ERYTHROMYCIN A 11,12-METHYLENE ACETAL

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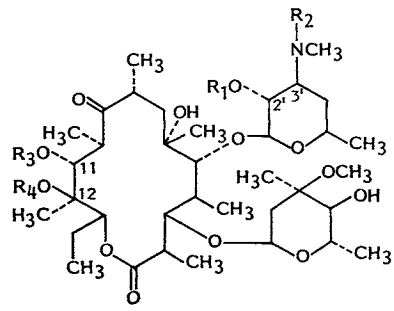
Erythromycin A 11,12-methylene acetal (**5**) and the corresponding 9-methoxime, 9-dihydro, and 8-hydroxy derivatives have been prepared and their antibacterial activities compared with those of erythromycin A and its 11,12-cyclic carbonate. The simple methylene acetal **5** showed excellent activity against Gram-positive organisms *in vitro*.

A number of 11,12-cyclic derivatives of the natural macrolide antibiotic erythromycin A (**1**) have been described. These include the 11,12-cyclic carbonate[1], the 11,12-cyclic sulfite[2], and the 11,12-cyclic amide acetals[3]. The best known of these derivatives is erythromycin A 11,12-carbonate (**3**), which as an antibacterial is about twice as active as erythromycin itself. The excellent *in vitro* activity of **3** attracted us to consider other 11,12-cyclic derivatives, and in this paper we describe the preparation and antibacterial activity of the cyclic methylene acetal **5** and some of its derivatives.

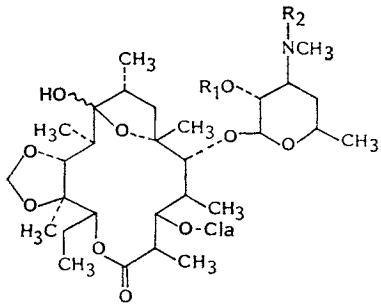
Since, for the preparation of **5**, we intended to use an alkylation-cyclisation process to introduce the methylene acetal group, we started the synthesis from the protected *N*-demethylerythromycin (**2**)[4]. Compound **2** was treated with chloroiodomethane and sodium hydride in *N*,*N*-dimethylformamide to give the desired 11,12-methylene acetal **4** and the 9,11-methylene acetal **8** (from the 6,9-hemiacetal tautomer of compound **2**). The usual[4] deprotection-reductive *N*-methylation sequence then converted compounds **4** and **8** into the free bases **5** and **9**. In fact, the *N*-protected 11,12-methylene acetal **4** existed not in the hydroxy-ketone form, but as a single 6,9-hemiacetal tautomer **6**† (as shown by ^{13}C NMR in $CDCl_3$). Likewise, the free base **5** when initially produced existed largely as a single 6,9-hemiacetal **7**, but this was converted, in methanol solution over several hours, into a mixture of the hydroxy-ketone **5** and two hemiacetals **7**. Similar behaviour had previously been noted for the 11,12-carbonate **3**[1].

For the preparation of the 8-hydroxy analogue of compound **5**, the *N*-protected enol ether **10** was converted into the 11,12-methylene acetal **11** by reaction with sodium hydride and chloroiodomethane. This was then treated with *m*-chloroperoxybenzoic acid in ethyl acetate-water[5] to give the 8-hydroxy derivative **12**. Deprotection and *N*-methylation of **12** then gave the 11,12-methylene acetal of 8-hydroxyerythromycin A (**13**). The 9-dihydro derivative of compound **5**

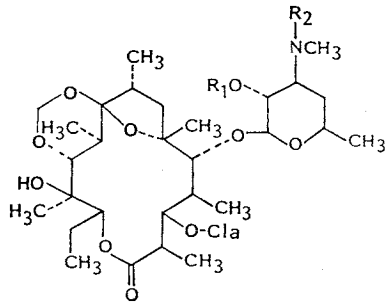


1	R_1=H	R_2=CH_3	R_3=R_4=H
2	R_1=R_2=$COOCH_2Ph$		R_3=R_4=H
3	R_1=H	R_2=CH_3	R_3+R_4=CO
4	R_1=R_2=$COOCH_2Ph$		R_3+R_4=CH_2
5	R_1=H	R_2=CH_3	R_3+R_4=CH_2

† The stereochemistry at C-9 is unknown.



