9,11-Secosterols from the Soft Corals *Sinularia lochmodes* and *Sinularia leptoclados*

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Chemical investigations on the EtOAc-soluble fractions from the EtOH extract of two Formosan soft corals afforded two new 9,11-secosteroids, 3β,11-dihydroxy-5β,6β-epoxy-24-methylene-9,11-secocholestan-9-one (**1**) and 3β,11-dihydroxy-24-methylene-9,11-secocholestan-9-one (**2**), from *Sinularia lochmodes* and *Sinularia leptoclados*, respectively, along with two known analogues (**3** and **4**) from *S. leptoclados*. The structures of the new metabolites were elucidated on the basis of extensive spectroscopic analysis and by comparison of their NMR data with those of the known compound **3**. The cytotoxicity of **2**−**4** toward a limited panel of cancer cell lines is also reported.

Studies on the chemical constituents of marine invertebrates have led to the isolation of various 9,11-secosterols from soft corals[1−7] and sponges.[8−12] These types of steroids were found to possess cytotoxic[4−6,11] and antihistamine[8] activities. Previous chemical investigation by our group on the genus *Sinularia* has afforded several bioactive norcembrane-[13−16] and β-caryophyllene-based diterpenes.[17] During our continuing investigation of the bioactive natural products from two Formosan soft corals, *Sinularia lochmodes* (Kolonko, 1926) *and S. leptoclados* (Ehrenberg, 1834), two new 9,11-secosterols (**1** and **2**), along with two known metabolites (**3** and **4**), were obtained. The structures of the new metabolites **1** and **2** were elucidated on the basis of extensive spectroscopic analysis and by comparison of their NMR features with those of the known compound **3**.[1] The cytotoxicity of these 9,11-secosterols toward several cancer cell lines was also evaluated.

The tissues of two soft corals *S. lochmodes and S. leptoclados* were homogenized exhaustively with EtOH. The EtOH extract of each were triturated separately with *n*-hexane followed by EtOAc. Each EtOAc-soluble fraction was concentrated under reduced pressure, and the residue was fractionated over silica gel column chromatography. The resulting fractions were further purified by normal-phase HPLC to yield metabolite **1** from *S. lochmodes* and **2**−**4** from *S. leptoclados* (see Experimental Section). The spectroscopic data of **3**[1] and **4**[1] were found to be in full agreement with those reported previously for two known marine 9,11-secosterols.[1−12]

Compound **1** was isolated as colorless needles. Its molecular formula, $C_{28}H_{46}O_4$, was established by HRFABMS (447.3472 *m*/*z*, $[M + H]^+$), implying six degrees of unsaturation. The presence of two hydroxy groups was suggested by a strong absorption band at 3437 cm^{-1} in the IR spectrum and further supported by the ion peaks at *m*/*z* 429 $(M + H - H_2O)^+$ and 411 $(M + H - 2H_2O)^+$ in the FABMS spectrum. The ^{13}C NMR spectral data of **1** (Table 1) indicated the presence of 28 carbon atoms, including five methyls, 10 sp^3 methylenes (including two oxygenated ones), one sp^2 methylenes, seven sp^3 methines (including two oxygenated ones), and five quaternary carbons. The quaternary carbon signal at δ 214.7 combined with the proton chemical shifts of H_3-18 (δ 0.68), H_3-19 (δ 1.30), H_3-21 (δ 0.97), H-3 (δ 3.87), and H_2-11 (δ 3.78 and 3.67) were found to be similar to those of 3-hydroxy-9,11-seco-9-oxosterol.[1−7,10−12] Moreover, the signals appearing at δ_C 58.1 (CH), 65.5 (C), 106.1 (CH_2), 156.6 (C), and 58.7 (CH_2) indicated the presence of an epoxy, an exomethylene, and a hydroxymethyl group

