CHEMICAL MODIFICATION OF ERYTHROMYCINS

III. SPIROKETALS OF 8-HYDROXYERYTHROMYCIN A*

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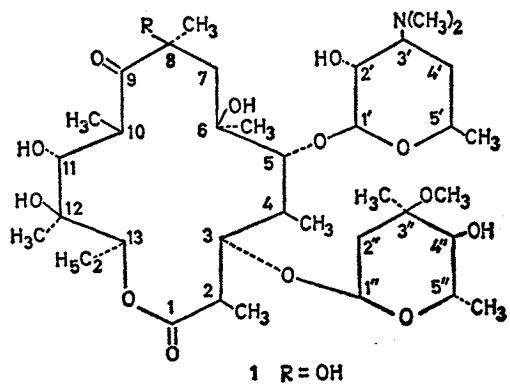
(Received for publication January 5, 1972)

Anhydration of 8-hydroxyerythromycin A (1) in aqueous medium at pH about 1.5 afforded 6^9 12-spiroketal 4, an analogue of anhydroerythromycin A (3). Hydroxylation of erythromycin A 8,9-anhydro-6^9-hemiketal (7) with *m*-chloroperbenzoic acid in chloroform solution yielded a mixture of the N-oxide of 8-hydroxyerythromycin A 6^9 12-spiroketal and the N-oxide of 8-hydroxyerythromycin A 6^9 11-spiroketal (8).

In a previous communication[1], the synthesis and properties of 8-hydroxyerythromycin A (1) have been reported. This compound (1) is more stable to acids than erythromycin A (2). Compound 2 is irreversibly converted into anhydroerythromycin A (3) even at pH 4, whereas compound 1 remains unchanged under these conditions. Acid degradation of compound 1 occurs only at pH 2~3 and at pH 1.5 is fairly rapid; after 1 hour, an anhydro compound $C_{37}H_{65}NO_{13}$ can be isolated in a yield of 80 %. Its structure was established as the 6^9 12-spiroketal of 8-hydroxyerythromycin A (4), an analogue of compound 3, from the following data. The IR and UV spectra showed no absorption for a ketone group. Moreover, in the IR spectrum, bands characteristic for a spiroketal function occurred at 945 and 920 cm^{-1}. Compound 4 consumed 1 mole of sodium metaperiodate with formation of the N-oxide. The lack of further reaction with $NaIO_4$ confirmed that one of the hydroxyl groups at C11-C12 has been engaged in the formation of the spiroketal system. Upon treatment with acetic anhydride in pyridine, compound 4 yielded the triacetate 5, undoubtedly utilizing the secondary OH groups at C2′, C4″ and C11.

Chart 1.

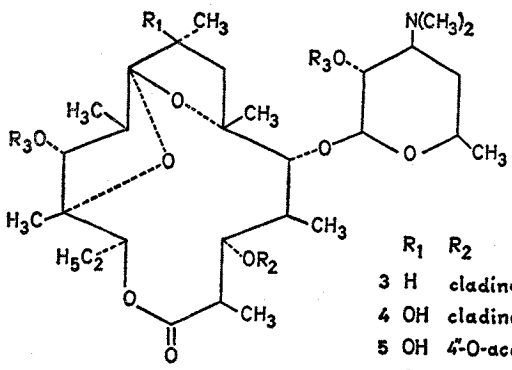


Heating compound 4 in boiling aqueous acetic acid split off the cladinose residue and gave 5-O-desosaminyl-6^9 12-spiroketal of 8-hydroxyerythronolide A (6). The structure of this compound was deduced from the analytical data, the lack of an NMR signal due to protons

* Taken from Ph. D. thesis of K. KROWICKI submitted to the Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, in May, 1970.

of a CH_3O group and the presence of bands at 954 and 925 cm^{-1} in IR. Among the products of hydrolysis, free cladinose was detected. It is noteworthy that with anhydroerythromycin A (3), splitting off the cladinose residue (*e.g.* by treatment with methanolic hydrogen chloride) is accompanied by elimination of a water molecule from position C10-C11 and formation of erythralosamine[2].

