## THE ISOLATION OF (-)-E-1-CHLOROTRIDEC-1-ENE-6,8-DIOL FROM A MARINE CYANOPHYTE

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Key Word Index—Schizothrix calcicola; Oscillatoria nigroviridis; Oscillatoriaceae; (-)-E-1-chlorotridec-1ene-6,8-diol.

Abstract—(-)-E-1-Chlorotridec-1-ene-6,8-diol is a major constituent in a mixture of cyanophytes, tentatively identified as primarily *Schizothrix calcicola* and *Oscillatoria nigroviridis*, from Enewetak Atoll. The absolute configuration of the diol is probably 6R,8R.

## INTRODUCTION

The shallow reef on the seaward side of Enewetak Atoll in the Marshall Islands is covered in some areas with a thick mat of blue-green algae which has been tentatively identified as primarily a mixture of *Schizothrix calcicola* and *Oscillatoria nigroviridis* [1]. The lipid extract of this algal mixture of Oscillatoriaceae is highly toxic to mice, but sublethal doses display activity against P:3881ymphocytic leukemia in mice. The major active compounds are debromoaplysiatoxin [1] and oscillatoxin A (31demethyldebromoaplysiatoxin) [2] with smaller amounts of the corresponding 17-bromo and 17,19-dibromotoxins [2].

In a study of this algal mixture we have now isolated a major non-toxic metabolite, which shows specific antibiotic activity against *Mycobacterium smegmatis*, and have identified it as (-)-*E*-1-chlorotridec-1-ene-6,8diol (1). It is not known, however, which of the two cyanophytes is responsible for its production.



**RESULTS AND DISCUSSION** 

Extraction of the frozen algal mixture with methylene chloride and methanol, followed by column chromatography of the lipid extract first on Florisil and then on Sephadex LH-20 yielded crystalline 1. The <sup>13</sup>C-NMR data indicated the presence of thirteen carbons where two were olefinic methine carbons, two were methine carbons bearing singly-bonded oxygen, eight were methylene carbons, and one was a methyl carbon. The IR and PMR spectra indicated the presence of —OH, and 1 had to be a diol as it could be readily converted to diacetate and dibenzoate derivatives. A Beilstein test showed that halogen was present and the molecular formula  $C_{13}H_{25}O_2Cl$  was established from both combustion and spectral data. A *trans* alkenyl chloride group was indicated by a doublet of triplets at  $\delta$  5.95 (J = 13.0, 6.5 Hz) for C-2 and a doublet of doublets at 6.13 ppm (J = 13.0, 0.8 Hz) for C-1 in the PMR spectrum [3].

Diol 1 gave a positive borax test, but failed to react with periodate suggesting that a 1,3 relationship existed between the two OH groups. The placement of the two hydroxyls on the linear carbon skeleton was accomplished by utilizing data from a lanthanide induced shift experiment. The relative magnitudes of the europium-induced chemical shifts ( $\Delta\delta$ , Table 1) of the PMR signals of 1 indicated that the 1,3 diol functionality was equidistant from the CHCl = and  $CH_2$  – terminal protons. The biggest induced shift (except for the hydroxyl proton) was experienced by a single CH, group, the one between the two hydroxyl-bearing methine groups. The  $\Delta\delta$  values for the other groups clustered in pairs, with smaller values for increased Eu-proton distances. Although complete sequential proton decoupling was not achieved, the decoupling experiments that were performed at various Eu:1 ratios demonstrated that the partial structures  $CHCl=CH-CH_2"-, CH_3CH_2-CH_2"'-,$ and

Table 1. Eu(fod)<sub>3</sub> induced proton chemical shift data for compound 1 in CDCl<sub>3</sub>

Position	Group	Δδ (ppm)
6 or 8	-OH	35.8
6 or 8	-OH	33.4
7	СН,	13.2
	1	
6 and 8	2 —C'H—	11.5
5 or 9	—СН,—	6.1
5 or 9	—СН,́—	5.1
4 or 10	-CH2-	3.2
4 or 10	-CH	2.7
3 or 11	—СН,́—	1.2
3 or 11	—СН <sup>*</sup>	1.1
2	=CH	0.70
12	-CH	0.53
1	CHCI <sup>2</sup>	0.25
13	CH.	0.15

Compound 1		Compound 2	
Chemical shift, $\delta^*$	Carbon position†	Chemical shift, $\delta^*$	Carbon position†
14.0	13	13.9	1 and 13
22.6	12	22.5	2 and 12
25.1	4		
25.4	10	25.4	4 and 10
30.7	3		
31.8	11	31.7	3 and 11
36.6	5		
37.4	9	37.4	5 and 9
42.4	7	42.3	7
68.6	6		
69.0	8	693	6 and 8
116.9	ĩ	0,10	o unu o
133.4	2		

Table 2. <sup>13</sup>C-NMR data for compounds 1 and 2.

