Ministry of Health of the Russian Federation

Volgograd State Medical University

Department of Pharmaceutical, Toxicological Chemistry
Pharmacognosy and Botany

SPECIAL PHARMACEUTICAL CHEMISTRY

Gestagens and Estrogens

Lesson 7

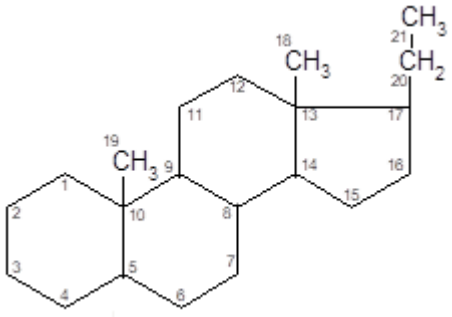
IX term

Volgograd, 2024

GESTAGENS AND THEIR SYNTHETIC ANALOGUES

According to modern concepts, gestagens include a group of natural hormones and their synthetic analogues that have the biological activity of progesterone.

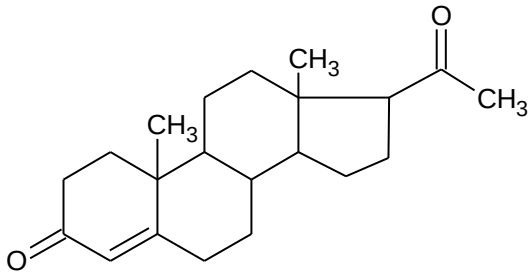
The chemical structure of progestins is based on the hydrocarbon pregnane.



pregnane

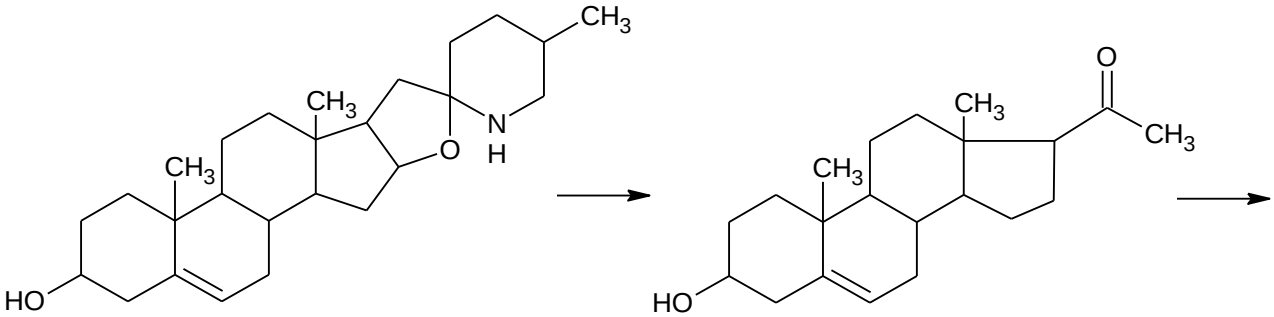
In medical practice, pharmaceutical preparations of the natural hormone progesterone and its semisynthetic analogues pregnane, norethisterone, and medroxyprogesterone acetate are used.

PROGESTERONE

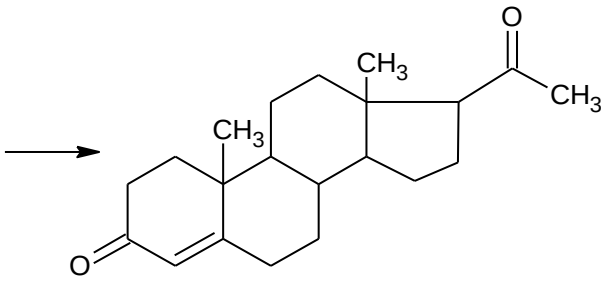


Obtaining

1. Release of hormones from the corpus luteum of the pig.
2. Semisynthetic route from solasodine as an intermediate of cortisone synthesis.

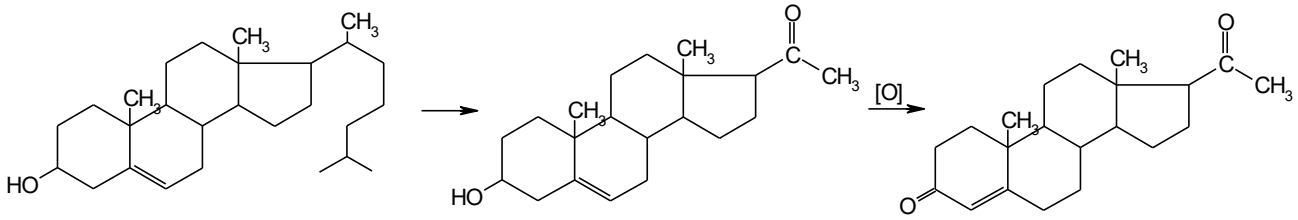


Solasodine *Pregnenolone*



Progesterone

3. Cholesterol, diosgenin, 17-ketosteroids can also serve as starting products for industrial synthesis. The intermediate product in the synthesis of progesterone from cholesterol is pregnenolone, which undergoes microbial dehydrogenation to progesterone:



холестерин прегненолон прогестерон

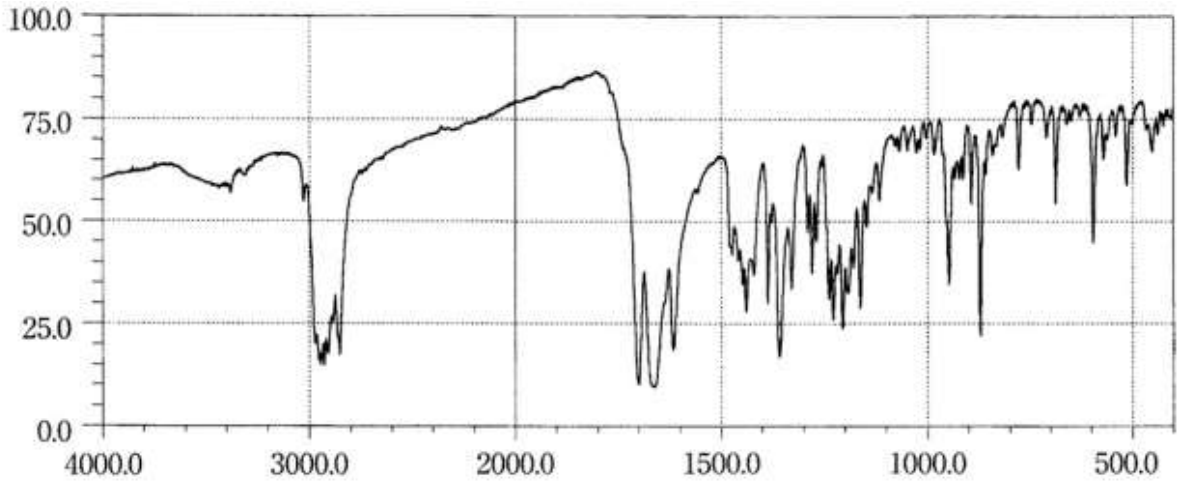
Physical properties

White or slightly yellowish crystalline powder. Melting point 127-131°C. Specific rotation from +190° to +200° (0.5% solution in ethanol). Practically insoluble in water, soluble in 95% alcohol and ether, very easily soluble in chloroform, hardly soluble in vegetable oils.

Identification

Instrumental methods

1. ***Infrared spectroscopy***. The infrared spectrum taken in vaseline oil in the region 3700-400 cm-1 should completely coincide with the spectrum figure attached to the pharmacopoeial monograph.



