specific for 3′ termini, and produces a derivative which is sufficiently stable to permit further manipulation and characterization of terminal fragments.

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5,6-Dideoxy-5-oxoerythronolide B, a Shunt Metabolite of Erythromycin Biosynthesis*

Jerry R. Martin and Richard S. Egan

ABSTRACT: A $C_{21}H_{36}O_6$ dihydroxydioxolactone accumulated in fermentation broth by a blocked mutant of *Streptomyces erythreus* was isolated. Chemical and physical evidence indicated the structure of the lactone to be 5,6-dideoxy-5-oxoerythronolide B. The new lactone was not converted into erythromycin when added to the fermentation medium of both erythromycin-producing and -blocked-nonproducing strains of *S. erythreus*. However, the lactone is probably formed *via* the erythromycin pathway but is an aberrant metabolite of the pathway.

Erythromycins A–C, antibiotic glycosides elaborated by *Streptomyces erythreus*, are characterized by a branched-chain, macrocyclic polyhydroxyoxolactone aglycone moiety, erythronolide (Gerzon *et al.*, 1956; Wiley *et al.*, 1957). Despite relatively extensive work on the biogenesis of erythronolide, most of the process remains obscure. Early work (Vaněk *et al.*, 1961; Grisebach *et al.*, 1960; Corcoran *et al.*, 1960) indicated that propionate was somehow involved in the biosynthesis and later (Friedman *et al.*, 1964; Kaneda and Corcoran, 1961; Wawszkiewicz and Lynen, 1964; Grisebach *et al.*, 1962) the important role of 2-methylmalonate was recognized. Friedman *et al.* (1964) concluded from a wide variety of data that erythronolide biosynthesis most likely involves a "primer" of propionyl-CoA[1] and the condensation of six molecules of 2-methylmalonyl-CoA probably forming a 21-carbon polyoxolactone (I) (Scheme I). Reduction of appropriate oxo functions could result in the formation of 6-deoxyerythronolide B (II), a progenitor of erythromycin biosynthesis recently isolated by Martin and Rosenbrook (1967).

In an attempt to elucidate the biosynthesis of erythromycin we are examining the metabolites of a number of blocked mutants of *S. erythreus*. In paper III of this series (Martin and Perun, 1968), we reported the structure of 5-deoxy-5-oxoerythronolide B, a shunt metabolite of erythromycin biogenesis, from the fermentation broth of a blocked mutant (*S. erythreus*, Abbott 4EB40). Preliminary evidence indicated

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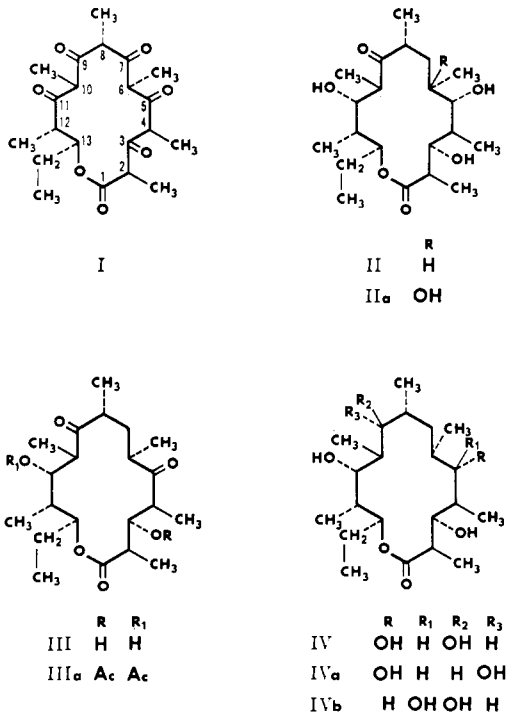
[1] The abbreviations used are: CoA, coenzyme A; TMSi, tetramethylsilane; ASIS, aromatic solvent-induced shifts.

SCHEME I



that this mutant elaborated into fermentation broth a number of unidentified macrolide compounds. In the present paper we present the isolation and structure of another of these macrolide metabolites, 5,6-dideoxy-5-oxoerythronolide B accumulating in fermentation broths of mutant 4EB40. The possible role of 5,6-dideoxy-5-oxoerythronolide B in erythromycin biosynthesis is unclear.

