ERYTHROMYCIN A OXIME 11,12-CARBONATE AND ITS OXIME ETHERS

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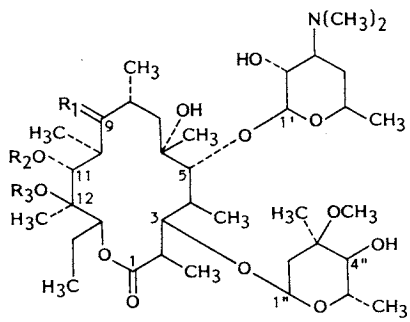
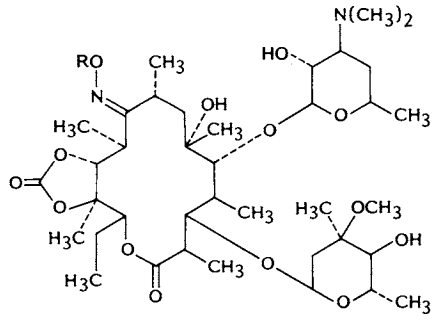
Erythromycin A oxime 11,12-carbonate (**5a**) and its oxime ethers **5b**~**5p** have been prepared and their antibacterial activities compared with those of erythromycin A (**1**) and its 11,12-carbonate **2**. The oxime **5a** and many of its oxime ether derivatives showed good activity *in vitro* against Gram-positive and the more permeable Gram-negative organisms, in some cases being even more active than the carbonate **2**.

Erythromycin A 11,12-carbonate (**2**), which is easily prepared from erythromycin A (**1**) and ethylene carbonate[1], shows excellent activity against Gram-positive organisms *in vitro*, being about twice as active as erythromycin itself. 11,12-Carbonates have also been described for various derivatives of erythromycin, including (9*S*)-9-dihydroerythromycin A[2], (9*S*)-erythromycylamine A[3], and (8*S*)-8-hydroxyerythromycin A[4], and like the carbonate **2**, some of these are quite active as antibacterials. We therefore became interested in extending this series to the 11,12-carbonates of erythromycin A oxime and its oxime ethers, in the hope that such compounds might have the good activity shown by **2** combined with the acid stability† of the 9-oximino derivatives. In this paper we describe the preparation and antibacterial activities of the oxime **5a** and the oxime ethers **5b**~**5p**††.

Our initial attempts to prepare the oxime **5a** and the methoxime **5b** by reaction of erythromycin A



1 $R_1=O$ $R_2=R_3=H$
2 $R_1=O$ $R_2+R_3=CO$
3 $R_1=NOH$ $R_2=R_3=H$
4 $R_1=NOCH_3$ $R_2=R_3=H$

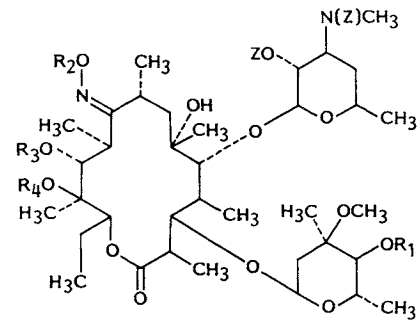


5a R=H
5b $R=CH_3$
5c~**5p** See Table 1

† Erythromycin A is very unstable to dilute mineral acid[8], and this property undoubtedly contributes to the rather poor pharmacokinetics of orally administered erythromycin base. Erythromycin A oxime (**3**) and its derivatives are much more stable to mineral acid[6].

†† A note on molecular structure is in order here. Although erythromycin A 11,12-carbonate is represented as the hydroxy-ketone **2**, in solution it exists largely as two tautomeric 6,9-hemiacetals[1]. This type of tautomerism is not observed for compounds **5a**~**5p**.

11,12-carbonate (**2**) with hydroxylamine and *O*-methylhydroxylamine, respectively, were unsuccessful. We therefore turned to the other obvious route to these compounds, namely the reaction of erythromycin A oxime (**3**) and methoxime (**4**) with ethylene carbonate; but these reactions also failed to give the desired compounds. In continuance of this approach, therefore, we investigated other reagents for introducing the 11,12-carbonate group, and found that, under the appropriate conditions, both phosgene and carbonyl diimidazole were well suited for this purpose.



