Ministry of Health of the Russian Federation

Volgograd State Medical University

Department of Pharmaceutical and Toxicological Chemistry

SPECIAL PHARMACEUTICAL CHEMISTRY

Vitamins of the alicyclic series. Pterin Vitamins

Lesson 11

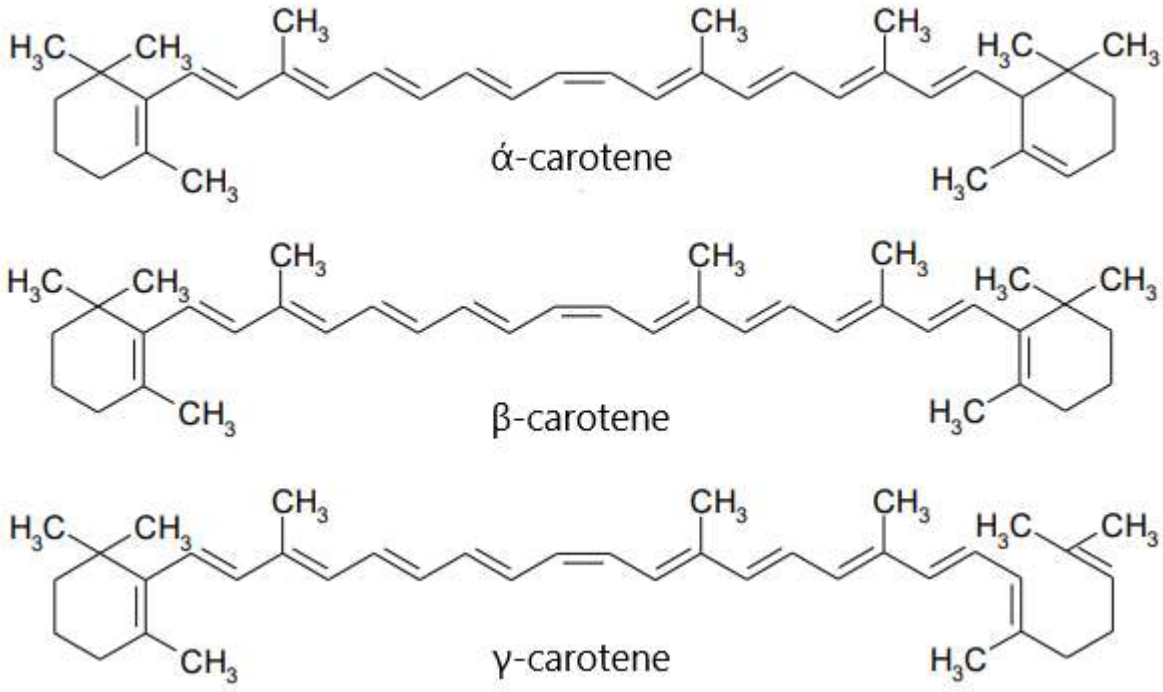
VII term

Volgograd, 2023

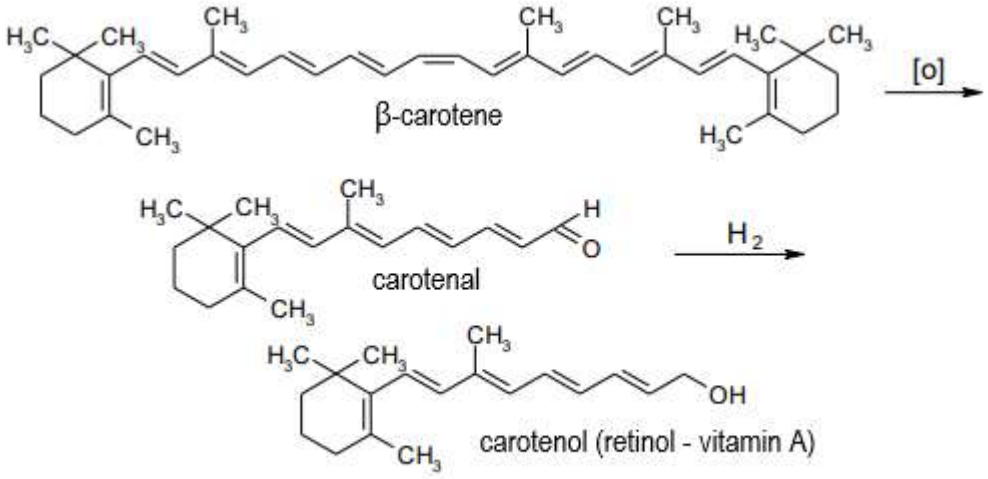
RETINOLS
(A VITAMINS)

Carotenes (ά, β, γ) and vitamin "A" (retinol) belong to a group of vitamins called carotenoids according to international nomenclature. Carotenes belong to the class of polyene hydrocarbons with conjugated double bonds, which accounts for their coloring.

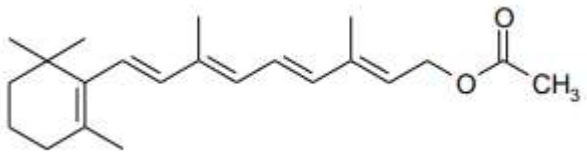
Carotenes contain a trimethylcyclohexyl cycle called the β-ion ring (ring A) and ring B, which differ in the location of the double bond, while in γ-carotene ring B is open. Rings A and B consist as if of two isoprene molecules, and the chain linking them consists of four isoprene fragments:



The presence of conjugated double bonds determines their coloring and ability to undergo various chemical transformations: they can easily hydrogenate, attach halogens (discoloration of bromine water), oxidize (discoloration of potassium permanganate in neutral solutions and formation of glycols). At deep oxidation, for example, KMnO4 in alkaline medium, the carbon chain can be broken to form aldehydes (carotenals). In this case, the carbon polyene chain of the resulting aldehyde can have different lengths. When carotenal was reduced, the alcohol carotinol, called vitamin "A", was obtained.



