Ministry of Health of the Russian Federation

Volgograd State Medical University

Department of Pharmaceutical, Toxicological Chemistry
Pharmacognosy and Botany

SPECIAL PHARMACEUTICAL CHEMISTRY

Corticosteroids

Lesson 5

IX term

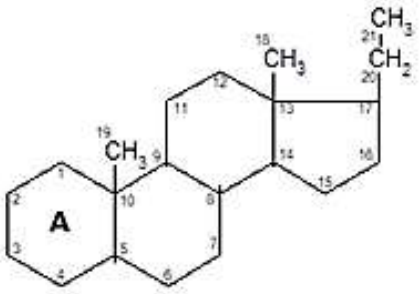
Volgograd, 2024

General characteristic

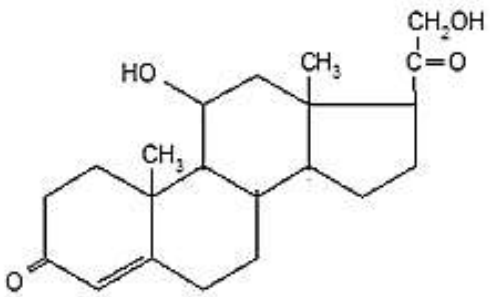
The adrenal glands are distinguished into cortical and medullary layers. Twenty-nine crystalline steroidal compounds called corticosteroids have been isolated from the adrenal cortex.

Corticosteroids [Latin *cortex* - cortex, Greek *stereos* - solid and *eidos* - species] are vertebrate hormones produced by the adrenal cortex and having a pronounced effect on water-salt, carbohydrate and protein metabolism.

They are all pregnan derivatives containing a ketone-containing group in the A ring and a ketone or dioxyacetone chain at position 17; some of them have a carbonyl or hydroxyl group at C11.



pregnan



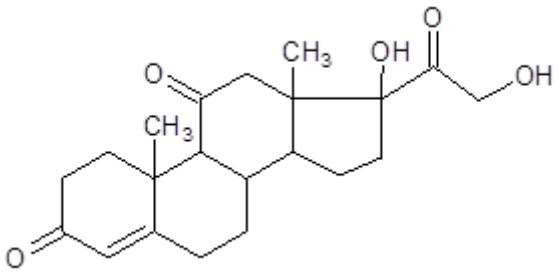
corticosterone

The main corticosteroids are hydrocortisone, corticosterone, and aldosterone. Their production is regulated by corticotropin-releasing hormone (corticoliberin), which is secreted by hypothalamic cells and transported with blood through portal vessels to the pituitary gland, where it causes secretion of adrenocorticotropic hormone, and the latter stimulates secretion of corticosteroids by the adrenal cortex.

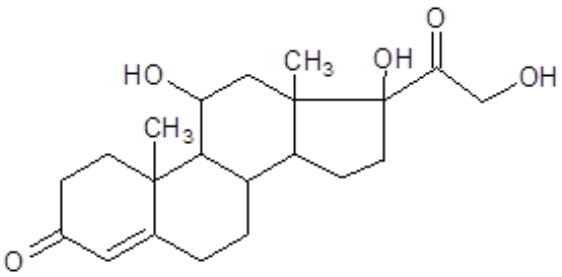
According to their action on the body, corticosteroids are conditionally divided into 2 groups:

- mineralocorticoids,
- glucocorticoids.

Glucocorticoids affect the metabolism of carbohydrates (in particular, increase blood glucose content), proteins and fats, have catabolic (promote protein breakdown) effects, have anti-inflammatory and immunosuppressive properties, cause involution of lymph nodes and thymus and lymphopenia. High concentration of glucocorticoids in the blood is observed under stress. The main glucocorticoids synthesised in the human body are hydrocortisone and cortisone.



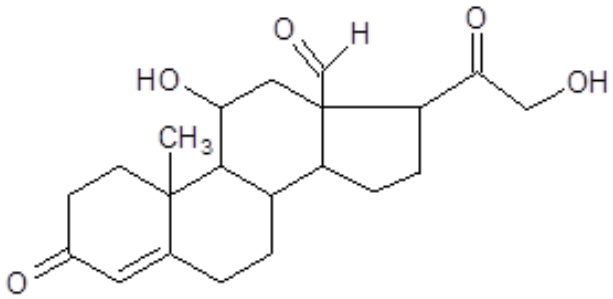
Cortisone



Hydrocortisone

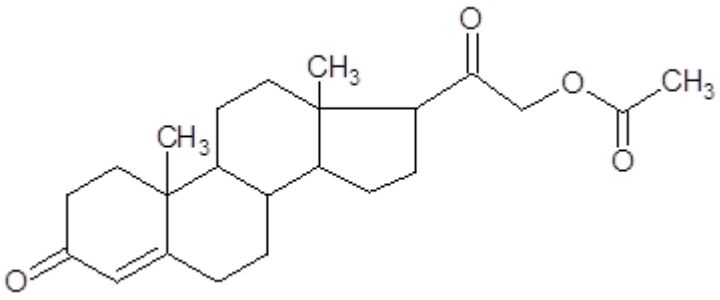
Because the most important glucocorticoid, hydrocortisone, has a hydroxyl group at the 17-position, they are sometimes considered 17-oxycorticosteroids.

Mineralocorticoids regulate electrolyte balance by stimulating potassium excretion and sodium retention. Mineralocorticoids such as aldosterone involved in the regulation of electrolyte metabolism do not contain an oxygen function at the 17-carbon atom and are therefore 17-deoxycorticosteroids.



Aldosterone

DEOXYCORTICOSTERONE ACETATE

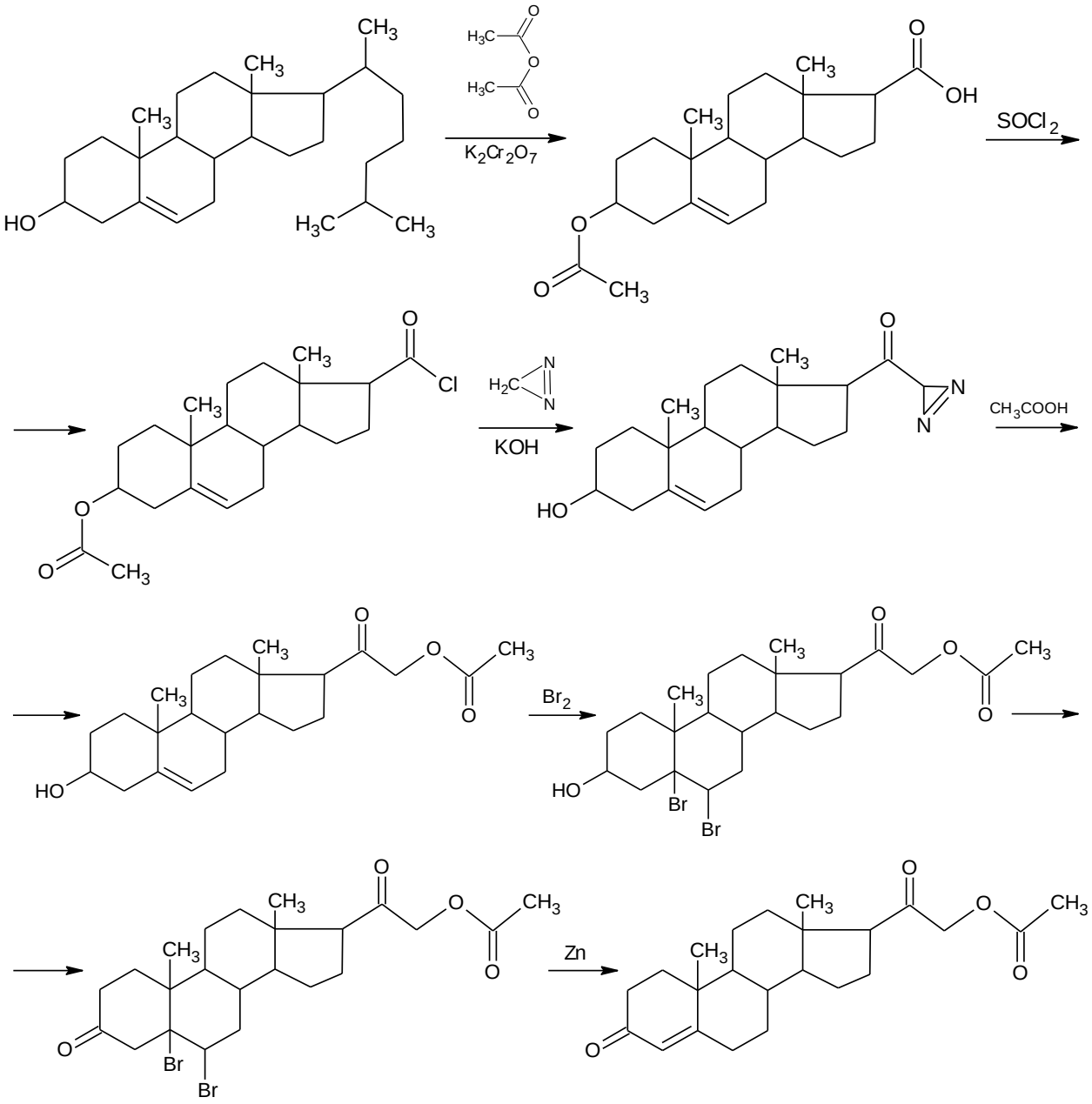


Obtaining

The source of deoxycorticosterone acetate is the adrenal glands of slaughtered cattle.

Deoxycorticosterone was obtained for the first time in 1937 not by organ isolation but by semi-synthetic synthesis (Steiger, Reichstein). Synthesis of

deoxycorticosterone acetate from cholesterol is multistep and includes acetylation, oxidation steps to obtain 3-acetoxyethiocholic acid, which is the initial product of deoxycorticosterone acetate production.



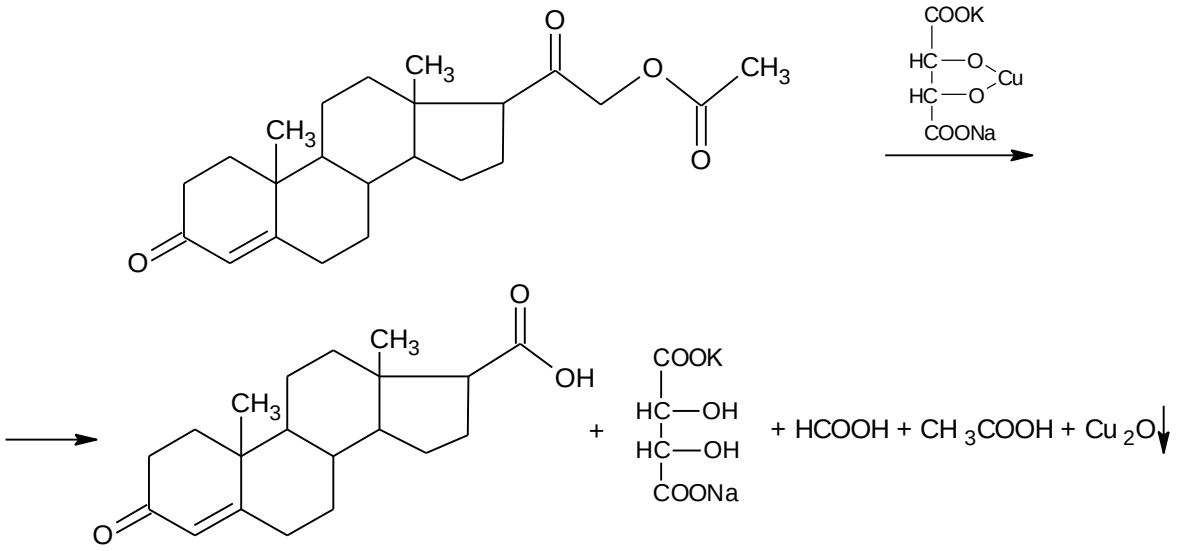
Physical properties

White or white odourless crystalline powder with a slight creamy tinge. Melting point 155-160°C. Specific rotation from +176 to +184° (1% solution in chloroform). Practically insoluble in water, hardly soluble in 95% alcohol, easily soluble in chloroform, soluble in acetone.

