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(8S)-8-HYDROXY-5,6-DIDEOXY-5-OXOERYTHRONOLIDE B, A SHUNT METABOLITE OF ERYTHROMYCIN BIOSYNTHESIS—V

STUDIES ON THE BIOSYNTHESIS OF THE ERYTHROMYCINS—V CONFORMATION OF MACROLIDE ANTIBIOTICS—V

J. R. MARTIN, T. J. PERUN and R. S. EGAN
Abbott Laboratories, Division of Antibiotics and Natural Products, North Chicago, Illinois 60064

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Abstract—The isolation and structure elucidation of a shunt metabolite of erythromycin biosynthesis, (8S)-8-hydroxy-5,6-dideoxy-5-oxoerythronolide B (**1a**), is described. NMR and CD data reveal that the title compound and related 5-oxo derivatives (**5** and **6**) are conformationally homogeneous with erythronolide B (**2**). Consistent with this observation is the hydroxyketone-hemiacetal equilibrium present in the case of **5**. The spectroscopic data further show that **1a**, (8S)8)hydroxyerythronolide B (**12**), and lankolide (**13**) have identical 8S stereochemistry.

ERYTHROMYCIN antibiotics, of which erythromycin A is perhaps the best known example, are produced by fermentation of *Streptomyces erythreus*. These antibiotics possess a 14-membered, polyfunctional macrocyclic ring aglycone, erythronolide, substituted with a neutral and a basic sugar moiety. Our search for possible biogenetic precursors of erythromycin has led to a number of blocked mutants of *S. erythreus*. In previous papers we have described the isolation and structure of both erythromycin progenitors[1,2] and aberrant metabolites.[3,4] This paper describes the isolation, structure, configuration, and solution conformation of an additional erythromycin shunt metabolite.

The ethyl acetate extract of the fermentation broth of a blocked mutant of *S. erythreus* (Abbott 4EB40) was separated into several fractions by chromatography on silica gel. The first fraction eluted (CF-1) contained three major components.[3] Additional chromatography on silica gel separated fraction CF-1 into three fractions: CF-1a, b, and c. The component in CF-1a (**6**) was the subject of a recent publication.[4] TLC analysis of CF-1c showed the material contained two components (R_f 0·59–0·67 and 0·56–0·64). These two components were readily separated into pure compounds by chromatography on Sephadex LH-20 prepared in chloroform.

The slowest moving compound and the subject of this paper, (8S)-8-hydroxy-5,6-dideoxy-5-oxoerythronolide B (**1a**), gave an elemental analysis, a result consistent with the empirical formula $C_{21}H_{36}O_7$. This was confirmed by the mass spectrum which showed a molecular ion peak at *m/e* 401. High resolution studies confirmed the elemental composition of this ion.

The NMR spectrum of the metabolite (**1a**; Fig 1; Table 1) showed a number of general features consistent with those previously described for macrocyclic lactones related to erythronolide B(**2**) an erythromycin biosynthetic intermediate.[5] The characteristic resonance of H-13, deshielded by the lactone, was observed at 5·51

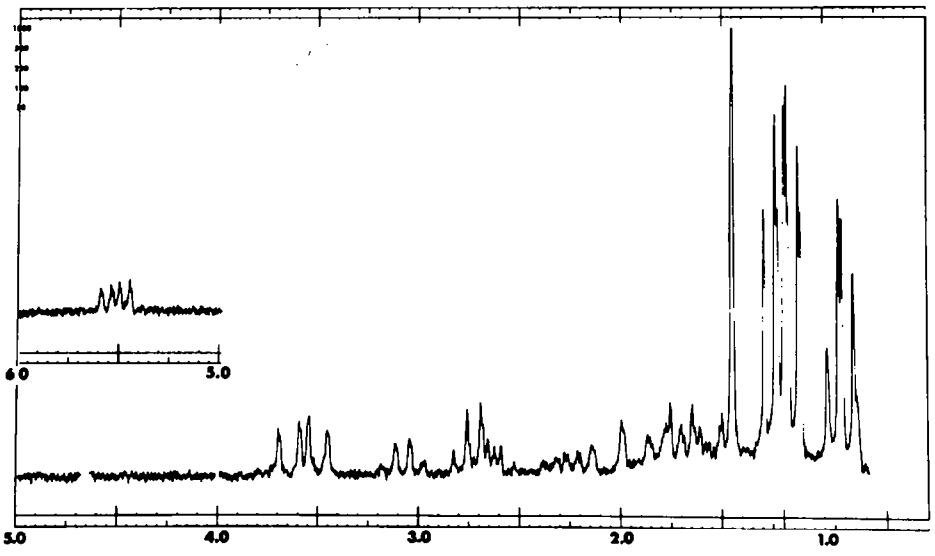
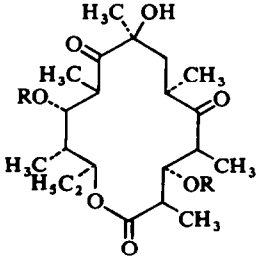


