

**KHARKIV NATIONAL MEDICAL UNIVERSITY  
DEPARTMENT OF MEDICAL AND BIOORGANIC CHEMISTRY**

A. O. Syrovaya, E. R. Grabovetskaya, V. N. Petiunina

**FUNDAMENTALS OF BIOORGANIC CHEMISTRY**

**Manual**

Kharkiv  
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**Reviewers:**

V.A. Georgiyanz, Pharm.D., Professor (National University of Pharmacy, Kharkiv)

A. V. Chubenko, PhD in Pharmacy, Associate Professor (Kharkiv Medical Academy of Postgraduate Education)

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The Manual includes basic topics in Bioorganic Chemistry according to ECTS: structure and chemical properties of biologically active compounds - carbohydrates, carboxylic acids and their functional derivatives, lipids,  $\alpha$ -amino acids, peptides, proteins, biologically active heterocycles, nucleosides, nucleotides, and nucleic acids. The current concepts on the theoretical fundamentals of electronic structure and reactivity of main classes of bioorganic compounds are presented. Much attention is paid to the biological activity of organic substances. The manual is intended for the students of the faculty of medicine and the faculty of dentistry.

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## **PREFACE**

We feel pleasure in presenting the manual in Bioorganic chemistry for the First year English medium students of Medical Universities. The manual is written strictly in accordance with the prescribed syllabus. We feel convinced that the students face a lot of difficulty in understanding the language and the contents of the chemistry books. Therefore, we have made an attempt to bring out a simplified, helpful, comprehensible book. The information has been given as detailed description of all theoretical and practical questions needed to complete the course of Bioorganic chemistry successfully in accordance with the modern examination system. The contents of the manual comprise all topics to be considered.

It is hoped that the manual will be received as an effective text book and English medium students would find mastering the basics of Bioorganic chemistry not such a difficult task after going through the contents.

# CLASSIFICATION, NOMENCLATURE, STRUCTURE, AND REACTIVITY OF ORGANIC COMPOUNDS

## *Characteristics of the subject*

When studying the chemical processes, which take place in the organism, you will meet numerous organic substances. To know all variety of substances, it is necessary to know their scientific classification and nomenclature.

Chemical properties of physiologically active substances (including their behavior in biochemical reactions) are determined by their composition, electronic and spatial structure and also by mutual influence of atoms in these substances.

## *Purposes*

To explain rules of IUPAC nomenclature and to be able to apply them in naming of bioorganic substances;

To make conclusions and analyze relation between structure, configuration and conformation of bioorganic substances;

To study dependence of bioorganic compounds reactivity on nature of chemical bond and mutual influence of atoms in a molecule;

To study mechanisms of reactions of different families of bioorganic compounds and their changes in biological systems.

## *Objectives*

1. To know the classification of organic substances according to the carbon skeleton and nature of functional group.
2. To know the nature of different types of isomerism of organic substances.
3. To be able to name organic substances using IUPAC nomenclature and rational nomenclature.
4. To know the electronic structure and spatial arrangement of bonds formed by carbon atoms in  $sp^3$ ,  $sp^2$  and  $sp$ -hybridization states.
5. To understand the characteristics of electronic structure of conjugated systems with opened and closed chains.
6. To be able to determine the sign and kind of electronic effects of substituents.
7. To know the main types and mechanisms of organic reactions.

### *Theoretical questions*

1. Structure and nomenclature of organic compounds. Electronic structure of carbon atom and its chemical bonds.
2. Space structure of carbon compounds. Conformational isomerism.
3. Conjugated systems.
4. The effects of substituent groups on the reactivity of compounds.
5. Main types and mechanisms of organic reactions.

## ***1. STRUCTURE AND NOMENCLATURE OF ORGANIC COMPOUNDS. ELECTRONIC STRUCTURE OF CARBON ATOM AND ITS CHEMICAL BONDS***

Organic chemistry was originally described as the chemistry of compounds found in living things – in plants and animals. All such naturally occurring compounds contain carbon, and it was thought that some "vital force" was needed for their formation. When Wöhler, in 1828, made urea from inorganic salt, ammonium cyanate,  $\text{NH}_4\text{CNO}$ , he changed the definition of organic chemistry. Today, the term organic chemistry refers to the chemistry of millions of carbon compounds.

Theory of chemical structure of organic compounds was formulated by Butlerov A.M. in 1861. The importance of this theory for organic chemistry is like the importance of Mendeleev's Periodic Table for inorganic chemistry. This theory enables to systematize all organic substances and to explain their properties.

*The main directions of Butlerov's theory:*

1. Atoms of elements, which form molecules, are combined in definite order according to their valence and all valences should be used to combine each other.
2. Properties of organic compound depend not only on its composition but on its structure as well, i.e. on the order of combining of atoms in a molecule and a character of atom bonds.
3. Atoms in a molecule influence each other and especially those, which are directly combined.

A number of conclusions concerning carbon and connected with its position in Periodic Table follows from the theory:

1. Carbon is tetravalent;

2. Carbon can be combined with both metals and nonmetals;
3. All carbon valences are identical;
4. Carbon atoms can be combined in long chains and rings;

There are some nomenclatures, systems, regulations that enable to name organic substances.

- Trivial. It appeared in ancient times and the names generally explain the origin of substances: formic, acetic, malic acids, etc.

- Rational. It is based on the trivial nomenclature. Greek letters mark location of substituents in a chain.

- International IUPAC. According to this nomenclature parent structure – the basis of a compound determines the name of organic substance. All other parts of a molecule are considered to be substituents. To be named according to IUPAC it is necessary:

1. To number the longest chain (or atoms in a ring), but to start from that end to which the substituent is closer. In heterocycles it should be started from a heteroatom.

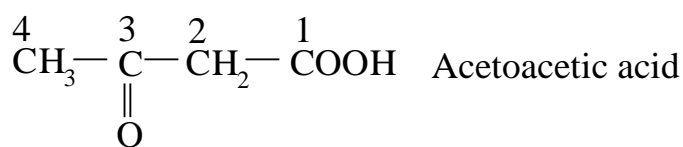
2. The numbers of carbon atoms have to be marked with figures.

3. To name the substituents.

4. To name a parent structure.

5. If there are several identical substituents in a formula, a number has to be written out in words before their names (di-, tri-, tetra-). Commas should separate the numbers of substituents.

6. The complete IUPAC name of organic compound consists of prefix, word root, primary suffix and secondary suffix.

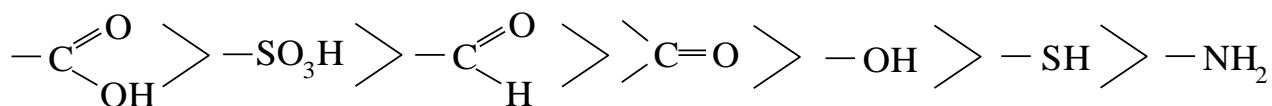


3-oxobutanoic acid

Prefix    Root    Primary suffix    Secondary suffix

CLASSES OF ORGANIC COMPOUNDS

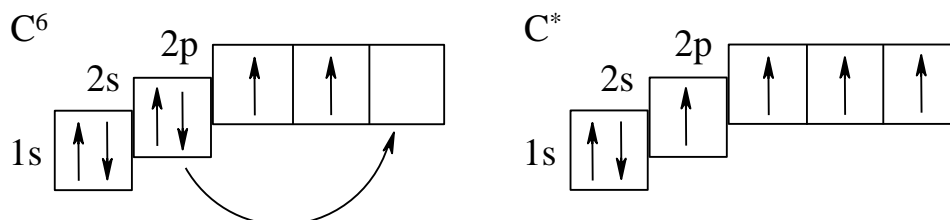
<i>Functional group</i>	<i>Name of class</i>	<i>General formula</i>	
-F, -Cl, -Br, -I (Hal) Halogens	Halogen derivatives	R-Hal	
-OH      Hydroxyl	Alcohols, phenols	R-OH	
-OR      Alkoxy	Ethers	R-OR	
-SH      Thiol	Thiols	R-SH	
-SR      Alkylthiol	Thioethers (sulphides)	R-SR	
-SO <sub>3</sub> H      Sulphonic	Sulphoacids	R-SO <sub>3</sub> H	
-NH <sub>2</sub> >NH >N-	Amino Amines	R-NH <sub>2</sub> R <sub>2</sub> NH R <sub>3</sub> N	
-NO <sub>2</sub> Nitro	Nitrocompounds	R-NO <sub>2</sub>	
-C≡N      Cyano	Nitriles	R-C≡N	
>C=O      Carbonyl	Aldehydes, ketones	$\begin{array}{l} \text{R}-\text{C} \begin{array}{l} \nearrow \text{O} \\ \searrow \text{H} \end{array} \\ \text{R}-\text{C}-\text{R} \\ \parallel \\ \text{O} \end{array}$	
$\begin{array}{l} \text{O} \\ \parallel \\ \text{—C} \\ \backslash \\ \text{OH} \end{array}$	Carboxyl	Carboxylic acids	$\text{R}-\text{C} \begin{array}{l} \nearrow \text{O} \\ \searrow \text{OH} \end{array}$
$\begin{array}{l} \text{O} \\ \parallel \\ \text{—C} \\ \backslash \\ \text{OR} \end{array}$	Alkoxycarbonyl	Esters	$\text{R}-\text{C} \begin{array}{l} \nearrow \text{O} \\ \searrow \text{OR} \end{array}$
$\begin{array}{l} \text{O} \\ \parallel \\ \text{—C} \\ \backslash \\ \text{NH}_2 \end{array}$	Carboxamide	Amides	$\text{R}-\text{C} \begin{array}{l} \nearrow \text{O} \\ \searrow \text{NH}_2 \end{array}$



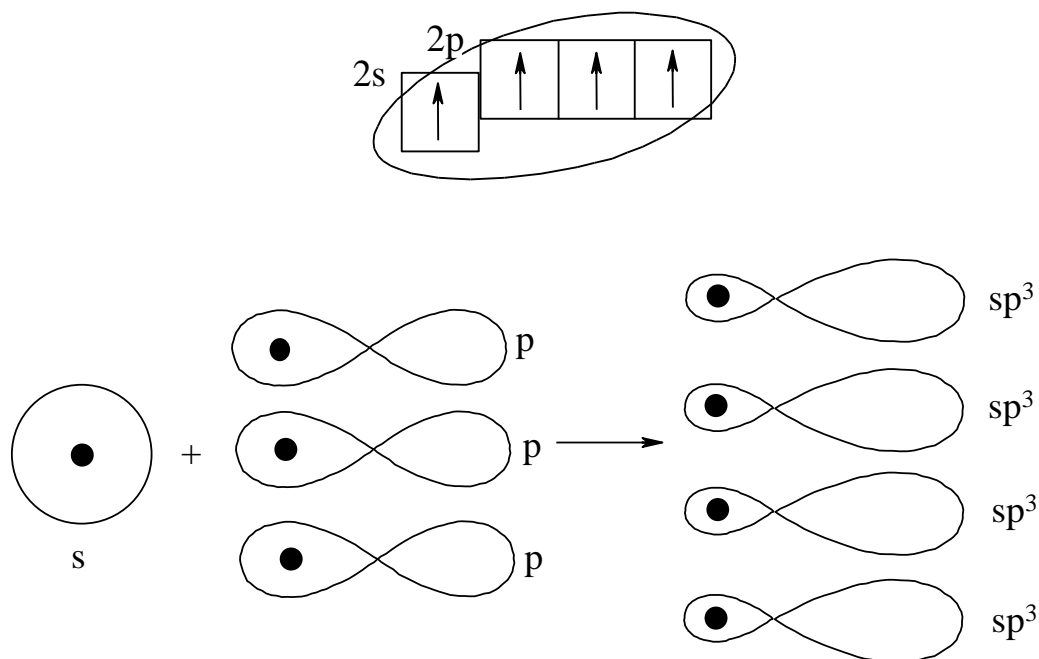


Carbon forms more compounds than any other element.

Let's consider the electronic configuration of carbon atom in its normal and excited,  $C^*$ , states.

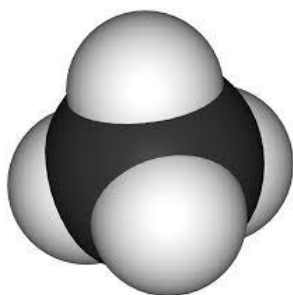
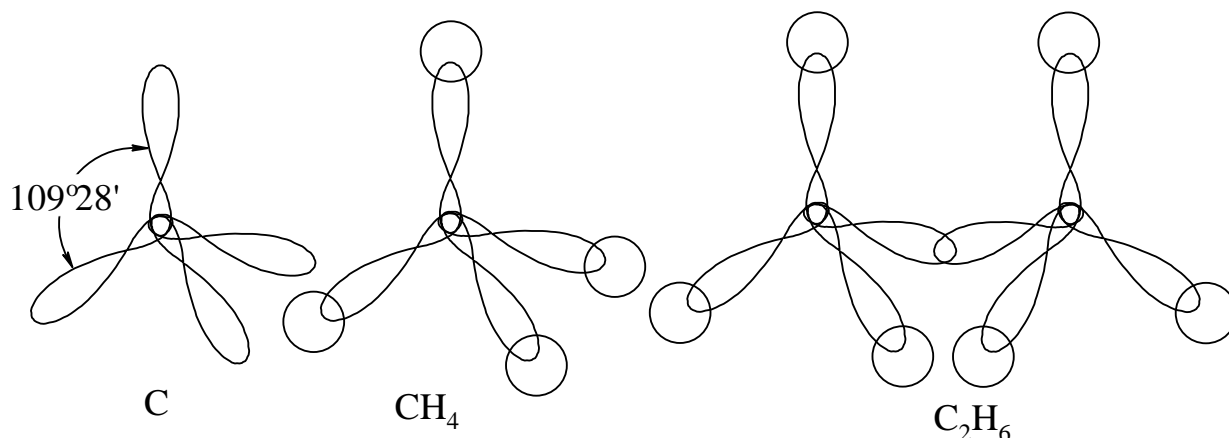


Each carbon atom has two unpaired electrons, and one might expect carbon to form two bonds. It would not then attain a stable outer octet of electrons (a neon-like structure): it needs to share four electrons to do this. A sharing of four electrons can be achieved by promoting one of the 2s electrons into the 2p level. (The atom must absorb energy in order to promote the electron and is described as an "excited" atom). The excited carbon atom might be expected to form two different kinds of bond, using one s orbital and three p orbitals. Actually, the electron density distributes itself evenly through four bonding orbitals, which are called  $sp^3$  hybrid orbitals. The  $sp^3$  atomic orbital is more concentrated in direction than a p orbital. An  $sp^3$  orbital is therefore able to overlap more extensively and form stronger bonds than a p orbital.

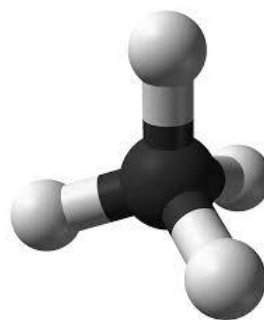


$sp^3$  orbitals have axes directed to the corners of a regular tetrahedron with the carbon nucleus at the center. The angle between the bonds is shown to be  $109^\circ 28'$ , pairs of electrons on the orbitals experience mutual repulsion. To minimize this

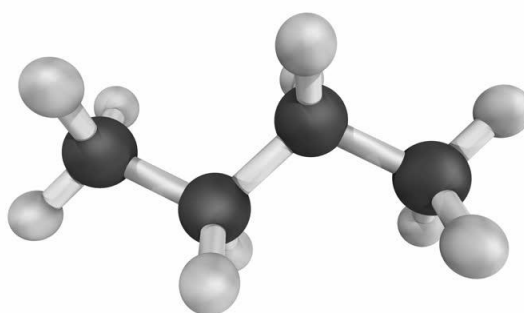
repulsion, the four electron orbitals adopt the spatial arrangement that maximizes the angle between the orbitals. This is the tetrahedral arrangement.  $sp^3$  orbitals lie as far apart from each other as possible in space due to electron repulsion.



Space-filling model of methane molecule

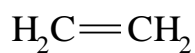


Ball-and-stick model of methane molecule

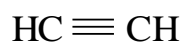


Ball-and-stick model of butane molecule

When a carbon atom combines with four hydrogen atoms, it forms a molecule of methane. If two carbon atoms join, each can still combine with three hydrogen atoms to form a molecule of ethane, etc. Compounds of C and H are called **hydrocarbons**. The compounds shown above are alkanes, they are said to be saturated as they contain only single bonds between carbon atoms. Alkenes and alkynes are **unsaturated** hydrocarbons: they contain multiple bonds between carbon atoms. Alkenes contain double bonds, alkynes – triple bonds:

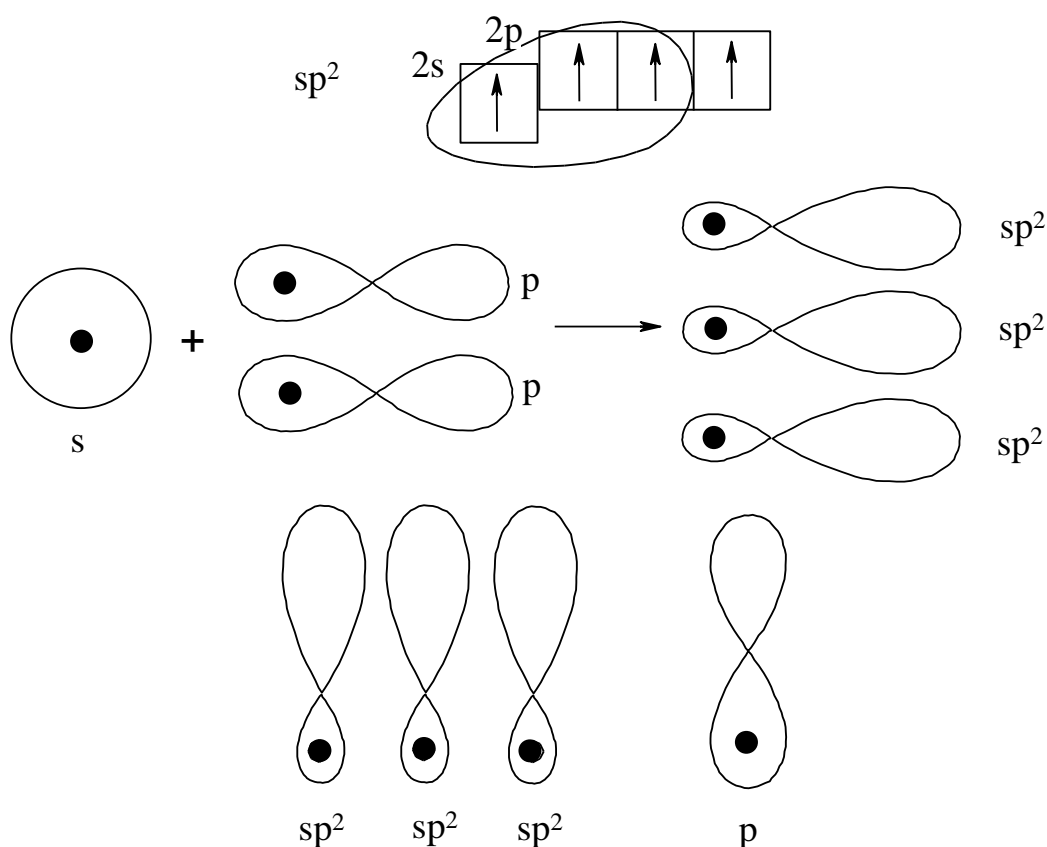


Ethylene

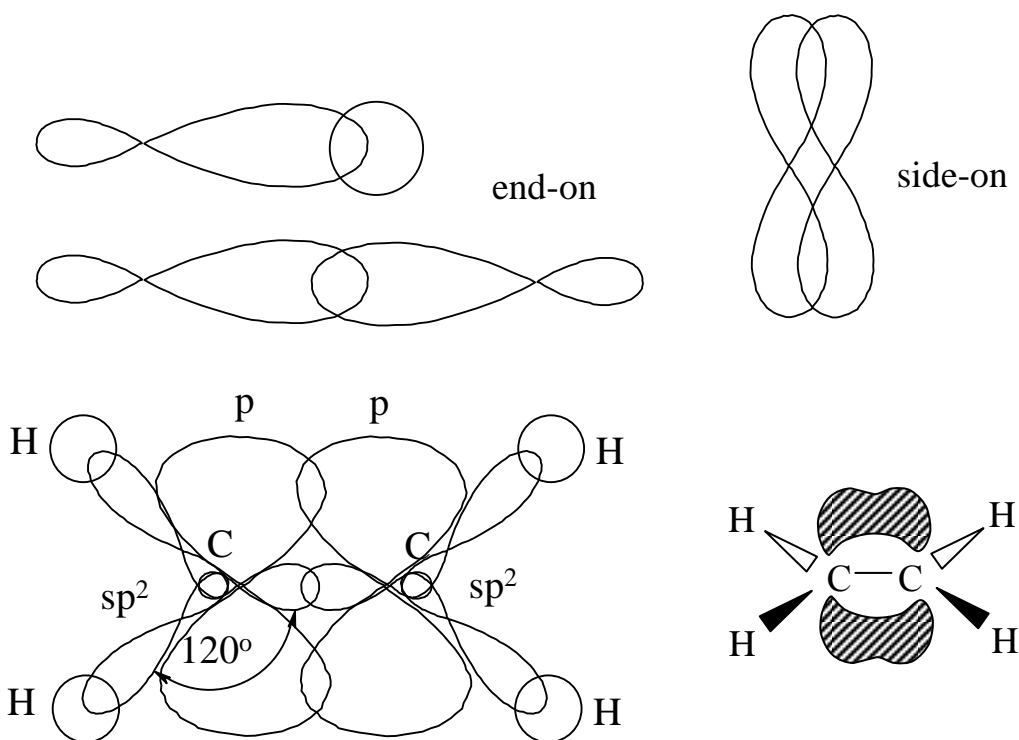


Acetylene

The double bond is not simply two single bonds. In a molecule of ethene (ethylene), each carbon atom uses a 2s orbital and two of three 2p orbitals to form three  $sp^2$  hybrid orbitals.

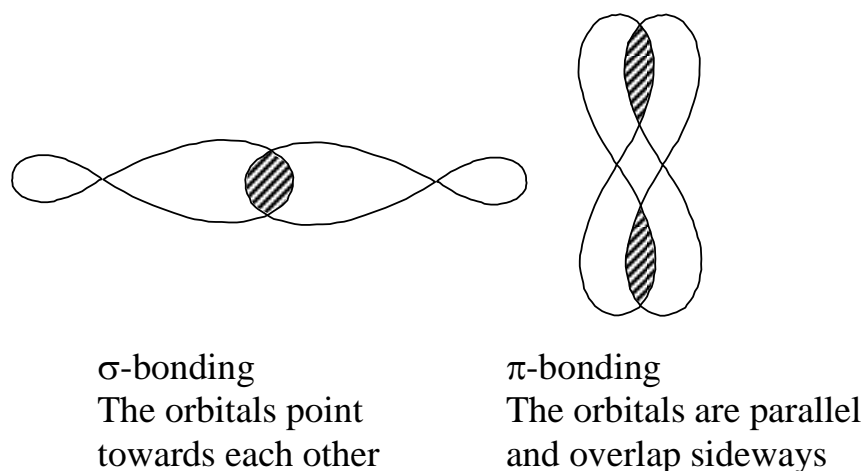


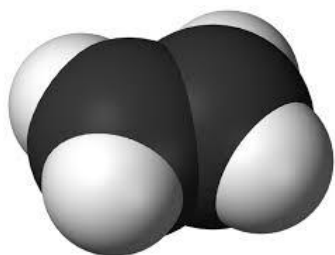
The carbon-carbon bond formed when the  $sp^2$  orbitals of neighboring carbon atoms overlap is called a  $\sigma$  bond. In  $\sigma$  bonds, e.g. any single bond, overlap of atomic orbitals occurs along the line joining the two bonded atoms (end-on overlapping). There is an unhybridized p orbital at right angle to the plane of the two  $sp^2$  orbitals, and the p orbitals on adjacent carbon atoms are close enough to overlap. The overlapping occurs at the sides of the orbitals (side-on overlapping).



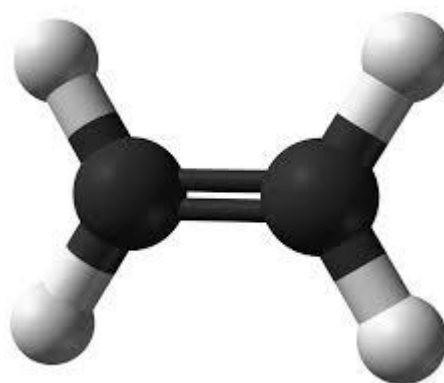
This type of bond, produced by sideways overlapping of  $p$  orbitals above and below the plane of  $sp^2$  bonds, is called a  $\pi$ , **pi**, bond. It is not as strong as a  $\sigma$  bond since there is less overlapping of orbitals.

Since overlapping of  $p$  orbitals on adjacent carbon atoms can occur only when the  $p$  orbitals are parallel, the two structures must be coplanar, i.e.  $\text{>C—}$  lies in the same plane. The  $\sigma$  bonds lie in the same plane at an angle of  $120^\circ$ . If one  $\text{CH}_2$  group twists with respect to the other, the amount of overlapping of  $p$  orbitals will decrease. And the  $\pi$  bond will be partially broken. Since it requires energy to break a bond, the most stable arrangement of the molecule is the one in which all six atoms lie in the same plane.



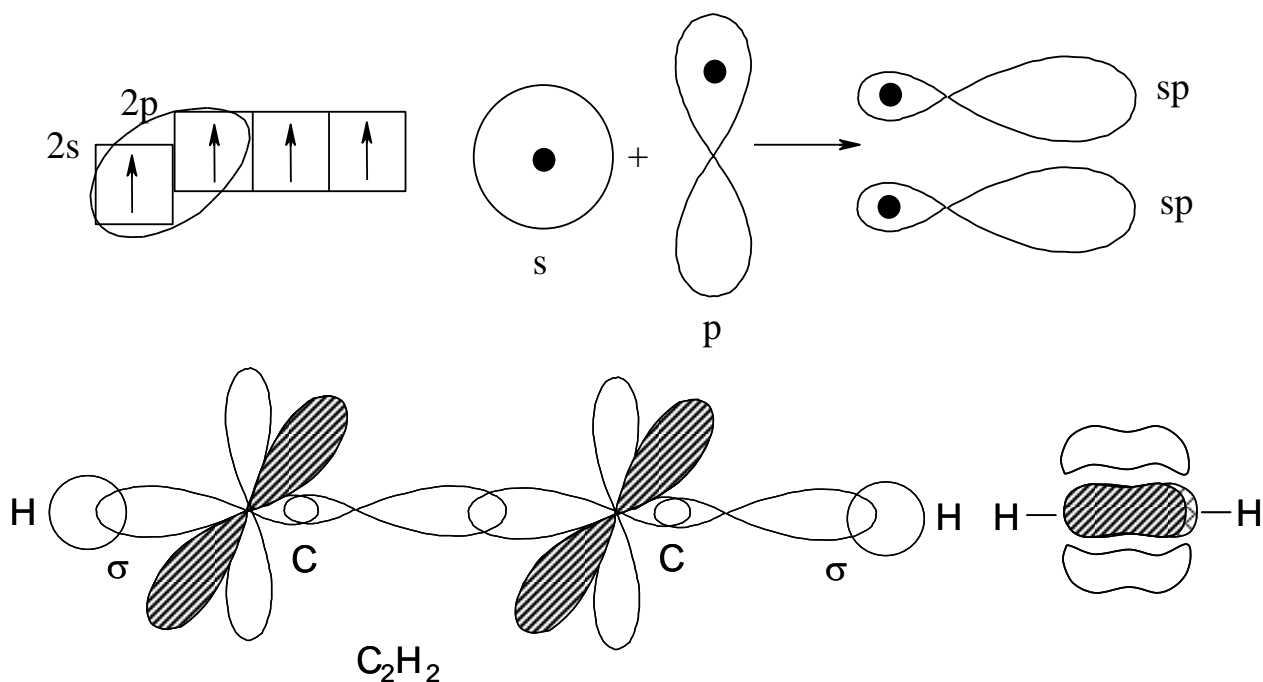


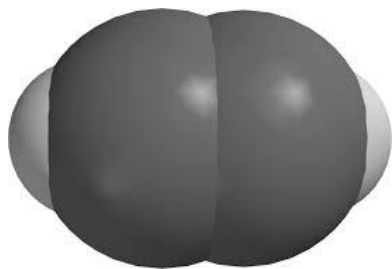
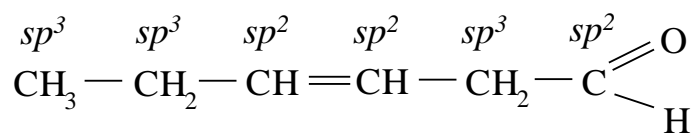
Space-filling model of ethene molecule



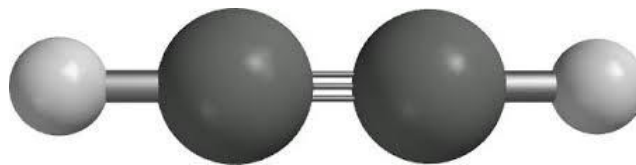
Ball-and-stick model of ethene molecule

When a carbon atom is bonded to only two other atoms, as in C<sub>2</sub>H<sub>2</sub> the two formed  $\sigma$  bonds use  $sp$  hybrid orbitals. In acetylene  $\sigma$  bonds are formed by the overlapping of  $sp$  hybrid orbitals of the two carbon atoms and by the overlapping of  $sp$  orbitals of each carbon atom with the  $1s$  orbital of a hydrogen atom. For maximum overlapping, the four atoms must lie in a straight line. The angle between the bonds is  $180^\circ$ , molecule is linear. Two  $\pi$  bonds are formed by overlapping of the remaining unhybridized  $p$  orbitals. There are two of these on each carbon atom at right angles to the  $sp$  hybrid orbitals. The two  $\pi$  bonds lie in mutually perpendicular planes, it can be said that one  $\pi$  bond is formed above and below the line of the  $\sigma$  bonds, the other in front and at the back.





Space-filling model of ethyne molecule



Ball-and-stick model of ethyne molecule

As it was mentioned,  $\pi$  bond is not as strong as a  $\sigma$  bond, so unsaturated hydrocarbons are more reactive than saturated ones.

## **2. SPACE STRUCTURE OF CARBON COMPOUNDS. CONFORMATIONAL ISOMERISM**

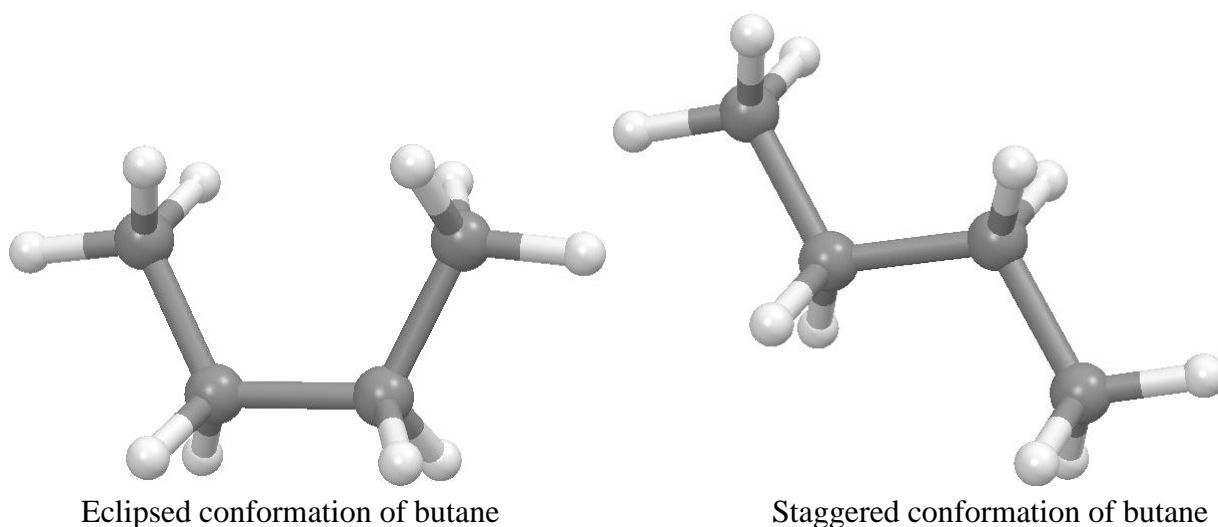
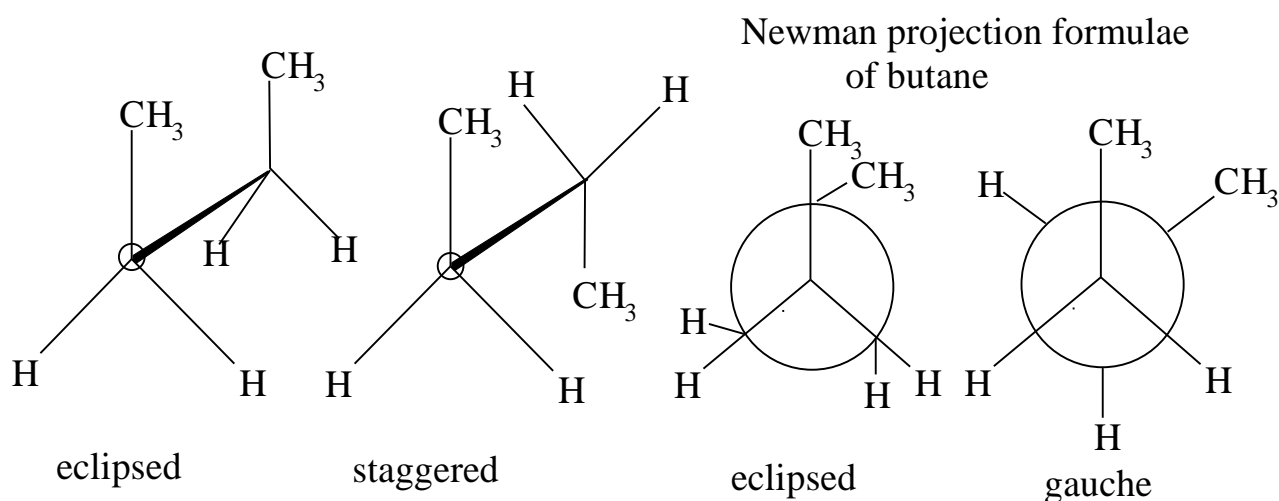
$\sigma$ -bond by means of which  $sp^3$ -hybridized carbon atoms are bonded cannot be classified as a rigid one. Rotation about this bond as it were around its axis is possible and a large variety of molecular forms named conformations are being formed in this process.

Conformations are different forms of molecules, which are formed as a result of atoms rotation about the single bonds.

Mutual conversions of conformations take place without breaking the bonds.

Of all the variety of conformations only those, which angle of rotation about  $\sigma$ -bond is  $60^\circ$  different are to be examined, i.e. six conformations in all. Newman projection formulae are used to represent them. Capability of alkanes to turn round in the homologous series starts with ethane.

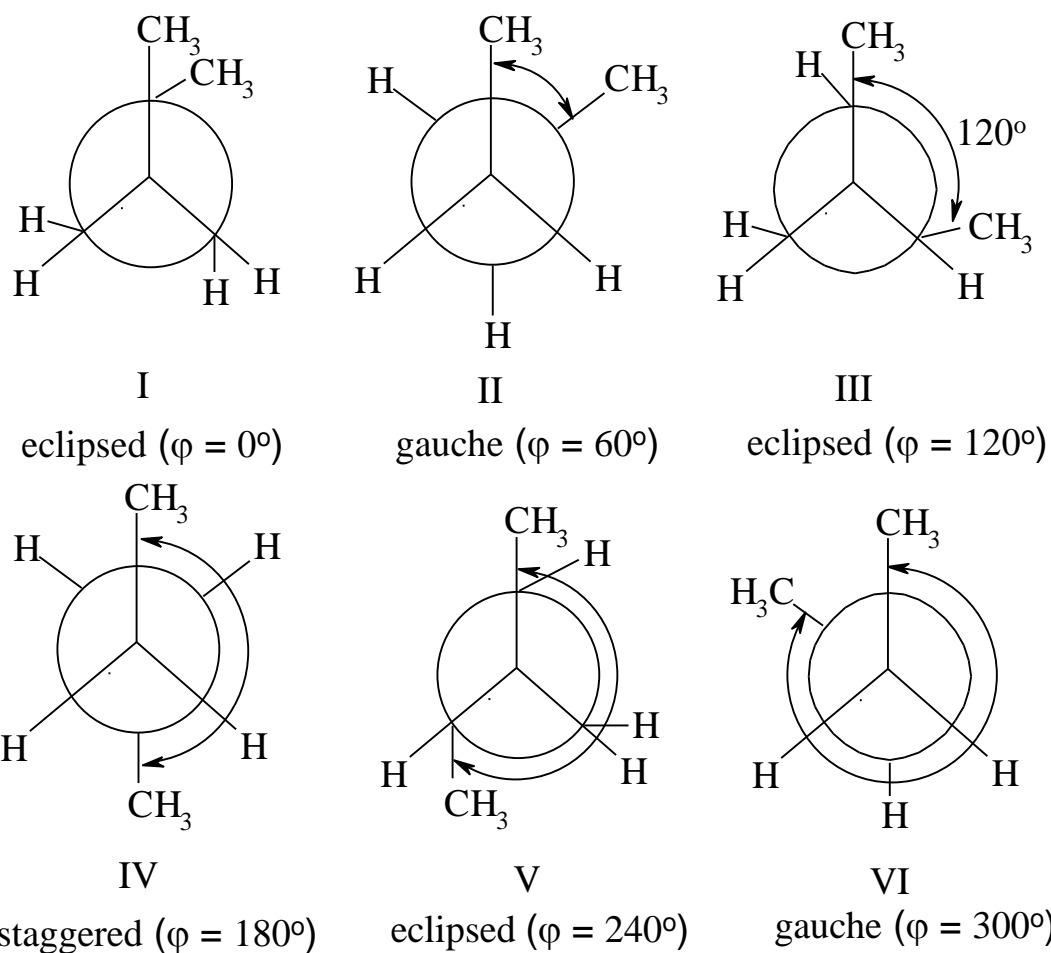
The molecule is being projected onto the plane along the C-C bond. The nearest to the examiner carbon atom is marked as a point in the center of the circle. The circle represents a rear atom. Three bonds of the nearest C-atom are indicated with the lines dispersed from the center of the circle while the bonds of the rear one – with the lines leaning out of the circle.



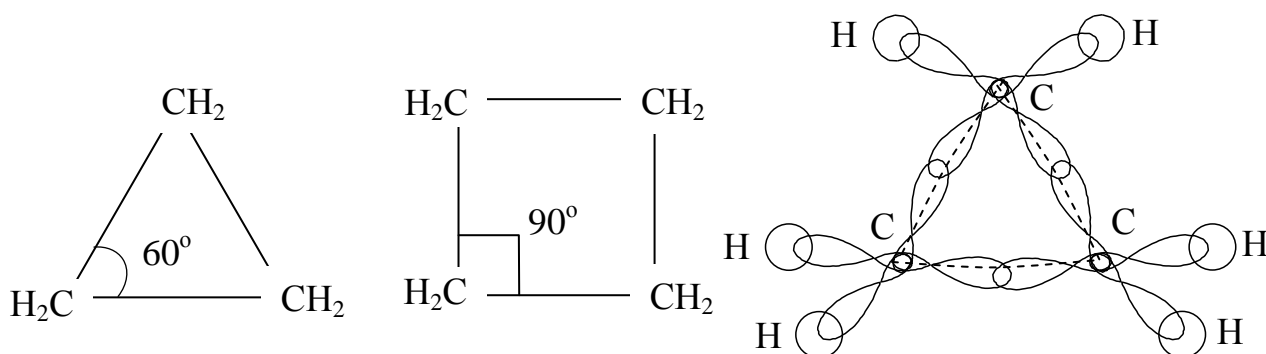
In the eclipsed conformation the substituents are located in a position closest to each other i.e. one group is by another. The distance between the atoms, which are not bonded, is minimal while the interaction is maximal. Such type of conformation is not stable.

In the staggered conformation (anti-conformation) the distance between the atoms, which are not bonded is maximal while the interaction is minimal. This type of conformation is stable. 69% of butane molecules exist as staggered conformation.

Conformations with the angle of  $60^\circ$  between  $\text{CH}_3$ - groups are called gauche conformations.



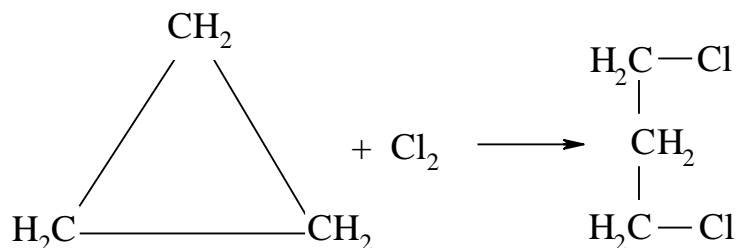
Cyclopropane as well as cyclobutane should be referred to small cycles. The angle between the bonds of cyclopropane, which molecule represents equilateral triangle, is  $60^\circ$  while the normal angle between  $sp^3$ -hybrid orbitals is to be equal to  $109^\circ 28'$ , that's why a strong angle strain arises in the molecule; cyclopropane is unstable. The bonds of cyclopropane differ from the regular  $\sigma$ -bonds. The overlap of the electron orbitals takes place not on the line connecting the centers of atoms (as it is when a regular  $\sigma$ -bond takes place) but above it. Such a bond is called "bent"-bond (or  $\tau$ -bond).



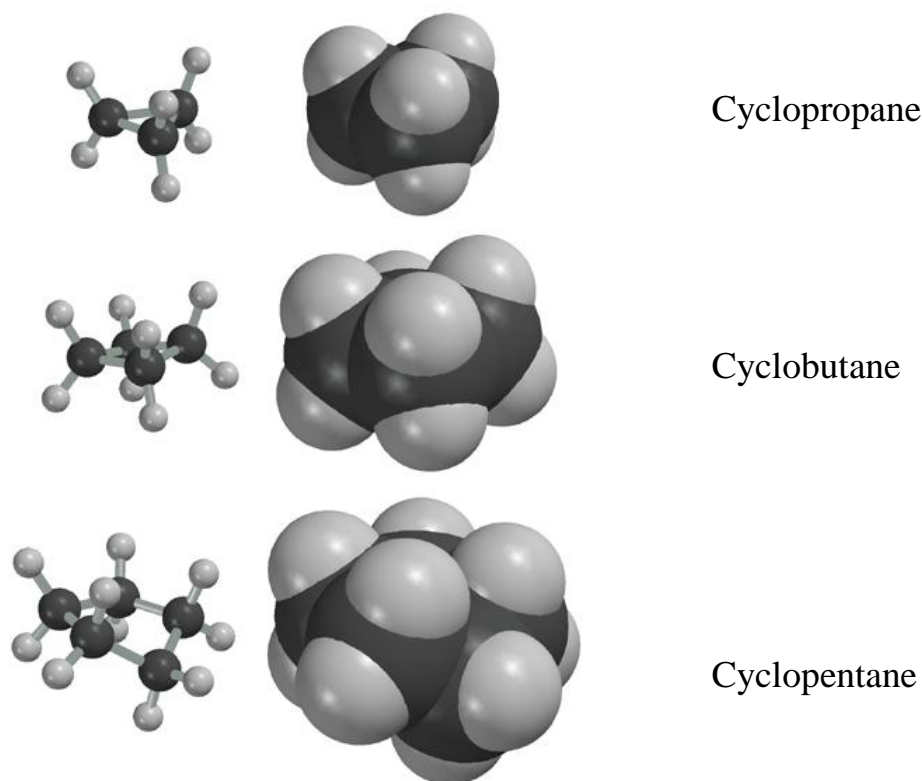


Valence angle in cyclobutane equals  $90^\circ$ , which means it also much differs from the normal tetrahedral angle of  $109^\circ28'$ . Owing to this the molecule of cyclobutane is unstable.

Since the “bent”-bond is similar to the  $\pi$ -bond the reactions of addition (not of substitution) are typical for the small cycles as well as for unsaturated hydrocarbons, the cycle being broken during the reaction.



Valence angles of cyclopentane (five-membered cycle) are equal to  $108^\circ$ , which is close to the normal value. Owing to this the molecule is stable and not strained.



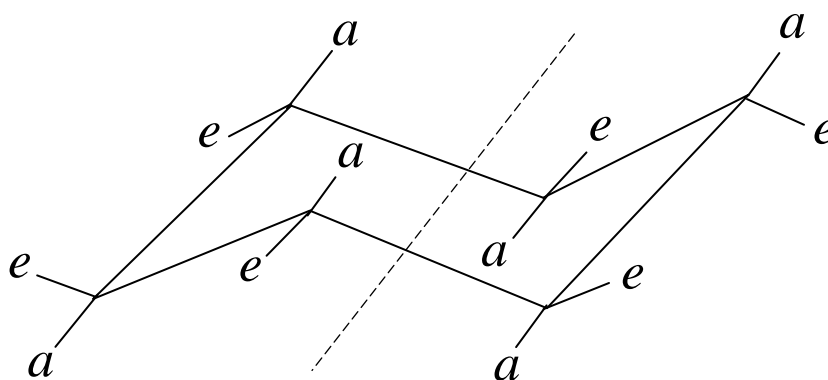
Cyclohexane couldn't represent a regular hexagon, as in this case the angle would be  $120^\circ$ . That's why rotation around  $\sigma$ -bonds in cyclohexane takes place in such way that the angle between the bonds is  $109^\circ28'$ . Due to such rotation cyclohexane assumes a chair and a boat conformation. These conformations are free

of angle strain. A chair conformation is more stable and, because of the greater stability of the chair, more than 99% of the molecules are estimated to be in a chair conformation at any given moment.

12 C–H bonds in cyclohexane molecule can be divided into two groups:

1. Axial. They are placed parallel to the axis of symmetry of the chair-like molecule and directed alternately up and down.

2. Equatorial. They are placed to the axis of symmetry at the tetrahedral angle of  $109^{\circ}28'$  and are also directed alternately up and down. We can say the equatorial bond is placed parallel to the line next nearest.

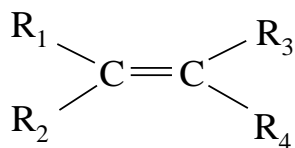


So, one bond with a hydrogen atom in every carbon atom of a chair-conformation of cyclohexane is axial and the other one is equatorial. During the reaction of substitution, the substituents often have equatorial location because it is more advantageous from the energetic point of view.

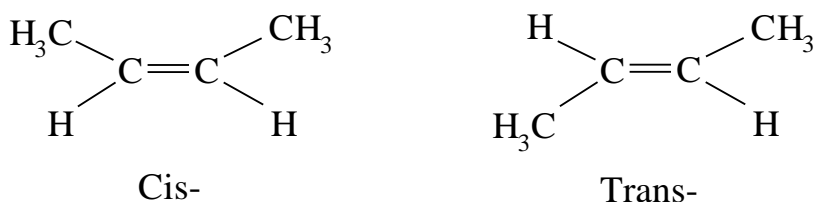


Ball-and-stick model of chair conformation of hexane

### *Cis – trans – isomerism*

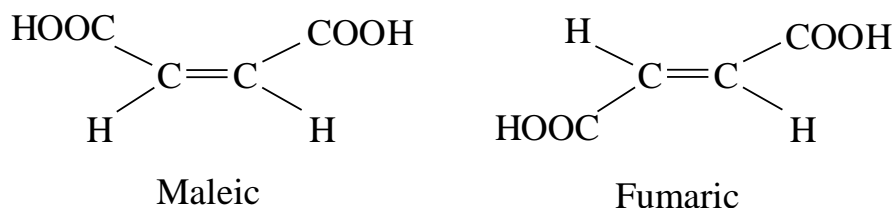


There is the planar arrangement of double bonds in  $R_2C=CR_2$  molecules. The  $CR_2$  groups are not free to rotate about the double bond. Rotation of the  $CR_2$  groups about the  $C=C$  bond is inhibited by the requirements for the formation of a  $\pi$  bond. The restriction of rotation about the  $C=C$  bond gives rise to cis-trans isomerism. For example, butene-2 has two isomers:

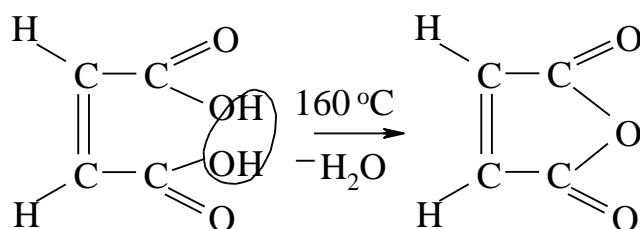


The geometry of the molecules is different. The isomer with both  $CH_3$ – groups on the same side of the double bond is called the cis-isomer, and the isomer with the  $CH_3$ – groups on opposite sides of the double bond is called trans-isomer. Cis-trans isomers have the same molecular formula and also the same structural formula, the difference between them is the arrangement of the bonds in space.

Cis-trans isomers do not have the same physical and chemical properties. For example, let's consider cis- and trans-butenedioic acid:



The isomers differ in melting temperature (cis -135°, trans- 287°), in solubility (cis being 100 times more soluble than trans). The cis acid (called Maleic) forms an anhydride on gentle heating, but the trans isomer (Fumaric acid) does not.



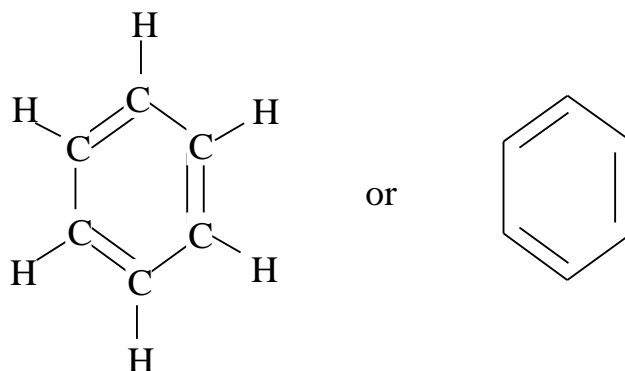
Fumaric acid is widely spread in nature and participates in the processes of metabolism. In particular, it is an intermediate in Krebs cycle.

### 3. CONJUGATED SYSTEMS

In the compounds discussed so far, the electrons in the  $\sigma$  and  $\pi$  bonds have been located in the region *between* nuclei of the bonded atoms. They are **localized** electrons. In some molecules, some of the electrons are **delocalized**: they do not remain between a pair of atoms.

#### *Benzene*

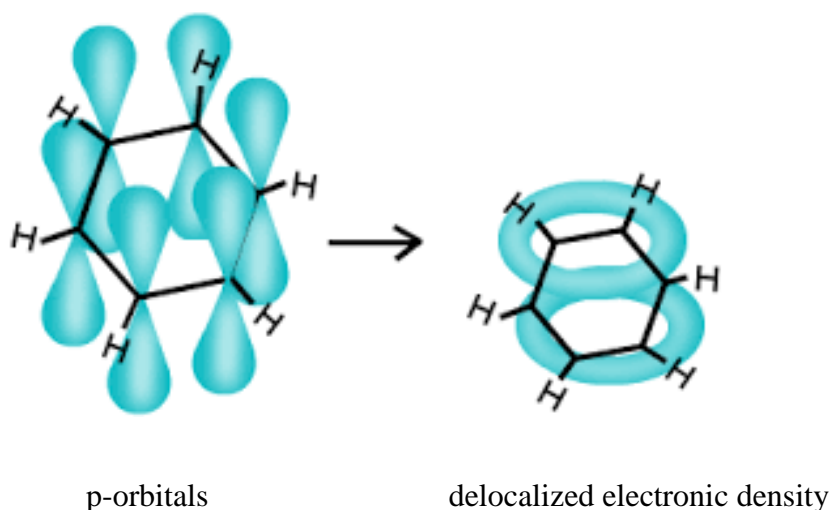
Benzene is an aromatic hydrocarbon. All carbon atoms are  $sp^2$  hybridized. Kekulé proposed the structural formula for benzene.



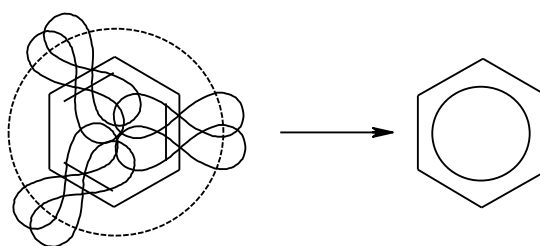
An alternating system of single and double bonds is called a **conjugated double bond system**. Between each pair of adjacent carbon atoms is a  $\sigma$  bond, formed by overlapping of  $sp^2$  hybrid orbitals. Since  $sp^2$  bonds are coplanar, all the carbon atoms lie in the same plane and form a regular hexagon. The unhybridized  $p$  orbitals of the carbon atoms are perpendicular to the plane of the hexagonal benzene "ring". As in ethene overlapping of  $p$  orbitals on adjacent carbon atoms gives rise to  $\pi$  bonds. In benzene, the  $p$  orbitals are able to overlap all round the ring. The electrons in the  $p$  orbitals cannot be regarded as located between any two carbon atoms: they are free to move between all the carbon atoms in the ring. They are described as **delocalized** and

are represented as an annular cloud of electron density above and below the plane of the molecule.

The phenomenon of delocalization of electrons is called conjugation. Electrons forming  $\pi$ -bonds are conjugated in benzene that's why such a conjugation is called  $\pi,\pi$ -conjugation. Energy is released during the formation of conjugated system and it makes the conjugated system stable (the less the store of the internal energy – the more stable the system is). This energy is called the **delocalization energy** of benzene. Because of the stability benzene is unreactive in comparison with the alkenes.



The formula of benzene is written as following to represent the delocalization of  $\pi$  electrons:



**Aromatic** hydrocarbons are related to benzene. The first isolated benzene compounds had pleasant aromas, and gave this group of hydrocarbons their name. Aromatic compounds resemble benzene in chemical behavior. They undergo substitution reactions rather than addition reactions. This characteristic behavior is called **aromatic character or aromaticity**.

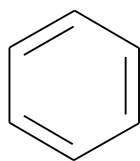
## Criteria of Aromaticity

An aromatic compound must have a planar cyclic molecule which:

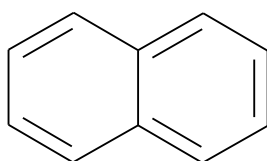
1. Contains a cyclic cloud of delocalized  $\pi$  electrons above and below the plane of the molecule.

2.  $\pi$ -electrons cloud must contain a total of  $(4n+2)$  electrons, where  $n$  is the number of cycles in molecule. *This is known as **Hückel's rule**.*

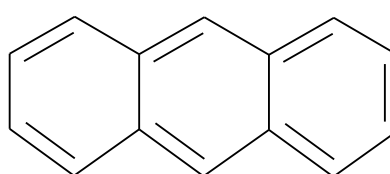
For example, according to the Hückel's rule, benzene have delocalized electron cloud of 6  $\pi$  electrons (sextet), naphthalene – 10  $\pi$  electrons, anthracene – 14  $\pi$  electrons, these are aromatic compounds.



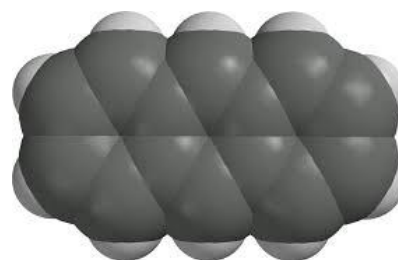
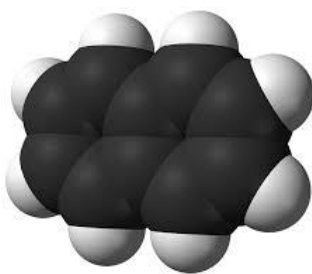
Benzene



Naphthalene

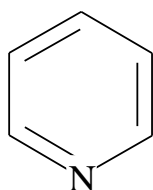


Anthracene

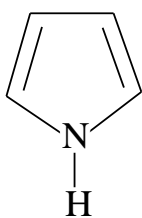


In molecules of many cyclic compounds an element other than carbon is present in the ring. These compounds are called *heterocyclic compounds*. Heterocyclic molecules are quite commonly encountered in nature.

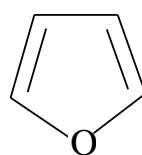
Heterocyclic compounds containing nitrogen, oxygen, or sulfur are by far the most common. Four important examples are given here in their Kekulé forms. These four compounds are all aromatic:



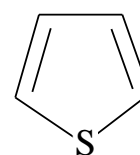
Pyridine



Pyrrole



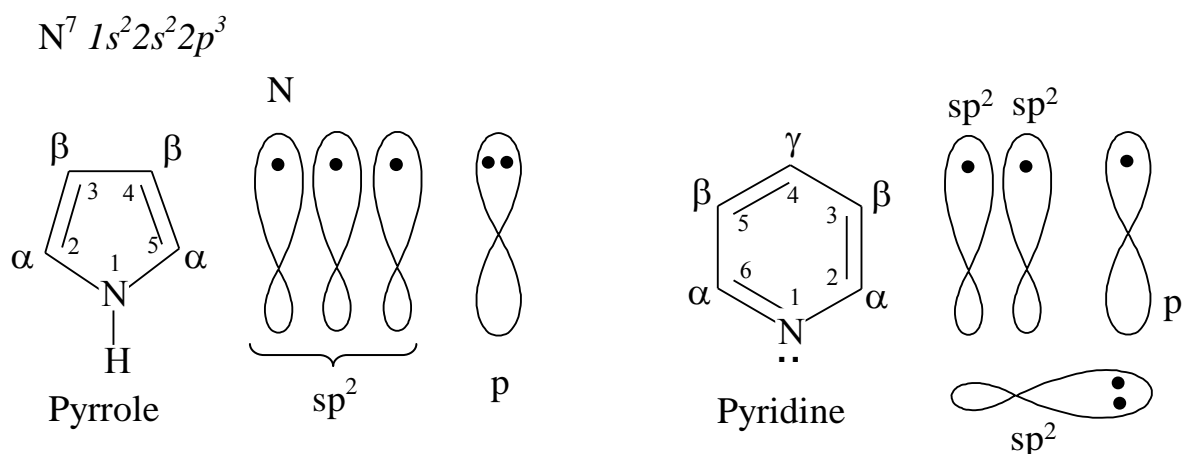
Furan



Thiophene

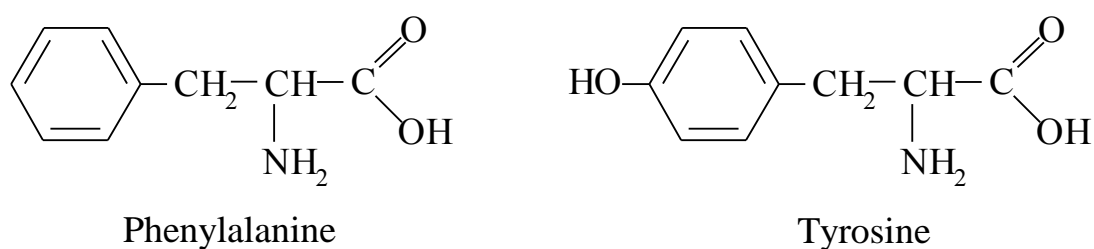
The nitrogen atoms in the molecules of both pyridine and pyrrole are  $sp^2$  hybridized. In pyridine the  $sp^2$ -hybridized nitrogen donates one bonding electron located on the  $p$  orbital to the  $\pi$  system. This electron, together with one from each of the five carbon atoms, gives pyridine a sextet of electrons like benzene. The two unshared electrons of the nitrogen of pyridine are in an  $sp^2$  orbital that lies in the same plane as the atoms of the ring. This  $sp^2$  orbital does not overlap with the  $p$  orbitals of the ring. The unshared pair on nitrogen is not a part of the  $\pi$  system, and these electrons confer on pyridine the properties of a weak base.

In pyrrole the electrons are arranged differently. Because only four  $\pi$  electrons are contributed by the carbon atoms of the pyrrole ring, the  $sp^2$ -hybridized nitrogen must contribute two electrons to give an aromatic sextet. Because these electrons are a part of the aromatic sextet, they are not available for donation to a proton. Thus, pyrrole is slightly acidic.



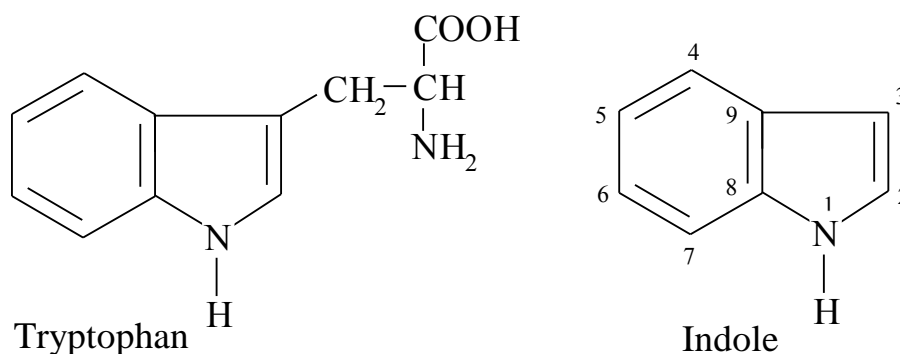
Furan and thiophene are structurally quite similar to pyrrole. The oxygen atom in furan and the sulfur atom in thiophene are  $sp^2$ -hybridized.

Compounds with aromatic rings occupy numerous and important positions in reactions that occur in living systems. Two amino acids necessary for protein synthesis contain the benzene ring:



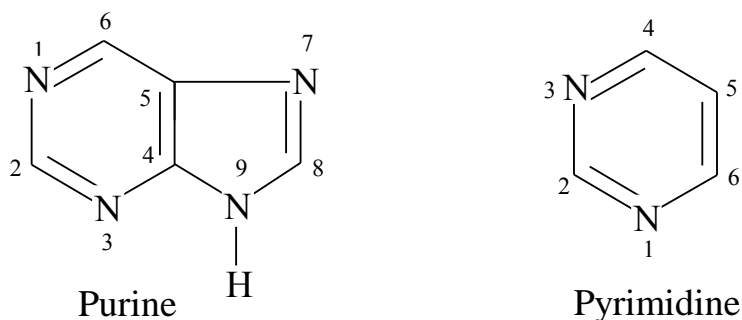
A third aromatic amino acid, tryptophan, contains a benzene ring fused to a

pyrrole ring. This aromatic ring system is called an indole system.



It appears that humans, because of the course of evolution, do not have the biochemical ability to synthesize the benzene ring. As a result, phenylalanine and tryptophan derivatives are essential in the human diet. Because tyrosine can be synthesized from phenylalanine in a reaction catalyzed by an enzyme known as phenylalanine hydroxylase, it is not essential in the diet as long as phenylalanine is present.

Heterocyclic aromatic compounds are also present in many biochemical systems. Derivatives of purine and pyrimidine are essential parts of DNA and RNA:



#### ***4. THE EFFECTS OF SUBSTITUENT GROUPS ON THE REACTIVITY OF COMPOUNDS***

Organic compounds mainly consist of covalent bonds. The electron pairs in these covalent bonds may undergo displacements under the influence of the substituents in the molecules. Two types of electron displacement are generally noticed in the mechanism of organic reactions. These are: inductive and mesomeric effects.

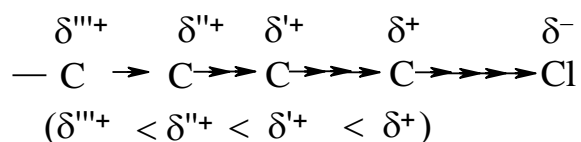
##### ***1. Inductive effect***

This is a permanent effect which arises whenever an electron displacing group (such as halogen) is attached to the end of a carbon chain. To understand this, let us consider a chain of carbon atoms having Cl atom at one end:





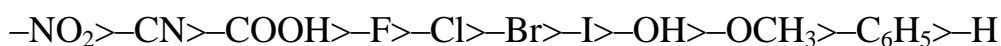
Chlorine is more electronegative than the Carbon. Due to a larger electronegativity of Cl, the electron pair shared between  $C_1$  and Cl will be displaced towards Cl atom. As a result of this, Cl acquires a partial negative charge ( $\delta^-$ ) and  $C_1$  acquires a partial positive charge ( $\delta^+$ ). This displacement is however, not limited to  $C_1$ -Cl bond but is transmitted to other carbon atoms along the chain. This is because the small positive charge on  $C_1$  will attract the  $\sigma$ -electrons of the  $C_1$ - $C_2$  bond towards it. As a result of this displacement, the positive charge on  $C_1$  is partially neutralized while a small positive charge is developed on  $C_2$ . The charge on  $C_2$  is less than that on  $C_1$  ( $\delta''^+ < \delta^+$ ). Similarly,  $C_3$  will acquire a small positive charge  $\delta'''^+$  ( $\delta'''^+ < \delta''^+$ ). Thus, *the process of electrons displacement along the chain of carbon atoms due to the presence of a polar covalent bond at one end of the chain is called **inductive effect** (or I-effect)*. This is a permanent effect and is generally represented by an arrow as shown below:



I-effect involves only single bonds. However, it may be noted that this effect decreases sharply as we move away from the atoms involved in the initial polar bond and becomes negligible from the fourth atom onwards.

For comparing the relative effects, hydrogen is taken as standard and the atoms or groups can be classified into two categories:

1. Atoms or groups of atoms having electron-attracting capacity more than hydrogen are referred to as having **-I (electron attracting) effect**. For example:



2. Atoms or groups of atoms having smaller electron-attracting power than hydrogen are referred to as having **+I (electron repelling) effect**, they are electron-donating. For example:



**Keep in mind: only alkyl groups have +I-effect!**

The inductive effect plays very significant role in organic chemistry in understanding reactions.

Let's recall that in the Brønsted-Lowry definition, an acid is a proton ( $\text{H}^+$ ) donor, and a base is a proton acceptor. Alcohols are acidic in nature. The acidic nature of alcohols is due to the presence of polar O–H bond. Oxygen is more electronegative than hydrogen and therefore, it withdraws the shared electron pair between O and H atoms towards itself. As a result, O–H bond becomes weak and loses a proton ( $\text{H}^+$ ). Therefore, alcohols behave as acids. However, alcohols are weak acids ( $K_a=10^{-16}$  to  $10^{-18}$ ) (do not react with bases) even weaker than water ( $K_a=10^{-14}$ ). This is quite expected because of the electron releasing inductive effect of the alkyl group. The alkyl group releases electrons towards oxygen atom and increases electron density around it. As a result, the tendency of oxygen to withdraw electrons in O–H bond towards itself decreases and therefore, the release of proton becomes difficult. On the other hand, there is no electron releasing alkyl group in water and the electron pair of O–H bond gets more attracted towards oxygen atom than in alcohol. Thus, the release of  $\text{H}^+$  from water is easier than from alcohol. Thus, *alcohols are weaker acids than water*.

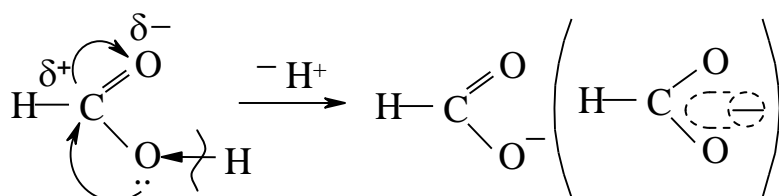


Acidic strength of alcohols decreases in homologous series, because larger alkyl groups have greater +I-effect.