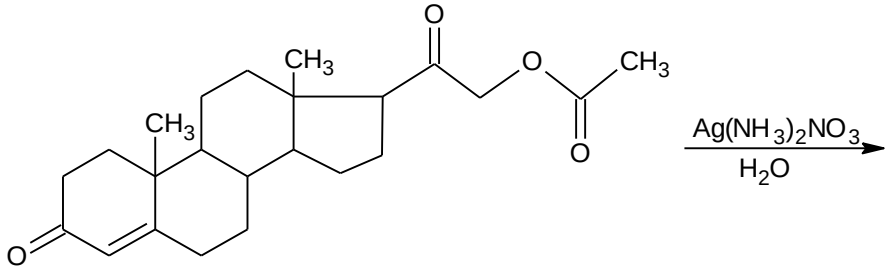
Identification

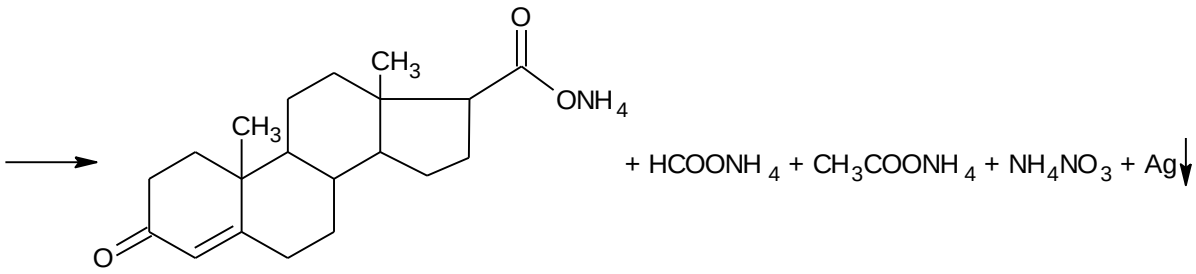
1. ***Infrared spectrometry.*** The infrared spectrum of deoxycorticosterone acetate should correspond to the spectrum of the standard sample or to the attached spectrum in the regulatory documentation.
2. ***UV spectrometry.*** The UV spectrum has a characteristic absorption band with a maximum at 240 nm, which is determined by the presence of the conjugated system of keto group and double bond.
3. ***Reaction for steroid cycle with concentrated sulphuric acid***. The drug gives a cherry colouring with green-brown fluorescence. After cooling and addition of chloroform, the lower layer stains yellow and the upper layer green.
4. ***Reactions with α-ketol groups***

   a) Interaction with Fehling's reagent. The drug is dissolved in methyl alcohol, added Fehling's reagent and heated in a water bath. An orange-red precipitate of copper (I) oxide is formed.

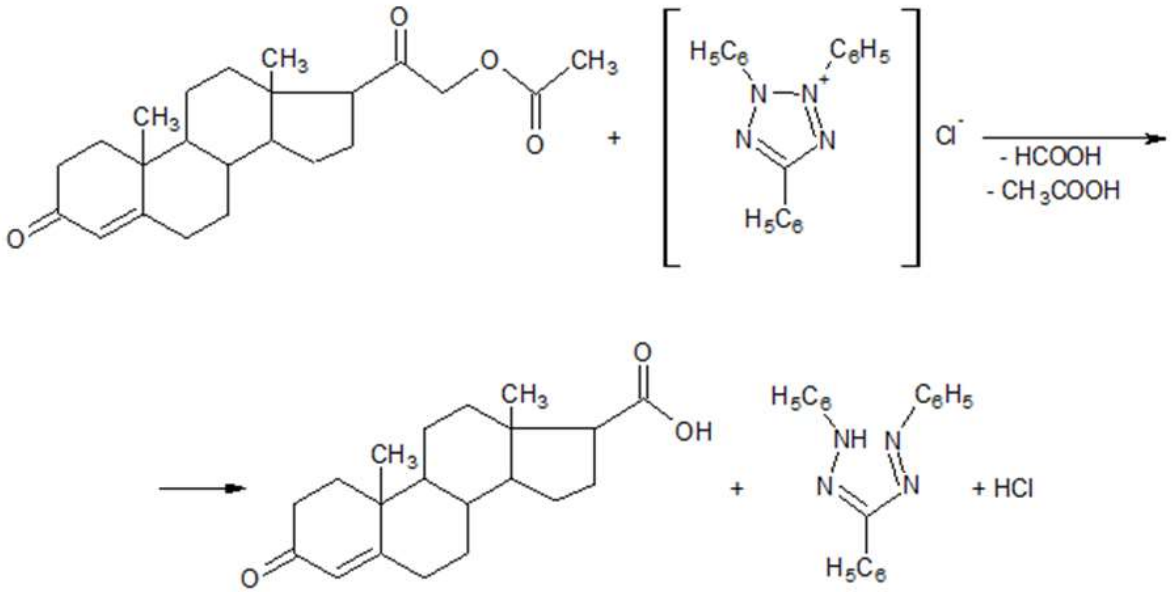


   b) Silver mirror reaction. When interacting with ammonia solution of silver nitrate, a black precipitate of metallic silver is formed.



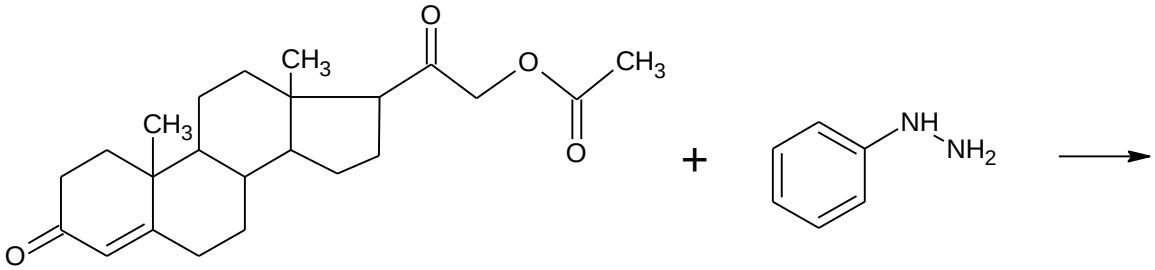


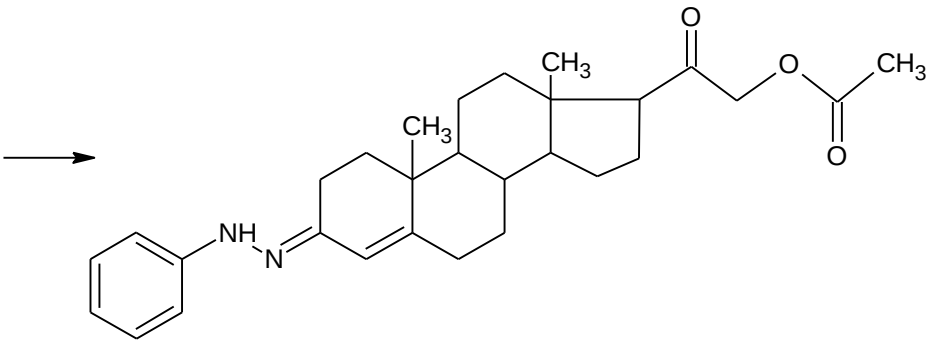
c) Interaction with 2,3,5-triphenyltetrazolium chloride. The reaction is carried out in ethanol in the presence of 10% tetramethylammonium solution. As a result, the tetrazolium salt is reduced to triphenylpharmazan of red colour and the cycle is opened.



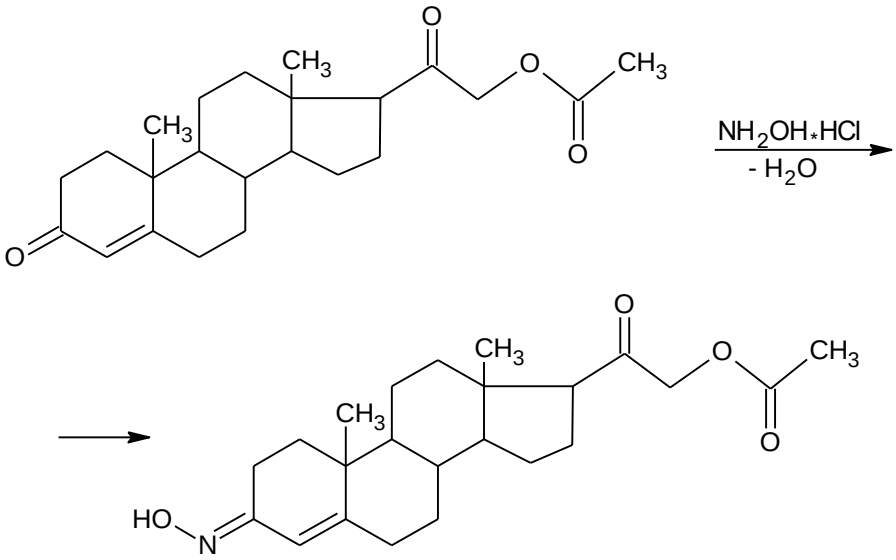
5. *Reactions to the carbonyl group in the 3rd position*

a) Interaction with phenylhydrazine. When an alcoholic solution of the preparation is heated with phenylhydrazine on a water bath, a yellow colouring appears due to the formation of phenylhydrazone.



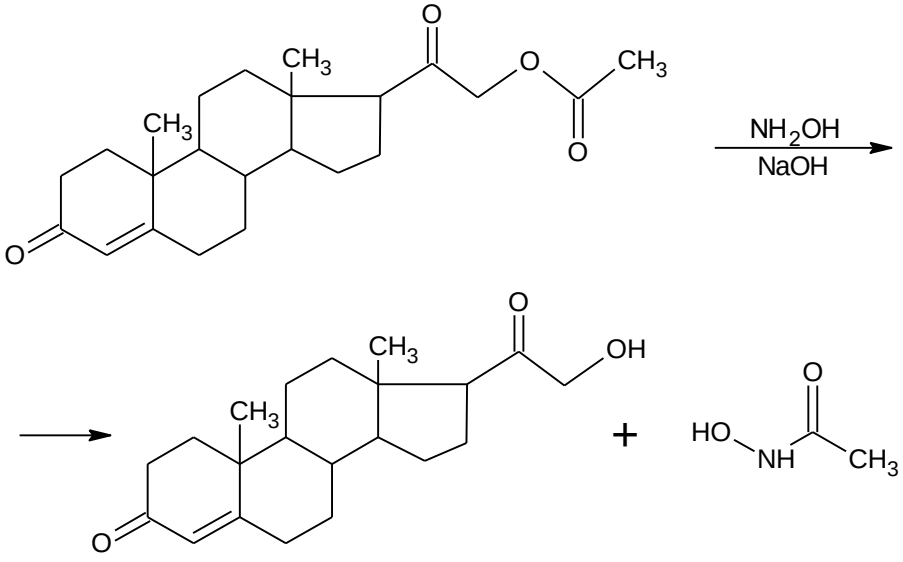


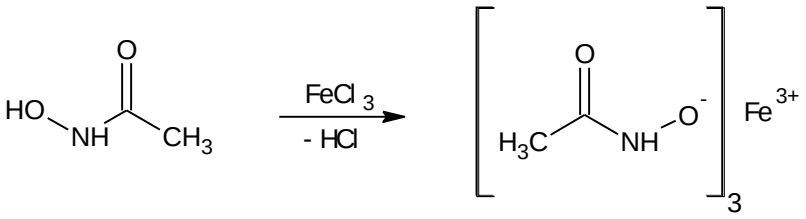
b) Formation of oxime. When an alcoholic solution of the drug interacts with hydroxylamine hydrochloride, an oxime having a certain melting point is formed.



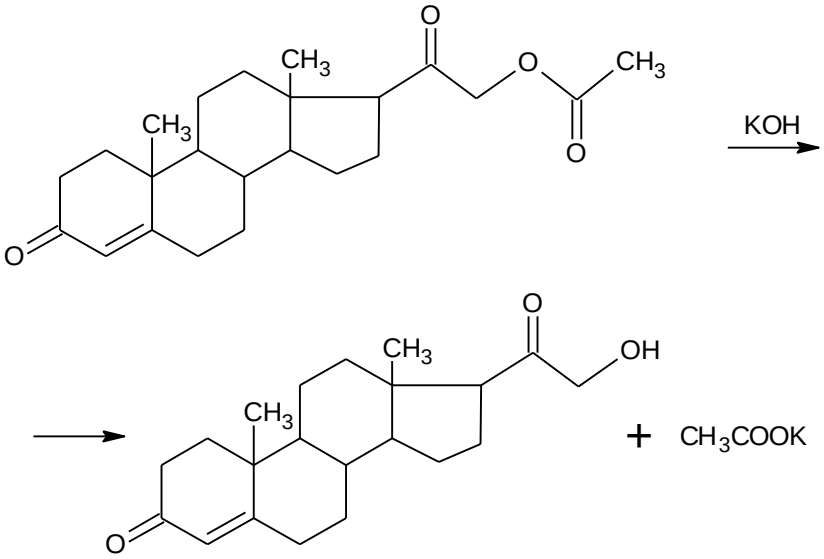
6. *Reactions with ester groups*

a) Hydroxamic acid reaction. Deoxycorticosterone acetate is dissolved in methanol and an alkaline solution of hydroxylamine is added. After 5 minutes, a dilute hydrochloric acid solution and iron (III) chloride solution are added. As a result, a red-brown coloured compound is formed.





b) Reaction of ethyl acetate formation. The acetyl group can be detected after hydrolysis of the preparation in an alcoholic solution of potassium hydroxide. Subsequent addition of concentrated sulphuric acid leads to the formation of ethyl acetate, which has a characteristic odour.



$$2CH_3COOK + H_2SO_4 + 2C_2H_5OH \longrightarrow 2CH_3COOC_2H_5 + K_2SO_4 + 2H_2O$$

Purity test

1. The presence of extraneous steroid impurities is determined by TLC.
2. Microcolumn HPLC. Determine the quantitative content of impurities.

