. Textbook for medical students / S. E. Zurabyan. 2006. P. 211–217.
2. *Solomons, T. W.* Fundamentals of Organic Chemistry / T. W. Solomons. John Wiley & sons, 1994. P. 972–979.

Problems for discussion:

1. Biogenic amino acids. Proteinogenic amino acids: classification, structures, stereochemistry.
2. Amphoteric properties of amino acids.
3. Reactions of amino acids on the carboxylic group.
4. Reactions of amino acids on the amino group.
5. Biologically important reactions of amino acids: deamination, transamination, decarboxylation, hydroxylation reactions.

Exercises

Write down the Fischer projections for the following compounds:

- a) L-glutaminic acid; b) L-threonine; c) L-tyrosine;
 d) D-isoleucine; e) L-isoleucine.

1. Show the structure of cysteine and alanine according to the R, S-nomenclature.

2. Write down the proteinogenic amino acids which possess charge at pH = 7,4 (ionogenic amino acids).

3. Write the hydrophobic amino acids (aliphatic and aromatic).

4. Write the structures of amino acids at the pH = 1, pH = 7,4, pH = 10:

- a) Ala; b) Glu; c) Asn; d) His; e) Arg; f) Tyr.

5. Write the oxidation reaction of cysteine.

6. Write down the reaction of methionine with:

- a) acetic anhydride; b) ethanol in the H⁺ presence; c) PCl₅.

7. Write down the schemas of biologically important reactions:

- a) pyruvic acid with Glu;
 b) α-oxoglutaric acid with Asp;
 c) decarboxylation of His;
 d) decarboxylation of 3,4-dihydroxyphenylamine;
 e) decarboxylation of Glu;
 f) oxidative deamination of Glu;
 g) oxidative deamination of 3,4-dihydroxyphenylamine;
 h) non-oxidative deamination of Asp;
 i) hydroxylation of Phe;
 j) hydroxylation of Tyr.

Test control

1. Select the hydrophilic amino acids:

- a) Met; b) Asp; c) Asn; d) Ser.

2. Select the hydrophobic amino acids:

- a) Met; b) Ile; c) Thr; d) Phe.

3. Select essential amino acids:

- a) Arg; b) Met; c) Phe; d) Pro.

4. Select amino acids with two chiral centers:

- a) Leu; b) Ile; c) Thr; d) Ser.

5. Select the ionogenic amino acids:

- a) Lys; b) His; c) Ala; d) Asp; e) Gln; f) Arg.

6. Which of the following tests are used to detect amino acids:

- a) xanthoproteinic test;
 b) Trommer's test;
 c) ninhydrine reaction;
 d) Schiff's test.

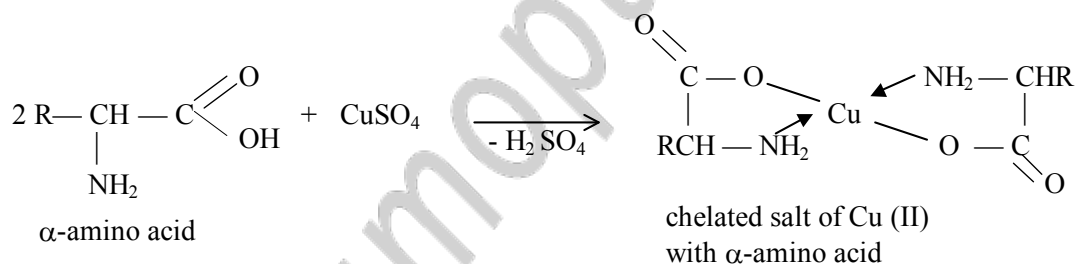
7. Which of the following amino acids can be detected with concentrated solution of nitric acid?

- a) Ala; b) Tyr; c) Phe; d) Trp.

Practical part

1. Reactions of amino acids with copper salts.

Amino acids as the amphoteric compounds form water — soluble chelated compounds with copper ions.

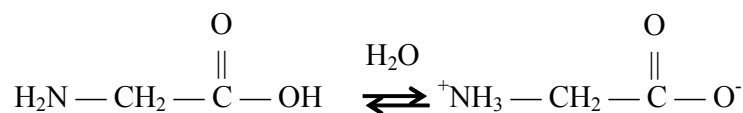


Accomplishment: add 1 copper (II) sulfate crystal (3) and 1 sodium acetate crystal (42) to 1 ml of 1 % glycine* solution. Shake the test-tube.