6 $R_1=R_3=R_4=H$ $R_2=Z$
7 $R_1=Z$ $R_2=H$ $R_3+R_4=CO$
$Z=COOCH_2C_6H_5$

Thus, the protected oxime **6** was treated with pyridine and an excess of phosgene (4.4 equiv) in dichloromethane, and when all of **6** had reacted the mixture was treated with benzyl alcohol. These reactions introduced the 11,12-carbonate and 4″-*O*-benzyloxycarbonyl groups. Mild base hydrolysis then removed the *O*-benzyloxycarbonyl protection from the oxime group to give the 11,12-carbonate derivative **7** in 58% yield. The remaining benzyloxycarbonyl groups were removed by catalytic hydrogenation and the deprotected product was reductively *N*-methylated[5] to give the oxime 11,12-carbonate **5a** (66%).

For the preparation of **5b** we used erythromycin A methoxime (**4**) and carbonyl diimidazole. The methoxime **4** was treated with carbonyl diimidazole and potassium carbonate to give the 4″-*O*-imidazol-1-ylcarbonyl derivative, and this was then brought into reaction with sodium hydride and more carbonyl diimidazole to produce the 11,12-carbonate. After treating the reaction mixture with ethylene glycol (to remove the imidazol-1-ylcarbonyl moiety from the 4″-hydroxyl) the methoxime 11,12-carbonate **5b** was isolated in 92% yield. Most other oxime ether 11,12-carbonates, as detailed in Table 1, were prepared by one of two methods: *O*-Alkylation of the oxime 11,12-carbonate derivative **7**, followed by deprotection and reductive *N*-methylation; or reaction of an erythromycin oxime ether [6,7] with phosgene in the presence of pyridine. Compounds **5e** and **5h** were prepared by catalytic hydrogenation of the unsaturated ethers **5d** and **5g**, respectively.

The antibacterial activities of the new derivatives are compared with those of erythromycin A (**1**) and its 11,12-carbonate **2** in Table 2. In general, all the compounds showed good activity against Gram-positive organisms, and against *Haemophilus influenzae* and *Branhamella catarrhalis*. Thus, the oxime **5a** showed activity which was at least as good as that shown by erythromycin A 11,12-carbonate (**2**), and, except against *Escherichia coli*, this good activity was also shown by the methoxime **5b**†. For the other simple *O*-alkyl oximes **5c**～**5h**, increasing the size of the alkyl group beyond methyl resulted in a slight loss of activity, but these compounds still compared very favourably with erythromycin (**1**). Likewise, the substituted alkyl ethers **5i**～**5n** showed a good level of activity, with the hydroxyethyl ether **5m** and the dimethylaminoethyl ether **5n** being particularly active. The two carbonyl-containing ethers, **5o** and **5p**, were, on the whole, slightly less active than the other oxime derivatives.

In conclusion, erythromycin A oxime 11,12-carbonate (**5a**) and many of its oxime ether derivatives

† The good activity of **5b** compared with that of **2** is in contrast to the situation in the analogous 11,12-methylene acetal series[9]. In that series, the methoxime derivative was significantly less active than erythromycin A 11,12-methylene acetal.

Table 1. Preparation and physical properties of 11,12-carbonate oxime ethers.

Compound	R	Method[a]	Yield (%)	MP (°C)	$[\alpha]_D$ (°)[b]	Molecular formula[c]	M^+
5c	Et	A	52	Foam	−18.7	—	802.485
5d	$CH_2=CHCH_2$-	B	91	117~118	−17.9	$C_{41}H_{70}N_2O_{14}\cdot\frac{1}{2}H_2O$	—
5e	Pr	C	80	Foam	−18.9	—	816.499
5f	$(CH_3)_2CH$-	B	57	184~186	−17.8	$C_{41}H_{72}N_2O_{14}\cdot\frac{1}{2}H_2O$	816.501
5g	$CH_2=C(CH_3)CH_2$-	B	80	108~110	−16.3	$C_{42}H_{72}N_2O_{14}\cdot\frac{1}{2}H_2O$	—
5h	$(CH_3)_2CHCH_2$-	C	73	Foam	−13.8	—	830.514
5i	CH_3OCH_2-	B	69	113~115	−27.0	$C_{40}H_{70}N_2O_{15}$	—
5j	$EtOCH_2$-	B	77	113~114	−38.5	$C_{41}H_{72}N_2O_{15}$	—
5k	$CH_3OCH_2CH_2OCH_2$-	A	52	Foam	−31.4	—	862.505
5l	$CH_3OCH_2CH_2$-	B	53	120~121	−34.3	$C_{41}H_{72}N_2O_{15}\cdot\frac{1}{2}H_2O$	—
5m	$HOCH_2CH_2$-	A	42	Foam	−26.3	—	818.479
5n	$(CH_3)_2NCH_2CH_2$-	B	63	219~220	−39.6	$C_{42}H_{75}N_3O_{14}$	—
5o	CH_3OCOCH_2-	B	83	Foam	−35.0	—	846.473
5p	$(CH_3)_2NCOCH_2$-	A	40	175~176	−36.4	$C_{42}H_{73}N_3O_{15}\cdot\frac{1}{2}H_2O$	—