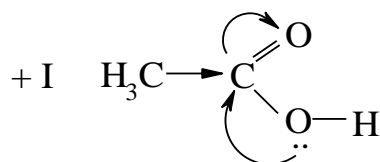


#### *Effect of substituents on acidic strength of acids*

Carboxylic acids are acidic because the carboxylate ions formed are stable and hence carboxylic acids have great tendency to loose proton. The carboxyl group is the conjugated system, it means that unshared electrons of the oxygen atom of the hydroxyl group are involved into conjugation with the double bond, therefore the oxygen atom of the hydroxyl group carries some positive charge. Consequently, the electron pair of the O–H is displaced towards oxygen atom. This displacement of electrons causes the release of a proton.

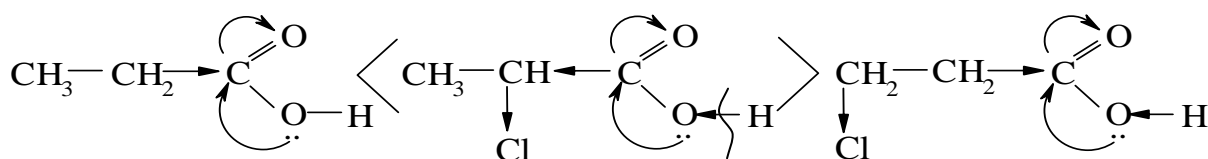


If the H atom of formic acid is replaced by  $-\text{CH}_3$  group to form acetic acid, the alkyl group will tend to increase the electron density on the O atom of the O–H bond due to the +I-effect. Consequently, the release of  $\text{H}^+$  ion in acetic acid will be more difficult as compared to formic acid or the former is a weaker acid. In general, the greater the +I effect of the alkyl group attached to the carboxyl group, lesser will be the acidic strength of the carboxylic acid. Therefore, acidic strength of acids decreases in homologous series.



The electron withdrawing substituents such as halogen atom possess  $-I$ -effect. Chlorine is an electron attracting atom, it withdraws the electrons from the carbon to which it is attached and this effect is transmitted throughout the chain. As a result the electrons are withdrawn more strongly towards O of O–H bond and this promotes the release of proton. Consequently, acidic strength increases. Therefore, 2-chloropropanoic acid is stronger acid than propanoic acid.

We know that inductive effect decreases rapidly with distance. Therefore, as the distance between the electron withdrawing group and the  $-\text{COOH}$  group increases, the electron withdrawing influence decreases. Beyond a few methylene groups, the effect becomes negligible. Therefore, 2-chloropropanoic acid is stronger acid than 3-chloropropanoic acid.



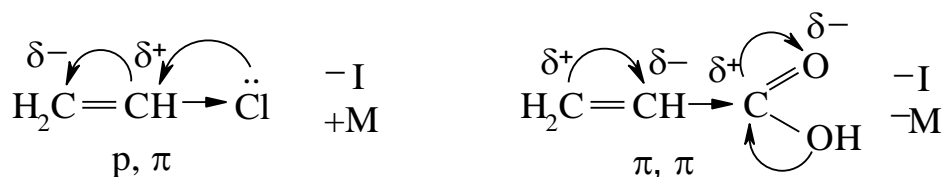
## 2. Mesomeric effect

When a substituent is attached to the conjugated system it enters into conjugation with this system. If a substituent possesses  $\pi$ -bond, the orbitals of this  $\pi$ -bond overlap with  $\pi$ -orbitals of conjugated system, in such case  $\pi,\pi$ -conjugation arises, i.e. the bonds of a substituent enter into conjugation with the bonds of the system. If a substituent possesses unhybridized  $p$ -orbital, this orbital enters into conjugation with the bonds of unsaturated system. In this case  $p,\pi$ -conjugation takes place.

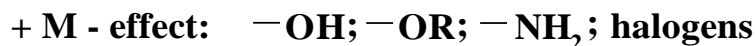
When a conjugation of  $\pi$ -bonds or  $p$ -orbitals of a substituent with molecule double bonds occurs we say the substituent influences the molecule. This influence is called mesomeric effect.

**Mesomeric effect** is a transfer of electronic influence of substituents over the conjugated system. At the same time a substituent itself is a member of conjugated system.

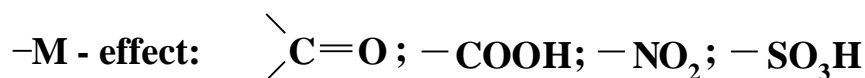
Mesomeric effect is shown by a curved arrow representing the movement of a pair of electrons.



A substituent that puts its  $p$ -orbital with a lone pair of electrons into the conjugation, i.e. when  $p,\pi$ -conjugation occurs, increases the electron density in the conjugated system. In this case a substituent shows +M effect. These substituents transfer a pair of electrons to the common conjugated system, i.e. they are electron donors. They activate the system.



If a substituent possesses a double bond ( $\pi,\pi$ -conjugation), it decreases the electrons density in the conjugated system. These types of substituents possess  $-M$ -effect and deactivate the system being electron acceptors.

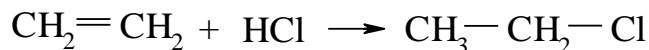


Electron effects of substituents have a substantial influence on the chemical properties of a molecule. Let's see how the reactive ability of a double bond changes

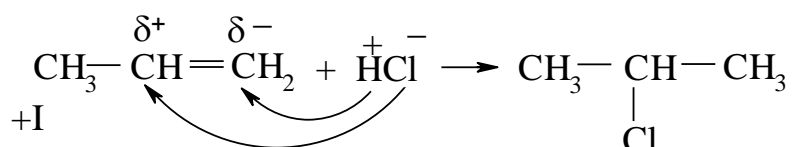
under the influence of different substituents.

The reactive ability of ethylene is determined by the double bond.

The reactions of addition are typical for alkenes as  $\pi$ -bond can be easily broken. There are no substituents in ethylene; hence there are no effects. Hydrogen can be added to any of the carbon atoms:

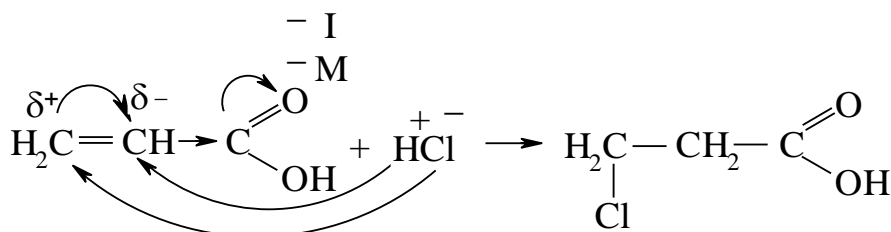


Propylene can be considered as ethylene, where one H atom is substituted by  $-\text{CH}_3$  group.



$-\text{CH}_3$  group doesn't possess M effect, as it has neither a double bond nor a lone pair of electrons. Still  $-\text{CH}_3$  group possesses +I effect and activates the system. Propylene is involved into reactions of addition more easily than ethylene. Because of the influence of  $-\text{CH}_3$  group on the double bond the electrons of  $\pi$ -bond are shifted from a substituent along the chain;  $\text{C}_1$  obtains a partial negative charge while  $\text{C}_2$  obtains a partial positive one. Hence,  $\text{H}^+$  goes to  $\text{C}_1$  while  $\text{Cl}^-$  goes to  $\text{C}_2$  during the reaction with HCl. *I-effect* phenomenon explains Markovnikov's rule: *during the addition across unsymmetrical double bond, the negative part of the attacking reagent joins to the carbon atom carrying lesser number of hydrogen atoms while the positive part goes to the carbon atom with more number of hydrogen atoms.*

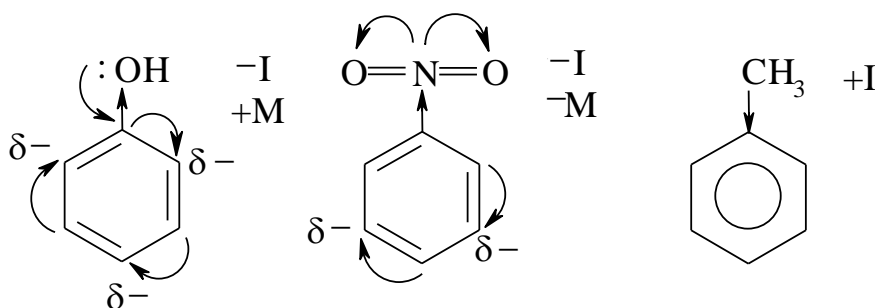
Reaction of propenoic acid with HCl takes place against the Markovnikov's rule.



It could be explained from the point of view of electron effects.  $-\text{COOH}$  group possesses  $-\text{M}$  effect and attracts  $\pi$ -electrons of double bond.  $\text{C}_2$  possesses  $\delta^-$  charge,  $\text{C}_3$  -  $\delta^+$  charge.  $\text{H}^+$  joins to  $\text{C}_2$  and  $\text{Cl}^-$  joins to  $\text{C}_3$  during the reaction of propenoic acid with HCl.  $-\text{COOH}$  deactivates a double bond. As a result the double bond in propenoic acid is less reactive than in propylene.

*The effect of substituent groups on the further substitution  
in the benzene ring*

The reagents which attack the benzene ring are mainly electrophilic reagents. If one of the H atoms in the benzene ring is replaced by a substituent, the compound formed will differ in reactivity from benzene. A substituent with a +I-effect or +M-effect donates electrons to the ring. It is said to activate the ring. A substituent with –I-effect or –M effect deactivates the ring. Let's consider the structure of phenol. It can be represented as benzene in which one H is substituted with the –OH group. –OH possesses –I effect and +M effect. The +M effect of the hydroxyl group is greater than the –I effect, and –OH therefore activates the benzene ring.

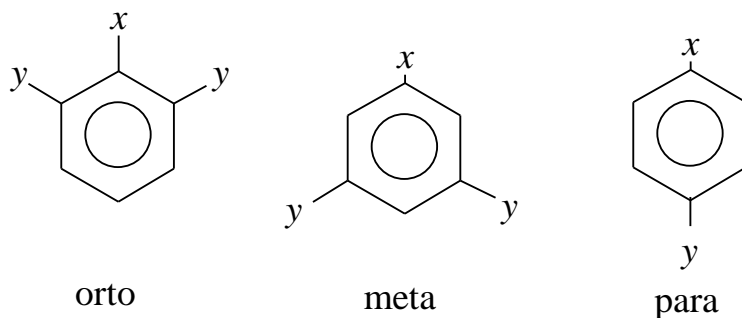


Activating substituents direct groups entering the benzene ring into the o- and p-positions. Deactivating substituents direct groups into the m-position in the ring.

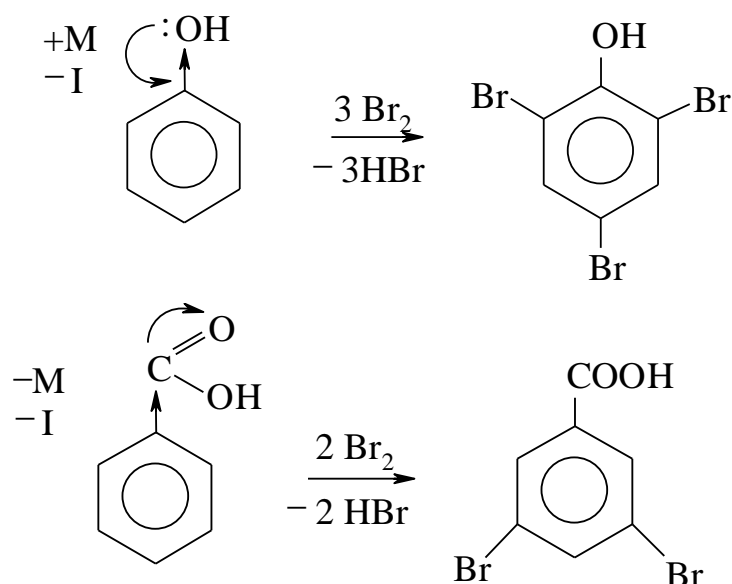
**Ortho.** The compound is said to be ortho if the two substituents are on adjacent carbon atoms. These are either called ortho or given the numbers 1,2–.

**Meta.** The compound is said to be meta if the two substituents are on alternate carbon atoms. These are given the numbers 1,3–.

**Para.** The compound is said to be para if the two substituents are on diagonally situated carbon atoms. These are given the numbers 1,4–.



Phenol is more reactive than benzene due to +M-effect of –OH group. Benzoic acid is less reactive than benzene due to the –M-effect of the –COOH group.

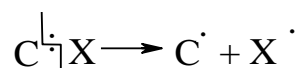


## 5. MAIN TYPES AND MECHANISMS OF ORGANIC REACTIONS

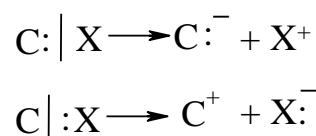
Reactivity of the molecules is determined by the functional composition, space structure and the type of chemical bond. Organic reactions involve making and breaking of covalent bonds. The fission of bonds can take place in two ways:

### *Types of bond fission*

**1. Homolytic fission.** In homolytic fission, the cleavage of covalent bond between two atoms takes place in such a way that each atom retains one electron of the shared pair. This is a symmetrical fission and leads to the formation of atoms or groups of atoms having unpaired electrons, called **free radicals**. Homolytic fission takes place if the bond between atoms is low polar, e.g. bonds of  $sp^3$ -hybridized carbon atom with hydrogen. (The free radicals are denoted by putting dot over the symbol of atom or group of atoms). Energy must be supplied, either as heat or light, to break the bond. Free radicals are very reactive because they have strong tendency to pair up their unpaired (odd) electron with another electron from wherever available ( $\text{Cl}^\bullet$ ,  $\text{HO}^\bullet$ ,  $\text{CH}_3^\bullet$ )



**2. Heterolytic fission.** Heterolytic fission is unsymmetrical. When the bond breaks, one of the bonded atoms takes both of the bonding electrons leaving none on the other. This results into two charged particles - anion and cation. An ion with a positively charged carbon atom is called a **carbocation**. An ion with a negatively charged carbon atom is called a **carbanion**.



Organic reactions have **different mechanisms** depending on the type of attacking reagent and the final result of reaction.

Depending upon the type of attacking reagent organic reactions can be classified into three groups: free radical, nucleophilic, electrophilic.

#### *Types of attacking reagents*

1. **Free radicals** have already been discussed. They attack non-polar covalent bonds.

If the covalent bond in organic compound is polar, the reagents which attack this compound seek out either the slightly positive ( $\delta^+$ ) end of the bond or the slightly negative ( $\delta^-$ ) end of the bond.

2. If ion attacks the electron-deficient end of a polar bond (positive end) it is called **nucleophilic reagent** or **nucleophile**. A nucleophile is a reagent containing an atom having unshared electrons. A nucleophile is electron rich and seeks electron deficient sites, i.e. nucleus (nucleus loving). ( $\text{OH}^-$ ,  $\text{CN}^-$ ,  $\text{RCOO}^-$ ,  $\text{Cl}^-$ ,  $\text{NH}_3$ ,  $\text{H}_2\text{O}$ ,  $\text{ROH}$ ,  $\text{ROR}$ ,  $\text{C}_6\text{H}_6$ ,  $\text{CH}_2=\text{CH}_2$ ).

3. A reagent which attacks a region where the electron density is high is called an **electrophile**. It is deficient of electrons and can accept a pair of electrons (electron loving). ( $\text{H}^+$ ,  $\text{Cl}^+$ ,  $\text{NO}_2^+$ ,  $\text{SO}_3$ ,  $\text{AlCl}_3$ ).

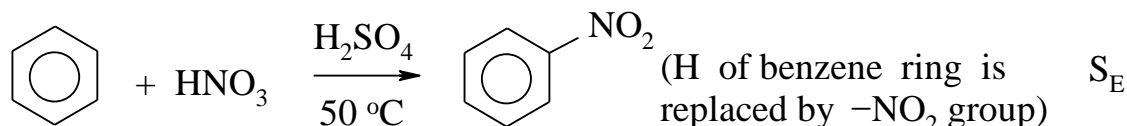
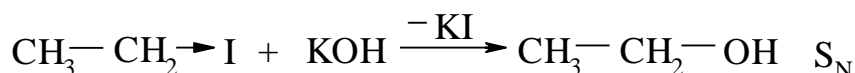
#### *Types of organic reactions*

Depending upon the final result of reaction three types of reactions are distinguished:

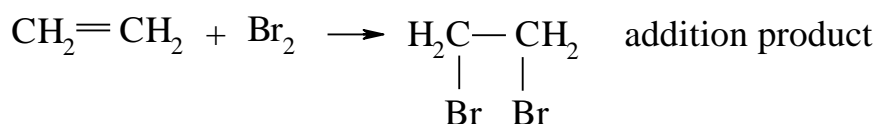
1. **Substitution reactions** (S-reactions). The reactions in which an atom or group of atoms in a molecule is replaced or substituted by different atoms or group of



atoms.

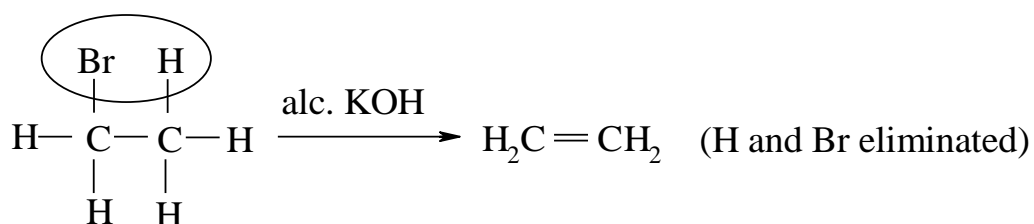


2. **Addition reactions** (A-reactions). The reactions in which two molecules react to form a single product.



The substitution or addition reactions are also called free radical, electrophilic or nucleophilic substitution or addition reactions depending upon the type of attacking reagent. For example, if the attacking reagent is nucleophile in substitution reaction, the reaction is called nucleophilic substitution ( $\text{S}_\text{N}$ -reaction).

3. **Elimination reactions** (E-reactions). The reactions in which a small molecule is removed from adjacent carbon atoms resulting in the formation of additional bond between them (multiple bond).



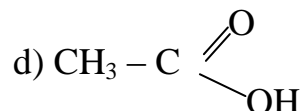
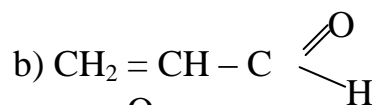
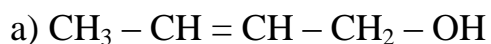
Different classes of organic compounds enter reactions of different mechanisms:  $\text{S}_\text{R}$ -reactions are typical for saturated hydrocarbons,  $\text{S}_\text{E}$ -reactions for aromatic compounds,  $\text{S}_\text{N}$  -reactions for alcohols and halogen derivatives,  $\text{A}_\text{E}$  -for unsaturated hydrocarbons,  $\text{A}_\text{N}$  - for aldehydes, ketones, and carboxylic acids.

## Revision exercises

1. Sulfanilamides which are used for infection diseases treatment are derivatives of benzene. Which group of compounds does benzene belong to?

- a) Acyclic    b) Heterocyclic    c) Aromatic    d) Acetylenic

2. In which of the following compounds all carbon atoms are  $sp^3$ - hybridized?



3. Choose isomers:

- a) Cis-butenedioic acid and trans- butenedioic acid  
b) Butenedioic acid and butanedioic acid  
c) Butenedioic acid and butenoic acid  
d) Butenoic acid and butanoic acid

Answers

1. c)  
2. c)  
3. a)

\* \* \*

# PROPERTIES OF MAIN CLASSES OF ORGANIC COMPOUNDS

## *Characteristics of the subject*

This subject is the basis for understanding the molecular level of the chemical processes taking place in living organisms during metabolism. This subject is important for conscious mastering the properties and characteristics of chemical behavior of biologically active compounds and their metabolites when studying biochemistry, normal physiology, pharmacology and other medico-biological subjects.

## *Purpose*

To study the chemical properties of main classes of organic compounds of different structures as well as the most important groups composing biologically active substances.

## *Objectives*

1. To be able to use the knowledge of electronic and spatial structure of hydrocarbon radicals and functional groups to characterize the properties of the compounds.

2. To be able to describe the mechanism of reactions:

- radical substitution ( $S_R$ )
- electrophilic substitution ( $S_E$ )
- electrophilic addition ( $A_E$ )
- nucleophilic substitution ( $S_N$ )
- nucleophilic addition ( $A_N$ )

## *Theoretical questions*

1. Properties of Saturated Compounds.
2. Properties of Unsaturated Compounds.
3. Properties of Aromatic Compounds.
4. Properties of Halogen Derivatives.
5. Properties of Hydroxy Compounds.
6. Properties of Aldehydes and Ketones.
7. Nomenclature and properties of Carboxylic acids.
8. Properties of Amines.

## 1. PROPERTIES OF SATURATED COMPOUNDS

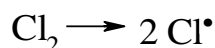
### *Free radical substitution reactions ( $S_R$ -reactions)*

All reactions of saturated hydrocarbons are substitution reactions. There are only  $sp^3$ -hybridized carbon atoms in saturated hydrocarbons molecules (alkanes, alicyclic hydrocarbons).  $\sigma$ -bonds (C–C and C–H) are non polar in these compounds. These bonds are comparatively strong and do not undergo heterolytic fission under the influence of nucleophilic or electrophilic reagents. Saturated hydrocarbons are not reactive in the most of heterolytic reactions. Free radical substitution reactions are typical for them ( $S_R$ -reactions). These are chain reactions.

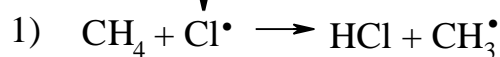
### *Mechanism of chlorination of methane*

Reaction takes place rapidly in sunlight or above  $300^\circ\text{C}$ .

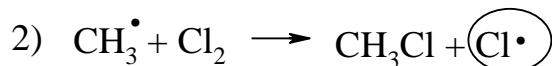
1. **Chain initiation.** Homolysis of the Cl–Cl bond. The energy for free radicals formation comes from the light absorbed or the heat supplied.



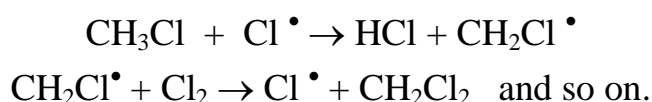
2. **Chain propagation.** The chlorine radicals formed are very reactive, they attack methane molecules:



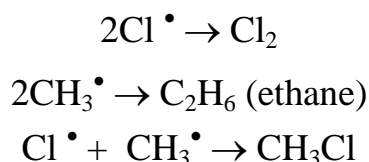
The reaction of  $\text{CH}_3^\bullet$  with  $\text{Cl}_2$  results in a chain reaction because the chlorine radical formed attack a new methane molecule:



1) and 2) steps are repeated many times, setting up a chain reaction.  $\text{CH}_3\text{Cl}$  can undergo further chlorination to  $\text{CCl}_4$  (tetra chloro methane):



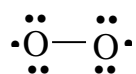
3. **Chain termination.** Thousands of molecules of chloromethane are formed for every photon of light absorbed. The high yield is due to the chain reaction. The reason why the yield is not higher is that radicals can combine with each other and bring the chain to an end.



The chain does not go on for ever. These reactions bring the chain to an end. Free radical processes take place in the organism; for example, peroxide oxidation of lipids is one of them. It is the main cause of cellular membranes injury (damage) particularly during the radiation sickness. Free radicals such as  $\text{HO}^\bullet$ , for example, are formed in the organism under the influence of various pathogenic effects (different types of radiation, carcinogenic substances, etc.). Free radicals are strong oxidizing agents able to interact with organic compounds. This process is called peroxide oxidation and develops according to the radical mechanism.

Free radicals attack unsaturated fatty acids, which are a part of lipids. Hydrogen atoms of methylene groups bounded with  $\text{sp}^2$  – hybridized carbon atoms form a place for radical attack. It can be explained by the resulting formation of a new radical, which is stable because of unpaired electron in it conjugated with  $\pi$ -electrons of double bond. Thus the reaction takes place in two methylene groups.

Under the influence of oxygen free radicals formed during this process form in its turn peroxide radicals (oxygen molecules are biradicals).

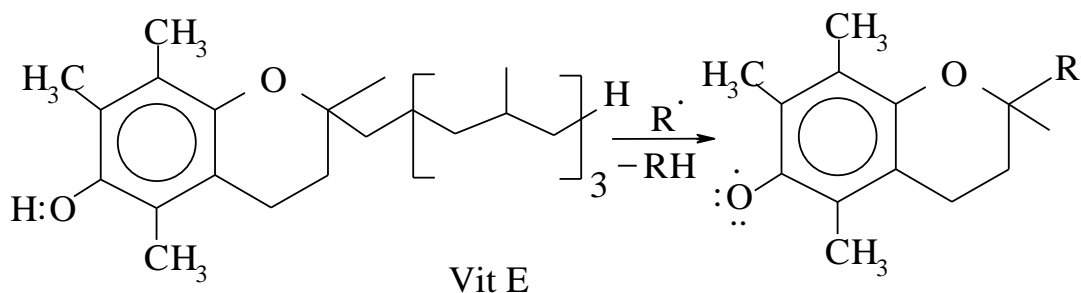


Peroxide radicals turn into hydroperoxides with the formation of new  $\bullet\text{OH}$  radicals able to destroy new molecules of fatty acid (chain propagation). Unstable hydroperoxides dissociate to aldehydes, which are oxidized with the formation of mono- and dicarboxylic acids.

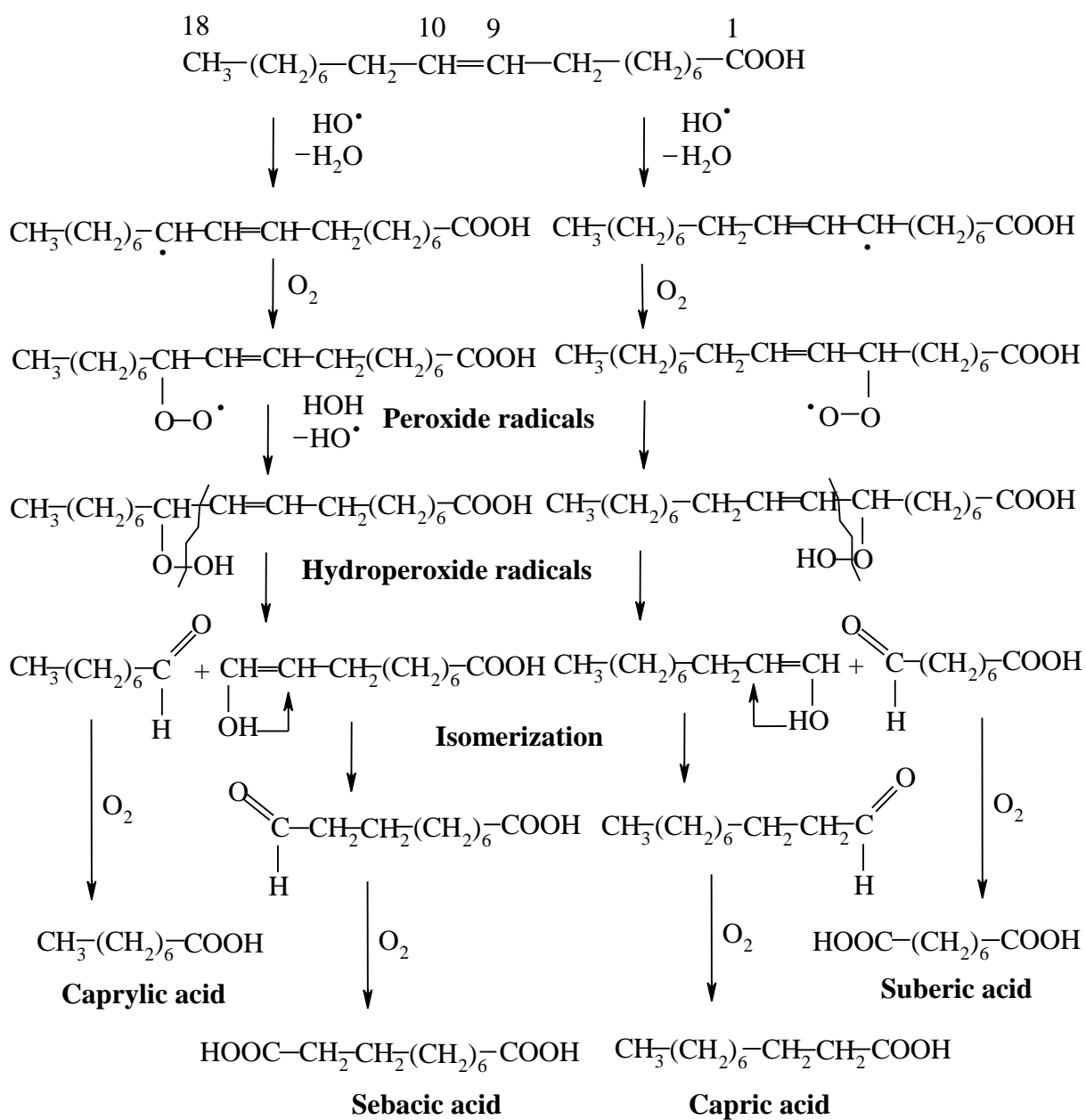
Hence, a long-chain acid is broken down into small pieces, a hole (breach) occurs in membrane causing a death of a cell.

There are substances the so-called antioxidants, which inhibit free radical oxidation. Vitamin E can serve the example of such a substance. It contains phenol fragment. Peroxide radicals attack O–H bond in the phenol fragment of vitamin E, tear H away from it and turn into stable molecules. The radical formed from vitamin

E being a conjugated system possesses low activity and is unable to react with the new molecule. Thus the chain process is terminated.



*Peroxide oxidation of oleic acid*

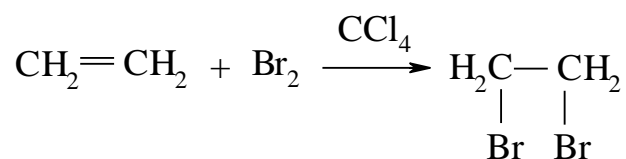


## 2. PROPERTIES OF UNSATURATED COMPOUNDS

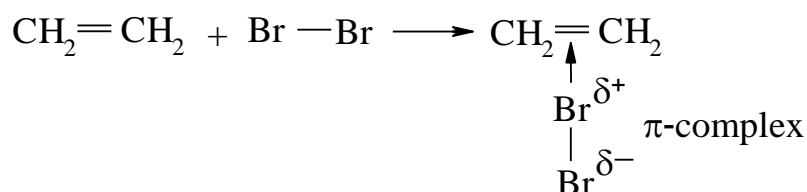
### *Electrophilic addition reactions (A<sub>E</sub>-reactions)*

Unsaturated hydrocarbons undergo electrophilic addition reactions (A<sub>E</sub>-reactions) as π electrons are susceptible to attack by an electrophilic reagent.

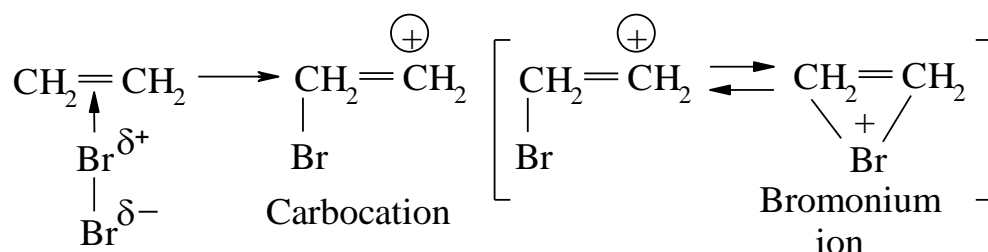
Halogens react with alkenes at room temperature, without catalysis. They add across the double bond. Ethene reacts with bromine in a polar solvents (such as tetrachloromethane) to give 1,2-dibromoethane.



*Mechanism:* 1. Ethene has both a σ bond and a π bond between the two carbon atoms. Bromine is an electrophile. When a bromine molecule approaches an ethene molecule, the π electron cloud interacts with the approaching bromine molecule, causing a polarization of the Br-Br bond (this structure is called π-complex):

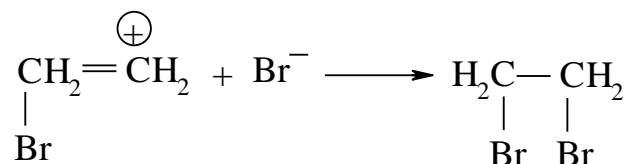


2. The π electrons become gradually more attached to the δ+ Br atom, and the electrons of the Br-Br bond become gradually more polarized until the association of ethene and bromine is transformed into a positive ion, called carbocation and a bromide ion:



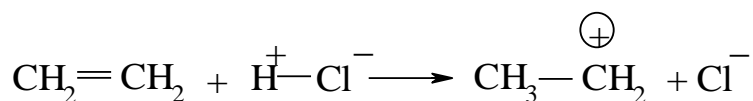
The negative charge resides on Br<sup>-</sup>.

3. The carbocation is immediately attacked by a bromide ion to form the product 1,2-dibromoethane:

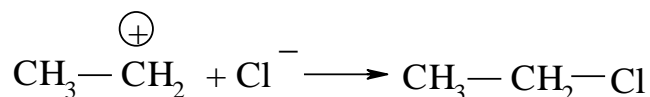


This reaction step is very fast compared with the other steps.

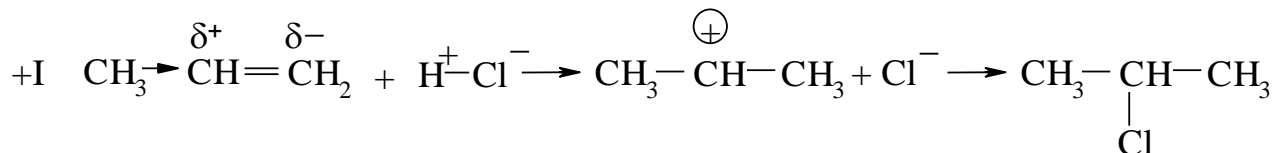
Addition of hydrogen halide to an alkene involves interaction of the electrophile with the double bond. A molecule of hydrogen halide is permanently polarized as  $\text{H}^{\delta+}-\text{Cl}^{\delta-}$ . The  $\pi$  electrons of the alkene bond to the electrophilic H atom:



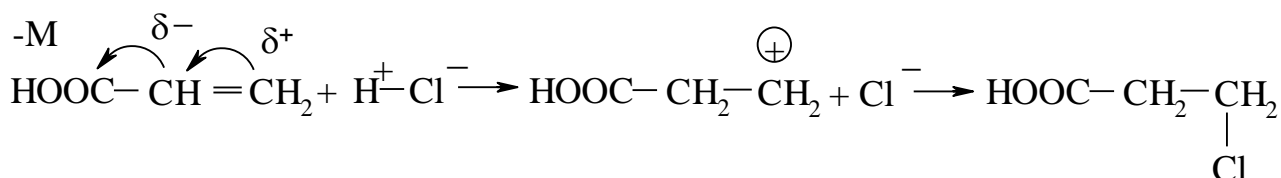
This is the slow step in the reaction. Once formed, the carbocation reacts rapidly with the chlorine ions:



Reactions of compounds containing unsymmetrical double bond:



Addition follows Markovnikov's rule (Markovnikov's rule provides that "if an unsymmetrical alkene combines with a hydrogen halide, the halide ion adds to the carbon with the fewer hydrogen atoms").



Reaction takes place against Markovnikov's rule. Addition across unsymmetrical double bond depends on electronic effects of substituents.

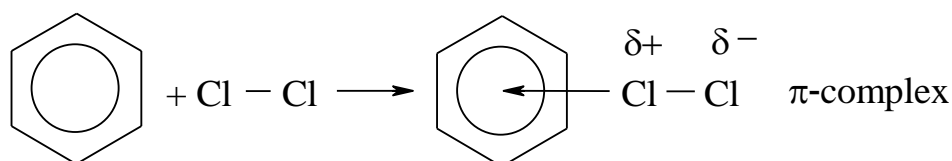


### 3. PROPERTIES OF AROMATIC COMPOUNDS

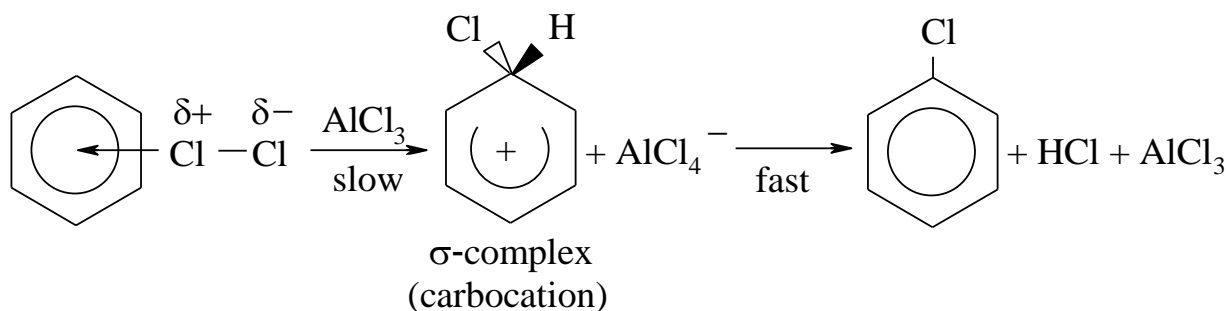
#### *Electrophilic substitution ( $S_E$ -reactions)*

Aromatic compounds are those which contain one or more benzene ring in them. The benzene ring is a planar hexagon with a cloud of delocalized  $\pi$  electrons lying above and below the ring. Since there are no single and double bonds in the molecule, it is a conjugated system that is known to be very stable, these compounds undergo substitution reactions rather than addition reactions. The reactions of benzene usually involve the attack of an electrophile on the cloud of  $\pi$  electrons. So, reactions of electrophilic aromatic substitution ( $S_E$ -reactions) are typical for aromatic compounds.

In the chlorination of benzene a chlorine molecule approaches a benzene molecule and encounters the annular cloud of delocalized  $\pi$  electrons above and below the plane of the ring. As the  $\pi$  electron cloud interacts with the chlorine molecule, the Cl–Cl bond becomes polarized:

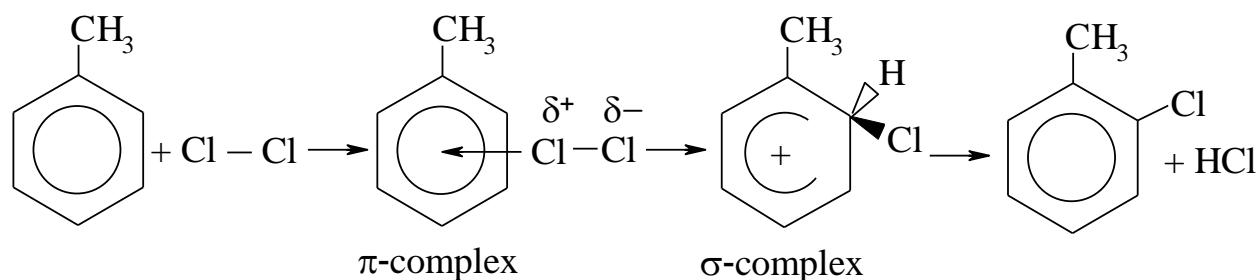


This will remind you the interaction between  $\text{Cl}_2$  and the  $\pi$  electrons of the C=C bond in alkenes. Benzene is less reactive than an alkene, and the reaction proceeds only if there is a Lewis acid (e.g.  $\text{AlCl}_3$ ) present. This accepts a pair of electrons from the  $\text{Cl}^{\delta-}$  atom in the polarized  $\text{Cl}_2$  molecule, and enables the Cl–Cl bond to split. A carbocation ( $\sigma$ -complex) and a  $\text{AlCl}_4^-$  complex ion are formed. The  $\sigma$ -complex rapidly loses proton to form chlorobenzene, with the regeneration of the catalyst,  $\text{AlCl}_3$ :

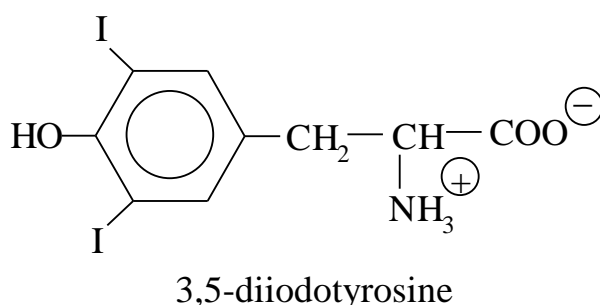


Activating substituents possessing +I or +M-effects (which donate electrons to

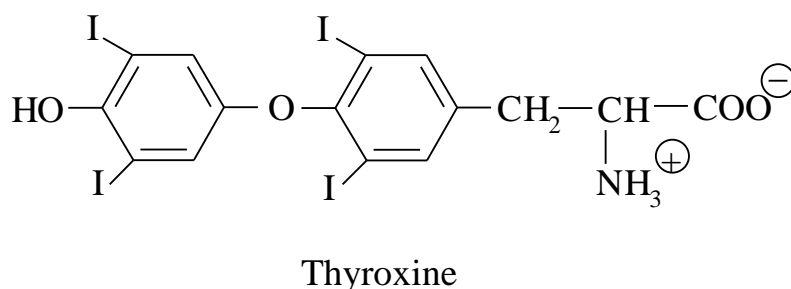
the ring) direct groups entering the benzene ring into the o- and p-positions. Deactivating substituents (which attract electron density from the ring) direct groups into the m-position in the ring.



The biosynthesis of thyroxine (hormone of thyroid gland) occurs by a biochemical version of electrophilic aromatic substitution. In this process iodine is introduced into tyrosine molecule. To introduce iodine into tyrosine, an enzyme called iodoperoxidase converts nucleophilic iodide anions from our diet (e.g. from iodized table salt) into an electrophilic iodine species. The electrophilic form of iodine reacts with tyrosine by electrophilic aromatic substitution mechanism. Reaction leads to incorporation of iodine at the 3 and 5 positions of the tyrosine rings.



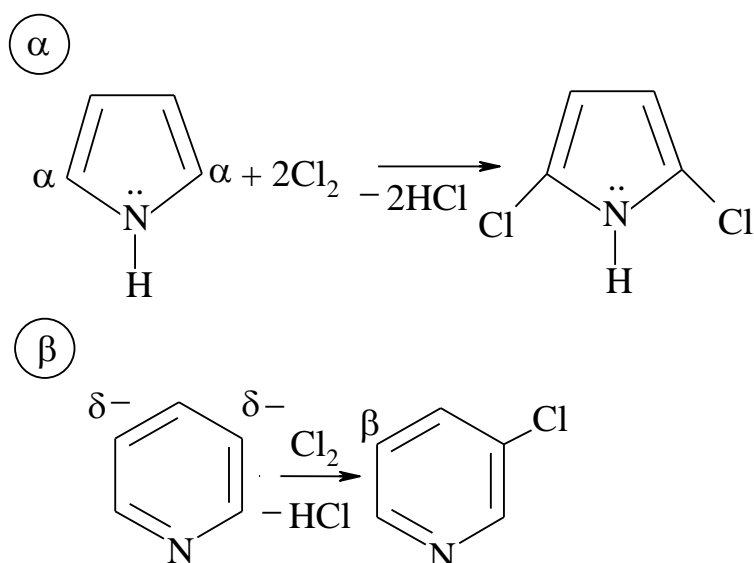
These are the positions ortho to the phenol hydroxyl group, precisely where we would expect electrophilic substitution to occur in tyrosine. Then coupling of two tyrosine units necessary to complete biosynthesis of thyroxine occurs.



Thyroxine is one of the key hormones involved in regulating metabolic rate. It causes an increase in the metabolism of carbohydrates, proteins, and lipids, as well as a

general increase in oxygen consumption by most tissues.

Heteroatoms in the heterocycles direct entering substituents into the certain positions:



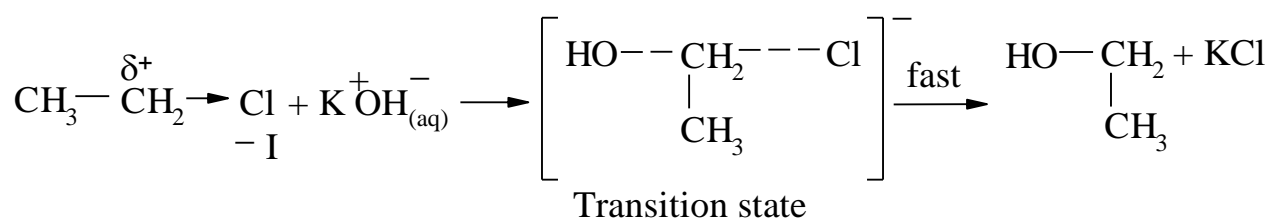
#### 4. PROPERTIES OF HALOGEN DERIVATIVES

##### *Nucleophilic substitution reactions ( $S_N$ -reactions)*

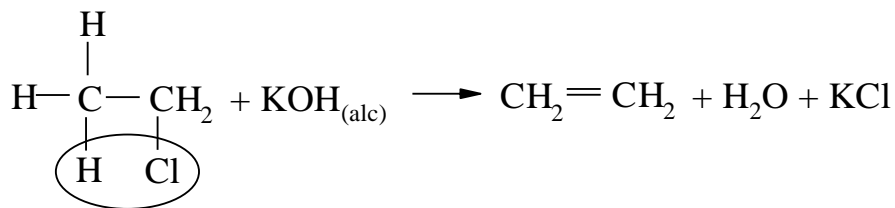
In halogen derivatives, alcohols and amines, the carbon atom is bonded to a more electronegative atom than the carbon. Consequently, the C–X bond is polar in nature. As a result, carbon gets partial positive charge. It makes it susceptible to attack by electron rich groups (nucleophiles). When a nucleophile approaches the positively charged carbon atom of a haloalkane, alcohol or amine molecule, the functional group along with its bonding electron pair gets displaced and a new bond with carbon and nucleophile is formed.

Haloalkanes are one of the most reactive classes of organic compounds.

##### 1. $S_N$ -reactions:

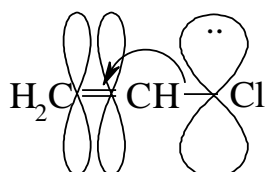


## 2. Elimination reactions:



Substitution and elimination are in competition. Substitution reactions are favoured by weakly basic nucleophiles, elimination by strongly basic reagents. A high concentration of base in non-aqueous solvent (KOH in  $\text{C}_2\text{H}_5\text{OH}$ ) at high temperature favours elimination.

Haloalkenes, e.g.  $\text{CH}_2=\text{CH}-\text{Cl}$  are very unreactive. They do not react with aqueous alkali and are not attacked by other nucleophiles. The reason is that the p orbitals of the doubly bonded carbon atoms overlap with the p orbitals of the chlorine atom to form a  $\pi$  bond (p, $\pi$  conjugation). This makes the C-Cl bond much stronger than that in chloroalkanes, where there is only a  $\sigma$  bond between carbon and chlorine.



The introduction of a halogen atom into the hydrocarbon molecule is accompanied by increasing of biological activity of the product. Halogen derivatives of alkanes are characterized by high narcotic activity, so some of them are used for anesthesia. Benzyl iodide  $\text{C}_6\text{H}_5-\text{CH}_2-\text{I}$  (tear gas) is lachrymatory due to the presence of halogen atom in the side chain attached to benzene ring.

Iodoform  $\text{CHI}_3$  is an antiseptic, it enters the composition of ointments, powders, and is used in dentistry.

Chloroethyl  $\text{C}_2\text{H}_5-\text{Cl}$  is applied topically, it boils at  $12^\circ\text{C} - 14^\circ\text{C}$ , evaporates from the skin and causes relieve of pain.

Chloroform  $\text{CHCl}_3$  is an agent for inhalation narcosis.

Halothane  $\text{CF}_3-\text{CHClBr}$  is an agent used for the combined inhalation narcosis.

Trichloroethylene  $\text{Cl}_2\text{C}=\text{CHCl}$  is used for short-term anesthesia.

## 5. PROPERTIES OF HYDROXY COMPOUNDS

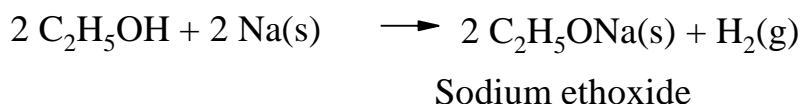
### Nucleophilic substitution reactions ( $S_N$ – reactions)

Alcohols are the compounds containing hydroxyl group attached to the alkyl group. Phenols are the compounds containing hydroxyl group attached to the aryl group (benzene ring). Phenols and related compounds occur widely in nature. E.g., tyrosine is an amino acid that occurs in proteins, estradiol is a female sex hormone, tetracyclines are important antibiotics, all these contain phenol fragment.

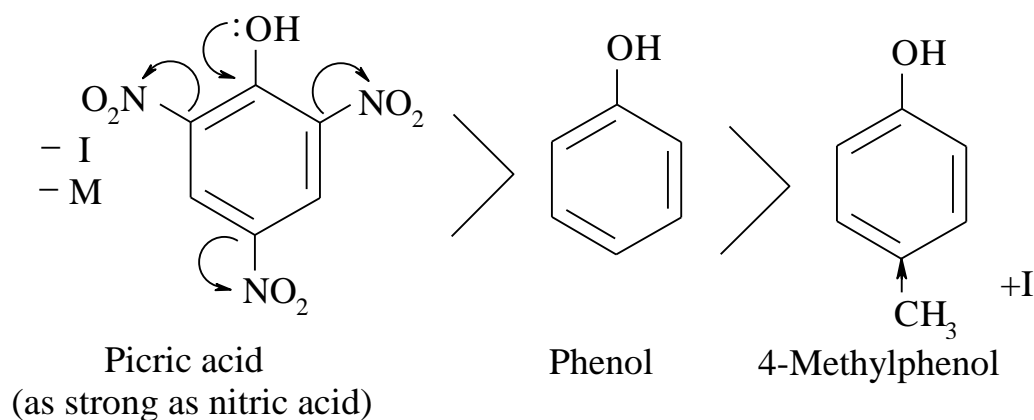
#### 1. Fission of RO–H bond (acidic properties)

##### a) Reaction with active metals.

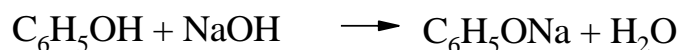
Alcohols are weakly acidic in nature and react with active metals (Na, K, Mg, Al, etc) to liberate hydrogen gas and form metal alkoxide:



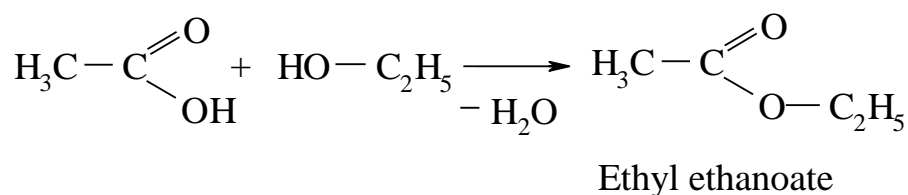
Phenols are stronger acids than alcohols. In the phenol, a  $p$  orbital of the oxygen atom overlaps with the  $\pi$  orbital of the ring carbon atoms ( $p, \pi$  conjugation), oxygen attracts electron pair from hydrogen, thus proton can be released easily. The presence of electron-withdrawing groups in the ring attracts electrons away from the oxygen atom and so increases the acid strength of phenol. This is why 2,4,6-trinitrophenol is a very much stronger acid than phenol. Electron-donating groups in the ring decrease the acid strength of phenol:



Because phenols are more acidic than alcohols, they can be converted to sodium phenoxides through the use of sodium hydroxide (rather than metallic sodium, the reagent used to convert alcohols to alkoxide ions):

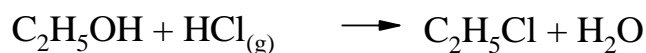


b) Esterification is a reaction with carboxylic acids:

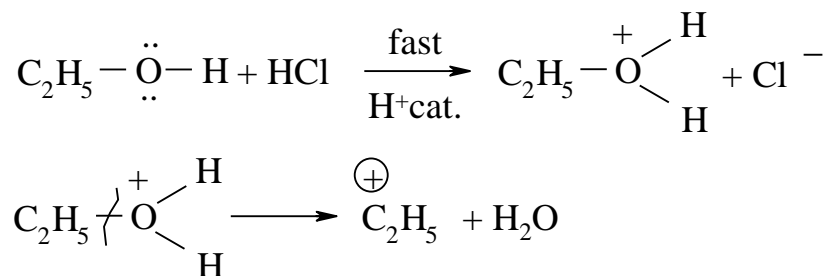


## 2. Fission of R-OH bond ( $S_N$ -reactions)

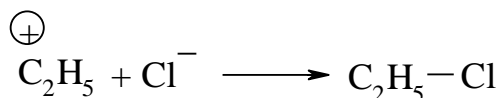
a) Chlorination. Dry HCl (g) or conc. HCl replaces OH-group by chlorine:



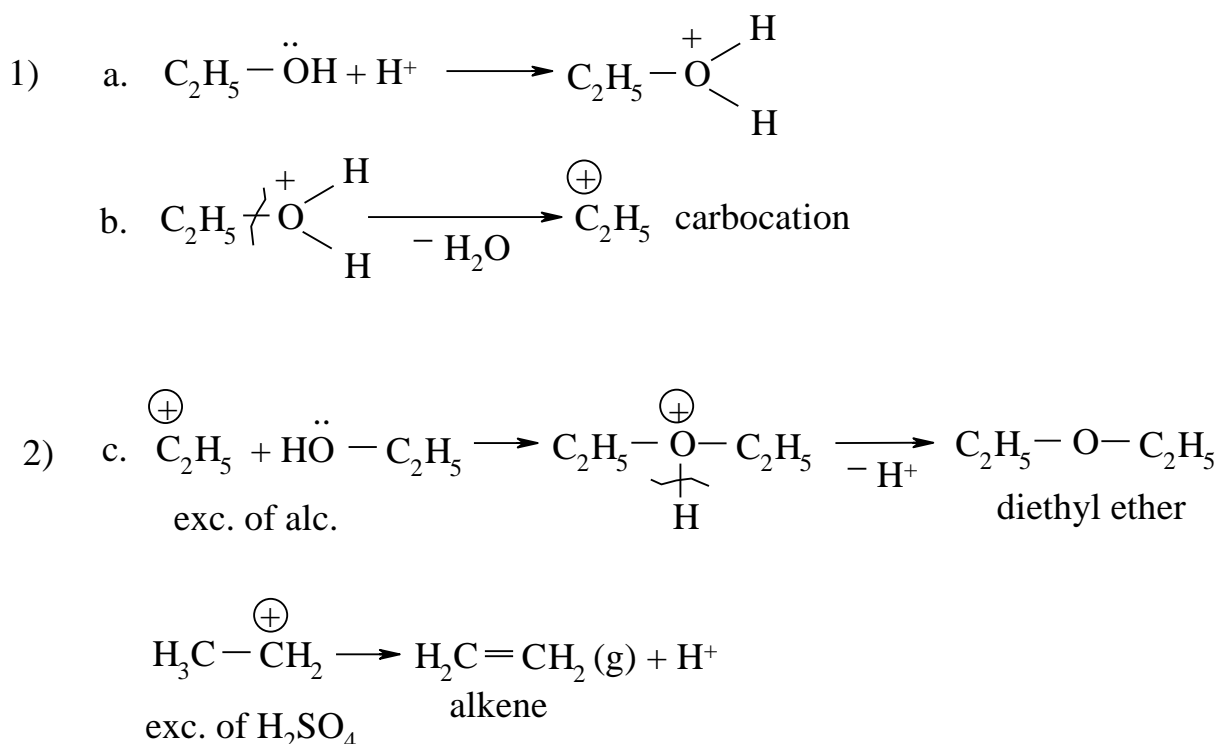
Mechanism: Oxygen atom in alcohol has 2 lone pairs of electrons, they combine with HCl to form protonated alcohol. The positive charge on oxygen weakens the C-O bond leading to its cleavage:



Carbocation formed then adjoin the  $\text{Cl}^-$  to produce the product:



b) Dehydration. Reaction with conc.  $\text{H}_2\text{SO}_4$  results in dehydration to give an ether (excess of alcohol,  $<170^\circ\text{C}$ ) or to give an alkene (excess conc.  $\text{H}_2\text{SO}_4$ ,  $>170^\circ\text{C}$ ):



Mechanism:

a) Alcohol combines with a proton from the acid to form a protonated alcohol.

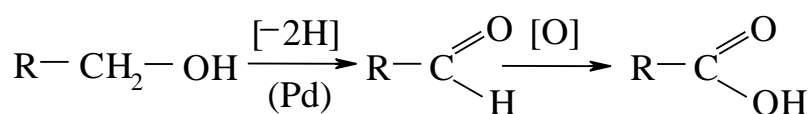
b) The protonated alcohol loses a water molecule to form carbocation.

c) The carbocation then eliminates a proton and undergoes rearrangement of electrons to form the alkene. If there is excess of alcohol, carbocation adjoin the molecule of alcohol and then unstable intermediate formed loses proton to form ether.

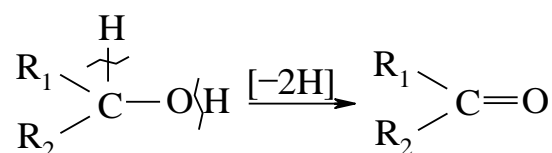
Phenols are unreactive toward  $\text{S}_{\text{N}}$ -reactions due to the conjugation of oxygen atom of the hydroxyl group with the ring. Phenols can't be directly converted into the halogen derivatives.

### 3. Oxidation

For organic substances, oxidation usually involves increasing its oxygen content or decreasing its hydrogen content. Reduction of an organic compound usually involves increasing its hydrogen content or decreasing its oxygen content. Primary alcohols are oxidized to aldehydes, they can be further oxidized to carboxylic acids:



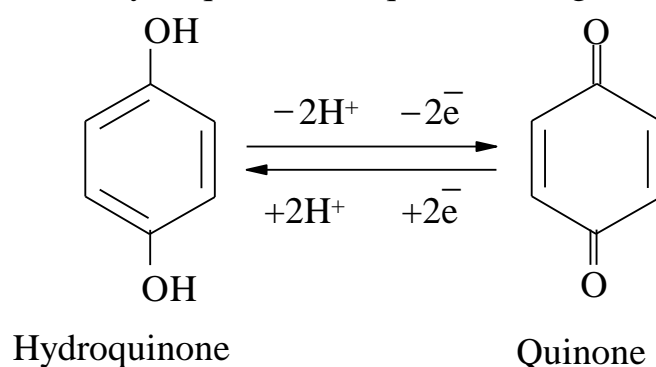
Secondary alcohols are oxidized to ketones:



Tertiary alcohols are resistant to oxidation. A powerful acidic oxidizing agent converts them into a mixture of carboxylic acids.

Hydroxyl group of phenols can be oxidized to carbonyl group.

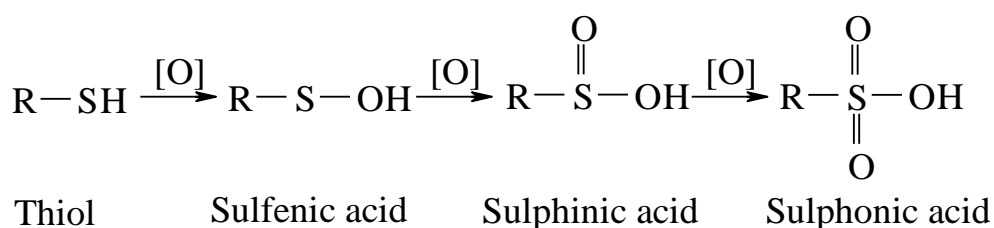
Mutual conversion of hydroquinone and quinone is of great biological importance.



Oxidation of hydroquinone to quinone amounts to the removal of a pair of electrons ( $2\text{e}^-$ ) and two protons from hydroquinone. This reaction is reversible; quinone is easily reduced to hydroquinone. Nature makes much use of this type of reversible oxidation-reduction to transport a pair of electrons from one substance to another in enzyme – catalyzed reactions. Important compounds in this respect are the compounds called ubiquinones (from ubiquitous + quinines are found within the inner mitochondrial membrane of every living cell). Ubiquinones are also called coenzymes Q (CoQ). In the electron transport chain, ubiquinones function by accepting two electrons and two hydrogen atoms to become a hydroquinone. The hydroquinone form carries the two electrons to the next acceptor in the chain.

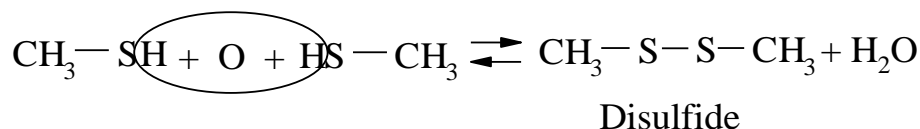
The sulfur counterpart of an alcohol is called a thiol or a mercaptan ( $\text{R-SH}$ ), as the  $-\text{SH}$  group is called the mercapto group. The sulfur atom is larger and more polarized than the oxygen atom. As a result, compounds containing  $-\text{SH}$  groups are stronger acids than their oxygen analogs and form salts – thiolates.

When thiols are treated with strong oxidizing agents, sulfur atom is oxidized.

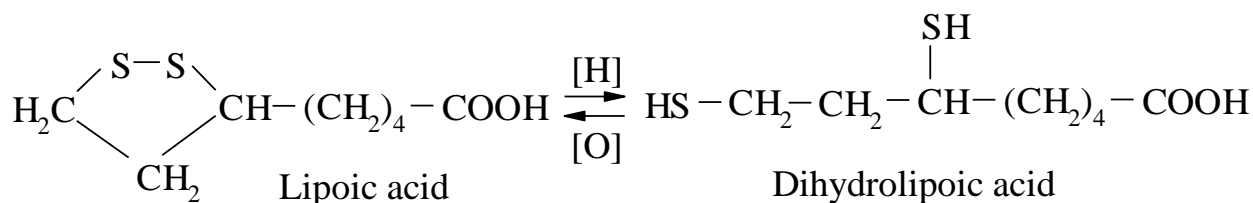




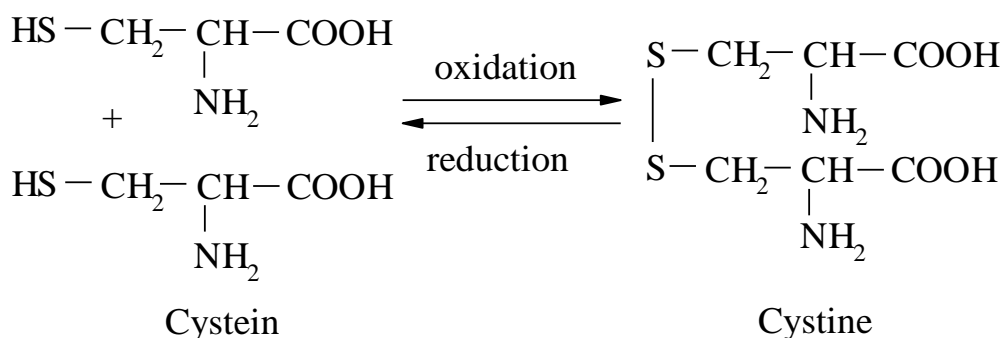
Thiols undergo an oxidative coupling reaction when they react with mild oxidizing agents; the product is disulfide:



Thiols and disulfides are important compounds in living cells, and in many biochemical oxidation – reduction reactions they are interconverted. Lipoic acid, for example, an important cofactor in biological oxidation, undergoes this oxidation – reduction reaction:



The amino acid cysteine and cystine are interconverted in a similar way:



The disulfide linkages of cystine units are important in determining the overall shapes of protein molecules.

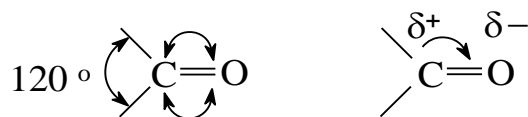
## 6. PROPERTIES OF ALDEHYDES AND KETONES

### *Nucleophilic addition reactions (A<sub>N</sub> - reactions)*

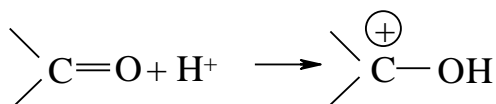
The chemical properties of aldehydes and ketones are due to the carbonyl group present in their molecules.

In the carbonyl group the carbon atom is sp<sup>2</sup> hybridized and, therefore, has 3 sp<sup>2</sup> hybrid orbitals and one unhybridized p-orbital. This means that carbon forms three single σ bonds (one with O atom and other two with two other atoms or groups). σ bonds lie in one plane at an angle of 120°, carbonyl group is planar. The

unhybridized p-orbital of the C atom overlaps with one p orbital of the oxygen atom to form a  $\pi$  bond. The oxygen atom is more electronegative than the C atom. As a result, O attracts the electron cloud of the  $\pi$ -bond towards itself and therefore, it acquires a small negative charge and carbon becomes slightly positively charged.

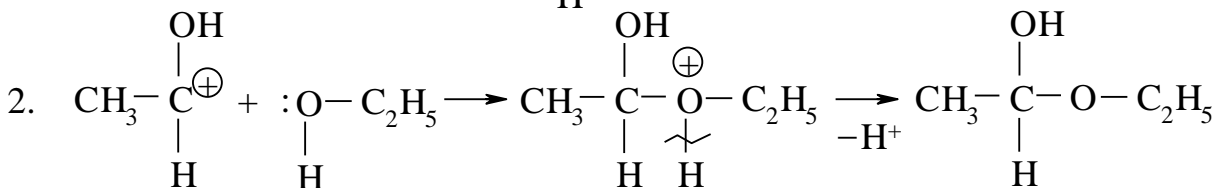
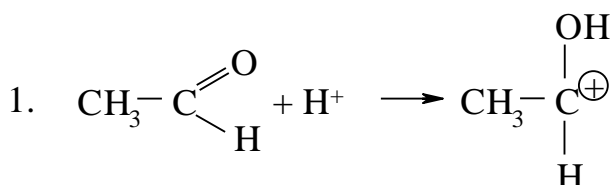


The positively charged carbon is easily attacked by a nucleophilic reagent forming a new bond between carbon and the nucleophile. So, **nucleophilic addition reactions** ( $A_N$ -reactions) are typical for carbonyl compounds. (*Aldehydes are more reactive than ketones. This is because the presence of two alkyl groups in ketones hinders the approach of attacking reagents to the carbonyl group. Another factor is that alkyl groups are electron-donating and reduce the partial positive charge on the carbon atom*). Many reactions of carbonyl groups involve an initial protonation of the oxygen in weakly acidic medium. The protonation increases the positive charge of the carbon so that it is more readily attacked by weaker nucleophiles.

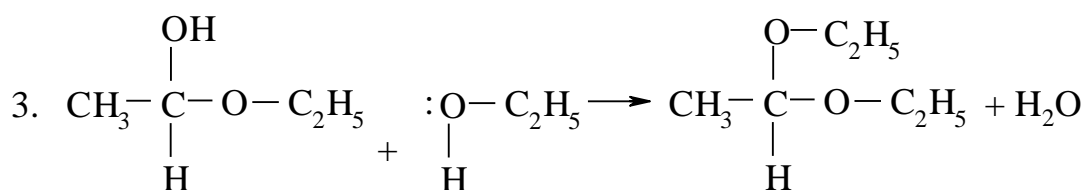


carbocation

**1. Addition of alcohols.** Aldehydes react with alcohols in the presence of dry HCl. The reaction takes place as:



Hemiacetal  
(1-ethoxy ethanol)



Acetal

In the first step, the addition of one molecule of alcohol to an aldehyde gives a hemiacetal.