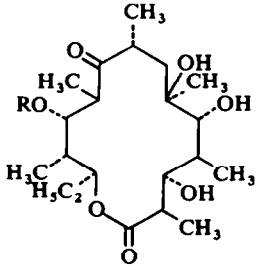
FIG 1. NMR spectrum of **1a** in $CDCl_3$ solution.

ppm (δ) in $CDCl_3$ solution. The Me region, 0·5–1·5 ppm, contained the resonances of seven C-Me groups—five doublets, one triplet and one singlet—consistent with the formulation of the structure as an erythronolide B derivative. Close inspection of the spectra revealed other relationships which permitted the proposal of a partial structure as a 2,4,6,8,10,12-hexamethylpentadecan-13-olide with O atoms as OH or CO functions at carbons 3,5,9 and 11 and with a 13-ethyl side chain.

The nature of the oxygen substituents was obtained largely from spectroscopic evidence. The IR spectra showed bonded OH adsorption and the CO region had maxima at 1722, 1700, and 1691 cm^{-1} consistent with the presence of a lactone and two ketone groups thus accounting for four O atoms. The NMR spectra in $CDCl_3$ solution contained three resonances which were exchangeable with D_2O; two doublets at 2·60 and 3·51 ppm and a singlet at 3·99 ppm. These resonances are evidence that the metabolite contained one tertiary and two secondary OH groups. This assignment was confirmed by the preparation of a diacetate (**1b**) whose IR spectra contained OH absorption. In addition two protons were present in the 3·3–5·0 ppm region of the



R
1a H
1b Ac



R
2 H
14 Ac

NMR spectrum characteristic of chemical shifts of protons geminal to OH groups or derivatives. These observations thus account for all seven O atoms.

Past experience with erythronolide related metabolites produced by *S. erythreus* has shown an unvarying oxygen substitution pattern at positions 3, 5, 9 and 11 as well as stereochemical identity at all asymmetric centers. Assuming maintenance of this pattern in the present case, the tertiary OH can be substituted at one of the ring carbons containing a Me group, namely 2, 4, 6, 8, 10, or 12. Detailed interpretation of the NMR spectra of the metabolite and its acetyl derivative allowed assignments of the position and nature of all oxygen substituents.

The positions of OH groups proved to be most readily assigned. The NMR spectra of the diacetate (**1b**; Fig 2) revealed the chemical shift of H-13 to be 5·10 ppm in $CDCl_3$ solution. This characteristic diamagnetic shift of H-13 to higher field on

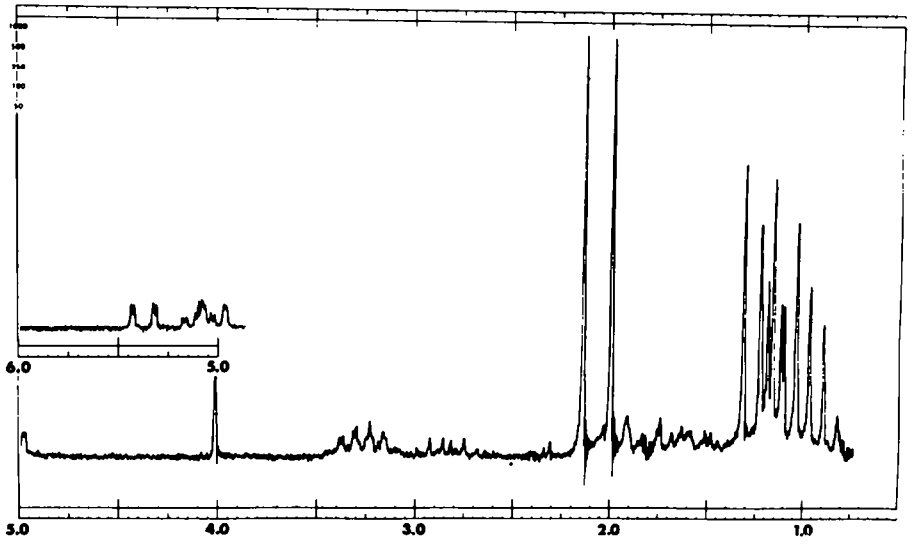
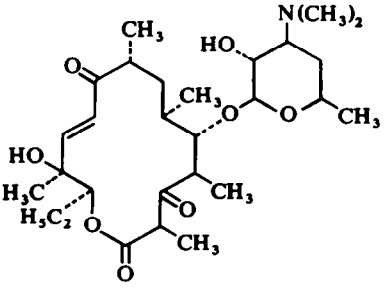


