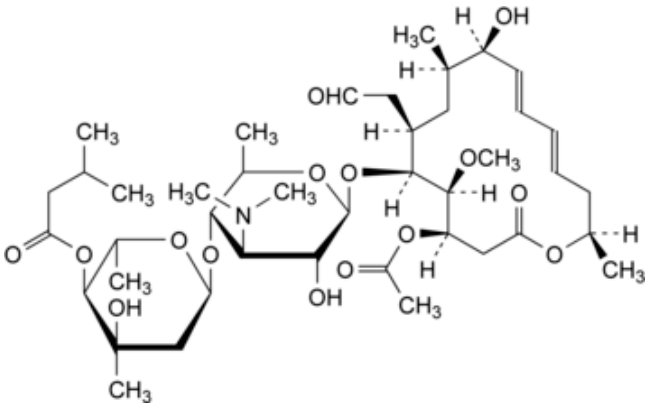
Edition: BP 2025 (Ph. Eur. 11.6 update)

Josamycin

General Notices

(Ph. Eur. monograph 1983)



$C_{42}H_{69}NO_{15}$ 828 *16846-24-5*

Action and use

Antibacterial.

Ph Eur

DEFINITION

Josamycin is a macrolide antibiotic obtained by fermentation using, for example, certain strains of *Streptomyces narbonensis* var. *josamyceticus* var. *nova*. The main component is (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-(acetyloxy)-6-[[3,6-dideoxy-4-*O*-[2,6-dideoxy-3-*C*-methyl-4-*O*-(3-methylbutanoyl)-α-L-*ribo*-hexopyranosyl]-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-10-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one.

Content

Minimum 900 Ph. Eur. U./mg (dried substance).

CHARACTERS

Appearance

White or slightly yellowish powder, slightly hygroscopic.

Solubility

Very slightly soluble in water, freely soluble in methanol and in methylene chloride, soluble in acetone.

IDENTIFICATION

First identification: A, C.

Second identification: A, B.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 0.10 g in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of this solution to 50.0 mL with *methanol R*.

Spectral range 220-350 nm.

Absorption maximum At 232 nm.

Specific absorbance at the absorption maximum 330 to 370.

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

Test solution Dissolve 10 mg of the substance to be examined in 2.5 mL of *methanol R*.

Reference solution (a) Dissolve 10 mg of *josamycin CRS* in 2.5 mL of *methanol R*.

Reference solution (b) Dissolve 10 mg of *josamycin propionate CRS* in 2.5 mL of *methanol R*.

Plate *TLC silica gel GF_{254} plate R*.

Mobile phase *methanol R*, *acetone R*, *ethyl acetate R*, *toluene R*, *hexane R* (8:10:20:25:30 *V/V/V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying At 100 °C for 10 min.

Detection Examine in ultraviolet light at 254 nm.

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) and different in position from the principal spot in the chromatogram obtained with reference solution (b).

C. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with the test solution is similar in position and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

The solution is clear (2.2.1) and not more intensely coloured than reference solution BY_4 (2.2.2, Method II).

Dissolve 2.0 g in *methanol R* and dilute to 20 mL with the same solvent.

Specific optical rotation (2.2.7)

-65 to -75 (dried substance).

Dissolve 1.000 g in *methanol R* and dilute to 100.0 mL with the same solvent. Allow to stand for 30 min before measuring the angle of rotation.

Related substances

Liquid chromatography (2.2.29).

Solvent mixture *acetonitrile R*, *water R* (30:70 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Reference solution (a) Dissolve 25.0 mg of *josamycin CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 20.0 mL with the solvent mixture.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 50.0 mL with the solvent mixture.

Reference solution (d) To 10 mL of the test solution add 0.1 mL of *strong hydrogen peroxide solution R* and heat in a water-bath for 10 min. Mix 1.0 mL of this solution and 1.0 mL of the test solution.

Reference solution (e) Dissolve 12.5 mg of *josamycin for peak identification CRS* (containing impurities A, B, C, D and E) in 5 mL of the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

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

— *temperature*: 45 °C.

