mg of dry lithium bromide and 190 mg of dry lithium carbonate. The mixture was heated under reflux for 45 min, then cooled, and poured into dilute hydrochloric acid. The precipitate was filtered, washed, and dried, giving 124 mg of crude V. Chromatography on silica gel, elution with 2% ether–hexane, and crystallization from methylene chloride–methanol gave 38 mg of 16-dehydro-16-methylpregnenolone acetate (V): mp 170–172°; $\lambda_{max}^{MeOH}$ 251 mμ (ε 8600); infrared spectrum matches that of an authentic sample.

**Registry No.**—II, 983-23-3; III, 13116-47-7; IV, 13116-48-8; V, 982-06-9; VI, 13116-50-2; VII, 13116-51-3; VIII, 13143-64-1; IX, 13116-52-4.

**Acknowledgments.**—We thank Dr. E. B. Hershberg for helpful suggestions in the course of this work. We are indebted to Miss C. Federbush for carrying out the microbiological transformations.

# Chemistry of Erythronolide B. Acid-Catalyzed Transformations of the Aglycone of Erythromycin B

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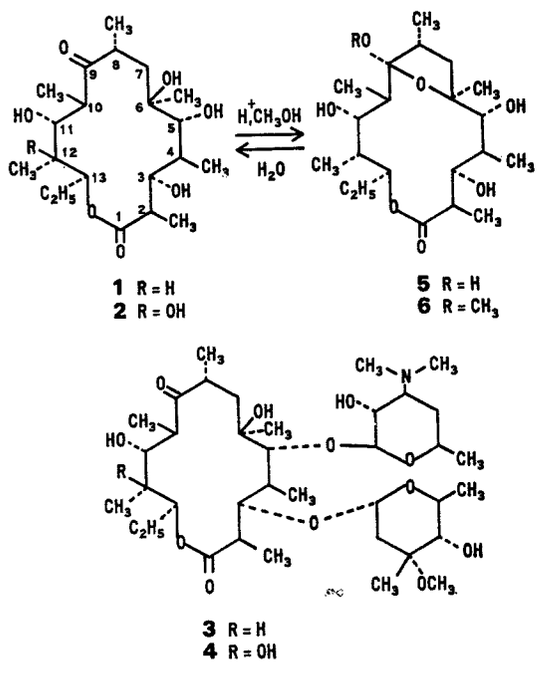
The treatment of erythronolide B and its derivatives with aqueous acid establishes an equilibrium between the hydroxy ketone and its corresponding 6,9-hemiketal or derived enol ether. An enol ether has been isolated from the treatment of triacetylerythronolide B. Further reaction of this enol ether with anhydrous acid caused elimination of the allylic acetoxy group giving a conjugated enol ether. Hydrolysis of this compound led to the formation of two anhydroerythronolide B derivatives, one of which was a member of the previously unknown 8-*epi*-erythronolide B series.

Erythronolide B (**1**)[1] is the 14-membered lactone portion of erythromycin B[4] (**3**), one of the macrolide family of antibiotics.[5] It has been shown to be an effective biological precursor of the erythromycins.[6] Even though erythromycin B has been shown to be more stable to acid than erythromycin A,[7] it is not possible to obtain erythronolide B by removal of the sugars, cladinose and desosamine, because the lactone ring is degraded during the severe hydrolysis procedure needed to remove the amino sugar. Similarly, erythronolide A (**2**) cannot be obtained from erythromycin A (**4**) because of extensive acid-catalyzed degradation.[8] Since we had available to us a large supply of erythronolide B from a fermentation procedure,[9] we decided to examine the nature of the acid-catalyzed degradation of this ring system (Scheme I).

It has been postulated that the acid-catalyzed degradation of erythromycin A involves the formation of a hemiketal bond between the carbonyl at C-9 and one of the tertiary hydroxyls at C-6 or C-12, followed by or concomitant with participation of the second tertiary hydroxyl giving a spiroketal.[8] Erythromycin B (and erythronolide B) does not have the tertiary hydroxyl at C-12, precluding the possibility of spiro ketal formation.[4] It should be possible, however, for a hemiketal bond to form between the carbonyl and the tertiary hydroxyl at C-6, and formation of the hemiketal **5** may be the first step in the acid-catalyzed degradation of the erythronolide B ring system. Since the ultraviolet absorption spectrum of erythronolide B contains an absorption maximum at 288 mμ (ε 39) due to the C-9 ketone, it was felt that the formation of a hemiketal could be followed by observing the disappearance of this peak.[10] Solutions were made of erythronolide B (1%) in methanol containing different concentrations of hydrochloric acid, and the absorbance of each solution at 288 mμ was recorded as a function of time. The rate of decrease in absorbance was very fast, even at low concentrations of acid. The half-life of the ketone function at room temperature in $10^{-4}$ *M* methanolic hydrochloric acid was slightly less than 4 min.

SCHEME I




(1) Stereochemistry is based on that determined for erythromycin A.[2,3] The structural formula used does not imply a particular conformation of the molecule but is merely a convenient planar representation of the 14-membered ring. Similarly, the double-bond geometry of the olefinic derivatives is not specified by the use of this planar structure.

(2) D. R. Harris, S. G. McGeachin, and H. H. Mills, *Tetrahedron Letters*, 679 (1965).

(3) W. D. Celmer, *J. Am. Chem. Soc.*, **87**, 1801 (1965).

(4) P. F. Wiley, M. V. Sigal, Jr., O. Weaver, R. Monahan, and K. Gerzon, *ibid.*, **79**, 6070 (1957).

(5) M. Berry, *Quart. Rev.* (London), **17**, 343 (1963).

(6) P. P. Hung, C. L. Marks, and P. L. Tardrew, *J. Biol. Chem.*, **240**, 1322 (1965).

(7) R. K. Clark, Jr., and M. Taterka, *Antibiot. Chemotherapy*, **5**, 206 (1955).

(8) P. F. Wiley, K. Gerzon, E. H. Flynn, M. V. Sigal, Jr., O. Weaver, U. C. Quarck, R. R. Chauvette, and R. Monahan, *J. Am. Chem. Soc.*, **79**, 6062 (1957).