2. ***UV** spectrophotometry* is based on the measurement of optical density at 241 nm (maximum light absorption).
3. *TLC*. The evaluation is made after the chromatograms are displayed by comparing the position, appearance and color intensity of the main stain of the test solution and the standard sample.
4. *HPLC* method on direct and reversed phase columns. The identity is confirmed by comparing the retention time of the main peak on the chromatogram of the test and standard samples.
5. *Polarimetry*. Determine the specific rotation, which should be between +190° and +200° (0.5% solution in 95% alcohol).

Chemical Methods

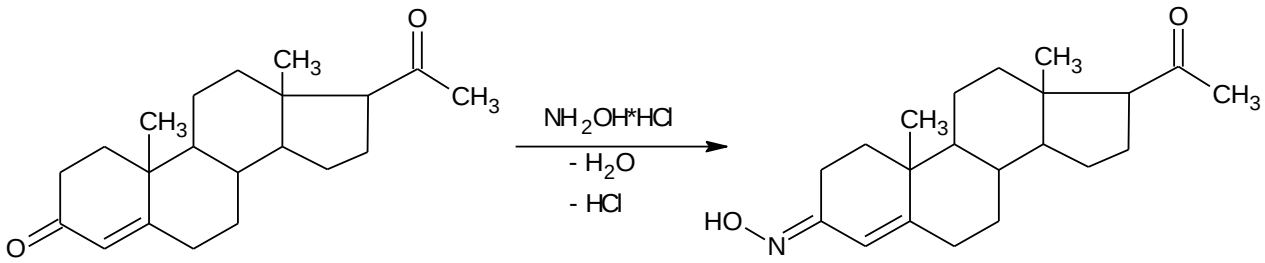
1. ***Reactions with the steroid cycle***.

a) Interaction with concentrated sulfuric acid. A yellow coloration with green fluorescence appears. Cool the solution, add 3 ml of chloroform and swirl; both layers are colorless.

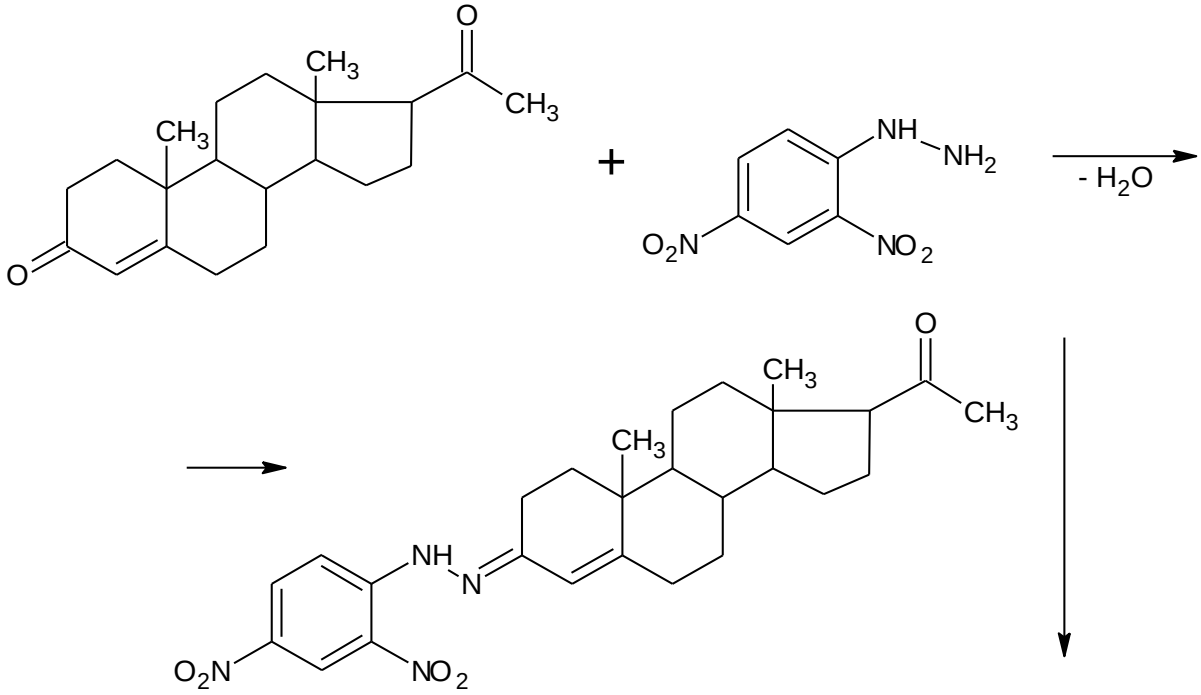
b) Boscott's reaction. A solution of the drug in a mixture of concentrated acetic acid and 88% phosphoric acid gives an intense yellow fluorescence.

2. Reactions to the carbonyl group in the 3rd position.

a) Oxime formation. When an alcoholic solution of the drug interacts with hydroxylamine hydrochloride, progesterone oxime is formed which has a melting point of 240°C.



b) Precipitation reaction of 2,4-dinitrophenylhydrazone. The decomposition temperature of the obtained 2,4-dinitrophenylhydrazone is 270-275°C. The reaction is carried out by boiling an alcoholic solution of progesterone with 2,4-dinitrophenylhydrazine in the presence of concentrated hydrochloric acid on a water bath.



3. Reactions due to the presence of a double bond in the A-ring.

a) Interaction with potassium permanganate solution. Discoloration of the solution is observed.

b) Interaction with bromine water. Discoloration of bromine water is observed.

4. Reaction with acetyl group at position 17.

a) Interaction with iodine. When heated with iodine in an alkaline medium, a yellow precipitate with a characteristic odor is formed - iodoform (CHI_3).

b) Color reaction with alkaline solution of m-dinitrobenzene. The preparation is dissolved in alcohol, the reagent is added, a pink color appears, which gradually changes to reddish-brown.

Purity Test

The presence of impurities in progesterone is detected by ***TLC***. The evaluation is made by comparing the position, appearance and color intensity of the main spot of the test solution and the standard sample. The acceptable level of foreign steroid impurities in progesterone should not exceed 1.5%.

Methods for the analysis of impurities in progesterone by ***HPLC*** on direct and reversed-phase columns are described.

Assay

1. ***Spectrophotometry at the absorption maximum (241 nm)*** using ethanol as solvent. The dry matter content is calculated from the predetermined specific absorbance (535).
2. ***HPLC method on columns with direct and reversed phases.*** According to the comparative retention time of the main peak on the chromatogram of the standard sample, which is used as an internal standard in the quantitative determination.
3. ***Gravimetry.*** Add 2,4-dinitrophenylhydrazine, 95% alcohol to the preparation and boil to dissolution on a water bath in a flask with a reflux condenser. Concentrated hydrochloric acid is then added and boiled for another 15 minutes. The contents of the flask are cooled to room temperature and filtered. The washed precipitate is dried at 100 °C to constant weight.

Storage

Progesterone is a light-sensitive substance. It should be stored in a dry place, in well-closed containers, away from light, at temperatures not exceeding 20-25°C.

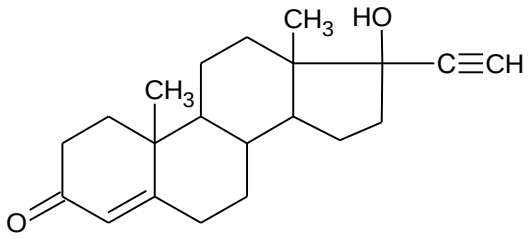
Dosage forms

0.5%, 1%, 2% oil solutions, 0.1 g capsules and 1% gel for topical application.

