

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
ХАРКІВСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ

**STRUCTURE, PROPERTIES AND BIOLOGICAL SIGNIFICANCE OF
DERIVATIVES OF CARBOXYLIC ACIDS
(HYDROXY-, KETO- AND PHENOLIC ACIDS).
AMINO ACID COMPOSITION OF PROTEINS AND PEPTIDES.
STRUCTURAL ORGANIZATION OF PROTEINS. DENATURATION.**

Methodical instructions for 1st year students' self-work
in biological and bioorganic chemistry

**БУДОВА, ВЛАСТИВОСТІ ТА БІОЛОГІЧНЕ ЗНАЧЕННЯ
ФУНКЦІОНАЛЬНИХ ПОХІДНИХ КАРБОНОВИХ КИСЛОТ
(ГІДРОКСИ-, КЕТО- ТА ФЕНОЛОКИСЛОТ).
АМІНОКИСЛОТНИЙ СКЛАД БІЛКІВ ТА ПЕПТИДІВ. СТРУКТУРНА
ОРГАНІЗАЦІЯ БІЛКІВ. ДЕНАТУРАЦІЯ.**

Методичні вказівки для самостійної роботи студентів 1-го курсу
з біологічної та біоорганічної хімії

Затверджено
Вченою радою ХНМУ
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Structure, properties and biological significance of derivatives of carboxylic acids (hydroxyl-, keto- and phenolic acids). Amino acid composition of proteins and peptides. Structural organization of proteins. Denaturation: methodical instructions for 1st year students' self-work / compiled by A.O. Syrovaya, O.S. Kalinenko, V.N. Petyunina et al. – 2nd edition, revised, corrected and expanded – Kharkiv: KhNMU, 2018. – 36 p.

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Будова, властивості та біологічне значення функціональних похідних карбонових кислот (гідрокси-, кето- та фенолокіслот). Амінокислотний склад білків та пептидів. Структурна організація білків. Денатурація: метод. вказ. для студентів 1-го курсу виклад. / уклад. Сирова Г.О., Каліненко О.С., Петюніна В.М. та ін. – 2-е вид., переробл., випр., доп. – Харків: ХНМУ, 2018. – 36 с.

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Subject 4 “Structure, properties and biological significance of derivatives of carboxylic acids (hydroxy-, keto- and phenolic acids). Amino acid composition of proteins and peptides. Structural organization of proteins. Denaturation”

Number of hours 4.

Material and methodological support.

Tables:

1. Scheme of structure of the subject.
2. Keto-enol form of the acetoacetic ester.
3. Transformation of acetoacetic acid in organism.
4. Optical isomers of tartaric acid.
5. The most important oxy acids.
6. Properties of hydroxy acids.
7. Models of asymmetric molecules of lactic acid.
8. Enantiomers.
9. Amino acid.
10. Non essential amino acid.
11. Metabolic transformation of amino acids.
13. Biochemical transformation of tryptophan.

Education literature:

1. Biological and Bioorganic chemistry: in two books: Textbook. Textbook 1. Bioorganic chemistry / B.S. Zimenkovsky, V.A.Muzyhenko, I.V. Nizhenkovska, G.O. Syrova; edited by B.S. Zimenkovsky, I.V. Nizhenkovska. – K.: AUP “Medicina”, 2017. – 288 p.
2. Fundamentals of bioorganic chemistry : manual / A.O. Syrovaya, E.R. Grabovetskaya, V.N. Petiunina. – Kharkiv : KhNMU, 2016. – 191 p.
3. Amino acids, peptides, proteins: methodical instructions for the 1st-year students’ self-work in biological and bioorganic chemistry (module 1) / compiled A.O. Syrovaya, L.G. Shapoval, V.N. Petiunina, E.R. Grabovetskaya, N.M. Tkachuk, V.A. Makarov, S.V. Andreeva, S.A. Nakonechnaya, R.O. Bachinskiy, S.N. Kozub, T. S. Tishakova, L.V. Lukyanova,

- O.L. Levashova, N.V. Vakulenko, N.N. Chalenko. – Kharkov: KNMU, 2013. – 26 p.
4. Hydroxy- and oxoacids. Heterofunctional compounds of benzene series. Metabolites and parent structures of medicines: methodical instructions for 1st year students' self-work in Biological and Bioorganic Chemistry (module 1) / compiled by A.O. Syrovaya, L.G. Shapoval, V.N. Petyunina et al. – Kharkiv: KhNMU, 2014. – 25 p.
 5. Structure, properties and biological significance of derivatives of carboxylic acids (hydroxyl-, keto- and phenolic acids). Amino acid composition of proteins and peptides. Structural organization of proteins: methodical instructions for 1st year students' self-work / compiled by A.O. Syrovaya, V.N. Petyunina, V.O. Makarov et al. – 2nd edition, revised, corrected and expanded – Kharkiv: KhNMU, 2018. – 34 p.
 6. Text of Lectures.

Motivational characteristics

Hydroxy-, oxoacids and amino acids in biological systems perform important physiological functions. Hydroxyacids (citric, isocitric, malic) and oxo-acids (oxaloacetic, α -ketoglutaric) transfer in the Krebs cycle that caters to the needs of human body in energy. Oxoacids take part in biosynthesis of nonessential amino acids (transamination). Salicylic acid and its derivatives are medicinal products.

α -Amino acids are the monomers of proteins – substances that form the basis of life. Biogenic amines are synthesized from amino acids in the organism. Some of them are neurotransmitters: serotonin, adrenalin, noradrenaline, dopamine etc. Derivatives of amino acids are lactams that are part of antibiotics, nootropics. Derivatives of p-aminobenzoic acid, sulfanilic acids are medicinal products.

Knowledge about the structure and properties of abovementioned substances helps future doctor to predict their behavior in biochemical transformations.

Objective

To study the structure, chemical properties of aliphatic heterofunctional compounds (hydroxy- and oxoacids), as the basis for understanding their metabolic conversions in the organism.

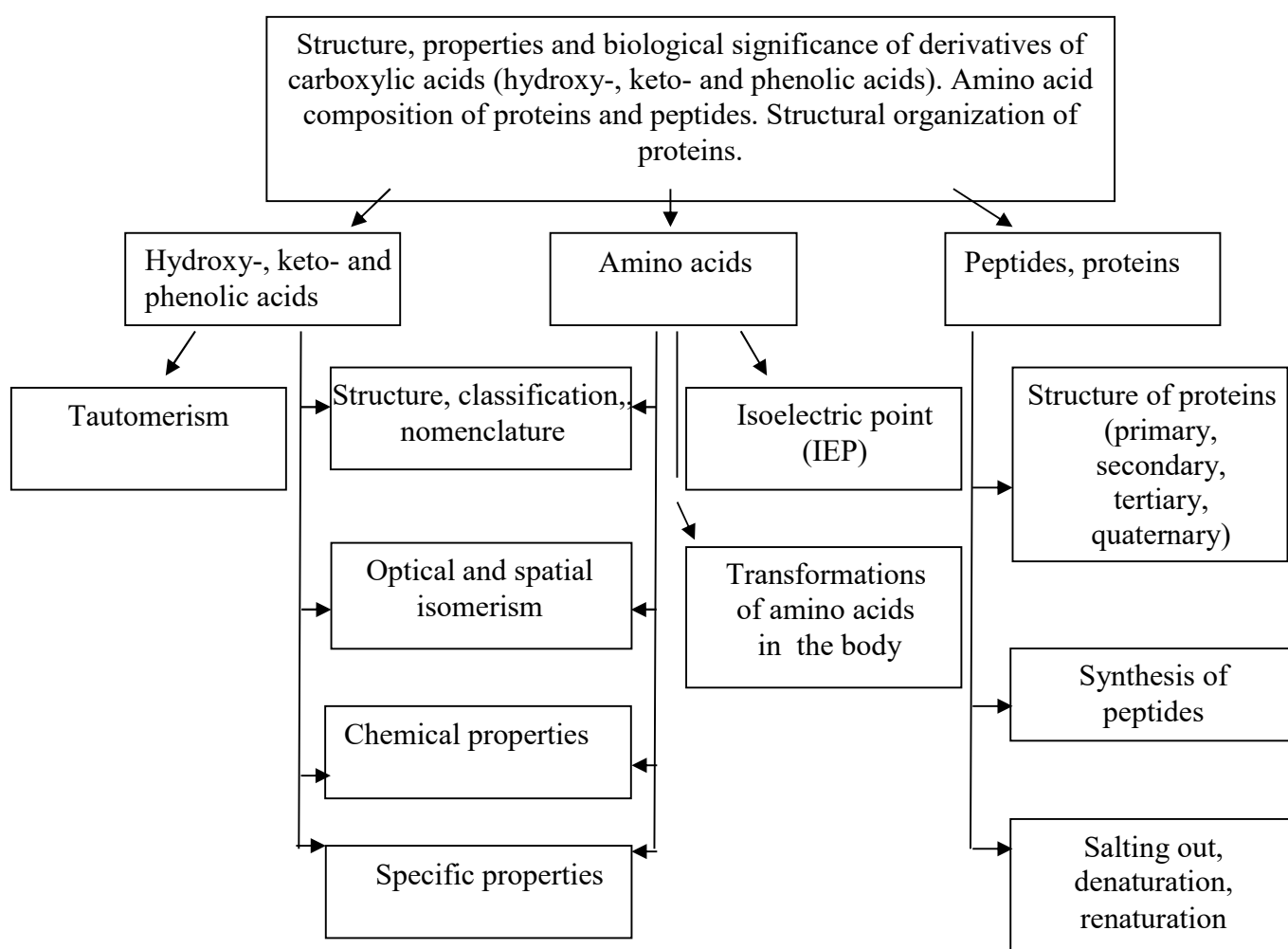
To study the chemical properties of amino acids associated with their structure and composition.

To study the structure and properties of peptides, chemical bases for structural organization of protein molecules as the basis for studying the biological properties of proteins at the molecular level.

Practical skills:

1. To be able to make the formulas of optical isomers of hydroxyacids and amino acids.
2. To be able to predict chemical properties of hydroxy- and amino acids depending on functional composition.
3. To be able to perform qualitative reactions on hydroxy-, oxo-, amino acids and proteins in biological liquids.

Scheme of structure of the subject



Plan of students' work.

No	Stage	Time, min	Educational and visual aids	Location
1.	Motivational characteristics and plan of the subject. Answers to the students' questions	15		Class room
2.	Incoming control	20	Tests of incoming control	
3.	Students' self-work with methodical literature, solution of learning tasks	95	Methodical instructions for students, lecture note, text book for students' self-work, posters	
4.	Laboratory work	20	Reagents, equipment	
5.	Final control	20	Tests of final control	
6.	Analysis and conclusions. Home work	10		

Training questions

1. Hydroxy-, oxo-acids and amino acids, their properties, classification, nomenclature.
2. Spatial (configurational) isomerism of hydroxy -acids and amino acids (enantiomerism, diastereomerism, mesophores, raemates). Optical activity. D- and L- stereochemical series.
3. Chemical properties of hydroxyacids and amino acids with the participation of corresponding functional groups.
4. Specific properties of α , β , γ - hydroxyacids and α , β , γ -amino acids.
5. Some transformations in the human body: decarboxylation, deamination, transamination.
6. Chemical properties of oxoacids as bifunctional compounds.
7. Keto-enol tautomerism of oxoacids.

8. Proteins: composition, primary, secondary, tertiary, quaternary structure.
Classification of proteins.

9. Methods of determination of qualitative composition and amino acid sequence in peptides and proteins.

10. Synthesis of peptides with specified structure: method of protection and activation of the functional groups.

11. Stability factors of protein existence in the solution.

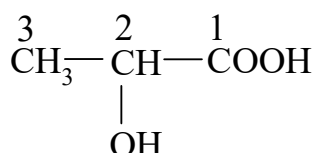
12. Salting out, denaturation, renaturation.

Teaching tasks

Task 1. What functional groups enter the content of oxoacids? Explain rules of the nomenclature of hydroxyacids.

Solution. Hydroxyacids are organic substances, which consist of carboxyl and hydroxyl groups. The quantity of carboxyl groups defines basicity of acids: monobasic acids, dibasic acids, tribasic acids, etc.

