52. Oxidation and Reduction of 8,9-Anhydroerythronolide B 6,9-Hemiacetal

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Zusammenfassung. Die Reaktion von Erythronolid B mit Säure lieferte das bisher unbekannte 8,9-Anhydroerythronolid-B-6,9-hemiacetal. Diese Verbindung wurde oxydativ in (8*S*,9*S*)-8,9-Anhydroerythronolid-B-6,9-hemiacetal 8,9-epoxid und (8*S*)-8-Hydroxyerythronolid B umgewandelt. Durch Reduktion der Doppelbindung im Enoläther wurde das (9*S*)-9-Dihydro-6,9-O-anhydro-erythronolid B erhalten.

During the conversion of erythronolide B (**1**) [1], the aglycone of erythromycin B[1]), to the corresponding oxime **2** with hydroxylamine hydrochloride and barium carbonate in methanol [3], an anhydro compound $C_{21}H_{36}O_6$ (**3a**) was consistently isolated. The reaction of **1** with hydroxylamine hydrochloride in methanol also gave **3a** in excellent yield, thus suggesting that **3a** was a product of acid degradation of **1**. This was confirmed when **3a** was prepared from **1** in acetic acid solution at room temperature [4]. In view of *Perun*'s work [2] it was of interest to investigate the structure of **3a**.

The structure of **3a** was determined from its 100 MHz NMR. spectra. The resonance of a vinyl-methyl group was evident at 1.59 ppm (δ) in the pyridine-d_5 solution spectra of **3a**. The absence of vinyl proton resonances between 6.0 and 8.0 ppm and the presence of a singlet methyl resonance at 1.48 ppm attributable to the tertiary C-6 methyl group eliminated the anhydroerythronolide B structures resulting from simple dehydration of a hydroxyl group. The vinyl-methyl resonance appeared as a triplet due to coupling with two allylic protons (J = 1.2 Hz). Irradiation of this resonance in a spin decoupling experiment located the resonances of the allylic protons as an AB quartet, the doublets of which were centered at 3.04 and 2.15 ppm, J_{AB} = 15.7 Hz. The magnitude of the coupling constant suggested that the interacting protons were geminal. Since the only available methylene group was that at C-7, the structure of the dehydration product was established as 8,9-anhydroerythronolide B 6,9-hemiacetal (**3a**). The remaining parameters determined for **3a** and the derived triacetate **3b** (Table) were in complete agreement with the structural assignments.

The definitive structural assignment of 8,9-anhydroerythronolide B 6,9-hemiacetal eliminated the structural ambiguity of a previously prepared triacetyl enol ether [2]. The hemiacetal **3a** was converted to the triacetate **3b** which proved to be identical with the compound prepared by *Perun* [2] by a different route. The enol ether structure **3a** was further confirmed by its conversion to **1** by treatment with aqueous methanolic hydrochloric acid.

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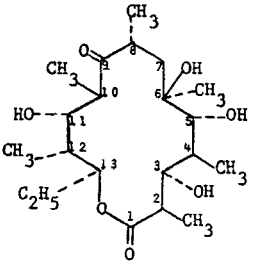
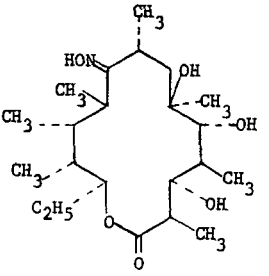
[1]) *T. J. Perun* [2] discussed the pertinent literature in his paper.

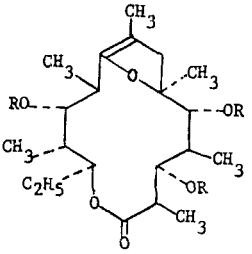
Two peaks in the mass spectrum of **3a** furnished additional support for the structural assignment. The structures of the ions were postulated on the basis of their possible formation from the molecular ion (M^+, *m/e* 384, 20% of base peak) and confirmed by determination of their molecular formulae by high resolution peak matching. A peak observed at *m/e* 287 (**a**, $C_{15}H_{27}O_5$, 10.5%) arose by cleavage of the 5,6- and 9,10-bonds with hydrogen transfer from the charged fragment to the neutral furan derivative. The sequential loss of two molecules of water from **a** gave rise to ions at *m/e* 269 ($C_{15}H_{25}O_4$, 5.1%) and *m/e* 251 ($C_{15}H_{23}O_3$, 4.7%), and the metastable ions at *m/e* 252.0 ($269^2/287 = 252.1$) and *m/e* 234.3 ($251^2/269 = 234.2$), respectively. This unusual cleavage was consistent with the location of the enolic double bond of **3a** between carbons 8 and 9. The ion $C_{14}H_{23}O_2$ (**b**, *m/e* 223, 11.9%) arose by cleavage of