Retinol acetate



OBTAINING

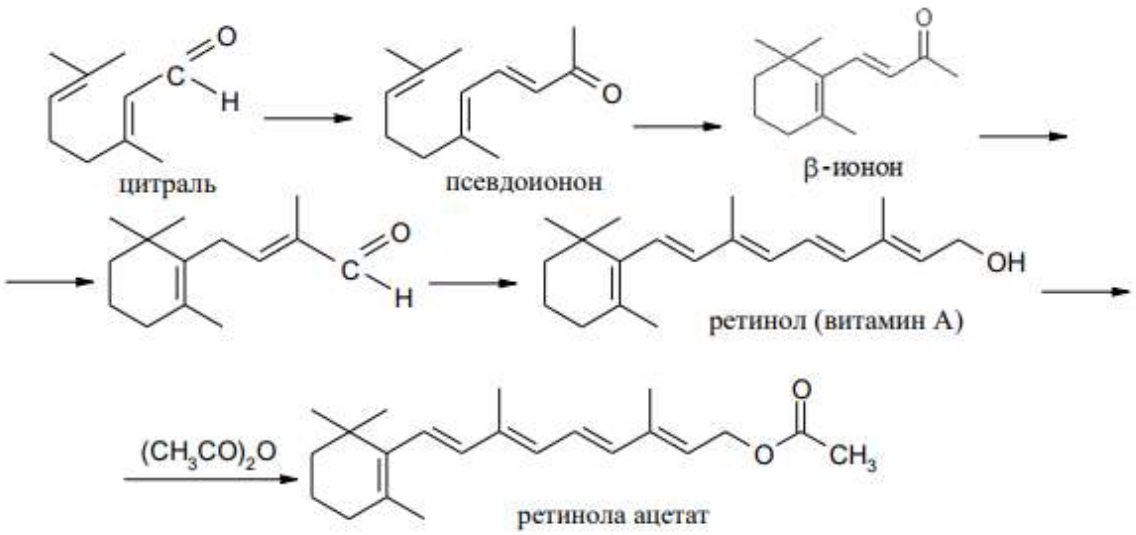
1. ***From natural raw materials***

   The starting point for the synthesis is retinol, which is isolated with a group of vitamins of the A complex from the main source of production - fish liver. Fresh or fresh-frozen liver is crushed, hydrolyzed with 25% caustic soda solution at 82-85 ºC and pH 9.0-10.0. As a result of hydrolysis, the bond between retinol and proteins is broken and it is extracted by liver fat. The resulting concentrate is purified by chromatography and retinol is extracted with dichloroethane. The solvent is extracted and the retinol is recrystallized. The retinol is then acetylated with acetic anhydride.

2. ***Industrial method***

   The synthetic method of retinol production from citral is of industrial importance. It is based on the sequential formation of β- ionic cycle and then side chain extension.

   General scheme of synthesis:



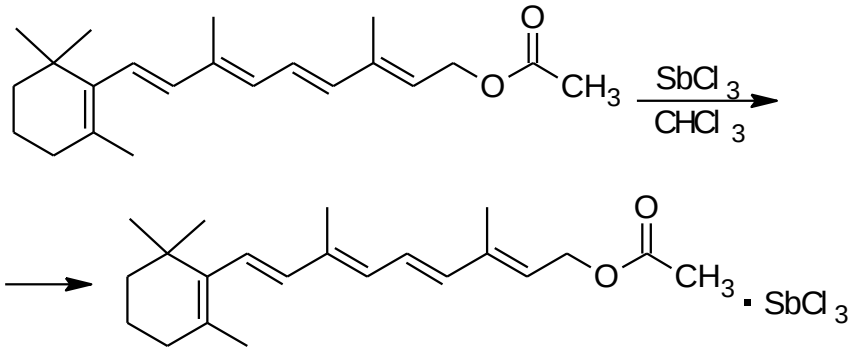
PHYSICAL PROPERTIES

Retinol acetate is white or pale yellow crystals with a faint odor. The melting point of retinol acetate is 53- 57 ºC. Soluble in chloroform, ether and fatty oils, insoluble in water.

IDENTIFICATION

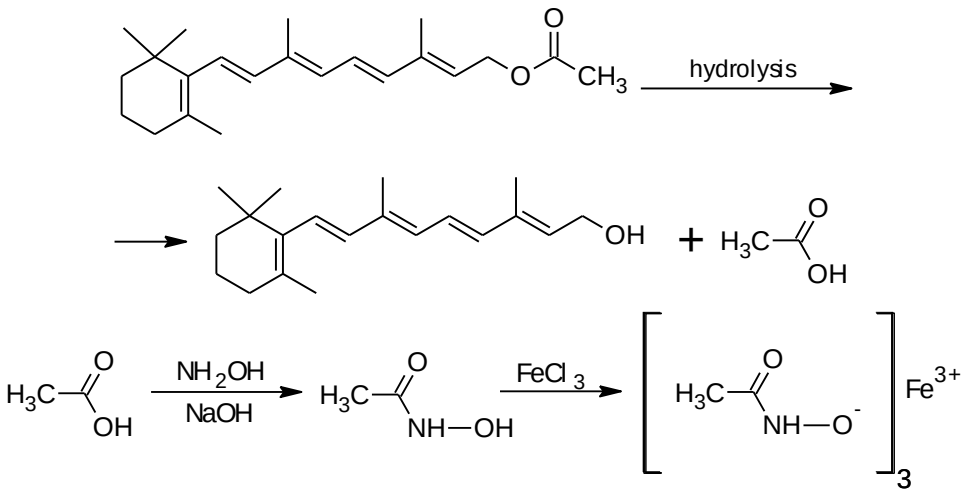
1. ***UV spectrophotometry***
2. ***Reaction with antimony chloride***

   The pharmacopoeial reaction is a color reaction based on interaction with trivalent antimony chloride in chloroform solution. An intermolecular complex is formed, colored intensely blue with a maximum of light absorption in the region of 620 nm.



3. ***Hydroxamic reaction***

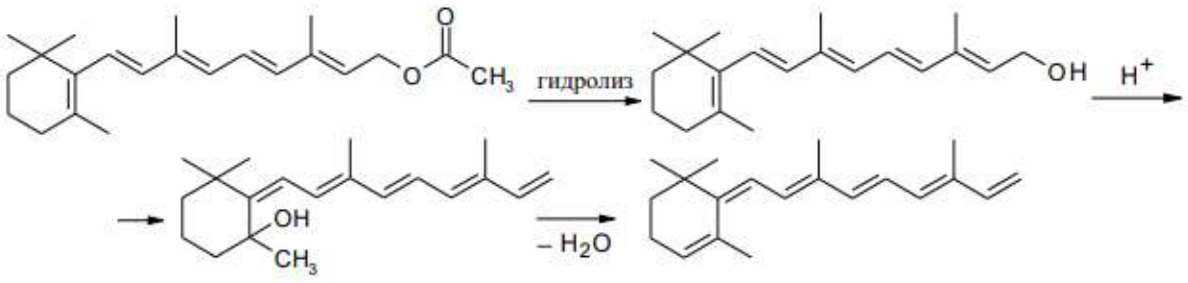
   The determination of acetic acid after hydrolysis is conducted by the formation of iron or copper hydroxamate:



Iron hydroxamate has a red coloration and copper hydroxamate has a blue-green coloration.

