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3-O-(2″,6″-DIDEOXY-α-L-*RIBO*-HEXOPYRANOSYL)-ERYTHRONOLIDE B AND 3-O-(2″,6″-DIDEOXY-α-L-*ARABINO*-HEXOPYRANOSYL)ERYTHRONOLIDE B, ABERRANT ERYTHROMYCIN BIOGENETIC METABOLITES WITH DEFECTIVE SUGAR MOIETIES

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Abstract—3-O-(2″,6″-Dideoxy-α-L-*ribo*-hexopyranosyl)erythronolide B (**6**) and 3-O-(2″,6″-dideoxy-α-L-*arabino*-hexopyranosyl)erythronolide B (**7**), aberrant metabolites of erythromycin biosynthesis, have been isolated from the fermentation broth of a blocked mutant of the erythromycin producing organism *Streptomyces erythreus*. The defective glycosides, when incubated with early blocked mutants of *S. erythreus* capable of converting authentic erythromycin progenitors to the complete antibiotic, were unmetabolized. The probable origin of the metabolites is discussed.

The naturally occurring erythromycins (**1–5**) macrocyclic aglycones linked to the deoxy sugars desosamine and either mycarose or cladinose, are antibiotic substances isolated from the fermentation broths of *Streptomyces erythreus*. In earlier reports we have communicated some of our studies on erythromycin biosynthesis using mutants of the producing organism. These studies have resulted in the isolation and identification of both erythromycin progenitors[1-3] and aberrant metabolites.[4-7] The aberrant structures previously reported were all the result of modifications on the 14-membered ring aglycone, erythronolide. We now wish to report the isolation, from fermentation broths of a blocked mutant, of two monoglycoside metabolites with abnormal sugar moieties. The defective glycosides, 3-O-(2″,6″-dideoxy-α-L-*ribo*-hexopyranosyl)erythronolide B (**6**) and 3-O-(2″,6″-dideoxy-α-L-*arabino*-hexopyranosyl)erythronolide B [3-O-(α-L-olivosyl)erythronolide B] (**7**), were not metabolized further when incubated with an early blocked mutant of *S. erythreus* capable of converting authentic erythromycin progenitors to the completed antibiotic.

A blocked mutant of the erythromycin producing organism *S. erythreus* (Abbott XE11) elaborates a number of previously identified erythromycin progenitors and aberrant metabolites. The authentic erythromycin biogenetic intermediates identified include 6-deoxyerythronolide B,[2] erythronolide B (**9**),[8] and 3-O-(α-L-mycarosyl)erythronolide B (**8**);[1] the shunt metabolites isolated were 5,6-dideoxy-5-oxoerythronolide B,[4] 5-deoxy-5-oxoerythronolide B,[5] and (8S)-8-hydroxy-5,6-dideoxy-5-oxoerythronolide B.[6] At least two of the sundry metabolites remained unidentified. The undefined components, $C_{27}H_{48}O_{10}$, now identified as 3-O-(2″,6″-dideoxy-α-L-*ribo*-hexopyranosyl)erythronolide B (**6**) and 3-O-(2″,6″-dideoxy-α-L-*arabino*-hexopyranosyl)erythronolide B (**7**) were isolated as colorless crystalline compounds.

The 100 MHz PMR spectra of the new metabolites (**6** and **7**, Table 1) were reminiscent of that of 3-O-(α-L-mycarosyl)erythronolide B (**8**). Monoglycosides were indicated since only one anomeric proton resonance was observed in the 4.0–5.5 ppm region. Also consistent with this proposal was the small magnitude of the $J_{4,5}$ couplings indicative of monoglycosides. The amino sugar desosamine, attached via a glycosidic linkage of erythronolide at C_5 in the erythromycins, was absent since no dimethylamino resonance or characteristic anomeric doublet at 4.4 ppm was observed in either spectrum. The presence of a sugar attached via a glycosidic bond was indicated by broad anomeric resonances at 5.04 ppm in **6** and 5.02 ppm in **7**; both resonances exhibited two small couplings. However, the OMe singlet usually associated with cladinose and the C-Me singlet at *ca.* 1.2 ppm associated with both mycarose and cladinose were absent suggesting that previously unencountered sugars were present.

