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Synthesis and Antibacterial Activities of Novel 12-*O*-Methylerythromycin A Derivatives

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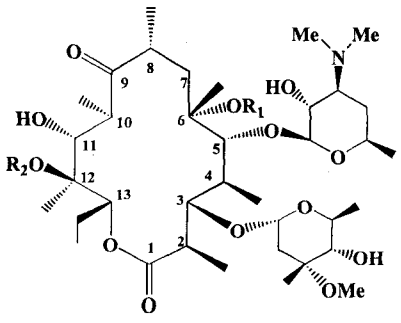
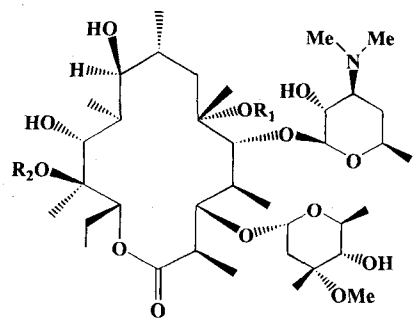
The novel erythromycin A derivatives: 9(*S*)-9-dihydro-12-*O*-methylerythromycin A (**6**) and 12-*O*-methyerythromycin A (**3**), have been prepared using a new synthetic approach. The critical step is the regioselective methylation of the 12-hydroxyl of the boron complex of 2′,4″-*O*-bis(trimethylsilyl)-9(*S*)-9-dihydroerythromycin A (**8**), which has unprotected 6-, 9- and 11-hydroxyl groups. The antibacterial activities of these new derivatives were compared with those of erythromycin A (**1**), clarithromycin (**2**) and 9(*S*)-9-dihydroclarithromycin (**5**).

Erythromycin A (**1**) is one of the most important macrolide antibiotics discovered. It is very effective in treating a variety of bacterial infections[1,2]. The major drawback to erythromycin A is its acid instability[3,4]. Since its discovery in 1952[5], numerous semi-synthetic erythromycin A derivatives have been prepared to improve the parent's antibacterial activity and pharmacokinetic profile[6~8]. One of the most successful examples is clarithromycin (**2**) (6-*O*-methylerythromycin A)[9]. The presence of a methoxyl group at the 6-position in clarithromycin successfully overcomes the internal ketalization problem between the 9-carbonyl and the 6-hydroxyl groups, which makes it more stable towards acid than erythromycin A and therefore a better antibiotic[10]. Similarly, 6,12-di-*O*-methyerythromycin A (TE-032) also exhibits the similar advantage[11].

The acid degradation of erythromycin A involves not only the 6-hydroxyl group but also the 12-hydroxyl group, which participates in the formation of a spiroketal between the 9-carbonyl and 6- and 12-hydroxyl groups. It is interesting to discover the effects that the methoxyl group at the 12-position has on the acid stability and antibiotic



1. $R_1 = H$, $R_2 = H$
2. $R_1 = Me$, $R_2 = H$
3. $R_1 = H$, $R_2 = Me$



4. $R_1 = H$, $R_2 = H$
5. $R_1 = Me$, $R_2 = H$
6. $R_1 = H$, $R_2 = Me$

activities of erythromycin A. To the best of our knowledge, the synthesis of 12-*O*-methylerythromycin A (**3**) has not been reported, and the effects of a methoxyl group at 12-position are not known. We wish to report here the first synthesis of 12-*O*-methyerythromycin A (**3**) and its antibiotic activities as well as the antibiotic activities of 9(*S*)-9-dihydro-12-*O*-methylerythromycin A (**6**).

