Studies on the Biosynthesis of the Erythromycins. III. Isolation and Structure of 5-Deoxy-5-oxoerythronolide B, a Shunt Metabolite of Erythromycin Biosynthesis*

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ABSTRACT: A $C_{21}H_{36}O_7$ polyhydroxydioxolactone, elaborated in small quantities, has been isolated from the fermentation broth of a blocked mutant of *Streptomyces erythreus*. Recovery and purification of the compound was effected by solvent extraction and column chromatography. The structure of the compound was shown by chemical and physical means to be 5-deoxy-5-oxoerythronolide B. The compound was not converted into erythromycin when added to the fermentation medium of several strains of *S. erythreus*. It was speculated that 5-deoxy-5-oxoerythronolide B is a shunt metabolite of erythromycin biogenesis.

Erythromycins are antibiotic glycosides elaborated during fermentation by *Streptomyces erythreus*. The aglycone moiety of the erythromycins consists of a macrocyclic polyhydroxyoxolactone (Gerzon *et al.*, 1956; Wiley *et al.*, 1957), of which the exact details of biogenesis remain obscure. In early work on the lactone biosynthesis, various investigators reported that *S. erythreus* incorporates [^{14}C]propionate into erythronolide, the aglycone portion of the antibiotic, as intact three-carbon subunits without randomization (Corcoran *et al.*, 1960; Grisebach *et al.*, 1960; Vaněk *et al.*, 1961; Kaneda *et al.*, 1962). Recently 2-methyl malonate was found to be an efficient precursor of the lactone ring (Kaneda and Corcoran, 1961; Friedman *et al.*, 1964). A hypothetical chain-forming scheme has been proposed involving a "priming" unit of propionyl-CoA condensing with a recipient 2-methylmalonyl-CoA molecule to form a dimer. The resulting dimer then becomes the donor which condenses with a second 2-methylmalonyl-CoA to form a trimer, etc., until a long-chain polyoxo acid is formed (Friedman *et al.*, 1964; Wawszkiewicz and Lynen, 1964). Lactonization of the acid could result in a 21-carbon polyoxolactone (I) (Chart I) (Spizek *et al.*, 1966; Corcoran and Chick, 1966). An alternate cyclization possibility (Celmer, 1966) envisions as a final intermediate a "tail-body" unit where the "head" is a linear ester bond with the "tail." As the ring-forming step, Celmer visualizes a body-head β-acyl condensation affording a lactone. Celmer (1966) speculated that some or all of the oxo functions on the developing chain might be "fashioned," *i.e.*, reduced, before ring-forming occurs. If the hypothetical C-21 polyoxolactone (I) or a partially fashioned lactone is indeed a precursor of erythronolide biosynthesis, one would expect several intermediate lactones between the hypothetical compounds and 6-deoxyerythronolide B (IIIb), an intermediate of erythromycin biosynthesis recently isolated by Martin and Rosenbrook (1967). However, at this time, no intermediates between the propionyl-CoA primer and its methylmalonyl-CoA chain extenders and 6-deoxyerythronolide B have been reported.

In an effort to identify possible intermediates, we are examining the metabolites of a number of blocked mutants of *S. erytherus*. In this communication we wish to report the isolation and structure of a C-21 polyhydroxydioxolactone, 5-deoxy-5-oxoerythronolide B (II), from a blocked mutant of *S. erythreus*. This compound appears to be a shunt metabolite of erythromycin biosynthesis.

Experimental Procedure and Results[1]

Fermentation Organism. The strain employed in this investigation was *S. erythreus* (Abbott 4EB40). This strain was derived by treatment of a high erythromycin B producing organism with ethylenimine followed by ultraviolet irradiation. The mutant has a complete block in the erythromycin biosynthetic pathway and elaborates into the fermentation broth a large quantity of erythronolide B (IIIa) plus smaller amounts of 5-deoxy-5-oxoerythronolide B and several other unidentified macrolide compounds, many in trace amounts.

