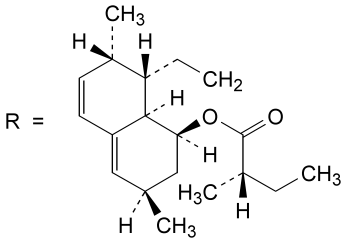
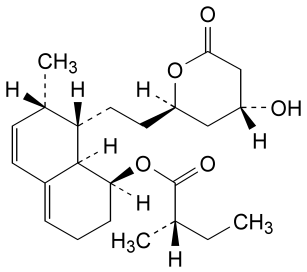
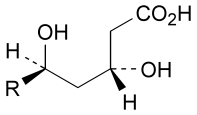
IMPURITIES

Specified impurities: *A, B, C, D.*

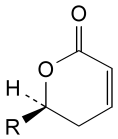




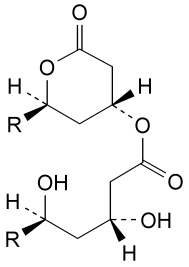
A. (1*S*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-7-methyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*S*)-2-methylbutanoate (mevastatin),



B. (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-2,6-dimethyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoic acid (hydroxyacid lovastatin),



C. (1*S*,3*R*,7*S*,8*S*,8a*R*)-3,7-dimethyl-8-[2-[(2*R*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]ethyl]-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*S*)-2-methylbutanoate (dehydrolovastatin),

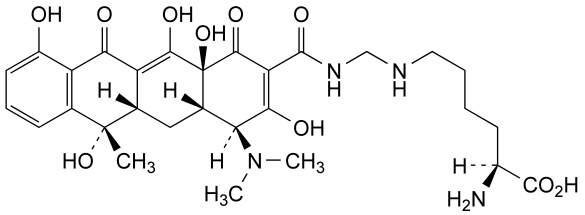


D. (2*R*,4*R*)-2-[2-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-2,6-dimethyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]ethyl]-6-oxotetrahydro-2*H*-pyran-4-yl (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-2,6-dimethyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoate (lovastatin dimer).

01/2008:1654

LYMECYCLINE

Lymecyclinum



$C_{29}H_{38}N_4O_{10}$ M_r 603

[302-17-0]

DEFINITION

(2*S*)-2-Amino-6-[[[[[(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracen-2-yl]carbonyl]amino]methyl]amino]hexanoic acid (reaction product of formaldehyde, lysine and tetracycline), semi-synthetic product derived from a fermentation product.

Content: 81.0 per cent to 102.0 per cent (equivalent to 60.0 per cent to 75.0 per cent of tetracycline) (anhydrous substance).

CHARACTERS

Appearance: yellow powder, hygroscopic.

Solubility: very soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a). Dissolve 5 mg of *tetracycline hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b). Dissolve 5 mg of *tetracycline hydrochloride CRS*, 5 mg of *demeclocycline hydrochloride R* and 5 mg of *oxytetracycline hydrochloride R* in *methanol R* and dilute to 10 ml with the same solvent.

Plate: *TLC octadecylsilyl silica gel F_{254} plate R* (2-10 µm).

Mobile phase: mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/l solution of *oxalic acid R* previously adjusted to pH 2.0 with *concentrated ammonia R*.

Application: 2 µl.

Development: over a path of 5 cm.

Drying: in air.

Detection: examine in ultraviolet light at 254 nm.

System suitability: reference solution (b):

– the chromatogram shows 3 clearly separated spots.

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 reference solution (a).

B. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 50 mg of the substance to be examined in 50 ml *water R*.

Reference solution (a). Dissolve 10 mg of *lysine hydrochloride CRS* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b). Dissolve 10 mg of *arginine CRS* and 10 mg of *lysine hydrochloride CRS* in *water R* and dilute to 25 ml with the same solvent.

Plate: *TLC silica gel plate R.*

Mobile phase: *concentrated ammonia R, 2-propanol R* (30:70 *V/V*).

Application: 5 µl.

Development: over 3/4 of the plate.

Drying: at 100-105 °C until the ammonia disappears completely.

Detection: spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

System suitability: reference solution (b):

– the chromatogram shows 2 clearly separated principal spots.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.2 g in 5 ml of *water R*, add 0.3 ml of *orthophosphoric acid R* and distil. To 1 ml of the distillate add 10 ml of *chromotropic acid-sulphuric acid solution R*. A violet colour is produced.

D. Specific optical rotation (see Tests).

TESTS

pH (*2.2.3*): 7.8 to 8.2.

Dissolve 0.1 g in 10 ml of *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*): – 180 to – 210 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 50.0 ml with the same solvent.

Free tetracycline: maximum 2.5 per cent (anhydrous and methanol-free substance).

To 0.5 g add 50 ml of *butyl acetate R* and allow to stand at 25 °C for 1 h. Filter and extract the filtrate with 2 quantities each of 25 ml of *0.1 M hydrochloric acid*. Combine the extracts and complete to 50.0 ml with *0.1 M hydrochloric acid*. Dilute 10.0 ml of this solution to 100.0 ml with *0.1 M hydrochloric acid*. The absorbance (*2.2.25*) measured at 355 nm is not greater than 0.64.

Light absorbing impurities: the absorbance (*2.2.25*) is not greater than 0.50 at 430 nm (anhydrous and methanol-free substance).

Dissolve 25.0 mg in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution. Dissolve 0.125 g of the substance to be examined in 5.0 ml of *water R*. Add 1.0 ml of a 40 g/l solution of *sodium metabisulphite R* and allow to stand without stirring at 20-25 °C for 16-24 h in the dark. Add 50 ml of *0.05 M hydrochloric acid*, shake to dissolve the precipitate and dilute to 100.0 ml with *water R*.

