Polyhydroxylated Sterols from the Octocoral *Dendronephthya gigantea*

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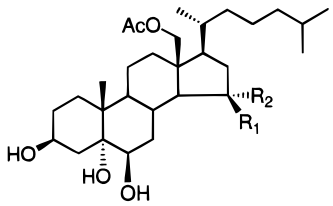
Two new polyhydroxylated sterols, dendronesterols A (**1**) and B (**2**), have been isolated from the octocoral *Dendronephthya gigantea*. The structures of **1** and **2** were proposed on the basis of extensive NMR experiments. Compound **2** was found to be weakly cytotoxic toward L1210 cells.

Extensive studies on sterols from marine invertebrates during the past decade have resulted in the isolation of steroids with unusual side chains and ones that are highly oxygenated.[1,2] However, only a few polyhydroxysterols have been reported from octocorals of the genus *Dendronephthya*.[3] As part of our study on the steroid metabolites of marine invertebrates, we have examined the sterol composition of *Dendronephthya gigantea* Verrill (Nephtheidae)[4] and have isolated two new sterols, dendronesterols A (**1**) and B (**2**). In this paper, the isolation and structure elucidation of **1** and **2** are described.



1 R_1, R_2= H
2 R_1= OH, R_2= H

A MeOH/CH_2Cl_2 (1:1) extract of *D. gigantea* collected off the coast of Tokushima Prefecture in Japan was partitioned between EtOAc and H_2O. The EtOAc-soluble portion was repeatedly chromatographed on a Si gel column. Final purification by reversed-phase C_{18} HPLC afforded two polyhydroxylated sterols, dendronesterols A (**1**) and B (**2**), together with three known sterols, (22*E*,24*S*)-24-methylcholesta-7,22-diene-3β,5α,6β,9α-tetrol (**3**),[5] (22*E*)-cholesta-7,22-diene-3β,5α,6β,9α-tetrol (**4**),[5] and (22*E*)-24-norcholesta-7,22-diene-3β,5α,6β-triol (**5**),[6] previously isolated from marine sponges.

Dendronesterol A (**1**) was obtained as an amorphous solid. The molecular formula $C_{29}H_{50}O_5$, as determined by HRCIMS (*m*/*z* 479.3634, M + H), suggested the presence of five degrees of unsaturation. Three successive losses of 18 mass units (*m*/*z* 461, 443, and 425) in the CIMS suggested the presence of three hydroxyl groups or more in the molecule. The absorptions in the IR spectrum at 3500, 1735, and 1230 cm^{-1} indicated the presence of hydroxyl and acetoxy groups. The ^{13}C NMR data and HMQC spectrum revealed the presence of an acetyl group [δ 170.9 (s) and 21.3 (q)], four oxygenated carbons [δ 77.1 (d), 76.7 (s), 68.2 (d), and 64.0 (t)], and four methyl carbons [δ 23.3 (q), 23.0 (q), 19.6 (q), and 17.5 (q)]. The ^1H NMR spectrum contained two methyl singlets at δ 2.08 and 1.64, three methyl doublets at δ 1.14 (d, J = 6.6 Hz) and 0.88 (6H, d, J = 6.6 Hz), and four carbinol protons at δ 4.86 (1H, m), 4.54 (1H, d, J = 11.8 Hz), 4.16 (1H, br s), and 4.09 (1H, d, J = 11.8 Hz). The foregoing spectral data and a literature survey provided evidence that **1** has a 3,5,6-triol cholestane skeleton, with an oxygenated methylene group (δ 64.0).[7] This methylene group was assigned to C-18, based on the absence of a methyl singlet (δ 0.70) assignable to the C-18 angular methyl and the presence of an AB doublet at δ 4.54 (J = 11.8 Hz) and 4.09 (J = 11.8 Hz). These showed HMBC cross-peaks to C-12 (δ 36.2), C-13 (δ 46.2), C-14 (δ 55.9), and C-20 (δ 36.6) and the carbonyl signal at δ 170.9. The relative stereochemistry of **1** was established by coupling constants and ROSEY experiments. Chair conformations of rings A and B and a trans-junction between them were elucidated from the coupling constant of H-3 ($W_{1/2}$=20 Hz) and NOEs of H_3-19 to H-2a, and H-4a. The α-OH group at the C-5 position could also be assigned from the deshielding effects of α-OH at C-5 on H-1a, H-3, H-7a, and H-9 (Table 1). The α-configuration of H-6 and thus the 6β-OH was proven by the sharp signal at δ 4.16 (br s). An NOE between the oxymethylene protons (H_2-18) and H-20 was in good agreement with the usual 17β-side-chain configuration.[7] Hence, the structure of dendronesterol A was established as **1**.