Fermentation and Extraction Procedures. Inoculum cultures of strain 4EB40 were prepared as described previously (Martin *et al.*, 1966). The fermentation medium and procedure has also been published (Martin and Rosenbrook, 1967). In a typical extraction, fermentation broth (4700 ml) was clarified and extracted with ethyl acetate as previously described (Martin *et al.*, 1966). The combined ethyl acetate extract was dried over anhydrous sodium sulfate and concentrated under reduced pressure to a small volume. After standing

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[1] Nuclear magnetic resonance spectra were determined using a Varian A-60 spectrometer with deuteriochloroform as solvent and tetramethylsilane as the internal reference. Infrared spectra were determined as chloroform solutions.

overnight, the bulk of the erythronolide B had crystallized. The crystals (10.9 g) were removed by filtration and the mother liquor was concentrated under reduced pressure to give 6.88 g of dark yellow oil. Thin-layer chromatography[2] of the oil showed the presence of a complex mixture containing at least six components.

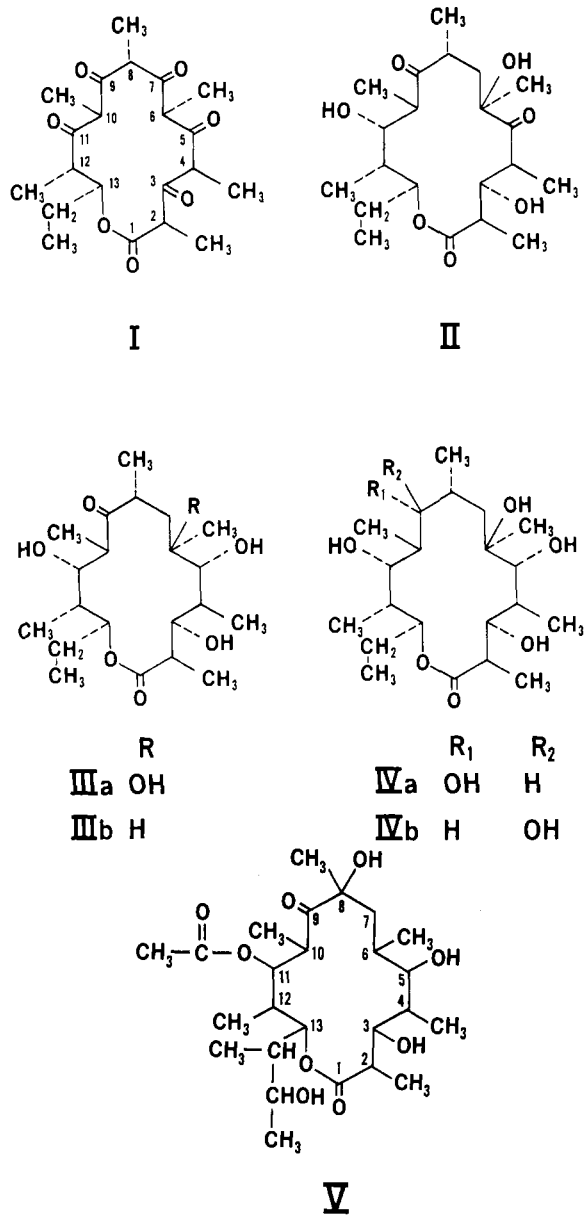
Isolation of 5-Deoxy-5-oxoerythronolide B. A portion of the yellow oil (3.5 g) was fractionated chromatographically using a silica gel column[3] (3.5 × 35 cm) prepared in chloroform. The column was successively eluted with chloroform–methanol in the indicated ratios: (1) 99.5:0.5, 500 ml; (2) 99.0:1.0, 1500 ml; and (3) 98.5:1.5, 2000 ml. The composition of each fraction was determined by thin-layer chromatography. Where appropriate, similar fractions were combined resulting in the following crude separations. Fraction CF-1, composed of the first yellow band eluted, contained three major components with R_F values of 0.78, 0.73, and 0.64. Fraction CF-2 contained 5-deoxy-5-oxoerythronolide B (R_F 0.52) and 6-deoxyerythronolide B (R_F 0.57). Fraction CF-3 was only slightly impure containing as a major component a material with R_F 0.47.