FIG 2. NMR spectrum of **1b** in $CDCl_3$ solution.

acetylation has been previously observed in cases where an 11-OH group is acetylated.[6]

Since no simple quartet resonance is present in the 3·5–4·5 ppm region of the NMR spectra of the metabolite or its diacetate derivative, no CH—CH_3 group is isolated between two CO groups. The NMR spectrum of picromycin (**3**) has an unsplit quartet at 3·90 ppm, assigned to H-2, characterizing this compound as a 3-oxo



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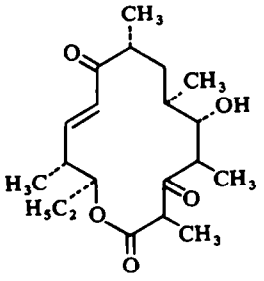
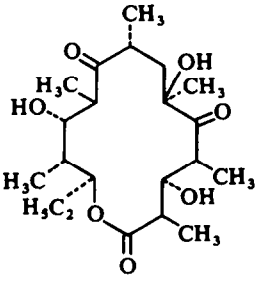


4

macrolide.[7] Narbonolide[8] (4), the 14-membered ring aglycone of narbomycin, a 3-oxo macrolide, has an unsplit quartet at 3·72 ppm also assigned to H-2. The absence of such a resonance in the present case rules out a 3-oxo structure. If the other secondary OH group is present at C-3 then the two ketones must be at positions 5 and 9. This result is not unexpected in view of the two 5-deoxy-5-oxoerythronolide B metabolites previously reported (5, 6).[3,4] Additional evidence for these assignments was obtained from the UV spectra which showed no absorbance at 294 nm after treatment of a ethanolic solution with alkali, characteristic of the spectra of picromycin.[7] In addition, the chemical shift of H-4 (3·07 ppm) is consistent only with a proton α to a C-5 CO group.



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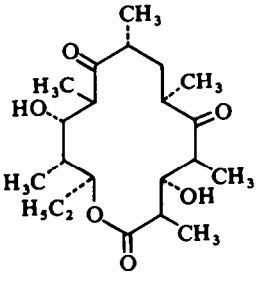

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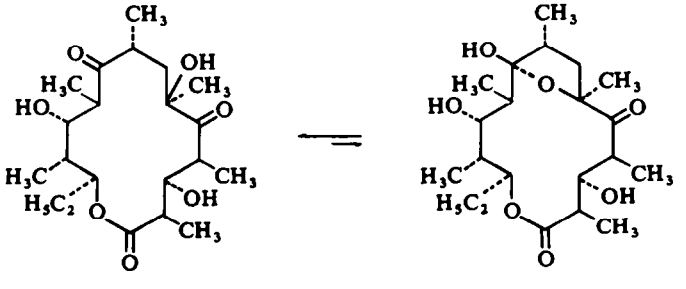
The inability of the metabolite to form a benzeneboronic acid derivative[9] should be noted here suggesting that one of the oxygen functions at C-3 or C-5 must be a ketone. It is well established that erythronolide structures having free C-3 and C-5



5a 5b

TABLE I. NMR PARAMETERS C_5D_5N SOLUTION[a]

	Chemical shifts					Coupling constants			
	1a	**2**[b]	**5**	**6**		**1a**	**2**	**5**	**6**
H-2	2·95	2·94	3·01	2·91	$J_{2,3}$	10·5	10·8	10·5	10·2
H-3	4·10	4·08	4·53	4·14	$J_{3,4}$	1·0	1·3	<1	1·2
H-4	3·20	2·45	3·98	3·13	$J_{4,5}$	—	2·0	—	—
H-5	–	4·07	—	—	$J_{5,6}$	—	—	—	—
H-6	2·70	—	—	2·8	$J_{6,7a}$	2·0	—	—	8·2
H-7a	2·30	2·23	2·36	2·10	$J_{6,7e}$	10·5	—	—	8·2
H-7e	~1·7	1·64	1·9	1·5	$J_{7a,e}$	15·0	15·2	14·0	14·3
H-8	—	3·09	~3·02	2·65	$J_{7a,8}$	—	7·0	9·0	3·0
H-10	3·30	3·08	3·19	3·09	$J_{7e,8}$	—	7·6	9·0	13·0
H-11	3·95	4·25	4·76	3·92	$J_{10,11}$	1·0	2·0	<1	1·5
H-12	~1·8	1·81	~1·8	~1·8	$J_{11,12}$	9·5	10·3	10·0	9·9
H-13	5·90	5·70	5·98	5·77	$J_{12,13}$	1·0	1·2	<1	1·1
H-14a	5·90	1·74	5·98	5·77	$J_{13,14a}$	8·9	8·8	9·0	8·7
H-14e	5·90	1·55	5·98	5·77	$J_{13,14e}$	5·2	6·6	5·0	5·2
					$J_{14a,14e}$	5·2	14·0	5·0	5·2

[a] Chemical shifts and coupling constants were measured after D_2O exchange of hydroxyl groups was completed. Chemical shifts are dependent on the amount of D_2O added and temperature. The spectra were recorded at the following temperatures **1a** = 60°, **2** = 110°, **5** = ambient, **6** = 80°.

