A New Epidioxy Sterol as an Antifouling Substance from a Palauan Marine Sponge, *Lendenfeldia chondrodes*

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Bioassay-guided fractionation of an extract of a marine sponge, *Lendenfeldia chondrodes*, has led to the isolation and identification of new epidioxy sterols **1a** and **1b** as an inseparable mixture. Two known epidioxy sterols **2** and **3** and three known sesterterpenes **4**−**6** were also isolated from the same extract. These compounds showed repellent activity against the blue mussel *Mytilus edulis galloprovincialis.*

Sponges of the order Dictyoceratida are known to produce sesterterpenes. As a part of our research aimed at the discovery of new active metabolites such as the antifouling substance from marine invertebrates, the sponge *Lendenfeldia chondrodes* de Laubenfels (family Spongiidae, order Dictyoceratida) was collected from a marine lake of Palau. The congeneric sponges, *Lendenfeldia frondosa*[1] collected in the Solomon Islands and *Lendenfeldia* sp.[2] collected in Western Australia, have been studied and shown to afford homosesterterpenes among their constituents. Our specimen contained a mixture of new epidioxy sterols **1a** and **1b**, together with two known epidioxy sterols **2** and **3**[3] and three known sesterterpenes **4**−**6**.[1,2,4,5]

The EtOAc-soluble fraction of the MeOH extract of marine sponge *L. chondrodes* was chromatographed on a Si gel column using eluents of increasing polarity ($CHCl_3$ and MeOH) to yield a mixture of compounds. The fractions containing sesterterpenes and sterols were further purified by an ODS column and ODS HPLC to yield compounds **1**−**6**. Fractionation was monitored using repellent activity against blue mussels.[6]

Compounds **1a** and **1b** were obtained as an inseparable mixture of C-24 stereoisomers in the form of a colorless solid of composition $C_{29}H_{46}O_5$ as determined by HRFABMS, together with two known compounds **2** and **3**. By comparison of the spectral data with literature data, compounds **2** and **3** were identified as 5α,8α-epidioxycholest-6-en-3β-ol (**2**) and 5α,8α-epidioxy-24-methylcholesta-6,9(11),24(28)-trien-3β-ol (**3**) previously isolated from the marine sponge *Tethya aurantia* by Gunatilaka et al.[3] In the ^{1}H NMR spectrum of **1**, two doublets at δ 6.24 (1H, d, J = 8.55 Hz) and 6.50 (1H, d, J = 8.55 Hz) were observed, characteristic of 5α,8α-epidioxysterols.[3] Other ^{1}H NMR spectral data were very similar to those of **2**; however, the signals for the sterol side chains were different, suggesting that compound **1** shares the 5α,8α-epidioxy 6-en-3β-ol nuclear moiety with **2**. The presence of a monosubstituted olefin was indicated by three olefinic proton signals [δ 5.729 and 5.731 (total 1H, each dd, J = 17.8, 11.4 Hz, H-28), 5.27 and 5.28 (total 1H, each dd, J = 11.4, 1.1 Hz, H-29), and 5.15 (1H, dd, J = 17.8, 1.1 Hz, H-29)] and two sets of two olefinic carbon signals [(δ 137.0 and 137.1, C-28) and (δ 116.3 and 116.4, C-29)] in the ^{1}H and ^{13}C NMR spectra of **1**. NMR signals for the methyl-21 protons and carbon appeared at δ 0.95 (3H, d, J = 7.5 Hz) and 18.8, respectively. Carbon signals for methyls-26 and -27 appeared as two pairs at δ 17.69 and 17.71 and 16.6 and 16.7, respectively. These data suggested that **1** is a mixture of epimers, probably at C-24. Consideration of these data with the molecular formula of **1** indicated that a hydroperoxy group and a vinyl group are attached at position-24 in both the *R* and *S* configurations, and the downfield chemical shifts of C-24 (δ 89.0 and 89.1) confirm this. HMBC correlation peaks (H-21−C-20,22,17; H-25−C-24,-28; H-26−C-25,27; H-28−C-24,28) were used to support the structure of the side chain. Thus, the structure 5α,8α-epidioxy-24-hydroperoxycholesta-6,28(29)-dien-3β-ol was proposed for **1**. The side chain of this compound was quite similar to that of the hydroperoxide derivative of the 3-oxo-