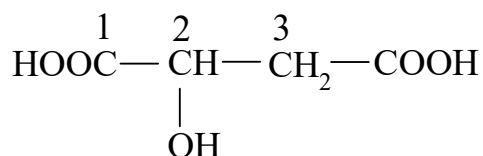
The rational and substitutive nomenclatures are used for making the names of hydroxyacids. However historical names of hydroxy- (oxo-) acids are more often used:



α -oxypropionic acid (rational)

2-oxypropanoic acid (substitutive)

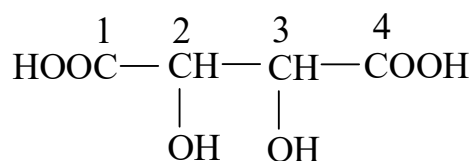
lactic acid (historical)



α -oxysuccinic acid (rational)

2-oxybutanedioic acid (substitutive)

malic acid (historical)



α, β -dioxysuccinic acid (rational)

2,3-dioxybutanedioic acid (substitutive)

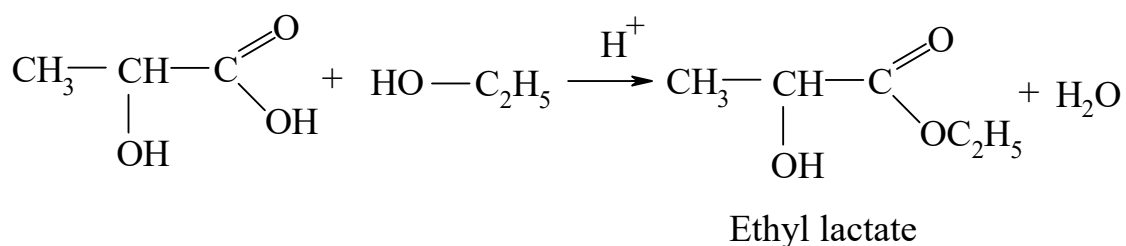
tartaric acid (historical)

Task 2. What chemical properties of hydroxyacids confirm their affiliation to the heterofunctional compounds?

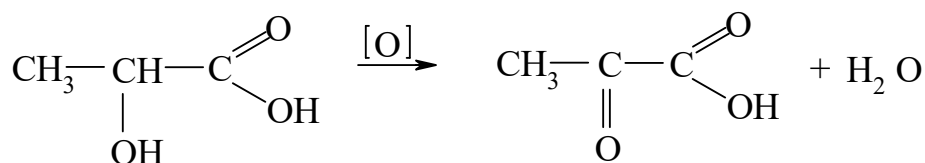
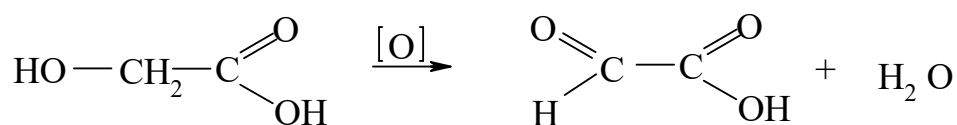
Solution. The hydroxyacids show all characteristic properties of alcohol and carboxylic acids. They have stronger acid properties than the one-basic acids with the same quantity of atoms of carbon that is caused by influence of the hydroxy groups.

The closer the hydroxyl is to the carboxyl group, the stronger acid.

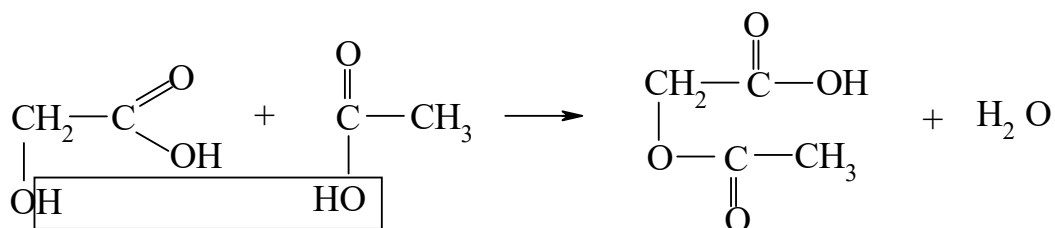
The carboxyl group of hydroxy- and oxoacids reacts with alcohols forming salts and/or esters.



The alcohol's hydroxyl of the oxoacids can be oxidized forming aldo- or ketoacids.

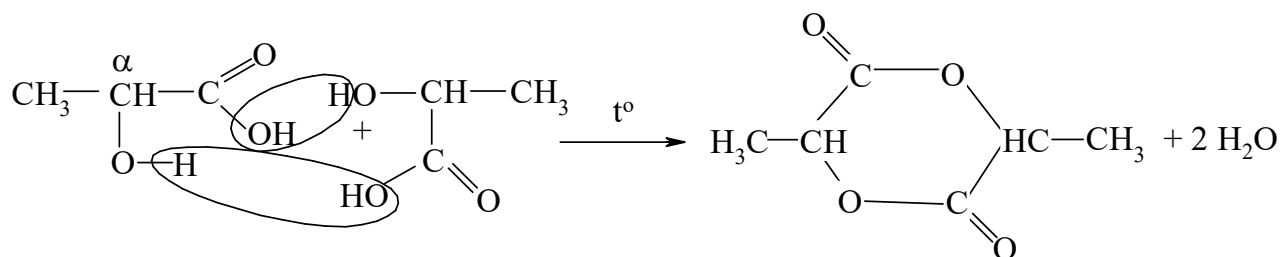


The interaction of the hydroxyl group of the hydroxyacid with carboxylic acid yields the formation of esters:

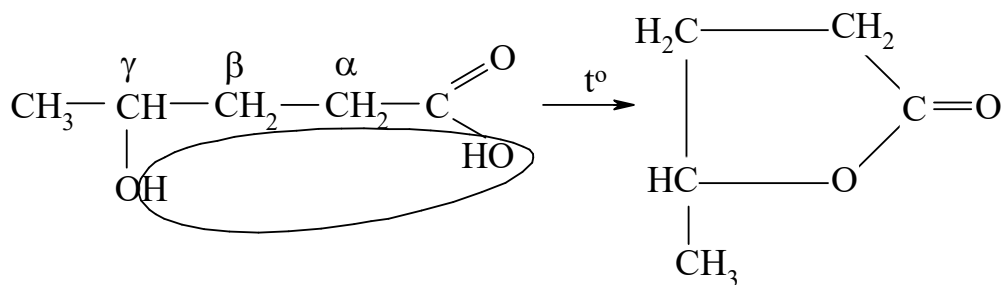


In addition, hydroxyacids can show properties, which are characteristic for alcohols and acids because of the presence of these functional groups in their structure. Heating of α -hydroxyacids is accompanied by the release of water and the

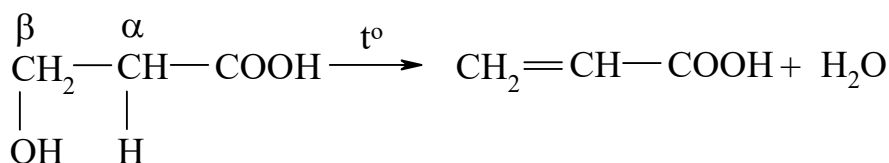
formation of cyclic esters – lactides:



Elimination of water from one molecule of an acid and formation of cyclic esters – lactones is observed at the heating of γ -hydroxyacids:



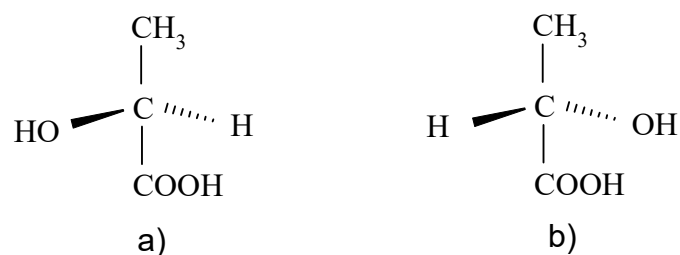
When heated, β -hydroxyacids form unsaturated acids by the intramolecular release of the water molecule:



These reactions are caused by high C-H acidity of hydrogen atoms of the methylene group in α - position.

Task 3. How can be explained optical activity of hydroxyacids? How do you define their affiliation to L-and to D-series?

Solution. Many hydroxyacids have optical activity, i.e. ability to rotate a plane polarized light. Polarization of a ray of light takes place under the passage through the crystals of tourmaline and the fluctuations of such ray of light are in one surface. Under the passage through a solution of such optically active substance, the surface of polarization deviates by certain angle. It has established, that in some cases the substances which rotate a plane of polarization to the right and to the left on the same angle, are isomers.



Optical activity of a substance arises if the molecule cannot be combined with its mirror image (chiral molecule). The molecule that can be combined with its mirror image is called achiral. The general attribute of chiral center is absence of symmetry surface or symmetry center in the molecule. Molecules, which have the carbon atom connected with four different substituents correspond to this condition. Such atom is called as dissymmetric atom or chiral center. For example, if lactic acid molecule is placed upward methyl groups, other substituents can be placed irregularly: a) hydrogen atom, carboxyl and hydroxyl – clockwise, b) hydrogen atom, carboxyl and hydroxyl – counter-clockwise.

Both molecules differ one from another, as an object and its image in a mirror. Chiral molecule and its mirror image are different substances, which are isomers. They are called mirror or optical isomers while they rotate a surface of a beam of polarized light by the same angle in the opposite directions.

To identify configurations of optical isomers D-, L-system is applied especially in the chemistry of carbohydrates and amino acids. Glyceraldehyde is used as the standard. Thus D-isomer was called a substance, which had group OH to the right from asymmetric carbon atom.



D-glyceraldehyde

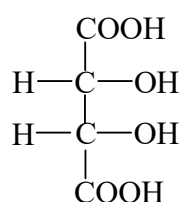
L- glyceric aldehyde

Substances which formulas can be formed of D-glyceraldehyde by the adjustment of a hydrocarbon chain from the side of aldehyde group belong to a D-series, and from L-glyceraldehyde – to L-series. Not in all cases substances of D-series rotate a surface of plane polarized light to the right, and substances of L-series – to the left. Letters D and L are used before the name of substance designation of a configuration and a direction of rotation of a plane of polarization. Also before name

of the substance must be written signs (+) and (-) corresponding to the rotation to the right or to the left.

Mixture of identical quantity of optical isomers (racemic mixture) can appear under formation of chiral molecules. Such mixture is optically inactive.

Compounds with several dissymmetric carbon atoms, can be optically inactive because they consist of identical quantity of oppositely constructed dissymmetric centers. For example, in a molecule of mesotartaric acid, accommodation of substituents at the top asymmetric atom causes the right rotation of a plane polarized light, and at the bottom – left rotation. Such compounds are called meso-forms or meso-isomers. Meso-isomers are achiral, because they have a plane of symmetry.

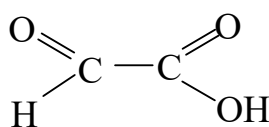


mesotartaric acid

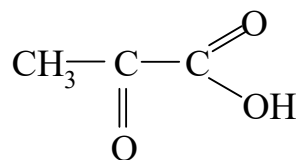
Optical activity of organic compounds is very important. Most of the natural compounds represent the one of the possible optical active forms and not racemic mixture. Optically active enzymes, which selectively interact with only one of optical isomers in the living organisms, that's why enzymes are highly specific.

Task 4. What are the structural features of aldehydoacids and ketoacids? Describe their chemical properties.