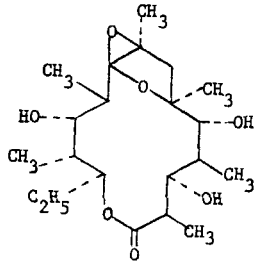
1



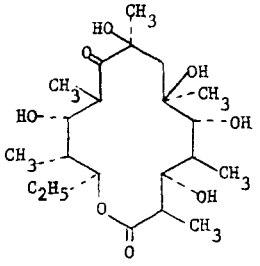
2



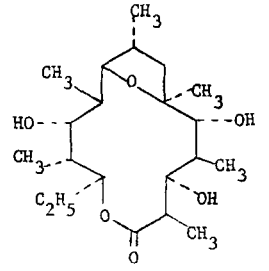
3a, R = H
3b, R = CH_3CO



4

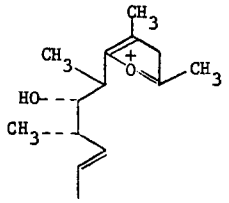


5



6

the 5,6-bond of the *McLafferty* rearrangement product of the molecular ion[2]) [5]. The loss of water from **b** afforded an ion at *m/e* 205 ($C_{14}H_{21}O$, 4.8%). Other fragments which were indicated in the mass spectrum of **3a** arose from α-cleavages of the carbon-carbon bonds next to the hydroxyl groups of the *McLafferty* rearrangement ion [5] formed from **3a**.



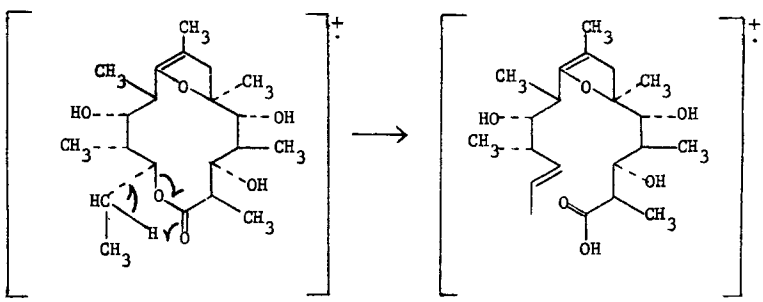
b

The mass spectrum of the triacetate **3b** was quite similar to that of the parent triol **3a**, with the important difference that the ion corresponding to **a** ($M - 97$) was not prominent. Likewise, the ions arising from the losses of one and two molecules of acetic acid from the $M - 97$ fragment gave rise to only minor peaks.

In view of the possible applicability of the above reaction to the erythromycin antibiotics, oxidative and reductive reactions of the intermediate **3a** were of interest. The hydrogen peroxide – osmium tetroxide oxidation [6] of **3a** led to the isolation of two new compounds. The first of these was identical with the *m*-chloroperbenzoic acid oxidation [7] product of **3a**, 8,9-anhydroerythronolide B 6,9-hemiacetal 8,9-epoxide (**4**). Based on the structure of the starting material **3a** and the microanalytical results, the second compound, **5**, was tentatively formulated as an 8-hydroxy-erythronolide B. The reduction of the double bond of **3a** was accomplished using a large amount of platinum, yielding 9-dihydro-6,9-O-anhydroerythronolide B (**6**). Supporting evidence for the structural assignments to the new compounds **4**, **5** and **6** was obtained from the studies of their NMR. and mass spectra.

The complete analysis of the NMR. spectra of **4** and **5** revealed the parameters collected in the Table. The absence of a vinyl-methyl resonance with allylic coupling in the spectra of both **4** and **5** confirmed the oxidation of the enol ether double bond. The introduction of an additional oxygen substituent was evidenced by the presence of two singlet methyl resonances (6-CH_3 and 8-CH_3) and the decrease of the number of multiplet methyl resonances by one from those present in **1**. The C-7 methylene proton resonances appeared as a simple *AB* quartet indicating that the substitution

[2]) The *McLafferty* rearrangement of the molecular ion of **3a** may be formulated as follows:



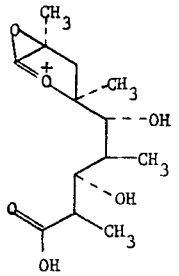
had occurred at C-8. Since the resonances of four protons remained in the 3.5–6.0 ppm region, the number of protons geminal to oxygen substituents was not altered.