[a] See Experimental section. Method A: *O*-Alkylation of oxime **7**, followed by deprotection and *N*-methylation. Method B: Oxime ether + phosgene. Method C: Catalytic hydrogenation of **5d** or **5g**.
[b] Determined for 1% solution in $CHCl_3$ at ambient temperature (20~25°C).
[c] Satisfactory microanalytical data were obtained.
—: Not measured.

Table 2. *In vitro* antibacterial activity for erythromycin A (**1**), its 11,12-carbonate **2** and the 11,12-carbonate oxime derivatives **5a~5p**.

Compound	MIC (μg/ml)					
	S.a.	*S.p.*	*S.f.*	*H.i.*	*B.c.*	*E.c.*
1	0.25	<0.015	0.5	2	0.13	16
2	0.13	<0.015	0.25	1	0.13	16
5a	0.13	<0.015	0.25	0.5	0.13	8
5b	0.13	<0.015	0.25	0.5	0.06	32
5c	0.25	0.03	0.5	2	0.25	64
5d	0.25	0.03	0.5	1	0.13	64
5e	0.25	0.03	0.5	1	0.25	64
5f	0.25	0.03	0.25	1	0.13	32
5g	0.25	0.03	0.5	1	0.25	64
5h	0.25	0.03	0.5	1	0.25	128
5i	0.13	<0.015	0.25	1	0.06	16
5j	0.25	0.015	0.5	1	0.13	32
5k	0.25	<0.015	0.5	1	0.25	32
5l	0.13	<0.015	0.5	1.5	0.13	64
5m	0.13	<0.015	0.5	0.5	0.13	16
5n	0.25	<0.015	0.25	0.5	0.06	4
5o	0.25	0.015	2	2	0.13	64
5p	0.13	0.06	1	2	0.13	32

Medium: Blood Agar Base + 5% lysed horse blood. Inoculum: 10^5~10^6 cfu per 1 μl spot. Incubation: 18 hours at 37°C.

Organisms: *S.a.*, *Staphylococcus aureus* Oxford; *S.p.*, *Streptococcus pneumoniae* 1761; *S.f.*, *Streptococcus faecalis* I; *H.i.*, *Haemophilus influenzae* Wy 21; *B.c.*, *Branhamella catarrhalis* 1502; *E.c.*, *Escherichia coli* NCTC 10418.

showed very good activity *in vitro* against Gram-positive and the more permeable Gram-negative organisms, and in this respect compared very favourably with erythromycin A 11,12-carbonate (**2**). Several of these derivatives were also superior to **2** in treating experimental infections in mice, and this will be the subject

of a future publication. Unfortunately, further evaluation of one of the more promising of these derivatives (**5f**) revealed a poor therapeutic ratio for this compound, and consequently further progression did not appear to be warranted.

Experimental

MP's were determined using a Köfler hot-stage apparatus. IR spectra and specific rotations were measured for solutions in $CHCl_3$. Electron impact (EI)-MS were determined using a VG ZAB 1F mass spectrometer operating at 8 kV with 70 eV electrons and a source temperature of 200°C. Fast atom bombardment (FAB)-MS were recorded on the same instrument operating at 6 kV accelerating voltage with Xe atoms as the collision beam accelerated to 8 kV, and using 3-nitrobenzyl alcohol and sodium acetate as the matrix. The structures of all compounds were confirmed by ^1H and/or ^{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.