[b] Some data from 220 MHz spectra.

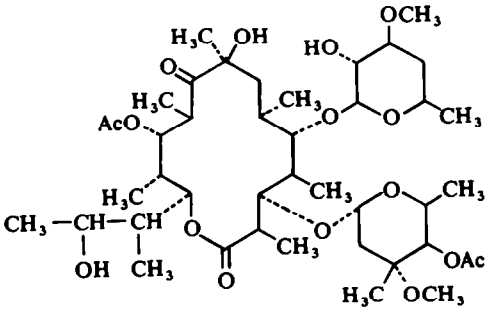
OH groups of natural configuration react with benzeneboronic acid to form the corresponding esters.[2,10] The failure of the compound to form a benzeneboronic acid derivative precludes a 3,5-dihydroxy structure.

Only the position of the tertiary OH group remained to be assigned. Spin-decoupling experiments revealed that the resonances of the two deshielded protons assigned to H-3 and H-11 each have two couplings (Table I). This is possible only if a proton is present at C-2, C-4, C-10, and C-12. If one of these positions were substituted by a OH group, the resonances of protons on an α-carbon, i.e., C-3 or C-11 would have a single coupling. Only two positions then remain to be considered, namely C-6 and C-8. Substitution at C-7, the only position not considered, would not result in the appearance of a tertiary Me singlet resonance. The compound having a tertiary OH at C-6, 5-deoxy-5-oxoerythronolide B (**5**), has been previously encountered[3] and its structure determined from both spectroscopic and chemical evidence. Therefore the metabolite must be 8-hydroxy-5,6-dideoxy-5-oxoerythronolide B (**1a**). (See discussion to follow which assigns stereochemistry).

The remarkable resistance of the C-8, C-9 bond of **1a** to periodate oxidation is not surprising since there are several examples of α-hydroxy ketones resistant to this oxidant. Lankamycin (**7**)[11] and 5-deoxy-5-oxoerythronolide (**5**)[3] are examples of 14-membered ring macrolides whose periodate resistant α-hydroxy ketone groups are entirely analogous to that of **1a**. Of particular interest is the fact that the surrounding C atoms of the α-hydroxy ketone function in the above examples are all substituted identically and probably have the same stereochemistry.[12]

(8S)-8-Hydroxy-5,6-dideoxy-5-oxoerythronolide B is biologically converted to a neutral compound of unknown structure which is not further metabolized when added to the fermentations of a number of early blocked mutants of *S. erythreus*. These

mutants are capable of converting known lactone precursors of erythromycin to the completed antibiotic. Thus, although the compound is not an intermediate of the erythromycin pathway, the biogenetic significance of this structure cannot be assessed at this time. The presence of an oxygen substituent at C-8 is not unknown in 14-mem-



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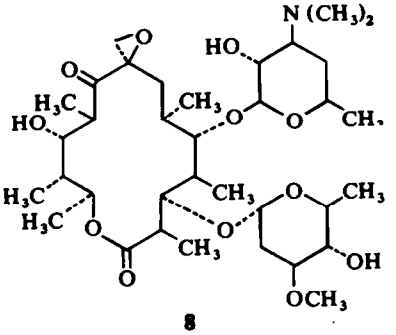

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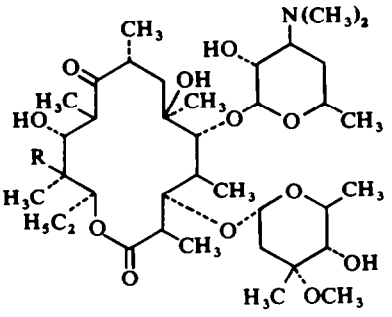
bered ring macrolide antibiotics. Lankamycin (7) a related neutral macrolide has a tertiary OH at C-8 and oleandomycin (8) is known to have an exocyclic epoxide at this position. Thus, if one can speculate, a point of special interest is the possibility of a distant common genetic lineage between *Streptomyces* strains producing 14-membered ring macrolide antibiotics.