Experimental Section and Results

General. Melting points, determined on a Kofler micro hot stage apparatus, are corrected. Ultraviolet spectra were recorded for 95% ethanol solutions with a Cary Model 11 spectrophotometer. Infrared spectra were determined as chloroform solutions with a Perkin-Elmer Model 521 grating spectrophotometer. Nuclear magnetic resonance spectra were obtained at 100 MHz using a Varian Associates HA-100 spectrometer. Chemical shifts are reported in parts per million (δ) downfield from internal TMSi. Optical rotations were determined with a Hilger and Watts polarimeter. Silica for thin-layer chromatography was Merck silica gel G after Stahl; that for column chromatography was Merck silica gel (70–325 mesh). Unless otherwise stated, the thin-layer chromatographic system used throughout this work consisted of 95% ethanol–chloroform (1:10, v/v). Compounds were visualized by spraying with the arsenomolybdate reagent of Nelson (1944) and heating for a few minutes on a hot plate.

Fermentation and Extraction Procedures. The strain of *S. erythreus* (Abbott 4EB40) employed in this investigation has been previously described (Martin and Perun, 1968). Conditions for the fermentation and extraction of fermentation broth have also been provided earlier (Martin *et al.*, 1966; Martin and Rosenbrook, 1967).

Isolation of 5,6-Dideoxy-5-oxoerythronolide B (III). A dark yellow, crystalline complex, fraction CF-1, obtained as described by Martin and Perun (1968), was the starting material for the isolation of 5,6-dideoxy-5-oxoerythronolide B. Fraction CF-1 (2.0 g) was chromatographed on a column of silica gel (3.0 × 35 cm) prepared in chloroform and eluted with 0.3% methanol in chloroform at a flow rate of 5–6 ml/hr. The effluent, collected in fractions of 20 ml each, was examined by thin-layer chromatography. The analysis revealed that a compound with R_F 0.70–0.78 was well separated from a minor component with R_F 0.67–0.75 (fraction CF-1b) and from two major components (R_F 0.59–0.67 and 0.56–0.64) which were eluted together (fraction CF-1c). The five fractions containing the compound with the greatest mobility were combined and concentrated to dryness, affording 617 mg of yellow crystalline residue. The crystalline mass was dissolved in methanol and decolorized with Darco G-60. Recrystallization from ethyl acetate–hexane gave 444 mg of colorless needles: mp 155–156°, $[\alpha]_D^{24}$ +64° (*c* 1.0, methanol). Molecular weight determination by mass spectrometry revealed a parent ion peak at *m/e* 385. The ultraviolet absorption in ethanol showed a maximum at 291 mμ (ε 108). The spectrum was unchanged by dilute and strong acid. On the addition of alkali to an ethanolic solution, bands at 279 and 233 mμ rapidly developed. These changes are not reversed by neutralization presumably due to decomposition. The infrared spectrum showed broad hydroxyl absorption at 3510 cm^{-1} and intense carbonyl bands at 1695 and 1720 cm^{-1}. Other significant bands occurred at 2965, 2930, 2570, 1450, 1375, 1355, 1330, 1170, 1100, 1030, 990, 970, and 890 cm^{-1}. The nuclear magnetic resonance spectrum is shown in Figure 1 and assignments and coupling constants are summarized in Table I.

Anal. Calcd for $C_{21}H_{36}O_6$: C, 65.60; H, 9.44. Found: C, 65.87; H, 9.63.

Acetylation of 5,6-Dideoxy-5-oxoerythronolide B. A solution of 100 mg of 5,6-dideoxy-5-oxoerythronolide B in 3.5 ml of pyridine was heated on a steam bath with 1.0 ml of acetic anhydride for 8 hr. The reaction mixture was poured onto cracked ice and the tan precipitate (120 mg) was collected by filtration and decolorized with Darco G-60. Crystallization from methanol–water gave 83 mg of diacetate (IIIa) as colorless prisms: mp 181–182°, $[\alpha]_D^{24}$ +105° (*c* 1.0, methanol). The infrared absorption spectrum lacked hydroxyl bands. Bands were seen at 2970, 2935, 1730, 1695, 1450, 1730, 1360, 1165, 1095, 1020, 1005, 985, 960, and 895 cm^{-1}. The nuclear magnetic resonance assignments are shown in Table I.

Anal. Calcd for $C_{25}H_{40}O_8$: C, 64.08; H, 8.60. Found: C, 64.03; H, 8.73.

Attempted Formation of a Phenylboronate Ester Derivative. A solution of 115.2 mg of 5,6-dideoxy-5-oxoerythronolide B in 5.0 ml of acetone was refluxed with 36.3 mg of phenylboronic acid for 4 hr. Concentration of the acetone and addition of water gave 78 mg of colorless needles (mp 153–155°) shown to be identical with the starting macrolide by mixture melting point (no depression) and infrared analysis (identical spectrum).