Mobile phase:

— *mobile phase A*: mix 3 volumes of a 67.9 g/L solution of *tetrabutylammonium hydrogen sulfate R*, 5 volumes of a 27.6 g/L solution of *sodium dihydrogen phosphate monohydrate R* adjusted to pH 3.0 with *dilute phosphoric acid R*, and 21 volumes of *acetonitrile R*, and dilute to 100 volumes with *water R*;

— *mobile phase B*: mix 5 volumes of a 27.6 g/L solution of *sodium dihydrogen phosphate monohydrate R* adjusted to pH 3.0 with *dilute phosphoric acid R*, and 50 volumes of *acetonitrile R*, and dilute to 100 volumes with *water R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 38	100	0
38 - 55	100 → 0	0 → 100

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 232 nm.

Injection 10 µL of the test solution and reference solutions (b), (c), (d) and (e).

Identification of impurities Use the chromatogram supplied with *josamycin for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities A, B, C, D and E.

Relative retention With reference to josamycin (retention time = about 35 min): impurity A = about 0.5; impurity B = about 0.8; impurity C = about 0.9; impurity D = about 1.2; impurity E = about 1.4.

System suitability Reference solution (d):

— *resolution*: minimum 1.7 between the 2 peaks due to josamycin and the peak eluted with a relative retention with reference to josamycin of about 1.1;

— *retention time of josamycin*: between 32 min and 38 min.

If necessary, adjust the concentration of acetonitrile in the mobile phases.

Limits:

— *impurities A, B, C, D, E* (any shoulder observed on the peak due to impurity A and/or the peak due to impurity B is not to be integrated separately): for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent);

— *any other impurity*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent);

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (20.0 per cent);

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

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 30.0 mg in 5 mL of *methanol R* and dilute to 100.0 mL with *water R*.

Carry out the microbiological assay of antibiotics (*2.7.2*).Use *josamycin CRS* as the chemical reference substance.

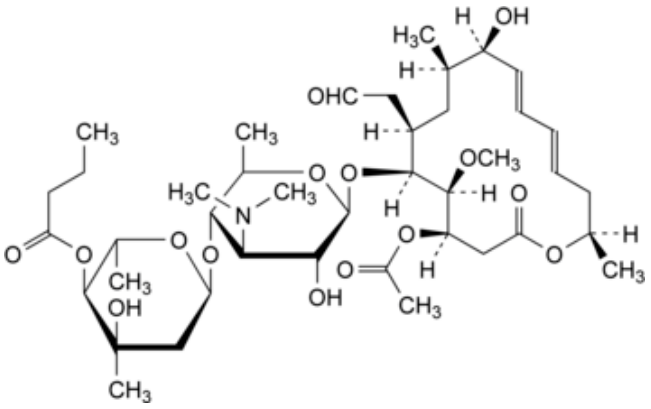
STORAGE

In an airtight container.

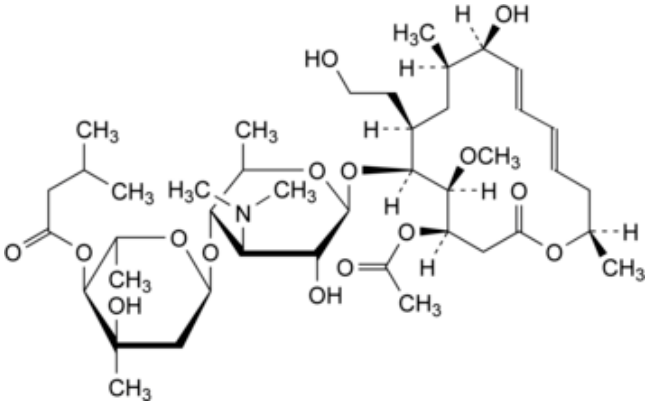
IMPURITIES

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

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) F, G, H, I, J, K.