Erythromycin A possesses three different hydroxyl groups on the aglycon ring. Selective methylation of one hydroxyl group in the presence of other unprotected hydroxyl groups is impracticable and leads to a complex reaction mixture of methylation products which includes 6-*O*-methyl, 11-*O*-methyl, 6,11-bis-*O*-methyl, and 6,12-bis-*O*-methyl erythromycin A[8,10]. Selective 6-*O*-methylation was achieved only with erythromycin A oxime derivatives[12], which may have a different conformation of the aglycon ring from erythromycin A. We have regioselectively methylated 12-hydroxyl with the boron complex of 2′,4″-*O*-bis(trimethylsilyl)-9(*S*)-9-dihydro-erythromycin A (**8**), the $NaBH_4$ reduction product that has unprotected 6-, 9- and 11-hydroxyl groups. Through this approach, the new 2′,4″-*O*-bis(trimethylsilyl)-9(*S*)-9-dihydro-12-*O*-methylerythromycin A (**9**) and 9(*S*)-9-dihydro-12-*O*-methylerythromycin A (**6**) have been successfully prepared[13].

With 2′,4″-*O*-bis(trimethylsilyl)-9(*S*)-9-dihydro-12-*O*-methylerythromycin A (**9**) in hand, we attempted to selectively oxidize the 9-hydroxyl group. Over-oxidation occurred under the conditions of Swern oxidation, Corey Kim oxidation, and Dess-Martin oxidation. Both 9-hydroxyl and 11-hydroxyl groups were oxidized and the mixture was very difficult to separate. The oxidation conditions using TPAP/NMO gave the best selectivity, although the oxidation reaction did not go to completion. Selective oxidation of 9-hydroxyl provided 2′,4″-*O*-bis(trimethylsilyl)-12-*O*-methylerythromycin A (**10**), which was purified by preparative HPLC chromatography. MS spectra indicated that the mono-oxidation product was obtained, *i.e.*, MS (ESI) $[M+H]^+$ *m/z* 892 and $[M+Na]^+$ *m/z* 914. Compound **10** exists as two forms in $CDCl_3$ solution: the 9-ketone form and the 9,6-hemiketal form, which are similar to erythromycin derivatives that have a free 6-hydroxyl group[14]. The chemical shift observed at 218.1 ppm in the ^{13}C NMR spectra is typical for the 9-ketone group in the macrolide ring[15]. In the long-range H-C correlation experiment, H7 (1.80, 1.65 ppm), H8 (3.01 ppm) and 8-methyl (where the protons are assigned by DQCOSY, ROESY, HSQC, HMBC experiments) all show long-range coupling to the carbon signal at 218.1 ppm. Therefore, the carbon signal at 218.1 ppm must be at the C9 position since it is not possible for those protons to couple with a carbonyl at the C11 position that is four and five bonds away. The C11 carbon in this compound appears at 72.6 ppm as an oxygenated methine carbon, which is a

Scheme 1.

