



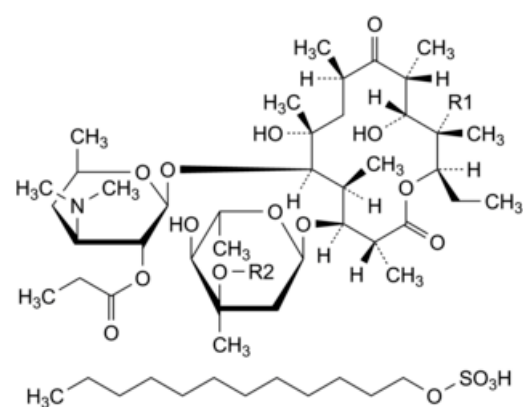
Edition: BP 2025 (Ph. Eur. 11.6 update)

Erythromycin Estolate



General Notices

(Ph. Eur. monograph 0552)



Erythromycin (estolate)	Mol. Formula	<i>M_r</i>	R1	R2
A	C ₅₂ H ₉₇ NO ₁₈ S	1056	OH	CH ₃
B	C ₅₂ H ₉₇ NO ₁₇ S	1040	H	CH ₃
C	C ₅₁ H ₉₅ NO ₁₈ S	1042	OH	H

Action and use

Macrolide antibacterial.

Preparation

Erythromycin Estolate Capsules

Ph Eur

DEFINITION

Mixture of the estolate esters of erythromycin.

Main component (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl- α -L-ribo-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-2-*O*-propanyl- β -D-xylo-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione dodecyl sulfate (erythromycin A 2"-propionate dodecyl sulfate).

Semi-synthetic product derived from a fermentation product obtained using a strain of *Streptomyces erythreus*.

Content

- *sum of erythromycins A, B and C expressed as estolates*: 93.0 per cent to 102.0 per cent (anhydrous substance);
- *erythromycin B estolate*: maximum 5.0 per cent (anhydrous substance);

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent), soluble in acetone. It is practically insoluble in dilute hydrochloric acid.

IDENTIFICATION

Infrared absorption spectrophotometry ([2.2.24](#)).

Comparison [erythromycin estolate CRS](#).

TESTS

Related substances

Liquid chromatography ([2.2.29](#)). *Prepare the solutions immediately before use (apart from the test solution).*

Solution A (hydrolysis solution). Dissolve 11.5 g of [dipotassium hydrogen phosphate R](#) in 900 mL of [water R](#), adjust to pH 8.0 with [dilute phosphoric acid R](#) and dilute to 1000 mL with [water R](#).

Test solution Dissolve 0.150 g of the substance to be examined in 25 mL of [methanol R](#). Add 20 mL of solution A, mix and allow to stand at room temperature for at least 12 h. Dilute to 50.0 mL with solution A.

Reference solution (a) Dissolve 40.0 mg of [erythromycin A CRS](#) in 12.0 mL of [methanol R](#) and dilute to 20.0 mL with solution A.

Reference solution (b) Dissolve 10.0 mg of [erythromycin B CRS](#) and 10.0 mg of [erythromycin C CRS](#) in 40.0 mL of [methanol R](#) and dilute to 100.0 mL with solution A.

Reference solution (c) Dilute 1.0 mL of reference solution (a) to 100.0 mL with a mixture of 4 volumes of solution A and 6 volumes of [methanol R](#).

Reference solution (d) Dissolve 4 mg of [erythromycin for system suitability CRS](#) (containing impurities A, B, C, D, E, F, H and L) in 0.4 mL of [methanol R](#) and add 0.6 mL of solution A.

Column:

— **size**: $l = 0.25$ m, $\varnothing = 4.6$ mm;

— **stationary phase**: [end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R](#) (3.5 μ m);

— **temperature**: 65 °C; preheating the mobile phase may be required, for instance by extending the inlet tubing in the oven to 30 cm.

Mobile phase:

— **mobile phase A**: [phosphate buffer solution pH 7.0 R7](#), [acetonitrile R1](#), [water for chromatography R](#) (5:35:60 V/V/V);

— **mobile phase B**: [phosphate buffer solution pH 7.0 R7](#), [water for chromatography R](#), [acetonitrile R1](#) (5:45:50 V/V/V);

Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - t_R	100	0
$t_R - (t_R + 2)$	100 → 0	0 → 100
$(t_R + 2) - (t_R + 15)$	0	100

t_R = retention time of erythromycin B, determined by injecting 20 µL of reference solution (b) and eluting with mobile phase A

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Autosampler Set at 4 °C.

Injection 200 µL.