(9) P. L. Tardrew and M. A. Nyman, U. S. Patent 3,127,315 (1964).

(10) This experiment was suggested by Dr. P. H. Jones. Preliminary observations of this effect were carried out by Dr. M. A. Nyman.

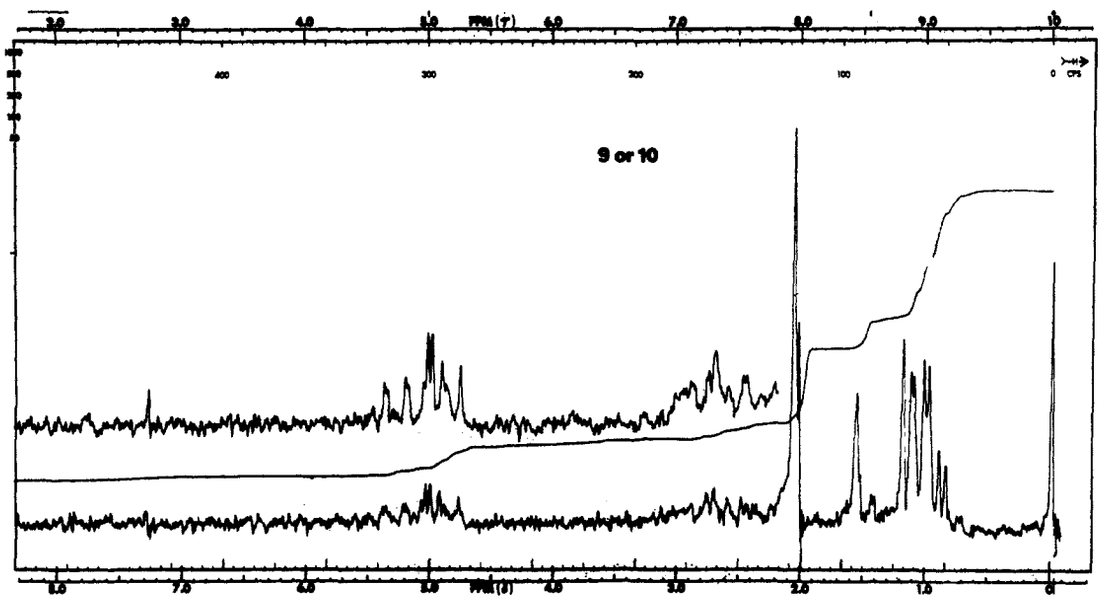



Figure 1.—Nmr spectrum of enol ethers 9 or 10.

The absorbance in all concentrations of methanolic acid solutions decreased to the same level which was approximately 5% of the original absorbance. When water was added to these solutions, the absorbance immediately increased to a new, higher level. The exact level depended upon the amount of water added. A solution of erythronolide B in 1:1 methanol–water containing $10^{-3}$ *M* hydrochloric acid was prepared, and the absorbance was observed to decrease slowly to a level which was 90% of the absorbance of erythronolide B in 1:1 methanol–water alone. When 1 drop of concentrated hydrochloric acid was added to the neutral standard solution, the absorbance quickly dropped to the level of the absorbance observed for the $10^{-3}$ *M* acid solution. Addition of more acid did not further reduce the absorbance. An equilibrium was evidently established between reactant and products which depended upon the concentration of water present but not on the concentration of acid.[11]

Attempts were made to isolate the hemiketal **5** (or its methyl ketal **6**) from the methanolic acid solution, but a complex mixture of products was isolated, and shown by thin layer chromatography (tlc) to contain no erythronolide B. The hydrolysis of this mixture with aqueous acid gave a 50% yield of erythronolide B, showing that no irreversible structural rearrangement occurred in the formation of at least half of the product material.

Since the secondary hydroxyls of erythronolide B were probably also involved in the acid-catalyzed reaction, attention was shifted to the study of the acid treatment of the triacetyl derivative **7**. The preparation of this compound was carried out by heating a pyridine solution of erythronolide B and acetic anhydride overnight at 100°. These fairly strenuous conditions were needed for complete acetylation because the secondary hydroxyls of erythronolide B were found to be somewhat unreactive. When **7** was refluxed with 0.01 *M* hydrochloric acid in aqueous alcohol, a new compound was isolated in 65% yield. This compound did not have the ketone absorption in the ultraviolet, nor the ketone or hydroxyl absorption in the infrared. It had the correct analysis for a compound formed by a loss of 1 mole of water from **7**. The nmr spectrum (Figure 1) contained a three-proton singlet at $\delta$ 1.55 due to a methyl group on a double bond. These data were consistent with either of the two structural formulations **9** or **10** which might be obtained by elimination of water from the hemiketal **8**.[12]

When the enol ether **9** or **10** was allowed to stand at room temperature in deuteriochloroform, the nmr spectrum was observed to change within 15 min. After the solution was allowed to stand overnight, a completely new nmr spectrum was obtained which showed the absence of the original compound, as evidenced by the complete loss of the methyl peak at $\delta$ 1.55. The odor of acetic acid was detected, and this was confirmed by an acetic acid methyl peak in the nmr spectrum. The observed reaction was apparently catalyzed by a trace of acid in the deuteriochloroform, for when the spectrum was obtained in deuteriochloroform which had been stored over sodium carbonate no change had occurred upon standing for 24 hr. This acid-catalyzed reaction was carried out on a preparative scale by refluxing a benzene solution of the enol ether with a catalytic amount of *p*-toluenesulfonic acid. A new compound was obtained in good yield and the nmr spectrum was identical with the spectrum obtained above. The compound had the correct analysis for the enol ether **9** or **10** minus 1 mole of acetic acid, and had no hydroxyl absorption in the infrared. The nmr spectrum (Figure 2) contained two peaks due to methyl groups attached to double bonds ($\delta$ 1.65 and 1.75), two peaks due to two acetyl methyls, and a multiplet due to an olefinic proton ($\sim\delta$ 5.2). This information indicated that the compound was the conjugated enol ether **11**. The ultraviolet spectrum contained a maximum at 254 m$\mu$ ($\epsilon$ 3100), showing that there had

(11) There was a possibility that the loss of ketone function was due to ketal formation with methanol alone, but this was ruled out by the observation that compound **15** (no C-6 hydroxyl) did not suffer any loss of ketone absorbance when dissolved in methanolic acid solution.