Chart 2.



	R_1	R_2	R_3
3	H	cladinosyl	H
4	OH	cladinosyl	H
5	OH	4"-O-acetylcladinosyl	CH_3CO
6	OH	H	H

Another spiroketal derived from 8-hydroxyerythromycin A was obtained by oxidizing erythromycin A 8,9-anhydro-6[9]-hemiketal (7) with *m*-chloroperbenzoic acid in chloroform solution. A mixture of two products, Rf 0.55 and 0.70*, was obtained. The first was identified as the above-mentioned N-oxide of 8-hydroxyanhydroerythromycin A. The second compound was considered to be the N-oxide of 6[9] 11-spiroketal of 8-hydroxyerythromycin A (8). Catalytic reduction of 8 yielded compound 9 containing one molecule of water less than 1, and indicating that 9 was an isomer of 6[9] 12-spiroketal 4.

Compound 8, like the N-oxide of compound 4, failed to react with $NaIO_4$, pointing to blocking of the glycol group at C11-C12. Compound 9, when treated in pyridine solution with an excess of acetic anhydride, yielded the diacetate 10. That one of the acetyl residues was situated in the desoamine moiety was indicated by a decrease in the pKa value obtained for diacetate compared with 9 (6.85 and 8.8, respectively, in 66% DMF). The second acetyl group was located in cladinose, since

Chart 3.

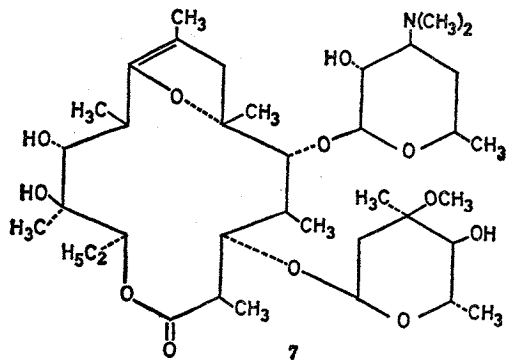


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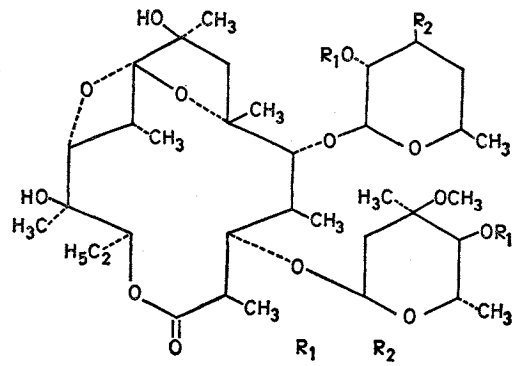
Chart 4.



	R_1	R_2
8	H,	$NO(CH_3)_2$,
9	H,	$N(CH_3)_2$,
10	CH_3CO,	$N(CH_3)_2$,

* TLC[3] in system - ethanol - ethyl ether - chloroform 5 : 25 : 70.

methanolysis of compound **10** afforded methyl 4-O-acetylcladinoside among the reaction products. The formation of the diacetate **10**, as opposed to that of the triacetate produced from the 6⁹ 12-spiroketal **4**, suggests that the secondary OH group at C11 in **9** is blocked. Moreover, an oxetane system is indicated by a band 960 cm^{-1} in the IR spectra of compounds **8**, **9** and **10**. According to BARROW and SEARLES, this ring exhibits a characteristic single or double band within the range 980~970 cm^{-1} [4].