The number and type of hydroxyl protons, as determined from the NMR. spectra before D_2O exchange, allowed differentiation of **4** and **5**. Five hydroxyl proton resonances were visible in the pyridine-d_5 solution spectra of **5**, three doublets (6.28, 5.81 and 4.09 ppm) arising from secondary hydroxyl protons coupled to the vicinal protons at C-3, C-5 and C-11, and two singlet hydroxyl resonances (5.66 and 5.25 ppm) assigned to the tertiary hydroxyl protons at C-6 and C-8. Only three hydroxyl proton resonances were visible in the dimethyl-d_6 sulfoxide spectra of **4** (4.29, 4.14 and 3.47 ppm). Consideration of these data and those collected in the Table confirmed the assigned structures of **4** and **5** and ruled out alternate possibilities.

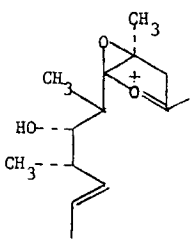
The absence of either vinyl-methyl or vinyl proton resonances in the NMR. spectra of **6** clearly indicated that the double bond had been reduced. The presence of a singlet methyl resonance at 1.37 ppm revealed that the oxygen substituent at C-6 was still present. Three doublet hydroxyl proton resonances at 4.90, 5.53 and 6.04 ppm, and the absence of an additional singlet hydroxyl proton resonance ruled out the presence of a tertiary hydroxyl group at C-6. The observation of five proton resonances downfield of 3.5 ppm, arising from H-(3), H-(5), H-(9), H-(11) and H-(13), completed the proof of structure **6**.

The most abundant ion above *m/e* 200 in the mass spectrum of **4** was at *m/e* 275 (**c**, $C_{13}H_{23}O_6$, 9.9%) which arose from the *McLafferty* rearrangement product of the molecular ion [5] by α-cleavage of the 9,10-bond, accompanied by cleavages or transannular cleavages in the epoxide region and transfer of two hydrogen atoms from the neutral fragment [8]. The sequential loss of two molecules of water from **c** resulted in fragments at *m/e* 257 ($C_{13}H_{21}O_5$, 7%, *m** at *m/e* 240.0, $257^2/275 = 240.2$) and *m/e* 239 ($C_{13}H_{19}O_4$, 2%, 60% of doublet). An important ion **d** at *m/e* 273 ($C_{13}H_{21}O_6$, 1.2%) arose from the α-cleavage of the 9,10-bond of the *McLafferty* rearrangement product of the molecular ion [5]; a fragment **e** at *m/e* 239 ($C_{14}H_{23}O_3$, 1.3%, 40% of doublet) was caused by α-cleavage of the 5,6-bond of the rearranged molecular ion.

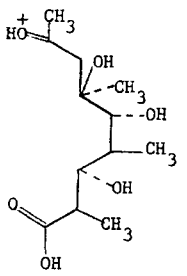
The ion **d** was of particular importance since it furnished evidence that the epoxide function in **4** was attached to C-8 and C-9, which in turn confirmed the location of the double bond in the enol ether **3a**. Ion **d** was formulated with the ether oxygen carrying the positive charge rather than the epoxide oxygen in view of the unusually low basicity of epoxides as hydrogen-bond acceptors and the higher ionization potential of ethylene oxide as compared to that of dimethyl ether [9].