Table 1. ^1H and ^{13}C NMR Data for Compounds **1** and **2**

	1			**2**		
C/H	δ_H[a]	δ_C[b]		δ_H[a]	δ_C[b]	
1	1.86 m	28.5	(CH_2)[d]	1.66 m	31.2	(CH_2)
	1.80 m			1.56 m		
2	2.02 m	30.4	(CH_2)	1.89 m	31.0	(CH_2)
	1.52 m			1.40 m		
3	3.87 m	68.0	(CH)	3.58 m	70.7	(CH)
4	2.14 m	38.8	(CH_2)	1.73 m	36.9	(CH_2)
	1.43 m			1.44 m		
5		65.5	(C)	1.44 m	46.1	(CH)
6	3.11 t (2.0)[c]	58.1	(CH)	1.74 m	28.5	(CH_2)
				1.48 m		
7	2.50 m	26.2	(CH_2)	2.00 m	33.0	(CH_2)
	2.28 m			1.32 m		
8	2.68 m	38.4	(CH)	2.83 m	44.6	(CH)
9		214.7	(C)		218.0	(C)
10		46.5	(C)		48.6	(C)
11	3.78 m	58.7	(CH_2)	3.77 m	59.5	(CH_2)
	3.67 m			3.67 m		
12	1.76 m	40.9	(CH_2)	1.72 m	40.8	(CH_2)
	1.44 m			1.36 m		
13		45.5	(C)		45.7	(C)
14	2.54 m	45.2	(CH)	2.58 m	42.3	(CH)
15	1.58 m	22.4	(CH_2)	1.53 m	23.8	(CH_2)
	1.32 m			1.32 m		
16	1.85 m	25.9	(CH_2)	1.84 m	25.8	(CH_2)
	1.37 m			1.35 m		
17	1.60 m	49.4	(CH)	1.62 m	49.5	(CH)
18	0.68 s	18.1	(CH_3)	0.67 s	17.5	(CH_3)
19	1.30 s	19.6	(CH_3)	1.19 s	16.0	(CH_3)
20	1.40 m	33.9	(CH)	1.39 m	34.5	(CH)
21	0.97 d (6.4)	19.5	(CH_3)	0.98 d (6.8)	19.6	(CH_3)
22	1.57 m	34.0	(CH_2)	1.54 m	34.3	(CH_2)
	1.10 m			1.14 m		
23	2.12 m	31.6	(CH_2)	2.10 m	32.0	(CH_2)
	1.90 m			1.88 m		
24		156.6	(C)		156.9	(C)
25	2.19 m	33.7	(CH)	2.22 m	34.0	(CH)
26	1.01 d (6.8)	22.0	(CH_3)	1.02 d (6.8)	22.2	(CH_3)
27	1.01 d (6.8)	21.8	(CH_3)	1.02 d (6.8)	22.1	(CH_3)
28	4.72 s	106.1	(CH_2)	4.72 s	106.4	(CH_2)
	4.65 s			4.65 s		

[a] Spectra recorded at 400 MHz in $CDCl_3$. [b] 100 MHz in $CDCl_3$. [c] *J* values (in Hz) in parentheses. [d] Attached protons determined by DEPT experiments.

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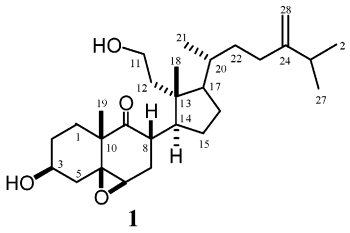

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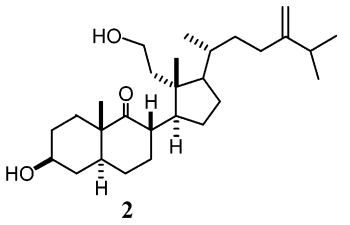

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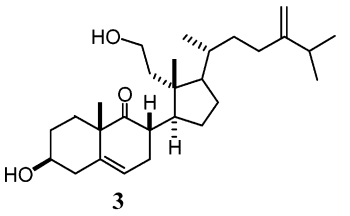

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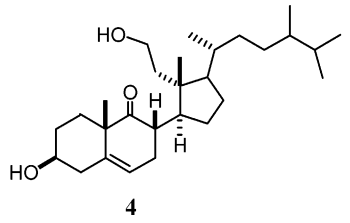