Assay

1. ***UV spectrometry.*** Deoxycorticosterone acetate is calculated from the specific absorbance in ethanol solution at an absorption maximum of 241 nm.

2. ***Spectrophotometry.*** It is based on the reaction with hydrazine of isatin. The optical density of the resulting hydrazone is measured in dioxane at a wavelength of 445 nm.

3. ***Photocolorimetry.*** It is based on the use of phenylhydrazine, 4-aminoantipyrine, isoniazid, sodium borohydride as reagents for the keto group at C-3 of the steroid cycle.

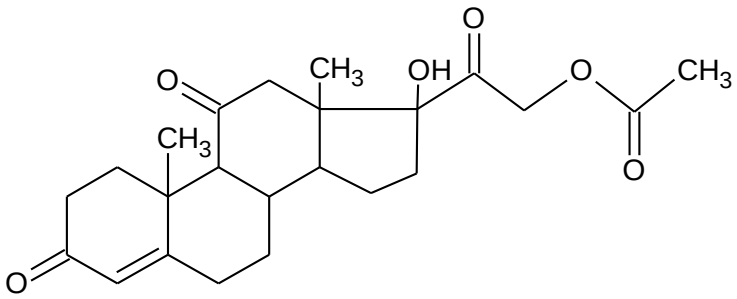
Storage

Store in well sealed containers, protected from light.

Medical use

Deoxycorticosterone acetate, which is a mineralocorticosteroid, is used in Addison's disease, myasthenia gravis, asthenia, general muscular weakness and other diseases. It is administered intramuscularly in the form of oil solutions.

CORTISONE ACETATE



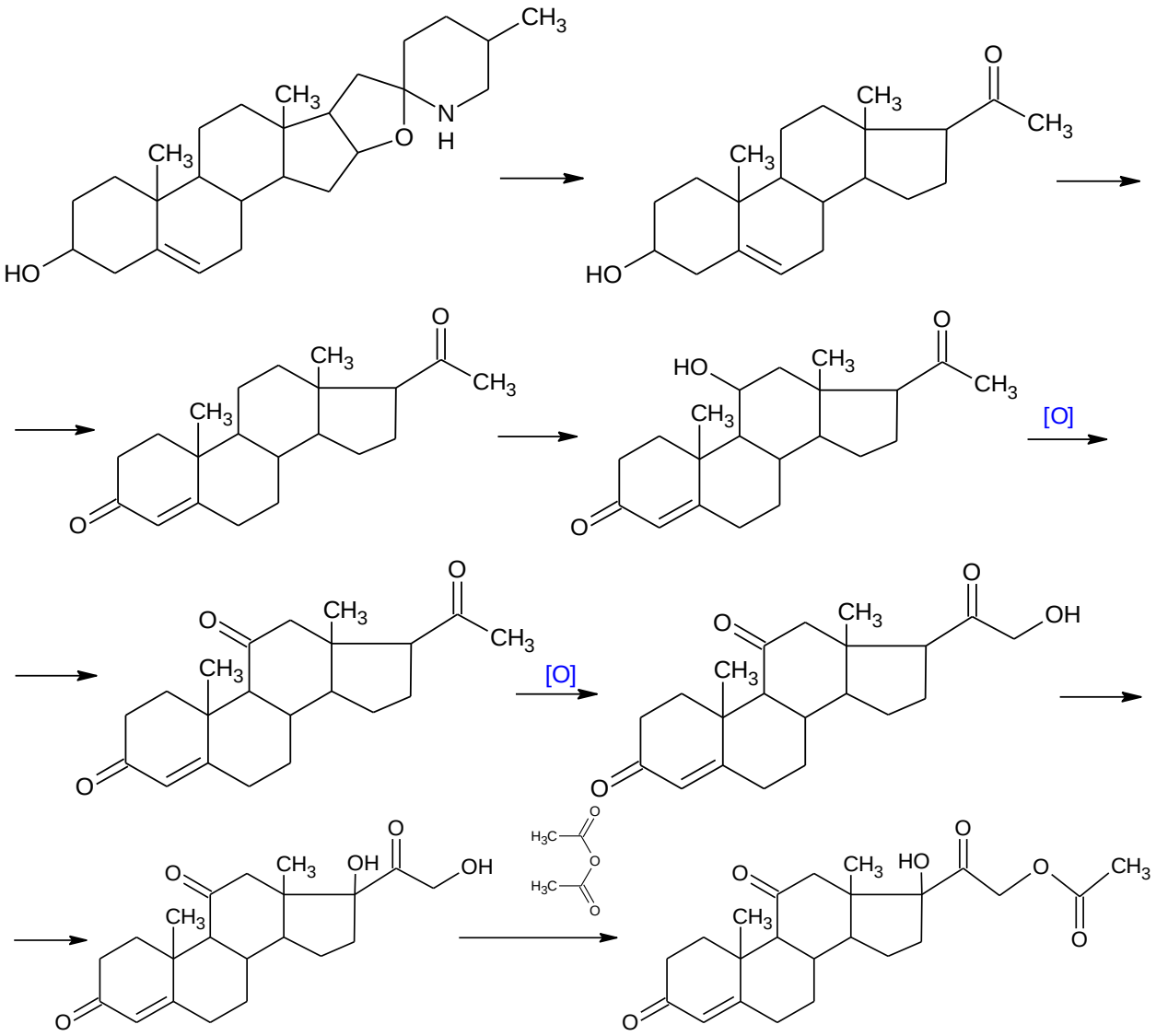
Obtaining

Cortisone was isolated in 1936 from the adrenal cortex simultaneously by Kendall and Wintersteiner in the USA and Reichstein in Switzerland. The difficulty in its synthesis is that there are no available steroidal compounds in nature containing a keto group at position 11. It is possible to introduce such a group by biochemical oxidation (by fungi, yeast, actinomycetes and various bacteria). The complete synthesis of cortisone was carried out in 1951 by Woodward (USA). It involves about 30 steps and, due to its complexity, is of theoretical interest only.

In 1956, N.N. Suvorov et al. showed the possibility of using solasodine, an aglycon of glucoalkaloid from bird's nightshade (Solanum aviculare), family Solanaceae, as a starting product for industrial production of cortisone.

The scheme consists of several steps: isolation of solasodine from plant material; preparation of pregnenolone and then progesterone from it; microbiological hydroxylation of progesterone to 11α-oxyprogesterone; sequential biochemical oxidation of 11α-oxyprogesterone and microbiological hydroxylation of 11-dehydrocorticosterone to form cortisone.

Scheme for the synthesis of cortisone from solasodine:

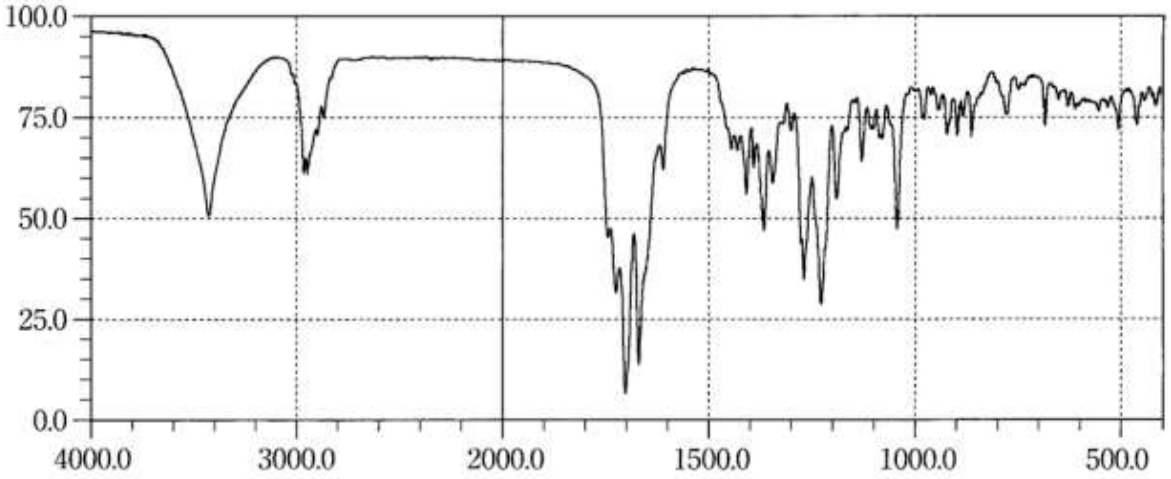


Physical properties

White or white with slight yellowish tinge crystalline powder. Melting point 238-243°C (with decomposition). Specific rotation from +178 to +194° (0.5% solution in acetone)Practically insoluble in water, very slightly soluble in 95% alcohol, easily soluble in chloroform, hardly soluble in acetone.

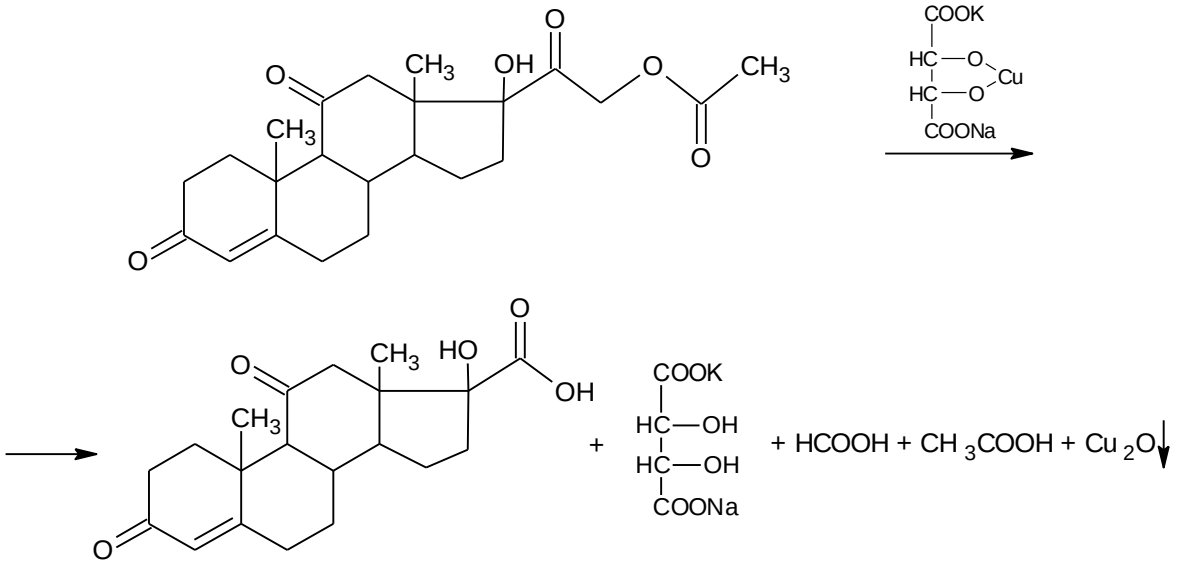
Identification

1. ***Infrared spectrometry.*** The infrared spectrum of cortisone acetate should correspond to the spectrum of the standard sample or to the attached spectrum in the regulatory documentation.



2. ***UV spectrometry.*** The UV-spectrum has a characteristic absorption band with a maximum at 238 nm (solvent - ethanol).
3. ***Reaction for steroid cycle with concentrated sulphuric acid.*** The drug gives an orange colour with yellow fluorescence.
4. ***Reactions with α-ketol groups***

   a) Interaction with Fehling's reagent. The preparation is dissolved in methyl alcohol, added Fehling's reagent and heated in a water bath. An orange-red precipitate of copper (I) oxide is formed.