Analysis of the sugar ring proton resonances by means of spin decoupling experiments and the observation of at least one proton at each ring position revealed that the sugars in both metabolites were 2,6-dideoxyhexopyranoses devoid of quarternary centers arising from C-Me or other substituents. In the spectrum of **6**, the magnitudes of the vicinal couplings of the sugar ring protons and in particular the small values of $J_{1',2a'}$ and $J_{2a',3'}$ couplings clearly indicate that both $C_{1'}$ and $C_{3'}$ substituents are axial. Conversely the large diaxial couplings of $J_{4',5'}$ require that the $C_{4'}$ and $C_{5'}$ substituents be equatorial. These observations establish the relative configuration of the sugar of **6** as *ribo*. In the spectrum of **7** the anomeric proton couplings are small indicating that the $C_{1'}$ substituent is axial. The $J_{3',4'}$ couplings are large and equal to $J_{4',5'}$ requiring that the $C_{3'}$, $C_{4'}$ and $C_{5'}$ substituents be equatorial. These observations define the relative configuration of the sugar moiety of **7** as *arabino*.

A total analysis of the aglycone ring proton resonances was possible and particularly informative were the coupling patterns of H_{11} and H_{13} which established that no C_{12} OH substituent was present in either metabolite. A singlet Me resonance and the observation of only one H_5 coupling showed that a C_6 OH substituent was present. These factors plus the observation that the remaining ring Me groups gave 5 doublets and a triplet resonance established the aglycone in both metabolites as erythronolide B (**9**).

From biological considerations it is reasonable to

assume that the dideoxyhexopyranose sugars identified above be attached at C_3 of erythronolide B. Thus, the PMR analysis suggests 3-O-(2″,6″-dideoxy-α-L-*ribo*-hexopyranosyl)erythronolide B (**6**) and 3-O-(2″,6″-dideoxy-α-L-*arabino*-hexopyranosyl)erythronolide B (**7**) as the structures of the aberrant metabolites.

The high resolution mass spectra of **6** and **7** supported the structures assigned from PMR studies. Spectra of both compounds were nearly identical and the highest signal was at *m/e* 514.3136 ($C_{27}H_{48}O_9$) but metastable defocusing and peak matching verified that these ions arose by loss of water from *m/e* 532. Characteristic ions formed by C–C bond cleavage of the McLafferty-rearrangement product of the lactone ring, erythronolide B, were present.[9,10] Most prominent were ions formed by cleavage between C_5–C_6 (*m/e* 241, $C_{14}H_{25}O_3$) and the subsequent loss of water (*m/e* 223, $C_{14}H_{23}O_2$). Other prominent fragments arose by cleavage at C_8–C_9 (*m/e* 155, $C_9H_{15}O_2$) and loss of water (*m/e* 137, $C_9H_{13}O$) and by scission at C_{11}–C_{12} (*m/e* 99, $C_6H_{11}O$). The sugar moiety of **6** and **7** was represented by prominent ions at *m/e* 131 ($C_6H_{11}O_3$) and the dehydration product *m/e* 113 ($C_6H_9O_2$).

It was anticipated that the acid catalyzed methanolysis of both **6** and **7** would yield erythronolide B (**9**) and 8,9-anhydroerythronolide B 6,9-hemiacetal (**10**)[11] and the α- and β-methyl glycosides of the respective glycosidically linked sugars. A TLC comparison of the hydrolysis products of both glycosides and 3-O-(α-L-mycarosyl)erythronolide B (**8**) indicated that this was indeed the case. These observations were further substantiated when erythronolide B (**9**) and 8,9-anhydroerythronolide B 6,9-hemiacetal (**10**) were isolated from hydrolysates of both metabolites. Paucity of **6** and **7** precluded isolation of the respective sugar moieties.

It is reasonable to assume that the aberrant erythromycin metabolites **6** and **7** result from defective mycarose biosynthesis. The sequence of biogenetic events that lead to mycarose is still largely hypothetical. Current evidence, obtained from isotope studies[12–17] indicates that mycarose is derived from D-glucose without cleavage of the glucose carbon skeleton; methionine supplies the C-Me group at C_3. In analogy with known biosynthetic schemes in other microorganisms the necessary transformations are thought to occur with the sugar attached to a nucleotide carrier. The nucleotide bound activated mycarose could then be attached via glycosidic linkage to the C_3 OH group of erythronolide B (**9**) to form 3-O-(α-L-mycarosyl)erythronolide B (**8**) both erythromycin intermediates.