Upon treatment with *p*-toluenesulphonic acid in chloroform or ethereal solution, the spiroketal **9** is readily transformed into the 6⁹ 12-spiroketal **4**, which is more stable thermodynamicaly. If **9** is treated with hot aqueous acetic acid, as well as spiroketals **4** and **6**, a small amount of 8-hydroxyerythromycin A is formed. These transformations can be interpreted as shown in Fig. 1.

Fig. 1. Reactions of 8-hydroxyerythromycin A 6⁹-11-spiroketal (**9**) leading to 8-hydroxyerythromycin A 6⁹ 12-spiroketal (**4**) and 8-hydroxyerythromycin A (**1**).

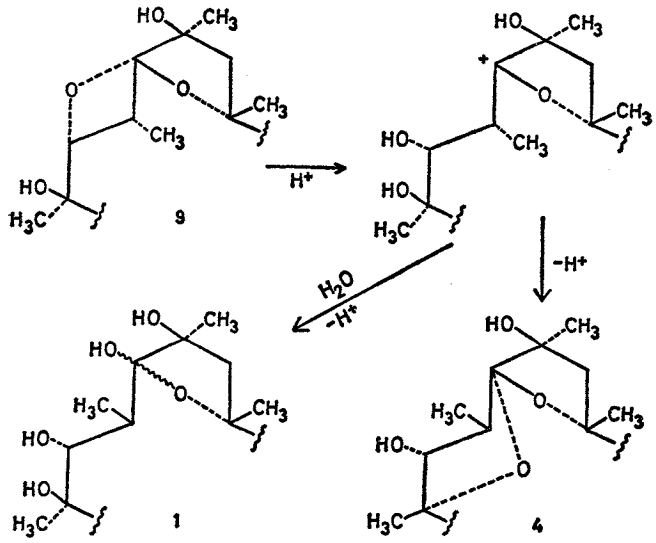
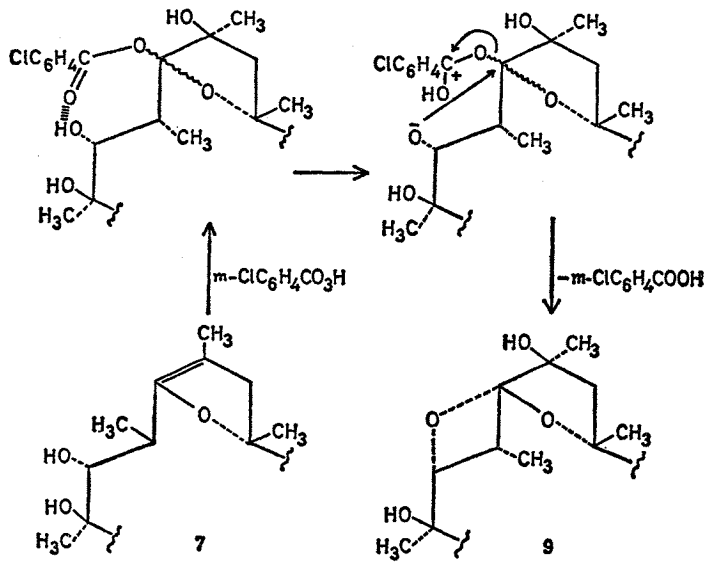


Fig. 2. Proposed scheme for 8-hydroxyerythromycin A 6⁹ 11-spiroketal (**9**) formation from 8,9-anhydro-6⁹-hemiketal of erythromycin A (**7**).



Some comment is needed upon the probable mechanism underlying the formation of N-oxides of spiroketals **4** and **9** during treatment of enol ether **7** with *m*-chloroperbenzoic acid. The first stage of this reaction involves the formation of the N-oxide group, with subsequent addition of peracid to the double bond to form the transient hydroxyester. Intramolecular alcoholysis by one of the OH groups, at C11 or C12, leads to closure

of the spiroketal system and elimination of *m*-chlorobenzoic acid. Formation of the 6[9] 11-spiroketal system is illustrated in Fig. 2.

