NOTES

8-Deoxylankolide from a Lankamycin Producing *Streptomyces* spp.

JEONGHO JU and YANG MO GOO*

Department of Pharmacy, Seoul National University, San 56-1, Shilim-Dong, Kwanack-Gu, Seoul 151-742, Korea

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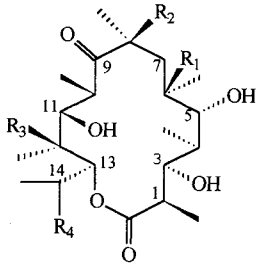
6-Deoxyerythronolide B (**1**) which is isolated from the culture broth of *Saccharopolyspora erythraea* has been proven to be an intermediate in the biosynthesis of erythromycins.[1] Actually, 6-deoxyerythronolide B, derived from propionyl CoA and six equivalents of methylmalonyl CoA, is oxidized by molecular oxygen to erythronolide B (**2**), which is then converted to the erythromycins.[2~7] Lankamycin, which was first isolated from *Streptomyces violaceoniger* in 1960[8] has an aglycone carbon skeleton similar to erythromycin except for the presence of a 2'-hydroxy-1'-methylpropionyl group at the C-13 position instead of the ethyl group.[9] Confirmation of 6-deoxyerythronolide as the precursor of erythronolides in the biosynthesis of erythromycins has raised the possibility of 8-deoxylankolide (**3**) as the corresponding intermediate in the biosynthesis of lankamycin. Compound **3** is expected to be converted to lankolide (**4**), most likely by a P450-dependent reaction involving molecular oxygen. Lankolide is converted to lankamycin. 8-Deoxylankolide (**3**) has not been isolated up to now.

Recently a *Streptomyces* spp. isolated from a soil sample from the north-side of Chuncheon, Korea showed production of lankamycin and lankacidin C. Careful examination of the extract of the culture broth of the organism allowed isolation of several more compounds. One of these, which showed no sugar moiety, has been shown to be 8-deoxylankolide. We wish to report the results in the present paper.

The *Streptomyces* spp. SNUS 9011-300 isolated from a soil sample which was obtained in Obong-mountain, Korea was cultured at 28°C for 4 days on a reciprocal shaker (180 rpm) in a medium containing soy bean flour 2%, glycerol 2%, NaCl 0.3%. The seed culture (500 ml) was used to inoculate 13 liters of the same medium. Production of antibiotics was achieved by culturing the organisms at 28°C for 5 days under agitation of 200 rpm and aeration of 10 liters/minute.

The fermentation broth (13 liters) was extracted with ethyl acetate. The extract residue was chromatographed over a column packed with silica gel by developing with chloroform - methanol (30 : 1). The eluted material was further purified by preparative TLC (silica gel) and HPLC (YMC-pack-SIL column) to give lankamycin (41.7 mg), lankacidin C (9.3 mg) and 8-deoxylankolide (8.0 mg). The physico-chemical properties of 8-deoxylankolide are summarized in Table 1. The ^{13}C and ^{1}H NMR data for 8-deoxylankolide and 6-deoxyerythronolide B are given in Tables 2 and 3. The NMR spectra of both compounds showed very similar chemical shifts.

8-Deoxylankolide shows a protonated molecular ion at *m/z* 431.5919 in the high resolution FAB mass spectrum (calcd. *m/z* 431.5927 for $C_{23}H_{43}O_7$). Two carbonyl stretching bands are observed at 1705 and 1715 cm^{-1} in the IR spectrum. The DEPT spectrum has revealed eight methyl, one methylene, and twelve methine carbon atoms. In the ^{1}H-^{1}H COSY spectrum, correlations are observed between the signals at δ 3.75 (dd, H-11) and those at δ 2.10 (br q, H-12) and δ 2.82 (br q, H-10) ppm, between the signals at δ 1.07 (d, 10-Me) and



	R_1	R_2	R_3	R_4
1	H	H	H	H
2	OH	H	H	H
3	H	H	H	$CHOHCH_3$
4	H	OH	H	$CHOHCH_3$

Table 1. Physico-chemical properties of 8-deoxylankolide.