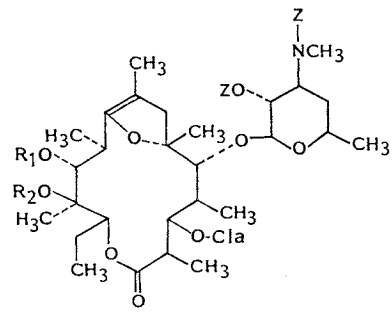
6 $R_1=R_2=COOCH_2Ph$
7 $R_1=H$ $R_2=CH_3$



8 $R_1=R_2=COOCH_2Ph$
9 $R_1=H$ $R_2=CH_3$

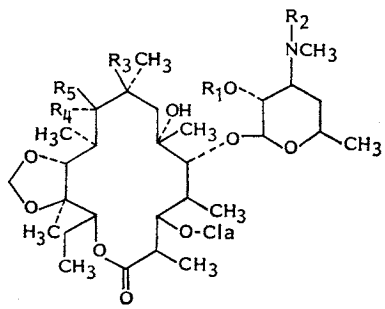
Cla: α-L-Cladinosyl

was prepared by way of reduction of **4** with sodium borohydride to give the 9-alcohol **14**, and then deprotection and *N*-methylation to give the desired 11,12-methylene acetal of 9-dihydroerythromycin A (**15**). Finally, the 9-methoxime of compound **5** was prepared from the *N*-protected methoxime **16**[6] by conversion into the methylene acetal **17** using sodium hydride and chloroiodomethane, and then deprotection and *N*-methylation to give the 11,12-methylene acetal of erythromycin A 9-methoxime (**18**).

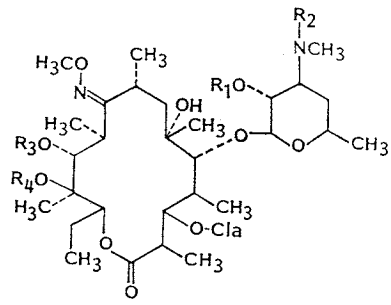


10 $R_1=R_2=H$ $Z=COOCH_2Ph$
11 $R_1+R_2=CH_2$ $Z=COOCH_2Ph$

The antibacterial activities of the new derivatives, compared with those of erythromycin (**1**) and its 11,12-carbonate **3**, are shown in Table 1. In general, erythromycin A 11,12-cyclic methylene acetal (**5**) showed excellent activity against Gram-positive organisms, being even more active than the cyclic carbonate **3** against *Staphylococcus aureus*. It was, however, less active than the carbonate **3** or erythromycin (**1**) against *Haemophilus influenzae*. The other derivatives showed less impressive activity. 9-Dihydroerythromycin A is about 10-fold less active than erythromycin A, and the same sort of relationship seems to hold for the 11,12-methylene acetals **5** and **15**. 8-Hydroxyerythromycin A is about



12 $R_1=R_2=COOCH_2Ph$ $R_3=OH$ $R_4+R_5=O$
13 $R_1=H$ $R_2=CH_3$ $R_3=OH$ $R_4+R_5=O$
14 $R_1=R_2=COOCH_2Ph$ $R_3=R_4=H$ $R_5=OH$
15 $R_1=H$ $R_2=CH_3$ $R_3=R_4=H$ $R_5=OH$



16 $R_1=R_2=COOCH_2Ph$ $R_3=R_4=H$
17 $R_1=R_2=COOCH_2Ph$ $R_3+R_4=CH_2$
18 $R_1=H$ $R_2=CH_3$ $R_3+R_4=CH_2$

Table 1. Antibacterial activity of erythromycin A (**1**), its 11,12-carbonate **3**, the 9,11-cyclic methylene derivative **9**, and the 11,12-cyclic methylene derivatives **5**, **13**, **15** and **18**.

Organism	MIC (μg/ml)						
	1	**3**	**5**	**9**	**13**	**15**	**18**
Staphylococcus aureus Oxford	0.25	0.13	0.03	2	1	1	0.25
S. aureus Russell	0.5	0.25	0.06	4	4	2	0.5
S. aureus T2	0.25	0.13	0.06	4	2	1	0.5
Streptococcus faecalis PAGE	1	0.5	0.5	16	4	4	4
S. pyogenes CN10A	0.03	$\leq$0.015	$\leq$0.015	$\leq$0.015	0.25	0.13	0.06
Streptococcus sp. 64/848C	0.03	0.03	$\leq$0.015	$\leq$0.015	0.25	0.13	0.06
Escherichia coli NCTC 10418	16	16	16	>128	>128	>128	64
Haemophilus influenzae Wy 21	2	1	4	64	64	16	4

Medium: Blood Agar Base +5% lysed horse blood, inoculum: $10^4 \sim 10^5$ cfu, incubation: 18 hours at 37°C.

half as active as erythromycin A, but in the 11,12-methylene acetal series the 8-hydroxy derivative **13** was considerably less active than its parent **5**. It seems that, as far as antibacterial activity is concerned, the 8-hydroxy group and the 11,12-methylene acetal function are not a good combination. Likewise, the methoxime **18** was less active than the simple methylene acetal **5**, but here the drop in activity was not so dramatic as with the introduction of the 8-hydroxy group. Except against certain *Streptococcus* organisms, the 9,11-cyclic methylene derivative **9** had rather poor activity.