CONFORMATIONAL ANALYSIS OF 5-DEOXY-5-OXOERYTHRONOLIDE B DERIVATIVES (1a, 5 AND 6)

NMR and CD studies have previously determined the solution conformation of the 14-membered aglycone ring, erythronolide B (2).[10, 13, 15] This conformation has also been shown to be applicable to a large number of derivatives of erythronolide B including the aglycone of the intact antibiotics erythromycin A and B (9a, b).[15] Comparison of the magnitude of the vicinal coupling constants of 1a with those of the other 5-deoxy-5-oxo metabolites (5 and 6) indicates that these compounds are conformationally homogeneous with erythronolide B (2; table I).

The conformation proposed for these compounds (Fig 3) places the 6-OH group, if present, in close proximity to the 9-keto group. This conformation also predicts that a 6,9-hemiacetal bridge can be formed without significant conformational reorganization. This prediction has been verified in the case of 5. Previous reports[3] have suggested



R
9a OH
9b H

that **5** exists as a mixture of the ketone and hemiacetal forms in $CDCl_3$ solution (**5a** and **5b**). Analysis of the $CDCl_3$ solution NMR spectra of **5** (Fig 4) confirms this suggestion. Separate resonances are observed for the 6-Me groups of both isomers and variable temperature experiments show that the relative amounts of the two isomers varies with temperature. The amount of hemiacetal present increases from 30 to 35% as the temperature increases from ambient (~30°) to 55° as shown by changes in the relative intensities of the 6-Me resonances. The major component was shown to be the hydroxyketone tautomer by the chemical shift of H-10 (2·98 ppm in $CDCl_3$ at ambient temperature) consistent only with a proton α to a ketone. The resonances of H-11 and H-13 of the hemiacetal were also observed under these conditions and they reveal coupling constants that are unchanged from those of the corresponding protons of the major isomer indicating that the hemiacetal bond has formed without significant conformational changes. The spectra of **5** in C_5D_5N solution indicate the presence of only the hydroxyketone tautomer in this solvent (Table I).

Table III compares the CD data for compounds **1a**, **5** and **6** with that of erythronolide B (**2**). The 5-oxoerythronolides have particularly distinctive CD spectra since the sign of the ketone transition is positive in all three derivatives whereas the curve is negative in the monoketone erythronolide series.[14,15] Since the NMR results have demonstrated conformational homogeneity in the mono- and diketo series, the

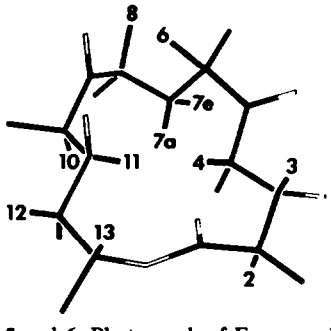


FIG 3. Conformation of **1**, **2**, **5** and **6**. Photograph of Framework Molecular Model construction. Solid lines represent C—C and C—H bonds while outlined lines represent C—O bonds. The position of ring protons is given by the appropriate numbers. For clarity, protons associated with Me and OH groups are not shown.

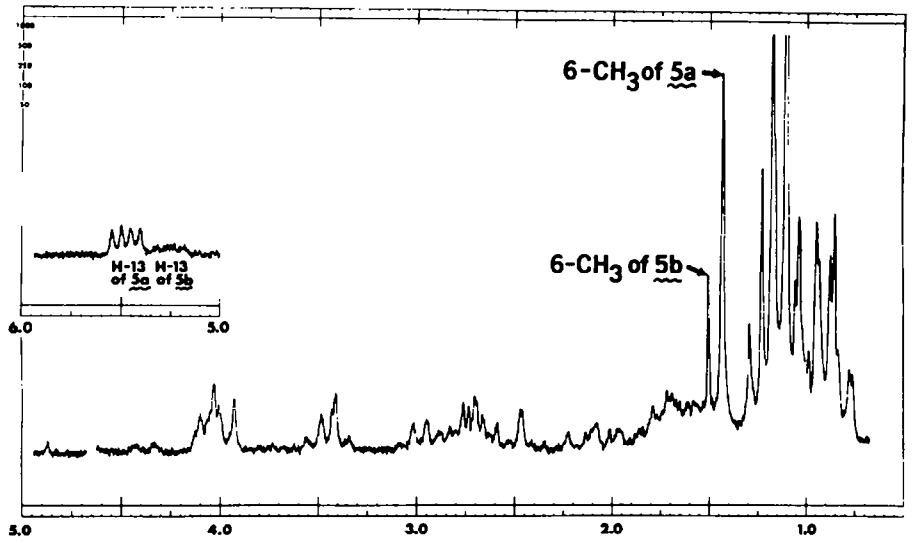


FIG 4. NMR spectrum of **5** in $CDCl_3$ solution.

presence of the second CO at C-5 must be responsible for the sign reversal. The dominance of the CO at C-5 is dramatically shown by the effect of added acid on the CD curves (Table III). In the case of **5**, acid treatment results in a loss of the

