

Article

Cembranoids with 3,14-Ether Linkage and a Secocembrane with Bistetrahydrofuran from the Dongsha Atoll Soft Coral *Lobophytum* sp.

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Abstract: Four new cembranoids, lobophylins A–D (**1–4**), and one novel secocembrane, lobophylin E (**5**) were isolated from a soft coral *Lobophytum* sp. The structures of new metabolites were elucidated on the basis of extensive spectroscopic methods. Among these metabolites, **1–4** are rarely found cembranoids possessing a tetrahydrofuran moiety with a 3,14-ether linkage. In addition, **5** is the first secocembrane possessing two tetrahydrofuran moieties with 3,14- and 4,7-ether linkages.

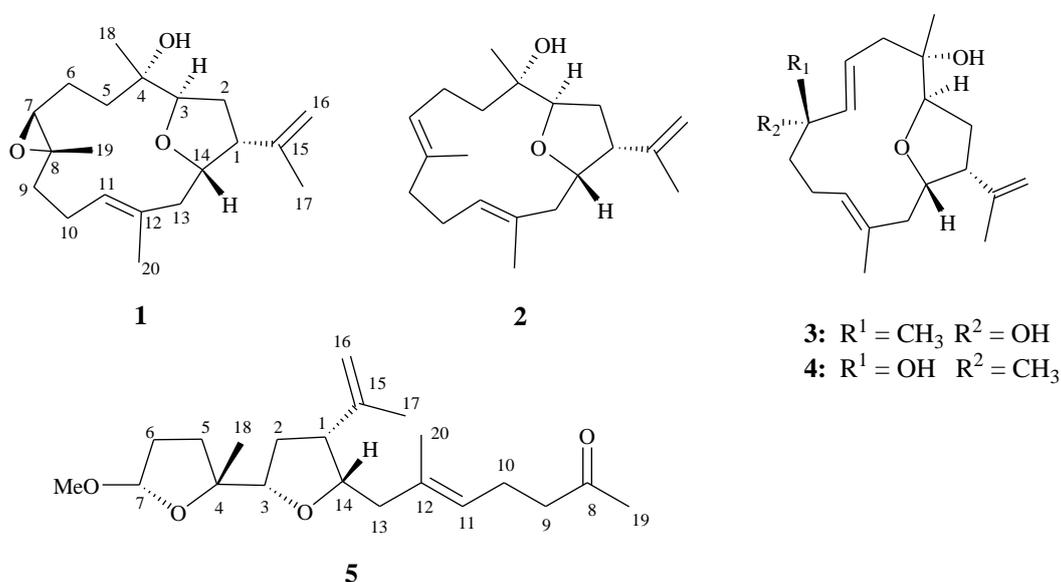
Keywords: soft coral; secocembrane; *Lobophytum*; tetrahydrofuran

1. Introduction

Soft corals have proven to be important sources of secondary metabolites with interesting biological activities [1]. In the investigation of the secondary metabolites from soft corals in Taiwan waters, a series of bioactive cembranoids have been isolated from octocorals (Alcyonaceae) belonging to the genera *Sinularia* [2–7], *Lobophytum* [8–10], *Sarcophyton* [11–16] and *Pachyclavularia* [17,18]. Some

of these metabolites have been shown to exhibit significant cytotoxic activity against the growth of various cancer cell lines [10,15–17], and/or anti-inflammatory activity [3,6,8,10,15,16]. Our previous chemical investigation on Dongsha Atoll soft coral *Lobophytum sarcophytooides* has led to the isolation of bioactive cembranoids [19]. In our continuing search for bioactive metabolites from Dongsha Atoll soft corals of the genus *Lobophytum*, we investigated the chemical constituents of *Lobophytum* sp. and succeeded in the isolation of four new cembranoidal lobophylins A–D (**1–4**) and a novel secocembrane, lobophylin E (**5**) (Chart 1). The structures of these compounds have been established by extensive spectroscopic analysis. The cytotoxicity of compounds **1–5** against four human cancer cell lines was investigated, however, none of these was found to possess useful biological activity.

Chart 1. Structures of metabolites **1–5**.