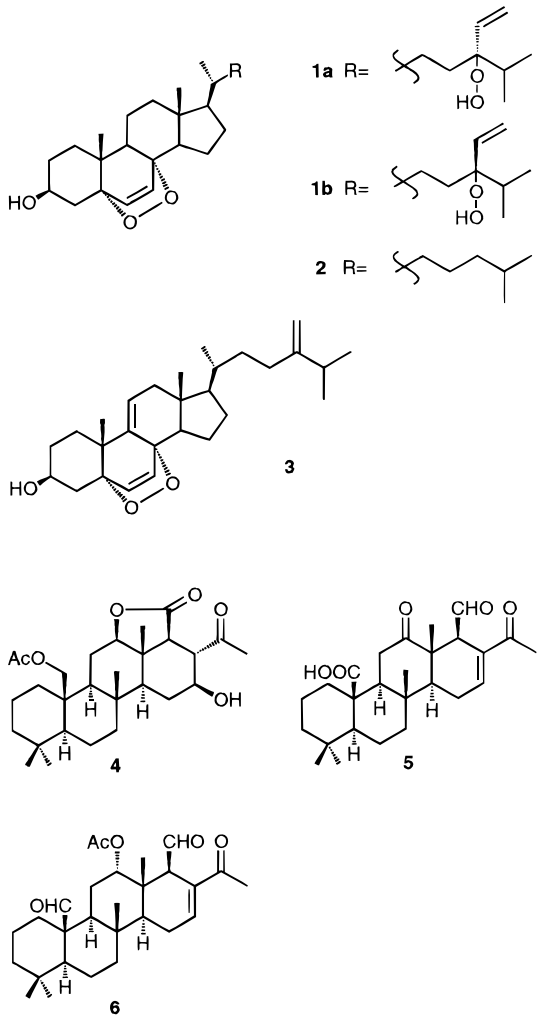

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Table 1. ^{1}H and ^{13}C NMR Data of Compound **1** (δ in $CDCl_3$, 500 and 125 MHz)

position	^{1}H	^{13}C
1		34.8
2		30.2
3	3.93 (1H, m)	66.5
4		37.0
5		82.1
6	6.24 (1H, d, J = 8.55 Hz)	135.4
7	6.50 (1H, d, J = 8.55 Hz)	130.7
8		79.4
9		51.2
10		37.0
11		20.7
12		39.5
13		44.8
14		51.6
15		23.4
16		28.2_5 and 28.2_7
17		56.1 and 56.3
18	0.81 (3H, s)	12.6_6 and 12.6_7
19	0.88 (3H, s)	18.2
20		35.4 and 35.7
21	0.95 (3H, d, J = 7.5 Hz)	18.8
22		28.3_6 and 28.3_9
23		28.5 and 28.7
24		89.0 and 89.1
25		32.0_1 and 32.0_2
26	0.86 and 0.87 (1.5H each, d, J = 7.5 Hz)	17.6_9 and 17.7_1
27	0.87_8 and 0.88_2 (1.5H each, d, J = 7.5 Hz)	16.6 and 16.7
28	5.72_9 and 5.73_1 (0.5H each, dd, J = 17.8, 11.5 Hz)	137.0 and 137.1
29	5.27 and 5.28 (0.5H each, dd, J = 11.5, 1.1 Hz) 5.15 (1H, d, J = 17.8, 1.1 Hz)	116.3 and 116.4

Table 2. Antifouling Activities of Compounds **1**−**6** against the Blue Mussel

concentration (ppm)	compound					
	1	**2**	**3**	**4**	**5**	**6**
1	0	0	0	90	95	10
10	20	0	0	100	100	55
100	95	0	0	100	100	75

4,6,8(14)-triunsaturated steroid[7] previously isolated by Kobayashi et al. They suggested that the hydroperoxy steroid is provably an artifact derived by an ene reaction from the 24-ethylidene derivative and singlet oxygen, during storage, and this is probably also the case for **1a** and **1b**. The fact that both isomers **1a** and **1b** were isolated supports this hypothesis. However, no Δ5,7 unsaturated sterols were isolated from a fresh sponge sample, indicating that **1a** and **1b** might be formed in the sponge during sample storage and extraction.