Reduction of 5,6-Dideoxy-5-oxoerythronolide B. ISOLATION OF (9*S*)- AND (9*R*)-9-DIHYDRO-6-DEOXYERYTHRONOLIDE B (IV, IVA) AND (9*S*)-9-DIHYDRO-5-*epi*-6-DEOXYERYTHRONOLIDE B (IVB). 5,6-Dideoxy-5-oxoerythronolide B (475 mg) was

TABLE I: Nuclear Magnetic Resonance Data.

	5,6-Dideoxy-5-oxoerythronolide B (III) and Diacetate (IIIa)									
	Chemical Shifts[a]					Coupling Constants				
	C_5D_5N III	$CDCl_3$ IIIa		C_5D_5N III	$CDCl_3$ IIIa		C_5D_5N III	$CDCl_3$ IIIa		C_5D_5N III	$CDCl_3$ IIIa
H-2	2.91	2.84	CH_3-2	1.43		$J_{2,3}$	10.2	10.9	J_2, CH_3	6.9	6.8
H-3	4.14	5.36	CH_3-4	1.17		$J_{3,4}$	1.2	1.4	J_4, CH_3	7.1	7.0
H-4	3.13	3.38	CH_3-6	1.36		$J_{6,7}$	8.2		J_6, CH_3	7.0	7.2
H-6	~2.8	2.67	CH_3-8	1.08		$J_{6,7}'$			J_8, CH_3	6.5	
H-7	2.10		CH_3-10	1.00		$J_{7,7}'$	14.3		J_{10}, CH_3	6.7	
H-7′	1.5–2.0		CH_3-12	0.97	1.04	$J_{7,8}$	3.0		J_{12}, CH_3	7.0	
H-8	2.65	3.4	CH_3-14	0.85	0.89	$J_{7}',_{8}$	13.0		J_{13}, CH_3	7.1	7.5
H-10	3.09	3.17				$J_{10,11}$	1.5	1.0	J_{14}', CH_3	7.1	7.5
H-11	3.92	4.96				$J_{11,12}$	9.9	10.6			
H-12	1.5–2.0					$J_{12,13}$	1.1	1.6			
H-13	5.77	5.11				$J_{13,14}$	5.2	6.4			
H-14	1.5–2.0					$J_{13,14}'$	8.7	8.9			
H-14′	1.5–2.0					$J_{14,14}'$					

[a] Chemical shifts are reported in parts per million (δ) downfield from internal TMSi. The spectra of III were determined in approximately 12% (w/v) solutions in pyridine-d_5 at approximately 80° after the addition of sufficient D_2O to remove hydroxyl resonances. Chemical shifts in pyridine-d_5–D_2O mixed solvent are dependent on the amount of D_2O added (approximately 0.02–0.03 ml was used) and temperature. The spectra of IIIa were determined in approximately 12% (w/v) solutions in $CDCl_3$ at ambient probe temperature (approximately 30°).

dissolved in 12 ml of anhydrous methanol and cooled in a salt–ice bath. Solid sodium borohydride (316 mg) was slowly added in small increments during a 2-hr period with stirring. The reaction mixture was stirred for an additional 0.5 hr, then neutralized with cold 1 N hydrochloric acid. The resulting solution was diluted with water and extracted three times with ethyl acetate. The combined ethyl acetate extract was washed with water and dried over sodium sulfate. After removal of the solvent 425 mg of colorless glassy residue was obtained. Thin-layer chromatography (chloroform–benzene–methanol–concentrated ammonium hydroxide, 80:20:5:5, v/v) showed the residue to contain two major and two minor components which moved in pairs. The slower moving major and minor spot from each pair (R_F 0.32–0.37 and 0.19–0.23, respectively) had the same R_F values as (9*S*)- and (9*R*)-9-dihydro-6-deoxyerythronolide B (Perun *et al.*, 1969b).

The residue was chromatographed on a column of silica gel (2.5 × 35 cm) prepared in chloroform. Slow elution (3–4 ml/hr) with 0.3% methanol in chloroform gave fractions containing only the faster moving component (R_F 0.36–0.42). Fractions containing homogeneous material were combined and the solvent evaporated to afford 111 mg of colorless oil. Crystallization from ethyl acetate–hexane gave 64 mg of plates (mp 197–199°). The infrared spectrum exhibited hydrogen-bonded and free hydroxyl absorption at 3470 and 3630 cm^{-1}, respectively. A strong carbonyl band was at 1700 cm^{-1}. Nuclear magnetic resonance studies indicated that the compound was (9*S*)-9-dihydro-5-*epi*-6-deoxyerythronolide B (IVb).

Anal. Calcd for $C_{21}H_{40}O_6$: C, 64.92; H, 10.38. Found: C, 64.81; H, 10.40.