2. Results and Discussion

The new metabolite lobophylin A (**1**) exhibited a protonated molecule peak in the HRESIMS at m/z 343.2251 [M + Na]⁺, establishing the molecular formula C₂₀H₃₂O₃ and five degrees of unsaturation. The IR spectrum suggested the presence of hydroxy group (ν_{\max} 3460 cm⁻¹) in **1**. The ¹³C NMR spectrum of **1** measured in CDCl₃ (Table 1) showed the presence of twenty carbon signals, which were assigned by the assistance of DEPT spectrum to four methyls, six sp³ methylenes, one sp² methylene, four sp³ methines (including three oxymethines), one sp² methine, and two sp³ quaternary and two sp² quaternary carbons. From the ¹H NMR spectroscopic data of **1** (Table 2), the presence of two hydroxy protons resonating at δ 3.98 (dd, $J = 9.6, 4.4$ Hz) and 4.37 (ddd, $J = 12.0, 3.6, 3.6$ Hz) were observed. Moreover, the ¹H NMR spectrum revealed the presence of two olefinic methylene protons at δ 4.87 (d, $J = 1.6$ Hz) and 4.81 (s) and one olefinic methine proton at δ 5.09 (t, $J = 6.8$ Hz). A proton signal appearing at δ 3.27 (¹H, d, $J = 6.8$ Hz) and correlating with a carbon signal at δ 64.7 in the HMQC spectrum was due to the proton of the trisubstituted epoxide. The planar structure and all of the assignments of ¹H and ¹³C NMR data of **1** were determined by the assistance of 2D NMR studies, including ¹H-¹H COSY and HMBC experiments (Figure 1). ¹H-¹H COSY spectrum revealed proton sequences from H-1 to H-3 and H-13 to H-1; H₂-5 to H-7; H₂-9 to H-11, as shown by the bold lines in

Figure 1. Key HMBC correlations of H-3 to C-4; H-7 to C-8; H₂-13 to C-11 and C-12; H₂-16 to C-1 and C-15; H₃-17 to C-1, C-15 and C-16; H₃-18 to C-3, C-4 and C-5; H₃-19 to C-7, C-8 and C-9; and H₃-20 to C-11, C-12 and C-13, permitted the connection of the carbon skeleton. Furthermore, the HMBC cross-peak from H-14 to C-3 suggested that C-3 and C-14 were linked through an oxygen to form a tetrahydrofuran ring. Thus, **1** was revealed as a cembranoid possessing a 3,14-ether linked tetrahydrofuran ring, on the basis of the above analysis.

Table 1. ¹³C NMR data for compounds **1–5**.

C#	1 ^a	2 ^a	3 ^a	4 ^b	5 ^b
1	50.2 (CH) ^c	49.0 (CH)	49.3 (CH)	49.3 (CH)	49.8 (CH)
2	29.1 (CH ₂)	27.4 (CH ₂)	26.7 (CH ₂)	26.7 (CH ₂)	30.9 (CH ₂)
3	77.5 (CH)	77.6 (CH)	77.8 (CH)	77.6 (CH)	82.2 (CH)
4	74.5 (C)	74.2 (C)	74.6 (C)	74.7 (C)	86.6 (C)
5	39.1 (CH ₂)	38.6 (CH ₂)	42.5 (CH ₂)	43.3 (CH ₂)	31.9 (CH ₂)
6	23.8 (CH ₂)	21.5 (CH ₂)	118.9 (CH)	121.8 (CH)	33.3 (CH ₂)
7	64.7 (CH)	126.6 (CH)	142.7 (CH)	141.5 (CH)	105.6 (CH)
8	60.3 (C)	132.8 (C)	73.6 (C)	72.6 (C)	208.9 (C)
9	38.1 (CH ₂)	38.2 (CH ₂)	44.4 (CH ₂)	43.7 (CH ₂)	43.7 (CH ₂)
10	23.9 (CH ₂)	24.4 (CH ₂)	23.5 (CH ₂)	22.2 (CH ₂)	22.5 (CH ₂)
11	126.5 (CH)	127.1 (CH)	129.4 (CH)	129.6 (CH)	124.2 (CH)
12	133.0 (C)	131.9 (C)	130.9 (C)	130.8 (C)	134.2 (C)
13	40.2 (CH ₂)	39.3 (CH ₂)	38.9 (CH ₂)	38.8 (CH ₂)	39.7 (CH ₂)
14	78.5 (CH)	76.7 (CH)	76.0 (CH)	76.0 (CH)	80.3 (CH)
15	141.6 (C)	142.4 (C)	142.2 (C)	142.3 (C)	144.0 (C)
16	111.3 (CH ₂)	111.0 (CH ₂)	111.2 (CH ₂)	111.1 (CH ₂)	112.2 (CH ₂)
17	25.0 (CH ₃)	23.5 (CH ₃)	23.5 (CH ₃)	23.5 (CH ₃)	22.5 (CH ₃)
18	24.6 (CH ₃)	23.1 (CH ₃)	21.6 (CH ₃)	21.9 (CH ₃)	24.2 (CH ₃)
19	19.8 (CH ₃)	16.3 (CH ₃)	29.6 (CH ₃)	28.3 (CH ₃)	29.9 (CH ₃)
20	17.3 (CH ₃)	15.4 (CH ₃)	15.4 (CH ₃)	15.5 (CH ₃)	16.5 (CH ₃)
OMe					54.3 (CH ₃)

