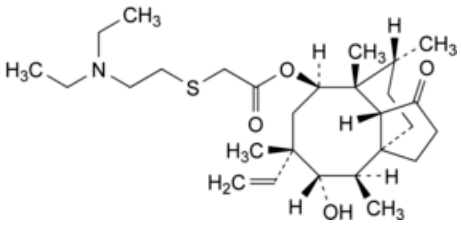
Edition: BP 2025 (Ph. Eur. 11.6 update)

Tiamulin

General Notices

(Tiamulin for Veterinary Use, Ph. Eur. monograph 1660)



$C_{28}H_{47}NO_4S$ 493.8 *55297-95-5*

Action and use

Antibacterial.

Ph Eur

DEFINITION

(3a*S*,4*R*,5*S*,6*S*,8*R*,9*R*,9a*R*,10*R*)-6-Ethenyl-5-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro-3a,9-propanocyclopenta[8]annulen-8-yl [[2-(diethylamino)ethyl]sulfanyl]acetate.

Semi-synthetic product derived from a fermentation product.

Content

96.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Sticky, translucent yellowish mass, slightly hygroscopic.

Solubility

Practically insoluble in water, very soluble in methylene chloride, freely soluble in anhydrous ethanol.

IDENTIFICATION

Infrared absorption spectrophotometry (2.2.24).

Comparison Ph. Eur. reference spectrum of tiamulin.

TESTS

Appearance of solution

The solution is clear (2.2.1) and its absorbance (2.2.25) at 420 nm is not greater than 0.050.

Dissolve 2.5 g in 50 mL of *methanol R*.

Related substances

Liquid chromatography (2.2.29).

Ammonium carbonate buffer solution pH 10.0 Dissolve 10.0 g of *ammonium carbonate R* in *water for chromatography R*, add 22 mL of *perchloric acid solution R* and dilute to 1000.0 mL with *water for chromatography R*. Adjust to pH 10.0 with *concentrated ammonia R1*.

Solvent mixture *acetonitrile R1*, ammonium carbonate buffer solution pH 10.0 (50:50 *V/V*).

Test solution Dissolve 0.200 g of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 0.250 g of *tiamulin hydrogen fumarate CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture.

Reference solution (c) Dilute 0.1 mL of *toluene R* to 100 mL with *acetonitrile R*. Dilute 0.1 mL of this solution to 100.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase *acetonitrile R1*, ammonium carbonate buffer solution pH 10.0, *methanol R2* (21:30:49 *V/V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 212 nm.

Injection 20 µL.

Run time 3 times the retention time of tiamulin.

Relative retention With reference to tiamulin (retention time = about 18 min): impurity A = about 0.22; impurity B = about 0.5; impurity C = about 0.66; impurity D = about 1.1; impurity F = about 1.6; impurity E = about 2.4.

System suitability Reference solution (a):

— baseline separation between the peaks due to tiamulin and impurity D.

Limits:

— *impurities A, B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *any other impurity*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent); disregard any peak present in the chromatogram obtained with reference solution (c).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 80 °C.

Bacterial endotoxins (*2.6.14, Method D*)

Less than 0.4 IU/mg, determined in a 1 mg/mL solution in *anhydrous ethanol R* (endotoxin free) diluted 1:40 with water for bacterial endotoxins test.

ASSAY

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

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{28}H_{47}NO_4S$, from the declared content of *tiamulin hydrogen fumarate CRS*.

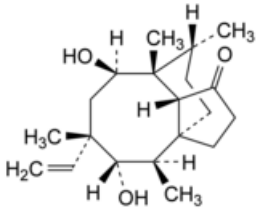
STORAGE

Protected from light.

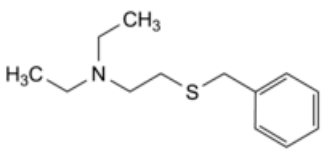
IMPURITIES

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

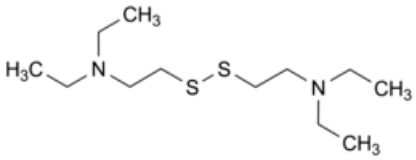
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *G, H, I, J, K, L, M, N, O, P, Q, R.*



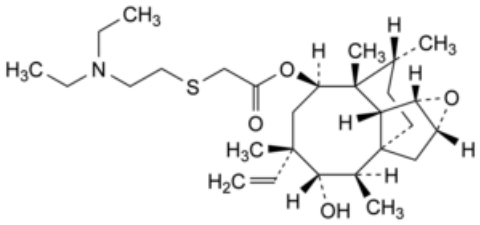
A. (3a*S*,4*R*,5*S*,6*S*,8*R*,9*R*,9a*R*,10*R*)-6-ethenyl-5,8-dihydroxy-4,6,9,10-tetramethyloctahydro-3a,9-propanocyclopenta[8]annulen-1(4*H*)-one (mutilin),