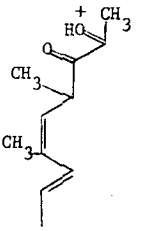
d



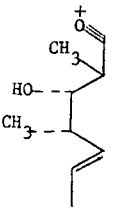
e



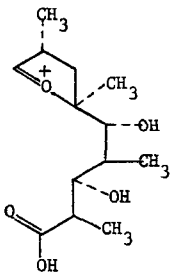
f



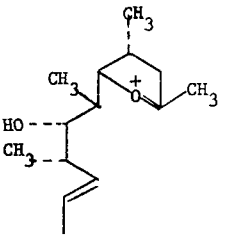
g



h



i



j

Among the cleavage products resulting from the electron impact fragmentation of **5**, five ions strongly supported its structural assignment. Three peaks were caused by fragments which were derived from the ion **f** ($C_{12}H_{23}O_6$, *m/e* 263, weak signal which could not be peak matched) by the sequential loss of one, two and three molecules of water, giving rise to fragments at *m/e* 245 ($C_{12}H_{21}O_5$, 2.4%), *m/e* 227 ($C_{12}H_{19}O_4$, 5.9%, *m** at *m/e* 210.2, $227^2/245 = 210.3$), and *m/e* 209 ($C_{12}H_{17}O_3$, 10.8%, *m** at *m/e* 192.5, $209^2/227 = 192.4$), respectively. The ion **g** (*m/e* 181, $C_{11}H_{17}O_2$, 4.1%) resulted from the α-cleavage of the 7,8-bond of the dehydrated *McLafferty* rearrangement product of the molecular ion [5], and the ion **h** (*m/e* 155, $C_9H_{15}O_2$, 9.0%) arose from α-cleavage of the 8,9-bond of the *McLafferty* rearrangement product of the molecular ion [5]. The observation of these ions strongly supported the formulation of **5** as an 8-hydroxy-9-ketone and in turn furnished further evidence for the assignment of the double bond to the 8,9-position in **3a**.

The mass spectrum of the reduction product **6** revealed ions which arose by α-cleavage of the 9,10- and 5,6-bonds of the *McLafferty* rearrangement product of the molecular ion [5]. The signal recorded at *m/e* 259 (**i**, $C_{13}H_{23}O_5$, 11.5%) was caused by a fragment which sequentially lost one, two and three molecules of water, giving rise to peaks at *m/e* 241 ($C_{13}H_{21}O_4$, 29.0%, *m** at *m/e* 224.3, $241^2/259 = 224.2$), *m/e* 223 ($C_{13}H_{19}O_3$, 39.7%, *m** at *m/e* 206.4, $223^2/241 = 206.3$), and *m/e* 205 ($C_{13}H_{17}O_2$, 1.6%, *m** at *m/e* 188.5, $205^2/223 = 188.5$). A peak observed at *m/e* 225 ($C_{14}H_{25}O_2$, 9.0%) corresponded to the ion **j**.

Stereochemistry. The UV. spectrum of 8-hydroxyerythronolide B (**5**) revealed a carbonyl absorption at 278 nm ($\varepsilon = 42$), while the equivalent absorption of erythronolide B (**1**) was at 288 nm ($\varepsilon = 39$) [2]. This hypsochromic shift of the carbonyl absorption of **5** relative to **1** required that the newly introduced 8-hydroxyl group be equa-

torial [10]. The circular dichroism spectrum[3]) of the α-hydroxyketone **5** further supports this contention. An equatorial hydroxyl group in the macrolide conformation proposed by *Perun & Egan*, and others [11–13] would bisect the carbonyl group at C-9 and have very little effect on the ketone absorption sign and intensity in reference to **1** in which the corresponding substituent is hydrogen. This was found to be the case (**1**, 290 nm $\theta = -12200$, 210 nm $\theta = -4300$; **5**, 282.5 nm $\theta = -9270$, 215 nm $\theta = -2690$). These results were consistent with an 8-hydroxyl group in **5** with the same stereochemistry as the 8-hydrogen in **1**. This sequence of reactions therefore led to a stereospecific substitution of the hydrogen at C-8 (**1**) by a hydroxyl group (**5**). The absolute stereochemistry at this carbon was identical to that present at the analogous position in the aglycones of oleandomycin [14], lankamycin [15] and the shunt metabolite 8-hydroxy-5,6-dideoxy-5-oxoerythronolide B recently isolated by *Martin* [16].

Examination of molecular models indicated that only one side of the double bond of the enol ether **3a** was readily accessible to an attacking reagent. This observation was consistent with the stereochemistry of the formation of the 8-hydroxy compound **5**. For this reason the epoxy group in **4** and the hydrogen atom in **6** were formulated with the same stereochemistry at C-8 as that of the hydrogen in **1** and the hydroxyl group in **5**.

The circular dichroism data used to assign the C-8 configuration of **5** was valid only if the conformation of the compound was unchanged from that previously determined for **1** and related derivatives [11–13]. The chemical shifts and coupling constants (Table) of **5** compared to those of **1** confirmed the conformational homogeneity of these compounds. Changes in conformationally susceptible couplings between vicinal ring protons were negligible. Changes in chemical shifts which were less definitive of conformational changes because of their susceptability to experimental conditions such as concentration, temperature, etc., were relatively small, or varied in a predictable manner. These changes arose from the introduction of an equatorial hydroxyl group as a result of electronic and aromatic solvent induced solvation effects [12].