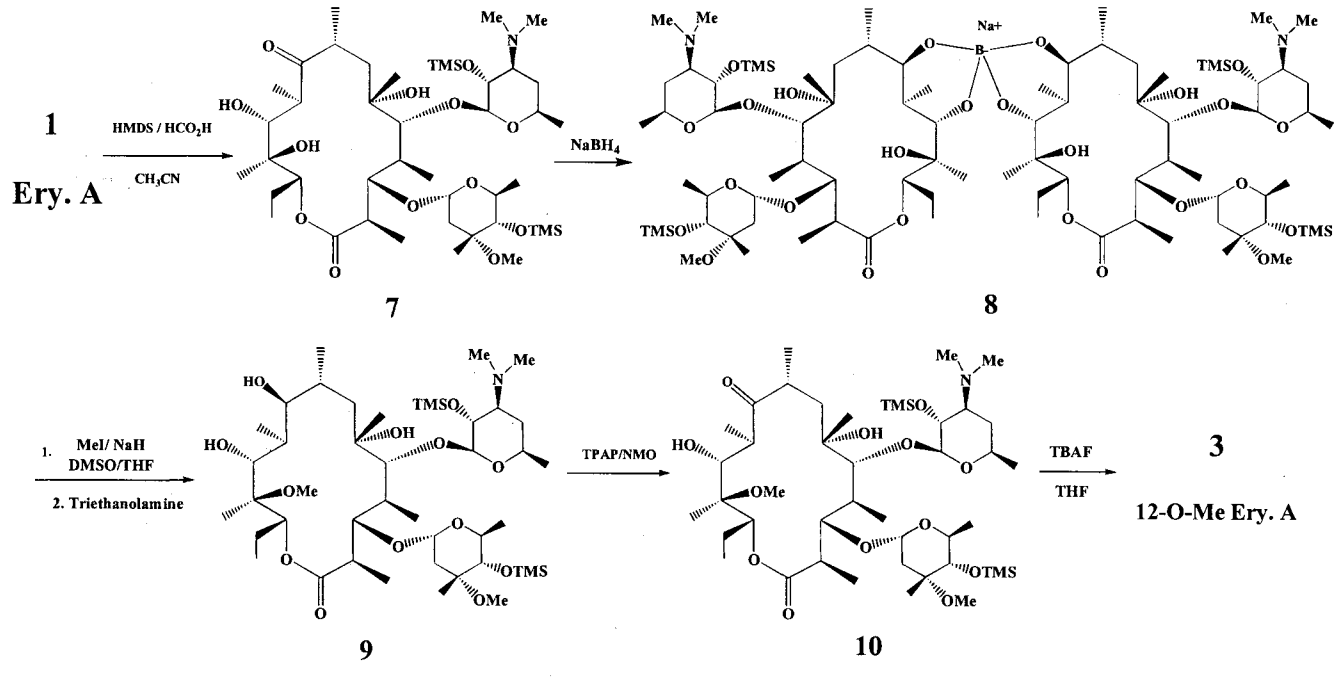


Table 1. Antibacterial activity of erythromycin A (**1**), clarithromycin (**2**), 12-*O*-methylerythromycin A (**3**), 9(*S*)-9-dihydroclarithromycin (**5**), 9(*S*)-9-dihydro-12-*O*-methylerythromycin A (**6**).

Organism	MIC (μg/ml)				
	1	**2**	**3**	**5**	**6**
Staphylococcus aureus 6538P	0.2	0.1	0.78	0.39	12.5
S. aureus A5177	6.2	0.78	25	6.2	>100
S. aureus CMX-642a	0.39	0.2	0.78	0.78	12.5
S. aureus NCTC 10649M	0.39	0.1	0.78	0.39	12.5
S. aureus CMX 553	0.39	0.1	0.78	0.78	25
S. epidermidis 3519	0.39	0.1	0.78	0.39	12.5
E. faecium ATCC8043	0.05	0.01	0.2	0.1	0.78
S. bovis A-5169	0.05	0.01	0.1	0.05	0.78
S. agalactiae CMX 508	0.05	0.005	0.1	0.1	0.78
S. pyogenes EES61	0.02	0.005	0.1	0.05	0.78
S. pyogenes PIU 2548	12.5	6.2	6.2	1.56	25
E. coli DC-2	>100	100	>100	>100	>100
E. coli H560	100	12.5	>100	50	>100
Pseudomonas aeruginosa BMH10	>100	>100	>100	50	>100
Acinetobacier calcoaceticus CMX 669	12.5	12.5	25	12.5	100

typical chemical shift for the C11 that attaches to a free hydroxyl group in the macrolide series compounds. The H11 at 3.98 ppm clearly shows scalar coupling to H10 at 3.16 ppm in the DQCOSY experiment. H10 and H11 also show H-C long-range coupling to C9 at 218.1 ppm. The final structure confirmation was made on the basis of 2D NMR experiments (DQCOSY, ROESY, HSQC, HMQC, and HMBC). Thus, the structure of the mono-oxidation product has been elucidated as 2′,4″-*O*-bis(trimethylsilyl)-12-*O*-methylerythromycin A (**10**) (Scheme 1).

Desilylation of 2′,4″-*O*-bis(trimethylsilyl)-12-*O*-methylerythromycin A (**10**) was carried out with TBAF/THF at room temperature. After purification, the new 12-*O*-methylerythromycin A (**3**) was obtained as a white solid. Structural confirmation was made on the basis of 2D NMR experiments (DQCOSY, ROESY, HSQC, HMQC, and HMBC). MS (FAB and APCI), $[M+H]^+$ *m/z* 748.

