Ministry of Health of the Russian Federation
Volgograd State Medical University

Department of Pharmaceutical and Toxicological Chemistry

SPECIAL PHARMACEUTICAL CHEMISTRY

Isoalloxazine derivatives.
Pyrrole derivatives.

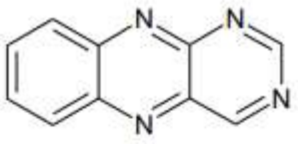
Lesson 17

VII term

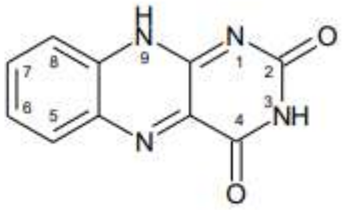
Volgograd, 2023

Isoalloxazine derivatives

The heterocyclic system isoalloxazine like pteridine includes two heterocycles: pyrazine and pyrimidine, but it also contains a benzene cycle, i.e. it is a partially hydrogenated derivative of benzopteridine. The pyrimidine nucleus of isoalloxazine has the character of a lactam cycle:



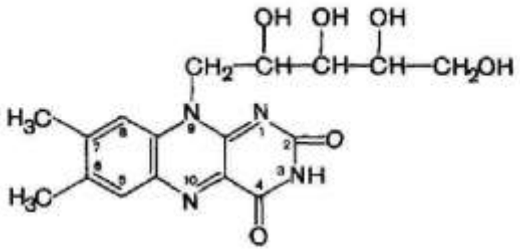
benzopteridine



isoalloxazine

Riboflavin

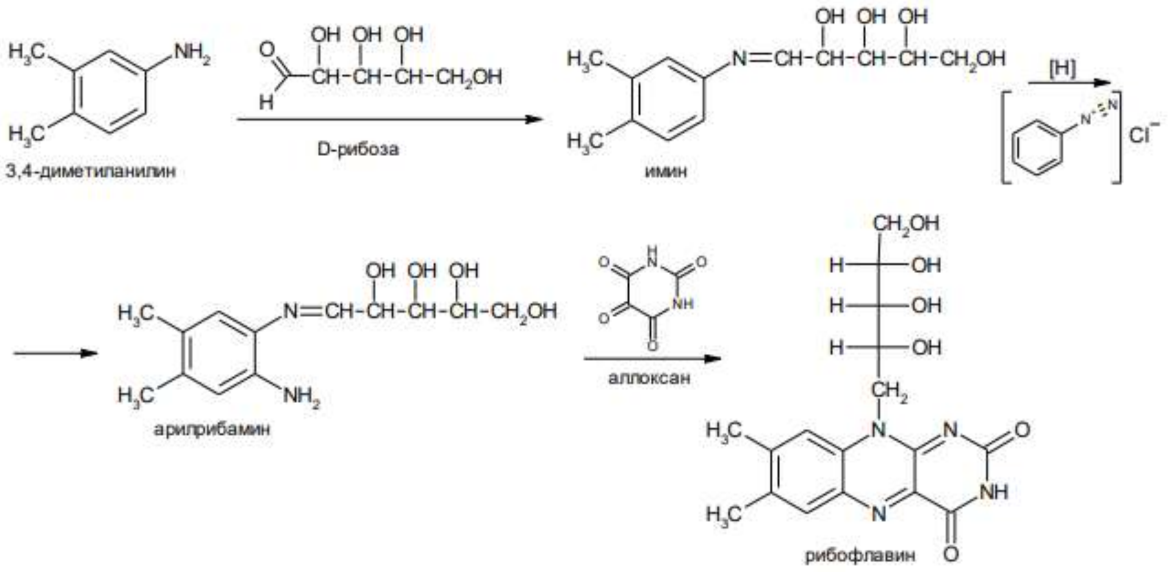
Vitamin B_2 (riboflavin)



Obtaining

Riboflavin can be obtained from animal or vegetable raw materials. However, this process is labor-intensive and yields very low yields. To extract 1.0 g of riboflavin, it is necessary to process 5.4 tons of whey.