The importance of **6** in studies of the conformation of erythronolide B (**1**) warranted additional experiments to deduce the magnitudes of the vicinal coupling constants. The conformation proposed for **1** and its derivatives [11–13] placed the tertiary 6-hydroxyl group in close proximity to the 9-keto group. This model predicted that a 6,9-hemiacetal bridge, or its equivalent, may be formed without significant conformational reorganization. However, the proton coupling constants of **3a** and **4** which possess such a bridge were significantly different from those of **1** or **5**, suggesting that a conformational change had occurred. Such a change could have been attributed to changes in the hybridization of the carbon atoms involved in double bonds or three-membered rings. It should be noted that the coupling constants determined for the vicinal protons of **6** were in excellent agreement with the corresponding couplings of **1** and **5**, thereby proving that a 6,9-bridge could be accomodated by the proposed conformational model [11–13].

[3]) The authors wish to express their thanks to Dr. *L. A. Mitscher* of the Ohio State University, Columbus, Ohio 43210, USA, for the recording and interpretation of the spectrum.

The conformational homogeneity of **6** and **1** indirectly offered evidence for the configuration assigned at C-8. A study of molecular models revealed that *cis*-addition of hydrogen from the hindered side of the double bond, resulting in an 8-*epi*-compound, would require conformational reorganization. Since no reorganization was evident, the 'natural' stereochemistry at C-8 was assigned. The new compounds derived from the enol ether **3a** were (8*S*,9*S*)-8,9-anhydroerythronolide B 6,9-hemiacetal 8,9-epoxide (**4**), (8*S*)-8-hydroxyerythronolide B (**5**), and (9*S*)-9-dihydro-6,9-O-anhydroerythronolide B (**6**).

Nuclear magnetic resonance parameters[a]

	Chemical shifts							*Coupling constants*					
	1[b]	**3a**	**3b**	**4**	**5**	**6**		**1**	**3a**	**3b**	**4**	**5**	**6**
H-(2)	2.94	2.87	2.88	2.98	2.87	2.84	$J_{2,3}$	10.8	10.2	10.0	7.2	10.2	10.0
H-(3)	4.08	3.88	5.54	~4.2	3.89	3.98	$J_{3,4}$	1.3	1.5	1.9	4.2	~1	1.5
H-(4)	2.45	2.39	2.75	2.15	2.56	2.63	$J_{4,5}$	2.9	~1	2.5	~1	2.8	<1
H-(5)	4.07	3.84	5.20	3.99	3.76	3.95	$J_{7a,7e}$	15.2	15.7	...	0[c]	15.0	...
H-(7)a	2.23	3.04	~2.9	1.81	2.45	~1.7	$J_{7a,8}$	7.0	–	–	–	–	...
H-(7)e	1.64	2.19	~2.1	1.81	2.16	~1.7	$J_{7e,8}$	7.6	–	–	–	–	...
H-(8)	3.09	–	–	–	–	~2.8	$J_{8,9}$	–	–	–	–	–	~9
H-(9)	–	–	–	–	–	~4.0	$J_{9,10}$	–	–	–	–	–	~9
H-(10)	3.08	2.76	2.98	3.19	3.21	1.67	$J_{10,11}$	2.0	8.2	8.9	4.5	~1	1.3
H-(11)	4.25	3.95	5.18	~4.2	4.36	4.30	$J_{11,12}$	10.3	8.2	8.9	...	10.0	8.6
H-(12)	1.81	1.87	2.28	2.39	~1.8	1.84	$J_{12,13}$	1.2	<1	2.0	3.0	~1	1.2
H-(13)	5.70	5.72	5.06	5.14	5.78	5.63	$J_{13,14a}$	8.8	8.2	7.6	8.5	8.8	8.4
							$J_{13,14e}$	6.6	6.0	6.3	5.0	6.0	5.8
CH_3-(2)	1.43	1.37	1.27	1.44	1.37	1.39							
CH_3-(4)	1.36	1.23	1.15	1.13	1.34	1.29	$J_{7a,8-CH_3}$	–	1.2	1.2	–	–	–
CH_3-(6)	1.52	1.48	1.17	1.41	1.42	1.37	$J_{7e,8-CH_3}$	–	1.2	1.2	–	–	–
CH_3-(8)	1.29	1.59	1.56	1.53	1.83	1.07							
CH_3-(10)	1.19	1.30	1.08	1.34	1.18	0.99							
CH_3-(12)	1.02	1.04	1.01	1.05	1.03	0.95							
CH_3-(14)	0.89	0.87	0.84	0.90	0.88	0.91							

[a] Reported parameters determined in pyridine-d_5 solution at 110° after D_2O exchange, unless otherwise noted.