^a Spectra recorded at 100 MHz in CDCl₃; ^b Spectra recorded at 125 MHz in CDCl₃; ^c Attached protons were deduced by DEPT experiments.

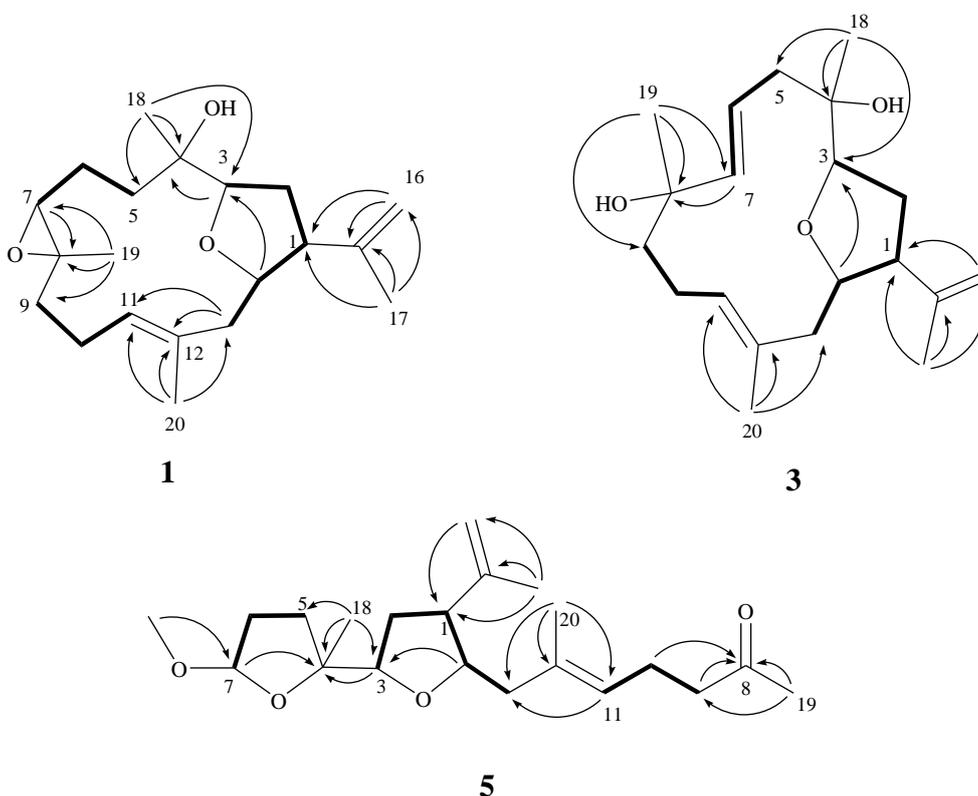
Table 2. ¹H NMR data for compounds **1–5**.

