Sesquiterpenoids from the Formosan Soft Coral *Sinularia leptoclados*

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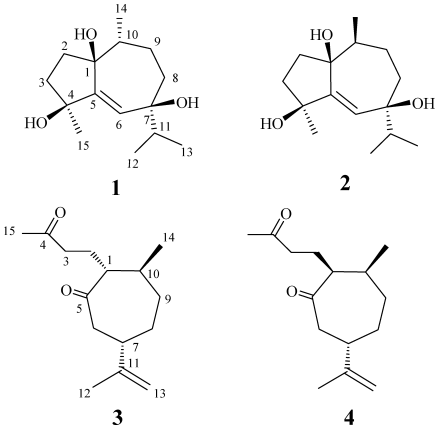
Chemical investigation of the soft coral *Sinularia leptoclados* has afforded three new sesquiterpenoids, leptocladols A (1), B (2), and 1-*epi*-chabrolidione A (3). The relative structures of 1—3 were determined on the basis of extensive spectroscopic analysis. The relative configuration of 1 was further confirmed by a single-crystal X-ray diffraction analysis.

Key words sesquiterpenoid; *Sinularia leptoclados*; cytotoxicity

Formosan soft corals of the genus *Sinularia* have been found to be important sources of structurally novel terpenoids[1—6] and polyhydroxylated steroids.[7] We previously isolated norcembranoids[8] and polyhydroxysteroids[9] from the soft coral *Sinularia leptoclados*. Our continuing investigation on the chemical constituents of the soft coral *S. leptoclados* has resulted in the isolation of two new guaiane-type sesquiterpenoids, leptocladols A (**1**) and B (**2**), and a new 4,5-seco-guaiane metabolite, 1-*epi*-chabrolidione A (**3**). The structures of **1**—**3** have been established by extensive spectroscopic analysis, including 2D NMR (^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond connectivity (HMBC), and nuclear Overhauser effect spectroscopy (NOESY)) experiments. The relative structure of **1** was further confirmed by X-ray diffraction analysis.[10] The cytotoxicity of compounds **1**—**3** against human liver carcinoma (Hepa59T/VGH), human oral epitheloid carcinoma (KB), human cervical epitheloid carcinoma (HeLa), and human medulloblastoma (Med) cells was studied, and the ability of **1** and **3** to inhibit up-regulation of the pro-inflammatory inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) proteins in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophage cells was also evaluated.

Leptocladol A (**1**) was obtained as colorless needles. HR-electrospray ionization (ESI)-MS (*m*/*z* 277.1781 $[M+Na]^+$) established its molecular formula to be $C_{15}H_{26}O_3$, requiring three degrees of unsaturation. The IR spectrum of **1** revealed the presence of a hydroxy (3385 cm^{-1}) group. The ^{13}C-NMR data of **1** (Table 1) showed the presence of 15 carbon signals, which were assigned with the assistance of the distortionless enhancement by polarization transfer (DEPT) spectrum to four methyls, four sp^3 methylenes, two sp^3 methines, one sp^2 methine, three quaternary sp^3 oxycarbons, and one sp^2 quaternary carbon. The ^{1}H-NMR spectrum of **1** displayed four methyl groups (Table 2) including one tertiary methyl (δ 1.26) and three secondary methyls (δ 0.93, 0.92, 0.89). In the lower field of the ^{1}H-NMR spectrum, the proton signal appearing at δ 5.91 (1H, s) indicated the presence of a trisubstituted olefin. By the analysis of ^{1}H–^{1}H COSY correlations (Fig. 1), it was possible to establish four partial structures of consecutive proton systems extending from H-2 to H-3; H_2-8 to H_2-9; H-10 to H_3-14; and H-11 to H_3-12 and H_3-13. Further analysis of the HMBC correlations was employed successfully to establish the gross structure of **1**, including the C-1, C-4, and C-7 positions of the three hydroxy groups and the C-5 and C-6 location of the double bond, as shown in Fig. 1. The relative configurations of four chiral centers at C-1, C-4, C-7, and C-10 in **1** were partly elucidated by NOE analysis (Fig. 2). It was found that H_3-14 (δ 0.89, d, J=7.0) showed



1 **2** **3** **4**

Table 1. ^{13}C-NMR Spectral Data for Compounds **1**—**3**[a] and **4**[b]