TABLE II. CHEMICAL SHIFTS AND AROMATIC SOLVENT INDUCED SHIFTS

	CHEMICAL SHIFTS[a]				
	$CDCl_3$		C_5D_5N		
	5	**1a**	**5**	**6**	**1a**
H-2	2·68	2·67	3·01	3·02	3·02
H-3	3·98	3·63	4·53	4·25	4·13
H-4	3·45	3·07	3·98	3·26	3·26
H-6	—	2·29	—	2·70	2·76
H-7a	2·15	2·06	2·36	2·16	2·35
H-8	2·75	—	3·02	2·88	—
H-10	2·98	2·72	3·19	3·16	3·34
H-11	4·06	3·49	4·76	4·05	4·03
H-13	5·47	5·51	5·98	5·93	6·04

	ASIS $\Delta = \delta_{CDCl_3} - \delta_{C_5D_5N}$	
	5	**1a**
H-2	−0·33	−0·35
H-3	−0·51	−0·50
H-4	−0·53	−0·19
H-6	—	−0·47
H-7a	−0·25	−0·29
H-8	−0·27	—
H-10	−0·21	−0·62
H-11	−0·70	−0·54
H-13	−0·51	−0·53

[a] Chemical shifts measured at ambient probe temperature (~30°).

C-9 ketone due to formation of the 6,9-hemiacetal or enol ether. The resultant curve arising from the lone CO at C-5 remains positive. In the case of **1a**, the acid treatment most likely results in loss of the CO at C-5 due to internal hemiacetal formation with the C-8 tertiary OH since a sign reversal occurs in the CD curve. The resultant negative curve is characteristic of the C-9 ketone.

TABLE III. CD DATA[a] IN MeOH

	Ketone	Ketone (after HCl)	Lactone
1a	292 nm, $[\theta]$ +17,500	300 nm, $[\theta]$ −11,500	210 nm, $[\theta]$ −9200
5	308 nm, $[\theta]$ +13,100	308 nm, $[\theta]$ +9500	212 nm, $[\theta]$ −5800
6	292 nm, $[\theta]$ +12,700	no change	210 nm, $[\theta]$ −7100
2	290 nm, $[\theta]$ −12,200		210 nm, $[\theta]$ −4300

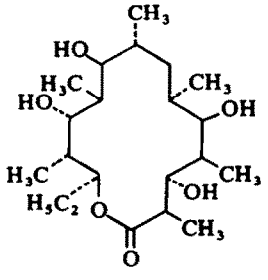
[a] We are indebted to Dr. L. A. Mitscher, Ohio State University for measurement and help in the interpretation of this data.

CONFIGURATIONAL ANALYSIS OF 5-DEOXY-5-OXOERYTHRONOLIDE B DERIVATIVES (1a, 5 AND 6)

Relative configuration is an important factor influencing ground state or lowest energy conformations. Introduction of an "unnatural" OH group in the related 5-*epi*-9S-9-dihydro-6-deoxyerythronolide B(**10**)[4] has been shown to result in substantial changes in conformation. The conformational homogeneity of **1a**, **5** and **6** as evidenced by the invariance of vicinal coupling constants suggests that these compounds are also configurationally homogeneous at asymmetric centers which can be characterized by coupling constants.

Unfortunately this criterion cannot be used with asymmetric centers which are not substituted by a proton (i.e., C-8 in **1a** and C-6 in **5**). Furthermore conformational differences in the C-6 to C-8 portion of the aglycone have been observed between compounds with identical configurations*; therefore, the magnitudes of C-7 methylene coupling constants cannot be unambiguously employed in configurational and/or conformational analyses. For these reasons, other methods were used to assign the configurations of tertiary asymmetric C atoms.

The stereochemistry of **5** and **6** has been rigorously proven to be the same as that



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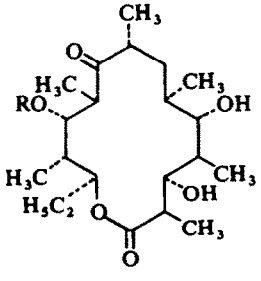


R
11 H
15 Ac

* Variations of C-7 methylene coupling constants will be discussed in a forthcoming publication in preparation.

of erythronolide B (**2**) and 6-deoxyerythronolide B (**11**) by the identity of reduction products.[3,4] However, the configuration of C-8 in **1a** remains to be proven.

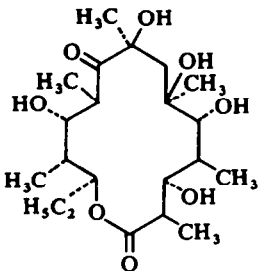
A number of meaningful correlations which offer evidence for the structure and conformation as well as the configuration of **1a** can be made when the chemical shifts of ring protons of **1a**, **5** and **6** are compared in $CDCl_3$ and C_5D_5N solution (Table II). Pyridine (aromatic) solvent induced chemical shift changes (ASIS values)[10,16,17] are also calculated whenever possible and presented in Table II. In the case of **6**, insufficient solubility in $CDCl_3$ prevented the calculation of ASIS values; however, correlations of the C_5D_5N solution chemical shifts are possible.