Medical use

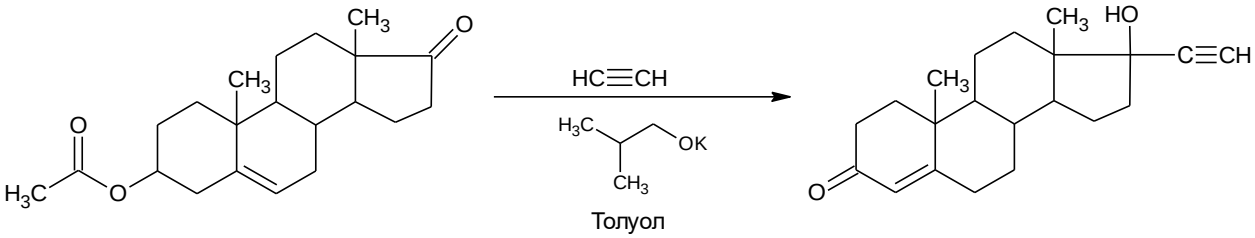
Progesterone is prescribed for amenorrhea, infertility associated with impaired function of the corpus luteum, habitual and threatening miscarriage, ovulatory uterine bleeding.

PREGNINE



Obtaining

Pregnine is prepared from 3-acetate of dehydroepiandrosterone-17 according to the following scheme:



Physical properties

White or white with yellowish tinge, odorless crystalline powder. Melting point 270-276°C. Specific rotation from +28 to +32° (0.5% solution in a mixture of equal volumes of 95% alcohol and chloroform). Practically insoluble in water, very slightly soluble in 95% alcohol and ether, slightly soluble in chloroform.

Identification

Instrumental methods

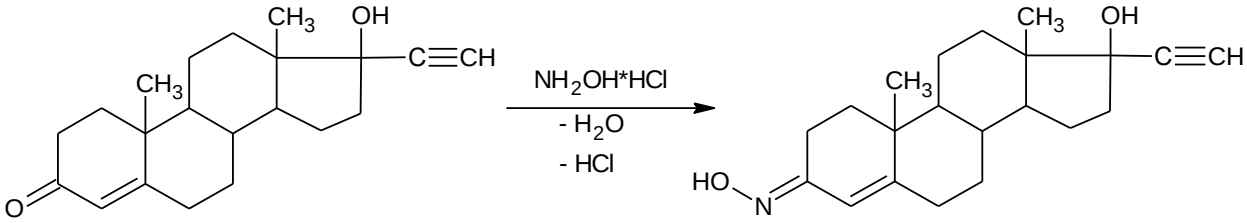
1. ***Infrared spectroscopy***. The infrared spectrum taken in vaseline oil in the region 3700-400 cm-1 should completely coincide with the spectrum figure attached to the pharmacopoeial monograph.
2. ***UV spectrophotometry*** is based on the measurement of optical density at 240 nm (maximum light absorption).
3. ***Polarimetry***. Determine the specific rotation, which should be from +28 to +32° (0.5% solution in a mixture of equal volumes of 95% alcohol and chloroform).

Chemical Methods

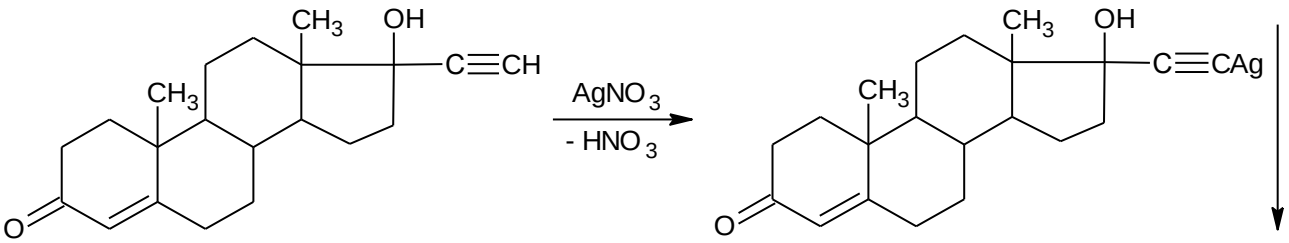
1. ***Interaction with concentrated sulfuric acid (for steroid cycle)***. The preparation is dissolved in concentrated sulfuric acid, water is added, shaken; a purple coloration with green fluorescence appears. If chloroform is added, the lower layer is orange, the upper layer is almost colorless.

2. ***Reaction with carbonyl group at 3 position***.

   Formation of oxime. With hydroxylamine hydrochloride in the presence of sodium acetate in the medium of methyl alcohol, oxime is formed which, after recrystallization from 70% methyl alcohol, melts at 226-232 °.



3. ***Reaction on the ethynyl group at position 17.*** When interacting with silver nitrate, a white precipitate of silver acetylenide is formed.



Purity Test

The purity of the preparation is determined by the absence of impurities (***sulfate as***h should not exceed 0.1% and ***loss on drying*** to constant weight at 100° -0.5%).

Impurities are also determined by ***TLC*** and ***HPLC*** methods.

Assay

Spectrophotometry. Pregnin in powder and tablet is determined spectrophotometrically at a wavelength of 241 nm (in relation to 0.001% solution of standard sample of pregnin).
Pregnin content in percent (X) is calculated by the formula:

$$X = \frac{D_1 \cdot C_0 \cdot 100}{D_0 \cdot C_1},$$

where D_1 - optical density of the test solution;
D_0 - optical density of the standard sample solution;
C_1 - concentration of the test solution;
C_o - concentration of the standard sample solution.

Storage

Store with care, in well corked jars, in a dry place, away from light.

Dosage forms

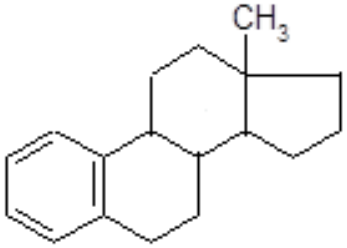
Tablets of 0.005-0.01.

Medical use

Pregnine is 5-6 times less effective than progesterone, but it is more convenient to introduce into the body. Pregnin is used internally. Indications: anovulatory menstrual cycle, secondary amenorrhea, metrorrhagia, dysmenorrhea, spontaneous abortion, threatened abortion.

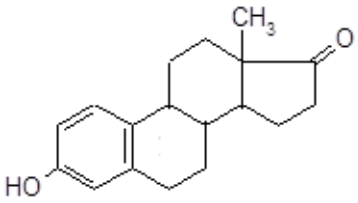
ESTROGENS

Estrogen is based on the hydrocarbon estran:

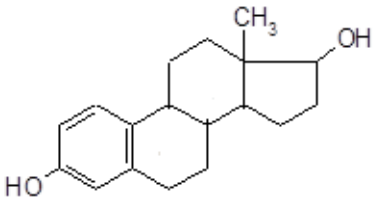


Estran

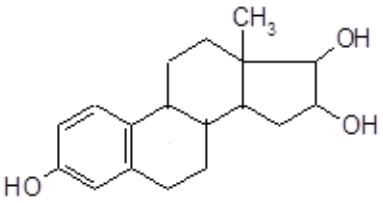
Three natural estrogenic hormones are known: estrone (folliculin), estradiol, and estriol:



эстрон



эстрадиол

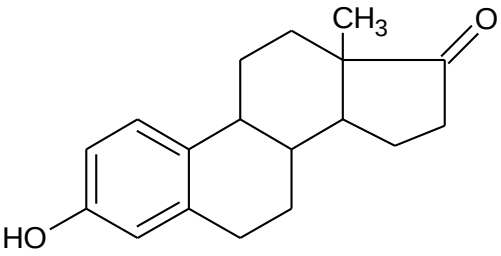


эстриол

For a long time, the natural hormone estrone (folliculin) was used in medicine in the form of oil solutions. Estradiol has twice the activity, but was not used because of its rapid inactivation. It was later shown that estradiol esters are more stable substances than estrone. They also have a longer action.