Compounds **4**−**6** were identified as 22-acetoxy-16β-hydroxy-24-methyl-24-oxoscalarano-25,12β-lactone (**4**), 24-methyl-12,24,25-trioxoscalar-16-en-22-oic acid (**5**), and 12α-acetoxy-24-methyl 24-oxoscalar-16-en-22,25-dial (**6**) by comparison of their MS and ^{1}H NMR spectral data with those of the previously isolated compounds[2,4,5] including those from *L. frondosa*[1] collected from the Solomon Islands. Antifouling activities of these compounds were tested against mussels by using the foot stimulant method[6] developed in our institute. These results are summarized in Table 2. Although the activity was not strong, it is of interest that among the epidioxy sterols isolated, only compound **1** showed any antifouling effect.

Experimental Section

General Experimental Procedures. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Unity 500 and 125 MHz NMR spectrometers, respectively, using $CDCl_3$. Mass spectra were measured on a JEOL JMS-SX102 mass spectrometer. Optical rotation was determined using a HORIBA SEPA-300 high-sensitivity polarimeter. IR spectra were measured with a JASCO FT/IR-7000 spectrophotometer.

Animal Material. *L. chondrodes* was collected in the Milky Way marine lake of Palau in July 1996, frozen on site, and stored at −20 °C before extraction. A voucher specimen is deposited at our laboratory. The sponge was identified by Professor P. R. Bergquist at the University of Auckland, New Zealand.

Extraction and Isolation. The frozen sample of *L. chondrodes* (1.2 kg) was extracted with acetone (2 L × three times). The extract was evaporated under reduced pressure to give a residue of 8.0 g. This was partitioned between EtOAc and H_2O. The organic layer was taken to dryness (8.0 g) and chromatographed on Si gel column using a $CHCl_3$−MeOH gradient system (from 0 to 5% MeOH in $CHCl_3$), and 10 major fractions were obtained. Each of the fractions positive in the blue mussel assay [3 (820 mg, crude **4** and **5**), 4 (3.0 g, crude **4**−**6**), and 8 (215 mg, crude **1**−**3**)] was fractionated on an ODS column (C_{18} column 10 × 250 mm; detector UV at 215 nm; flow 3 mL/min) with a mixed solvent system: H_2O−MeOH gradient (from 40% to 100% MeOH in H_2O) to give an inseparable mixture of **1a** and **1b** and pure compounds **2**−**6**.

Compounds 1a and 1b. An inseparable mixture of **1a** and **1b** (2.8 mg) was obtained as a white solid: $[\alpha]^{24}_D$ +5.2°(*c* 0.175, MeOH); IR ($CHCl_3$) ν_{max} 3440, 2958, 2880, 1458, 1381, 1075, 1044, 1025, 957, 754 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; HRFABMS *m/z* 497.3235 [M + Na]$^+$ (calcd for $C_{29}H_{46}O_5Na$, 497.3243).

Compound 2. 5α,8α-Epidioxycholest-6-en-3β-ol (5.2 mg) was obtained as a white solid. IR and ^{1}H NMR spectra of **2** taken in $CHCl_3$ and C_6D_6, respectively, were identical with those reported: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.73(3H, s, H-18), 0.79 (3H, d, J = 6.6 Hz, H-27), 0.80 (3H, d, J = 6.6 Hz, H-26), 0.81(3H, s, H-19), 0.83 (3H, d, J = 6.6 Hz, H-21), 3.90 (1H, m, H-3), 6.18 (IH, d, J = 8.6 Hz, H-6), 6.42 (1H, J = 8.6 Hz, H-7); EIMS *m/z* 416 [M]$^+$.