	1	2	3	4
1	83.8 (C)[c]	80.9 (C)	58.1 (CH)	55.8 (CH)
2	36.3 (CH_2)	36.0 (CH_2)	24.5 (CH_2)	20.8 (CH_2)
3	39.3 (CH_2)	38.4 (CH_2)	41.8 (CH_2)	42.2 (CH_2)
4	79.7 (C)	79.6 (C)	208.6 (C)	208.6 (C)
5	155.6 (C)	160.4 (C)	214.0 (C)	214.3 (C)
6	131.4 (CH)	128.6 (CH)	48.8 (CH_2)	48.8 (CH_2)
7	75.0 (C)	74.3 (C)	43.8 (CH)	43.3 (CH)
8	25.8 (CH_2)	35.1 (CH_2)	28.2 (CH_2)	31.7 (CH_2)
9	26.4 (CH_2)	28.6 (CH_2)	32.6 (CH_2)	33.0 (CH_2)
10	39.4 (CH)	42.3 (CH)	34.8 (CH)	33.6 (CH)
11	39.5 (CH)	40.0 (CH)	148.6 (C)	149.0 (C)
12	17.5 (CH_3)	17.1 (CH_3)	21.1 (CH_3)	20.2 (CH_3)
13	16.1 (CH_3)	16.6 (CH_3)	109.7 (CH_2)	109.7 (CH_2)
14	14.9 (CH_3)	17.1 (CH_3)	20.7 (CH_3)	18.3 (CH_3)
15	25.8 (CH_3)	28.3 (CH_3)	29.9 (CH_3)	29.9 (CH_3)

a) Spectra recorded at 125 MHz in $CDCl_3$. *b*) ^{13}C-NMR data, see ref. 11. *c*) Attached protons were deduced using DEPT experiments.

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Table 2. ^{1}H-NMR Spectral Data for Compounds **1**—**3**[a] and **4**[b]

	1	2	3	4
1			2.32 m	2.60 m
2	1.88 m; 1.84 m	1.79 m; 1.50 m	1.78 m; 1.95 m	1.67 m; 1.94 m
3	1.95 m; 1.61 m	1.81 m; 1.94 m	2.34 m; 2.43 m	2.38 m; 2.52 m
6	5.91 s	5.99 s	2.50 m	2.42 m; 2.62 m
7			2.30 m	2.34 m
8	1.70 m; 1.48 m	1.69 m	1.65 m; 1.72 m	1.56 m; 1.82 m
9	2.59 m; 1.40 m	2.51 m; 1.51 m	1.55 m; 1.62 m	1.34 m; 1.78 m
10	2.00 m	1.53 m	1.58 m	2.03 m
11	1.81 m	1.76 m		
12	0.92 d (7.0)[c]	0.97 d (7.0)	1.74 s	1.71 s
13	0.93 d (6.5)	0.94 d (6.5)	4.74 s	4.70 s; 4.71 s
14	0.89 d (7.0)	0.96 d (7.0)	1.10 d (6.0)	0.91 d (7.0)
15	1.26 s	1.25 s	2.12 s	2.12 s

a) Spectra recorded at 500 MHz in $CDCl_3$. *b*) ^{1}H-NMR data, see ref. 11. *c*) *J* values (in Hz) are given in parentheses.

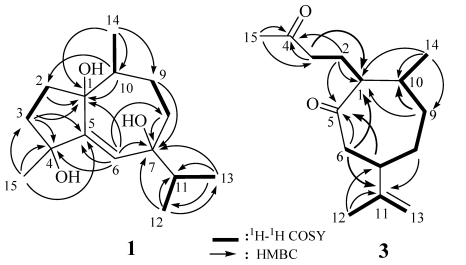

Fig. 1. The ^{1}H–^{1}H COSY and HMBC Correlations for **1** and **3**

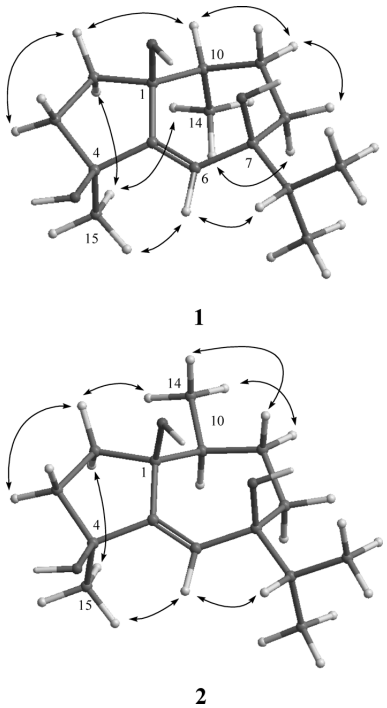

Fig. 2. Selective NOESY Correlations of **1** and **2**

NOE interactions with H_3-15 (δ 1.26, s). Thus both H_3-14 and H_3-15 were assumed to be positioned on the α face. Furthermore, single-crystal X-ray diffraction analysis was carried out to confirm the molecular structure of **1** (Fig. 3). The X-ray structure of **1** established the β-orientation for all of the three hydroxy groups at C-1, C-4, and C-7. On the basis of the above analyses, the relative structure of **1** was unambiguously established.