[b] NMR. parameters reported are those obtained at 220 MHz and confirmed by spin decoupling at 100 MHz [11].

[c] Magnitude due to chemical shift equivalence; in tetrachloroethylene solution at 110°, $J_{7a,7e}$ = 12.5 Hz, H-(7)a 1.76; H-(7)e 1.55 ppm δ.

The authors are indebted to Dr. *M. Levenberg* and Mrs. *Sandra L. Mueller* for mass spectra, to Mrs. *Ruth Stanaszek* for her assistance in obtaining the NMR. spectra, to Mrs. *Brigitte Fruehwirth* and Mr. *D. Sahr* for IR. spectra, to Mr. *D. E. Williamson* for the UV. spectra, to Mrs. *Evelyn Baker* for thin-layer chromatographic analyses, to Mr. *D. A. Dunnigan* for the hydrogenation, and to Mr. *V. Rauschel* and his associates for microanalyses. We wish to thank Drs. *Jack Tadanier* and *Peter H. Jones* for stimulating discussions during the course of this investigation.

Experimental Part

General Remarks. The melting points were determined on a *Fisher-Johns* melting point apparatus. Optical rotations were measured with a *Hilger* and *Watts* polarimeter using methanol solutions, and the IR. spectra were obtained with a *Perkin-Elmer* Model 421 grating spectrophotometer with chloroform solutions unless stated otherwise. The UV. spectra were recorded using

methanol solutions. The NMR. spectra were determined at 100 MHz with a *Varian* HA-100 spectrometer. Chemical shifts were reported in ppm δ from internal tetramethylsilane (0 δ) and coupling constants were reported in Hz. Simple first order analysis was used to obtain the reported parameters. Unless otherwise stated, the spectra were determined at elevated temperature (C_5D_5N, 110°) after the addition of several drops of D_2O to exchange hydroxyl protons. Whenever possible, chemical shifts and coupling constants were confirmed with appropriate spin decoupling experiments performed with a *Hewlett-Packard* audio-oscillator, Model 200 AB. The mass spectra were recorded on an *A.E.I.* MS-902 high resolution mass spectrometer under standard conditions.

8,9-Anhydroerythronolide B 6,9-Hemiacetal (**3a**) *and Erythronolide B Oxime* (**2**). A mixture of 6.60 g of erythronolide B (**1**), 6.00 g of hydroxylamine hydrochloride, and 8.53 g of barium carbonate in 70 ml of methanol was warmed with stirring to a gentle reflux for 24 hrs according to the procedure successfully employed in the preparation of erythromycin A oxime [3]. The suspension was filtered through a short column of silica gel, the residue was washed with several small portions of hot methanol, the methanolic washes were combined with the original filtrate and evaporated to dryness. The residue was purified by chromatography on 600 g of silica gel. Evaporation of the early ethyl acetate eluates gave a residue of 2.10 g of a colorless oil which was recrystallized from benzene to yield 1.12 g of **3a**, m.p. 98–102°. A portion of this sample was recrystallized from benzene for analysis: m.p. 100–102°; $[\alpha]_D^{26} = +38°$ ($c = 0.976$); $\tilde{\nu}_{max}$ 3612, 3490, 1710 cm^{-1}; λ_{max} 209 nm ($\varepsilon = 6920$).

$C_{21}H_{36}O_6$ (384.50) Calc. C 65.59 H 9.44 O 24.97% Found C 65.69 H 9.35 O 25.01%

A sample of the enol ether **3a** (0.055 g) was quantitatively converted to erythronolide B (**1**) by treatment with a solution of 50 ml of methanol, 49.5 ml of water, and 0.5 ml of 2N hydrochloric acid within $3^1/_2$ hrs, as shown by monitoring of the reaction mixture by thin-layer chromatography.

The residues from the later fractions eluted with ethyl acetate amounted to 3.99 g of the crude oxime **2**. After two recrystallizations from benzene, 2.59 g of *erythronolide B oxime* (**2**) was isolated, m.p. 204–206°. An analytical sample had the following physical constants: m.p. 206–207°; $[\alpha]_D^{25} = -21°$ ($c = 1.112$); $\tilde{\nu}_{max}^{Nujol}$ 3550–3200, 1726, 1629 cm^{-1}.