Observed changes: _____

Conclusion: _____

2. Glycine has neutral medium.



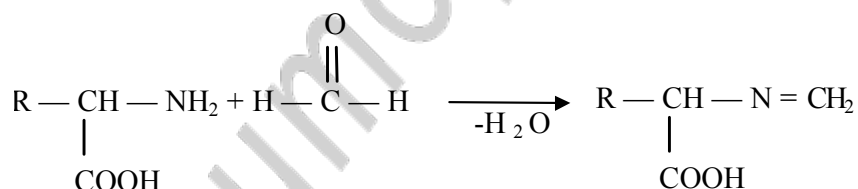
Accomplishment: add 1 drop of 0,2 % methyl red indicator* solution to 3 drops of 0,2 % glycine* solution.

Observed changes: _____

Conclusion: _____

3. Reactions of amino acid with formaldehyde.

Formaldehyde is able to react in the A_N reaction (nucleophilic addition with the following water elimination) with amines and amino acids. At the same time the amino group of amino acid transforms into the methylenamino group (substituted imine). A free carboxylic group of the methylenamino acid causes pH medium change (the medium becomes acidic). It may be proved by the indicator color change.



This reaction is the basis of amino acid quantitative detection in the biological substrates (formalin titration with alkali according to the Serensen method).

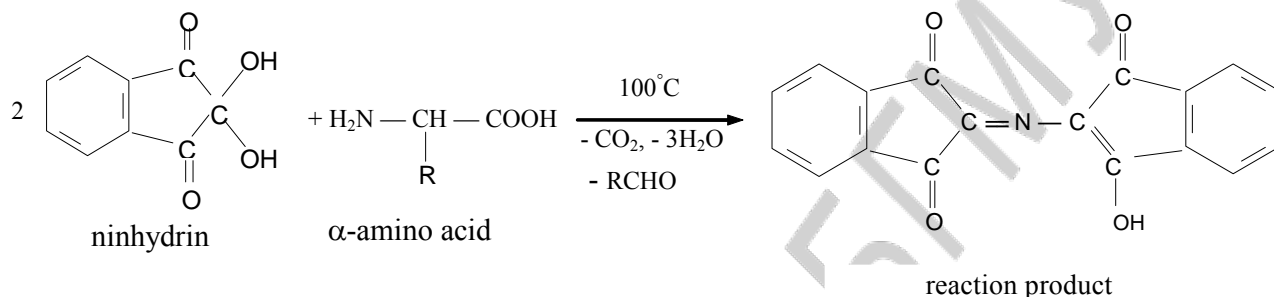
Accomplishment: add 1 drop of 0,2 % methyl red indicator* solution to 3 drops of 40 % of formaldehyde solution (32). Add (carefully) 2N NaOH* solution to neutral medium of solution (fix color change). Then add 3 drops of 0,2 N glycine* solution (fix color change again).

Observed changes: _____

Conclusion: _____

4. Ninhydrin reaction.

This reaction is characterized for amino groups of free amino acids and α -amino groups of amino acids that are the part of peptide and protein structure. A ninhydrin reaction is used for α -amino acid detection in the biological liquids under consideration.



Accomplishment: add 1 drop of 0,03 % ninhydrin* solution to 1 ml of 1 % glycine* solution. Heat the mixture to boil.

Observed changes: _____

Conclusion: _____

LABWORK № 15

Theme: Peptides. Their structure, Reactivity and Importance. The levels of protein organization

Objective: to form knowledge about organization levels of protein molecules, stereochemical features of peptide bond and types of interactions in protein molecule formation.

Recommended literature:

1. *Zurabyan, S. E.* Fundamentals of Bioorganic Chemistry. Textbook for medical students / S. E. Zurabyan. 2006. P. 211–224.
2. *Solomons, T. W.* Fundamentals of Organic Chemistry / T. W. Solomons. John Willey&sons, 1994. P. 986–1005.

Problems for discussion:

1. Peptides: structure and functions. Gluthathion, aspartam, insulin.
2. Peptide bond.
3. Proteins. Primary structure of peptides and proteins.
4. Artificial peptide synthesis.
5. Secondary structure of proteins.
6. Tertiary and quaternary structures of proteins. Hemoglobin.
7. Denaturation of proteins.