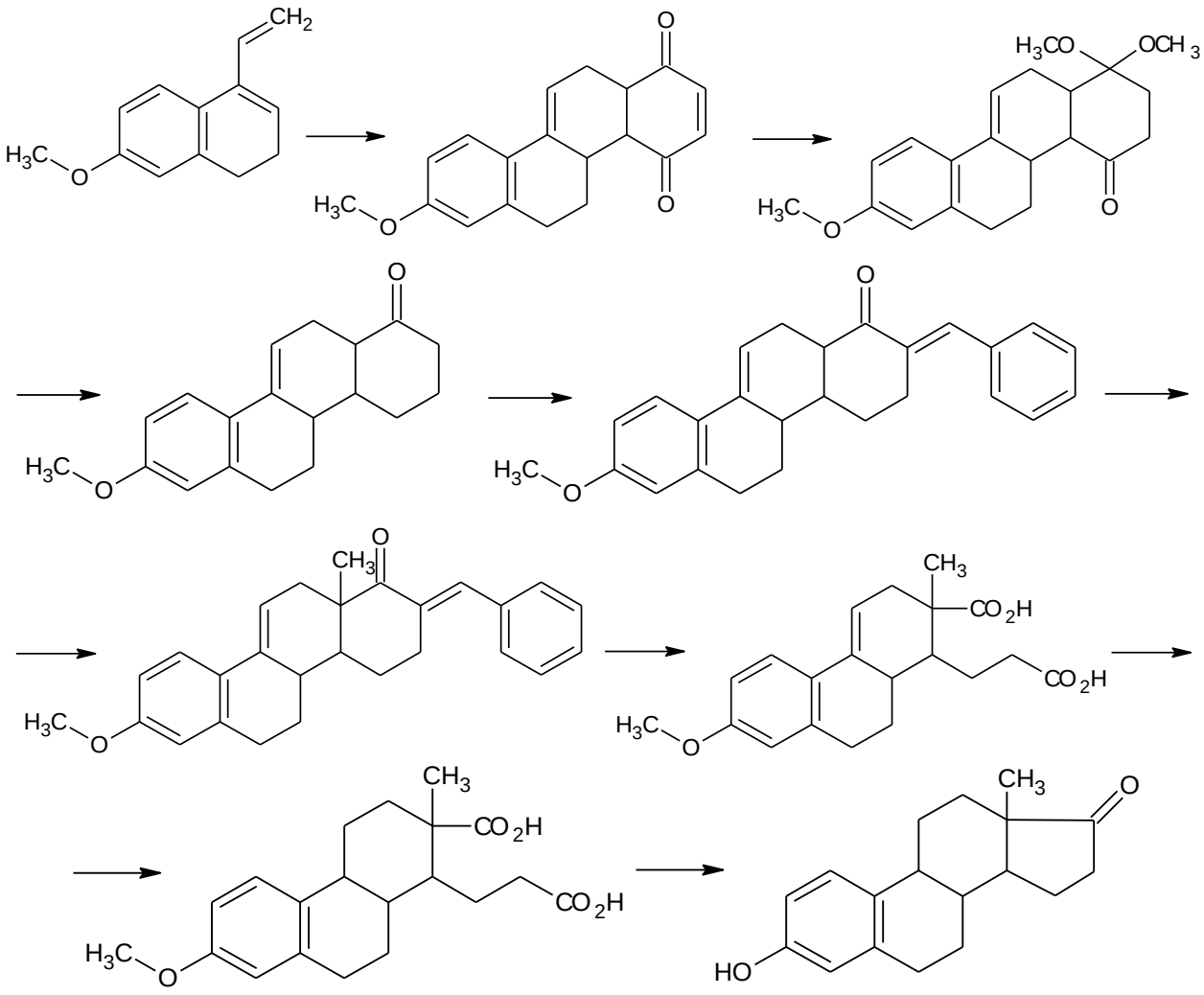
Of the semisynthetic estradiol analogues, ethinyl estradiol, mestranol, and estradiol dipropionate are used as drugs. Ethinylestradiol and mestranol are characterized by the presence of ethinyl radical (as in Pregnin) at position 17 in the molecule, which led to a several-fold increase in estrogenic activity compared to estrone and its retention when administered orally.

ESTRONE



Obtaining

The synthesis of estrone was carried out by W. Johnson. The method proposed by him is the simplest and most stereospecific:



For medical purposes, estrone (folliculin) is obtained from the urine of pregnant women or pregnant animals. During pregnancy, the production of follicular hormone increases significantly and large amounts are excreted in the urine.

Physical properties

Small crystals of white color or white with creamy tint crystalline powder. Practically insoluble in water, soluble in alcohol, ether, acetone, dioxane, vegetable oils.