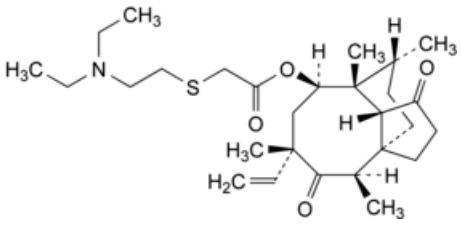
B. 2-(benzylsulfanyl)-*N*,*N*-diethylethan-1-amine,



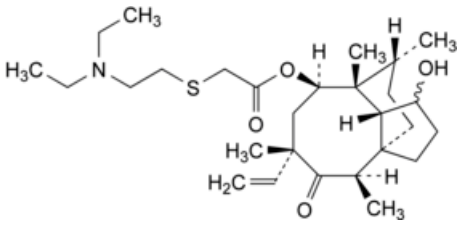
C. 2,2′-disulfanediylbis(*N*,*N*-diethylethan-1-amine),



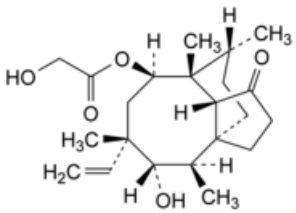
D. (1a*S*,1b*R*,2*R*,3*R*,5*S*,6*S*,7*R*,7a*S*,8a*R*,11*R*)-5-ethenyl-6-hydroxy-2,5,7,11-tetramethyldecahydro-2,7a-propanocycloocta[3,4]cyclopenta[1,2-*b*]oxiren-3-yl [[2-(diethylamino)ethyl]sulfanyl]acetate,



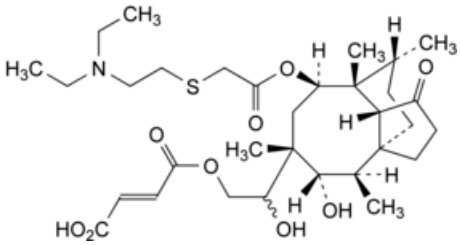
E. (3a*S*,4*R*,6*S*,8*R*,9*R*,9a*R*,10*R*)-6-ethenyl-4,6,9,10-tetramethyl-1,5-dioxodecahydro-3a,9-propanocyclopenta[8]annulen-8-yl [[2-(diethylamino)ethyl]sulfanyl]acetate (11-oxotiamulin),



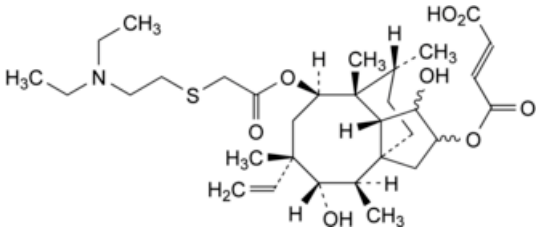
F. (1Ξ,3a*R*,4*R*,6*S*,8*R*,9*R*,9a*R*,10*R*)-6-ethenyl-1-hydroxy-4,6,9,10-tetramethyl-5-oxodecahydro-3a,9-propanocyclopenta[8]annulen-8-yl [[2-(diethylamino)ethyl]sulfanyl]acetate (1-hydroxy-11-oxotiamulin),



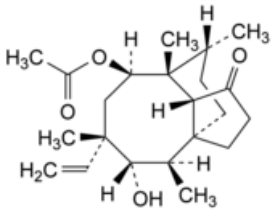
G. (3a*S*,4*R*,5*S*,6*S*,8*R*,9*R*,9a*R*,10*R*)-6-ethenyl-5-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro-3a,9-propanocyclopenta[8]annulen-8-yl hydroxyacetate (pleuromutilin, pleuromulin),



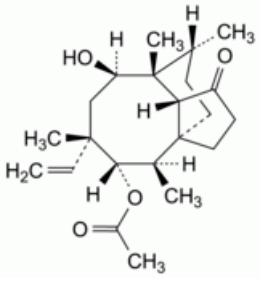
H. (2*E*)-4-[(2Ξ)-2-[(3a*S*,4*R*,5*S*,6*R*,8*R*,9*R*,9a*R*,10*R*)-8-[[[[2-(diethylamino)ethyl]sulfanyl]acetyl]oxy]-5-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro-3a,9-propanocyclopenta[8]annulen-6-yl]-2-hydroxyethoxy]-4-oxobut-2-enoic acid (19,20-dihydroxytiamulin 20-fumarate),