(12) Similar eliminations of hemiketals and ketals to give enol ethers have been observed with sapogenin-related compounds: H. Hirschmann and F. B. Hirschmann, *Tetrahedron*, **3**, 243 (1958).

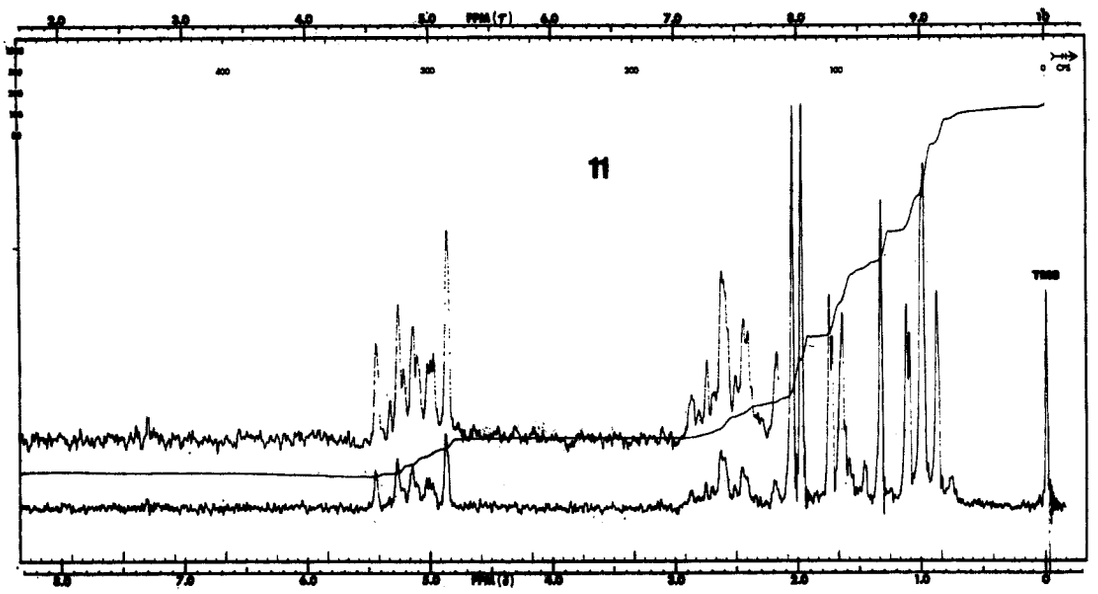
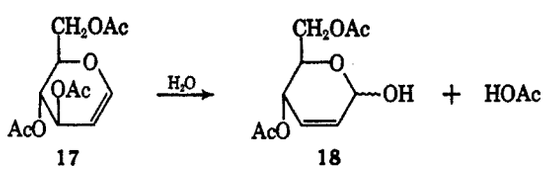



Figure 2.—Nmr spectrum of conjugated enol ether 11.

been no elimination of the C-3 acetoxy group $\beta$ to the carbonyl to give a conjugated lactone.

The position of this second double bond was also confirmed by hydrolysis of the conjugated enol ether **11** to the $\alpha,\beta$-unsaturated ketone **12**. The infrared spectrum of **12** showed the $\alpha,\beta$-unsaturated ketone absorption at 1655 $cm^{-1}$, while the ultraviolet spectrum showed a maximum at 232 m$\mu$ ($\epsilon$ 12,100). The nmr spectrum contained a peak at $\delta$ 1.78 due to the methyl attached to the conjugated double bond, and a doublet at 6.71 due to the conjugated olefinic proton. The hydrolysis of the enol ether to give **12** could be reversed by treatment of **12** with acid under anhydrous conditions. The re-formation of **11** from **12** showed that no structural change or isomerization had occurred in the hydrolysis reaction (Scheme II).

The reaction producing **11** by a facile elimination of the acetoxy group is somewhat analogous to the reaction of triacetyl-D-glucal **17** with water giving acetyl-D-pseudoglucal **18**.[13] This type of allylic rearrangement has also recently been reported for a furanose-related




**17** **18**

glycal.[14] These reactions were carried out without the presence of acid,[15] but it has been found that the presence of acid catalyzes this rearrangement with other compounds in the glycal series.[16,17]

The formation of the conjugated enol ether **11** may be formulated as an elimination of the protonated acetoxy group of **9** to form the allylic cation **19**, followed by loss of the C-8 proton (Scheme III). The preference for elimination of a proton from C-8 to give an enol ether rather than solvolysis of the carbonium ion (as in the glycal series) may be due to the presence of steric interactions between substituents on the five-membered ring when C-9 is tetrahedral. The interactions between substituents on C-9 and C-8 are relieved by going to the enol ether even though this structure appears to be somewhat strained.

The extreme ease of the acetic acid elimination under very mild conditions is consistent with the intervention of the stable allylic oxonium ion intermediate **19** formed *via* a transition state in which there is considerable charge delocalization of the type present in **19**.

Although this facile reaction is accommodated by a mechanism proceeding through the enol ether structure **9**, a definite structural assignment to the isolated enol ether is precluded by the possibility of a ready interconversion of **9** and **10** *via* a prototropic shift in the reaction medium. At present there is insufficient evidence to make a choice between structures **9** and **10**, but it is hoped that a detailed analysis of 100-Mc nmr spectra[18] may allow an unehuivocal assignment.