Assignment of the (9*S*)-5-*epi* configuration was made primarily on the basis of changes in coupling constants (Table II). Examination of the $CDCl_3$ solution nuclear magnetic resonance spectra (Figure 2) of IVb revealed that the resonance assigned to H-5 in IV and IVa, having two small couplings (in cycles per second) ($J_{4,5}$ = 1.0 and $J_{5,6}$ = 5.5) was absent, and an additional higher field resonance (J = 10.0 and J = 1.0) was present. The chemical shifts (Table II) of the remaining deshielded protons were relatively unchanged from those of IV and indicated that the configuration at C-9 is *S* (Perun *et al.*, 1969b; Demarco, 1969a,b).

The conformations of IV and IVa having the natural

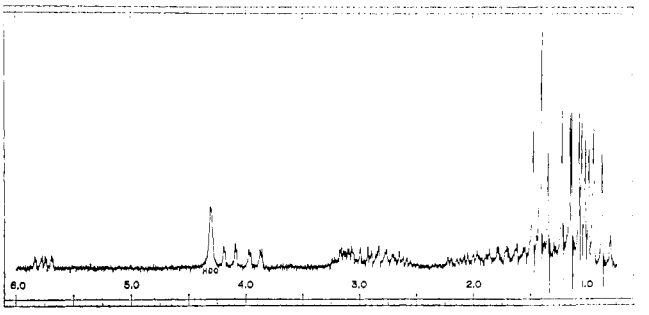


FIGURE 1: 100-MHz nuclear magnetic resonance spectrum of 5,6-dideoxy-5-oxoerythronolide B in deuteriopyridine.

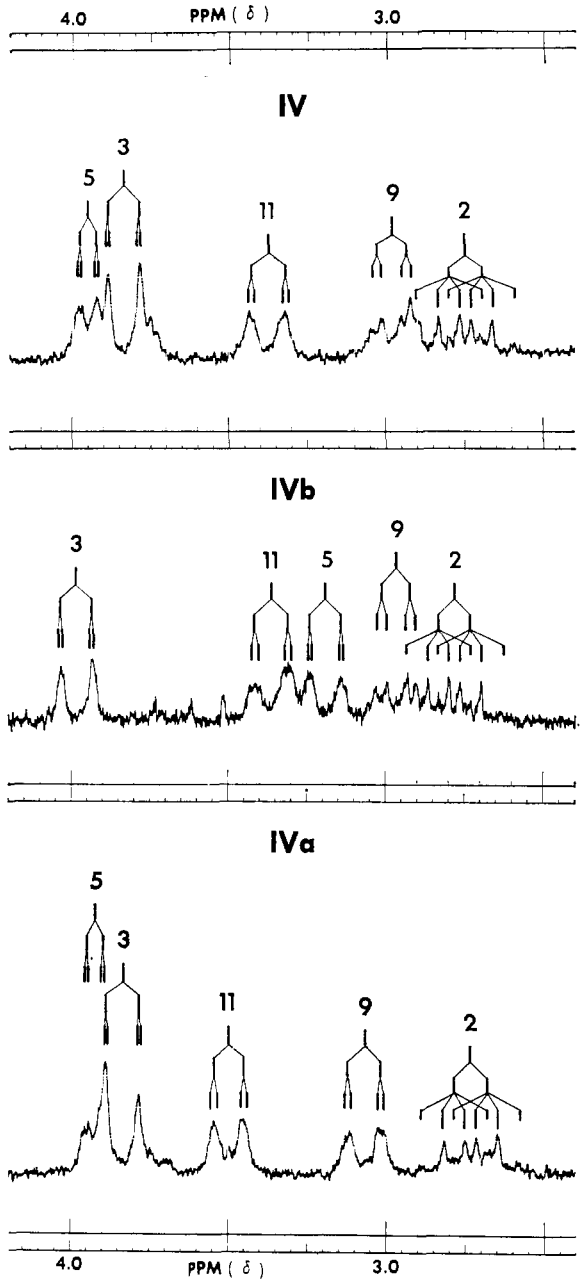


FIGURE 2: Portions of the 100-MHz nuclear magnetic resonance spectra of IV, IVa, and IVb in deuteriochloroform.

TABLE II: Nuclear Magnetic Resonance Parameters of 9-Dihydro-6-deoxyerythronolides (IV–IVb).

