Notes

CHEMICAL MODIFICATION OF ERYTHROMYCINS

V. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 4″-*O*-METHYL DERIVATIVES OF ERYTHROMYCIN A 11,12-CYCLIC CARBONATE

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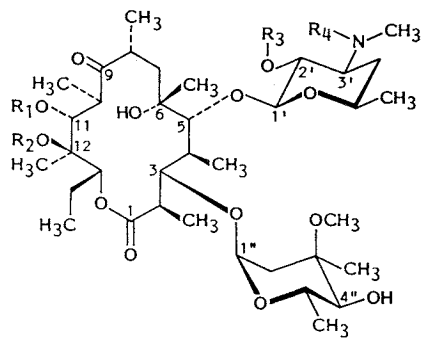
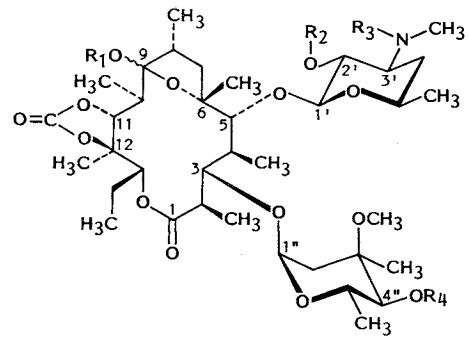
Erythromycin A (**1**) is a macrolide antibiotic used for treatment of infections caused by Gram-positive bacteria and *Mycoplasma* sp.[1] Its 11,12-cyclic carbonate derivative (**2**),[2,3] formed by reaction of **1** with ethylene carbonate, has greater antibacterial activity than **1**. While **1** and **2** induced strongly macrolide-resistance in *Staphylococcus aureus*,[4] introduction of an acetyl or some sulfonyl groups at the C-4″ position of **1** or **2** resulted in a decrease of inducer activity.[4,5] On the other hand, it has been reported that 4″-deoxy derivatives of **1** exhibited no difference with **1** in the inducibility test.[6] Consequently, we undertook the methylation of the 4″-hydroxyl group. Herein we report the synthesis and antibacterial activity of 4″-*O*-methyl derivatives of erythromycin A 11,12-cyclic carbonate.

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethylerythromycin A (**3**)[7] was reacted with ethylene carbonate in the presence of K_2CO_3 to yield (9*S*)-hemiacetal (**4a**) and its C-9 isomer (**4b**) in the ratio of *ca.* 3:1. The NMR data suggested that **4b** was a mixture of the C-9 keto form and (9*R*)-hemiacetal in $CDCl_3$.[8] The isomer **4b** could be converted to **4a** by the treatment with dimethyl sulfate (1 equiv) in *N*,*N*-dimethylformamide. Compound **4a** was methylated with CH_3I and NaH in *N*,*N*-dimethylformamide to give *O*-methylated products A (60%) and B (7%). The product A was hydrogenated to remove the benzyloxycarbonyl groups, and subsequent reductive *N*-methylation with HCHO yielded the corresponding dimethylamino derivatives **5**, **6** and **7** in the yield of 36, 6 and 9% from **4a**, respectively. By the same procedure, the product B provided **5** (4% from **4a**).

The structures of **5**, **6** and **7** were established by comparison of their mass and NMR spectra with those of **2**.[9] The ^1H and ^{13}C NMR chemical shifts of these compounds were assigned by 2D-NMR spectroscopy, including H/C COSY and heteronuclear multiple-bond correlation (HMBC). The mass spectra of **5**, **6** and **7** represented the molecular ions at *m/z* 773, 787 and 787, respectively, indicating the introduction of one or two methyl groups to **2**. The ^1H NMR spectrum of **5** showed a new methoxy signal at 3.47 ppm and the doublet methine signal due to 4″-H at 2.61 ppm. Its ^{13}C NMR spectrum showed a new methoxy signal at 61.9 ppm with a significant downfield shift of C-4″ compared to that of **2**. Other ^{13}C chemical shifts for **5** were almost similar to those for **2** (Table 1).