Possible reaction sequences for the biosynthetic conversion of D-glucose to L-mycarose have been suggested.[15,18] Both of these schemes, although greatly differing, visualize a nucleoside disphosphate sugar intermediate—either the enol form of a 4-keto-6-deoxy hexose[15] or a 2,6-dideoxy-2,3-anhydro hexose[18]—that is C_3 methylated by S-adenosyl methionine. 3-O-(2″,6″-dideoxy-α-L-*ribo*-hexopyranosyl)erythronolide B (**6**) and 3-O-(2″,6″-dideoxy-α-L-*arabino*-hexopyranosyl)erythronolide B (**7**) presumably result from defective one carbon introduction at C_3. In the proposals mentioned, the hexose chiral center at C_3 was eliminated by double bond formation and then stereospecifically regenerated and locked by C_3 methylation. In the case here, defective methylation would allow only epimerization at C_3. Presumably the remaining necessary biosynthetic events for the synthesis of the defective sugars are performed by enzymes of broad specificity. We have previously found that the enzyme(s) responsible for erythronolide C_3 glycosidation have broad specificity in contrast to the rigid substrate requirements necessary for desosamine glycosidation at C_5.

It should be mentioned that Celmer[19] recently reported the isolation of 2,6-dideoxy-L-*arabino*-hexose, termed olivose, from O-demethyloleandomycin, an antibiotic co-produced in the oleandomycin fermentation. Presumably the O-demethyloleandomycin is methylated to form oleandomycin. It is interesting that a previously studied blocked mutant of *S. erythreus* (Abbott 4EB40) derived from the same parent as *S. erythreus* (Abbott XEI1), produced small amounts of deoxyoleandolide, the deoxy aglycone of oleandomycin. Now we find a *S. erythreus* mutant producing an aberrant metabolite having an oleandomycin sugar moiety.

With only limited success we have previously attempted to prepare modified intact erythromycin analogs by incubation of aberrant or chemically derived 14-membered lactone ring structures with blocked mutants of the erythromycin producing organism.[7,20–23] Our previous failures to prepare significant quantities of diglycoside erythromycin derivatives by biological glycosidation were repeated when 3-O-(2″,6″-dideoxy-α-L-*ribo*-hexopyranosyl)erythronolide B (**6**) and 3-O-(2″,6″-dideoxy-α-L-*arabino*-hexopyranosyl)erythronolide B (**7**) were incubated with blocked mutants of *S. erythreus* capable of converting erythromycin progenitors to the complete antibiotic. TLC examination of clarified fermentation broths indicated that much of the added substrate (**6** or **7**) remained intact after 120 hr incubation and that new basic compounds did not accumulate in fermentation broths. In addition, fermentation broths had only low levels of antibiotic activity.

EXPERIMENTAL

General. Instrumental methods of analysis have been previously described.[3] TLC was performed on Analtech precoated silica gel GF plates using CH_2Cl_2–5% aqueous MeOH–conc NH_4OH 90:10:1 v/v as the developing solvent. Compounds were detected with the arsenomolybdate reagent of Nelson.[24] Silica gel for column chromatography was that of Merck, 70–230 mesh.