	$CDCl_3$			C_5D_5N		
	IV	IVb	IVa	IV	IVv	IVa
			Coupling Constants[a]			
$J_{2,3}$	10.4	10.2	10.3	10.2	10.3	10.0
$J_{3,4}$	~1	~1	~1	1.3	~1	0.5
$J_{4,5}$	1.0	~1	1.5	3.0	{6.5}[c]	3.0
$J_{5,6}$	5.5	10.0	5.0	4.5	{4.5}	5.0
$J_{8,9}$[b]	9.5	9.2	{1.5}	9.5	10.0	{2.0}
$J_{9,10}$	2.8	3.5	{10.5}	2.6	2.5	{10.0}
$J_{10,11}$	2.2	2.0	~1	1.6	~1	2.0
$J_{11,12}$	10.5	10.5	9.5	10.0	10.0	10.0
$J_{12,13}$	1.0	1.2	1.0	1.3	~1	1.5
			Chemical Shifts[d]			
H-2	2.75	2.78	2.73	3.01	3.40	3.02
H-3	3.84	3.99	3.84	4.18	4.53	4.19
H-5	3.96	3.20	3.93	4.22	3.65	4.22
H-9	2.99	2.97	3.07	3.25	3.25	3.60
H-11	3.37	3.36	3.51	3.88	4.00	3.88
H-13	5.04	5.00	5.12	5.75	5.71	5.63

[a] Coupling constants which are expressed as approximate values were not resolved but were detectable from spin-decoupling experiments. [b] The magnitude of $J_{8,9}$ and $J_{9,10}$ has been confirmed by appropriate spin-decoupling experiments for IV and IVb (Perun *et al.*, 1969b); those of IVa are logically assigned but not confirmed. [c] The magnitude of $J_{4,5}$ and $J_{5,6}$ of IVb in $CDCl_3$ has been confirmed by spin-decoupling experiments; however, assignments in C_5D_5N are arbitrary. [d] Chemical shifts were obtained in 5% (w/v) solutions at ambient probe temperature (~30°) in the appropriate solvent after the addition of sufficient D_2O to exchange hydroxyl protons and are expressed in parts per million downfield from internal TMSi (0).

absolute configuration at C-5 are shown in Figure 3.[2] In these conformations, the *gauche* relationships (dihedral angle 60°) between H-5 and both H-4 and H-6, results in the small couplings between these protons (Perun and Egan, 1969; Perun *et al.*, 1969a). Epimerization at C-5 without concomitant conformational reorganization (Figure 3, IVb′) places these same protons in *anti* relationships (dihedral angle 180°). This requires two large couplings between H-5 and both H-4 and H-6 (9.0–13.0 Hz) which are not observed. However, conformational studies of racemic and *meso*-2,4-pentanediol (Fukuroi *et al.*, 1968) have shown that an intramolecular hydrogen bond, possible only between *syn*-axial[3] hydroxyl groups offers sufficient stabilization so that conformations incorporating this relationship predominate in $CDCl_3$ solution. In a conformation of IVb in which a stabilizing *syn*-axial relationship exists between the 3- and 5-*epi*-hydroxyl groups (Figure 3, IVb), H-4 and H-5 are now *gauche* while H-5 and H-6 remain *anti*. This conformation has proton relationships which are

[2] It should be noted that the conventions of the Chan–Ingold–Prelog system of configurational nomenclature (Cahn *et al.*, 1956) specifies C-5 as S in IV, IVa, and II, but R in IIa which lacks the C-6 hydroxyl. However, the absolute configurations at C-5 are the same (natural) for all of these compounds. We appreciate a referee calling this to our attention.

[3] The term *syn*-axial does not designate the orientation of the interacting groups as axial *vs.* equatorial, but rather is used in a general sense to indicate two groups, oriented with parallel bond axes, on mutually 1,3 atoms. We acknowledge a personal communication from Professor E. L. Eliel (Notre Dame University) confirming that in a 14-membered ring *syn*-axial relationships can exist between groups with equatorial orientations.

consistent with observed coupling constants ($J_{4,5}$ = 1.0 and $J_{5,6}$ = 10.0).

Spin-decoupling experiments were performed in $CDCl_3$ solution to corroborate the predicted couplings of H-5. Resonance overlap makes decoupling experiments difficult; however, evidence that the $J_{4,5}$ and $J_{5,6}$ coupling were correctly assigned was obtained. The H-3 multiplet can be unequivocally assigned on the basis of its coupling with H-2, the only deshielded proton unaffected by reduction coupled to a methyl group (Tables I and II). Irradiation at 1.95 ppm removed the small coupling from H-3, known to arise from H-4 (Perun and Egan, 1969; Demarco, 1969a,b), as well as the small couplings of the remaining downfield resonances (H-5, -9, and -11) without affecting the large coupling of any resonance. It was also possible to remove the large couplings from these multiplets by irradiating at 1.70 and 1.45 ppm without affecting the resonance of H-3. Then, regardless of the specific assignments of H-5, -9, and -11, the large coupling of H-5 cannot arise from H-4 and must therefore be due to H-6.