Identification of impurities Use the chromatogram supplied with [erythromycin for system suitability CRS](#) and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities A, B, C, D, E, F and L; use the chromatogram obtained with reference solution (b) to identify the peaks due to erythromycins B and C.

Relative retention With reference to erythromycin A (retention time = about 23 min): impurity A = about 0.4; impurity B = about 0.5; erythromycin C = about 0.55; impurity L = about 0.63; impurity C = about 0.9; impurity D = about 1.61; erythromycin B = about 1.75; impurity F = about 1.81; impurity E = about 2.3.

System suitability Reference solution (d):

- **resolution**: minimum 1.2 between the peaks due to impurity B and erythromycin C;
- **peak-to-valley ratio**: minimum 1.5, where H_p = height above the baseline of the peak due to impurity F and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin B; minimum 2.0, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to erythromycin A. If necessary, adjust the concentration of [acetonitrile R1](#) in the mobile phases and/or the gradient to obtain the required separation.

Calculation of percentage contents:

- **correction factors**: multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 2; impurity E = 0.08; impurity F = 0.08; impurity L = 0.11;
- for each impurity, use the concentration of erythromycin A in reference solution (c).

Limits:

- **impurity C**: maximum 3.0 per cent;
- **impurities A, B**: for each impurity, maximum 2.0 per cent;
- **impurities D, F**: for each impurity, maximum 1.0 per cent;
- **impurities eluting with relative retention between 2.1 and 2.4**: not more than 4 impurities; for 1 impurity, maximum 3.5 per cent; for each other impurity, maximum 1.0 per cent;
- **impurity L**: maximum 0.4 per cent;
- **any other impurity**: for each impurity, maximum 0.4 per cent;
- **total**: maximum 7.0 per cent;
- **reporting threshold**: 0.2 per cent; disregard the peaks due to erythromycins B and C and any peak with a relative retention to erythromycin A of less than 0.3 (hydrolysis peaks).

Free erythromycin

Liquid chromatography ([2.2.29](#)). Prepare the solutions immediately before use.

Test solution Dissolve 0.250 g of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution Dissolve 75.0 mg of [erythromycin A CRS](#) in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 5.0 mL of the solution to 25.0 mL with [acetonitrile R](#).

Column:

- **size:** $l = 0.25$ m, $\varnothing = 4.6$ mm;
- **stationary phase:** [end-capped octylsilyl silica gel for chromatography R](#) (5 μ m);
- **temperature:** 30 °C.

Mobile phase Mix 35 volumes of [acetonitrile R1](#) and 65 volumes of a solution containing 3.4 g/L of [potassium dihydrogen phosphate R](#) and 2.0 g/L of [triethylamine R](#), previously adjusted to pH 3.0 with [dilute phosphoric acid R](#).

Flow rate 1 mL/min.

Detection Spectrophotometer at 195 nm.

Injection 20 μ L.

Run time Twice the retention time of erythromycin A for the reference solution and 4.5 times the retention time of the 1st peak of erythromycin propionate for the test solution.

Retention time Erythromycin A = about 5 min; 1st peak of erythromycin propionate = about 10 min.

Limit:

- **free erythromycin:** not more than the area of the principal peak in the chromatogram obtained with the reference solution (6.0 per cent).

Dodecyl sulfate

23.0 per cent to 25.5 per cent of $C_{12}H_{26}O_4S$ (anhydrous substance).

Dissolve 0.500 g in 25 mL of [dimethylformamide R](#). Titrate with [0.1 M sodium methoxide](#) using 0.05 mL of a 3 g/L solution of [thymol blue R](#) in [methanol R](#) as indicator.

1 mL of [0.1 M sodium methoxide](#) is equivalent to 26.64 mg of $C_{12}H_{26}O_4S$.

[Water \(2.5.12\)](#)

Maximum 4.0 per cent, determined on 0.300 g.

Use a 100 g/L solution of [imidazole R](#) in [anhydrous methanol R](#) as the solvent.

[Sulfated ash \(2.4.14\)](#)

Maximum 0.5 per cent, determined on 0.5 g.

ASSAY

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

Injection Test solution and reference solutions (a) and (b).

System suitability Reference solution (a):

- **symmetry factor:** maximum 2.5 for the peak due to erythromycin A;
- **repeatability:** maximum relative standard deviation of 1.0 per cent determined on 6 injections.