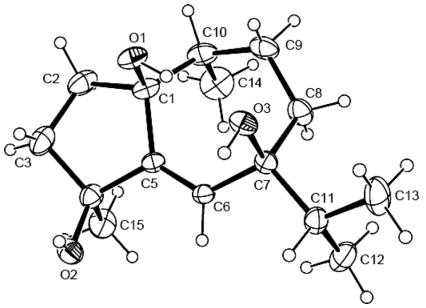

Fig. 3. Molecular Structure of **1** Based on X-Ray Analysis

HR-ESI-MS and NMR spectroscopic data (Tables 1, 2) revealed that leptocladol B (**2**) has the same molecular formula, $C_{15}H_{26}O_3$, as that of **1**. By analysis of 2D NMR spectra, including ^{1}H–^{1}H COSY, HMQC, and HMBC, compound **2** was shown to possess the same molecular framework as that of **1**. Careful investigation of the NOESY spectrum of **2** (Fig. 2) revealed that one proton (δ 1.50, m) of CH_2-2 showed NOE interaction with H_3-15 (δ 1.25, s) and was assigned as H-2α. H-2β (δ 1.79, m) was found to correlate with H_3-14 (δ 0.96, d, J=7.0 Hz), revealing the β-orientation of H_3-14. Further analysis of other NOE interactions revealed that **2** possessed the same relative configurations at C-1, C-4, and C-7 as those of **1**. Therefore **2** was found to be the C-10 epimer of **1**.

Metabolite **3** was obtained as a colorless oil. HR-ESI-MS indicated the molecular formula $C_{15}H_{24}O_2$, with four degrees of unsaturation. The IR spectrum suggested the presence of a carbonyl group (1712 cm^{-1}). In the 2D NMR spectra, including ^{1}H–^{1}H COSY and HMBC (Fig. 1), three isolated consecutive proton spin systems, H-1 to H_2-3, H_2-6 to H-10 that further connected with H_3-14 at CH-10, and the allylic protons H_3-12 to vinylic proton H-13, were found in the ^{1}H–^{1}H COSY correlations. The detailed analysis of HMBC correlations further established the planary structure of **3**. On the basis of the above results, compound **3** was shown to possess the same molecular framework as **4**, which was isolated previously from the soft coral *Nephthea chabrolii*.[11] The relative structure of **3** was elucidated by the analysis of NOE correlations. As shown in Fig. 4, the NOE correlations between H_3-14 and those of H-1 and H-8a (δ_H 1.65) indicated that all of these protons adapt the β-orientation. Also, both protons at C-8 exhibited NOE correlations with H-7, revealing the β-orientation of H-7. The carbon shifts of **3** were found to be shifted downfield at C-1 ($\Delta\delta_C$ +2.3 ppm), C-2 ($\Delta\delta_C$ +3.7 ppm), C-10 ($\Delta\delta_C$ +1.2 ppm), and C-14 ($\Delta\delta_C$ +2.4 ppm) relative to those of **4** (Tables 1, 2) and revealed that **3** is the C-1 epimer of **4**. The biosynthetic pathway of **3** could arise from the oxidative cleavage of the 4,5-chemical bond of the related guaiane-type metabolite.

The cytotoxicity of compounds **1**—**3** against the proliferation of a limited panel of cancer cell lines, including Hepa59T/VGH (human liver carcinoma), KB (human oral epitheloid carcinoma), HeLa (human cervical epitheloid carcinoma), and Med (human medulloblastoma), was studied. The results showed that **1**—**3** are not cytotoxic toward the above cancer cells (ED_{50}>20 μg/ml). The *in vitro* antiinflammatory effects of compounds **1** and **3** were tested. In this

