CHEMICAL MODIFICATION OF ERYTHROMYCINS

V. CYCLIC CARBONATES OF 8-HYDROXYERYTHROMYCIN A

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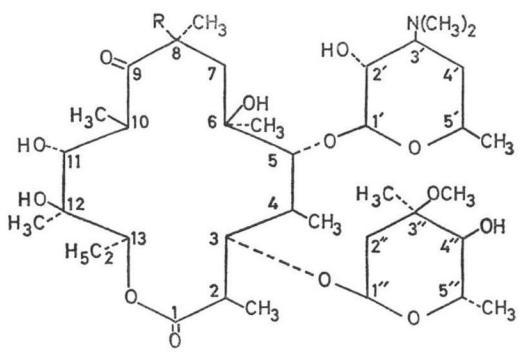
By treatment of 8-hydroxyerythromycin A in an aprotic solvent with ethylene carbonate in the presence of K_2CO_3, two cyclic carbonates of 8-hydroxyerythromycin A with molecular formulae of $C_{38}H_{65}NO_{15}$ and $C_{39}H_{63}NO_{16}$, respectively, were obtained. Analytical, spectral and chemical data indicated their structures to be the 11,12-cyclic carbonate of 8-hydroxyerythromycin A and the 8,9; 11,12-dicyclic dicarbonate of 8-hydroxyerythromycin A 6[9]-hemiketal, respectively. The respective compounds have antibacterial activities against *Bacillus pumilus* (in erythromycin A units) corresponding to 500 μg/mg and 1,250 μg/mg. Similar treatment of the methyl[9] 6-ketal of 8-hydroxyerythromycin A yields the 11,12-cyclic carbonate. Acid hydrolysis of the latter is another route providing the 11,12-cyclic carbonate of 8-hydroxyerythromycin A, mentioned above.

In a previous paper[1], the synthesis and properties of the semisynthetic antibiotic 8-hydroxyerythromycin A (**1**) have been reported. This compound is more stable to acids but half as active as erythromycin A (**2**) against *Bacillus pumilus*. In 1968 H.W. MURPHY, V.C. STEPHENS and J.W. CONINE obtained the 9,11-cyclic carbonate of erythromycin A 6[9]-hemiketal[2] which is 2.5 times more active than the parent antibiotic (**2**). Thus it was of interest to investigate the formation of cyclic carbonates of 8-hydroxyerythromycin A (**1**).

When 8-hydroxyerythromycin A (**1**) is treated for a short time (half an hour) in a benzene, ethyl acetate or dimethyl carbonate solution with ethylene carbonate in the presence of K_2CO_3 at 80°C, the main product is the 11,12-cyclic carbonate of 8-hydroxyerythromycin A (**3**). The molecular ion 775 is in agreement with the calculated molecular weight 775.9 for $C_{38}H_{65}NO_{15}$. The IR spectrum shows a prominent maximum of the carbonate group at 1800 cm^{-1}. Compound **3** treated with an excess of acetic anhydride in pyridine solution gave the diacetate. Both acetate groups were located in the sugar moieties; methanolysis of this diacetate in the presence of *p*-toluenesulphonic acid gave methyl acetylcladinoside. The diacetate of **3** is less basic (pK_a 6.50 in 66 % methanol) than the parent compound **3** (pK_a 8.2 in 66 % methanol), an observation compatible with the presence of an acetate group at the alcohol function of desosamine. Undoubtedly, if the C11-secondary hydroxyl group were not engaged

Chart 1



1 R=OH
2 R=H

in the cyclic carbonate ring, the acetylation would give a triacetate.

The IR and UV spectra of the carbonate of 8-hydroxyerythromycin A **3** lack the maxima corresponding to a ketone group at C9, indicating the compound to be in the ^{9}6-hemiketal form.

Similarly, the reaction of 8-hydroxyerythromycin A methyl ^{9}6-ketal with ethylene carbonate in the presence of K_2CO_3 yielded the 11,12-cyclic carbonate of the 8-hydroxyerythromycin A methyl ^{9}6-ketal **4**. The IR spectrum revealed the presence of a carbonate group, 1790 cm^{-1}, and the NMR spectrum showed two OCH_3 groups at δ3.35 and δ3.50. Acetylation of **4** provided a diacetate with both acetate groups in the sugar moieties.