Exercises

1. Describe the formation reaction of peptide alanyllysine.
2. Describe the acidic hydrolysis reaction of dipeptide Thr-Phe.
3. Write down the schema of hydrolysis reaction of the following peptides:
a) Pro-Gln; b) Asp-His; c) Trp-Pro-Val; d) Tyr-Arg-Cys.
4. Show the following peptides in the ionized form:
a) Gln-Asp-Ile; c) Ser-Gly-His;
b) Met-Pro-Glu; d) Arg-Tyr-Asn.
5. Write the glutathione structure and its oxidation reaction.
6. Describe the synthesis reaction of aspartame with activation and protection of corresponding groups.
7. Write down the interaction reaction between 2,4-dinitrofluorobenzene and dipeptide Ala-Val. Write the hydrolysis reaction of obtained product. This reaction is based on the Sanger method.
8. Write down the interaction reaction between phenylisothiocyanate and Gly-Ser to obtain phenylthiohydantoin. This reaction is used for detection of primary peptide structures with the Edman method.

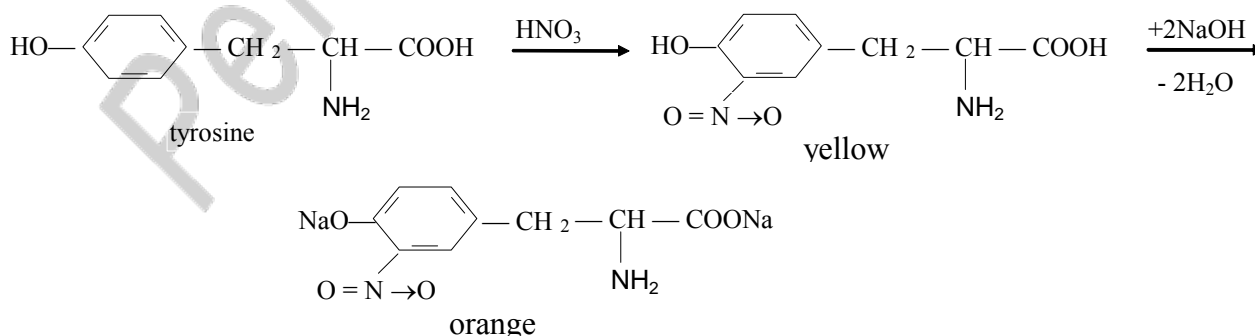
Test control

1. What charge do peptide Arg-Val have at $\text{pH} = 7,4$?
a) positive; b) negative; c) has't charge.
2. Select the correct statements about peptide bond:
a) carbon, nitrogen and oxygen atoms are sp^2 -hybridized;
b) peptide bond has $\text{p}-\pi$ -conjugation;

- c) rotation along C-N bond is possible;
 d) rotation along C-N bond isn't possible.
3. Primary structure of proteins is formed with:
 a) disulfide bonds; c) hydrogen bonds;
 b) ionic bonds; d) peptide bonds.
4. Secondary structure of proteins is formed with:
 a) hydrogen bonds;
 b) peptide bonds;
 c) hydrophobic interactions;
 d) ionic bonds.
5. Tertiary structure of proteins is formed with:
 a) hydrogen bonds;
 b) peptide bonds;
 c) hydrophobic interactions;
 d) ionic bonds.
6. Peptide bonds are found with:
 a) xantoproteinic reaction; c) ninhydrin reaction;
 b) biuretic test; d) lead acetate.

Practical part

- 1. Xantoproteinic reaction** proves the presence of aromatic and heterocyclic α -amino acids such as tryptophane, phenylalanine, tyrosine, histidine in protein structure. When reacted HNO_3 concentrated solution with protein solution nitro-compound is formed. When alkali is added to protein solution the ionization of phenol OH-group occurs.

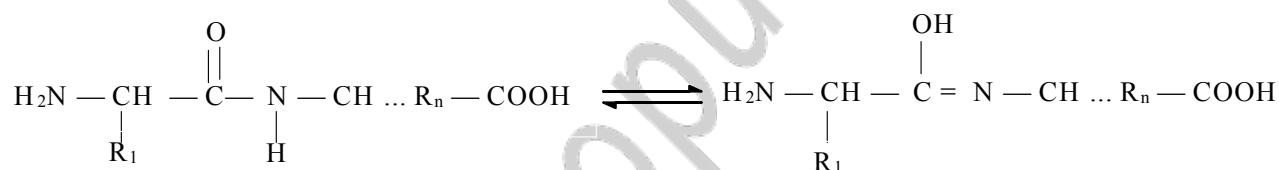


Accomplishment: to 1 ml of protein solution (28) add drop by drop concentrated solution of HNO₃* to form sediment (of what color?). Then heat carefully this test-tube (fix the change of color). Add some NaOH (21) solution (fix the change of color again).