(9*E*)-3′-*N*,2′-*O*,4″-*O*-Tris(benzyloxycarbonyl)-*N*-demethylerythromycin A Oxime 11,12-Carbonate (**7**)

3′-*N*,2′-*O*-Bis(benzyloxycarbonyl)-*N*-demethylerythromycin A 9-(*O*-benzyloxycarbonyl)oxime (**6**) (1.14 g) in dry dichloromethane (10 ml) was treated with pyridine (0.8 ml). The solution was cooled to 0°C and phosgene (3.5 ml of a 12.5% w/w solution in toluene) was added with stirring. The solution was warmed to room temperature and kept for 100 minutes. Benzyl alcohol (0.8 ml) was added and the solution was kept for a further 1 hour. The solution was diluted with $CHCl_3$, and was washed with water, dilute citric acid, and satd $NaHCO_3$. The solution was dried, the solvent was removed, and the resulting residue was dissolved in MeOH - water - Et_3N (90 : 10 : 4, 30 ml). After 4 hours, the mixture was diluted with EtOAc and washed with water, dilute citric acid, and satd $NaHCO_3$. The solution was dried, the solvent was removed, and the residue was chromatographed using EtOAc - hexane (1 : 2) to give the carbonate **7** as a white foam (0.68 g, 58%). Crystallisation from MeOH - water gave colourless crystals: MP 125~128°C; FAB-MS *m/z* 1,185 (MNa^+).

Anal Calcd for $C_{61}H_{82}N_2O_{20}$: C 62.98, H 7.10, N 2.41.
Found: C 62.91, H 7.19, N 2.39.

(9*E*)-Erythromycin A Oxime 11,12-Carbonate (**5a**)

(9*E*)-3′-*N*,2′-*O*,4″-*O*-Tris(benzyloxycarbonyl)-*N*-demethylerythromycin A oxime 11,12-carbonate (**7**) (0.5 g) in EtOH (45 ml) and acetate buffer (pH 4.8, 0.73 M, 5 ml) was shaken with 10% palladium - carbon (0.1 g) under hydrogen (1 atmosphere) for 30 minutes. 37% Formaldehyde (0.5 ml) was added and hydrogenation was continued for 1 hour. The catalyst was removed by filtration and was washed with EtOH and water. The EtOH was removed from the filtrate under reduced pressure, the aqueous residue was brought to pH 10 using K_2CO_3, and the solution was extracted with EtOAc. The organic solution was washed with water and dried. The solvent was removed and the residue was chromatographed using 35% aq NH_3 - MeOH - CH_2Cl_2 (0.75 : 7.5 : 92) to give the oxime 11,12-carbonate **5a** as a white foam (0.22 g, 66%): $[\alpha]_D^{22}$ −24.3° (*c* 1.0); IR ν_{max} cm^{-1} 3550, 1800, 1730, 1600, 1450; EI-MS *m/z* 774 (M, found 774.4537; calcd for $C_{38}H_{66}N_2O_{14}$ 774.4514).

(9*E*)-Erythromycin A Methoxime 11,12-Carbonate (**5b**)

(9*E*)-Erythromycin A methoxime (**4**) (600 mg) in dry THF (6 ml) was treated with powdered potassium carbonate (600 mg) and carbonyl 1,1′-diimidazole (500 mg), and the mixture was stirred for 1.5 hours. The mixture was diluted with EtOAc (50 ml) and was washed with water (3 × 30 ml). The solution was dried and the solvent was removed to give (9*E*)-4″-*O*-(imidazol-1-ylcarbonyl)erythromycin A methoxime as a white foam. The foam was dissolved in dry THF (5 ml) and the solution was treated with carbonyl 1,1′-diimidazole (500 mg) and sodium hydride (50% dispersion in oil, 80 mg). The mixture was stirred at 60°C for 20 minutes. The mixture was cooled to room temperature, ethylene glycol (1 ml) was added, and stirring was continued for 30 minutes. The mixture was diluted with EtOAc (50 ml) and was washed with

water (3 × 30 ml). The solution was dried, the solvent was removed, and the residue was chromatographed using 35% aq NH_3 - MeOH - CH_2Cl_2 (1 : 9 : 90) to give the methoxime 11,12-carbonate **5b** as a white solid (570 mg, 92%). Crystallisation from CH_2Cl_2 - hexane gave colourless prisms: MP 230 ~ 231°C; $[\alpha]_D^{20}$ −20.1° (*c* 1.0); IR ν_{max} cm^{-1} 3550, 3450, 1795, 1730; EI-MS *m/z* 788 (M, found 788.4657; calcd for $C_{39}H_{68}N_2O_{14}$ 788.4671).

