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New Fatty Monoesters of Erythromycin A

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New fatty polyenic (linoleic, linolenic, arachidonic, linoelaidic) mono esters of erythromycin A have been synthesized by using various reagents such as acyl chloride, carboxylic acid anhydride, and mixed carbonic anhydride. These different ways of activating the fatty acid allowed a regioselectivity of esterification at position 2′ of the desosamine ring or position 4″ of the cladinose ring of erythromycin A. The *in vitro* antibacterial properties of these new esters against members of the resident flora of the human skin were determined and compared with those of erythromycin A. The number and the stereochemistry of the double bonds seem to play a crucial role in the expression of the *in vitro* antibacterial activity.

Keywords erythromycin ester; polyenic fatty acid; regioselective acylation; antibacterial activity

Among numerous antibiotics active against gram-positive strains, erythromycin, a macrolide,[1] is very often recommended due to its efficiency[2] and its low toxicity.[3] Accordingly erythromycin is used in the treatment of acne to reduce inflammation caused mainly by the presence of *Propionibacterium acnes*,[4] a resident strain of the skin. Though the etio-pathogeny of acne is still unknown,[5] the efficacy of erythromycin in this disease is well-established.[6] This antibiotic was initially administered by the oral route and, later topically, avoiding systemic effect and degradation at low pH.[7] However, the efficacy of erythromycin is limited by its low penetration into pilo-sebaceous follicles which host the microaerophilic *Propionibacterium acnes*.[8] In addition, long-term clinical use of erythromycin tends to induce resistance of this bacterium towards this class of antibiotic.[9]

Numerous derivatives of erythromycin A have been synthesized, especially esters with short hydrocarbon chains.[10] A U.S. patent claims that fatty saturated and monounsaturated esters of this antibiotic[11] improve its bitter taste and its stability after oral administration.

We wish to describe herein the synthesis and the *in vitro* antibacterial evaluation of new fatty polyunsaturated esters of erythromycin A. Our initial goal was to confer upon erythromycin A a more lipophilic balance to enhance its penetration through the oily medium of the pilo-sebaceous duct. As far as the lipidic moiety was concerned, we decided to employ essential fatty acids[12] indispensable for a healthy skin; among them, linoleic acid shows a high affinity for the sebaceous structure, when applied topically[13] and is suspected to play a beneficial role in the etiology of acne.[14] Different ways of activating the fatty acid chain were examined. Principally acyl chlorides, carboxylic acid anhydrides and mixed carbonic anhydrides were synthesized, as shown in Charts 1 and 2.

Linoleoyl chloride (**1**) was prepared with phosphorus oxychloride in toluene, linoleic, α-linolenic and arachidonic anhydrides (**2**, **3**, **4**) with *N*,*N*′-dicyclohexylcarbodiimide in *N*,*N*-dimethylformamide or tosyl chloride in pyridine[15] and linoleic mixed anhydride (**5**) with ethyl chloroformate in tetrahydrofuran. These activated forms were prepared just before the esterification and were not isolated. In order to assess the importance of the configuration of the chain, linoelaidic anhydride (**6**), the *trans–trans* isomer of the linoleic chain with no biological activity, was also prepared, as well as oleic anhydride (**7**) which allowed the synthesis of the oleic ester of erythromycin A previously described in a U.S. patent.[11]

The reaction of esterification must overcome several difficulties (Chart 3): the problems of possible degradation of erythromycin in anhydroerythromycin hemiketal[7] (**8**) in an acidic medium, and the epimerization of carbons at the α,α′ positions of the ketone or the elimination of the hydroxy group at position 11 to form the α,β-unsaturated ketone. The migration, in a basic medium, of the dou-