1 $R_1=R_2=R_3=H$ $R_4=CH_3$
2 $R_1+R_2=CO$ $R_3=H$ $R_4=CH_3$
3 $R_1=R_2=H$ $R_3=R_4=COOCH_2C_6H_5$



4a $R_1=H(9S)$ $R_2=R_3=COOCH_2C_6H_5$ $R_4=H$
5 $R_1=H(9S)$ $R_2=H$ $R_3=R_4=CH_3$
6 $R_1=CH_3(9S)$ $R_2=H$ $R_3=R_4=CH_3$
7 $R_1=CH_3(9R)$ $R_2=H$ $R_3=R_4=CH_3$

The above data suggest structure **5** which is 4″-*O*-methylerythromycin A 11,12-cyclic carbonate. The ^{1}H NMR spectra of **6** and **7** showed peaks due to 4″-OCH_3 at 3.47 and 3.56 ppm, respectively. In addition, these spectra contained another methoxy peaks at 3.20 and 3.22 ppm, respectively, whose corresponding ^{13}C chemical shifts were centered at 49.4 and 48.8 ppm, respectively, with the downfield shifts of C-9 and C-6 compared to those of **2** (Table 1). The above data indicated that both **6** and **7** were 9-deoxo-6-deoxy-6,9-epoxy-9-methoxy-4″-*O*-methylerythromycin A 11,12-cyclic carbonates. The NOE difference spectrum of **6** showed NOE enhancements between the proton of 9-OCH_3 and each proton of 11-H and 6-CH_3, while for **7** NOE's were observed between the proton of 9-OCH_3 and each proton of 10-H and 10-CH_3, and between the proton of 6-CH_3 and each proton of 8-H and 11-H (Fig. 1). These results established the stereochemistry of the C-9 for **6** and **7** as 9*S*- and 9*R*-configurations, respectively.

Against erythromycin-susceptible bacteria, the *in vitro* activity of compounds **5**, **6** and **7** was equal to or slightly less active than that of **1**. However, these compounds were 8 to 256-fold more active than **1** against some of erythromycin-resistant

Table 1. ^{13}C NMR chemical shifts of 4″-*O*-methyl derivatives of erythromycin A 11,12-cyclic carbonate.

Carbon	Chemical shift (δ, ppm)[a]				Carbon	Chemical shift (δ, ppm)[a]			
	2[b]	**5**	**6**	**7**		**2**[b]	**5**	**6**	**7**
1	177.5	177.6	177.4	178.7	12-CH_3	16.7	16.6	14.1	15.5
2	41.8	43.8	44.2	48.1	9-OCH_3	—	—	49.4	48.8
3	82.6	82.4	78.2	73.5	1′	103.1	102.7	102.9	105.0
4	38.4	38.2	40.4	44.8	2′	70.3	70.2	70.3	70.2
5	82.4	82.4	83.1	85.6	3′	65.4[c]	65.4	65.2	65.1
6	86.2	86.1	87.8	85.8	4′	28.5	28.3	28.5	29.1
7	39.6	39.5	43.2	43.7	5′	69.6	69.1	68.8	69.2
8	43.9	41.6	42.7	39.2	5′-CH_3	21.3	21.2	21.2	21.1
9	107.3	107.2	111.2	108.5	3′N(CH_3)$_2$	40.4	40.1	40.1	40.3
10	36.7	36.5	37.2	35.0	1″	95.3	95.3	96.5	95.3
11	75.8	75.9	79.8	82.5	2″	35.0	35.3	35.4	35.0
12	86.4	86.3	85.5	85.8	3″	73.0	73.7	73.6	73.7
13	78.6	78.2	76.9	80.0	4″	78.1	88.9	88.8	89.0
14	22.8	22.6	21.6	23.2	5″	66.0[c]	64.4	64.4	64.2
15	10.5	10.4	10.1	10.0	3″-CH_3	21.7	21.3	21.2	21.3
2-CH_3	12.8	13.0	14.0	13.5	5″-CH_3	18.2	18.2	17.8	17.5
4-CH_3	12.1	12.0	12.4	11.9	3″-OCH_3	49.6	49.7	49.5	49.5
6-CH_3	26.5	26.3	29.9	30.6	4″-OCH_3	—	61.9	61.9	62.0
8-CH_3	12.6	12.4	17.4	14.0	C=O	152.9	152.9	153.5	153.8
10-CH_3	12.3	12.3	9.5	9.6					

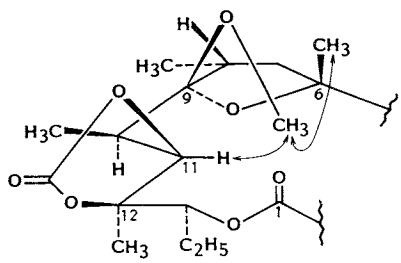
[a] Chemical shifts are in ppm downfield of TMS. ^{13}C NMR spectra were taken in $CDCl_3$ on a Jeol JNM-GX 400 spectrometer.

[b] Assignments for **2** were based on ref 9.

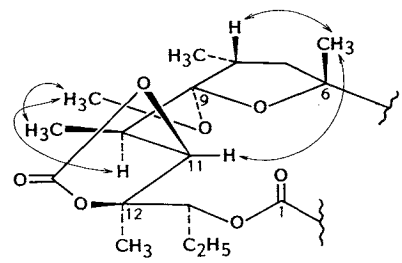
[c] Values at C-3′ and C-5″ are different from ref 9.