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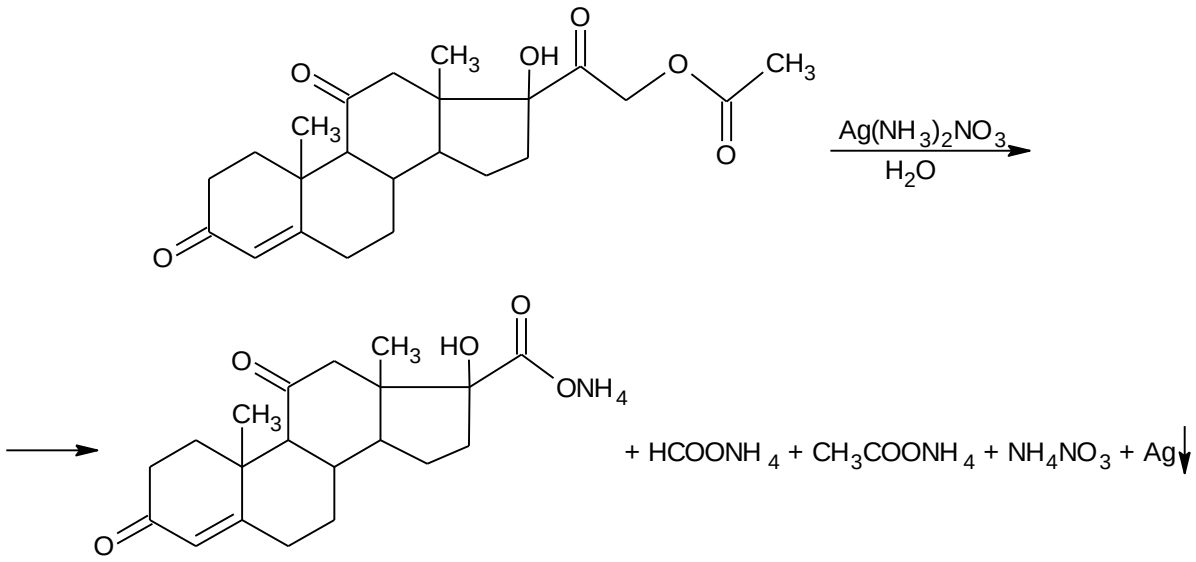
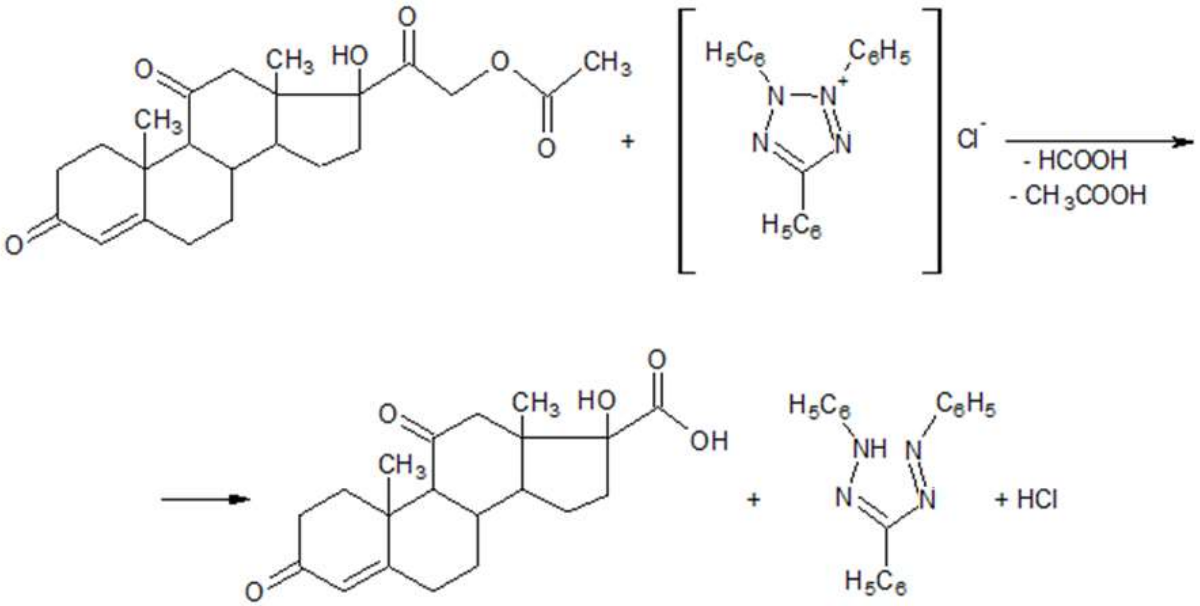
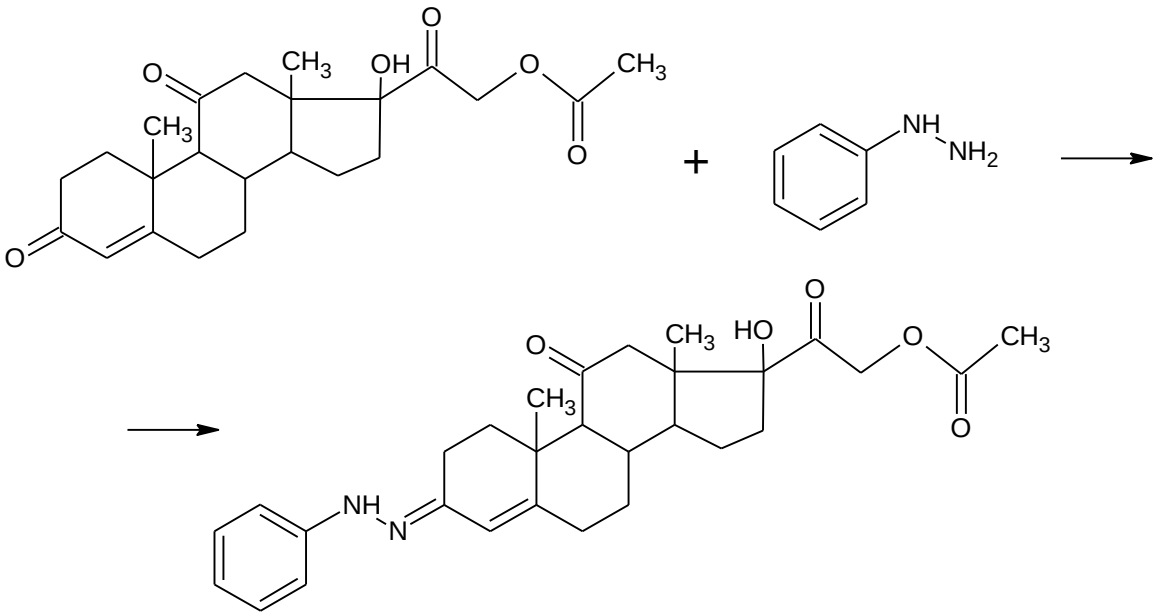


c) Interaction with 2,3,5-triphenyltetrazolium chloride. The reaction is carried out in ethanol in the presence of 10% tetramethylammonium solution. As a result, the tetrazolium salt is reduced to triphenylpharmazan of red colour and the cycle is opened.

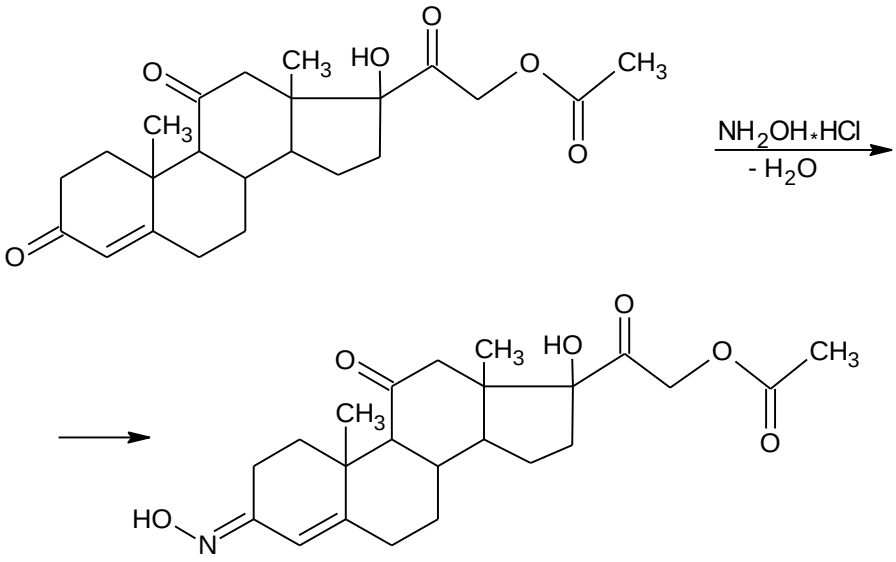


5. *Reactions to the carbonyl group in the 3rd position*

a) Interaction with phenylhydrazine. When an alcoholic solution of the preparation is heated with phenylhydrazine on a water bath, a yellow colouring appears due to the formation of phenylhydrazone.

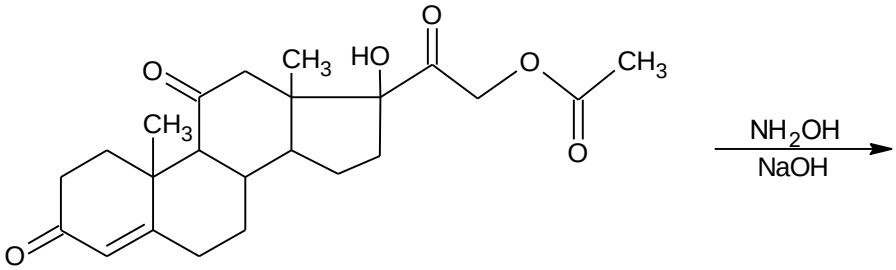


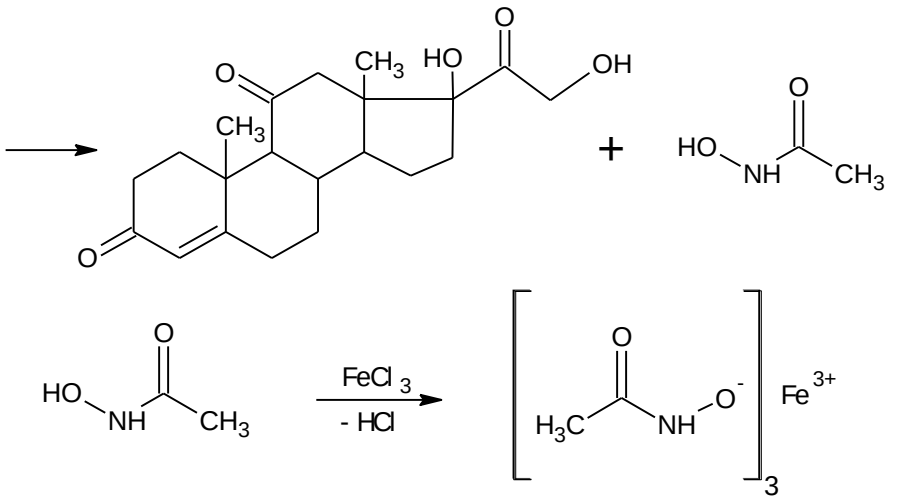
b) Formation of oxime. When an alcoholic solution of the drug interacts with hydroxylamine hydrochloride, oxime is formed, which has a certain melting point.



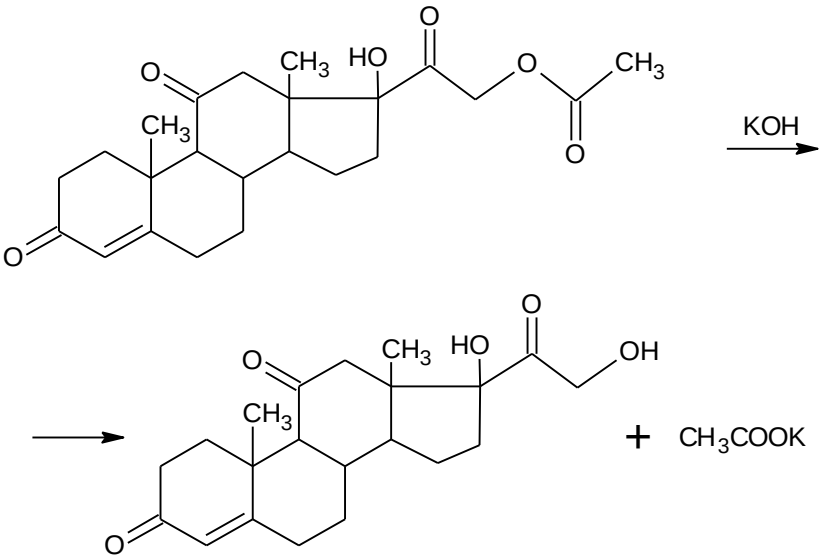
6. Reactions with ester groups

a) Hydroxamic reaction. Cortisone acetate is an ester. The drug is dissolved in methanol, an alkaline solution of hydroxylamine is added. After 5 minutes, dilute hydrochloric acid and iron (III) chloride solution are added. A dark cherry-coloured compound is formed.





b) Reaction of ethyl acetate formation. The acetyl group can be detected after hydrolysis of the preparation in an alcoholic solution of potassium hydroxide. Subsequent addition of concentrated sulphuric acid leads to the formation of ethyl acetate, which has a characteristic odour.



$$2CH_3COOK + H_2SO_4 + 2C_2H_5OH \longrightarrow 2CH_3COOC_2H_5 + K_2SO_4 + 2H_2O$$

7. ***Colour reaction.*** A solution of cortisone acetate in ethanol evaporated to dryness in vacuo, after heating to 70°C for 30 min, with 1 M sodium hydroxide solution acquires a yellow colouration with intense absorption at 370 nm.

