CHEMICAL MODIFICATION OF ERYTHROMYCINS
IV. 8-HYDROXYERYTHROMYCIN B*

KRZYSZTOF KROWICKI and ALEKSANDER ZAMOJSKI

Institute of Organic Chemistry, Polish Academy of Sciences,
Warsaw, ul. Kasprzaka 44/52, Poland

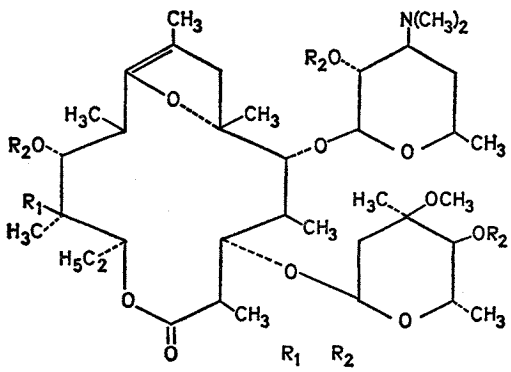
(Received for publication January 5, 1972)

Treatment of erythromycin B (2) with tetraacetylglucosyl bromide in nitromethane and pyridine afforded the 8,9-anhydro-6⁹-hemiketal of erythromycin B (4) in yields above 80 %. Hydroxylation of compound 4 with *m*-chloroperbenzoic acid, irrespective of the solvent used, yielded the N-oxide of 8-hydroxyerythromycin B 6⁹11-spiroketal (6). After reduction of the N-oxide function of compound 6 and subsequent hydrolysis with aqueous acetic acid, 8-hydroxyerythromycin B was obtained. The antibacterial activity of this antibiotic against *Bacillus pumilus* (*in vitro*) was a half that exhibited by erythromycin B.

We have previously shown[1] that under certain conditions erythromycin A (1) undergoes dehydration, forming the enol ether 3. Compound 3 proved to be a convenient intermediate for the synthesis of 8-hydroxyerythromycin A[2]. We thought it of interest to investigate the behavior of the closely related macrolide, erythromycin B (2), and its derivatives under conditions used for the transformation of compounds 1 and 3.

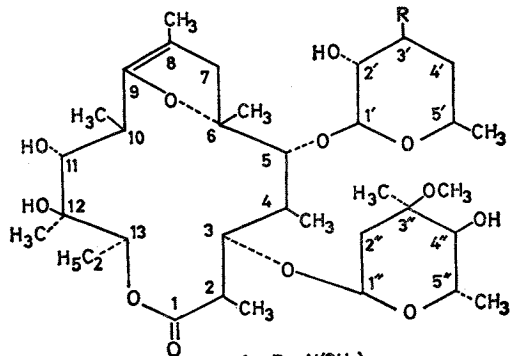
Upon heating of erythromycin B in nitromethane solution with tetraacetylglucosyl bromide in the presence of pyridine, the anhydro compound 4 was formed in better than 80 % yield. This compound contained a double bond, and its IR and UV

Chart 1.



	R_1	R_2
3	OH,	H,
4	H,	H,
5	H,	CH_3CO,

Chart 2.



1 R= $N(CH_3)_2$
2 R= $NO(CH_3)_2$

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

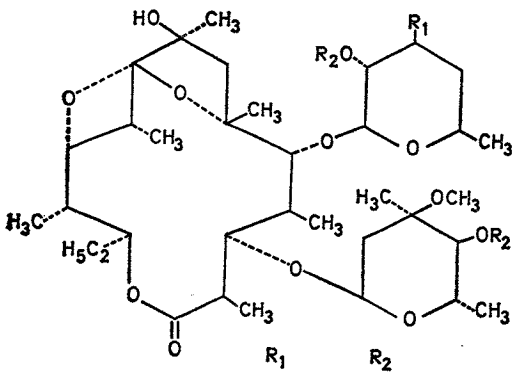
spectra exhibited no absorption corresponding to a ketone group. Mild acid hydrolysis of compound 4 regenerated erythromycin B (2) in good yield. These results, as well as the close analogy to the preparation of compounds 3 and 4 identified the latter as erythromycin B 8,9-anhydro-6⁹-hemiketal. Acetylation of 4 with acetic anhydride in pyridine yielded 2′,4″,11-triacetate 5.

In 1971 KURATH *et al.* obtained erythromycin B 8,9-anhydro-6⁹-hemiketal by another route, when treated erythromycin B with glacial acetic acid[3]. Chemical and physical properties are in agreement with ours except melting points which differ as much as 46°C.