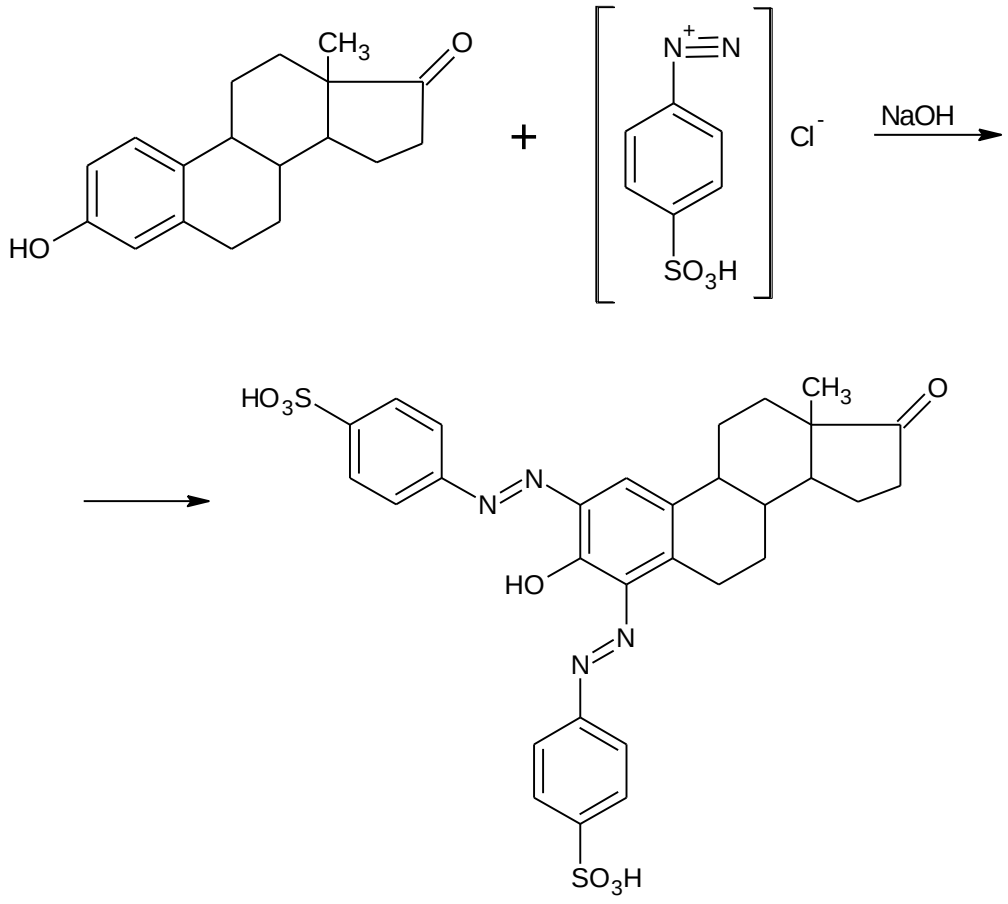
Identification

Instrumental methods

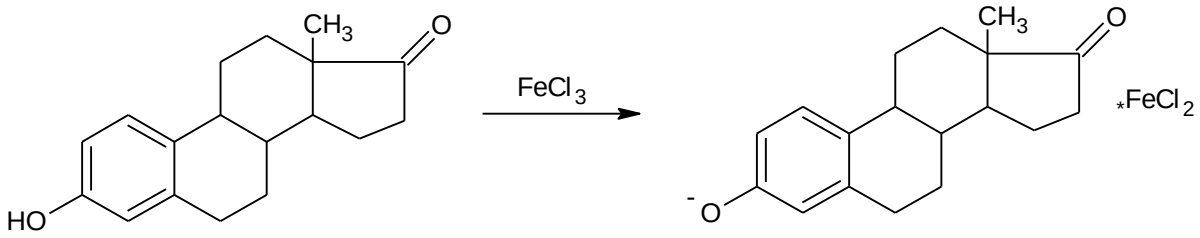
1. ***Infrared spectrometry***. The infrared spectrum taken in vaseline oil in the region from 4000 to 200 cm-1 should not differ from the spectrum figure attached to the pharmacopoeial monograph.
2. ***UV spectrometry***.
3. ***Chromatographic methods*** (HPLC and TLC).
4. ***Mass spectrometry***.

Chemical methods

1. ***Reaction for steroid cycle with concentrated sulfuric acid***. The preparation takes on a straw yellow color, changing to orange and red-brown.
2. ***Phenolic hydroxyl reactions***.
   (a) *Formation of azo dye*. When estrone is combined with diazonium salt in an alkaline strelod, a pale yellow coloration gradually appears.



(b) ***Salt formation***. When interacting with ferric (III) chloride, a blue coloration appears.



3. ***Reactions for the keto group at position 17***. The drug is dissolved in sodium hydroxide and 2% alcoholic solution of m-dinitrobenzene is added. After 5-8 min a red coloration develops.

Purity test

The TLC method is used to determine the impurity content of other steroids.

Assay

1. ***Photocolorimetry***. The technique is based on the reaction of azo dye formation.
2. ***HPLC***

Storage

Store with caution in well capped containers, in a dry place protected from light.

Dosage form

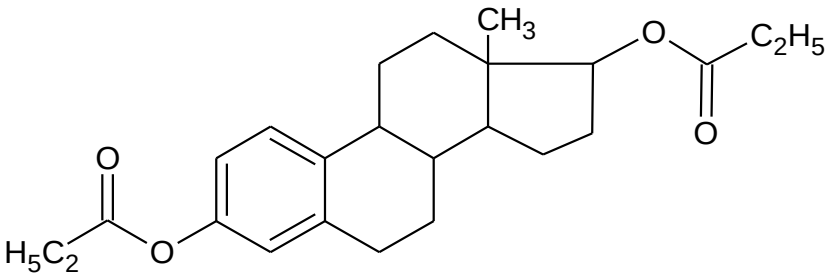
Oil solution in ampoules of 1 ml (5000 units) in a package of 6 pieces; 1 ml (10 000 units) in a package of 3 pieces.

Medical use

Conditions caused by insufficient ovarian function: primary and secondary amenorrhea, hypoplasia of genital organs and insufficient development of secondary sexual characteristics, menopausal and post-

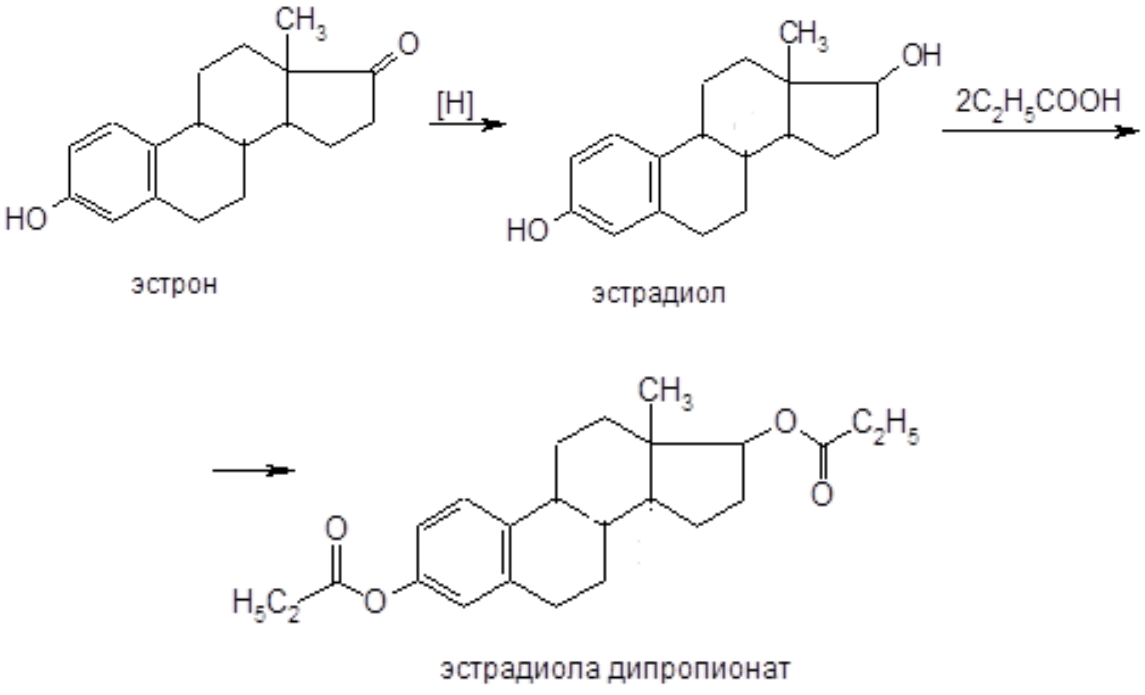
castration disorders, infertility due to decreased ovarian estrogenic function, weakness of labor activity, postpartum pregnancy.

ESTRADIOL DIPROPIONATE



Obtaining

Estradiol and estradiol dipropionate are synthesized from estrone by hydrogenation of the 17 keto group to estradiol followed by acylation of the 3- and 17β-oxy groups:



Physical properties

White crystalline powder without odor. Melting point 104-108°C. Specific rotation from +37 to +41° (1% solution in dioxane). Insoluble in water, soluble in alcohol, ether, vegetable oils.

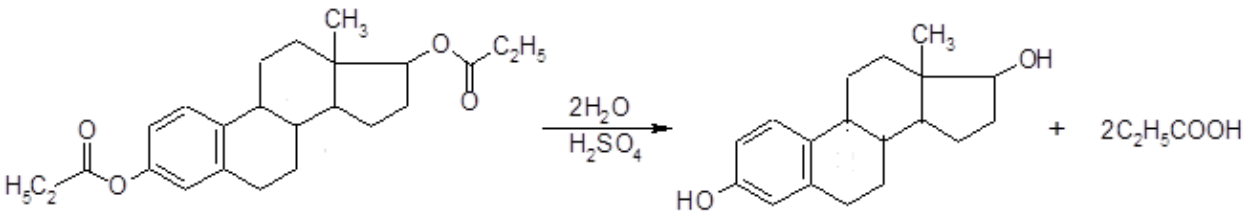
Identification

Instrumental methods

1. ***Infrared spectrometry***. The IR spectrum taken in petroleum jelly in the region from 4000 to 200 cm-1 should not differ from the spectrum figure attached to the pharmacopoeial monograph.
2. ***UV spectrometry***. Estradiol dipropionate is identified by UV-spectrum of 0.01% solution in ethanol, which in the region of 220-350 nm should have two absorption maxima (at 269 and 276 nm).
3. ***Chromatographic methods*** (HPLC and TLC).
4. ***Mass spectrometry.***