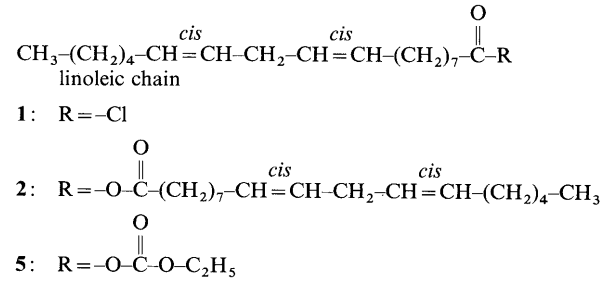


Chart 1

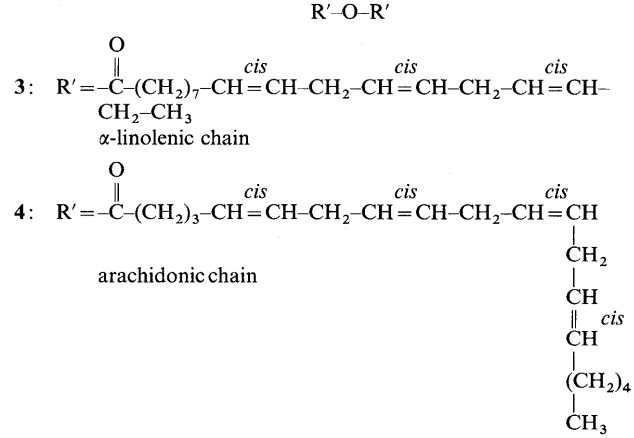
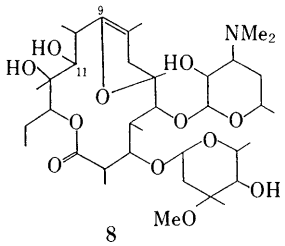


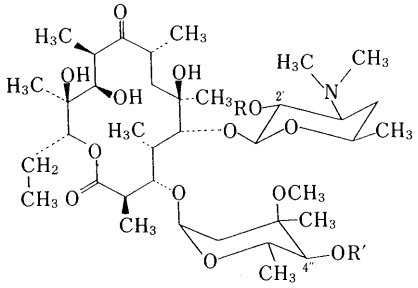
Chart 2



8

8,9-anhydroerythromycin A-6,9-hemiketal

Chart 3



9 : R=linoleic chain, R′=H
10 : R=arachidonic chain, R′=H
11 : R=linoelaidic chain, R′=H
12 : R=H, R′=linoleic chain
13 : R=H, R′=linoelaidic chain
14 : R=-C(=O)-O-C_2H_5, R′=H
15 : R=H, R′=α-linolenic
16 : R=oleic chain, R′=H

Chart 4

ble bonds of linoleic, linolenic and arachidonic chains, leading to conjugation, is also an important possible route of degradation of fatty polyunsaturated acids.

These problems could be largely overcome by esterification at pH between 6 and 8, avoiding traces of anhydro ester at pH 5 and the beginning of α,α′ isomerization of the ketone at pH 9.

Subject to these restrictions, 2′ esters of erythromycin (Chart 4) with linoleic, arachidonic and linoelaidic chains were synthesized by the anhydride and the mixed carbonic anhydride methods.[16] The anhydride method gave yields of at least 60% of 2′ ester (**9**, **10**, **11**) and 15% of 4″ ester (**12**, **13**) for all these chains (Chart 4). The mixed carbonic anhydride method gave yields of 78% of 2′ linoleic ester (**9**) with a reduced amount of 4″ ester (**12**) (yield less than 5%). In this case, the synthesis of 5% of 2′ carbonic ethyl ester (**14**) (Chart 4) resulting from the attack of erythromycin A on the carbonic function of the anhydride is noteworthy. This way of activation is preferred because of its high regioselectivity. The use of acyl chloride led to the formation of the 4″ ester predominantly in the case of the linoleic chain (65% yield of 4″ (**12**) and 25% of 2′ (**9**)) and the α-linolenic chain (65% yield of 4″ (**15**)). The positions of esterification were determined on the basis of the C-1′, C-3′ and C-5″ chemical shifts in the carbon-13 nuclear magnetic resonance (^{13}C-NMR). C-1′ and C-3′ resonate at a lower field (−2 ppm) in the case of 2′ esterification and C-5″ at lower field (−2 ppm) in the case of 4″ esterification.[17]

The *in vitro* microbiological properties of these monoesters are indicated in Table I. These new esters are less active than erythromycin (**17**) against a strain of *Propionibacterium acnes*[18] sensitive to erythromycin ($P37E_s$)

Table I. Values of Minimal Inhibitory Concentration (μg/ml) of Monofatty Esters of Erythromycin A (**17**) against *P. acnes in Vitro*