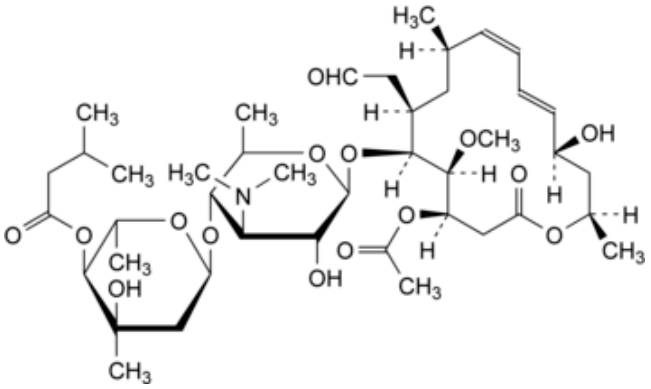


A. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-(acetyloxy)-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-4-*O*-butanoyl-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-10-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one,

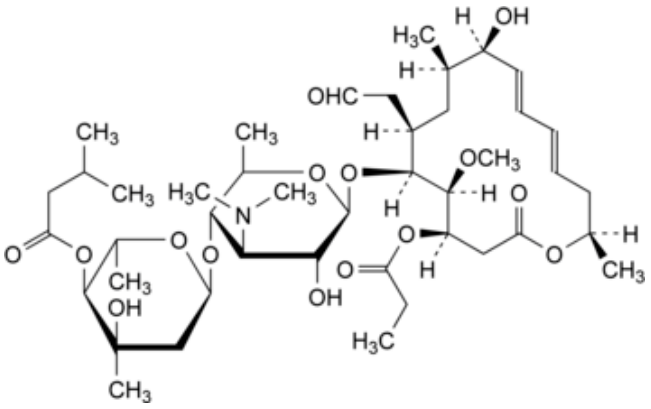


B. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-(acetyloxy)-6-[[3,6-dideoxy-4-*O*-[2,6-dideoxy-3-*C*-methyl-4-*O*-(3-methylbutanoyl)-α-L-*ribo*-hexopyranosyl]-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-10-hydroxy-7-(2-hydroxyethyl)-5-methoxy-9,16-dimethyloxacyclohexadeca-11,13-dien-2-one,

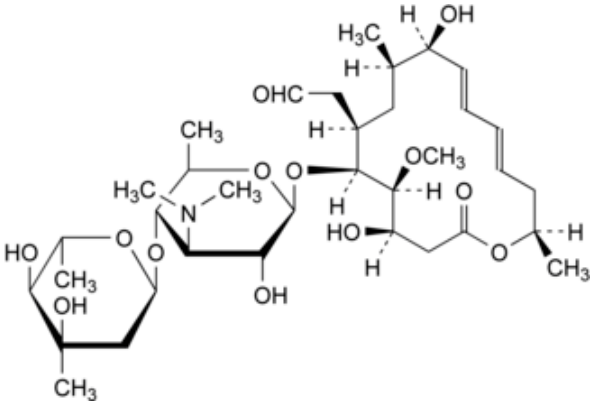
C. unknown structure,



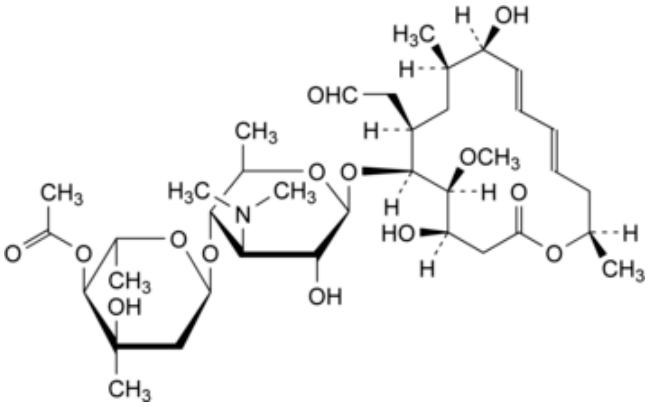
D. (4*R*,5*S*,6*S*,7*R*,9*R*,10*Z*,12*E*,14*R*,16*R*)-4-(acetyloxy)-6-[[3,6-dideoxy-4-*O*-[2,6-dideoxy-3-*C*-methyl-4-*O*-(3-methylbutanoyl)-α-L-*ribo*-hexopyranosyl]-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-14-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-10,12-dien-2-one (isojosamycin),