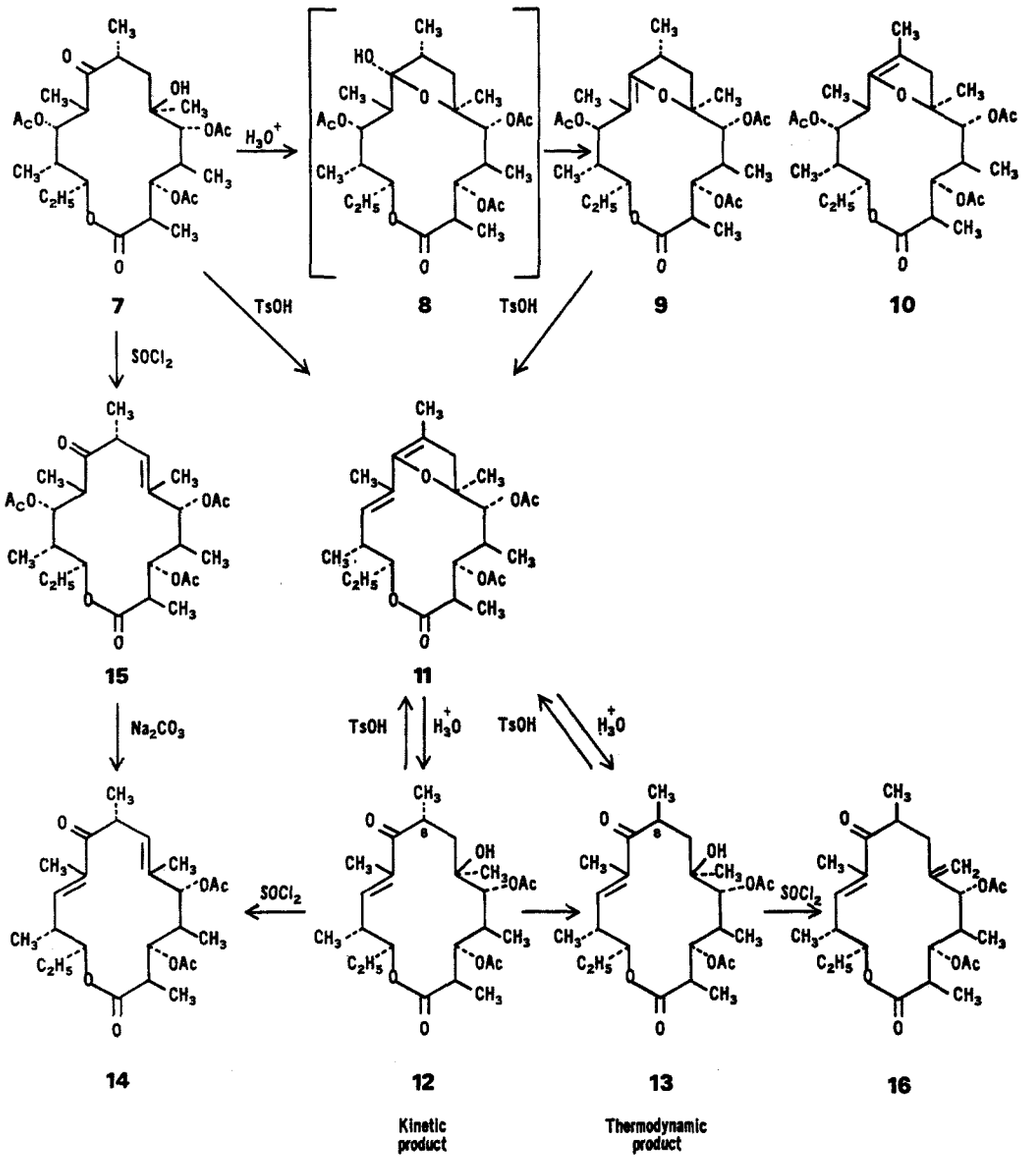
The conjugated enol ether **11** could also be obtained in good yield directly from triacetylerythronolide B, **7** under the conditions used for the conversion of the enol ether **9** or **10** to **11**. Evidently, under these anhydrous acid conditions, when any of the enol ether is formed in the reaction it immediately undergoes elimination to the conjugated enol ether.

It was somewhat surprising that the elimination of acetic acid from **9** or **10** which occurred so readily under anhydrous conditions, did not occur at all in the aqueous acid treatment. In fact, when the enol ether was dissolved in a 0.02 *M* aqueous hydrochloric acid solution and heated for a short time, the product obtained was found to consist mainly of starting material along with some triacetylerythronolide B. Thus, in

(13) B. Helferic, *Advan. Carbohydrate Chem.*, **7**, 209 (1952).
(14) R. K. Ness and H. G. Fletcher, Jr., *J. Org. Chem.*, **28**, 435 (1963).
(15) R. J. Ferrier, *J. Chem. Soc.*, 5443 (1964).
(16) R. J. Ferrier, W. G. Overend, and G. H. Sankey, *ibid.*, 2830 (1965).
(17) R. U. Lemieux, D. R. Lineback, M. L. Wolfrom, F. B. Moody, E. G. Wallace, and F. Komitsky, Jr., *J. Org. Chem.*, **30**, 1092 (1965).

(18) A detailed examination of the nmr spectra of the compounds in this series is being carried out in collaboration with Dr. M. Levenberg and R. Egan using the Varian HA-100 instrument with attached spin decoupler. Preliminary results show that the coupling constants of many of the ring protons remain fairly constant throughout the series. This indicates that the erythronolide B ring system has a stable conformation which is particularly favorable even in solution. A possible conformation of the macrolide antibiotic oleandomycin (and by analogy, erythromycin) has been suggested by Celmer.[19]
(19) W. D. Celmer, *Antimicrobial Agents Chemotherapy*, 144, (1965).