The mechanism is following:

1. Aldehyde combines with a proton from the acid to form the carbocation.
2. The alcohol that is nucleophile attacks the protonated carbonyl group. This gives rise to an intermediate having a positively charged oxygen.
3. The intermediate then eliminates proton to form an addition product – hemiacetal.

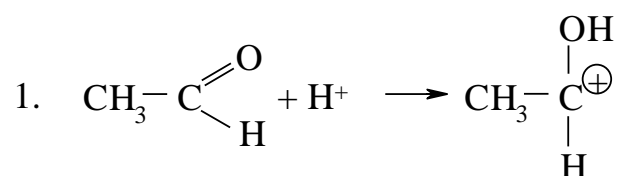
The hemiacetal contains both an ether and alcohol functional group. It is an unstable compound and reacts further with another molecule of alcohol to form stable acetal. Reaction of hemiacetal and acetal formation plays very important role in chemistry of carbohydrates.

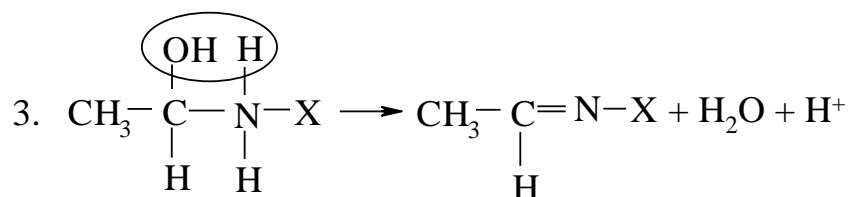
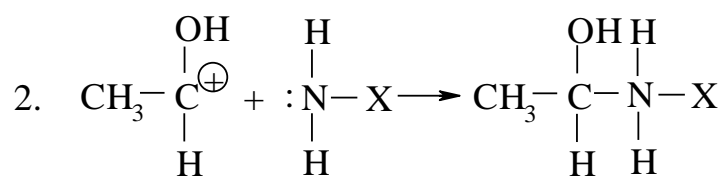
Ketones do not form acetals with monohydric alcohols. They combine with diols to give cyclic ketals.

**2. Addition-elimination (or condensation) reactions.** Aldehydes and ketones react with a number of ammonia derivatives in a weakly acidic medium with the elimination of water molecule. The ammonia derivatives contain a lone pair of electrons and, therefore, their reaction with aldehydes and ketones involves the nucleophilic attack by the basic nitrogen compound on the carbonyl carbon. The ammonia derivatives are weak nucleophiles and, therefore, the reaction is catalyzed by weakly acidic medium. In acidic medium carbonyl oxygen gets protonated. Due to the presence of positive charge, the protonated carbonyl group undergoes nucleophilic attack of ammonia derivatives easily.

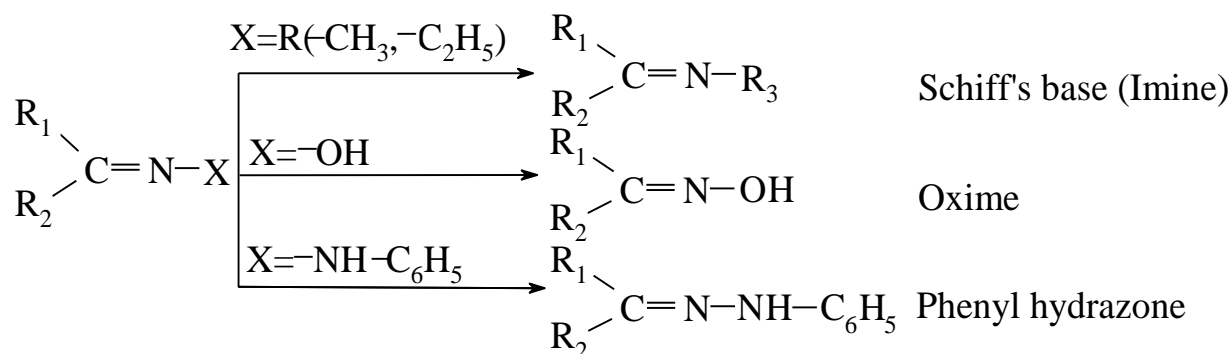
The mechanism is following:

1. Aldehyde combines with a proton from the acid to form the carbocation.
2. The ammonia derivative attacks the protonated carbonyl group. Unstable intermediate is formed.
3. The intermediate then eliminates water to form the product.



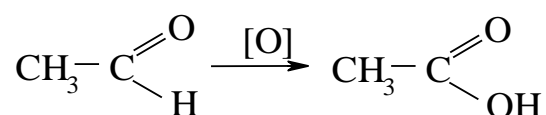


Some of the products are crystalline and are used for the characterization of aldehydes and ketones.



Imines are involved in biological chemistry. For example, pyridoxal phosphate (PLP) that is a very important coenzyme derived from vitamin B<sub>6</sub>, contains imine functional group when it is involved in biochemical reactions. When amino group of amino acid reacts with aldehyde group of PLP imine is formed. Some enzymatic reactions that involve PLP include transaminations, which convert amino acids to ketones for use in the citric acid cycle and other pathways; decarboxylation of amino acids for biosynthesis of neurotransmitters such as histamine, dopamine, and serotonin. All of the reactions of PLP are wonderful examples of how biological processes exemplify organic chemistry in action.

**3. Oxidation.** Aldehydes can be easily oxidized to carboxylic acids on treatment with common oxidizing agents like KMnO<sub>4</sub> and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>. The carboxylic acids formed contain the same number of carbon atoms as the aldehyde.

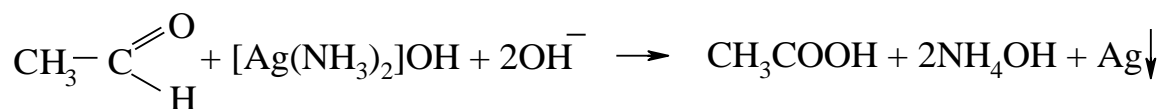


Ketones are not easily oxidized. However, with powerful oxidizing agents such as conc.  $\text{HNO}_3$ ,  $\text{KMnO}_4/\text{H}_2\text{SO}_4$  or  $\text{K}_2\text{Cr}_2\text{O}_7/\text{H}_2\text{SO}_4$ , cleavage of carbon-carbon bond takes place giving a mixture of carboxylic acids having less number of carbon atoms than the original ketone.



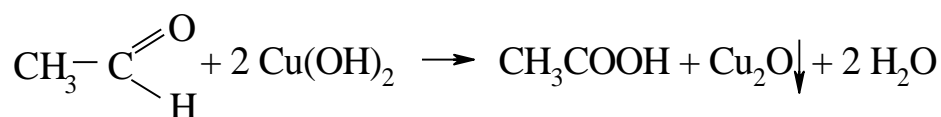
Thus, oxidation reaction can be used to distinguish between aldehydes and ketones. The basis of this test is that aldehydes are very readily oxidized not only by strong oxidizing agents but also by weak oxidizing agents like *Tollen's reagent* and *Fehling solution*. Ketones are not oxidized by these reagents.

When heated with *Tollen's reagent* (ammoniacal silver nitrate solution), aldehydes reduce silver ions to metallic silver and a bright silver mirror is produced on the inner side of the test tube.



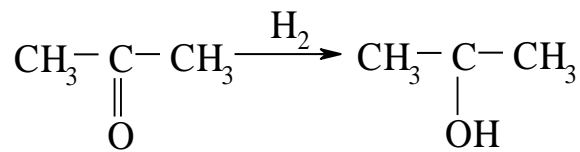
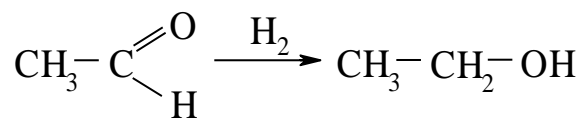
This reaction is also known as *silver mirror test*. The silver mirror formed indicates the presence of aldehydic group in the molecule.

*Fehling solution* is an alkaline solution of  $\text{CuSO}_4$  containing some sodium potassium tartrate (Rochelle salt).

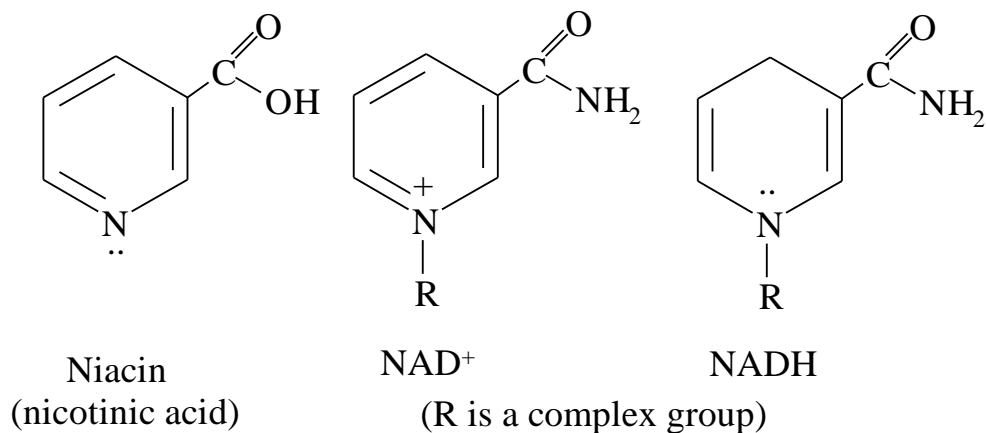


Ketones do not give these tests.

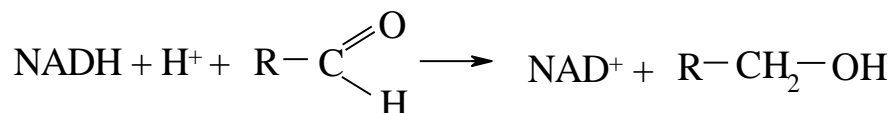
**4. Reduction.** Aldehydes and ketones on reduction give alcohols. Aldehydes give primary alcohols while ketones give secondary alcohols. (*Catalytic hydrogenation occurs in the presence of Ni, Pt, Pd or chemically by lithium aluminium hydride ( $\text{LiAlH}_4$ ) or sodium borohydride ( $\text{NaBH}_4$ ).*)



In biochemical systems the reduction of carbonyl compounds is catalysed by the enzymes – dehydrogenases, in which nicotinamide adenine dinucleotide serve as a coenzyme. Coenzymes are molecules that are part of the organic machinery used by some enzymes to catalyze reactions. The role of many vitamins in our diet is to become coenzymes for enzymatic reactions. The vitamins niacin (nicotinic acid) and its amide nicotinamide (vitamin **PP**, **B<sub>3</sub>**) are precursors to the coenzyme nicotinamide adenine dinucleotide.

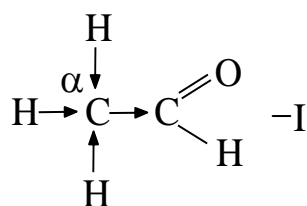


This coenzyme exists in two forms. In its oxidized form it is called NAD<sup>+</sup>, while in its reduced form it is known as NADH. In glycolysis, the citric acid cycle, and many other biochemical pathways, NAD<sup>+</sup> serves as an oxidizing agent. On the other hand, in the electron transport chain and other metabolic processes, NADH is a reducing agent that acts as an electron donor.



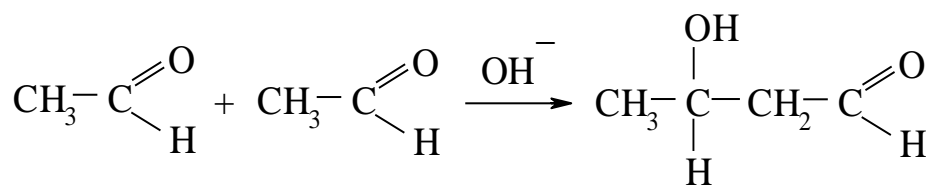
Aldehydes and ketones act as intermediates of monosaccharides, fatty acids, amino acids metabolism.

5. **Aldol condensation.** Carbonyl group is the electron acceptor, it possesses -I-effect.



It withdraws the electron density towards itself. As a result, bonds between C and H become weak and proton can be released. Therefore, compound containing  $\alpha$ -hydrogen (H atom attached to the C atom adjacent to the carbonyl group) behaves as acid. This phenomenon is known as CH-acidity and compound is said to be CH-acid.

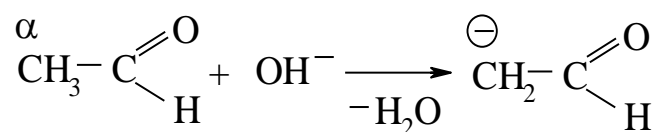
Aldehydes and ketones containing  $\alpha$ -hydrogen undergo **condensation** in the presence of a dilute alkali. This reaction is called **aldol condensation**. The word "aldol" is derived from the combination of words aldehyde and alcohol – the two functional groups present in the product ald + ol = aldol).



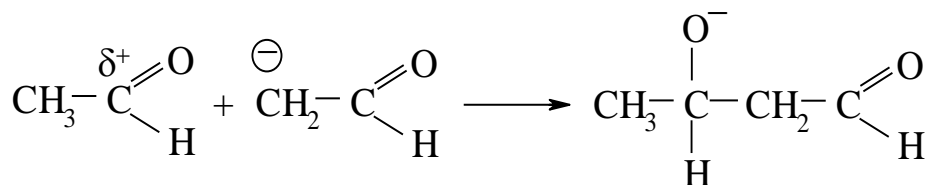
3-hydroxy butanal

Mechanism:

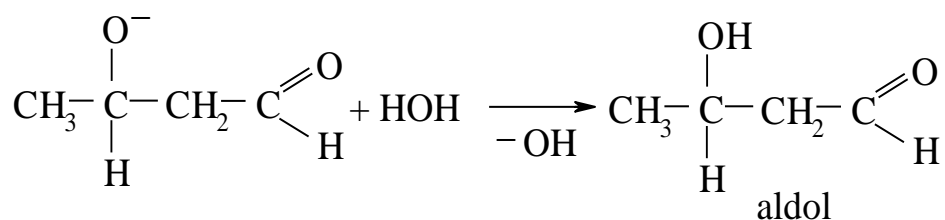
1. Carbonyl compound containing  $\alpha$ -hydrogen loses proton in the presence of alkali and becomes carboanion.



2. Carboanion combines with the other molecule of carbonyl compound.

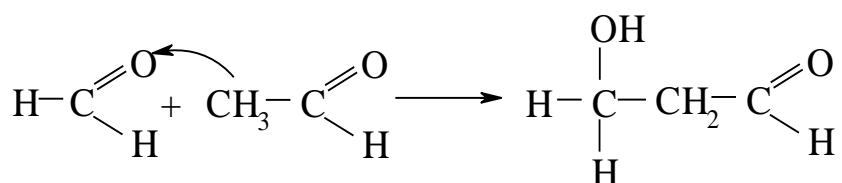


3. Anion formed combines with the proton from water molecule to form the product – aldol.

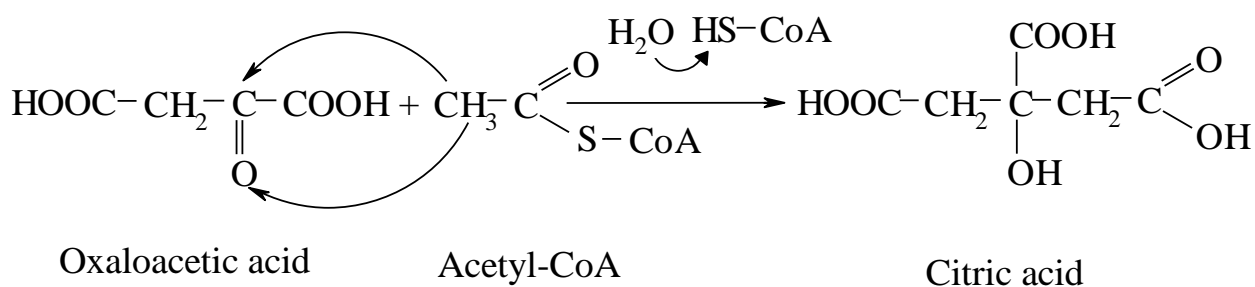


The condensation of molecules of the same carbonyl compound is called **self condensation**. The condensation of two different carbonyl compounds (one of which must have one  $\alpha$ -hydrogen) is known as **cross aldol condensation**.

If the aldehyde or ketone does not contain an  $\alpha$ -hydrogen, it will not undergo self aldol condensation. Formaldehyde and benzaldehyde can serve as example. But they may undergo cross aldol condensation with other aldehydes and ketones which contain  $\alpha$ -hydrogen.

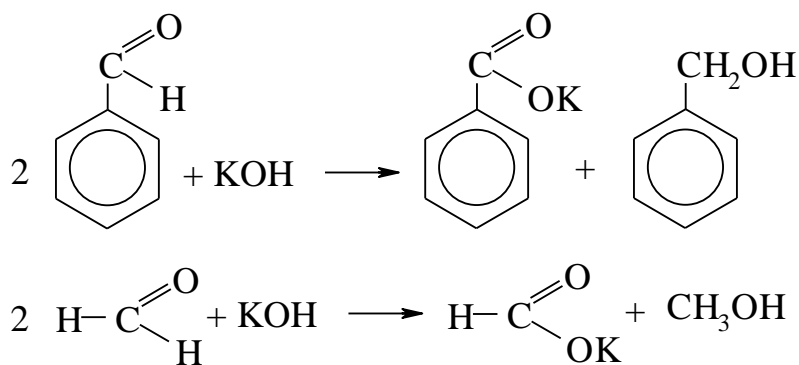


Aldol condensation reactions are of great importance in biochemistry, especially in carbohydrates metabolism. In the organism such reactions take place in the presence of thioesters – derivatives of coenzyme A. For example, in Krebs cycle acetyl-CoA undergoes aldol condensation with  $\alpha$ -keto acid (oxaloacetic acid) to yield citric acid.



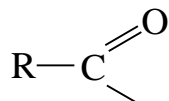
Aldehydes, which do not contain any  $\alpha$ -hydrogen atom undergo self oxidation and reduction reaction on treatment with concentrated alkali. In this reaction, one molecule is oxidized to acid while another is reduced to alcohol. Thus, a mixture of an alcohol and a salt of carboxylic acid is formed. This is **Cannizzaro reaction**.





## 7. NOMENCLATURE AND PROPERTIES OF CARBOXYLIC ACIDS

Carboxylic acids are the compounds containing carboxyl group in their molecules. The common formula is R-COOH. The residue of carboxyl group without the hydroxyl group is called acyl:



According to the number of carboxyl groups carboxylic acids are classified as:

### 1. Monobasic acids, e.g.:

HCOOH – formic (methanoic) acid

CH<sub>3</sub>COOH – acetic (ethanoic) acid

CH<sub>3</sub>CH<sub>2</sub>COOH – propionic (propanoic) acid

CH<sub>3</sub>(CH<sub>2</sub>)<sub>2</sub>COOH – butyric (butanoic) acid

CH<sub>3</sub>(CH<sub>2</sub>)<sub>3</sub>COOH – valeric (pentanoic) acid

CH<sub>3</sub>(CH<sub>2</sub>)<sub>4</sub>COOH – caproic (hexanoic) acid

CH<sub>3</sub>(CH<sub>2</sub>)<sub>5</sub>COOH – enanthic (heptanoic) acid

### 2. Dibasic acids, e.g.:

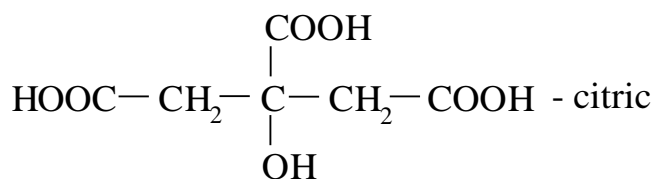
HOOC-COOH – oxalic (ethanedioic) acid

HOOC-CH<sub>2</sub>-COOH – malonic (propanedioic) acid

HOOC-(CH<sub>2</sub>)<sub>2</sub>-COOH – succinic (butanedioic) acid

HOOC-(CH<sub>2</sub>)<sub>3</sub>-COOH – glutaric (pentanedioic) acid

### 3. Tribasic acids, e.g.:

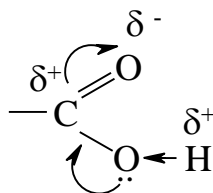


(3-carboxy-3-hydroxy-pentanedioic) acid

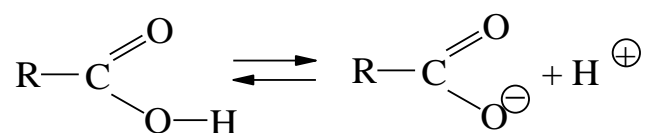
According to the structure of hydrocarbon radical acids are classified as:

1. Aliphatic (saturated or unsaturated (e.g. acrylic acid – propenoic acid, crotonic acid – but-2-enoic acid);
2. Aromatic (e.g. benzoic acid);
3. Heterocyclic (e.g. nicotinic acid)

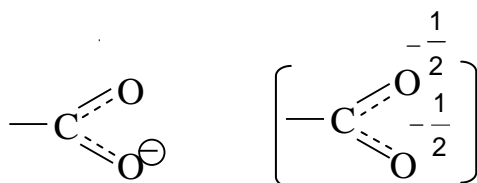
The carboxyl group is made up of carbonyl and hydroxyl group. The two groups influence each other and they do not react as they do in carbonyl compounds and alcohols. The polar carbonyl group attracts electrons away from the –O–H bond, and makes it easier for the hydrogen atom to ionize than in the O–H bond in an alcohol. The flow of electrons from the –OH group towards the carbonyl carbon atom reduces the  $\delta^+$  charge on the carbon atom, with the result that it is not attacked by the nucleophiles that attack carbonyl compounds.



Carboxylic acids are distinctly acidic. They ionize in water:



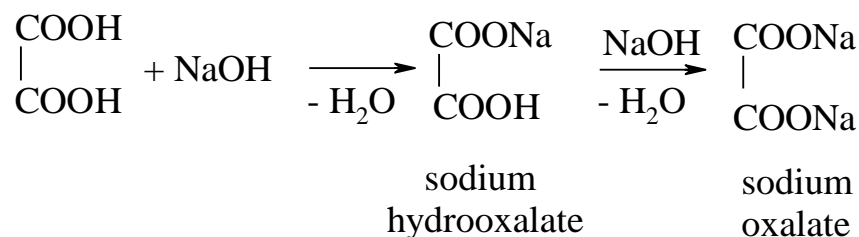
Carboxylic acids are acidic because the carboxylate ions formed are stable and hence carboxylic acids have greater tendency to ionize to form stable carboxylate ions. Carboxylate ion is stable because the negative charge on the ion is shared equally between two oxygen atoms. Unhybridized  $p$  orbital of carbon atom can overlap sidewise with either of the  $p$ -orbital of oxygen atom forming  $\pi$  bond. The resulting  $\pi$ -orbital cloud is spread over both oxygen atoms and carbon atom. This delocalization gives stability to the carboxylate ion.



### 1. Reactions due to the hydrogen atom (acidic character)

a) All carboxylic acids turn blue litmus red.

b) Carboxylic acids react with active metals, alkalis, salts. In the case of polycarboxylic acids acid salts and neutral salts may be formed:



### 2. Reactions involving the –OH group ( $S_N$ -reactions)

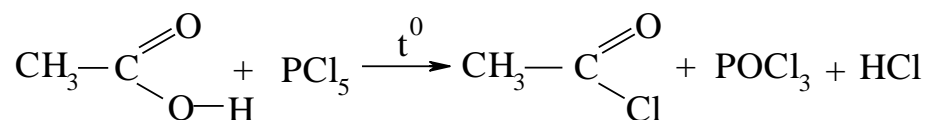
The following  $S_N$  reactions of carboxylic acids are important in biochemical systems:

- Substitution of –OH group by alkoxy group (–OR), i.e. esterification reaction, which is essential in the biosynthesis of lipids.

- Substitution of –OH group by amino group (–NH<sub>2</sub>), i.e. amide formation. The reaction of an amine with an acyl chloride or an ester is often the best method for synthesizing an amide.

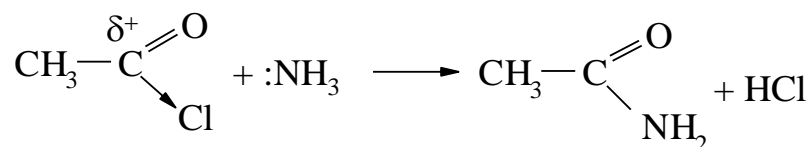
- Substitution of –OH group by phosphate or pyrophosphate group, i.e. acyl phosphates formation.

a) Formation of acid halides (acyl halides). Carboxylic acids can be converted into acid halides by treatment with strong halogenating agents (phosphorous halides, thionyl chloride SOCl<sub>2</sub> in pyridine).

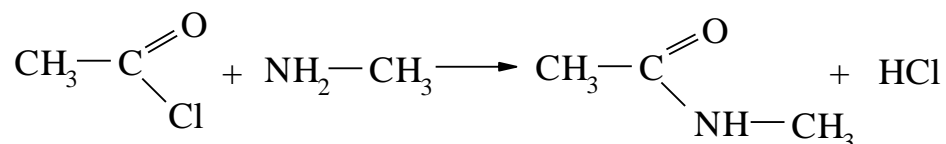


Among the different derivatives of carboxylic acids acyl halides (Acyl chlorides are more common because these are more stable) are the most reactive compounds. Electronegative chlorine atom has strong –I-effect, therefore the electron density on the carbonyl carbon diminishes. As a result, acyl chlorides undergo **nucleophilic substitution reactions** in which –Cl is replaced by a nucleophile readily. As acyl halides are very reactive they are used for preparation of many compounds.

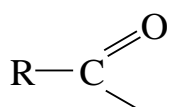
The acyl chlorides react with ammonia to form acid amides:



In reactions with amines acyl chlorides give substituted amides:

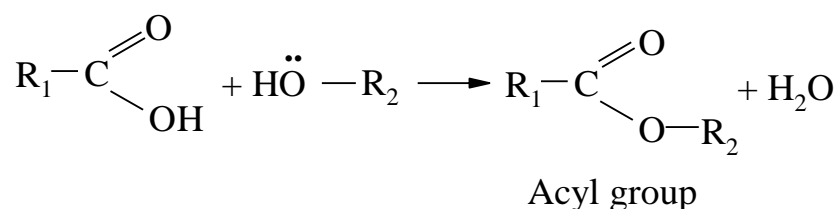


Acyl chloride is the acylating agent in this reaction, and the process is called acylation.

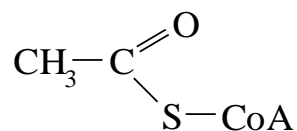


Acyl group

b) Formation of esters (esterification):

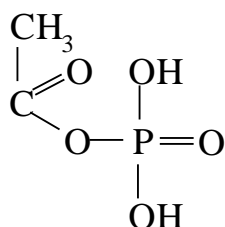


Thiol esters are of great importance in syntheses that occur within living cells. One of the important thiol esters in biochemistry is “acetyl-coenzyme A”. It has a complicated structure and is usually abbreviated as follows:



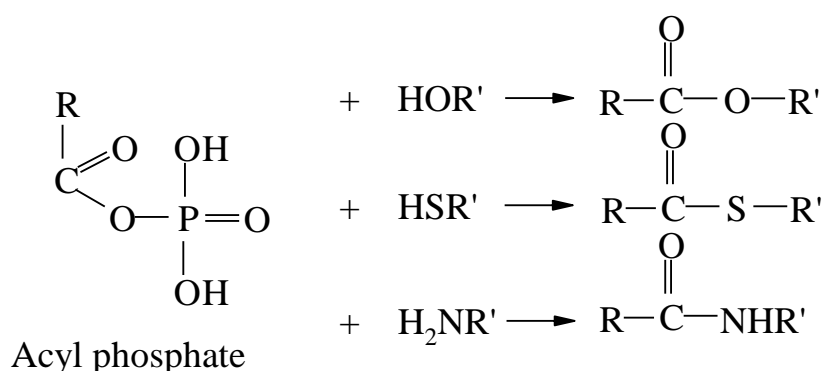
And coenzyme A is abbreviated CoA – SH. In certain biochemical reactions, an acyl-coenzyme A operates as an acylating agent; it transfers an acyl group to another nucleophile.

Formation of mixed anhydrides of carboxylic acids and phosphoric acid is of great importance in biochemistry.



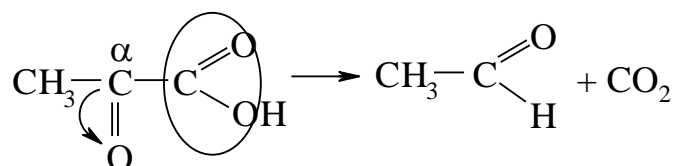
Acetyl phosphoric acid

Being in the form of acyl phosphates acyl groups can be transferred to the hydroxyl, thiol and amino groups of the different biological compounds.



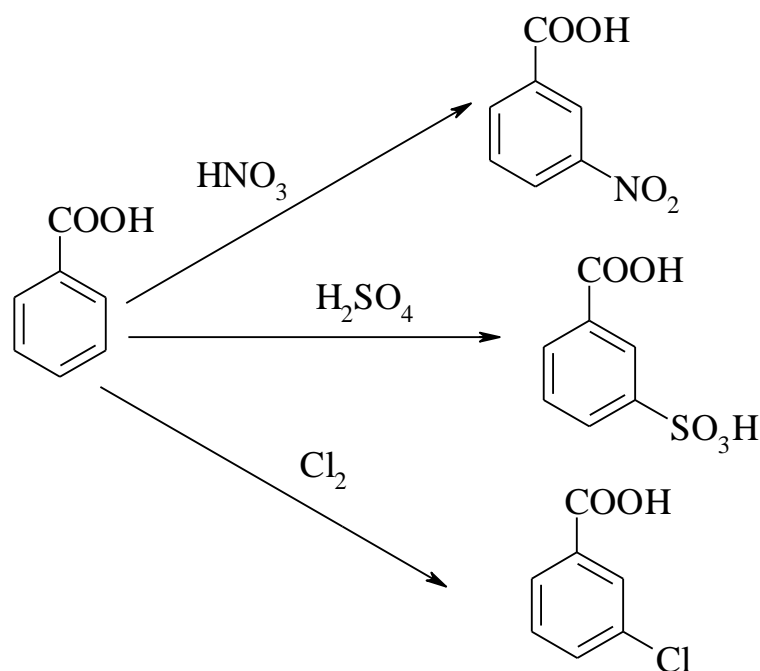
### 3. Reactions involving the carboxyl group.

a) Decarboxylation. Carboxylic acids get decarboxylated i.e. lose carbon dioxide. Carboxylic acids containing electron withdrawing groups in  $\alpha$ - and  $\beta$ -positions undergo decarboxylation more easily than carboxylic acids which do not contain any substituents.



In the process of decarboxylation of carboxylic acids carbon dioxide which is one of the final products of organic substances oxidation is formed.

**Benzoic acid** is an aromatic acid which can be found in many berries, fruits and herbs. It is reactive toward electrophilic substitution. Carboxyl group possesses negative mesomeric effect, and therefore directs electrophile in the meta-position:



Benzoic acid is one of the oldest medicines. It causes local irritating, and antiseptic action, and it is also applied as an external antimicrobial and antifungal agent. Benzoic acid is used internally in the form of sodium salt as an expectorant.

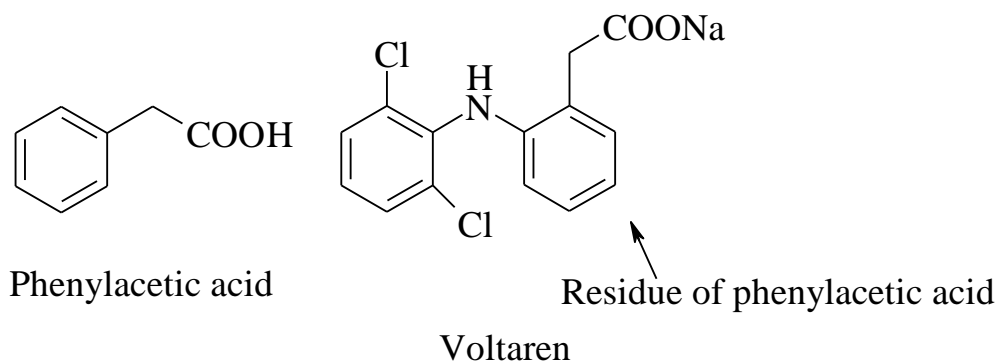
In the organism, benzoic acid is the final product of the oxidation of fatty acids with odd number of carbon atoms. Benzoic acid exists also in the bound form, for example in human and herbivores urine it exists in the form of hippuric acid  $\text{C}_6\text{H}_5\text{CONHCH}_2\text{COOH}$ . Hippuric acid consists of residues of benzoic acid and amino-acetic acid. It forms in the course of detoxification of aromatic substances.

Introduction of different chemical groups (amino, nitro, etc.) into the benzene ring results in formation of substituted aromatic acids. Reduction of o-nitrobenzoic leads to formation of o-aminobenzoic acid (anthranilic acid) which is an important primary product for the synthesis of many drugs (e.g. furosemide).

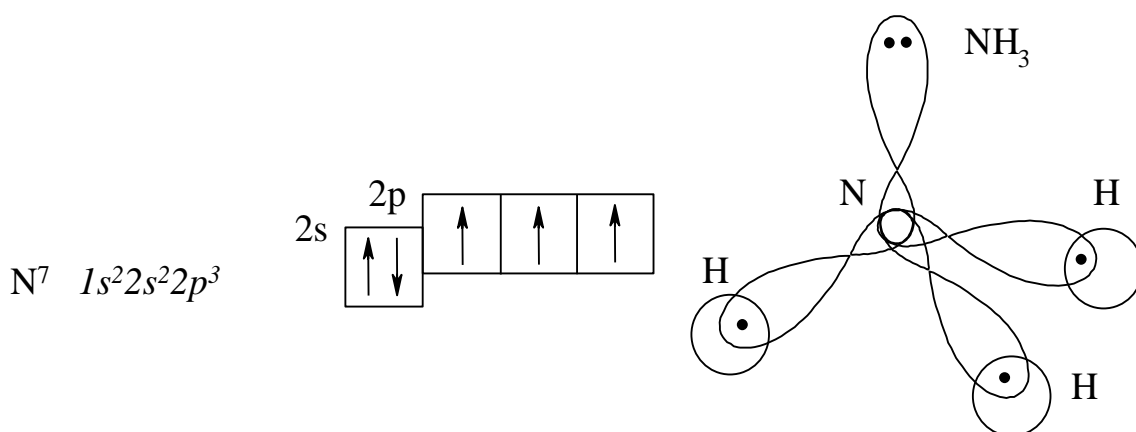
Introduction of such structural fragments as glycine, pyrazole, pyridine to the benzoic acid molecule allows to expect that these derivatives will exhibit anti-inflammatory and analgesic effects. It can be explained by the fact that combination of two biologically active centers using cation-anion bond within the same molecule enhances the action of components and increases the solubility.

Some aromatic acids contain carboxyl group in the side chain (phenylacetic acid). Benzoic as well as phenylacetic acid are important products of metabolism.

They enter the composition of many drugs, e.g. diclofenac sodium (Voltaren) which serves as the "gold standard" in the treatment of inflammatory processes.



## 8. PROPERTIES OF AMINES

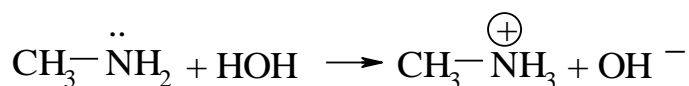


Amines are derivatives of ammonia in which one or more hydrogen atoms have been replaced by alkyl or aryl group. N involves  $sp^3$  hybridization. One of the four hybrid orbitals contains a lone pair. Due to the presence of lone pair on N-atom of the  $-NH_2$  group, the aliphatic amines are basic in nature – they have tendency to donate this lone pair of electrons to electron deficient compounds.

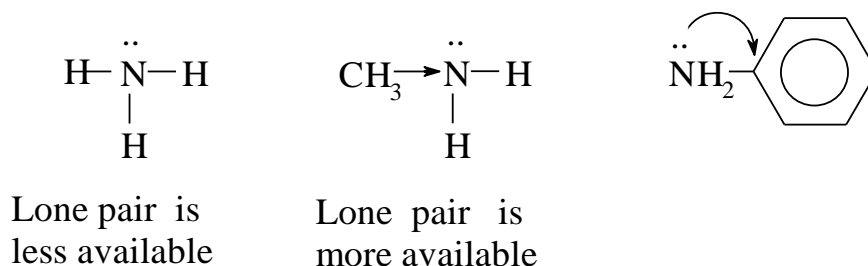
### 1. Basic character.

a) Reaction with water.

When amines are dissolved in water they combine with proton from the water and medium becomes basic.

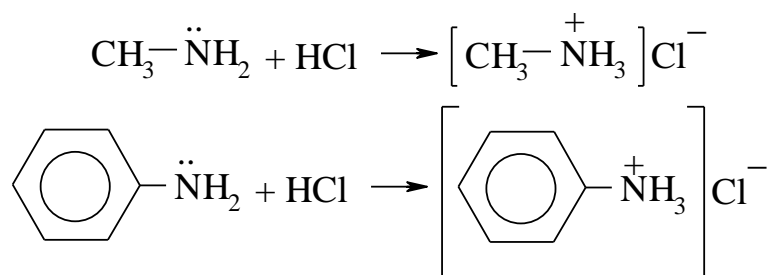


Aliphatic amines are stronger bases than ammonia, because of +I-effect of the alkyl group.



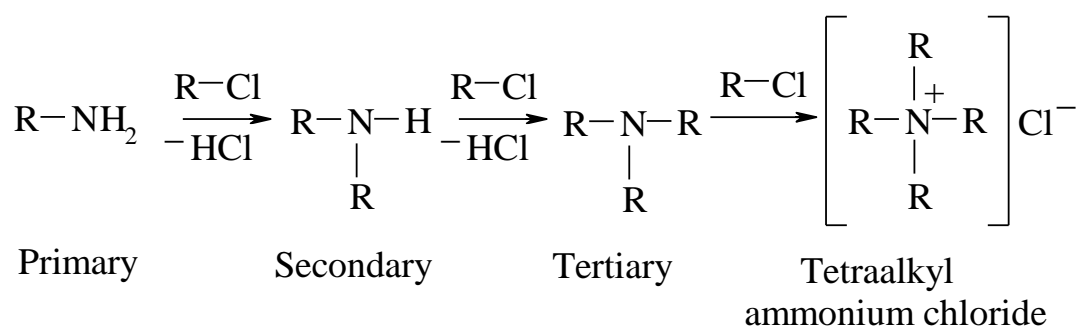
Electron releasing alkyl group increases the electron density on the N and therefore, aliphatic amines can donate electron pair more easily than ammonia. Aromatic amines are weaker bases than ammonia because lone pair of electrons is involved in conjugation (delocalized) with the ring and becomes less available for protonation. Substituents in the ring influence the basic character of aromatic amines. Electron releasing groups increase the basic strength while electron withdrawing groups decrease the basic strength.

b) Reaction with acids. Amines react with acids to form salt.



## 2. Alkylation

Halogenoalkanes (alkyl halides) react with amines, replacing the hydrogen atoms by alkyl groups:

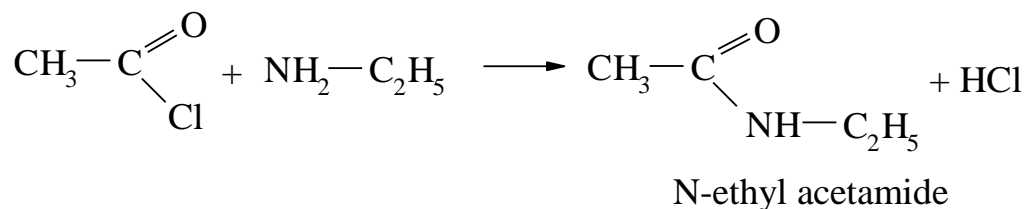


Primary amines are converted into secondary amines and secondary amines into tertiary amines. The final product is a quaternary ammonium salt.



### 3. Acylation

Acylation is the reaction with acid chlorides and acid anhydrides (acylating agents). Primary and secondary amines (which contain replaceable hydrogen atoms) react with acylating agent to form substituted amides.



#### Revision exercises

- What is the product of reaction:  $\text{CH}_3 - \text{CH}_2 - \text{CH} = \text{CH}_2 + \text{HBr} \rightarrow ?$ 
  - $\text{CH}_3 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2\text{Br}$
  - $\text{CH}_3 - \text{CH}_2 - \text{CHBr} - \text{CH}_3$
  - $\text{CH}_2\text{Br} - \text{CH}_2 - \text{CH} = \text{CH}_2$
  - $\text{CH}_3 - \text{CH}_2 - \text{CHBr} - \text{CH}_2\text{Br}$
- Which reaction illustrates acidic properties of propanol?
  - $\text{C}_3\text{H}_7\text{OH} \rightarrow \text{C}_3\text{H}_6 + \text{H}_2\text{O}$
  - $\text{C}_3\text{H}_7\text{OH} + \text{HBr} \rightarrow \text{C}_3\text{H}_7\text{Br} + \text{H}_2\text{O}$
  - $\text{C}_3\text{H}_7\text{OH} + \text{HOC}_2\text{H}_5 \rightarrow \text{C}_3\text{H}_7 - \text{O} - \text{C}_2\text{H}_5 + \text{H}_2\text{O}$
  - $2\text{C}_3\text{H}_7\text{OH} + 2\text{K} \rightarrow 2\text{C}_3\text{H}_7\text{OK} + \text{H}_2$
- Electrophilic substitution reactions are typical for:
  - Saturated hydrocarbons
  - Unsaturated hydrocarbons
  - Aromatic hydrocarbons
  - Alcohols

#### Answers

- b)
- d)
- c)

\* \* \*

## **ACID-BASE PROPERTIES OF ORGANIC COMPOUNDS**

### ***Characteristics of the subject***

Acidity and basicity are among the most important properties of the substances which determines their action in the organism.

### ***Purpose***

To study acidity and basicity of organic compounds, and also main properties determining the majority of chemical processes in the living organisms.

### ***Objectives***

1. To be able to explain the acidic properties of alcohols, phenols and carboxylic acids, their dependence on the number of functional groups and the presence of substituents in the molecule.

2. To understand the phenomenon of C-H acidity and its influence on the properties of compounds.

3. To explain basic properties of aliphatic and aromatic amines as well as nitrogen-containing heterocycles.

4. To know the classes of chemical compounds which have amphoteric properties. To be able to give an example.

### ***Theoretical questions***

1. Acidic properties of organic compounds. Types of acidity.

2. Basic properties of organic compounds.

According to the Bronsted protolytic theory, acids - are neutral molecules or ions capable of donating a hydrogen proton (donors of protons). Bases are neutral molecules capable to accept hydrogen proton (proton acceptors).

Acidity and basicity are not absolute, but relative properties of compounds: the acidic properties manifest themselves only in the presence of bases and basic - in the presence of acids.

### ***1. ACIDIC PROPERTIES OF ORGANIC COMPOUNDS. TYPES OF ACIDITY***

Acids are organic compounds containing hydrogen atoms connected to the more electronegative atom (oxygen, sulfur, nitrogen, and carbon). Depending upon

the element to which hydrogen is bonded to, the following types of acids are distinguished:

- OH-acids (alcohols, phenols, carboxylic acids);
- SH-acids (thiols);
- NH-acids (amines, amides);
- CH-acids (hydrocarbons and their derivatives).

For elements of the same period the ability to detach a proton increases with increase in electronegativity. Therefore Bronsted acids having the same radicals can be arranged in the following order of increasing acidity:

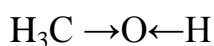


Let us consider acidic properties of alcohols, phenols and carboxylic acids.

The polarity of the O - H bond in the alcoholic hydroxyl group is the reason of its ability to heterolytic cleavage. This type of bond cleavage occurs in reactions with active metals with formation of solid, alcohol-soluble products – alcoholates (alcoxides) with ionic bond between oxygen and metal:

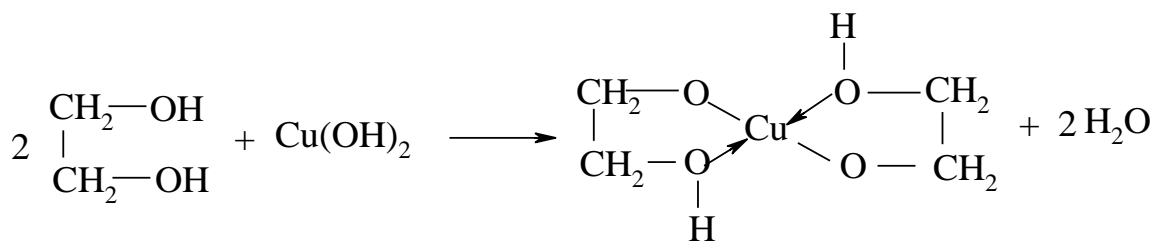


Positive inductive effect (+ I) of a carbon radical decreases the polarity of the O – H bond and therefore reduces the acidic properties of alcohols.



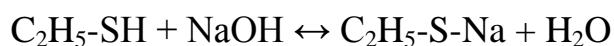
So, alcohols are even weaker acids than water. For the same reason, the acidity of alcohols decreases with increasing of the number of carbon atoms in the radical.

Acidity of polyhydric alcohols is higher than that of monohydric ones, due to negative inductive effect (-I) of hydroxyl groups. Hydrogen atoms of polyhydric alcohols are easily replaced by some heavy metals with formation of chelates.

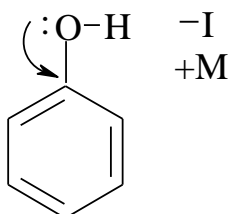


Chelates have bright coloration and their formation is used for the qualitative determination of polyhydric alcohols.

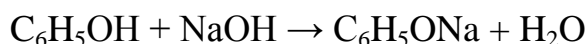
The acidic properties of **thiols** are stronger than that of alcohols due to the higher polarization ability of the sulfur atom as compare to oxygen. Therefore thiols form salts (mercaptides) with aqueous alkali solutions:



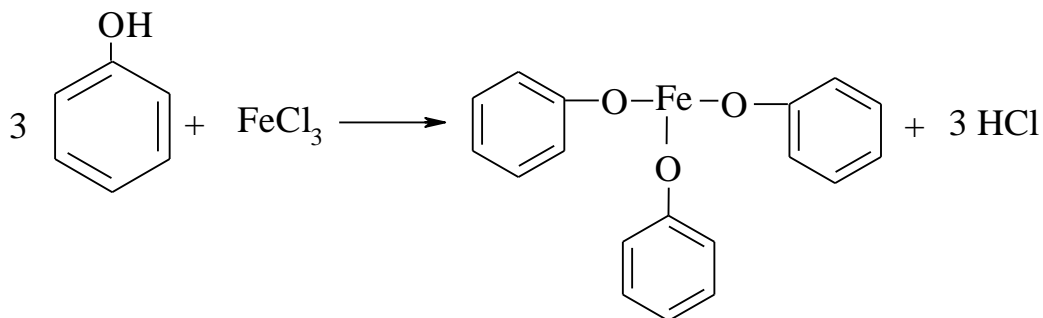
The acidity of **phenols** is stronger than that of alcohols. This is because the electron pair of the oxygen atom is shifted to the aromatic ring (+ M effect), which leads to increasing of polarization of O-H bond.



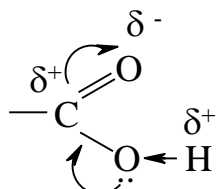
Unlike aliphatic alcohols phenol easily reacts with alkalis forming salts - phenolates:



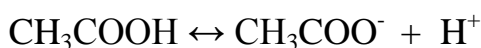
Phenols also react with metals and salts, showing all of the chemical properties of acids. Qualitative reaction to the phenolic hydroxyl group is the reaction with the ferric salts:



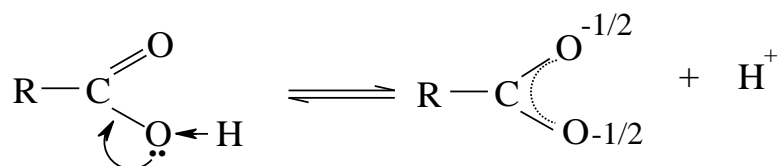
Introduction of electron-accepting substituents into aromatic ring increases the acidity of phenols, and introduction of electron-donating substituents reduces it. Acidic properties are mostly expressed in the series of carboxylic acids - compounds containing carboxyl group -COOH. The presence of the carbonyl group near to hydroxyl group causes the conjugation of paired electrons of oxygen of the hydroxyl group with electrons of the carbonyl  $\pi$ -bond:



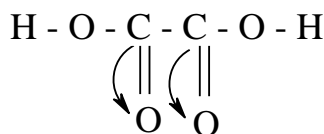
As a result the electronegativity of oxygen atoms increases, which leads to increasing polarization of the O - H bond and facilitates the removal of a proton. In aqueous solution, the lower carboxylic acids significantly dissociate to produce protons.



Carboxylate anion is a delocalized three-centered system in which bonds of carbon atom with two oxygen atoms are equalized.

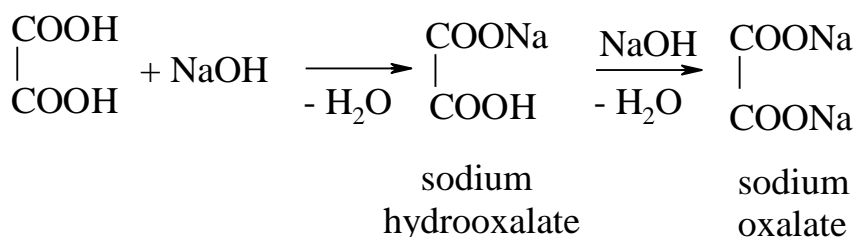


Carboxylic acids are weak electrolytes. The more stable the anion formed by the dissociation, the greater equilibrium of dissociation process is shifted to the right. Therefore, the greater is the stability of the anion, the stronger is the acid. Stability of the anion, in its turn, is determined by the degree delocalization of negative charge. Therefore, the factors facilitating the delocalization of the charge, increase acidity and those factors which inhibit delocalization reduce acidity. This fact is also true for other classes of compounds exhibiting acidic properties. In particular, the dicarboxylic acids are stronger acids than monobasic ones with the same number of carbon atoms due to the -I effect of the second carboxyl group.



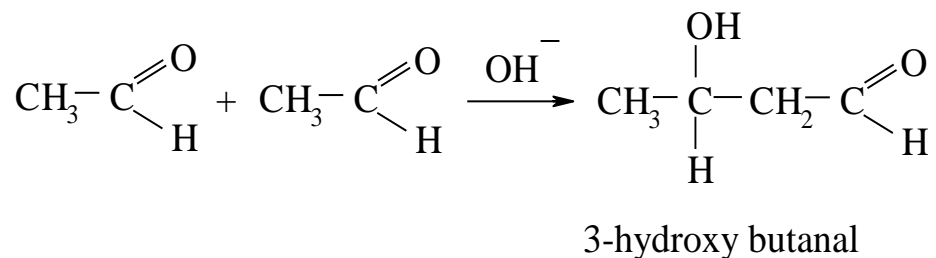
Carboxylic acids change the coloration of the indicator and show all properties of acids, interacting with metals, bases, basic oxides, and salts. Acidic strength decreases in homologies series of monobasic carboxylic acids due to increasing +I-effect of hydrocarbon radical. It means that formic acid is the strongest in this series.

Dibasic carboxylic acids which, by the way, play an important role in the biochemical transformations, can form acid salts and neutral salts:

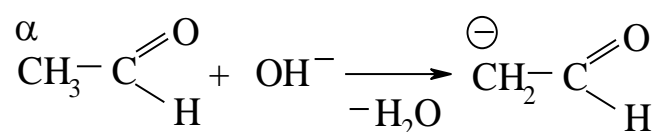


Polarity of the **C-H bond** in hydrocarbons is negligible and therefore their acidic properties are expressed very poorly. The presence of the electron-accepting substituents significantly increases the CH-acidity. In CH-acids hydrogen proton detaches from  $\alpha$ -carbon atom (carbon adjacent to the functional group). Aldol

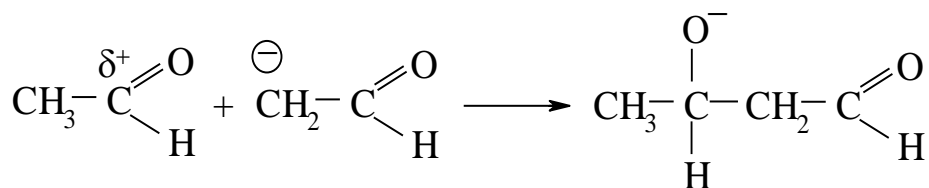
condensation reaction can serve as example of CH-acidity. Aldol condensation is the reaction between molecules of aldehydes in aqueous alkaline solution.



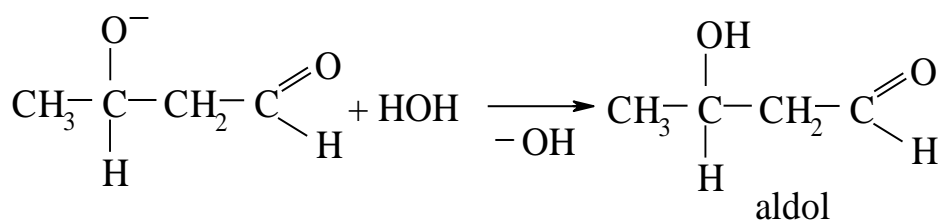
Hydroxyl group of alkali attracts hydrogen proton from  $\alpha$ -carbon atom and molecule becomes carboanion:



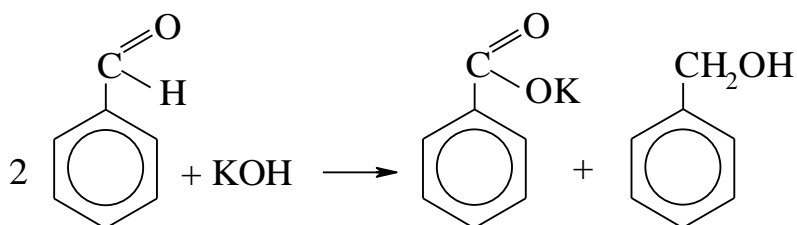
This anion is rather stable because  $\alpha$ -carbon atom becomes  $sp^2$  hybridized and enters into conjugation with  $\pi$ -bond of carbonyl group. Carboanion combines with the other molecule of carbonyl compound.



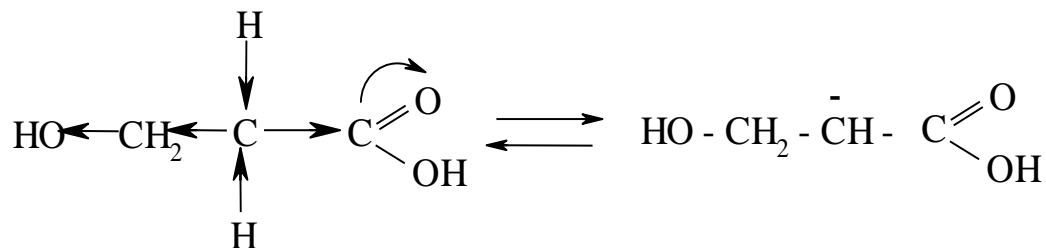
Anion formed is unstable due to the absence of conjugation and stabilizes itself by combination with the proton from water molecule:



C-H acidity can be performed only by substances having hydrogen atoms at  $\alpha$ -carbon atom. For example, there are no  $\alpha$ -carbons in benzaldehyde molecule, so, under the influence of alkali benzaldehyde does not form aldols. Under the same conditions it enters Cannizzaro reaction in which one molecule of the aldehyde is reduced to benzyl alcohol, and the second is oxidized to benzoic acid. Benzoic acid in basic medium gives salt.



The CH-acidity is manifested strongly in compounds containing methyl group between two electron-accepting substituents: in  $\beta$ -hydroxy acids,  $\beta$ -amino acids, etc. In this case, the hydrogen proton at the carbon atom can be released easily and the molecule is cleaved into a carbanion:

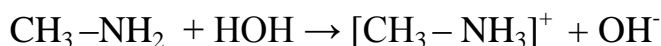


This phenomenon causes specific reactions of these compounds.

## 2. BASIC PROPERTIES OF ORGANIC COMPOUNDS

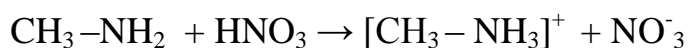
According to the Bronsted protolytic theory bases are acceptors of protons. Organic compounds can accept proton either by the unshared electrons or by electrons of  $\pi$ -bond.

Basic properties are mostly expressed in the series of amines - compounds containing substituents  $-\text{NH}_2$ ,  $-\text{NHR}$  or  $\text{R}_1\text{-N-R}_2$ . Similar to ammonia the chemical properties of amines are substantially determined by the presence of unshared electrons of nitrogen. So, amines join protons when dissolved in water and accumulation of hydroxyl groups makes solution basic.

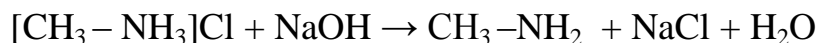


Ion  $[\text{R-NH}_3]^+$  can be considered as a complex ion containing nitrogen as the central atom. Coordination number of nitrogen is 4, and hydrogen atoms or alkyl groups are located in the inner coordination sphere.

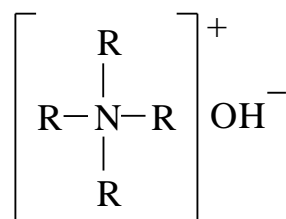
Basic properties of amines are also manifested by their ability to interact with acids to form salts:



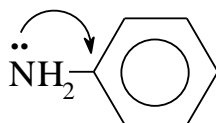
Salts of amines are crystalline solids, readily soluble in water. Alkalies being stronger bases than amines replace amines from their salts:



Amines are stronger bases than ammonia due to the +I effect of hydrocarbon radicals. Introduction of the third alkyl group (in tertiary amines) decreases basicity due to steric hindrance caused by the shielding effect of the three alkyl groups. Quaternary ammonium bases possess strong basic properties which are comparable in strength with alkali.

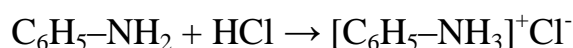


The basic properties of aromatic amines are significantly weaker than that of aliphatic ones. This is due to the conjugation of the lone pair of electrons of the nitrogen atom with the  $\pi$ -electron system of the ring:



As a result, the electronic density of the nitrogen atom is reduced and the ability to combine proton decreases. Addition of hydrogen proton in this case leads to reducing of number of atoms participating in conjugation, and as a result, to less delocalization of electronic density which is not favorable from energetic point of view.

An aqueous solution of aromatic amines does not change color of indicators, they do not form salts with weak acids. They react with strong acids to produce salts:

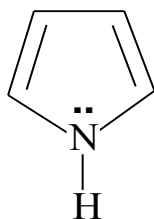


Electron-accepting substituents reduce basicity of aromatic amines and electron-donating substituents increase it.

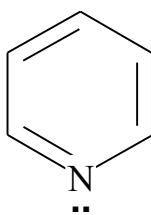
Basic properties are also inherent to nitrogen-containing heterocyclic compounds. In these compounds basicity depends on whether unshared electrons of nitrogen enter in conjugation with the electrons of the atoms of carbon cycle or not. In pyrrole basic properties are expressed very weakly, because unshared electrons of



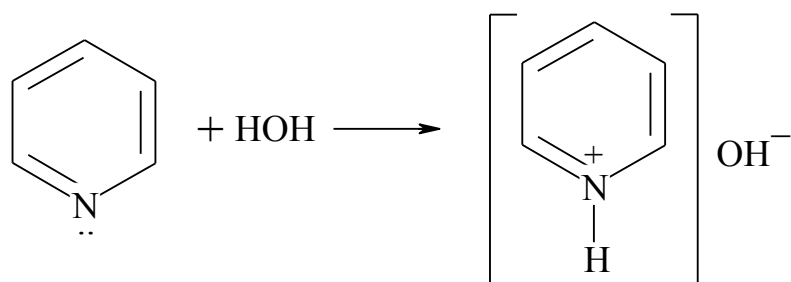
nitrogen are involved in conjugation with the ring to form aromatic system. So, hydrogen proton can not be accepted by pyrrolic nitrogen.



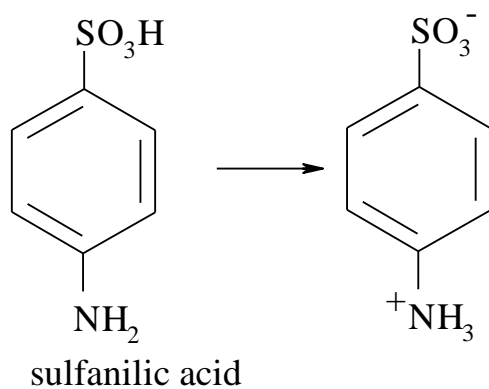
In the pyridine molecule conjugated system is formed without electrons of nitrogen, so pyridine behaves similar to aliphatic amines.



Aqueous solution of pyridine is basic:

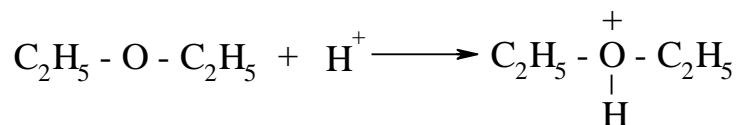


Compounds containing both acidic and basic groups are capable to form internal salts in which proton formed by the dissociation of acidic group is bind by basic group.



Amino acids and some other compounds are also capable to form internal salts.

Proton can also be accepted by unshared electrons of the oxygen atom in alcohols, aldehydes, ethers, etc. In this case oxonium ions - alkyl derivatives of hydronium ion are formed:



Just as the hydronium ion, oxonium bases are unstable - they serve as intermediates in many reactions, especially in case of acidic catalysis.

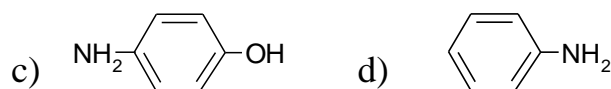
### Revision exercises

1. Which compounds are basic in nature?

- a) Saturated hydrocarbons      b) Aromatic hydrocarbons  
c) Amines      d) Alcohols

2. Which of the following compounds is more basic?

- a)  $\text{CH}_3 - \text{NH}_2$       b)  $\text{C}_3\text{H}_7 - \text{NH}_2$



3. Which of the following is the weakest acid?

- a) Formic acid      b) Acetic acid  
c) Propionic acid      d) Butyric acid

### Answers

1. c)  
2. b)  
3. d)

\* \* \*

# **STRUCTURE, PROPERTIES AND BIOLOGICAL IMPORTANCE OF CARBOXYLIC ACIDS DERIVATIVES (HYDROXY ACIDS, OXOACIDS, AND PHENOLIC ACIDS)**

## ***Characteristics of the subject***

Numerous redox processes in the organism are associated with formation and participation of hydroxy- and oxo- acids (oxidation of carbohydrates and fatty acids in the body, formation of citric acid in Krebs cycle, etc.). A number of these compounds or their derivatives are drugs. E.g. salts of lactic acid (lactates) are used in anemia treatment, citric acid is used in blood preservation, aspirin, which is the ester of salicylic and acetic acids is used as analgesic and antipyretic.

## ***Purpose***

To study the structure, chemical properties and spatial structure of carboxylic acids derivatives (hydroxy-, oxoacids, and phenolic acids) as the basis for understanding their metabolic conversions in the organism.

## ***Objectives***

1. To know the structure of the most important hydroxy- and oxo- acids.
2. To be able to explain the properties of hydroxy- and oxo- acids as heterofunctional compounds, as well as specific reactions of  $\alpha$ ,  $\beta$  and  $\gamma$  hydroxy-acids.
3. To know transformation of hydroxy- and oxo- acids in Krebs cycle.
4. To be able to explain keto-enol tautomerism on the example of acetoacetic acid.
5. To be able to make formulas of enantiomers of L- and D- series. To understand the difference in their properties and biological activity.
6. To know the peculiarities of isomerism of molecules with two chiral centers.

## ***Theoretical questions***

1. Hydroxy acids: representatives, chemical properties, optical isomerism.
2. Oxoacids: representatives, keto-enol tautomerism.
3. Krebs cycle.
4. Phenolic acids.

Hydroxy acids are polyfunctional compounds containing both hydroxyl and carboxyl groups.

Oxoacids are compounds containing both carboxyl and aldehyde or keto groups. According to this we distinguish aldoacids and keto acids.

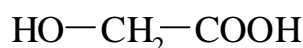
Basing on the number of carboxyl groups, monobasic, dibasic, tribasic, etc. acids can be distinguished.

Basing on the number of hydroxyl groups monohydroxy acids (possessing one -OH group), dihydroxy and etc. can be distinguished.

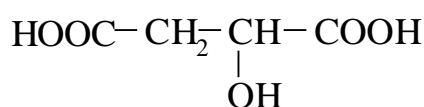
## **1. HYDROXYACIDS: REPRESENTATIVES, CHEMICAL PROPERTIES, OPTICAL ISOMERISM**

### **Representatives**

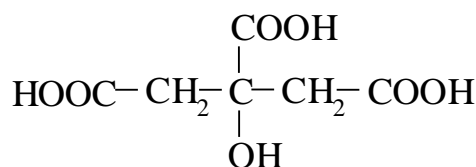
Glycolic acid (monohydroxy-monobasic)



Malic acid (monohydroxy-dibasic)



Citric acid (monohydroxy-tribasic)



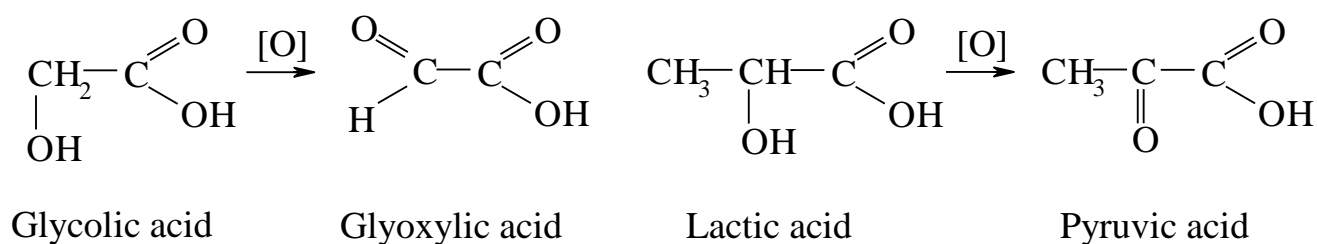
Hydroxy acids possess all chemical properties typical for carboxyl group and hydroxyl group as well as the new properties typical only for hydroxy acids.

