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Two New Cembranes from a Formosan Soft Coral *Sinularia facile*

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Two new hydroxycembranes **1** and **2** and two known epoxycembrane **3** and isocembrol **4** have been isolated from a Formosan soft coral *Sinularia facile*. The structures of new metabolites were elucidated on the basis of extensive spectroscopic analysis and cytotoxic activity of **1**–**4** against the proliferation of a limited panel of cancer cell lines was measured.

Formosan soft corals of the genus *Sinularia* have been found to be a rich source of bioactive secondary metabolites, such as cembranoids[1–4] and steroids.[5,6] Although the chemical constituents of the soft coral *Sinularia facile* (Durivault) have rarely been studied, two cembranes were isolated from this marine organism.[7] Our previous chemical investigation on the Formosan soft coral *S. facile* have afforded five new polyoxygenated steroids.[8] Our continuing chemical investigation on this soft coral has again led to the isolation of two new hydroxylated cembranes **1** and **2**, along with two known metabolites **3** (diepoxycembrane A)[9] and **4** (isocembrol).[10] The structures of two new metabolites were determined on the basis of extensive spectroscopic analysis, including 2D NMR (^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY) spectroscopy. Cytotoxicity of metabolites **1**–**4** against a limited panel of human tumor cell lines including liver (Hep G2 and Hep G3B), breast (MDA-MB-23), and gingival (Ca9-22) carcinoma cells are also studied. We report herein the isolation, structure elucidation, and bioactivity of these compounds.

Table 1. ^{1}H NMR Data for Sterols **1** and **2**

No.	**1**[a)]	**2**[a)]
1		1.97 m
2	α 2.45 m	2.01 m
	β 2.20	
3	5.27 t (7.5)[b)]	5.06 t (6.9)
5	2.23 m	α 2.03 m
		β 2.19 m
6	2.23 m	2.21 m
7	4.94 t (5.3)	5.04 t (6.0)
9	α 2.01 m	2.65 m
	β 2.10 m	
10	2.10 m	5.54 dt (15.7, 7.1)
11	5.06 t (6.5)	5.39 d (15.7)
13	1.84 m	α 1.28 m
		β 1.54 m
14	1.70 m	α 1.05 m
	1.85 m	β 1.52 m
16	1.79 s	1.66 s
17	4.88 s	4.71 s
	5.01 s	4.73 s
18	1.58 s	1.56 s
19	1.57 s	1.67 s
20	1.59 s	1.27 s

a) Spectra recorded at 300 MHz in $CDCl_3$. b) J values (in Hz) in parentheses.

The sliced bodies of the soft coral *S. facile* were extracted exhaustively with EtOH, and then the concentrated EtOH extract was partitioned between EtOAc and H_2O. The combined EtOAc-soluble fraction was concentrated under reduced pressure and the residue was repeatedly chromatographed to yield metabolites **1**–**4**.

Compound **1** was isolated as a colorless oil. Its molecular formula, $C_{20}H_{32}O$, was established by HR-ESI-mass spectrum (m/z 311.2352 $[M + Na]^+$) and ^{13}C NMR spectroscopic data, implying five degrees of unsaturation. The IR absorption was observed at 3368 cm^{-1}, suggesting the presence of a hydroxy group. The structure of this compound was deduced from its ^{13}C NMR and DEPT spectra, which showed that the compound has 20 carbons, including four methyls, eight methylenes (including one sp^2 CH_2), three sp^2 methines, and one sp^3 quaternary carbon. From ^{1}H and ^{13}C NMR spectra (Tables 1 and 2), **1** was found to possess three trisubstituted olefinic groups [δ_H 5.27 (t, $J = 7.5$ Hz), δ_C 137.9 (qC), 120.4 (CH); δ_H 4.94 (t, $J = 5.3$ Hz), δ_C 133.7 (qC), 125.9 (CH); δ_H 5.06 (t, $J = 6.5$ Hz), δ_C 136.1 (qC), 122.5 (CH)], and one 1,1-disubstituted carbon–carbon double bond [δ_C 149.3 (C) and 110.5 (CH_2); δ_H 4.88 and 5.01, 2H, s]. A hydroxy group was observed by IR absorption at 3368 cm^{-1} and carbon resonance at δ 77.6 (C). Detailed analysis of the ^{1}H–^{1}H COSY and HMBC correlations (Figure 1) further established the planar structure of **1** as a cembrane-type compound bearing a hydroxy group at