	1 ^a	2 ^a	3 ^a	4 ^b	5 ^b
1	2.77 dt (8.8, 8.0) ^c	2.73 dt (11.2, 7.2)	2.73 dt (8.0, 8.8)	2.74 dt (9.0, 8.5)	2.78 dt (7.5, 8.5)
2	2.16 m; 1.92 m	2.08 m; 1.90 m	2.04 m; 1.86 m	2.05 m; 1.86 m	1.96 m; 1.91 m
3	3.98 dd (9.6, 4.4)	3.97 dd (9.6, 4.5)	3.82 dd (10.0, 4.8)	3.82 dd (9.5, 4.5)	3.98 dd (7.5, 7.5)
5	1.97 m; 1.70 m	1.94 m; 1.53 m	2.40 dd (14.0, 10.0); 2.05 m	2.40 dd (14.0, 10.0); 2.10 m	2.40 dd (14.0, 10.0); 1.94 m
6	2.05 m; 1.31 m	2.25 m; 2.06 m	5.60 ddd (15.2, 10.0, 5.2)	5.51 ddd (15.5, 10.0, 5.0)	2.02 m; 1.94 m
7	3.27 d (6.8)	5.17 dd (6.0, 6.0)	5.70 d (15.6)	5.75 d (15.5)	5.00 d (4.5)
9	1.86 m; 1.52 m	2.14 m; 1.96 m	1.92 m; 1.58 m	1.95 m; 1.58 m	2.45 dd (8.0, 7.0)
10	2.21 m; 1.88 m	2.32 m; 2.04 m	2.19 m; 2.10 m	2.56 m; 1.96 m	2.27 dd (7.5, 7.5)

Table 2. Cont.

11	5.09 t (6.8)	4.89 d (8.0)	4.96 d (9.6)	4.94 d (10.0)	5.12 dd (7.0, 6.5)
13	1.95 m; 1.68 m	1.88 m; 1.72 m	1.91 m; 1.64 m	1.92 m; 1.64 m	2.00 m; 1.97 m
14	4.37 ddd (12.0, 3.6, 3.6)	4.36 ddd (11.6, 5.2, 4.8)	4.33 ddd (11.6, 6.0, 5.2)	4.33 ddd (12.0, 6.0, 5.5)	4.14 ddd (9.0, 4.5, 3.5)
16	4.87 d (1.6); 4.81 s	4.85 d (1.2); 4.78 s	4.86 d (1.6); 4.80 s	4.86 d (1.0); 4.80 s	4.83 s; 4.72 s
17	1.77 s	1.75 s	1.76 s	1.76 s	1.75 s
18	1.15 s	1.09 s	1.11 s	1.13 s	1.28 s
19	1.24 s	1.65 s	1.28 s	1.37 s	2.13 s
20	1.61 s	1.57 s	1.67 s	1.70 s	1.65 s
OMe					3.34 s

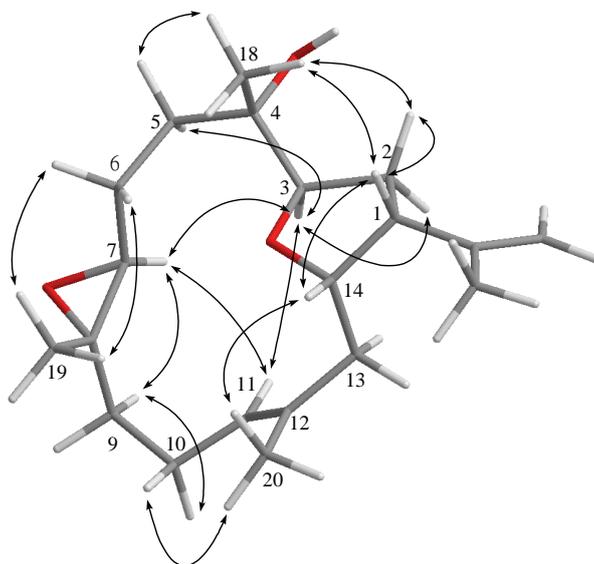
^a Spectra recorded at 400 MHz in CDCl₃; ^b Spectra recorded at 500 MHz in CDCl₃; ^c *J* values (in Hz) in parentheses.

Figure 1. Selected ¹H-¹H COSY (—) and HMBC (→) correlations of **1**, **3** and **5**.