The fraction containing 5-deoxy-5-oxoerythronolide B (CF-2) was concentrated under reduced pressure affording a light yellow oil. The oil (906 mg) was placed on a column of silica gel (3.0 × 35 cm) prepared in chloroform. Elution with chloroform–methanol (99.5:0.5, 500 ml; 99.3:0.7, 500 ml; and 99.0:1.0, 1000 ml) gave elutes exhibiting only a single spot of 5-deoxy-5-oxoerythronolide B on thin-layer plates. These fractions were combined and evaporated to dryness under reduced pressure to give a light yellow residue. The residue was dissolved in methanol and treated with charcoal (Darco G60). Crystallization from ethyl acetate–hexane gave 220 mg of fine colorless needles: mp 197–199°, $[\alpha]_D^{25}$, +92° (*c* 1.0, methanol). The ultraviolet absorption spectrum in ethanol showed a maximum at 297 mμ (log ε 80). The infrared spectrum showed absorption at the following frequencies: 3485, 2970, 2935, 2875, 1700, 1450, 1370, 1355, 1330, 1175, 1085, 1050, 1030, 1020, 1000, 970, 940, and 890 cm^{-1}. The nuclear magnetic resonance spectrum is shown in Figure 1. The mass spectrum of 5-deoxy-5-oxoerythronolide B typically does not exhibit a mass peak for the molecular ion. The peak at highest mass (*m/e* 382) corresponds to the loss of one molecule of water. An active hydrogen determination established the presence of three hydroxyl groups.

Anal. Calcd for $C_{21}H_{36}O_7$: C, 62.97; H, 9.06. Found: C, 63.23; H, 9.04.

Reduction of 5-Deoxy-5-oxoerythronolide B. ISOLATION OF (9*S*)- AND (9*R*)-9-DIHYDROERYTHRONOLIDE B. To an ice bath stirred solution of 5-deoxy-5-oxoerythronolide B (200 mg) in methanol (6 ml), solid sodium borohydride (200 mg) was slowly added in small increments during the course of 15 min. The mixture was stirred for an additional 30 min, then it was neutralized with cold 1 N hydrochloric acid. Water (100 ml) was added and the solution was extracted with two 100-ml portions of ethyl acetate. The ethyl acetate extracts were combined, washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure to give a white crystalline solid. Thin-layer chromatography of the resulting solid revealed four components with the following R_F values: 0.37, 0.32, 0.23, and 0.16. The mixture was chromatographed on a 2.5 × 35 cm column of silica gel prepared in chloroform. Elution with increasing concentrations of methanol in chloroform gave fractions containing the compound with R_F 0.37. These fractions were combined, concentrated to dryness, and decolorized with carbon (Darco G60) to give a colorless oil from which crystals (mp 181–182°)

Chart I



[2] Thin-layer chromatography was carried out on silica gel G plates (250 μ) using the solvent system chloroform–95% ethanol (10:1); components were detected with the arsenomolybdate reagent of Nelson (1944).

[3] Silica gel used for column chromatography refers to that of the Davison Chemical Co., grade 923, 100–200 mesh.

