CHEMICAL MODIFICATION OF ERYTHROMYCINS

I. 8,9-ANHYDRO-6⁹-HEMIKETAL OF ERYTHROMYCIN A*

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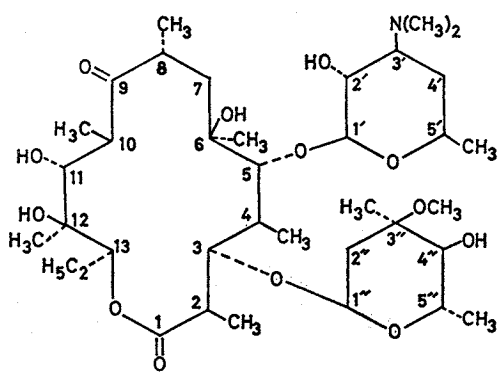
By treatment of erythromycin A with compounds containing a mobile halogen atom (tetraacetylglucosyl bromide, *t*-butyl bromide, triphenylmethyl chloride) in aprotic solvents of high dielectric constant (nitromethane, acetonitrile) and in the presence of pyridine, an anhydrocompound $C_{37}H_{65}NO_{12}$ was obtained in up to 95% yield. Analytical, spectral, and chemical data indicated that the compound was erythromycin A 8,9-anhydro-6⁹-hemiketal (2).

Relatively little attention has so far been given to the chemical transformation of erythromycins, especially in comparison with other antibiotics, *e.g.* penicillin, rifamycin, lincomycin. If studies on the erythromycin ester formation are excluded, there are only a few paper dealing with the modification of erythromycins. These describe changes in the substituents at the nitrogen atom in the desosamine residue[1], COPE degradation of erythromycin N-oxide[2], transformation of these macrolides containing an unsaturated sugar residue[3], and the preparation of erythromycin A hydrazone[4], oxime[5] and erythromycylamine[4]. The antibacterial activity of the modified antibiotics in no case exceeded that of its parent substance 1.

The studies reported in this series of papers[6] were designed to continue research on the modification of erythromycins and, at the same time, to obtain a deeper insight into their chemistry.

Heating erythromycin A (1) in nitromethane and pyridine with tetraacetylglucosyl bromide afforded in very good yield compound 2, $C_{37}H_{65}NO_{12}$. This composition corresponds to a loss of one molecule of water from the parent antibiotic. Compound 2 differs from the well-known anhydroerythromycin A (4) which is readily formed from 1 in acid medium (pH 4). The arguments which led to assignment of the erythromycin A 8,9-anhydro-6⁹-hemiketal structure to 2 are given below.

Chart 1.



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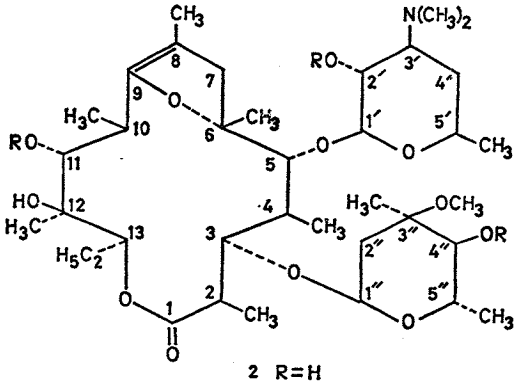
The positive reaction of compound 2

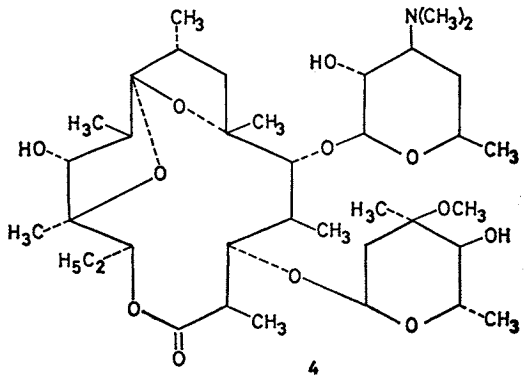
* Taken from Ph. D. thesis of K. KROWICKI submitted to the Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, in May, 1970.