The values collected in Table II indicate that the chemical shifts of protons distant from the tertiary hydroxyls are not influenced by the positions of these hydroxyls. For example, the ASIS value of H-2, H-3, and H-13 are the same within experimental error in **1a** and **5**.

When the 6-OH group is removed, a decrease in the ASIS of H-11 is observed (i.e., $-0{\cdot}54$ in **1a** compared to $-0{\cdot}70$ in **5**). This result is consistent with the proposed conformation, since the 6-OH group and H-11, although on opposite sides of the aglycone ring, are internally substituted and therefore proximate. Although ASIS values are not available for **6**, the C_5D_5N solution chemical shift of H-11 in **1a** and **6** is identical within experimental error and different in **5** and **6** indicating that the 8-OH group in **1a** is not responsible for the decrease in the ASIS of H-11. Similar changes in the chemical shift of H-11 are encountered in other erythronolides—the chemical shift of H-11 in 6-deoxyerythronolide B (**11**) is $\sim 4{\cdot}2$ ppm while in erythronolide B (**2**) the resonance appears at 4·42 ppm when both compounds are examined in C_5D_5N solution. Analogous reasoning can be used to explain the decrease in the ASIS value of H-4 when the 6-OH group is not present. The correlations offer independent spectroscopic evidence that **1a** and **6** are 6-deoxy compounds. Similarly, the large ASIS value of H-10 in **1a** combined with the small value in **5** is consistent with the substitution of the tertiary OH at C-8 in **1a**. The large ASIS value is attributable to the 8-OH with no large change caused by the lack of a 6-OH group as evidenced by the similarity of the C_5D_5N solution chemical shifts of H-10 in **5** and **6**.

A similar effect can be observed in compounds lacking the 5-oxo group. The chemical shift of H-10 in erythronolide B (**2**) in C_5D_5N solution at ambient temperature is 3·15 ppm. Under the same conditions the chemical shift of H-10 in **5** is 3·19 ppm indicating that the C-5 ketone is not significantly contributing to its chemical shift. The chemical shift of H-10 in (8S)-8-hydroxyerythronolide B (**12**)[21] under the same conditions is 3·32 ppm while H-10 of **1a** resonates at 3·34 ppm. This strongly suggests that the configurations of C-8 in **1a** and **12** are both 8S. Furthermore, the C_5D_5N solution chemical shift of H-10 in lankolide (**13**) (an 11-acetate) is 3·43 ppm which is essentially identical to the 3·44 ppm chemical shift of the 11-acetate **1b** also suggesting that the C-8 configurations in these compounds are 8S. In the absence of an 8-OH group the chemical shift of H-10 is 3·24 ppm in 11-acetylerythronolide B (**14**) and 3·22 ppm in 11-acetyl-6-deoxyerythronolide B (**15**). These considerations offer the first evidence that the predicted C-8 configuration of lankamycin (**7**)[12] is correct and further specify that this configuration is also present in **1a**. It is reasonable to expect that the C-8 Me group of **1a** has the same configuration present in **5** and **6** since most microbiological hydroxylations proceed with retention of configuration.[18]

The CD data for the 5-keto analogs **1a**, **5**, and **6** are not particularly useful in the



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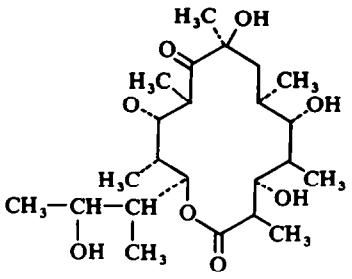

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determination of the configuration at C-8 in **1a**. OH groups introduced α to a ketone normally result in a bathochromic shift of the ketone transition when the OH is axial, and produce a hypsochromic shift when equatorial.[20] This rule was useful in the erythronolide series in determining the stereochemistry at C-8 in (8S)-8-hydroxy-erythronolide B (**12**),[21] but is not applicable in the 5-ketoerythronolides. As shown in Table III, the position of the ketone transition is identical in the case of **6** and its 8-hydroxy derivative **1a**. On the other hand the introduction of an α-OH at C-6 (**5**) results in a pronounced bathochromic shift as would be predicted by the presence of this axial substituent. This is further evidence for the dominant role played by the CO at C-5 in the CD spectra.

