BIOTRANSFORMATION OF LANKAMYCIN, DARCANOLIDE, AND 11-ACETYLLANKOLIDE BY A BLOCKED MUTANT OF THE ERYTHROMYCIN PRODUCING ORGANISM *STREPTOMYCES ERYTHREUS*

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The biotransformation of lankamycin and congeners darcanolide and 11-acetyllankolide by a blocked mutant of the erythromycin-producing organism *Streptomyces erythreus*, which cannot synthesize erythromycin without supplementation with erythromycin precursors, was investigated. Darcanolide and 11-acetyllankolide were converted into the corresponding 15-deoxy-15-oxo derivatives. Lankamycin was transformed to 15-deoxy-15-oxolankamycin, 4″-deacetyl-15-deoxy-15-oxolankamycin and 3′-de-*O*-methyllankamycin. None of the derivatives possessed high antimicrobial activity.

Erythromycin and lankamycin (**1**) are 14-membered ring macrolide antibiotics substituted at C_3 and C_5 with sugar moieties. Erythromycin, substituted at C_5 with an amino sugar and at C_3 with a neutral sugar, has high antibacterial activity in contrast to the low activity displayed by lankamycin substituted both at C_3 and C_5 with neutral sugars. The diminished activity of lankamycin might be attributed in part to the lack of an amino sugar moiety at C_5.

Mutants of the erythromycin-producing organism *Streptomyces erythreus*, which cannot make erythromycin unless supplemented with erythromycin precursors, have been utilized to produce novel glycosides related to the erythromycins.[1~4] These studies have shown the rather loose specificity exhibited by the enzyme(s) responsible for addition of the neutral sugar mycarose to the C_3 carbon of the macrocyclic lactone in contrast to the rigid requirements necessary for addition of the C_5 amino sugar desosamine. Although this latter specificity limits the utility of the mutant-modified precursor system for formation of new macrolide antibiotics, several *bis*-glycosides and a host of novel C_3 monoglycosides have been prepared.

The availability of 11-acetyllankolide (**2**) prompted us to apply the mutant-modified precursor technique in an attempt to obtain a novel lankamycin-related structure. Our initial efforts were directed toward preparing a compound having the lankamycin aglycone, 11-acetyllankolide, substituted at C_3 and C_5 with the respective erythromycin sugars, mycarose (or cladinose) and desosamine. Although this goal was not realized, several unexpected biotransformations, related here, were observed.

Materials and Methods

General

Instrumental methods of analysis have been previously described.[5] Tlc was performed on Merck silica gel G after Stahl using 95% ethanol - chloroform 1: 10 v/v as the developing solvent. Compounds were visualized by spraying with anisaldehyde reagent (95% ethanol - conc. H_2SO_4 - anisaldehyde, 10: 1: 1 v/v) and then heating. Silica gel for column chromatography was that of Merck (Darmstadt), 70~230 mesh.

Fermentation and Product Isolation

A previously described organism,[6] *S. erythreus* (Abbott 2NU153), was used in all fermentations. This organism, a blocked mutant, synthesizes erythromycin only in the presence of progenitors containing a lactone ring. The complex fermentation medium and culture conditions were previously given.[6] In a typical experiment, 25 mg of finely divided substrate was added to each 500-ml Erlenmeyer flask containing 50 ml of a 48-hour culture of strain 2NU153. Incubation was continued for an additional 120 hours, and the crude product isolated from clarified broth by ethyl acetate extraction.[7]

Biotransformation of 11-Acetyllankolide (2). Isolation of 11-Acetyl-15-deoxy-15-oxolankolide (3)

Tlc examination of fermentation broths fed 11-acetyllankolide (**2**) indicated that the added lactone was partially converted to a new, faster moving compound. Addition of 250 mg of 11-acetyllankolide (**2**) gave, after solvent extraction, 138 mg of viscous yellow-brown oil. The new component was separated cleanly from residual **2** by column (1.0×32 cm) chromatography on silica gel prepared in chloroform. Elution with increasing concentration of methanol in chloroform gave 11-acetyl-15-deoxy-15-oxolankolide (**3**) (16 mg). Crystallization from methanol - chloroform gave needles, mp 158~160°C; $[\alpha]_D^{24}$ +3° (*c* 0.55, CH_3OH); $\lambda_{max}^{CH_3OH}$ 275 nm (ε 130); IR, 3610, 3460, 1720 and 1704 cm^{-1}; PMR see Table 1.