Appearance	Pale yellowish oil
$[\alpha]_D^{25}$	−10.42 (*c* 0.25, MeOH)
FAB-MS (*m/z*)	431 $(M+H)^+$
HRFAB-MS (*m/z*)	
Found:	431.5919
Calcd:	431.5927 for $C_{23}H_{43}O_7$
UV λ_{max} ($\log\varepsilon$) nm in MeOH	289 (1.50)
IR ν_{max} cm^{-1} in $CHCl_3$	3400, 1715, 1705, 1460
Rf value on TLC	0.20
	Plate; Silica gel F_{254}
	Solvent; $CHCl_3$ - MeOH (20 : 1)

Table 2. Assignment of the ^{13}C NMR signals (δ, ppm) observed in 8-deoxylankolide and the reported chemical shifts value of carbon atoms in 6-deoxyerythronolide.

Position	8-Deoxylankolide[a]	6-Deoxyerythronolide[b]
1	176.4	178.7
2	44.0	43.7
3	79.3	79.6
4	37.6	37.8
5	76.4	76.5
6	35.5	35.6
7	37.4	37.8
8	39.5	40.0
9	213.7	214.9
10	43.3	44.2
11	71.1	71.2
12	37.7	40.8
13	76.8	76.5
14	40.0	25.6
15	66.7	10.7
2-Me	14.7	14.8
4-Me	6.9	7.0
6-Me	16.6	16.8
8-Me	13.4	13.4
10-Me	6.5	6.3
12-Me	9.5	9.2
14-Me	9.6	
15-Me	21.1	

[a] The ^{13}C NMR spectrum (125 MHz) of 8-deoxylankolide was measured in $CDCl_3$.

[b] The ^{13}C NMR spectrum (55 MHz) of 6-deoxyerythronolide was measured in $CDCl_3$-CD_2Cl_2 (4:1). Assignments are based on those previously reported.[11]

Table 3. Assignment of the ^1H NMR signals (δ, ppm; J, Hz) of 8-deoxylankolide and the reported chemical shifts (δ, ppm) and the coupling constants (J, Hz) of 6-deoxyerythronolide.

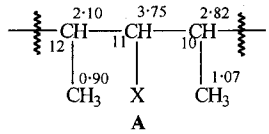
Position	8-Deoxylankolide[a]		6-Deoxyerythronolide[b]	
2	2.81	$J_{2,3}=10.4$	2.78	$J_{2,3}=10.5$
3	3.94	$J_{3,4}=\sim 0$	3.90	$J_{3,4}=<1$
4	1.87	$J_{4,5}=6.3$	1.87	$J_{4,5}=2.5$
5	4.01	$J_{5,6}=2.0$	3.98	$J_{5,6}=4.7$
6	2.03	$J_{6,7a}=3.5$	2.01	$J_{6,7a}=4.7$
7	1.23	$J_{6,7e}=6.6$	1.25	$J_{6,7e}=10.2$
	1.71	$J_{7a,7e}=14.0$	1.70	$J_{7a,7e}=15.0$
8	2.66	$J_{7a,8}=9.1$	2.65	$J_{7a,8}=13.0$
10	2.82	$J_{7e,8}=\sim 0$	2.77	$J_{7e,8}=4.0$
11	3.75	$J_{11,12}=11.2$	3.69	$J_{10,11}=2.0$
12	2.10	$J_{12,13}=\sim 0$	1.74	$J_{11,12}=10.2$
13	5.24	$J_{13,14}=9.7$	5.15	$J_{12,13}=1.5$
14	1.90		1.82, 1.54	$J_{13,14a}=8.9$
15	4.01			$J_{13,14e}=4.6$
2-Me	1.33			$J_{14a,14e}=14.0$
4-Me	1.10			
6-Me	1.08			
8-Me	1.09			
10-Me	1.07			
12-Me	0.90			
14-Me	0.93			
15-Me	1.25			

[a] The ^1H NMR spectrum (600 MHz) of 8-deoxylankolide was measured in $CDCl_3$.

