CHEMICAL MODIFICATION OF ERYTHROMYCINS

IV. SYNTHESIS AND BIOLOGICAL PROPERTIES OF 6-*O*-METHYLERYTHROMYCIN B

SHIGEO MORIMOTO, TAKASHI ADACHI, YOKO MISAWA, TAKATOSHI NAGATE, YOSHIAKI WATANABE and SADAFUMI OMURA

Research Center, Taisho Pharmaceutical Co., Ltd.,
1-403 Yoshino-cho, Ohmiya-shi, Saitama 330, Japan

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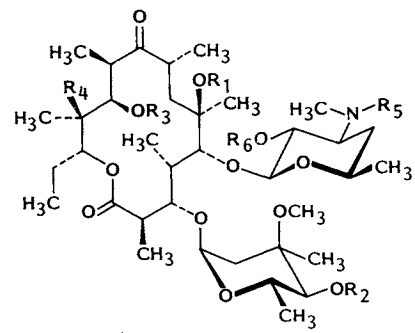
6-*O*-Methylerythromycin B has been synthesized from erythromycin B *via* regioselective methylation of the 6-hydroxyl group in 71% overall yield. This compound shows *in vitro* antibacterial activity comparable to erythromycins A and B and exhibits superior *in vivo* activity with improved pharmacokinetic properties.

In the previous paper we have described the synthesis and the antibacterial activity of *O*-alkyl derivatives of erythromycin A (**1**, EM-A)[1, 2]. Among them, 6-*O*-methylerythromycin A (**2**, clarithromycin, TE-031) showed more potent antibacterial activity than **1** and superior pharmacokinetic properties. Erythromycin B (**3**, EM-B, 12-deoxyerythromycin A), a biosynthetic precursor of **1**, is produced as a minor product by *Streptomyces erythraeus*[3]. We were interested in the synthesis and the biological properties of 6-*O*-methylerythromycin B (**4**). Methylation of 2'-*O*,3'-*N*-bis(benzyloxycarbonyl)-*N*-demethylerythromycin B (**8**), derived from **3**, afforded the 6-*O*-methylated compound (**9**) predominantly, whereas methylation of the corresponding EM-A derivative (**13**) afforded the 11-*O*-methylated compound as the major and the 6-*O*-methylated compound as the minor product.

This paper describes the synthesis and the biological properties of 6-*O*-methylerythromycin B and its related compounds.



Compound	R_1	R_2	R_3	R_4	R_5	R_6
1	H	H	H	OH	CH_3	H
2	CH_3	H	H	OH	CH_3	H
3	H	H	H	H	CH_3	H
4	CH_3	H	H	H	CH_3	H
5	H	H	CH_3	H	CH_3	H
6	CH_3	CH_3	H	H	CH_3	H
7	CH_3	CH_3	CH_3	H	CH_3	H
8	H	H	H	H	Z	Z
9	CH_3	H	H	H	Z	Z
10	H	H	CH_3	H	Z	Z
11	CH_3	CH_3	H	H	Z	Z
12	CH_3	CH_3	CH_3	H	Z	Z
13	H	H	H	OH	Z	Z

Z=$COOCH_2C_6H_5$.

Chemistry

Reaction of EM-B (**3**) with benzyl chloroformate and $NaHCO_3$ afforded 2'-*O*,3'-*N*-bis(benzyloxycarbonyl)-*N*-demethylerythromycin B (**8**) in 88% yield[4]. Methylation of **8** with methyl iodide (MeI, 2.5 equiv)/KOH powder (1.5 equiv) in dimethyl sulfoxide (DMSO)-1,2-dimethoxyethane (1:1) afforded **9** (87%), the 11-*O*-methylated compound (**10**, 6%) and the 6,4''-di-*O*-methylated compound (**11**, 4%). Methylation of **8** with MeI (10 eq)/NaH

Table 1. ^1H and ^{13}C NMR chemical shifts (δ)[a] of **3** and **4**.