Anal. Calcd for $C_{25}H_{42}O_9$: C, 61.71; H, 8.70.
Found: C, 61.47; H, 8.78.

Biotransformation of Darcanolide (4). Isolation of 15-Deoxy-15-oxodarcanolide (5)

Tlc examination of fermentation broths fed darcanolide (**4**) indicated that added glycoside was partially converted to a new, faster-moving compound. Addition of 275 mg of **4** gave, after solvent extraction, 243 mg of viscous yellow-brown oil. The new component was cleanly separated from

Table 1. PMR parameters of 11-acetyllankolide (**2**), 11-acetyl-15-deoxy-15-oxolankolide (**3**), darcanolide (**4**) and 15-deoxy-15-oxodarcanolide (**5**)

	Chemical shifts (ppm*)			
	2[a]	**3**[b]	**4**[a]	**5**[b]
H-2	2.67	2.66	2.66	2.64
H-3	3.72	3.70	3.77	3.76
H-5	3.70	3.70	3.68	3.69
H-10	3.16	3.08	3.14	3.09
H-11	4.96	4.89	5.00	4.98
H-13	4.96	5.25	4.95	5.25
H-14	1.84	2.85	2.1	2.85
H-15	3.68	—	3.65	—
8-CH_3	1.34	1.34	1.34	1.31
15-CH_3	0.84	2.18	0.86	2.11
11-OAc	2.07	2.15	2.07	2.15
H-1	—	—	4.33	4.33
3′-OCH_3	—	—	3.39	3.39

	Coupling constants (Hz*)			
	2[a]	**3**[b]	**4**[a]	**5**[b]
$J_{2,3}$	9.5	9.5	9.5	10.0
$J_{3,4}$	1.7	1	1	1
$J_{4,5}$	1.7	1	1	1.5
$J_{5,6}$	6	6	3.5	4.0
$J_{10,11}$	1	1	1	1
$J_{11,12}$	10.0	10.0	10.0	10.0
$J_{12,13}$	1	1	1	1
$J_{13,14}$	8	10.5	7.5	10.5
$J_{14,15}$	6	—	6.5	—
$J_{1',2'}$	—	—	7.1	7.5

* Measured at [a] 300 MHz [b] 100 MHz.

residual **4** by column (1.0×32 cm) chromatography on silica gel prepared in chloroform. Elution with increasing concentration of methanol in chloroform gave 15-deoxy-15-oxodarcanolide (**5**) (42 mg). Crystallization from ethyl acetate-hexane gave needles, mp 182~184°C; $[\alpha]_D^{23}$ −5° (*c* 1.0, CH_3OH); $\lambda_{max}^{CH_3OH}$ 278 nm (ε 96); IR, 3583, 3470, 1745, 1730, and 1703 cm^{-1}; PMR see Table 1.

Anal. Calcd for $C_{32}H_{54}O_{12}$: C, 60.93; H, 8.63.
Found: C, 60.75; H, 8.89.

Continued elution with higher concentration of methanol gave darcanolide (**4**) (107 mg) identified by comparison with authentic material.

Biotransformation of Lankamycin (**1**). Isolation of 15-Deoxy-15-oxolankamycin (**6**), 4″-Deacetyl-15-deoxy-15-oxolankamycin (**7**), and 3′-De-*O*-methyllankamycin (**8**)

Lankamycin (**1**) (1.0 g) was incubated as before and the crude product isolated by solvent extraction (873 mg). Tlc examination revealed the presence of at least 3 components together with residual starting material. Chromatography on a column (1.5×24 cm) of silica gel prepared in chloroform and eluted with increasing concentration of methanol in chloroform separated the individual components. The first compound eluted, 15-deoxy-15-oxolankamycin (**6**) (64 mg), on crystallization from ethyl acetate gave needles, identical with an authentic sample of 15-deoxy-15-oxolankamycin (**6**).[8]