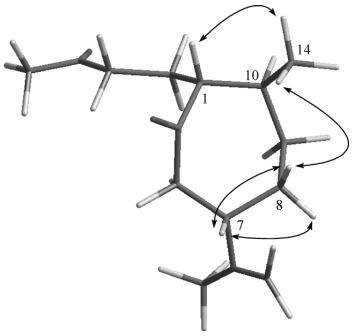

Fig. 4. Selective NOESY Correlations of **3**

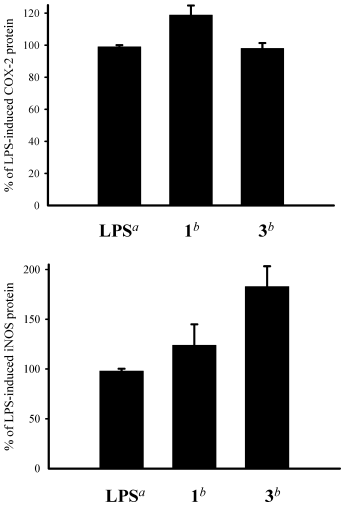

Fig. 5. Effect of Compounds **1** and **3** on LPS-Induced iNOS and COX-2 Proteins Expression in RAW264.7 Macrophage Cells by Immunoblot Analysis: under the Same Experimental Condition CAPE (Caffeic Acid Phenylethyl Ester, 10 μM) Reduced the Levels of the iNOS and COX-2 to 2.5±3.7% and 67.2±13.4%, Respectively: *a*) Stimulated with LPS, *b*) Stimulated with LPS in the Presence of **1** and **3** (10 μM)

assay, the up-regulation of the pro-inflammatory iNOS and COX-2 proteins of LPS-stimulated RAW264.7 macrophage cells was evaluated using immunoblot analysis. At a concentration of 10 μM, compounds **1** and **3** did not inhibit COX-2 and iNOS expression relative to the control cells stimulated with LPS alone (Fig. 5). In contrast, compound **3** was found to enhance iNOS expression.

Experimental

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/IR-4100 infrared spectrophotometer. NMR spectra were recorded on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$. Low-resolution MS data were obtained by ESI on a Bruker APEX II mass spectrometer. HR-MS were recorded on ESI on a Bruker APEX II mass spectrometer. Si gel 60 (Merck, 230—400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.2 mm) were used for analytical TLC. HPLC was performed on a Hitachi L-7100 HPLC apparatus with a Merck Hibar Si-60 column (250×21 mm, 7 μm).

Animal Material The soft coral *S. leptoclados* was collected by hand using scuba off the coast of the southernmost tip of Taiwan in April 2004 at depths of 5 to 10 m and stored in a freezer until extraction. A voucher sample was deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

Extraction and Isolation The frozen bodies of *S. leptoclados* (1.5 kg, wet weight) were minced and exhaustively extracted with EtOH (11×4). The organic extract was concentrated to an aqueous suspension and was further partitioned between EtOAc and H_2O. The EtOAc layer was dried with anhydrous Na_2SO_4. After removal of the solvent *in vacuo*, the residue (15.0 g) was subjected to column chromatography on silica gel and eluted with EtOAc in *n*-hexane (0—100% EtOAc, gradient) to yield 20 fractions. Fraction 8, eluted with EtOAc–*n*-hexane (1 : 20), was further separated with normal-phase HPLC using acetone–*n*-hexane (1 : 15) to yield **3** (3.5 mg). Fraction 16, eluted with EtOAc–*n*-hexane (1 : 2), was further purified with normal-phase HPLC using acetone–*n*-hexane (1 : 3) to afford **1** (9.9 mg) and **2** (1.9 mg), respectively.

Leptocladol A (**1**): Colorless crystal (9.9 mg); mp 144—146 °C; $[\alpha]_D^{25}$ +12.5 (c=2.0, $CHCl_3$); IR (neat) ν_{max} 3385, 2964, 1637, 1379, 1298 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz); ESI-MS *m*/*z* 277 (100, $[M+Na]^+$); HR-ESI-MS *m*/*z*: 277.1781 (Calcd for $C_{15}H_{26}O_3Na$, 277.1780).

Leptocladol B (**2**): White solid (1.9 mg); mp 130—135 °C; $[\alpha]_D^{25}$ +42.5 (c=0.8, $CHCl_3$); IR (neat) ν_{max} 3422, 2966, 1638, 1375 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz); ESI-MS *m*/*z*: 277 (100, $[M+Na]^+$); HR-ESI-MS *m*/*z*: 277.1781 (Calcd for $C_{15}H_{26}O_3Na$, 277.1780).

1-*epi*-Chabrolidione A (**3**): Colorless oil; $[\alpha]_D^{25}$ −10 (c=1.1, $CHCl_3$); IR (neat) ν_{max} 2930, 1712, 1645, 1375 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz); ESI-MS *m*/*z*: 259 (100, $[M+Na]^+$); HR-ESI-MS *m*/*z*: 259.1676 (Calcd for $C_{15}H_{24}O_2Na$, 259.1674).