The *in vitro* antibacterial activities of the new 9(*S*)-9-dihydro-12-*O*-methylerythromycin A (**6**) and 12-*O*-methylerythromycin A (**3**) are compared with those of erythromycin (**1**), clarithromycin (**2**), and 9(*S*)-9-dihydroclarithromycin (**5**)[15]. The MICs are reported in Table 1, which shows that the antibacterial activities of 12-*O*-methyerythromycin A (**3**) are less than that of both erythromycin A (**1**) and clarithromycin (**2**). Similarly, the 9(*S*)-9-dihydro-12-*O*-methylerythromycin A (**6**) is less active than 9(*S*)-9-dihydroclarithromycin (**5**). These results suggest substitution at the 12-hydroxyl reduces the antibacterial activities of its parent erythromycin A (**1**), which is contrary to the fact that substitution at 6-hydroxyl (clarithromycin) enhances the antibacterial activities[10]. In addition, the antibacterial activities of 9(*S*)-9-dihydro-12-*O*-methylerythromycin A (**6**) are less than that of 12-*O*-methylerythromycin (**3**), which is consistent with the previously reported results that converting the 9-ketone of erythromycin A to the 9-hydroxyl reduces the activities, *i.e.*, 9(*S*)-9-dihydroerythromycin A (**4**) is less active than that of erythromycin A (**1**) and 9(*S*)-9-dihydroclarithromycin (**5**) is less active than that of clarithromycin (**2**)[16].

Experimental

Antibacterial Activity

The *in vitro* antibacterial activity was determined by agar dilution using Brain Heart Infusion agar. Tests were incubated in ambient air at 37°C for 18 to 20 hours. Erythromycin A (**1**) was used as the control antibiotic and gave the expected results. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of drug inhibiting visible growth of the bacterial strain.

Synthesis

The NMR spectra were recorded on a Varain Unity 500 MHz instrument at 500.5 MHz for ^{1}H and 125.9 MHz for ^{13}C. All NMR spectra were collected at 30°C in $CDCl_3$. The chemical shifts of the protons were measured relative to the residual $CHCl_3$ at 7.27 ppm, and the chemical shifts of carbon signals were referenced to 77.0 ppm of the $CDCl_3$. The 2D experiments, including DQCOSY, ROESY, HMQC and HMBC, were acquired for all the compounds, and their proton and carbon chemical shifts were assigned completely. The electrospray ionization (ESI) mass spectra were obtained using a Finnigan 7000 spectrometer, and fast atom bombardment (FAB) mass spectra were obtained using a JEOL SX102A spectrometer. The 2′,4″-*O*-bis(trimethylsilyl)-12-*O*-methyerythromycin A (**9**) was prepared as previously described[13]. Flash chromatography was performed with silica gel (230~400 mesh) using the solvent system of 1% Et_3N, 2% MeOH in CH_2Cl_2.