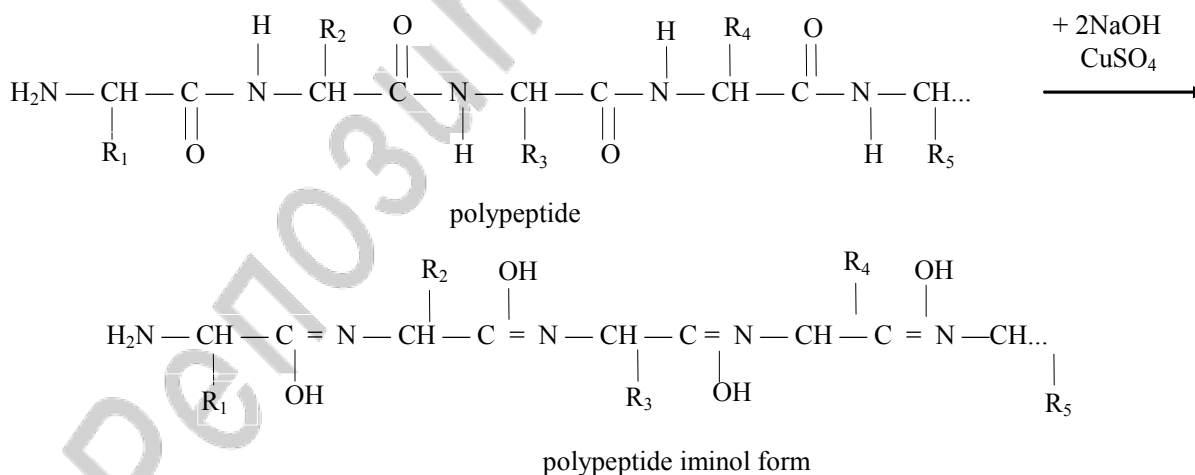
Observed changes: _____

Conclusion: _____

2. Biuretic reaction determines the peptide bond in the solution of analysed compound. Complex compound of Cu with protein peptide group is formed as a result of biuretic reaction. Commonly peptide bond is presented in amide (or keto-form) in peptides and protein, but in alkaline medium it turns to iminol (enol) form.



Biuretic reaction proceeds in such way:



Accomplishment: to 1 ml of protein solution (28) add 1 ml of NaOH (21) concentrated solution and then by degrees on the test-tube side pour 1–2 drops of 0,5 % solution of CuSO₄ (26).

Observed changes: _____

Conclusion: _____

3. Precipitation of proteins with sulfosalicylic acid.

It is the example of irreversible protein precipitation. Proteins can not be soluble in the same solvent. Irreversible reactions are protein precipitation reactions with heavy metals, mineral (inorganic) and organic acids, alkaloid reagents and when boiled.

Accomplishment: pour 10 drops of 20 % sulfosalicylic acid* solution to 0,5 ml of protein solution (28). Solution turbidity occurs.

Observed changes: _____

Conclusion: _____

4. Precipitation of proteins with dehydrating agents (alcohol or acetone).

It is the example of reversible protein precipitation. It's called graining that means precipitation process with the concentrated salt solutions (NaCl, $(\text{NH}_4)_2\text{SO}_4$, MgSO_4) or denaturants (alcohol, acetone). Hydration of protein polar group decreases and charge disappearance leads to aggregation and precipitation of proteins. Obtained precipitate can be dissolved with dilution or dialysis that's why it is the reversible precipitation.

Accomplishment: to 0,5 ml of protein (28) solution pour 1 drop of acetone*. Solution turbidity occurs.

Observed changes: _____

Conclusion: _____

Labwork № 16

Theme: Purine and Pyrimidine Bases. Nucleosides. Nucleotides. Nucleic Acids

Objective: to form knowledge about structure and properties of purine and pyrimidine bases, nucleosides and nucleotides, nucleic acids; to develop skills to carrying out of qualitative reactions on structural components of nucleotides.