Despite its good activity *in vitro*, the methylene acetal **5** was only marginally more active than erythromycin (**1**) in treating experimental infections in mice, and did not appear to merit further progression.

Experimental

MP's were determined using a Kofler hot-stage apparatus. Specific rotations were measured for solutions in $CHCl_3$. Electron impact mass spectra (EI-MS) were obtained on a VG ZAB 1F mass spectrometer at 8kV using 70 eV electrons and a source temperature of 200°C. The structures of all compounds were confirmed by ^1H and ^{13}C NMR spectra for solutions in $CDCl_3$.

Solutions were dried with anhydrous sodium sulfate and solvents were evaporated using a rotary evaporator with bath temperature below 30°C. Merck Silica gel 60 was used for column chromatography.

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-11-*O*,12-*O*-methyleneerythromycin A (**4**) and 2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-9-*O*,11-*O*-methyleneerythromycin A 6,9-Hemiacetal (**8**)

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethylerythromycin A (**2**)[4] (2.0 g) in dry DMF (15 ml) was treated with chloroiodomethane (2 ml) and the solution was cooled to 0°C and stirred while 50% sodium hydride dispersion (250 mg) was added in one portion. The mixture was stirred at 0°C with exclusion of moisture for 50 minutes, and was then allowed to warm to room temperature during 20 minutes. The mixture was diluted with EtOAc (100 ml) and was washed with water three times. The solution was dried, the solvent was removed, and the residue was chromatographed using EtOAc-hexane to give, in order of elution, compound **4** and compound **8**. Compound **4** was obtained as a white foam (150 mg): MP 92~97°C; $[\alpha]_D^{24}$ −56.8° (*c* 1.0).

Anal Calcd for $C_{53}H_{77}NO_{17}$: C 63.65, H 7.76, N 1.40.
Found: C 64.02, H 7.56, N 1.43.

Compound **8** was also obtained as a white foam (450 mg): MP 95~98°C; $[\alpha]_D^{23}$ −65.7° (*c* 1.0).

Anal Calcd for $C_{53}H_{77}NO_{17}$: C 63.65, H 7.76, N 1.40.
Found: C 64.01, H 7.94, N 1.43.

11-*O*,12-*O*-Methyleneerythromycin A (**5**)

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-11-*O*,12-*O*-methyleneerythromycin A (**4**) (150 mg) in EtOH (10 ml) and acetate buffer (pH 4.8) (1 ml) was shaken with 10% palladium-carbon (50 mg) under hydrogen (1 atm) for 25 minutes. 38% Formaldehyde solution (1 ml) was added and the hydrogenation was continued for 1.5 hours. The catalyst was removed by filtration and was washed with EtOH and water. The EtOH was evaporated from the filtrate under reduced pressure and the aqueous residue was brought to pH 10 using K_2CO_3 and extracted with EtOAc. The extract was washed with water and dried, and the solvent was removed to give the title compound **5** as a white foam (100 mg): MP 109~114°C; $[\alpha]_D^{24}$ −37.7° (*c* 1.0); EI-MS *m/z* 745 (M, found 745.4612; calcd for $C_{38}H_{67}NO_{13}$ 745.4596).

Anal Cacld for $C_{38}H_{67}NO_{13}$: C 61.19, H 9.05, N 1.89.
Found: C 60.45, H 9.11, N 1.82.

9-*O*,11-*O*-Methyleneerythromycin A 6,9-Hemiacetal (**9**)

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-9-*O*,11-*O*-methyleneerythromycin A 6,9-hemiacetal (**8**) (190 mg) was converted into the title compound **9** using the process described for the preparation of compound **5**. Compound **9** was obtained as a white foam (120 mg): MP 91~94°C; $[\alpha]_D^{25}$ −49.1° (*c* 1.0); EI-MS *m/z* 745 (M, found 745.4604; calcd for $C_{38}H_{67}NO_{13}$ 745.4596).

Anal Calcd for $C_{38}H_{67}NO_{13}$: C 61.19, H 9.05, N 1.89.
Found: C 61.12, H 9.21, N 1.75.

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-8,9-anhydro-11-*O*,12-*O*-methyleneerythromycin A 6,9-Hemiacetal (**11**)

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-8,9-anhydroerythromycin A 6,9-hemiacetal (**10**) (500 mg) was converted into the title compound **11** using the process described for the preparation of compounds **4** and **8**. Crystallisation from Et_2O - hexane gave compound **11** as colourless needles (240 mg): MP 190~191°C; $[\alpha]_D^{22}$ −48.2° (*c* 1.0); EI-MS *m/z* 981 (M, found 981.5103; calcd for $C_{53}H_{75}NO_{16}$ 981.5089).