The relative configuration of **1** elucidated mainly by NOESY spectrum was compatible with that of **1** offered by using the MM2 force field calculations which suggested the most stable conformations as shown in Figure 2. In the NOESY spectrum, it was found that H-1 (δ 2.77, dt, *J* = 8.8, 8.0 Hz) showed NOE interactions with H-14 and H₃-18 (δ 1.15, s); therefore, assuming the β -orientation of H-1, H-14 and H₃-18 should also be positioned on the β face. One of the methylene protons at C-2 (δ 1.92) exhibited NOE correlations with H-1 and was characterized as H-2 β , while the other (δ 2.16) was assigned as H-2 α . NOE correlations observed between H-2 α and H-3 (δ 3.98, dd, *J* = 9.6, 4.4 Hz), and H-3 and H-7 (δ 3.27, d, *J* = 6.8 Hz), reflected the α -orientations of both protons H-3 and H-7. Also, H₃-19 was found to interact with H₂-6, but not with H-7, revealing the *trans* geometry of the

trisubstituted epoxide. Furthermore, the NOE correlations observed H₃-20 and H-10 (δ 2.21), but not with H-11, reflected the *E* geometry of double bond at C-11. On the basis of the above findings and other detailed NOE correlations (Figure 2), the relative structure of **1** was determined.

Figure 2. Computer-generated model for **1** using MM2 force field calculations and key NOE correlations.



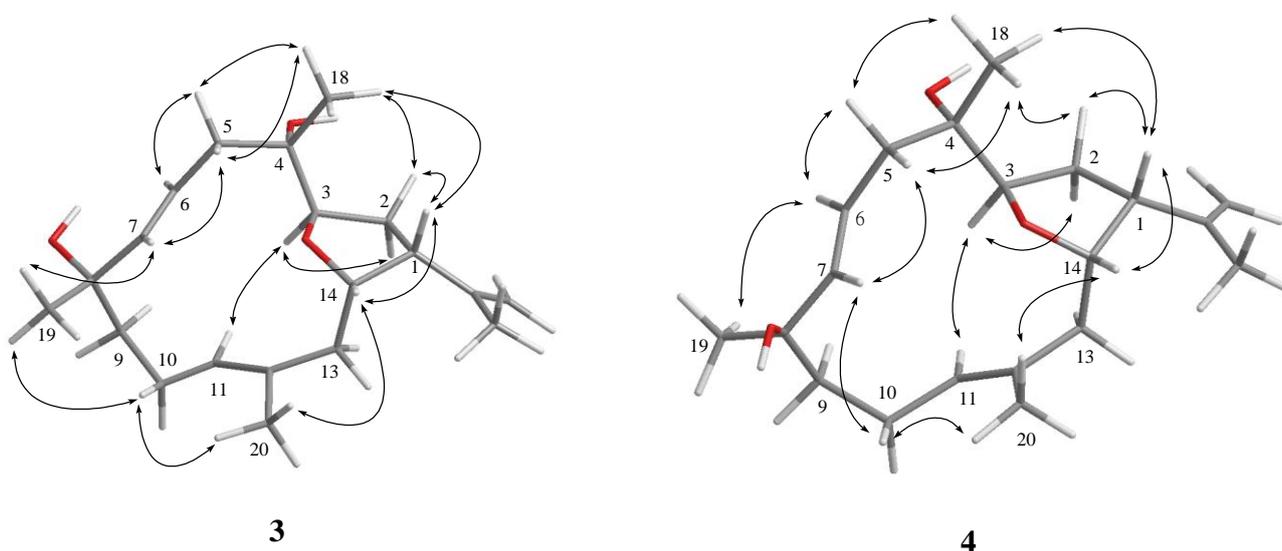
HRESIMS analysis of lobophylin B (**2**) provided a molecular formula of C₂₀H₃₂O₂ ([M + Na]⁺ *m/z* 327.2301). The ¹H and ¹³C NMR spectroscopic data of **2** were very close to those of **1** (Tables 1 and 2), except for the replacement of the two carbon signals of the epoxide moiety in **1** by the signals of a trisubstituted double bond in **2** (δ 126.6, CH, C-7 and 132.8, C, C-8). This double bond was positioned at C-7/C-8 due to the ¹H-¹H COSY correlation found between the H-6 and H-7, the HMBC correlations observed from the olefinic methyl protons at δ 1.65 (3H, s) to C-7, C-8 and C-9. Furthermore, the *E* geometry of the 7,8-double bond was deduced from the NOE correlation of H₃-19 with H₂-6 and not with H-7. Thus, the structure of **2** was determined unambiguously. Literature review revealed a known compound similar to compound **2** but possessing a rare 3,13-bridged tetrahydropyran ring [20].