EXPERIMENTAL

General. M.ps were taken on a microscope hot stage and are corrected. UV spectra were recorded for 95% EtOH solns with a Cary Model II spectrophotometer. Optical rotations were measured on 1% solutions in MeOH with a Hilger and Watts polarimeter. IR spectra were recorded from $CHCl_3$ solns on a Perkin–Elmer Model 521 instrument. CD spectra were obtained with a Durrum-Jasco model ORD/UV-5 instrument equipped with a CD attachment and operating at ambient temp in spectra-grade MeOH solvent. Mass spectra were recorded with an AEI-MS-9 mass spectrometer with an ionization energy of 70 eV. Samples were introduced into the source by the direct inlet system. NMR spectra were obtained at 100 MHz using a Varian Associates HA-100 spectrometer. Chemical shifts are reported in ppm (δ) downfield from internal TMS (0δ). Coupling constants were obtained by direct measurement and are reported in Hz. NMR parameters were determined from first order analysis. Spectra were determined from approx. 12% w/v solns after the addition of sufficient D_2O to exchange OH resonances (approx 0·02 ml required). Chemical shifts in $CDCl_3$ are temp dependent and in C_5D_5N both temp and amount of D_2O should be considered. Coupling constants reported were measured at highest temp obtained for increased precision. Chemical shifts and coupling constants reported in approximate values could not be directly observed but were determined from spin-decoupling experiments. Chemical shift and coupling constant assignments were confirmed by appropriate spin-decoupling experiments whenever possible. TLC was carried out on Merck silica gel G after Stahl using 95% EtOH—$CHCl_3$; 1:10 as the developing solvent. Compounds were visualized by spraying with the arsenomolybdate reagent of Nelson[19] and then heating on a hot plate.

Isolation of (8S)-8-*hydroxy*-5,6-*dideoxy*-5-*oxoerythronolide B* (**1a**). A light yellow crystalline complex fraction CF-1c, obtained as previously described,[3] was the starting material for the isolation of **1a**. Fraction CF-1c, composed of two compounds with R_f 0·59–0·67 and 0·56–0·64 (800 mg), was chromatographed on a column (2·5 × 90 cm) of Sephadex LH-20 prepared in $CHCl_3$. Slow elution with $CHCl_3$ (~6 ml/hr) cleanly separated the two components. Fractions containing the slowest moving material (R_f 0·56–0·64) were combined and evaporated to dryness *in vacuo* to give 231 mg of crystalline solid. Recrystallization from EtOAc-hexane gave 170·3 mg of colourless crystals, m.p. 155–157°; $[\alpha]_D^{24}$ +99°; (*c*, 1·0, MeOH), γ_{max} 3500, 1722, 1700 and 1691 cm^{-1}. (Found: C, 62·91; H, 8·83. $C_{21}H_{36}O_7$ requires: C, 62·98; H, 9·06%).

Preparation of 3,11-*diacetyl*-(8S)-8-*hydroxy*-5,6-*dideoxy*-5-*oxoerythronolide B* (**1b**). A soln of **1a** (100 mg) in 10 ml pyridine and 2·0 ml Ac_2O was gently heated on a steam bath for 8 hr. The soln was cooled and poured into ice–water. The resulting pale brown ppt was collected and crystallized from EtOAc-hexane to give 59 mg of **1b** as colourless prisms, m.p. 183°–185°; γ_{max} 3465, 1743, 1707 cm^{-1}. (Found: C, 61·72; H, 8·19; M^+-HOAc *m/e* 424·2495. $C_{25}H_{40}O_9$ requires: C, 61·97; H, 8·32; M^+-HOAc *m/e* 424·2461).

Attempted formation of a 3,5-*phenyl boronate derivative of* (8S)-8-*hydroxy*-5,6-*dideoxy*-5-*oxoerythronolide B*(**1a**). A soln of 80 mg of **1a** and 24·2 mg phenylboronic acid in 10 ml acetone was heated under reflux for 4 hr. The mixture was reduced in volume and water was added. The resulting colourless needles were identical in m.p., mixture m.p., TLC and spectrometric behavior to starting **1a**.

Action of periodate on (8S)-8-*hydroxy*-5,6-*dideoxy*-5-*oxoerythronolide B* (**1a**), *lankolide* (**13**) *and erythronolide B* (**2**). A soln of 1 mg of **1a** in 0·5 ml MeOH and 0·5 ml of 0·01 M $NaIO_4$ was kept at room temp in the dark for 4 days. Since a sample which was removed for TLC showed no change, the soln was heated on a steam bath. Chromatographic comparison showed only starting material after 2 and 6 hr of heating. A 1 mg sample of **13** treated as above was also inert to periodate oxidation. A control sample of 1 mg of **2** in 0·5 ml MeOH and 0·5 ml of 0·01 M $NaIO_4$ was completely oxidized to a new compound in 6 hr at room temp.

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