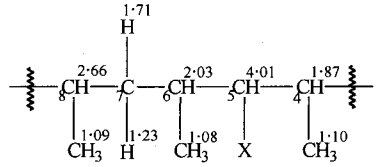
[b] The ^1H NMR spectrum (220 MHz) of 6-deoxyerythronolide was measured in $CDCl_3$. Assignments of the proton chemical shifts and the coupling constants of 6-deoxyerythronolide were based on those previously reported.[10]

those at δ 2.82 (br q, H-10) ppm, and between the signal at δ 0.90 (d, 12-Me) and those at δ 2.10 (br q, H-12) ppm. Partial structure **A** is deduced from these correlations. Clear observation of correlations of the signals at δ 2.66 (dq, H-8) and δ 2.03 (m, H-6) ppm with those at δ 1.71 (ddd, H-7) and δ 1.23 (dd, H-7) ppm, of the signals at δ 4.01 (dd, H-5) ppm with those at δ 2.03 (m, H-6) and δ 1.87 (dq, H-4) ppm, and of the signals of methyl groups at δ 1.10 (d, 4-Me), δ 1.08 (d, 6-Me), and δ 1.09 (d, 8-Me) ppm with the signals at δ 1.87, δ 2.03 and δ 2.66, respectively, implied partial structure **B**. Furthermore, the correlations of the signals at δ 1.90 (m, H-14) ppm with those at δ 5.24 (d, H-13) and δ 4.01 (dq, H-15) ppm, and of the signals of methyl groups at δ 0.93 (d, 14-Me), δ 1.25 (d, 15-Me) with the signals at δ 1.90 and δ 4.01, respectively, suggested partial structure **C**. The correlations of the signals at δ 2.81 (dq, H-2) ppm with those at δ 3.94 (d, H-3) ppm and of the signals of methyl group at δ 1.33 (d, 2-Me) ppm with the signals at δ 2.81 ppm suggested partial structures **D**. The ^{13}C signals and the proton signals of 8-deoxylankolide were assigned by analyzing the ^1H-^{13}C heteronuclear COSY 2D NMR spectrum. The chemical shifts of protons and

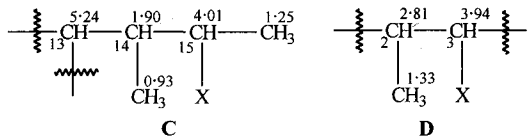
Fig. 1. Structural fragments derived from the cross peaks in ^1H-^1H COSY spectrum of 8-deoxylankolide.



A

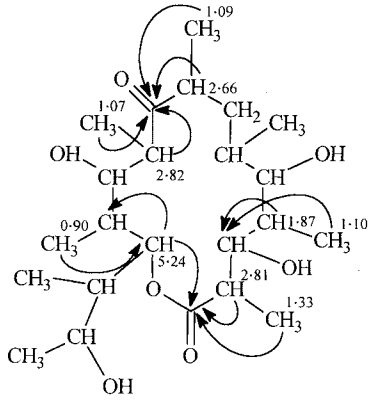


B



C **D**

Fig. 2. HMBC correlations of 8-deoxylankolide.



carbons are summarized in Tables 2 and 3, respectively. In the heteronuclear multiple-bond correlation (HMBC) spectrum, the ^{13}C signal of the carbonyl of C-9 at δ 213.7 ppm gives cross peaks with the signals at δ 1.09 (8-Me), δ 1.07 (10-Me), δ 2.66 (H-8), and δ 2.82 (H-10) ppm, and the carbonyl of C-1 at δ 176.4 with those at δ 5.24 (H-13), δ 2.81 (H-2), and δ 1.33 (2-Me). Also, the ^{13}C signals at δ 37.7 (C-12), δ 76.8 (C-13) and δ 79.3 (C-3) ppm show cross peaks with the proton signals at δ 5.24 (H-13), δ 0.90 (12-Me), and at δ 1.10 (4-Me) and δ 1.87 (H-4), respectively. The observed cross peaks allow the connectivities between the partial structures to give the final structure, **3**. The HMBC spectral data are summarized in Fig. 2.