Fig. 1. Stereochemistry of C-9 in **6** and **7**.

⟶NOE.



6 (9*S*)



7 (9*R*)

Table 2. *In vitro* antibacterial activity of 4″-*O*-methyl derivatives of erythromycin A 11,12-cyclic carbonate.

Organisms	MIC (μg/ml)				
	1	**2**	**5**	**6**	**7**
Staphylococcus aureus 209P-JC	<0.05	<0.05	<0.05	<0.05	0.10
S. aureus Smith 4	0.20	0.10	0.20	0.39	0.78
S. aureus TPR 20	6.25	1.56	6.25	12.5	50
S. aureus TPR 23[a]	100	25	3.13	3.13	6.25
S. aureus TPR 25[a]	50	12.5	0.78	1.56	3.13
S. aureus TPR 27[a]	>100	>100	>100	>100	100
S. epidermidis IID 866	0.39	0.10	0.39	0.39	0.78
S. epidermidis TPR 13[a]	25	3.13	0.78	1.56	3.13
Enterococcus faecalis ATCC 8043	<0.05	<0.05	<0.05	<0.05	0.1
Micrococcus luteus NIHJ	<0.05	<0.05	<0.05	<0.05	<0.05
Bacillus subtilis ATCC 6633	0.20	<0.05	0.20	0.39	0.39
B. subtilis EM-R[a]	>100	>100	0.39	0.39	0.78
Escherichia coli NIHJ JC-2	100	25	100	100	>100

[a] Erythromycin-resistant strain.
Medium: Mueller-Hinton agar.
Inoculum size: 10^6 cfu/ml.

bacteria (Table 2). These 4″-*O*-methyl cyclic carbonates did not induce resistance to josamycin in the inducibility test by the disk assay[10] with the use of *Bacillus subtilis* EM-R which is highly resistant to **1**. However, the unmodified cyclic carbonate **2** showed a strong inducer activity as well as **1**. When administered orally, the ED_{50} value for **5** was 0.578 mg/mouse against the mice systemic infection of *S. aureus* Smith 4, whereas that for **1** was 1.22 mg/mouse.

Experimental

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethylerythromycin A 11,12-Cyclic Carbonate (**4a**) and its Isomer (**4b**)

A mixture of **3** (50 g, 0.05 mol), ethylene carbonate (50 g, 0.57 mol) and K_2CO_3 (25 g, 0.18 mol) in dry benzene (300 ml) was heated under reflux for 2 hours. The mixture was cooled to ambient temperature and poured into water. The organic layer was separated, washed with satd NaCl soln, dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The residue was chromatographed over silica gel column with EtOAc-hexane (1:2) to give **4a** and **4b**. Recrystallization of **4a** from Et_2O gave 24.3 g (47%) of colorless crystals: MP 197.5~199.0°C; TLC (EtOAc-hexane, 1:1) Rf 0.59; FD-MS *m/z* 1,013 (M); IR (KBr) cm^{-1} 1805, 1755, 1705; $[\alpha]_D^{24}$ −60.1° (*c* 0.526, EtOH); ^1H NMR (200 MHz, $CDCl_3$) δ 2.85, 2.81 (3H, NCH_3), 1.63 (12-CH_3), 1.34 (6-CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 107.1 (C-9); *Anal* Calcd for $C_{53}H_{75}NO_{18}$: C 62.77, H 7.45, N 1.38. Found: C 62.80, H 7.55, N 1.33. Recrystallization of **4b** from EtOAc gave 7.5 g (15%) of colorless crystals: MP 186.5~188.5°C; TLC (EtOAc-hexane, 1:1) Rf 0.51; FD-MS *m/z* 1,014 (M+H); IR (KBr) cm^{-1} 1815, 1735, 1675; $[\alpha]_D^{24}$ −66.6° (*c* 0.344, EtOH); ^1H NMR (200 MHz, $CDCl_3$) δ 2.87, 2.82 (3H, NCH_3), 1.60 (12-CH_3), 1.50 (6-CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 214.6 (C-9 keto), 110.1 (C-9 hemiacetal); *Anal* Calcd for $C_{53}H_{75}NO_{18}$: C 62.77, H 7.45, N 1.38. Found: C 62.47, H 7.39, N 1.34.