QUANTIFICATION:

1. ***Spectrophotometry and photocolorimetry in absolute alcohol***
   The reaction of formation of the colored complex of retinol acetate with antimony trichloride is the basis of the spectrophotometric and photocolorimetric method in absolute alcohol (at a wavelength of 326 nm).
2. ***Spectrophotometry***
   Spectrophotometric quantification of retinol is also performed in chloroform solution using 50% perchloric acid solution (at 543 nm.) as a reagent.
3. ***Spectrophotometric determination conversion of retinol to anhydrovitamin A***
   Spectrophotometric determination can be performed after conversion of retinol to anhydrovitamin A (n-toluene sulfonic acid is used for dehydrogenation):



STORAGE

Retinol acetate is stored in sealed ampoules, at temperature not higher than +5 ºC, protected from light (due to slight oxidizability). Retinol acetate solution in oil is

stored in well-corked orange glass vials filled to the top. Fish oil should be stored under identical conditions.

MEDICAL USE

Vitamin A is of great importance for the body. It is necessary for cell reproduction, promotes normal metabolism, growth and development of the body, normal function of the organs of vision, tear, sweat, sebaceous glands, increases resistance to infection. With avitaminosis A first comes chicken blindness, and with a lack of vitamins A for a long time can come the process of keratinization of the cornea of the eye (it becomes dry and dull). This disease is called xerophthalmia, and vitamin A is called anti-xerophthalmic, anti-infectious vitamin that protects the epithelium.

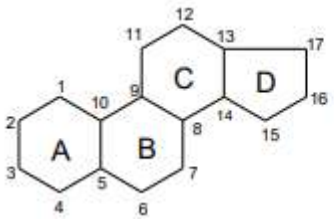
Indications for use are avitaminosis A, hypovitaminosis, infectious and cold diseases, skin lesions and diseases, diseases of the eyes and digestive organs. Prophylactic dose of vitamin A for an adult is 15 mg (5000 IU) per day, therapeutic dose up to 10 mg (33000 IU), but not more than 30 mg (to prevent hypervitaminosis).

Retinol acetate is prescribed in various dosage forms (dragees, granules, oil solution) orally, intramuscularly, topically.

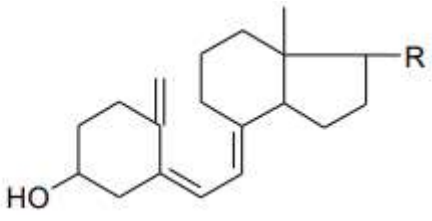
CALCYFEROLS
(VITAMIN "D")

The D vitamins are known as calciferols or antirachitic vitamins. To date, several D vitamins have been discovered: D1-7. Vitamin D_2 (ergocalciferol) and vitamin D_3 (cholicalciferol) are of practical importance as drugs. They are similar to each other in chemical structure, in physicochemical properties and in action on the body.

The structure of calciferols is genetically related to the structure of sterols, which are derivatives of cyclopentaphenanthrene. The difference between these two groups of compounds is that calciferols have an open B cycle:



Cyclopentanophenanthrene

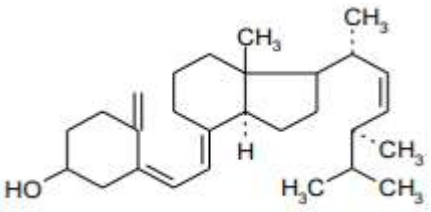


general formula of calciferols

Rings A and C are connected by an ethylene bridge with two double exocyclic bonds, causing cis-trans isomerism. Natural calciferols have a trans configuration.

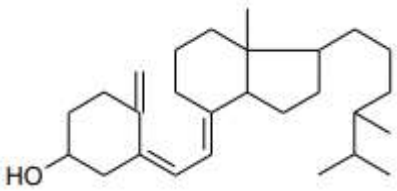
Vitamin D drugs

Ergocalciferol (vitamin D_2)



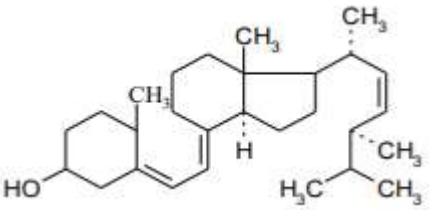
White crystalline powder, odorless. Oxidizes in the light. Melting point 113 - 118 °C. Practically insoluble in water, slightly soluble in ethanol.

Cholecalciferol (vitamin D_3)



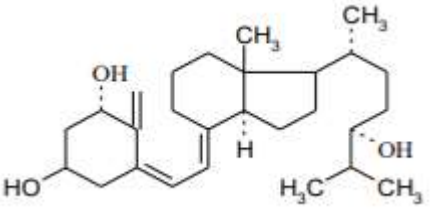
Colorless needle-like crystals. Insoluble in water, soluble in ethanol.

Oxydevit



White crystalline powder, odorless. Not stable to light and air oxygen. Melting point 133 - 140 °C. Practically insoluble in water, slightly soluble in ethanol.

Dioxidevit

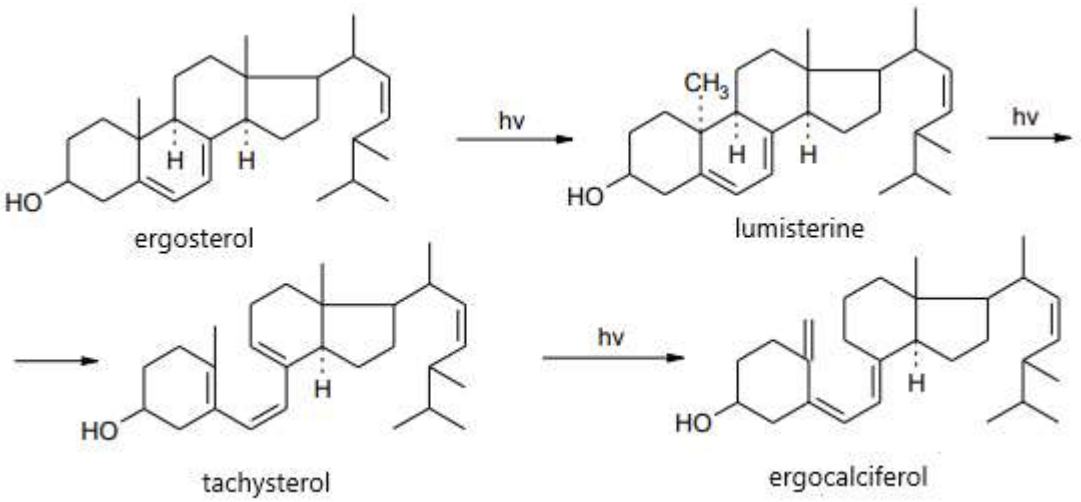


White crystalline powder without odor. Oxidizes with oxygen in air. Practically insoluble in water, slightly soluble in ethanol.