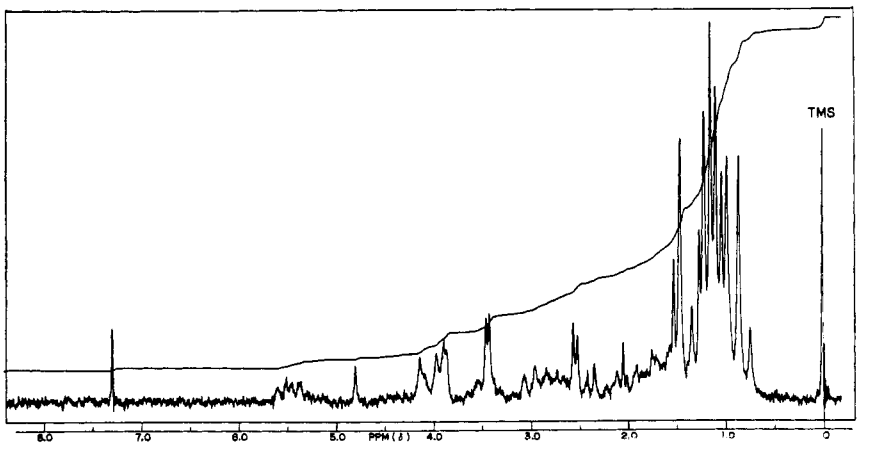


FIGURE 1: Nuclear magnetic resonance spectrum of 5-deoxy-5-oxoerythronolide B (10% solution in deuteriochloroform).

appeared upon addition of acetonitrile. Recrystallization from ethyl acetate–hexane gave 83 mg of colorless prisms: mp 186–187°, $[\alpha]_D^{23}$ +6.8° (*c* 1.0, methanol). This material was identical in its infrared spectrum with (9*S*)-9-dihydroerythronolide B (IVb) (mp 185–186°, $[\alpha]_D^{24}$ +5.7° (*c* 1.0, methanol)) prepared by the sodium borohydride reduction of erythronolide B (T. J. Perun and J. R. Martin, in preparation). A mixture melting point (186–187°) was undepressed.

Anal. Calcd for $C_{21}H_{40}O_7$: C, 62.35; H, 9,97. Found: C, 62.15; H, 9.74.

Further elution of the column gave fractions containing the compound with R_F 0.32. These fractions after the usual work-up gave 43 mg of colorless residue. Although thin-layer chromatography showed only a single component, initial efforts to crystallize the residue from several solvents gave only gels. Eventual slow evaporation of an ethyl acetate solution gave a colorless crystalline material, mp 149–151°. The infrared spectrum was very similar to that of (9*S*)-9-dihydroerythronolide B with strong hydroxyl absorption at 3400 cm^{-1} and carbonyl absorption at 1690 cm^{-1}.

Anal. Calcd for $C_{21}H_{40}O_7$: C, 62.35; H, 9.97. Found: C, 62.09; H, 9.67.

The next compound eluted had R_F 0.23. These fractions were combined and worked up as above affording 18 mg of colorless oil. Crystallization from ethyl acetate–hexane gave 11 mg of colorless prisms, mp 189–190°. This compound was identical in all respects (melting point, infrared spectrum, and thin-layer chromatography) with (9*R*)-9-dihydroerythronolide B (IVa) (mp 189–190°, $[\alpha]_D^{24}$ +9.7° (*c* 0.90, methanol)) obtained from the sodium borohydride reduction of erythronolide B (T. J. Perun and J. R. Martin, in preparation).

Anal. Calcd for $C_{21}H_{40}O_7$: C, 62.35; H, 9.97. Found: C, 62.33; H, 9.84.

Finally elution with 3% methanol in chloroform gave fractions yielding 18 mg of the component with R_F 0.16. After the usual work-up the small amount of material remaining could not be crystallized.

Monoacetylation of 5-Deoxy-5-oxoerythronolide B. 5-Deoxy-5-oxoerythronolide B (141 mg) was dissolved in 3.0 ml of dry pyridine, and 0.4 ml of acetic anhydride was added. The reaction mixture was allowed to stand at room temperature for 48 hr, and then it was poured onto cracked ice. The solution was extracted with two portions of chloroform. The combined chloroform extract was washed with water and dried over anhydrous sodium sulfate. The chloroform was removed under reduced pressure and the residue was dissolved in benzene. Evaporation of the benzene under reduced pressure gave a light yellow oil (144 mg) free of pyridine. Crystallization from ether–hexane afforded 126 mg of monoacetate which had mp 148–150°, $[\alpha]_D^{25}$ +115° (*c* 1.0, methanol). The infrared spectrum exhibited hydroxyl absorption at 3600 and 3500 cm^{-1} and carbonyl bands at 1740 and 1720 cm^{-1}. In the nmr spectrum a singlet at δ 2.03 characteristic of an acetoxyl group was evident.