In industry, riboflavin is synthesized by condensation of 3,4-dimethylaniline with D-ribose. The resulting imine is hydrogenated, then arylribamine is formed through an azo-combination reaction (with reduction of the azo group) and condensed with alloxane:



Currently, riboflavin is produced by microbial synthesis. The use of co-modern advances in the field of microbial physiology and genetic engineering has made it possible to increase the yield of riboflavin biosynthesis by 4-5 thousand times.

Physical properties

Yellow-orange crystalline powder with a weak specific odor. Not stable in the light. Specific rotation from –115 to –135° (0.5% solution in an alcoholic solution of potassium hydroxide).

Riboflavin is practically insoluble in water, practically insoluble in ethanol and chloroform. Riboflavin is soluble in solutions of acids and alkalis, because is an amphoteric compound.

Identification

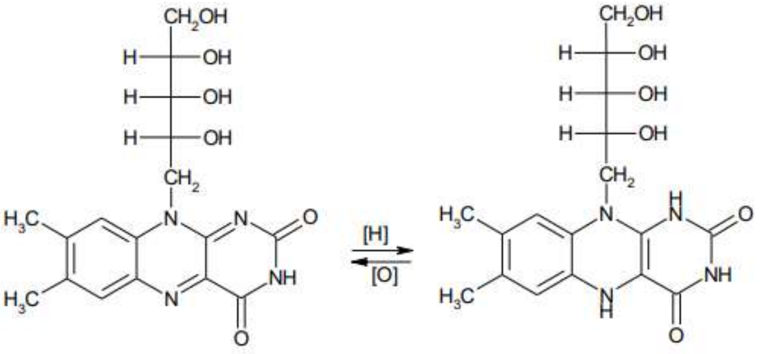
1. ***IR spectroscopy***

   The IR spectrum must match the spectrum obtained with its standard sample or the reference spectrum

2. ***UV spectrometry***

3. ***Color and fluorescence of solution***

   The authenticity of *riboflavin* is determined by its characteristic bright greenish-yellow color and intense green fluorescence of the aqueous solution (in ultraviolet radiation). Fluorescence disappears when solutions of hydrochloric acid or alkali are added. If sodium hydrosulfite is added to an aqueous solution of riboflavin, the color and fluorescence disappear due to the formation of leucoriboflavin:



riboflavin leucoriboflavin

4. ***Interaction with concentrated sulfuric acid***

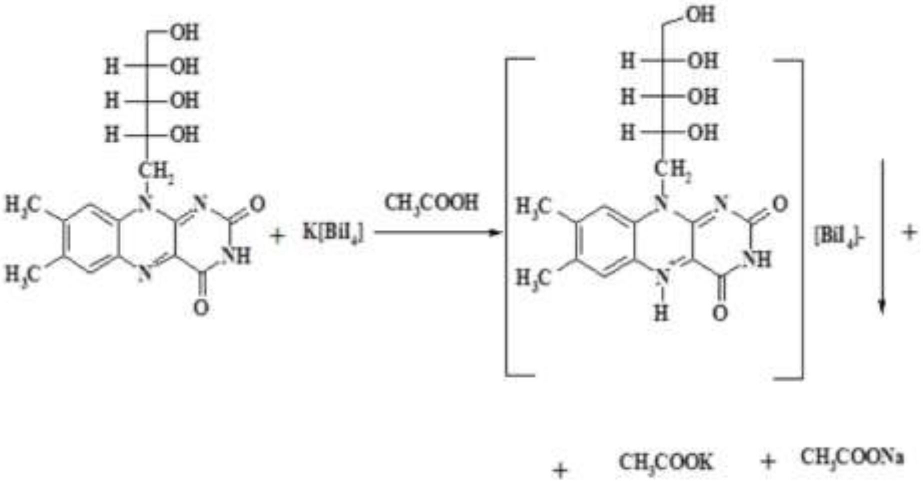
When wetted with concentrated sulfuric acid, a grain of riboflavin acquires a cherry-red coloration.

5. ***Interaction with ninhydrin solution***

A solution of ninhydrin forms a green color in the presence of riboflavin when boiled in an alkaline medium.