2′,4″-*O*-Bis(trimethylsilyl)-12-*O*-methylerythromycin A (**10**): To a solution of 2′,4″-*O*-bis(trimethylsilyl)-12-*O*-methyl-9(*S*)-9-dihydroerythromycin A (**9**)[13] (100 mg, 0.11 mmol) in CH_2Cl_2 (1 ml) and CH_3CN (0.2 ml) was added 4Å powdered sieves and 4-methylmorpholine *N*-oxide (39 mg, 0.34 mmol), followed by tetrapropylammonium perruthenate (3 mg, 0.01 mmol). The mixture was stirred for 3 hours at ambient temperature under N_2. The reaction mixture was then diluted with EtOAc and filtered through a Whatman syringe filter (0.2 μm). The filtered solution was then passed through a bed of silica gel (230~400 mesh) with excess EtOAc. The EtOAc solution was concentrated to give a crude product of 2′,4″-*O*-bis(trimethylsilyl)-12-*O*-methylerythromycin A (**10**) (100 mg, 100%). The crude product was purified by prep HPLC to give 30 mg (30.1% yield) of pure (**10**). ^{1}H NMR: ($CDCl_3$, ppm) (This compound has two forms: 9-ketone and 9,6-hemiketal, in $CDCl_3$ solution with a ratio of about 3 : 2. Only the major form, the 9-ketone form, is reported here): δ 5.38 (dd, 1H, J=10.0, 2.2), 4.80 (d, 1H, J=4.7), 4.54 (d, 1H, J=6.8), 4.40 (br. s, 1H), 4.23 (m, 1H), 3.98 (br. s, 1H), 3.67 (m, 1H), 3.63 (d, 1H, J=7.0), 3.34 (s, 3H), 3.32 (s, 3H), 3.20 (m, 1H), 3.16 (m, 2H), 3.01 (m, 1H), 2.66 (m, 1H), 2.41 (d, 1H, J=15.0), 2.23 (s, 6H), 1.87 (m, 1H), 1.80 (m, 1H), 1.76 (m, 1H), 1.65 (m, 2H), 1.59 (m, 1H), 1.48 (dd, 1H, J=15.0, 4.8), 1.31 (s, 3H), 1.25~1.13 (7 methyl groups), 1.10 (d, 3H, J=7.2), 0.93 (t, 3H, J=7.2), 0.14 (s, 9H), 0.09 (s, 9H). ^{13}C NMR: ($CDCl_3$) δ 218.1 (C-9), 176.5 (C-1), 102.3 (C-1′), 96.7 (C-1″), 80.7 (C-4″), 80.6 (C-5), 80.2 (C-12), 78.1 (C-3), 75.6 (C-6), 75.3 (C-13), 73.1 (C-2′), 73.0 (C-3″), 72.6 (C-11), 68.0 (C-5′), 65.3 (C-5″), 65.0 (C-3′), 52.0 (C-12-OMe), 49.4 (C-3″-OMe), 44.3 (C-2), 44.2 (C-10), 42.9 (C-4), 40.9 (C-3′-NMe), 40.8 (C-8), 39.7 (C-7), 35.5 (C-2″), 29.7 (C-4′), 26.7 (C-6-Me), 22.7 (C-14), 22.3 (C-3″-Me), 21.4 (C-6′), 19.0 (C-6″), 18.3 (C-12-Me), 17.8 (C-8-Me), 13.3 (C-2-Me), 11.8 (C-10-Me), 11.3 (C-15), 10.5 (C-4-Me), 0.9 (C-OTMS), 0.8 (C-OTMS). MS (ESI), $(M+H)^+$, *m/z* 892, and $(M+Na)^+$, *m/z* 914, MW 891.