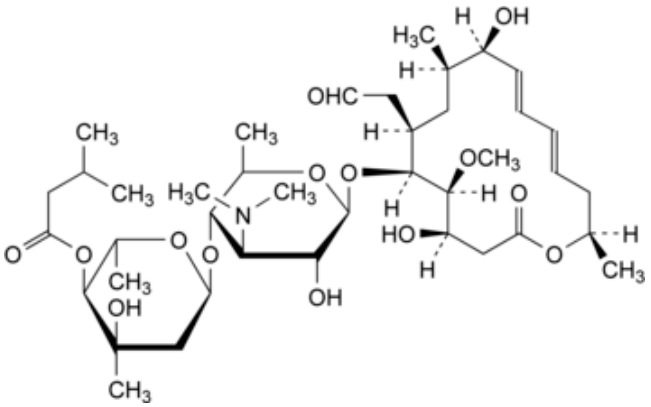
E. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[3,6-dideoxy-4-*O*-[2,6-dideoxy-3-*C*-methyl-4-*O*-(3-methylbutanoyl)-α-L-*ribo*-hexopyranosyl]-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-10-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)-4-(propanoyloxy)oxacyclohexadeca-11,13-dien-2-one,



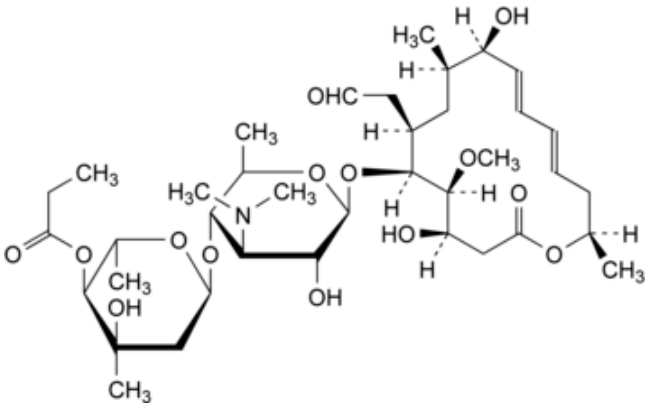
F. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-4,10-dihydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one,



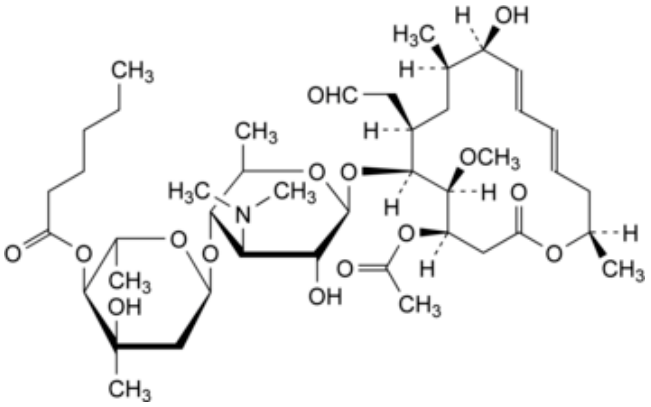
G. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[4-*O*-(4-*O*-acetyl-2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3,6-dideoxy-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-4,10-dihydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one,



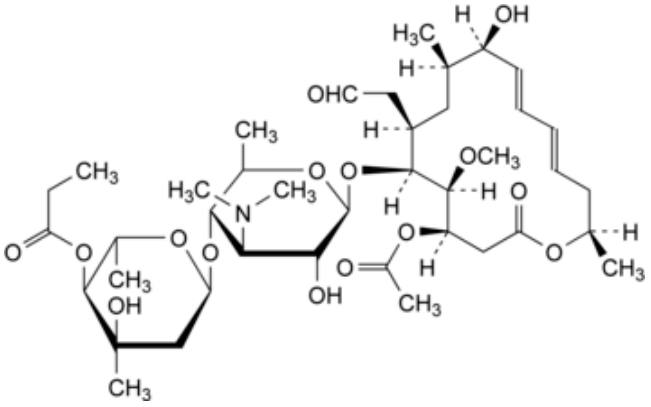
H. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[3,6-dideoxy-4-*O*-[2,6-dideoxy-3-*C*-methyl-4-*O*-(3-methylbutanoyl)-α-L-*ribo*-hexopyranosyl]-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-4,10-dihydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one,



I. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-4-*O*-propanoyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-4,10-dihydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one,



J. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-(acetyloxy)-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-4-*O*-hexanoyl-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-10-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one,



K. (4*R*,5S,6S,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-(acetyloxy)-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-4-*O*-propanoyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-10-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one.

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