Chemical Methods

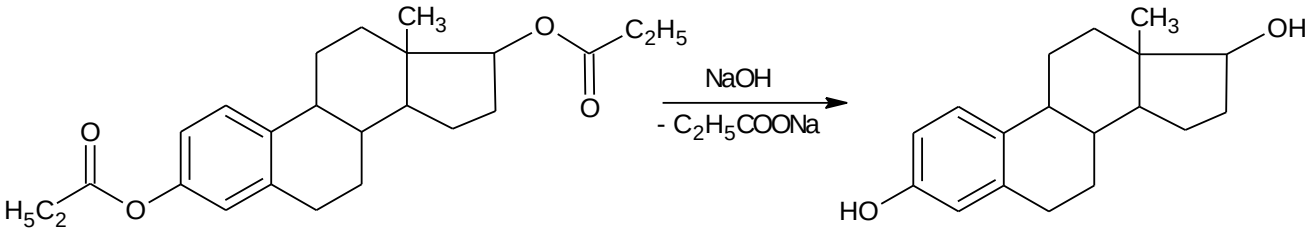
1. ***Reaction for steroid cycle with concentrated sulfuric acid***. When concentrated sulfuric acid is added to the preparation, a brown color with characteristic greenish fluorescence appears on heating. When this mixture is diluted with water, a pink color appears.
2. ***Reaction with propionic acid residue***. Estradiol dipropionate is hydrolyzed under the action of concentrated sulfuric acid to form estradiol and propionic acid. Subsequent heating in the presence of ethanol leads to the formation of propionic acid ethyl ester, which has a characteristic odor:



эстрадиола дипропионат эстрадиол

3. ***Estradiol detection***. After alkaline hydrolysis (followed by purification from impurities) of estradiol dipropionate, estradiol

is identified by the formation of estradiol, which has a melting point of 173-179°C.

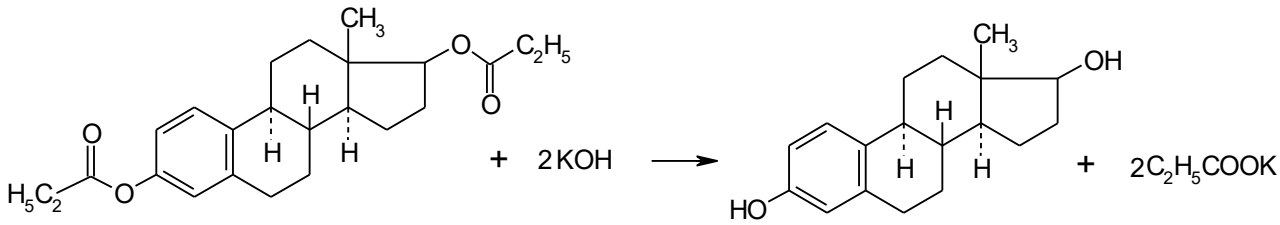


Purity test

Impurities of extraneous steroids are determined by TLC. HPLC and mass spectrometry can also be used to determine impurities.

Assay

1. ***Neutralization***. For quantitative determination of estradiol dipropionate use alkaline hydrolysis reaction exactly measured amount of 0.1 M alcoholic potassium hydroxide solution, excess of which is titrated with 0.1 M hydrochloric acid solution (indicator phenolphthalein):



эстрадиола дипропионат эстрадиол

$$KOH + HCl \longrightarrow KCl + H_2O$$

2. ***HPLC***
3. ***Mass spectrometry***

Storage

Estradiol dipropionate should be stored with caution in tightly closed containers at a dry place away from light.

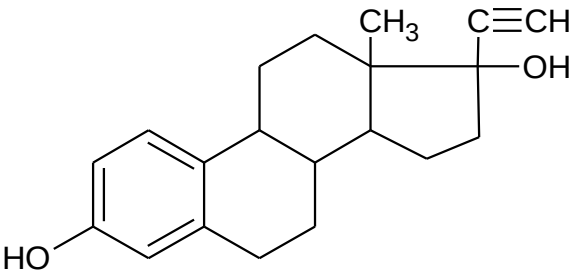
Dosage forms

In ampoules of 1 ml of 0.1% oil solution in a package of 3 pieces.

Medical use

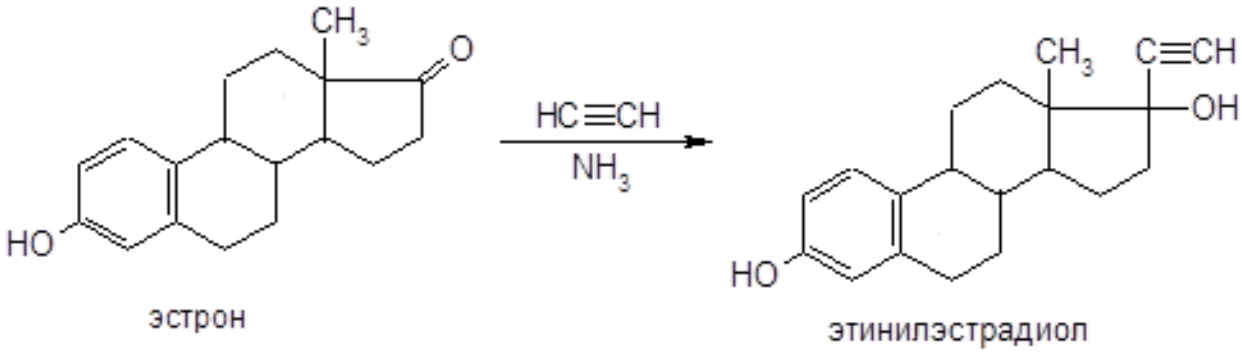
- Diseases accompanied by insufficient ovarian function
- Underdevelopment or shrinking of the ovaries and mammary glands
- Absence or irregularity of menstruation
- Painful conditions associated with menopause or castration
- Infertility
- To Induce labor
- To induce labor in case of prolonged pregnancy.
- Skin diseases: acne, hypertrichosis, etc.

ETHINYL ESTRADIOL



Obtaining

Ethinyl estradiol is synthesized by the action of acetylene on estrone:



эстрон

этинилэстрадиол

Physical properties

White with creamy tint to light cream color fine-crystalline powder. Melting point 181 to 186°C. Specific rotation from -27 to -31° (0.4% solution in pyridine). Practically insoluble in water, soluble in 95% alcohol and chloroform, readily soluble in acetone, dioxane and ether. Insignificantly soluble in alkali solutions.