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

No.	**1**[a)]	**2**[a)]
1	77.6 (C)[b)]	48.4 (CH)
2	37.9 (CH_2)	33.5 (CH_2)
3	120.4 (CH)	125.5 (CH)
4	137.9 (C)	134.8 (C)
5	39.5 (CH_2)	39.4 (CH_2)
6	24.9 (CH_2)	24.1 (CH_2)
7	125.9 (CH)	123.2 (CH)
8	133.7 (C)	134.1 (C)
9	39.9 (CH_2)	41.2 (CH_2)
10	23.6 (CH_2)	127.2 (CH)
11	122.5 (CH)	137.8 (CH)
12	136.1 (C)	73.6 (C)
13	32.4 (CH_2)	40.8 (CH_2)
14	42.7 (CH_2)	26.3 (CH_2)
15	149.3 (C)	148.4 (C)
16	19.2 (CH_3)	18.9 (CH_3)
17	110.5 (CH_2)	110.5 (CH_2)
18	15.4 (CH_3)	14.8 (CH_3)
19	15.1 (CH_3)	17.9 (CH_3)
20	17.2 (CH_3)	25.8 (CH_3)

a) 75 MHz in $CDCl_3$. b) Attached protons were deduced by DEPT experiments.

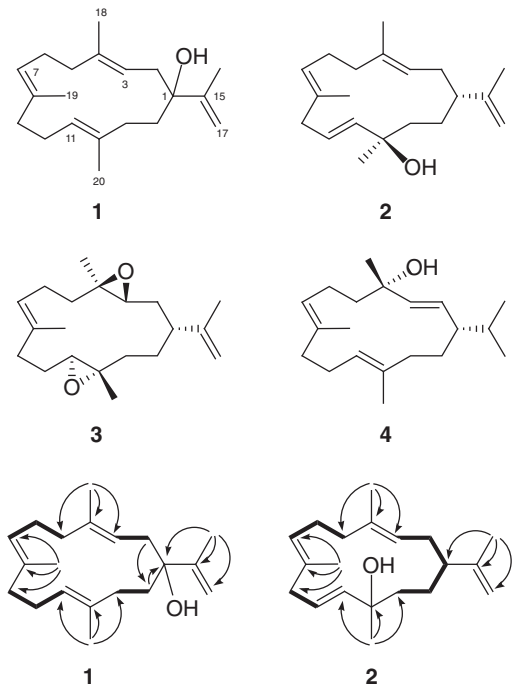

Figure 1. ^{1}H–^{1}H COSY and HMBC correlations for **1** and **2**.

C-1, three trisubstituted double bond at C-3/C-4, C-7/C-8, and C-11/C-12, and a 1,1-disubstituted carbon–carbon double bond at C-15/C-17. The geometries of trisubstituted double bonds at C-3/C-4, C-7/C-8, and C-11/C-12 are all *E*, as the chemical shifts for C-18, C-19, and C-20 were upfield shifted to 15.1–17.2 ppm, as further confirmed by NOESY correlations of H_3-18 with one proton of H_2-2 (δ_H 2.45), but not with H-3; H_3-19 with H_2-6 and one proton of H_2-9 (δ_H 2.10), but not with H-7; and H_3-20 with both H_2-10 and H_2-13, but not with H-11. The absolute configuration of the chiral center of C-1 was not able to be elucidated. On the basis of these results, the structure **1** was elucidated to be (3*E*,7*E*,11*E*,15*E*)-1-hydroxycembra-3,7,11,15-tetraene.

The HR-ESI-MS of compound **2** showed the pseudomolecular ion at m/z 311.2352 ($[M + Na]^+$), which indicated the molecular formula $C_{20}H_{32}O$. Thus, **1** and **2** have the same molecular formula and are isomers. It was shown that the NMR spectroscopic data of **2** (Tables 1 and 2) is largely similar to those of **1**, except for those of the replacemant of the 11,12-double bond in **1** to a 10,11-double bond in **2** and the conversion of the oxygenated quaternary carbon C-1 in **1** to an oxygenated quaternary carbon C-12 in **2**. Moreover, the hydroxy substituent at C-1 (δ 77.6) in **1** was found to be replaced by a hydrogen group in **2** (δ 48.4 for C-1), as confirmed by the ^{1}H–^{1}H COSY correlations between H-1 and both H_2-2 and H_2-14; H_2-9 and H-10, and HMBC correlations from H_3-20 (δ_H 1.27, s) to C-11 (δ_C 137.8, CH), C-12 (δ_C 73.6, qC), and C-13 (δ_C 40.8, CH_2). The geometries of trisubstituted double bonds at C-3/C-4 and C-7/C-8 are all *E*, as suggested on the basis of the NOE correlations observed for H_3-18 with H_2-2; H_3-19 with H_2-6, in addition to the *E* geometry of the double bond at C-10/C-11, confirmed by the large coupling constant 15.7 Hz between H-10 and H-11. The relative configuration of **2** was determined by the NOE correlations observed in a NOESY experiment and also with the aid of molecular modeling using MMF94 force field calculations. The NOE correlations between the assumed H-1β with one proton (δ 1.52) at C-14, and one proton (δ 1.54) at C-13 and H-3 suggested the β-orientations of both above-mentioned protons at C-13 and C-14. Also, NOE correlations of H_3-20 with the other proton (δ 1.05) at C-14 and one proton (δ 1.28) at C-13 suggested that H_3-20 is α-oriented. On the basis of these results, **2** was found to possess the (1*R**,12*R**,3*E*,7*E*,10*E*,11*E*)-12-hydroxycembra-3,7,10,15-tetraene.