Purity test

1. The presence of extraneous steroid impurities is determined by TLC.
2. Microcolumn HPLC. Determine the quantitative content of impurities.

Assay

1. UV spectrometry. Cortisone acetate is calculated from the specific absorbance in ethanol solution at an absorption maximum of 238 nm.

2. Photocolorimetry. It is based on the use of phenylhydrazine, 4-aminoantipyrine, isoniazid, sodium borohydride as reagents for the keto group at C-3 of the steroid cycle.

Storage

Store in well corked containers, protected from light.

Medical use

Cortisone acetate is a glucocorticoid with strong anti-inflammatory, desensitising and anti-allergic action, antishock and antitoxic properties.

It inhibits the development of lymphoid tissue; having immunosuppressive effect, inhibits the growth and development of connective tissue, reduces capillary permeability, inhibits the synthesis and accelerates the breakdown of protein.

Cortisone acetate is used in

- diseases of connective tissue and joints: collagenosis, rheumatism, non-specific infectious polyarthritis, systemic lupus erythematosus, dermatomyositis, periarteritis nodosa, sarcoidosis, psoriatic arthritis.

- Allergic diseases: bronchial asthma, drug reactions.

- Blood diseases: leukaemia (acute lymphoblastic, myeloblastic leukaemia), infectious mononucleosis. Haemolytic anaemia.

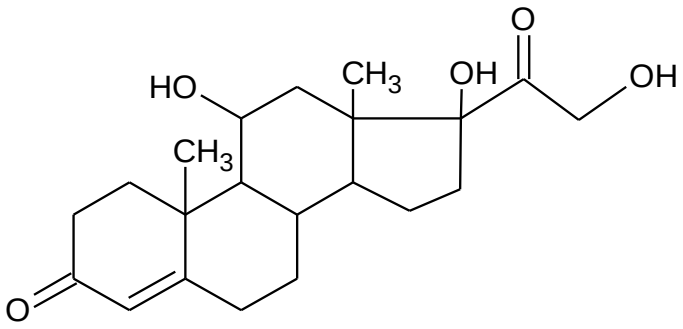
- Skin diseases: neurodermatitis, eczema, true vesicles, haemoderma, severe erythema multiforme exudative erythema and other persistent widespread dermatoses that cannot be treated by other methods.

- Addison's disease, acute adrenal insufficiency, glomerulonephritis, acute pancreatitis.

- shock and collapse during surgical interventions;

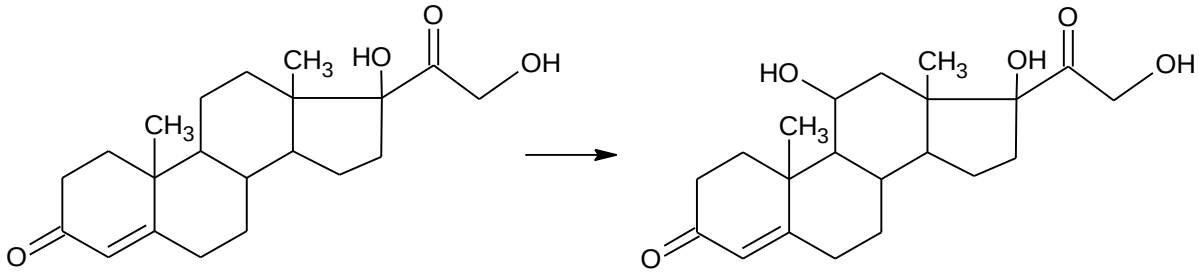
- to suppress tissue rejection in organ homotransplantation.

HYDROCORTISONE



Obtaining

Hydrocortisone (cortisol) is obtained biotechnologically by means of Cuervulagia Linata from the substance S Reichstein.

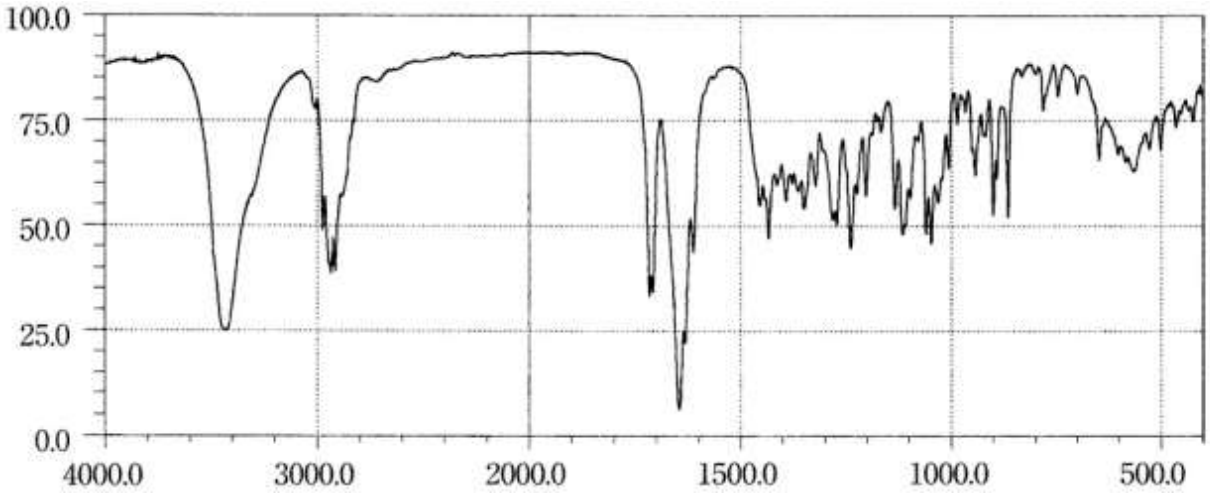


Physical properties

White or almost white crystalline powder. Practically insoluble in water, slightly soluble in chloroform, very slightly soluble in alcohol 96 %.

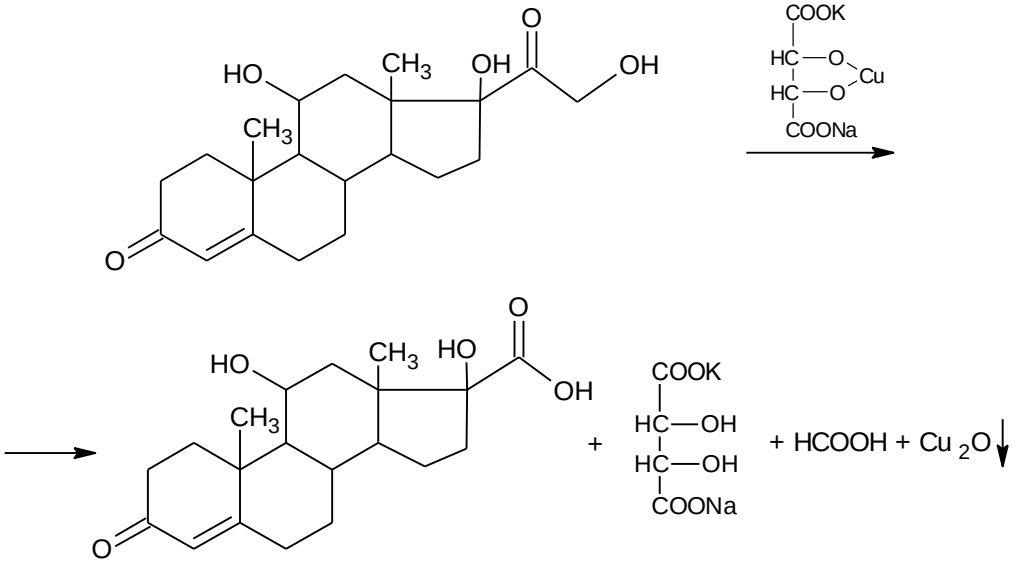
Identification

1. ***Infrared spectrometry*.** The infrared spectrum of the substance, taken in a disc of potassium bromide, in the region from 4000 to 400 cm-1 in the position of the absorption bands should correspond to the spectrum of a standard sample of hydrocortisone.



2. ***UV Spectrometry.*** The UV spectrum of the test solution in the wavelength region from 200 to 300 nm should have an absorption maximum at 241 nm.
3. ***Reaction for steroid cycle with concentrated sulphuric acid.*** The preparation gives yellow colouring, changing after 5 minutes to red colouring with yellow-green fluorescence (after addition of water it changes to green).
4. ***Reactions with α-ketol groups***

a) Interaction with Fehling's reagent. The preparation is dissolved in methyl alcohol, added Fehling's reagent and heated in a water bath. An orange-red precipitate of copper (I) oxide is formed.



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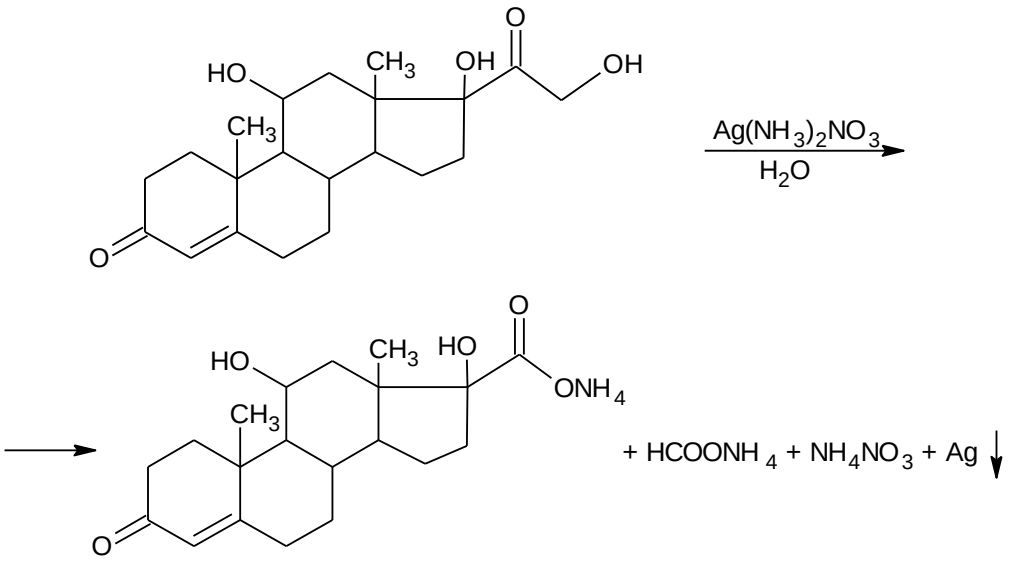
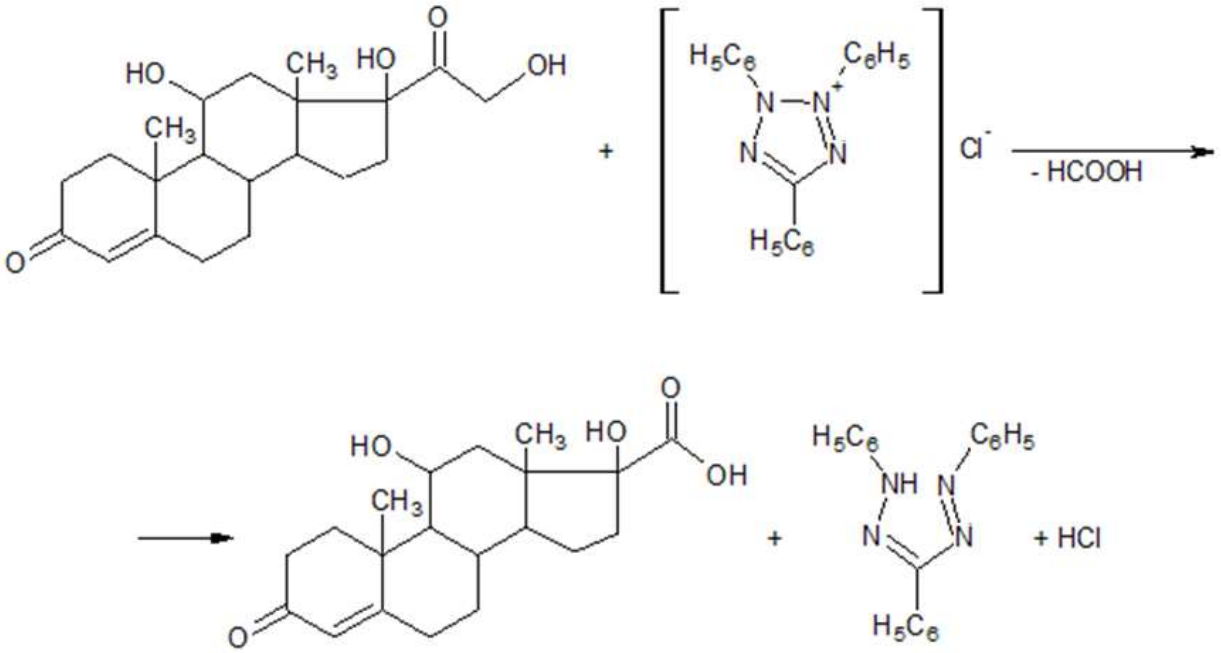


c) Interaction with 2,3,5-triphenyltetrazolium chloride. The reaction is carried out in ethanol in the presence of 10% tetramethylammonium solution. As a result, the tetrazolium salt is reduced to triphenylpharmazan of red colour and the cycle is opened.