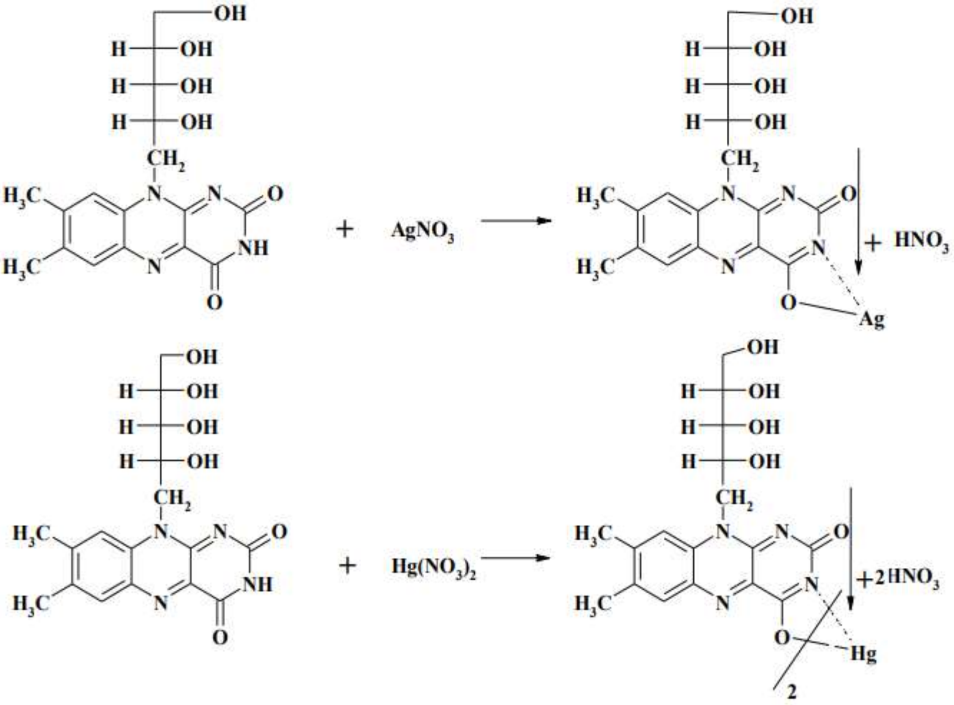
6. ***Interaction with common alkaloid reagents***

For example, it forms an orange precipitate with Dragendorf's reagent



7. ***Complexation***

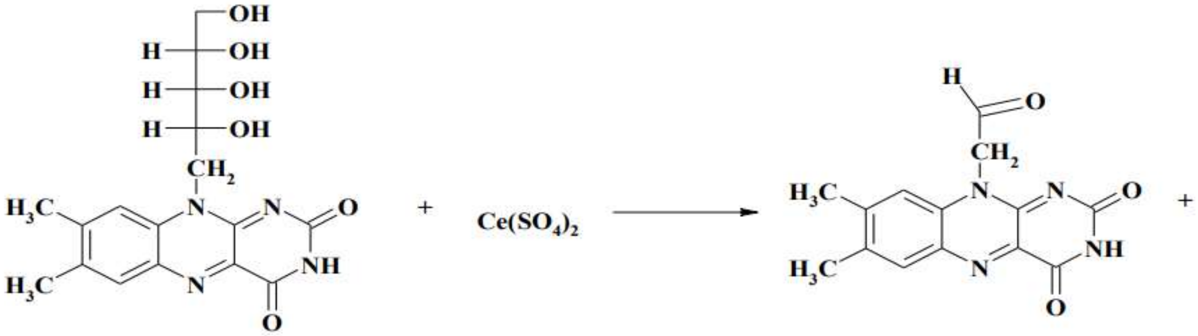
With metal salts (silver, cobalt, copper, mercury, etc.) riboflavin forms insoluble colored complex compounds. For example, with silver nitrate solution - orange-red, turning to red, and with mercury (II) salts - orange color.