Acid hydrolysis of **4** produced the 11,12-cyclic carbonate of 8-hydroxyerythromycin A **3**, thereby confirming the structure of the latter.

The monocyclic carbonate of 8-hydroxyerythromycin A obtained by acid hydrolysis of the methyl ^{9}6-ketal **4** has a higher content of keto form than that obtained by the direct esterification of 8-hydroxyerythromycin A. For this reason they differ in their optical rotations $[\alpha]_D^{26}$ $-36\pm1°$ and $[\alpha]_D^{26}$ $-41.5\pm1°$, in melting points 149 ~ 152°C and 150 ~ 152°C (after solidifying second melting points are 215~221°C and 219~221°C), and in the ultraviolet spectra $-\varepsilon$ 20 at 279 nm and a negligible absorption at about 280 nm, respectively. The two substances are identical in their IR and NMR spectra, in TLC, as well as in IR, NMR and TLC of their diacetates. Their antibacterial activities against *Bacillus pumilus* are also identical, namely 500 μg/mg.

Chart 2

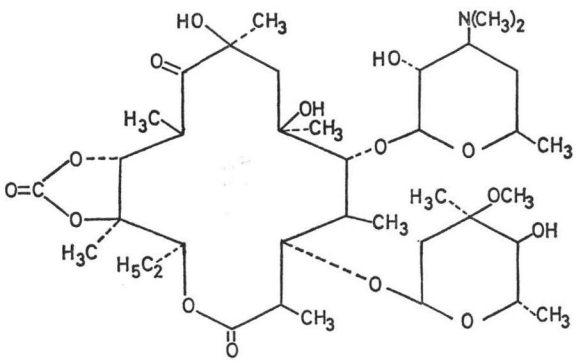


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Chart 3

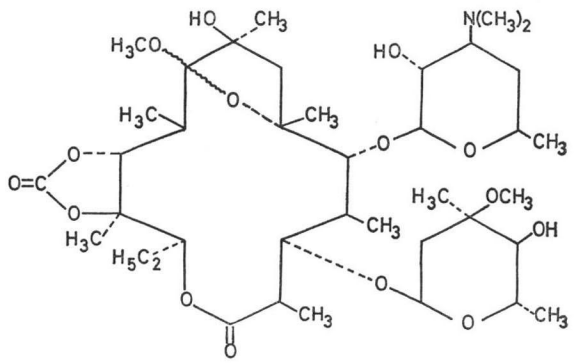

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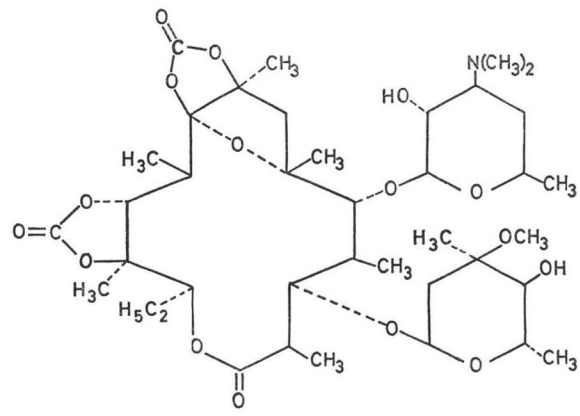
Chart 4



5

Further reaction of the carbonate **3**, obtained by the direct method or from **4**, with ethylene carbonate gave a dicarbonate of 8-hydroxyerythromycin A. This dicarbonate, acetylated with an excess of acetic anhydride and pyridine, formed the diacetate which contained no hydroxyl groups (IR spectrum) and the two acetate groups were located in the sugar moieties. This proves the C8–C9 position of the second carbonate ring. These data correspond to structure **5**,

the second carbonate ring formation being possible only if the new asymmetric center possesses R configuration.

The same compound **5** was obtained by a direct reaction of 8-hydroxyerythromycin A with ethylene carbonate in the presence of K_2CO_3, after 16 hours heating at 80°C in a benzene, ethyl acetate or dimethyl carbonate solution.

When the various 8-hydroxyerythromycin A carbonates were treated with an equivalent amount of acetic anhydride in the presence of pyridine, the corresponding 2′-monoacetates were obtained.

