CHEMICAL MODIFICATION OF ERYTHROMYCINS

VI. STRUCTURE AND ANTIBACTERIAL ACTIVITY OF ACID DEGRADATION PRODUCTS OF 6-*O*-METHYLERYTHROMYCINS A

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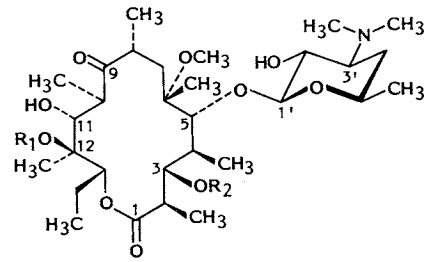
Erythromycin A, a useful macrolide antibiotic, is extremely unstable to acid and when administered orally undergoes dehydration *in vivo* to anhydroerythromycin A, an inactive 6,9; 9,12-spiroketal metabolite.[1] In preceding paper,[2] we have reported that 6-*O*-methylerythromycin A (**1**) was more stable to acid than erythromycin A due to the presence of 6-*O*-methyl group, which blocks the formation of 6,9; 9,12-spiroketal derivative.

Nevertheless, **1** loses gradually its antibacterial activity in dilute HCl solution. In present paper we describe the structure and antibacterial activity of the degradation products of **1** and its derivatives under acidic conditions.

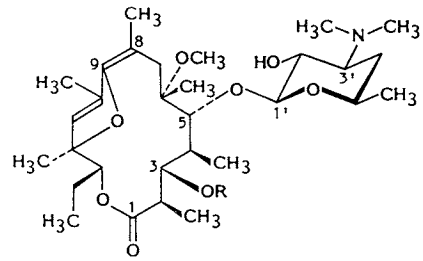
When **1** was allowed to stand in aqueous acid solution (0.2% HCl or 9% AcOH), a cleavage of the cladinosyl moiety proceeded gradually to yield **3** which was also reported as one of metabolites of **1** in human or animals.[3,4] Treatment of **1** and **3** with glacial AcOH gave the conjugated enol ether **5** (67%) and **6** (71%), respectively. When heated at 70°C in AcOH-pyridine (1:3), erythromycin A provided pseudoerythromycin A derivatives *via* translactonization between the 11-hydroxyl group and the lactone group;[5] compound **1** yielded **5** under the same condition. Treatment of **1** with 1% HCl-MeOH gave two isomers **6** (25%) and **7** (72%); **3** also gave both **6** (41%) and **7** (40%). 6,12-Di-*O*-methylerythromycin A (**2**)[6], however, provided the decladinosyl derivative **4** (75%) without dehydration in the aglycone ring.

Absence of the cladinose sugar in **3** was indicated by the absence of the corresponding absorptions in the ^1H and ^{13}C NMR spectra and by the mass spectrum with *m/z* 590 (M+H). The ^{13}C NMR spectrum of **3** also showed a δ value of 88.2 ppm for C-5 compared to 80.8 ppm in **1**, suggesting the presence of hydrogen bonding of the oxygen at C-5 with the free hydroxyl group at C-3 (Table 1).

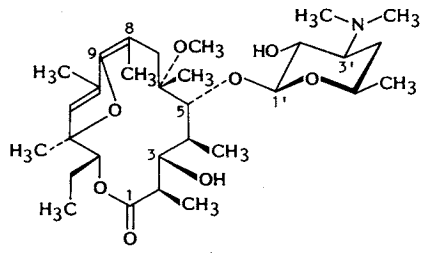
The ^{13}C NMR chemical shifts of **3** and **4** were essentially the same except for the presence of a new methoxy signal at 53.4 ppm in **4** with a significant



1 $R_1=H$ $R_2=$Cladinose
2 $R_1=CH_3$ $R_2=$Cladinose
3 $R_1=R_2=H$
4 $R_1=CH_3$ $R_2=H$



5 R=Cladinose
6 R=H



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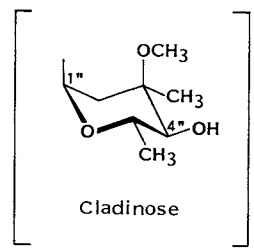

downfield shift of C-12 (δ_{Δ} +4.9 ppm) compared to that of **3** (Table 1).