Anal Calcd for $C_{39}H_{68}N_2O_{14}$: C 59.37, H 8.69, N 3.55.
Found: C 59.59, H 8.82, N 3.52.

(9*E*)-Erythromycin A Ethoxime 11,12-Carbonate (**5c**)

Tetrabutylammonium hydroxide (25% w/v in MeOH, 0.2 ml) was added to a solution of (9*E*)-3′-*N*,2′-*O*,4″-*O*-tris(benzyloxycarbonyl)-*N*-demethylerythromycin A oxime 11,12-carbonate (**7**) (230 mg) in dry THF (5 ml) under nitrogen. After 5 minutes, ethyl iodide (0.1 ml) was added and the mixture was stirred for 30 minutes. The mixture was diluted with diethyl ether and filtered. The filtrate was washed with satd NaCl. The solution was dried, the solvent was removed, and the residue was chromatographed using EtOAc - hexane (1 : 3) to give (9*E*)-3′-*N*,2′-*O*,4″-*O*-tris(benzyloxycarbonyl)-*N*-demethylerythromycin A ethoxime 11,12-carbonate as a colourless gum (143 mg, 61%).

The above product (120 mg) was converted into the title compound using the process for the conversion of **7** into **5a**. The ethoxime 11,12-carbonate **5c** was obtained as a white foam (70 mg, 86%): $[\alpha]_D^{22}$ −18.7° (*c* 1.0); IR ν_{max} cm^{-1} 1795, 1735; EI-MS *m/z* 802 (M, found 802.4850; calcd for $C_{40}H_{70}N_2O_{14}$ 802.4827).

Compounds **5k**, **5m**, and **5p** were prepared similarly.

(9*E*)-Erythromycin A *O*-(Prop-2-enyl)oxime 11,12-Carbonate (**5d**)

(9*E*)-Erythromycin A *O*-(prop-2-enyl)oxime (400 mg) in CH_2Cl_2 (10 ml) was stirred at 0°C under nitrogen while pyridine (0.4 ml) and then phosgene (2.2 ml of a 12.5% w/w solution in toluene) were added. The mixture was stirred at 0°C for 90 minutes. Water (5 ml) was added and stirring was continued at 0°C for 10 minutes. The layers were separated and the organic layer was washed with satd NaCl. The solution was dried, the solvent was removed, and the residue was chromatographed using 35% aq NH_3 - MeOH - Et_2O (0.5 : 5 : 95) to give the 11,12-carbonate **5d** as a white foam (380 mg, 91%). Crystallisation from Me_2CO - water gave colourless crystals: MP 117 ~ 118°C; $[\alpha]_D^{22}$ −17.9° (*c* 1.0); IR ν_{max} cm^{-1} 1795, 1735; FAB-MS *m/z* 837 (MNa^+).

Anal Calcd for $C_{41}H_{70}N_2O_{14} \cdot \frac{1}{2}H_2O$: C 59.78, H 8.51, N 3.40.
Found: C 59.89, H 8.54, N 3.21.

Compounds **5f**, **5g**, **5i**, **5j**, **5l**, **5n**, and **5o** were prepared similarly.

(9*E*)-Erythromycin A *O*-(Propyl)oxime 11,12-Carbonate (**5e**)

(9*E*)-Erythromycin A *O*-(prop-2-enyl)oxime 11,12-carbonate (**5d**) (200 mg) in EtOH (15 ml) was shaken with 10% palladium - carbon (50 mg) under hydrogen (1 atmosphere) for 3 hours. The catalyst was removed by filtration and was washed with EtOH. The solvent was removed from the filtrate and the resulting residue was chromatographed using 35% aq NH_3 - MeOH - CH_2Cl_2 (1 : 9 : 90) to give the 11,12-carbonate **5e** as a white foam (160 mg, 80%): $[\alpha]_D^{20}$ −18.9° (*c* 1.0); IR ν_{max} cm^{-1} 1795, 1740; EI-MS *m/z* 816 (M, found 816.4992; calcd for $C_{41}H_{72}N_2O_{14}$ 816.4984).

Compound **5h** was similarly prepared from **5g**.

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