Enol ether 4 was treated with *m*-chloroperbenzoic acid. In contrast to the case of the enol ether 3[2,4], the reaction product was always N-oxide compound 6, irrespective of the solvent used (chloroform, methanol, ethyl acetate with water). Elemental analysis indicates the addition of two oxygen atoms in the conversion of 4 to compound 6. Compound 6 failed to react with $NaIO_4$. After reduction of the N-oxide function, a basic compound 7 was obtained. Acetylation of the latter yielded the diacetate 8; it could be shown that one acetyl group was situated in each sugar residue, *i.e.* in cladinose and desosamine (cf assignment of the acetyl groups positions in the analogous 2′,4″-diacetate of 8-hydroxyerythromycin A 6⁹ 11-spiroketal[4]. This reaction pointed to blocking of the hydroxyl group at C 11. The IR spectrum of compound 8 at high dilution in CCl_4 showed a single band for a tertiary OH group at 3618 cm^{-1}. In the IR spectra of compounds 6,7 and 8, a split band at about 960 cm^{-1} suggested an oxetane system[5]. From this information and because of the great similarity of the IR spectra of hydrorhodanides obtained from compound 7 and 8-hydroxyerythromycin A 6⁹ 11-spiroketal[4] (Fig. 1), compound 7 was assigned an analogous structure as 8-hydroxyerythromycin B 6⁹11-spiroketal.

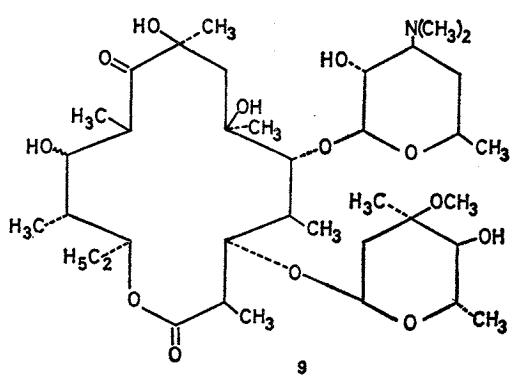
The formation of compound 6 from enol ether 4 doubtless should be interpreted in the same way as the formation of erythromycin A derivatives[4]. Intramolecular alkoholysis of transient 8-hydroxy-9-*m*-chlorobenzoate probably proceeds much more rapidly than the reaction with water or methanol, since the OH at C 11 is not

Chart 3.



	R_1	R_2
6	$NO(CH_3)_2$,	H,
7	$N(CH_3)_2$,	H,
8	$N(CH_3)_2$,	CH_3CO,

Chart 4.



9

Fig. 1. IR spectra of hydrorhodanides of 8-hydroxyerythromycin A (I) and B (II) 6⁹11-spiroketals in KBr.