Recommended literature:

1. *Zurabyan, S. E.* Fundamentals of Bioorganic Chemistry. Textbook for medical students / S. E. Zurabyan. 2006. P. 225–237.
2. *Solomons, T. W.* Fundamentals of Organic Chemistry / T. W. Solomons. John Wiley & Sons, 1994. P. 1017–1039.

Problems for discussion:

1. Structural components of nucleic acids: heterocyclic bases, pentoses.
2. Nucleosides, nucleotides: their structure and properties.
3. Primary structure of DNA and RNA.
4. Secondary structure of DNA.
5. Types of RNA.
6. Nucleotide derivatives: cyclic AMP, cyclic GMP, ATP.
7. NAD^+ coenzyme.

Exercises

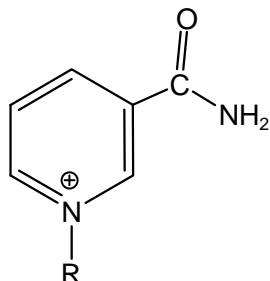
1. Write down the heterocyclic bases: uracil, thymine, guanine, adenine, cytosine. What types of tautomerism are characterized for each of them? Write tautomeric forms?
2. Write the structural formulas showing the hydrogen bonds in complementary base pairs of DNA and RNA:
 - a) uracil – adenine;
 - b) cytosine – guanine;
 - c) thymine – adenine.
3. Write the formulas of the following nucleotides:
 - a) guanosine;
 - b) thymidine;
 - c) deoxyadenosine.
4. Write the hydrolysis reaction of 2'-deoxycytidine-5'-monophosphate in the cases of:
 - a) $\text{pH} = 4$;
 - b) $\text{pH} = 1$;
 - c) $\text{pH} = 9$.
5. Show the formulas of 3',5'-cyclic adenilic acid and 3',5'-cyclic guanilic acid.
6. Write the alkaline hydrolysis reaction of ATP to obtain ADP.

7. Write the activation reaction of ala with ATP.

8. Write the activation reaction of D-glucose with ATP.

9. Show the structure of nicotinamide adeninedinucleotide.

10. Write the oxidation reaction of lactic acid with NAD^+ . Use the following short form of NAD^+ .



11. Write the structure of DNA with the nucleotide sequence 5'-end G-C-T 3'-end.

12. Show the structure of RNA with the nucleotide sequence 5'-end C-U-A 3'-end.

Test control

1. Heterocyclic bases are formed as a result of DNA hydrolysis:

- a) thymine; b) adenine; c) uracil; d) guanine.

2. Nucleic acids contain the following components:

- a) sulfate; b) phosphate; c) heterocyclic bases; d) ribose.

3. The types of bond are found in the nucleic acids:

- a) peptide; c) glycosidic;
b) disulfide; d) 3', 5'-phosphodiesteric.

4. The products are formed as a result of an alkaline hydrolysis of cytidine-5'-monophosphate:

- a) ribose; b) cytidine; c) cytosine; d) phosphate.

5. Which nucleotide sequence of the one chain of DNA chain accords to GTTACTG:

- a) CATTGAC; c) CAATGAC;
b) GAATCAG; d) CTTAGTC.

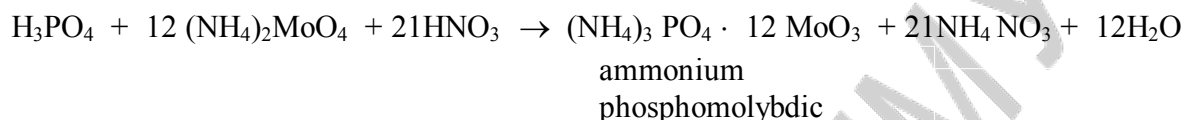
6. The end products of an acidic hydrolysis of 2'-deoxyadenosine-5'-monophosphate are:

- a) 2-deoxyribose; c) adenosine;
 b) phosphate; d) adenine.

Practical part

To investigate chemical composition of nucleotides baker's yeast hydrolyzate is used.