Fermentation and isolation

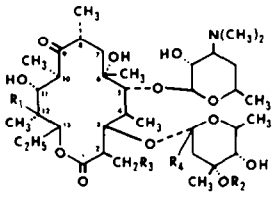
(a) 3-O-(2″,6″-*Dideoxy*-α-L-*ribo*-*hexopyranosyl*)*erythronolide B* (**6**). *Streptomyces erythreus* (Abbott XEI1) was fermented as described previously.[1,2] The whole fermentation broth (71) was clarified as given earlier[1] and extracted two times with 500 ml portions of chloroform. The combined chloroform extracts were washed with water and dried ($MgSO_4$). Evaporation of the major portion of the chloroform gave 2.83 g of crystalline **8**. Further concentration of chloroform gave an additional 0.38 g of **8**. The mother liquor was concentrated to dryness to leave a dark yellow–brown residue (3.64 g). Examination by TLC indicated the presence of at least 8 components. Comparison with authentic samples of previously isolated *S. erythreus* metabolites allowed identification of erythronolide B (**9**),[8] 6-Deoxyerythronolide B[2], 3-O-(α-L-mycarosyl)erythronolide B (**8**),[1] 5-deoxy-5-oxoerythronolide B,[3] 5,6-dideoxy-5-oxoerythronolide B[4] and (8S)-8-hydroxy-5,6-dideoxy-5-oxoerythronolide B.[6] Two major components remained unidentified—a material which was more polar than erythronolide B and a compound which migrated slightly ahead of erythronolide B.

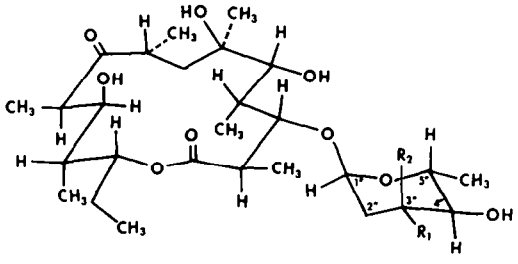
The array of compounds were easily separated and isolated by column chromatography on silica gel. In a typical experiment 3.13 g of residue was chromatographed on a column (2.9 × 40 cm) of silica gel prepared in chloroform. Slow elution with increasing concentrations of MeOH in chloroform allowed separation of all the compounds mentioned above in order of decreasing R_f values. Of the unidentified compounds, that which migrated ahead of

Table 1. PMR parameters of 3-O-(2",6"-dideoxy-α-L-*ribo*-hexopyranosyl)erythronolide B (**6**), 3-O-(2",6"-dideoxy-α-L-*arabino*-hexopyranosyl)erythronolide B (**7**) and 3-O-(α-L-mycarosyl)erythronolide B (**8**)[a]

	Chemical Shifts ppm				Coupling Constants Hz		
n	**6**	**7**	**8**		**6**	**7**	**8**
H-2	2.77	2.76	2.79	$J_{2,3}$	10.0	11.0	10.5
H-3	3.66	3.60	3.68	$J_{3,4}$	<1	<1	0
H-4	2.2	2.2	2.16				
H-5	3.52	3.51	3.53	$J_{4,5}$	2.0	2	2.5
H-7a			1.91	$J_{7a,7e}$			14.6
H-7e			1.46	$J_{7a,8}$			11.4
H-8	2.7	2.7	2.70				
H-10	2.96	2.97	2.97	$J_{7e,8}$			2.2
H-11	3.81	3.84	3.82	$J_{10,11}$	1	<1	1
H-12	1.7	1.7	1.67				
H-13	5.46	5.47	5.47	$J_{11,12}$	10.0	9.5	10.0
				$J_{12,13}$	<1	<1	<1
H-1"	5.04	5.02	5.50	$J_{13,14a}$	9.5	9.0	9.5
H-2a"		1.7	1.82				
H-2e"	2.25		2.14	$J_{13,14}$	4.5	5.5	4.8
H-3"	3.94	3.8	—	$J_{14a,14e}$			14.0
H-4"	3.15	3.09	2.97				
H-5"	4.03	3.93	3.91				
				$J_{1",2a"}$	<1	1	1
2-CH_3	1.21	1.20	1.21	$J_{1",2e"}$	2.0	3.0	3.8
4-CH_3	1.02	1.00	1.01				
6-CH_3	1.38	1.38	1.39	$J_{2a",2e}$	15.0		14.4
8-CH_3	1.13	1.13	1.13	$J_{2e",3"}$	<1		—
10-CH_3	0.98	0.99	0.98	$J_{2a",3"}$	2.0		—
12-CH_3	0.89	0.91	0.91				
14-CH_3	0.86	0.87	0.87	$J_{3",4"}$	2.0	9.0	—
				$J_{4",5"}$	9.5	9.0	9.6
5"-CH_3	1.32	1.31	1.33				
3"-CH_3	—	—	1.24				

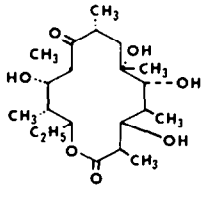
[a] Obtained from 100 MHz spectra in $CDCl_3$ by direct measurement using first order rules and reported in ppm downfield from internal TMS.