HREI-MS and elemental analysis of both **6** and **7** are in agreement with the empirical formula $C_{30}H_{51}NO_8$. Their UV spectra showed strong absorptions due to the conjugated diene structure at 274 (ε_{max} 12,400) and 277 nm (ε_{max} 12,500). The ^{1}H NMR spectrum of **6** showed a peak (δ 5.82) due to the olefinic proton 11-H and two peaks (δ 1.92 and 2.04) due to methyl groups attached to the conjugated double bonds which were assigned to 8-CH_3 and 10-CH_3, respectively. Similarly, the ^{1}H NMR spectrum of **7** showed peaks due to 11-H, 8-CH_3 and 10-CH_3 at δ 5.74, 1.73 and 2.03, respectively.

The both ^{13}C NMR spectra of **6** and **7** showed resonances of four olefinic carbons attributed to C-8, C-9, C-10 and C-11 in the range of δ 101.4 to 154.5, with the downfield shifts of C-12 (δ_{Δ} +14.1 and +14.3 ppm in **6** and **7**, respectively) and 12-CH_3 (δ_{Δ} +8.5 and +6.7 ppm in **6** and **7**, respectively) compared to those of **3** (Table 1). In the NOE

Table 1. ^{13}C NMR chemical shifts of **3**~**7**.

Carbon	Chemical shift (δ, ppm)[a]					
	1	**3**	**4**	**5**	**6**	**7**
1	175.9	175.0	175.1	175.6	176.3	175.6
2	45.1	44.5	44.7	45.5	44.2	45.0
3	78.5	78.8	78.6	80.6	78.2	77.5
4	39.3	37.4	36.3	39.3	37.4	37.7
5	80.8	88.2	88.1	84.4	95.4	95.2
6	78.5	78.0	78.8	81.4	82.5	81.7
7	39.4	38.7	38.3	37.0	37.4	32.4
8	45.3	45.4	45.0	102.8	101.4	104.1
9	221.1	220.7	218.7	154.3	154.5	154.1
10	37.3	35.8	38.9	135.6	134.7	134.7
11	69.1	70.2	72.3	129.9	131.7	132.4
12	74.3	74.2	79.1	87.7	88.3	88.5
13	76.7	76.6	75.2	77.3	76.1	78.9
14	21.1	21.3	21.7	24.6	23.6	23.8
15	10.6	10.4	10.6	10.6	10.4	10.2
2-CH_3	16.0	15.1	15.3	15.7	15.2	16.0
4-CH_3	9.1	8.2	8.3	9.1	7.9	7.8
6-CH_3	19.8	18.7	19.0	25.8	20.1	21.1
8-CH_3	18.0	17.7	17.9	19.5	18.2	15.1
10-CH_3	12.3	12.6	11.5	16.7	16.6	16.6
12-CH_3	16.0	16.1	17.1	24.1	24.6	22.8
6-OCH_3	50.7	49.5	49.8	47.4	48.1	47.0
12-OCH_3	—	—	53.4	—	—	—
1′	102.9	106.6	106.5	103.9	107.0	107.2
2′	71.0	70.6	70.7	71.3	70.3	70.3
3′	65.6	65.6	65.8	65.5	65.3	65.5
4′	28.6	28.0	28.4	29.1	28.3	28.3
5′	68.8	69.7	70.2	68.8	69.5	69.5
3′-$N(CH_3)_2$	40.3	40.2	40.3	40.5	40.1	40.2
5′-CH_3	21.5	21.2	21.3	21.4	21.2	21.3
1″	96.1			96.1		
2″	34.9			35.0		
3″	72.7			72.6		
4″	78.0			78.1		
5″	65.8			65.0		
3″-CH_3	21.5			21.6		
5″-CH_3	18.7			18.4		
3″-OCH_3	49.5			49.4		

[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. Assignments were determined by 2D NMR techniques.

Table 2. *In vitro* antibacterial activity of acid degradation product (**5**) of 6-*O*-methylerythromycin A.