Quantitation

1. ***Cerimetry with iodometric termination*.**

   An excess of titrated solution of cerium (IV) sulfate is added to the drug solution, and the ribityl fragment is oxidized. Potassium iodide solution is added to the excess of unreacted cerium sulfate and the released iodine is titrated with sodium thiosulfate (indicator - starch):



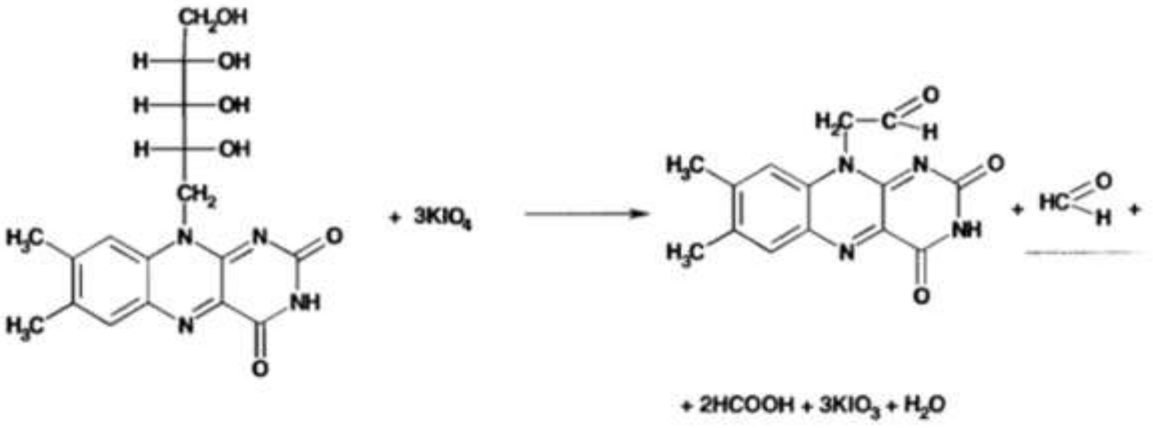
$$+ \ 2\,HCOOH + HCOH + Ce_2(SO_4)_3$$

$$2\,Ce(SO_4)_2 + 2\,KI \longrightarrow I_2 + K_2SO_4 + Ce_2(SO_4)_3$$

$$I_2 + Na_2S_2O_3 \longrightarrow NaI + Na_2S_4O_6$$

2. ***Alkalimetry after oxidation***

   Oxidation with 0.02 M potassium periodate solution in neutral medium at room temperature to form formic acid:

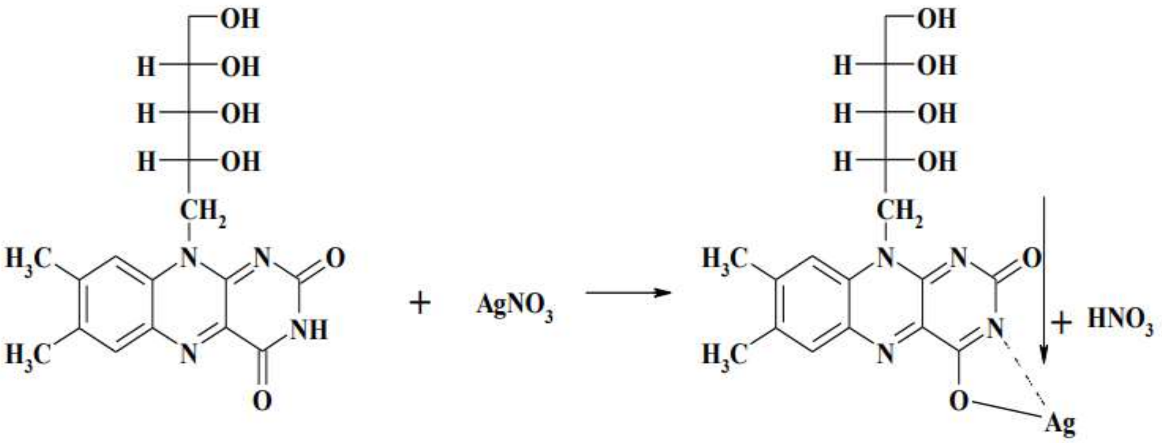


$$2HCOOH + 2NaOH \longrightarrow 2HCOONa + 2H_2O$$

The released formic acid is titrated alkalimetrically.

