## 8,8A-DEOXYOLEANDOLIDE: ELABORATED BY A BLOCKED MUTANT OF THE ERYTHROMYCIN-PRODUCING ORGANISM *STREPTOMYCES ERYTHREUS*

Sir:

During our investigations on the biosynthesis of the erythromycins we have searched the fermentation broths of several blocked mutants of the erythromycin-producing organism *Streptomyces erythreus* in an effort to identify possible biogenetic progenitors. These studies have resulted in the isolation and identification of several biosynthetic precursors[1,2] and a number of aberrant metabolites.[3,4,5] We now wish to report the isolation and identification of small quantities of 8,8a-deoxyoleandolide (**1**), the 14-membered deoxy aglycone lactone of oleandomycin,[6] from a blocked mutant of the erythromycin-producing organism.

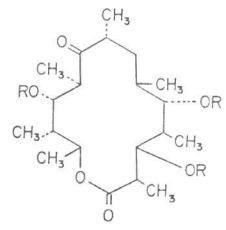
Submerged cultures of *S. erythreus* (Abbott 4EB40), a blocked mutant of a high erythromycin-producing strain, were grown as previously described.[2] The fermentation mash was clarified and extracted as before[1] to leave a heavy yellow oil. Fractionation of the oil by silica gel and Sephadex LH-20 chromatography indicated the presence of small amounts of a component that behaved similarly to the also present 6-deoxyerythronolide B (**2**) an erythromycin progenitor previously isolated.[2] Repeated chromatography resulted in the separation of **1** from **2** as a light yellow oil that defied all attempts of crystallization. Treatment of **1** with acetic anhydride in pyridine gave the triacetyl derivative **3**, devoid of hydroxyl absorption in the infrared (ir), which crystallized from methanol-water, m.p. 152~155°C; circular dichroism (cd) (MeOH) $[\theta]_{292}$ −6640, $[\theta]_{220}$ +2822*; ir ($CDCl_3$) 1733, 1704(sh) $cm^{-1}$; nmr (see Table 1); *Anal.* Calcd. for $C_{26}H_{42}O_8$: mol wt, 498.2828. Found: mol wt (high resolution mass spec.), 498.2828.

The nmr spectra of **1** and **3** were characteristic of 14-membered macrolide aglycone compounds and strikingly similar to the spectrum of **2** and its triacetyl derivative **4**. Pertinent chemical shifts and coupling constants which document this similarity are collected in Table 1. Most significant is the resonance of H–13 which is shown to be directly coupled to a methyl group in **1** and **3**. This established that **1** and **3** have a methyl group at the terminus of the lactone and are therefore related to the aglycone of oleandomycin. An analysis of the methyl region of **1** between 0.5~1.5 ppm reveals 7 doublets but no triplet or singlet methyl resonances. The absence of singlet resonances rules out possible structures with hydroxyl substitution at C–6 and/or C–12 as found in the erythromycin aglycones, the erythronolides. The number of methyl resonances requires that all alternate ring carbons have methyl substituents thereby suggesting that an 8-methyl group is present rather than an epoxide. No resonances attributable to an exocyclic epoxide are recognized in the spectrum.

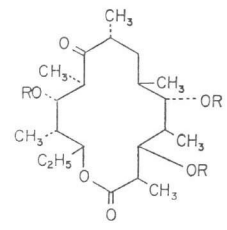
Further evidence for the assigned structures of **1** and **3** was provided through comparison of their high resolution mass spectra with those of **2** and **4**. Molecular ions were observed as noted in Table 2. Compounds **1**~**4** all exhibited a high intensity signal at *m/e* 223 ($C_{13}H_{19}O_3$) resulting from lactone ring cleavage by a McLafferty rearrangement, scission at $C_9$–$C_{10}$ and sequential loss of two molecules of either $H_2O$ or HOAc from $C_3$ and $C_5$.[7,8]

Another structurally significant fragment in the spectra of **1** and **2** was observed at *m/e* 317 ($C_{16}H_{29}O_6$, ~ 3%) arising from cleavage of $C_{11}$–$C_{12}$ following ring opening of the lactone.[7,8]

It is now well established that the initiation of erythronolide biosynthesis begins with a primer of propionate or a biological equivalent.[9] The remaining portion of the lactone ring is then presumably fashioned from linear assembly of 6 units of methylmalonate. The



| | R |
|---|---|
| 1 | H |
| 3 | Ac |



| | R |
|---|---|
| 2 | H |
| 4 | Ac |

* This reading is approximate due to proximity to instrument cut off. We are indebted to Professor L. A. MITSCHER and Dr. M. S. BATHALA, Ohio State University, for cd data.