Product	*P. acnes* strain	
	$P37E_s$	$P37E_r$
9	4	16
10	6.25	>50
11	25	100
12	3.5	28
13	25	100
15	4.5	37
16	5.0	100
17	0.78	>50

Table II. Values of Minimal Inhibitory Concentration (μg/ml) of 2′-Linoleic Ester of Erythromycin A (**9**) and Erythromycin A (**17**) against *S. epidermidis in Vitro*

Product	*S. epidermidis* strain: ATCC 9491
9	>70
17	6.25

but show a significant activity towards a strain resistant to erythromycin ($P37E_r$) in the case of linoleic and α-linolenic chains. This point is of importance, since many subjects harbor erythromycin-resistant strains. This resistance is probably caused by widespread usage of this macrolide and/or by cross-resistance to other antibiotics such as clindamycin.[9] The linoleic (**9**, **12**) and linolenic (**15**) esters are the most active against these two strains. The *in vitro* activities of arachidonic (**10**) and oleic (**16**) esters illustrate the importance of the number of double bonds. The weak activity of linoelaidic esters (**11**, **13**) emphasizes the significance of the *cis* stereochemistry of the double bonds.

The location of the ester bond (2′ or 4″) does not seem to influence the activity towards *Propionibacterium acnes*. Table II shows the *in vitro* microbiological properties of the 2′ linoleic ester (**9**) and erythromycin against *Staphylococcus epidermidis*, another resident aerobic strain of the skin which, up to now, has not been suspected of playing a role in the development of acne. We observed some activity of erythromycin, while its esters were inactive. This selectivity in microbiocidal activity of the esters is of interest, because it may be possible to avoid a desequilibrium in the skin flora, thereby better maintaining the ecology of the resident flora of the skin.

Experimental

Melting points were determined on a Reichert hot-plate apparatus and are uncorrected. Optical rotations were measured on a "Quick" Roussel and Jouan polarimeter. Infrared (IR) spectra were obtained on a Perkin-Elmer 297 spectrometer. ^{13}C-NMR spectra were obtained on Bruker WM 250 (62.9 MHz) and AC 100 (25.18 MHz) instruments. Chemical shifts (δ) are reported with reference to tetramethylsilane. Thin-layer chromatography (TLC) was performed on Schleicher and Schüll silica gel plates (60F254); the plates were initially examined under ultraviolet (UV) light (254 and 366 nm) then developed with appropriate spray reagents. Column chromatography was carried out under low pressure, using Merck Kieselgel (type 60); the eluant is given in parentheses. Evaporations were performed below 40 °C using a Büchi rotary evaporator.

Preparations of 2′ and 4″ Monoesters by the Carboxylic Acid Anhydride Method (I) A mixture of carboxylic fatty acid anhydride (10.8 mmol), 4-dimethylaminopyridine (0.033 mmol), erythromycin A (2.7 mmol) and anhydrous pyridine (75 ml) was stirred under nitrogen at 20 °C for 8 h.

The solution was poured into water (60 ml) containing sodium hydrogen carbonate (200 mg) and extracted with ethyl acetate. The organic phase was dried with magnesium sulfate and evaporated *in vacuo* to dryness. The residue was chromatographed on a silica gel column using dichloromethane–methanol (98 : 2) as the eluant to give the 2′ monoester (1.6 mmol, yield 60%) and 4″ monoester (0.4 mmol, yield 15%).

Preparations of 4″ and 2′ Monoesters by the Acyl Chloride Method (II) A mixture of fatty acid chloride (10.8 mmol), 4-dimethylaminopyridine (0.033 mmol), erythromycin A (2.7 mmol) and anhydrous pyridine (75 ml) was stirred under nitrogen at 20 °C for 8 h. The solution was poured into water (60 ml) containing sodium hydrogen carbonate (200 mg) and extracted with ethyl acetate. The organic phase was dried with magnesium sulfate and evaporated *in vacuo* to dryness. The residue was chromatographed on a silica gel column using dichloromethane–methanol (98 : 2) as the eluant to give the 4″ monoester (1.75 mmol, yield 65%) and 2′ monoester (0.67 mmol, yield 25%).

