ACID DEGRADATION STUDIES OF 6-DEOXYERYTHROMYCIN A

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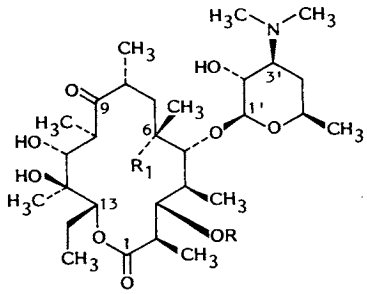
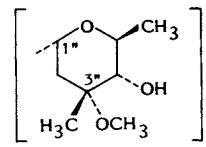
Erythromycin A (**1**), a well-established macrolide antibiotic, is unstable under acidic conditions. When administered orally, **1** is in equilibrium with its 8,9-anhydro-6,9-hemiketal and is simultaneously converted directly to the antibacterially inactive 6,9:9,12-spiroketal[1]. A number of research groups in recent years have prepared new derivatives of erythromycin A by modification of the functional groups which participate in the degradation reaction, namely the ketone at C-9, the hydroxyl group at C-6, the hydrogen at C-8 and the diol at C-11,12[2]. Recently a series of erythromycin derivatives lacking the hydroxyl group at C-6, therefore more stable to acid than erythromycin A, were isolated by one of us[3]. Nevertheless **2**, the most active compound in the series, slowly loses its antibacterial activity in dilute acidic solution. In this paper, we describe the structure and antibacterial activity of the acid degradation products of **2**.

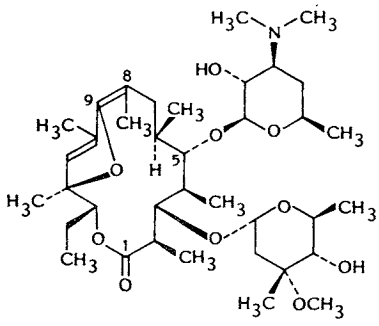
When **2** was treated with 10% AcOH, a loss of the cladinose moiety was observed to give **3**. Treatment of **2** with glacial AcOH at room temperature after four hours gave the conjugated enol ethers **4** and **5** in 83 and 7% isolated yields, respectively. The loss of the cladinose in **3** was indicated by the mass spectrum with *m/z* 560 (M+H) and by the absence of corresponding resonances in the ^1H and ^{13}C NMR spectrum. HREI-MS of **4** and **5** are in agreement with the empirical formula $C_{37}H_{63}NO_{10}$. The ^{13}C NMR spectra of **4** and **5** showed resonances of four olefinic carbons, C-8, C-9, C-10 and C-11 in the range δ 105.6 to 153.2. In the NOE difference spectra, strong NOE's were observed between 8-CH_3 and 10-CH_3 in **5** and between 8-CH_3 and 5-H and a weak NOE between 10-CH_3 and one of 7-H, establishing the stereochemistry of the diene system for **4** and **5** as (8*E*,10*Z*) and (8*Z*,10*Z*). In a similar study, MORIMOTO *et al.*[4] observed conjugated enol



1 R=Cladinose R_1=OH
2 R=Cladinose R_1=H
3 R=R_1=H



Cladinose



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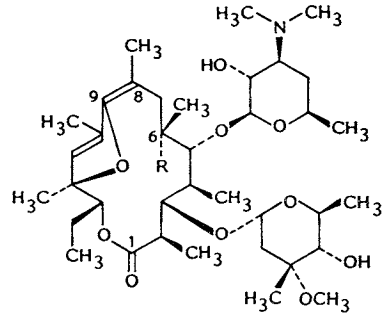

5 R=H
6 R=OCH_3

Table 1. ^{1}H NMR chemical shifts[a] of **2**, **4** and **5**.

Proton No.	Multiplicity	δ (ppm)		
		2	**4**	**5**
2-H	t	2.80	2.54	2.70
3-H	dd	3.65	3.65	3.70
4-H	ddq	1.60	1.89	1.86
5-H	d	3.48	3.22	3.49
6-H	m	1.36	2.01	2.17
7-H_{eq}	m	1.69	2.41	3.10
7-H_{ax}	m	1.53	1.70	1.41
8-H	ddq	2.63	—	—
9-H	—	—	—	—
10-H	ddq	3.07	—	—
11-H	t	3.40	5.63	5.67
12-H	—	—	—	—
13-H	dd	4.93	5.10	4.89
14-H	ddq	1.50, 1.93	1.45, 1.67	1.38, 1.61
15-H	t	0.89	0.89	0.87
16-H	d	1.19	1.13	1.13
17-H	d	1.17	0.96	1.14
18-H	d	1.17	1.04	1.11
19-H	d	1.18	2.00	1.69
20-H	d	1.15	2.02	2.15
21-H	s	1.10	1.28	1.31
1′-H	d	4.23	4.17	4.33
2′-H	dd	3.25	3.32	3.37
3′-H	ddd	2.48	2.55	2.77
4′-H_{ax}	ddd	1.25	1.28	1.36
4′-H_{eq}	ddd	1.67	1.69	—
5′-H	ddq	3.46	3.47	3.51
6′-H	d	1.24	1.23	1.25
3′-N(CH_3)$_2$	s	2.29	2.33	2.49
1″-H	d	4.88	4.80	4.88
2″-H_{ax}	dd	1.55	1.51	1.54
2″-H_{eq}	dd	2.36	2.32	2.35
4″-H	dd	3.00	2.99	3.00
5″-H	dq	3.92	3.95	3.89
6″-H	d	1.28	1.26	1.29
7″-H	s	1.12	1.21	1.20
3″-OCH_3	s	3.28	3.25	3.27