$C_{21}H_{39}NO_7$	Calc.	C 60.41	H 9.42	N 3.36	O 26.82%
(417.53)	Found	,, 60.33	,, 9.31	,, 3.48	,, 26.60%

8,9-Anhydroerythronolide B 6,9-Hemiacetal (**3a**). – *A) Preparation with Hydroxylamine Hydrochloride:* A solution of 6.60 g of erythronolide B (**1**) and 0.60 g of hydroxylamine hydrochloride in 70 ml of methanol was warmed and stirred to a gentle reflux for $1^1/_2$ hrs. The reaction mixture was evaporated to dryness under reduced pressure to leave a residue of 7.29 g. After chromatographic purification on silica gel and recrystallization from benzene, 4.95 g (78% yield) of the enol ether **3a**, m.p. 99–103°, was isolated. An analytical sample had m.p. 100–103°; $[\alpha]_D^{26} = +35°$ ($c = 1.086$); $\tilde{\nu}_{max}$ 3612, 3490, 1710 cm^{-1}. A mixture melting point determination with the above sample showed no m.p. depression.

$C_{21}H_{36}O_6$ (384.50) Calc. C 65.59 H 9.44% Found C 65.69 H 9.71%

B) Preparation with Acetic Acid: A suspension of 2.74 g of erythronolide B (**1**) in 20 ml of glacial acetic acid was warmed gently on the steam bath to obtain a clear solution which was allowed to stand at room temperature for 2 hrs [4]. The solvent was evaporated on the steam bath under reduced pressure, the residue was dissolved in 100 ml of chloroform and poured into 60 ml of saturated sodium hydrogencarbonate solution. The layers were separated and the aqueous phase was extracted once more with 100 ml of chloroform. The chloroform solutions were washed with saturated sodium hydrogencarbonate solution, dried over anhydrous magnesium sulfate, filtered, combined, and evaporated to leave a residue of 2.58 g of solid. After chromatographic purification and recrystallization from benzene, 1.70 g (62% yield) of **3a**, m.p. 98–104°, was obtained. The identity of this material with the above samples of **3a** was confirmed by IR. and thin-layer chromatography.

3,5,11-Tri-O-acetyl-8,9-anhydroerythronolide B 6,9-Hemiacetal (**3b**). The enol ether **3a** (0.50 g) was warmed on the steam bath with 10 ml of pyridine and 2 ml of acetic anhydride for 16 hrs [2]. The reaction mixture was poured into 100 ml of ice water, the gum was extracted with chloroform, the chloroform solution was washed with several small portions of water, dried over

anhydrous magnesium sulfate, filtered, and evaporated to leave 0.64 g of crystals which were purified by chromatography on 65 g of silica gel. The residues obtained from the eluates with benzene – ethyl acetate (4:1) were combined and recrystallized from acetone-heptane to yield 0.51 g of **3b**, m.p. 162–163°. – An analytical sample was prepared by further recrystallization: m.p. 164–165°; $[\alpha]_D^{26} = +85°$ ($c = 1.10$); $\tilde{\nu}_{max}$ 1745 cm^{-1}; λ_{max} 208 nm ($\varepsilon = 7910$) (Lit. [2]: m.p. 164–165°; $[\alpha]_D^{23} = +85°$). A mixture melting point determination of this sample with that of *Perun* [2] showed no m.p. depression.

$C_{27}H_{42}O_9$ (510.61) Calc. C 63.51 H 8.29 O 28.20% Found C 63.28 H 8.33 O 28.06%

Hydrogen Peroxide – Osmium Tetroxide Oxidation of 8,9-Anhydroerythronolide B 6,9-Hemiacetal (**3a**). A solution of 8.46 g of the enol ether **3a** in 45 ml of *t*-butyl alcohol, 27 ml of hydrogen peroxide – *t*-butyl alcohol reagent [6], and 3 ml of an 0.5% osmium tetroxide solution in *t*-butyl alcohol was allowed to stand at room temperature for 10 days. The yellow solution had turned colorless. The solvent was evaporated under high vacuum at room temperature; twice small amounts of *t*-butyl alcohol were added and likewise evaporated. After careful drying under high vacuum a residue of 9.74 g was obtained which was chromatographed in two portions of 4.85 g and 4.89 g, respectively, on two columns of 450 g of silica gel. The early fractions of both chromatograms [benzene – ethyl acetate (1:1) and ethyl acetate eluates] contained substances which could not be obtained crystalline. Further elution of the columns with ethyl acetate led to the isolation of 3.12 g of a substance which formed gels upon attempted crystallization from acetone-heptane. A part of this gelatinous material, 2.32 g, was rechromatographed on 230 g of silica gel. The residues from the ethyl acetate eluates, 2.18 g, appeared to be pure in several thin-layer chromatographic systems. A part of this compound, 1.19 g, was recrystallized twice from benzene to yield 0.90 g of *(8S,9S)-8,9-anhydroerythronolide B 6,9-hemiacetal 8,9-epoxide* (**4**). The substance was further recrystallized from benzene to a constant m.p. 184–186° (capillary, after drying at 144° under high vacuum for several days to remove the benzene of crystallization); $[\alpha]_D^{25} = +45°$ ($c = 0.567$); $\tilde{\nu}_{max}$ 3600, 3550, 3500–3300, 1720 cm^{-1}.