Lobophylin C (**3**) showed a protonated molecule peak [M + Na]⁺ at *m/z* 343.2248 in the HRESIMS, corresponding to the molecular formula C₂₀H₃₂O₃ and five degrees of unsaturation. The IR spectrum showed the presence of hydroxy (3377 cm⁻¹) group. ¹H and ¹³C NMR spectroscopic data (Tables 1 and 2) of **3** showed the structural unit of a 3,14-oxa-bridged tetrahydrofuran, too. ¹H-¹H COSY and HMBC (Figure 1) further revealed that **3** possesses a 1,2-disubstituted double bond (δ 118.9 and 142.7, each CH) at C-6 and C-7 and a quaternary oxycarbon at C-8 (δ 73.6, C). On the basis of the above observations, and by the assistance of additional 2D NMR (¹H-¹H COSY and HMBC) correlations, it was possible to establish the planar structure of **3** as illustrated in Figure 1. The relative configurations of the five chiral centers at C-1, C-3, C-4, C-8 and C-14 in **3** were thus determined on the basis of NOE correlations (Figure 3). By careful inspection on the NOESY spectrum of **3**, it was found that one proton (δ 2.40) of H₂-5 showed NOE interaction with both H₃-18 and H-7, and H-7 was NOE correlated with H₃-19. Therefore, H₃-18 and H₃-19 are situated on the same β -face. Furthermore,

NOESY spectrum showed correlation of H₃-20 with one proton (δ 2.19) of CH₂-10, but not with H-11, revealing the *E*-configurations of the 11,12-trisubstituted double bond. The above finding, together with *J* values for both H-6 (15.2 Hz) and H-7 (15.6 Hz), confirmed the *E*-configuration of the 6,7-double bond. Further NOE analysis revealed that **3** possessed the same configurations at C-1, C-3, C-4 and C-14, as in compound **1** (Figure 3). Based on the above results, the structure of **3** was established.

The HRESIMS spectrum of lobophylin D (**4**) showed a molecular formula of C₂₀H₃₂O₃, the same as that of **3**. By analysis 2D NMR spectra, including ¹H-¹H COSY, HMQC and HMBC, **4** was shown to possess the same molecular framework as that of **3**. Furthermore, it was found that the NMR data of **4** were very similar to those of **3** (Tables 1 and 2), revealing that **4** might be an isomer of **3**. However, the significant downfield shift at C-6 ($\Delta\delta_C$ +2.9 ppm) and the upfield shift at C-7 ($\Delta\delta_C$ -1.2 ppm), C-8 ($\Delta\delta_C$ -1.0 ppm) and C-19 ($\Delta\delta_C$ -1.3 ppm), relative to those of **3** (Table 2), suggested that **4** might be the C-8 epimer of **3**. From NOESY spectrum, it was found that one proton (δ 2.56, m) of H₂-10 of **4** showed NOE correlations with H-7 (δ 5.75, d, *J* = 15.5 Hz) and H₃-20 (δ 1.70, s), while H-6 (5.51, ddd, *J* = 15.5, 10.0, 5.0 Hz) was NOE correlated with H₃-19 (δ 1.37, s) (Figure 3). Therefore, both H-7 and H₃-20 are situated on the β -face, and in contrast, H-6 and H₃-19 should be positioned on the α -face. This inferred the *R** configuration at C-8. Further analysis of other NOE interactions revealed that **4** possessed the same relative configurations at C-1, C-3, C-4 and C-14 as those of **3** (Figure 3). Therefore, **4** was found to be the C-8 epimer of **3**.