Dendronesterol B (**2**) was isolated as an amorphous solid and was determined to have a molecular formula of $C_{29}H_{50}O_6$ by HRCIMS (*m*/*z* 495.3617, M + H), 16 mass units more than that of **1**. On comparison of the ^{13}C NMR spectra of **2** and **1**, hydroxylation shifts[8] were observed at C-8 (−4.1 ppm), C-14 (+5.5 ppm), C-15 (+43.6 ppm), C-16 (+13.1 ppm), and C-17 (+1.7 pm) in **2** (Table 1). The remaining carbon signals were nearly identical with those of **1**. These findings showed that the extra hydroxyl group was located at C-15. The β-position for this group was based on two findings. The first was the deshielding effects of the β-OH at C-15 on H-8, H-16a, and H_2-18 as compared with **1** (Table 1). The second, ROESY correlations were observed between H-9/H-14, H-14/H-15, H-15/H-16b, H-16b/H-17, H-17/H-12b, and H-18/H-20 (Figure 1). Consequently, the structure of dendronesterol B was suggested to be **2**. Dendronesterol B exhibited cytotoxic activity against L1210 lymphocytic leukemia cells in vitro with IC_{50} value of 5.2 μg/mL.

Experimental Section

General Experimental Procedures. The following instruments were used: JASCO FT/IR-5300 (IR), JASCO DIP-1000 polarimeter (optical rotation), JEOL JMS-HX-100 mass spectrometer (HRMS), and Varian UNITY 600 spectrometer (^1H and ^{13}C NMR).

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Table 1. ^{1}H and ^{13}C NMR Data of Dendronesterols A (**1**) and B (**2**) in C_5D_5N

position	**1** H (δ)	**1** C (δ)	**2** H (δ)	**2** C (δ)
1a	2.20 (m)	33.6 (t)	2.24 (m)	33.3 (t)
1b	1.56 (m)		1.60 (m)	
2a	2.27 (m)	32.8 (t)	2.38 (m)	32.5 (t)
2b	2.06 (m)		2.05 (m)	
3	4.86 (m)	68.2 (d)	4.83 (m)	68.2 (d)
4a	2.94 (dd, 11.4, 12.7)	43.1 (t)	2.95 (dd, 11.5, 12.6)	42.8 (t)
4b	2.33 (dd, 4.9, 12.7)		2.34 (dd, 4.8, 12.6)	
5		76.7 (s)		75.9 (s)
6	4.16 (br s)	77.1 (d)	4.21 (br s)	76.3 (d)
7a	2.22 (m)	36.1 (t)	2.53 (m)	34.7 (t)
7b	1.72 (m)		2.39 (dt, 3.0, 11.4)	
8	2.24 (m)	31.8 (d)	2.87 (dq, 3.9, 11.4)	27.7 (d)
9	2.06 (m)	46.2 (d)	2.16 (dt, 4.5, 11.4)	46.2 (d)
10		39.4 (s)		39.3 (s)
11a	1.66 (m)	22.1 (t)	1.70 (m)	21.7 (t)
11b	1.58 (m)		1.63 (m)	
12a	2.53 (dt, 3.3, 13.2)	36.2 (t)	2.73 (dt, 3.0, 12.9)	36.1 (t)
12b	1.66 (m)		1.22 (m)	
13		46.2 (s)		46.3 (s)
14	1.35 (m)	55.9 (d)	1.32 (m)	61.4 (d)
15a	1.66 (m)	24.6 (t)	4.49 (br t, 5.6)	68.2 (d)
15b	1.04 (m)			
16a	1.86 (m)	28.5 (t)	2.48 (m)	41.6 (t)
16b	1.50 (m)		1.76 (m)	
17	1.25 (m)	55.7 (d)	1.24 (m)	57.4 (d)
18a	4.54 (d, 11.8)	64.0 (t)	5.00 (d, 12.5)	64.3 (t)
18b	4.09 (d, 11.8)		4.91 (d, 12.5)	
19	1.64 (s)	17.5 (q)	1.64 (s)	17.1 (q)
20	1.57 (m)	36.6 (d)	1.72 (m)	36.4 (d)
21	1.14 (d, 6.6)	19.6 (q)	1.22 (d, 6.3)	19.6 (q)
22a	1.38 (m)	36.8 (t)	1.43 (m)	36.5 (t)
22b	1.06 (m)		1.10 (m)	
23a	1.38 (m)	24.2 (t)	1.43 (2H, m)	23.9 (t)
23b	1.18 (m)			
24	1.35 (2H, m)	28.5 (t)	1.50 (2H, m)	28.2 (t)
25	1.14 (m)	40.1 (d)	1.14 (m)	39.7 (d)
26	0.88 (d, 6.6)	23.0 (q)	0.87 (d, 6.6)	22.7 (q)
27	0.88 (d, 6.6)	23.3 (q)	0.87 (d, 6.6)	22.9 (q)
Ac	2.08 (s)	21.3 (q)	2.08 (s)	21.0 (q)
		170.9 (q)		170.9 (q)