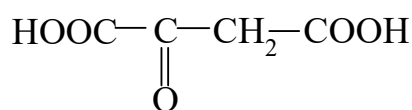
Solution. Aldoacids and ketoacids are compounds which contain two functional groups, carboxylic and aldo- or keto- groups. The formulas of aldoacids and ketoacids are given:



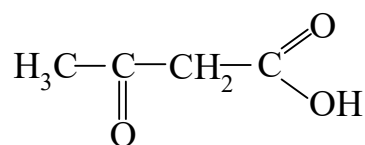
Glyoxalic acid



Pyruvic acid
(α -ketobutyric acid)

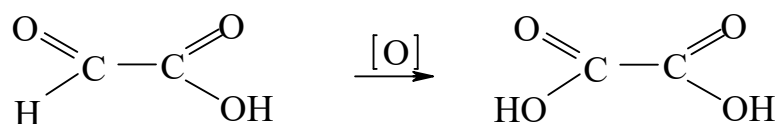


Oxaloacetic acid



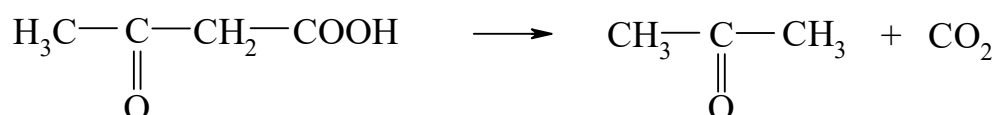
Acetoacetic acid (β -ketobutyric acid)

Aldo- and ketoacids have the properties of carboxylic acids and aldehydes or ketones. They form salts, esters, etc. on carboxyl group; on carbonyl group they enter into the addition reactions, forming oxide, cyanhydride, etc. Aldoacids are easily oxidized with formation of the dibasic acids:



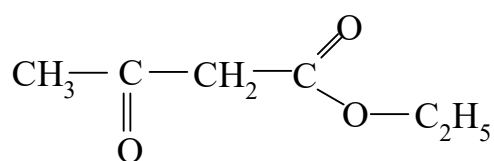
Pyruvic acid, which is an intermediate product of transformation of carbohydrates and proteins in the living organism, has the important biological significance.

Acetoacetic acid is unstable and easy decomposes on acetone and carbon dioxide (IV) (decarboxylation):



Task 5. Explain the keto-enol tautomerism phenomenon on the example of acetoacetic ester.

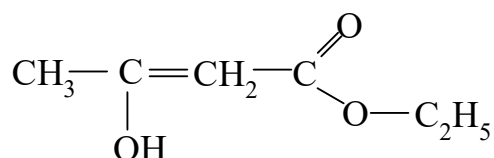
Solution. Esters of acetoacetic acid are stable and they are extensively used for chemical synthesis. An ethyl ester has particular interest:



acetoacetic ester

(1)

The ethyl ester shows the basic properties of ketones, and at the same time it interacts with sodium as alcohol, and attaches bromine as unsaturated compounds. These properties can be explained if ester is considered as ester of hydroxycrotonic acids:



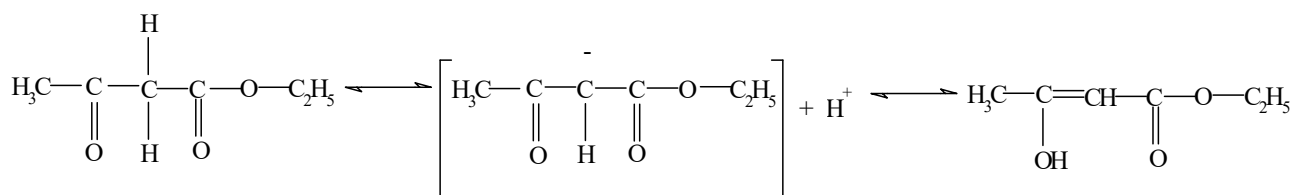
(2)

Researches show that acetoacetic ester is a mixture of isomers, with corresponding formulas (1) and (2). These isomers are called ketonic form or enol form respectively at the presence the ketonic or enol groups. At a room temperature acetoacetic ether contains 92,3 % ketonic form and 7,7 % enol form. Both the keto and the enol forms are in equilibrium through a process of tautomerism, continuous interconversion between two forms This phenomenon is called tautomerism. Forms which turn one into another are tautomers and their mutual transition are tautomeric transformations.

If tautomers are substances with carbonyl and enol groups (for example, isomerism of acetoacetic ester), than tautomerism is called keto-enol.

Tautomerism of acetoacetic ester is caused by hydrogen atoms of methylene group being in between of two carbonyl groups which withdraws electron density from the adjacent C-H bonds and increase its C-H acidity. Hydrogen atoms possess high mobility and can be removed in the form of a proton (C – H acidity).

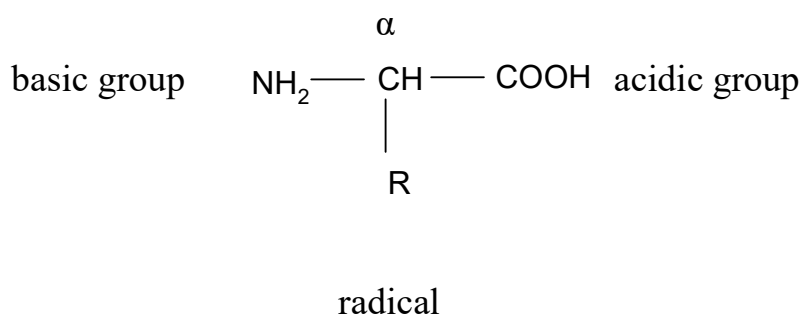
Depending on the place of proton attaching to the formed ion, the last is transformed into keto- or enol form:



Conjugation is a factor that stabilizes the enol form.

Task 6. Give examples of proteogenic amino acids.

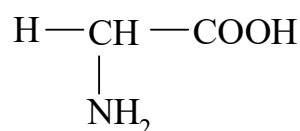
Solution. Amino acids are heterofunctional compounds contain carboxylic group and amino group.



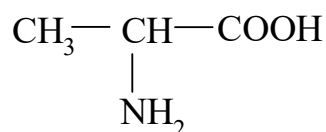
Names of α -amino acids are made by IUPAC nomenclature, but in biochemistry trivial names are often used. In bioorganic chemistry and biochemistry it is accepted to use three- and one- letters abbreviations of trivial names which are used for writing peptides and proteins. According the chemical nature of a radical amino acids are classified as aliphatic, aromatic and heterocyclic.

Aliphatic amino acids (number of carbon atoms no more than 6):

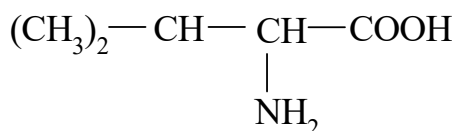
Glycine (Gly)
(aminoacetic)



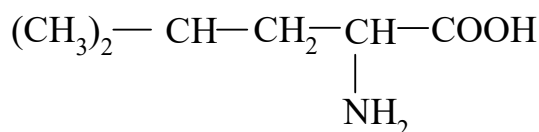
Alanine (Ala)



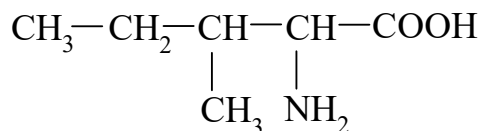
Valine (Val)



Leucine (Leu)

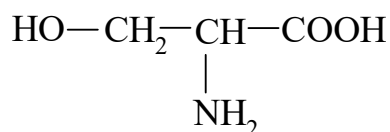


Isoleucine (Ile)

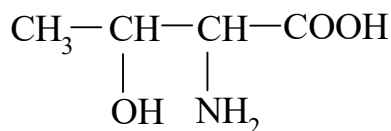


The amino acids, containing OH-group:

Serine (Ser)

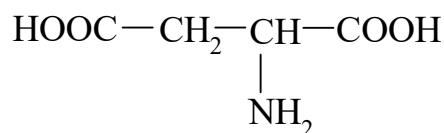


Threonine (Thr)

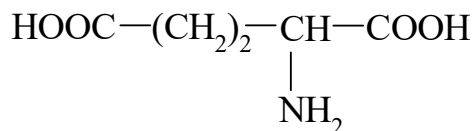


The amino acids containing COOH-group:

Aspartic acid (Asp)

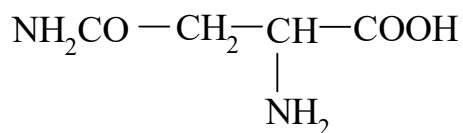


Glutamic acid (Glu)

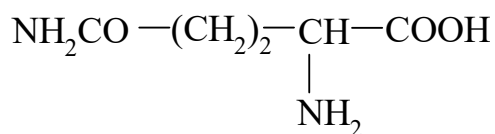


Amino acids containing NH₂CO-group:

Asparagine (Asn)

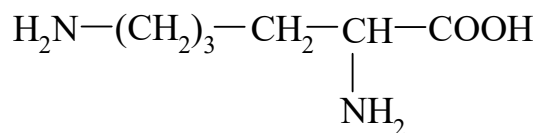


Glutamine (Gln)

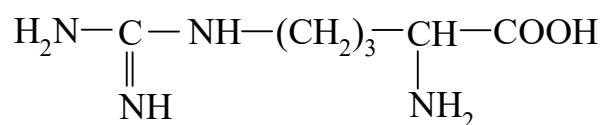


Amino acids containing NH₂- group:

Lysine (Lys)

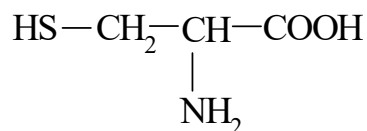


Arginine (Arg)

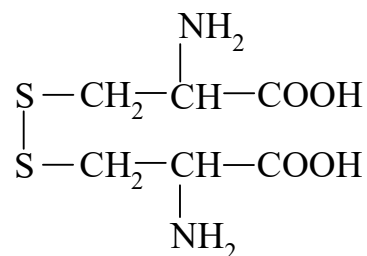


Amino acids containing sulphur:

Cysteine (Cys)

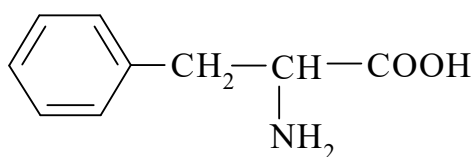


Cystine (Cys)

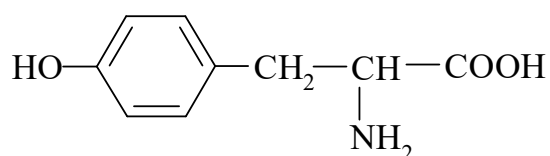


Aromatic amino acids:

Phenylalanine (Phe)

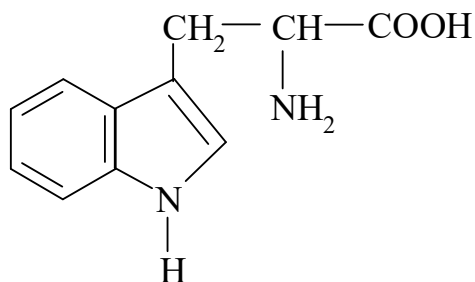


Tyrosine (Tyr)

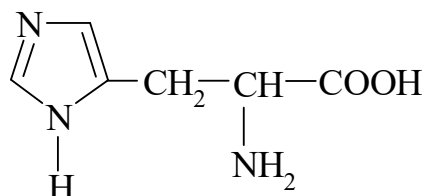


Heterocyclic amino acids:

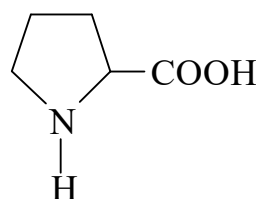
Tryptophan (Trp)



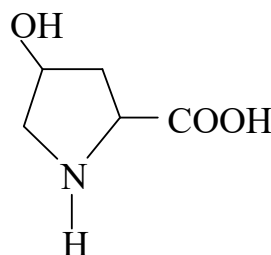
Histidine (His)



Proline (Pro)

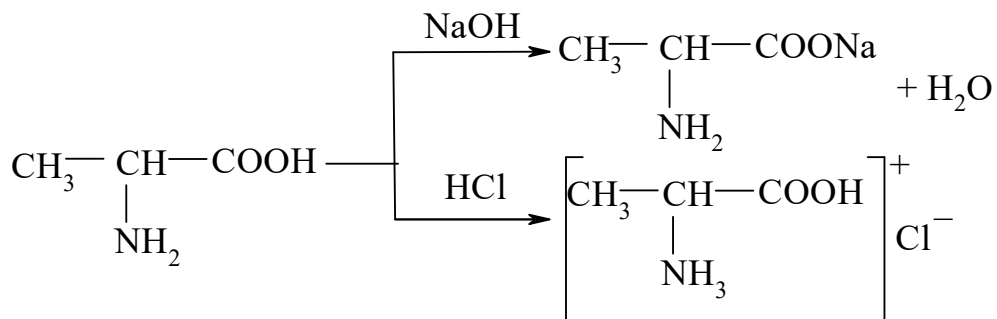


Hydroxyproline (Hyd)

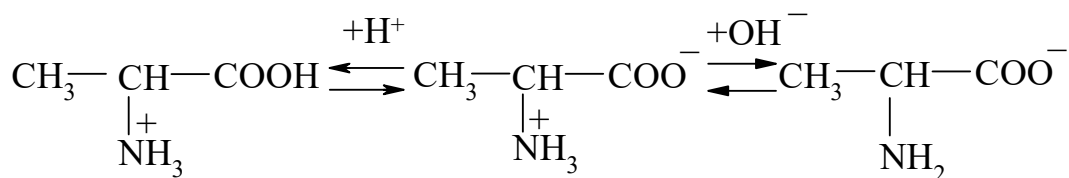


Task № 7. Write the equation for interaction of alanine with sodium hydroxide, hydrochloric acid, and copper hydroxide.