\* In ppm from TMS.

† Calculated chemical shifts [5] support the assignments.

-CHOH-CH<sub>2</sub>-CHOH-CH<sub>2</sub>-CH<sub>2</sub>'- where CH<sub>2</sub>' is coupled to either CH<sub>2</sub>" or CH<sub>2</sub>" were present. Compound 1 was therefore an *E*-1-chlorotridec-1-ene-6,8diol. This structure was further supported by the presence of intense fragment ion peaks at m/e 177 and 179, 145, and 101 in its field desorption MS.

Assignment of the stereochemistry at the two asymmetric centres was achieved by catalytic hydrogenation of 1 to the 6,8-tridecanediol. The <sup>13</sup>C-NMR spectrum of the product exhibited only seven signals as expected for a 6,8-tridecanediol and the observed chemical shifts Table 2) agreed with calculated values. Since the hydrogenation product was optically active, the meso stereochemistry could be eliminated from further consideration. From the sign of the optical rotation,  $[\alpha]_D - 16.3^{\circ}$  (MeOH, c 3.1), the 6,8-tridecanediol appeared to be the 6*R*,8*R* enantiomer (2) since dextrorotatory 2,4-pentanediol,  $[\alpha]_D + 59.1^{\circ}$  (MeOH, c 2.5), had been reported to have the 2*S*,4*S* configuration [4]. If this assumption is correct then compound 1, which is also levorotatory, should have the 6*R*,8*R* configuration as shown in 1.

## **EXPERIMENTAL**

PMR and <sup>13</sup>C-NMR spectra were obtained on a Varian XL-100 spectrometer equipped with a Digilab Fourier transform system. Chemical shifts are reported in  $\delta$  units (ppm relative to TMS ( $\delta = 0$ ). Optical rotations were determined on a ETL-NPL (Ericsson Telephone Unlimited) automatic polarimeter. Mp's are uncorrected. MS were obtained at 70 eV. Elemental analyses were performed by the Chemical Analytical Services, University of California, Berkeley.

Isolation. Frozen Oscillatoria nigroviridis-Schizothrix calcicola (8 kg), collected from the seaward side of Enewetak, Marshall Islands, was homogenized and extracted with  $CH_2Cl_2$ -MeOH (1:2). After filtration,  $H_2O$  was added to the filtrate; the resulting  $CH_2Cl_2$  layer was dried over dry  $Na_2SO_4$  and evap. to give a crude oily residue (14.5 g). CC of the extract (13.8 g) on Florisil yielded three fractions (total 3.2 g) containing **1**, which were eluted with hexane-CHCl<sub>1</sub> (3:1-1:1). Gel filtration of one fraction (1.3 g) on Sephadex LH-20 with CHCl<sub>3</sub>-MeOH (1:1) yielded a pale yellow oil (0.98 g) which crystallized from pentane as white needles, mp 57.5-58.0°C.  $[\alpha]_D^{27} = -12.2^{\circ}$  (MeOH, c, 3.3); IR v<sup>CHCl3</sup> cm<sup>-1</sup>: 3610 (m), 3470 (br), 2990 (m), 2930 (s), 2860 (s), 1640 (w), 1025 (m), 940 (m); field desorption MS m/e 251, 249, 179, 177, 145, 101: PMR (CDCl<sub>3</sub>):  $\delta 0.86$  (3H, t, J = 7 Hz), 1.1-1.7 (14H), 2.05 (2H, m), 2.94 (2H, br), 3.90 (2H, m), 5.8-6.0 (2H, m); PMR (actone-d<sub>6</sub>):  $\delta 0.88$  (3H, t, J = 7 Hz), 1.2-1.6 (14H), 2.12 (2H, m), 3.65 (OH, d, J = 5.5 Hz), 5.95 (1H, dt, J = 13.0, 7.0 Hz), 6.13 (1H, dd, J = 13.0, 0.5 Hz). (Found: C, 62.9: H, 10.0: Cl, 14.1. Calcd. for C<sub>13</sub>H<sub>25</sub>CIO<sub>2</sub>: C, 63.0; H, 10.2: Cl, 14.3 %).