Preparations of 2′ Monoesters by the Carboxylic Acid Mixed Carbonic Anhydride Method (III) A solution of fatty acid mixed carbonic anhydride (5.4 mmol), anhydrous pyridine (5 ml), erythromycin (2.7 mmol) and anhydrous tetrahydrofuran (70 ml) was stirred under nitrogen at 20 °C for 10 h. The solution was poured into water (60 ml) and extracted with ethyl acetate. The organic phase was dried with magnesium sulfate and evaporated *in vacuo* to dryness. The residue was chromatographed on a silica gel column using dichloromethane–methanol (98 : 2) as the eluant to give the 2′ monoester (2.1 mmol, yield 78%).

2′-*O*-Linoleoylerythromycin A (**9**) was synthesized according to methods I and III. mp 80 °C (acetonitrile–water), $[\alpha]_D^{20} = -55°$ ($c = 1.3$, dichloromethane). IR 1730 cm^{-1} (ester). *Anal.* Calcd for $C_{55}H_{97}NO_{14}$: C, 66.3; H, 9.81; N, 1.4. Found: C, 66.32; H, 9.86; N, 1.4. ^{13}C-NMR ($CDCl_3$): 100.9 (C-1′), 63.69 (C-3′), 65.69 (C-5″).

2′-*O*-Arachidonoylerythromycin A (**10**) was synthesized according to method I. $[\alpha]_D^{20} = -53°$ ($c = 1.5$, dichloromethane). IR 1730 cm^{-1} (ester). *Anal.* Calcd for $C_{57}H_{97}NO_{14}$: C, 67.09; H, 9.58; N, 1.37. Found: C, 67.05; H, 9.61; N, 1.31. ^{13}C-NMR ($CDCl_3$): 100.9 (C-1′), 63.78 (C-3′), 65.68 (C-5″).

2′-*O*-Linoelaidoylerythromycin A (**11**) was synthesized according to method I. mp 54—56 °C (acetonitrile–water), $[\alpha]_D^{20} = -53°$ ($c = 1$, dichloromethane). IR 1730 cm^{-1} (ester). *Anal.* Calcd for $C_{55}H_{97}NO_{14}$: C, 66.3; H, 9.81; N, 1.4. Found: C, 66.15; H, 9.8; N, 1.51. ^{13}C-NMR ($CDCl_3$): 101 (C-1′), 63.8 (C-3′), 65.68 (C-5″).

4″-*O*-Linoleoylerythromycin A (**12**) was synthesized according to method II. mp 81—83 °C (acetonitrile–water) $[\alpha]_D^{20} = -52°$ ($c = 3.5$, dichloromethane). IR 1730 cm^{-1} (ester). *Anal.* Calcd for $C_{55}H_{97}NO_{14}$: C, 66.3; H, 9.81; N, 1.4. Found: C, 66.35; H, 9.85; N, 1.43. ^{13}C-NMR ($CDCl_3$): 102.4 (C-1′), 65.31 (C-3′), 63.1 (C-5″).

4″-*O*-Linoelaidoylerythromycin A (**13**) was synthesized according to method I. mp 57—59 °C (acetonitrile–water), $[\alpha]_D^{20} = -51°$ ($c = 2.2$, dichloromethane). IR 1730 cm^{-1} (ester). *Anal.* Calcd for $C_{55}H_{97}NO_{14}$: C, 66.3; H, 9.81; N, 1.4. Found; C, 66.16; H, 9.8; N, 1.51. ^{13}C-NMR ($CDCl_3$): 102.4 (C-1′), 65.3 (C-3′), 63 (C-5″).

4″-*O*-α-Linolenoylerythromycin A (**15**) was synthesized according to method II. mp 68—71 °C (acetonitrile–water), $[\alpha]_D^{20} = -58°$ ($c = 1.2$, dichloromethane). IR 1730 cm^{-1} (ester). *Anal.* Calcd for $C_{55}H_{95}NO_{13} \cdot H_2O$: C, 63.03; H, 9.62; N, 1.34. Found: C, 63.1; H, 9.31; N, 1.46. ^{13}C-NMR ($CDCl_3$): 102.39 (C-1′), 65.29 (C-3′), 63.08 (C-5″).

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