Here are the properties depended on the presence of carboxyl group:

1. Salt formation
2. Esterification
3. Acid halides formation
4. Amides formation
5. Decarboxylation

Here follow the depended on the presence of hydroxyl group:

1. Oxidation (if the hydroxyl is primary, aldoacid is formed, if it is secondary - keto acid)



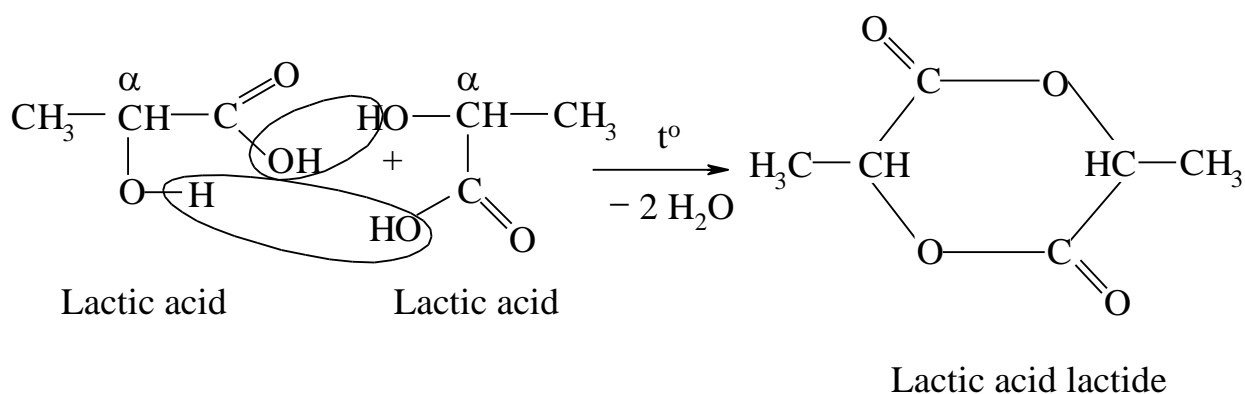
2. Alkoxide formation (acidic character - reaction with the active metals).
3. Esterification (reaction with carboxylic acid)

4. *Etherification* (reaction with alcohol)
5. *Hemiacetal formation*
6. *Replacing of hydroxyl group by halogen*

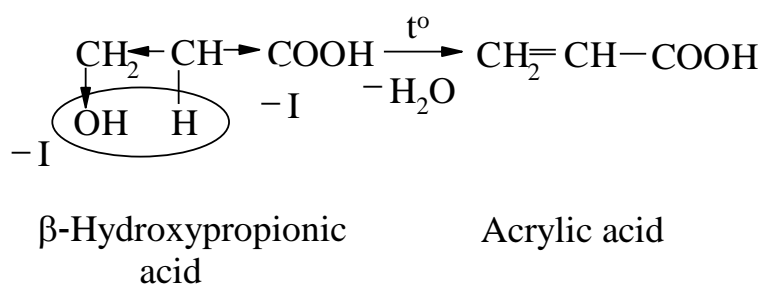
### Specific reactions

As molecules of hydroxy acids contain  $-\text{COOH}$  and  $-\text{OH}$  groups, these groups can react with each other.

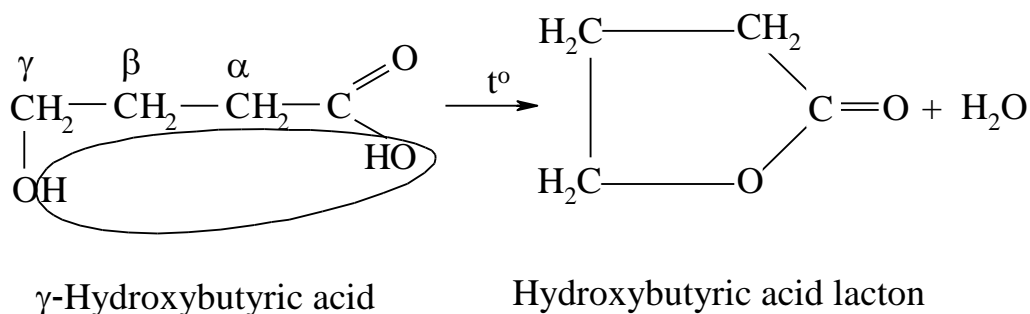
While heating  $\alpha$ -hydroxy acids in the acidic medium, cyclic ester consisting of two molecules of  $\alpha$ -hydroxy acids (two molecules of water are eliminated from two molecules of acid) is being formed. The substance produced is called ***lactide*** and is very stable being six-membered cycle.



Elimination of one molecule of  $\text{H}_2\text{O}$  from one molecule of acid is typical for  $\beta$ -hydroxy acids when under the same conditions. ***Unsaturated acid*** is formed.



Elimination of one molecule of  $\text{H}_2\text{O}$  from one molecule of acid is typical for  $\gamma$ - and  $\delta$ -hydroxy acids.  $\text{H}_2\text{O}$  molecule is formed owing to  $-\text{COOH}$  and  $-\text{OH}$  groups of the same molecule. The formation of cyclic ester (***lactone***) is the result of it: five-membered cyclic ester is formed from  $\gamma$ -acid and six-membered cyclic ester - from  $\delta$ -acid. Both cycles are stable.



### *Optical isomerism*

One of the types of stereoisomerism called optical isomerism can often be found in the group of hydroxyacids.

*Optical isomerism* is a kind of stereoisomerism in which the isomers differ in the spatial arrangements of the atoms or group of atoms. Optical isomers have the same properties but they differ in their behaviour towards plane polarized light.

***Optical activity.*** A beam of ordinary light has waves vibrating in all planes in space. When ordinary light is passed through a Nicol prism (crystalline  $\text{CaCO}_3$  (calcite)) it becomes plane polarized light. In the prism, vibrations in all other planes except in one plane are cut off.

When the solutions of certain organic compounds are placed in the path of a plane polarized light, they rotate the path of the plane polarized light either to the left or to the right. Such substances, which rotate the plane polarized light, are called ***optically active substances***. The common examples are: lactic acid, tartaric acid, glucose, fructose, etc. This property of a substance by virtue of which it rotates the plane of polarized light, is called ***optical activity***. The effect is measured in an instrument called *polarimeter*, which sends a beam of plane polarized light through a solution of a substance.

All optically active substances do not rotate the plane of polarized light in the same direction. Some rotate the plane of polarized light to the right (clockwise) while some rotate it to the left (anti-clockwise). A substance, which rotates the plane of polarized light to the right, is called ***dextro-rotatory***. Such compounds are designated by the sign (+). A substance, which rotates the plane of polarized light towards the left, is called ***laevo-rotatory***. Such compounds are designated by the sign (–).

***Optical activity and Chirality.*** The study of structure of optically active

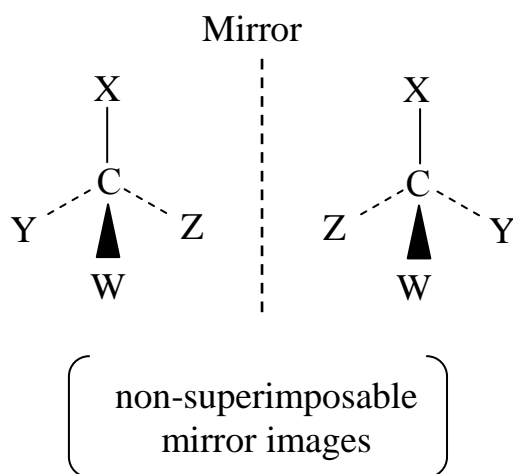
compounds reveals that all of them are chiral or dissymmetric in nature. A *molecule, which is not superimposable on its mirror image, is called a **chiral** or **dissymmetric molecule***. The dissymmetric molecule does not have any plane of symmetry. *The property of non-superimposability of a structure on its mirror image is called **chirality** or **dissymmetry***. On the other hand, a *molecule, which is superimposable on its mirror image, is called achiral (not chiral) or **symmetric molecule** and this property is called **achirality** or **symmetry***.

**Dissymmetry and Plane of Symmetry.** A plane which divides a compound into two symmetrical halves is called **plane of symmetry**. A plane of symmetry is an imaginary plane, which bisects the molecule in such a way that the two halves of the molecule are mirror images of each other. Chiral molecules cannot be divided into two equal and identical halves. Thus, chiral molecules do not have any plane of symmetry and therefore, are called **dissymmetric**. On the other hand, achiral molecules have plane of symmetry and are called **symmetric**.

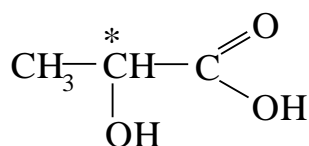
The dissymmetry is the essential condition for the optical activity.

The chirality or dissymmetry in organic compounds was studied by Van't Hoff and Le Bel. They observed that dissymmetry in organic molecules is due to tetrahedral nature of organic compounds.

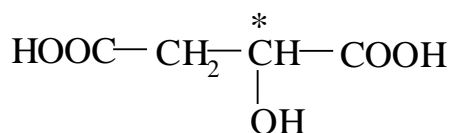
It was observed that most of the organic compounds, which are chiral in nature, have at least one carbon atom, which is bonded to four different atoms or group of atoms. *The carbon, which is bonded to four different atoms or group of atoms, is called **chiral** or **asymmetric carbon atom***. It is generally indicated as C\*. For example, consider a hypothetical molecule CWXYZ. If we place this molecule before a mirror, it is observed that it is not superimposable on its mirror image. We may twist or turn them as much as we wish (without breaking bonds) but it is observed that the two structures are not superimposable.



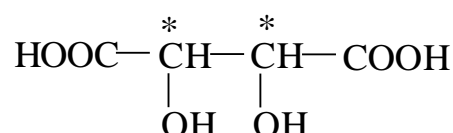
Molecules having one or more asymmetric or chiral carbon atom are:



Lactic acid



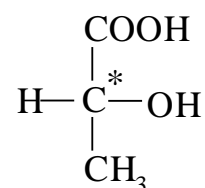
Malic acid



Tartaric acid

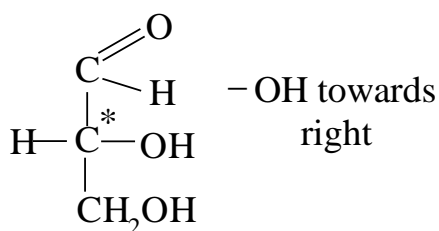
**Enantiomers.** These non-superimposable mirror images are called *enantiomers*. These enantiomers will rotate the plane polarized light equally but in opposite directions. Thus, *the isomers whose molecular structures are non-superimposable mirror images of each other and which rotate the plane polarized light equally but in opposite directions are called enantiomers*. It may be noted that the enantiomers are due to the non-superimposability of mirror images and this also gives them the property of optical activity. Therefore, enantiomers are also referred as optical isomers. It may be noted that the fundamental condition for enantiomerism is dissymmetry.

Fischer projection formulae are often used to depict enantiomers on the surface. Hydrocarbon chain of the compound should be located as vertical line in such way that the principal group would be on the top.

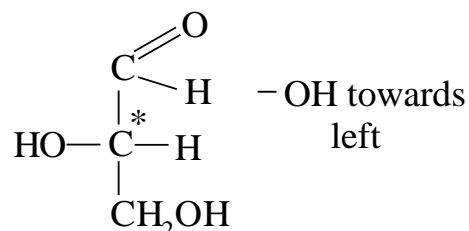


Lactic acid

**D- and L-designations.** The optical active substances are divided into two families: the D-family and L-family, which have definite configurations. These configurations are represented with respect to glyceraldehyde as the standard. The glyceraldehydes may be represented by two forms as:



D-Glyceraldehyde

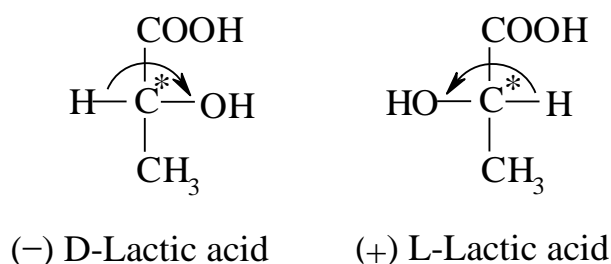


L-Glyceraldehyde

The D-configuration has  $-\text{OH}$  attached to the chiral carbon atom on the **right** while L-configuration has  $-\text{OH}$  attached to the chiral carbon atom on the **left**. The compounds are called D- or L- depending upon whether the configuration of the molecule is related to D-glyceraldehyde or L-glyceraldehyde. By convention, *a molecule is assigned D- configuration if the functional group ( $-\text{OH}$ ,  $-\text{NH}_2$ , halogen) attached to*



the **upper** chiral carbon adjacent to the principal group in the correct written Fischer projection formula is on the right hand side irrespective of the position of other groups. I.e. in the D-hydroxy acid molecule H, COOH and OH groups in Fischer projection formula are located clockwise. On the other hand, the molecule is assigned L-configuration if the functional group attached to the **upper** chiral carbon adjacent to the principal group is on the left. I.e. in the L-hydroxy acid –H, –COOH and –OH groups are located anti-clockwise.



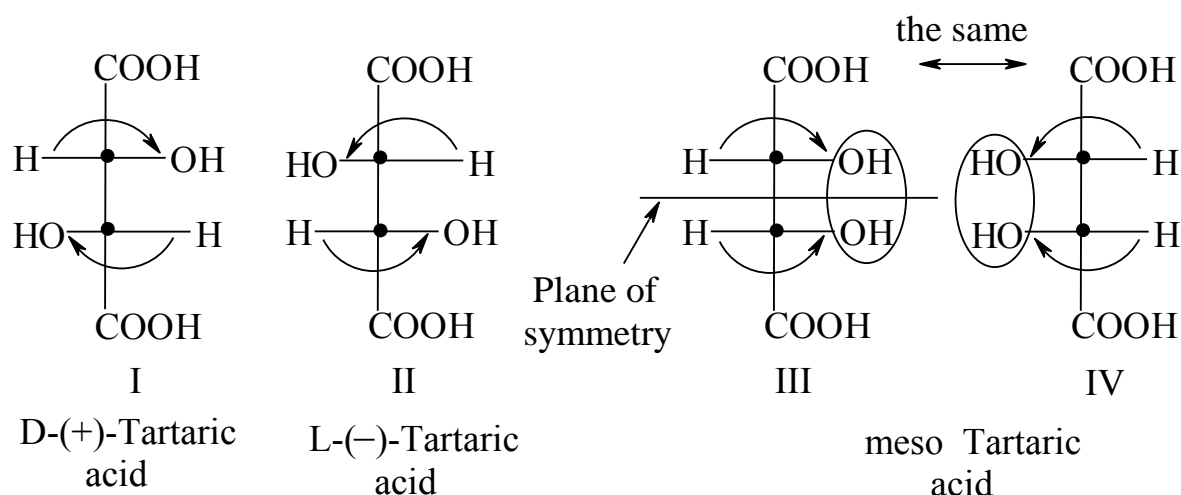
However, it may be noted that D– and L– do not represent dextrorotatory or laevorotatory. The optical activity of the molecule is represented by (+) and (–) which represent the direction of rotation of plane polarized light whether dextrorotatory or laevorotatory. The direction of rotation can be determined only experimentally. There is no any direct connection between the direction of rotation and configuration. It is not the common practice for D-isomer to rotate always to the right as well as for L– isomer – to the left. For example, D–lactic acid rotates to the left while L–lactic acid rotates to the right.

Belonging to stereochemical series shows the real location of groups in the space, i.e. their **absolute configuration**.

Enantiomers react in the same way in chemical reactions. They may differ in biochemical reactions. An enzyme and its substrate fit together like a key and a lock. The geometry (absolute configuration) of the substrate is important, and enzymes can distinguish between enantiomers.

**Racemic mixtures.** *An equimolar mixture of the enantiomers (dextro and laevo forms) is called **racemic mixture**.* It may be represented as  $\pm$  forms and will be optically inactive.

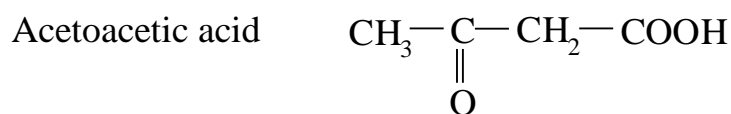
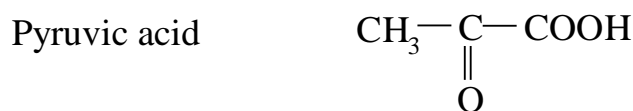
**Meso compounds and diastereomers.** The presence of asymmetric carbon atom is important for enantiomerism but it is not the essential and sufficient condition. There are certain molecules, which contain more than one asymmetric carbon atoms but are optically inactive. Consider a molecule of tartaric acid, which has two asymmetric carbon atoms. Since there are two chiral atoms, we expect four stereomers having the structures I, II, III and IV as:



Structures I and II are non-superimposable mirror images and thus are enantiomers. Structures III and IV are mirror images but one structure can be superimposed on the other. Thus the molecule represented by the structures III and IV cannot be dissymmetric as there is a plane of symmetry in the molecule: one half of its molecule is a mirror image of the other half. Thus, the molecule is achiral and hence is optically inactive although it contains two asymmetric carbon atoms. Such a stereomer, which is optically inactive, is called a **meso compound**. Thus, tartaric acid exists in three optical isomers.

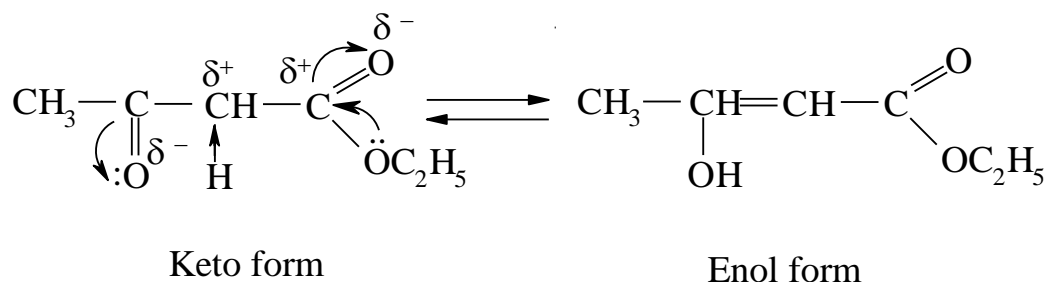
**Diastereomers.** Any pair of stereoisomers which are not mirror images of each other (not enantiomers) are called **diastereomers**. For example, structures I and III and II and IV and II and III are diastereomers.

## 2. OXOACIDS: REPRESENTATIVES, KETO-ENOL TAUTOMERISM



Oxoacids possess all properties typical for carboxylic acids, aldehydes and ketones. As oxo compounds they exhibit **keto-enol tautomerism**. **Tautomerism** is a special type of functional isomerism in which the isomers differ in the arrangement of

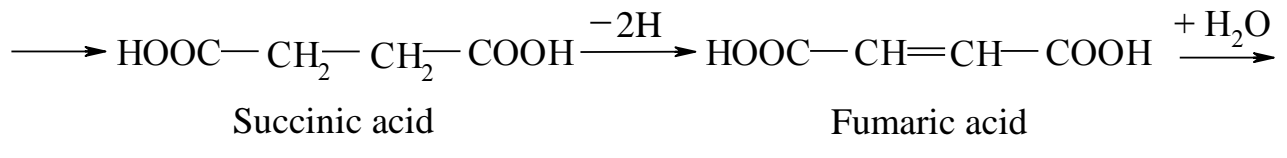
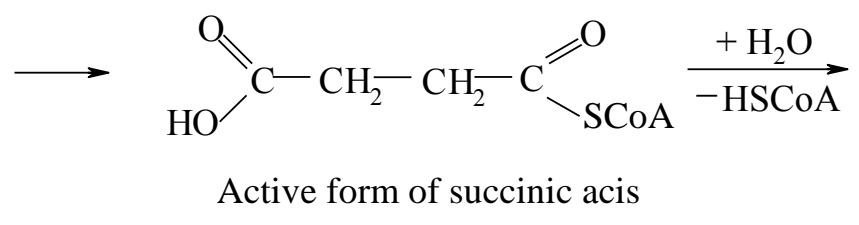
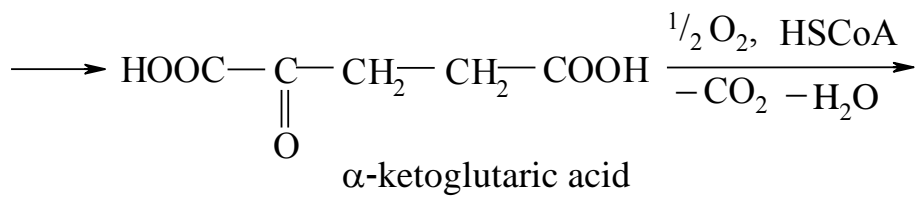
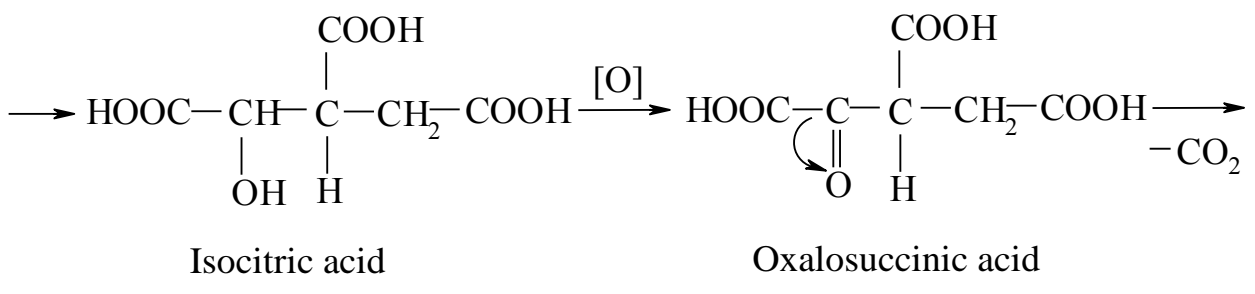
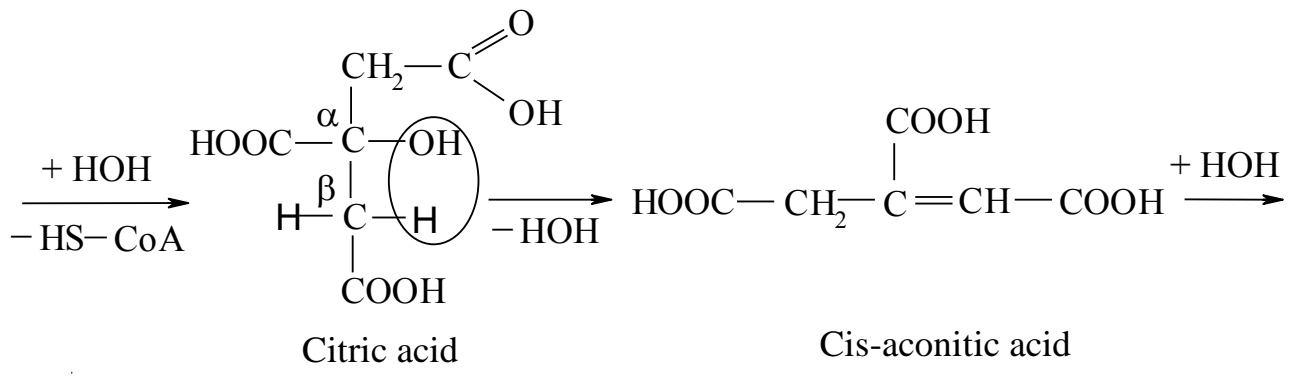
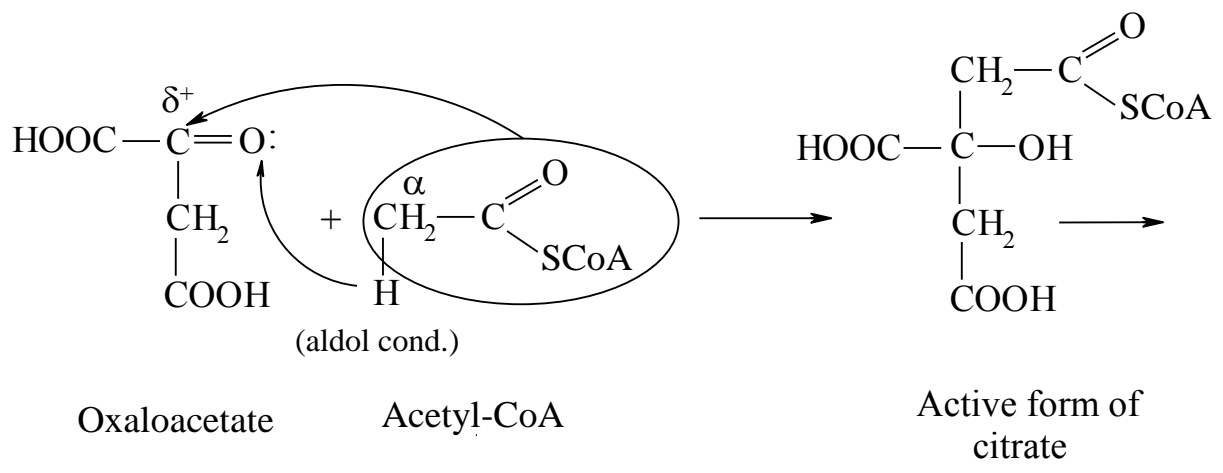
atoms but *they exist in dynamic equilibrium with each other*. For example, acetoacetic ester exists in two forms as:

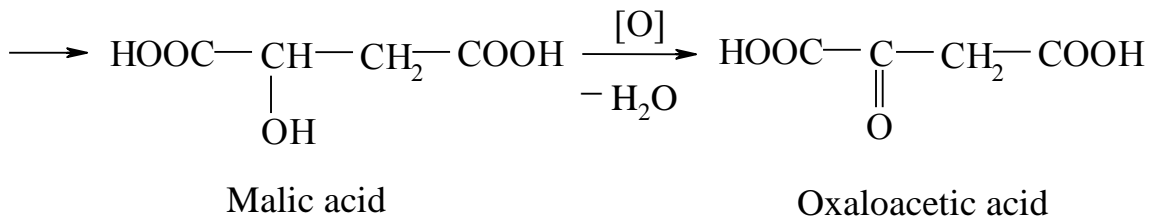


The tautomers differ in the position of a hydrogen atom. They are called keto form (for the  $> \text{C}=\text{O}$  group in ketones) and enol form (ene for  $-\text{C}=\text{C}-$  and ol for the  $-\text{OH}$  group). The equilibrium mixture of tautomers gives the reactions of alkenes, carbonyl compounds and alcohols. There is only a small fraction of the enol present (for acetoacetic ester there is 7% of enol form at room temperature). The enol has a different set of reactions: it has the reactions of the hydroxyl group and the reactions of the alkene group. If the small fraction of enol present is used up in a reaction, more will be formed from the keto-tautomer to maintain equilibrium.

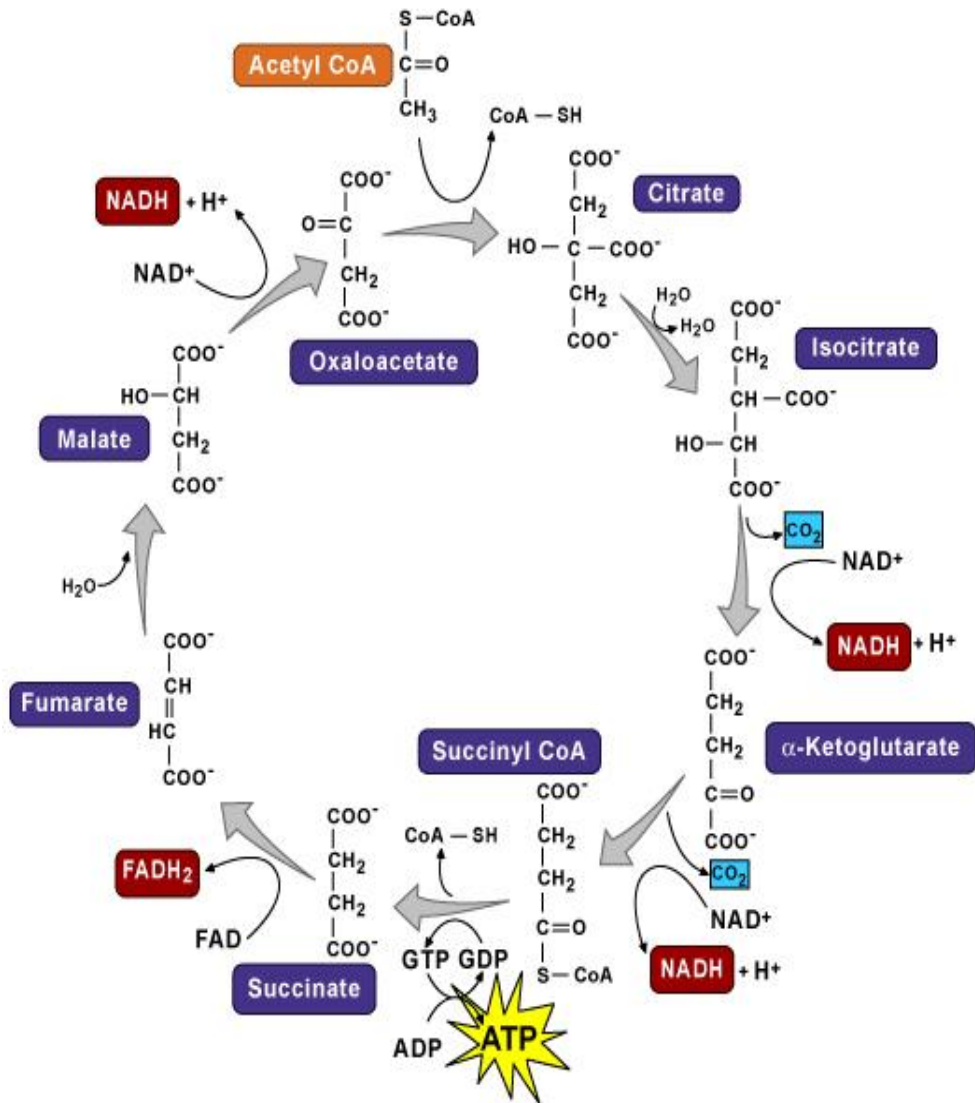
### 3. *KREBS CYCLE*

Hydroxy- and Oxoacids participate in **Krebs cycle (citric acid cycle)**. Krebs cycle is a complex process consisting of the series of consecutive reactions including specific enzymes. This process takes place in mitochondria under aerobic conditions only and is of universal significance for animal and plant world. All nutrients (proteins, fats and carbohydrates) are decomposed to the final product - acetyl-CoA in the process of catabolism. Acetyl-CoA joins the citric acid cycle. There it is being broken up with the formation of two  $\text{CO}_2$  molecules and four pairs of hydrogen atoms. In the beginning acetyl-CoA combines with oxaloacetate to form citrate, which loses two molecules of carbon dioxide stepwise. In the last step of the cycle, oxaloacetate is regenerated for initiating a new cycle by reaction with another molecule of acetyl-CoA. The end products of the citric acid cycle are two molecules of carbon dioxide. During the oxidation of acetate to  $\text{CO}_2$ , some coenzyme molecules ( $\text{NAD}^+$  and  $\text{FAD}^+$ ) are also reduced. The later are regenerated via a series of reactions, which involve transfer of hydrogen atoms and electrons from acetate and other intermediates **to oxygen**. This series of oxidation-reduction reactions called **electron transport chain (ETC)** produces most of the ATP formed in the oxidation of nutrients. For example, for each molecule of glucose breaking down into smaller fragments, 38 molecules of ATP are produced.



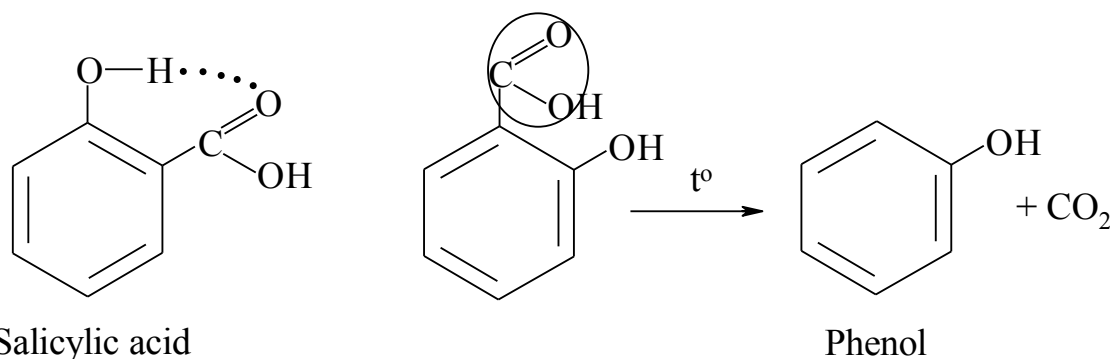


*Scheme of Krebs cycle*



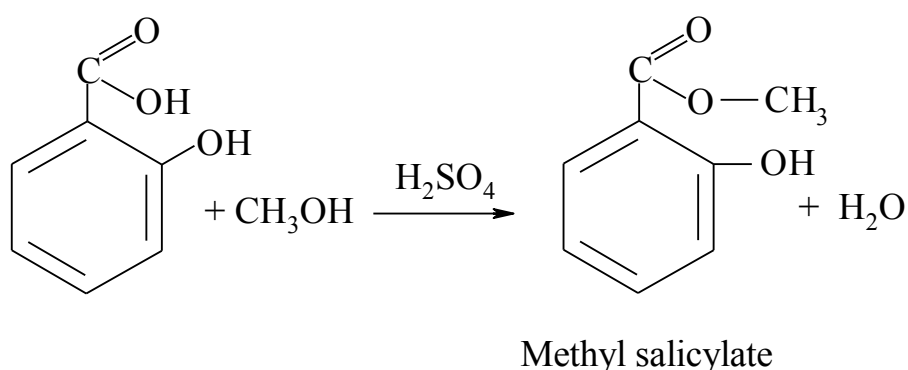
#### 4. PHENOLIC ACIDS

**Salicylic acid** is the phenolic acid. It is stronger acid than benzoic one. This is due to formation of intramolecular hydrogen bond which stabilizes carboxylate-anion. Similar to the other  $\alpha$ -hydroxy acids salicylic acid undergoes decarboxylation easily with phenol formation.

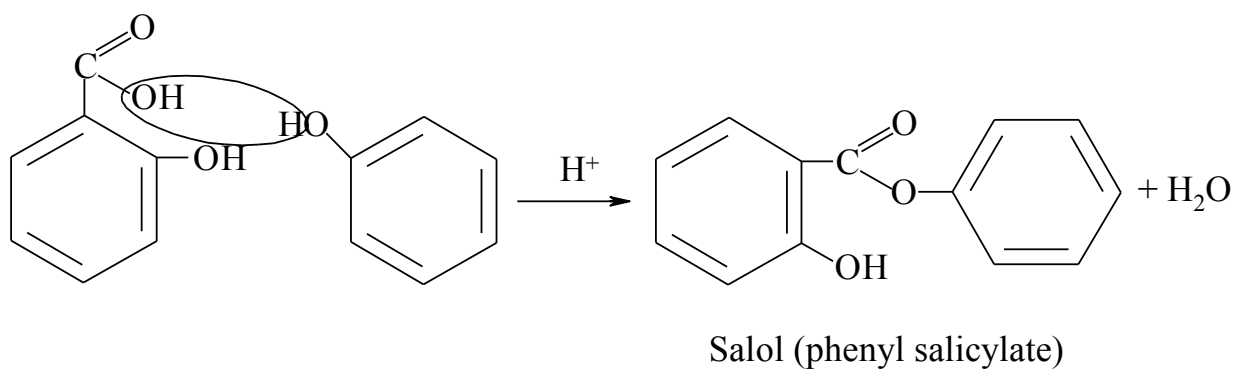


Salicylic acid is water soluble. It gives violet color with  $\text{FeCl}_3$  due to the presence of free phenolic hydroxyl group. It possesses analgesic, antipyretic, anti-inflammatory properties but can be applied only for external use.

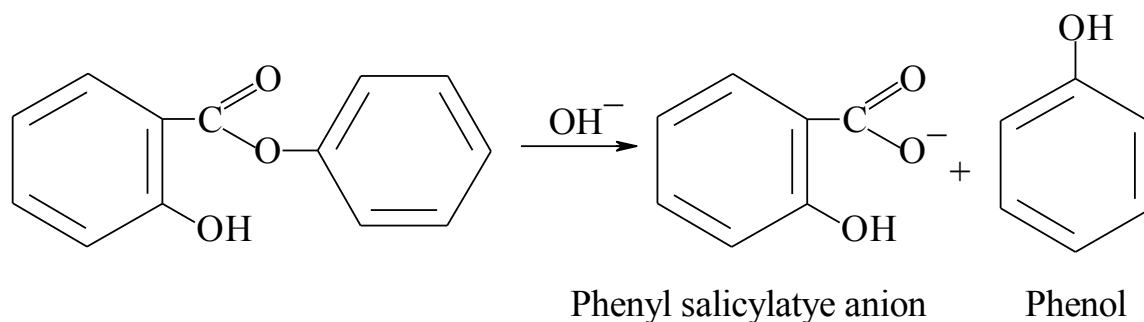
From the chemical point of view, salicylic acid derivatives (salicylates) are esters. When salicylic acid is heated with methanol in the presence of sulfuric acid it gives methyl salicylate. Methyl salicylate is a colourless liquid with pleasant aroma. It possesses irritant, analgesic, and anti-inflammatory properties. It is used for compresses with oil in case of rheumatic disease.



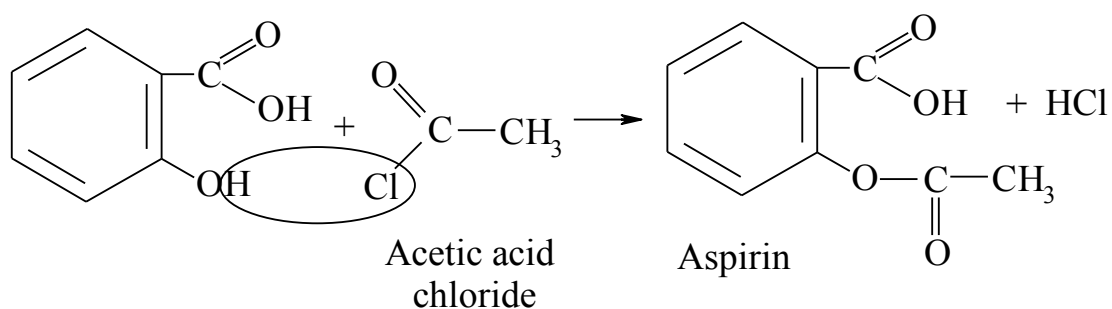
Phenyl salicylate (salol) is an ester of salicylic acid and phenol.



Phenyl salicylate is insoluble in acidic medium of stomach but in the basic medium of intestine salol is hydrolyzed with formation of salicylic acid and phenol possessing healing properties. Salol is used as antiseptic for intestine diseases treatment.



Phenolic hydroxyl group is involved in esterification reactions with acids. Ester of salicylic acid and acetic acid (aspirin) can be obtained in reaction of salicylic acid with acetic acid anhydride or acetic acid chloride:



Aspirin is a white crystalline powder slightly soluble in water possessing antipyretic and analgesic properties. It is used in the form of powders and tablets.

### Revision exercises

1. Which of the following acids is optically active?  
a) Oxaloacetic b) Salicylic c) Lactic d) Pyruvic
2. Which acid can give Schiff's base?  
a) Glyoxalic b) Lactic c) Butyric d) Salicylic
3. Which derivative of salicylic acid involves its hydroxyl group?  
a) Methyl salicylate b) Sodium salicylate c) Salol d) Aspirin

### Answers

1. c)
2. a)
3. d)

\*\*\*



# CARBOHYDRATES

## *Characteristics of the subject*

Carbohydrates are widely spread in nature. They participate in metabolism and maintain energetic balance in the organism of humans and animals. They play structural role and can be found in the composition of complex biologically active compounds. Monosaccharides are structural components of animal starch, heparin and other important biopolymers. They participate in biological oxidation and serve as the main source of energy for the living creatures. Polysaccharides are important biopolymers, they can be found in numerous organs, tissue and systems of the organism. Disturbance in carbohydrates metabolism results in severe diseases, e.g. diabetes mellitus.

## *Purposes*

To consolidate the knowledge about classification, nomenclature, structure, tautomeric balance, chemical and biological properties of important monosaccharides. To study the principles of structure and main chemical properties of oligosaccharides, homo- and heteropolysaccharides associated with their biological functions.

## *Objectives*

1. To know the classification of monosaccharides according to the number of carbon atoms and functional composition.
2. To know the classification of monosaccharides depending on the size of the cycle and location of hemiacetal hydroxyl.
3. To study the most stable conformations of hexose.
4. To know the properties associated with the presence of hemiacetal (glycoside) hydroxyl.
5. To be able to illustrate the properties of monosaccharides associated with the presence of carbonyl and hydroxyl groups (oxidation, reduction, formation of esters and ethers, epimerization of hexoses in alkaline medium).
6. To know monosaccharide composition, structure and conformations of the most important disaccharides (maltose, lactose, cellobiose, sucrose).
7. To study fraction composition and properties of cellulose and starch, linear and spatial structure of amylose and amylopectin, as well as an animal starch - glycogen.
8. To know the composition and structure of main heteropolysaccharides (hyaluronic acid, heparin, chondroitin sulfates).

### *Theoretical questions*

1. Classification of Carbohydrates.
2. Biological functions of Carbohydrates.
3. Photosynthesis and Carbohydrates metabolism.
4. Monosaccharides: classification, structure and properties.
5. Oligosaccharides and Polysaccharides: structure and properties.

Carbohydrates are a class of naturally occurring organic compounds of carbon, hydrogen and oxygen. These contain the polyhydric aldehydes, polyhydric ketones and large polymeric substances, which can be broken down to polyhydric aldehydes or ketones. These include glucose, fructose, cellulose, sucrose, starch, etc.

In the *earlier days*, the carbohydrates were regarded as the *hydrates of carbon* with the general formula  $C_x(H_2O)_y$ . These days carbohydrates are defined as polyhydroxy aldehydes or polyhydroxy ketones or compounds which give such compounds on hydrolysis.

### **1. CLASSIFICATION OF CARBOHYDRATES**

Carbohydrates are *systematically classified* into three principal classes :

**1. Monosaccharides.** These are the simplest carbohydrates, which cannot be hydrolyzed into simpler compounds. About 20 monosaccharides occur naturally. They contain from 3 to 10 carbon atoms. They have the general formula  $(CH_2O)_n$ . If the carbohydrates contain aldehydic group, they are named as **aldoses** and if they have ketonic group, they are commonly called **ketoses**. The common examples are: ribose  $C_5H_{10}O_5$ , glucose  $C_6H_{12}O_6$ , fructose  $C_6H_{12}O_6$ , etc.

**2. Oligosaccharides.** These are the carbohydrates, which give two to nine monosaccharide molecules on hydrolysis. These are further classified as disaccharides, trisaccharides, tetrasaccharides, etc. depending upon the number of monosaccharide units present in their molecules. For example, **Disaccharides**: sucrose, lactose, maltose. All these have the molecular formula  $C_{12}H_{22}O_{11}$ .

**Trisaccharide:** raffinose  $C_{18}H_{32}O_{16}$ .

**Tetrasaccharides:** stachyose  $C_{24}H_{42}O_{21}$ .

Upon hydrolysis, the oligosaccharides give monosaccharides.

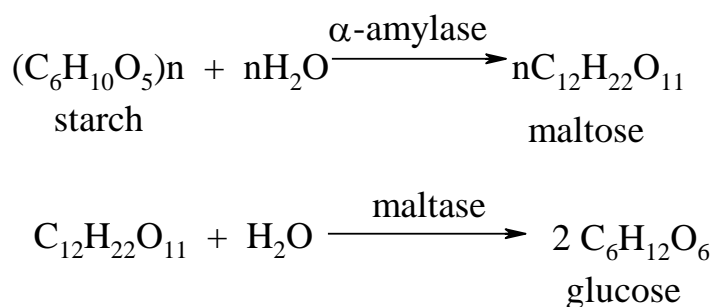
**3. Polysaccharides.** Polysaccharides, also known as **glycans**, consist of monosaccharide joined together by glycosidic linkages. Polysaccharides that are polymers of a single monosaccharide are called **homopolysaccharides**; those made up of more than one type of monosaccharide are called **heteropolysaccharides**. Three

important homopolysaccharides, all of which consist of glucose units, are starch, glycogen, and cellulose. Important heteropolysaccharides are constituent part of connective tissues. These are hyaluronic acid, chondroitin sulphates, heparin.

## **2. BIOLOGICAL FUNCTIONS OF CARBOHYDRATES**

Carbohydrates, along with proteins and lipids, are major chemical compounds of living organisms. The important functions of carbohydrates are:

1. Carbohydrates (with the exception of cellulose) work as body fuels and act as the main *sources of energy*. Polysaccharides first undergo hydrolysis to give glucose, which then supplies the energy. Starch and sugars get hydrolyzed to glucose by the enzymes present in the various juices secreted by different organs in the human and animal digestive systems.



2. The carbohydrates act as storage of energy for the functioning of living organisms. In case of emergency like illness or fasting, they supply energy.

3. Some carbohydrates such as sugar and starch serve as food.

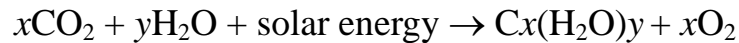
4. They form structural materials for cells. For example, cellulose is present in the cell walls of the plant cells.

5. Carbohydrates play protective role in the organism (involvement of immunoglobulin carbohydrate components in the maintenance of immunity).

6. Carbohydrates are used in the synthesis of nucleic acids (ribose and deoxyribose); they are constituents of the nucleotide coenzymes and mixed carbohydrate – containing biopolymers: glycoproteins, glycolipids, lipopolysaccharides, glycolipoproteins.

### **3. PHOTOSYNTHESIS AND CARBOHYDRATES METABOLISM**

Carbohydrates are synthesized in green plants by photosynthesis – a process that uses solar energy to reduce, or “fix”, carbon dioxide. Photosynthesis in algae and higher plants occurs in cell organelles called chloroplasts. The overall equation for photosynthesis can be written as follows:



Photosynthesis begins with the absorption of light by the green pigment of plants, chlorophyll. The green color of chlorophyll and, therefore, its ability to absorb sunlight in the visible region are due primarily to its extended conjugated system. As photons of sunlight are trapped by chlorophyll, energy becomes available to the plant in a chemical form that can be used to carry out the reactions that reduce carbon dioxide to carbohydrates and oxidize water to oxygen.

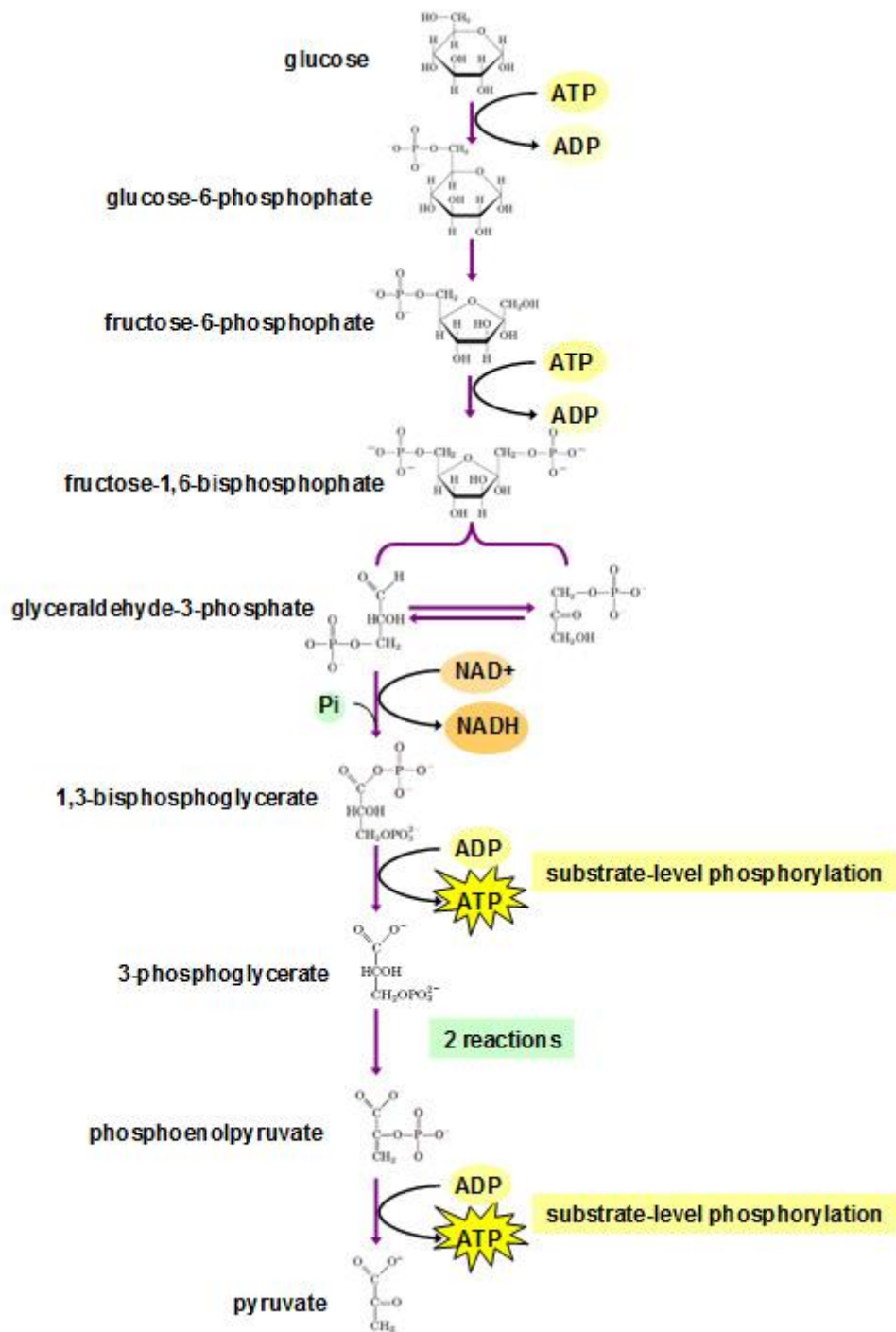
Carbohydrates act as a major chemical repository for solar energy. Their energy is released when animals or plants metabolize carbohydrates to carbon dioxide and water:



In the beginning of the carbohydrates metabolism, carbohydrates present in the food are converted into various monosaccharides by different enzymes during digestion. These monosaccharides are finally converted into glucose by several steps and release a large amount of energy. Thus, oxidation of glucose is most important reaction in the living cells which gives useful cellular energy. The mechanism of glucose oxidation can be divided into two stages: glycolysis and cellular respiration.

**1. Glycolysis** is a process in which a glucose molecule is broken down into smaller molecules of pyruvate. This occurs in 10 successive steps. This is also accompanied by generation of 2 ATP molecules per molecule of glucose. Glycolysis occurs in cytoplasm of cells and does not require oxygen. The ATP molecules in the anaerobic cells (those that live without oxygen) are formed by glycolysis.

## Scheme of glycolysis



**2. Cellular respiration.** This stage of glucose oxidation requires oxygen and therefore, aerobic. This process converts pyruvate molecules into  $\text{CO}_2$  and  $\text{H}_2\text{O}$  and takes place in the mitochondria of cells. The pyruvate formed during glycolysis is oxidized with loss of its carboxyl group to  $\text{CO}_2$ , to form acetyl coenzyme A. This then participates in a series of cyclic reactions known as **citric acid cycle (Krebs**

**cycle).** Products of Krebs cycle (reduced forms of some coenzymes) then enter the **electron transport chain** in which ATP molecules are synthesized from adenosine diphosphate (ADP) and inorganic phosphate.

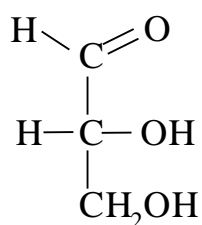
Although some of the energy released in the oxidation of carbohydrates is inevitably converted to heat, much of it is conserved in the bonds of ATP. Plants and animals can use the conserved energy of ATP to carry out all of their energy – requiring processes.

#### **4. MONOSACCHARIDES: CLASSIFICATION, STRUCTURE AND PROPERTIES**

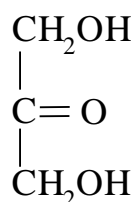
Monosaccharides are polyhydric aldehydes and ketones which cannot be hydrolysed into simpler carbohydrates. The monosaccharides with an aldehydic group (–CHO) are called aldoses while those with ketonic group (>C=O) are called ketoses. They are farther classified as *trioses*, *tetroses*, *pentoses*, *hexoses*, etc. depending upon the number of carbon atoms they contain. Therefore, while naming these monosaccharides, the prefix indicating the number of carbon atoms like *tetra*–(4), *penta*–(5), *hexa*–(6) etc., is incorporated in the term aldose or ketose. For example, an *aldopentose* means that it is an aldehydic carbohydrate containing five carbon atoms. Similarly, *ketohexose* means a ketone containing six carbon atoms. The carbon atoms of an aldose are **numbered** starting from the aldehyde group (–CHO) and that of ketose from that end which is closest to the ketonic group.

Most of the monosaccharides occur in nature. They are colourless, crystalline solids, soluble in water and have sweet taste. These are quite stable and do not get hydrolyzed.

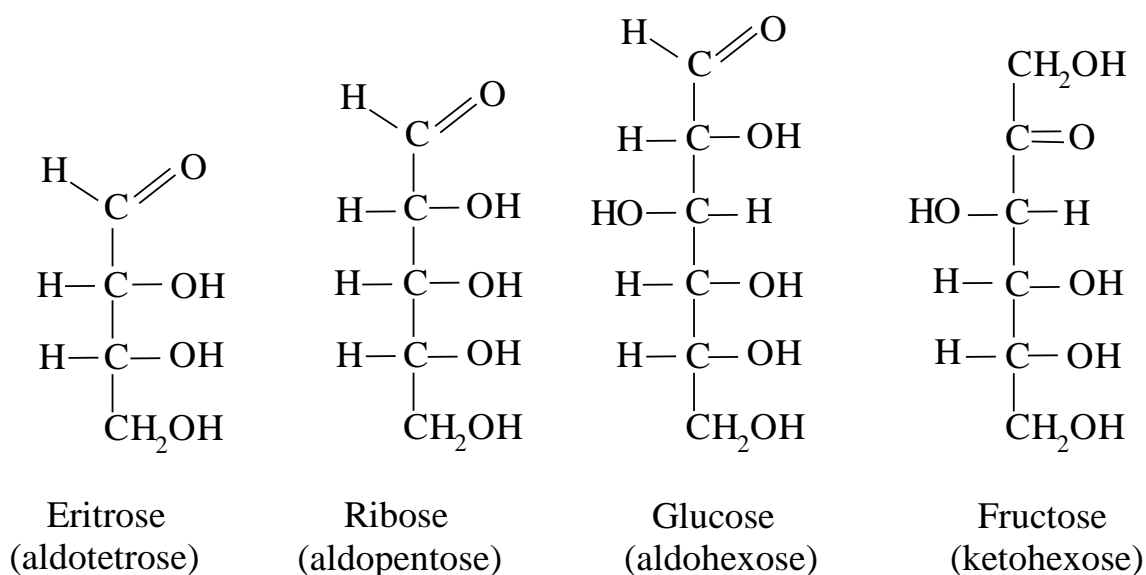
##### *Representatives*



Glyceraldehyde  
(aldotriose)

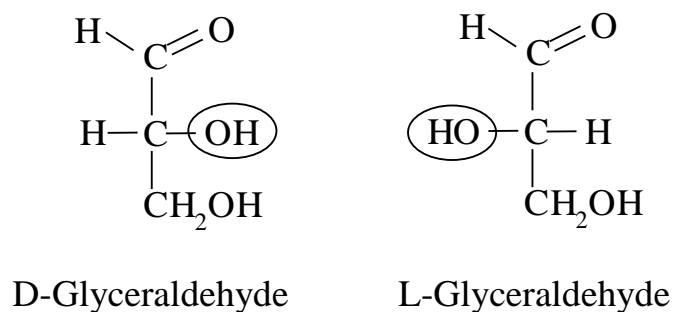


Dihydroxyacetone  
(ketotriose)



### *D- and L-Designations*

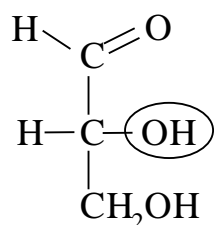
The sugars are divided into two families: the *D-family* and *L-family* which have definite configurations. These configurations are represented with respect to *glyceraldehyde* as the standard. Glyceraldehyde contains a chiral carbon - the carbon, which is bonded to four different atoms or group of atoms. Glyceraldehyde exists, therefore, in two enantiomeric forms that are known to have the absolute configuration shown here:



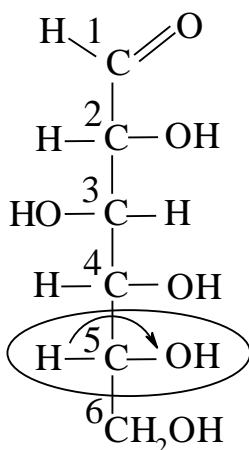
The D-configuration has —OH attached to the carbon adjacent to —CH<sub>2</sub>OH on the **right** while L-configuration has —OH attached to the carbon adjacent to —CH<sub>2</sub>OH on the **left**. The sugars are called D- or L- depending upon whether the configuration of the molecule is related to D-glyceraldehyde or L-glyceraldehyde. By **convention**, a molecule is assigned D-configuration if the —OH group attached to the carbon adjacent to the —CH<sub>2</sub>OH group (last chiral carbon) is on the right hand side irrespective of the position of other groups. On the other hand, the molecule is assigned L-configuration if the —OH group attached to the carbon adjacent to the —CH<sub>2</sub>OH group is on the left.

It has been found that all naturally occurring sugars belong to D-series e.g.

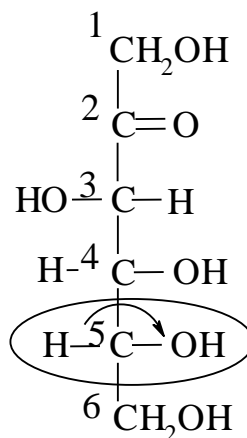
D-glucose, D-ribose and D-fructose.



D-Glyceraldehyde

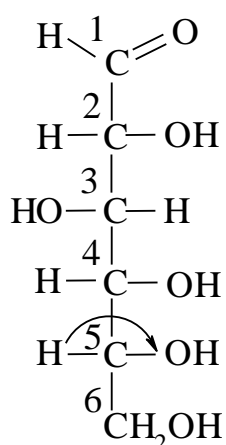


D-Glucose

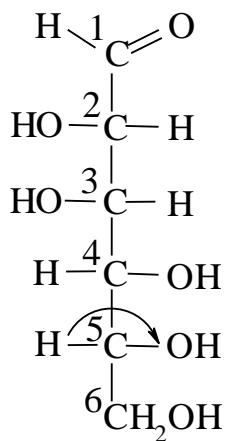


D-Fructose

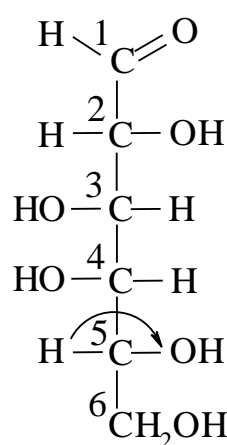
However, it may be noted that D- and L- do not represent dextrorotatory or laevorotatory. The optical activity of the molecule is represented by (+) and (-) which represent the direction of rotation of plane polarized light whether dextrorotatory or laevorotatory. Thus, the letter D- and L- refer to absolute configuration around asymmetric carbon atom while (+) and (-) refer to the direction of the plane polarized light which is a measured physical constant and cannot be obtained by looking at the formula. On carefully examining the monosaccharide molecules, we observe that they contain one or more chiral carbon atoms. For example, glucose has four chiral carbon atoms (carbons 2, 3, 4 and 5). If there are n chiral carbon atoms in a molecule, it will have  $2^n$  optical isomers. Therefore, glucose has  $2^4$  or sixteen optical isomers. Three of these sixteen aldohexoses are D-glucose, D-galactose and D-mannose. Only these three occur in nature.



D-Glucose



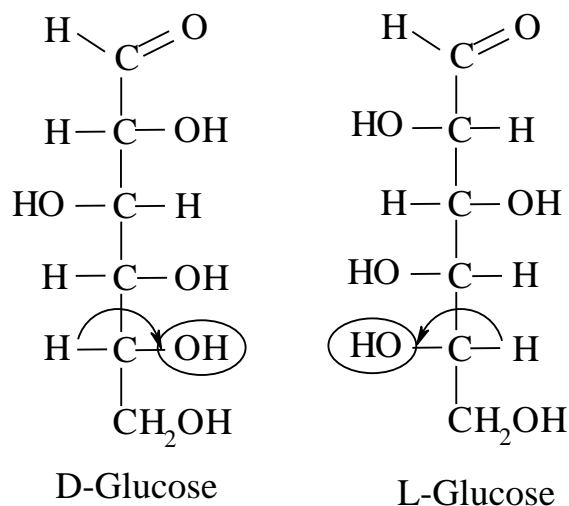
D-Mannose



D-Galactose



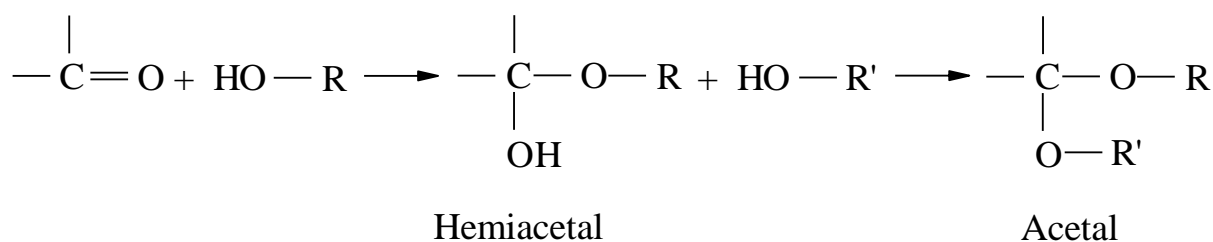
It may be noted in all these three molecules, the configuration at **C-5** is same (–OH is on the right) therefore, they belong to D-family. Enantiomer of L-family with the **opposite configuration of all chiral centers** corresponds to every aldose of D-family. D-glucose and L-glucose are enantiomers. It means that they bear the relation of object and its mirror image. They have same degree of rotation but in opposite direction.



D-galactose and L-glucose, for example, are diastereomers (stereoisomers that are not mirror images of each other). D-mannose and D-galactose are said to be epimers of D-glucose. Epimers are the pair of diastereomers which differ in configuration at only one chiral carbon atom.

### *Cyclic Structures of Monosaccharide*

The monosaccharides give the characteristic reactions of alcohols and carbonyl group (aldehydes and ketones). It has been found that these monosaccharides exist in the form of *cyclic structures*. We know that aldehydes and ketones react with the hydroxyl group to form hemiacetals and acetals, as

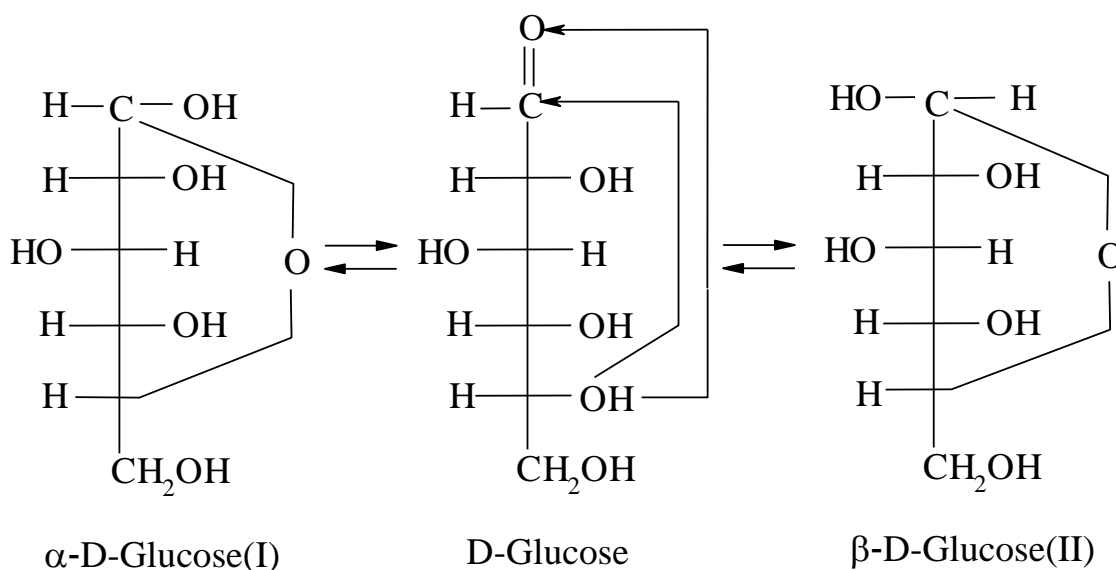


Monosaccharides contain a number of –OH groups and an aldehyde or a

keto group. Therefore, they can undergo *intramolecular reactions* (within the molecule) to form hemiacetals which result in *cyclic structures*. In cyclization, the —OH groups (generally of C-5 or C-4 in aldoses and C-5 or C-6 in ketoses) combine with the aldehyde or keto groups. As a result, cyclic structures of five or six membered rings (which are known to be stable) containing one oxygen atom are formed. In this process one more hydroxyl group called **glycosidic hydroxyl** is formed.

### *Cyclic Structure of Glucose*

**Anomers.** Glucose forms a hemiacetal between the —CHO group and the —OH group on the C-5 atom. As a result, C-1 becomes asymmetric (chiral) and forms two isomers (I) and (II). These two isomers differ in the orientation of H and glycosidic hydroxyl groups around C-1 atom. These isomers are known as  $\alpha$ -D-glucose and  $\beta$ -D-glucose. Such pairs of optical isomers, which differ in the configuration only around C-1 atom are called anomers. The C-1 atom is known as anomeric carbon atom.

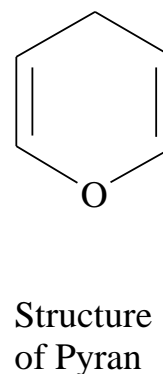
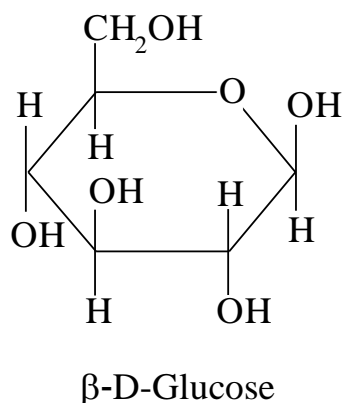
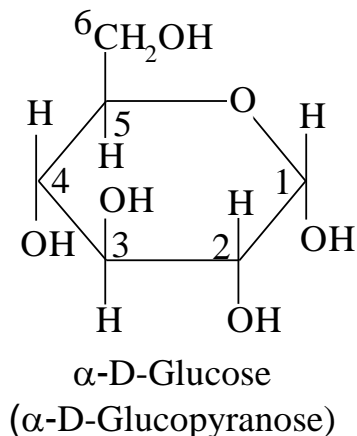


The above representations, as you know, are called **Fischer projection formulae**.