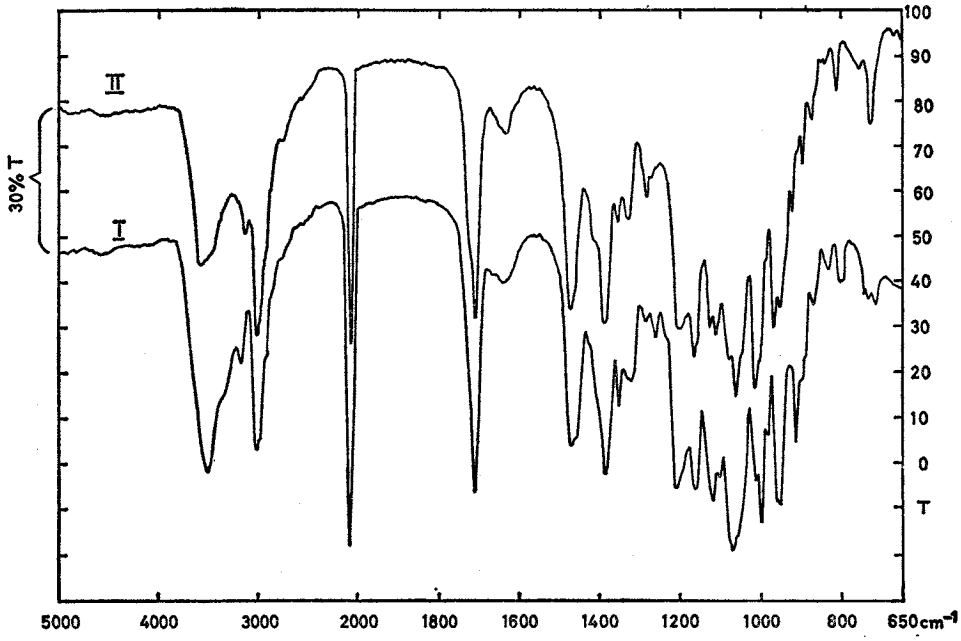
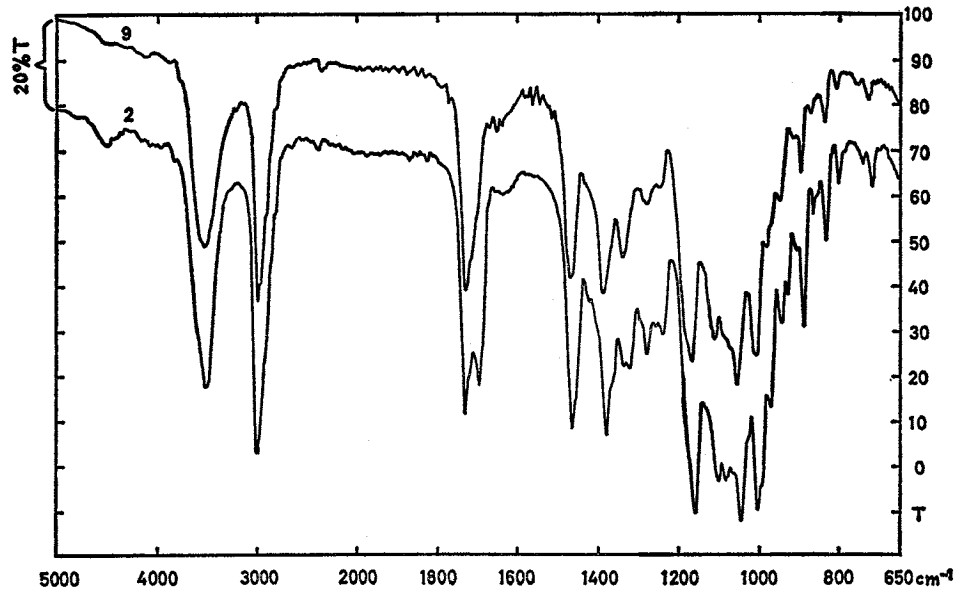


Fig. 2. IR spectra of 8-hydroxyerythromycin B (9) and erythromycin B (2) in KBr.



deactivated by a hydrogen bond with the OH at C 12, as it is in the enol ether of erythromycin A.

Short heating of spiroketal **7** with aqueous acetic acid afforded in about 50 % yield the hydrolysis product of the oxetane ring, *viz.* 8-hydroxyerythromycin B (**9**). Compound **9** consumed only two moles of $NaIO_4$ (1 mole for the formation of an N-oxide). The IR spectrum of compound **9** is very similar to that of erythromycin B (Fig. 2).

The UV spectrum exhibits a band at 279 nm (ε 40.5), pointing to the presence of a free ketone group at C 9. The CD spectrum of compound **9** showed a maximum of the COTTON effect at 284 nm, indicating a hypsochromic shift, as compared with erythromycin B (290 nm); this points to configuration S at C 8, as in the case of 8-hydroxyerythromycin A[2]. The molar amplitudes of compounds **9** and **2** amount to −79 and −137, respectively. The difference is considerable and suggests that in compound **9** the configuration at C 11 may be changed. Cleavage of the oxetane ring can take place at C 9 or C 11 and it is evident that the attack at C 11 brings about a change in configuration at this carbon atom.

Table 1. The antibacterial spectra (*in vitro*) of erythromycin A (**1**) and 8-hydroxyerythromycin B (**9**).

Strain	Minimum inhibitory concentration μg/ml	
	1	**9**
Staphylococcus aureus FDA 209 P	1.95	3.9
〃 〃 111	<1.95	<1.95
〃 〃 penicillin resistant	<1.95	<1.95
Enterococcus 93	0.97	0.97
Escherichia coli 466	125	250
〃 〃 $O_{55}B_6$	125	250
〃 〃 866	125	250
Proteus OX_{22}	500	500
Bacillus subtilis 729	<1.95	<1.95
Klebsiella pneumoniae 559	125	250
Salmonella paratyphi A 192	125	250
〃 〃 B 217	31.2	31.2
〃 〃 C	31.2	31.2
Sarcina lutea	<1.95	<1.95
Bacillus cereus ATCC	3.9	<1.95
Shigella shigae	62.5	62.5

The antibacterial activity of 8-hydroxyerythromycin B is a half that shown by erythromycin B (against *Bacillus pumilus*, cylinder method). The comparative antibacterial spectra of erythromycin A (**1**) and 8-hydroxyerythromycin B (**9**) are presented in Table 1.