Calculate the percentage content of erythromycin A ($C_{37}H_{67}NO_{13}$) using the chromatogram obtained with reference solution (a). Calculate the percentage contents of erythromycin B ($C_{37}H_{67}NO_{12}$) and erythromycin C ($C_{36}H_{65}NO_{13}$) using the

chromatogram obtained with reference solution (b). Express the result as erythromycin A estolate, erythromycin B estolate and erythromycin C estolate by multiplying the percentage content of erythromycin A by 1.4387, the percentage content of erythromycin B by 1.4485 and the percentage content of erythromycin C by 1.4472.

For the calculation of content of erythromycin estolate, use the sum of erythromycins A, B and C expressed as estolates as described above.

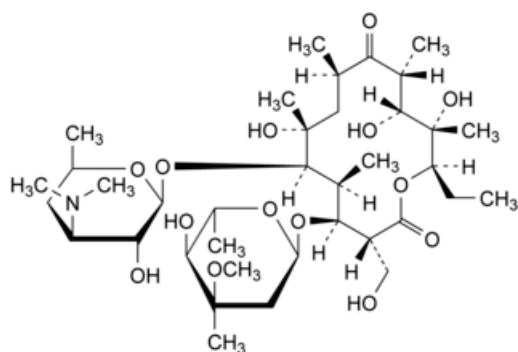
STORAGE

Protected from light.

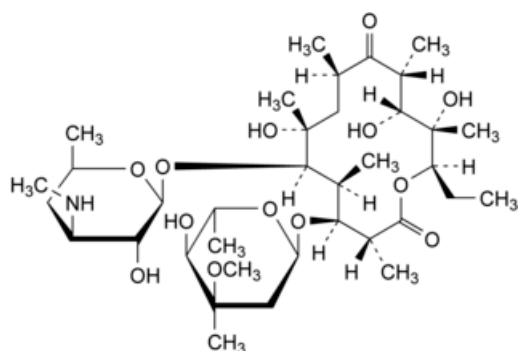
IMPURITIES

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

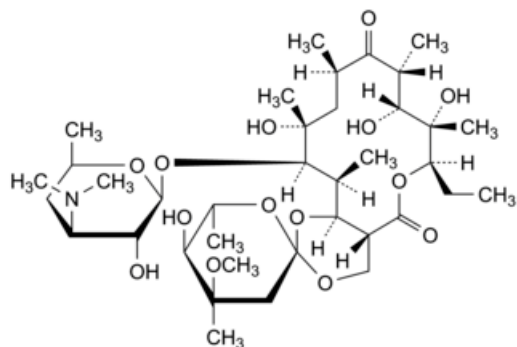
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for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



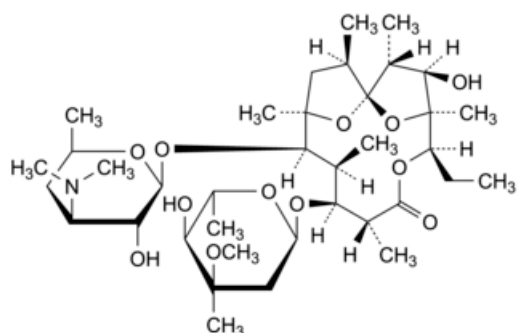
A. (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribo-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3-(hydroxymethyl)-5,7,9,11,13-pentamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)- β -*D*-xylo-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione (erythromycin F),



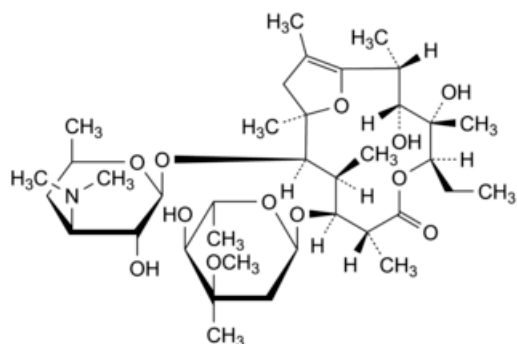
B. (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribo-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(methylamino)- β -*D*-xylo-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione (3'-*N*-demethylerythromycin A),