OBTAINING

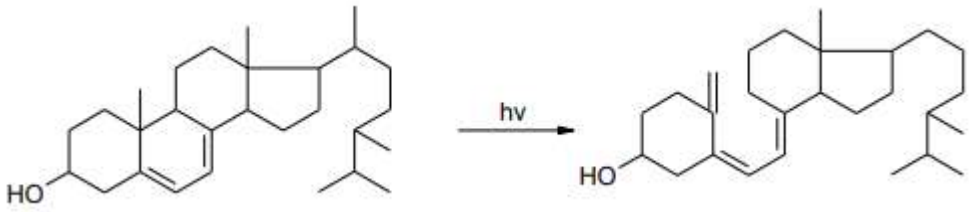
Ergocalciferol (vitamin D_2)

A cheap source of ergosterol is mycelium, a waste product of penicillin production, containing about 0.5% sterols. UV irradiation (photolysis) of ergosterol produces a number of products, including ergocalciferol:



Cholecalciferol (vitamin D_3)

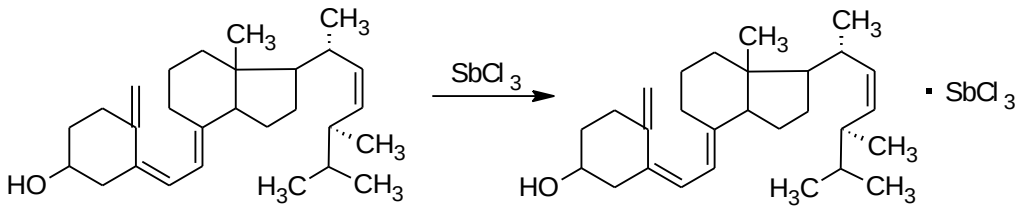
Vitamin D3 (cholicalciferol) is obtained from cholesterol in a similar way:



IDENTIFICATION

1. ***IR and UV spectrometry***
2. ***Reaction with antimony chloride***

   The detection reagent for ergocalciferol is a solution of trivalent antimony chloride. The complex salt has an orange-yellow coloration:

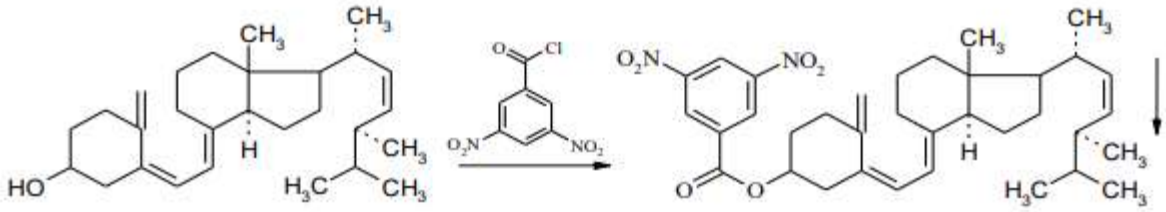


   The State Pharmacopoeia recommends adding 2% acetyl chloride solution to the reagent when performing the reaction for ergocalciferol; in this case an orange-pink coloration appears.

   The solution of oxidevit in chloroform after addition of two drops of antimony chloride solution acquires pink coloring.

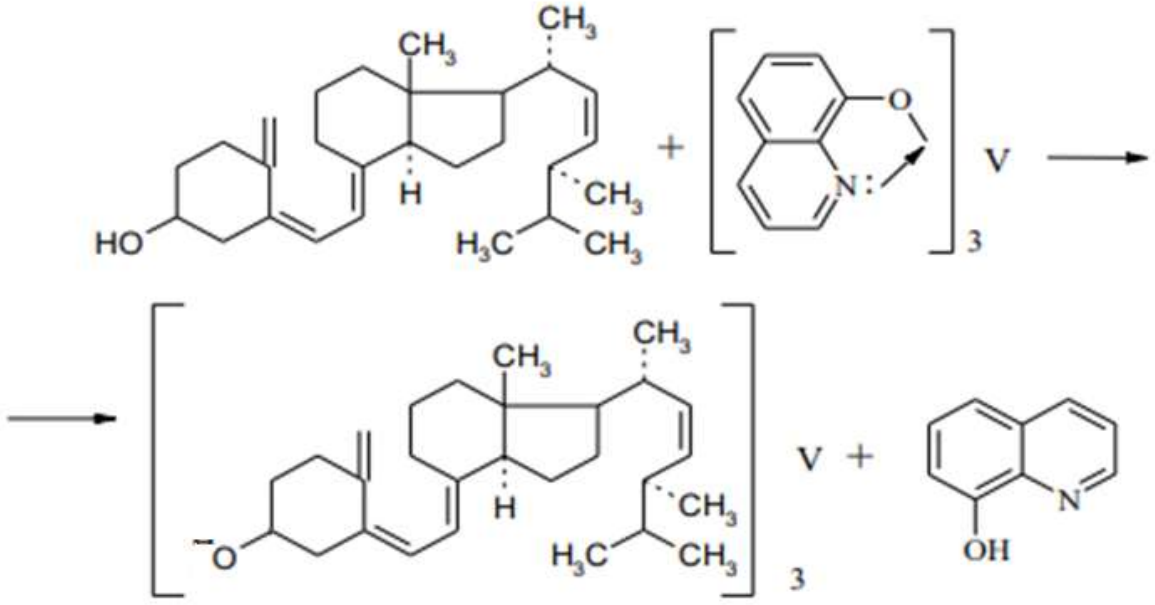
3. ***Reaction with 3,5-dinitrobenzoyl chloride***

Reaction with 3,5-dinitrobenzoyl chloride in anhydrous pyridine medium on heating gives ergocalciferyl-3,5-dinitrobenzoate, melting point of which is about 148 ºC.



4. ***Reaction with a benzene vanadium (III) quinolinate solution.***

When heated on a water bath to 60 ºC, the gray-green color of the reagent changes to red:

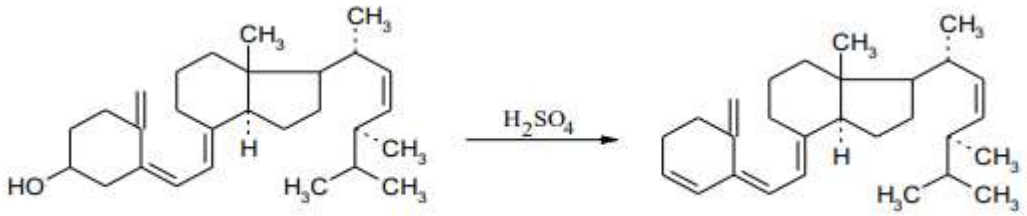


5. ***Interaction with acetic anhydride and sulfuric acid***

A solution of ergocalciferol in chloroform when shaken with acetic anhydride and sulfuric acid acquires a red color, changing to violet, and then to blue and finally to green.