12-*O*-Methylerythromycin A (**3**): To a stirred solution of 2′,4″-*O*-bis(trimethylsilyl)-12-*O*-methylerythromycin A (**10**) (51 mg, 0.06 mmol) in THF (2 ml) was added tetrabutylammonium fluoride (0.4 ml, 0.43 mmol). The mixture was stirred for 1 hour at ambient temperature under N_2. Half-saturated NaCl solution was added and the product was extracted with *i*-PrOAc. The organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated to yield crude product (57 mg, 133%). Pure product was purified by prep HPLC to yield 12-*O*-methylerythromycin A (**3**) (38 mg, 89%). ^{1}H NMR: ($CDCl_3$, ppm) (This compound has two forms, 9-ketone and 9,6-hemiketal, in $CDCl_3$ solution with a ratio about 1 : 1. The chemical shifts of both forms are reported here). The ketone form: δ 5.43 (br. d, 1H, J=9.8), 4.84 (d, 1H, J=4.8), 4.47 (d, 2H, J=5.5) (overlaps with the hemiketal form), 4.11 (d, 1H, J=6.7), 3.99 (m, 1H), 3.84 (d, 1H, J=1.8), 3.60 (d, 1H, J=6.8), 3.49 (m, 2H), 3.35 (s, 3H), 3.31 (s, 3H), 3.20 (m, 2H) (overlaps with the hemiketal form), 3.12 (qd, 1H, J=7.0, 1.5), 2.98 (d, 2H, J=9.4) (overlaps with the hemiketal form), 2.89 (m, 1H), 2.77 (m, 1H), 2.42 (m, 2H) (overlaps with the hemiketal form), 2.35 (d, 2H, J=15.2) (overlaps with the hemiketal form), 2.26 (s, 3H), 1.91 (m, 1H), 1.77 (dd, 1H, J=14.8, 7.5), 1.77 (m, 1H), 1.66~1.62 (m, 4H) (overlaps with the hemiketal form), 1.56 (m, 2H) (overlaps with the hemiketal form), 1.35 (s, 3H), 1.26 (s, 3H), 1.21~1.18 (m, 22H, and 15H, 5 methyl groups) (overlaps with the hemiketal form), 1.12~1.08 (12H, 4 methyl groups) (overlaps with the hemiketal form), 0.87 (t, 3H, J=7.3). The hemiketal form (the peaks overlap with the ketone forms are not reported, see the ketone form): 5.17 (dd, 1H, J=11.4, 2.8), 4.68 (s, 1H), 4.42 (s, 1H), 4.35 (d, 1H, J=7.3), 4.03 (m, 1H), 3.74 (d, 1H, J=7.4), 3.30 (s, 3H), 3.19 (s, 3H), 2.65 (qd, 1H, J=7.2, 1.2), 2.42 (m, 2H), 2.25 (s, 3H), 2.16 (m, 1H), 2.14 (m, 1H), 1.99 (m, 1H), 1.73 (m, 1H), 1.61 (s, 3H), 1.50 (dd, 1H, J=15.5, 5.1), 1.44 (m, 1H), 1.25 (s, 3H), 0.98 (d, 3H, J=6.7), 0.95 (d, 3H, J=7.2), 0.82 (t, 3H, J=0.73). ^{13}C NMR: ($CDCl_3$), the ketone form: δ 218.8 (C-9), 176.1 (C-1), 102.8 (C-1′), 96.1 (C-1″), 82.8 (C-5), 79.7 (C-12), 79.1 (C-3), 78.0 (C-4″), 75.3 (C-13), 75.2 (C-6), 73.0 (C-3″), 72.0 (C-11), 70.9 (C-2′), 69.0 (C-5′), 65.9 (C-5″), 65.3 (C-3′), 52.4 (C-12-OMe), 49.4 (C-3″-OMe), 43.6 (C-2), 42.1 (C-10), 42.1 (C-8), 41.0 (C-4), 40.3

(C-3′-NMe), 38.8 (C-7), 34.9 (C-2″), 28.7 (C-4′), 26.4 (C-6-Me), 22.1 (C-14), 21.6 (C-3″-Me), 21.2 (C-6′), 18.4~18.1 (C-6″, C-8-Me, C-10Me), 17.5 (C-12-Me), 14.6 (C-2-Me), 10.9 (C-15), 9.6 (C-4-Me). The hemiketal form: δ 176.1 (C-1), 111.0 (C-9), 102.7 (C-1′), 95.0 (C-1″), 85.1 (C-6), 82.5 (C-12), 81.0 (C-5), 77.8 (C-4″), 76.5 (C-3), 76.0 (C-13), 72.6 (C-3″), 72.9 (C-11), 70.7 (C-2′), 68.8 (C-5′), 65.8 (C-5″), 65.2 (C-3′), 49.4 (C-3″-OMe), 48.9 (C-12-OMe), 44.5 (C-2), 43.8 (C-4), 41.8 (C-7), 41.0 (C-8), 40.3 (C-3′-NMe), 36.3 (C-10), 34.8(C-2″), 30.1 (C-6-Me), 28.6 (C-4′), 24.9 (C-14), 21.5 (C-3″-Me), 21.2 (C-6′), 18.4~18.1 (C-6″, C-8-Me), 16.5 (C-12-Me), 11.7 (C-10-Me), 11.7 (C-2-Me), 10.7 (C-15), 9.3 (C-4-Me). FAB-HRMS: calcd for $C_{38}H_{70}O_{13}N$ $(M+H)^+$ 748.4847, found 748.4844.

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