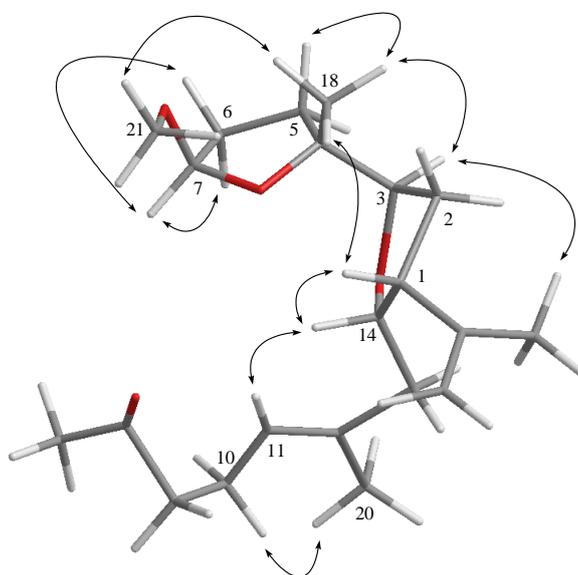
Figure 3. Computer-generated model for **3** and **4** using MM2 force field calculations and key NOE correlations.



Lobophylin E (**5**) was assigned a molecular formula of C₂₁H₃₄O₄, according to the HRESIMS and NMR spectroscopic data (Tables 1 and 2). The IR absorption band at 3444 cm⁻¹ revealed the presence of hydroxy group. By the analysis of ¹³C and DEPT spectroscopic data, the carbons signals were assigned into five methyls (including one methoxy methyl resonating at δ_C 54.3), six sp³ methylenes, one sp² methylene, four sp³ methines (including two monooxygenated carbons resonating at δ_C 82.2 and 80.3 and an acetal carbon resonating at δ_C 105.6), one sp² methine, one sp³ quaternary carbons

and three sp^2 quaternary carbons (including a normal ketone resonating at δ_C 208.9). From the 1H - 1H COSY spectrum of **5**, it was possible to identify three different structure units, which were assembled with the assistance of an HMBC experiment. Key HMBC correlations between H-3 to C-4; H₂-9 and H₂-10 to C-8 (carbonyl carbon); H-11 to C-13; H₂-16 to C-1 and H₃-17 to C-1, C-15 and C-16; H₃-18 to C-3, C-4 and C-5; H₃-19 to C-8 and C-9; and H₃-20 to C-11, C-12 and C-13 permitted the connection of the carbon skeleton (Figure 1). Furthermore, the HMBC correlation observed from the methoxy protons (δ 3.34, 3H, s) to the carbon resonating at δ 105.6 positioned a methoxy group at C-7. In considering the degrees of unsaturation and molecular formula, two oxa-bridged ether linkages were placed between C-3/C-14 and C-4/C-7 by HMBC correlations from H-14 to C-3 and H-7 to C-4. The relative configuration of **5** was determined by the interpretation of the NOESY correlations (Figure 4). It was found that H₃-18 showed NOE interactions with H-1, H-3 and methoxy protons (H₃-21). Thus, by considering a molecular model as shown in Figure 4 and assuming the β -orientation of H₃-18, all of H-1, H-3 and methoxy group should be positioned on the β face. The NOE correlation observed between H-1 and H-14 also reflected the β -orientation of H-14. Furthermore, NOESY spectrum showed NOE interaction of H₃-20 with H-10, but not with H-11, revealing the *E* geometry of the C-11/C-12 double bond. From the above evidence and the other NOE correlations (Figure 4) the relative configurations at chiral centers of **5** was assumed to be 1*R**, 3*R**, 4*R**, 7*R** and 14*S**. On the basis of the above analysis, the structure of **5** was established.

Figure 4. Computer-generated model for **5** using MM2 force field calculations and key NOE correlations.



It is worth noting that metabolites **1–4** are rare cembranoids possessing a tetrahydrofuran moiety with a 3,14-ether linkage, which has been discovered previously in the soft coral *Sinularia gibberosa* [5,21]. In addition, **5** is the first secocembrane possessing two tetrahydrofuran moieties with 3,14- and 4,7-ether linkages. Our study thus adds the structure diversity of cembranoidal natural compounds.

The cytotoxicity of compounds **1–5** against the proliferation of a limited panel of cancer cell lines, including K562 (human chronic myelogenous leukemia), DLD-1 (human colon adenocarcinoma) and

HepG2 and Hep3B (human liver carcinoma), was studied. The results showed that **1–5** are not cytotoxic toward the above cancer cells ($IC_{50} > 20 \mu\text{g/mL}$).