6. ***Interaction with concentrated sulfuric acid***

The solution of ergocalciferol in ethanol after the addition of concentrated sulfuric acid acquires a red coloration:



QUANTIFICATION

Quantitative determination of ergocalciferol and oxydevit is performed by spectrophotometric method at wavelengths of 265, 251 and 265 nm, respectively. The solvent is ethanol. The activity of the drug is measured in international units (IU). The international unit of vitamin D is 0.025 μg or 0.000025 mg of chemically pure crystalline vitamin D.

STORAGE

Vitamin D in all its dosage forms should be stored according to List B, in geometrically closed, up to the top filled orange glass vials, in a dry place, protected from light, at a temperature not exceeding 10 ºC.

MEDICAL USE

The main function of vitamin D is that it regulates the metabolism of potassium and phosphorus in the body, facilitates the absorption of these substances in the intestine and their deposition in growing bones. Therefore, vitamin D is a specific remedy against rickets.

In addition to its main purpose as an anti-rachitic agent, vitamin D is effective for the prevention and treatment of all forms of lupus of the skin and diseases of the mucous membranes. In addition, vitamin D is used in some forms of tuberculosis.

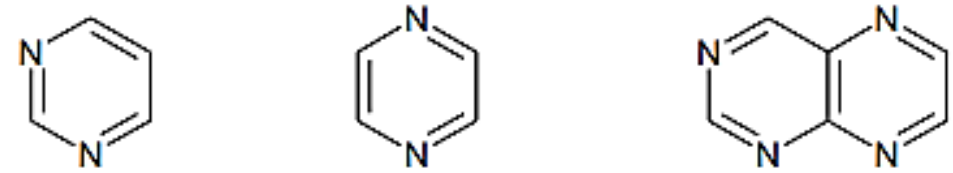
For the treatment of rickets ergocalciferol is prescribed about 100 000-15 000 IU daily for 30-60 days. Dihydrotachysterol is prescribed to regulate phosphorus-potassium metabolism in the form of 0.1% solution in oil 20 drops 3 times a day.

Ergocalciferol (vitamin D) is available in the following dosage forms: dragees, solution in oil, solution in alcohol.

PTERIN VITAMINS

General characteristic

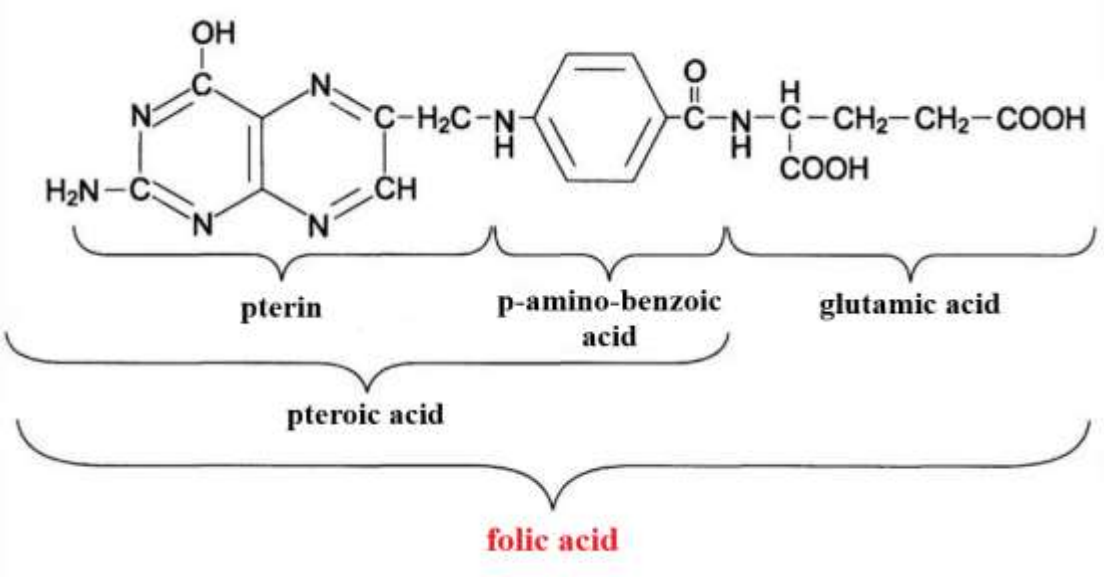
The chemical structure of folic acid was established in 1946. It is based on a heterocyclic system - pteridine, consisting of two fused heterocycles - pyrimidine and pyrazine:



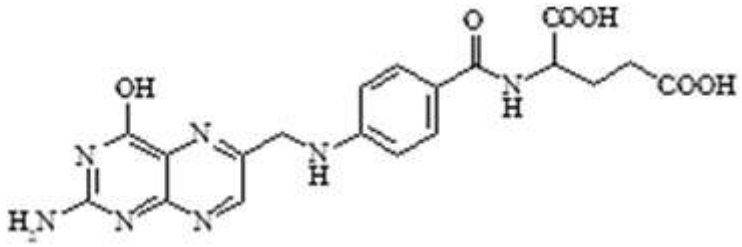
The pteridine derivative 2-amino-4-hydroxypteridine is known as pterin.

Pterin is part of the folic acid molecule, which is why this group of vitamins is called pteric. In addition to pterin, the folic acid molecule contains p-amino-benzoic acid and one or more glutamic acid residues. Pterin linked by a methyl group to p-aminobenzoic acid forms pteroic acid.

Folic (pteroylglutamic) acid, containing one glutamic acid residue, was first isolated from spinach leaves. Its rational name is N-{4'-[(2-amino-4-hydroxy-6-pteridyl)methyl]amino} benzoyl-L(+)-glutamic acid:

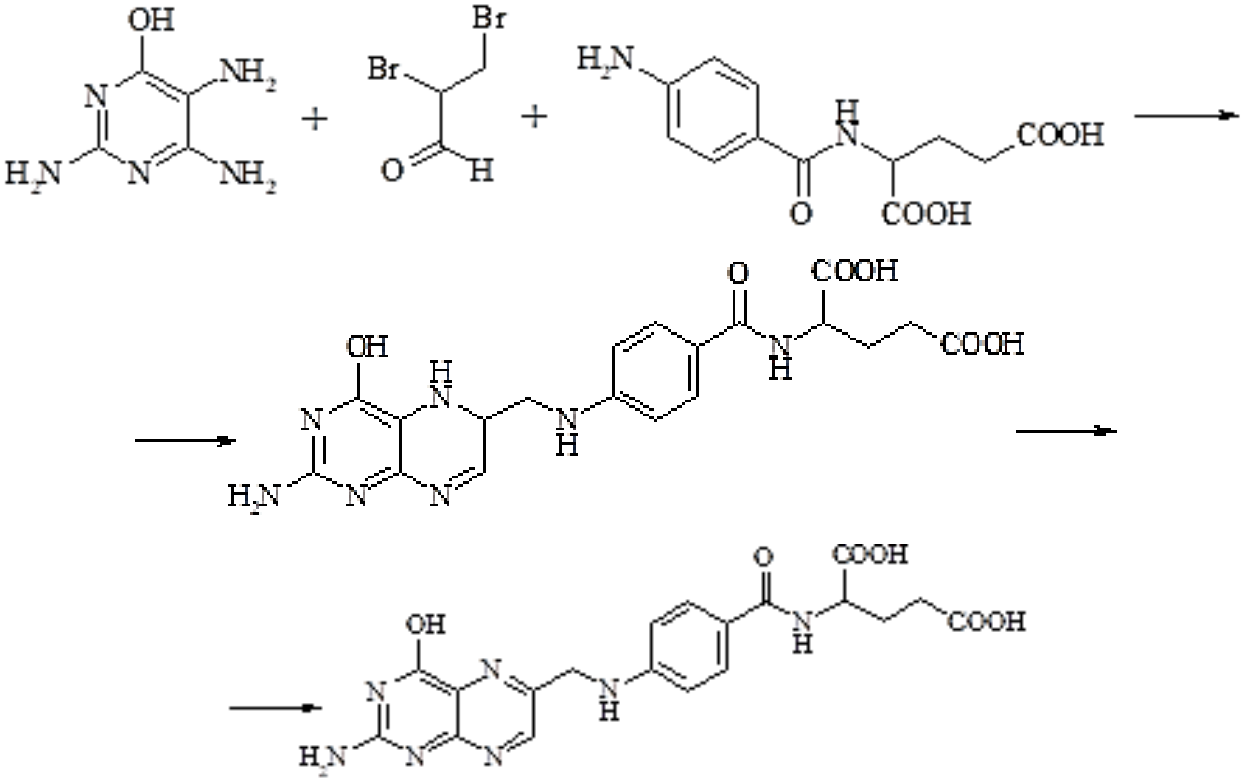


FOLIC ACID



OBTAINING

Folic acid is obtained by condensation of equimolecular amounts of 2,4,5-triamino-4-oxypyrimidine, α,β-dibromopropionic aldehyde and p-aminobenzoyl-L(+)-glutamic acid:



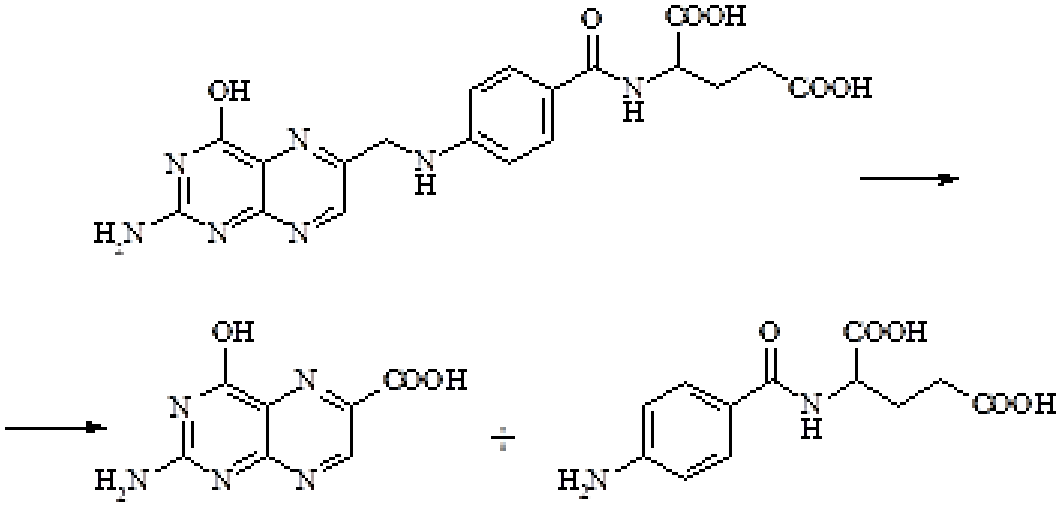
Physical properties

Folic acid is a crystalline substance of yellow or yellow-orange color. The drug is soluble in dilute mineral acids (better when heated) and easily soluble in solutions of caustic alkalis. In the molecule of folic acid there is an amino group in the pyrimidine cycle, which causes its weak basic properties. For this reason, folic acid does not form salts.

IDENTIFICATION

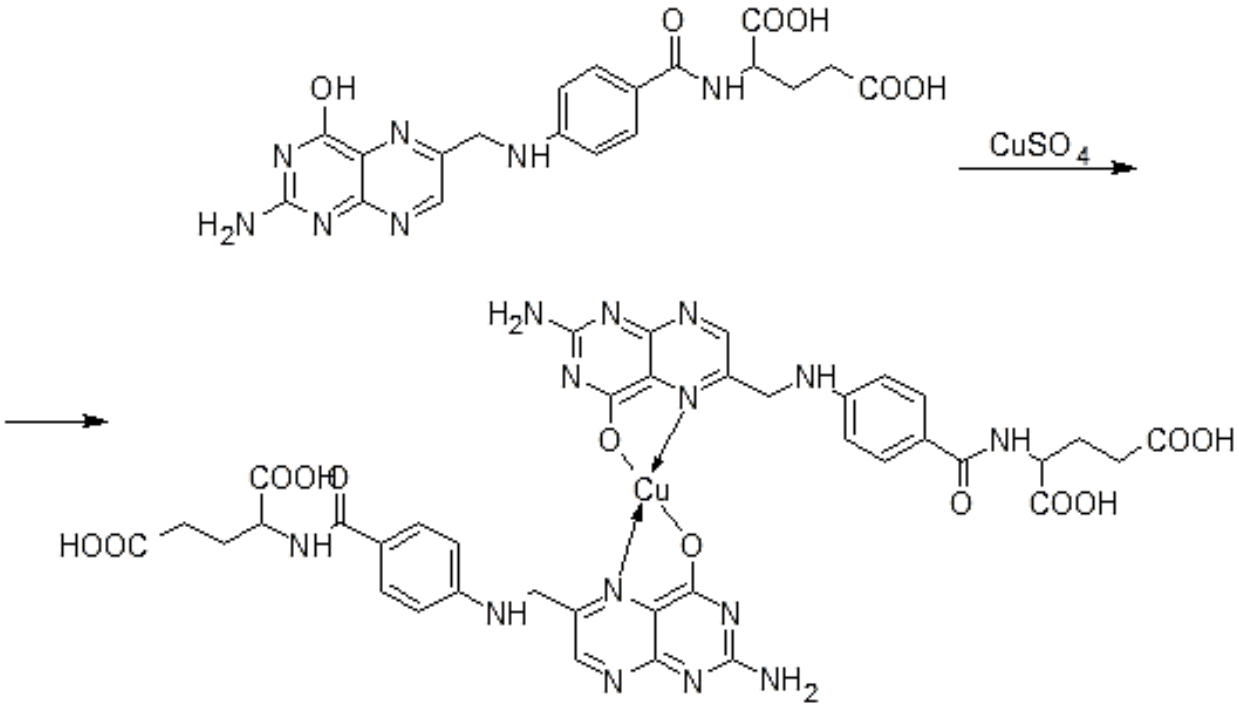
1. ***Ultraviolet spectrophotometry***
2. ***Oxidation.***