Experimental

Pure erythromycin B was obtained from a crude erythromycin A (Polfa) as follows. The bulk of erythromycin A was separated by crystallization from diisopropyl ether. Evaporation of mother liquor gave residue consisted in 60 % of erythromycin B. After crystallization from acetone near pure erythromycin was obtained. To remove traces of erythromycin A, the crystals were dissolved in aqueous acetic acid; several hours later the solution was made basic with aqueous ammonia and extracted with CH_2Cl_2. After evaporation of the extracts, the residue was again crystallized from acetone, yielding pure erythromycin B, m.p. 208°C, $[\alpha]_D^{20}$ −95° (*c* 1, methanol).

The preparation of tetraacetylglucosyl bromide, as well as information on equipment used in spectral studies and gels used for TLC and column chromatography have been described in Part I[1].

1. 8,9-Anhydro-6[9]-hemiketal of erythromycin B (3).

Compound **2** (2 g) and tetraacetylglucosyl bromide (1.64 g) dissolved in nitromethane (40 ml) and pyridine (10 ml) were heated for 2 hours at 110°C. The mixture was cooled and shaken with aqueous Na_2CO_3. The aqueous layer was extracted with CH_2Cl_2 and the extract combined with the nitromethane layer. After evaporation under reduced pressure, the residue in a small volume of ethanol was left to crystallize. 1.6 g (82 %) of compound **3**, m.p. 126~128°C, was obtained. IR ($CHCl_3$): 3640, 3560 and 3460 (OH); 1720 cm^{-1} (CO of lactone). UV: no absorption above 250 nm. NMR: 1.58 (s, 3 H)-CH_3 at C 8; 2.50 (s, 6 H)-$N(CH_3)_2$; 3.41 (s, 3 H)-CH_3O. $[\alpha]_D^{20}$ −35±1° (*c* 1, methanol). pKa 8.8 (66 % DMF).

Anal. Calcd. for $C_{37}H_{65}NO_{11}$ (699.89): C 63.49, H 9.36 %
Found. C 63.36, H 9.45 %

Antibacterial activity (*in vitro*) against *B. pumilus*: 24 μg/mg.

2. 2′,4″,11-Triacetate of erythromycin B 8,9-anhydro-6⁹-hemiketal (5).

Compound **3** (560 mg) was acetylated according to the method described in Part I, Paragraph 2. (1) to obtain 630 mg (95 %) of compound **5**, m.p. 108~111°C. IR ($CHCl_3$): 1730 (CO of lactone and acetate), 1245 cm^{-1} (CH_3COO). NMR: 1.59 (s, 3 H)-CH_3 at C 8; 2.06 (s, 3 H), 2.11 (s, 3 H) and 2.14 (s, 3 H)-3 CH_3COO; 2.45 (s, 6 H)-$N(CH_3)_2$; 3.35 (s, 3 H)-CH_3O.

Anal. Calcd. for $C_{43}H_{71}NO_{14}$ (825.99): C 62.52, H 8.66 %
Found: C 62.46, H 8.63 %

3. N-Oxide of 8-hydroxyerythromycin B 6⁹11-spiroketal (6).

To compound **3** (700 mg) in CH_2Cl_2 (30 ml) 447 mg of 77 % *m*-chloroperbenzoic acid were added. After 2 hours at room temperature, the mixture was washed with diluted aqueous KOH. After evaporation, crystallization of the residue from methanol - dissopropyl ether afforded 428 mg (58 %) of compound **6**, m.p. 182~184°C. IR (KBr): 3500 (OH), 1730 (CO of lactone), 960 and 950 cm^{-1} (oxetane ring). NMR: 1.52 (s, 3 H)-CH_3 at C 8; 3.21 (s, 6 H)-$NO(CH_3)_2$; 3.43 (s, 3 H)-CH_3O.

Anal. Calcd. for $C_{37}H_{65}NO_{13}$ (731.89): C 60.71, H 8.95 %
Found: C 60.75, H 8.97 %

Compound **6** did not react with $NaIO_4$ during one hour[6].

4. 6⁹11-Spiroketal of 8-hydroxyerythromycin B (7).