3. ***Alkalimetric determination after reaction with silver nitrate***

Under the action of silver nitrate, hydrogen substitution of the imino group by silver ion occurs with the release of an equivalent amount of nitric acid, which is titrated with alkali (indicator bromthymol blue).



$$HNO_3 + NaOH \longrightarrow NaNO_3 + H_2O$$

4. ***Kjeldahl method***

Based on mineralization of riboflavin with concentrated sulfuric acid:

From the formed ammonium hydrosulfate ammonia is extracted by the action of sodium hydroxide on heating:

$$\text{Riboflavin} + H_2SO_{4\,\text{concentrated}} \rightarrow NH_4HSO_4 + CO_2 + H_2O$$

$$NH_4HSO_4 + NaOH \rightarrow NH_3 + Na_2SO_4 + H_2O$$

$$NH_3 + H[B(OH)_4] \rightarrow NH_4[B(OH)_4]$$

$$NH_4[B(OH)_4] + HCl \rightarrow NH_4Cl + H_2O + H_3BO_3$$

Ammonia is absorbed with boric acid solution, and the resulting complex is titrated with hydrochloric acid in the presence of a mixed indicator.

5. *UV spectrophotometry*

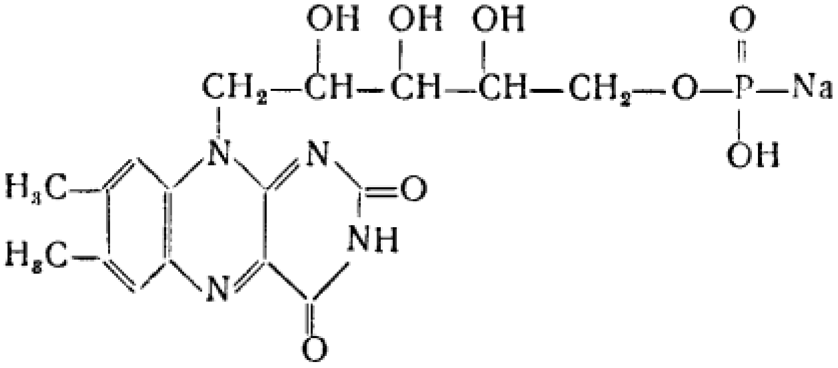
Storage

Riboflavin should be stored in well-corked orange glass jars, taking into account its property of easy oxidation and decomposition under the action of light with the formation of biologically inactive lumichrome and lumiflavin.

Medical use

Riboflavin makes up for vitamin B_2 deficiencies in the body. It is especially important for the normal function of vision. It is administered orally in tablets and dragees for hypo- and avitaminosis, various eye diseases, long non-healing wounds and ulcers, radiation sickness, Botkin's disease, etc.