Anal. Calcd for $C_{23}H_{38}O_8$: C, 62.42; H, 8.65. Found: C, 62.29; H, 8.86.

Diacetylation of 5-Deoxy-5-oxoerythronolide B. 5-Deoxy-5-oxoerythronolide B (435 mg) was dissolved in 25 ml of dry pyridine and 5 ml of acetic anhydride was added. The reaction mixture was heated on a steam bath for 29 hr, then cooled, and poured onto cracked ice. Work-up as above gave a dark oil which was dissolved in methanol, treated with charcoal (Norit), and the solution was evaporated to yield 500 mg of yellow oil. Crystallization from 1:1 ethanol–water gave 265 mg of diacetate: mp 182–183°, $[\alpha]_D^{25}$ +108° (*c* 1.0, methanol). In the ultraviolet a maximum was observed at 295 mμ (log ϵ 80). The infrared spectrum exhibited hydroxyl absorption at 3600 and 3500 cm^{-1} and carbonyl bands at 1740 and 1720 cm^{-1}. In the nmr spectrum the acetoxyl peaks appeared at δ 2.03 and 2.12.

Anal. Calcd for $C_{25}H_{40}O_9$: C, 61.96; H, 8.32; O, 29.72. Found: C, 61.80; H, 8.29; O, 29.99.

Attempted Formation of a Phenylboronate Ester. 5-Deoxy-5-oxoerythronolide B (80 mg) and benzeneboronic acid (24 mg) were dissolved in 5 ml of dry acetone and refluxed on a steam bath for 4 hr. Analysis of the reaction mixture by thin-layer chromatography showed only starting material. Concentration of the mixture under reduced pressure and addition of hexane afforded crystalline material (45 mg). Recrystallization from ethyl acetate–hexane gave colorless needles, mp 194–196°. The infrared spectrum was identical with that of 5-deoxy-5-oxoerythronolide B.

Attempted Periodate Oxidation of 5-Deoxy-5-oxoerythronolide B. To a suspension of 100 mg of 5-deoxy-5-oxoerythronolide B in 50 ml of sodium periodate solution (0.00998 M), 30 ml of acetone was added and the solution was allowed to stand in the dark at room temperature. Samples were withdrawn at intervals and titrated according to the method of Dyer (1956). Simultaneously, a blank was run under the same conditions. The consumption of periodate over a 100-hr period was very slow and proceeded at the same rate as that of the blank. By contrast, under the same conditions, erythronolide B (IIIa) consumed a theoretical amount of periodate in less than 10 hr.

The remaining reaction mixture was allowed to stand in the dark at room temperature for 2.5 months, then was extracted with chloroform. The chloroform extract after evaporation gave a 65% yield of a residue which was identical by thin-layer chromatography with starting material. Crystallization of the residue from ethyl acetate–hexane gave needles, mp 197–199°. The infrared spectrum was identical with that of 5-deoxy-5-oxoerythronolide B. A mixture melting point was undepressed (197–199°).