The second material cleanly eluted from the column was identified as lankamycin (**1**) (67 mg) by comparison with a known sample. Further elution gave a mixture of **1** and 4″-deacetyl-15-deoxy-15-oxolankamycin (**7**) (105 mg) followed by fractions containing pure 4″-deacetyl-15-deoxy-15-oxolankamycin (**7**) (45 mg) which could not be crystallized. $[\alpha]_D^{20}$ −57.8° (*c* 0.95, CH_3OH); IR, 3576, 3485, 1750, 1738, 1731 and 1705 cm^{-1}; PMR see Table 2; mass spectrum see Table 3.

Further elution gave fractions containing the most polar compound, 3′-de-*O*-methyllankamycin (**8**) (21 mg) which failed to crystallize from several solvents; $[\alpha]_D^{23}$ −75.5° (*c* 1.23, CH_3OH); IR, 3595, 3475, 1750, 1730 and 1710 cm^{-1}; PMR see Table 2; mass spectrum see Table 3.

Table 2. PMR parameters of lankamycin and related compounds

	Chemical shifts (ppm*)			
	1[a]	**6**[b]	**7**[b]	**8**[b]
H-2	2.80	2.82	2.8	2.75
H-3	3.96	3.93	3.96	3.97
H-5	3.53	3.5	—	—
H-10	3.15	3.09	—	—
H-11	4.90	4.83	4.83	4.9
H-13	4.87	5.18	5.14	4.9
H-14	—	2.84	2.8	—
H-15	3.70	—	—	—
8-CH_3	1.34	1.31	1.30	1.30
15-CH_3	0.85	2.08	2.07	0.82
11-OAc	2.07	2.15	2.14	2.03
H-1′	4.32	4.30	4.30	4.29
3′-OCH_3	3.40	3.39	3.42	—
H-1″	5.04	5.04	4.99	5.04
H-4″	4.68	4.69	3.05	4.68
H-5″	4.48	4.48	4.51	4.49
3″-OCH_3	3.44	3.44	3.27	3.27
4″-OAc	2.12	2.11	—	2.09

	Coupling constants (Hz*)			
	1[a]	**6**[b]	**7**[b]	**8**[b]
$J_{2,3}$	5.5	6.5	6.0	6
$J_{3,4}$	2.4	2.0	2.5	2
$J_{4,5}$	6.0	—	—	—
$J_{5,6}$	1	—	—	—
$J_{10,11}$	1.5	1.5	1	—
$J_{11,12}$	10	9.5	9.5	—
$J_{12,13}$	1	1.5	1	—
$J_{13,14}$	9	10.0	10.0	—
$J_{14,15}$	6.0	—	—	—
$J_{1',2'}$	7.0	7.0	7.0	7.5
$J_{1'',2a''}$	4.5	4.5	4.5	4.5
$J_{1'',2e''}$	1	1	1	1
$J_{4'',5''}$	1.5	1.5	1.5	1.5

* Measured at [a] 220 MHz [b] 100 MHz.

Table 3. Diagnostic ions of **1**, **6**, **7** and **8** observed by high resolution mass spectrometry*

Origin of peak	Formula	*m/e*	Relative intensity (%)			
			1	**6**	**7**	**8**
4-Acetylarcanose						
–OH	$C_{10}H_{17}O_4$	201	59	49	--	58
201–CH_3COOH	$C_8H_{13}O_2$	141	30	19	14	20
201–CH_3OH	$C_9H_{13}O_3$	169	100	100	--	100
4-Deacetylarcanose	$C_8H_{15}O_3$	159	—	—	33	—
Chalcose						
–OH	$C_7H_{13}O_3$	145	18	11	21	—
145–H_2O	$C_7H_{11}O_2$	127	15	11	49	—
145–CH_3OH	$C_6H_9O_2$	113	20	16	30	—
3-De-*O*-methylchalcose						
–OH	$C_6H_{11}O_3$	131	—	—	--	15
131–H_2O	$C_6H_9O_2$	113	—	—	--	21
11-Acetyllankolide	$C_{25}H_{44}O_9$	487	2	—	--	2
11-Acetyl-15-deoxy-15-oxolankolide	$C_{25}H_{41}O_9$	485	—	<0.1	<0.1	—
Darcanolide	$C_{32}H_{56}O_{12}$	632	<0.1	—	--	—

* Electron impact mass spectra of compounds in the lankamycin series have very weak molecular ions which do not provide exact mass measurement.