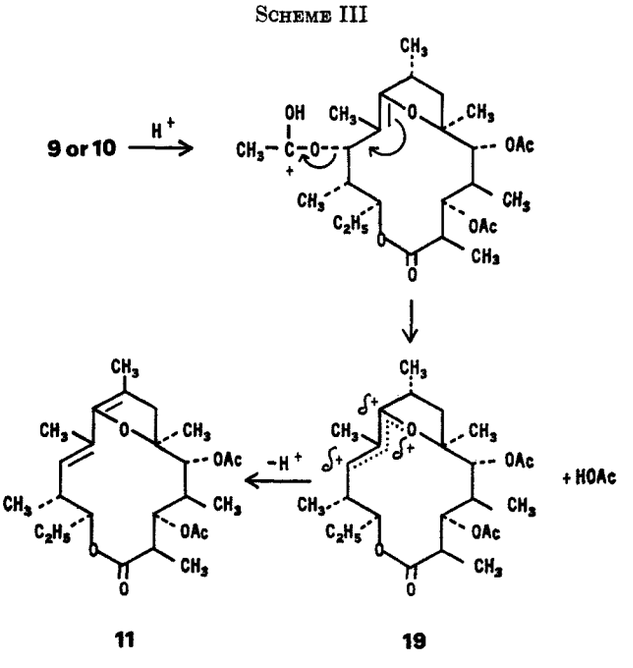
SCHEME II




aqueous acid, there must be an equilibrium established between the hydroxy ketone **7** and the enol ether **9** or **10**. With triacetylerythronolide B, the equilibrium in *aqueous* acid lies on the side of the enol ether whereas with erythronolide B, the ultraviolet study discussed earlier showed that in *aqueous* acid, the equilibrium lies on the side of the hydroxy ketone. No compound corresponding to the hemiketal intermediate **8** was ever isolated or seen on thin layer plates. In an attempt to effect the transformation **9** or **10** → **11** in aqueous acid, the enol ether was treated with more concentrated (0.1 *M*) hydrochloric acid solution. The product obtained was mainly starting material, but thin layer chromatography showed the presence of the unsaturated ketone **12**. This most probably resulted from hydrolysis of a small amount of conjugated enol ether **11** which had formed in the reaction. The conversion of **11** to **12** occurs readily under these condition.

As mentioned above, the conjugated enol ether **11** was hydrolyzed with aqueous acid to give the conjugated ketone **12**. It seemed likely that the first step

SCHEME III



in this hydrolysis was the protonation at C-8, and thus the two C-8 epimers of **12** might be formed, even though only one isomer was isolated. Examination of the crude reaction product by thin layer chromatography showed that two compounds different from starting material were indeed present. These studies also showed that, when the reaction was allowed to continue for longer periods of time, the amount of the new, minor compound increased at the expense of the other major one. This indicated that the isolated major compound was the kinetically favored product of the reaction, but that the minor compound was the thermodynamically more stable isomer. That this was the case was shown by the treatment of the major isomer **12** with 0.1 *M* aqueous acid over an 11-day period. It was converted in 60% yield to the second isomer **13**. This isomer (**13**) could also be obtained directly from the conjugated enol ether **11** by prolonged aqueous acid treatment. Compounds **12** and **13** were shown to be isomeric by their elemental analyses, and by comparison of their infrared, ultraviolet, and nmr spectra. The infrared spectrum of each compound showed absorption peaks due to an $\alpha,\beta$-unsaturated ketone, a lactone carbonyl, and a hydroxyl group, and the ultraviolet spectrum contained a maximum due to the $\alpha,\beta$-unsaturated ketone. The nmr spectrum of each contained peaks corresponding to a methyl on a double bond, and a conjugated vinyl proton. Based on their mode of formation it was assumed that these compounds are epimeric at C-8. Confirmatory evidence that these compounds are C-8 epimers was obtained by treating the isomer **13** with *p*-toluenesulfonic acid in benzene. The product of this reaction was the same conjugated enol ether **11** from which **12** and **13** were derived. The formation of **11** from both **12** and **13** not only shows that no structural rearrangement occurred in the epimerization reaction, but also clearly shows that the two isomers must be epimeric at C-8 since this is the only carbon atom that loses its asymmetry in the formation of **11**.

The stereochemistry of the epimers **12** and **13** at C-8 was determined by a high-dilution infrared absorption study. The spectrum of **12** as a 0.0025 *M* solution shows absorption bands due to both free and intramolecular hydrogen-bonded hydroxyls. The spectrum of **13** shows an intense, free hydroxyl band but only a trace of intramolecular hydrogen-bonded hydroxyl absorption. Models show that the intramolecular hydrogen bond of **12** is between the C-6 hydroxyl and the C-9 carbonyl. The lack of the hydrogen-bonded hydroxyl absorption in the spectrum of **13** is due to the steric interference of the methyl group at C-8 with formation of this intramolecular bond, and is consistent with a *cis*-1,3 relationship of the C-8 methyl and C-6 hydroxyl.

These stereochemical results show that epimer **12** has the same stereochemistry at C-8 as does erythronolide B and thus belongs to the "normal" erythromycin series. Epimer **13** is the first known member of the C-8 "*epi*" erythromycin series.[20]

Epimer **12** was correlated directly with erythronolide B by its conversion to a compound derived from triacetylerythronolide B by another route. Treatment of **12** with thionyl chloride in pyridine effected endocyclic elimination of the tertiary C-6 hydroxyl giving the diene **14**. This same compound could be obtained from **7** by a two-step synthesis. Treatment of **7** with thionyl chloride in pyridine gave a high yield of the anhydro derivative **15**. Treatment of **15** with 5% sodium carbonate in aqueous ethanol effected $\beta$ elimination of the C-11 acetoxy group to give **14**, identical in all respects with the compound obtained above. The evidence for the endocyclic double bond in compounds **14** and **15** was obtained from the nmr spectra. The spectrum of **14** contained doublets at $\delta$ 1.55 and 1.76 due to the methyls on the unconjugated and conjugated double bonds, and doublets at 5.12 ($J_{7,8}$ = 10.5 cps) and 6.38 ($J_{11,12}$ = 9 cps) due to the C-7 unconjugated and C-11 conjugated vinyl protons. The spectrum of **15** contained a doublet at $\delta$ 1.63 due to the methyl on the double bond, and a doublet at $\delta$ 5.05 ($J_{7,8}$ = 11 cps) due to the vinyl proton at C-7.

In contrast to **12**, epimer **13** did not undergo endocyclic elimination of the tertiary hydroxyl when treated with thionyl chloride in pyridine. The nmr spectrum of the product contained a multiplet at $\delta$ 5.10 due (in part) to two vinyl protons and a doublet at 6.34 due to the conjugated vinyl proton. The spectrum also contained a peak at $\delta$ 1.87 due to the methyl on the conjugated double bond, but a peak corresponding to a methyl on the unconjugated double bond was absent. This indicated that the elimination was exocyclic giving the anhydro derivative **16**. This was confirmed by the presence of an exocyclic methylene absorption peak in the infrared at 3080 $cm^{-1}$.[21] Evidently the change in stereochemistry at C-8 causes a change in the conformation of this part of the ring and makes the transition state leading to the endocyclic double bond less favorable than that leading to the exocyclic double bond.