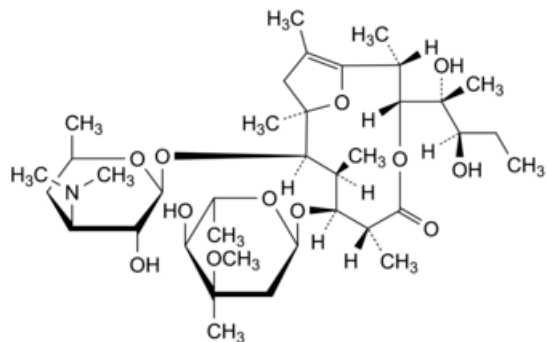
C. (2*S*,4*aR*,4'*R*,5'*S*,6'*S*,7*R*,8*S*,9*R*,10*R*,12*R*,14*R*,15*R*,16*S*,16*aS*)-7-ethyl-5',8,9,14-tetrahydroxy-4'-methoxy-4',6',8,10,12,14,16-heptamethyl-15-[[[3,4,6-trideoxy-3-(dimethylamino)-β-*D*-xylo-hexopyranosyl]oxy]]hexadecahydrospiro[5*H*,11*H*-1,3-dioxino[5,4-*c*]oxacyclotetradecin-2,2'-pyrane]-5,11-dione (erythromycin E),



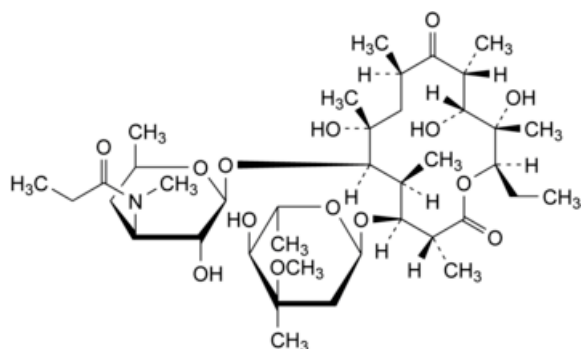
D. (1*S*,2*R*,3*R*,4*S*,5*R*,8*R*,9*S*,10*S*,11*R*,12*R*,14*R*)-9-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-*L*-ribo-hexopyranosyl)oxy]-5-ethyl-3-hydroxy-2,4,8,10,12,14-hexamethyl-11-[[[3,4,6-trideoxy-3-(dimethylamino)-β-*D*-xylo-hexopyranosyl]oxy]]-6,15,16-trioxatricyclo[10.2.1.1^{1,4}]hexadecan-7-one (anhydroerythromycin A),



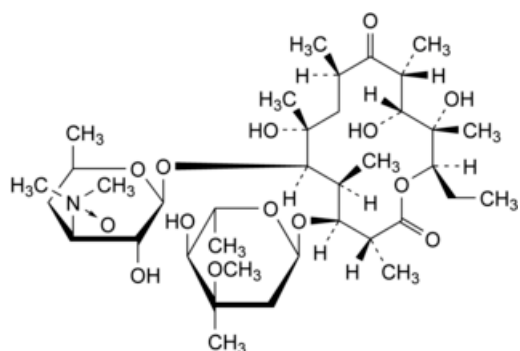
E. (2*R*,3*R*,4*S*,5*R*,8*R*,9*S*,10*S*,11*R*,12*R*)-9-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-*L*-ribo-hexopyranosyl)oxy]-5-ethyl-3,4-dihydroxy-2,4,8,10,12,14-hexamethyl-11-[[[3,4,6-trideoxy-3-(dimethylamino)-β-*D*-xylo-hexopyranosyl]oxy]]-6,15-dioxabicyclo[10.2.1]pentadec-1(14)-en-7-one (erythromycin A enol ether),



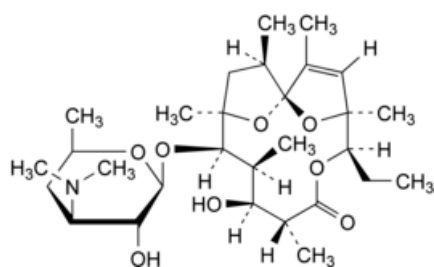
F. (2*R*,3*R*,6*R*,7*S*,8*S*,9*R*,10*R*)-7-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribo-hexopyranosyl)oxy]-3-[(1*R*,2*R*)-1,2-dihydroxy-1-methylbutyl]-2,6,8,10,12-pentamethyl-9-[[3,4,6-trideoxy-3-(dimethylamino)- β -*D*-xylo-hexopyranosyl]oxy]-4,13-dioxabicyclo[8.2.1]tridec-1(12)-en-5-one (pseudoerythromycin A enol ether),



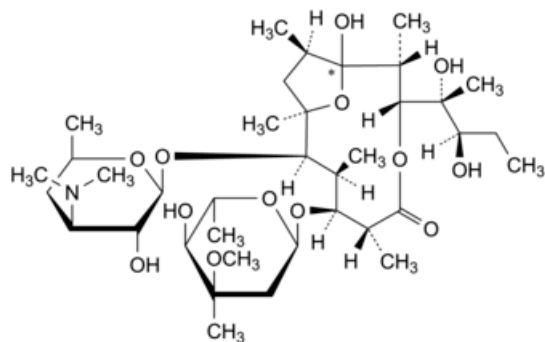
G. (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribo-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(methylpropanoylamino)- β -*D*-xylo-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione (3''-*N*-demethyl-3''-*N*-propanoylerythromycin A),