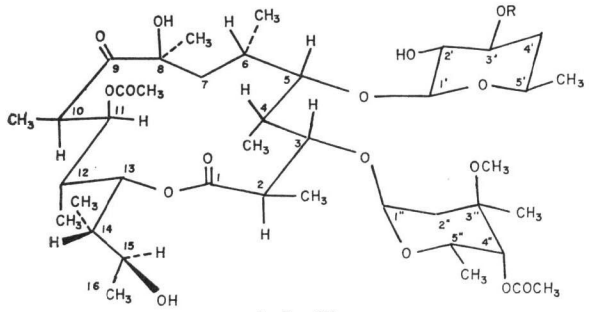
Acid Catalyzed Hydrolysis of 15-Deoxy-15-oxolankamycin (**6**)

15-Deoxy-15-oxolankamycin (**6**) (335 mg) in 13.4 ml of methanol was treated with 6.7 ml of 0.2 N HCl. After 21 hours the reaction mixture was reduced in volume and poured into 200 ml of cold 5% sodium hydrogen carbonate solution. Chloroform extraction gave 326 mg of colorless glass which was chromatographed on a column (1.5 × 30 cm) of silica gel prepared in chloroform and eluted with increasing concentration of methanol in chloroform. The first fractions contained methyl acetylarcanose, α- and β-acetylarcanose and starting material. Later fractions gave 15-deoxy-15-oxodarcanolide (**5**) (152 mg) identical with the material prepared by biological oxidation of darcanolide (**4**).

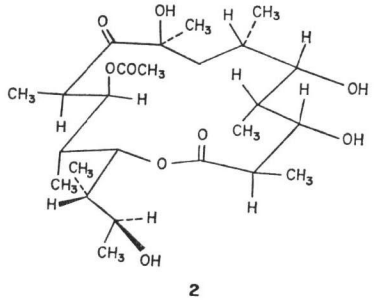
Results and Discussion

When 11-acetyllankolide (**2**) was incubated with *S. erythreus* (Abbott 2NU153), starting **2** and two, new, faster moving compounds were observed on tlc examination of fermentation broths. The new compounds gave a distinctive yellow-orange color when visualized with anisaldehyde reagent. Column chromatography cleanly separated the individual compounds. The fastest moving component, produced in only small quantities, was not examined further. The second new component was characterized as 11-acetyl-15-deoxy-15-oxolankolide (**3**). The PMR spectrum of **3** (Table 1) was reminiscent of 11-acetyllankolide (**2**) but displayed two prominent 3-proton singlets at δ 2.15 and 2.18 previously seen in the spectrum of 15-deoxy-15-oxolankamycin (**6**) and assigned to the C_{11} acetoxyl methyl and the C_{16}-C_{17} $COCH_3$ protons. In several tlc systems, **3** was identical with a major product observed on acid catalyzed hydrolysis of 15-deoxy-15-oxodarcanolide (**5**) by both Rf value and yellow-orange color, when visualized with anisaldehyde reagent.