Organisms	MIC (μg/ml)		Organisms	MIC (μg/ml)	
	1	**5**		**1**	**5**
Bacillus subtilis ATCC 6633	0.05	0.78	*S. epidermidis* IID 866	0.10	1.56
Staphylococcus aureus 209P-JC	0.05	1.56	*Enterococcus faecalis* CSJ 1212	0.78	25
S. aureus Smith 4	0.10	3.13	*Micrococcus luteus* ATCC 9341	0.025	0.39
S. aureus Terajima	0.10	3.13	*Branhamella catarrhalis* ATCC 25238	0.10	1.56
S. aureus BB	0.10	3.13	*Escherichia coli* NIHJ JC-2	100	>100
S. aureus CSJ 1923	0.10	3.13	*E. coli* CSJ 1922	100	>100
S. aureus J-109	>100	>100	*E. coli* K-12	12.5	>100
S. aureus B1	>100	>100			
S. aureus C1	0.78	25			

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

difference spectra, strong NOE's were observed between 8-CH_3 and 10-CH_3 in **6**, and between 8-CH_3 and 5-H in **7**, establishing that the stereochemistry on the diene system for **6** and **7** was (8*Z*,10*Z*) and (8*E*,10*Z*), respectively. Further, the ^{13}C NMR chemical shifts of **5** have been established as shown in Table 1, indicating that **5** differs from **6** in having the cladinose moiety at the C-3 position.

The antibacterial activity of **3**~**7** was determined using the agar dilution method. Despite the pronounced structural change in the aglycone ring, **5** demonstrated activity 16 to 32-fold less potent than that of **1** (Table 2). On the other hand, the decladinosyl derivatives **4**, **6** and **7** exhibited no activity against all strains tested (MIC >100 μg/ml), suggesting that the presence of cladinose moiety is indispensable for the antibacterial activity of erythromycin.

Experimental

5-*O*-Desosaminyl-6-*O*-methylerythronolide A (**3**)

A solution of **1**[2] (2 g, 2.7 mmol) in 100 ml of 0.2% HCl was allowed to stand for 24 hours at ambient temperature. The reaction mixture was poured into satd Na_2CO_3 soln and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with brine, dried ($MgSO_4$), and evaporated *in vacuo* to afford a foam (1.96 g). The product was purified by silica gel column chromatography ($CHCl_3$-MeOH, 15:1) and by crystallization from ether to give 1.1 g (70%) of **3** as colorless crystals: MP 237~240°C; IR (KBr) cm^{-1} 1730, 1685; FAB-MS *m/z* 590 (M+H); ^1H NMR (200 MHz, $CDCl_3$), δ 2.97 (3H, s, 6-OCH_3), 2.27 (6H, s, $N(CH_3)_2$), 1.37 (3H, s, 6-CH_3); ^{13}C NMR: See Table 1.

5-*O*-Desosaminyl-6,12-di-*O*-methylerythronolide A (**4**)

A solution of **2**[6] (100 mg, 0.1 mmol) in 1% HCl-MeOH (1 ml) was allowed to stand for 1 day at ambient temperature. The mixture was diluted with EtOAc and washed with satd Na_2CO_3 soln and water. The organic layer was dried ($MgSO_4$) and evaporated to dryness *in vacuo*. The residue was purified by silica gel column chromatography (Me_2CO-hexane-triethylamine, 3:10:0.2) to give 59 mg (75%) of **4** which was crystallized from Me_2CO-hexane: MP 193~194°C; HREI-MS *m/z* 603.3984 (M, calcd *m/z* for $C_{31}H_{57}NO_{10}$: 603.3982); IR (KBr) cm^{-1} 3532, 1716, 1689; ^1H NMR (400 MHz, $CDCl_3$) δ 3.45 (3H, s, 12-OCH_3), 3.00 (3H, s, 6-OCH_3), 2.36 (6H, s, $N(CH_3)_2$), 1.37 (3H, s, 6-CH_3), 1.16 (3H, s, 12-CH_3). ^{13}C NMR: See Table 1.