5. *Reactions to the carbonyl group in the 3rd position*

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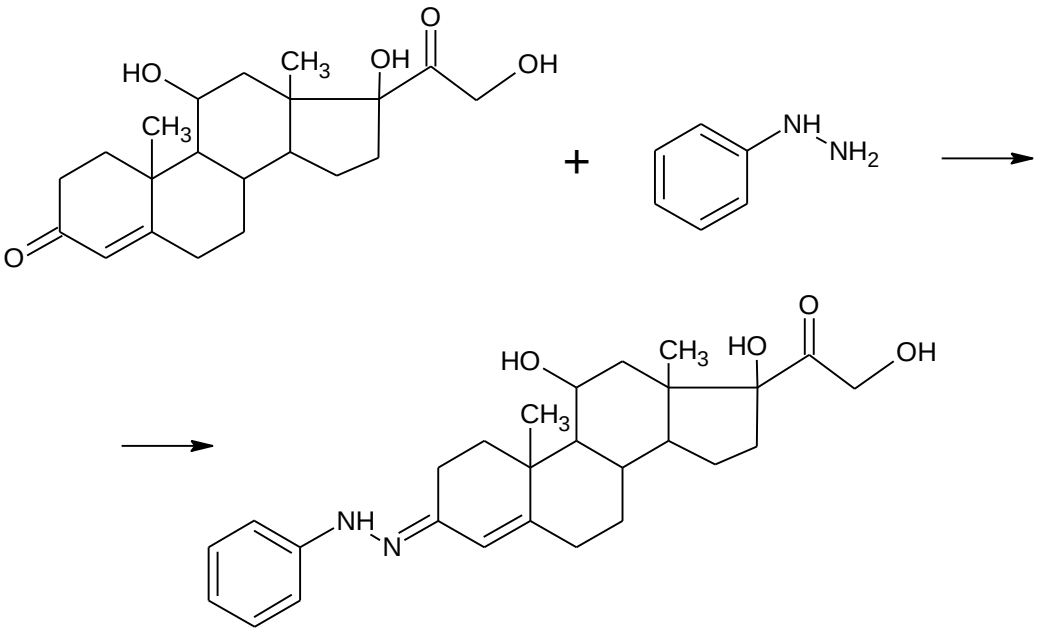
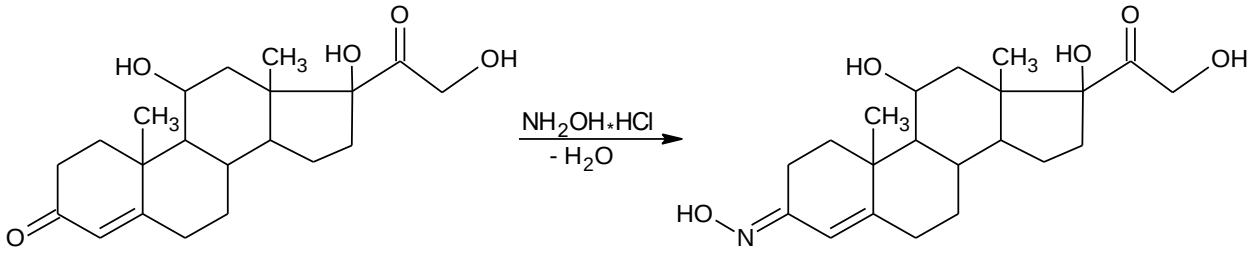


b) Formation of oxime. When an alcoholic solution of the drug interacts with hydroxylamine hydrochloride, oxime is formed, which has a certain melting point.



Purity test

1. The presence of extraneous steroid impurities is determined by TLC.
2. Microcolumn HPLC. Determine the quantitative content of impurities.

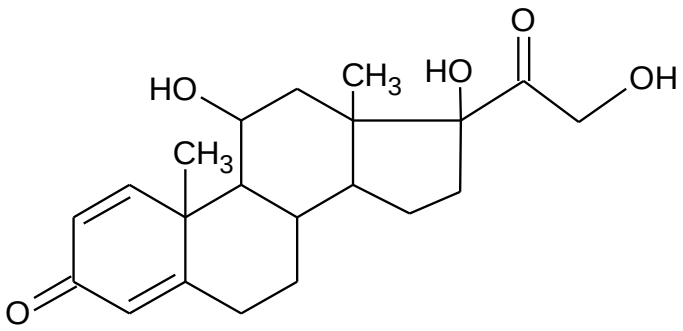
Assay

1. ***UV spectrometry.*** The hydrocortisone content is calculated from the specific absorbance in ethanol solution at an absorption maximum of 238 nm.
2. ***Photocolorimetry.*** It is based on the use of phenylhydrazine, 4-aminoantipyrine, isoniazid, sodium borohydride as reagents for the keto group at C-3 of the steroid cycle.
3. ***The HPLC method*** in direct-phase and reverse-phase versions is used for quantitative determination of hydrocortisone in ointments. Chloroform-methanol mixture (93:3) is used for analysis in the forward phase and methanol in the reverse phase.

Storage
Store in well corked containers, protected from light.

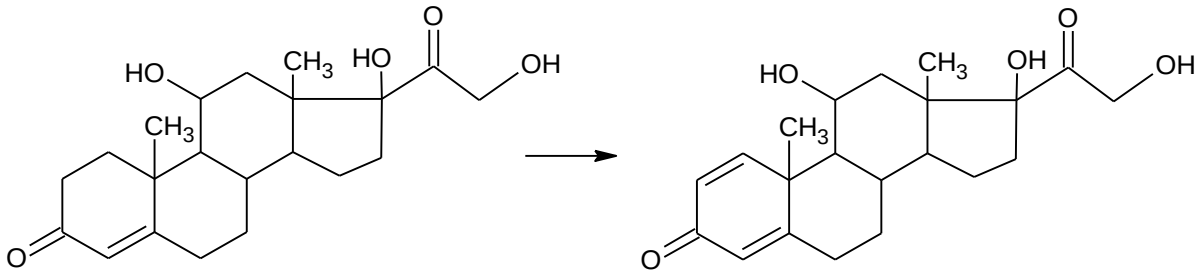
Medical use
Hydrocortisone is a synthetic glucocorticoid, it has anti-inflammatory, anti-allergic, immunosuppressive, antipruritic, antishock, antiexudative effect. In medical practice, it is used for systemic and topical application.

PREDNISOLONE



Obtaining

Prednisolone is obtained by oxidation of hydrocortisone using Arthrobacter simplex cell culture. The process is carried out in neutral medium at 28°C for 120 h.

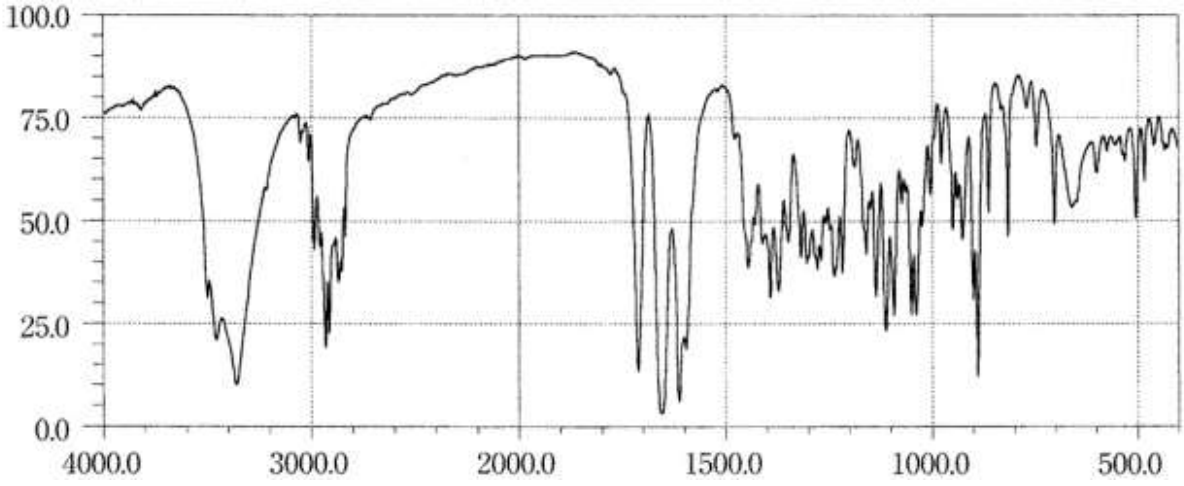


Physical properties

White or white with a faint yellowish tinge, odourless crystalline powder. Anhydrous or contains 1-1/2 moles of crystallising water. Melting point 227-230° (with decomposition). Specific rotation from +96° to + 104° (1% solution in dioxane, from a dried suspension). Practically insoluble in water, soluble in methyl and 95% ethyl alcohols, hardly soluble in acetone and dioxane, slightly soluble in chloroform.

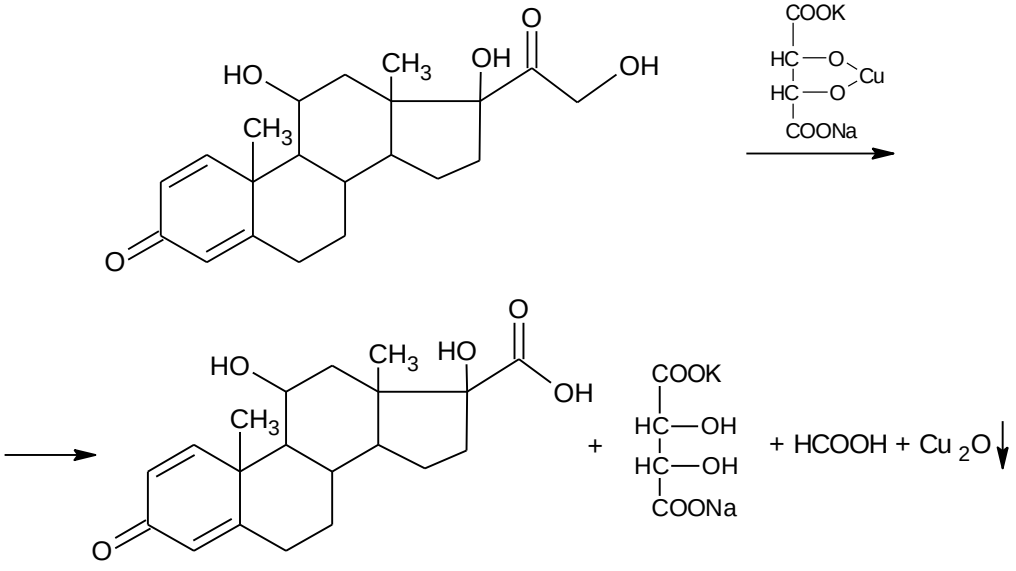
1. ***Infrared spectrometry.*** The infrared spectrum of the substance, taken in vaseline oil, in the region from 4000 to 400 cm-1 in the position of the

absorption bands should correspond to the spectrum of a standard sample of prednisolone.