Solution. Amphoteric character of α -amino acids is caused by the presence of functional groups of acidic (-COOH) and basic (NH₂-) nature. That's why amino acids form salts both with acids and bases.



In a water solution α -amino acids exist in the form of bipolar ion. In acidic medium amino acids exist in the form of cation, in basic medium then it is in the form of anion.

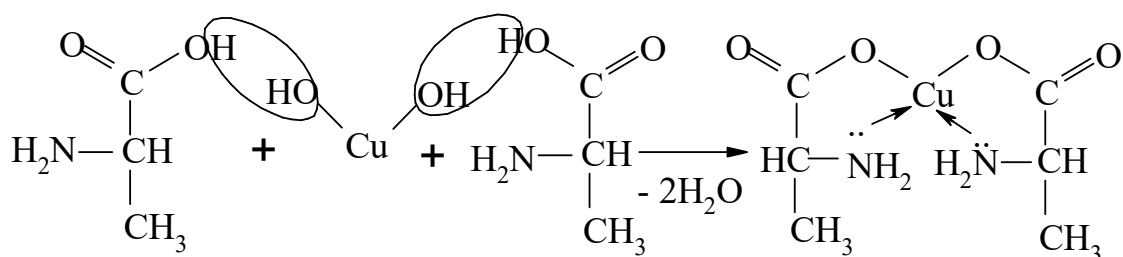


Bipolar ion (zwitterion)

Equilibrium position depends on pH:

acidic medium \leftarrow pH medium \rightarrow basic medium

Specific property of amino acids is ability to form complex copper salts of dark blue coloration. Amino group due to lone-electron pair forms coordination bond with an ion of bivalent copper.

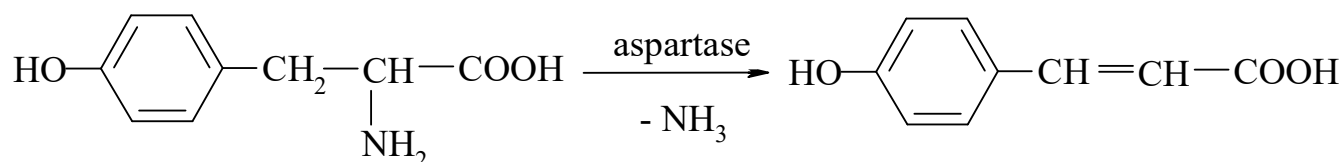


Complexes of amino acids, as well as polyhydric alcohols, have chelate structure.

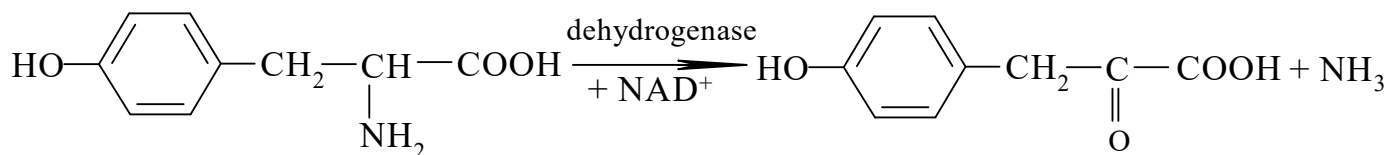
Task № 8. What products are formed at deamination of tyrosine *in vitro* and *in vivo*.

Solution. As a result of deamination excess of α -amino acids is decreased in an organism. Deamination *in vivo* can be reductive (bacteria, fungi) and oxidative.

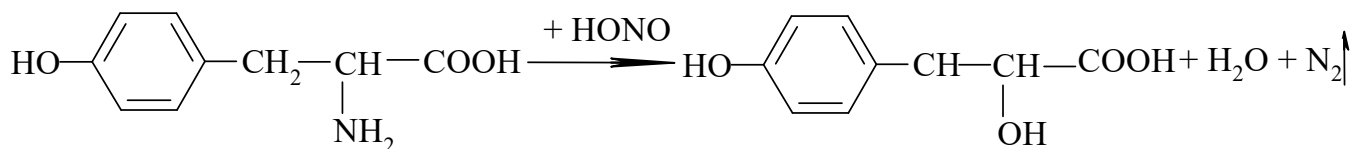
The process of reductive deamination occurs without participation of oxygen with the participation of enzymes. As a result α -unsaturated acid (p-oxyphenylakrylic) and ammonia are formed.



The process of oxidative deamination occurs with the participation of dehydrogenase enzymes and NAD^+ coenzyme. As a result α -oxo acid (p-oxyphenylpyruvic) and ammonia are formed.



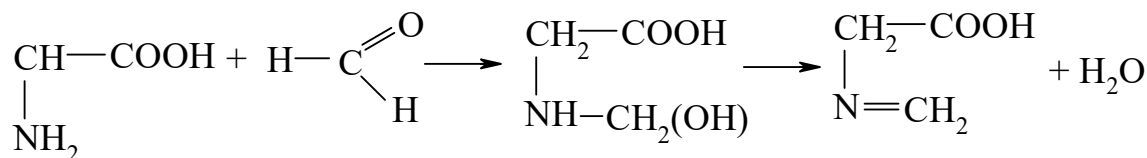
The process of deamination by the nitrous acid takes place *in vitro*. Amino acid is transformed into corresponding hydroxy acid and gaseous nitrogen is liberated:



This reaction is used for quantitative determination of amino acids (Van Slyke method).

Task № 9. Write the reaction equation for interaction of glycine with formaldehyde.

Solution. At interaction of α -amino acids with aldehydes substituted imines (Shiff's base) are formed through a formation of carbinolamines.

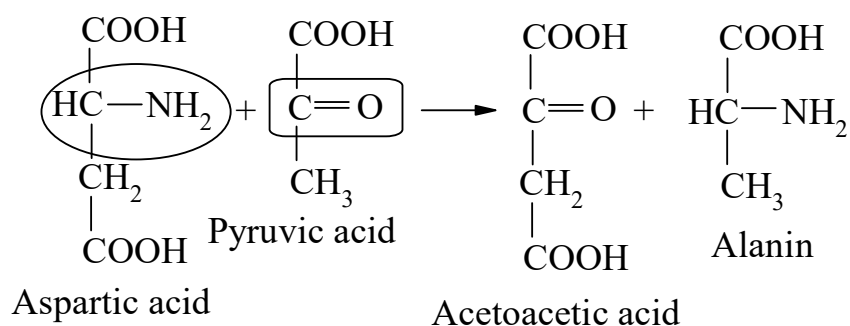


Basic amino group is neutralized, and the amino acids content can be determined by means of carboxyl groups titration (Serensen method (formol titration)).

Task № 10. What products are obtained in transamination of aspartic and pyruvic acids?

Solution. In living organisms under the influence of enzymes amino acid experience a number of biochemical transformations. Transamination – is the main way of biosynthesis of α -amino acids.

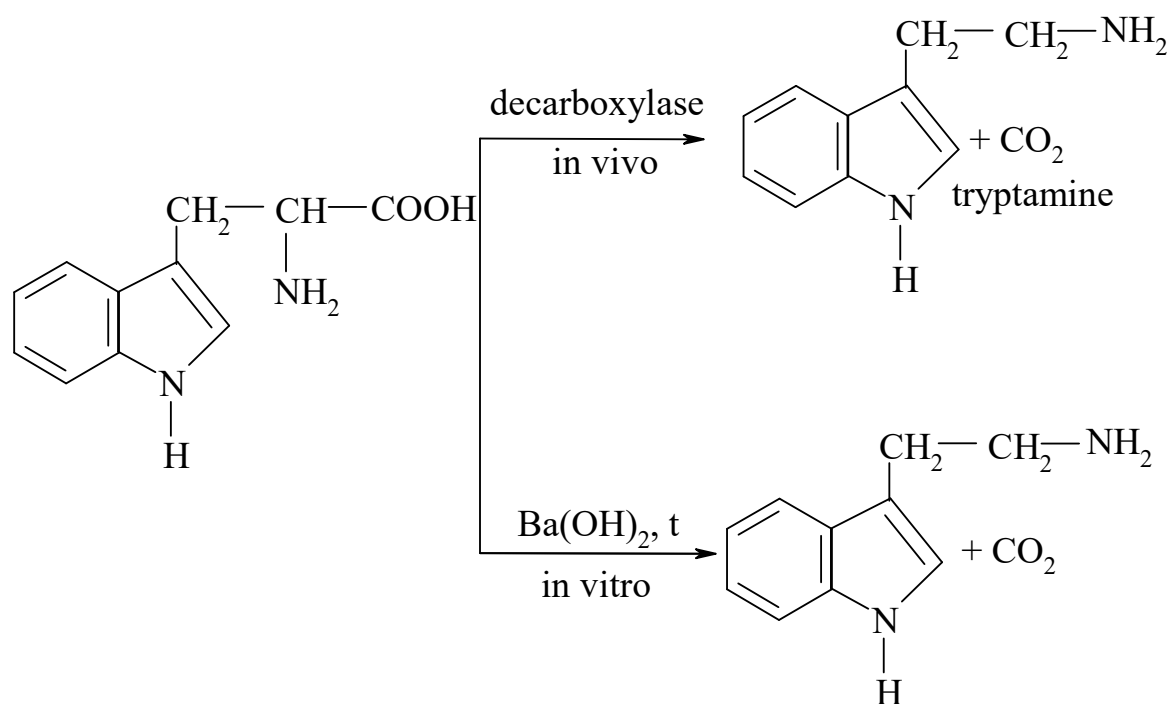
The necessary amino acid-II for an organism is synthesised from α -amino acid-I which are available in an organism in sufficient or superfluous quantity, by carrying over of an amino group from one acid to α -oxoacid.



The process takes place in the presence of enzymes – transaminases and coenzyme – pyridoxal phosphate (vitamin B₆).

Task № 11. Write the scheme of tryptophan decarboxylation reaction. What are the conditions for this reactions *in vivo* and *in vitro*?

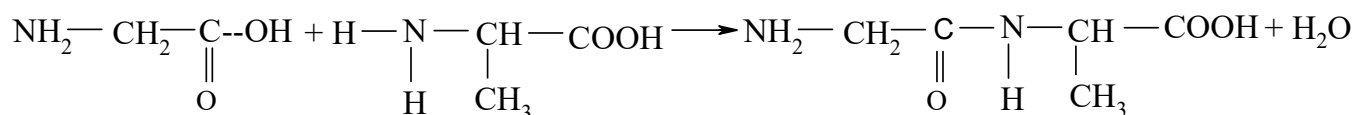
Solution. The presence of electron-accepting group NH₂ promotes stabilization of COO⁻-ion. As a result of decarboxylation of acids corresponding amines form.



Task № 12. Draw the structure of dipeptide Gly-Val. Show peptide bond. Determine its isoelectric point.