### **Pyranose Structures**

The structures of  $\alpha$ -D-glucose and  $\beta$ -D-glucose may be drawn in a simple six membered ring form called **pyranose structures**. These resemble pyran, which is a six membered heterocyclic ring containing five carbon atoms and one oxygen atom. These structures were suggested by English chemist W.N. Haworth and are known as

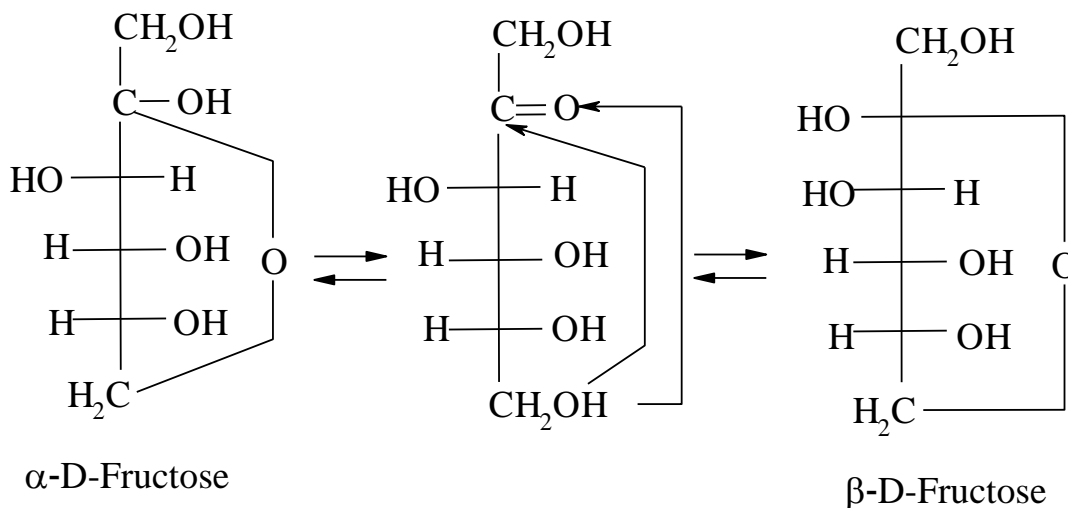
Haworth projection formulae or pyranose structures.



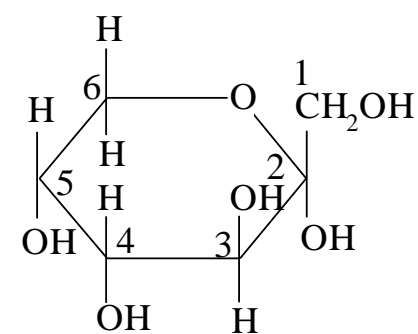
To write pyranose structure for any monosaccharide ( $\alpha$ - and  $\beta$ -D-glucose), draw a hexagon with its oxygen atom at the upper right hand corner. The terminal  $\text{—CH}_2\text{OH}$  group is always placed above the plane of the hexagon ring (in D-series). Place all the groups (on C-1, C-2, C-3, and C-4, which are present on the left hand side in Fischer projection *above the plane of the ring* and all those groups on the right hand side *below the plane of the ring*).

### *Cyclic Structure for Fructose*

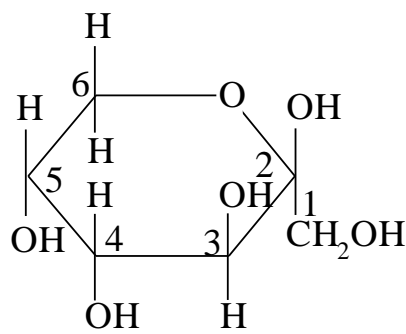
Like glucose, fructose has also six membered hemiacetal ring structure. The hemiacetal is formed by the intramolecular combination of keto group and  $\text{—OH}$  group of C-6 atom. As a result, C-2 atom becomes asymmetric and therefore, D-fructose has two possible isomers as  $\alpha$ -D-fructose and  $\beta$ -D-fructose, which differ in the arrangement of  $\text{CH}_2\text{OH}$  and  $\text{OH}$  groups around  $\text{C}_2$ . These are shown below:



The above structures may be written in the Haworth forms as pyranose ring structures as:

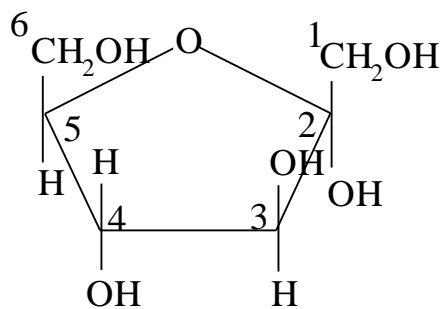


$\alpha$ -D-Fructose  
( $\alpha$ -D-Fructopyranose)

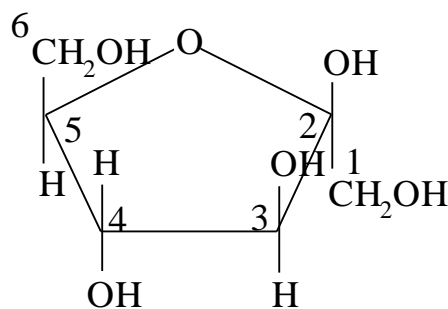


$\beta$ -D-Fructose

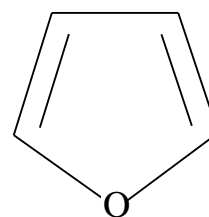
In the free state D-fructose exists as a six membered ring or as pyranose ring. However, in the combined state as a component of disaccharides, it exists in the furanose form (five membered hemiketal). This structure is similar to furan ring, which is a five membered heterocyclic ring with one oxygen atom. The furanose structure can be obtained by internal ketal formation by combining keto group (of C-2) and -OH group of C-5, as shown below:



$\alpha$ -D-Fructose  
( $\alpha$ -D-Fructofuranose)



$\beta$ -D-Fructose



Structure  
of Furan

These cyclic structures can also be written in the same way as for glucose.

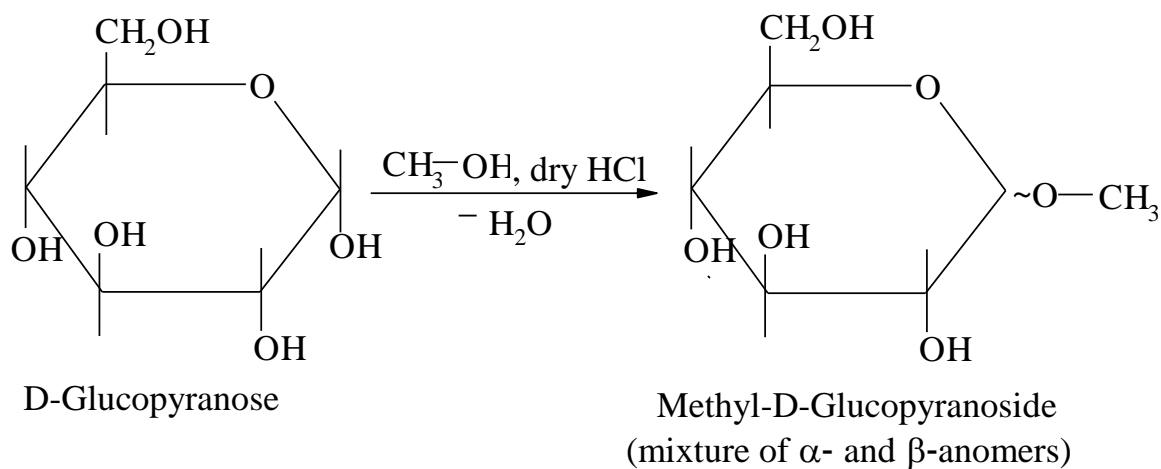
## Chemical properties

**1. Tautomerism.** In the solid state monosaccharides exist as a cyclic form. In a solution monosaccharides exist as an equilibrium mixture of tautomers - cyclic and open chain forms. For example, the two forms of glucose ( $\alpha$  and  $\beta$ ) exist in separate

crystalline forms and have different melting points and optical rotations. In water solution either of these forms get converted into the other form and an equilibrium mixture is formed with small amount of the open chain form. The reason is that the ring opens and then closes either in the inverted position or in the original position giving a mixture of  $\alpha$ -glucose, open chain form and  $\beta$ -glucose. This process is called *ring-chain tautomerism*.



**2. Glycosides formation.** Glycosidic hydroxyl exhibits a high reactivity and is liable to replacement by other groups in the reactions with alcohols, carboxylic acids, phenols and other species. Cyclic acetals called glycosides are formed. Depending on the dimension of the cycle, glycosides are divided into pyranosides and furanosides. Glucose acetals are called glucosides, fructose acetals – fructosides, acetals of mannose are mannosides, and so on. For example, the reaction of methanol with glucose in the presence of inorganic acids yields an alkylated product:



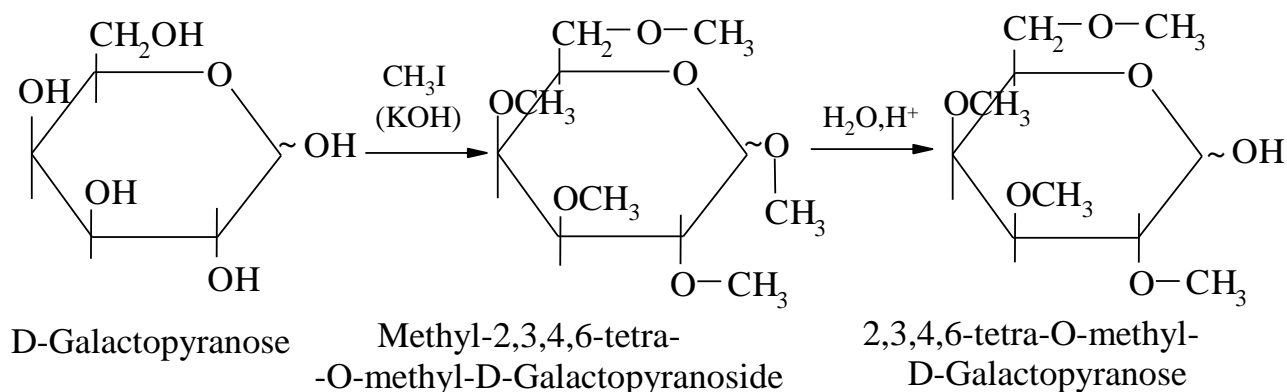
The glycoside molecule consists of carbohydrate moiety and **aglycone** moiety. The other hydroxyl groups of monosaccharides are also subject to methylation but this reaction can be accomplished under more severe conditions. In the cases where alcohols, phenols, or carboxylic acids enter into the reaction, the derived products are referred as O-glycosides. Methyl-D-glucopyranoside is therefore O-glycoside (the bonding is effected via oxygen). The native O-glycosides are mainly produced in the course of the vital activity of plants. Oligo- and polysaccharides chemically are O-glycosides.

An important glycoside class is N-glycosides in which the glycoside bond is effected through nitrogen, rather than oxygen. N-glycosides are regarded as

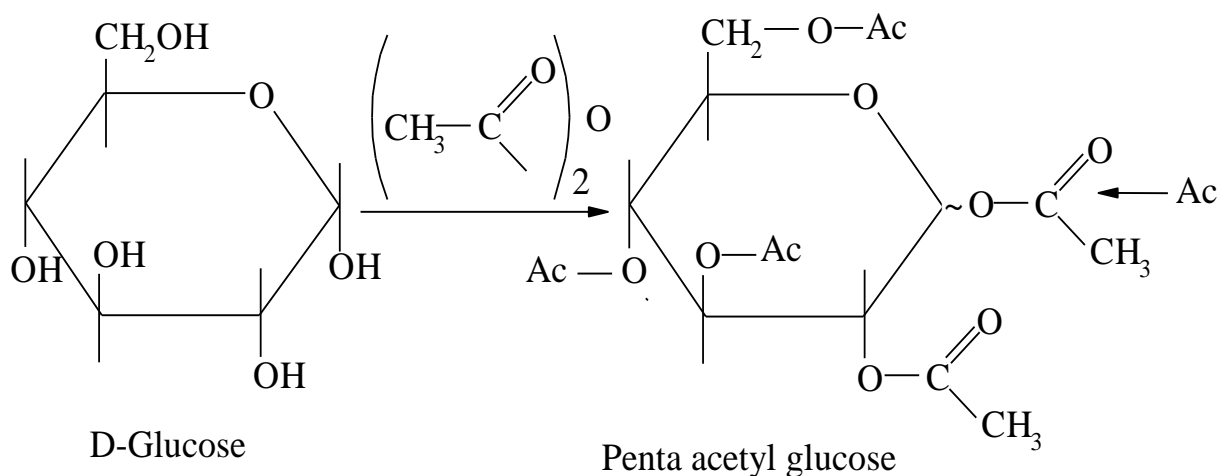
monosaccharide derivatives in whose molecules the glycosyl moiety is linked through a nitrogen atom to an organic radical which is not a carbohydrate residue. Similar to O-glycosides, the N-glycosides are built like pyranosides or furanosides and can exist in the  $\alpha$ - or  $\beta$ -form. To the class of N-glycosides belong the products of supreme importance in metabolism, derived from cleaved nucleic acids and nucleoproteins (nucleotides and nucleosides), ATP, NAD, NADP, certain antibiotics, and other compounds.

S-glycosides are also known which are derived from the cyclic forms of thiosugars; in these, the hydrogen atom of mercapto group ( $-\text{SH}$ ) at position C-1 is replaced by a radical. S-glycosides are encountered in certain plants (for example, mustard, Adonis flower, and hawthorn). Glycosides are easily hydrolyzed in acidic medium but they are alkaline hydrolysis – resistant.

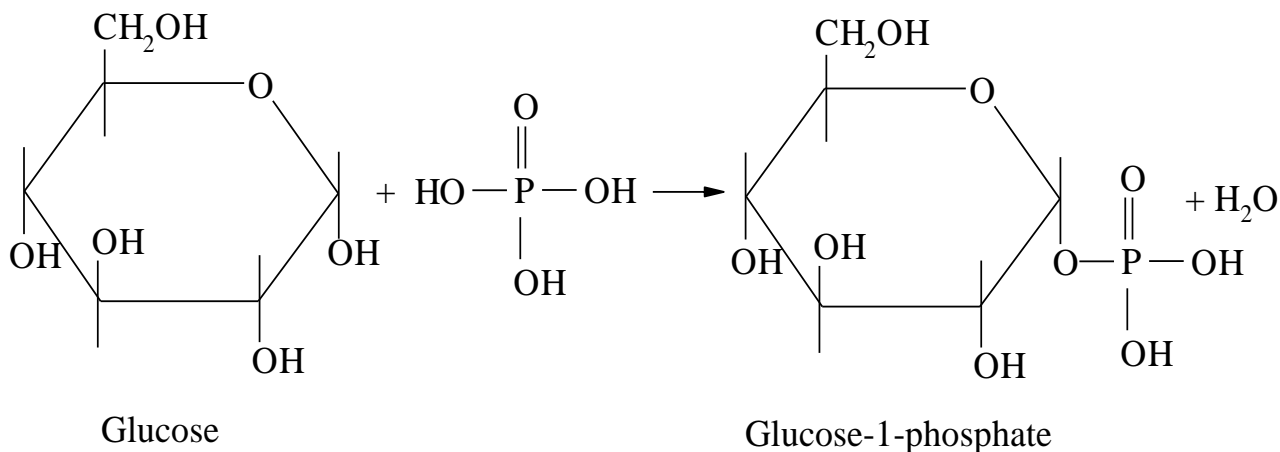
**3. Ethers formation.** Ethers are formed due to the interaction of alcoholic hydroxyl groups of monosaccharides with alkyl halides. Glycosidic hydroxyl also takes part in the reaction with the formation of glycoside. Ethers cannot be hydrolyzed in the acidic medium.



**4. Esters formation.** Monosaccharides are easily acylated with anhydrides (halides) of organic acids. As a result esters are formed. All hydroxyl groups of monosaccharide take part in this reaction.

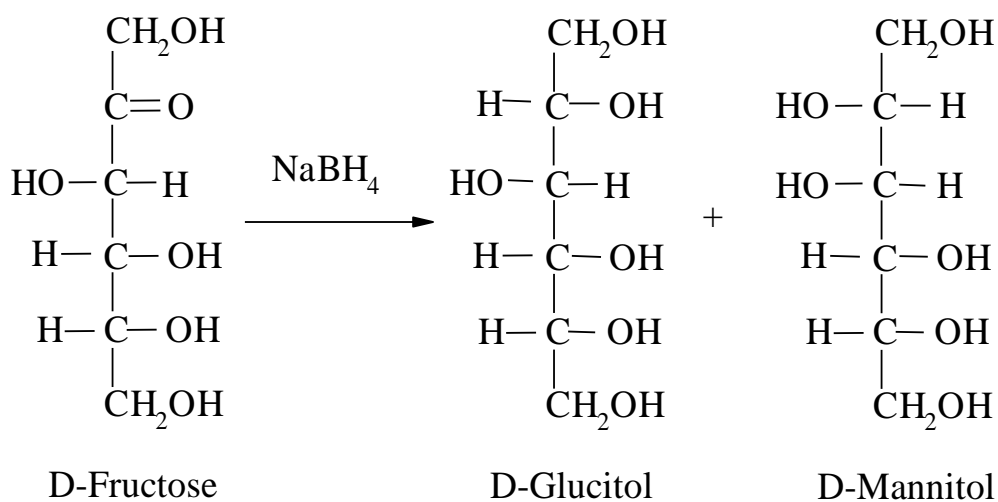


Esters of phosphoric acid (phosphates) are of great importance. All plant and animal organisms contain phosphates, which represent metabolically active forms of monosaccharides. For example, glucose-1-phosphate is formed during the process of glycogen hydrolysis, glucose-6-phosphate is formed on the initial stage of glycolysis. Ribose and deoxyribose phosphates are structural elements of nucleic acids and of some coenzymes.



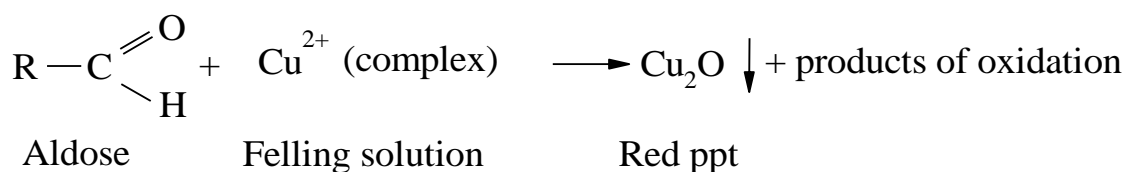
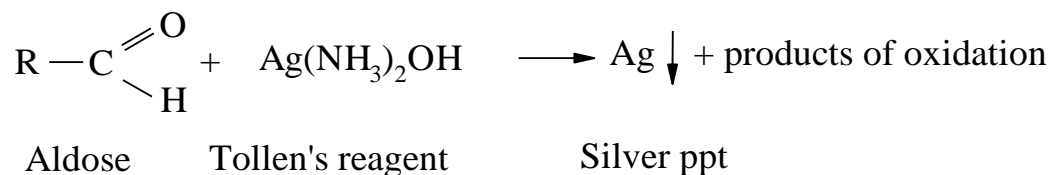
Esters of sulfuric acid are structural components of biologically active heteropolysaccharides of connective tissue. For example, N-acetylgalactosamine-4-sulphate is a component of chondroitine-4-sulphate, N-acetylgalactosamine-6-sulphate is a component of chondroitine-6-sulphate, N-acetylglucosamine-6-sulphate is a component of heparin.

**5. Reduction.** Polyhydric alcohols (alditols) are formed during the process of monosaccharides reduction. Aldehyde or keto group undergoes reduction. Alditols have sweet taste and are often used as artificial sweeteners. Glucose is reduced to glucitol, galactose - to dulcitol, mannose - to mannitol, fructose - to the mixture of glucitol and mannitol.



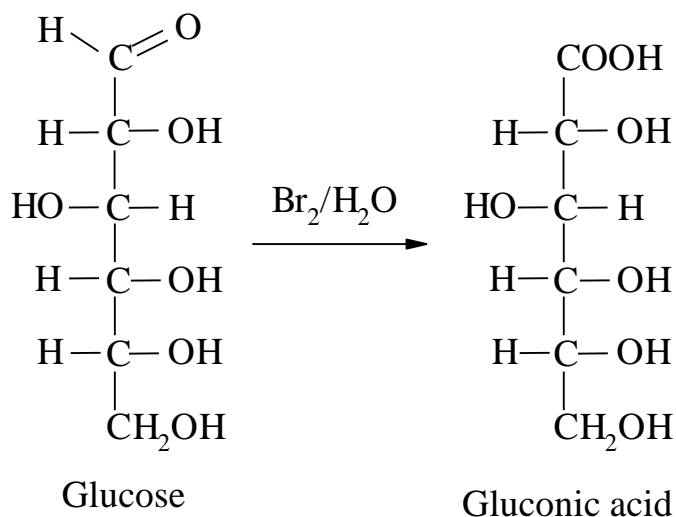
**6. Oxidation.** The oxidation of monosaccharides gives a variety of products depending upon the nature of the oxidizing agent used.

a) Oxidation in alkaline medium. Monosaccharides are unstable in the alkaline medium. With Tollen's reagent (ammoniacal silver nitrate solution) or Fehling solution (alkaline solution of  $\text{CuSO}_4$  containing sodium potassium tartrate) monosaccharides undergo oxidative cleavage giving mixture of acids.



These reactions can be used for determination of glucose in biological fluids.

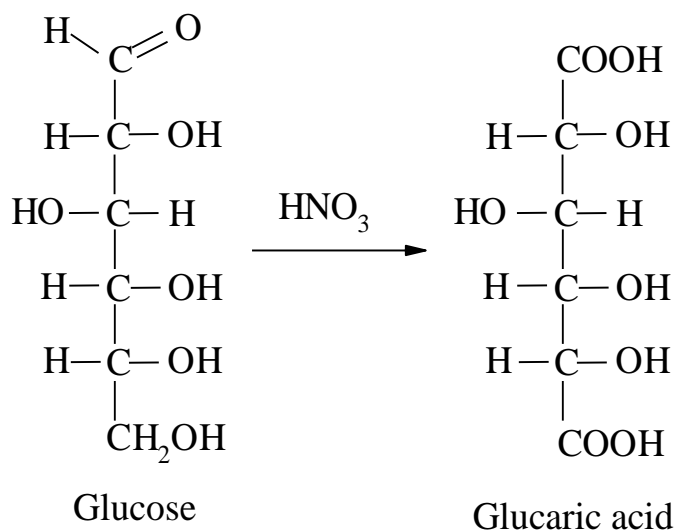
b) Oxidation in neutral medium. Aldehyde group can be oxidized with mild oxidizing agents like bromine water to the carboxyl group without involving any other groups. As a result **aldonic acids** are formed.



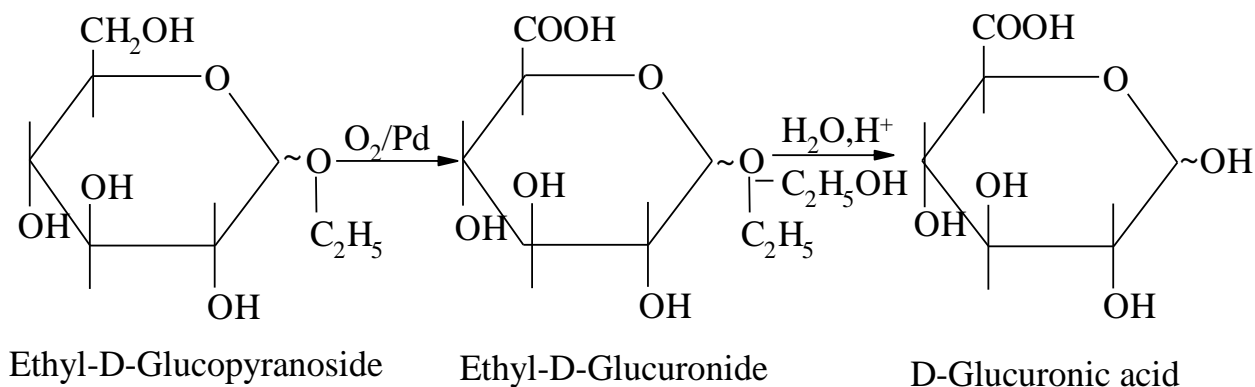
Calcium salt of gluconic acid (calcium gluconate) finds its use in medicine.

c) Oxidation in acidic medium. With a strong oxidizing agents like  $\text{HNO}_3$  terminal groups of monosaccharides (aldehyde and primary alcohol) get oxidized simultaneously to the carboxyl groups. As a result **saccharic acids** are formed. Glucose is oxidized to glucaric acid, galactose - to galactaric acid.

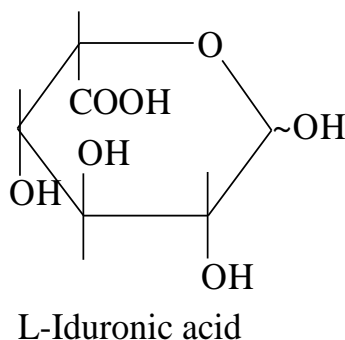




Monosaccharide derivatives in which the  $-\text{CH}_2\text{OH}$  group at C-6 has been specifically oxidized to a carboxyl group are called **uronic acids**. Their names are based on the monosaccharide from which they are derived. For example, specific oxidation of C-6 of glucose to a carboxyl group converts glucose to glucuronic acid. In the same way, specific oxidation of C-6 of galactose would yield galacturonic acid. Direct oxidation of an aldose affects the aldehyde group first, converting it to a carboxylic acid. Synthesis of an uronic acid from an aldose requires protecting this group from oxidation. If glycoside is taken, uronic acid can be obtained:



D-glucuronic acid and L-iduronic acid are structural components of heparin.



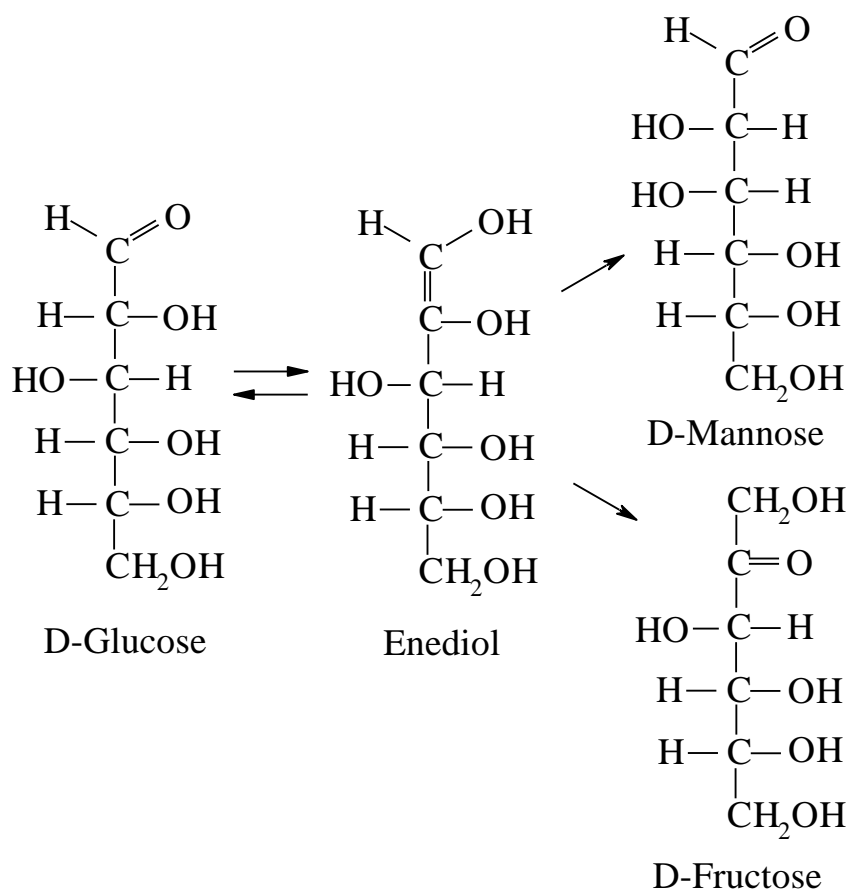
Uronic acids play important role in the organism. They produce water-soluble glycosides with different toxic substances and being in this form harmful species are excreted with urine.

**7. Interconversions of aldoses and ketoses.** Isomerization of monosaccharides (i.e. formation of several different monosaccharides from one monosaccharide) takes place in the weak alkaline medium at room temperature. For example, the following mixture can be obtained from D-glucose in five days:

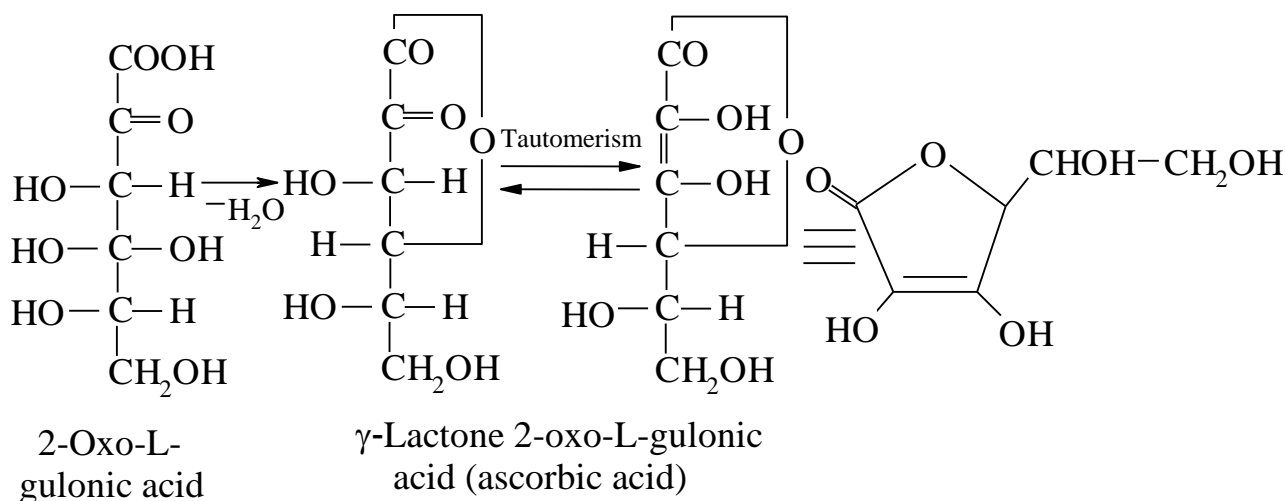
D-glucose - 63,5%, D-mannose - 2,5%, D-fructose - 31%.

C-2 represents C-H acidic center due to which enediol is formed. During the conversion of enediol form back to oxo-form different variations are possible to obtain.

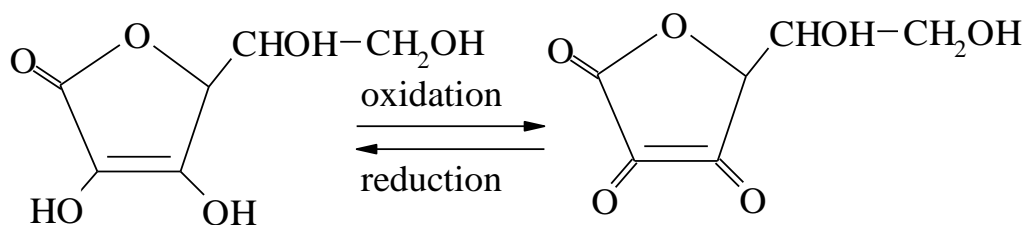
Therefore a mixture of several sugars is formed.



L-ascorbic acid (Vitamin C) is the lactone (cyclic ester) of an acid structurally similar to L-glucose. For the adult human, the daily requirement in vitamin C is 75 mg. Vitamin C is synthesized in plants from L-gulonic acid.



Ascorbic acid takes part in oxidation–reduction reactions.



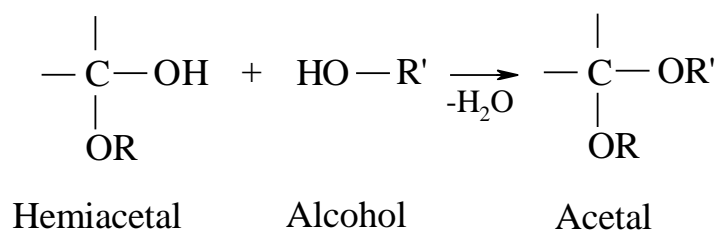
Vitamine C participates in the reactions for hydroxylation of proline and lysine in the synthesis of collagen, in the synthesis of adrenocortical hormones (corticosteroids) and the amino acid tryptophan, and, possibly, in other hydroxylation reactions. Vitamin C deficiency leads to scurvy.

## **5. OLIGOSACCHARIDES AND POLYSACCHARIDES: STRUCTURE AND PROPERTIES**

### *Disaccharides*

Disaccharides are the carbohydrates which on hydrolysis give two same or different monosaccharides. Their general formula is  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ . The important members belonging to disaccharides are sucrose, maltose and lactose.

The disaccharides are made up of two molecules of monosaccharides linked to each other by condensation reaction. The linking is formed just as hemiacetals react with alcohols to form acetals with the elimination of a water molecule.

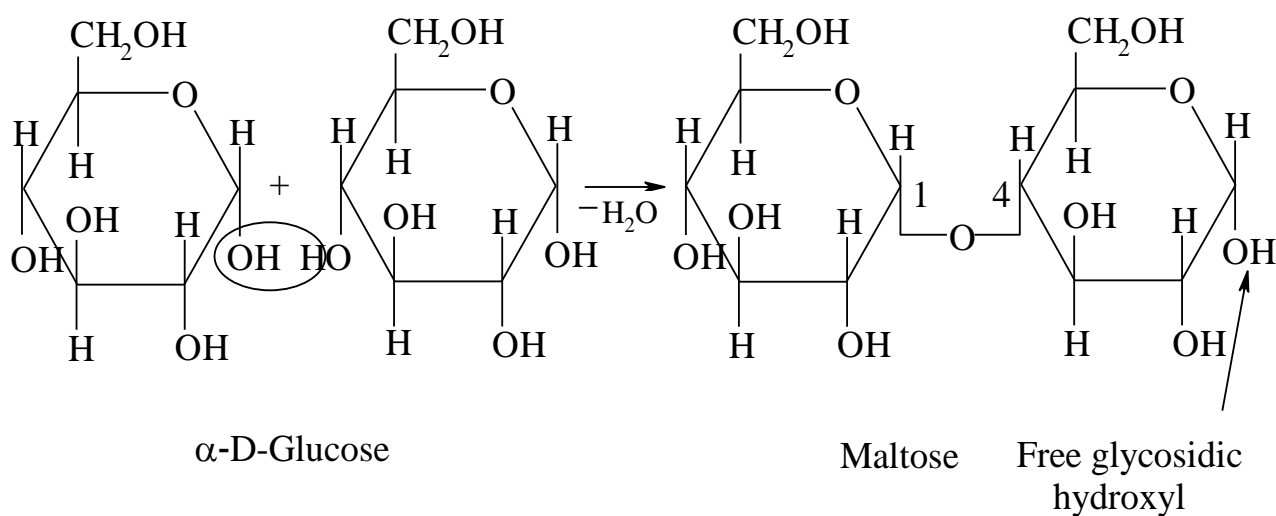


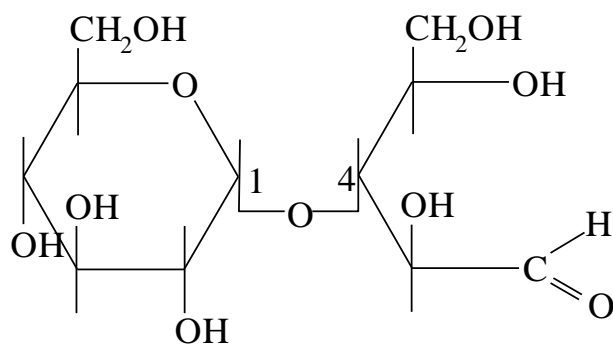
In a similar way, the hydroxyl groups of two monosaccharide units condense to form disaccharide. The two monosaccharide units are linked to each other by a bond called **glycosidic linkage**. There are two types of monosaccharide linking:

1. Due to the glycosidic OH-group of one monosaccharide unit and any alcoholic OH-group of the other one (this is a group **of reducing sugars**).
2. Due to the glycosidic OH-groups of both monosaccharide units (this is a group **of non-reducing sugars**).

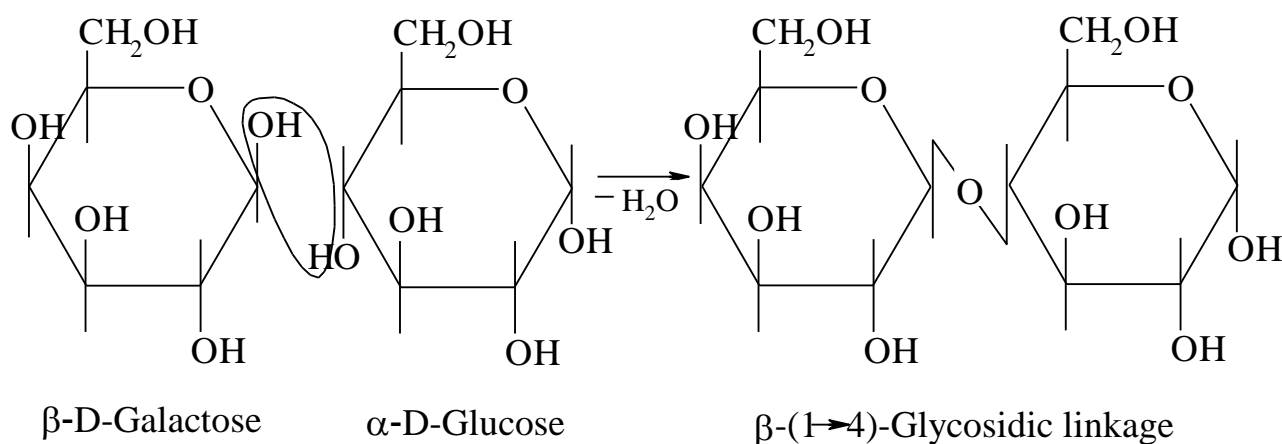
A molecule of reducing sugars contains a free glycosidic hydroxyl due to which the ability to open a cycle keeps well. An open chain form contains an aldehyde group which possesses reducing properties. That's why reducing sugars reduce such chemicals as Fehling solution and Tollen's reagent.

**1. Maltose.** It is known as malt sugar. It is the principal disaccharide obtained by the hydrolysis of starch. It is composed of two  $\alpha$ -D-glucose units (pyranose rings), which are condensed together through C<sub>1</sub> of one unit and C<sub>4</sub> of the other unit ( $\alpha$ -(1→4) glycosidic linkage). Maltose belongs to **reducing sugars** because of the presence of free glycosidic hydroxyl.

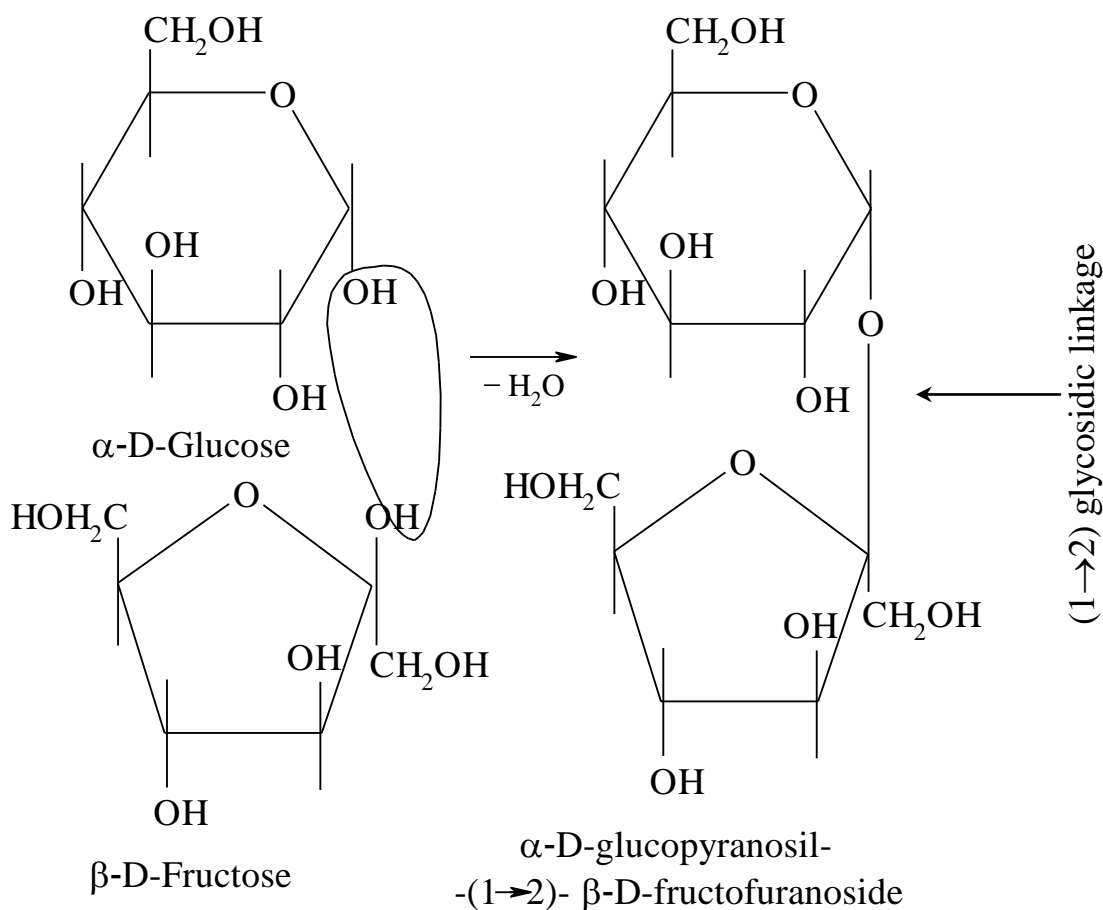




**2. Lactose.** It occurs in the milk of all mammals. It is composed of  $\beta$ -D-galactose and  $\alpha$  or  $\beta$ -D-glucose units (pyranose rings). These units are held together by glycosidic linkage between  $C_1$  of galactose and  $C_4$  of the glucose unit ( $\alpha$ -(1 $\rightarrow$ 4) glycosidic linkage). Lactose is **reducing sugar**.



**3. Sucrose.** It is the most common disaccharide and is present in the sugar cane juice. It is composed of  $\alpha$ -D-glucose and  $\beta$ -D-fructose. These units are held together by  $\alpha,\beta$ -glycosidic linkage between  $C_1$  of the glucose unit (pyranose ring) and  $C_2$  of the fructose unit (furanose ring) ((1 $\rightarrow$ 2) or (2 $\rightarrow$ 1) glycosidic linkage).



Free hemiacetal (glycosidic) hydroxyls are absent in sucrose, that's why it isn't capable of ring-chain tautomerism. Therefore sucrose doesn't possess reducing properties, i.e. it belongs to **non-reducing sugars**.

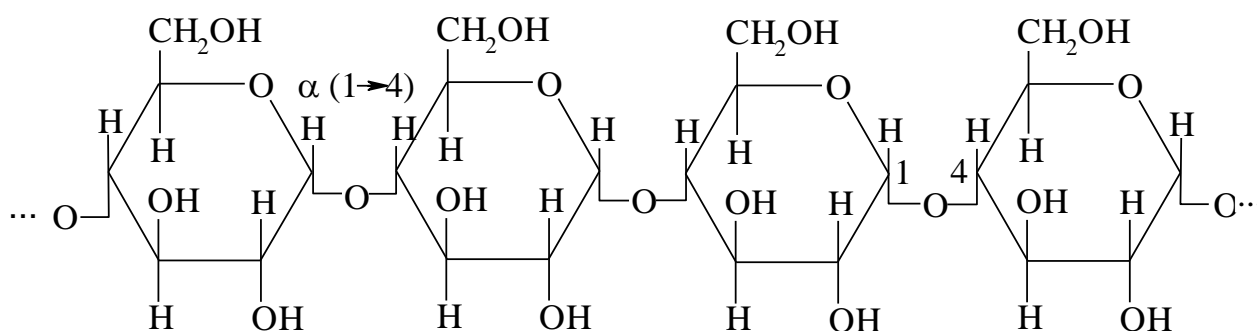
### *Homopolysaccharides*

These are neutral polymeric compounds in which a large number of monosaccharide units are joined by *glycosidic linkages*. They have the general formula  $(\text{C}_6\text{H}_{10}\text{O}_5)_n$ , where  $n$  has very large value. They are colourless, tasteless and are insoluble in water. They play very important role in plant and animal life as food storage and structural components. They are usually made up of pentoses or hexoses. The important polysaccharides are *cellulose*, *starch* and *glycogen*.

**Starch** is the main storage polysaccharide of plants. It is a polymer of  $\alpha\text{-D}$ -glucose (pyranose ring) and consists of two components: (20%) *amylose* and (80%) *amylopectin*.

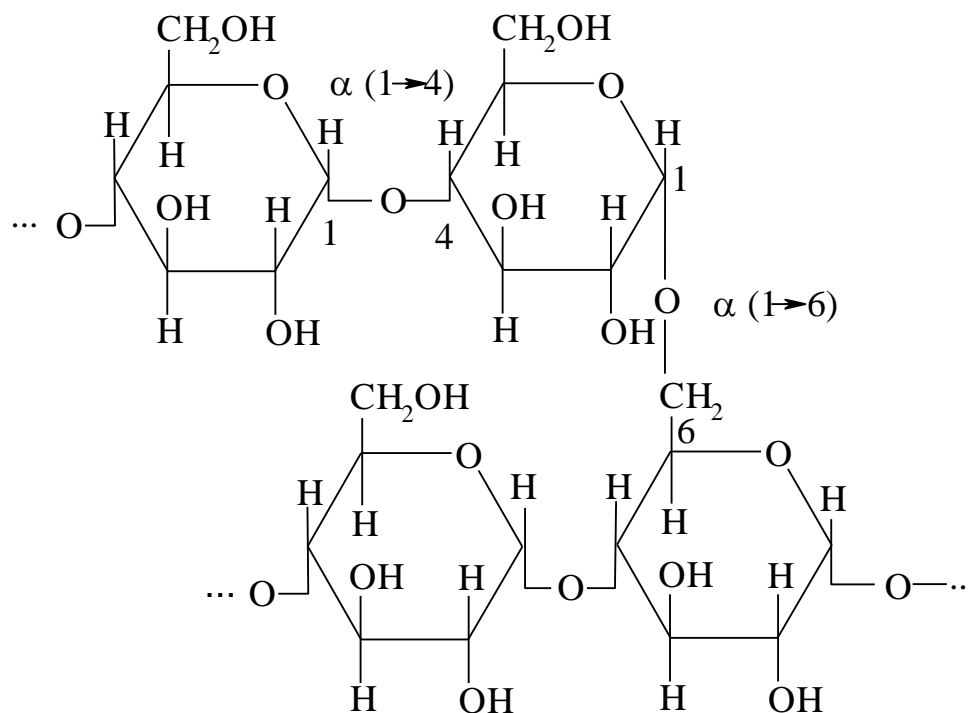
a) Amylose is a water soluble fraction. It is a linear polymer of  $\alpha\text{-D}$ -glucose. It contains about 200-1000 glucose units which are linked to one another through  $\alpha$ -linkage involving C-1 of one glucose and C-4 of other ( $\alpha\text{-(1}\rightarrow\text{4)}$  glycosidic linkage)

as shown below:



Amylose gives a deep blue color with iodine. Chains of amylose tend to assume a helical arrangement. This arrangement results in a compact shape for the amylose molecule even though its molecular weight is quite large (150000 – 600000).

b) Amylopectin is water insoluble fraction. It consists of branched chains of  $\alpha$ -D-glucose involving about 1000-3000 units per molecule. In this case the main chain involves  $\alpha$ -linkages between C-1 of one  $\alpha$ -D-glucose unit and C-4 of the other ( $\alpha$ -(1 $\rightarrow$ 4) glycosidic linkage). The C-1 of terminal glucose in each chain is further linked to C-6 of the other glucose unit in the next chain through  $\alpha$ -(1 $\rightarrow$ 6) glycosidic linkage. This gives highly branched structure as shown below.

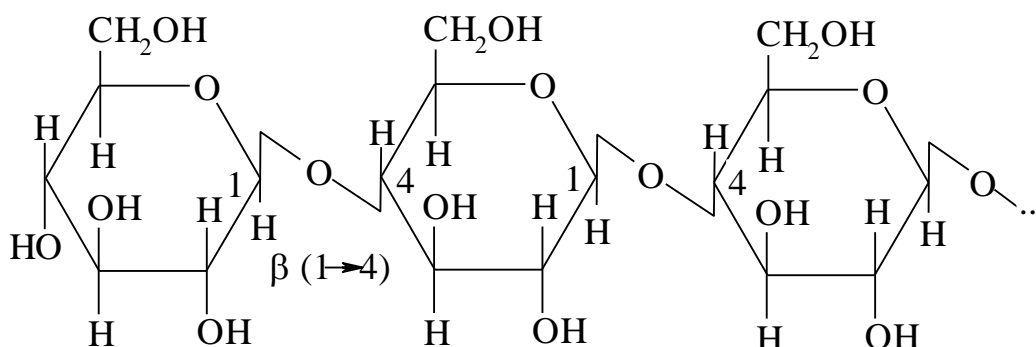


Branching occurs at interval of 20 – 25 glucose units. Amylopectin has a

molecular weight of 1 – 6 million.

Starch is used as the principal food storage of glucose energy. It comprises the major part of roots, seeds and corns.

**Cellulose** is also major structural polysaccharide in higher plants where it constitutes the bulk of cell wall. It is probably the most abundant organic substance found in nature. Over 50% of the total organic matter in the living world is cellulose. Dry leaves contain 10-20% cellulose, wood contains 50% and cotton contains 90% cellulose. Cellulose forms the fibrous component of plant cell walls. Structurally, its molecules are made up of a large number of  $\beta$ -glucose units. The glucose units are present in pyranose form and the two glucopyranose units are linked to each other through the  $\beta$ -anomeric carbon (C-1) of one and C-4 of the other ( $\beta$ -(1 $\rightarrow$ 4) glycosidic linkage). This configuration of the anomeric carbon atoms makes cellulose chains essentially linear; they do not tend to coil into helical structures as do glucose polymers when linked in an  $\alpha$ (1 $\rightarrow$ 4) manner. The chains are arranged to form bundles and are held together by hydrogen bonds between glucoses of adjacent chains.



Cellulose

The digestive enzymes of humans cannot attack its  $\beta$ (1 $\rightarrow$ 4) linkages. Hence, cellulose cannot serve as a food source for humans, as can starch.

Cellulose is an industrially important compound. It finds uses in textiles, paper and plastic industries. When treated with a wide variety of chemicals, it forms many useful products, celluloid, rayon, gun cotton (an explosive), cellulose acetate (plastics and wrapping films), methyl cellulose (fabric sizing, pastes and cosmetics) ethyl cellulose (plastic coals and films) etc.

**Glycogen** has a structure very much like that of amylopectin; however, in glycogen the chains are much more highly branched (approximately twice more). There is one end group for every 10 – 12 glucose units; branches may occur as often



as every 6 units. Glycogen has a very high molecular weight. Studies of glycogen isolated under conditions that minimize the likelihood of hydrolysis indicate molecular weights as high as 100 million.

The size and structure of glycogen beautifully suits its function as a reserve carbohydrate for animals. First, its size makes it too large to diffuse across cell membranes; thus, glycogen remains inside the cell, where it is needed as an energy source. Second, the localization of glucose units within a large, highly branched structure simplifies one of cell's problems: that of having a ready source of glucose when cellular glucose concentrations are low and of being able to store glucose rapidly when cellular glucose concentrations are high. There are enzymes within the cell that catalyze the reactions by which glucose units are detached from (or attached to) glycogen. These enzymes operate at end groups by hydrolyzing (or forming)  $\alpha$ -(1 $\rightarrow$ 4) glycosidic linkages. Because glycogen is so highly branched, a very large number of end groups is available at which these enzymes can operate.

Amylopectin presumably serves a similar function in plants. The fact that amylopectin is less highly branched than glycogen is, however, not a serious disadvantage. Plants have a much lower metabolic rate than animals – and plants, of course, do not require sudden bursts of energy.

Animals store energy as fats as well as glycogen. Fats, because they are more highly reduced, are capable of furnishing much more energy. The metabolism of a typical fatty acid liberates more than twice as much energy per carbon as glucose. But glucose (from glycogen) is readily available and is highly water soluble. Glucose, as a result, diffuses rapidly through the aqueous medium of the cell and serves as an ideal source of "ready energy". Long – chain fatty acids, by contrast, are almost insoluble in water, and their concentration inside the cell could never be very high. They would be a poor source of energy if the cell were in an energy pinch. On the other hand, fatty acids (as triacylglycerols), because of their caloric richness, are an excellent energy repository for long energy storage.

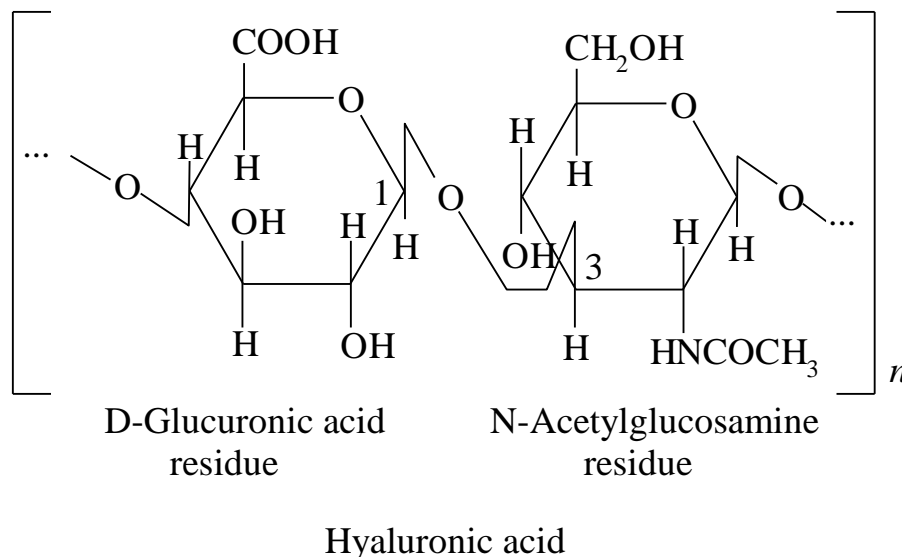
### *Heteropolysaccharides*

Heteropolysaccharides consist of different monosaccharide units. In the organism, heteropolysaccharides are included in the composition of proteoglycans. Proteoglycans are high-molecular compounds of carbohydrate and protein, found in animal structural tissues. They form the ground substance of the intracellular matrix

of connective tissue. Proteoglycans account for about 30% of connective tissue (in dry weight). The polysaccharide moiety in proteoglycans is called mucopolysaccharides or glycosaminoglycans.

The glycosaminoglycans of connective tissue are linear unbranched polymers built of regularly repeating disaccharide units. In a free state, that is, as “pure” carbohydrates, the glycosaminoglycans are never encountered in the organism; they are always bound, to a varied extent, with protein molecules.

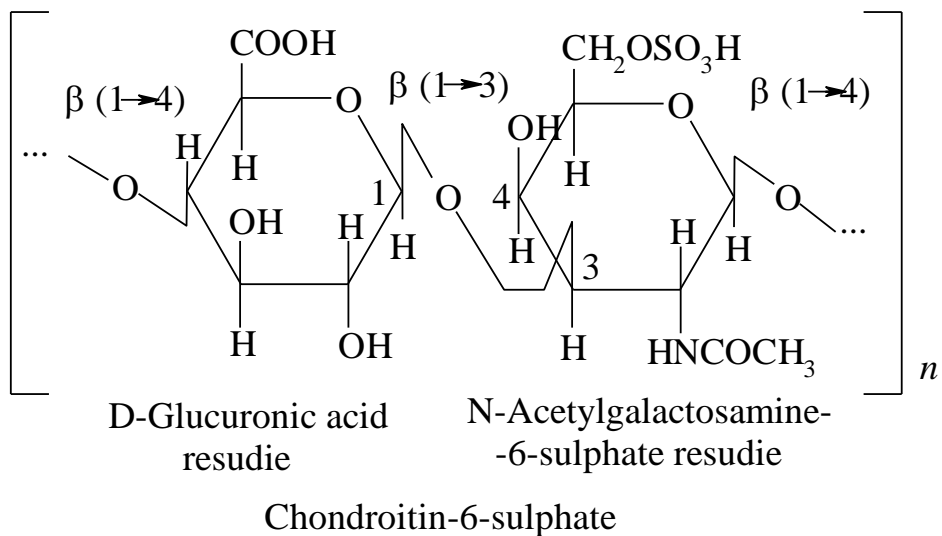
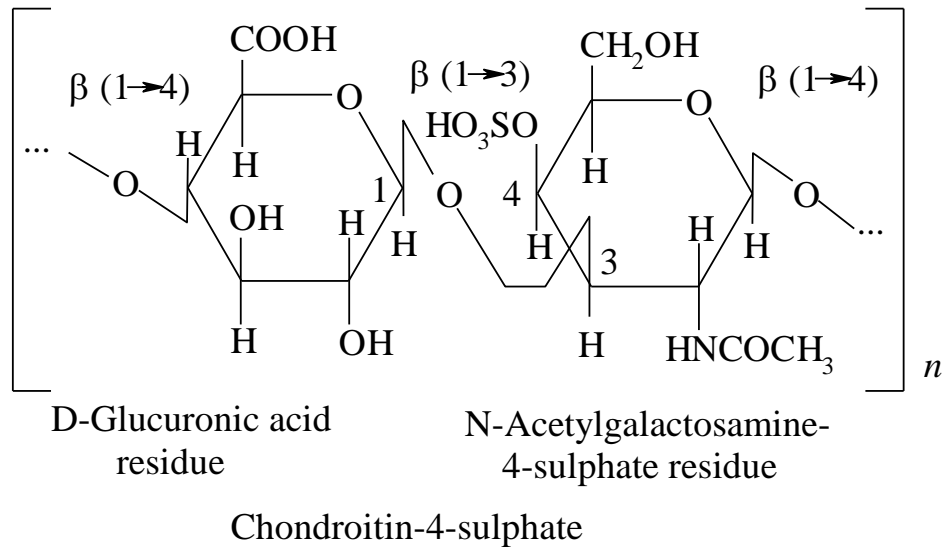
**Hyaluronic acid** was first discovered in the vitreous body of the eye. Of all the glycosaminoglycans, hyaluronic acid has the largest molecular mass ( $10^5 - 10^7$  Da). Hyaluronic acid consists of the repeating disaccharide units which are bonded by  $\beta(1 \rightarrow 4)$  linkages. Each disaccharide unit is composed of residues of D-glucuronic acid and N-acetyl-D-glucosamine which are connected through  $\beta(1 \rightarrow 3)$  glycosidic linkage.



It is commonly believed that the major function of hyaluronic acid in the connective tissue is to bind water. On uptake of water, the intracellular substance turns into a gel-like matrix capable of “supporting” the cells suspended therein. Hyaluronic acid plays also an important role in the regulation of tissue permeability. It is mainly localized in skin, cartilages, tendons, umbilical cord, vitreous body, synovial fluid, cardiac valves, bones, embryonic cartilages.

**Chondroitin 4-sulphate** and **chondroitin 6-sulphate** are structural congeners, the only distinction being the site for localization of sulphate group. Chondroitin 4-sulphate consists of the repeating disaccharide units which are bonded by  $\beta(1 \rightarrow 4)$  linkages. Each disaccharide unit is composed of residues of D-glucuronic acid and

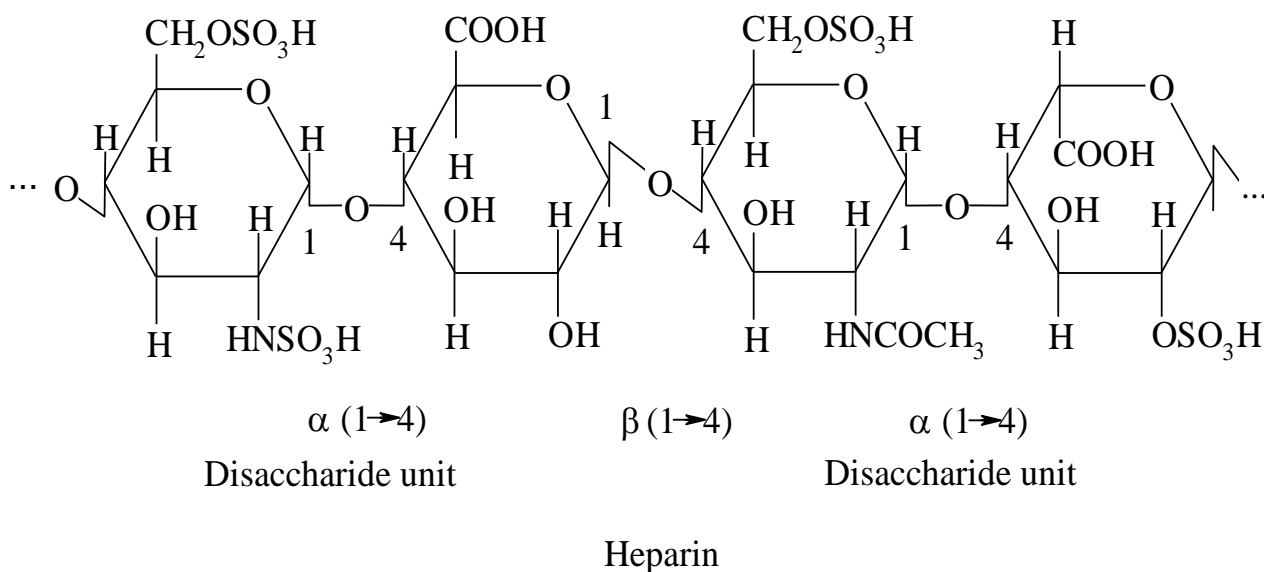
N-acetyl-D-galactosamine-4 sulphate which are connected through  $\beta$ -(1 $\rightarrow$ 3) glycosidic linkage. Chondroitin 6-sulphate contains N-acetyl-D-galactosamine-6 sulphate.



Despite this apparently minor structural dissimilarity, the two species differ significantly in their physico – chemical properties and in the occurrence in different types of connective tissue. Chondroitin 4-sulphate can be found in cartilages, bones, embryonic cartilages, and cornea. Chondroitin 6-sulphate can be found in skin, tendons, ligaments, umbilical cord, and cardiac valves.

**Heparin** is primarily known as an anticoagulant. Nonetheless, this species should be assigned to glycosaminoglycans, since it is synthesized by mast cells which are a variety of the cellular elements of connective tissue. Heparin consists of repeating disaccharide units of two types. Disaccharide unit contains D-glucosamine and D-glucuronic acid, or D-glucosamine and L-iduronic acid. Within the

disaccharide unit monomers are bonded through  $\alpha$ -(1 $\rightarrow$ 4) glycosidic linkage. Disaccharide units are bonded either through  $\alpha$ -(1 $\rightarrow$ 4) glycosidic linkage or  $\beta$ (1 $\rightarrow$ 4) linkage. Some hydroxyl and amino groups are sulphated or acylated.



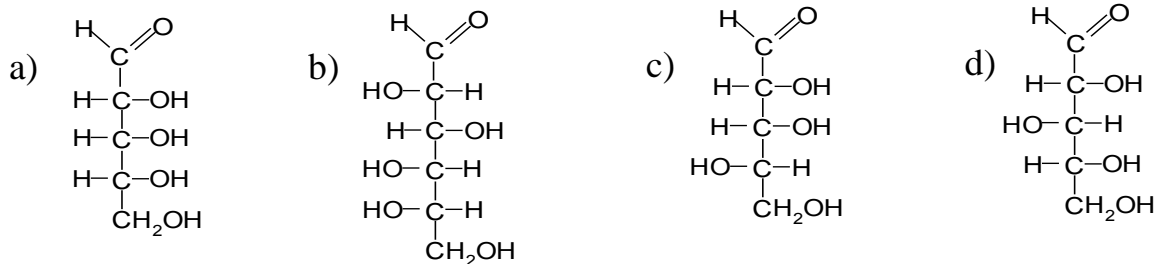
Heparin can be found in liver, lungs, and vascular walls. Heparin occurs in intracellular granules of mast cells that line arterial walls, where, when released through injury, it inhibits the clotting of blood. Its purpose seems to be to prevent runaway clot formation. Heparin is widely used in medicine to prevent blood clotting in postsurgical patients.

### Revision exercises

1. How many chiral carbon atoms are there in galactose molecule?

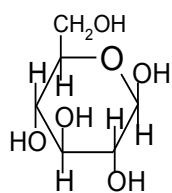
- a) 2 chiral carbon atoms                      b) 3 chiral carbon atoms  
 c) 4 chiral carbon atoms                      d) 5 chiral carbon atoms.

2. Which if the following is L-aldopentose?

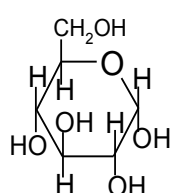


3. Which of the following is  $\beta$ -D-glucopyranose?

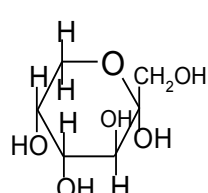
a)



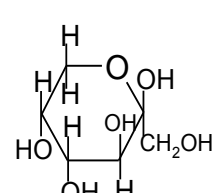
b)



c)



d)



Answers

1. c)
2. c)
3. a)

\*\*\*

## HIGHER FATTY ACIDS. LIPIDS. PHOSPHOLIPIDS

### *Characteristics of the subject*

The structure and chemical properties of saponifiable lipids and their components are a chemical base for studying the structure of biological membranes and lipid metabolism.

### *Purposes*

To study the structure and regularities of chemical behavior of esters and saponifiable lipids. To consider the composition and structure of phospholipids, sphingomyelins, glycolipids, non-saponifiable lipids and their biological role.

### *Objectives*

1. To know the composition and structure of higher fatty acids which are components of lipids.
2. To know the electronic mechanism of esterification reaction and the most important chemical properties of esters (hydrolysis, interaction with ammonia and amines, etc.).
3. To know the structure, physical properties, analytical characteristics and chemical properties of fats. To understand their biological functions.
4. To learn the structure of phospholipids (cephalins and lecitins), to understand their biological role.
5. To study the structure of waxes, sphingolipids, glycolipids.
6. To know the peculiarities of chemical behavior of thioethers, formation and properties of esters of co-enzyme A.

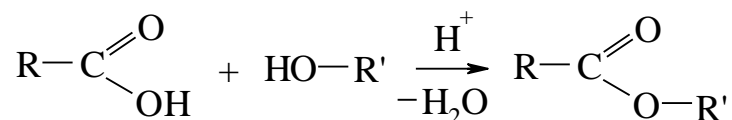
### *Theoretical questions*

1. Reaction of formation, classification, and biological functions of lipids.
2. Simple lipids.
3. Lipids digestion and metabolism ( $\beta$ -oxidation).
4. Complex lipids.
5. Non-saponifiable lipids.

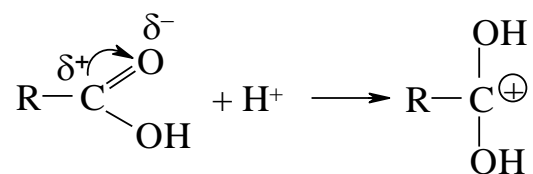
## **1. REACTION OF FORMATION, CLASSIFICATION, AND BIOLOGICAL FUNCTIONS OF LIPIDS**

Lipids are oily, fatty or waxy substances present in living organisms.

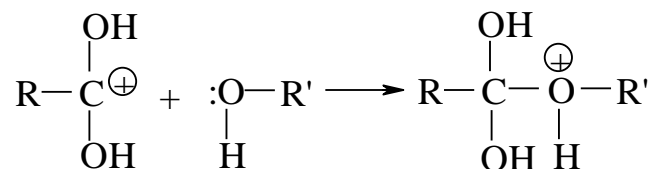
Chemically, lipids are esters of long chain fatty acids and alcohols. Let's see the mechanism of the reaction of esterification in details. Heating of carboxylic acids with alcohols in acid medium produces esters.



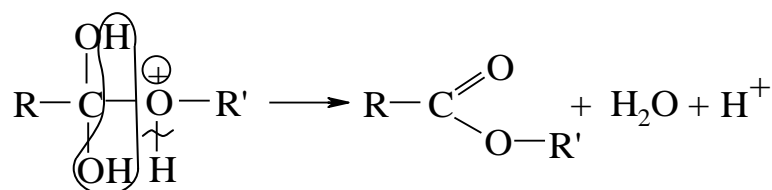
1. First a proton joins carboxyl group in the presence of mineral acid with the formation of carbocation.