Position	3		4		Position	3		4	
	^1H	^{13}C	^1H	^{13}C		^1H	^{13}C	^1H	^{13}C
1		176.1		176.1	20 (10-CH_3)	0.99	9.2	0.97	9.9
2	2.90	44.8	2.94	44.9	21 (12-CH_3)	0.86	9.1	0.84	9.1
3	4.04	80.2	3.78	78.9	6-OCH_3			3.10	50.9
4	2.10	39.3	2.04	38.7	1′	4.42	102.7	4.46	102.7
5	3.57	83.5	3.71	80.6	2′	3.22	71.1	3.19	71.1
6		75.1		78.7	3′	2.46	65.6	2.41	65.6
7	1.64/1.99	38.0	nd/1.90	38.7	4′	1.13/1.65	28.6	nd/nd	28.6
8	2.74	44.8	2.60	45.5	5′	3.50	68.7	3.48	68.7
9		219.6		219.7	6′ (5′-CH_3)	1.22	21.5	1.23	21.5
10	2.98	38.9	2.92	37.8	3′-$N(CH_3)_2$	2.29	40.3	2.28	40.3
11	3.82	69.3	3.73	69.5	1″	4.90	96.2	4.94	96.2
12	1.65	39.8	nd	40.3	2″	1.58/2.38	35.0	1.60/2.37	35.0
13	5.35	74.9	5.39	74.8	3″		72.7		72.7
14	1.47/1.67	25.5	1.46/1.72	25.7	4″	3.01	78.0	3.03	78.0
15 (14-CH_3)	0.88	10.3	0.88	10.5	5″	4.04	65.8	4.02	65.8
16 (2-CH_3)	1.19	15.5	1.20	16.0	6″ (5″-CH_3)	1.29	18.4	1.31	18.4
17 (4-CH_3)	1.14	9.1	1.12	9.1	7″ (3″-CH_3)	1.24	21.5	1.26	21.5
18 (6-CH_3)	1.47	27.2	1.41	20.0	3″-OCH_3	3.22	49.5	3.33	49.5
19 (8-CH_3)	1.14	18.4	1.10	18.8					

[a] δ values in ppm from $(CH_3)_4$ Si, measured in $CDCl_3$ at 400 MHz for ^1H and 100.4 MHz for ^{13}C; as determined from a ^1H-^1H homonuclear and ^1H-^{13}C heteronuclear 2D shift correlated experiments.
nd: Not determined because of the complexities of the spectra.

dispersion (2 eq) in *N*,*N*-dimethylformamide (DMF) afforded **9** (21%), **11** (51%) and the 6,11,4″-tri-*O*-methylated compound (**12**, 10%). When large amounts of NaH dispersion were used in DMF, **12** was obtained in 93% yield.

Catalytic hydrogenation of **9**, **10**, **11** and **12** using Pd-Black in EtOH containing 2.5 M acetate buffer (pH 5.0) and reductive *N*-methylation with formaldehyde afforded **4** (93%), 11-*O*-methylerythromycin B (**5**, 61%), 6,4″-di-*O*-methylerythromycin B (**6**, 69%) and 6,11,4″-tri-*O*-methylerythromycin B (**7**, 48%), respectively.

The molecular formula of **4** was determined as $C_{38}H_{69}NO_{12}$ from elemental analysis, FAB-MS and ^{13}C NMR spectra, indicating the introduction of a methyl group to **3**. The ^1H and ^{13}C NMR spectra of **4** were directly compared with those of **3** (Table 1). The ^1H NMR spectrum of **4** is similar to that of **3** except for the new *O*-methyl signal at 3.10 ppm. In the ^{13}C NMR spectrum, C-6 (78.7 ppm) of **4** is 3.6 ppm further downfield than that of **3**. Upfield shifts of C-5 (−2.9 ppm) and C-18 (−7.2 ppm) were also observed. The spectral data of **4** are consistent with the published substituent effect in **2**[2]. The structure of **4** is therefore determined to be 6-*O*-methylerythromycin B.

The molecular formulae of **6** and **7** were determined as $C_{39}H_{71}NO_{12}$ and $C_{40}H_{73}NO_{12}$, respectively, from FAB-MS, ^{13}C NMR spectra and elemental analyses, indicating the introduction of two and three methyl groups to **3**, respectively. Compound **6** exhibits the new *O*-methyl signal at 3.54 ppm in addition to the 6-*O*-methyl signal at 3.10 ppm. The ^1H NMR spectrum of **7** showed one more *O*-methyl signal at 3.44 ppm besides two *O*-methyl signals at 3.54 and 3.16 ppm which were similar to those observed in **6**. In the ^{13}C NMR spectrum of **6**, a typical downfield shift of C-4″ (+11.0 ppm) was observed together with the new *O*-methyl signal at 62.1 ppm compared to **3**. Compound **7** showed downfield shifts of C-11 (+9.4 ppm) and C-4″ (+11.1 ppm) together with the *O*-methyl signals at 60.1 and 62.2 ppm compared to

3. The structures of **6** and **7** were therefore determined to be 6,4″-di-*O*-methyl- and 6,11,4″-tri-*O*-methylerythromycins B, respectively.