Experimental

8-Hydroxyerythromycin A (**1**) was prepared by the method given in Part II of this series[1]. 8,9-Anhydro-6[9]-hemiketal of erythromycin A (**7**) was obtained by the method reported in Part I[5] which also describes the equipment used for spectral studies and gels employed for TLC and column chromatography.

1. 6[9] 12-Spiroketal of 8-hydroxyerythromycin A (**4**).

Compound **1** (750 mg) in water (10 ml) was acidified with aqueous HCl to pH 1.5. After 1 hour the solution was made basic with aqueous Na_2CO_3, extracted with CH_2Cl_2 and the extract was evaporated. The residue was crystallized from ligroin, b.p. 80~100°C, the pure product remaining in the filtrate. Evaporation of ligroin gave 585 mg (80 %) of compound **4**, m.p. 153~156°C. IR (KBr): 3350 (OH), 1740 (CO of lactone), 920 cm^{-1} (spiroketal).

Anal. Calcd. for $C_{37}H_{65}NO_{13}$ (731.89) : C 60.71, H 8.95 %
Found : C 60.85, H 8.88

TLC[9] : ethanol - methylene chloride - ethyl ether 5 : 60 : 35, Rf 0.3.

2. N-Oxide of 8-hydroxyerythromycin A 6[9] 12-spiroketal.

Compound **4** (858 mg) in water (10 ml) was treated with $NaIO_4$ (250 mg). After 10 minutes, the solution was extracted 5 times with 10 ml portions of CH_2Cl_2. Evaporation of the extracts gave 857 mg (98 %) of N-oxide of **4**, m.p. 183~185°C (ethyl acetate). IR (KBr): 3500 (OH), 1725 (CO of lactone), 920 cm^{-1} (spiroketal). NMR: 1.58 (s, 3H)-CH_3 at C8; 3.26 (s, 6H)-$NO(CH_3)_2$; 3.40 (s, 3H)-CH_3O).

3. 2′,4″,11-Triacetate of 8-hydroxyerythromycin A 6[9]12-spiroketal (**5**).

Compound **4** (732 mg) was acetylated by the procedure described in Part I, Paragraph 2. (1)[5]. After crystallization of the crude product from ligroin, b.p. 60°C, 770 mg (90 %) of compound **5**, m.p. 131~135°C, were obtained. IR (KBr): 3600 (OH), 1740 (CO of lactone and acetate), 1235 (CH_3COO), 905 cm^{-1} (spiroketal). IR of 0.0005 M solution in CCl_4: 3563 cm^{-1} (OH); upon further dilution, the position of this band was unchanged. NMR: 1.61 (s, 3H)-CH_3 at C8; 2.04 (s, 3H)-CH_3COO; 2.12 (s, 6H)-$2CH_3COO$; 2.25 (s, 6H)-$N(CH_3)_2$; 3.27 (s, 3H)-CH_3O.

Anal. Calcd. for $C_{43}H_{71}NO_{16}$ (858.0) : C 60.19, H 8.37 %
Found : C 59.90, H 8.18

4. 5-O-Desosaminyl-6[9] 12-spiroketal of 8-hydroxyerythronolide A (**6**).

Compound **4** (366 mg) in water was acidified with acetic acid to pH 3 and refluxed for 15 minutes. The solution was cooled, made basic with Na_2CO_3 and extracted with CH_2Cl_2. During extraction a white precipitate hindering the separation of the layers was formed. The precipitate was filtered and combined with the evaporated extracts. Crystallization from acetone gave 230 mg (80 %) of compound **6**, m.p. 233~236°C. IR (KBr): 3550 (OH), 1730 (CO of lactone), 945, 925 and 900 cm^{-1} (spiroketal). NMR: 1.50 (s, 3H)-CH_3 at C8; 2.26 (s, 6H)-$N(CH_3)_2$; lack of the CH_3O protons, no olefinic protons. pKa 8.95±0.1 (water). One mole of the compound **6** used up during 1 hour 1.0 mole of $NaIO_4$[6] (for formation of N-oxide).