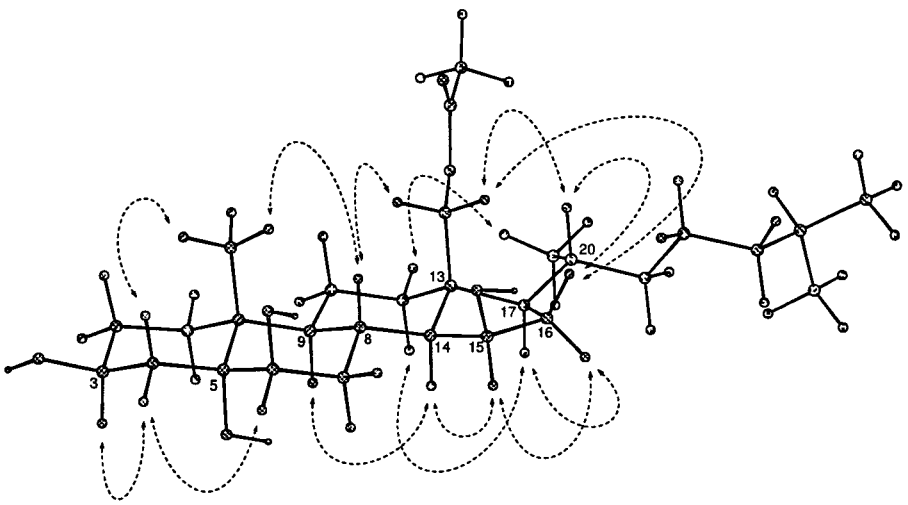

Figure 1. Relative stereochemistry of **2** (ROESY correlations).

Animal Material. The octocoral, *Dendronephthya gigantea* (10 kg, wet wt), was collected off the coast of Tokushima prefecture, Japan, and identified by Mr. Y. Imahara (The Wakayama Prefectural Museum of Natural History, Japan). The voucher sample (TB7001) of the organism under consideration is deposited in the herbarium of the Department of Pharmacognosy, Tokushima Bunri University, Tokushima, Japan.

Extraction and Isolation. Fresh samples (10 kg) were exhaustively extracted twice with MeOH and then twice with MeOH/CH_2Cl_2 (1:1). The latter extracts were concentrated, and the resulting residue extracted with EtOAc (500 mL × 3). The EtOAc-soluble portion (24 g) was repeatedly subjected to Si gel flash column chromatography (using increasing concentrations of MeOH in CH_2Cl_2 as eluent), followed by reversed-phase HPLC (85% MeOH, ODS) to give **1** (9.5 mg), **2** (8.0 mg), **3** (9.5 mg), **4** (9.5 mg), and **5** (8.0 mg).

Dendronesterol A (1): amorphous solid; $[\alpha]^{25}_{D}$ +3.42° (*c* 1.0, MeOH); FTIR (dry film) 3420 (br), 1740, 1230 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; COSY (H/H) 1/2, 2/3, 3/4, 6/7, 7/8, 8/9, 9/10, 11/12, 8/14, 14/15, 15/16, 16/17, 17/20, 20/21, 20/22, 22/23, 23/24, 25/26, 25/27; HMBC (H/C) 4/2, 4/3, 4/5, 4/10, 6/8,

6/10, 7/5, 17/13, 17/14, 17/20, 18/12, 18/13, 18/14, 18/17, 19/1, 19/4, 19/9, 19/10, 21/20, 21/22, 26/24, 26/25, 27/24, 27/25; ROESY (H/H) 1a/3, 2a/19, 2b/3, 3/4b, 4a/19, 4b/6, 6/7a,b, 8/18, 8/19, 18/20; HRCIMS *m*/*z* $[M + H]^+$ 479.3634 (calcd for $C_{29}H_{50}O_5 + H$, 479.3659).

Dendronesterol B (2): amorphous solid; $[\alpha]^{25}_D$ +1.00° (*c* 0.9, MeOH); FTIR (dry film) 3420 (br), 1740, 1230 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; COSY (H/H) 1/2, 2/3, 3/4, 6/7, 7/8, 8/9, 9/10, 11/12, 8/14, 14/15, 15/16, 16/17, 17/20, 20/21, 20/22, 22/23, 23/24, 25/26, 25/27; HMBC (H/C) 4/2, 4/3, 4/5, 4/10, 6/8, 6/10, 7/9, 15/13, 16/13, 16/17, 18/12, 18/17, 19/1, 19/4, 19/9, 19/10, 21/17, 21/20, 21/22, 26/24, 26/25, 27/24, 27/25; HRCIMS *m*/*z* $[M + H]^+$ 495.3617 (calcd for $C_{29}H_{50}O_6 + H$, 495.3616).

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