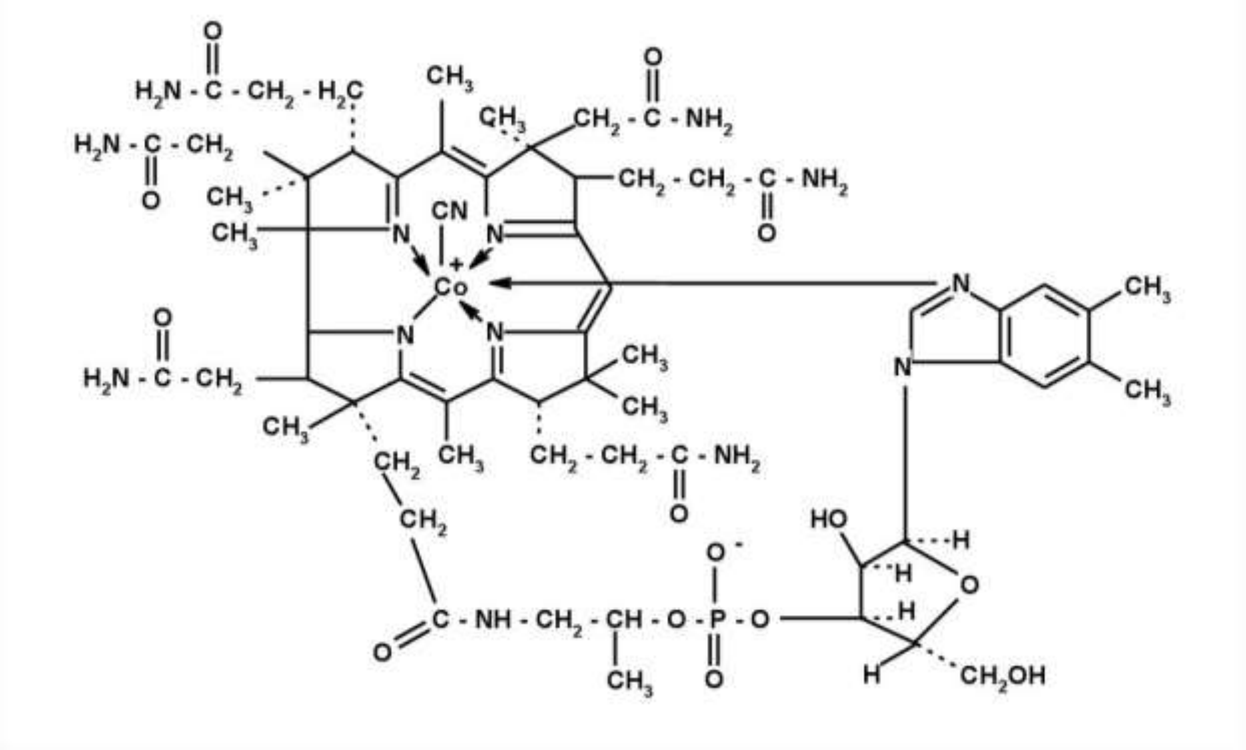
Riboflavin mononucleotide



Yellow-orange crystalline powder, odorless. Unstable in the light. Hygroscopic. Specific rotation from +37 to +43° (1.5% solution in 5 M hydrochloric acid solution).

Pyrrole derivatives

Cyanocobalamin



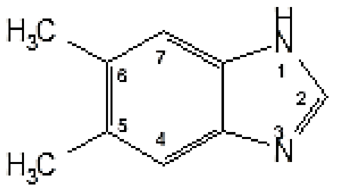
Sources

The most vitamin B12 is found in animal products such as liver, beef, pork, lamb, eggs and turkey breast. Other important foods containing vitamin B12 are milk, cheese, beef kidneys, cod, chum salmon, mackerel, shrimp and mussels.

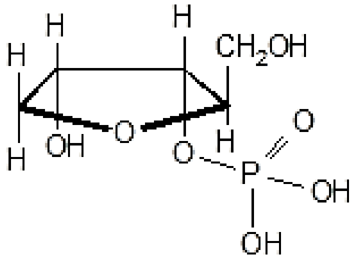
Structure

The cyanocobalamin molecule consists of two related parts: the cobalt complex of the benzimidazole nucleotide and the macrocyclic corrin system, i.e., it is Coα-[α(5,6-dimethylbenzimidazolyl)]-Coβ-cyanocobamide.

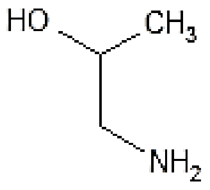
The nucleotide includes the following linked structural elements: 5,6-dimethylbenzimidazole, α-D-ribofuranose phosphate (phosphoric ester of α-D-ribose), and D-1-aminopropanol-2:



5,6-dimethylbenzimidazole



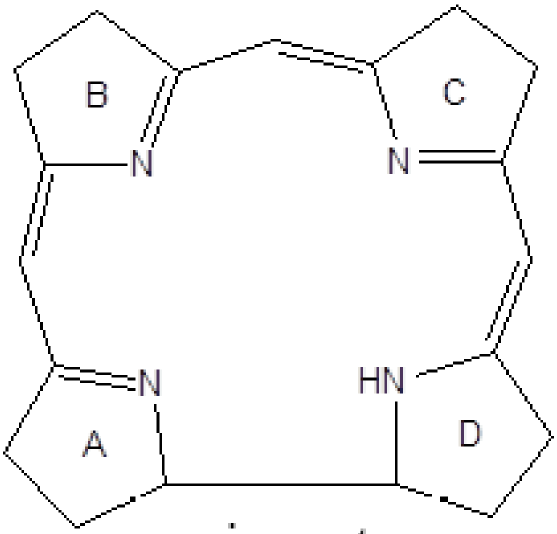
α-D-ribofuranose phosphate



D-1-aminopropanol-2

The nucleotide is connected to the corrin system by a peptide bond. The nitrogen atom of 5,6-dimethylbenzimidazole in position 3 is linked by a coordination bond with a cobalt (III) atom. Cobalt forms a chelate compound with the cyano group and with nitrogen atoms of hydrogenated pyrrole cycles of the corrin system.

The corrin system has six conjugated double bonds and nine asymmetric carbon atoms:



corrin system

Obtaining

Cyanocobalamin is commercially obtained by bacterial fermentation.

Physical properties

Crystals or crystalline powder of dark red color, odorless. Hygroscopic. Decomposes at a temperature of 200 °C.

Cyanocobalamin is moderately soluble in water and ethanol, practically insoluble in ether and chloroform.

Identification

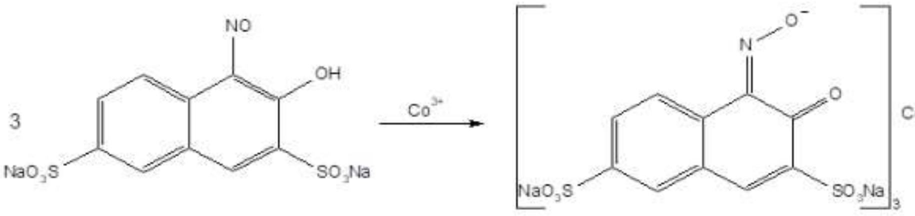
1. ***UV spectrophotometry***

2. ***Drip method***

   If oxalic acid is added to an aqueous solution of cyanocobalamin in a test tube and carefully heated, a blue stain appears on filter paper moistened with a mixture of copper acetate solution and benzidine, with which the test tube is covered.

3. ***Determination of cobalt***

    - Cyanocobalamin is fused with potassium hydrosulfate. The cobalt ion is then exposed. The reaction is based on the formation of a red-colored intra-complex compound of cobalt with nitroso-R-salt (dinuclear salt of 1-nitroso-2-naphthol-3,6-disulfonic acid):



    - After evaporation and calcination of cyanocobalamin with potassium sulfate and 2 drops of sulfuric acid. A saturated solution of ammonium rhodanide in acetone is added to the residue; a blue-green coloring appears:

$$Co^{2+} + 2NH_4NCS \longrightarrow Co(NCS)_2 + 2NH_4^+$$

Quantificification

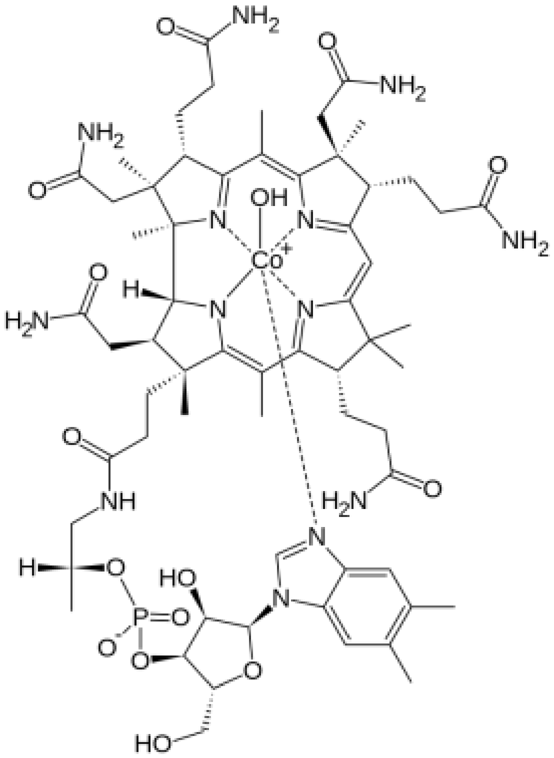
1. ***UV spectrophotometry***
2. ***Spectrophotometry***
3. ***Atomic absorption method***