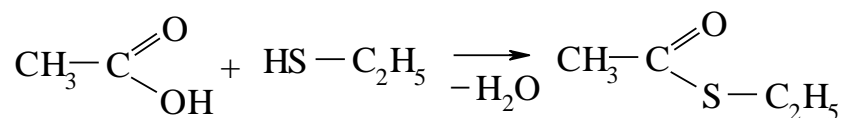
2. A molecule of alcohol joins the formed carbocation due to the unshared electrons oxygen atom.



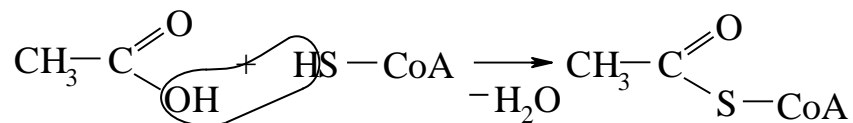
3. The addition compound is stabilized with the elimination of proton and water molecule.



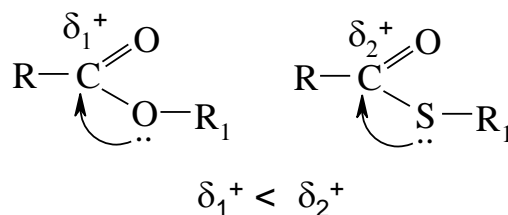
Carboxylic acids form thioesters interacting with thiols.



Carboxylic acids in the organism form thioesters with coenzyme A. CoA-SH is a thiol, it consists of 2-aminoethanethiol, pantothenic acid (vit B<sub>3</sub>) and ADP. Carboxylic acids participate in a large number of biochemical processes just being in the form of thioesters (acetyl coenzyme A).



Thioesters are more reactive than esters themselves as the overlapping of orbitals of C and S atoms is less effective than of the C and O ones. That's why  $\delta^+$  on the C atom of carboxyl group of thioester is greater than in ester.



***Lipids main functions are:***

1. Energetic function – mainly triacylglycerides store energy for the cell.
2. Structural function – phospholipids, sphingolipids form part of the structure of biological membranes.
3. Protecting function – lipids form mechanical coating around inner organs. They protect organs from injury and overcooling (thermo regulating) function.
4. Lipids act as a source of endogenic water.

They are soluble in organic solvents such as chloroform, ether, etc. and are insoluble in water. Lipids can be divided into **three main classes**:

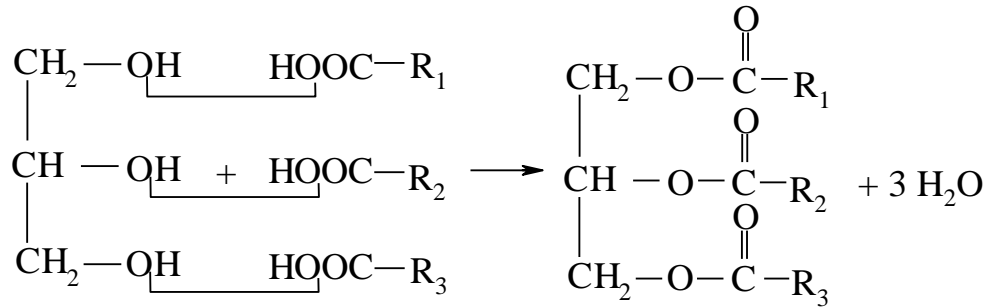
1. Simple lipids, which consist of long chain fatty acids and alcohols.
2. Complex lipids, which give fatty acids, alcohols and other compounds on hydrolysis.
3. Non-saponifiable lipids (derived lipids). Steroids like cholesterol, fat soluble vitamins like vitamins A,D,E and K are the examples of derived lipids.

Simple and complex lipids are called **saponifiable lipids** as they undergo alkaline hydrolysis (saponification).



## 2. SIMPLE LIPIDS

Oils and fats are simple lipids. These are esters of glycerol and three fatty acids. These are also called triglycerides.



a) The three fatty acids may be identical (a simple glyceride) or different (mixed glyceride). The naturally occurring fatty acids may be saturated or unsaturated.

### *Saturated fatty acids*

Lauric acid:  $\text{C}_{11}\text{H}_{23}\text{COOH}$

Myristic acid:  $\text{C}_{13}\text{H}_{27}\text{COOH}$

Palmitic acid:  $\text{C}_{15}\text{H}_{31}\text{COOH}$

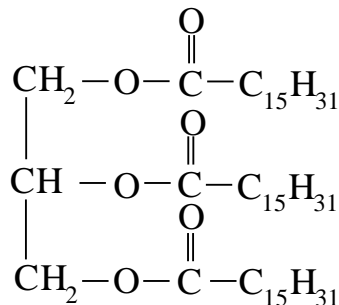
Stearic acid:  $\text{C}_{17}\text{H}_{35}\text{COOH}$

*Unsaturated fatty acids* (contains one or more double bonds),

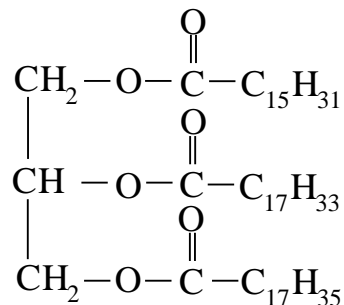
Oleic acid:  $\text{C}_{17}\text{H}_{33}\text{COOH}$  (one double bond)

Linoleic acid:  $\text{C}_{17}\text{H}_{31}\text{COOH}$  (two double bonds)

Linolenic acid:  $\text{C}_{17}\text{H}_{29}\text{COOH}$  (three double bonds)



Tripalmitine



Palmito-oleo-stearate  
(1-palmitoyl-2-oleoyl-  
-3-stearoylglycerine)

**Waxes** are also esters and they are simple lipids. They are fatty acids esters of long chain monohydric alcohols and may be represented by the general formula RCOOR' where R and R' are long hydrocarbon chains.

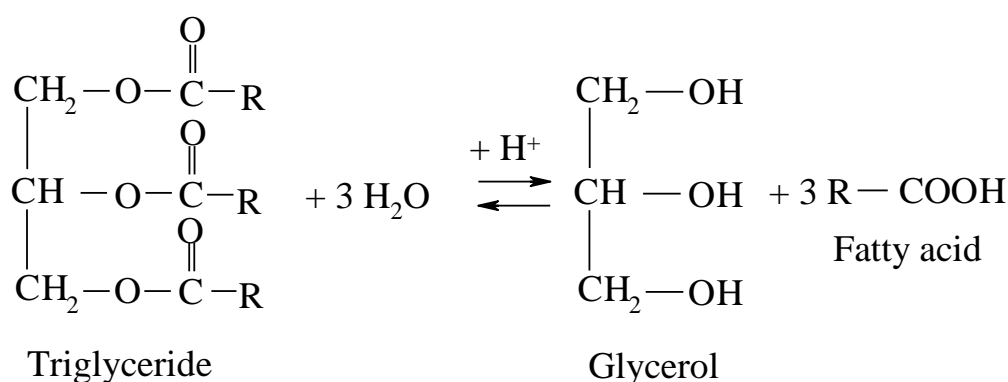
$C_{15}H_{31}COO C_{30}H_{61}$  – in bees wax (myricyl palmitate)

$C_{15}H_{31}COO C_{16}H_{33}$  – in spermaceti (cetyl palmitate)

In fact, they occur as mixtures. The waxes are widely spread in nature and play an important role as a protective coatings on fruits, leaves and animals skin.

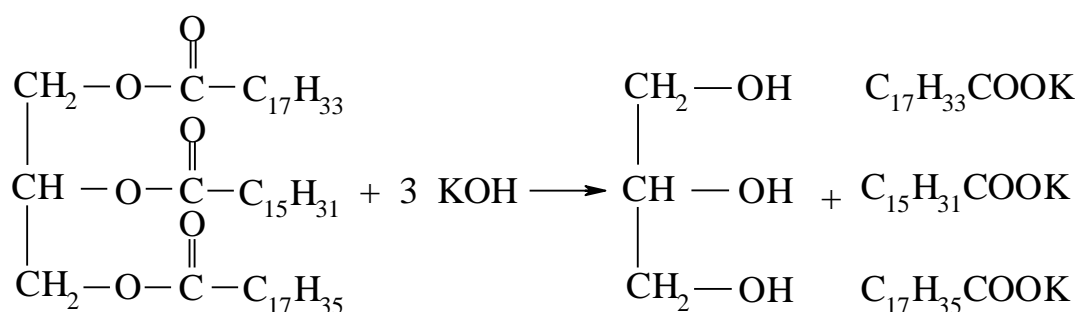
They protect the surface from loss of water and attack of micro-organisms.

b) Simple lipids undergo hydrolysis to give one molecule of glycerol and three molecules of fatty acids per molecule of oil or fat.



Hydrolysis

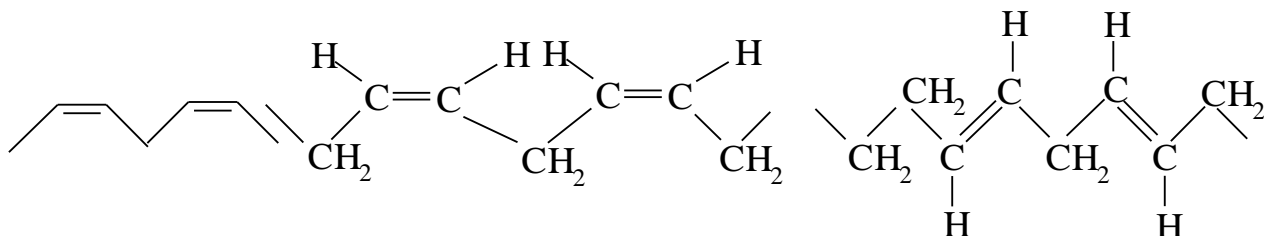
Hydrolysis is catalyzed by acids or basis. Acidic hydrolysis can be shown as process reverse to the reaction of esterification. The alkaline hydrolysis or **saponification** of fats gives glycerol and the sodium or potassium salt of the carboxylic acid. This is a soap.



The alkaline hydrolysis is irreversible owing to the formation of precipitating salts. Sodium soaps are solid soaps, potassium soaps are liquid (shampoos).

The glycerides containing large proportion of saturated acids are **solids** at room temperature and called **fats**. The glycerides containing large proportion of unsaturated

acids are **liquids** at room temperature and called **oils**. The difference in melting temperatures arises from the fact that saturated hydrocarbon chains can pack more closely together than unsaturated chains. In these esters, the configuration at the double bond is always cis, so that the molecules are bent, and cannot pack closely.



The oils can be converted into fats by hydrogenation in the presence of nickel as a catalyst. Margarine is made in this way from corn oil and soya bean oil. Saturated fats are very stable compounds. Unsaturated oils undergo oxidation by air. This is what happens when fats and oils turn rancid.

c) *Iodine number and alkali neutralization number (saponification number)* are analytical characteristics of fat. **Iodine number** is a quantity of grams of iodine that may be added to 100g of fat.

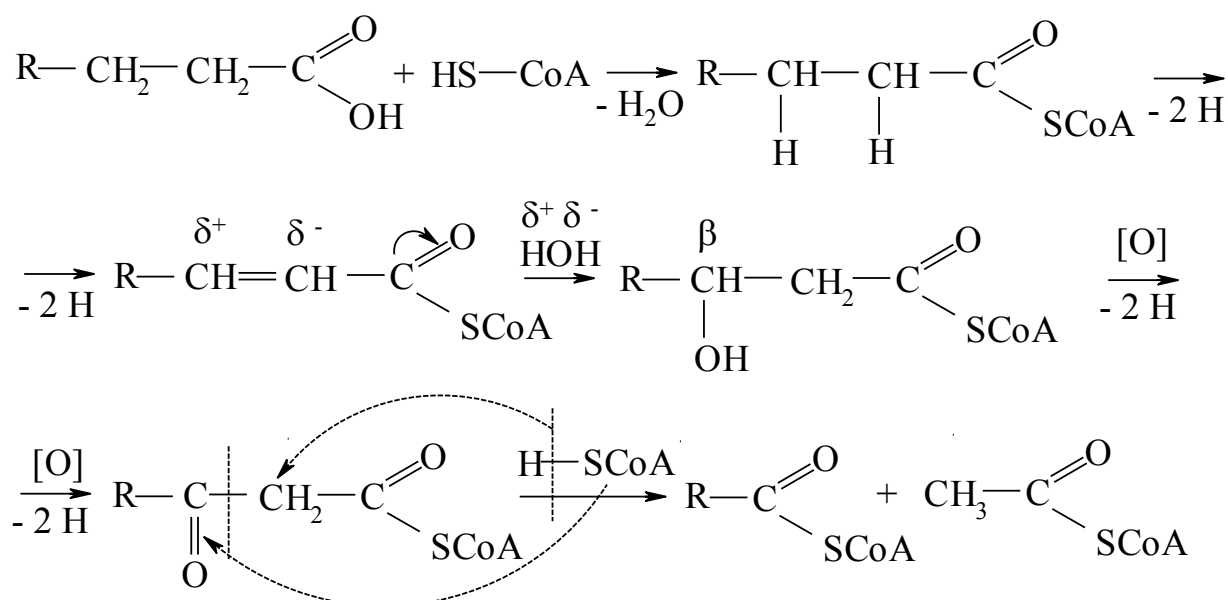
Iodine adds across double bonds in acids, that is why iodine number serves as an index of fat unsaturation. The greater the iodine number is the more unsaturated acids are present in the composition of fat. Iodine number of fat in a human being is 64 (which means 100g of fat join 64g of iodine). Iodine number enables to judge about the aggregative state of fat. If the iodine number is less than 70 – the fat is solid, if it is greater – the fat is liquid.

**Saponification number** is a quantity of KOH in mg necessary to saponify 1 g of fat. Saponification number characterizes the length of the chain in alkyl group of fatty acid. During the process of neutralization one molecule of KOH is used up for each molecule of fatty acid. The greater the molecular weight of fatty acids in the composition of the fat is, the fewer molecules of these fatty acids are liberated from 1g of the fat during the process of its hydrolysis and the fewer KOH molecules are needed for their neutralization. Hence, the smaller the alkali neutralization number is, the bigger the molecular weight of fatty acids in the composition of the fat will be.

*Triglycerides are very important and find uses in soaps, paints, varnishes, ointments, creams. Fatty acids are major sources of energy of the cell. Linoleic and linolenic acids help the body to synthesize a very important group of compounds called prostaglandins. These control almost all physiological activities of the body.*

### 3. LIPIDS DIGESTION AND METABOLISM ( $\beta$ -OXIDATION)

The digestion of lipids takes place in small intestine. The food mixes up with the bile fluid. In this case, the fats get emulsified and allow enzymes such as lipases to hydrolyse fat into glycerol and fatty acids which then pass into blood stream. Most of the energy in fats is contained in the long hydrocarbon chains of fatty acids. They are the main energy reserves of the body. Fatty acids are transferred to the cells by the blood stream and are oxidized in mitochondria. This oxidation is called  $\beta$ -oxidation. During this process, two carbon fragments are removed sequentially to form acetyl coenzyme A, which enters citric acid cycle (Krebs cycle) for production of ATP. For example, palmitic acid produces 130 molecules of ATP.



### 4. COMPLEX LIPIDS

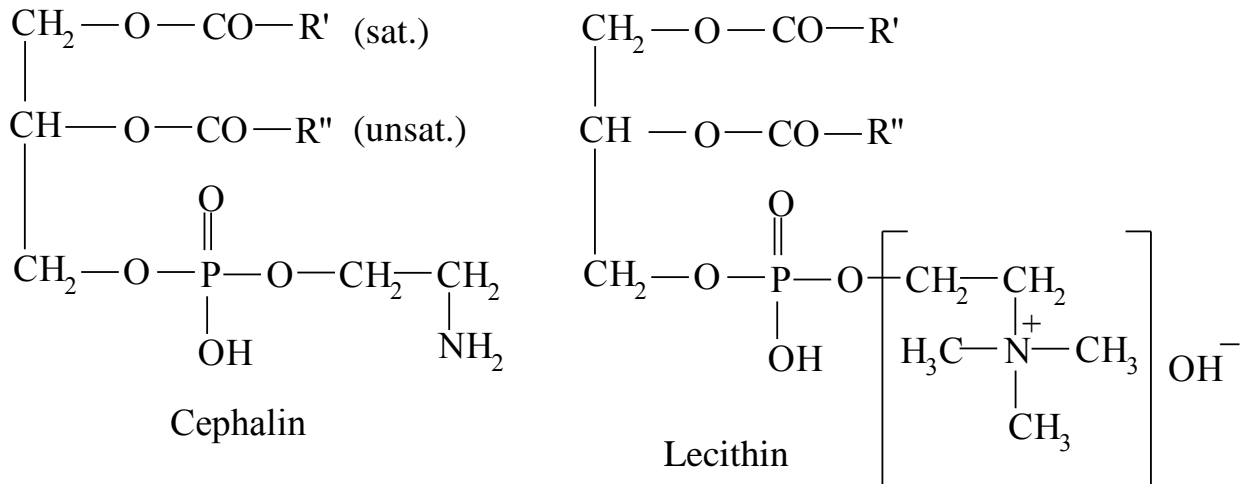
The complex lipids on hydrolysis give other substances in addition to alcohols and fatty acids. Phospholipids are examples of complex lipids.

#### *Phospholipids*

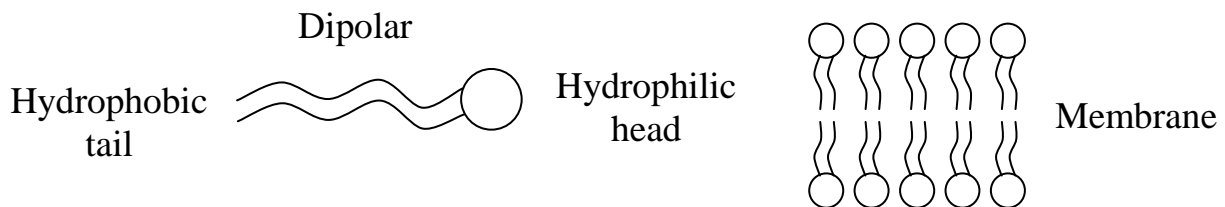
Phospholipids are mixed glycerides of higher fatty acids and phosphoric acid in which two  $-\text{OH}$  groups of glycerol are esterified by fatty acids and third by some derivatives of phosphoric acid. The common examples of phospholipids are *lecithins* and *cephalins* which are found principally in the brain, nerve cells and liver of

animals. These are also found in egg yolks, yeast, soya beans, etc.

Lecithins are derivatives of choline,  $\text{HOCH}_2\text{CH}_2\text{N}(\text{CH}_3)_3$ , cephalins are derivatives of ethanolamine,  $\text{HOCH}_2\text{CH}_2\text{NH}_2$ .



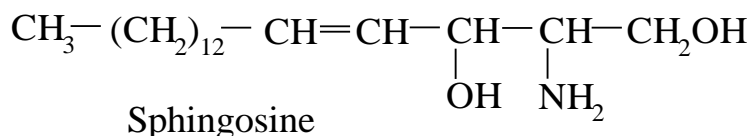
The phosphate group forms a polar hydrophilic head on the molecule and the two fatty acid chains constitute the non-polar hydrophobic (water repelling) tail. So, the phosphoglyceride may be shown as:



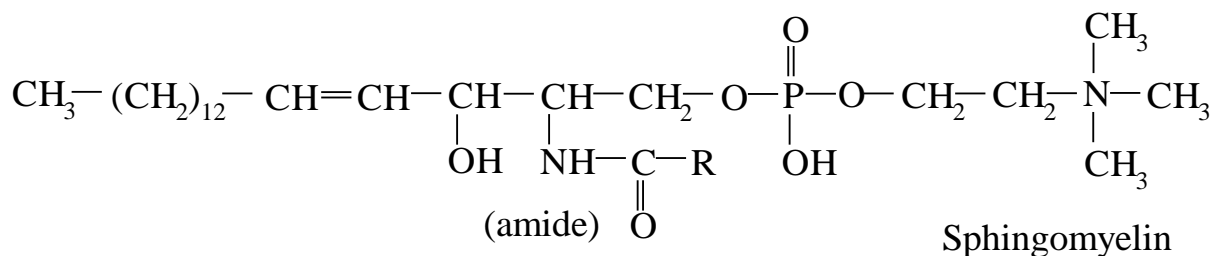
Cellular membranes are constituted mainly of phospholipids. They are arranged in a double layer (bilayer) with their hydrophilic (polar) heads pointing outside and hydrophobic (non-polar) tails into the interior of the membrane. This bilayer arrangement allows the polar head groups (phosphate ester) to interact with aqueous surroundings (both inside and outside the cell) and non-polar tails to be away from the aqueous medium.

## *Sphingolipids*

Sphingolipids are contained in nerve tissue. Sphingolipids contain sphingosine, an unsaturated long-chain amino alcohol, instead of glycerin.

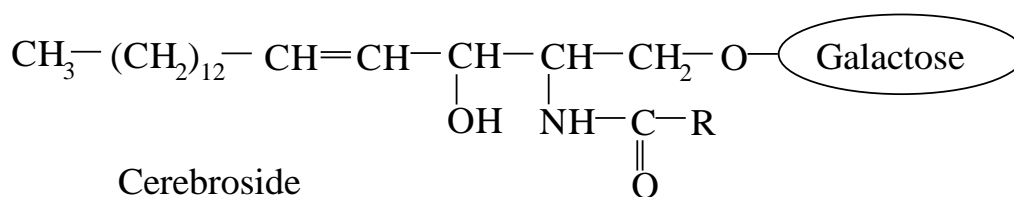


Sphingomyelins compose an important group of shingolipids. Sphingomyelin contains sphingosine and the residue of fatty acid, phosphoric acid and choline:



Glycolipids can be found in brain in large quantities. Cerebrosides and gangliosides are typical specimens of glycolipids. Cerebrosides are composed of sphingosine, galactose and fatty acids.

Gangliosides differ from cerebrosides as they contain oligosaccharides instead of monosaccharides.



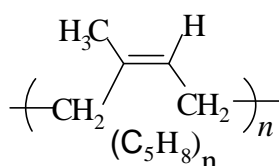
R – C<sub>23</sub>H<sub>45</sub>COOH – nervonic acid

C<sub>23</sub>H<sub>47</sub>COOH – lignoceric acid

## 5. NON-SAPONIFIABLE LIPIDS

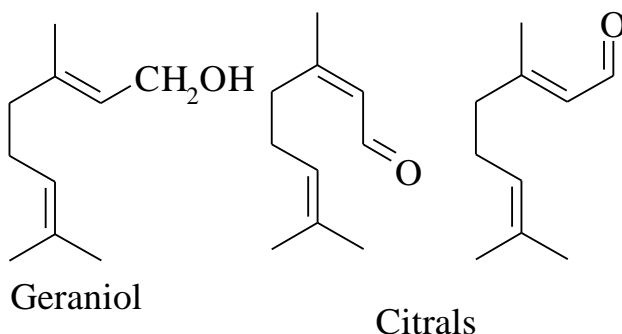
Non-saponifiable lipids do not undergo hydrolysis. They are represented by two types of compounds: steroids and terpenes. Steroids are mainly of animal origin while terpenes can be found in lipids of plants. They are very similar to each other as they are both built of identical isoprene five-carbon fragments.

Isoprenoids, prostaglandins and fat-soluble vitamins are important non-saponifiable lipids. Isoprenoids are built of the residues of conjugated diene - isoprene. The most common isoprenoid is the natural rubber.



Natural rubber

Biologically important isoprenoids include terpenes, carotinoids and steroids. According to the structure of the hydrocarbon skeleton terpenes are classified as acyclic (aliphatic) and cyclic.



This alcohol-terpenoids can be found in the flowers of geranium and roses. On oxidation they give aldehydes - citral A and citral B. Geraniol and citrals are pheromones.

Pheromones are volatile compounds that influence the behavior of living organisms. They evoke feelings of fear, anxiety, and sexual desire. An example of such a "chemical communication" is the secretion of geraniol and citral by bees to attract other bees to the source of food. These pheromones are called attractants (attraho (Latin) – to attract). Pheromones that repel other organisms are called repellents (repello (Latin) – to scare of). Citral serves as repellent for some insects. Not all pheromones are terpenes: the working bee has pheromone of alarm which is isoallylic ester of acetic acid  $\text{CH}_3\text{-COOCH}_2\text{-CH}_2\text{-CH}(\text{CH}_3)_2$ . After sting bee secretes this pheromone that attracts other bees to do the same.

### Revision exercises

1. How to determine molecular mass of high molecular fatty acids?

- a) By carboxylation                      b) By  $\beta$ -oxidation  
c) By Iodine number determination    d) By saponification.

2. Cephalin enters the composition of biological membranes. Which group of complex lipids does it belong to?

- a) Phospholipids                      b) Sphingolipids  
c) Glycolipids                         d) Derived lipids

3. How many molecules of acetyl-CoA are formed in the process of stearic acid  $\beta$ -oxidation?

- a) 3    b) 6    c) 9    d) 17

### Answers

1. d)  
2. a)  
3. c)

\*\*\*



# **AMINO ACIDS, PEPTIDES, PROTEINS: STRUCTURE, PROPERTIES, BIOLOGICAL FUNCTIONS**

## ***Characteristics of the subject***

Amino acids are the simplest compounds in the structure of high-molecular substances, i.e. proteins, the most important compound for building the tissues of the organism. Proteins are the most significant compounds necessary for building the tissues of the human body. Proteins are involved in a large variety of functions characteristic of the living organism; the main functions of proteins are: catalytic, nutritive (reserve), transport, protective, contractile, structural, hormonal.

## ***Purposes***

To study the chemical properties of amino acids associated with their structure and composition. To study the structure and properties of peptides, chemical basis for structural organization of protein molecules as the basis for understanding the biological properties of proteins at the molecular level.

## ***Objectives***

1. To learn the structure, composition and classification of amino acids.
2. To be able to characterize the most important chemical properties of amino acids associated with their structure.
3. To know the methods of qualitative and quantitative determinations amino acids and to be able to make these reactions.
4. To study the most important transformations of amino acids in the organism: transamination, deamination, decarboxylation.
5. To be able to write the schemes for oligopeptide formation from 2 amino acids.
6. To characterize the chemical properties of oligopeptides with the account of the nature of their constituents (amino acids).
7. To understand main techniques for determining primary structure of natural peptides.
8. To study the principles of synthesis of peptides with the known structure. Methods of protection and activation of the functional groups.

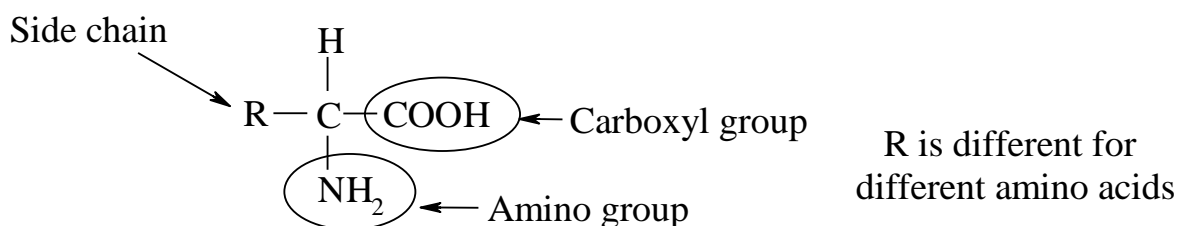
## ***Theoretical questions***

1. Composition, structure and classification of amino acids.
2. Chemical properties of amino acids.

3. Biologically important chemical reactions.
4. Qualitative and quantitative reactions for amino acids.
5. Amino acids metabolism.
6. Proteins as biopolymers.
7. Classification of proteins.
8. Structure of proteins.
9. Forces that stabilize protein structures.
10. Analytical methods for amino acid sequence determination in peptides and proteins.
11. Synthesis of peptides.

### ***1. COMPOSITION, STRUCTURE AND CLASSIFICATION OF AMINO ACIDS***

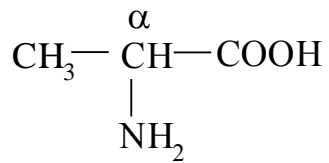
Amino acids are organic compounds containing both an amino group and carboxylic group. They are represented by the general formula:



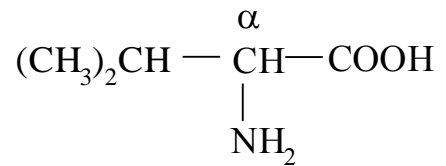
The amino group may be attached to any carbon atom other than that of carboxylic group. They are referred to as  $\alpha$ ,  $\beta$ ,  $\gamma$  depending upon whether the amino group is present on a  $\alpha$ ,  $\beta$  or  $\gamma$  carbon atom relative to carboxyl group. Nearly all the naturally occurring amino acids are  $\alpha$ -amino acids, i.e. containing amino group on the adjacent carbon atom to carboxylic group. These amino acids are very important because these are the building blocks of proteins.

The currently accepted rational classification for amino acids is based on the polarity of their constituent radicals, i.e. on their ability to react with water. Within this classification scheme, the amino acids are divided into 4 classes:

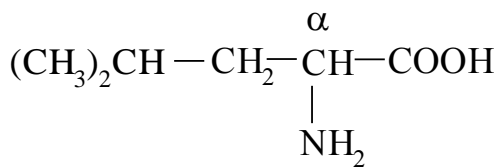
## 1. Nonpolar (hydrophobic):



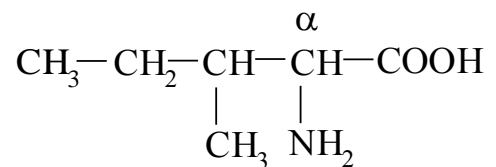
Alanine (Ala)



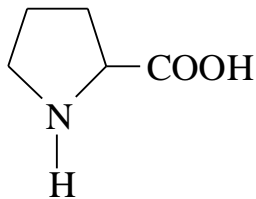
Valine (Val)



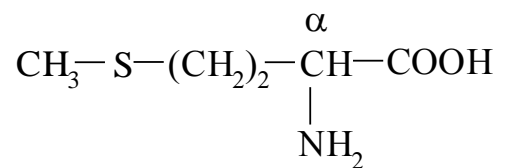
Leucine (Leu)



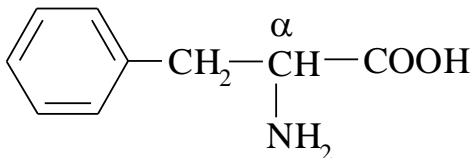
Isoleucine (Ile)



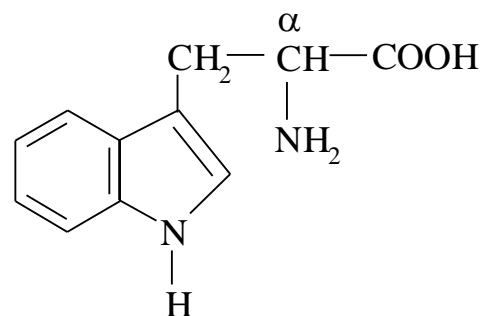
Proline (Pro)



Methionine (Met)

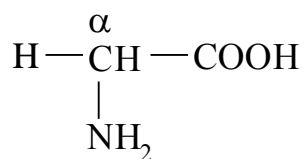


Phenylalanine (Phe)

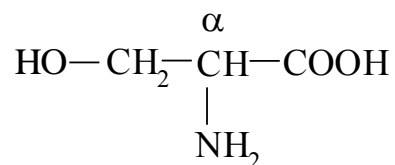


Tryptophan (Trp)

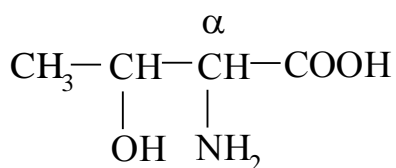
## 2. Polar non-charged (hydrophilic):



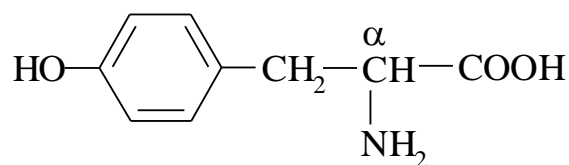
Glycine (Gly)



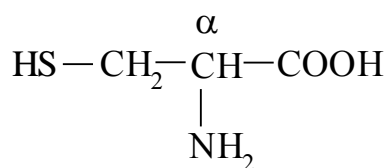
Serine (Ser)



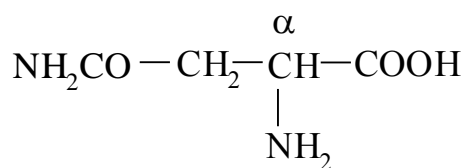
Threonine (Thr)



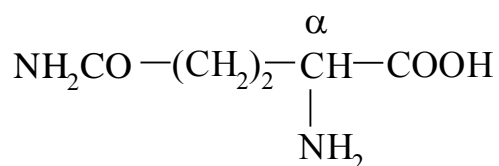
Tyrosine (Tyr)



Cysteine (Cys)

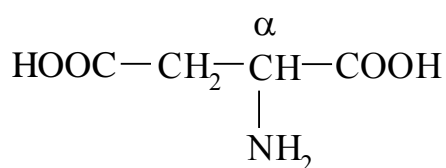


Asparagine (Asn)

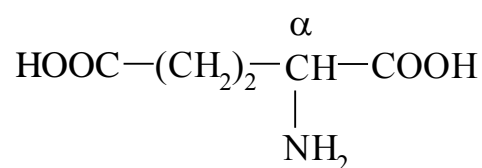


Glutamine  
(Gln)

## 3. Polar negatively charged:

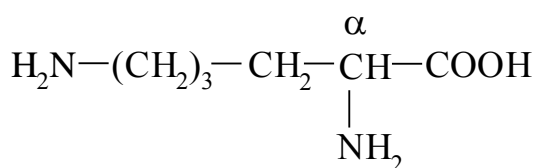


Aspartic acid (Asp)

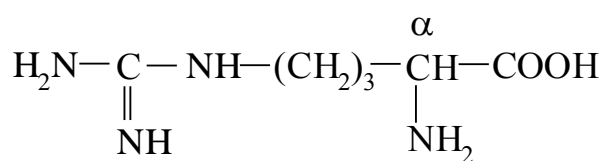


Glutamic acid (Glu)

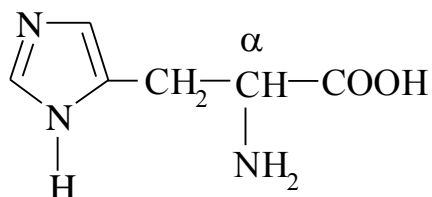
#### 4. Polar positively charged:



Lysine (Lys)

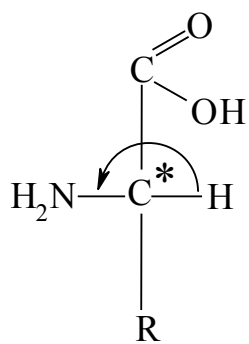


Arginine (Arg)

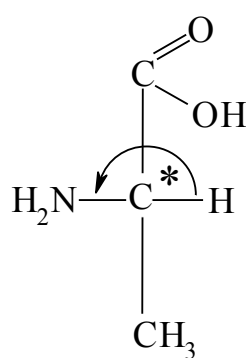


Histidine (His)

With the exception of glycine, all other  $\alpha$ -amino acids have chiral carbon atom and have two optically active isomers. However, all naturally occurring amino acids belong to L-series.



L-amino acid



L-Alanine

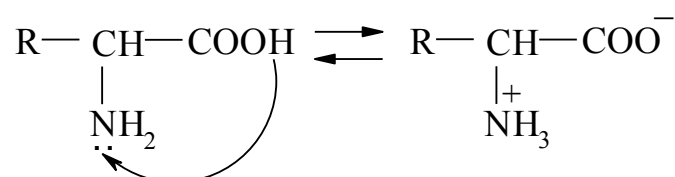
Certain amino acids can be made by human bodies and therefore, people do not require them in their diet. These are called ***non-essential amino acids***. The human body can synthesize 12 out of 20 amino acids found in proteins. Therefore, other must be supplied to our diet and these are called ***essential amino acids***. Essential amino acids are: *Valine, Leucine, Isoleucine, Threonine, Lysine, Methionine, Phenylalanine, Tryptophan*.

Although amino acids can be named according to IUPAC system, they are generally known by their common names. For the sake of simplicity, each amino acid has

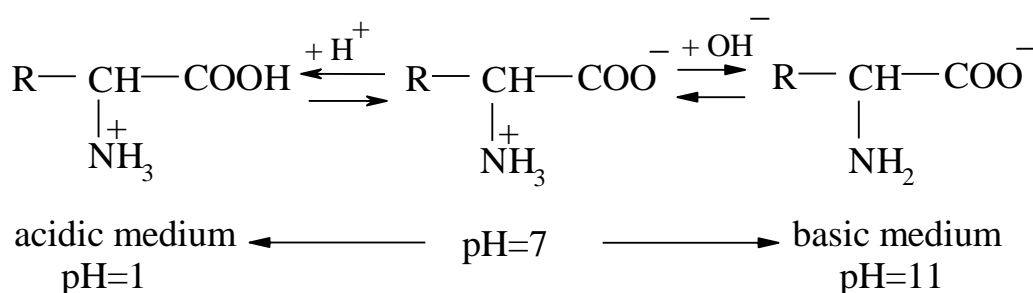
been given an abbreviation, which generally consists of the first three letters of the common name. For example, glycine may be abbreviated as *Gly*, alanine may be represented as *Ala*.

There are two functional groups,  $-\text{NH}_2$  and  $-\text{COOH}$ , each of which is responsible for typical set of reactions.

The amino acids exist as dipolar ion called a **zwitterion**. It has positive as well as negative ends within the same molecule. In the formation of zwitterion, the proton goes from the carboxyl group to the amino group. The zwitterion structure of  $\alpha$ -amino acid may be written as:



The dipolar structure is also called **internal salt**. The pH at which amino acid exists in the form of zwitterion is called **isoelectric point**. It differs for different amino acids. If pH in solution is less than pI, amino acid exists in the form of cation. If pH is greater than pI then it is in the form of anion.



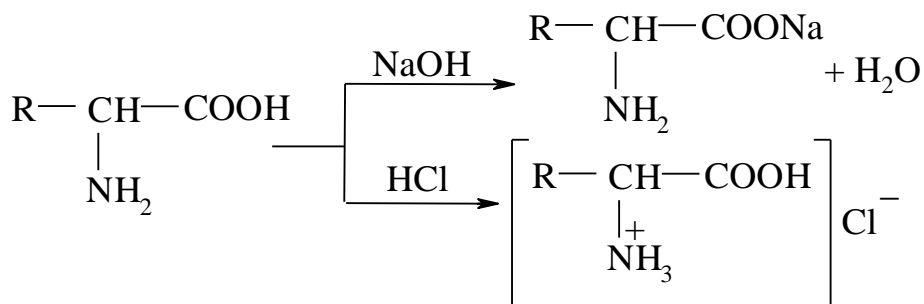
Since amino acids can accept both  $\text{H}^+$  ions and  $\text{OH}^-$  ions, they possess a buffering action. When being in the form of zwitterion, amino acids are slightly soluble in water and precipitate, in the organism amino acids exist as anions or cations.

*In electrophoresis, an amino acid will move towards the cathode or the anode, depending on the pH. This behavior is the basis of the electrophoretic method of separating amino acids according to their isoelectric points. Another method of separating amino acids is chromatography. The positions of the colorless amino acids on the chromatogram are revealed by spraying the paper with ninhydrin. This reagent gives a blue-violet color with amino acids.*

## 2. CHEMICAL PROPERTIES OF AMINO ACIDS

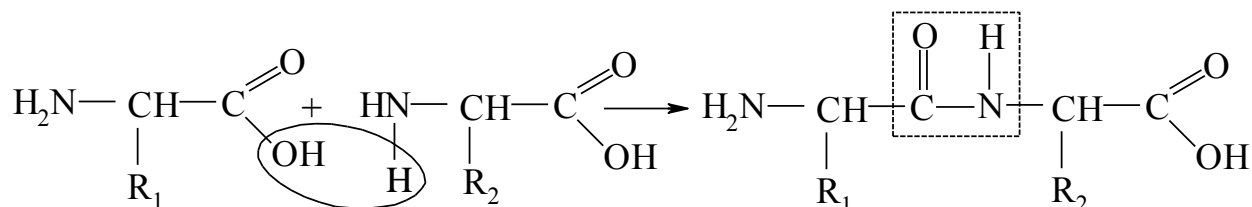
Amino acids possess all chemical properties typical for carboxyl group and amino group as well as the new properties typical only for amino acids.

**1. Amphoteric character** of amino acids is caused by the presence of functional groups of acidic and basic nature. That's why amino acids form salts both with bases and acids.

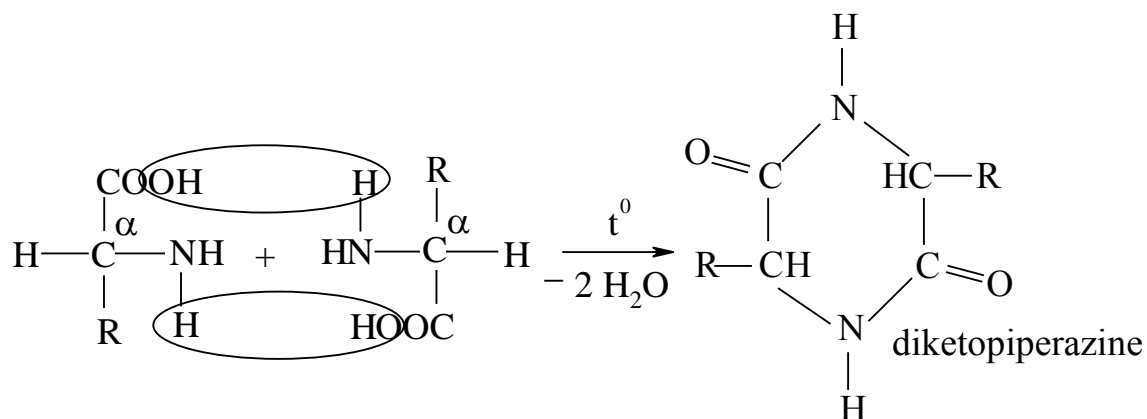


**2. Specific reactions of  $\alpha$ ,  $\beta$  and  $\gamma$  amino acids.** One amino acid can interact with another, as they are heterofunctional compounds. But for all that one of amino acids will conduct itself as an acid and the other one – as amine.

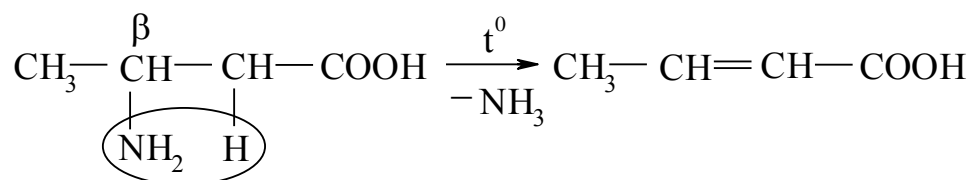
a) Elimination of one water molecule from two molecules of amino acids is typical for  $\alpha$ -amino acids. As a result a dipeptide is formed.



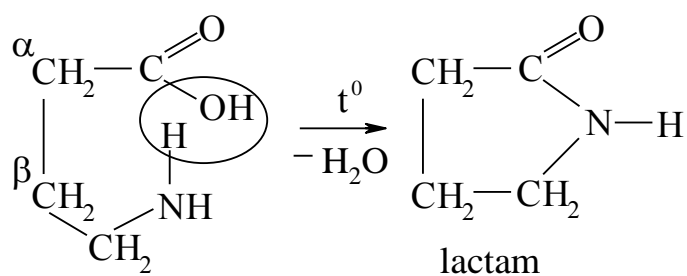
b) Elimination of two water molecules from two molecules of  $\alpha$ -amino acids is possible. The cyclic product – diketopiperazine (cyclic amide) is formed.



c) Elimination of ammonia from one molecule of amino acid is typical for  $\beta$ -amino acids. As a result an unsaturated carboxylic acid is formed.

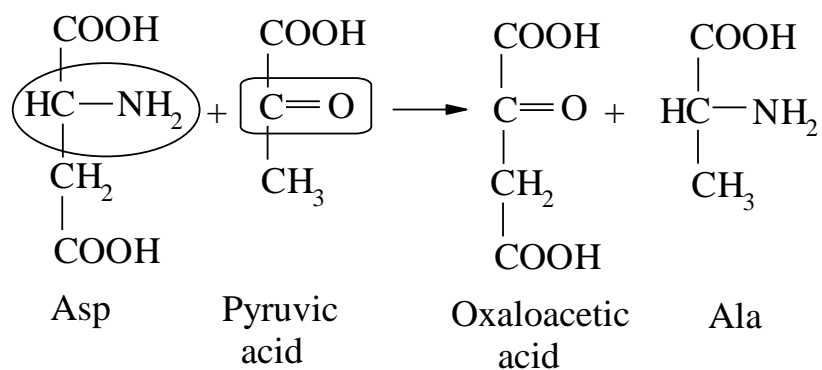


d) In the case of  $\gamma$ -amino acids intramolecular cyclization occurs and one molecule of water is eliminated from one molecule of amino acid. As a result a lactam (cyclic amide) is formed.



### 3. BIOLOGICALLY IMPORTANT CHEMICAL REACTIONS

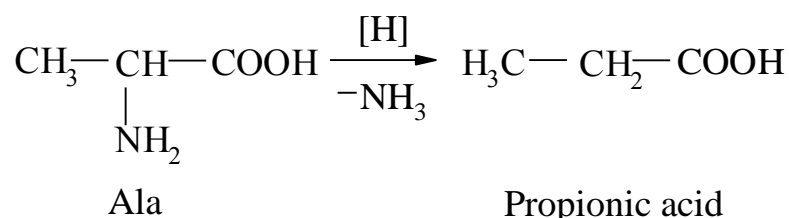
1. *Transamination.* Transamination – is the main way of biosynthesis of  $\alpha$ -amino acids from  $\alpha$ -oxo acids. The donor of amino group is  $\alpha$ -amino acid, the content of which in a cell is high, the acceptor is  $\alpha$ -oxo acid. In this case  $\alpha$ -amino acid turns into  $\alpha$ -oxo acid while  $\alpha$ -oxo acid turns into a corresponding  $\alpha$ -amino acid. The process takes place in the presence of enzymes – transaminases and coenzyme – pyridoxal phosphate (vit B<sub>6</sub>).



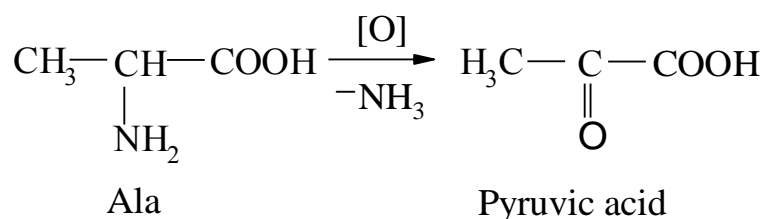
2. *Deamination.* As a result of deamination acids and ammonia are formed in the organism. There are several ways of deamination:

a) The process of reductive deamination takes place in the intestines, where pH is basic.

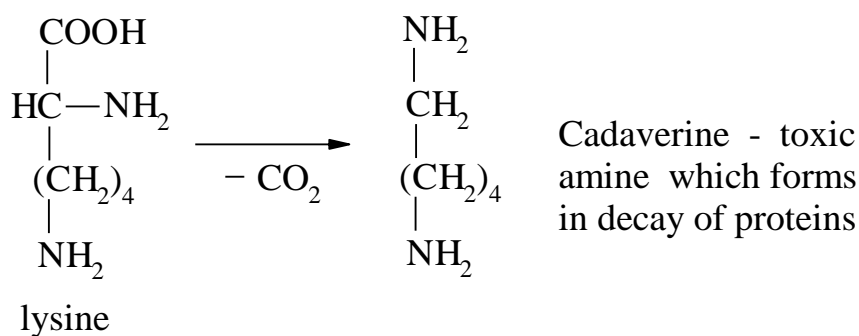




b) The process of oxidative deamination takes place in the tissues of the organism with the participation of dehydrogenase enzymes and  $\text{NAD}^+$  coenzyme. As a result  $\alpha$ -keto acid and ammonia are formed.



3. *Decarboxylation.*  $\alpha$ -amino acids contain electron-accepting amino group in  $\alpha$ -position, hence they are easily decarboxylized. The process of decarboxylation takes place in the organism under the influence of enzymes called decarboxylases and pyridoxal phosphate coenzyme (vitamin  $\text{B}_6$ ). As a result biogenic amines, e.g. histamine from histidine, serotonin from tryptophan, carrying out important biological functions are formed.

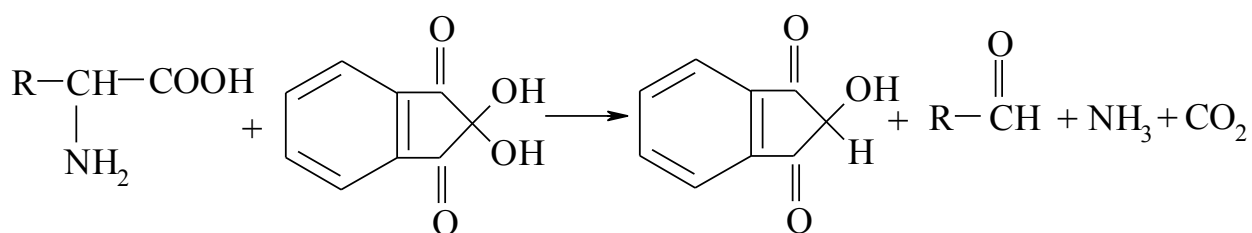


#### 4. QUALITATIVE AND QUANTITATIVE REACTIONS FOR AMINO ACIDS

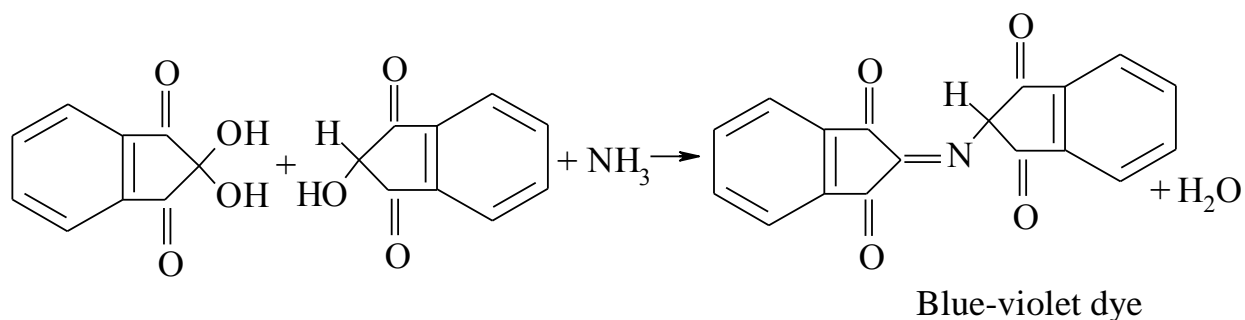
There are some color reactions commonly employed for identification of both individual amino acids and those constitutive of proteins. These reactions are specific of the chemical nature of amino acid side-chain radicals.

1. *Ninhydrin reaction* is characteristic for  $\alpha$ -amino acids. At the first stage of this reaction, a reduced ninhydrin is formed by oxidative deamination of amino acids

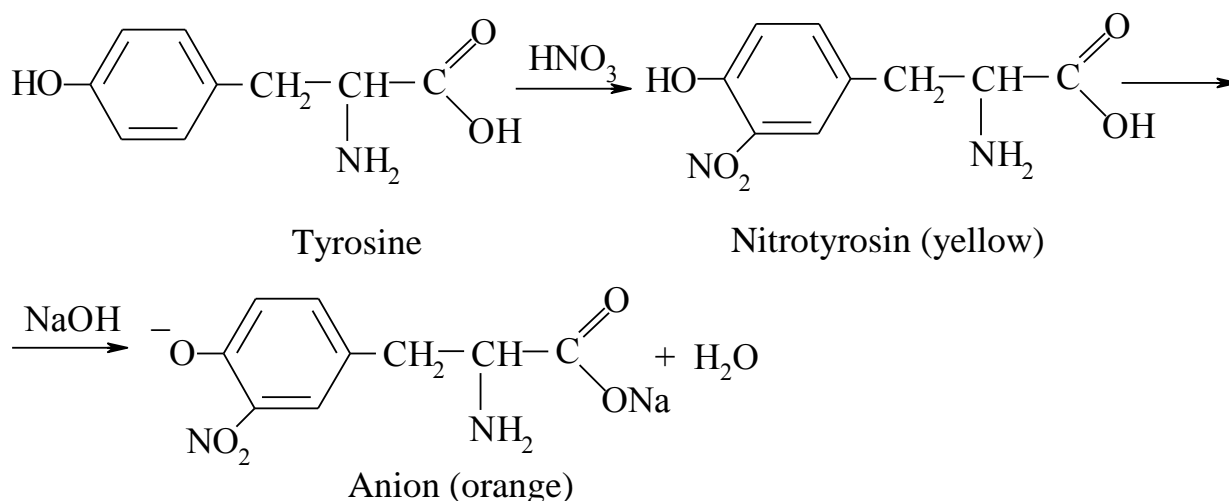
(simultaneously, the amino acid decarboxylation takes place):



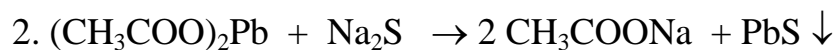
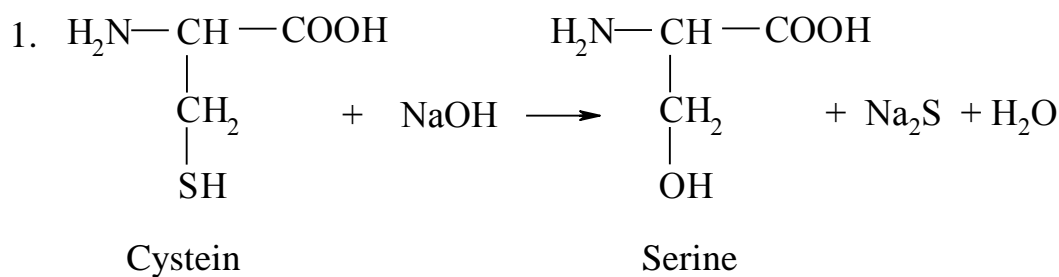
At the second stage, the ammonia produced by deamination reacts with equimolar quantities of oxidized and reduced ninhydrin to yield a blue-violet dye.



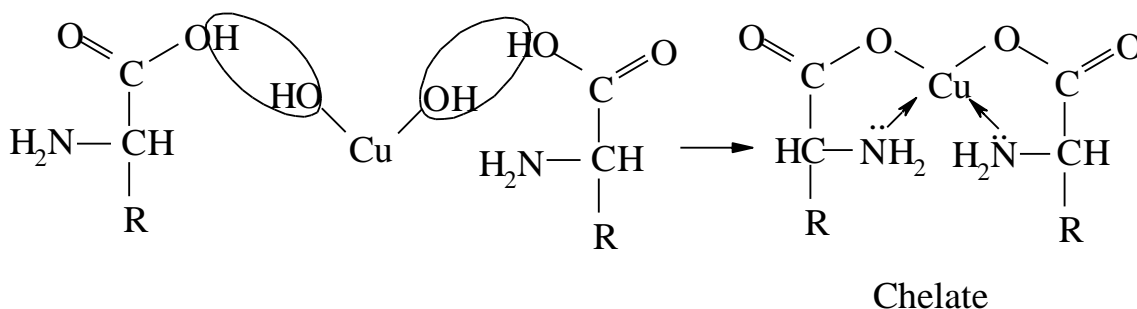
2. *Xanthoprotein reaction* is characteristic for aromatic amino acids – phenylalanine, tyrosine, histidine, tryptophan. These amino acids give yellow coloration when heated with concentrated nitric acid. In basic medium the coloration becomes orange due to formation of quinoid structure salt.



3. *Fol's reaction* is characteristic for sulfur containing amino acids. When sulfur containing amino acids - cystein, cystin, methionine, and proteins containing these amino acids are heated with lead acetate in alkaline medium the black ppt of lead sulfide forms.

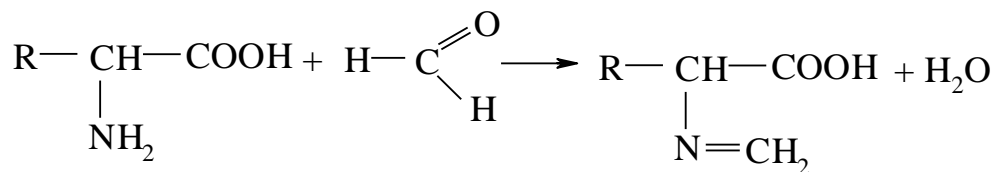


4.  $\alpha$ -amino acids give blue coloration with copper hydroxide due to the formation of complex salt (chelate).



For **quantitative** determination of amino acids following methods are used:

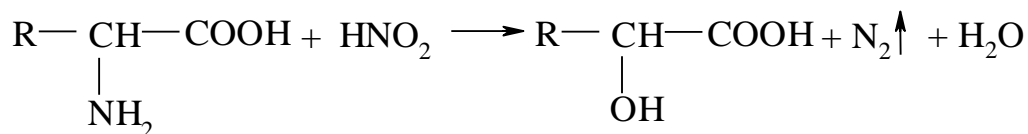
1. Serensen method (formol titration):



Basic amino group is neutralized, and the amino acid content can be determined by means of carboxyl groups titration.

2. Van Slyke method:

The method is based on deamination by nitrous acid. Amino acid is transformed into hydroxy acid and gaseous nitrogen is liberated. The initial quantity of amino acid can be determined according to the volume of nitrogen evolved.

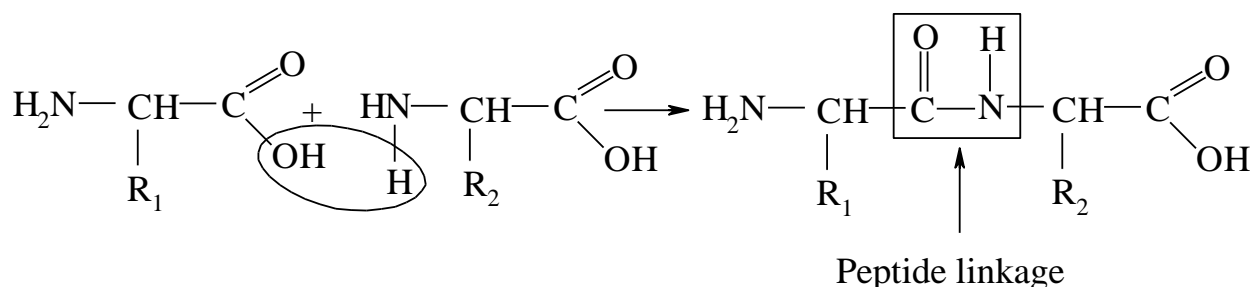


## 5. AMINO ACIDS METABOLISM

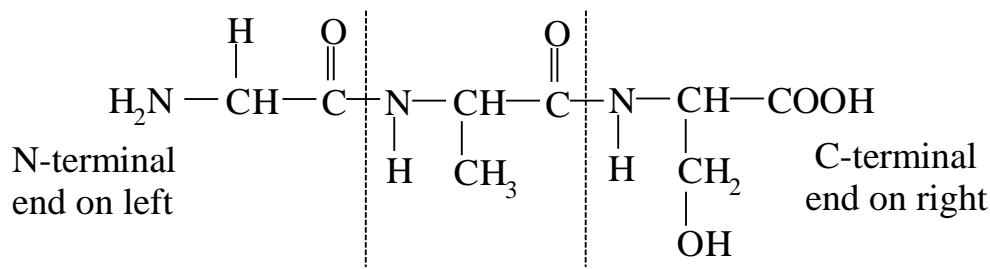
Amino acids are present in intestine and are absorbed into blood and have many uses in the body. They are the source of the nitrogen and used in the formation of new cells or repair of old cells, synthesis of the other amino acids, enzymes, hormones, antibodies and non-protein molecules such as nucleic acids. Unlike carbohydrates, there is no storage of amino acids. Some amino acids are metabolized to pyruvate, some to acetyl-CoA and others to various intermediates in glucose metabolism. Energy is obtained by their breakdown into carbon dioxide and water. All these reactions are catalyzed by enzymes and the energy released during these oxidation reactions is conserved in the form of ATP.

## 6. PROTEINS AS BIOPOLYMERS

Peptides are compounds formed by the condensation of two or more same or different  $\alpha$ -amino acids. The carboxyl group of one amino acid and amino group of another amino acid get condensed with the elimination of water molecule. The resulting linkage is called a *peptide linkage*.



If a large number of  $\alpha$ -amino acids (hundreds to thousands) are joined by peptide bonds, the resulting polyamide is called polypeptide. 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*. The structure is generally written with N-terminal end to the left and C-terminal end to the right. The name of the peptide is written from the names of the amino acids as they appear from left to right starting from N-terminal amino acid. For example, the tripeptide formed by glycine, alanine and serine is written as:



Glycylalanylserine (Gly-Ala-Ser)

For the sake of convenience, the names of amino acids are generally abbreviated.

All kinds of organisms contain peptides. They act as antibiotics, toxins, and hormones. Depending upon the number of amino acids residues per molecule, the peptides are called dipeptide, tripeptide, polypeptide etc. The formation of peptide bonds can continue until a molecule containing several hundred thousands amino acids is formed. Such a molecule is called polypeptide or protein. By convention a peptide having molecular weight up to 10000 is called polypeptide while a peptide having a molecular weight more than 10000 is called a protein.

## 7. CLASSIFICATION OF PROTEINS

1. Classification of proteins on the basis of molecular structure. Proteins can be classified into two broad classes on the basis of molecular structure as:

a) fibrous proteins; b) globular proteins

a) *Fibrous proteins*. These types of proteins consist of linear thread like molecules, which tend to lie side by side to form fibres. The molecules are held together at many points by hydrogen bonds. These are usually insoluble in water. The common examples are keratin, in skin, hair, nails and wool, collagen in tendons, fibroin in silk, myosin in muscle, etc. These proteins serve as the main structural materials of animal tissues.

b) *Globular proteins*, in this type of proteins, the molecules are folded together into compact units forming spheroidal shapes. The peptide chains in globular proteins are also held by hydrogen bonds but these forces are comparatively weak. These are soluble in water. Examples are enzymes, hormones etc.

2. Classification of proteins on the basis of hydrolysis products. Based on the type of products formed on hydrolysis, the proteins may be classified as:

a) Simple proteins; b) Conjugated proteins

a) *Simple proteins* give amino acids only on hydrolysis with acids or enzymes.

Examples are: albumins, globulins, keratin etc.

b) *Conjugated proteins* on hydrolysis give a non-protein part and  $\alpha$ -amino acids. Thus, these are formed by the combination of simple proteins with some non-proteins substance. The non-proteins part is called **prosthetic group** and it controls the biological functions of the protein. For example, nucleo proteins contain nucleic acids as prosthetic group, glyco proteins contain sugars (carbohydrates), lipo proteins contain lipids such as lecithin, phospho proteins contain phosphoric acid residues, chromo proteins contain pigment having some metals such as Fe, Cu (hemoglobin).

## 8. STRUCTURE OF PROTEINS

The structure of proteins may be discussed as follows:

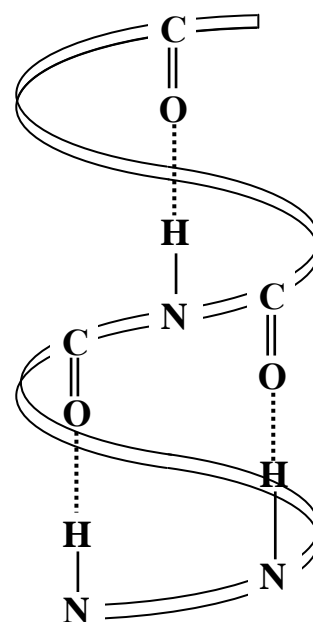
**1. Primary structure.** The sequence in which the amino acids are linked in polypeptide chain is called the primary structure of protein. The amino acid sequence of a protein determines its function and is critical to its biological activity. Frederic Sangar determined the primary structure of a protein (insulin) for the first time in 1953.

The importance of a primary structure of a protein lies in the fact that even a change of one amino acid can change drastically the properties of the entire protein. For example, normal hemoglobin has 574 amino acid units changing just one amino acid in the sequence results in defective hemoglobin found in patients suffering from *sickle cell anemia*. In these patients the defective hemoglobin in red blood cells precipitates causing the cells to sickle and sometimes even burst leading ultimately to the death.

**2. Secondary structure.** The secondary structure gives the manner in which the polypeptide chains are folded or arranged. Therefore, it gives the shape or conformation of the protein molecule. This arises from the plane geometry of the peptide bond and hydrogen bonding between the C=O and N-H groups of different peptide bonds.

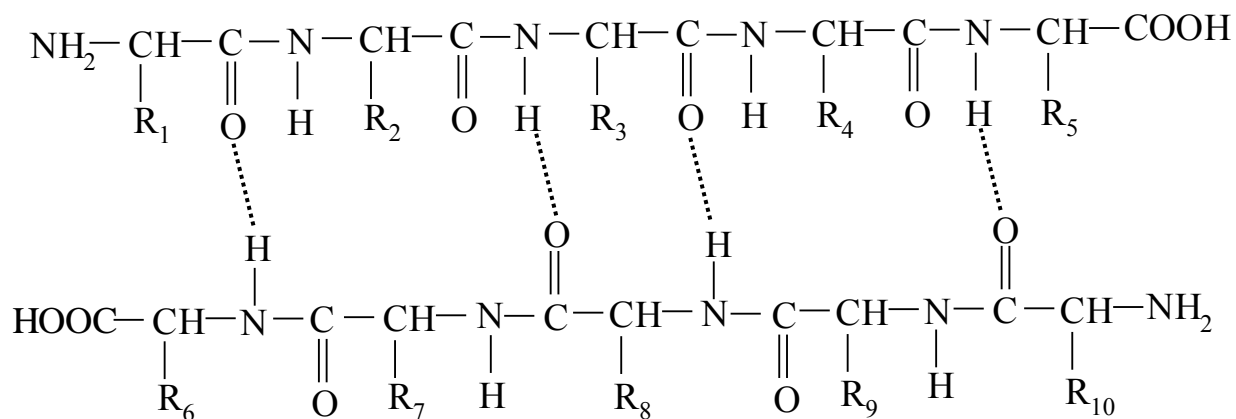
Pauling and Corey investigated the structure of many proteins with the help of X-rays patterns. It was observed that there are two common types of structures:

a)  **$\alpha$ -Helix structure.** In this structure, formation of hydrogen bonding between amide groups within the same chain causes the peptide chains to coil up into a spiral structure. This is called  $\alpha$ -helix. This structure can be imagined as if one can coil a polypeptide chain around an invisible cylinder.  $\alpha$ -helix



structure is found in many proteins such as myosin (found in muscles), keratin (hair, wool, nails).

b) ***β-pleated sheet structure.*** In this structure, the long peptide chains lie side by side in a zigzag manner to form a flat sheet. Each chain is held to the two neighboring chains by hydrogen bonds. These sheets are stacked one upon another to form a three dimensional structure called β-pleated sheet structure. Silk fibroin has this type of structure.