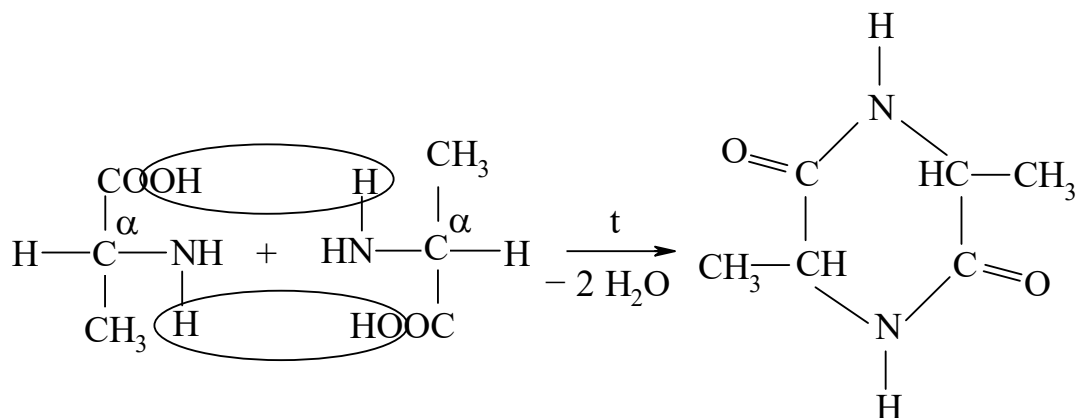
Solution. The amino group of one amino acid and carboxylic group of another α-amino acid can get condensed with formation of dipeptide.

The group of the atoms $\begin{array}{c} \text{O} \quad \text{H} \\ \parallel \quad | \\ -\text{C}-\text{N}- \end{array}$ is called a *peptide linkage*. Dipeptides, as well as amino acids, contain free carboxylic and an amino group. Therefore, on reaction with amino acid molecule, they can form tripeptide, etc. Substances consisting of great number of amino acid residues are called polypeptides. Incorporating among themselves, polypeptides chains form molecules of proteins. Presence of acid and basic groups defines pH of the medium. In this case medium is neutral.

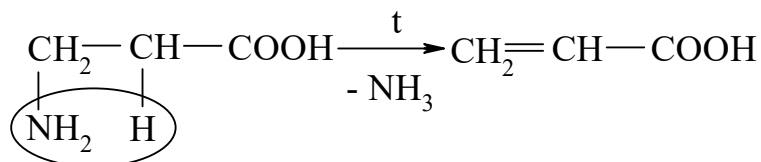


Task № 13. Give examples of intermolecular dehydration of α , β , γ -amino acids at heating.

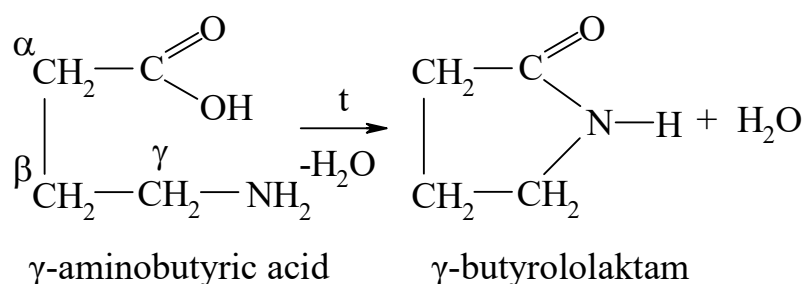
Solution. Elimination of one water molecule from two molecules of amino acids is typical for α -amino acids. As a result a diketopiperazine is formed:



Elimination of ammonia from one molecule of amino acids is typical for β -amino acids. As a result an unsaturated carboxylic acid is formed:

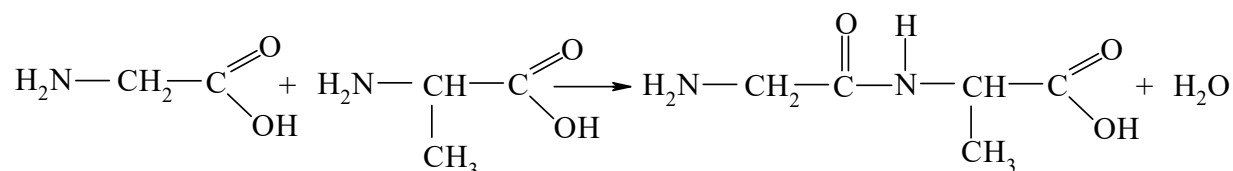


In the case of γ -amino acids intramolecular cyclization occurs and one water eliminates from one molecule of amino acids. As a result a lactam (cyclic amide) is formed:

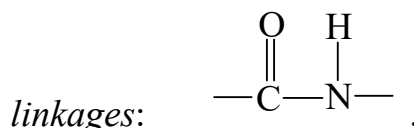


Task № 14. Draw the structure of dipeptide Gly-Ala and determine its isoelectric point.

Solution. At studying of amino acids it was specified that peptides are compounds formed by condensation of two or more same or different molecules α -amino acids:

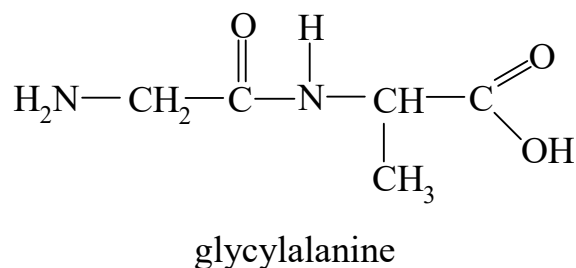


In 1891 A. Y. Danilevsky suggested that amino acids in peptides and proteins are joined by peptide bonds. The carboxyl group of one amino acid and amino group of another amino acid are condensed. This type of amide linkages is called *peptide*



As the carbon atom in this group is in sp^2 -hybridization state all atoms forming peptide linkages are located in one plane.

Depending upon the number of amino acids residues per molecule, the peptides are called oligopeptides and polypeptides. The names of the peptides are made from the names of corresponding amino acids. The amino acids which gives in formation of peptide chain its carboxyl group, receive a suffix -yl:



Usually abbreviations (Gly-Ala) are used for naming peptides.

Each polypeptide chain has a free amino group at one end and the free carboxylic group at the other end. The amino group end is called amino or *N-terminal end* while the end having free $-\text{COOH}$ group is called *C-terminal end*.

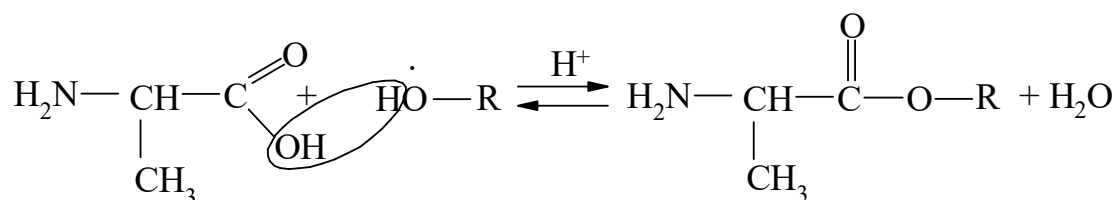
As in our case the peptide has one amino group and one carboxylic group isoelectric point – will be at $\text{pH} < 6$.

Value of $\text{pI} = (\text{pK}_{\text{COOH}} + \text{pK}_{\text{NH}_2})$ is a characteristic constant of peptides. The isoelectric point of the most of peptides of animal tissue lies within 5,5-7,0 that testifies partial prevalence of acidic amino acids. However in nature there are peptides which pI lies at extreme values of pH . In particular, pI of pepsin (enzyme of gastric juice) is equal to 1, and that of salmin (the main peptide from milk of a salmon) – nearly 12.

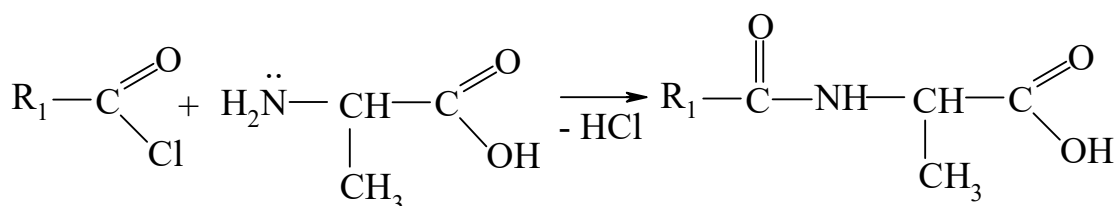
From the given amino acids three more peptides: Gly-Gly, Ala-Gly, Ala-Ala can be received. When a protein or polypeptide is treated with 6 M hydrochloric acid for 24 h, hydrolysis of all the peptide linkages usually takes place, liberating its constituent amino acids as a mixture. Chromatographic separation and quantitative analysis of the resulting mixture can then be used to determine which amino acids comprised the intact polypeptide and their relative amounts.

To synthesize a peptide with a given structure we need to deactivate (protect) some functional groups and activate the others (technique of activation – protection of functional groups). Functional groups forming peptide bond are to be active (carboxyl group of the first amino acid and amino group of the second one). Besides, carboxyl group of the first amino acid should be activated as carboxylic acids usually react with amine forming salts.

Carboxyl group of the second amino acid should be protected by esterification:

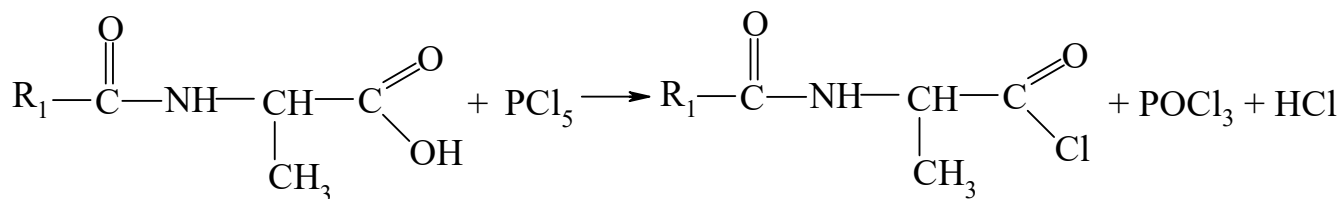


Amino group of the first amino acid should be protected by acylation:

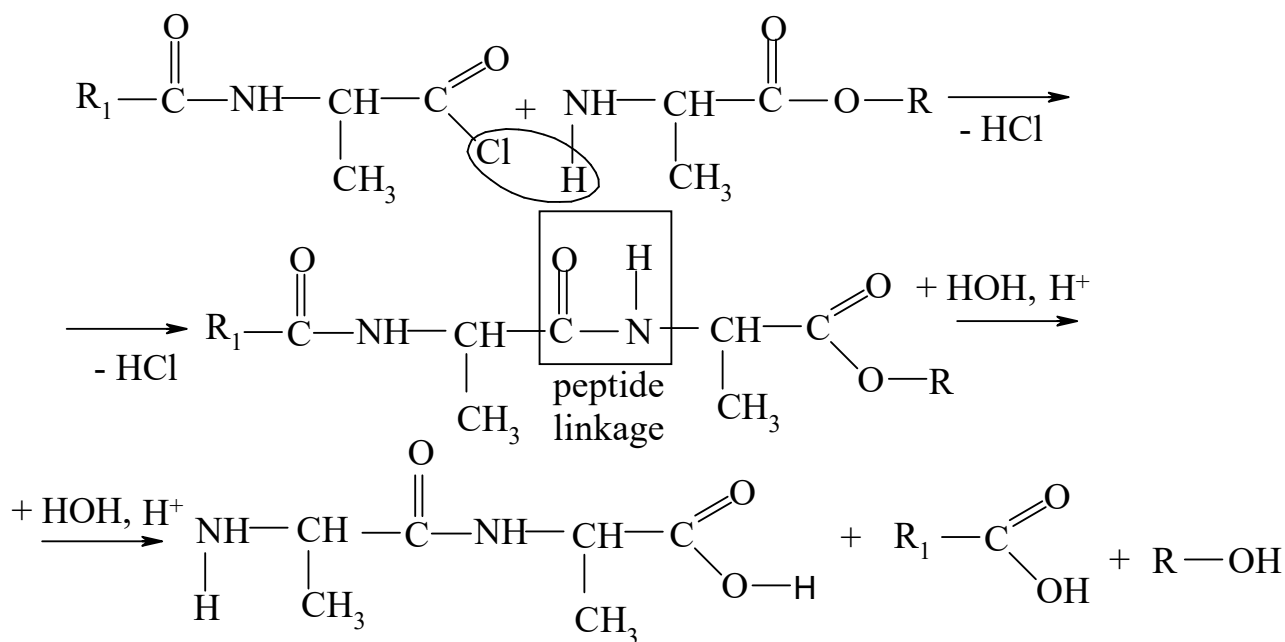


Carboxyl group of the first amino acid should be activated by changing into

halide:



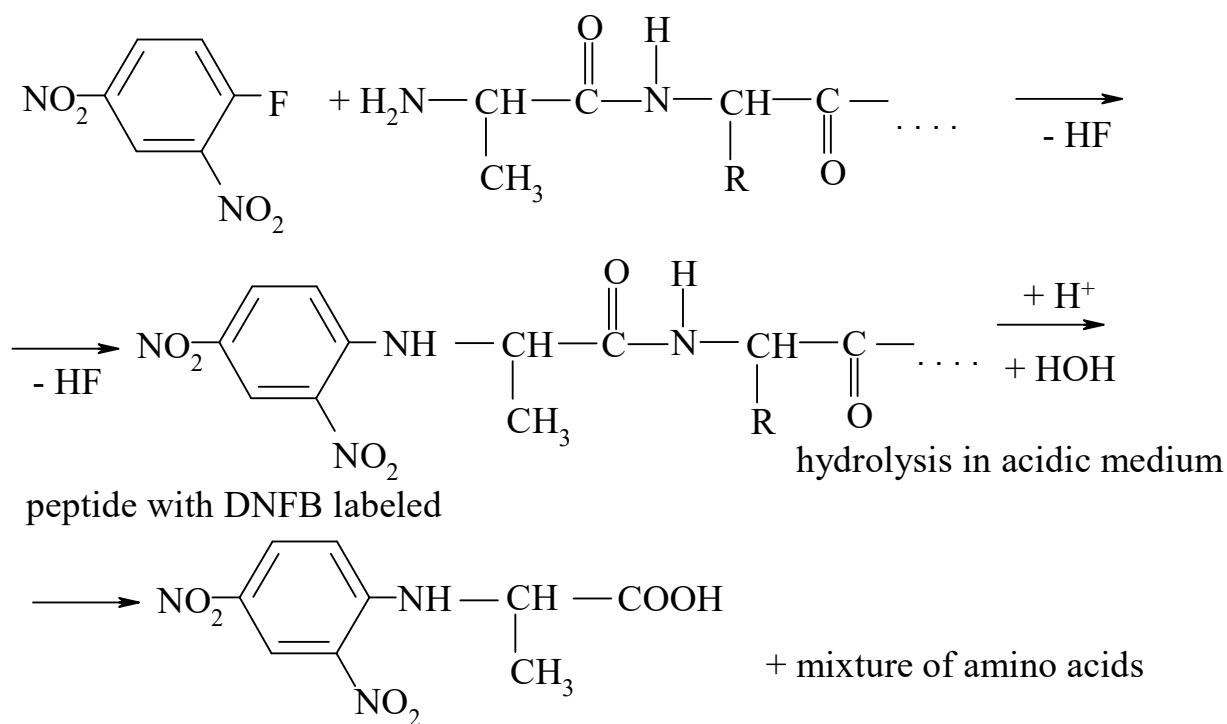
Formation of peptide bond:



Protection is to be removed by hydrolysis in the acidic medium.

Apparently, synthesis of dipeptide under according to this scheme consists of several stages, and for synthesis of polypeptides the number of stages is necessary.

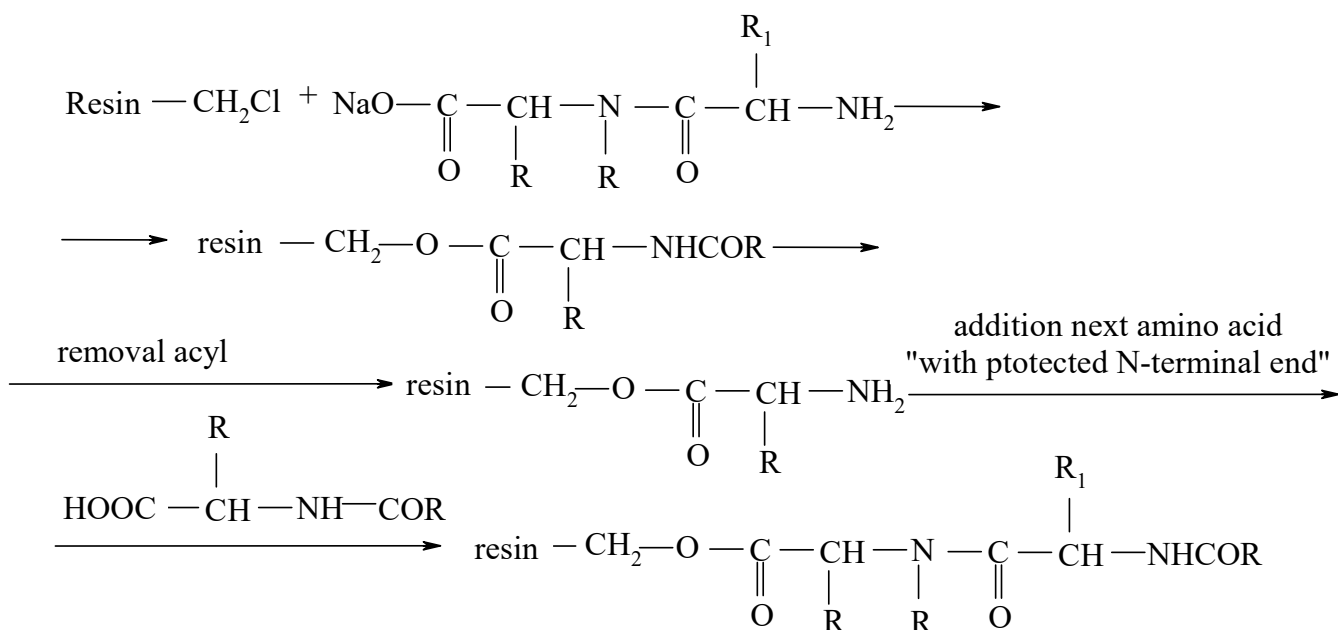
There is a variety of methods available to determine the sequence of amino-acid residues. One method for N-terminal sequence analysis is the Sanger method (or DNFB method), based on the use of 2,4-dinitrofluorobenzene (DNFB) in weak basic medium. DNFB reacts with NH_2 - group of N-terminal amino acid. Subsequent hydrolysis of the polypeptide gives a mixture of amino acids in which the N-terminal amino acid is labeled with a 1,4-dinitrophenyl group. After separating this amino acid from the mixture it can be identified by comparison with known standards. This amino acid may be linked to phenyl isothiocyanate $\text{C}_6\text{H}_5\text{N}=\text{C}=\text{S}$ (Edman degradation method).



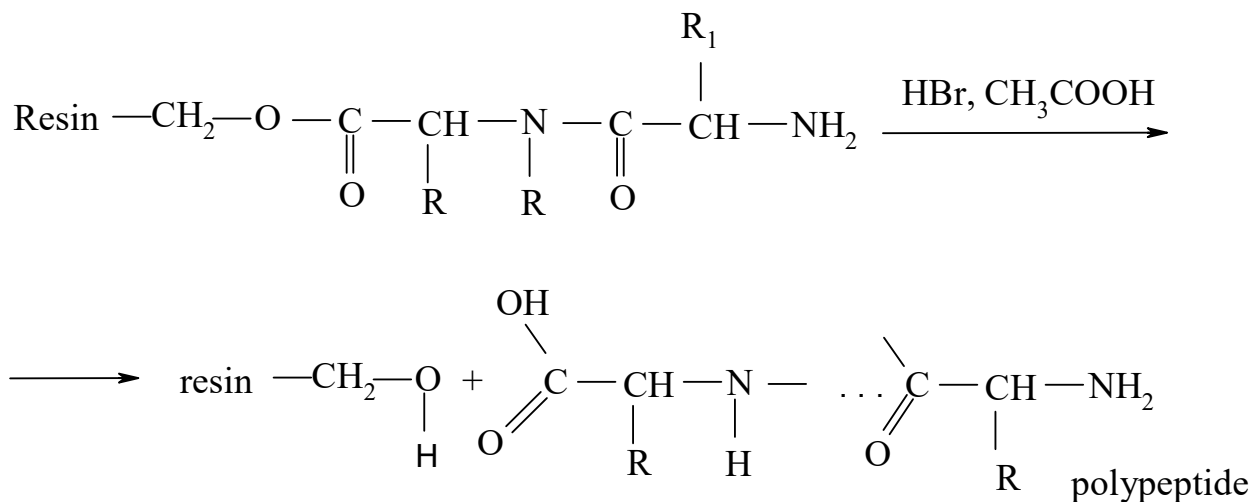
For determination of the sequences of amino acids partial acidic or enzymatic hydrolysis is also used.

Task № 15. Give the scheme of solid-phase synthesis of polypeptide.

Solution. The perspective method of polypeptides synthesis Merifield offered in 1963. Later this method has received the name of solid-phase synthesis of polypeptide. It is conducted on the ion-exchange resin containing CH_2Cl groups. At the first stage salt of amino acid with the protected group joins to solid carrier with formation of so-called "anchor bond".



If protection is removed, synthesis can be conducted further. After formation of peptide necessary length the "anchor" bond is hydrolyzed in the presence of hydrobromic acid and acetic acid:



Nowadays three-phase synthesis is performed in an automatic synthesizer. All reactions pass in the programmed sequence in one reactionary chamber in which micropumps give necessary reactants in certain order. In such synthesizer it is possible to attach six amino acid residues to growing polypeptide chain within 24 hours.

Polypeptides are structural components of protein molecules.

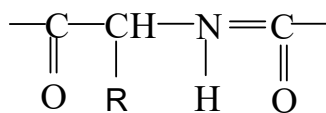
Task № 16. Describe types of interaction which can arise in polypeptide chain with following amino acid sequence: Cys-Glu-Gly-Ala-Val-Ser-Lys-Phe-Cys.

Solution. Types of the interactions stabilizing a spatial structure, i.e. secondary, tertiary and quaternary structures of peptides and proteins, are determined first of all by their primary structure.

Any polypeptide chain is constructed of alternating monomeric units α -amino acids connected by peptide groups (primary structure) between which hydrogen bonds are formed. Such bonds are formed between hydrogen atom connected with

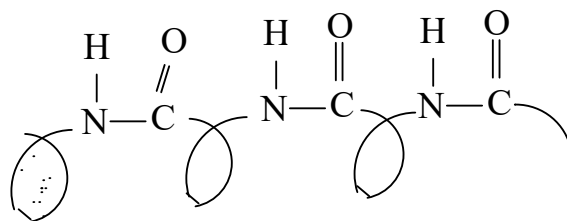
nitrogen atom, and atom of oxygen carbonyl group: $\text{>N-H} \dots \text{O}=\text{C}<$.

Such hydrogen bonds stabilize secondary structure of protein molecule in the form of a helix. Such connections could arise between Glu (1) and Ser (5), Gly (1) and Lys (5), Ala (1) and the Phe (5) in the considered polypeptide chains. As α -helix is

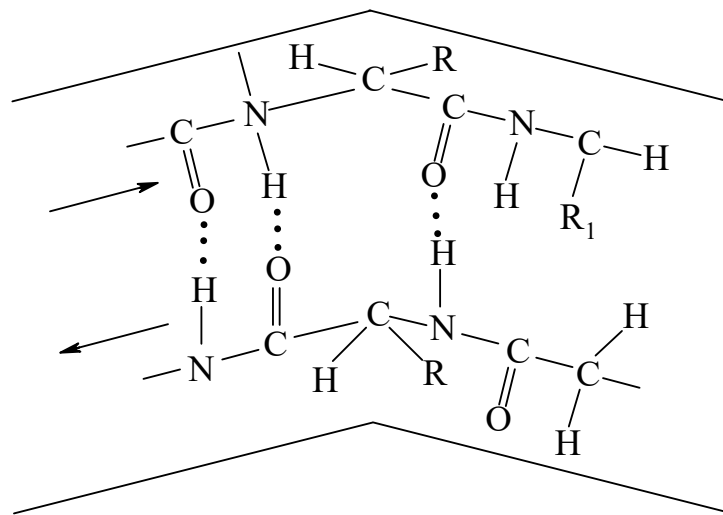


formed from one type of links

Is sizes are constant enough, one coil includes 3,7 amino acid residues, the distance between separate coils is 5,44 Å. If α -helix was unique type of secondary structure of proteins they would be rigid, rod-like formations. But as polypeptide chains possess sufficient flexibility, it is necessary to conclude that α -helix makes only separate sites of polypeptide chains. The deviation from α -helix form can be caused by various factors, in particular, by the presence of proline, oxyproline and valine in peptide chains. After formation peptide bonds amide hydrogen is absent in the residues proline and oxyproline and they cannot participate in formation of hydrogen bonds.