Darcanolide (**4**) fed to *S. erythreus* gave a single new compound of characteristic yellow-orange color when visualized with anisaldehyde reagent. The structure of the new compound was established



1 R = CH_3
8 R = H



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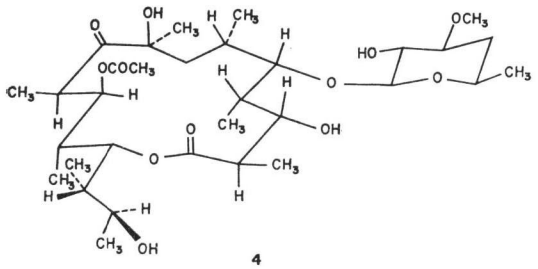

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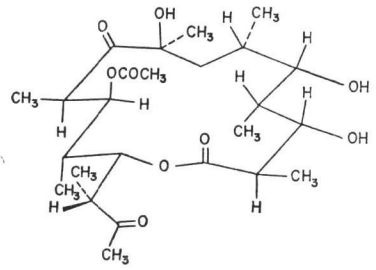

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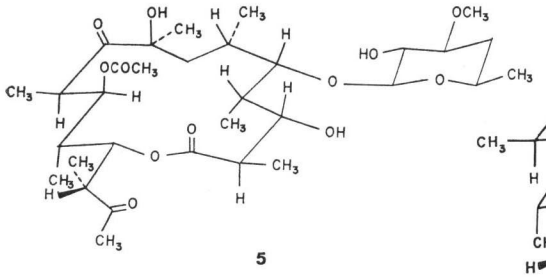
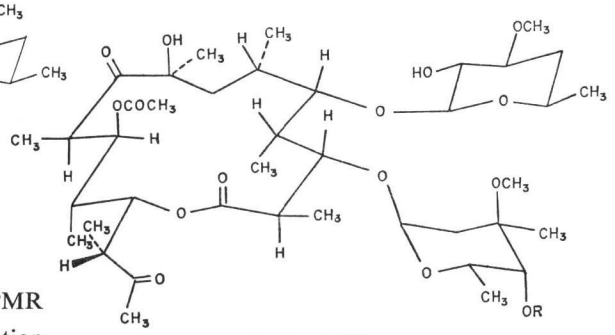


5



6 R = $COCH_3$
7 R = H

as 15-deoxy-15-oxodarcanolide (**5**) by PMR spectroscopy (Table 1) and confirmed by isolation of the same compound from the partial acid catalyzed hydrolysis of 15-deoxy-15-oxolankamycin (**6**).

Although the desired glycosides were not realized when 11-acetyllankolide and darcanolide were fed to *S. erythreus*, the results clearly indicated that the C_{15} hydroxyl group of intact lankamycin might be amenable to biological oxidation. This was confirmed on addition of lankamycin (**1**) to fermentations of strain 2NU153. Rather unexpectedly, tlc examination of fermentation beers indicated that added **1** was converted into a complex mixture of at least 3 new components, two of which gave the characteristic yellow-orange color with anisaldehyde reagent.

The complex was easily separated into the individual components by column chromatography. The fastest moving compound, of yellow-orange color on visualization with anisaldehyde, was identified as 15-deoxy-15-oxolankamycin (**6**) by comparison with a known sample.[8]

Residual lankamycin was eluted next, followed by 4″-deacetyl-15-deoxy-15-oxolankamycin (**7**) giving the distinctive yellow-orange color with anisaldehyde, which by now suggested the presence of a C_{15} oxo function. The PMR spectrum of **7** (Table 2) was markedly similar to that of 15-deoxy-15-oxolankamycin (**6**) but showed only two 3-proton resonances in the 2.0~2.15 ppm region in contrast

to the three such resonances seen in the spectrum of **6**. The large upfield shift of the $H_{4''}$ proton in the spectrum of **7** clearly indicated the absence of a $C_{4''}$ acetoxyl grouping. These observations established the structure as 4''-deacetyl-15-deoxy-15-oxolankamycin (**7**). Confirmatory evidence was obtained from consideration of the mass spectrum (Table 3). A prominent ion at *m/e* 159 (33%) was formed from 4-deacetylarcanose. This ion was absent in the spectra of **1**, **6** and **8**.