Anal Calcd for $C_{53}H_{75}NO_{16}$: C 64.81, H 7.70, N 1.43.
Found: C 64.63, H 7.51, N 1.45.

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-8-hydroxy-11-*O*,12-*O*-methyleneerythromycin A (**12**)

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-8,9-anhydro-11-*O*,12-*O*-methyleneerythromycin A 6,9-hemiacetal (**11**) (240 mg) was dissolved in EtOAc (6 ml) and water (1.5 ml) was added to the solution. The mixture was stirred while *m*-chloroperoxybenzoic acid (55 mg) was added over 30 minutes, and then stirring was continued for 2 hours. The mixture was diluted with EtOAc (50 ml) and was washed with water, satd $NaHCO_3$, and water. The solution was dried, the solvent was removed, and the residue was chromatographed using EtOAc - hexane to give the title compound **12** as a colourless gum (220 mg). From Et_2O - hexane, **12** was obtained as colourless crystals: MP 210~212°C; $[\alpha]_D^{21}$ −49.0° (*c* 1.0).

Anal Calcd for $C_{53}H_{77}NO_{18}$: C 62.63, H 7.64, N 1.38.
Found: C 62.23, H 7.57, N 1.35.

8-Hydroxy-11-*O*,12-*O*-methyleneerythromycin A (**13**)

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-8-hydroxy-11-*O*,12-*O*-methyleneerythromycin A (**12**) (170 mg) was converted into the title compound **13** using the process described for the preparation of compound **5**. Compound **13** was obtained as a white foam (120 mg): MP 104~110°C; $[\alpha]_D^{22}$ −37.1° (*c* 1.1); EI-MS *m/z* 761 (M, found 761.4569; calcd for $C_{38}H_{67}NO_{14}$ 761.4562).

Anal Calcd for $C_{38}H_{67}NO_{14}$: C 59.90, H 8.86, N 1.84.
Found: C 60.01, H 9.06, N 1.84.

9-Dihydro-11-*O*,12-*O*-methyleneerythromycin A (**15**)

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethyl-11-*O*,12-*O*-methyleneerythromycin A (**4**) (180 mg) was dissolved in dry 1,2-dimethoxyethane (7 ml) and the solution was treated with sodium borohy-

dride (100 mg). The mixture was stirred at 55°C for 8 hours. The mixture was cooled to room temperature and acetic acid (0.2 ml) was added. After 5 minutes, the mixture was diluted with EtOAc and the solution was washed with water, satd $NaHCO_3$, and water. The solution was dried, the solvent was removed and the residue was chromatographed to give 2′-*O*,3′-*N*-bis(benzyloxycarbonyl)-*N*-demethyl-9-dihydro-11-*O*,12-*O*-methyleneerythromycin A (**14**) as a colourless gum (110 mg).

Compound **14** was then converted into the title compound **15** using the process described for the preparation of compound **5**. Compound **15** was obtained as a white foam (75 mg): MP 103~106°C; $[\alpha]_D^{25}$ −37.5° (*c* 1.0); EI-MS *m/z* 747 (M, found 747.4783; calcd for $C_{38}H_{69}NO_{13}$ 747.4768).

Anal Calcd for $C_{38}H_{69}NO_{13}$: C 61.02, H 9.30, N 1.87.
Found: C 61.03, H 9.25, N 1.84.

11-*O*,12-*O*-Methyleneerythromycin A 9-Methoxime (**18**)

2′-*O*,3′-*N*-Bis(benzyloxycarbonyl)-*N*-demethylerythromycin A 9-methoxime[6] (**16**) (300 mg) was converted into the 11-*O*,12-*O*-methylene derivative **17** using the process described for the preparation of compounds **4** and **8**. Compound **17** was obtained as a colourless gum (160 mg): EI-MS *m/z* 1,028 (M, found 1028.5473; calcd for $C_{54}H_{80}N_2O_{17}$ 1028.5461).

Compound **17** was converted into the title compound **18** using the process described for the preparation of compound **5**. Compound **18** was obtained as a white foam (120 mg): MP 94~99°C; $[\alpha]_D^{22}$ −45.1° (*c* 1.0); EI-MS *m/z* 774 (M, found 774.4862; calcd for $C_{39}H_{70}N_2O_{13}$ 774.4881).

Anal Calcd for $C_{39}H_{70}N_2O_{13}$: C 60.44, H 9.10, N 3.61.
Found: C 60.65, H 9.09, N 3.37.

Acknowledgements

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