The pyridine-d_5 spectra of IVb offered further corroboration of this interpretation. In that solvent the couplings of H-5 are markedly changed from the values in $CDCl_3$ solution. This is in agreement with the findings of Fukuroi *et al.* (1968) that the most populated conformer of racemic and *meso*-2,4-pentanediol, in pyridine solution, is no longer one with *syn*-axial hydroxyl groups but rather one in which the hydroxyl groups have a *syn*-clinal (60°) relationship. This corresponds to conformation IVb′ (Figure 3) and is evidenced by an increase of $J_{4,5}$. A similar although smaller change is evident in the pyridine-d_5 spectra of IV and IVa. This is also consistent with the observation (Fukuroi *et al.*, 1968) that the populations of the *syn*-clinal conformers of *meso*-2,4-pentanediol (analogous by configuration to IV and IVa) are considerably smaller than those of racemic 2,4-pentanediol (analogous by configuration to IVb).

Chemical shifts are known to be conformationally dependent (Eliel *et al.*, 1966). Since the conformations of IV and IVa are shown to be different than those of IVb, which also shows a large solvent dependence, attempts to use the δ_{CDCl_3}–$\delta_{C_5D_5N}$ ASIS shifts (Demarco *et al.*, 1968) previously applied to IV and IVa (Perun *et al.*, 1969b) and related compounds (Demarco, 1969a,b) were not made.

Continued elution gave a series of heterogeneous fractions containing IV and the compound with R_F 0.32–0.37. The fractions were followed with tubes containing a single homogeneous component with R_F 0.32–0.37. Fractions containing only this material were combined and the solvent evaporated to give 73 mg of colorless oil which crystallized on standing. Recrystallization from ethyl acetate–hexane gave 43 mg of fine, colorless needles (mp 195–198°). The infrared and nuclear magnetic resonance spectra and the thin-layer mobility in several systems were identical with those of an authentic sample of (9*S*)-9-dihydro-6-deoxyerythronolide B (IV). A mixture melting point was undepressed.

Anal. Calcd for $C_{21}H_{40}O_6$: C, 64.92; H, 10.38. Found: C, 64.90; H, 10.28.

Further elution with gradually increasing concentrations of methanol in chloroform gave fractions containing 145 mg of an oil composed mainly of IV with smaller amounts of a compound with R_F 0.23–0.27. Attempts to recover the minor component by additional chromatography were not rewarding.

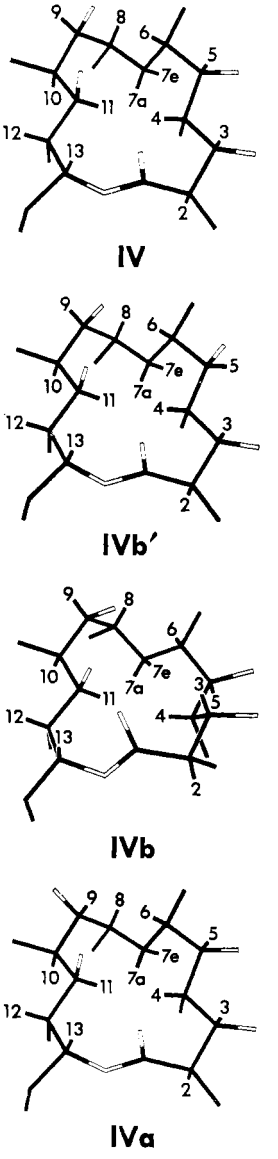


FIGURE 3: Proposed solution conformations of IV, IVa (Perun *et al.*, 1969b), and IVb. These are photographs of Framework Molecular Models.[4] Solid lines represent C—C and C—H bonds while outlined lines represent C—O bonds. The position of ring protons is given by the appropriate numbers. Protons associated with methyl and hydroxyl groups are not shown for clarity.

The last fractions collected contained the cleanly separated compound with thin-layer mobilities identical with (9*R*)-9-dihydro-6-deoxyerythronolide B (IVa). These fractions were combined and evaporated to dryness *in vacuo* to give 73 mg of oily residue. Crystallization from ethyl acetate–hexane gave 18.5 mg of colorless prisms (mp 106–113°). The infrared spectrum of this material was identical with that of an authentic sample of (9*R*)-9-dihydro-6-deoxyerythronolide B (Perun *et al.*, 1969b) and a mixture melting point was undepressed thus establishing the congruency of the two compounds.

Anal. Calcd for $C_{21}H_{40}O_6$: C, 64.92; H, 10.38. Found: C, 65.10; H, 10.33.