4

in the structure of **1**. This was further supported by the ^{1}H NMR signals of epoxy, exomethylene, and oxymethylene protons at δ 3.11 (1H, t, J = 2.0 Hz), 4.72 and 4.65 (each 1H, s), and 3.67 and 3.78 (each 1H, m), respectively. Careful analysis of the ^{1}H−^{1}H COSY correlations of **1** also led to the establishment of four partial structures as shown in Figure 1. The connectivities of these partial structures together with the location of the ketone, epoxy, hydroxyl, hydroxymethyl, methyl, and exomethylene groups were achieved by careful investigation of HMBC correlations (Figure 1), and thus the 9,11-secosteroidol skeleton of **1** was proposed (Figure 1). The ^{1}H−^{13}C long-range correlations found from H_3-19 (δ 1.30) to C-5 (δ 65.5 C) and from the oxymethine proton at δ 3.11 to C-4 (δ 38.8, CH_2) unambiguously established the C-5, C-6 position of the epoxy group. This was further supported by comparison of the ^{1}H and ^{13}C NMR data of **1** with those of **3**, which revealed that the double bond at C-5/C-6 in **3** was replaced by an epoxide group in **1**.

The relative stereochemistries at C-3, C-8, C-10, C-13, C-14, C-17, and C-20 in **1** were found to be the same as those of **3** (Figure 2). Key NOE correlations for **1** showed interactions between H-3/H-4α (δ 1.43) and H-4α/H-6. Also, H-7α (δ 2.50) showed NOE responses with both H-6 and H-14. Thus, H-6 should be located on the α-face and the oxygen of the epoxide must be positioned on the β-face. The above data established the structure of compound **1** as 3β,11-dihydroxy-5β,6β-epoxy-24-methylene-9,11-secocholestan-9-one.

Compound **2** was obtained as a white amorphous solid and exhibited a pseudomolecular ion peak at m/z 455.3501 in the HRESIMS, appropriate for a molecular formula of $C_{28}H_{48}O_3$ [M + Na]$^+$. Its EIMS showed peaks at m/z 414 [M − H_2O]$^+$ and 396 [M − 2 H_2O]$^+$, suggesting the presence of two hydroxy groups. The spectroscopic data of **2** (IR, UV, ^{1}H and ^{13}C NMR) were similar to those of **1**, except for the absence of the 5,6-epoxide signals, which were replaced by signals of a carbon−carbon single bond [δ 46.1, CH, C-5 and 28.5, CH_2, C-6] in **2**. This was further

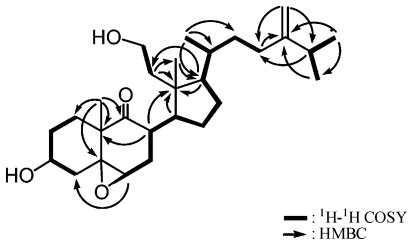

Figure 1. Key ^{1}H−^{1}H COSY and HMBC correlations for **1**.

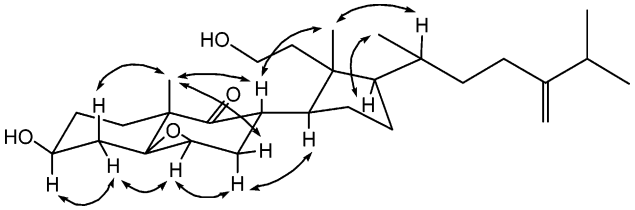

Figure 2. Selected NOESY correlations observed for **1**.

Table 2. Cytotoxicity of Compounds **2**−**4**

	cancer cell line(IC_{50}, μg/mL)			
compound	Hep G2	A-549	MDA-MB-231	MCF-7
2	8.2	9.8	11.3	9.2
3	9.1	9.1	9.9	4.5
4	14.1	16.8	15.0	15.7
doxorubicin	0.3	0.4	0.2	0.4

confirmed by HMBC correlations observed from H_3-19 (δ 1.19, s) to C-5 (δ 46.1). The relative stereochemistry of compound **2** was established by NOESY correlations in comparison with those of **1**. The NOE correlations observed between H-5 and H-3, but not with H_3-19, confirmed the α-orientation of H-5. On the basis of the above results, the structure of compound **2** was established as 3β,11-dihydroxy-24-methylene-9,11-secocholestan-9-one.