Both cyclic carbonates **3** and **5** have antibacterial activities against *Bacillus pumilus*, respectively, 500 μg/mg and 1,250 μg/mg (cylinder method). The latter compound is more active than the parent 8-hydroxyerythromycin A (**1**). So, the original concept of this work is confirmed.

Table 1. Antibacterial spectra of the 8-hydroxyerythromycin A monocarbonate **3** and dicarbonate **5** (*in vitro*).

Strain	Minimum inhibitory concentration (μg/ml)	
	3	**5**
Staphylococcus aureus FDA 209 P	1.95	0.2
Staphylococcus aureus penicillin resist.	1.95	3.13
Enterococcus 93	250	0.39
Escherichia coli 466	>1,000	>400
Proteus OX_{22}	>1,000	>400
Salmonella paratyphi A	>1,000	>400
Klebsiella pneumoniae 559	>1,000	>400
Shigella shigae	>1,000	400
Bacillus cereus ATCC	1.95	1.56
Sarcina lutea	1.95	<0.2
Bacillus subtilis 729	0.97	<0.2

Antibacterial spectra of the carbonates **3** and **5** are shown in Table 1.

Experimental

8-Hydroxyerythromycin A and its methyl [9]6-ketal were obtained by methods described in the literature[1]. For TLC, Kieselgel (Serva) and Kieselguhr (Merck) were employed; the plates were impregnated with formamide[3]. IR spectra were recorded on Unicam SP-200, and UV spectra on a Unicam SP-700 spectrophotometer. The NMR spectra were obtained on Jeol JNM-4H-100, in $CDCl_3$ solution with TMS as internal standard; the chemical shifts are reported on a δ scale (TMS=0 ppm).

(**1**) 11,12-Cyclic carbonate of 8-hydroxyerythromycin A **3**.

To dry 8-hydroxyerythromycin A (100 mg) in ethyl acetate (1 ml) 50 mg of dry K_2CO_3 and 100 mg of ethylene carbonate were added. The mixture was stirred and heated at 80°C for half an hour. After evaporation of solvent, the residue was washed twice with 0.25 ml portions of water. The dry solid was dissolved in ethyl ether (20 ml), filtered, and evaporated to a volume of 1 ml. Eighty mg (77.6 %) of carbonate **3** crystallized, m.p. 150~152°C. $[\alpha]_D^{28}$ −41.5 ±1° (*c* 1, methanol). pK_a 8.2±0.05 (66 % methanol), pK_a 8.55±0.05 (66 % DMF). IR spectrum: 3560 and 3540 (OH): 1800 (CO of carbonate), 1730 cm^{-1} (CO of lactone). NMR spectrum: 1.64 (s,3H)—CH_3 at C8; 2.34 (s,6H)—$N(CH_3)_2$; 3.34 (s,3H)—CH_3O. Molecular ion 775.

Anal. Calcd. for $C_{38}H_{65}NO_{13}$ (775.90): C 58.82, H 8.44, N 1.80 %
Found: C 58.64, H 8.42, N 1.80 %

TLC: ethanol-methylene chloride-ethyl ether-ligroin (b.p. 60°C), 5:35:30:30, Rf 0.5. One mole of the compound **3** used up 2.0 moles of $NaIO_4$ during 1 hour.[4]

(**2**) 2′-Acetate of 11,12-cyclic carbonate of 8-hydroxyerythromycin A.

Carbonate **3** (114 mg) in anhydrous pyridine (1 ml) was treated with 18 mg of acetic anhydride and left for 1 day at room temperature. After evaporation under reduced pressure, the residue was treated with water (2 ml) and made basic with $NaHCO_3$. The mixture was extracted twice with methylene chloride (10 ml altogether). The solvent was removed, an addition of ethanol

(1 ml) was made and then 90 mg (75 %) of acetate crystallized, m.p. 152~153°C. pK_a 6.50±0.05 (66 % methanol). IR spectrum ($CHCl_3$): 3550 (OH), 1800 (CO of carbonate), 1735 (CO of lactone and acetate), 1240 cm^{-1} (CH_3COO). NMR spectrum: 1.63 (s,3H)—CH_3 at C8; 2.12 (s,3H)—CH_3COO; 2.32 (s,6H)—$N(CH_3)_2$; 3.36 (s,3H)—CH_3O.