Cembranes possessing hydroxy group at C-1, like **1**, have rarely been discovered.[11,12] A literature search showed that **1** is the first discovered 1-hydroxycembratetraene. Also, in order to explore the potential biological activity, cytotoxicity of compounds **1**–**4** against the proliferation of a limited panel of cancer cell lines, including human liver (Hep G2 and Hep G3B), breast (MDA-MB-23), and gingival (Ca9-22) carcinoma cells, was evaluated. The results showed that compound **3** exhibited cytotoxicity toward Hep G2 cancer cell line with IC_{50} value of 12.9 μg mL^{-1}. New hydroxycembranes **1**, **2**, and known compound **4**, however, were found to be not cytotoxic toward these four cancer cell lines.

Experimental

General Experimental Procedures. IR spectra were recorded on a Jasco FT/IR-4100 infrared spectrophotometer. Optical rotations were measured on a Jasco P-1020 polarimeter. NMR spectra were recorded on a Bruker AMX-300 FT-NMR at 300 MHz for ^{1}H and 75 MHz for ^{13}C, in $CDCl_3$. LRMS and HRMS were obtained by ESI on a Bruker APEX II mass spectrometer. Silica 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. High-performance liquid chromatography (HPLC) was performed on

a Shimadzu LC-10AT VP apparatus equipped with a Shimadzu SPD-10A VP UV detector. The columns used in HPLC separation are YMC-Pack Pro C18 (reverse-phase column, 250 × 10 mm, 5 μm) and Varian Dynamax, Si-60 (normal-phase column, 250 × 21.4 mm, 100 Å, 5 μm).

Animal Material. The soft coral *S. facile* was collected by hand using SCUBA off the coast of Pingtung County, located in southern Taiwan, in July 2001, at depths of 2–5 m and stored in a freezer until extraction. A voucher sample (20010719-1) was deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

Extraction and Isolation. The frozen bodies of *S. facile* (1.05 kg, wet wt) were sliced and exhaustively extracted with EtOH (1 L × 4). The combined organic layer was filtered and concentrated with a rotorary evaporator, and the residue of the resulting aqueous suspension was partitioned between EtOAc and H_2O. The EtOAc extract was dried with anhydrous Na_2SO_4. After removal of solvent in vacuo, the residue (15 g) was subjected to column chromatography on Si gel and eluted with EtOAc in *n*-hexane (0–100% of EtOAc, gradient) to yield 26 fractions. Fraction 6, eluted with *n*-hexane–EtOAc (10:1), was rechromatographed over a Sephadex LH-20 column, using acetone as the mobile phase to afford five subfractions (A1–A5). Subfraction A3 was separated by normal-phase HPLC (*n*-hexane–acetone, 1:30) to afford compound **1** (3.1 mg). Fraction 7, eluted with *n*-hexane–EtOAc (5:1), was rechromatographed over a Sephadex LH-20 column, using acetone as the mobile phase to afford five subfractions (B1–B5). Subfraction B4 was separated by reverse-phase HPLC (CH_3CN–H_2O, 3:1) to afford compound **2** (8.0 mg), **3** (2.2 mg), and **4** (10.1 mg).

(3*E*,7*E*,11*E*,15*E*)-1-Hydroxycembra-3,7,11,15-tetraene (**1**): colorless oil; $[\alpha]_D^{25}$ −23 (*c* 0.3, $CHCl_3$); IR (neat) ν_{max} 3368 cm^{-1}; ^{13}C and ^1H NMR data (300 MHz; $CDCl_3$), see Tables 1 and 2; ESIMS *m*/*z* 311 $[M + Na]^+$; HRESIMS *m*/*z* 311.2352 $[M + Na]^+$ (calcd for $C_{20}H_{32}ONa$, 311.2351).

(1*R**,12*R**,3*E*,7*E*,10*E*,11*E*)-12-Hydroxycembra-3,7,10,15-tetraene (**2**): colorless oil; $[\alpha]_D^{25}$ −14 (*c* 0.8, $CHCl_3$); IR (neat) ν_{max} 3651 cm^{-1}; ^{13}C and ^1H NMR data (300 MHz; $CDCl_3$), see Tables 1 and 2; ESIMS *m*/*z* 311 $[M + Na]^+$; HRESIMS *m*/*z* 311.2352 $[M + Na]^+$ (calcd for $C_{20}H_{32}ONa$, 311.2351).