	R_1	R_2	R_3	R_4
1	OH	CH_3	H	H
2	H	CH_3	H	H
3	OH	H	H	H
4	H	H	H	H
5	OH	CH_3	—O—	

	R_1	R_2
6	H	OH
7	OH	H
8	CH_3	OH



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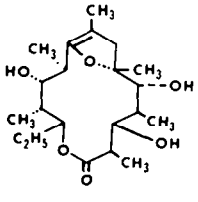

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erythronolide B eluted first. Pure homologous fractions were pooled and concentrated to dryness to yield 269 mg of pale yellow oil. The oil was treated with carbon (Darco G60) in MeOH. Crystallization from EtOAc-hexane gave 164 mg of **6** as colorless fine needles, m.p. 192–194°; $[\alpha]_D^{23}$ −62.7° (*c* 1.0, MeOH); λ_{max}^{MeOH} 287 nm (ϵ36); IR, 3605, 3510, 1713 and 1689 cm^{-1}; PMR see Table 1; Mass spectrum, M^+-H_2O, 514.3131. (Found: C, 61.00; H, 9.25. Calc. for $C_{27}H_{48}O_{10}$: C, 60.88; H, 9.08%).

(b) 3-O-(2″,6″-*Dideoxy-α-*L-*arabino-hexopyranosyl)erythronolide B* (**7**). Continued elution with increasing concentrations of MeOH in chloroform gave erythronolide B and finally fractions containing only **7**. These fractions were collected and concentrated to dryness to give 101 mg of straw colored oil. After treatment with Darco G60 in MeOH, crystallization from EtOAc-hexane gave 49.6 mg of colorless needles, m.p. 131–135°; $[\alpha]_D^{23}$ −86.8° (*c* 1.0, MeOH); λ_{max}^{MeOH} 280 nm (ϵ49); IR, 3605, 3460, 1712 and 1690 cm^{-1}; PMR see Table 1; Mass spectrum, M^+-H_2O, 514.3142. (Found: C, 61.00: H, 9.35. Calc. for $C_{27}H_{48}O_{10}$: C, 60.88; H, 9.08%).

Acid catalyzed methanolysis of 3-O-(2″,6″-*dideoxy-α-*L-*ribo-hexopyranosyl)erythronolide B* (**6**) *and* 3-O-(2″,6″-*dideoxy-α-*L-*arabino-hexopyranosyl)erythronolide B* (**7**)

Isolation of erythronolide B (**9**) *and* 8,9-*anhydroerythronolide B* 6,9-*hemiacetal* (**10**). Compound **6** (80 mg) was treated with 5 ml of 1% methanolic HCl and allowed to stand at ambient temp. for 6 hr. Examination of the mixture by TLC and comparison with authentic samples showed the presence of **9**, **10** and two unidentified spots thought to be the α- and β-methyl glycosides of the 2″,6″-dideoxy-*ribo*-hexopyranose. The mixture was slowly added to 50 ml of cold $NaHCO_3$aq and then extracted with chloroform. The chloroform extract was washed, dried ($MgSO_4$), and evaporated leaving a pale yellow residue (72 mg). The residue was chromatographed on a silica gel column (1.8 × 30 cm) prepared in chloroform. Elution with increasing concentrations of MeOH in chloroform gave **10** as an oil (17 mg), identical with an authentic sample prepared by Kurath[9] (TLC, IR and PMR). Further elution yielded **9**[9] as needles (14 mg), m.p. 235–237°, identical by TLC, IR and PMR with an authentic sample.

Compound **7** (32 mg) treated with 1% methanolic HCl as above and examined by TLC showed the presence of **9**, **10** and two unidentified spots presumably the α- and β-methyl glycosides of the 2″,6″-dideoxy-arabino-hexopyranose. Column chromatography of the products of the mixture gave 7 mg of **10** and 5 mg of **9** identical in all respects with authentic samples.

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