Chart 2.



2 R=H
3 R=CH_3CO

Chart 3



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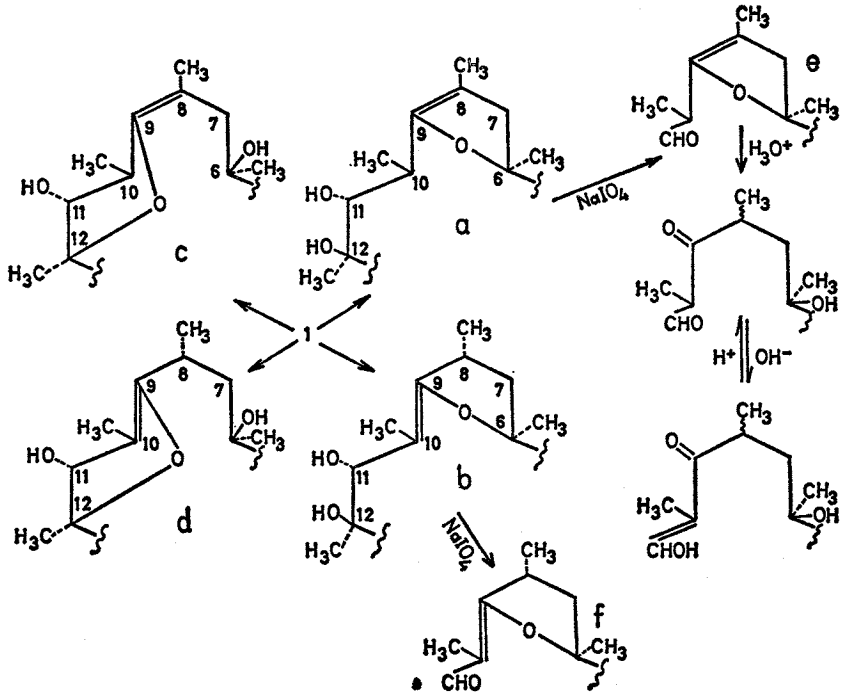
with tetranitromethane, as well as the three proton singlet found in the NMR spectrum at δ 1.56 (C8-vinylmethyl group) indicated that a double bond was present. The IR and UV spectra of compound 2 lacked the maxima for a ketone group at C9. When treated with dilute acetic acid (pH 3), compound 2 was transformed into anhydroerythromycin A (4).

The lack of participation of secondary OH groups at C2′, C4″ and C11 in the dehydration was proved as follows: Four acetyl esters of erythromycin A (2′-monoacetate, 4″-monoacetate, 2′,4″-diacetate and 2′,4″,11-triacetate) were treated with tetraacetylglucosyl bromide in nitromethane and pyridine, yielding the respective

Fig. 1. Four possible routes of dehydration of erythromycin A hemiketal, and glycol cleavage with sodium periodate.



anhydrocompounds. Further acetylation of these anhydromonoacetates and diacetate led to the same compound which was also formed from the 2′,4″,11-triacetate of erythromycin A, in turn, this compound was identical with triacetate **3** obtained by direct acetylation of compound **2**.

Erythromycin A is known to form hemiketals or ketals readily[7]. In these the ketone group at C9 and the tertiary OH groups at C6 and/or C12 are engaged. Dehydration of a hemiketal (Fig. 2) would afford compound **2** and two possibilities for splitting off the elements of water have to be considered: for both the 6^9- and 12^9-hemiketals the double bond could occur either in position C8-C9 or C9-C10 (Fig. 1).

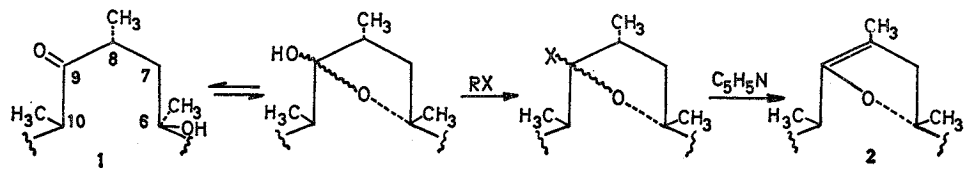
It is evident that enol ethers **a**-**d** should readily undergo acid hydrolysis to the hemiketal with subsequent conversion to anhydroerythromycin A (4).