4″-*O*-Methylerythromycin A 11,12-Cyclic Carbonate (**5**) and 9-Deoxo-6-deoxy-6,9-epoxy-9-methoxy-4″-*O*-methylerythromycin A 11,12-Cyclic Carbonates (9*S*-epimer **6** and 9*R*-epimer **7**)

To a cooled solution of **4a** (19 g, 0.019 mol) and CH_3I (12 ml, 0.193 mol) in dry DMF (100 ml) was added by portions 60% NaH dispersion (1.7 g, 0.043 mol) with stirring at 0~5°C. The mixture was stirred for a further 2.5 hours at 0~5°C and then triethylamine (40 ml) was added to quench the reaction. The reaction mixture was poured into satd $NaHCO_3$ soln, and extracted with EtOAc. The EtOAc layer was washed with satd NaCl soln, dried ($MgSO_4$) and evaporated to dryness *in vacuo*. The residue was chromatographed over silica gel column with EtOAc-hexane (1:2) to give, in order of elution, 11.8 g of product A and 1.4 g of product B. The suspension of the product A (11 g) and palladium black (1.0 g) in a mixture of Na_2CO_3 (1.4 g), AcOH (0.88 ml), water (40 ml) and EtOH (200 ml), was stirred for 5 hours at ambient

temperature under a gentle hydrogen stream. Formaldehyde (37%, 25 ml) was added to the reaction mixture, and the hydrogenation was continued for a further 4 hours. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to remove almost of the EtOH. The residue was diluted with water (100 ml), basified (pH 10) using Na_2CO_3, and extracted with CH_2Cl_2 (2×100 ml). The combined extracts were washed with satd NaCl soln and dried ($MgSO_4$). The solvent was evaporated and the residue was purified by silica gel column chromatography ($CHCl_3$-MeOH, 100:2~100:5), affording 4.8 g of **5** (36% from **4a**), 0.86 g of **6** (6% from **4a**) and 1.3 g of **7** (9% from **4a**) as a colorless foam, respectively.

For Compound **5**: MP 122~126°C; TLC ($CHCl_3$-MeOH, 4:1) Rf 0.40; FAB-MS *m/z* 774 (M+H); IR (KBr) cm^{-1} 1805, 1735; $[\alpha]_D^{24}$ −44.8° (*c* 0.580, EtOH); ^1H NMR (400 MHz, $CDCl_3$) δ 4.52 (1H, dd, J=9.8 and ~1 Hz, 3-H), 3.55 (1H, d, J=6.5 Hz, 5-H), 3.47 (3H, s, 4″-OCH_3), 3.25 (3H, s, 3″-OCH_3), 2.61 (1H, d, J=9.6 Hz, 4″-H), 2.20 (6H, s, $N(CH_3)_2$), 1.54 (3H, s, 12-CH_3), 1.33 (3H, s, 6-CH_3); ^{13}C NMR: See Table 1.

For Compound **6**: MP 121~124.5°C; TLC ($CHCl_3$-MeOH, 4:1) Rf 0.47; FAB-MS *m/z* 788 (M+H); IR (KBr) cm^{-1} 1810, 1735; $[\alpha]_D^{24}$ −58.9° (*c* 0.557, EtOH); ^1H NMR (400 MHz, $CDCl_3$) δ 4.03 (1H, dd, J=4.6 and 3.6 Hz, 3-H), 3.49 (1H, d, J=7.8 Hz, 5-H), 3.47 (3H, s, 4″-OCH_3), 3.22 (3H, s, 3″-OCH_3), 3.20 (3H, s, 9-OCH_3), 2.61 (1H, d, J=9.6 Hz, 4″-H), 2.21 (6H, s, $N(CH_3)_2$), 1.48 (3H, s, 6-CH_3), 1.33 (3H, s, 12-CH_3); ^{13}C NMR: See Table 1. *Anal* Calcd for $C_{40}H_{69}NO_{14}$: C 60.97, H 8.83, N 1.78. Found: C 60.58, H 8.83, N 1.83.

For Compound **7**: MP 120.5~123.5°C; TLC ($CHCl_3$-MeOH, 4:1) Rf 0.44; FAB-MS *m/z* 788 (M+H); IR (KBr) cm^{-1} 1810, 1735; $[\alpha]_D^{24}$ −38.4° (*c* 0.258, EtOH); ^1H NMR (400 MHz, $CDCl_3$) δ 4.22 (1H, dd, J=2.1 and 1.9 Hz, 3-H), 3.37 (1H, d, J=10.6 Hz, 5-H), 3.56 (3H, s, 4″-OCH_3), 3.29 (3H, s, 3″-OCH_3), 3.22 (3H, s, 9-OCH_3), 2.70 (1H, d, J=9.2 Hz, 4″-H), 2.32 (6H, s, $N(CH_3)_2$), 1.49 (3H, s, 12-CH_3), 1.42 (3H, s, 6-CH_3); ^{13}C NMR: See Table 1.

According to the same procedure described for the product A, the product B (2 g) provided 0.75 g of **5** (4% from **4a**) which was identical in all respects to the sample prepared as noted above.

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