Compound 3. 5α,8α-Epidioxy-24-methylcholesta-6,9(11),-24(28)-trien-3β-ol (10.3 mg) was obtained as a white solid. IR and ^{1}H NMR spectra of **3** taken in $CHCl_3$ and C_6D_6, respectively, were identical with those reported: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.71(3H, s, H-18), 1.00 (3H, d, J = 7.1 Hz, H-27), 1.01(3H, d, J = 6.8 Hz, H-26), 1.07(3H, s, H-19), 0.92 (3H, d, J = 6.3 Hz, H-21), 4.02 (1H, m, H-3), 4.64 (1H, s, H-28β),4.71 (1H, s, H-28α), 5.40 (1H, d, J = 5.6 Hz, H-11), 6.29 (IH, d, J = 8.8 Hz, H-6), 6.60 (1H, J = 8.8 Hz, H-7); EIMS *m/z* 426 [M]$^+$.

Compound 4. 22-Acetoxy-16β-hydroxy-24-methyl-24-oxoscalarano-25,12β-lactone (28.5 mg) was obtained as a white solid: IR ($CHCl_3$) ν_{max} 3450, 1754, 1720, 1220, 1104, 735, 598 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.81 (3H, s, CH_3), 0.86 (3H, s, CH_3), 0.97 (3H, s, 2 × CH_3), 2.04 (3H, s, AcO), 2.15 (1H, d, J = 11.5 Hz, H-18), 2.40 (3H, s, 24-CH_3), 2.86 (IH, dd, J = 10.6, 9.7 Hz, H-17), 3.66 (1H, d, J = 10.6, 3.5 Hz, H-12), 3.88 (1H, m, H-16), 4.08 (1H, d, J = 12.4 Hz, H-22β), 4.61 (1H, d, J = 12.4 Hz, H-22α); FABMS *m/z* 475 [M + H]$^+$.

Compound 5. 24-Methyl-12,24,25-trioxoscalar-16-en-22-oic acid (3.4 mg) was obtained as a white solid: IR ($CHCl_3$) ν_{max} 2936, 1740, 1719, 1670, 1458, 1390, 1245, 913, 731 cm^{-1}; ^{1}H −NMR (500 MHz, $CDCl_3$) δ 0.88 (3H, s, CH_3), 0.92 (3H, s, CH_3), 1.02 (1H, m, H-7α), 1.06 (3H, s, CH_3),1.15 (3H, s, CH_3), 1.64 (1H, m, H-6α), 1.96 (1H, dt, J = 12.8, 3.4 Hz, H-7β), 2.27 (3H, s, 24- CH_3), 2.31 (1H, m, H-6β), 2.52 (2H, bd, J = 12.5 Hz, H-1), 3.81 (1H, m, H-18), 6.94 (1H, bs, H-16), 10.18 (1H, d, J = 2.0 Hz, H-25); FABMS *m/z* 429 [M + H]$^+$.

Compound 6. 12α-Acetoxy-24-methyl-24-oxoscalar-16-en-22,25-dial (15.8 mg) was obtained as a white solid: IR ($CHCl_3$) ν_{max} 1740, 1720, 1660, 1377, 1241, 1038, 731, 607 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.77 (3H, s), 0.81 (3H, s), 0.84 (3H, s), 0.86 (3H, s), 2.17 (3H, s), 2.26 (3H, s), 3.51 (1H, m), 4.77 (1H, m), 7.05 (1H, m), 9.39 (1H, d, J = 3.9 Hz), 10.09 (1H, s); FABMS *m/z* 457 [M + H]$^+$.

Bioassay. Candidate antifouling substances were dripped onto the foot of the blue mussel (*Mytilus edulis galloprovincialis*), and the animal's reactions were observed. When an

active liquid stimulus, such as a solution of $CuSO_4$ was dripped on the foot, the mussel contracted its foot immediately. After the drip of an inactive substance, the foot of the mussel either showed no reaction or contracted after a few seconds. The number of responding mussels was counted. The foot was immediately washed with 1 mL of artificial seawater (ASW) after the observation and allowed to rest for 15 min. The concentration of the test solution was raised and the experiment was repeated from the point of dripping the ASW as control until all the mussels showed a reaction. The reactivities of the test compounds were indicated by the percentage of mussels showing a reaction at each concentration of test solution, calculated as follows: 100 × number of reactive mussels/(number of reactive mussels + unreactive mussels). Ten mussels were prepared for each sample.

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