X-Ray Diffraction Analysis of Leptocladol A (1)[10] A suitable colorless crystal (0.7×0.6×0.6 mm^3) of **1** was grown by slow evaporation of the EtOAc solution. Diffraction intensity data were acquired with a Rigaku AFC7S single-crystal X-ray diffractometer with graphite-monochromated Mo$K\alpha$ radiation (λ=0.71073 Å). Crystal data for **1**: $C_{15}H_{26}O_3$ (formula weight 254.36); approximate crystal size, 0.7×0.6×0.6 mm^3; orthorhombic, space group, $P2_12_12_1$ (# 19), T=298(2) K, a=7.3207(15) Å, b=11.514(2) Å, c=17.760(4) Å, V=1497.0(5) Å^3, D_c=1.129 Mg/m^3, Z=4, F(000)=560, $\mu_{(MoK\alpha)}$=0.076 mm^{-1}. A total of 2284 reflections were collected in the range 2.11°<θ<26.00°, with 2107 independent reflections; completeness to θ_{max} was 100%; psi-scan absorption correction was applied; full-matrix least-squares refinement on F^2, the number of data/restraints/parameters was 2107/0/176, respectively; goodness-of-fit on F^2=1.049; final R indices [$I>2\sigma(I)$], R_1=0.0373, wR_2=0.0937; R indices (all data), R_1=0.0504, wR_2=0.0994; and largest difference peak and hole, 0.121 and −0.197 e/Å^3, respectively.

Cytotoxicity Testing Cell lines were purchased from the American Type Culture Collection. Cytotoxicity assays of test compounds **1**—**3** were performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[12,13]

***In Vitro* Anti-inflammatory Assay** The assay procedure was as previously reported.[14,15]

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References and Notes

1) Chao C.-H., Hsieh C.-H., Chen S.-P., Lu C.-K., Dai C.-F., Wu Y.-C., Sheu J.-H., *Tetrahedron Lett.*, **47**, 2175—2178 (2006).
2) Chen S.-P., Ahmed A. F., Dai C.-F., Lu C.-K., Hu W.-P., Wang J.-J., Sheu J.-H., *Tetrahedron*, **62**, 6802—6806 (2006).
3) Tseng Y.-J., Ahmed A. F., Dai C.-F., Chiang M. Y., Sheu J.-H., *Org. Lett.*, **7**, 3813—3816 (2005).
4) Ahmed A. F., Tai S.-H., Wen Z.-H., Su J.-H., Wu Y.-C., Hu W.-P., Sheu J.-H., *J. Nat. Prod.*, **71**, 946—951 (2008).
5) Chen S.-P., Su J.-H., Yeh H.-C., Ahmed A. F., Dai C.-F., Wu Y.-C., Sheu J.-H., *Chem. Pharm. Bull.*, **56**, 162—166 (2009).
6) Ahmed A. F., Su J.-H., Kuo Y.-H., Sheu J.-H., *J. Nat. Prod.*, **67**, 2079—2082 (2004).
7) Ahmed A. F., Tai S.-H., Wu Y.-C., Sheu J.-H., *Steroids*, **72**, 368—374 (2007).
8) Ahmed A. F., Shiue R.-T., Wang G.-H., Dai C.-F., Kuo Y.-H., Sheu J.-H., *Tetrahedron*, **59**, 7337—7344 (2003).
9) Su J.-H., Tseng Y.-J., Huang H.-H., Ahmed A. F., Lu C.-K., Wu Y.-C., Sheu J.-H., *J. Nat. Prod.*, **69**, 850—852 (2006).
10) Crystallographic data for compound **1** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 720012). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ,

U.K. (fax: +44–1223–336033 or e-mail: deposit@ccdc.cam.ac.uk).
11) Su J.-H., Dai C.-F., Huang H.-H., Wu Y.-C., Sung P.-J., Sheu J.-H., *Chem. Pharm. Bull.*, **55**, 594—597 (2007).
12) Alley M. C., Scudiero D. A., Monks A., Hursey M. L., Czerwinski M. J., Fine D. L., Abbott B. J., Mayo J. G., Shoemaker R. H., Boyd M. R., *Cancer Res.*, **48**, 589—601 (1988).
13) Scudiero D. A., Shoemaker R. H., Paull K. D., Monks A., Tierney S., Nofziger T. H., Currens M. J., Seniff D., Boyd M. R., *Cancer Res.*, **48**, 4827—4833 (1988).
14) Ho F.-M., Lai C.-C., Huang L.-J., Kuo T.-C., Chao C.-M., Lin W.-W., *Br. J. Pharmacol.*, **141**, 1037—1047 (2004).
15) Ahmed A. F., Hsieh Y.-T., Wen Z.-H., Wu Y.-C., Sheu J.-H., *J. Nat. Prod.*, **69**, 1275—1279 (2006).