The formation of enol ethers of this type in the erythromycin series has been reported by PERUN[8]. By heating the triacetate of erythronolide B with 0.01 M hydrochrolic acid in methanol-water solution, he obtained a compound to which he assigned the 6^9-enol ether structure, without, however, determining the position of the double bond (C8-C9 or C9-C10). That was pointed to be C8-C9 by KURATH and EGAN[9].

From compound **2** we obtained the N-oxide which was found to react with sodium periodate. This fact suggested the presence of a 1,2-glycol system in compound **2**. The only group of this type occurs at C11-C12 of the macrolide ring. Consequently, it could be assumed that the hydroxyl group at C12 cannot be involved in the formation of enol ether **2**, and, in turn, formulae **c** and **d** for **2** could be ruled out. To choose between formulae **a** and **b**, we employed the above-mentioned reaction with periodate. The cleavage of diol **a** ought to afford $\beta\gamma$-unsaturated aldehyde **e**, whereas oxidation of diol **b** would provide an $\alpha\beta$-unsaturated aldehyde **f**. The products, *i.e.* **e** and **f**, should be readily distinguishable by their UV spectra. The product obtained by oxidation of the N-oxide of compound **2** with periodate showed a weak maximum, $\varepsilon=130$ at 278 nm. Upon acidification of the sample, the extinction increased to 640 at 277 nm, and in basic solution to 1170 at 274 nm. This result is consistent with formula **a** which upon cleavage with sodium metaperiodate would afford two isolated carbonyl groups. Treatment with aqueous acid would bring out hydration of the double bond of the enol ether with formation of a β-ketoaldehyde, enolization of the latter would increase the extinction. These data are consistent with compound **2**, being erythromycin A 8,9-anhydro-6^9-hemiketal.

In 1971 KURATH *et al.*[10] reported the right assignment of the erythromycin A 8,9-anhydro-6^9-hemiketal structure to a compound obtained much earlier by STEPHENS

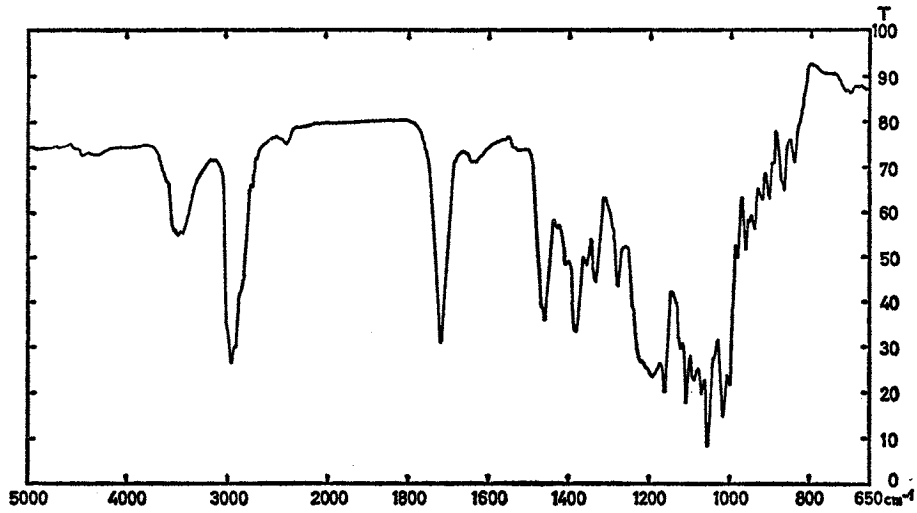
Fig. 2. The formation of erythromycin enol ether (2).



and CONINE[11], when treated erythromycin A with glacial acetic acid, and so-called "erythromycin hemiketal". Chemical properties and optical rotation for this compound are in agreement with ours. Some deviation of melting points may be due to different crystallization solvents used.

