ASSAY

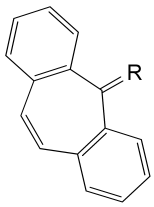
Dissolve 0.250 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *alcohol R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 32.39 mg of $C_{21}H_{22}ClN$.

STORAGE

Store protected from light.

IMPURITIES



A. R = H_2: dibenzo[*a,d*]cycloheptene,

B. R = O: 5*H*-dibenzo[*a,d*]cyclohepten-5-one (dibenzosuberone).

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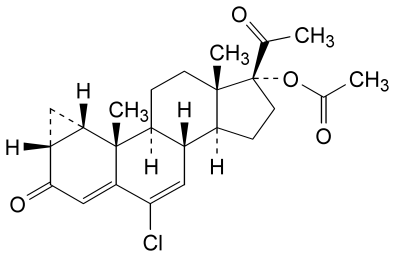
CYPROTERONE ACETATE

Cyproteroni acetas



$C_{24}H_{29}ClO_4$ M_r 416.9
[427-51-0]

DEFINITION

6-Chloro-3,20-dioxo-1β,2β-dihydro-3′*H*-cyclopropa-[1,2]pregna-1,4,6-trien-17-yl acetate.

Content: 97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, very soluble in methylene chloride, freely soluble in acetone, soluble in methanol, sparingly soluble in anhydrous ethanol.

mp: about 210 °C.

IDENTIFICATION

First identification: A.

Second identification: B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *cyproterone acetate CRS*.

B. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve 20 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.
Reference solution. Dissolve 10 mg of *cyproterone acetate CRS* in *methylene chloride R* and dilute to 5 ml with the same solvent.
Plate: *TLC silica gel F_{254} plate R*.
Mobile phase: *cyclohexane R, ethyl acetate R* (50:50 *V/V*).
Application: 5 µl.
Development: twice over a path of 15 cm; dry the plate in air between the 2 developments.
Drying: in air.
Detection: examine in ultraviolet light at 254 nm.
Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. To about 1 mg add 2 ml of *sulphuric acid R* and heat on a water-bath for 2 min. A red colour develops. Cool. Add this solution cautiously to 4 ml of *water R* and shake. The solution becomes violet.

D. Incinerate about 30 mg with 0.3 g of *anhydrous sodium carbonate R* over a naked flame for about 10 min. Cool and dissolve the residue in 5 ml of *dilute nitric acid R*. Filter. To 1 ml of the filtrate add 1 ml of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

E. It gives the reaction of acetyl (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*): + 152 to + 157 (dried substance).

Dissolve 0.25 g in *acetone R* and dilute to 25.0 ml with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 10.0 mg of the substance to be examined in *acetonitrile R* and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile R*.

Reference solution (b). Dissolve 5 mg of *medroxyprogesterone acetate CRS* in *acetonitrile R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with reference solution (a).

Column:

– *size*: *l* = 0.125 m, Ø = 4.6 mm;

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase: *acetonitrile R, water R* (40:60 *V/V*).

Flow rate: 1.5 ml/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µl.

Run time: twice the retention time of cyproterone acetate.

System suitability: reference solution (b):

– *resolution*: minimum 3.0 between the peaks due to cyproterone acetate and medroxyprogesterone acetate.

Limits:

– *total*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

– *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying at 80 °C at a pressure not exceeding 0.7 kPa.

Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

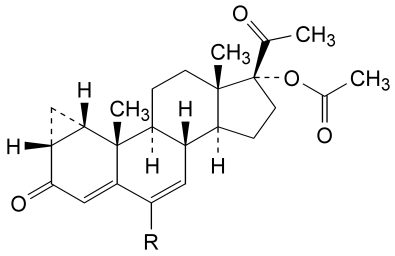
Dissolve 50.0 mg in *methanol R* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *methanol R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 282 nm.

Calculate the content of $C_{24}H_{29}ClO_4$ taking the specific absorbance to be 414.

STORAGE

Protected from light.

IMPURITIES



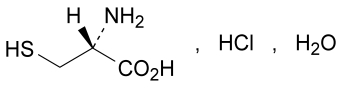
A. R = H: 3,20-dioxo-1β,2β-dihydro-3′*H*-cyclopropa-[1,2]pregna-1,4,6-trien-17-yl acetate,

B. R = OCH_3: 6-methoxy-3,20-dioxo-1β,2β-dihydro-3′*H*-cyclopropa[1,2]pregna-1,4,6-trien-17-yl acetate.

01/2008:0895
corrected 6.0

CYSTEINE HYDROCHLORIDE MONOHYDRATE

Cysteini hydrochloridum monohydricum



$C_3H_8ClNO_2S,H_2O$ M_r 175.6
[7048-04-6]

DEFINITION

Cysteine hydrochloride monohydrate contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (2*R*)-2-amino-3-sulfanylpropanoic acid hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, slightly soluble in alcohol.

IDENTIFICATION

First identification: A, B, E.

Second identification: A, C, D, E.

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *cysteine hydrochloride monohydrate CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour, and size to the principal spot in the chromatogram obtained with reference solution (b).

D. Dissolve about 5 mg in 1 ml of *dilute sodium hydroxide solution R*. Add 1 ml of a 30 g/l solution of *sodium nitroprusside R*. An intense violet colour develops which becomes brownish-red and then orange. Add 1 ml of *hydrochloric acid R*. The solution becomes green.

E. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S. Dissolve 2.5 g in *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution. Dilute 10 ml of solution S to 20 ml with *water R*. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*). Dissolve 2.00 g in *hydrochloric acid R1* and dilute to 25.0 ml with the same acid. The specific optical rotation is + 5.5 to + 7.0, calculated with reference to the dried substance.

Ninhydrin-positive substances. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel plate R*.

Test solution (a). Dissolve 0.20 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent. To 5 ml of the solution add 5 ml of a 40 g/l solution of *N-ethylmaleimide R* in *alcohol R*. Allow to stand for 5 min.

Test solution (b). Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a). Dissolve 20 mg of *cysteine hydrochloride monohydrate CRS* in *water R* and dilute to 10 ml with the same solvent. Add 10 ml of a 40 g/l solution of *N-ethylmaleimide R* in *alcohol R*. Allow to stand for 5 min.

Reference solution (b). Dilute 2 ml of reference solution (a) to 10 ml with *water R*.

Reference solution (c). Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (d). Dissolve 10 mg of *tyrosine CRS* in 10 ml of reference solution (a) and dilute to 25 ml with *water R*.

Apply separately to the plate 5 µl of each test solution and reference solutions (b), (c), and (d). Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Dry the plate at 80 °C for 30 min. Spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows 2 clearly separated principal spots.

Sulphates (*2.4.13*). Dilute 10 ml of solution S to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium (*2.4.1*). 50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm NH_4) R*.

Iron (*2.4.9*). In a separating funnel, dissolve 0.50 g in 10 ml of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min