Attempted Biological Conversion of 5,6-Dideoxy-5-oxoerythronolide B into Erythromycin by Strains of S. erythreus.

In order to follow the metabolic fate of 5,6-dideoxy-5-oxoerythronolide B, the lactone was incubated under conventional fermentation conditions with a number of nonerythromycin-producing and erythromycin-producing strains of *S. erythreus* capable of efficiently converting the known erythromycin intermediates into the amino glycoside. A few of the test strains and the fermentation procedure have been previously described (Martin and Perun, 1968). Finely divided lactone (25 mg) was added to 50 ml of a 24-hr culture of each of the test strains and the progress of the fermentation followed for 144 hr by thin-layer chromatography of ethyl acetate extracts of the fermentation broth and by anti *Bacillus subtilis* activity using the agar diffusion method of assay. Strains capable of synthesizing erythromycin produced essentially the same level of antibacillus activity irrespective of the addition of 5,6-dideoxy-5-oxoerythronolide B. Blocked strains, producing antibacterial activity only when erythromycin precursors are fed, failed to produce antibiotic. In every case, much of the added lactone could be recovered unaltered after 6-days incubation, although thin-layer chromatography indicated some degradation had occurred in all test systems.

Discussion

Elemental analysis of the *S. erythreus* metabolite was consistent with the empirical formula $C_{21}H_{36}O_6$. This was confirmed by the mass spectrum which showed a molecular ion peak at *m/e* 385. The compound is optically active and its ultraviolet absorption in ethanol shows a maximum of 291 mμ (ε 108). The spectrum was unchanged by acid but the addition of alkali led to decomposition. The infrared spectrum showed hydrogen-bonded hydroxyl absorption at 3510 cm^{-1} and carbonyl absorption at 1720 and 1695 cm^{-1}.

Evidence that the compound was probably a macrocyclic lactone related to erythronolide B was gained by examination of the nuclear magnetic resonance spectrum (Figure 1 and Table I). The characteristic resonance of H-13 appeared at 5.77, indicating the possible lactone nature of the compound. A total of seven methyl groups was shown by the resonances, six doublets and one triplet, in the 0–1.5 region. The lack of a singlet methyl resonance indicated the absence of a tertiary hydroxyl group. These data suggest the presence of the 14-membered macrolide ring with a 13-ethyl side chain, which characterizes previously isolated members of the erythronolide group.

The presence of the erythronolide carbon skeleton was confirmed and delineation of oxygen substitution was supplied by characterization of the reduction products. Sodium borohydride reduction of the metabolite gave rise to a mixture of two major and two minor diastereoisomers. The diastereoisomers are separated on thin plates and can be isolated by column chromatography on silica gel. The principal product of the reduction and the fastest moving by thin-layer chromatography is the previously unknown (9*S*)-9-dihydro-5-*epi*-6-deoxyerythronolide B (IVb) which was fully delineated by nuclear magnetic resonance.

The second most abundant diastereoisomer was identical in all respects with (9*S*)-9-dihydro-6-deoxyerythronolide B (IV). Of the two minor disastereoisomers formed by the reduction only the slowest moving could be crystallized. Infrared spectroscopy, thin-layer chromatography, and melting point behavior established the structure as (9*R*)-9-dihydro-6-deoxyerythronolide B (IVa). Isolation of these known substances confirms the presence of a 2,4,6,8,10,12-hexamethylpentadecan-13-olide skeleton with oxygen atoms as hydroxyl or carbonyl functions at C-3, -5, -9, and -11 and identifies the stereochemistry at each asymmetric center. The isolation of the alcohols isomeric at C-9 suggested that a ketone group occupied this position. Presumably, another ketone was located at C-5 because of the isolation of an epimer at that center. These postulations were then confirmed independently.

The nuclear magnetic resonance spectrum obtained before deuterium oxide was added to remove exchangeable protons indicated two hydroxyl proton resonances at 6.79 and 5.62, and two proton resonances appear in the characteristic position for protons attached to a carbon bearing a hydroxyl function (3.5–4.5). The metabolite reacted in acetic anhydride–pyridine to afford a diacetate (IIIa) which lacked hydroxyl absorption in the infrared. These data suggest that the compound must have two hydroxyl groups and must then have two ketone functions. The molecular formula, $C_{21}H_{36}O_6$, requires four degrees of unsaturation which are satisfied by the macrocyclic ring and three carbonyl groups (lactone and two ketones). The magnitude of the ultraviolet absorption (ε 108) is over double that of erythronolide B (ε 39) and 6-deoxyerythronolide B (ε 56) consistent with an additional ketone carbonyl. The identity of the products isolated from sodium borohydride reduction eliminate the possibility that the additional degree of unsaturation is a double bond.