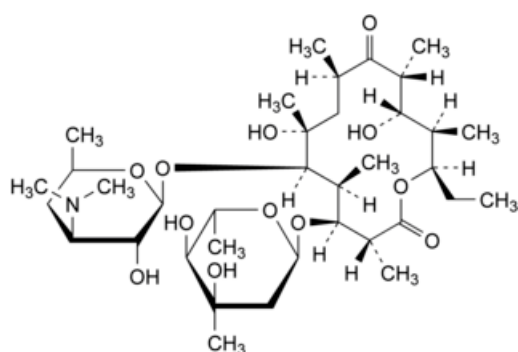
H. (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribo-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)- β -*D*-xylo-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione *N*-oxide (erythromycin A 3''-*N*-oxide),



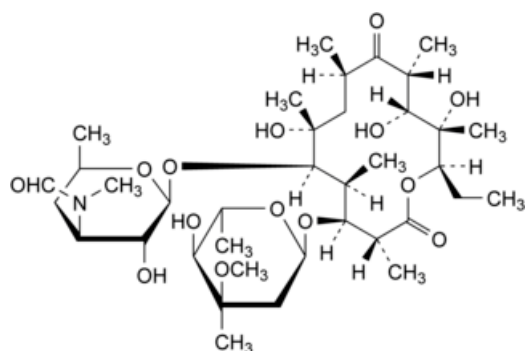
I. (1*S*,4*S*,5*R*,8*R*,9*S*,10*S*,11*R*,12*R*,14*R*)-5-ethyl-9-hydroxy-2,4,8,10,12,14-hexamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- β -*D*-xylo-hexopyranosyl]oxy]-6,15,16-trioxatricyclo[10.2.1.1^{1,4}]hexadec-2-en-7-one (erythralosamine),



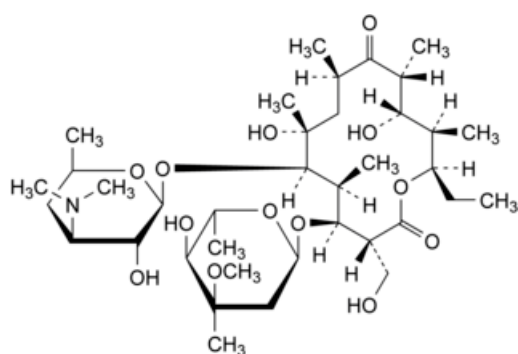
J. (1*RS*,2*R*,3*R*,6*R*,7*S*,8*S*,9*R*,10*R*,12*R*)-7-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribo-hexopyranosyl)oxy]-3-[[1*R*,2*R*]-1,2-dihydroxy-1-methylbutyl]-1-hydroxy-2,6,8,10,12-pentamethyl-9-[[3,4,6-trideoxy-3-(dimethylamino)- β -*D*-xylo-hexopyranosyl]oxy]-4,13-dioxabicyclo[8.2.1]tridecan-5-one (pseudoerythromycin A hemiketal),



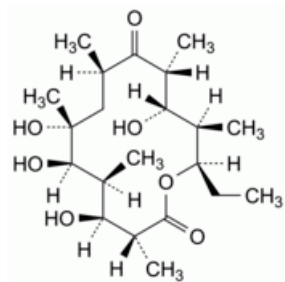
K. (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*S*,13*R*,14*R*)-4-[(2,6-dideoxy-3-*C*-methyl- α -*L*-ribo-hexopyranosyl)oxy]-14-ethyl-7,12-dihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)- β -*D*-xylo-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione (erythromycin D),



L. (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribo-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(formylmethylamino)- β -*D*-xylo-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione (3''-*N*-demethyl-3''-*N*-formylerythromycin A),



M. (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*S*,13*R*,14*R*)-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribo-hexopyranosyl)oxy]-14-ethyl-7,12-dihydroxy-3-(hydroxymethyl)-5,7,9,11,13-pentamethyl-6-[[[3,4,6-trideoxy-3-(dimethylamino)- β -*D*-xylo-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione (erythromycin G),



N. (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*S*,13*R*,14*R*)-14-ethyl-4,6,7,12-tetrahydroxy-3,5,7,9,11,13-hexamethyloxacyclotetradecane-2,10-dione (erythronolide B).

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