Reference solution (a). Dissolve 25.0 mg of *tetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (b). Dissolve 12.5 mg of *4-epitetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 50.0 ml with the same acid.

Reference solution (c). Dissolve 10.0 mg of *anhydrotetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid.

Reference solution (d). Dissolve 10.0 mg of *4-epianhydrotetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 50.0 ml with the same acid.

Reference solution (e). Mix 1.0 ml of reference solution (a), 2.0 ml of reference solution (b) and 5.0 ml of reference solution (d) and dilute to 25.0 ml with *0.01 M hydrochloric acid*.

Reference solution (f). Mix 40.0 ml of reference solution (b), 20.0 ml of reference solution (c) and 5.0 ml of reference solution (d) and dilute to 200.0 ml with *0.01 M hydrochloric acid*.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,

– *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm) with a pore size of 10 nm,

– *temperature*: 60 °C.

Mobile phase: weigh 80.0 g of *2-methyl-2-propanol R* and transfer to a 1000 ml volumetric flask with the aid of 200 ml of *water R*. Add 100 ml of a 35 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 8.0 with *dilute phosphoric acid R*, 200 ml of a 10 g/l solution of *tetrabutylammonium hydrogen sulphate R* adjusted to pH 8.0 with *dilute sodium hydroxide solution R* and 10 ml of a 40 g/l solution of *sodium edetate R* adjusted to pH 8.0 with *dilute sodium hydroxide solution R*; dilute to 1000.0 ml with *water R*.

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µl of the test solution and reference solutions (e) and (f).

Run time: 5 times the retention time of the principal peak in the chromatogram obtained with the test solution.

Relative retention with reference to tetracycline (retention time = about 8 min): impurity E = about 0.50; impurity F = about 0.68; impurity A = about 0.75; impurity B (eluting on the tail of the principal peak) = about 1.2; impurity D = about 1.45; impurity G = about 1.45; impurity C = about 2.95.

System suitability: reference solution (e):

– *resolution*: minimum 3.0 between the 1st peak (impurity A) and the 2nd peak (tetracycline) and minimum 5.0 between the 2nd peak and the 3rd peak (impurity D); adjust the concentration of 2-methyl-2-propanol in the mobile phase if necessary;

– *symmetry factor*: maximum 1.25 for the peak due to tetracycline.

Limits:

– *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (5.0 per cent),

– *impurity B*: not more than 0.1 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.5 per cent),

– *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (1.0 per cent),

– *sum of impurities D and G*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.5 per cent),

– *impurities E, F*: for each impurity, not more than 0.1 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.5 per cent),
– *any other impurity*: for each impurity, not more than 0.04 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.2 per cent),
– *total*: not more than 1.6 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (8.0 per cent),
– *disregard limit*: 0.02 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.1 per cent).

Methanol (*2.4.24, System A*): maximum 1.5 per cent.

Water (*2.5.12*): maximum 5.0 per cent, determined on 0.200 g.

Sulphated ash (*2.4.14*): maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection: test solution and reference solution (a).

System suitability:

– *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections of reference solution (a).

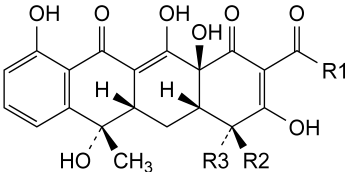
Calculate the percentage content of tetracycline and multiply it by 1.3560 to obtain the percentage content of lymecycline.

STORAGE

In an airtight container, protected from light.

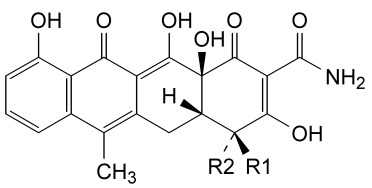
IMPURITIES

Specified impurities: A, B, C, D, E, F.



A. R1 = NH_2, R2 = H, R3 = $N(CH_3)_2$: (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epitetracycline),

B. R1 = CH_3, R2 = $N(CH_3)_2$, R3 = H: (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-2-acetyl-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyltetracycline),



C. R1 = $N(CH_3)_2$, R2 = H: (4*S*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydrotetracycline),

D. R1 = H, R2 = $N(CH_3)_2$: (4*R*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (4-epianhydrotetracycline),

E. unknown structure,

F. unknown structure,

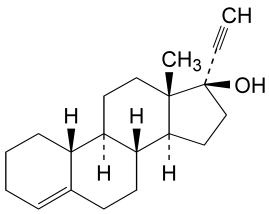
G. chlortetracycline,

H. tetracycline.

01/2008:0558
corrected 6.0

LYNESTRENOL

Lynestrenolum



$C_{20}H_{28}O$ M_r 284.4
[52-76-6]

DEFINITION

Lynestrenol contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 19-nor-17α-pregn-4-en-20-yn-17-ol, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, soluble in acetone and in alcohol.

IDENTIFICATION

First identification: B.

Second identification: A, C.

A. Melting point (*2.2.14*): 161 °C to 165 °C.
B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *lynestrenol CRS*.
C. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with test solution (b) is similar in position, fluorescence and size to the principal spot in the chromatogram obtained with reference solution (b).

TESTS

Appearance of solution. Dissolve 0.2 g in *alcohol R* and dilute to 10 ml with the same solvent. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*). Dissolve 0.900 g in *alcohol R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is – 9.5 to – 11, calculated with reference to the dried substance.