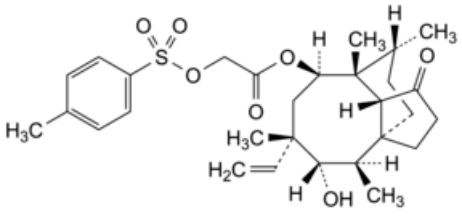
I. (2*E*)-4-[[(1Ξ,2Ξ,3a*S*,4*R*,5*S*,6*S*,8*R*,9*R*,9a*R*,10*R*)-8-[[[[2-(diethylamino)ethyl]sulfanyl]acetyl]oxy]-6-ethenyl-1,5-dihydroxy-4,6,9,10-tetramethyldecahydro-3a,9-propanocyclopenta[8]annulen-2-yl]oxy]-4-oxobut-2-enoic acid (2,3-dihydroxytiamulin 2-fumarate),



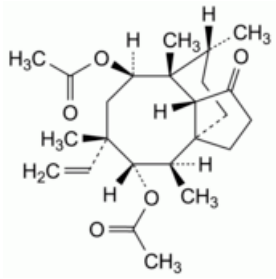
J. (3a*S*,4*R*,5*S*,6*S*,8*R*,9*R*,9a*R*,10*R*)-6-ethenyl-5-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro-3a,9-propanocyclopenta[8]annulen-8-yl acetate (mutilin 14-acetate),



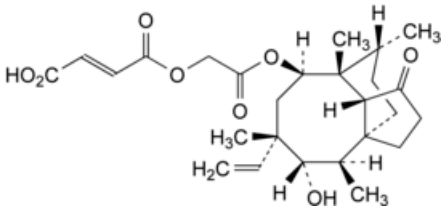
K. (3a*S*,4*R*,5*S*,6*S*,8*R*,9*R*,9a*R*,10*R*)-6-ethenyl-8-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro-3a,9-propanocyclopenta[8]annulen-5-yl acetate (mutilin 11-acetate),



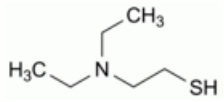
L. (3a*S*,4*R*,5*S*,6*S*,8*R*,9*R*,9a*R*,10*R*)-6-ethenyl-5-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro-3a,9-propanocyclopenta[8]annulen-8-yl [(4-methylbenzene-1-sulfonyl)oxy]acetate (pleuromutilin 22-tosylate),



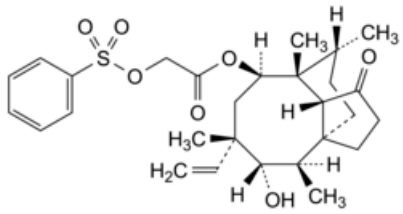
M. (3a*S*,4*R*,5*S*,6*S*,8*R*,9*R*,9a*R*,10*R*)-6-ethenyl-4,6,9,10-tetramethyl-1-oxodecahydro-3a,9-propanocyclopenta[8]annulen-5,8-diyl diacetate (mutilin 11,14-diacetate),



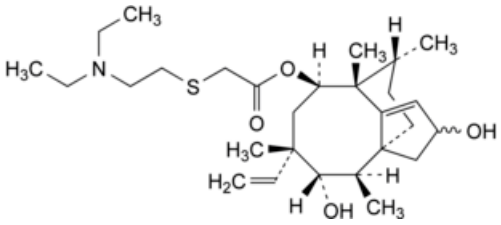
N. (2*E*)-4-[2-[[(3a*S*,4*R*,5*S*,6*S*,8*R*,9*R*,9a*R*,10*R*)-6-ethenyl-5-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro-3a,9-propanocyclopenta[8]annulen-8-yl]oxy]-2-oxoethoxy]-4-oxobut-2-enoic acid (pleuromutilin 22-fumarate),



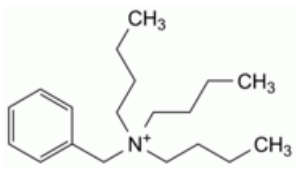
O. 2-(diethylamino)ethane-1-thiol,



P. (3a*S*,4*R*,5*S*,6*S*,8*R*,9*R*,9a*R*,10*R*)-6-ethenyl-5-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro-3a,9-propanocyclopenta[8]annulen-8-yl [(benzenesulfonyl)oxy]acetate,



Q. (2*Ξ*,3a*S*,4*R*,5*S*,6*S*,8*R*,9*R*,10*R*)-6-ethenyl-2,5-dihydroxy-4,6,9,10-tetramethyl-2,3,4,5,6,7,8,9-octahydro-3a,9-propanocyclopenta[8]annulen-8-yl [[2-(diethylamino)ethyl]sulfanyl]acetate (3,4-didehydro-2-hydroxytiamulin),



R. *N*-benzyl-*N*,*N*-dibutylbutan-1-aminium.

Ph Eur