$C_{21}H_{36}O_7$ (400.50) Calc. C 62.98 H 9.06 O 27.96% Found C 63.29 H 9.11 O 28.03%

The residues from the later fractions eluted with ethyl acetate of the original columns contained 3.21 g of a crystalline substance which could be recrystallized from acetone-heptane to yield 1.53 g of *(8S)-8-hydroxyerythronolide B* (**5**), m.p. 227–228°. A sample of this material was recrystallized to a constant m.p. 228–230°; $[\alpha]_D^{26} = -24°$ ($c = 1.072$); $\tilde{\nu}_{max}$ 3600, 3470, 1718, 1692 (shoulder) cm^{-1}; λ_{max} 278 nm ($\varepsilon = 42$).

$C_{21}H_{38}O_8$ (418.51) Calc. C 60.26 H 9.16 O 30.58% Found C 60.47 H 9.00 O 30.37%

(8S,9S)-8,9-Anhydroerythronolide B 6,9-Hemiacetal 8,9-Epoxide (**4**). A solution of 3.84 g of 8,9-anhydroerythronolide B 6,9-hemiacetal (**3a**) in 50 ml of methylene chloride was added to a stirred suspension of 5.16 g of *m*-chloroperbenzoic acid in 35 ml of methylene chloride over a period of 5 min [7]. Stirring at room temperature was continued overnight, the solution was diluted to 250 ml with methylene chloride, extracted with two 250-ml portions of a 2% sodium hydrogensulfite solution, three ice-cold portions of saturated sodium hydrogencarbonate solution and one 200-ml portion of saturated sodium chloride solution. The aqueous washes were extracted with two additional 200-ml portions of methylene chloride. The organic extracts were dried over anhydrous magnesium sulfate, filtered, combined, and evaporated to leave 3.37 g of residue. The latter was crystallized and recrystallized twice from benzene to yield a first crop of 1.97 g, m.p. 123–130°; concentration of the mother liquors afforded a second crop of 0.871 g, m.p. 126–131° (the crystals of both crops still contained benzene of crystallization).

A part of the first crop of **4** was recrystallized from benzene for analysis. The sample was dried at 144° under high vacuum: m.p. 181–183°; $[\alpha]_D^{25} = +44°$ ($c = 0.600$); $\tilde{\nu}_{max}$ 3600, 3550, 3500–3300, 1720 cm^{-1}.

$C_{21}H_{36}O_7$ (400.50) Calc. C 62.98 H 9.06 O 27.96% Found C 63.25 H 9.20 O 27.71%

The sample was found to be identical with the above obtained epoxide (thin-layer chromatogram, IR., UV. and mass spectra).

(9S)-9-Dihydro-6,9-O-anhydroerythronolide B (**6**). A solution of 1.00 g of 8,9-anhydroerythronolide B 6,9-hemiacetal (**3a**) in 150 ml of glacial acetic acid containing 5 drops of tri-

fluoroacetic acid was hydrogenated over platinum, prepared by reducing 1.00 g of platinum oxide, over a period of 1 h. The suspension was filtered and evaporated under reduced pressure. The residue was dissolved in 300 ml of chloroform, washed with two 100-ml portions of saturated sodium hydrogencarbonate solution and a small volume of saturated sodium chloride solution; the aqueous washes were extracted twice with 200 ml of chloroform. The chloroform solutions were dried over anhydrous magnesium sulfate, filtered, and evaporated to leave 0.82 g of residue which was purified by chromatography on 100 g of silica gel. Upon evaporation of the ethyl acetate eluates 0.48 g of product was obtained which upon recrystallization from acetone-heptane yielded 0.32 g of **6**, m.p. 222–223°. – An analytical sample had the following physical constants: m.p. 222–223°; $[\alpha]_D^{25} = +77°$ ($c = 0.805$); $\tilde{\nu}_{max}$ 3600, 3470, 1722 cm^{-1}.

$C_{21}H_{38}O_6$ (386.51) Calc. C 65.25 H 9.91 O 24.84% Found C 65.28 H 9.93 O 24.76%

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