[a] δ Values in ppm from TMS, measured in $CDCl_3$ at 500 MHz as determined from ^{1}H-^{1}H 2D homonuclear shift correlated experiments.

ether **6** upon treatment of 6-*O*-methylerythromycin A with glacial AcOH. Under no conditions was a compound with the 8*E*,10*Z* stereochemistry and an intact cladinosyl substituent observed. Hence, the present study provides an opportunity to assess the effect of diene stereochemistry on antibacterial activity.

The antibacterial activity of **2**~**5** was determined by the agar dilution method using brain heart infusion agar. Despite the major structural change in the macrolactone ring, **4** demonstrated considerable activity, albeit 2- to 20-fold less potent than that of **2** (Table 2). On the other hand, **3** and **5** did not show any activity against all strains tested (MIC ≥ 100 μg/ml), suggesting that the presence of cladinose and the stereochemistry of the diene contribute to the antibacterial activity. Interestingly, and in contrast to **5**, compound **6** was reported[4] to have significant antibacterial activity.

Table 2. Antibacterial activity of 6-deoxyerythromycin A **2** and **4**.

Organism	MIC (μg/ml)	
	2	**4**
Staphylococcus aureus ATCC 6538P	1	6.2
S. aureus NCTC 10649	1	12.5
S. aureus CMX 553	0.5	12.5
S. epidermidis 3519	1	6.2
Micrococcus luteus ATCC 9341	0.06	0.78
Streptococcus agalactiae CMX 508	0.12	0.39
S. pyogenes EES61	0.25	0.39
Escherichia coli JUHL	>100	>100
E. coli SS	2	6.2

Experimental

Acid Hydrolysis of 6-Deoxyerythromycin A (2)

A solution of **2** (220 mg, 0.3 mmol) in 15 ml of glacial acetic acid was stirred for 4 hours at room temperature. After this time, the mixture was evaporated to dryness *in vacuo*, diluted with 100 ml of CH_2Cl_2 and washed once with 100 ml of 8% solution of sodium bicarbonate and twice with water (2 × 100 ml), then dried (Na_2SO_4). The solvent was evaporated *in vacuo* and the residue was purified by flash chromatography ($CHCl_3$-MeOH-NH_4OH, 95 : 5 : 0.5) to give 172 mg (83%) of **4**: MP 86~88°C: HREI-MS *m/z* 681.4458 (M, calcd *m/z* for $C_{37}H_{63}NO_{10}$: 681.4452); $[\alpha]_D^{25}$ +32.5° (*c* 1.01, $CHCl_3$); TLC-Rf 0.41; ^{1}H NMR: see Table 1. *Anal.* calcd for $C_{37}H_{63}NO_{10}$: C 65.17, H 9.31. Found C 65.20, H 9.30 and 14 mg (7%) of **5**: MP 93~94°C: HREI-MS *m/z* 681.4450 (M, calcd *m/z* for $C_{37}H_{63}NO_{10}$: 681.4452); $[\alpha]_D^{25}$ −16.5° (*c* 0.8, $CHCl_3$); TLC-Rf 0.53; ^{1}H NMR: see Table 1. *Anal.* calcd for $C_{37}H_{63}NO_{10}$: C 65.17, H. 9.31. Found C 65.10, H 9.29.

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References

1) CACHET, T.; G. VAN DEN MOOTER, R. HAUCHECORNE, C. VINCKIER & J. HOOGMARTENS: Decomposition kinetics of erythromycin A in acidic aqueous solutions. Int. J. Pharm. 55: 59~65, 1989
2) KIRST, H. A. & G. D. SIDES: New directions for macrolide antibiotics: structural modifications and *in vitro* activity. Antimicrob. Agents Chemother. 33: 1413~1418, 1989
3) WEBER, J. M.; J. O. LEUNG, S. J. SWANSON, K. B. IDLER & J. B. MCALPINE: An erythromycin derivative produced by targeted gene disruption in *Saccharopolyspora erythraea*. Science 252: 114~117, 1991
4) MORIMOTO, S.; Y. MISAWA, T. ASAKA, H. KONDOH & Y. WATANABE: Chemical modification of erythromycins. VI. Structure and antibacterial activity of acid degradation products of 6-*O*-methylerythromycins A. J. Antibiotics 43: 570~573, 1990