Since 8-deoxylankolide and lankamycin show similar coupling constants except those at C-3 and C-5, we may presume that 8-deoxylankolide has the same stereochemistry as lankamycin. The protons of H-11 and H-12 show a large coupling constant ($J=11.2$ Hz) and they may have a dihedral angle near to 180°.[10] However, the protons of H-3 and H-4, and those of H-12 and H-13 shows very small coupling constants ($J=\sim 0$ Hz) and their dihedral angles may be around 90°. 8-Deoxylankolide showed very weak antibacterial activities (Table 4). The present spectral analysis and comparison of their spectral data with those of 6-deoxyerythronolide clearly prove that the third compound isolated with lankamycin and lankacidin C is 8-deoxylankolide.

Table 4. Antibacterial activity of 8-deoxylankolide.

Test organism	MIC (μg/ml)[a]
Bacillus subtilis ATCC 6633	128
Bacillus cereus ATCC 27348	128
Micrococcus luteus ATCC 9341	128
Streptococcus faecalis ATCC10541	128
Staphylococcus aureus ATCC 65389	128
Staphylococcus aureus SP-N2	128
Escherichia coli ATCC 25922	256
Escherichia coli IFO 13168	256
Klebsiella pneumoniae ATCC 10031	128
Pseudomonas aeruginosa ATCC 25619	256
Pseudomonas putida ATCC 17426	256
Shigella sonnei ATCC 9290	256
Shigella flexneri ATCC 9199	256
Shigella dysenteriae ATCC 9752	256
Enterobacter colacae ATCC 27508	128
Serratia marcescens ATCC 27117	256
Salmonella typhimurium ATCC 13311	256
Salmonella paratyphi ATCC 11511	256
Proteus mirabilis ATCC 25933	256
Proteus vulgaris ATCC 25619	256

[a] MICs were determined by the microdilution method with Mueller-Hinton medium (Difco).

Acknowledgment

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References

1) MARTIN, J. R. & W. ROSENBROOK: Studies on the biosynthesis of erythromycins. II. Isolation and structure of a biosynthetic intermediate, 6-deoxyerythronolide B. Biochem. 6: 435~440, 1967
2) FRIEDMAN, S. M.; T. KANEDA & J. W. CORCORAN: Antibiotic glycosides V. A comparison of 2-methylmalonate and propionate as precursor of C21 branched chain lactone in erythromycin. J. Biol. Chem. 239: 2386~2391, 1964
3) CANE, D. E. & C. YANG: Macrolide biosynthesis. 4. Intact incorporation of a chain-elongation intermediate into erythromycin. J. Am. Chem. Soc. 109: 1255~1257, 1987
4) VYGANTAS, A. M. & J. W. CORCORAN: Hydroxylation of 6-deoxyerythronolide B by a soluble enzyme system from *Streptomyces erythreus*. Fed. Proc., Fed. Am. Soc. Exp. Biol. 33: 1233, 1974
5) CORCORAN, J. W. & A. M. VYGANTAS: Hydroxylation steps in erythromycin biosynthesis. Fed. Proc., Fed. Am. Soc. Exp. Biol. 36: 663, 1975
6) CANE, D. E.; H. HASLER & T. LIANG: Macrolide biosynthesis. Origin of oxygen atoms in the erythromycins. J. Am. Chem. Soc. 103: 5960~5962, 1981
7) CANE, D. E.: Polyketide biosynthesis: Molecular recognition or genetic programming. Science 263: 338~340, 1994
8) GAUMANN, E.; R. HUTTER, W. KELLER-SCHIERLEIN, L. NIPPE, V. PRELOG & H. ZAHNER: Stoffwechselprodukte von Actinomyceten Lankamycin und Lankacidin. Helv. Chim. Acta 43: 601~606, 1960
9) EGAN, R. S. & J. R. MARTIN: Structure of lankamycin. J. Am. Chem. Soc. 92: 4129~4130, 1970
10) EGAN, R. S.; T. J. PERUN, J. R. MARTIN & L. A. MITSCHER: The conformation of erythronolide, the 14-membered aglycone ring of the erythromycin antibiotics. Tetrahedron 29: 2525~2538, 1973
11) NOURSE, J. G. & J. D. ROBERTS: Nuclear magnetic resonance spectroscopy. Carbon-13 spectra of some macrolide antibiotics and derivatives. Substituent and conformational effect. J. Am. Chem. Soc. 97: 4584~4594, 1975