1. Phosphoric acid detection in products of nucleoprotein hydrolysis (hydrolyzates).



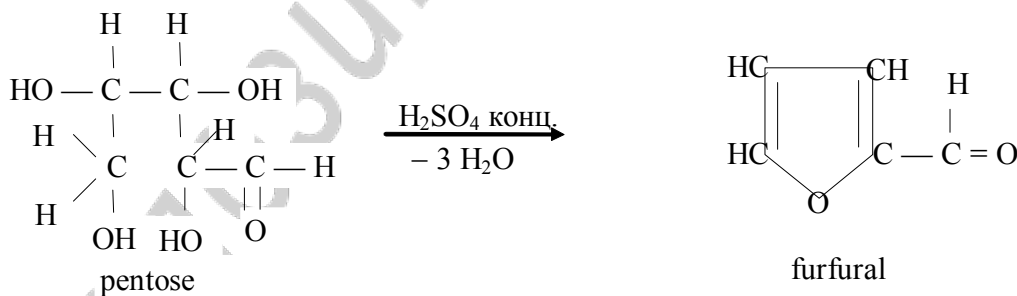
Accomplishment: add 5 drops of molybdenic reagent* to 3–5 drops of yeast hydrolyzate* and boil some minutes.

Observed changes: _____

Conclusion: _____

2. Pentose detection in products of nucleoprotein hydrolysis (the Bial's test).

When reacted with H₂SO₄ concentrated solution or dilute HCl pentoses are dehydrated to form furfural which is condensed with orcinol.

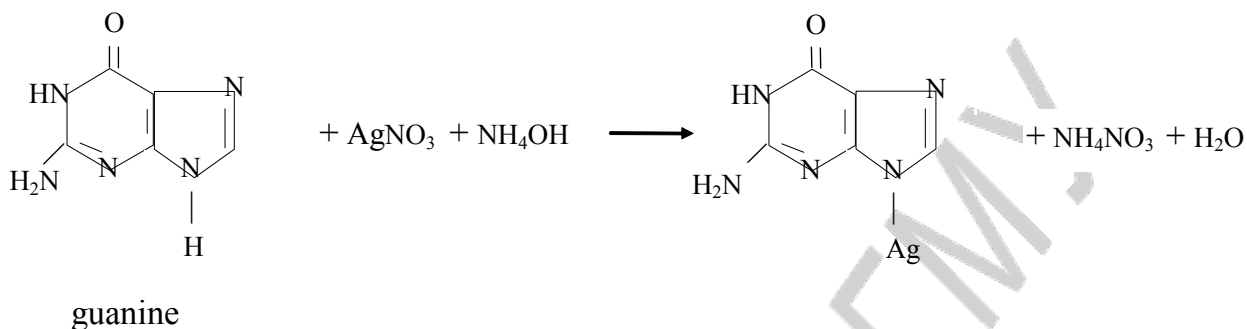


Accomplishment: add 10 drops of the Bial's reagent* (orcinol solution in HCl with FeCl₃) to 10 drops of yeast hydrolyzate* and boil 1–2 minutes.

Observed changes: _____

Conclusion: _____

3. Purine base detection in products of nucleoprotein hydrolysis.



Accomplishment: add 1 drop of concentrated solution of ammonia and 5 drops of 1 % solution of AgNO_3^* to 10 drops of yeast hydrolyzate*. Leave the test-tube for 3–5 minutes without mixing.

Observed changes: _____

Conclusion: _____

Labwork № 17
Concluding Test

“Biopolimers And Their Structural Components”

Remind the program material from the theme № 9 to № 16.

Recommended literature:

Study the literature from the theme № 9 to № 16.

Labwork № 18
Theme: Low-Molecular Bioregulators.
Steroids. Alkaloids

Objective: to develop knowledge of a stereochemical structure, consider important properties of steroids and alkaloids and develop skills to carry out qualitative reactions on steroids.

Recommended literature:

1. *Zurabyan, S. E.* Fundamentals of Bioorganic Chemistry. Textbook for medical students / S. E. Zurabyan. 2006. P. 251–258.
2. *Solomons, T. W.* Fundamentals of Organic Chemistry / T. W. Solomons. John Willey&sons, 1994. P. 951–961.

Problems for discussion:

1. Steroids: their structure, nomenclature and classification.
2. Stereochemistry of steroids. 5α and 5β series of steroids.
3. Sex hormones: estrane and androstane derivatives.
4. Pregnane derivatives: corticosteroids and progestins.
5. Cholic acid. Bile acids.
6. Cholesterol. Biological importance.
7. Vitamins D_2 and D_3 .