Identification

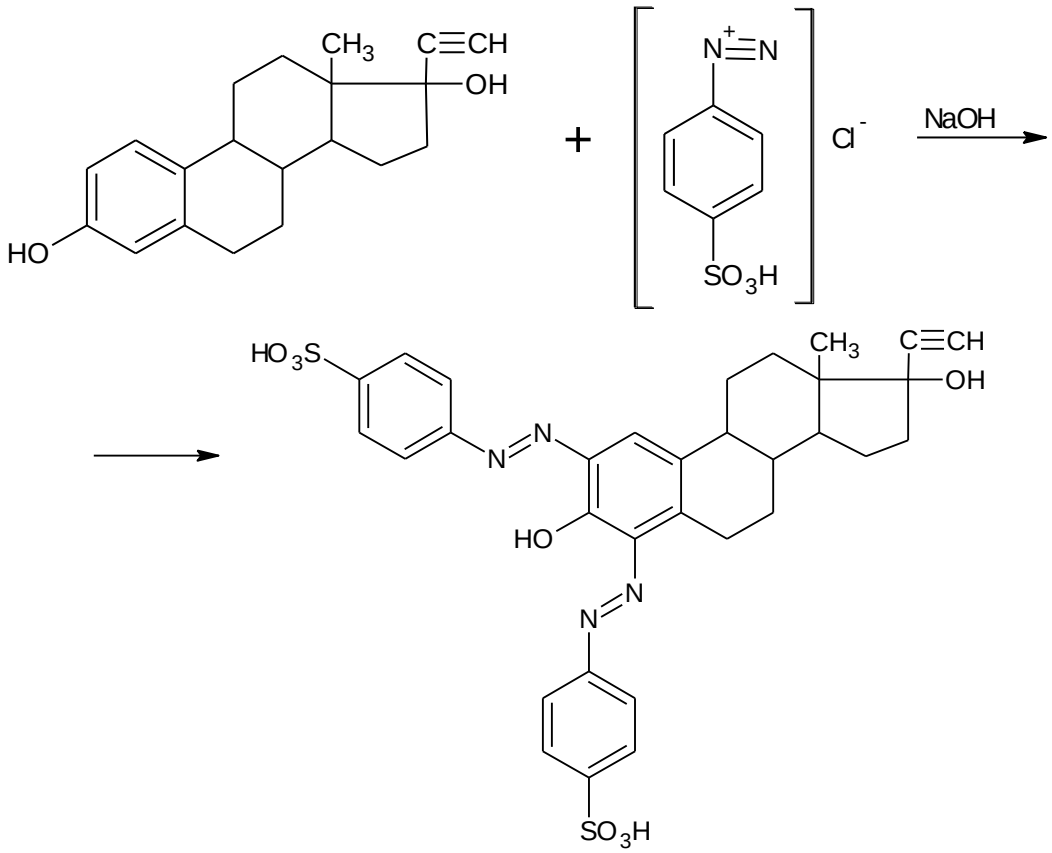
Instrumental methods

1. ***Infrared spectroscopy***. The IR spectrum taken in petroleum jelly in the region from 4000 to 200 cm-1 should not differ from the spectrum pattern attached to the pharmacopoeial monograph.
2. ***UV spectroscopy***. The UV absorption spectrum of a solution of ethinyl estradiol in a mixture of ethanol and sodium hydroxide in the region of 220 to 330 nm has absorption maxima at 241 and 299 nm and absorption minima at 226 and 271 nm, while a solution in ethanol has an absorption maximum at 280 nm. Ethinyl estradiol can be distinguished by the specific absorbance of a 0.005% alcohol solution at 280 nm. It should be 65-69.
3. ***Chromatographic methods*** (HPLC and TLC).
4. ***Mass spectrometry***.

Chemical methods

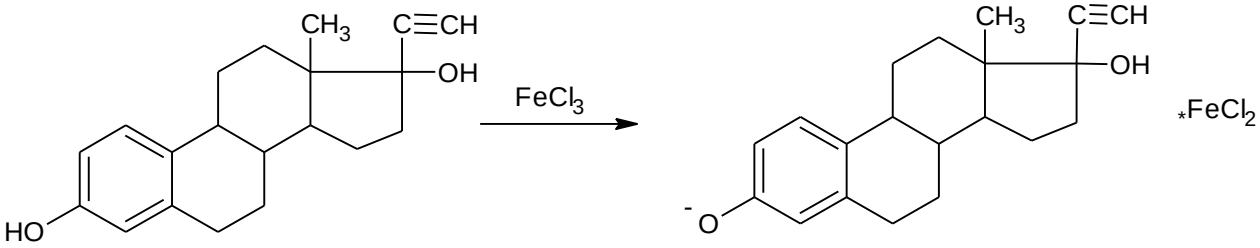
1. ***Reaction for steroid cycle with concentrated sulfuric acid***. This results in an orange-red colored solution with yellowish green fluorescence.
2. ***Reactions on phenolic hydroxyl***

   (a) *Formation of azo dye*. When ethinyl estradiol is combined with diazonium salt in an alkaline strelod, a dark red colored solution is formed.

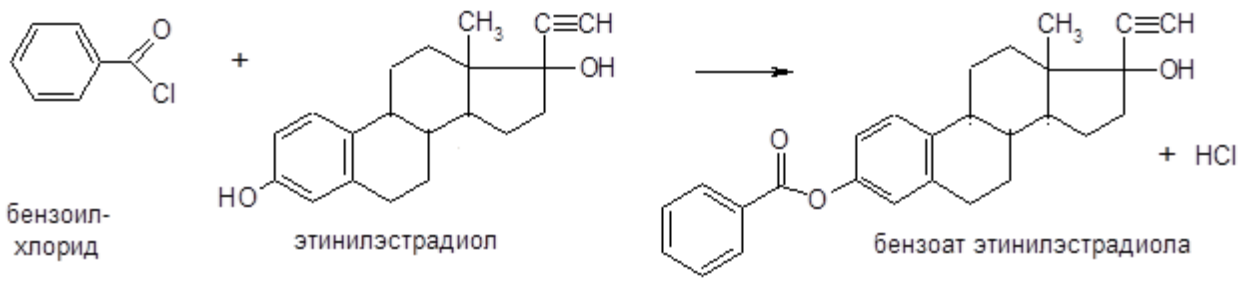


(b) Formation of auric dye. With Markey's reagent (formaldehyde in concentrated sulfuric acid) an auric dye colored crimson or violet is formed.

(c) Interaction with ferric (III) chloride. A blue coloring appears.



(d) Formation of esters. When the drug interacts with benzoyl chloride, ethinyl estradiol benzoate is formed, which has a melting point of 199-202°C.



бензоил-хлорид

этинилэстрадиол

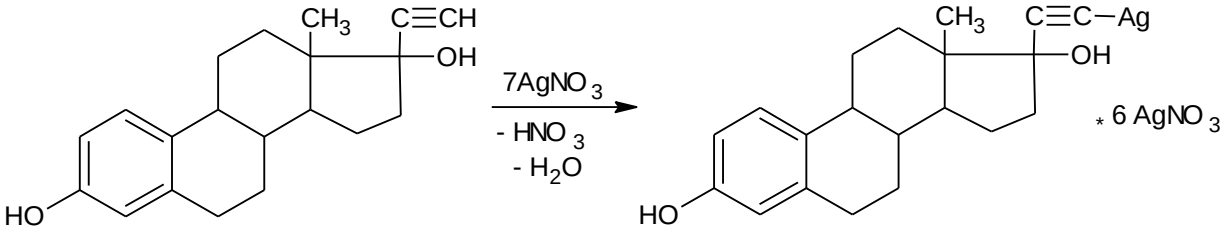
бензоат этинилэстрадиола

Purity test

Impurities are determined by the TLC method.

Assay

1. ***Indirect neutralization***. The property of acetylene derivatives to form salts with silver is used. Tetrahydrofuran purified from peroxide compounds is used as a solvent. Nitric acid released after addition of silver nitrate is titrated with 0.1 M sodium hydroxide solution by potentiometric method with glass indicator electrode. Ethinylestradiol forms a double salt with silver nitrate, which consists of the silver salt of ethinylestradiol and six molecules of silver nitrate:



$$HNO_3 + NaOH \rightarrow NaNO_3 + H_2O$$

2. ***Spectrophotometry*** in anhydrous ethanol medium at 281 nm.
3. ***Photocolorimetric methodology*** for the determination of ethinyl estradiol is based on the reaction of azo dye formation (see identity reactions).
4. ***HPLC***

Storage

In well corked orange glass jars.