Folic acid is easily oxidized by potassium permanganate. The excess of the reagent is removed by the action of hydrogen peroxide solution, the mixture is filtered and the characteristic blue fluorescence of the filter in ultraviolet rays is observed. The test is based on the destruction of the folic acid molecule and the formation of p-aminobenzoylglutamic and pterinic acids:



3. *Complexation.*

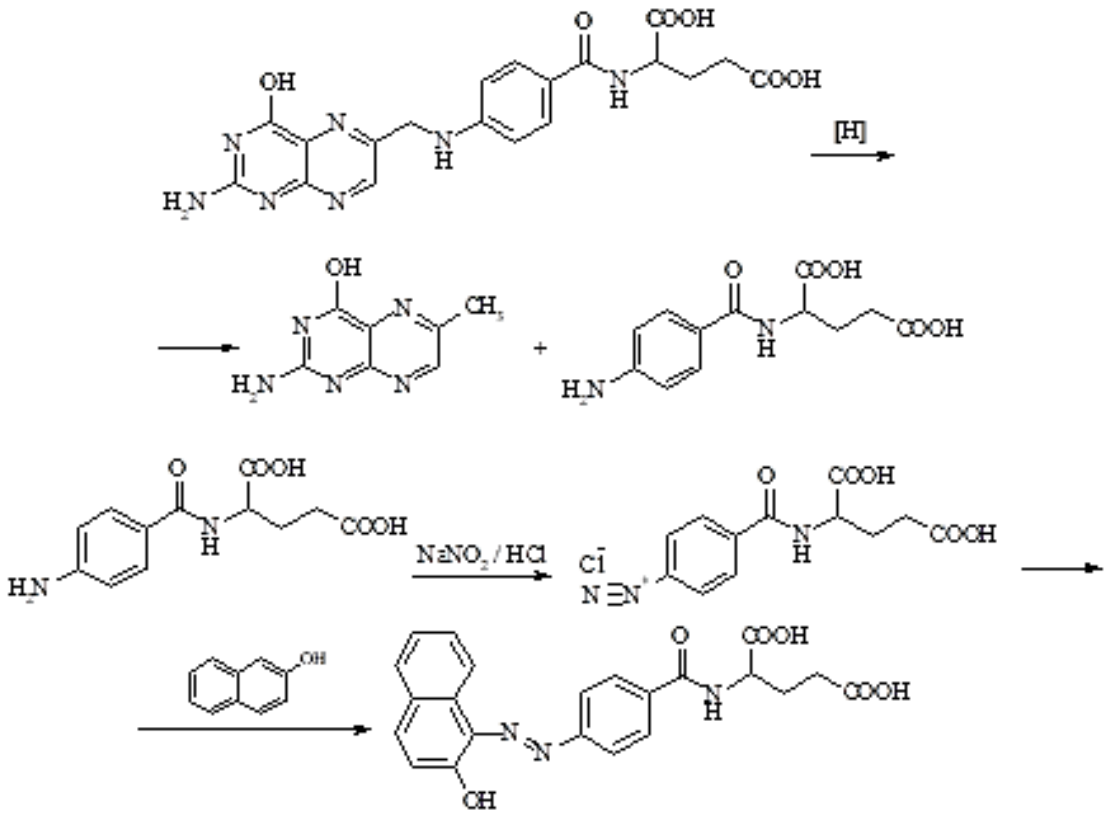
Lead acetate produces a lemon-yellow precipitate, copper (II) sulfate - green, silver nitrate - yellow-orange, cobalt nitrate - dark yellow, iron (III) chloride - red-yellow, zinc sulfate - white.



4. *Formation of azo dye*

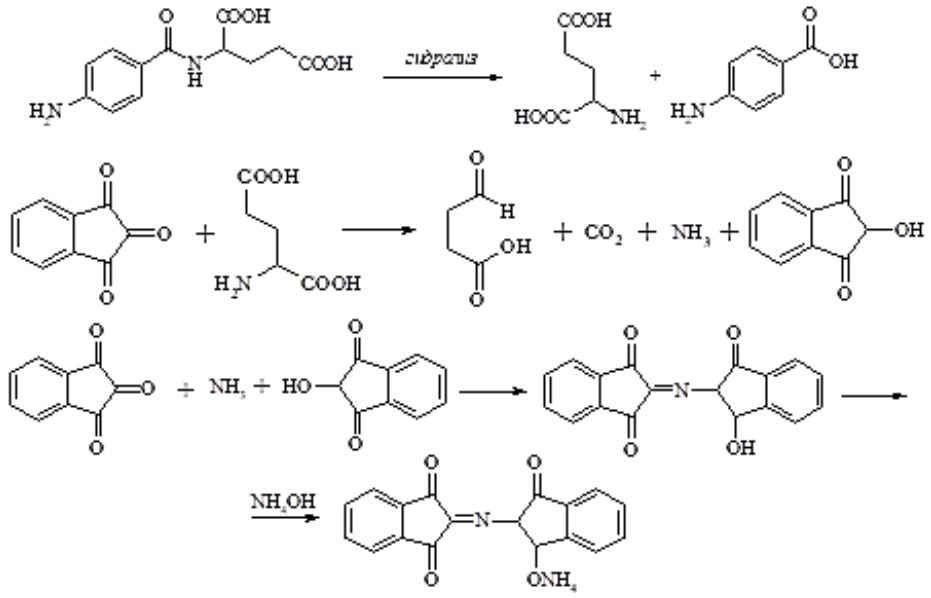
This reaction are based on the preliminary reduction of folic acid by zinc in alkaline medium to 6-methylpterin and p-aminobenzoylglutamic acid.