Except the intrachained bonds there are the hydrogen bonds arising between different chains (interchained hydrogen bonds). They stabilize other kind of secondary structure, so-called β -pleated sheet or β -conformation. It arises between antiparallel chains.



β -conformation is found in β -keratine and silk fibroin.

Tertiary structure of a protein is total conformation or spatial orderliness of separate sites of polypeptide chains as a whole.

While the secondary structure of a protein is defined by hydrogen bonds, numerous bends of polypeptide chain, giving proteins tertiary structure, depend not only from peptide and hydrogen bonds, but also on other types of interaction, namely electrostatic interactions between carboxyl groups and amino groups which do not participate in formation of peptide bonds: disulfide bonds, in cysteine, hydrophobic interactions.

The principal interactions which stabilize secondary and tertiary structures of peptides and proteins are as follows:

Covalent	Noncovalent	
disulfide bonds	ionic bonds	hydrogen bonds
between spatially aproxaded cysteine residues	are formed due to attraction between the oppositely charged groups	between peptide groups
		interchain - folded structure
stabilize tertiary structure		stabilize secondary and tertiary structure

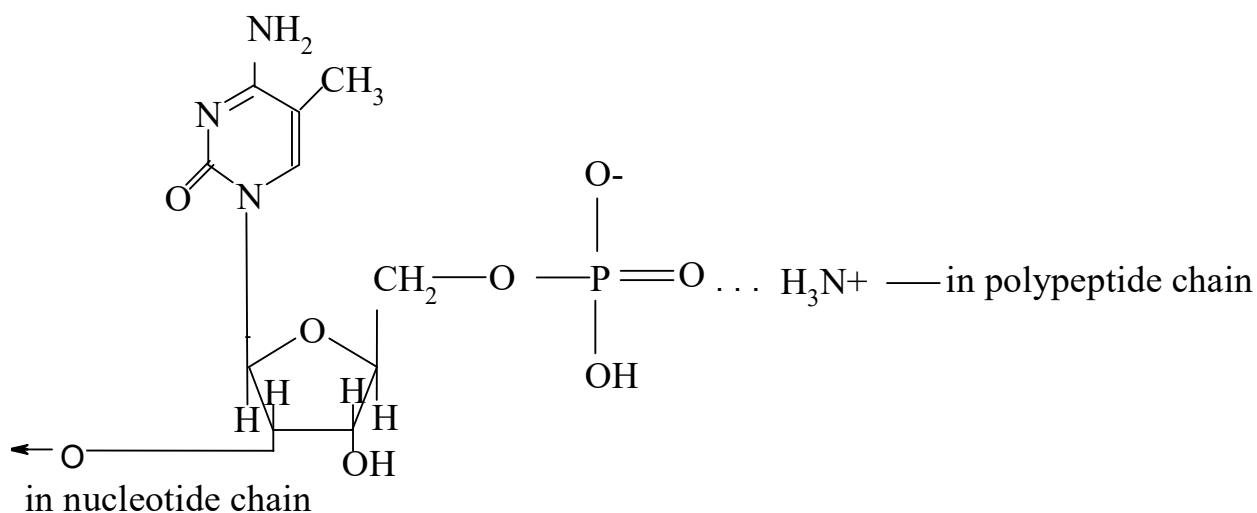
For display of biological activity some proteins should form quaternary structures – a macrocomplex consisting of several protein molecules. Thus each protein serves as monomer, and quaternary structure defines degree of association of such monomers in biologically active material.

Task № 17. Give the structure of biologically active nucleoproteins.

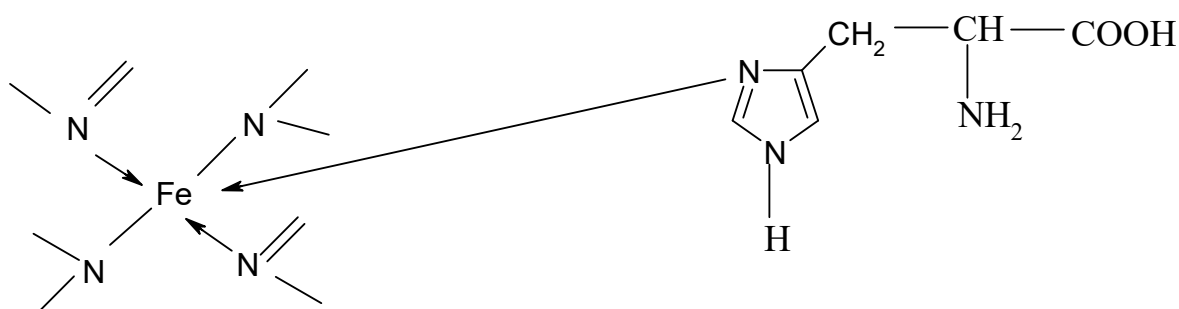
Solution. Till now the proteins consisting only from amino acids were considered. These proteins are called simple. The structure of complex proteins, except amino acids, includes non-protein substances, so-called prosthetic groups – carbohydrates, lipids, nucleic acids etc.

The most important in the biological relation to proteins concern nucleotides – cell fission processes, storage and transfer of hereditary properties are connected with them.

In animal organisms there are two types of nucleoproteins: ribonucleoproteins and deoxyribonucleoproteins. Proteins make up about 50 % of weight of nucleoproteins. Phosphoric acid and amino groups of diamine acids play connecting role in interaction of molecules of DNA and RNA with protein molecules .



Example of complex proteins (chromoproteins) is hemoglobin. Heme is its non-protein part. The protein part – globin – contains four polypeptide chains. Bond of heme with peptide chain is accomplished by iron ion which is connected with histidine residue of peptide chains:



Myoglobin is a chromoprotein – protein of muscular tissues. Some enzymes – catalase, peroxidase, etc. are also chromoproteins. Most of chromoproteins contain

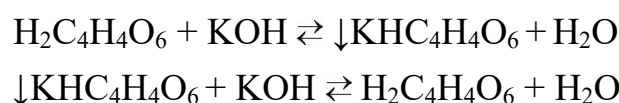
metal (iron, copper, molybdenum etc.). Metalloproteins play an important role in processes of biological oxidation in tissues.

Glycoproteins contain various derivatives of carbohydrates as prosthetic groups: D- glycosamine, D-glucaric acid; lipoproteins contain lipids, phosphatids, sterols. Formation of a complex of proteins with lipids promotes solubility of the last and causes their transportation in tissues.

LABORATORY WORK

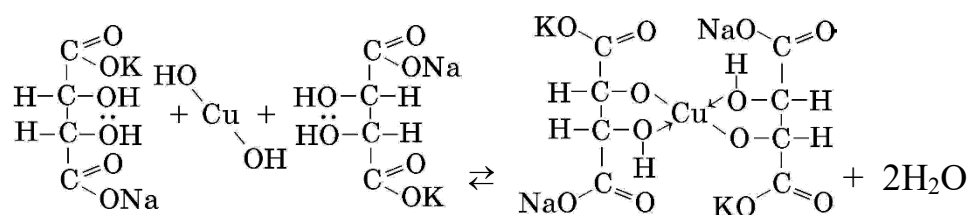
Experiment 1. Proof of presence of two carboxylic groups in tartaric acid.

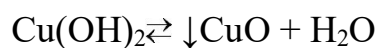
Place 1 drop of 15% tartaric acid solution, 2 drops of 5% potassium hydroxide solution into a test tube and shake it. White crystal precipitate of potassium salt of tartaric acid (potassium hydrogen tartrate) forms gradually. If precipitate does not form, cool the test tube under running tap (cold) water and scratch the inside of the test tube by rubbing with a glass rod. Add 4 – 5 drops of potassium hydroxide solution into the same test tube. Precipitate dissolves gradually because freely soluble potassium tartrate forms. Keep obtained solution for next experiment.



Experiment 2. Proof of presence of hydroxyl groups in tartaric acid.

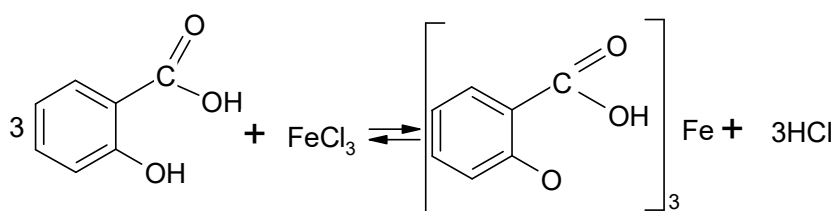
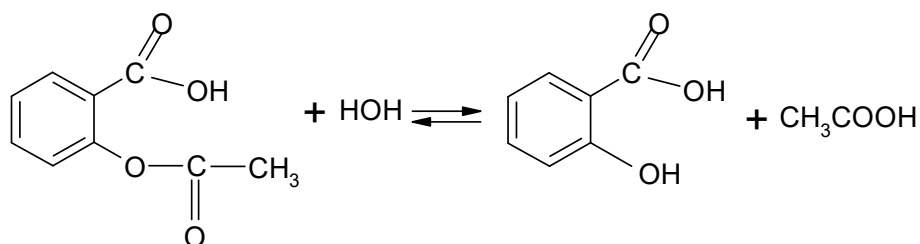
Take 2 test tubes and place in every test tube 2 drops of copper (II) sulfate and 10% sodium hydroxide solution. Blue precipitate of copper (II) hydroxide forms. In the first test tube add solution of potassium tartrate obtained in the previous experiment. Precipitate of copper (II) hydroxide dissolves with the formation of blue solution. Bring the content of test tubes to a boil. In the first test tube colour of solution does not change but in the second test tube blue precipitate passes to a black precipitate of copper (II) oxide.





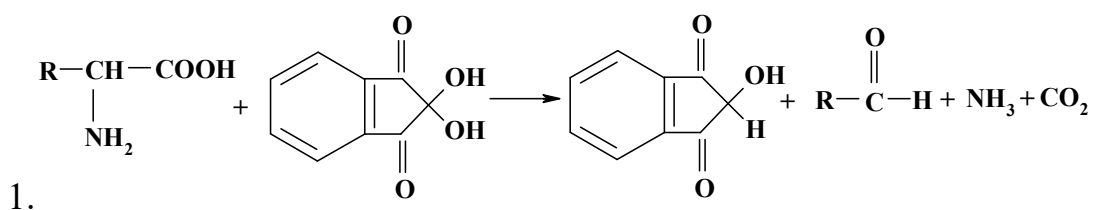
Experiment 3. Proof of absence of phenolic hydroxyl in acetylsalicylic acid and its hydrolysis.

Place several crystals of acetylsalicylic acid and 5 – 6 drops of water into a test tube. Shake the test tube to accelerate dissolution. Pour out part of obtained solution into another test tube. Add 1 – 2 drops of iron (III) chloride solution into one of the test tubes. Content of other test tube bring to a boil and then add 1 drop of iron (III) chloride solution. Blue coloration appears (reaction on phenolic hydroxyl).



Experiment 4. Ninhydrin reaction with α -aminoacids, peptides, proteins.

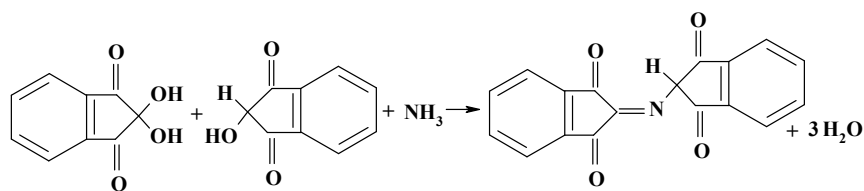
Place 20 drops of α -aminoacid, protein into each of two test tubes. Add 5 drops of ninhydrin solution in every test tube and heat a composition of test tubes.



1.

Ninhydrin

Reduced
ninhydrin



2.