Anal. Calcd. for $C_{40}H_{67}NO_{16}$ (817.94): C 58.73, H 8.26, N 1.71 %
Found: C 58.60, H 8.20, N 1.70 %

TLC: ethanol-methylene chloride-ligroin (b.p. 60°C), 5:35:60, Rf 0.40.

(3) 2′,4″-Diacetate of 11,12-cyclic carbonate of 8-hydroxyerythromycin A.

Monocarbonate **3** (150 mg) in 0.5 ml of acetic anhydride and pyridine (1:1) was left for 1 day at room temperature. After evaporation of solvent under reduced pressure, the residue was treated with water (1 ml) and made basic with $NaHCO_3$. 120 mg (72 %) of the diacetate precipitated, m.p. 144~147°C. pK_a 6.50±0.05 (66 % methanol). IR spectrum ($CHCl_3$): 3570 (OH), 1800 (CO of carbonate), 1735 (CO of lactone and acetate), 1240 cm^{-1} (CH_3COO). NMR spectrum: 1.65 (s,3H)—CH_3 at C8; 2.10 (s,3H) and 2.21 (s,3H)—$2CH_3COO$; 2.34 (s,6H)—$N(CH_3)_2$; 3.40 (s,3H)—CH_3O.

Anal. Calcd. for $C_{42}H_{69}NO_{17}$ (859.98): C 58.65, H 8.09, N 1.53 %
Found: C 58.36, H 8.06, N 1.59 %

TLC: ethanol-benzene-ligroin (b.p. 60°C), 5:45:50, Rf 0.55.

This diacetate was dissolved in methanol with addition of *p*-toluenesulphonic acid and refluxed for 3 minutes. The formation of methyl acetylcladinoside was demonstrated by TLC on Kieselgel in system benzene-ethyl ether, 1:1. In this system cladinose, methyl cladinoside and methyl acetylcladinoside can be distinguished.

(4) 11,12-Cyclic carbonate of 8-hydroxyerythromycin methyl ^{9}6-ketal **4**.

To methyl ^{9}6-ketal of 8-hydroxyerythromycin A (1.6 g) in ethyl acetate (20 ml) ethylene carbonate (4 ml) and K_2CO_3 (0.8 g) were added. The mixture was stirred 16 hours at 90°C. The solvent was removed and the residue, washed twice with water and dried by distillation with benzene, was dissolved in ethyl ether (100 ml) and evaporated to a volume of 5 ml. The crystalline carbonate of 8-hydroxyerythromycin A methyl ^{9}6-ketal was filtered off and washed with some ethyl ether and ligroin mixture to give 1.2 g (73 %), m.p. 185~187°C. $[\alpha]_D^{26}$ −51±1° (*c* 1, methanol). pK_a 8.60±0.05 (66 % DMF). IR spectrum: 3550 (OH), 1970 (CO of carbonate), 1730 cm^{-1} (CO of lactone). NMR spectrum: 1.57 (s,3H)—CH_3 at C8; 2.35 (s,6H)—$N(CH_3)_2$; 3.35 (s,3H) and 3.50 (s,3H)—$2CH_3O$.

Anal. Calcd. for $C_{39}H_{67}NO_{15}$ (789.93): C 59.29, H 8.55, N 1.77 %
Found: C 59.39, H 8.77, N 1.73 %

TLC: ethanol-methylene chloride-ligroin (b.p. 60°C), 5:45:50, Rf 0.95.

(5) 2′,4″-Diacetate of 11,12-cyclic carbonate of 8-hydroxyerythromycin A methyl ^{9}6-ketal.

Carbonate **4** (200 mg) in 0.5 ml of acetic anhydride and pyridine (1:1) was left for 1 day at room temperature. After evaporation of solvent under reduced pressure, the residue was treated with water (1 ml) and made basic with $NaHCO_3$. 172 mg (80 %) of the diacetate precipitated, m.p. 131~133°C. pK_a 6.70±0.05 (66 % DMF). NMR spectrum: 1.55 (s,3H)—CH_3 at C8; 2.10 (s,3H) and 2.20 (s,3H)—$2CH_3COO$; 2.30 (s,6H)—$N(CH_3)_2$; 3.40 (s,3H) and 3.47 (s,3H)—$2CH_3O$.