Subsequent diazotization and treatment of p-aminobenzoylglutamic acid with an alkaline solution of β-naphthol gives a red-colored azo dye:



5. *Detection of glutamic acid after hydrolysis*

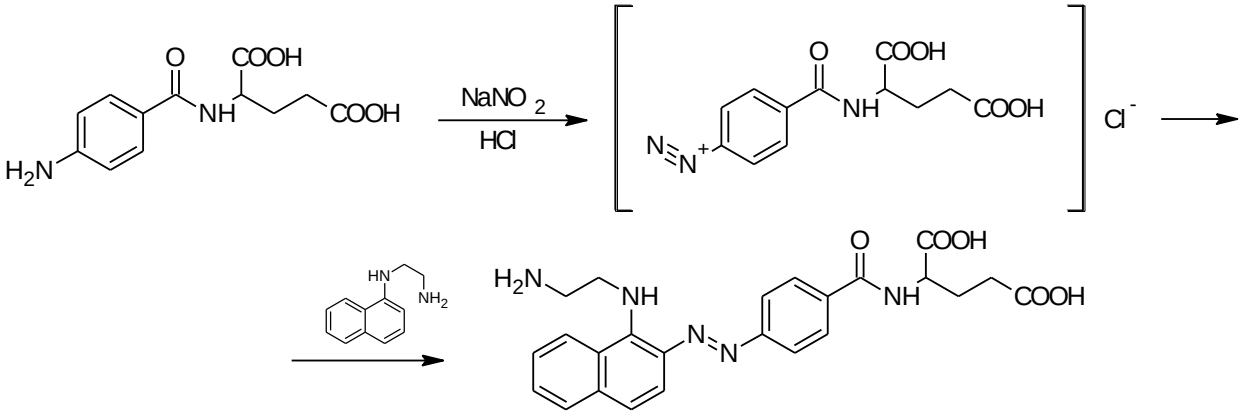
Glutamic acid obtained after hydrolysis of p-aminobenzoyl-glutamic acid is α-amino acid, which interacting with ninhydrin gives a condensation product of blue color, the intensity of which increases with the addition of ammonia solution:



QUANTIFICIFICATION

1. Photocolorimetric determination.

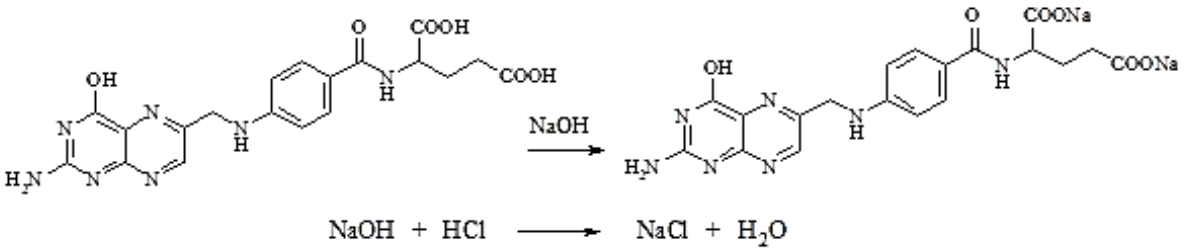
The determination is based on the preliminary oxidation of the preparation with potassium permanganate to pterinic and p-aminobenzoylglutamic acids. The p-aminobenzoylglutamic acid is then diazotized with sodium nitrite solution and combined with N-(1-naphthyl) ethylenediamine:



The color intensity of the formed azo dye is measured on a photoelectrocolorimeter

2. Neutralization method.

The reverse alkalimetric determination is used. A sample of the preparation is dissolved in an excess of 0.1 M sodium hydroxide solution and then titrated with 0.1 M hydrochloric acid solution. A mixed indicator (phenolphthalein with methylene blue) is used. In parallel, the content of free p-aminobenzoylglutamic acid is determined, which is extracted from folic acid with ethanol.

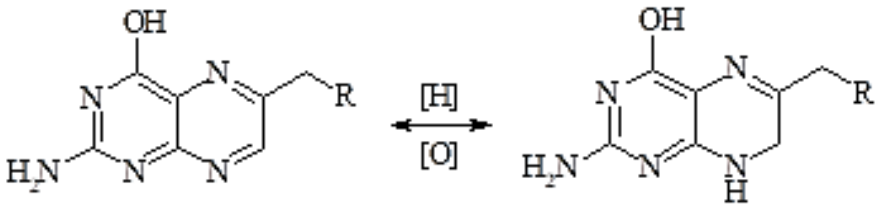


3. Spectrophotometric determination.

Spectrophotometric determination of folic acid can be performed at a wavelength of 365 nm (solvent 0.1 M sodium hydroxide solution) or 320 nm (solvent 5 M sulfuric acid solution).

4. Polarographic determination

Polarographic determination of folic acid is based on its ability to be easily reduced in sodium carbonate medium to 7,8-dihydrofolic acid. The reverse process is easily accomplished even under the influence of air oxygen:



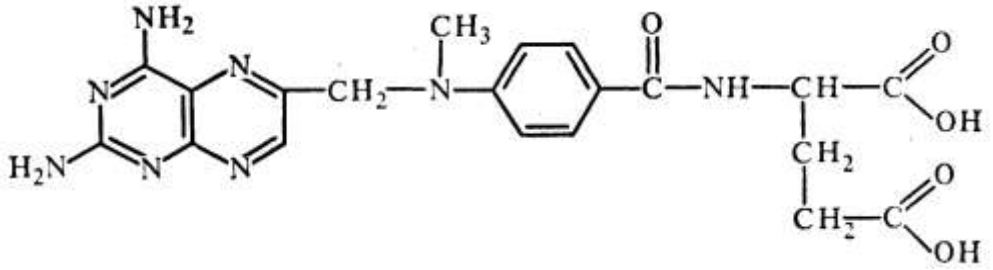
STORAGE

The drug is stored in well-corked containers, in a dry, dark place, as folic acid is hygroscopic and decomposes under the influence of light. This process inactivates folic acid and produces fluorescence due to the formation of pterinic acid. More stable solutions at pH 5.0-10.0.

MEDICAL USE

The use of folic acid in medicine is caused by the important role of this vitamin in the process of hematopoiesis, metabolism in cells (including choline). Together with vitamin B_{12} folic acid stimulates erythropoiesis, is involved in the synthesis of amino acids.

METHOTREXATE



A structural analogue and antagonist of folic acid is the drug methotrexate.

PHYSICAL PROPERTIES

Yellow or orange-yellow fine crystalline powder. Practically insoluble in water, ethanol, ether. Easily soluble in solutions of alkalis and alkali metal carbonates. Specific rotation from -19 to +24° (1% solution of the drug in sodium carbonate solution).

Methotrexate is practically insoluble in water, ethanol, ether and chloroform. However, it is easily soluble in solutions of alkalis and alkali metal carbonates.

IDENTIFICATION

1. ***IR spectroscopy***
2. ***UV spectrometry***
3. ***Thin layer chromatography***

4. Oxidation

Methotrexate, like folic acid is oxidized by potassium permanganate, producing blue fluorescence in UV light.

QUANTIFICATION

Quantification can be performed by high-performance liquid chromatography.

STORAGE

Methotrexate is stored in tightly sealed containers protected from light at temperatures from +5 to +10 oC. Methotrexate should be handled with care, avoiding contact with skin and mucous membranes.

MEDICAL USE

Methotrexate is used for treatment of acute leukemia, malignant diseases of uterus, mammary gland, lung cancer and others.