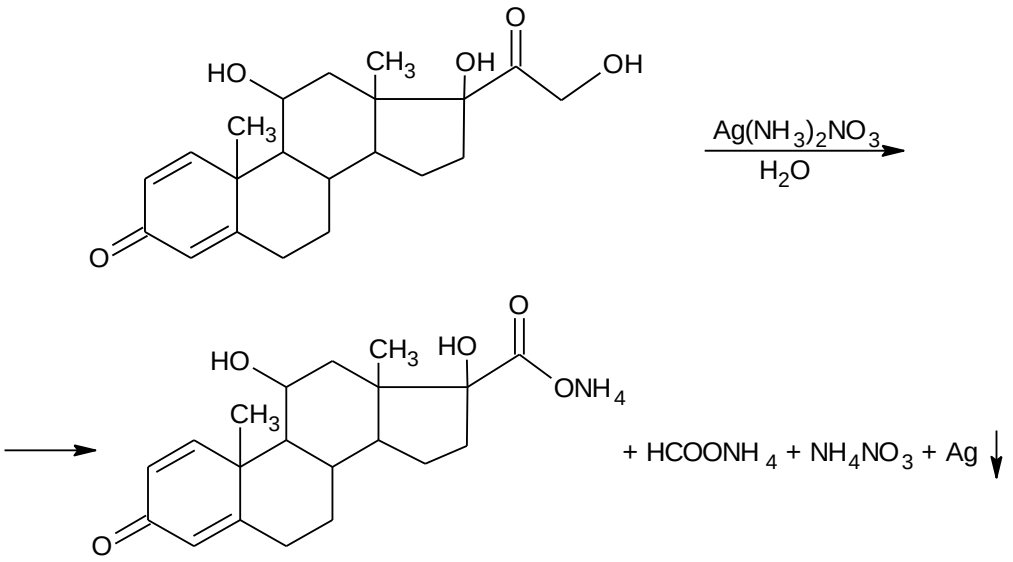


2. ***UV Spectrometry.*** The UV spectrum of the test drug methanol in the wavelength region of 200 to 300 nm should have an absorption maximum at 242 nm.
3. ***Reaction for steroid cycle with concentrated sulphuric acid.*** The preparation gives a green colour changing to red. Fluorescence is absent.
4. ***Reactions with α-ketol groups***

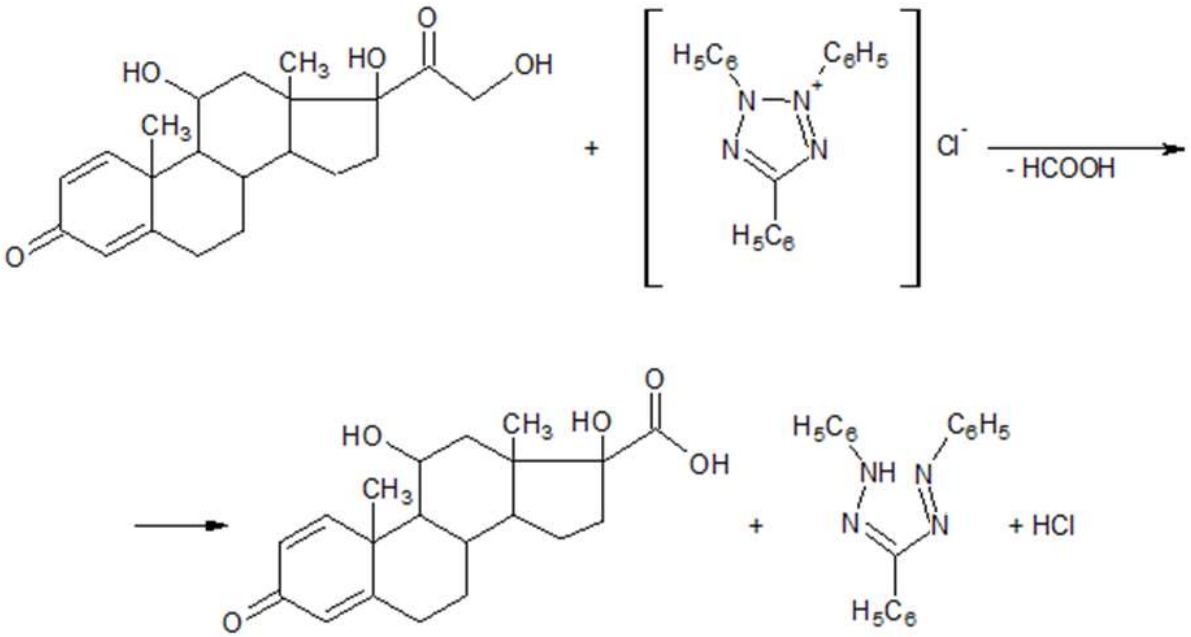
a) Interaction with Fehling's reagent. The preparation is dissolved in methyl alcohol, added Fehling's reagent and heated in a water bath. An orange-red precipitate of copper (I) oxide is formed.



b) Silver mirror reaction. When interacting with ammonia solution of silver nitrate, a black precipitate of metallic silver is formed.

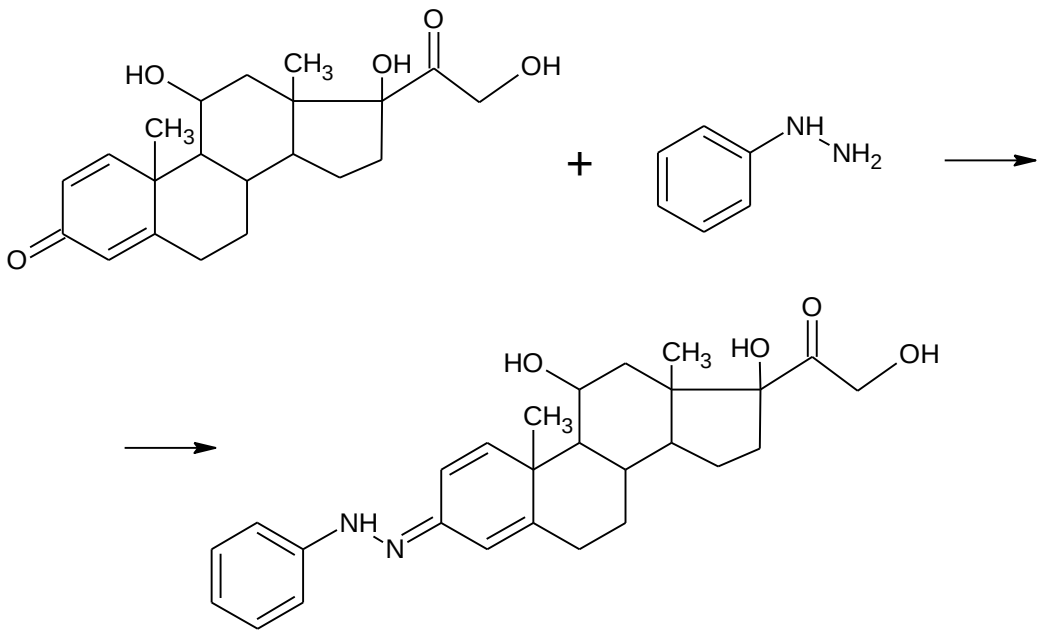


c) Interaction with 2,3,5-triphenyltetrazolium chloride. The reaction is carried out in ethanol in the presence of 10% tetramethylammonium solution. As a result, the tetrazolium salt is reduced to triphenylpharmazan of red colour and the cycle is opened.

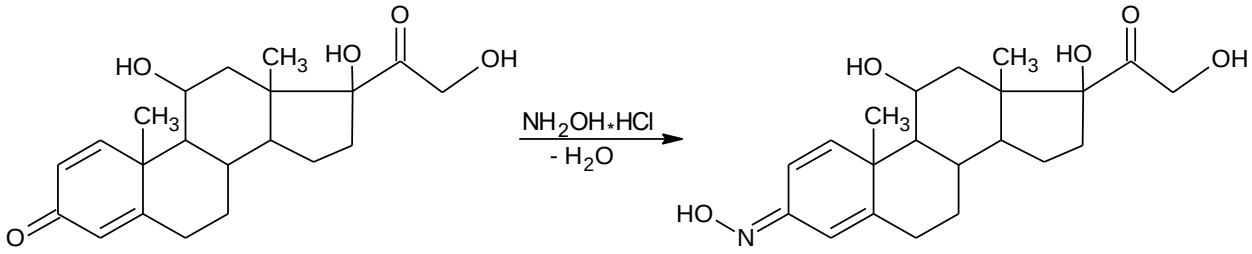


5. *Reactions to the carbonyl group in the 3rd position*

a) Interaction with phenylhydrazine. When an alcoholic solution of the preparation is heated with phenylhydrazine on a water bath, a yellow colouring appears due to the formation of phenylhydrazone.



b) Formation of oxime. When an alcoholic solution of the drug interacts with hydroxylamine hydrochloride, oxime is formed, which has a certain melting point.



6. ***Colour reaction.*** Solution of prednisolone in ethanol, evaporated to dryness in vacuum, after heating to 70°C for 30 min, with 1M sodium hydroxide solution acquires weak yellow colouring.

Purity test

1. The presence of extraneous steroid impurities is determined by TLC.
2. Microcolumn HPLC. Determine the quantitative content of impurities.

Assay

1. ***UV spectrometry.*** The hydrocortisone content is calculated from the specific absorbance in ethanol solution at an absorption maximum of 238 nm.
2. ***Photocolorimetry.*** It is based on the use of phenylhydrazine, 4-aminoantipyrine, isoniazid, sodium borohydride as reagents for the keto group at C-3 of the steroid cycle.

3. ***The HPLC method*** in direct-phase and reverse-phase versions is used for quantitative determination of prednisolone in ointments. Chloroform-methanol mixture (93:3) is used for analysis in the forward phase, and methanol in the reverse phase.

Storage

Store in well corked containers, protected from light.

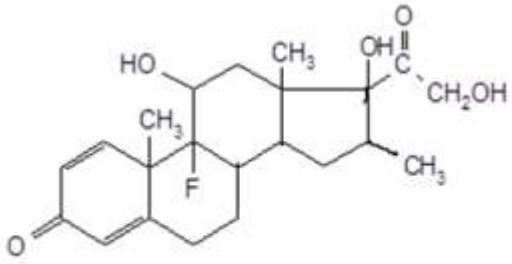
Medical use

Prednisolone is a synthetic drug from the group of glucocorticoid hormones. The drug has anti-inflammatory, antishock, anti-allergic and immunosuppressive effect. In medical practice, it is used for systemic and topical application.

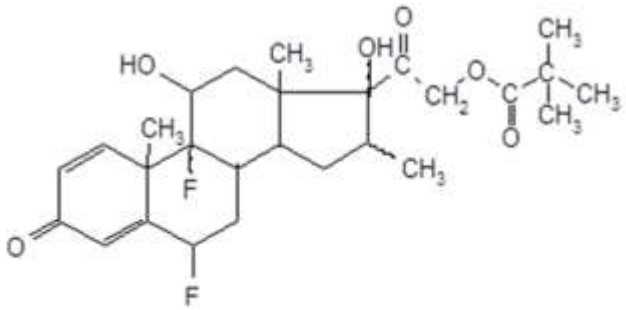
FLUOROSUBSTITUTED CORTICOSTEROID COMPOUNDS

As a result of the study of the influence of halogens introduced into the corticosteroid molecule on their pharmacological activity, mono- and difluoro derivatives of prednisolone were synthesised.

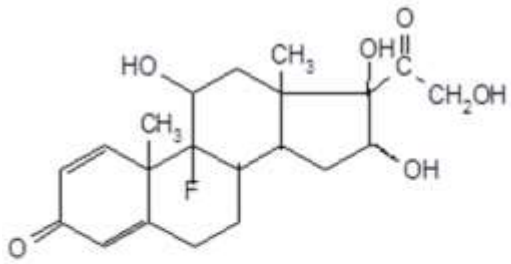
They contain one fluorine atom at position 9 - Dexamethasone, Triamcinolone or two fluorine atoms at positions 6 and 9 - Flumethasone Pivalate and Fluocinolone Acetonide:



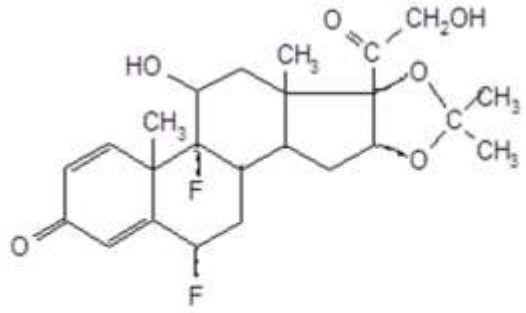
Dexamethasone



Flumethasone Pivalate



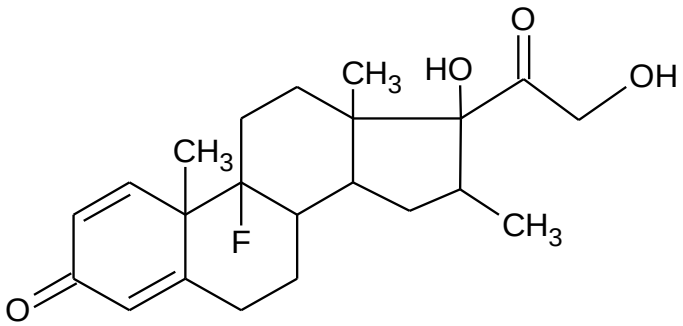
Triamcinolone



Fluocinolone Acetonide

Fluorine derivatives are characterised by more active anti-inflammatory, anti-allergic action than prednisolone. They are highly effective when applied topically. Dexamethasone is 35 times more active than cortisone.