The molecular formula of **5** was determined as $C_{38}H_{69}NO_{12}$ from elemental analysis, FAB-MS and ^{13}C NMR spectra, indicating the introduction of a methyl group to **3**, which was the same as that of **4**. In the ^{1}H NMR spectrum of **5**, the *O*-methyl signal was newly observed at 3.28 ppm. In the ^{13}C NMR spectrum, the *O*-methyl signal (59.9 ppm) and C-11 (79.3 ppm) in **5** were similar to the 11-*O*-methyl signal (60.1 ppm) and C-11 (78.7 ppm) in **7**, respectively. The structure of **5** was therefore determined to be 11-*O*-methylerythromycin B.

Selectivity for methylation of EM-B derivative **8** is considerably different from that of EM-A derivative **13**. Methylation of **13** with MeI/KOH in DMSO-1,2-dimethoxyethane afforded the 11-*O*-methylated compound as the major product, some 6-*O*-methylated compound, and small amounts of 6,11- and 6,12-di-*O*-methylated compounds[2]. On the other hand, the 6-hydroxyl group in **8** is much more reactive than the 11-hydroxyl and the 4″-hydroxyl groups. Compound **8** is different from **13** only by the lack of the 12-hydroxyl group. The above significant difference for methylation is caused by the neighboring group effect of the 12-hydroxyl group. We are studying the reason for the selectivities by theoretical calculation using molecular mechanics (MM2′) and molecular orbital method (MNDO).

Biological Property

Table 2 shows the *in vitro* antibacterial activities of 6-*O*-methylerythromycins B (**4**, **6** and **7**) and 11-*O*-methylerythromycin B (**5**) compared to those of **1**, **2** and **3** against a variety of standard strains. 6-*O*-Methylerythromycin B (**4**) is equal to or 2-fold more active than **3**, and equal to or 2-fold less active than **2**. 11-*O*-Methylerythromycin B (**5**) is equal to or 2-fold less active than **4**. Di- and tri-*O*-methylerythromycins B (**6** and **7**) are much less active than **4**. The *in vivo* activities of **1**, **2**, **3** and **4** are listed in Table 3. Compound **4** is 2-fold more effective than **1** and **3**, but less effective than **2** against experimental infections by *Staphylococcus aureus* Smith 4. The hydroxyl group at C-12 plays an important role for *in vivo* antibacterial activity.

Pharmacokinetic properties of **1**, **2**, **3** and **4** were compared after oral administration to rats in Table

Table 2. *In vitro* antibacterial activities.

Strain	MICs (μg/ml)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Staphylococcus aureus 209P-JC	0.10	0.10	0.20	0.10	0.20	1.56	1.56
S. aureus BB	0.10	0.10	0.20	0.39	0.39	3.13	3.13
S. aureus Smith 4	0.20	0.10	0.20	0.20	0.39	3.13	3.13
S. aureus Terajima	0.10	0.10	0.20	0.20	0.78	1.56	3.13
S. aureus J-109	>100	>100	>100	>100	>100	>100	>100
S. aureus B1	>100	>100	>100	>100	>100	>100	>100
S. epidermidis IID 866	0.20	0.10	0.20	0.10	0.20	1.56	1.56
S. epidermidis sp-al-1	0.39	0.20	0.39	0.20	0.20	1.56	3.13
Bacillus subtilis ATCC 6633	0.10	0.05	0.10	0.10	0.10	0.78	1.56
Micrococcus luteus ATCC 9341	0.025	0.012	0.05	0.025	0.025	0.10	0.10
Escherichia coli NIHJ JC-2	100	100	>100	>100	>100	>100	>100
E. coli K-12	25	12.5	25	25	25	>100	>100
Klebsiella pneumoniae IFO 3317	100	100	>100	>100	>100	>100	>100

Inoculum size: 10^6 cfu/ml.
Medium: Sensitivity Test Agar (Eiken).

Table 3. *In vivo* antibacterial activities of **1**, **2**, **3** and **4** in mouse protection test.

	1	**2**	**3**	**4**
ED_{50} (mg/mouse)	0.666 0.652[2)]	0.086[2)]	0.661	0.358

Compounds **1**, **2**, **3** and **4** were administered orally 1 hour after infection of *Staphylococcus aureus* Smith 4.

4. AUC's of **4** and **2** are significantly higher than those of **3** and **1**, which could explain the above excellent *in vivo* activity.