Attempted Biological Conversion of 5-Deoxy-5-oxoerythronolide B into Erythromycin by Strains of S. erythreus. Attempts to convert 5-deoxy-5-oxoerythronolide B into erythromycin by certain strains of *S. erythreus* were unsuccessful. Both nonerythromycin-producing and erythromycin-producing strains were selected for these experiments. Varients 2NU153 and 9EI41 were derived from high erythromycin-producing strains. Strain 2NU153 has a complete block early in the erythromycin pathway and is unable to synthesize erythromycin *de novo*. However, the organism can synthesize erythromycin if certain intermediates, *e.g.*, erythronolide B, 6-deoxyerythronolide B, or 3-*O*-(α-L-mycarosyl)erythronolide B are added to the fermentation medium. Strain 9EI41 is similar to 2NU153 but is a leaky mutant producing 6–12 μg of erythromycin/ml of fermentation medium. Mutant 9EI41 does not accumulate macrolide precursors of erythromycin in fermentation beers. However, this strain is capable of converting the three erythromycin precursors mentioned above into the antibiotic at four times the rate of 2NU153. Strain ER598 is a high erythromycin-producing organism.

When 25 mg of finely divided 5-deoxy-5-oxoerythronolide B was added to 50 ml of a 24-hr culture of each of the above strains in fermentation medium, the broths at 168 hr had the same level of antibacillus activity as unsupplemented control fermentations. Thus, the broth of the supplemented fermentation of 2NU153 had no antibiotic activity against *Bacillus subtilis*, that of 9EI41 had a normal 6–12 μg/ml of activity, and finally the supplemented fermentation broth of the high erythromycin-producing strain ER598 showed no increase in antibacillus activity.

Thin-layer chromatography of ethyl acetate extracts of the fermentation broths indicated that 5-deoxy-5-oxoerythronolide B was converted by all three strains into a new compound with R_F 0.57. This compound has the same R_F value as 6-deoxyerythronolide B but gives a deep purple color with arsenomolybdate reagent. 6-Deoxyerythronolide B gives a bright blue color thus indicating that the two compounds are different.

Discussion

During a systematic study of the biosynthesis of erythromycins, a neutral crystalline compound was isolated from the fermentation broth of a blocked mutant of *S. erythreus*. A molecular formula, $C_{21}H_{36}O_7$, with at least three hydroxyl groups was indicated from elemental analysis and active hydrogen determination. Two of the hydroxyl groups could be acetylated with acetic anhydride in pyridine. The ultraviolet spectrum in ethanol contained a maximum at 297 mμ with a molar extinction coefficient of 80. The infrared spectrum revealed intramolecular hydrogen-bonded hydroxyl absorption near 3500 cm^{-1} and strong ketone absorption at 1700 cm^{-1}.

The nmr spectrum (Figure 1) showed many features in common with the nmr spectra of erythronolide B (IIIa) and 6-deoxyerythronolide B (IIIb). The typical low-field broad multiplet at δ 5.5 (1 H) due to the proton of the carbon carrying the alkyl oxygen of the lactone was evident. Further upfield a series of resonance peaks centered at δ 3.9 (2 H) was assigned to the protons on carbon atoms bearing hydroxyl groups. The methyl region had a series of peaks at δ 0.7–1.5 which were shown by integration to arise from seven methyl groups. Deuterium exchange indicated that doublets centered at δ 3.4 and 2.5 were due to exchangeable hydrogens. The sharp singlet at δ 1.46 was assigned to the tertiary methyl at C-6. The extra peak at δ 1.52 apparently was due to the presence (in solution) of a 6,9-hemiketal in equilibrium with the hydroxy ketone (Perun, 1967). This peak was not present in the nmr spectrum obtained in pyridine. The sharp peak at δ 4.82 which integrated for less than one proton and which underwent deuterium exchange was presumably the hemiketal hydroxyl proton. Integration of the entire spectrum gave ~34 hydrogen atoms.