Storage

Cyanocobalamin is stored, taking into account hygroscopicity, in well-corked containers, observing aseptic conditions, because microflora absorbs vitamin B_{12}. It is destroyed by the action of oxidizing and reducing agents. It is also necessary to protect from the action of light.

Medical use

Cyanocobalamin is prescribed for treatment of malignant anemia, various types of anemia, radiation sickness, liver diseases, nervous system, skin and other diseases.

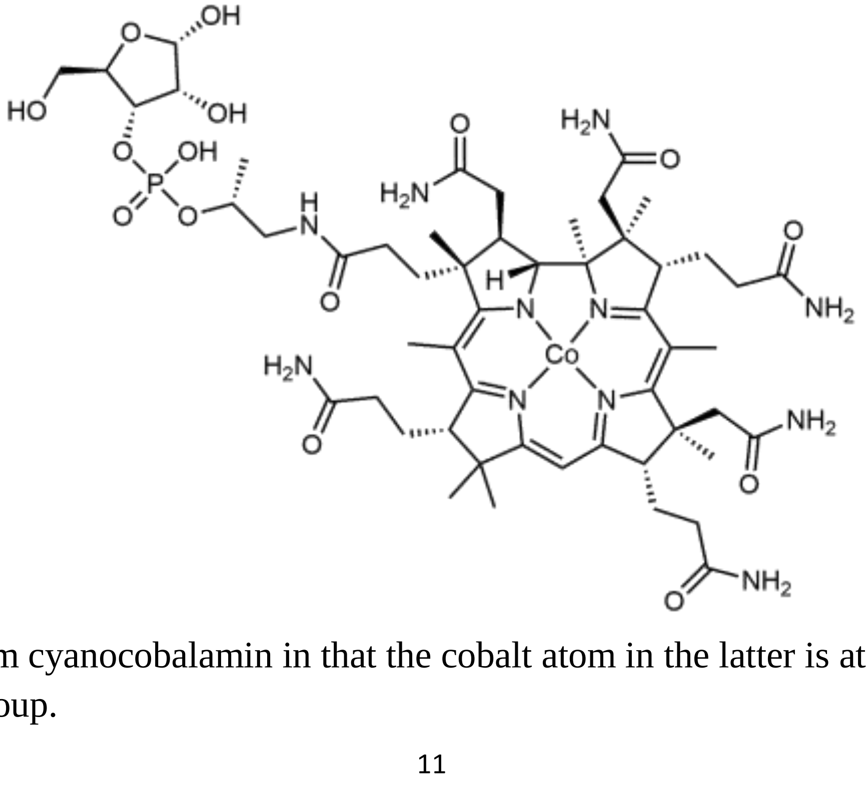


Hydroxocobalamin ((OH)Kbl, or B12a) is the natural form of vitamin B12, and the main member of the cobalamin family. It is this form of the vitamin that is mass produced commercially by bacteria.

A metabolite of cyanocobalamin. It is involved in the process of hematopoiesis, formation of epithelial cells, and functioning of the nervous system.

Cobalamide (Adenosylcobalamin (AdoCbl))

A vitamin also known as adenosylcobalamin and dibencoside. Together with methylcobalamin (MeCbl), it is one of the active forms of vitamin B12.[1] As a dietary supplement, it is available in tablet form, but (unlike cyancobalamin, methylcobalamin and hydroxocobalamin) it cannot be injected directly into the bloodstream.



It differs from cyanocobalamin in that the cobalt atom in the latter is attached to the cyano group.