DEXAMETASONE



Obtaining

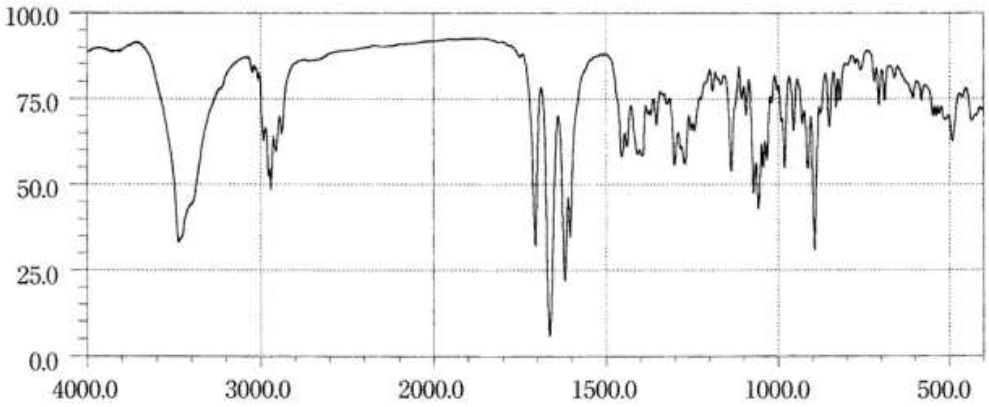
The introduction of fluorine atom at position 9α is carried out by hydrogen fluoride action, and the formation of double bonds at positions 1-2 is carried out microbiologically.

Physical properties

White or almost white odourless crystalline powder. Specific rotation from +86 to + 92°(1% solution of substance in anhydrous ethanol). Moderately soluble in acetone, ethanol, chloroform, practically insoluble in water.

Identification

1. ***Infrared spectrometry.*** The infrared spectrum of the substance, taken in a disc of potassium bromide, in the region from 4000 to 400 cm-1 should correspond in position of the absorption bands to the spectrum of a standard sample of dexamethasone.



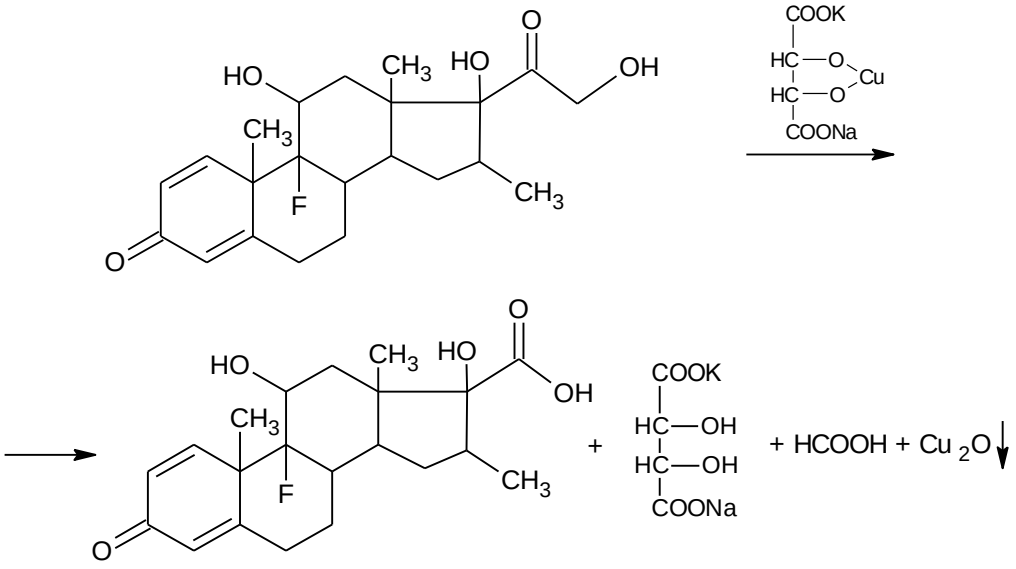
2. ***UV Spectrometry.*** The UV spectrum of the test drug methanol in the wavelength region of 200 to 300 nm should have an absorption maximum at 242 nm.

3. ***TLC.*** The test is carried out on plates with a layer of silica gel F254. The adsorption zone of the test sample must coincide with the adsorption zone of the standard sample.

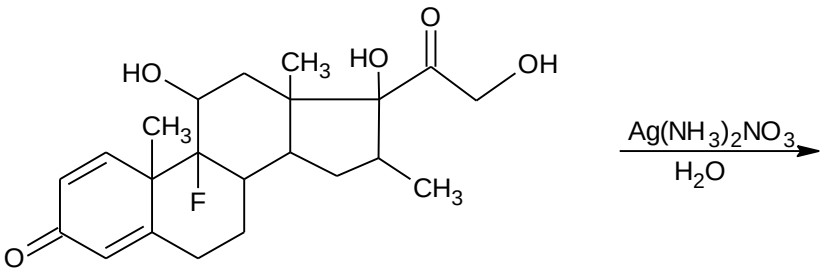
4. ***Reaction for steroid cycle with concentrated sulphuric acid***. After dissolving the drug in concentrated sulphuric acid for 5 minutes, a pale reddish-brown colouration should appear.

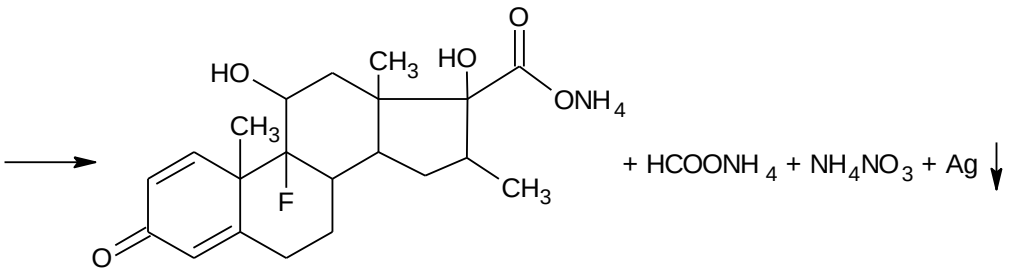
5. ***Reactions with α-ketol groups***

a) Interaction with Fehling's reagent. The preparation is dissolved in methyl alcohol, added Fehling's reagent and heated in a water bath. An orange-red precipitate of copper (I) oxide is formed.



b) Silver mirror reaction. When interacting with ammonia solution of silver nitrate, a black precipitate of metallic silver is formed.





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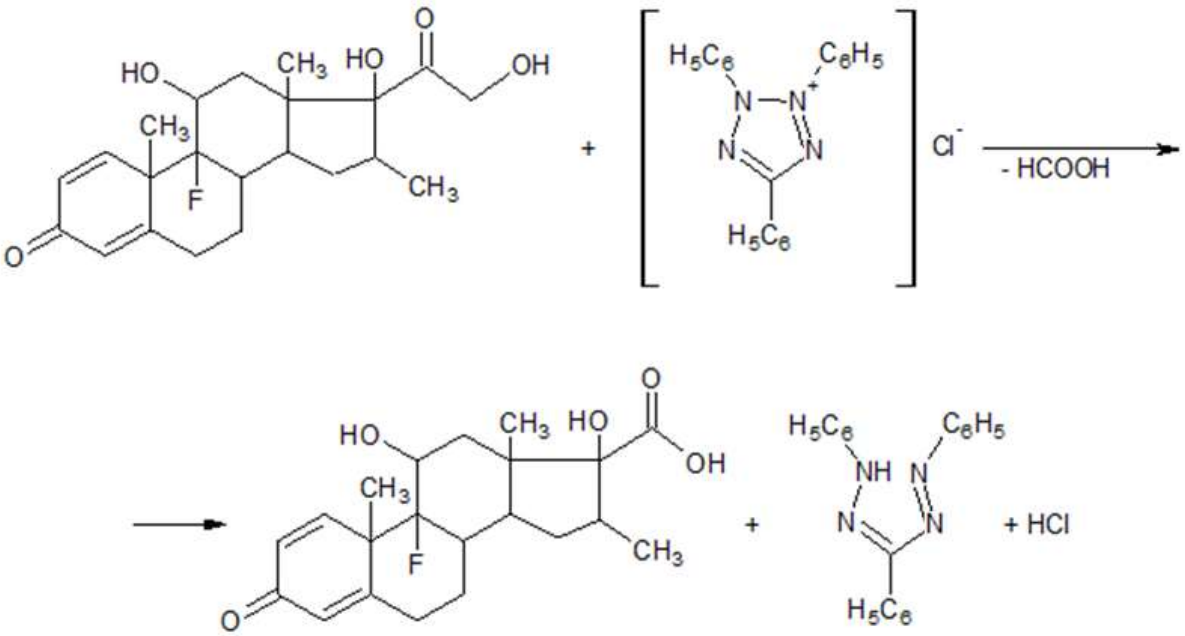
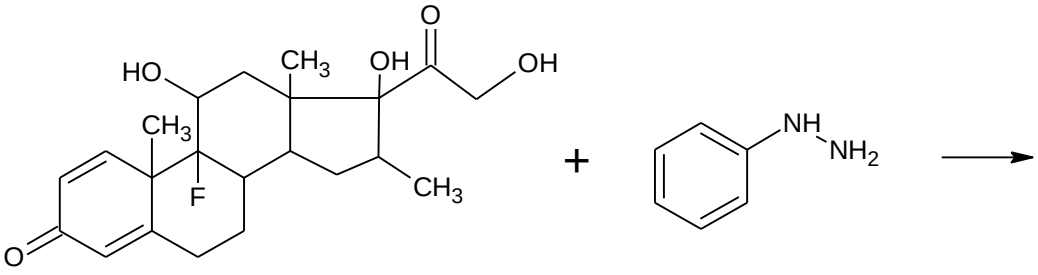
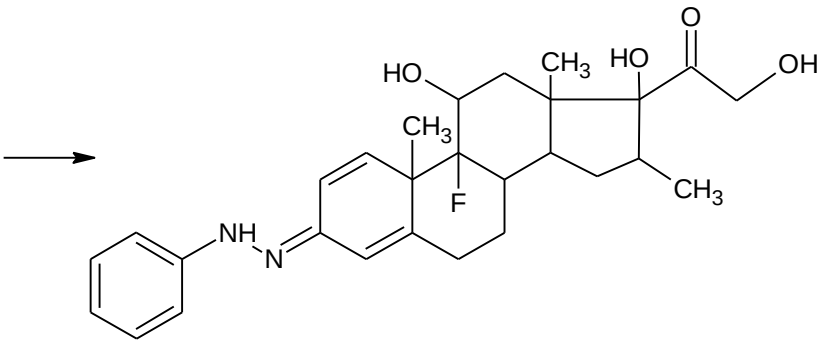


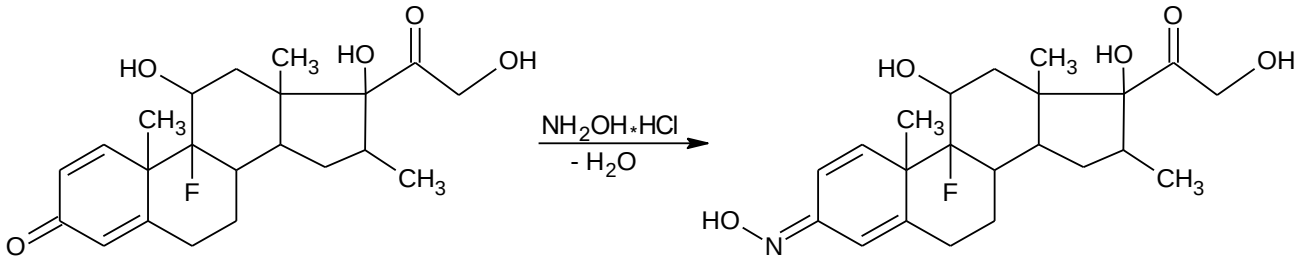
6. *Reactions to the carbonyl group in the 3-position*

a) Interaction with phenylhydrazine. When an alcoholic solution of the preparation is heated with phenylhydrazine on a water bath, a yellow colouring appears due to the formation of phenylhydrazone.





b) Formation of oxime. When an alcoholic solution of the drug interacts with hydroxylamine hydrochloride, oxime is formed, which has a certain melting point.



Purity test

The content of related impurities in the preparation is determined by HPLC. In addition, the content of heavy metals and sulphate ash is determined.

Storage

Store in well corked containers, protected from light.

Medical use

Synthetic glucocorticosteroid with anti-inflammatory and immunosuppressive action along with the ability to penetrate into the CNS. Due to these properties it can be used in the treatment of patients with cerebral oedema and inflammatory eye diseases. It has a pronounced anti-inflammatory, anti-allergic and anti-exudative effect.