Compound **6** (226 mg) was reduced with H_2/Pt in methanol. After evaporation of the solvent, the crude product was treated with water (1 ml), made neutral with acetic acid and some solution of KSCN was added to obtain 172 mg (60 %) of hydrorhodanide of compound **7**, m.p. 163~164°C. IR (KBr): 3570 (OH), 2070 (SCN), 1710 (CO of lactone), 970 and 950 (oxetane ring) (Fig. 1). This hydrorhodanide (172 mg) was made basic with aqueous ammonia and extracted with CH_2Cl_2. After evaporation of the extracts, crystallization of the residue from acetone gave 112 mg (70 %) of compound **7**, m.p. 216~220°C. IR (KBr): 3540 (OH), 1700 (CO of lactone), 965 and 955 cm^{-1} (oxetane ring). NMR: 1.51 (s, 3 H)-CH_3 at C 8; 2.29 (s, 6 H)-$N(CH_3)_2$; 3.35 (s, 3 H)-CH_3O.

5. 2′,4″-Diacetate of 8-hydroxyerythromycin B 6⁹11-spiroketal (8).

Compound **7** (716 mg) was acetylated according to the method described in Part I, Paragraph 2. (1)[1]. Crystallization of the crude product from ligroin, b.p. 60°C, gave 720 mg (90 %) of compound **8**, 173~177°C. IR (KBr): 3570 (OH), 1740 and 1705 (CO of lactone and acetate), 1240 (CH_3COO), 960 cm^{-1} (oxetane ring). IR of 0.0005 M solution in CCl_4: 3618 cm^{-1} (*tert.* OH); upon further dilution the band did not change the position. NMR: 1.43 (s, 3 H)-CH_3 at C 8; 1.98 (s, 3 H) and 2.05 (s, 3 H)-2CH_3COO; 2.23 (s, 6 H)-$N(CH_3)_2$; 3.27 (s, 3 H)-CH_3O. pKa 6.9 (66 % DMF).

Anal. Calcd. for $C_{41}H_{69}NO_{14}$ (799.96): C 61.50, H 8.69 %
Found: C 61.75, H 8.89 %

TLC[7]: ethanol - benzene - ligroin, b.p. 60°C, 5 : 20 : 75, Rf 0.75.

6. 8-Hydroxyerythromycin B (9).

Compound **7** (716 mg) in water was brought to pH 3 with acetic acid and refluxed for 3~5 minutes. The solution was cooled, made basic with Na_2CO_3 and extracted with CH_2Cl_2. Column chromatography, with ethanol - methylene chloride - ethyl ether (5 : 60 : 35), on Kieselgel impregnated with formamide[7], gave 370 mg (50 %) of pure **9**, m.p. 124~127°C. IR (KBr): 3510 (OH), 1730 cm^{-1} (CO of lactone and ketone) (Fig. 2). UV: λ_{max} 279 nm, ε 40.5 (methanol). CD: **a** −79 at 284 nm (methanol). NMR: 1.63 (s, 3 H)-CH_3 at C 8; 2.27 (s, 6 H)-$N(CH_3)_2$; 3.27 (s, 3 H)-CH_3O. $[\alpha]_D^{20} -69 \pm 1°$ (*c* 1, methanol). pKa 9.2 (water).

Anal. Calcd. for $C_{37}H_{67}NO_{13}$ (733.91): C 60.56, H 9.20 %
Found: C 60.63, H 9.35 %

One mole of **9** in 1 hour consumed 1.92 mole of $NaIO_4$[6]. Antibacterial activity against *B. pumilus* was 440 μg/mg (cylinder-plate method).

References

1) KROWICKI, K. & A. ZAMOJSKI: Chemical modification of erythromycins. I. 8,9-Anhydro-6^9-hemiketal of erythromycin A. J. Antibiotics 26: 569~574, 1973
2) KROWICKI, K. & A. ZAMOJSKI: Chemical modification of erythromycins. II. 8-Hydroxyerythromycin A. J. Antibiotics 26: 575~581, 1973
3) KURATH, P.; P. H. JONES, R. S. EGAN & T. J. PERUN: Acid degradation of erythromycin A and erythromycin B. Experientia 27: 362, 1971
4) KROWICKI, K. & A. ZAMOJSKI: Chemical modification of erythromycins. III. Spiroketals of 8-hydroxyerythromycin A. J. Antibiotics 26: 582~586, 1973
5) BARROW, G. M. & S. SEARLES: Effect of ring size of the infrared spectra of cyclic ethers. Characteristic frequencies of trimethylene oxides and tetrahydrofurans. J. Am. Chem. Soc. 75: 1175~1177, 1953
6) NEUMÜLLER, G. & E. VASSEUR: The influence of pH on the periodate oxidation of carbohydrates. Arkiv Kemi 5: 235~245, 1953
7) BANASZEK, A.; K. KROWICKI & A. ZAMOJSKI: Thin-layer and column chromatography of erythromycins and some degradation products. J. Chromatog. 32: 581~583, 1968