CHEMICAL MODIFICATION OF ERYTHROMYCINS. I. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 6-*O*-METHYLERYTHROMYCINS A

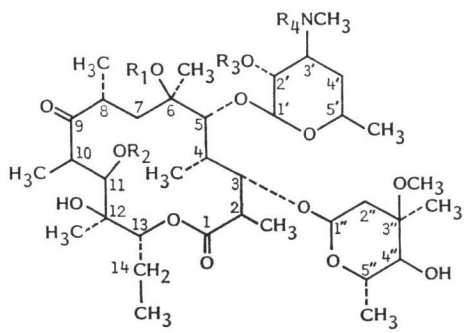
Sir:

Erythromycin A (**1**) is the best known of the medicinally important macrolide antibiotics. Among lots of its chemical modification, the *O*-alkylation of **1** has not been reported, except for 11-*O*-alkylation[1] of erythromycin B (12-deoxyerythromycin A).

In this paper we describe the preparation and the antibacterial activity of *O*-methyl derivatives; 6-*O*-methylerythromycin A (**3**) and 6,11-di-*O*-methylerythromycin A (**4**).

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethylerythromycin A (**2**)[2] was methylated with CH_3I (8 equiv) and NaH (1.2 equiv) in dimethyl sulfoxide - tetrahydrofuran (1: 1) under ice-cooling for 30 minutes. The mixture, thus obtained, was subjected to silica gel column chromatography using ethyl acetate - hexane (1: 2) as an eluent to afford two products A (35% yield) and B (42% yield). Rf values of A and B were 0.16 and 0.09, respectively, by silica gel thin-layer chromatography using ethyl acetate - hexane (1: 1).

Product A was hydrogenated with Pd-black as catalyst in ethanol in the presence of a sodium acetate - acetic acid buffer (pH 5) to remove benzyloxycarbonyl groups, followed by the reductive methylation with formaldehyde and hydrogen to yield the corresponding dimethylamino derivative. This substance was recrystallized from chloroform - isopropyl ether to give the mixture of **3** and **4** as colorless needles (95% yield, the ratio of **3** to **4** (4: 1) was determined by ^1H NMR or high performance liquid chromatography analysis).



	R_1	R_2	R_3	R_4
1	H	H	H	CH_3
2	H	H	$CO_2CH_2C_6H_5$	$CO_2CH_2C_6H_5$
3	CH_3	H	H	CH_3
4	CH_3	CH_3	H	CH_3

The crystals were extracted repeatedly with ethyl ether, and the ether extract and ether-insoluble solid were separated respectively. The ether extract was evaporated and the residue was recrystallized twice from ethanol to yield **3** (14.3% from **2**) as needles: mp 222~225°C; $[\alpha]_D^{24}$ −90.4° (*c* 1.0, $CHCl_3$); IR (KBr) 3450, 1730, 1690 cm^{-1}; UV ($CHCl_3$) 288 nm (ε 27.9); ^1H NMR ($CDCl_3$) δ 0.84 (t, 7.3, 14-CH_3), 1.14 (s, 6-CH_3), 2.28 (s, $N(CH_3)_2$), 3.03 (s, 6-OCH_3), 3.19 (dd, 10.2, 7.2, H-2′), 3.33 (s, 3″-OCH_3), 3.67 (d, 7.4, H-5), 3.76 (d, 1.5, H-11), 3.77 (dd, 9.0, ~1, H-3), 4.44 (d, 7.2, H-1′), 4.93 (dd, 4.6, ~1, H-1″), 5.06 (dd, 10.9, 2.2, H-13); *Anal* Calcd for $C_{38}H_{69}NO_{13}$: C 61.02, H 9.30, N 1.87. Found: C 60.57, H 9.13, N 1.82.

The ether-insoluble solid was subjected to the reversed phase silica gel column chromatography (Merck, silica gel 60 silanized) using phosphate buffer (0.1 M, pH 7) - methanol (2: 3) to give **3** (Rf 0.42 by TLC using silanized silica gel plate/above cited solvent, 1.1% yield from **2**) and **4** (Rf 0.39, 3% yield from **2**). Recrystallization of **4** from dichloromethane - ethyl ether gave colorless needles: mp 252~256°C (dec); $[\alpha]_D^{24}$ −102.8° (*c* 1.0, $CHCl_3$); IR (KBr) 3485, 3430, 1724, 1703 cm^{-1}; UV ($CHCl_3$) 292 nm (ε 23.6); ^1H NMR ($CDCl_3$) δ 0.83 (t, 7.3, 14-CH_3), 1.41 (s, 6-CH_3), 2.28 (s, $N(CH_3)_2$), 3.10 (s, 6-OCH_3), 3.19 (dd, 10.2, 7.2, H-2′), 3.33 (s, 3″-OCH_3), 3.44 (d, ~1, H-11), 3.58 (s, 11-OCH_3), 3.71 (dd, 10.6, ~1, H-3), 3.76 (d, 7.1, H-5), 4.48 (d, 7.2, H-1′), 4.94 (dd, 4.6, ~1, H-1″), 4.97 (dd, 10.3, 2.2, H-13); *Anal* Calcd for $C_{38}H_{71}NO_{13}$: C 61.47, H 9.39, N 1.84. Found: C 61.65, H 9.54, N 1.81.