The location of hydroxyl and ketone substituents was determined by both chemical and spectroscopic means. The nuclear magnetic resonance spectra of the diacetate in deuteriochloroform (Table I) showed a resonance at 5.11 ascribable to H-13. This unusually high chemical shift has been correlated with acetylation of the 11-hydroxyl group in related erythronolide compounds (T. J. Perun and R. S. Egan, unpublished results). Additional insight into the location of the hydroxyl groups was obtained by the inability of the metabolite to form a benzeneboronic acid derivative (Sugihara and Bowman, 1958). The ability of numerous erythronolide structures containing free C-3 and C-5 hydroxyl groups of natural configuration to form benzeneboronic esters is well established (Martin and Rosenbrook, 1967; Perun *et al.*, 1969b). The failure of the compound to form a benzeneboronic acid derivative precludes a 3,5-dihydroxy structure and supports the assignment of one hydroxyl group at C-11.

From a biogenetic consideration it seemed reasonable to regard one oxo function at C-9. The validity of this assignment was confirmed by the identical nature of two of the diastereoisomers isolated from the reduction with the two C-9 dihydro epimers formed by the sodium borohydride reduction of 6-deoxyerythronolide B. In addition, the lack of a benzeneboronic acid derivative also ruled out a (9*S*)-11-dihydroxy structure which form such derivatives (Perun *et al.*, 1969b). Spectroscopic evidence for the 9-oxo assignment was also obtained by the chemical shift of H-8 and H-10 in the nuclear magnetic resonance spectra. The resonances of both protons appear in the 2.5–3.5 region characteristic for protons α to a carbonyl group (Perun and Egan, 1969). The assignments of these protons are unambiguous as spin-decoupling experiments clearly establish the H-11

and H-10 resonances as well as H-8 which exhibits coupling to both protons of the C-7 methylene.

Having then assigned an 11-hydroxyl-9-oxo structure, it was necessary to assign the second ketone to C-3 or C-5. The isolation of (9*S*)-9-dihydro-5-*epi*-6-deoxyerythronolide B (IVb) after borohydride reduction suggests the second carbonyl was at C-5. The possibility of a 3-oxo structure was eliminated by consideration of the ultraviolet and nuclear magnetic resonance spectra. Rickards and Smith (1968) observed that picromycin, a 14-membered ring macrolide with an oxo function at C-3, gives an absorption maximum at 294 mμ which they ascribe to the enolate anion OCO-CMe=C—O$^-$ on addition of alkali to an ethanolic solution. However, Muxfeldt *et al.* (1968) concluded that picromycin did not enolize since H-2 could not be exchanged under mildly basic conditions. Nevertheless, a similar ultraviolet peak was not observed for the new macrolide and no ring protons exhibited any exchange in D_2O–C_5D_5N milieu at 110° after several hours, suggesting that the second ketone function must be at C-5.

The chemical shift of H-4 (3.13) deshielded from its resonance position in erythronolide B (2.57) confirms the presence of an α-carbonyl group. That this carbonyl must be at C-5 and not C-3 is demonstrated by the chemical shift of H-2 (2.91) inconsistent with a proton α to two carbonyl groups, and by the coupling of H-2 as well as all other protons in this region to at least one other ring proton. The nuclear magnetic resonance spectrum of picromycin (Rickards and Smith, 1968) containing a 3-oxo group exhibits a quartet at 3.90 assigned to H-2. Thus, to account for all the physical and chemical properties of the metabolite the only possible formulation is 5,6-dideoxy-5-oxoerythronolide B (III).

To ascertain the metabolic rate of 5,6-dideoxy-5-oxoerythronolide B, the compound was incubated under conventional fermentation conditions with blocked and erythromycin-producing strains of *S. erythreus*. None of the systems tested, with strains capable of efficiently converting known erythromycin progenitors into the completed glycoside, converted the dioxo compound into erythromycin and most of the added macrolide could be recovered from fermentation broth unchanged after 6-days incubation. However, thin-layer chromatography of ethyl acetate extracts of fermentation broth indicated that some degradation and/or transformations had taken place in all test systems. The nature of these products, found only in minor quantities, was not examined further.

The failure of *S. erythreus* to convert 5,6-dideoxy-5-oxoerythronolide B into erythromycin was a disappointment. Although negative results in biosynthetic experiments with intact cell systems must be treated with caution due to permeability factors that may prevent access of the precursor to the synthetic site, our feeling is that the compound is not an erythromycin intermediate. However, it seems fairly certain that the lactone is formed *via* the erythronolide pathway and is a shunt product from that pathway.

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