Table 4. Pharmacokinetic properties of **1**, **2**, **3** and **4** by po administration in rats.

Compound	Dose (mg/kg)	Plasma level		AUC (μg·hour/ml)
		Cmax (μg/ml)	$T_{1/2}$ (hours)	
1	50	0.64	2.81	2.37
2	50	2.40	2.47	11.17
3	50	1.20	1.78	4.03
4	50	1.70	1.15	15.2

Conclusion

EM-A (**1**) has been one of the most useful macrolide antibiotics for the past three decades[5,6)]. We have synthesized clarithromycin (**2**), which is expected to supersede EM-A as a new antibiotic. This compound exhibits excellent antibacterial activity and preferable pharmacokinetic properties, which were brought by 6-*O*-methylation[2)].

6-*O*-Methylerythromycin B (**4**) was synthesized from EM-B (**3**) in 71% overall yield. Methylation of the 6-hydroxyl group in **8** is considerably regioselective, which could not be presumed from methylation of EM-A derivative (**13**). Methylation of the 6-hydroxyl group does not affect the *in vitro* antibacterial activity significantly, but improves the *in vivo* activity due to the preferable pharmacokinetic properties. 6-*O*-Methylerythromycin B (**4**) is expected to be a promising antibiotic as well as clarithromycin (**2**).

Experimental

MP's are uncorrected. IR and UV spectra were recorded with a Perkin-Elmer 1760 FT-IR spectrometer and a Shimadzu UV 240 spectrophotometer, respectively. Optical rotations were measured on a Jasco DIP-360 digital polarimeter. NMR spectra were recorded with a Jeol JNM-GX 400 spectrometer. Mass spectra were measured on a Jeol JMS-SX102 spectrometer equipped with a Jeol JMA-DA6000 data system using FAB techniques.

2'-*O*,3'-*N*-Bis(benzyloxycarbonyl)-*N*-demethylerythromycin B (**8**)

To a vigorously stirred suspension of $NaHCO_3$ (270 g) in carbobenzoxy chloride (500 g) was added **3** (135 g) in small portions at 40~50°C for 1 hour. The mixture was stirred for 1.5 hours at the same temperature, and CH_2Cl_2 (350 ml) was added. The reaction mixture was filtered, and the solid was washed with CH_2Cl_2 (500 ml). The filtrate was evaporated under reduced pressure. Crystallization of the residue from ethyl ether-petroleum ether (100 ml/4.5 liters) afforded **8** (161.8 g, 88%) as colorless needles, mp 212~213.5°C; $[\alpha]_D^{25}$ −112.7° (*c* 0.5, EtOH); UV λ_{max}^{EtOH} nm (ε) 214 (503.8), 252 (298.1), 258 (273.3), 263 (326.7), 267 (sh), 289 (32.9); IR (KBr) cm^{-1} 3446, 1751, 1727, 683; FAB-MS *m/z* 972 (M+H); *Anal* Calcd for $C_{52}H_{77}NO_{16}$: C 64.24, H 7.98, N 1.44. Found: C 63.96, H 8.03, N 1.35.

2'-*O*,3'-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-6-*O*-methylerythromycin B (**9**) and 2'-*O*,3'-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-11-*O*-methylerythromycin B (**10**)

To a stirred solution of **8** (24.3 g) and MeI (3.9 ml, 2.5 equiv) in DMSO-1,2-dimethoxyethane (1:1, 500 ml) was added 85% KOH powder (2.47 g, 1.5 equiv) at 0~5°C in one portion. After the reaction mixture was stirred for 2.5 hours, triethyl amine (10 ml) was added. The above mixture was poured into 5% $NaHCO_3$ soln, and extracted with EtOAc. The organic layer was washed with satd NaCl soln, dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was chromatographed on a silica gel column using EtOAc-*n*-hexane (1:2) to afford **9** (21.4 g, 87%), **10** (1.6 g, 6%) and **11** (1.0 g, 4%). Crystallization of **9** from $CHCl_3$-petroleum ether afforded colorless prisms, mp 102~106°C; $[\alpha]_D^{26.5}$ −114.0°

(*c* 0.5, EtOH); UV λ_{max}^{EtOH} nm (ε) 213 (488.8), 252 (294.7), 258 (373.8), 263 (328.4), 267 (sh), 289 (31.8); IR (KBr) cm^{-1} 3515, 1751, 1730, 1704; FAB-MS *m/z* 986 (M+H); *Anal* Calcd for $C_{53}H_{79}NO_{16}$: C 64.55, H 8.07, N 1.42. Found: C 63.93, H 8.13, N 1.33.