Exercises

1. Show the basic classes of steroids: estrane, androstane, pregnane, cholane, cholestane.
2. Draw the conformations of 5α and 5β steroids.
3. Write the formulas of:
 - a) 1,3,5(10)-estratriene-3,17 β -diol (estradiol);
 - b) 3-hydroxy-1,3,5(10)-estratrien-17-one (estrone);
 - c) 17 β -hydroxy-4-androsten-3-one (testosteron);
 - d) 3 α -hydroxy-5 α -androstan-17-one (androsterone);
 - e) 17 α , 21-dihydroxy-4-pregnene-3,11,20-trione (cortisone);
 - f) 17 α , 11 β , 21-trihydroxy-4-pregnene-3,20-dione (cortisol);
 - g) 4-pregnene-3,20-dione (progesterone);

- h) $3\alpha, 7\alpha, 12\alpha$ -trihydroxy- 5β -cholan-24-oic acid;
- i) $3\alpha, 12\alpha$ -dihydroxy- 5β -cholan-24-oic acid;
- j) 5-cholesten- 3β -ol (cholesterol);
- k) 24-methyl-5,7,22-cholestatriene- 3β -ol (ergosterone);
- l) 7-dehydrocholesterol;
- m) vitamin D₃.

Test control

1. Structural base of steroids is:
 - a) phenanthrene;
 - b) sterane;
 - c) perhydrophenanthrene cyclopentane;
 - d) pyrrole.
2. Structure of steroids is characterized:
 - a) plane structure;
 - b) non-plane structure;
 - c) sterane has chiral centers;
 - d) sterane has no chiral centers.
3. The parent structures of the sex hormones are:
 - a) androstane;
 - b) pregnane;
 - c) cholane;
 - d) estrane.
4. Bile acids contain:
 - a) androstane;
 - b) pregnane;
 - c) cholestane;
 - d) cholane.
5. Select the correct statements about estradiol:
 - a) it contains oxo-group at 3 carbon atom;
 - b) it contains hydroxy-group at 3 carbon atom;
 - c) it has basic properties;
 - d) it has acidic properties.
6. Select the correct statements about cholesterol:
 - a) it has oxo-group at 3 carbon atom;
 - b) it is base to form sex hormone;
 - c) it is base to form bile acids;
 - d) it is a component of biological membranes.

Practical part

Color reaction on the cholesterol

Accomplishment: in the dry test-tube pour 1 drop of FeCl₃ solution in acetic acid* and 5–8 drops of concentrated solution of H₂SO₄*. Carefully shake the test-tube and add 5 drops of cholesterol solution in acetic acid*.

Observed changes: _____

Conclusion: _____

Репозиторий БГМУ

Contents

Plan of laboratory classes	3
Thematic plan of lectures	4
Safety rules during the work in the chemical laboratory	5
Labwork № 1. Classification and nomenclature of organic compounds. Spatial structure of organic molecules. Configuration and conformations	8
Labwork № 2. Stereoisomerism, its role for biological activity demonstration	10
Labwork № 3. Chemical bond structure and atom effects in the organic molecules	14
Labwork № 4. Acid-base properties of organic compounds. Oxidation reactions.....	16
Labwork № 5. Classification and mechanisms of reactions in organic chemistry. Reactions of radical substitution (S_R). Electrophilic addition reactions (A_E).....	22
Labwork № 6. Electrophilic substitution reaction of aromatic compounds. Nucleophilic substitution at sp^3 -hybridized carbon atom. Competitive elimination reactions.....	26
Labwork № 7. Carbonyl compounds and their biologically important reactions	29
Labwork № 8. Concluding test “Theoretical fundamentals of basic classes of organic compound structure and reactivity”	37
Labwork № 9. Heterofunctional compounds of aliphatic series. The structure and reactivity oxy-, oxo- and amino acids	37
Labwork № 10. Biologically active heterofunctional compounds of benzene and heterocyclic series, metabolites and bioregulators	43
Labwork № 11. Lipids. Structure, properties. Lipid peroxidation	47
Labwork № 12. Carbohydrates. Monosaccharides.....	51
Labwork № 13. Oligo- and polysaccharides	56
Labwork № 14. Structure and reactivity of amino acids acting as heterofunctional compounds	61
Labwork № 15. Peptides. their structure, reactivity and importance. The levels of protein organization.....	66
Labwork № 16. Purine and pyrimidine bases. Nucleosides. Nucleotides. Nucleic acids	71
Labwork № 17. Concluding test “Biopolimers and their structural componens”	74
Labwork № 18. Low-molecular bioregulators. Steroids. Alkaloids.....	75

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ПРАКТИЧЕСКИЙ КУРС**

**BIOORGANIC CHEMISTRY
PRACTICAL COURSE**

Практикум

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