3. ***Tertiary structure.*** The tertiary structure arises due to folding, coiling and bending of polypeptide chains producing three-dimensional structures. This structure gives the overall shape of proteins. The tertiary structure of a protein can be obtained due to folding and superimposition of various secondary structures. The examples of the proteins having tertiary structures are globular proteins.

## 9. FORCES THAT STABILIZE PROTEIN STRUCTURES

The following types of forces stabilize the protein structure:

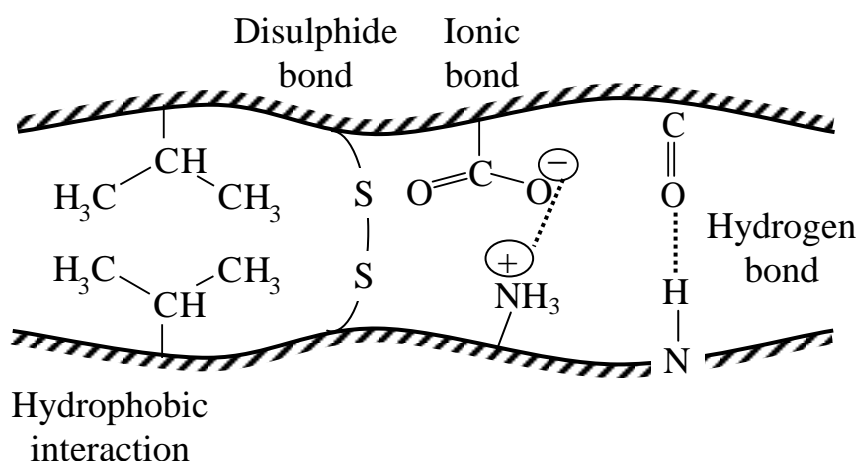
1. ***Hydrogen bonding.*** These are weak forces and arise between partially positive hydrogen and a partially negative atom such as oxygen or nitrogen on the same or different molecule.

2. ***Ionic bonding.*** Ionic bonding can take place between anionic and cationic side chains resulting side chain cross linking.

3. ***Covalent bonding.*** The most common form of inter-chain bonding is the disulfide bond formed between the sulfur atoms of two cysteine residues. The insulin consists of two polypeptide chains linked together by covalent bonding.

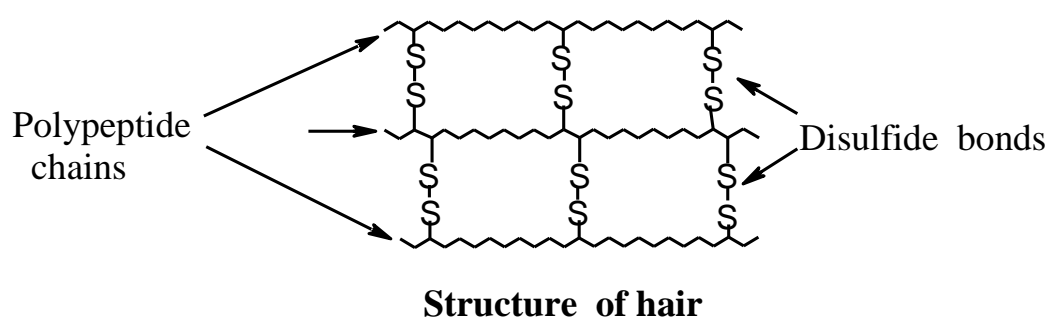
4. ***Hydrophobic bonding.*** Many amino acid residues have hydrophobic side chains. Proteins in aqueous solutions fold so that most of the hydrophobic chains become clustered inside the folds. The polar side chains, which are hydrophilic, lie on

the outside or the surface of the protein.



### *The structure of hair*

Hair is made of fibrous protein (keratin). It contains a large number of amino acid cysteine. Oxidation of cysteine residues results in formation of disulfide bonds linking the polypeptide chains. These bonds determine a unique form of human hair (curly, straight). Disulfide bonds can be destroyed by means of reducing agents. After action of reducing agent it is possible to give a different shape to the hair. This new shape can be fixed by action of oxidizing agent. New disulfide bonds that give the hair the desired shape will be formed.



## **10. ANALYTICAL METHODS FOR AMINO ACID SEQUENCE DETERMINATION IN PEPTIDES AND PROTEINS**

When a protein or polypeptide is refluxed with 6M 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

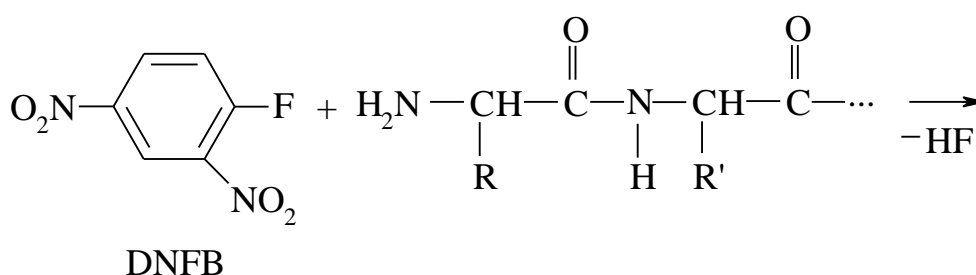


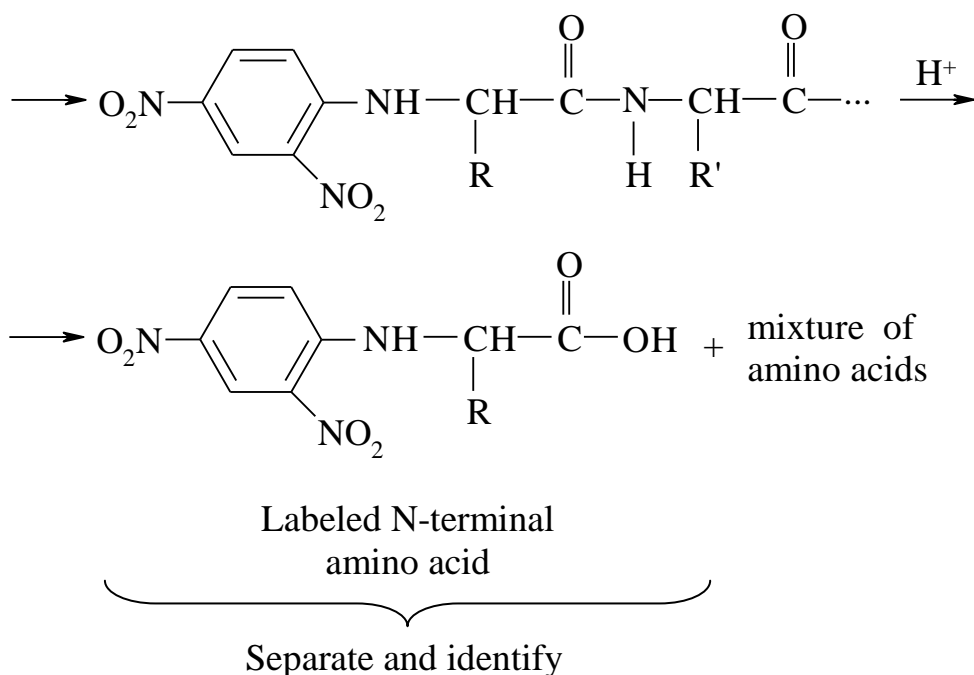
the resulting mixture can then be used to determine which amino acids comprised the intact polypeptide and their relative amounts.

One chromatographic method for separation of a mixture of amino acids is based on the use of cation-exchange resins, which are insoluble polymers containing sulfonate groups. If an acidic solution containing a mixture of amino acids is passed through a column packed with a cation-exchange resin, the amino acids will be absorbed by the resin because of attractive forces between the negatively charged sulfonate groups  $-\text{SO}_3$  and the positively charged amino acids (in acidic medium amino acids are positively charged). If the column is then washed with a buffered solution at a given pH, the individual amino acids move down the column at different rates and ultimately become separated.

There are a variety of methods available to determine the sequence of amino acids in a polypeptide. The most widely used procedure for identifying the N-terminal amino acid in a peptide is the **Edman degradation** method. The machines called amino acid sequencers have been developed to carry out the Edman degradation process in automated cycle. The chemistry of the Edman degradation is based on a labeling reaction between the N-terminal amino group and phenyl isothiocyanate,  $\text{C}_6\text{H}_5\text{N}=\text{C}=\text{S}$ . Phenyl isothiocyanate reacts with the N-terminal amino group to form a phenylthiocarbonyl derivative, which is then cleaved from the peptide chain by acid. The result derivative is introduced directly to a high-performance liquid chromatograph (HPLC) and identified by comparison of its retention time with known amino acid. The cycle is then repeated for the next N-terminal amino acid.

Another method for N-terminal sequence analysis is the **Sanger method**, based on the use of 2,4-dinitrofluorobenzene (DNFB). DNFB reacts with  $\text{NH}_2$ -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.





## 11. SYNTHESIS OF PEPTIDES

The structure of many peptides has been decoded, a lot of biologically active peptides can be synthesized nowadays. The first synthesized peptides were hormones oxytocin, vasopressin and insulin. The complexity of peptide synthesis is connected with the necessity to provide the strictly defined sequence of amino acids. As amino acids are heterofunctional compounds even in the simplest case of dipeptide synthesis from, for example, *Ala* and *Val*, one can obtain 4 variations of dipeptide *Ala-Ala*, *Val-Val*, *Ala-Val*, *Val-Ala*.

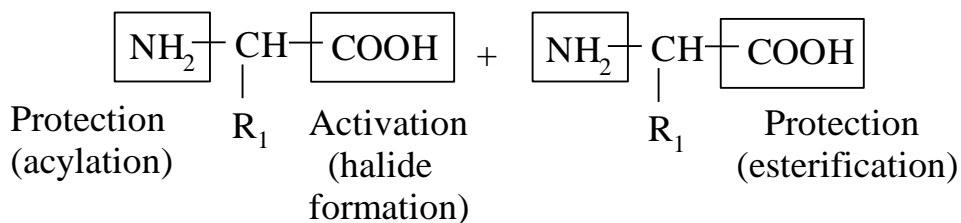
6 variations of tripeptide can be obtained from 3 amino acids, 20 variations of tetrapeptide - from 4 amino acids,  $10^{14}$  variations from 20 amino acids, etc., i.e. almost endless number of combinations can be formed. This makes possible a large variety of peptides and proteins.

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).

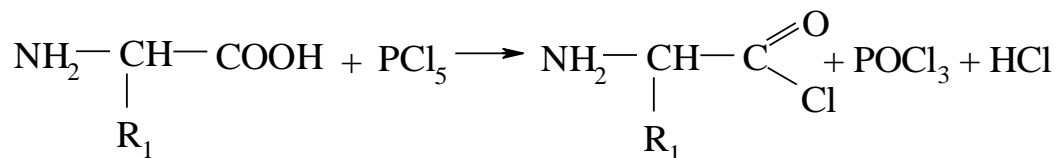
Functional groups forming peptide bond are to be active (carboxyl group of the first amino acid and amino group of the second one). Carboxyl group of the first amino acid should be activated by changing into halide.

Amino group of the first amino acid should be protected by acylation and carboxyl group of the second amino acid - by esterification.

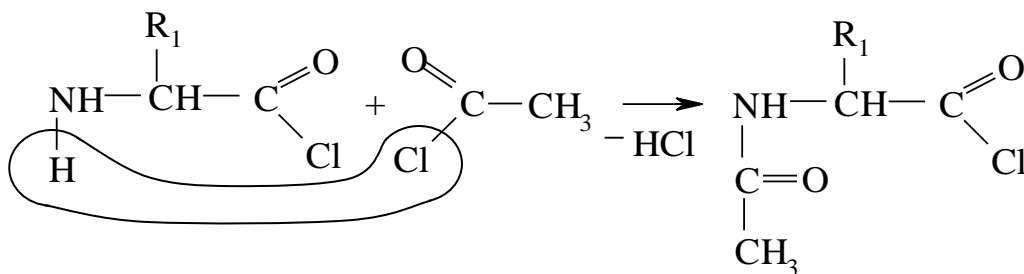
After a dipeptide has already been formed a protection is to be removed by hydrolysis.



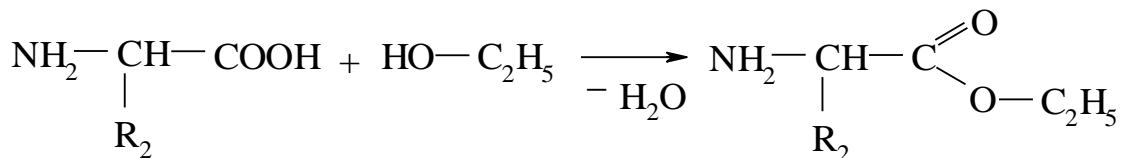
1. Activation of -COOH group:



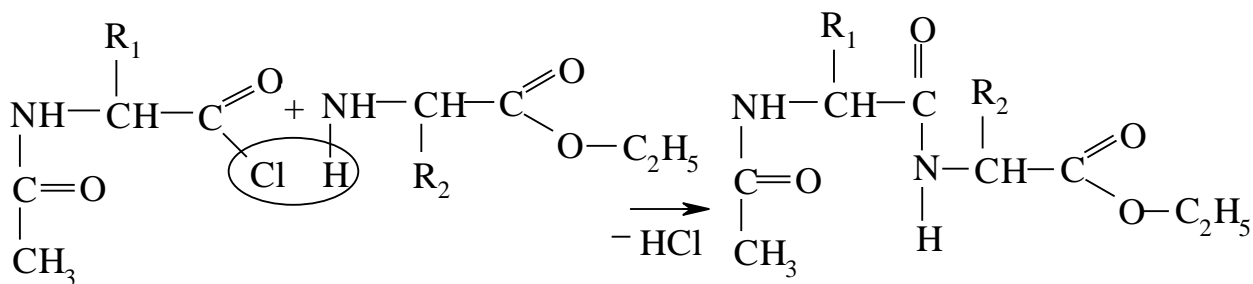
2. Protection of -NH<sub>2</sub> group:



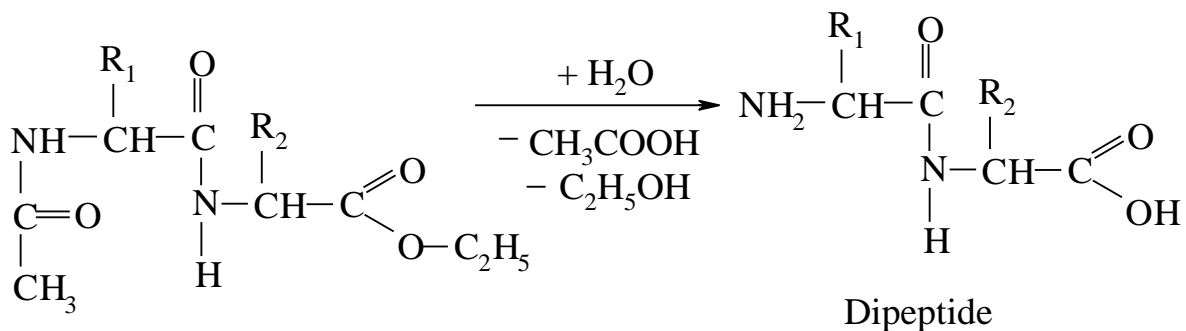
3. Protection of -COOH group:



4. Dipeptide formation:



5. Protection removing:



Revision exercises

- In aqueous solution at  $\text{pH} \approx 7$  monobasic monoamino acid exists as  
a) Anion b) Cation c) Zwitterions d) Non polar form
- Which of the following is essential amino acid?  
a) Alanine b) Aspartic acid c) Serine d) Tryptophan
- The main bonds which stabilize secondary structure of proteins are:  
a) Peptide bonds b) Hydrogen bonds c) Ionic bonds d) Hydrophobic bonds

Answers

- c)
- d)
- b)

\*\*\*

# HETEROCYCLIC COMPOUNDS. STRUCTURE, PROPERTIES, AND BIOLOGICAL FUNCTIONS OF NUCLEIC ACIDS

## *Characteristics of the subject*

Heterocyclic structures are in the basis of numerous natural and biologically active substances and drugs. Alkylated pyrrole rings are the parent structures of important biologically active compounds, i.e. hem, chlorophyll, vitamin B<sub>12</sub>. Heterocyclic molecules (indol, imidazole, etc.) are included in some essential amino acids: e.g. tryptophan, histidine. A lot of drugs also contain heterocycles: non-narcotic analgesics contain pyrazolon-5, vitamin PP and anti-tuberculosis drugs contain pyridine.

To know the structure and properties of biopolymers (nucleic acids) is necessary to understand the essence of normal processes and pathology, the origin of hereditary diseases and problems of vital activity regulation.

## *Purpose*

To learn the structure and chemical behavior of biologically active heterocyclic compounds.

To study the structure of nucleic acids, important structural elements of the cell at the level of their primary and secondary structure.

## *Objectives*

1. To study the structure, classification and chemical properties of biologically active heterocycles.
2. To be able to explain the dependence between reactivity and structure for heterocyclic compounds.
3. To explain the role of heterocycles in the composition of vitamins, hormones, co-enzymes, and also different drugs.
4. To know the composition and structure of the component of nucleic acids: ribose, deoxyribose as well as pyrimidine and purine bases.
5. To be able to write formulas of nucleosides and nucleotides.
6. To know the structure and composition of the most important nucleotides (ATP, NAD<sup>+</sup>, NADP<sup>+</sup>) and to understand their biological role.
7. To know the linear and spatial structure and biological role of nucleic acids.

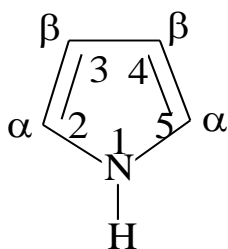
### ***Theoretical questions***

1. Five-membered heterocycles.
2. Six-membered and conjugated heterocycles.
3. Alkaloids.
4. Nucleosides, Nucleotides, and Nucleic acids.

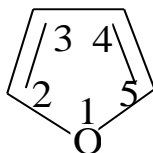
### ***1. FIVE-MEMBERED HETEROCYCLES***

In molecules of many cyclic compounds an element other than carbon is present in the ring. These compounds are called ***heterocyclic compounds***. Heterocyclic molecules are quite commonly encountered in nature. Heterocycles are classified according to the dimension of the cycle, number and nature of heteroatoms, degree of saturation. Compounds with aromatic rings occupy numerous and important positions in reactions that occur in living systems. ***Five and six-membered*** heterocyclic aromatic compounds containing ***nitrogen, oxygen, or sulfur*** are by far the most common.

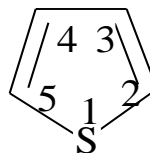
#### ***Five-membered heterocycles containing one heteroatom.***



Pyrrole

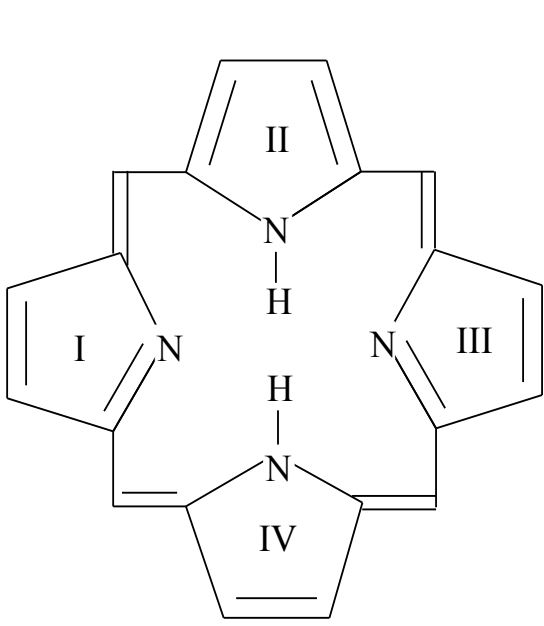


Furan

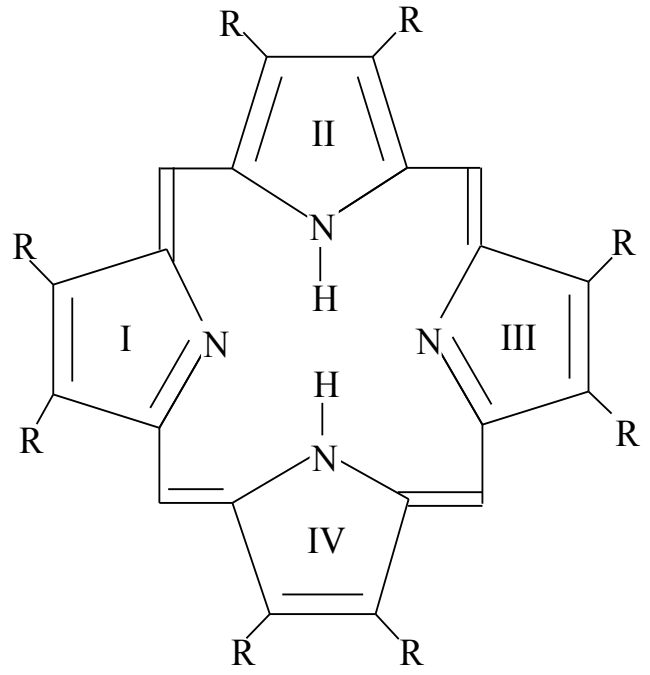


Thiophene

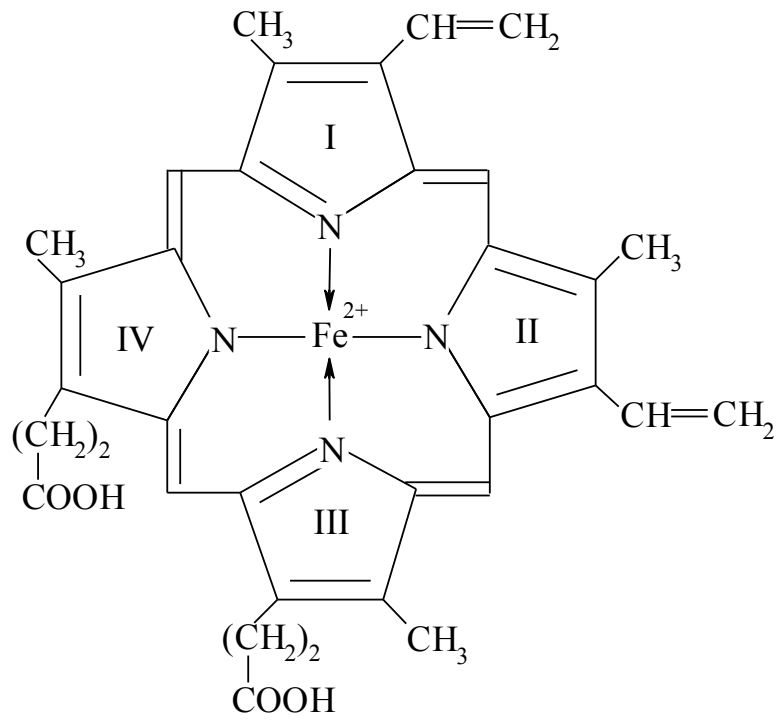
All atoms in the composition of these compounds are  $sp^2$  hybridized. Pyrrole is the most important among five-membered cycles. Four pyrrole rings fused together constitute the flat macro cycle which is a component of porphyrins. Porphyrins are encountered in nature as complexes with metals. Complex of porphyrine with magnesium is in composition of ***chlorophyll***. Complex of porphyrine with iron +2 is called ***heme*** which is prosthetic group of hemoglobin. Porphyrine is also contained in vit B<sub>12</sub>, cytochromes and some other enzymes.



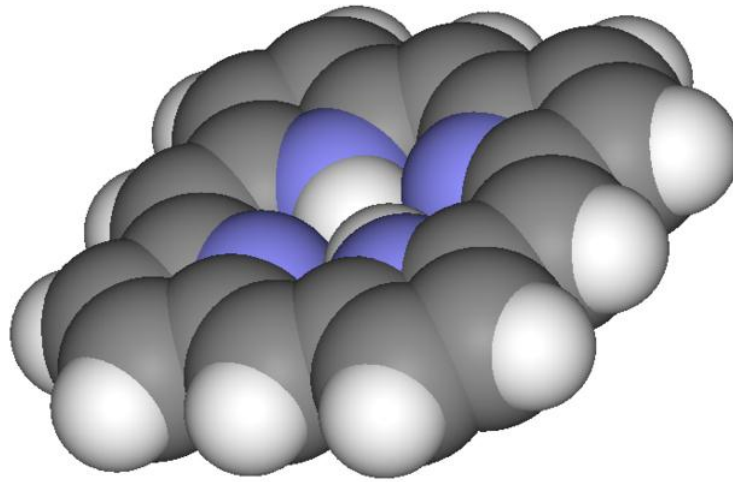
Porphyne



Porphyrine

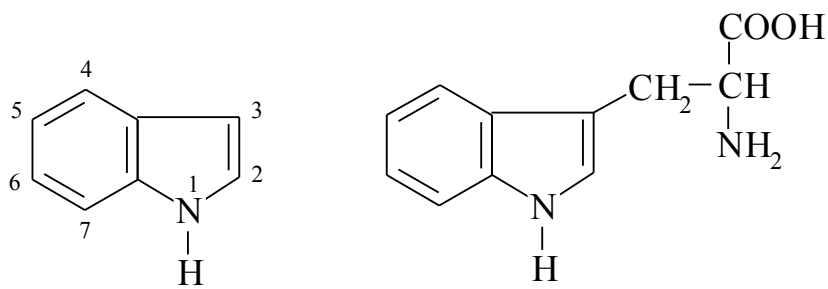


Heme



Space-filling model of porhyne

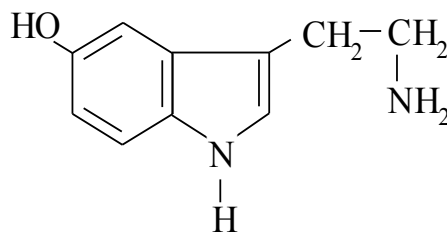
**Indole** contains a benzene ring fused to a pyrrole ring. The main biologically active derivatives of indole are amino acid **tryptophan** and its metabolites.



Indole

Tryptophan

Tryptophan is an essential amino acid. The product of tryptophan hydroxylation and decarboxylation is 5-hydroxytryptamine (serotonin).

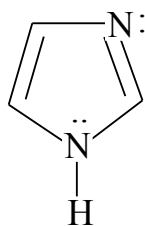


Serotonin

Serotonin is a brain neuromediator. The disturbance of serotonin metabolism results in schizophrenia.

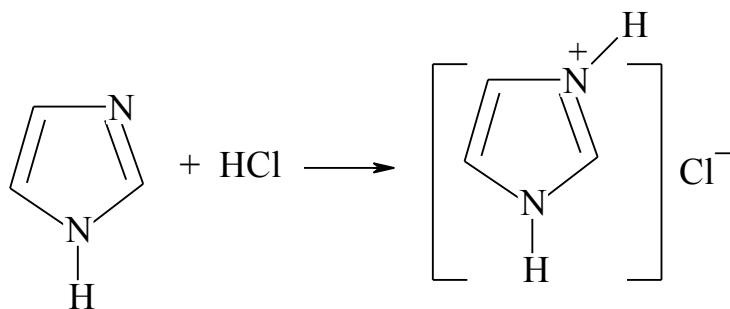


**Five-membered heterocycles containing two heteroatoms.** Imidazole is a five-membered heterocycle containing two nitrogen atoms.

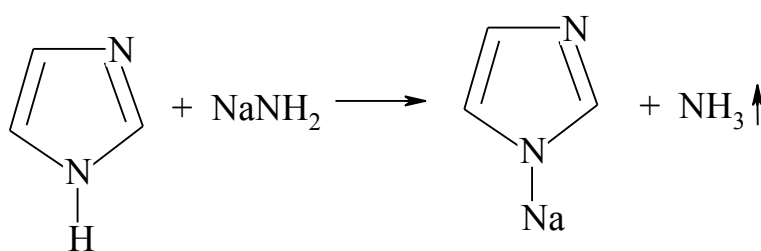


Imidazole

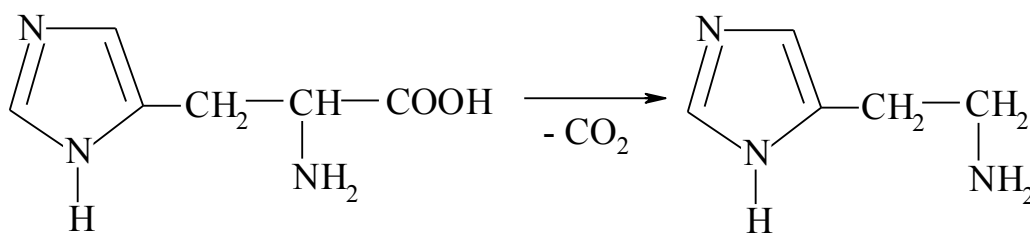
One nitrogen atom in imidazole provides one electron to the conjugated system and, therefore, this is pyridic nitrogen. It imparts basic properties to imidazole. The second one provides two electrons to the conjugated system and is, therefore, pyrrolic nitrogen. This imparts weak acidic properties to imidazole. So, imidazole is amphoteric in nature. The main biologically active derivatives of imidazole are amino acid *histidine* and the product of its decarboxylation *histamine*.



Imidazolium chloride



Sodium imidazole

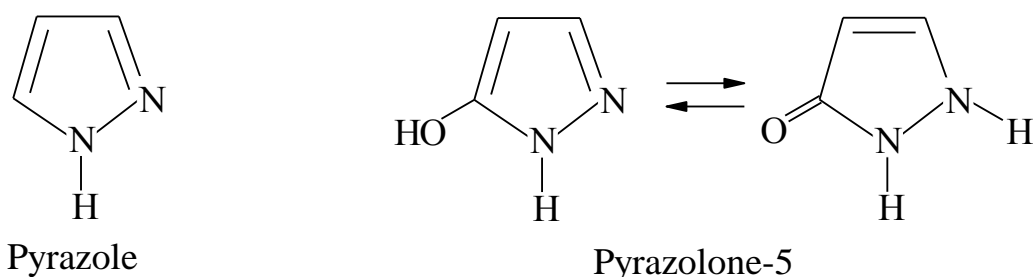


Histidine

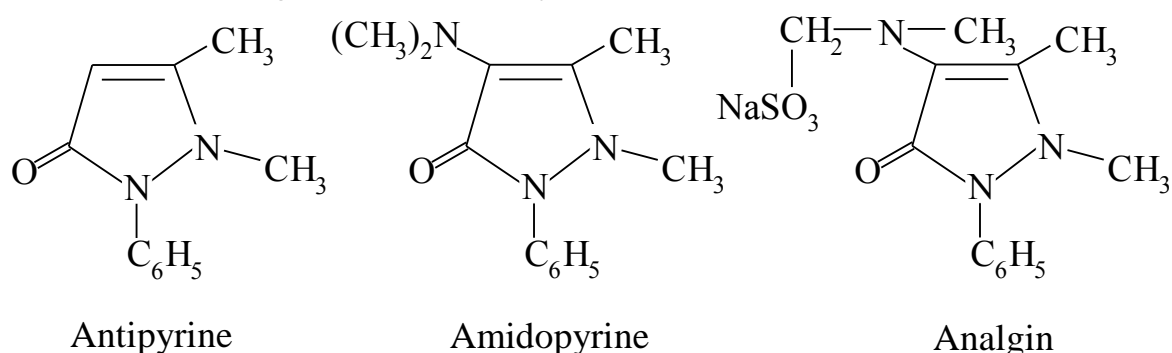
Histamine

Histamine exhibits a wide spectrum of biological action. It acts as a vasodilator. Histamine, by producing a vasodilatory action, facilitates the afflux of leucocytes and activates thereby the defence function of the organism. Histamine is also ascribed the role of an algetic and allergic mediator. Histamine is directly involved in the effects of sensitization and desensitization. The patients with histamine hypersensitivity are prescribed antihistamine preparations (naphthysine, dimedrol, diazolinum), which exert an action on vascular receptors.

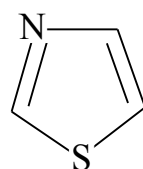
Pyrazole is an isomer of imidazole. Its derivatives are not found in nature. The most common derivative of pyrazole is pyrazolone-5.



Many derivatives of pyrazolone have been prepared and tested for antipyretic as well as for analgesic effects. Among these compounds antipyrine, amidopyrine (pyrimidon), and analgin have been very successful.



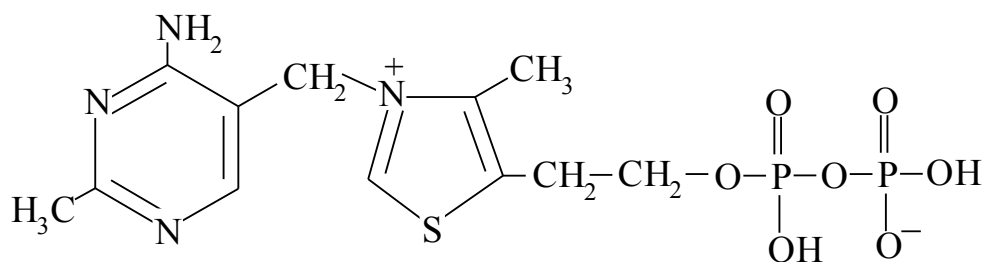
Thiazole is a five-membered heterocycle containing two heteroatoms – nitrogen and sulfur.



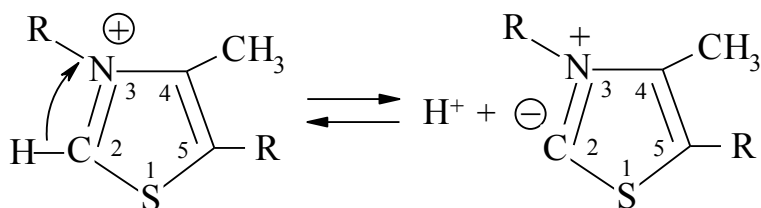
Thiazole

Thiazole is in composition of Vitamine B<sub>1</sub> (thiamine). Chemical structure of thiamine is composed of a pyrimidine ring and a thizole ring linked through a

methylene bridging bond. In the organism Vitamine B<sub>1</sub> is converted into its active form - thiamine pyrophosphate (TPP):

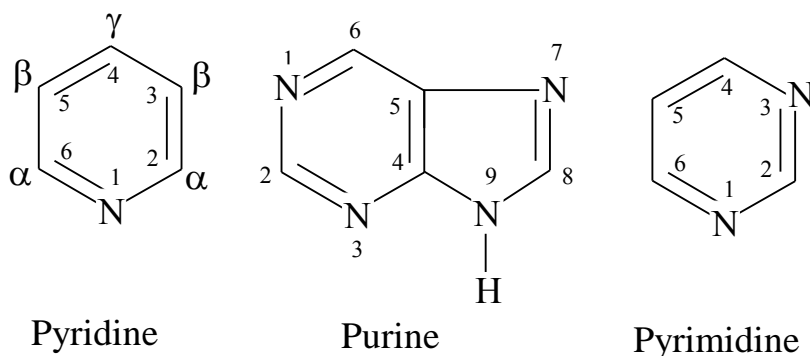


TPP is known to make part of multienzyme systems, pyruvate- and  $\alpha$ -ketoglutarate-dehydrogenase complexes that catalyze the oxidative decarboxylation of pyruvic and  $\alpha$ -ketoglutaric acids. The product of pyruvic acid decarboxylation is acetyl coenzyme A. Conversion of pyruvate to acetyl coenzyme A is a step that makes link between glycolysis and other metabolic pathways. Pyruvate is the end product of glycolysis, and acetyl coenzyme A is the start point for other critical biochemical processes, including Krebs cycle. Reactive site of TPP molecule is second carbon atom in thiamine fragment. Reactivity is due to C-H acidity of this position.

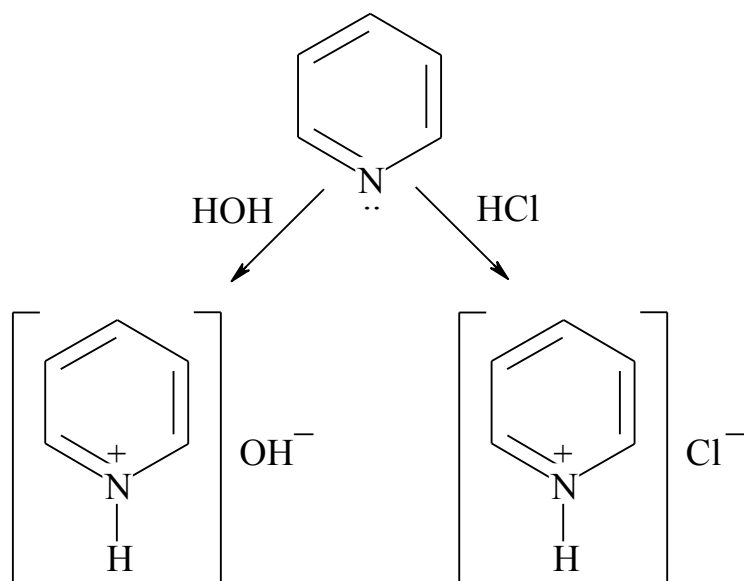


## 2. SIX-MEMBERED AND CONJUGATED HETEROCYCLES

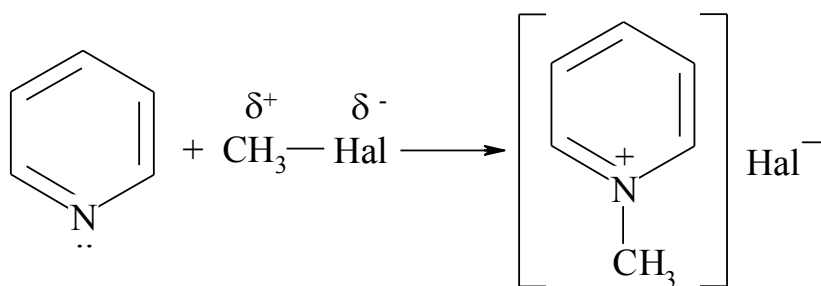
*Six-membered and conjugated nitrogen containing heterocycles* are also present in many biological systems.



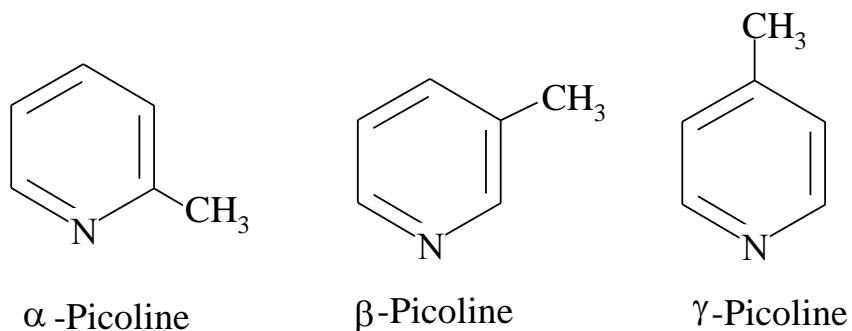
Chemical properties of pyridine are caused by its electronic structure. Due to the presence of unshared electrons on nitrogen atom, pyridine can accept proton i.e. pyridine is basic in nature; it reacts with water and acids.



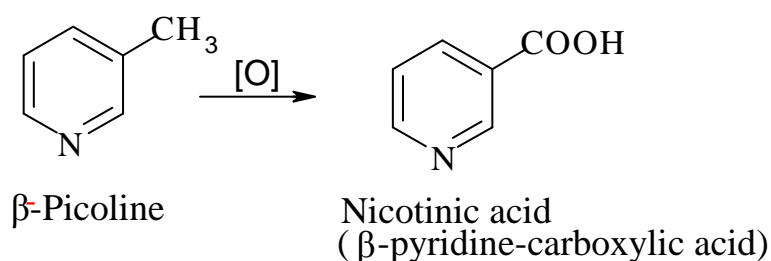
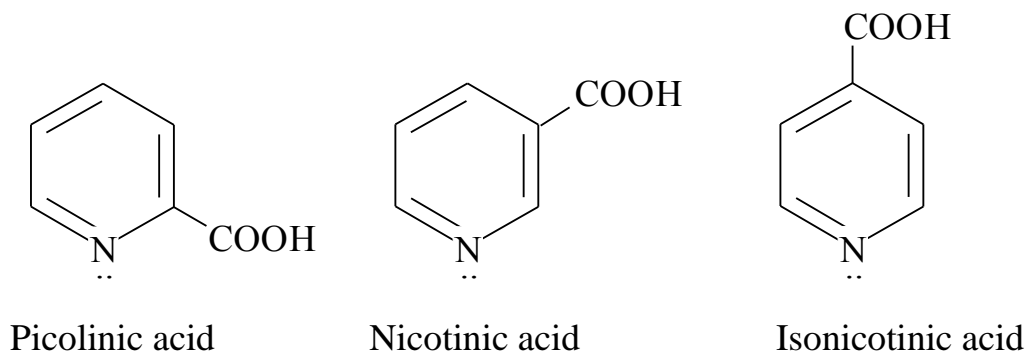
As a nucleophile, pyridic nitrogen attacks electrophilic sites in alkyl halide molecules with formation of alkyl pyridinium salts:



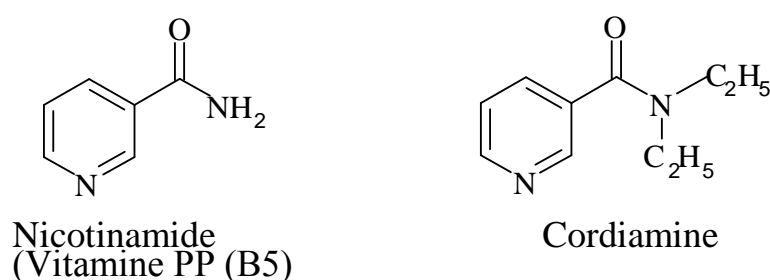
Homologues of pyridine (methyl pyridine, picolines) are:



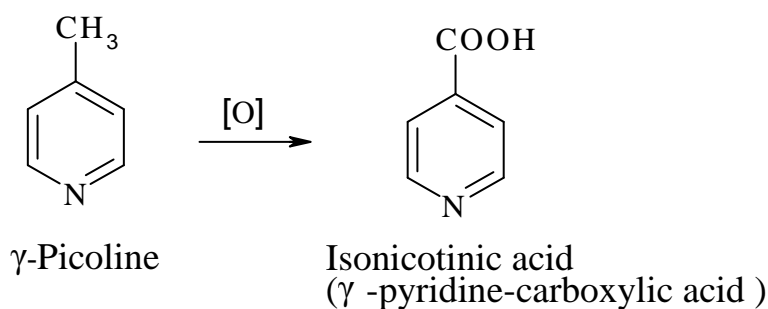
Side-chains in homologues of pyridine can be easily oxidized with formation of  $\alpha$ ,  $\beta$ ,  $\gamma$  - pyridine-carboxylic acids. The most important of these are nicotinic acid and isonicotinic acid.



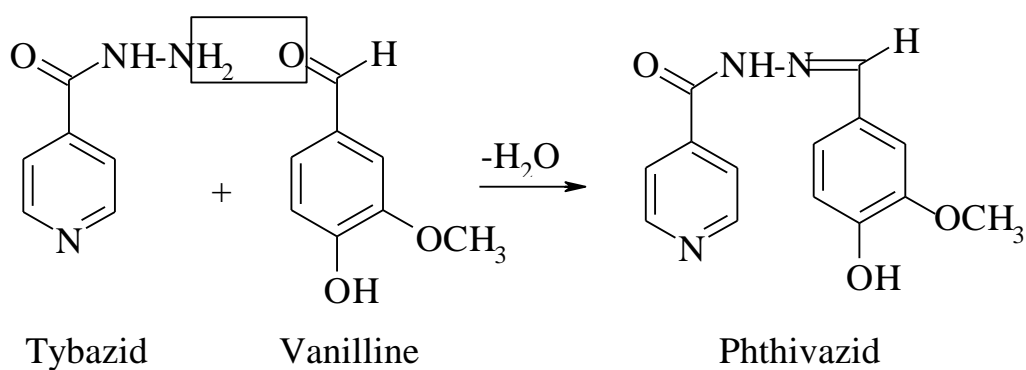
Nicotinic acid and its amide exhibit vitamin activity, they are used in medicine as antipellagric drugs. Nicotinamide (Vit PP(B5)) is a structural component and reactive site of the coenzyme  $\text{NAD}^+$ . Nicotinic acid diethylamide called cordiamine is a medicine used in case of heart failure.



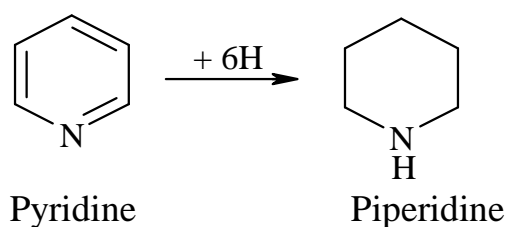
Physiologically important derivatives of isonicotinic acid are compounds used as medicines in tuberculosis treatment. Isonicotinic acid hydrazide is called isoniazid (tybazid). Isoniazid is a highly effective antitubercular drug.



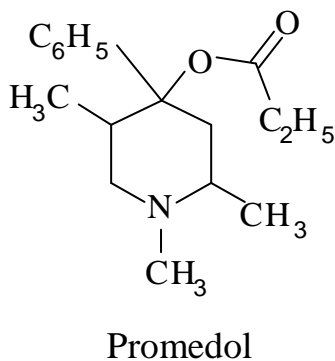
Isoniazid condensed with vanilla has lower toxicity as compare to pure isoniazid. The product is called phthivazid, which is also a highly effective antitubercular drug.



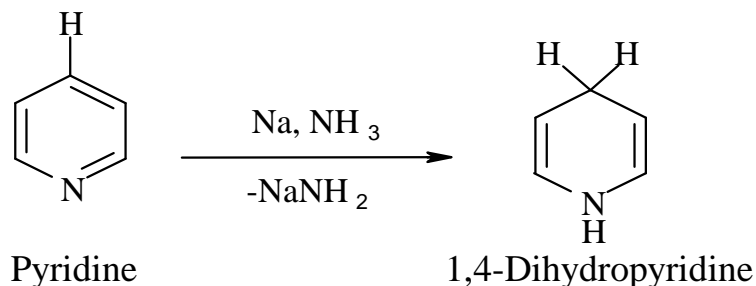
Complete hydrogenation of pyridine gives piperidine.



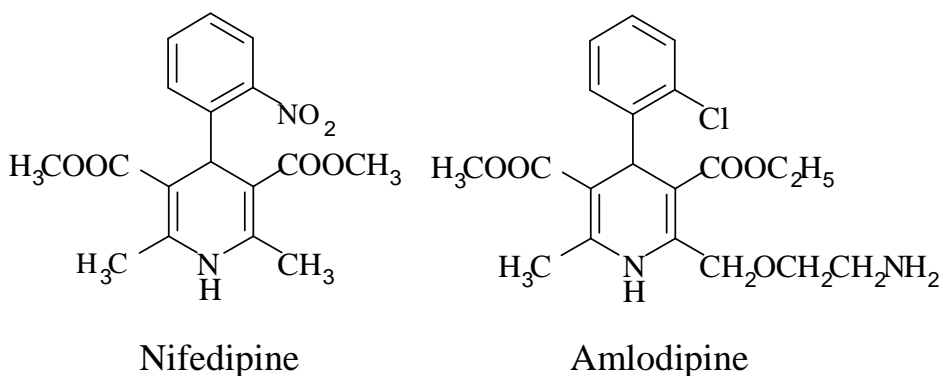
Piperidine derivative - promedol is a narcotic analgesic which causes addiction.



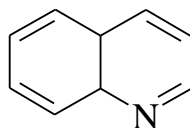
Piperidine ring is in composition of the alkaloids atropine, cocaine, lobeline. Reduction of pyridine with sodium metal in the presence of ammonia gives 1,4-dihydropyridine.



Derivatives of 1,4 - dihydropyridine are calcium ions antagonists. Some of them are used in medical practice for the treatment of cardiovascular disease, hypertension. Nifedipine and amlodipine block the penetration of calcium ions in the smooth muscle cells of heart, causing them to expand and lowering blood pressure.



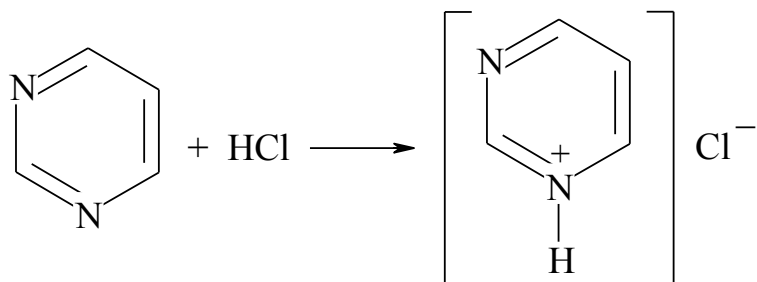
The fused heterocycle quinoline composed of pyridine and benzene rings is in composition of some natural alkaloids: quinine, morphine, codeine, heroin, and of some synthetic antimicrobial drugs: nitroloxinum, enteroseptolum, etc.



Quinoline

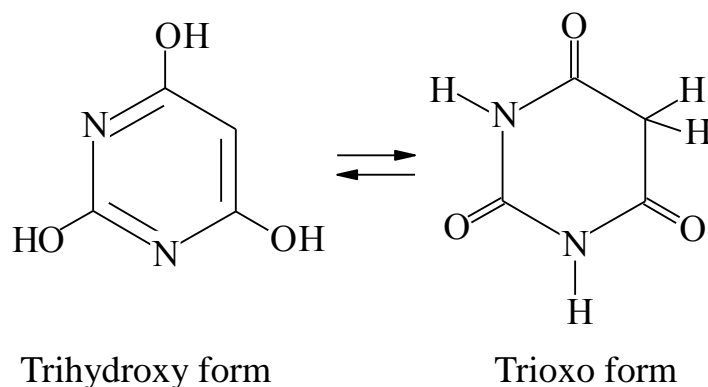
Among six-membered heterocycles with two nitrogen atoms pyrimidine and its derivatives are most abundant. Pyrimidine is an aromatic system containing two pyridic nitrogens. The presence of two electronegative nitrogen atoms results in decreasing of electronic density in the ring and reduces affinity of the ring towards

electrophilic substitution reactions. Moreover, notwithstanding the presence of the two basic centers, basic properties of pyrimidine are weaker than those of pyridine – diazines react only with one equivalent of acid with formation of a salt:

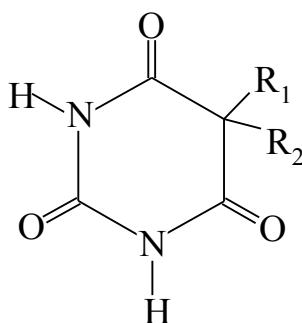


Derivatives of pyrimidine present in living systems are preferentially hydroxy- and aminopyrimidines which are in composition of nucleotides of nucleic acids, vitamins and coenzymes, so called nitrogen bases.

Barbituric acid (2,4,6-trihydroxypyrimidine) is a basis for some medicines. Barbituric acid in water solutions can exist being in some tautomeric forms. Lactam-lactim tautomerism is caused by migration of hydrogen atom between NH- and carbonyl groups, keto-enol tautomerism is due to migration of hydrogen atom between methylene – CH<sub>2</sub> - and carbonyl groups:



Barbiturates are drugs synthesized on the basis of barbituric acid. They are known to have hypnotic and sedative properties. The two hydrogen atoms at position-5 in barbituric acid are highly reactive and can be replaced by various alkyl or aryl radicals to give a variety of products as in therapeutics.



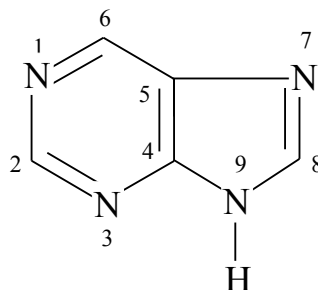


## Barbiturates

Phenobarbital (Luminal):  $R_1 = C_2H_5$ ;  $R_2 = C_6H_5$

Barbital (Veronal):  $R_1 = R_2 = C_2H_5$

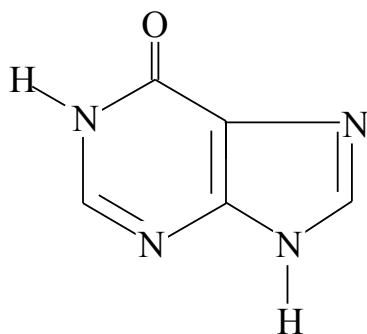
Purine – is a fused aromatic system consisting of pyrimidine and imidazole:



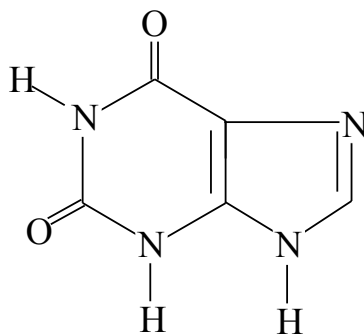
Purine

Hydroxy- and amino purines participating in life activity processes are the most important purine derivatives.

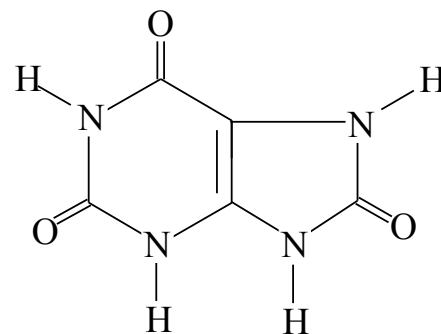
Hydroxy purines – hypoxanthine, xanthine and uric acid are formed in the organism in the nucleic acid metabolism.



Hypoxanthine

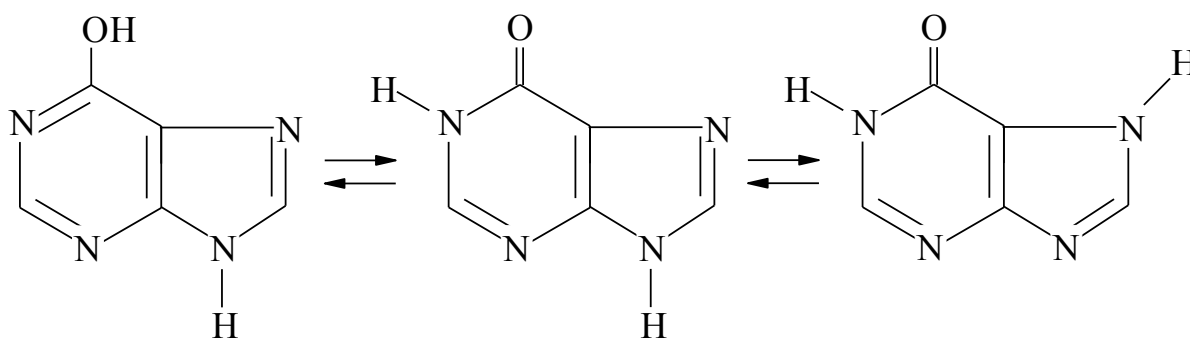


Xanthine



Uric acid

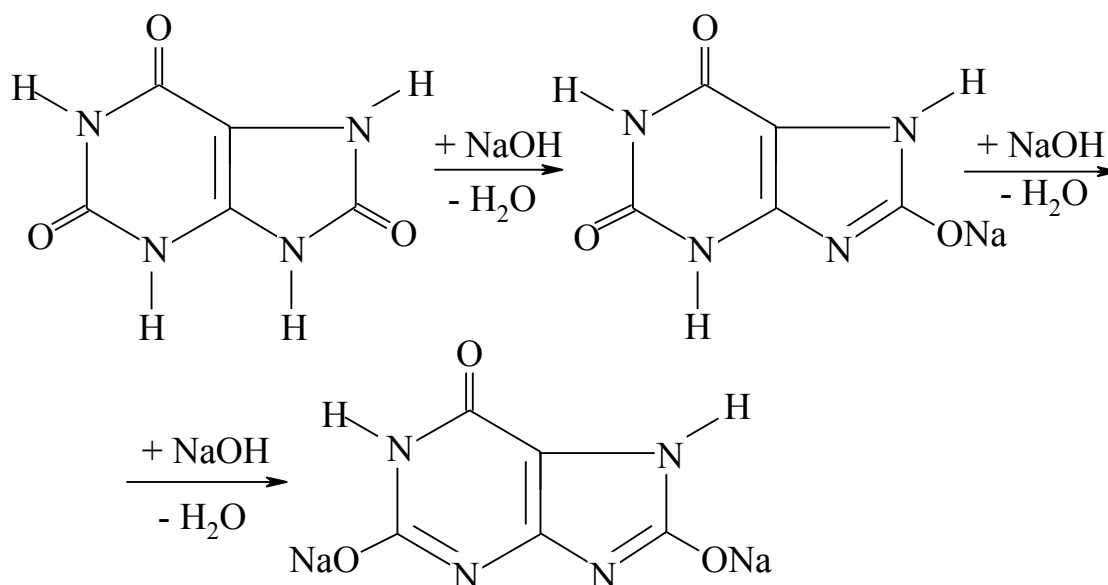
For purine containing compounds lactim-lactam tautomerism is typical, as well as migration of hydrogen proton between nitrogen atoms at positions 7 and 9 of imidazole ring (prototropic tautomerism):



Lactim-lactam tautomerism  
of hypoxanthine

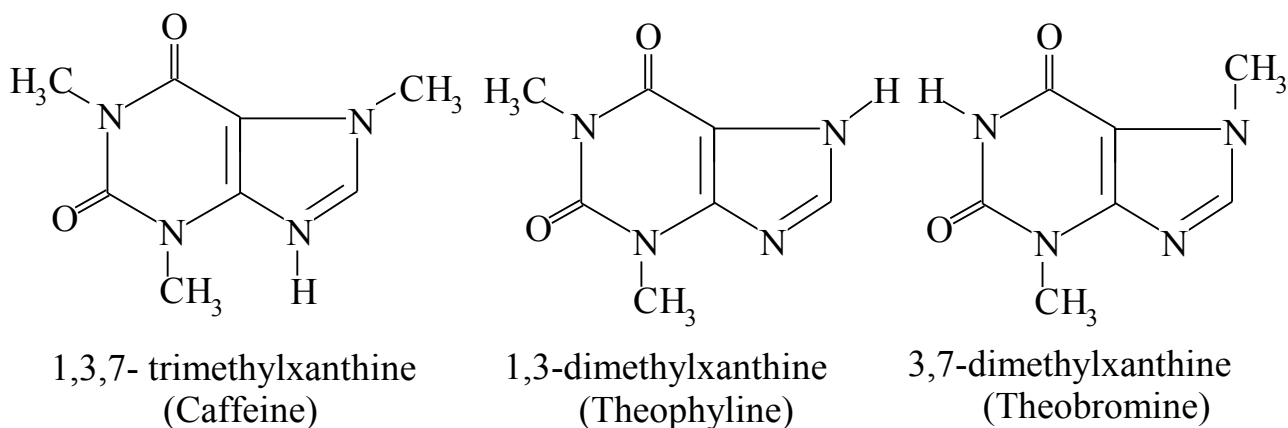
Prototropic tautomerism  
of hypoxanthine

Uric acid is the end product of purines metabolism in the organism. The average urinary excretion of uric acid is about 0.5-1 g per day. Uric acid is dibasic, poorly soluble in water, but well soluble in bases with formation of acidic and neutral salts – urates:



Salts of uric acid, especially acidic ones, are deposited in joints in case of gout and as kidney stones.

N-methyl substituted xanthine derivatives consist of alkaloids – caffeine, theophylline, and theobromine which are used as drugs:



The natural source of these alkaloids is the tea leaves, coffee and cacao beans. Caffeine lessens fatigue and enhances mental alertness. It is used in morphine poisoning and drowsiness due to sedatives. It stimulates cerebral cortex and heart activity. Theophylline causes less stimulation than caffeine. Theobromine is devoid of stimulating properties. These alkaloids possess also diuretic effect. Caffeine is not clinically used as a diuretic because of its strong CNS stimulant effect which overshadows the former effect. Theobromine is less effective than theophylline and theophylline is three times as potent as caffeine.

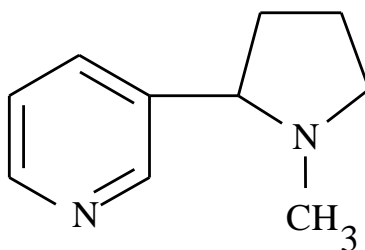
6-aminopurine (adenine) and 2-amino-6-hydroxypurine (guanine) are the most important purines as they are components of nucleic acids.

Derivatives of purine and pyrimidine are essential parts of DNA and RNA. DNA is the molecule responsible for the storage of genetic information, and RNA is prominently involved in the synthesis of proteins.

### 3. ALKALOIDS

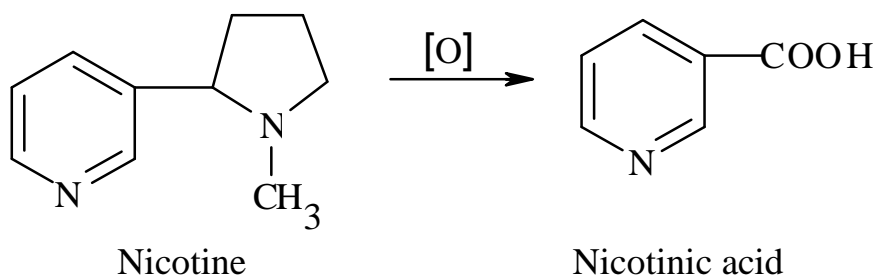
Alkaloids are nitrogen-containing organic compounds of plant origin mainly that have basic properties and high biological activity. Alkaloids are toxic compounds but in small doses they are used as medicines. Most of them have a heterocyclic structure.

1. **Alkaloids - derivatives of pyridine and piperidine.** The most important of this group are nicotine and lobeline. The nicotine is contained in tobacco leaves. The molecule consists of two heterocyclic fragments, one of them is pyridine and the second is N-methyl derivative of completely hydrogenated pyrrole - pyrrolidine.



Nicotine

Nicotine is diacidic base. Pyridic nitrogen determines the basic properties, and N-methylpyrrolidine ring can be considered as a tertiary amine which provides a relatively high basicity to nicotine molecule. Nicotine gives nicotinic acid on oxidation.

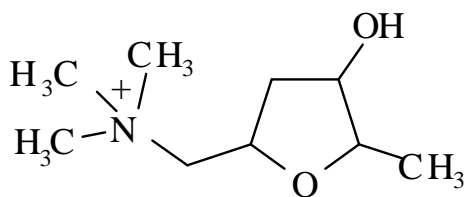


Nicotine

Nicotinic acid

There are cholinergic receptors in a human organism. Some of them are stimulated by acetylcholine and muscarine (M-cholinergic receptors).

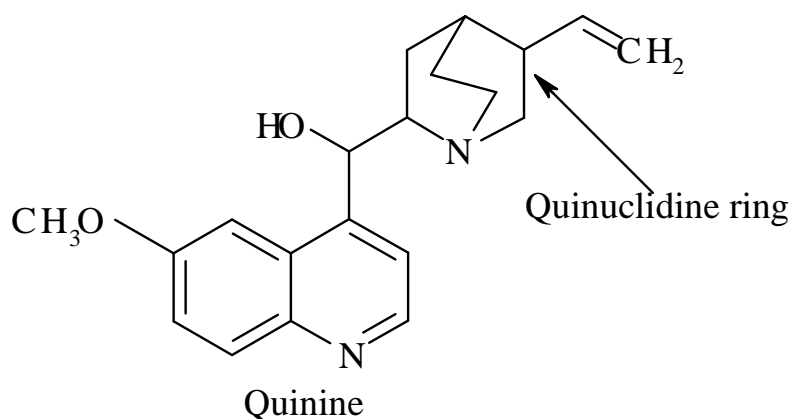
Muscarine (contains tetrahydrofuran ring) is a toxin of amanita mushroom.



Muscarine

There are also muscarinic and nicotinic (N-cholinergic) receptors.

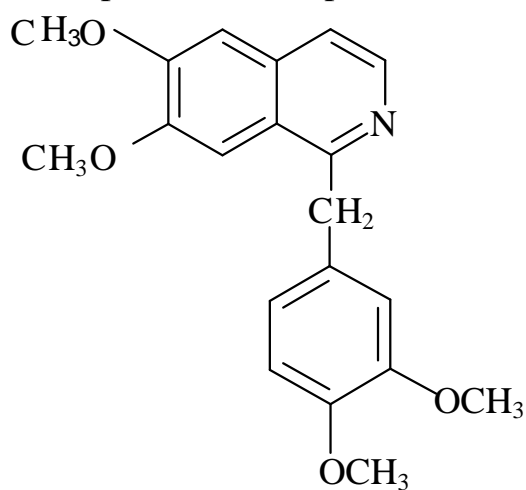
2. **Alkaloids - derivatives of quinoline.** This group contains alkaloids isolated from the bark of the cinchona tree (the most important of these is quinine).



Quinine, similar to nicotine, is diacidic base. Pyridine ring provides weak basic properties, while quinuclidine ring is responsible for strong basicity. Due to the vinyl radical quinoline discolors bromine water, and the secondary alcoholic hydroxyl group causes the properties of secondary alcohols. Quinine is an effective antimalarial drug, it is also able to stimulate labors.

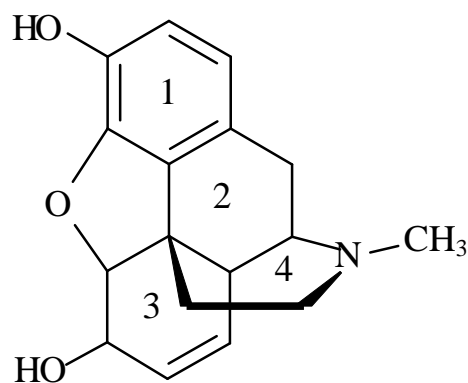
### 3. Alkaloids – derivatives of isoquinoline and phenanthrenizoquinoline.

Papaverine is an alkaloid, which was initially isolated from the soporific poppy (*Papaver somniferum*). It possesses antispasmodic and vasodilatory effects.



Papaverine

Morphine is a complex polycyclic system. Rings 1,2 and 3 compose partially hydrogenated phenanthrene ring, and the rings 3 and 4 form the hydrogenated isoquinoline ring.

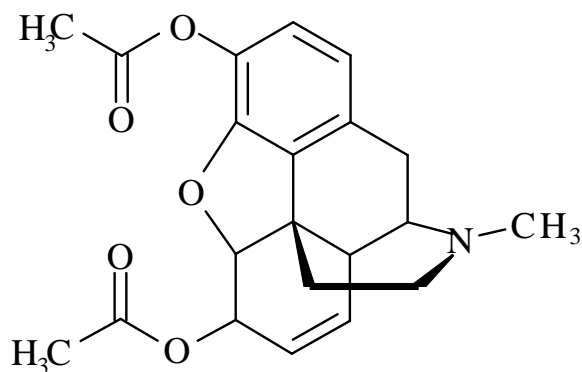


Morphine

Morphine contains phenolic hydroxyl group (therefore forms phenolates with alkalis, and complex compounds of blue coloration with FeCl<sub>3</sub>), alcoholic hydroxyl group (therefore behaves as a secondary alcohol), and also tertiary amino group (therefore possesses basic properties forming salts with mineral acids).