The cytotoxicity of compounds **2**−**4** was assessed against the following cancer cell lines: human hepatocellular carcinoma Hep G2, human breast carcinomas MCF-7 and MDA-MB-231, and human lung carcinoma A-549. Compound **3**, the most potent of compounds **2**−**4**, exhibited the best cytotoxicity toward the MCF-7 cancer cell line (IC_{50} 4.5 μg/mL) (Table 2). Also, metabolites **2** and **4** showed moderate to weak cytotoxicity toward the above four cancer cells.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were recorded on a Jasco FT-5300 infrared spectrophotometer. NMR spectra were recorded on a Bruker AMX 400 FT NMR at 400 MHz for ^{1}H and 100 MHz for ^{13}C, in $CDCl_3$. Low-resolution mass spectral data were obtained by EI or FAB with a VG Quattro GC/MS spectrometer. HRMS were recorded by ESI FT-MS on a Bruker APEX II mass spectrometer or by FAB on a VG 70-250S GC/MS spectrometer. Silica gel (Merck, 230−400 mesh) was used for CC. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC.

Animal Material. The soft corals *Sinularia lochmodes* and *S. leptoclados* were collected by hand using scuba off the coast of Southern Taiwan, in July 2000 at depths of 15 to 20 m and in April 2004 at depths of 5 to 10 m, respectively, and stored in a freezer until extraction. Two voucher samples were deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

Extraction and Separation. The tissues of the soft coral *S. lochmodes* (1.9 kg, wet wt) were exhaustively extracted with EtOH. The organic layer was filtered and concentrated under vacuum to afford a dark brown viscous residue (64.4 g). The residue was partitioned between *n*-hexane and H_2O, then between H_2O and EtOAc. The combined EtOAc-soluble layer was evaporated under vacuum to yield

an oily residue (2.1 g), which was subjected to CC on Si gel, using *n*-hexane and EtOAc mixtures of increasing polarity. Elution by *n*-hexane−EtOAc (2:1) afforded a fraction containing compound **1**, which was further purified by NP HPLC using *n*-hexane−EtOAc (3:1) to yield **1** (1.0 mg).

The tissues of *S. leptoclados* (1.5 kg, wet wt) were homogenized with EtOH and filtered and treated similarly as above to afford a dark brown viscous residue (15 g). This extract was triturated with *n*-hexane followed by EtOAc. The EtOAc fraction (10.5 g) was subjected to CC on Si gel using *n*-hexane and EtOAc mixtures of increasing polarity. Elution by *n*-hexane−EtOAc (4:1) afforded a fraction containing a mixture of compounds **2**−**4**. This mixture were further separated by NP HPLC by using *n*-hexane−acetone (6:1) to give compounds **2** (6.5 mg), **3** (15 mg), and **4** (5.0 mg).

3*β*,11-Dihydroxy-5*β*,6*β*-epoxy-24-methylene-9,11-secocholestan-9-one (1): white powder; mp 151−152 °C; $[\alpha]^{25}_{D}$ −27 (*c* 0.40, $CHCl_3$); IR (neat) ν_{max} 3437, 2928, 1711, and 1381 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; FABMS *m*/*z* 447 ($[M + H]^+$, 0.6), 429 ($[M + H - H_2O]^+$, 0.3), 411 ($[M + H - 2H_2O]^+$, 0.3); HRFABMS *m*/*z* 447.3472 $[M + H]^+$ (calcd for $C_{28}H_{47}O_4$, 447.3462).

3*β*,11-Dihydroxy-24-methylene-9,11-secocholestan-9-one (2): white powder; mp 130−132 °C; $[\alpha]^{25}_{D}$ −46 (*c* 0.78, $CHCl_3$); IR (neat) ν_{max} 3395, 2957, 1701, 1641, and 1379 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 432 ($[M]^+$, 0.7), 414 ($[M - H_2O]^+$, 2), 396 ($[M - 2H_2O]^+$, 0.2); HRESIMS *m*/*z* 455.3501 $[M + Na]^+$ (calcd for $C_{28}H_{48}O_3Na$, 455.3503).

Cytotoxicity Testing. Cytotoxicity assays of compounds **2**−**4** were performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[19]

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