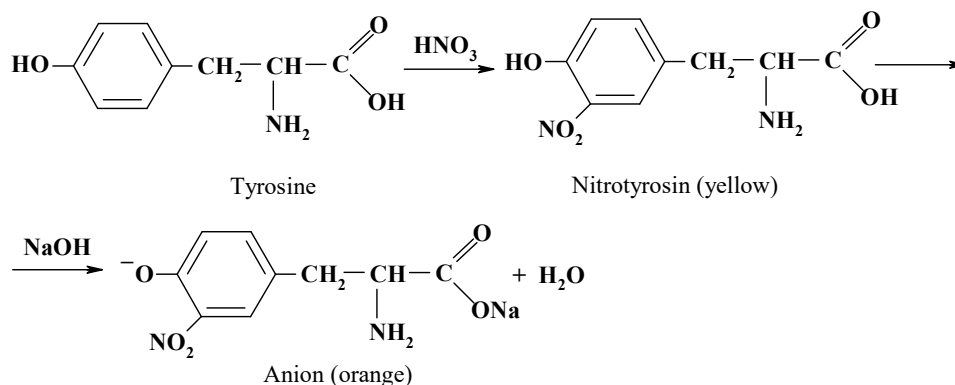
Oxidized ninhydrin

Reduced ninhydrin

Colored product of condensation

Experiment 5. Xanthoprotein reaction with aromatic acids.

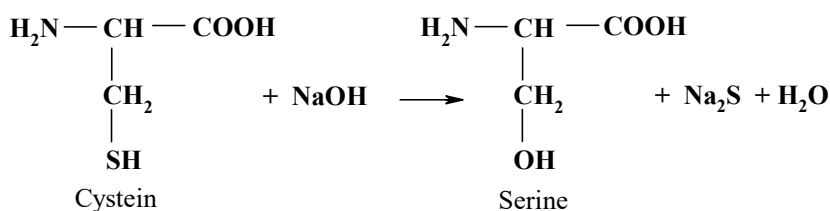
Pour 5 drops of into a test tube. Add 3 drops of nitric acid to each tube. Heat carefully. The solution turns yellow only in the first tube. After cooling add 10 drops of ammonia or NaOH solution. The color turns orange.



Experiment 6. Fohl's reaction for sulfur-containing amino acids.

Pour 5 drops of protein into a test tube and add 5 drops of NaOH solution and 1 drop of $(\text{CH}_3\text{COO})_2\text{Pb}$. St Bring the content of the test tube to a boil.

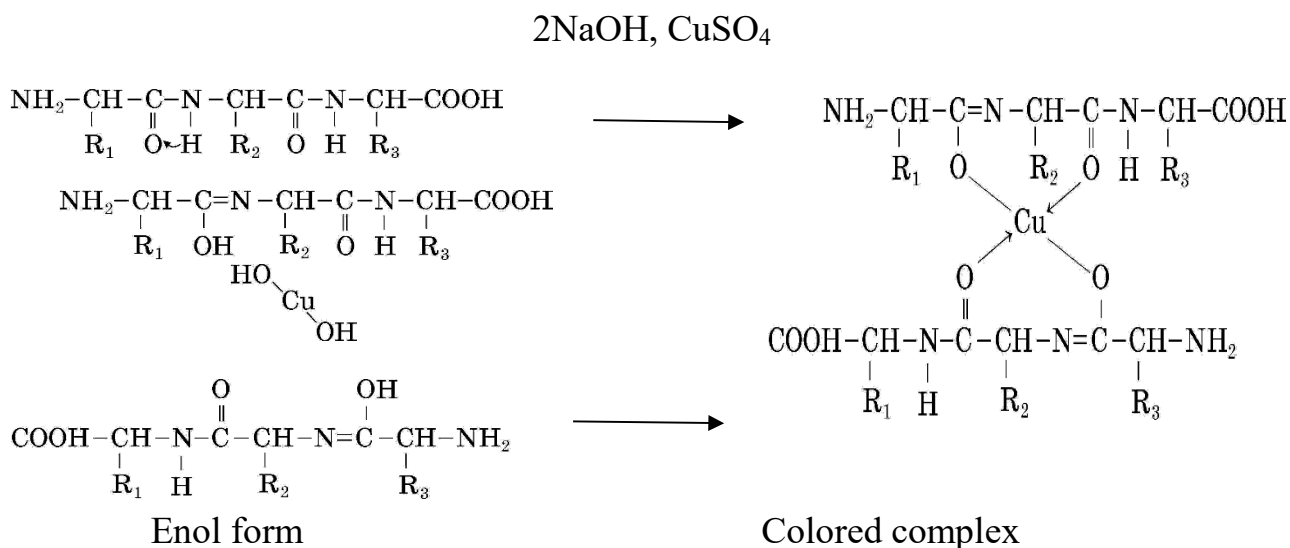
1.



Experiment 7. Biuret reaction for peptides and proteins.

Place 20 drops of protein into a test tube, add 20 drops of NaOH solution and 2 drops of CuSO_4 . Shake. The solution turns violet.

Reaction with polypeptide:



Revision exercises

№ 1

1. What color reaction to proves presence of a benzene ring in amino acid?
2. Give the definition of enantiomers. Draw projection formulas of β -hydroxybutyric acids enantiomers, determine dissymmetric carbon atom and establish whether it is a D or L isomer?
3. Draw the structure of tripeptide His-Trp-Lys and define its isoelectric point.

№ 2

1. Write the equation for the formation reaction of salicylic ester of an acetic acid. Name obtained medical preparation, what is its use in medicine?
2. Write the reactions equations of γ -hydroxyvaleric acid with NaOH solution at heating (specific reaction).
3. Draw the structure of tripeptide Gly-Val-Phe and define its isoelectric point.

№ 3

1. Obtain an ethyl ether of p-aminobenzoic acid. What is the name of medicinal preparation? What is its using in medicine?
2. Give the scheme of glyoxalic acid oxidation with ammoniac solution silver hydroxide.
3. Write a structure of tripeptide Gln-Cys-Gly and define its isoelectric point.

№ 4

1. Write the equation for the qualitative reaction on phenols by the example of salicylic acid.
2. Write the reaction equations of pyruvic acid and ethyl ester.
3. Write the scheme of dipeptide Val-Ala-Tyr and define its isoelectric point..

№ 5

1. Write the reaction equation for hydrolysis of aspirin (acetylsalicylic acid), proceeding at its storage. How can you check purity of aspirin?
2. Write projection formulas of the lactic acid and key substance on which the affiliation to a stereochemical series is defined.
2. Write the scheme of dipeptide Глі-Ала-Ser and define its isoelectric point..

№ 6

1. Write the reaction equation of electrophilic substitution for salicylic acid.
2. Write the reaction equation of acidic hydrolysis of α -hydroxyvaleric acid lacton.

3. Write the scheme of tripeptide Gly-Phe-Met formation and define its isoelectric point.

№ 7

1. Write the reaction equation for ester formation of salicylic acid with phenol. Name the product obtained. What is the application of this compound in medicine?

2. Write projection formulas of malic acid enantiomers. Define the dissymmetric carbon atoms and specify an affiliation to series.

3. Write the scheme of dipeptide Тир-Цис-Gly synthesis and define its isoelectric point.

№ 8

1. Write the reaction equation of interaction between salicylic acid and methanol. Name the product obtained. What is its application in medicine?

2. Give the definition of enantiomers. Write projection formulas of enantiomers of glycerine aldehyde, define dissymmetric carbon atoms and specify their affiliation to the stereochemical series.

3. Draw the structure of tripeptide Val-Ala-Gly and define its isoelectric point.

№ 9

1. Write the reaction equation of salicylic acid decarboxylation.

2. Write the schemes of reactions that prove presence of aldehyde group in glyoxalic acid.

3. Draw the structure of tripeptide Leu-Glu-Cys and define its isoelectric point..

№ 10

1. Obtain methyl ester of p-aminobenzoic acid. Name obtained medicinal preparation. What is its application in medicine?

2. Write projection formulas of β -oxybutyric acid enantiomers. Point asymmetric carbon atom and specify its affiliation to series.

3. Draw the structure of tripeptide Arg-Ser-Gly and define its isoelectric point.

№ 11

1. Write the reaction equation of methyl salicylate hydrolysis.

2. Write the reaction equation of interaction for lactic acid with ethanol, and write the specific reaction taking place at heating.

3. Write the scheme of dipeptide Arg-Val-Ser formation and define its isoelectric point..

№ 12

1. Write the reaction equations for the following transformations:
 $\text{p-aminophenol} + \text{ethanol} \rightarrow ? + \text{acetic acid chloride} \rightarrow ?$
2. Write the scheme of interaction between γ -oxybutyric acids and sodium (in excess).
3. Draw the structure of tripeptide Thr-Asp-Val and define its isoelectric point.

№ 13

1. Write qualitative reaction for salicylic acid with FeCl_3 .
2. Write the reaction equation of malic acid dehydration and name the product.
3. Write the scheme of dipeptide Gly-Tyr-Val formation and define its isoelectric point.

№ 14

1. Write esterification reaction mechanism for p-amino-benzoic acid.
2. Write the reaction equation of pyruvic acid with HCl. What is the mechanism of this reaction?
3. Draw the structure of tripeptide Thr-Gly-Ala and define its isoelectric point..

№ 15

1. Write the reaction equation for novocain formation as a result of interaction between p-aminobenzoic acid and diethylaminoethanol. Write the structure of novocain hydrochloride used in medicine.
2. What is the difference between lactic acid and pyruvic acid?
3. Write the scheme of dipeptide Gly-Tyr-Ala formation and define its isoelectric point..

EDUCATION LITERATURE

1. Biological and Bioorganic chemistry: in two books: Textbook. Textbook 1. Bioorganic chemistry / B.S. Zimenkovsky, V.A.Muzyhenko, I.V. Nizhenkovska, G.O. Syrova; edited by B.S. Zimenkovsky, I.V. Nizhenkovska. – K.: AUP “Medicina”, 2017. – 288 p.
2. Fundamentals of bioorganic chemistry : manual / A.O. Syrovaya, E.R. Grabovetskaya, V.N. Petiunina. – Kharkiv : KhNMU, 2016. – 191 p.
3. Amino acids, peptides, proteins: methodical instructions for the 1st-year students' self-work in biological and bioorganic chemistry (module 1) /

- compiled A.O. Syrovaya, L.G. Shapoval, V.N. Petiunina, E.R. Grabovetskaya, N.M. Tkachuk, V.A. Makarov, S.V. Andreeva, S.A. Nakonechnaya, R.O. Bachinskiy, S.N. Kozub, T.S. Tishakova, L.V. Lukyanova, O.L. Levashova, N.V. Vakulenko, N.N. Chalenko. – Kharkov: KNMU, 2013. – 26 p.
4. Hydroxy- and oxoacids. Heterofunctional compounds of benzene series. Metabolites and parent structures of medicines: methodical instructions for 1st year students' self-work in Biological and Bioorganic Chemistry (module 1) / compiled by A.O. Syrovaya, L.G. Shapoval, V.N. Petyunina et al. – Kharkiv: KhNMU, 2014. – 25 p.
 5. Structure, properties and biological significance of derivatives of carboxylic acids (hydroxyl-, keto- and phenolic acids). Amino acid composition of proteins and peptides. Structural organization of proteins: methodical instructions for 1st year students' self-work / compiled by A.O. Syrovaya, V.N. Petyunina, V.O. Makarov et al. – 2nd edition, revised, corrected and expanded – Kharkiv: KhNMU, 2018. – 34 p.
 6. Text of Lectures.

SUGGESTED READINGS

1. Biologically important classes of bioorganic connections. Biopolymers and their structural components: Theoretical course of biological and bioorganic chemistry, Module 1 / A. O. Syrovaya, E. R. Grabovetskaya, N. M. Tkachuk, L. G. Shapoval, V. N. Petiunina, S. A. Nakonechnaya. – X.: «Цифровая типография № 1». – 2013. – 183 p.
2. Zurabyn S. E. Fundamentals of Bioorganic Chemistry. – M.: GEOTAR-MED, 2003. – 320 p.

Навчальне видання

Будова, властивості та біологічне значення функціональних похідних карбонових кислот (гідрокси-, кето- та фенолокислот). Амінокислотний склад білків та пептидів. Структурна організація білків

Методичні вказівки для самостійної роботи студентів 1-го курсу з біологічної та біоорганічної хімії (Модуль 1)

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