Table 1. Nmr parameters of 1~4[a)]

| | Chemical shifts | | | | | Chemical shifts | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | 1 | 2 |
| H-2 | 2.72 | 2.78 | 2.78 | 2.76 | $CH_3$-2 | 1.25 | 1.29 |
| H-3 | 3.84 | 3.90 | 5.22 | 5.22 | $CH_3$-4 | 1.05 | 1.08 |
| H-4 | ~1.8 | 1.87 | ~2.2 | ~2.2 | $CH_3$-6 | 1.03 | 1.07 |
| H-5 | 3.93 | 3.98 | 4.80 | 4.82 | $CH_3$-8 | 1.05 | 1.08 |
| H-6 | ~2.0 | 2.01 | ~2.0 | ~2.0 | $CH_3$-10 | 1.00 | 1.04 |
| H-8 | ~2.6 | 2.65 | ~2.7 | ~2.8 | $CH_3$-12 | 0.91 | 0.91 |
| H-10 | 2.78 | 2.77 | 3.08 | 3.09 | $CH_3$-13 | 1.30 | — |
| H-11 | 3.66 | 3.69 | 4.94 | 4.92 | | | |
| H-12 | ~1.6 | 1.74 | ~1.8 | ~1.8 | | | |
| H-13 | 5.50 | 5.15 | 5.19 | 5.06 | | | |

| | Coupling constants | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| $J_{2,3}$ | 10.5 | 10.5 | 10.5 | 10.6 |
| $J_{3,4}$ | <1 | <1 | 1.5 | 1.4 |
| $J_{4,5}$ | 2.5 | 2.5 | 6.0 | 6.2 |
| $J_{5,6}$ | 4.5 | 4.7 | 2.0 | 2.0 |
| $J_{10,11}$ | 2.0 | 2.0 | 1.5 | 1.5 |
| $J_{11,12}$ | 10.5 | 10.2 | 10.0 | 10.0 |
| $J_{12,13}$ | 1.5 | 1.5 | 1.5 | 1.3 |

a) Measured on HA-100 spectrometer at ambient probe temperature in $CDCl_3$ solutions.

Table 2. Molecular ions of 1~4 observed by high resolution mass spectrometry

| Structure | Molecular ion | | Formula | Relative intensity (Percent) |
|---|---|---|---|---|
| | Measured | Calculated | | |
| 1 | 372.2496 | 372.2511 | $C_{20}H_{36}O_6$ | ~5 |
| 2 | 386.2673 | 386.2667 | $C_{21}H_{38}O_6$ | ~5 |
| 3 | 498.2828 | 498.2828 | $C_{26}H_{42}O_9$ | ~1 |
| 4 | 512.3006 | 512.2984 | $C_{27}H_{44}O_9$ | ~1 |

biosynthetic scheme for oleandolide can be visualized as involving an acetate or equivalent followed by assembly of chain extending $C_3$ units analogous to erythromycin biosynthesis. The isolation of **1** and **3** from fermentation broth of the same organism further strengthens suggestions that they are biogenetically related.[10)]

When **1** was added to fermentations of an early blocked mutant of *S. erythreus* (Abbott 2NU153), which is unable to synthesize erythromycin *de novo* but is capable of converting lactone ring containing erythromycin progenitors to the complete antibiotic, high antibiotic titers were realized with the concomitant disappearance of added **1**. The small quantities of **1** available for fermentation feeding precluded any attempt to isolate and identify the resulting antibiotics. However, the experiment infers that the antibiotic activity results from the biological conversion of the added 8,8a-deoxyoleandolide. It is reasonable to expect that the unknown antibiotics contain a hydroxyl substituted oleandolide aglycone with the erythromycin sugars, cladinose or mycarose and desosamine, glycosidically attached at $C_3$ and $C_5$, respectively. The hydroxyl substitution probably mimics that which occurs in normal biosynthesis when 6-deoxyerythronolide B (**3**) is added to 2NU153 fermentations, *i.e.*, hydroxylation of $C_6$ and possibly at $C_{12}$ on the added **1** moiety.[11)]

JERRY R. MARTIN
RICHARD S. EGAN
ALMA W. GOLDSTEIN
SANDRA L. MUELLER
ESTHER A. HIRNER
RUTH S. STANASZEK

Abbott Laboratories,
Division of Antibiotics and
Natural Products,
North Chicago,
Illinois 60064,
U.S.A.

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