The fact that the same dianhydro derivative **14** was obtained from both **7** and **12** is evidence that the stereochemical as well as structural integrity of this system was maintained in the reaction sequence **7** → **15** → **14**. Further evidence for this was obtained by comparison of the nmr spectra of **15** and **14**. The doublet corresponding to the vinyl proton at C-7 can be seen clearly in the nmr spectra of both compounds. The chemical shift and coupling constant of this proton with the proton at C-8 are remarkably constant in both spectra.[18] This indicates that there has been no change in the basic geometry of this part of the lactone ring and argues against epimerization at C-8 during the $\beta$-acetoxy elimination.

Special note should be made of the unusual chemical and physical characteristics of some of the compounds obtained. The ultraviolet absorption spectra of compounds **12**, **13**, **14**, and **16** each contain a maximum due to the $\alpha,\beta$-unsaturated ketone at a somewhat shorter wavelength (228–232 m$\mu$) than was predicted (237 m$\mu$).[22] The values agree quite well, however, with the shorter wavelengths observed for similarly substituted cyclopentenones.[23] This implies that the ground-state or excited-state energy of this system is dif-

(20) *Cf.* W. D. Celmer in "Biogenesis of Antibiotic Substances," Z. Vanek and Z. Hostalek, Ed., Academic Press Inc., New York, N. Y., 1965, p 106.

(21) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed, Methuen and Co. Ltd., London, 1960, p 34.

(22) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp. New York, N. Y., 1959, p 19.

(23) H. S. French, *J. Am. Chem. Soc.*, **74**, 514 (1952).

ferent from that of the corresponding cyclohexenones or open-chain, unsaturated ketones.[24]

The ultraviolet absorption spectrum of compound **15** contained a maximum at 286 mμ (ε 92). This is in the normal region for the $n \rightarrow \pi^*$ carbonyl transition, but the increased intensity indicates there is some interaction between the nonconjugated carbonyl and double-bond chromophores.[25,26] The double bond in this compound appears to be in an unusually stable position. It was not isomerized into conjugation with the ketone during the base-catalyzed preparation of **14** from **15**, and was unaffected by treatment with acid.[27] The double bond was also stable to catalytic hydrogenation in either neutral or acidic solvents.

## Experimental Section

Melting points were determined with a Fisher-Johns apparatus. Infrared spectra were obtained by Mr. W. H. Washburn and associates on a Perkin-Elmer Model 421 spectrophotometer. Nmr spectra were recorded by Mrs. R. Stanaszek and Mr. R. Kriese on a Varian A-60 instrument as deuteriochloroform solutions with tetramethylsilane as the internal standard. Ultraviolet spectra were obtained by Messrs. J. Sutherland and D. Williamson. Thin layer chromatography was carried out by Mrs. E. Baker and associates. Microanalyses were performed by Mr. O. Kolsto and staff.

**Treatment of Erythronolide B with Methanolic Hydrochloric Acid.**—Solutions of erythronolide B (1%) in methanolic hydrochloric acid were prepared in cuvettes, and the absorbance at 288 mμ was measured and recorded as a function of time by a Beckman DU spectrophotometer with an attached Gilford Model 2000 multiple sample absorbance recorder.

**Attempted Preparation of the Hemiketal 5.**—Erythronolide B (1.0 g) was dissolved in 25 ml of 0.01 *M* methanolic hydrochloric acid. The solution was allowed to stand at room temperature for 2 hr and was then neutralized with silver carbonate. The filtered solution was evaporated giving a colorless, glassy solid. Thin layer chromatography (benzene–methanol, 85:15) showed the presence of six components. The mixture was dissolved in hot ethanol–water and 1 drop of concentrated hydrochloric acid was added. The solution was heated for 1 hr then cooled to give 0.5 g of crystals, mp 217–220°. The infrared spectrum was identical with that of erythronolide B.

**3,5,11-Triacetylerythronolide B (7).**—Erythronolide B (10.0 g) was dissolved in 200 ml of anhydrous pyridine with 40 ml of acetic anhydride. The solution was heated on a steam bath for 16 hr, then cooled and poured into ~500 ml of ice–water. The solid obtained was recrystallized from 1:1 ethanol–water giving 9.5 g (73%) of colorless needles: mp 190–191°; $[\alpha]^{23}D$ −15° (*c* 1, $CH_3OH$); $\tilde{\nu}_{max}$ ($CHCl_3$) 1700, 1730, 3510, 3590 $cm^{-1}$; $\lambda_{max}$ 288 mμ (ε 26).

*Anal.* Calcd for $C_{27}H_{44}O_{10}$: C, 61.34; H, 8.39; O, 30.27. Found: C, 61.07; H, 8.48; O, 30.23.

**9,10-Anhydro-3,5,11-triacetylerythronolide B 6,9-Hemiketal (9) or 8,9-Anhydro-3,5,11-triacetylerythronolide B 6,9-Hemiketal (10).**—Triacetylerythronolide B (4.0 g) was dissolved in 130 ml of hot 0.01 *M* hydrochloric acid in 1:1 ethanol–water, and the solution was refluxed for 3 hr. The cooled solution gave 2.5 g (65%) of crystals: mp 164–165° $[\alpha]^{23}D$ +85° (*c* 1, $CH_3OH$); $\tilde{\nu}_{max}$ ($CHCl_3$) 1690 (sh), 1735 $cm^{-1}$.

*Anal.* Calcd for $C_{27}H_{42}O_9$: C, 63.51; H, 8.29; O, 28.20. Found: C, 63.45; H, 8.31; O, 28.23.

When this reaction was conducted over a longer period of time or with more concentrated acid (0.1 *M*), the crude material obtained after the isolation of the enol ether was found (tlc, diethyl ether–diisopropyl ether, 1:1) to be a mixture of compounds **12**, **13**, and the enol ether **9** or **10**.