(8*Z*,10*Z*)-8,9; 10,11-Dianhydro-6-*O*-methylerythromycin A 9,12-Hemiketal (**5**)

A solution of **1** (500 mg, 0.7 mmol) in glacial AcOH (2 ml) was allowed to stand for 4 days at ambient temperature. The reaction mixture was poured into water, basified (pH 10) using 2 N NaOH, and extracted with EtOAc. The EtOAc layer was washed with water, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (Me_2CO-hexane-triethylamine, 3:10:0.2) to give 320 mg (67%) of **5** as colorless foam: MP 123~127°C: FAB-MS *m/z* 712 (M+H); UV λ_{max}^{EtOH} nm (ε) 279 (10,500): IR (KBr) cm^{-1} 3440, 1730, 1650, 1625; $[\alpha]_D^{24}$ +26.7° (*c* 0.27, EtOH); ^1H NMR (200 MHz, $CDCl_3$) δ 5.69 (1H, q, *J*=1 Hz, 11-H), 3.28 (3H, s, 3″-OCH_3), 3.15 (3H, s, 6-OCH_3), 2.30 (6H, s, $N(CH_3)_2$), 2.05 (3H, s,

8-CH_3), 2.05 (3H, d, J=1 Hz, 10-CH_3), 1.32 (3H, s, 6-CH_3), 1.27 (3H, s, 12-CH_3). ^{13}C NMR: See Table 1.

(8*Z*,10*Z*)-5-*O*-Desosaminyl-8,9; 10,11-dianhydro-6-*O*-methylerythronolide A 9,12-Hemiketal (6)

Compound **3** (3 g, 5 mmol) was treated with AcOH (30 ml) as described for **5** to give 2 g (71%) of **6** which was crystallized from EtOAc: MP 198~200°C; HREI-MS m/z 553.3628 (M, calcd m/z for $C_{30}H_{51}NO_8$: 553.3615); UV λ_{max}^{EtOH} nm (ε) 274 (12,400): IR (KBr) cm^{-1} 3400, 1720, 1640, 1620; $[\alpha]_D^{24}$ +71.6° (c 0.5, EtOH); ^1H NMR (400 MHz, $CDCl_3$) δ 5.82 (1H, q, J=1 Hz, 11-H), 3.17 (3H, s, 6-OCH_3), 2.27 (6H, s, $N(CH_3)_2$), 2.04 (3H, d, J=1 Hz, 10-CH_3), 1.92 (3H, s, 8-CH_3), 1.44 (3H, s, 6-CH_3), 1.27 (3H, s, 12-CH_3); ^{13}C NMR: See Table 1. *Anal* Calcd for $C_{30}H_{51}NO_8$: C 65.07, H 9.28, N 2.53. Found: C 64.81, H 9.33, N 2.48.

(8*E*,10*Z*)-5-*O*-Desosaminyl-8,9; 10,11-dianhydro-6-*O*-methylerythronolide A 9,12-Hemiketal (7)

A solution of **3** (4 g, 6.8 mmol) in 1% HCl - MeOH (100 ml) was allowed to stand for 4 days at ambient temperature. One-half volume of MeOH was distilled off *in vacuo* and the resultant mixture was poured into water, basified (pH 10) using 2 N NaOH, and extracted with EtOAc. The EtOAc layer was washed with water, dried ($MgSO_4$), and evaporated to afford crystals. The resulting crystals were collected on a filter, washed with petroleum ether and crystallized from EtOAc to give 1.4 g (37%) of **7** as colorless crystals. The filtrate and washing were combined and evaporated *in vacuo*. The residue was chromatographed over silica gel column with MeOH - $CHCl_3$ (3:97) to give 1.6 g (41%) of **6** and 0.1 g (3%) of **7**. For compound **7**: MP 224~226°C; HREI-MS m/z 553.3608 (M, calcd m/z for $C_{30}H_{51}NO_8$: 553.3615); UV λ_{max}^{EtOH} nm (ε) 277 (12,500): IR (KBr) cm^{-1} 3440, 1730, 1645, 1620; $[\alpha]_D^{24}$ −8.8° (c 0.5, EtOH); ^1H NMR (400 MHz, $CDCl_3$) δ 5.74 (1H, q, J=1 Hz, 11-H), 3.15 (3H, s, 6-OCH_3), 2.25 (6H, s, $N(CH_3)_2$), 2.03 (3H, d, J=1 Hz, 10-CH_3), 1.73 (3H, s, 8-CH_3), 1.31 (3H, s, 6-CH_3), 1.31 (3H, s, 12-CH_3); ^{13}C NMR: See Table 1. *Anal* Calcd for $C_{30}H_{51}NO_8$; C 65.07, H 9.28, N 2.53. Found: C 64.95, H 9.46, N 2.53.

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