The slowest moving lankamycin, 3'-de-*O*-methyllankamycin (**8**) eluted last. Comparison of the PMR spectrum of **8** with the spectra of other lankamycins fully supported the structural assignment (Table 2). The only outstanding differences between the spectra of **8** and lankamycin (**1**) was the lack of a resonance attributable to 3'-OCH_3 in the spectrum of **8**. The chemical shifts of diagnostically important lactone ring protons and methyl groups were very nearly identical with those of lankamycin. Resonances due to $H_{1''}$, $H_{4''}$, $H_{5''}$ and 4''-$OCOCH_3$ of acetylarcanose were also in good agreement with those observed for lankamycin. The slight variance of the chemical shift of the 3''-OCH_3 protons is presumably the consequence of perturbation from the neighboring sugar moiety and has been previously observed.[9] These observations confirm the presence of acetylarcanose and 11-acetyllankolide moieties. The presence of a second sugar moiety, presumably attached at C_3, was accounted for by a one proton doublet at 4.25 ppm ($J_{1',2'}=7.5$ Hz). Since the spectrum of **8** is missing the resonance attributable to the 3'-OCH_3 protons of lankamycin, it was concluded that the D-chalcose moiety was de-*O*-methylated.

Confirming evidence for the structure was obtained from a detailed study of the high resolution mass spectrum (Table 3). Although the spectrum did not display a molecular ion, the fragmentation pattern supported the structure assignment. Most prominent were the ions expected of 4-acetylarcanose. Prominent ions from the 3-de-*O*-methylchalcose moiety of **8** were, as expected, 14 amu lower than the corresponding ions derived from chalcose of lankamycin. As anticipated, ions derived from the aglycone moiety, 11-acetyllankolide, were identical with those seen in the spectrum of lankamycin. From these considerations the structure of **8** was formulated.

Antimicrobial activities of the lankamycins described above, against several organisms, are tabulated in Table 4. All compounds possessed low *in vitro* activities.

Table 4. Antibacterial activities of lankamycin and derivatives*

Organism	**1**	**6**	**7**	**8**
Staphylococcus aureus 9144	12.5	25	100	>100
Streptococcus faecalis 10541	25	50	>100	>100
Klebsiella pneumoniae 10031	>100	>100	>100	>100
Bacillus subtilis Univ. II 10707	6.25	6.25	12.5	>100
Shigella sonnei 9290	>100	>100	>100	>100

* Determined by an agar dilution method.

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References

1) MARTIN, J. R.: Antipyretic agent. U. S. Patent No. 3,740,425, June 19, 1973
2) MARTIN, J. R.; R. S. EGAN, T. J. PERUN & A. W. GOLDSTEIN: 8-*epi*-Erythronolide B and its metabolic

fate in fermentations of *Streptomyces erythreus*. Tetrahedron 29: 935~940, 1973

3) MARTIN, J. R.; R. S. EGAN, A. W. GOLDSTEIN, S. L. MUELLER, E. A. HIRNER & R. S. STANASZEK: 8,8a-Deoxyoleandolide: Elaborated by a blocked mutant of the erythromycin-producing organism *Streptomyces erythreus*. J. Antibiotics 27: 570~572, 1974
4) MARTIN, J. R.: Unpublished observations.
5) MARTIN, J. R.; R. S. EGAN, A. W. GOLDSTEIN & P. COLLUM: Extension of the erythromycin biosynthetic pathway. Isolation and structure of erythromycin E. Tetrahedron 31: 1985~1989, 1975
6) MARTIN, J. R. & W. ROSENBROOK: Studies on the biosynthesis of the erythromycins. II. Isolation and structure of a biosynthetic intermediate, 6-deoxyerythronolide B. Biochemistry 6: 435~440, 1967
7) MARTIN, J. R.; T. J. PERUN & R. L. GIROLAMI: Studies on the biosynthesis of the erythromycins. I. Isolation and structure of an intermediate glycoside, 3-α-L-mycarosylerythronolide B. Biochemistry 5: 2852~2856, 1966
8) MARTIN, J. R.; R. S. EGAN, A. W. GOLDSTEIN, R. S. STANASZEK, J. TADANIER & W. KELLER-SCHIERLEIN: New minor lankamycin related antibiotics from *Streptomyces violaceoniger*. Helv. Chim. Acta, in press.
9) EGAN, R. S.: The conformation of erythromycin and related antibiotics. Abstr. Papers, 166th Meet. Am. Chem. Soc., Chicago, August, 1973