When the enol ether **9** or **10** was dissolved in 0.02 *M* hydrochloric acid and the solution heated at 100° for 1.5 hr, the crude material obtained was found (tlc, chloroform–ethanol, 97:3) to consist of the enol ether as the major component and triacetylerythronolide B (**7**) as the minor component.

**8,9–10,11-Dianhydro-3,5-diacetylerythronolide B 6,9-Hemiketal (11). A. From the Enol Ether 9 or 10.**—Two crystals of *p*-toluenesulfonic acid monohydrate were dissolved in 50 ml of hot benzene (~$10^{-4}$ *M*), and 1.0 g of the enol ether was added. The solution was boiled for 1 hr, then cooled and shaken with portions of 0.1% sodium carbonate, water, and saturated sodium chloride solution. The benzene solution was evaporated and the viscous oil obtained was dissolved in hot ethanol–water. The cooled solution gave 0.6 g (68%) of colorless needles, mp 132–134°. A second recrystallization gave material: mp 135–136°; $[\alpha]^{26}D$ −71° (*c* 1, $CH_3OH$); $\tilde{\nu}_{max}$ ($CHCl_3$) 1675 (sh), 1735 $cm^{-1}$; $\lambda_{max}$ 254 mμ (ε 3100).

*Anal.* Calcd for $C_{25}H_{38}O_7$: C, 66.64; H, 8.50; O, 24.86. Found: C, 66.42; H, 8.45; O, 24.89.

**B. From 3,5,11-Triacetylerythronolide B (7).**—A 7.5-g amount of **7** was added to 125 ml of benzene containing a few crystals of *p*-toluenesulfonic acid monohydrate, and the solution was stirred and refluxed for 1 hr while the water produced was separated with a Dean–Stark trap. The benzene solution was worked up as above to give 4.5 g (70%) of crystals, mp 133–135°. The infrared spectrum and tlc analysis (benzene–diethyl ether–diisopropyl ether, 1:1:1) showed that this material was identical with **11** obtained in procedure A.

**C. From 3,5-Diacetyl-10,11-anhydroerythronolide B (12).**—A 100-mg amount of **12** was dissolved in benzene containing a crystal of *p*-toluenesulfonic acid monohydrate and the solution was heated on a steam bath for 2 hr. The solution was worked up as above to give 50 mg of crystals with mp 132–133.5°. The infrared spectrum showed it to be identical with **11** obtained in procedure A.

**D. From 3,5-Diacetyl-10,11-anhydro-8-*epi*-erythronolide B (13).**—A 300-mg amount of **13** was dissolved in benzene containing two crystals of *p*-toluenesulfonic acid monohydrate, and the solution was heated on a steam bath for 30 min. The solution was worked up as above to give 150 mg of crystals, mp 131–134°. Material from a second recrystallization had mp 132–134°. The infrared spectrum and tlc analysis showed that this material was identical with **11** obtained in procedure A.

**3,5-Diacetyl-10,11-anhydroerythronolide B (12).**—A 2.0-g amount of the conjugated enol ether **11** was dissolved in 60 ml of hot 0.01 *M* hydrochloric acid in 1:1 ethanol–water, and the solution was heated at steam-bath temperature for 1.5 hr. The crystalline product obtained from the cooled solution amounted to 1.6 g (77%), mp 202–204°. A second recrystallization gave material: mp 205–206°; $[\alpha]^{25}D$ +50° (*c* 1, $CH_3OH$); $\tilde{\nu}_{max}$ ($CHCl_3$) 1655, 1725, 3500, 3595 $cm^{-1}$; $\tilde{\nu}_{max}$ (0.0025 *M* $CCl_4$) 3530, 3600 $cm^{-1}$; $\epsilon_b/\epsilon_f$ = 0.6; $\lambda_{max}$ 232 mμ (ε 12,100). The nmr spectrum contained peaks at δ 1.78 ($CH_3C{=}C$), 2.02, 2.16, (2 $CH_3COO$), and 6.71 (vinyl proton).

*Anal.* Calcd for $C_{25}H_{40}O_8$: C, 64.08; H, 8.60; O, 27.32. Found: C, 63.95; H, 8.57; O, 27.51.

**3,5-Diacetyl-10,11-anhydro-8-*epi*-erythronolide B (13).**—A 1.0-g amount of **12** was dissolved in 80 ml of 0.1 *M* hydrochloric acid in 2:1 ethanol–water. The solution was allowed to stand at room temperature for 13 days, then was reduced to approximately one-half volume. The material obtained (0.6 g) was recrystallized twice giving crystals: mp 164–165°; $[\alpha]^{25}D$ +2.1° (*c* 1, $CH_3OH$); $\tilde{\nu}_{max}$ 1640 (sh), 1660, 1735, 3500 (sh), 3600 $cm^{-1}$; $\tilde{\nu}_{max}$ (0.0025 *M* $CCl_4$) 3530, 3600 $cm^{-1}$, $\epsilon_b/\epsilon_f$ = 0; $\lambda_{max}$ 228 mμ (ε 13,900). The nmr spectrum contained peaks at δ 1.87 ($CH_3C{=}C$), 2.08 (2 $CH_3COO$), and 6.30 (vinyl proton).

*Anal.* Calcd for $C_{25}H_{40}O_8$: C, 64.08; H, 8.60; O, 27.32. Found: C, 64.22; H, 8.69; O, 27.38.

This compound could also be prepared directly from the conjugated enol ether **11** by conducting the hydrolysis in 0.1 *M* hydrochloric acid and allowing the solution to stand for 11–13 days.

**3,5-Diacetyl-6,7-10,11-dianhydroerythronolide B (14). A. From 3,5-Diacetyl-10,11-anhydroerythronolide B (12).**—A solution of **12** (0.7 g) in 20 ml of anhydrous pyridine was stirred at 0° while a solution of 0.7 ml of thionyl chloride in 5 ml of pyridine was added dropwise. Stirring was continued for 45 min; then the solution was poured onto cracked ice. This cold mixture was allowed to stand for a few hours, and the crystalline precipitate was collected and washed with water to give 0.55 g. A recrystallization from ethanol–water gave 0.4 g (60%) of jagged crystals:

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mp 170–171°; $[\alpha]^{26}D$ +6.4° (*c*, 1 $CH_3OH$); $\bar{\nu}_{max}$ 1645 (sh), 1660, 1730 $cm^{-1}$; $\lambda_{max}$ 229 mμ (ε 12,000).