Crystallization of **10** from EtOAc-*n*-hexane afforded colorless prisms, mp 129~133°C; $[\alpha]_D^{25}$ −118.3° (*c* 0.5, EtOH); UV λ_{max}^{EtOH} nm (ε) 214 (510.3), 252 (301.4), 258 (380.3), 263 (331.0), 267 (sh), 289 (31.8); IR (KBr) cm^{-1} 3523, 1752, 1729, 1703; FAB-MS *m/z* 986 (M+H); *Anal* Calcd for $C_{53}H_{79}NO_{16}$: C 64.55, H 8.07, N 1.42. Found: C 64.07, H 8.12, N 1.33.

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-6,4″-di-*O*-methylerythromycin B (**11**)

To a stirred solution of **8** (0.80 g) and MeI (0.8 ml, 16 equiv) in DMF (6.4 ml) was added 60% NaH dispersion (64 mg, 2.0 equiv) at 0~5°C in one portion, and the reaction mixture was stirred for 1.5 hours. Purification as described above afforded **12** (80 mg, 10%), **11** (420 mg, 51%), and **9** (170 mg, 21%). Compound **11** was obtained as a colorless glass, mp 94~98°C; $[\alpha]_D^{27}$ −108.4° (*c* 0.5, EtOH); UV λ_{max}^{EtOH} nm (ε) 219 (603.7), 252 (381.5), 258 (438.7), 263 (381.3), 267 (sh), 289 (sh); IR ($CHCl_3$) cm^{-1} 3509, 1745, 1723, 1693; FAB-MS *m/z* 1,000 (M+H); *Anal* Calcd for $C_{54}H_{81}NO_{16}$: C 64.84, H 8.16, N 1.40. Found: C 64.17, H 8.10, N 1.30.

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-6,11,4″-tri-*O*-methylerythromycin B (**12**)

To a stirred solution of **8** (1.0 g) and MeI (1 ml, 16 equiv) in DMF (8 ml) was added 60% NaH dispersion (192 mg, 4.9 equiv) at 0~5°C, and the reaction mixture was stirred for 0.5 hour. Similar treatment and purification as described above afforded **12** (972 mg, 93%). Crystallization of **12** from ethyl ether-petroleum ether afforded colorless needles, mp 187~189°C; $[\alpha]_D^{25}$ −129.6° (*c* 0.5, EtOH); UV λ_{max}^{EtOH} nm (ε) 211 (527.0), 252 (303.3), 258 (386.4), 263 (335.3), 267 (sh), 289 (28.4); IR(KBr) cm^{-1} 1750, 1726, 1708; FAB-MS *m/z* 1,014 (M+H); *Anal* Calcd for $C_{55}H_{83}NO_{16}$: C 65.13, H 8.25, N 1.38. Found: C 64.87, H 8.34, N 1.32.

6-*O*-Methylerythromycin B (**4**)

To a solution of **9** (21.42 g) in 2.5 M acetate buffer (pH 5.0, 24 ml) and EtOH (200 ml) was added Pd-black (1 g) and the mixture was stirred under hydrogen atmosphere for 3 hours at room temperature. After the complete removal of the benzyloxycarbonyl groups, 37% formaldehyde soln (40 ml) was added and hydrogenation was continued for further 3 hours. The catalyst was filtered off, and ice water (1 liter) was added to the filtrate. The resulting mixture was adjusted to pH 10~10.5 with 2 N NaOH soln under stirring to give the crude crystals. The crystals, collected by filtration, were washed with 2% $NaHCO_3$ soln and water and dried. Crystallization from $CHCl_3$-petroleum ether afforded **4** (14.84 g, 93%). Recrystallization from EtOH gave needles for analyses, mp 219~220°C; $[\alpha]_D^{26.5}$ −104.0° (*c* 0.5, EtOH); UV λ_{max}^{EtOH} nm (ε) 217 (381.2), 289 (30.9); IR (KBr) cm^{-1} 3468, 1729, 1692; FAB-MS *m/z* 732 (M+H); *Anal* Calcd for $C_{38}H_{69}NO_{12}$: C 62.36, H 9.50, N 1.91. Found: C 62.70, H 9.40, N 1.74. ^1H and ^{13}C NMR data are listed in Table 1.