Acetylation of 1. Dry  $C_5H_5N$  (1.0 ml) and  $Ac_2O$  (1.0 ml) were added to 1 (19.3 mg) with cooling. The reaction mixture was allowed to sit at room temperature for 2 hr, after which the excess reagents were removed under red. pres. to give the diacetate (24.7 mg). PMR (CDCl\_3):  $\delta$  0.86 (3H, t, J = 7 Hz), 1.1–1.6 (two broad peaks, 12H), 1.71 (2H, t, J = 6.5 Hz), 2.00 (3H, s, acetate Me), 2.01 (3H, s, acetate CH<sub>3</sub>), 2.04 (obscured q, 2H), 4.92 (2H, quin, J = 6.5 Hz), 5.91 (2H, m,); MS m/e (rel. int.): no molecular ion, 274 (2.5), 272 (7, loss of CH<sub>3</sub>CO<sub>2</sub>H), 43 (100); high resolution MS m/e 272.15402 (calcd. for C<sub>1.5</sub>H<sub>2.5</sub>O<sub>2</sub> <sup>35</sup>Cl, 272.154311).

Benzoylation of 1. Dry  $C_5N_5N$  (1.5 ml) and benzoyl chloride (0.5 ml) were added to 1 (19.8 mg). The mixture was heated at 75° for about 1 min, after which H<sub>2</sub>O (5 ml) followed by 5%, Na<sub>2</sub>CO<sub>3</sub> soln (5 ml) were added. The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 15 ml) and the extract was washed with 5%, Na<sub>2</sub>CO<sub>3</sub> and H<sub>2</sub>O, dried over dry Na<sub>2</sub>SO<sub>4</sub> and evap. under red. pres. The residual oil was purified by gel filtration on Sephadex LH-20 using CHCl<sub>3</sub>-MeOH (1:1) to yield the pure dibenzoate (34.4 mg) as a colourless oil,  $[\alpha]_D^{-2} = -50.8^\circ$  (MeOH, c, 0.59): PMR (CHCl<sub>3</sub>):  $\delta$  0.85 (3H, t. J = 7 Hz), 1.1–1.9 (12H), 2.09 (4H, m), 5.28 (2H, m), 5.90 (2H, m), 7.2–7.6 (6H), 7.97 (4H, m): MS m/e 456, 458, 336, 334 (base peak), 176, 105: high resolution MS m/e 334.169905 (calcd. for  $C_{20}H_2$ ;  $O_2$  <sup>3×</sup>Cl, 334.169901), 176.156145 (calcd. for  $C_{13}H_{20}$  176.156504). Catalytic hydrogenation of 1. To a soln of 1 (50 mg) in 15 ml

Catalytic hydrogenation of 1. To a soln of 1 (50 mg) in 15 ml MeOH was added 7 mg PtO<sub>2</sub>. The mixture was stirred under a H<sub>2</sub> atmos for 105 mm at room temp and filtered. The filtrate was evap. under red. pres. to give a white solid (43.8 mg). mp 82.0-83.0°C;  $[\alpha]_D^{2^{7}} = -16.3^{\circ}$  (MeOH, c 3.1); PMR (CDCl<sub>3</sub>):  $\delta$  0.86 (6H, t, J = J Hz), 1.1–1.5 (16H), 1.58 (2H, dd, J = 5 and 6 Hz), 1.8 (2H, br), 3.93 (2H, br quin, J = 5-6 Hz); MS m/e (rel. nt.) 216 (1), 199 (1), 198 (1), 181 (1.5), 180 (1.5), 156 (1.5), 155 (2), 145 (55), 127 (49), 109 (62), 101 (55), 98 (100), 83 (39), 70 (25), 56 (28). (Found: C, 71.9; H, 13.0. Calcd. for C<sub>1.3</sub>H<sub>2.8</sub>O<sub>2</sub> <sup>•</sup> C, 72.2; H, 13.0%).

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