*Anal.* Calcd for $C_{25}H_{38}O_7$: C, 66.64; H, 8.50; O, 24.86. Found: C, 66.45; H, 8.75; O, 24.78.

**B. From 3,5,11-Triacetyl-6,7-anhydroerythronolide B (15).**—A 1.0-g amount of **15** was dissolved in 25 ml of ethanol and 25 ml of 5% sodium carbonate solution was added. The cloudy solution was heated to reflux for 45 min then stirred at room temperature for 3 hr. Concentration of the solution gave a solid which was collected and recrystallized yielding 0.35 g, mp 168–170°. Material from a second recrystallization had mp 170–171°. This compound was shown to be identical with **14** obtained in procedure A by a mixture melting point determination, infrared spectrum, and thin layer chromatographic comparison (carbon tetrachloride–ether–ethyl acetate, 8:1:1).

**3,5,11-Triacetyl-6,7-anhydroerythronolide B (15).**—A solution of 3,5,11-triacetylerythronolide B (**7**, 5.0 g) in 50 ml of anhydrous pyridine was stirred at 0° while a solution of 5 ml of thionyl chloride in 25 ml of pyridine was added dropwise. When the addition was complete, the reaction was stirred at 0° for 1 hr then poured onto cracked ice. The mixture was allowed to stand overnight in the cold, and the fluffy solid obtained was collected and washed with water to give 4.3 g (89%), mp 147–148.5°. A recrystallization from ethanol–water gave long needles: mp 148–149°; $[\alpha]^{23}D$ −69° (*c* 1, $CH_3OH$); $\bar{\nu}_{max}$ 1710, 1735 $cm^{-1}$; $\lambda_{max}$ 286 mμ (ε 92).

*Anal.* Calcd for $C_{27}H_{42}O_9$: C, 63.51; H, 8.29; O, 28.20. Found: C, 63.77; H, 8.18; O, 28.02.

**3,5-Diacetyl-6-deoxy-6-demethyl-6-methylene-10,11-anhydro-8-*epi*-erythronolide B (16).**—A solution of 3,5-diacetyl-10,11-anhydro-8-*epi*-erythronolide B (**13**, 0.4 g) in 10 ml of anhydrous pyridine was stirred at 0° while a solution of 0.5 ml of thionyl chloride in 5 ml of pyridine was added dropwise. Stirring was continued for 50 min; then the solution was poured onto cracked ice. The mixture was allowed to stand in the cold overnight, and the crystalline solid (0.25 g) which formed was collected, washed with water, and recrystallized from ethanol–water to give tabular crystals: mp 133–134°; $[\alpha]^{26}D$ +66° (*c* 0.7, $CH_3OH$); $\bar{\nu}_{max}$ 1640, 1670, 1740, 3080 $cm^{-1}$; $\lambda_{max}$ 231 mμ (ε 13,500).

*Anal.* Calcd for $C_{25}H_{38}O_7$: C, 66.64; H, 8.50; O, 24.86. Found: C, 66.72; H, 8.71; O, 24.96.

**Registry No.**—**1**, 3225-82-9; **7**, 13143-78-7; **9**, 13135-38-1; **10**, 13118-61-1; **11**, 13143-79-8; **12**, 13118-62-2; **13**, 13143-80-1; **14**, 13118-63-3; **15**, 13118-64-4; **16**, 13118-65-5.

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# The Absolute Configuration of Sarkomycin[1]

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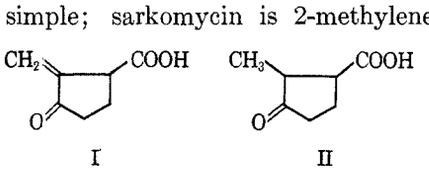
Analysis of the mixture resulting from Mannich condensation on ethyl 3-ketocyclopentanecarboxylate shows that reaction occurs at both positions α to the ketone. As a result, a published assignment of absolute configuration of the antitumor antibiotic sarkomycin, based on the assumption that the Mannich reaction occurs specifically at C-2, is shown to be invalid. Unambiguous proof of configuration is provided by relating (+)-dihydrosarkomycin to standards of absolute configuration in two independent ways. In the first, dihydrosarkomycin is converted to (1*R*;2*R*)-1,2-dimethylcyclopentane, which is in turn prepared from (*R*)-(+)-pulegone. In the second, the Wolff–Kishner reduction product of dihydrosarkomycin is related to (*R*)-(+)-3-methylcyclohexanone. These results establish the (*R*) configuration for sarkomycin, in contrast to the previous assignment.

In 1953 Umezawa, *et al.*,[2] discovered that *Streptomyces erythrochromogenes*, a soil microorganism found in Japan, produces an antibiotic, sarkomycin, which possesses a powerful inhibitory effect on Ehrlich ascites tumors in mice. Subsequent pharmacological studies[3] revealed that sarkomycin caused specific destruction of tumor cells, and a preparation of this substance is now marketed[4] in Japan as a prescription drug against cancer. Sarkomycin selectively inhibits DNA synthesis; it has been suggested that the site of inhibition is DNA polymerase, probably at the sulfhydryl group.[3b]

Hooper and co-workers[5] at the Bristol Laboratories showed that the structure of this antibiotic was surprisingly simple; sarkomycin is 2-methylene-3-ketocyclopentanecarboxylic acid (I). Synthetic support was provided by showing that dihydrosarkomycin was identical[5–7] with the known[8,9] 2-methyl-3-ketocyclopentanecarboxylic acid (II), and several syntheses of sarkomycin itself have subsequently been reported.[10–12]




I II

Sarkomycin possesses a single asymmetric center and is levorotatory.[5,13] Because of the influence of optical configuration on biological activity, it was clearly of interest to determine the absolute configuration of

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