11-*O*-Methylerythromycin B (**5**)

By the method described above, **10** (200 mg) was hydrogenated and *N*-methylated. The reaction mixture was filtered, and the filtrate was poured into $NaHCO_3$ soln and extracted with EtOAc. The organic layer was washed with satd NaCl soln, dried over $MgSO_4$ and evaporated to give the crude product. Column chromatography of the crude product on silica gel with $CHCl_3$-MeOH-conc NH_4OH (20:1:0.1) afforded **5** (91 mg, 61%) as crystalline solid, mp 178~182°C; $[\alpha]_D^{27}$ −103.2° (*c* 0.25, EtOH); UV λ_{max}^{EtOH} nm (ε) 213 (726.4),. 285 (47.7); IR ($CHCl_3$) cm^{-1} 3452, 1722; FAB-MS *m/z* 732 (M+H); *Anal* Calcd for $C_{38}H_{69}NO_{12}$: C 62.36, H 9.50, N 1.91. Found: C 62.09, H 9.32, N 1.75. ^1H NMR (400 MHz, $CDCl_3$) δ 2.28 (6H, s, 3′-N(CH_3)$_2$), 2.99 (1H, dd, 4″-H), 3.28 (3H, s, 11-OCH_3), 3.31 (3H, s, 3″-OCH_3), 3.99 (1H, dd, 11-H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 40.4 (3′-N (CH_3)$_2$), 49.4 (3″-OCH_3), 59.9 (11-OCH_3), 79.3 (C-11), 74.7 (C-6), 77.9 (C-4″).

6,4″-Di-*O*-methylerythromycin B (**6**)

By the method described above, **11** (600 mg) was hydrogenated and *N*-methylated. Column

chromatography of the crude product on silica gel with $CHCl_3$-MeOH (10:1) and crystallization from EtOH afforded **6** (327 mg, 69%) as needles which contained 1 mol of EtOH, mp 200~201°C; $[\alpha]_D^{26.5}$ −95.8° (*c* 0.47, EtOH); UV λ_{max}^{EtOH} nm (ε) 216 (397.0), 289 (34.2); IR (KBr) cm^{-1} 3466, 1728, 1693; FAB-MS *m/z* 746 (M+H); *Anal* Calcd for $C_{39}H_{71}NO_{12} \cdot C_2H_5OH$: C 62.17, H 9.80, N 1.77. Found: C 61.82, H 9.73, N 1.74. ^1H NMR (400 MHz, $CDCl_3$) δ 2.28 (6H, s, 3'-$N(CH_3)_2$), 2.66 (1H, d, 4''-H), 3.10 (3H, s, 6-OCH_3), 3.33 (3H, s, 3''-OCH_3), 3.54 (3H, s, 4''-OCH_3), 3.73 (1H, dd, 11-H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 40.2 ($N(CH_3)_2$), 49.7 (3''-OCH_3), 50.9 (6-OCH_3), 62.1 (4''-OCH_3), 69.5 (C-11), 78.7 (C-6), 89.0 (C-4'').

6,11,4''-Tri-*O*-methylerythromycin B (**7**)

Similarly **12** (830 mg) was hydrogenated and *N*-methylated. The crude product was chromatographed on silica gel column with $CHCl_3$-MeOH (20:1) and crystallized from EtOH to afford **7** (316 mg, 48%), as needles which contained 1 mol of EtOH, mp 228~230°C; IR (KBr) cm^{-1} 3455, 1726, 1715, 1696; $[\alpha]_D^{26.5}$ −108.4° (*c* 0.47, EtOH); FAB-MS *m/z* 760 (M+H); UV λ_{max}^{EtOH} nm (ε) 217 (433.0), 287 (40.0); *Anal* Calcd for $C_{40}H_{73}NO_{12} \cdot C_2H_5OH$: C 62.58, H 9.88, N 1.74. Found: C 62.42, H 9.65, N 1.70; ^1H NMR (400 MHz, $CDCl_3$) δ 2.28 (6H, s, 3'-N $(CH_3)_2$), 2.66 (1H, d, 4''-H), 3.16 (3H, s, 6-OCH_3), 3.33 (3H, s, 3''-OCH_3), 3.44 (3H, s, 11-OCH_3), 3.54 (3H, s, 4''-OCH_3), 3.51 (1H, dd, 11-H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 40.3 (3'-$N(CH_3)_2$), 49.7 (3''-OCH_3), 50.7 (6-OCH_3), 60.1 (11-OCH_3), 62.2 (4''-OCH_3), 78.7 (C-11), 79.3 (C-6), 89.1 (C-4'').

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