Anal. Calcd. for $C_{42}H_{73}NO_{16}$ (848.01): C 59.48, H 8.68, N 1.65 %
Found: C 59.43, H 8.43, N 1.60 %

(6) 8,9;11,12-Dicyclic dicarbonate of 8-hydroxyerythromycin A 6^9-hemiketal **5**.

To dry 8-hydroxyerythromycin A (100 mg) or its monocarbonate **3** (103 mg) in ethyl acetate (1 ml) 50 mg of dry K_2CO_3 and 100 mg of ethylene carbonate were added. The mixture was stirred and heated at 80°C for 16 hours. After evaporation of solvent, the residue, washed with water (5 ml) and dried, was dissolved in methylene chloride (1 ml) and the solvent removed. The solid was treated with ethyl acetate (1 ml)—after dissolving, 90 mg (84 %) of dicarbonate **5** precipitated quickly in a crystalline form, m.p. 253~255°C. $[\alpha]_D^{26}$ −52.2±1° (*c* 1, methanol).

pK_a 8.20±0.05 (66 % methanol). IR spectrum: 3550 (OH), 1800 (CO of carbonate), 1735 cm^{-1} (CO of lactone). NMR spectrum: 1.70 (s,6H)—$2CH_3$ at C8 and C12; 2.34 (s,6H)—$N(CH_3)_2$; 3.38 (s,3H)—CH_3O. In the UV spectrum no absorption appears above 250 nm. Molecular ion is 801.

Anal. Calcd. for $C_{39}H_{63}NO_{16}$ (801.91): C 58.40, H 7.92, N 1.75 %
Found: C 58.13, H 8.00, N 1.73 %

TLC: ethanol-methylene chloride-ethyl ether-ligroin (b.p. 60°C), 5:35:30:30, Rf 0.9.

(7) 2′-Acetate of 8,9;11,12-dicyclic dicarbonate of 8-hydroxyerythromycin A 6⁹-hemiketal.

Dicarbonate **5** (108 mg) in anhydrous pyridine (1 ml) was treated with 18 mg of acetic anhydride and left for 1 day at room temperature. Then water (5 ml) was added and the mixture was made basic with $NaHCO_3$. The precipitate was filtered off and crystallized from ethanol. 92 mg (80.7 %) of monoacetate were obtained, m.p. 240~242°C. pK_a 6.40±0.05 (66 % methanol). IR spectrum ($CHCl_3$): 3550 (OH), 1800 (CO of carbonate), 1735 (CO of lactone and acetate), 1240 cm^{-1} (CH_3COO). NMR spectrum: 1.69 (s,3H) and 1.74 (s,3H)—$2CH_3$ at C8 and C12; 2.12 (s,3H)—CH_3COO; 2.31 (s,6H)—$N(CH_3)_2$; 3.40 (s,3H)—CH_3O.

Anal. Calcd. for $C_{41}H_{65}NO_{17}$ (843.94): C 58.35, H 7.76, N 1.66 %
Found: C 58.15, H 7.66, N 1.60 %

TLC: ethanol-methylene chloride-ligroin (b.p. 60°C), 5:35:60, Rf 0.52.

(8) 2′,4″-Diacetate of 8,9;11,12-dicyclic dicarbonate of 8-hydroxyerythromycin A 6⁹-hemiketal.

Dicarbonate **5** (150 mg) in pyridine (1 ml) and acetic anhydride (0.25 ml) was left for 1 day at room temperature. After evaporation of solvent under reduced pressure, the residue was treated with water (1 ml) and made basic with $NaHCO_3$. 150 mg of the diacetate were filtered off, m.p. 249~252°C. pK_a 6.40±0.05 (66 % methanol). IR spectrum ($CHCl_3$): 1800 (CO of carbonate), 1735 (CO of lactone and acetate), 1240 cm^{-1} (CH_3COO). NMR spectrum: 1.67 (s,3H) and 1.72 (s,3H)—$2CH_3$ at C8 and C12; 2.09 (s,3H) and 2.15 (s,3H)—$2CH_3COO$; 2.32 (s,6H)—$N(CH_3)_2$; 3.40 (s,3H)—CH_3O.

Anal. Calcd. for $C_{43}H_{67}NO_{18}$ (885.97): C 58.29, H 7.62, N 1.50 %
Found: C 58.26, H 7.41, N 1.49 %

TLC: ethanol-benzene-ligroin (b.p. 60°C), 5:45:50, Rf 0.73.

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