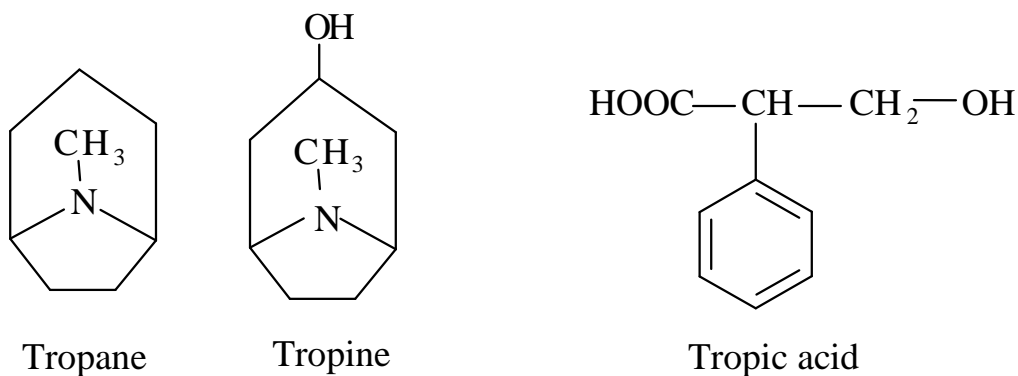
Morphine was isolated from opium first. In medical practice morphine is applied in case of intense pain as a narcotic analgesic. Morphine addiction develops rapidly (morphinism).

Full acetic ester of morphine is called heroin, it is the most common drug which causes strong addiction (heroinism).

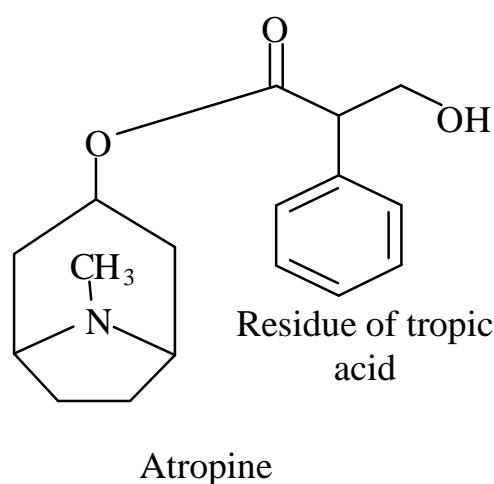


Heroin

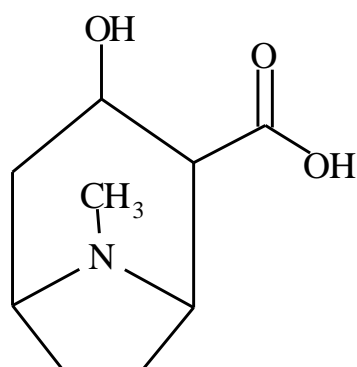
4. **Alkaloids – derivatives of tropane.** The most important representatives of this group of alkaloids are atropine and cocaine. The parent structure is tropane ring.



Atropine is an ester of alcohol tropine and tropic acid. It is a toxic substance. Atropine is contained in the plant belladonna - *Atropa belladonna* (Latin). In small doses it is used in medicine as an antispasmodic and M-anticholinergic drug (blocks M-cholinergic receptors). In ophthalmology it is used for pupil dilation in eyeground exam (causes mydriasis).

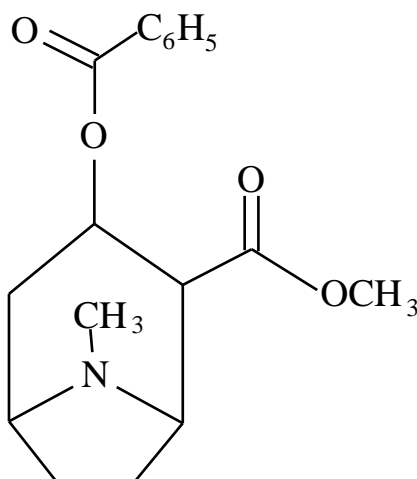


Cocaine is a double ester of ecgonine (ecgonine is a tropane derivative, hydroxy acid).



Ecgonine

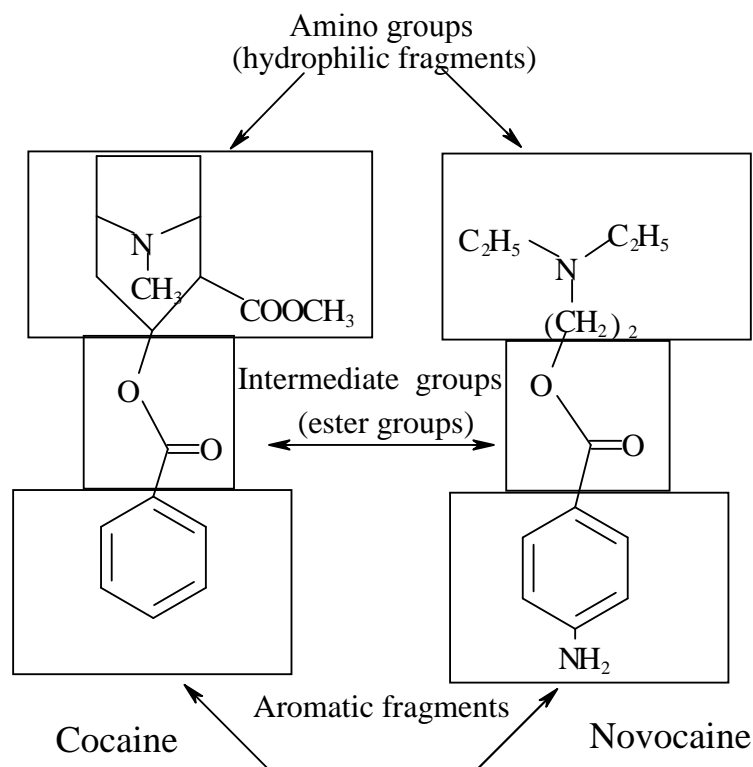
Ecgonine in one case serves as an alcohol and like acid in the other. Alcoholic hydroxyl group of ecgonine is esterified with benzoic acid, and carboxyl group is esterified with methyl alcohol.



Cocaine

Cocaine can be obtained from the bush Coca. It shows strong analgesic effect, stronger than that of benzocaine and novocaine. Similar to morphine, cocaine causes cocaineism. Cocaine is rarely used in ophthalmology and for anesthesia of the mouth and nose mucous membranes and also in surgery practice. Analgesic effect of novocaine and cocaine is due to the presence of identical fragments in their molecules.

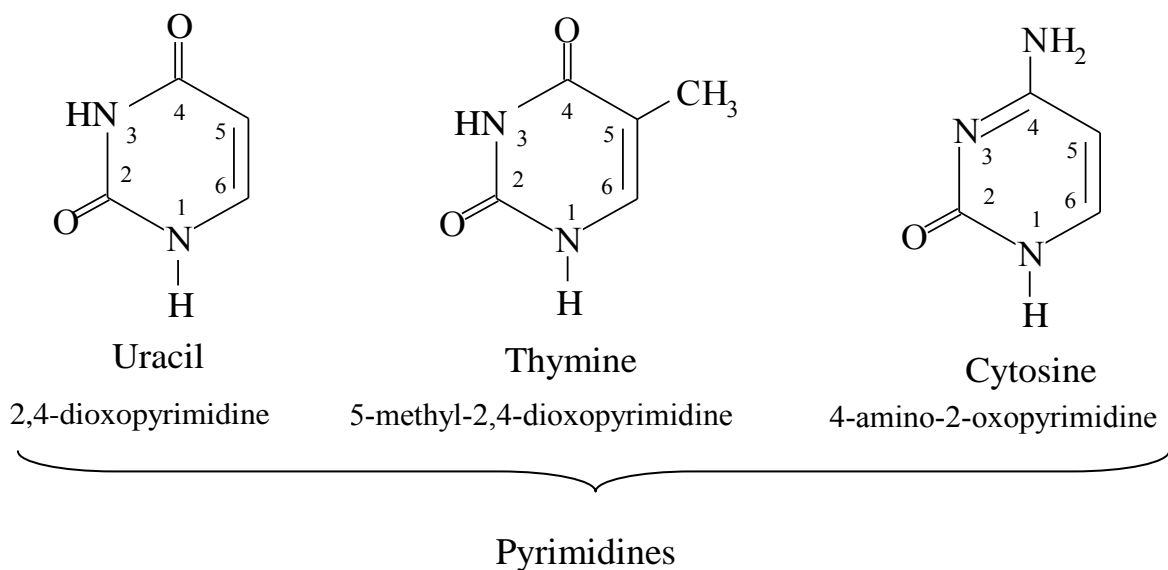


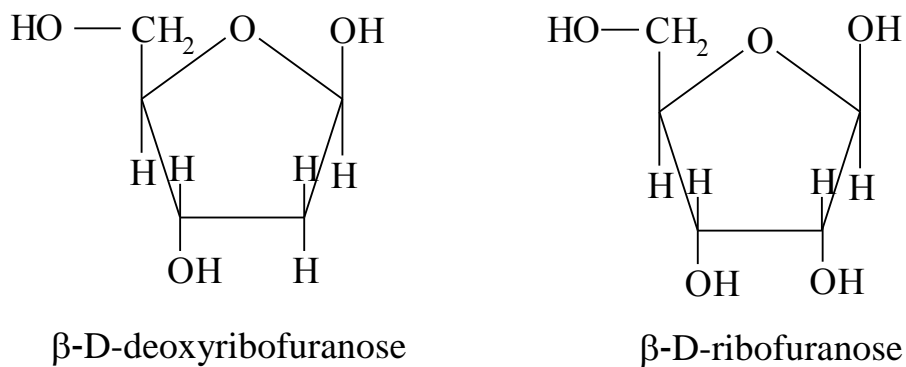
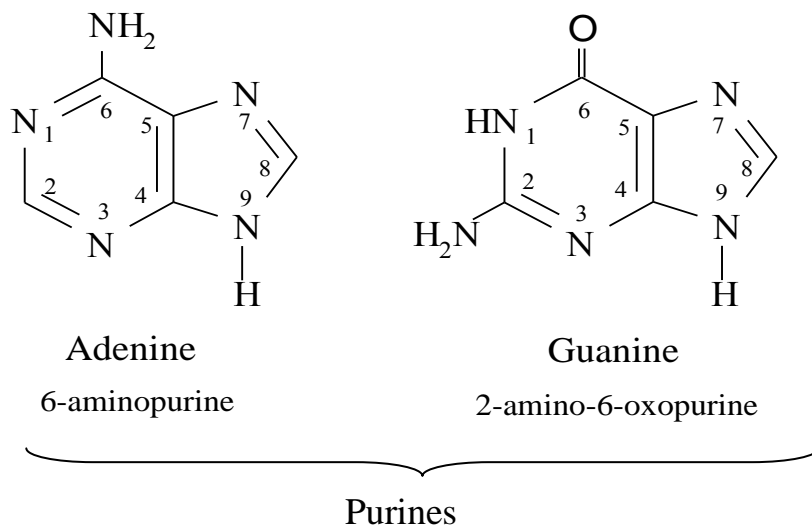


#### 4. NUCLEOSIDES, NUCLEOTIDES, AND NUCLEIC ACIDS

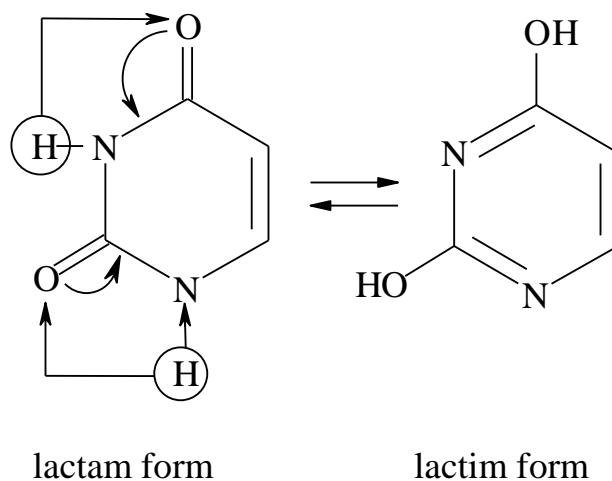
Nucleic acids are biological polymers comprised of monomeric units called *nucleotides*. Complete hydrolysis of a nucleotide furnishes:

1. A nitrogen containing heterocycle (nitrogen base) from either purine or pyrimidine family.
2. A five-carbon monosaccharide that is either D-ribose or 2-deoxy-D-ribose.
3. A phosphate ion.



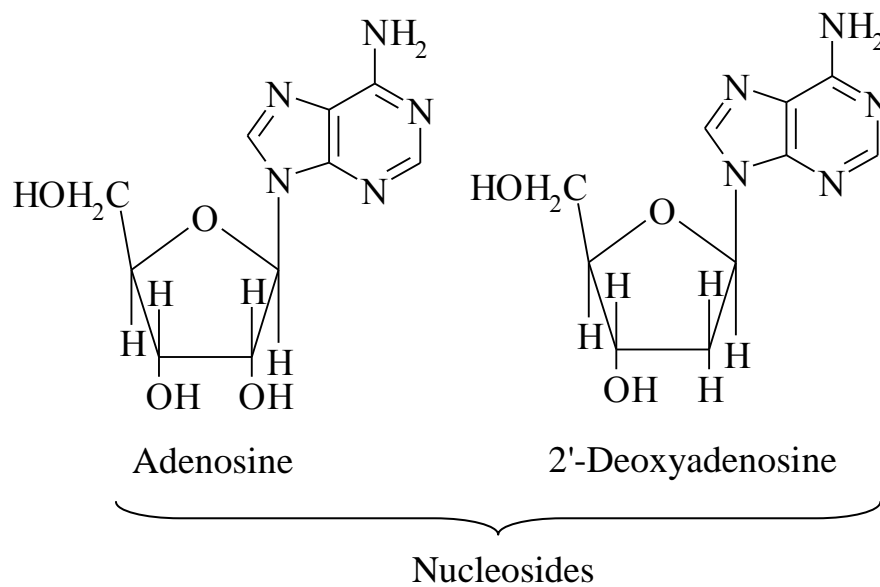


Nitrogen bases show lactim-lactam tautomerism but in the composition of nucleic acids nitrogen bases exist only being in lactam form.

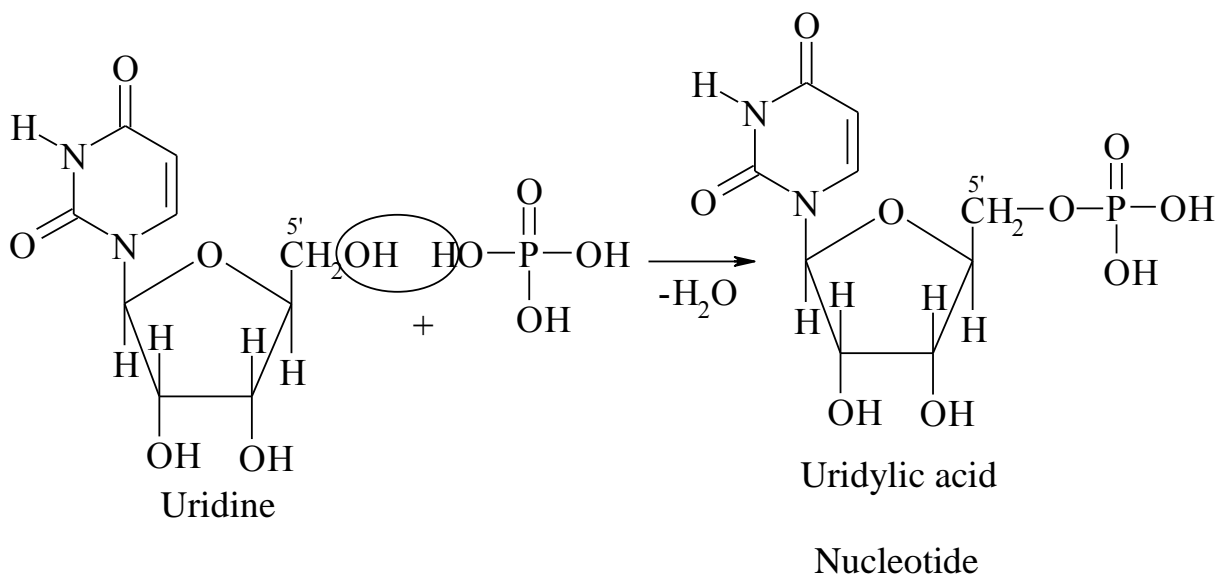


The central portion of the nucleotide is the monosaccharide, and it is always present as a five-membered ring, that is, as a furanoside. The heterocyclic base of a nucleotide is attached through an N-glycosidic linkage to C1' of the ribose or deoxyribose unit and this linkage is always  $\beta$ . The phosphate group of a nucleotide is

present as a phosphate ester and may be attached at C5' or C3'. (In nucleotides, the carbon atoms of the monosaccharide portion are designated with primed numbers, i.e. 1', 2', 3', etc.) Removal of the phosphate group of a nucleotide converts it to a compound known as a *nucleoside*. The nucleosides that can be obtained from DNA all contain 2-deoxy-D-ribose as their sugar component and one of four heterocyclic bases, adenine, guanine, cytosine, or thymine. The nucleosides obtained from RNA contain D-ribose as their sugar component and adenine, guanine, cytosine, or uracil as their heterocyclic base.



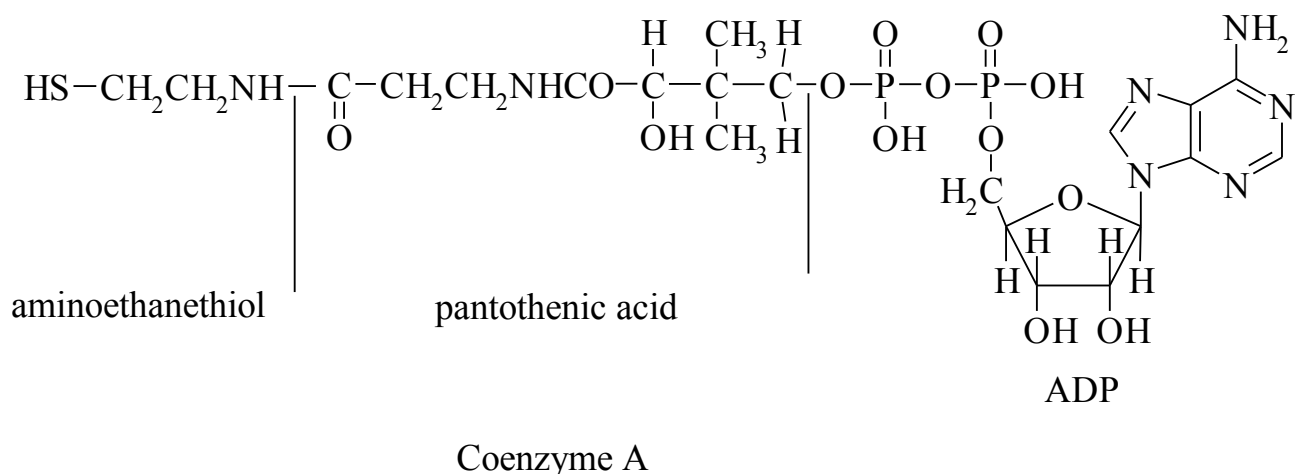
In naming of nucleosides pyrimidine bases have suffix *-idine* and purine bases have suffix *-osine*.



Nucleotides are named in several ways. Adenylic acid, for example, is usually called AMP, for adenosine monophosphate. The position of the phosphate group is sometimes explicitly noted by use of the names adenosine 5'-monophosphate or 5'-adenylic acid. Uridylic acid is usually called UMP, for uridine monophosphate, although it can also be called uridine 5'-monophosphate or 5'-uridylic acid. If a nucleotide is present as a diphosphate or triphosphate, the names are adjusted accordingly, such as ADP for adenosine diphosphate or GTP for guanosine triphosphate.

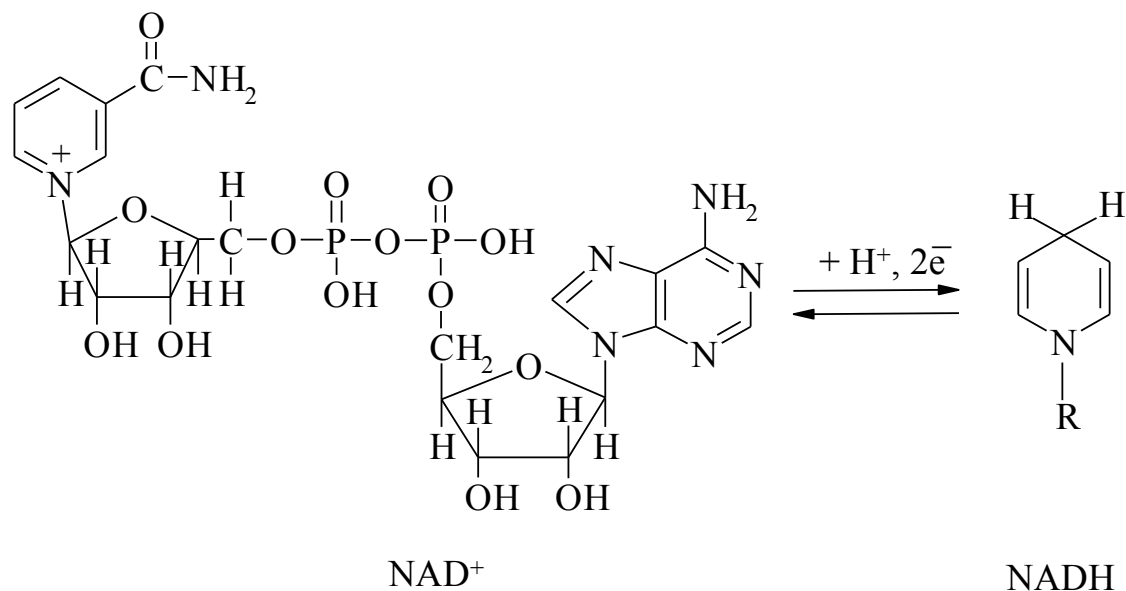
Nucleosides and nucleotides are found in places other than as part of the structure of DNA and RNA. For example, adenosine units are part of the structure of two important coenzymes, NADH and coenzyme A.

Coenzyme A (the coenzyme of acylation) participates in enzymatic reactions involving both the activation and the transport of acid residues (acyls). Acyl-CoA is an active form of carboxylic acid in the organism. CoA-SH consists of 3'-phosphoadenosine-5'-diphosphate residue, pantothenic acid (vit B3) and aminoethanethiol.



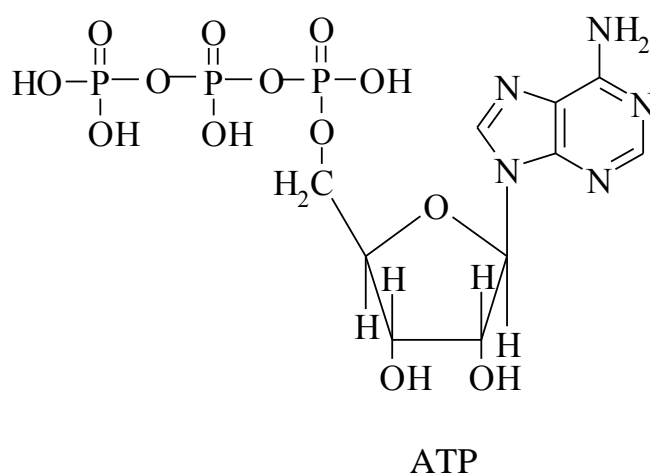
The SH- group is the reactive site of coenzyme A molecule. CoA-SH forms thiol esters with carboxylic acids. CoA-SH participates in major biochemical processes – oxidation and biosynthesis of higher fatty acids, oxidative decarboxylation of  $\alpha$ -keto acids (e.g.  $\alpha$ -ketoglutarate in Krebs cycle), biosynthesis of fats, phospholipids, steroid hormones, hemoglobin, acetylcholine, and others.

NAD<sup>+</sup>, nicotinamide adenine dinucleotide, one of the most important coenzymes in biological oxidations and reductions, includes both a pyridine derivative (nicotinamide) and a purine derivative (adenine) in its structure.

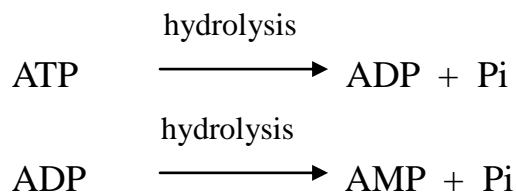


$\text{NAD}^+$  is the oxidized form that contains the pyridinium aromatic ring. The reduced form of the coenzyme is  $\text{NADH}$ , in which the pyridine ring is no longer aromatic due to presence of an additional hydrogen and two electrons in the ring. A key role of  $\text{NAD}^+$  in metabolism is to serve as a coenzyme for enzymes called dehydrogenases in glycolysis and then in Krebs cycle, the pathways by which nutrients are broken down for energy production. When glucose in glycolysis and acetyl-CoA are oxidized concurrently the  $\text{NAD}^+$  is reduced to its higher energy form,  $\text{NADH}$ . The chemical energy stored in  $\text{NADH}$  is used in the mitochondria for the production of ATP.

ATP, the 5'-triphosphate of adenosine is the important energy source. ATP is a nucleotide consisting of adenine, ribose and three molecules of phosphate.

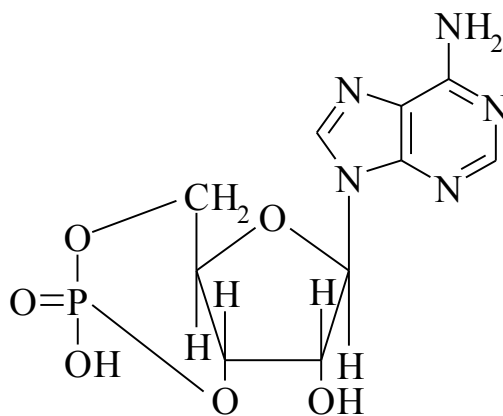


When ATP is hydrolyzed a large amount of energy is released. The hydrolysis of ATP proceeds in the following steps as:



These energy producing reactions are coupled to carry out some other chemical reactions which are otherwise not energetically possible. For example, the energy released by the hydrolysis of ATP can be used to carry out various cell functions such as movement of muscles, export of molecules of all cell activity, uptake of nutrients, transmitting nerve impulses etc. Thus, ATP acts as the center of all activities of cell.

Another important nucleotide is *cyclic AMP (3',5'-cyclic adenylic acid)*. This is an important regulator of hormone activity. cAMP is a universal “second messenger” between the hormones and various functions of the cells susceptible to the action by these hormones.



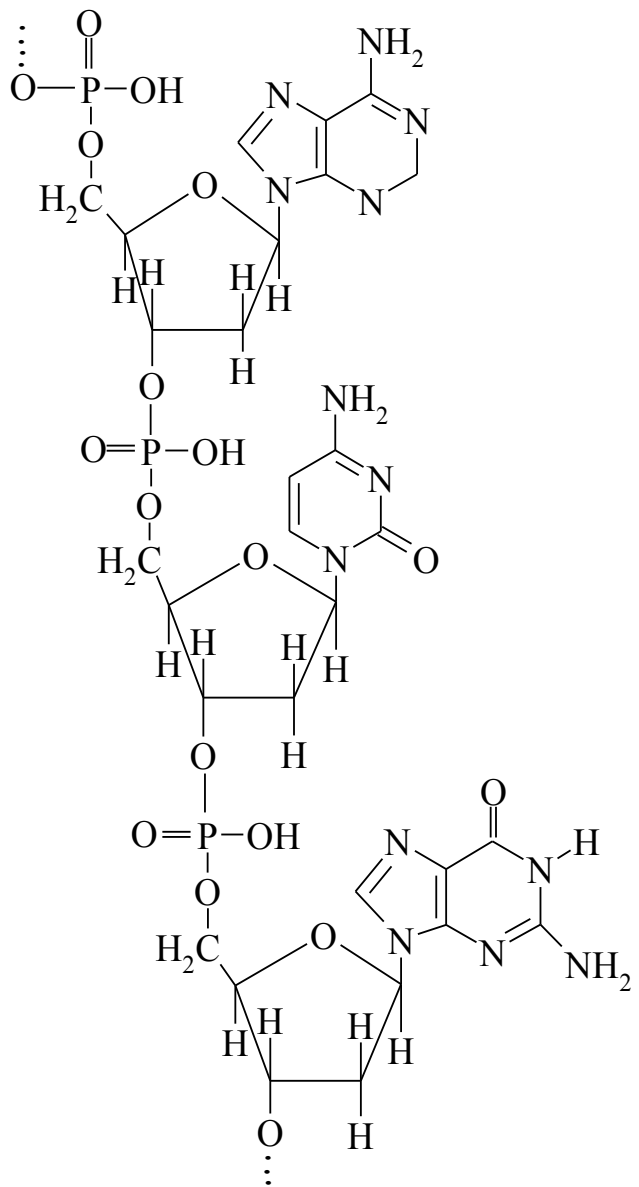
Cyclic AMP

Cells synthesize this compound from ATP through the action of an enzyme, adenylate cyclase.

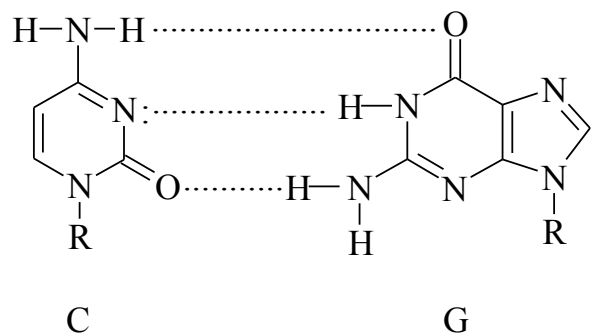
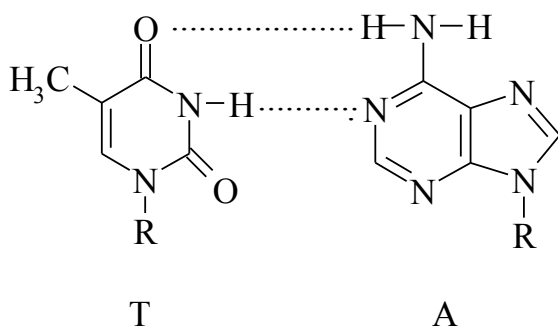
**Primary structure of DNA.** DNA consists of nucleotides linked by phosphate ester linkages. Phosphate esters link the 3'-OH of one ribose (or deoxyribose) with the 5'-OH of another. It is the base sequence along the chain of DNA that contains the encoded genetic information

**Secondary structure of DNA.**

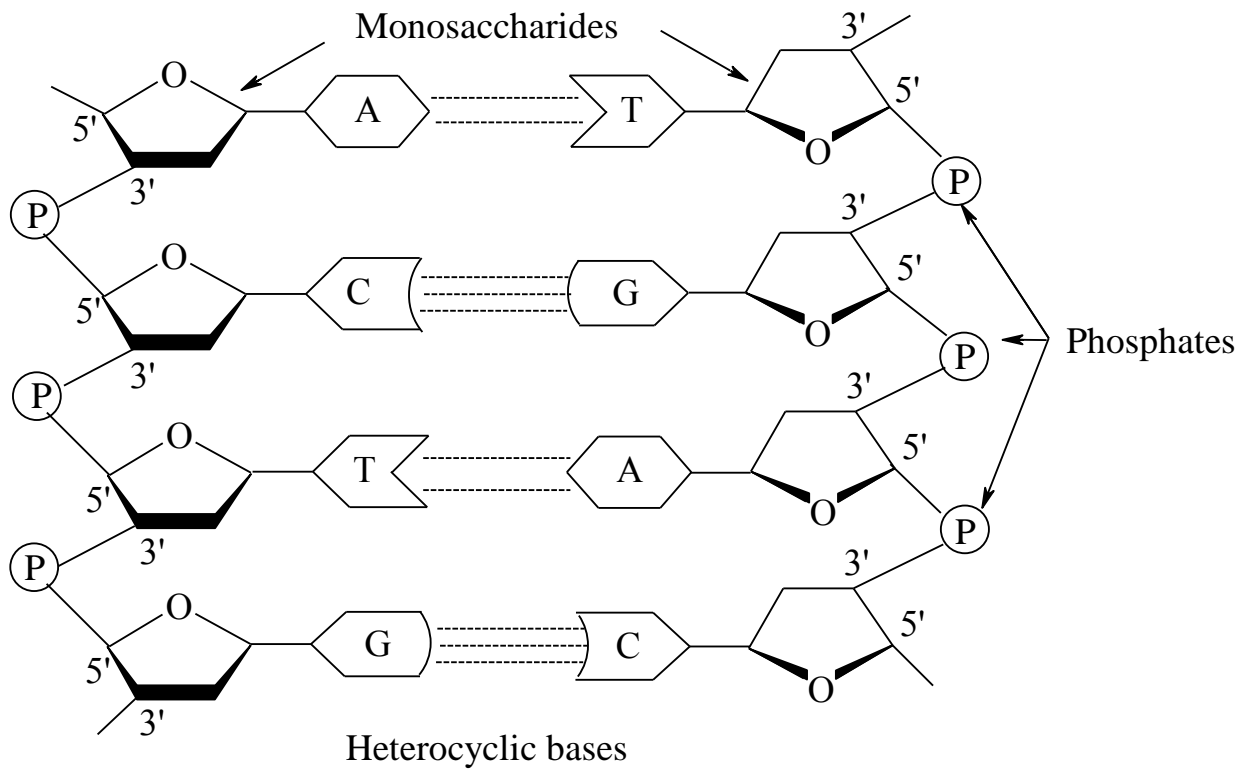
Watson and Crick proposed a double helix as a model for the secondary structure of DNA. According to this model, two nucleic acid chains are held together by hydrogen bonds between base pairs on opposite strands. Base pairing occurs in only a specific way: adenine (A) with thymine (T) and cytosine (C) with guanine (G). A is linked with T by means of two bonds, C with G by three bonds. Specific base pairing means that two chains of DNA are complementary. Wherever A appears in one chain, T must appear opposite it in the other; wherever C appears in one chain, G must appear in the other.



**Primary structure of DNA**



The structure of RNA is similar to that of DNA except that it is a single strand structure.



The important biological functions of nucleic acids are replication and protein synthesis.

Replication is the property of a molecule to synthesize another molecule. DNA has a unique property to duplicate or replicate itself. Replication of DNA is an enzyme catalyzed process. In this process, the two strands of DNA helix unwind and each strand serves as a pattern for the synthesis of a new strand. Due to unique specificity of base pairing, the newly synthesized complementary strand in each case is an exact copy of the originally separated from it. As a result, two double stranded DNA molecules are formed called two daughter DNA molecules. One of the strands comes from the parent DNA molecule and the other is newly synthesized. Each DNA is exactly replica of the parent. In this way hereditary effects are transmitted from one cell to another.

DNA molecules also perform an important function of synthesizing proteins, which serve as machinery of the living cell. In this process, the genetic information coded in DNA in the form of specific base sequence is translated and expressed in the form of amino acid which result in the synthesis of specific proteins which perform various functions in the cell. This is brought about in two steps: transcription and translation.

The transcription involves copying of DNA sequences into a complementary RNA molecule called *messenger RNA (mRNA)*. A portion of DNA double helix strand is unwound and one of the two DNA strands acts as the template for the synthesis of



mRNA molecule. This process is similar to replication process. However, it differs in the following respects:

1. In mRNA synthesis, ribose nucleotide assemble along the uncoiled template instead of deoxyribonucleotide which is assemble in replication of DNA.

2. In this case, the base uracil is substituted for the base thymine.

Transcription is catalyzed by an enzyme called RNA polymerase which recognizes certain base sequence as the starting point of transcription and binds to the DNA near the site where unwinding occurs. After transcription mRNA detaches from the DNA molecule and moves from the nucleus of the cell to a ribosome in cytoplasm where it serves as a template for protein synthesis.

During translation, mRNA directs protein synthesis in the cytoplasm of cell with the involvement of another type of rRNA molecule namely *transfer RNA (tRNA)* and the ribosomes which consist of protein and so called *ribosomal RNA (rRNA)*. The process occurs with the attachment of mRNA to the ribosomes in the cytoplasm. The mRNA then gives the message of the DNA and dictates the specific amino acid sequence for the synthesis of protein. The four bases in mRNA act in the form of triplets and each triplet acts as a code for a particular amino acid. Each triplet of nucleotides is called a *codon* and it specifies one amino acid. It may be noted that there may be more than one triplet combination codes for the same amino acid.

The code expressed in mRNA is read by tRNA and is translated into an amino acid sequence. tRNA transfers the desired amino acid to the proper position on the ribosome. This process is repeated again and again and thus proteins are synthesized. When the synthesis of a specific protein is completed, it is released from ribosome.

## Revision exercises

1. Vitamin PP is a derivative of:

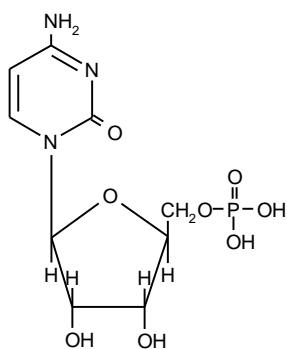
- a) Pyrrole   b) Pyridine   c) Pyrimidine   d) Purine

2. Biogenic amines serotonin and tryptamine are derivatives of

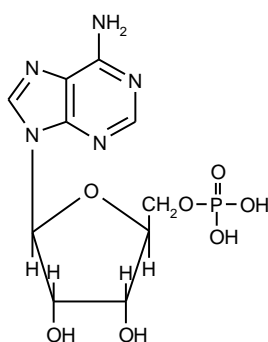
- a) Indole   b) Imidazole   c) Pyridine   d) Pyrrole

3. Which of the following is adenylic acid?

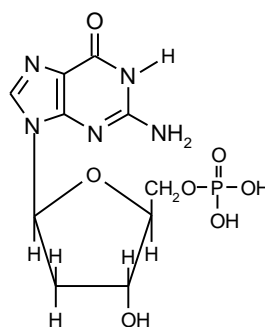
a)



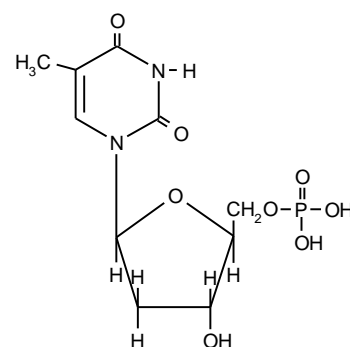
b)



c)



d)



## Answers

1. b)
2. a)
3. b)

\*\*\*

## **BIOLOGICALLY IMPORTANT HETEROFUNCTIONAL COMPOUNDS**

### ***Characteristics of the subject***

Heterofunctional compounds are ancestors of the significant groups of drugs and physiologically active compounds: hormones, non-narcotic analgesics, local anesthetics, and antibiotics.

### ***Purpose***

To consolidate the knowledge about the structure and chemical properties of the most significant biologically active heterofunctional compounds.

### ***Objectives***

1. To know the structure and properties of amino alcohols and their biologically important representatives – choline, acetylcholine, catecholamines.
2. To know the structure and properties of p-aminobenzoic acid. To learn the formulae and activity of drugs – derivatives of p-aminobenzoic acid (anesthesine, novocaine) and their use.
3. To know the structure and properties of sulfanilic acid. To study the formulae and activity of antibacterial drugs - sulfanilamides and their ancestor – sulfanilamide (white streptocide).

### ***Theoretical questions***

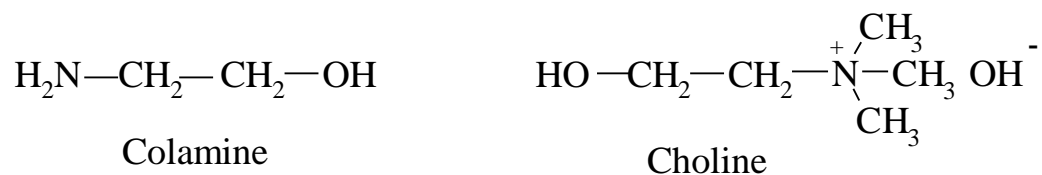
1. Amino alcohols.
2. Sulfanilic acid, p-aminobenzoic acid and their derivatives.
3. Natural and artificial odors.

Heterofunctional compounds are derivatives of hydrocarbons which contain two or more different functional groups. The most important classes of heterofunctional compounds include amino alcohols, amino phenols, and amino sulfoacids.

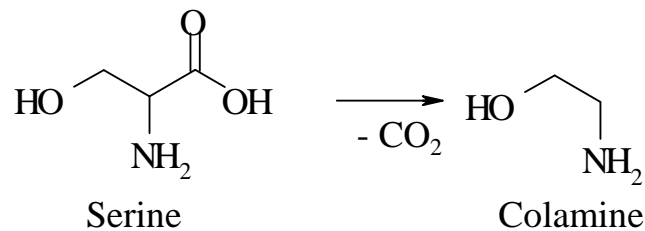
### ***1. AMINO ALCOHOLS***

Amino alcohols contain both alcoholic hydroxyl group and amino group, so they show properties of both alcohols and amines. The most important representatives of amino alcohols are colamine (2-amino-ethanol) and choline (2-hydroxyethyltrimethylammonium hydroxide). These compounds are important

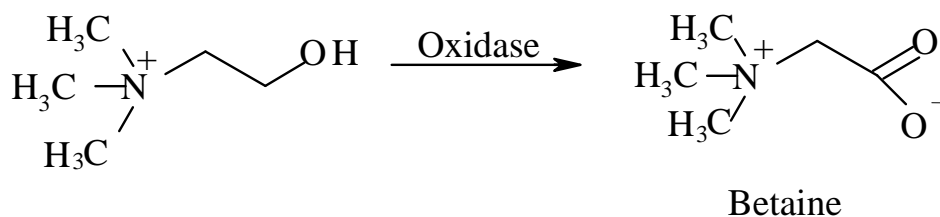
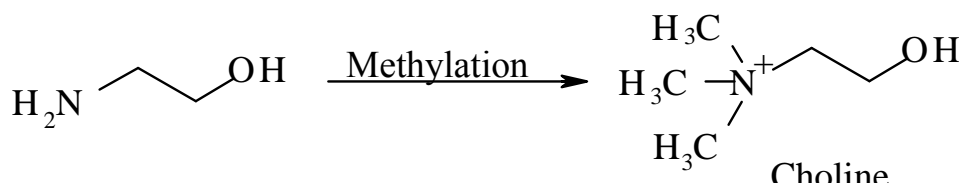
products of metabolism. They enter the composition of phospholipids and also they serve as structural fragments of some catecholamines and many drugs.



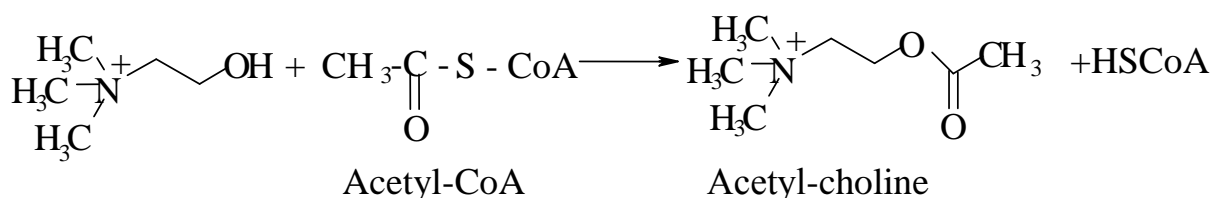
Colamine forms in the organism by the decarboxylation of amino acid serine which contains three different functional groups (carboxyl, amino and hydroxyl ones).



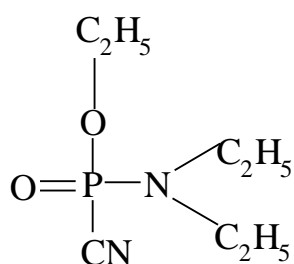
Methylation of colamine in the organism gives choline which is oxidized by enzyme oxidase to form a dipolar ion betaine. Betaine in the organism serves as a source of methyl groups for the methylation of many products of metabolism.



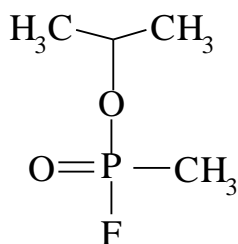
In the organism choline undergoes acylation by acetyl-CoA with formation of acetylcholine which is an ester of amino alcohol choline and acetic acid.



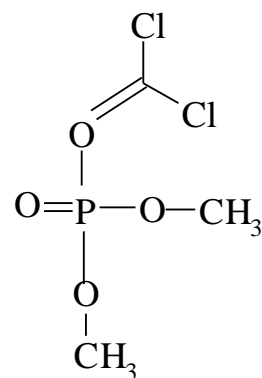
Acetylcholine is one of the most important neurotransmitters (the main neurotransmitter of the parasympathetic nervous system), it interacts with acetylcholine receptors and activates them, it causes contraction of muscles and the organism performs various movements. Acetylcholine is hydrolyzed by the enzyme acetylcholine esterase. Introduction of compounds that block acetylcholine esterase in the organism causes the accumulation of significant amounts of acetylcholine which results in strong stimulation of the nervous system and continuous muscle contractions lead to the death of the organism. This fact is the reason of toxicity of chemical weapons (sarin, tabun), and insecticides (dichlorvos). They interact with the amino acid residues serine, which is a constituent part of acetylcholine esterase and block its activity.



Tabun



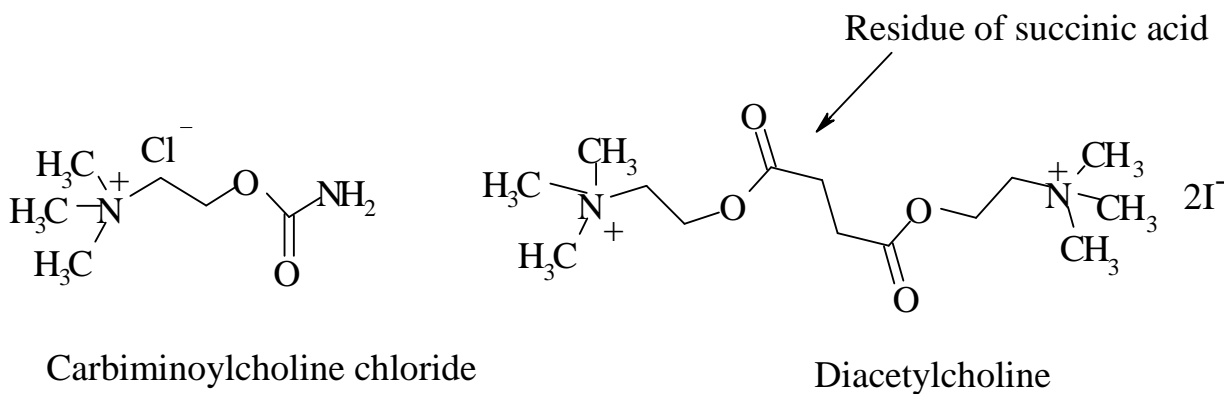
Sarin



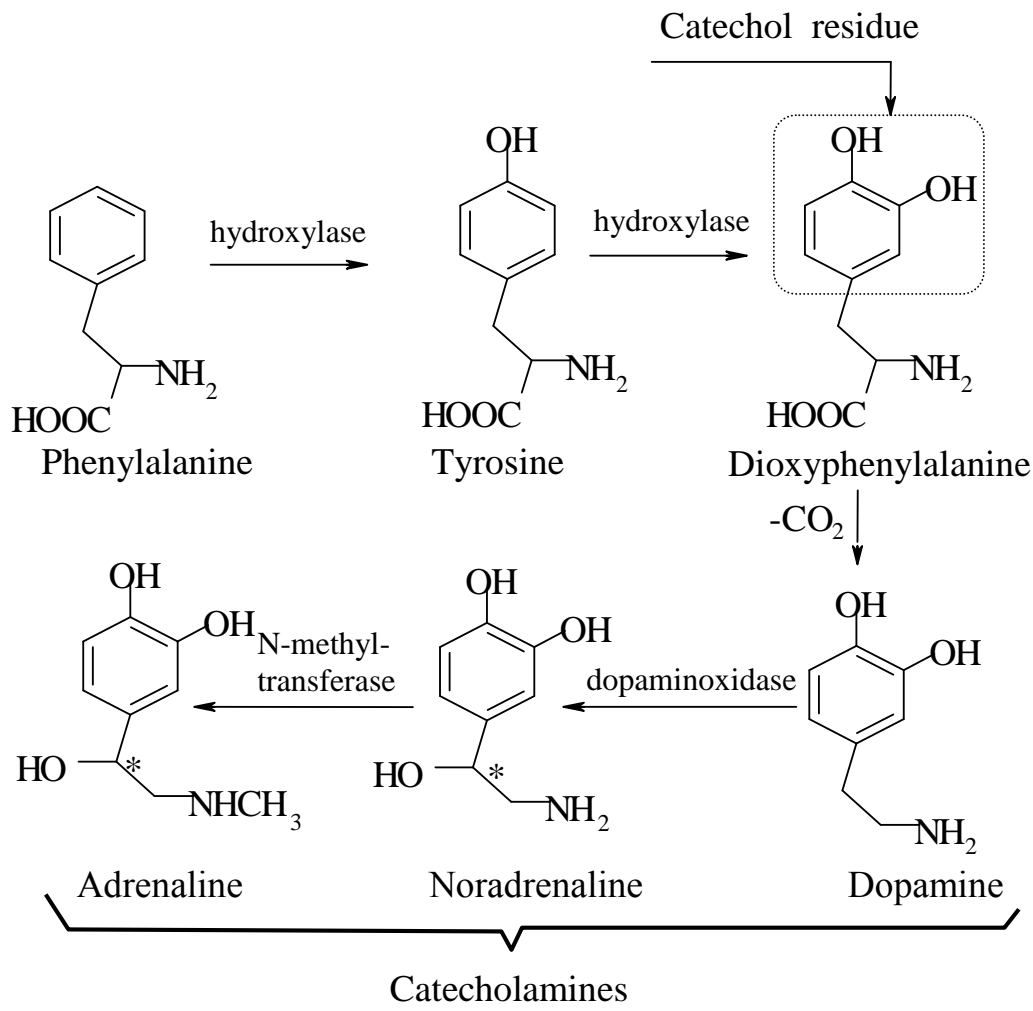
Dichlorvos

Derivatives of choline are used in clinical practice. Acetylcholine chloride slows the heart rate, dilates peripheral blood vessels, and lowers blood pressure.

Carbiminylcholine chloride is an ester of carbamic acid and choline, it does not undergo hydrolysis under the action of acetylcholine esterase, and because of this has more prolonged effect than acetylcholine. Diacetylcholine is an ester of choline iodide and succinic acid, it is used to relax muscles during short-term operations and orthopedic surgery.



Colamine residue is a structural component of noradrenalin and adrenalin. In the organism catecholamine is formed from the essential amino acid phenylalanine.

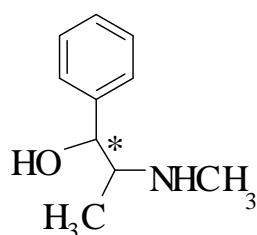


Adrenaline is a hormone of the adrenal medulla. In stressful situations it is released in large quantities in the blood and causes feeling of anxiety and fear. That is why it is called the hormone of fear. Adrenaline effects heart activity constricts blood vessels, increases blood pressure. In medical practice the drug epinephrine

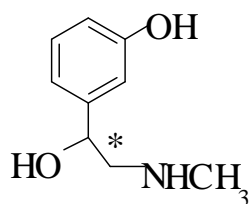
hydrochloride is used. Noradrenalin is the major neurotransmitter of sympathetic nervous system.

Noradrenaline and adrenaline molecules contain asymmetric carbon atom, so both of these compounds exist in the form of two spatial isomers - one of them is physiologically active, while the other is inactive. The inactive one can not interact with the receptors of cells, because their functional groups are not compatible with the atomic groups of the compound).

Medicines ephedrine and phenylephrine (in the form of hydrochloride) are similar to adrenaline according to the structure and physiological effect.

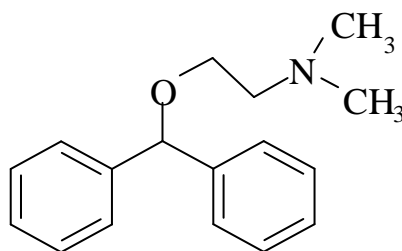


Ephedrine



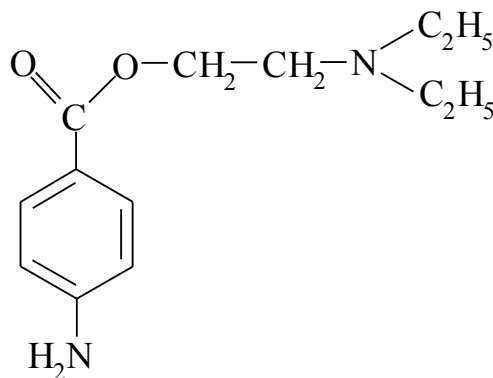
Phenylephrine

Residue of colamine enters the composition of the antihistamine drug dimedrol which possesses hypnotic action.



Dimedrol

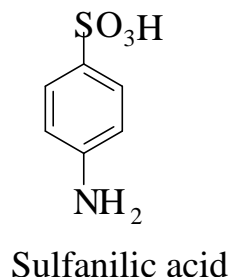
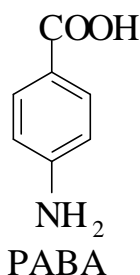
Residues of aminoethanol are structural fragments of some local anesthetic drugs (e.g., novocaine is the ester of amino alcohol diethylaminoethanol).



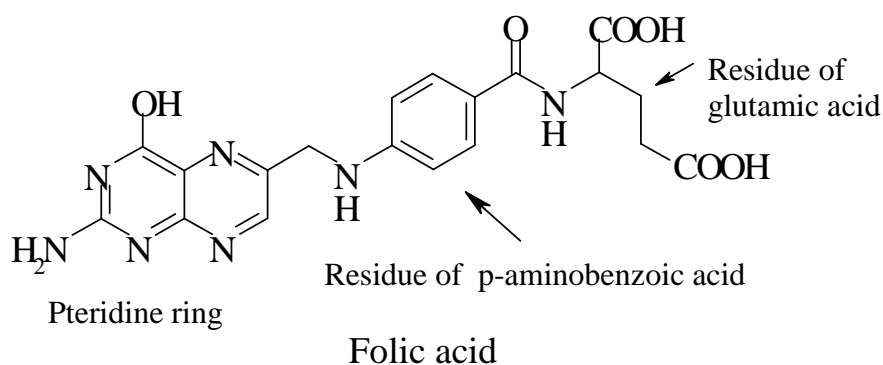
Novocaine

## 2. SULFANILIC ACID, *p*-AMINOBENZOIC ACID AND THEIR DERIVATIVES

Sulfanilic acid and *p*-aminobenzoic acid (PABA) belong to heterofunctional compounds containing acidic and amino groups.

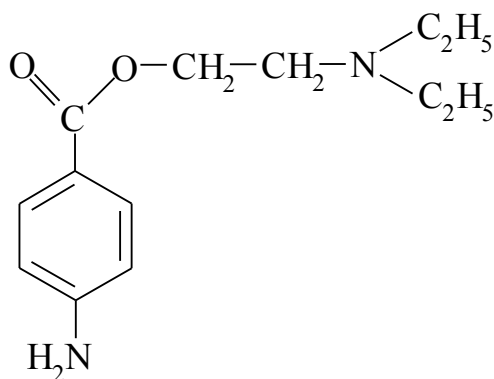
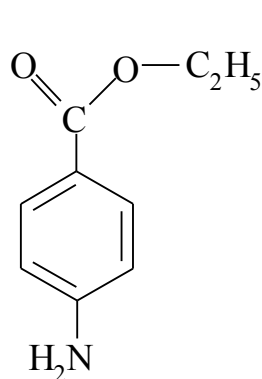


PABA is an important metabolite which is in composition of the folic acid. Folic acid is present in large amounts in the leaves of spinach, in carrots and other plants. It is not synthesized in the organism and comes with food. The name "folic" comes from Latin "folium" which means "leaf".



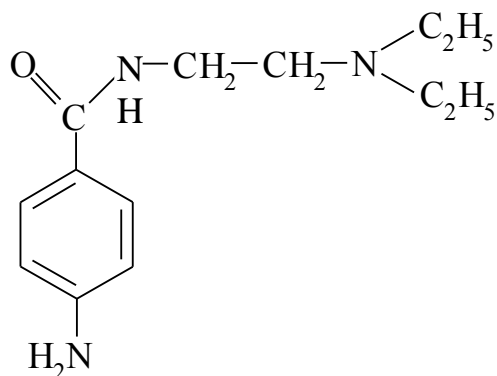
Pteridine ring bonded with PABA residue forms pterioic acid.

Anesthesine, dicaine, and novocaine are esters of PABA. They are used in anesthesia practice. Novocaine causes mild anti-arrhythmic action.





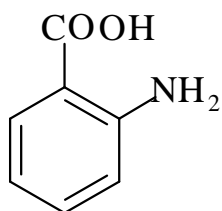
Novocaine is  $\beta$ -diethylaminoethyl ester of p-aminobenzoic acid. It is applied in the form of salt (hydrochloride). Novocainamide is an active antiarrhythmic agent with local anesthetic activity.



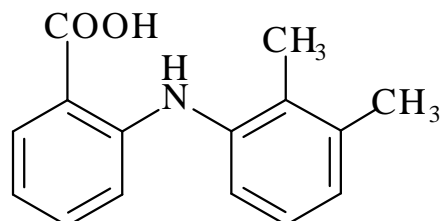
Novocainamide

Novocainamide is  $\beta$ -diethylaminoethylamide of p-aminobenzoic acid. It is applied in the form of salt (hydrochloride).

o-Aminobenzoic acid (anthranilic acid) is the basis of the structure of anti-inflammatory drug - mefenamic acid.



Anthranilic acid

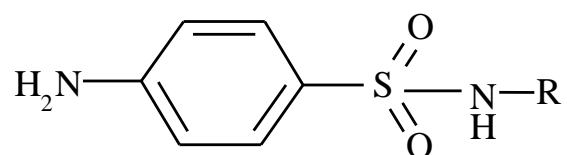


Mefenamic acid

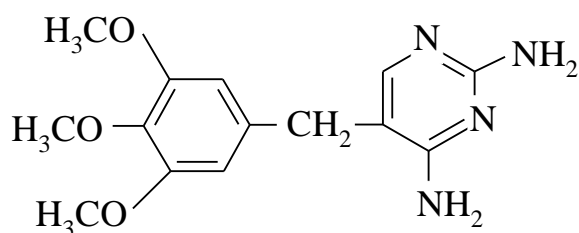
Mefenamic acid contains the elements which are structurally similar to salicylic acid, so mefenamic acid shows similar pharmacological effects - anti-inflammatory, analgesic, antipyretic.

Most of the modern NSAIDs contain a carboxyl group, which in this case serves as anti-inflammatory pharmacophore.

Sulfanilic acid also refers to the amino acids, in which sulfo group acts as acidic center. Sulfanilic acid forms dipolar ions. Sulfanilic acid amide is used in medical practice named White Streptocide. Streptocide is the parent structure of a large group of synthetic chemotherapeutic agents - sulfonamides having the general formula:

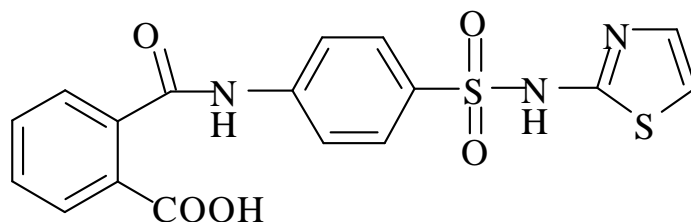


One of the hydrogen atoms of the amino group, located at C<sub>4</sub> in benzene ring can also be substituted by various radicals. The combination of sulfamethoxazole and derivative of diaminopyrimidine - trimethoprim in the drug Biseptol (Bactrim) contributes to its bactericidal (cido (Greek) - to kill) action.



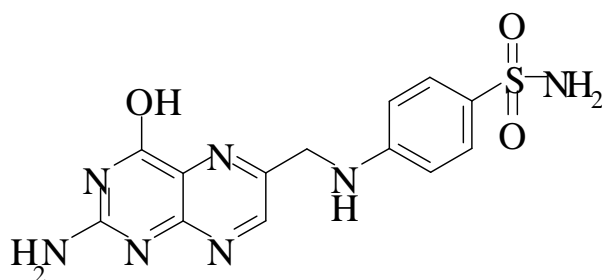
Trimethoprim (2,4-diamino-5-(3,4,5 - trimethoxybenzyl)-pyridine

Substitution of one of the hydrogen atoms at C<sub>4</sub> in the radical results in formation of intestinal sulfanilamide drugs (phthalazole is used in case of dysentery, enterocolitis, colitis).

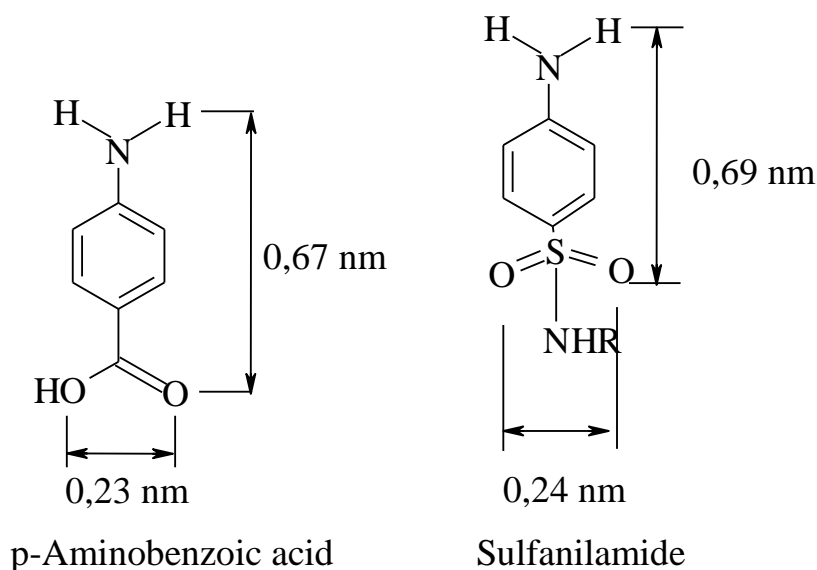


Phthalazole

If in the molecule of sulfonic acid amide the amino group at C<sub>4</sub> is replaced with the other group then this drug does not exhibit antimicrobial activity. Ortho- and meta-isomers of streptocide have no antimicrobial effect. The same phenomenon is observed when additional substituents are introduced in benzene ring of sulfonamides. The antimicrobial action of sulfa drugs is explained by the fact that in the organism they compete with the structurally similar PABA on the stage of synthesis of pteric acid forming a compound (that is, they are anti-metabolites of PABA):



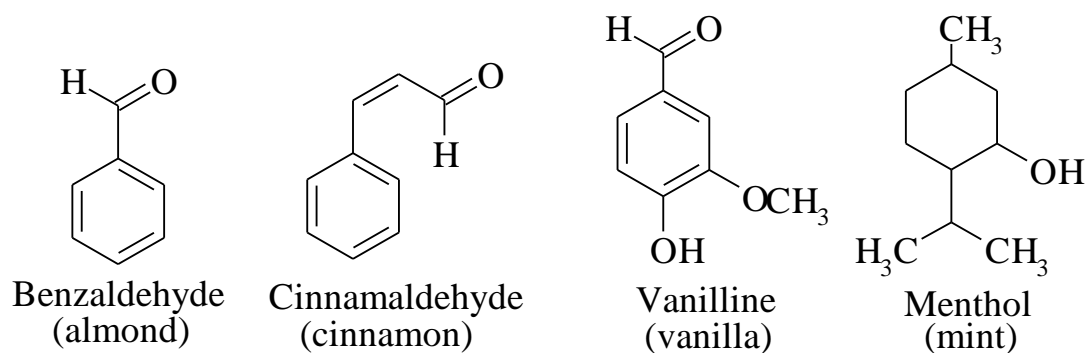
In this case, the synthesis of folic acid stops which leads to the death of microbial cells.



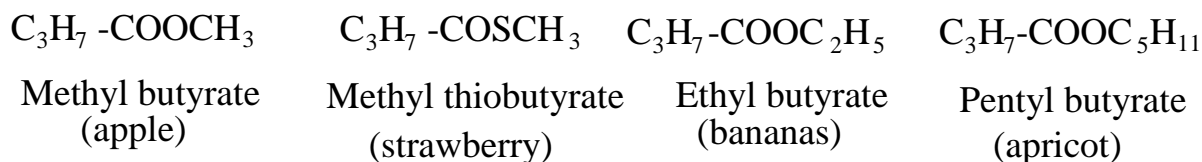
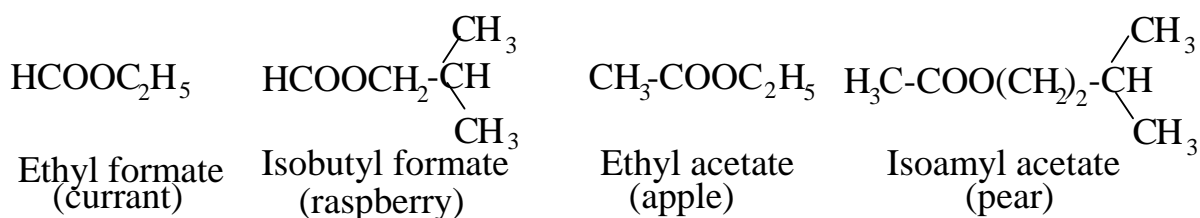
### 3. NATURAL AND ARTIFICIAL ODORS

We can distinguish the various fruits, berries, spices, etc., by their unique odors. These odors are caused by different heterofunctional compounds.

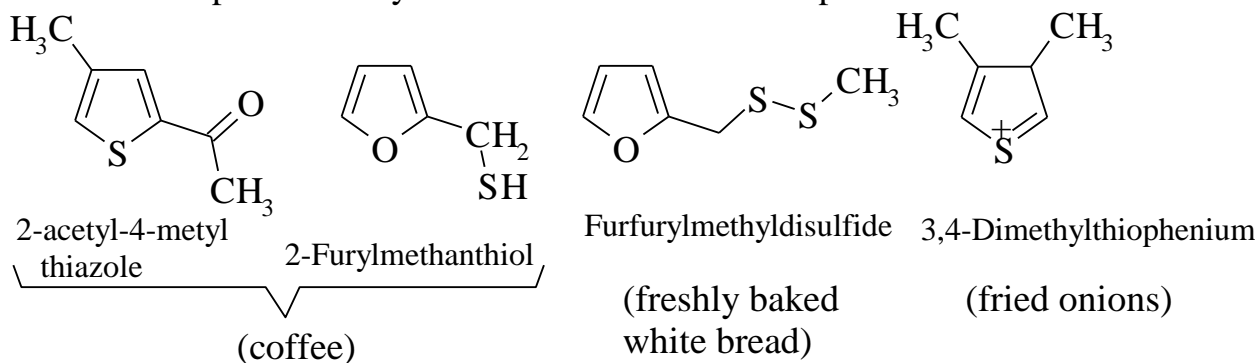
Representatives of aldehydes and phenols having odors:



Representatives of esters having odors:



Often, the smell and taste of natural products are determined not just by one chemical compound but by the mixture of several compounds.



### Revision exercises

- Colamine in the organism forms as a result of decarboxylation of:
  - Threonine
  - Serine
  - Alanine
  - Histidine
- Adrenal medulla hormones - catecholamines (adrenaline, noradrenaline) in the organism form from:
  - Proline
  - Valine
  - Phenylalanine
  - Lysine
- In the organism choline forms from colamine in the reaction of:
  - Alkylation
  - Acylation
  - Oxidation
  - Decarboxylation

Answers

- b)
- c)
- a)

\*\*\*

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Academic Edition

Syrovaya Anna Olegovna  
Grabovetskaya Evgeniya Romanovna  
Petiunina Valentina Nikolaevna

**Fundamentals of Bioorganic Chemistry**

Manual