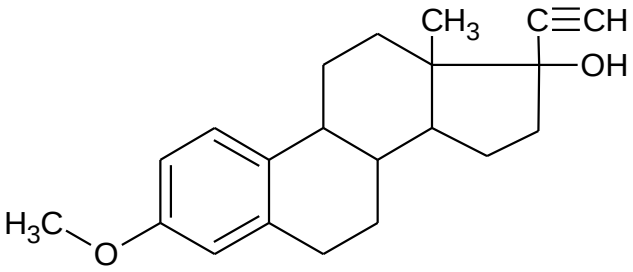
Dosage form

Tablets 0.00001 and 0.00005 g; forte tablets 0.05 g. Included in the contraceptives nonovlon, ovidone, etc. Injectable forms are known.

Medical use

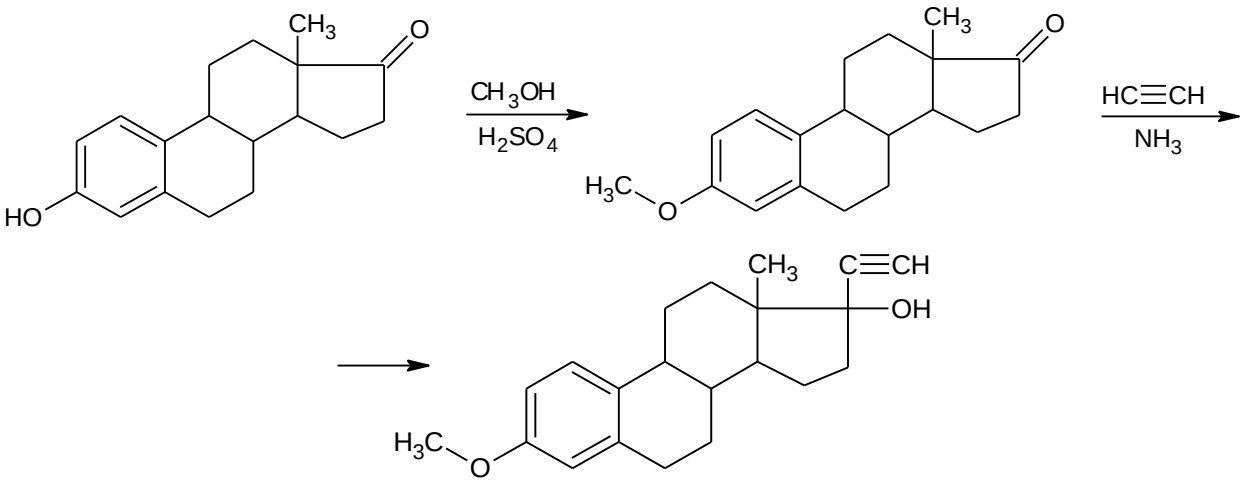
Some forms of endocrine infertility, severe forms of pathological menopause, dysfunctional uterine bleeding, androgen-dependent prostate cancer, some forms of breast cancer. Used as part of some contraceptives.

MESTRANOL



Obtaining

Methylation of estrone by various methylating agents (diazomethane, methanol with sulfuric acid) followed by acetylation with ammonia.



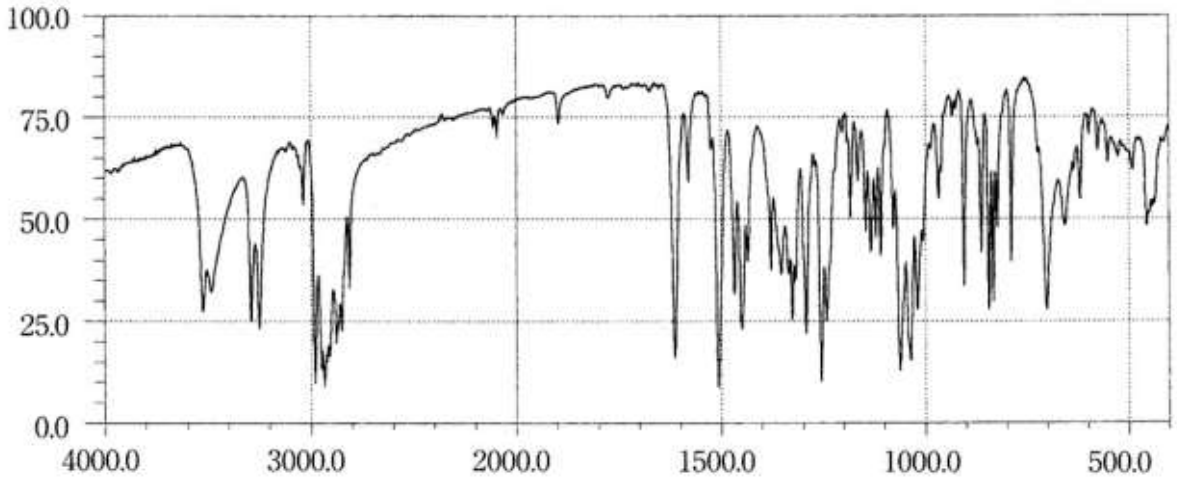
Physical properties

White or white with a creamy tint crystalline powder. Melting point149-154°C. Specific rotation from +2 to + 8° (2% solution in chloroform). Practically insoluble in water, easily soluble in chloroform, moderately soluble in ethanol.

Identification

Instrumental methods

1. ***Infrared spectroscopy***. The infrared spectrum taken in vaseline oil in the region from 4000 to 200 cm-1 should not differ from the spectrum figure attached to the pharmacopoeial monograph.



2. ***UV spectroscopy***. The UV spectrum of a solution of mestranol (0.005% solution in ethanol or methanol) has a maximum at 279 nm and a specific absorption index of 59 to 64.
3. ***Chromatographic methods*** (HPLC and TLC).
4. ***Mass spectrometry***.

Chemical methods

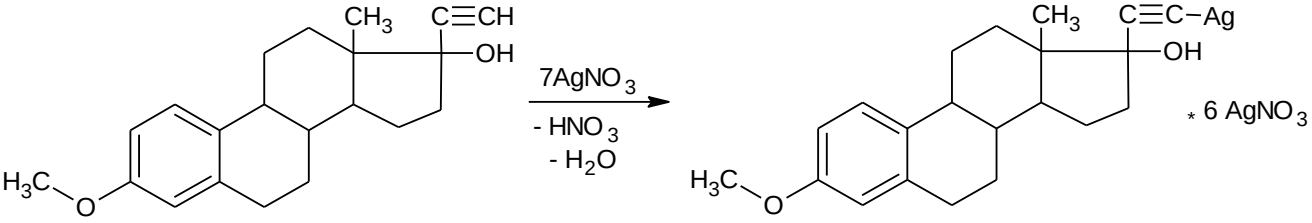
Reaction To the steroid cycle with concentrated sulfuric acid. Mestranol with concentrated sulfuric acid forms a blood red staining with yellow-green fluorescence.

Purity test

Impurities of extraneous steroids are determined by TLC. The total content of steroid impurities - not more than 2%.

Assay

1. ***Indirect neutralization***. The property of acetylene derivatives to form salts with silver is used. Tetrahydrofuran purified from peroxide compounds is used as a solvent. Nitric acid released after addition of silver nitrate is titrated with 0.1 M sodium hydroxide solution by potentiometric method with glass indicator electrode.



$$HNO_3 + NaOH \rightarrow NaNO_3 + H_2O$$

2. ***HPLC***

Storage

In well corked orange glass jars.

Dosage form

Tablets of 0.05 and 0.08 g. Included in the contraceptive drug infecundin.

Medical use

Secondary amenorrhea, cyclic additional bleeding of a functional nature, postpartum endometritis, endometritis after abortion, menopausal syndrome.