The skeleton of the new macrolide was confirmed as follows. Sodium borohydride reduction of the unknown compound gave a mixture, presumably a mixture of four diastereoisomers, albeit in different proportions. The diastereoisomers are separated on chromatoplates (R_F values of 0.37, 0.32, 0.23, and 0.16) and can be isolated by column chromatography. The principal product of the sodium borohydride reduction (R_F 0.37) was identical in all respects with (9*S*)-9-dihydroerythronolide B (IVb) obtained by the sodium borohydride reduction of erythronolide B (T. J. Perun and J. R. Martin, in preparation). This confirmed the presence of a 2,4,6,-8,10,12-hexamethylpentadecan-13-olide skeleton with oxygen atoms as hydroxyl or carbonyl functions at positions 3, 5, 9, and 11, and a tertiary hydroxyl group at C-6 and identifies the stereochemistry at each asymmetric center.

From the foregoing observations it can be concluded that the unknown macrolide contains two carbonyl functions. Biogenetic considerations permit a tentative assignment of one ketone at C-9. The validity of the assignment was shown by the identical nature of two of

the four diastereoisomers formed on reduction of the new macrolide with the two C-9 epimeric dihydro compounds obtained on reduction of erythronolide B with sodium borohydride. The diastereoisomer with R_F 0.23 was shown by direct comparison (melting point, mixture melting point, infrared spectrum, and thin-layer chromatography) to be identical with (9*R*)-9-dihydroerythronolide B (IVa) obtained by the sodium borohydride reduction of erythronolide B (T. J. Perun and J. R. Martin, in preparation). The isolation of (9*S*)- and (9*R*)-9-dihydroerythronolide B determines unambiguously the position of one ketone function at C-9.

At this stage the only remaining feature of the structural elucidation of the unknown macrolide was the location of the second carbonyl group. There were three possible sites for the location of this group: C-3, C-5, or C-11. The observation that the compound did not form a 3,5-cyclic ester derivative with benzeneboronic acid as does erythronolide B and 6-deoxyerythronolide B (Martin and Rosenbrook, 1967; T. J. Perun, unpublished observations), tentatively places the carbonyl at C-3 or C-5. Analogous with similar shifts in steroid spectra (Cookson and Dandegaonker, 1955), the shift of the ultraviolet carbonyl absorption to a longer wavelength relative to erythronolide B (Perun, 1967) is indicative of an α-hydroxy ketone group. It was therefore probable that a carbonyl function was at C-5.[4] Thus it was exciting to find that the macrolide was not oxidized, even after prolonged treatment, by periodate. These observations establish that the second carbonyl must be at C-5 and the compound has structure II. If the carbonyl were at C-3, C-5 would have to carry a hydroxyl group giving a C-5,C-6-diol which would be readily oxidized by periodate as is erythronolide B which has a C-5,C-6-diol grouping.[5]

The inability of periodate to oxidize 5-deoxy-5-oxoerythronolide B even after prolonged treatment is somewhat surprising since α-hydroxy ketones are known to react with this reagent. However, the resistance of the C-5,C-6 bond to periodate cleavage is consistent with the observation of Keller-Schierlein and Roncari (1964). These investigators found that the C-8,C-9 bond of lankamycin and its aglycone, monoacetyllankolide (V), is not readily cleaved by periodate. Clearly C-6 through C-10 of monoacetyllankolide are substituted identically with C-4 through C-8 of 5-deoxy-5-oxoerythronolide B. The stereochemistry of each corresponding center is also probably the same (Celmer, 1966).

Periodate oxidations are notably subject to steric effects (Sklarz, 1967) and apparently the bulkiness of the highly substituted carbons adjacent to the α-hydroxy ketone grouping in 5-deoxy-5-oxoerythronolide B prevents its oxidation by periodate. Other examples of periodate-resistant α-hydroxy ketones can be found in the steroid literature. von Euw and Reichstein (1941) reported that the 17,20-hydroxy ketone group of a pregnanediolone is resistant to periodate oxidation under mild conditions, presumably because of the highly hindered tertiary hydroxyl.