We have examined the formation of enol ether 2 from erythromycin A, when changing two variables: the nature of the halogen compound and of the solvent. Good yields of product 2 were obtained with substances containing a mobile halogen atom; tetraacetylglucosyl bromide could be replaced by *tert*-butyl bromide or triphenylmethyl chloride. The application of primary and secondary halides, such as *n*-butyl

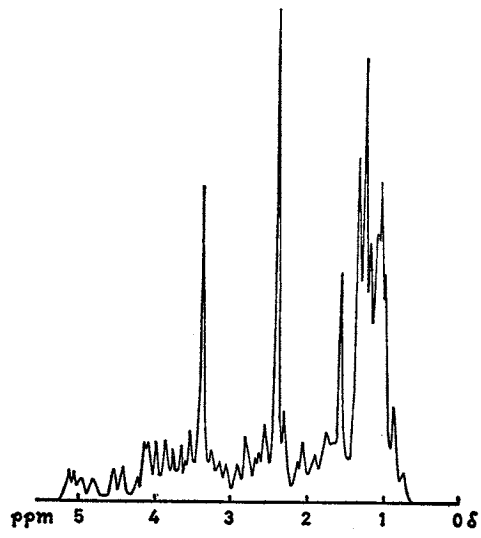
Fig. 3. The IR spectrum of 8,9-anhydro-6⁹-hemiketal of erythromycin A (2) in $CHCl_3$.



bromide, 2-bromopentane, diphenylchloromethane, greatly reduced the yield. If a solvent with low dielectric constant, *e.g.* ethyl acetate, was used instead of nitromethane, only traces of the product were obtained. On the other hand, in the acetonitrile the yield of enol ether 2 was nearly quantitative. Pyridine is an indispensable component of the reaction mixture; when it was replaced by piperidine 2 was not formed. From these results we conclude that the formation of enol ether 2 (Fig. 2) occurs by:

(1) Replacement of the hemiketal hydroxyl group in erythromycin A hemiketal by a halogen atom, this being promoted by solvents with high dielectric constant, while

Fig. 4. The NMR spectrum of 8,9-anhydro-6⁹-hemiketal of erythromycin A (2) in $CDCl_3$, 60 MHz.



pyridine enhances the mobility of the halogen ions by complexing with the halogen compound.

(2) Pyridine-induced elimination of the elements of hydrogen halide.

Erythromycin A 8,9-anhydro-6[9]-hemiketal (2) is virtually devoid of antibacterial activity; the value of the latter amounts to only 33 μg/mg in the test using *Bacillus pumilus* (cylinder-plate method). Compound **2** is a useful intermediate for chemical modifications of erythromycin A[6].

Experimental

Pure erythromycin A was obtained from the commercial product (Polfa) by crystallization from diisopropyl ether and, subsequently, from nitromethane, m.p. 135~140°C, $[\alpha]_D^{20}$ −73.4° (*c* 1, methanol), homogeneous in TLC[12]. Acetyl esters of erythromycin A were prepared according to literature procedures[7b]. Tetraacetylglucosyl bromide was obtained from glucose and acetyl bromide[13]. The ramaining halogen compounds were commercial products.

Column chromatography was performed using Kieselgel für Chromatographie, unter 0.08 mm (Merck) and Kieselguhr (Merck). For TLC, Kieselgel (Serva) and Kieselguhr (Merck) were employed; the plates were impregnated with formamide[12].

The IR spectra were recorded on Hilger H-800 and Unicam SP-200 units, and UV spectra on a Unicam SP-700 spectrophotometer. The NMR spectra were obtained on Varian HR-60/IL and Jeol JNM-4H-100 units, in $CDCl_3$ solution with TMS as internal standard; the chemical shifts are reported in δ scale (TMS=0 ppm).

1. 8,9-Anhydro-6[9]-hemiketal of erythromycin A (2).