Molecular Mechanics' Calculations. The minimum energy conformation of **1** and **2** was determined using the ChemBio3D version 2010 molecular modeling package[13] incorporating an empirical force field, MMF94, on an ASUS P6TD Deluxe PC. Molecular mechanics was utilized to investigate the minimization and minimum energy was calculated by a conjugate gradient method until minimum RMS gradient with a gradient less than 0.001 kcal mol^{-1} $Å^{-1}$. The conformers shown in Figure 2 are the lowest energy conformation for **1** and **2**.

Cytotoxicity Testing. Cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays were performed in duplicate using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[14,15]

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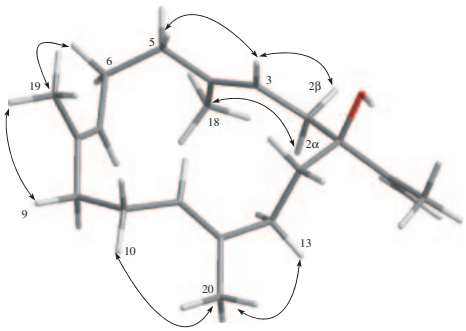

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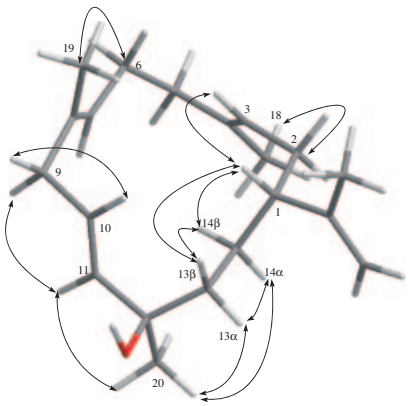

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Figure 2. Key NOE correlations and computer-generated perspective model using MMF94 force field calculations for **1** and **2**.

References

1 A. F. Ahmed, S.-H. Tai, Z.-H. Wen, J.-H. Su, Y.-C. Wu, W.-P. Hu, J.-H. Sheu, *J. Nat. Prod.* **2008**, *71*, 946.
2 Y. Lu, C.-Y. Huang, Y.-F. Lin, Z.-H. Wen, J.-H. Su, Y.-H. Kuo, M. Y. Chiang, J.-H. Sheu, *J. Nat. Prod.* **2008**, *71*, 1754.
3 Y.-S. Lin, C.-H. Chen, C.-C. Liaw, Y.-C. Chen, Y.-H. Kuo, Y.-C. Shen, *Tetrahedron* **2009**, *65*, 9157.
4 S.-Y. Cheng, K.-J. Huang, S.-K. Wang, Z.-H. Wen, P.-W. Chen, C.-Y. Duh, *J. Nat. Prod.* **2010**, *73*, 771.
5 J.-H. Su, Y.-J. Tseng, H.-H. Huang, A. F. Ahmed, C.-K. Lu, Y.-C. Wu, J.-H. Sheu, *J. Nat. Prod.* **2006**, *69*, 850.
6 A. F. Ahmed, Y.-T. Hsieh, Z.-H. Wen, Y.-C. Wu, J.-H. Sheu, *J. Nat. Prod.* **2006**, *69*, 1275.
7 B. F. Bowden, J. C. Coll, S. J. Mitchell, R. Kazlauskas, *Aust. J. Chem.* **1981**, *34*, 1551.
8 B.-W. Chen, J.-H. Su, C.-F. Dai, Y.-C. Wu, J.-H. Sheu, *Bull. Chem. Soc. Jpn.* **2008**, *81*, 1304.
9 M. Hérin, M. Colin, B. Tursch, *Bull. Soc. Chim. Belg.* **1976**, *85*, 801.
10 N. K. Kashtanova, A. I. Lisina, V. A. Pentegova, *Chem. Nat. Compd.* **1968**, *4*, 45.
11 M. Kobayashi, K. Osabe, *Chem. Pharm. Bull.* **1989**, *37*, 631.
12 E. Klein, H. Obermann, *Tetrahedron Lett.* **1978**, *19*, 349.
13 S. M. Kerwin, *J. Am. Chem. Soc.* **2010**, *132*, 2466.
14 M. C. Alley, D. A. Scudiero, A. Monks, M. L. Hursey, M. J. Czerwinski, D. L. Fine, B. J. Abbott, J. G. Mayo, R. H. Shoemaker, M. R. Boyd, *Cancer Res.* **1988**, *48*, 589.
15 D. A. Scudiero, R. H. Shoemaker, K. D. Paull, A. Monks, S. Tierney, T. H. Nofziger, M. J. Currens, D. Seniff, M. R. Boyd, *Cancer Res.* **1988**, *48*, 4827.