Anal. Calcd. for $C_{29}H_{51}NO_{10}$ (573.70) : C 60.71, H 8.96 %
Found : C 60.61, H 9.01

5. N-Oxide of 8-hydroxyerythromycin A 6[9] 11-spiroketal (**8**).

To compound **7** (10 g) in chloroform (100 ml) 6.24 g of 77 % *m*-chloroperbenzoic acid in chloroform (100 ml) were added. After 1 hour the mixture was shaken with aqueous $NaHCO_3$ and the chloroform layer evaporated. The residue was separated by column chromatography on a mixture of 9 parts silica gel and 1 part basic Al_2O_3 (Woelm)

impregnated with formamide[3], using as eluent ethanol - chloroform - ethyl ether, 5 : 63 : 32. Two main products were obtained. Crystallization of one from ethyl acetate gave 2 g of the previously described N-oxide of **4**. Crystallization of the other from methanol gave 2 g of compound **8**, m. p. 213~217°C. IR (KBr): 3540 (OH), 1725 (CO of lactone), 960 (oxetane ring), 920 cm^{-1} (spiroketal). NMR: 1.53 (s, 3H)-CH_3 at C8; 3.20 (s, 6H)-NO$(CH_3)_2$; 3.38 (s, 3H)-CH_3O. There was no preceptible reaction of compound 8 with $NaIO_4$ during 1 hour[6].

6. 6[9] 11-Spiroketal of 8-hydroxyerythromycin A (9).

Compound 8 (748 mg) was reduced with H_2/Pt in methanol. Crystallization of the crude product from acetonitrile gave 700 mg (90 %) of monosolvate of compound **9**, m.p. 249~251°C. IR (KBr): 3620 and 3550 (OH), 1725 (CO of lactone), 960 (oxetane ring), 920 cm^{-1} (spiroketal). NMR: 1.53 (s, 3H)-CH_3 at C8; 1.96 (s, 3H)-CH_3CN; 2.25 (s, 6H)-$N(CH_3)_2$; 3.32 (s, 3H)-CH_3O. pKa 8.8 (66 % DMF).

Compound **9** neutralized with acetic acid, in concentrated aqueous solution, after addition of aqueous KSCN afforded a crystalline hydrorhodanide, m.p. 188~191°C. IR (KBr): 3500 (OH), 2070 (SCN), 1720 (CO of lactone), 965 and 960 (oxetane ring).

7. 2′, 4″-Diacetate of 8-hydroxyerythromycin A 6[9] 11-spiroketal (10).

Compound **9** (732 mg) was acetylated by the procedure described in Part I, Paragraph 2. (1)[5]. Crystallization of the crude product from ligroin, b.p. 60°C, gave 735 mg (90 %) of compound **10**, m.p. 157~160°C. IR (KBr): 3550 (OH), 1740 (CO of lactone and acetate), 1240 (CH_3COO), 960 (oxetane ring), 915 cm^{-1} (spiroketal). IR of 0.0005 M solution in CCl_4: 3618 cm^{-1} (*tert.* OH), 3513 cm^{-1} (OH intramolecularly bound); further dilution caused no shift of bands. NMR: 1.50 (s, 3H)-CH_3 at C8; 2.00 (s, 3H)-CH_3COO; 2.05 (s, 3H)-CH_3COO; 2.25 (s, 6H)-$N(CH_3)_2$; 3.29 (s, 3H)-CH_3O. pKa 6.85 (66 % DMF).

Anal. Calcd. for $C_{41}H_{69}NO_{15}$ (815.96) : C 60.34, H 8.52 %
Found : C 60.27, H 8.71

TLC[3]: ethanol - benzene - ligroin, b.p. 60°C, 5 : 20 : 75, Rf 0.09.

Compound **10** 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.

References

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