Erythromycin A (245 mg) and triphenylmethyl chloride (140 mg) were heated at 80°C for 2 hours in acetonitrile (2.5 ml)-pyridine (0.5 ml) solution. With nitromethane instead of acetonitrile as the solvent, a higher temperature for the reaction mixture was required (~110°C). After evaporation at room temperature under reduced pressure, the residue shaken with a few millilitres of 10 % aqueous Na_2CO_3 was extracted with CH_2Cl_2. The CH_2Cl_2 layer was dried over $MgSO_4$ and the solvent removed. After addition of a small amount of ethanol, 215 mg (90 %) of compound **2** crystallized, m.p. 138~140°C. The IR spectrum is shown in Fig. 3, and the NMR spectrum in Fig. 4. In the UV spectrum no absorption appears above 250 nm. $[\alpha]_D^{20}$ −45±1° (*c* 1, methanol). pKa 8.8 (66 % DMF).

Analysis Calcd. for $C_{37}H_{65}NO_{12}$ (715.89) : C 62.07, H 9.15 %
Found : C 61.98, H 9.14 %

2. 2',4'',11-Tri-O-acetyl-8,9-anhydro-6[9]-hemiketal of erythromycin A (3).

(1) Compound **2** (560 mg) was acetylated at room temperature with 2 ml of acetic anhydride - pyridine mixture (1 : 1). After 72 hours, the solution was evaporated to dryness under reduced pressure, the residue was dissolved in a small amount of acetone and poured into aqueous $NaHCO_3$; 625 mg of compound **3**, m.p. 122~125°C, precipitated.

Anal. Calcd. for $C_{43}H_{71}NO_{15}$ (841.99) : C 61.33, H 8.50 %
Found : C 61.35, H 8.42 %

IR ($CHCl_3$): 3610, 3500 (OH), 1740 (CO), 1250 cm^{-1} (CH_3COO). NMR: 1.64 (s, 3H)-CH_3 at double bond; 2.15 (s, 3H), 2.19 (s, 6H)-3CH_3COO; 2.44 (s, 6H)-$N(CH_3)_2$; 3.43 (s, 3H)-OCH_3.

(2) 2',4'',11-Tri-O-acetylerythromycin A (0.5 g) and tetraacetylglucosyl bromide (0.41 g) were heated at 110°C for 2 hours in nitromethane (20 ml)-pyridine (5 ml) solution. After treatment as described in Paragraph 1, the crude product was separated on a silica gel column impregnated with formamide[12] (eluent, *n*-hexane - benzene - ethanol 65 : 30 : 5), yielding 250 mg of triacetate identical (TLC, IR) with the compound **3** described in Paragraph 2 (1).

3. N-Oxide of erythromycin A 8,9-anhydro-6⁹-hemiketal.

Compound **2** (1.125 g) was dissolved in CH_2Cl_2 (20 ml) and treated with an equivalent amount of perbenzoic acid in the same solvent. After 10 minutes, the mixture was shaken with aqueous dil. KOH. The CH_2Cl_2 layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. After treatment with a small amount of acetone, 960 mg (80 %) of N-oxide of compound **2**, m.p. 219~220°C, crystallized. IR (KBr): 3500 (OH), 1730 cm^{-1} (CO of lactone). NMR: 1.52 (s, 3H)-CH_3 at double bond; 3.15 (s, 6H)-$NO\cdot(CH_3)_2$; 3.32 (s, 3H)-OCH_3.

Anal. Calcd. for $C_{37}H_{65}NO_{13}$ (731.89) : C 60.71, H 8.95 %
Found : C 60.79, H 9.29 %

The N-oxide of compound **2** (36.6 mg) dissolved in 20 % methanol-water solution (2 ml) was treated with $NaIO_4$ (10.7 mg). After 12 hours at room temperature, the solution was diluted with methanol to 10 ml, freed from inorganic salts by filtration and its UV absorption examined. The extinction was $\varepsilon=130$ at 278 nm. One drop of concentrated HCl was added to the solution. After a few minutes, the extinction increased to 640 at 277 nm. Then several drops of concentrated aqueous ammonia were added and the extinction increased to 1170 at 274 nm.

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