The ^1H NMR spectra of both **3** and **4** showed the presence of new methoxy signals at 3.03 ppm in **3** and at 3.10, 3.58 ppm in **4**. FD mass spectra of **3** and **4** showed $[MH]^+$ at *m/z* 748 and 762, respectively. In addition prominent ions attributed to desosamine and cladinose (*m/z* 158 and 159, respectively) were present in EI mass spectra, indicating that the new methoxy groups in both compounds were substituted on the

Table 1. ^{13}C NMR chemical shifts* of erythromycin A (**1**)**, 6-*O*-methylerythromycin A (**3**) and 6,11-di-*O*-methylerythromycin A (**4**).

Carbon number	Chemical shifts			Carbon number	Chemical shifts		
	1	**3**	**4**		**1**	**3**	**4**
1	175.9	175.9	175.7	12-Me	16.2	16.0	17.5
2	45.1	45.1	45.0	14-Me	10.7	10.6	10.5
3	80.0	78.4	78.7	6-OMe		50.6	50.6
4	39.5	39.2	38.3	11-OMe			60.8
5	83.6	80.8	79.8	1′	103.3	102.9	102.6
6	74.9	78.4	79.1	2′	71.0	71.0	71.1
7	38.5	39.4	37.7	3′	65.6	65.6	65.6
8	44.9	45.3	45.5	4′	28.7	28.6	28.6
9	221.7	221.0	217.4	5′	68.9	68.8	68.7
10	38.0	37.2	38.0	3′-NMe_2	40.3	40.3	40.3
11	68.9	69.1	77.8	5′-Me	21.5	21.5	21.5
12	74.8	74.3	76.1	1″	96.3	96.1	96.4
13	77.0	76.6	77.5	2″	35.0	34.9	35.1
14	21.1	21.0	21.5	3″	72.6	72.7	72.7
2-Me	16.0	16.0	16.1	4″	78.0	78.0	78.0
4-Me	9.2	9.1	9.3	5″	65.6	65.7	65.9
6-Me	26.8	19.8	20.4	3″-Me	21.4	21.5	21.5
8-Me	18.3	18.0	19.4	5″-Me	18.6	18.7	18.8
10-Me	12.0	12.3	12.8	3″-OMe	49.5	49.5	49.5

* Chemical shifts (ppm downfield from Me_4Si) were measured at 50.3 MHz in $CDCl_3$.
** For the ^{13}C NMR assignment of erythromycin A (**1**), see ref 3.

Table 2. Antibacterial activities of 6-*O*-methylerythromycin A (**3**) and 6,11-di-*O*-methylerythromycin A (**4**).

Test organism	Medium	Minimum inhibitory concentration (μg/ml)		
		Erythromycin	**3**	**4**
Staphylococcus aureus Smith	I	0.2	0.1	0.4
Staphylococcus aureus Terajima	I	0.1	0.1	0.2
Staphylococcus epidermidis IID 866	I	0.2	0.1	0.2
Staphylococcus epidermidis sp-al-1	I	0.2	0.1	0.2
Streptococcus faecalis ATCC 8043	I	≦0.05	≦0.05	≦0.05
Bacillus subtilis ATCC 6633	I	0.1	≦0.05	0.1
Micrococcus luteus ATCC 9341	I	≦0.05	≦0.05	≦0.05
Escherichia coli NIHJ JC-2	I	50	50	100
Klebsiella pneumoniae IFO 3317	I	25	12.5	50
Mycoplasma pneumoniae IID Wada	II	0.015	0.007	0.03
Mycoplasma pneumoniae No. 112	II	0.007	0.007	0.015

Medium I: Mueller-Hinton agar.
II: PPLO broth and agar (horse serum 17%).

erythronolide moiety.

The ^{13}C NMR chemical shifts (Table 1) showed that *O*-methylation caused the downfield shifts of α-carbon (C-6 in **3** and **4**, C-11 in **4**) and the upfield shifts of β-carbon (C-5 & C-6Me in both **3** and **4**). By the application of the reported considerations with regard to *O*-methylation shift,[4,5] it was assigned that the methoxy group at 50.6 ppm in **3** is attached pseudo-axially at C-6 position and those at 50.6 and 60.8 ppm in **4** are located at C-6 and C-11 positions, respectively. Furthermore, the upfield shift of ^{13}C NMR signal of C-9 ($\Delta\delta_C$ −4.3 compared with that of **1**) and the IR peak of $\nu_{C=O}$ at 1703 cm^{-1} ($\nu_{C=O}$ 1690 cm^{-1} in **1** and **3**) in **4**, suggested the absence of the hydrogen bonding between 9-carbonyl and 6- and 11-hydroxyl groups.[4,6] Thus, the structure of **3** and **4** has been elucidated as 6-*O*-methyl-

and 6,11-di-*O*-methylerythromycin A, respectively.

On the other hand, product B was hydrogenated in the similar procedure as product A to give a white glass (38% yield). Crystallization from acetone gave colorless needles, mp 182~185°C. The structure of this compound was deduced from its spectral data to be 11-*O*-methylerythromycin A. Detailed description of the structure determination and *in vitro* antibiotic activity of this compound will be forthcoming.

6-*O*-Methylerythromycins (**3** and **4**) are far more acid-resistant. After 30-minute exposure of **1**, **3** and **4** to a dilute hydrogen chloride solution (pH 2), their retained biological activities against *Staphylococcus aureus* 209P were 0.5, 95 and 95%, respectively.

Table 2 shows the *in vitro* activity of 6-*O*-methylerythromycins A (**3** and **4**) compared to that of erythromycin against a variety of bacteria and *Mycoplasma* species. Especially, the activity of **3** is higher than that of erythromycin.

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