The biological conversion or nonconversion of an erythronolide structure into a normal erythromycin by *S. erythreus* should enable certain conclusions to be drawn concerning its place in the erythromycin biosynthetic pathway. Attempts to convert 5-deoxy-5-oxoerythronolide B into erythromycin by either blocked or erythromycin-producing strains of *S. erythreus* were unsuccessful. However, in fermentation systems which are capable of efficiently converting 6-deoxyerythronolide B, erythronolide B, and 3-*O*-(α-L-mycarosyl)erythronolide B into erythromycin, 5-deoxy-5-oxoerythronolide B was metabolized to an unidentified neutral metabolite. Preliminary evidence indicates that the compound is not further metabolized.

Erythromycin biosynthesis probably proceeds at defined centers (either inside the cell or on the membrane surface) and an exogenous biosynthetic intermediate must have free access to the site of antibiotic synthesis. Therefore, the cell may be unable to utilize a compound offered to it even though the substance may be a true intermediate. Although a permeability barrier could explain the inability of *S. erythreus* to convert 5-deoxy-5-oxoerythronolide B into erythromycin, it is likely that the compound is not a genuine erythromycin intermediate since a number of true intermediates of similar structure are readily converted into erythromycin (Martin *et al.*, 1966; Martin and Rosenbrook, 1967; Hung *et al.*, 1965), indicating that these compounds have free access to the site of antibiotic synthesis. These results suggest that 5-deoxy-5-oxoerythronolide B, although not an intermediate of erythromycin biosynthesis, is probably a shunt metabolite of antibiotic biogenesis.

It has been suggested (Corcoran, 1965) that erythronolide biosynthesis may involve an integrated complex of enzymes similar to the fatty acid synthetase system (Lynen, 1967). If such is the case, the whole sequence of reactions necessary for the formation of 6-deoxyerythronolide B may be accomplished by a multienzyme complex. The developing erythronolide molecule would be bound to the complex. Therefore, it is possible that an incomplete structure if once removed from the enzyme complex may no longer be a suitable substrate. The consequence, if the C-6 hydroxylase enzyme is of broad specificity, could be a compound such as 5-deoxy-5-oxoerythronolide B.

There are, of course, alternate explanations for the formation of erythronolide shunt metabolites. For example, in the series of reactions necessary for erythromycin biosynthesis, one or more of the steps may be mediated by enzymes with restricted specificity effectively controlling the pathway sequence even though some of the reactions necessary for biosynthesis may be

[4] At the suggestion of a referee, the ultraviolet spectrum of 5-deoxy-5-oxoerythronolide B was obtained in alkaline solution in order to compare it with the expected spectrum of a compound with a ketone at C-3. A bathochromic shift of the absorption maximum was not observed, ruling out a β-dicarbonyl structure. A pronounced irreversible hypsochromic shift was seen indicating that some unknown structural rearrangement had occurred. In acidic solution no change in the spectrum was noted.

[5] Comparison of the Varian HA-100 nuclear magnetic resonance spectrum of 5-deoxy-5-oxoerythronolide B with the detailed spectra of erythronolide B and its derivatives (T. J. Perun, in preparation) confirms the location of the carbonyl at C-5.

mediated by enzymes with broad specificity. Thus, if certain of the steps are performed out of sequence, the resulting metabolite may no longer be able to reenter the normal pathway of erythromycin biosynthesis. In the case of 5-deoxy-5-oxoerythronolide B a hydroxyl group may have been introduced out of sequence at C-6 effectively diverting the compound from the erythromycin pathway.

Currently there are no criteria to indicate the time of cyclization or the sequence of reductions necessary to convert the hypothetical polyoxo intermediate or an acyclic polyoxo compound into 6-deoxyerythronolide B. While reduction of the C-5 carbonyl group might be considered to be the final reductive step in the formation of 6-deoxyerythronolide B from its progenitors, the isolation of 5-deoxy-5-oxoerythronolide B does not allow this prediction with certainty.

A detailed examination of the metabolites of mutant 4EB40 is now in progress. It is hoped that these investigations will yield insight into the still relatively obscure reaction sequence of erythronolide biosynthesis.

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