3. Experimental Section

3.1. General Experimental Procedures

The melting points were determined using a Fisher-Johns melting point apparatus. Optical rotation values were measured with a JASCO P-1010 digital polarimeter. IR spectra were recorded on a VARIAN DIGLAB FTS 1000 Fourier transform infrared spectrophotometer. The NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR (or Varian Unity INOVA 500 FT-NMR) instrument at 400 MHz (or 500 MHz) for ^1H NMR and 100 MHz (or 125 MHz) for ^{13}C NMR, respectively, in CDCl_3 . ESIMS were recorded 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 F254, 0.25 mm) and precoated RP-18 F254S plates (Merck, 1.05560) were used for TLC analysis. High-performance liquid chromatography (HPLC) was performed on a Hitachi L-7100 pump equipped with a Hitachi L-7400 UV detector at 210 nm. A semipreparative reversed-phase column (250 × 10 mm, 5 μm) and a preparative normal phase column (250 × 21.2 mm, 5 μm) was used for HPLC.

3.2. Animal Material

The soft coral *Lobophytum* sp. was collected by hand using SCUBA off the coast of Dongsha Atoll, in April, 2007, at a depth of 10 m, and stored in a freezer until extraction. A voucher specimen (Specimen No. DA2007-04-20) was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

3.3. Extraction and Separation

The frozen soft coral (1.5 kg, fresh wt) was minced and extracted exhaustively with EtOAc (5 × 1 L). The organic extract was evaporated to yield a residue (21.9 g), which was fractionated by open column chromatography on silica gel using *n*-hexane–EtOAc and EtOAc–MeOH mixtures of increasing polarity to yield 16 fractions. Fraction 5, eluting with *n*-hexane–EtOAc (15:1), was further separated by silica gel column chromatography with gradient elution (*n*-hexane–EtOAc, 15:1 to 5:1) to yield five subfractions (5A–5E). Subfraction 5C was subjected to normal phase HPLC (*n*-hexane–EtOAc, 15:1) to obtain compound **2** (2.5 mg). Fractions 7 and 8, eluting with *n*-hexane–EtOAc (5:1), were combined and further separated over silica gel column chromatography (*n*-hexane–EtOAc, gradient elution, 5:1 to 1:1) to give four subfractions (7A–7D). Subfraction 7A was further purified by RP-18 HPLC (CH_3CN – H_2O , 3:2) to yield compound **5** (2.2 mg). In the same manner, compound **1** (4.2 mg) was obtained from subfraction 7B using RP-18 HPLC (CH_3CN – H_2O , 5:2). Fraction 11, eluting with *n*-hexane–EtOAc (1:1), was further separated by silica gel column chromatography with gradient elution (*n*-hexane–EtOAc, 1:1 to 1:5) to yield five subfractions (11A–11E). Subfraction 11C was further purified by RP-18 HPLC (CH_3CN – H_2O , 1:1) to yield compounds **3** (3.0 mg) and **4** (2.5 mg).

Lobophylin A (**1**): colorless oil; $[\alpha]_D^{25} = -39$ (*c* 0.3, CHCl₃); IR (neat) ν_{\max} 3460, 2926, 1649, 1458, 1381 and 1215 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS *m/z* 343 [100, (M + Na)⁺]; HRESIMS *m/z* 343.2251 (calcd for C₂₀H₃₂O₃Na, 343.2249).

Lobophylin B (**2**): colorless oil; $[\alpha]_D^{25} = -35$ (*c* 0.3, CHCl₃); IR (neat) ν_{\max} 3445, 2926, 1649, 1456, 1376 and 1265 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS *m/z* 327 [100, (M + Na)⁺]; HRESIMS *m/z* 327.2301 (calcd for C₂₀H₃₂O₂Na, 327.2300).

Lobophylin C (**3**): white powder; mp 76–78 °C; $[\alpha]_D^{25} = +30$ (*c* 0.1, CHCl₃); IR (neat) ν_{\max} 3377, 2927, 1649, 1459, 1377 and 1269 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS *m/z* 343 [100, (M + Na)⁺]; HRESIMS *m/z* 343.2248 (calcd for C₂₀H₃₂O₃Na, 343.2249).

Lobophylin D (**4**): white powder; mp 68–70 °C; $[\alpha]_D^{25} = +22$ (*c* 0.2, CHCl₃); IR (neat) ν_{\max} 3425, 2924, 1640, 1455, 1379 and 1240 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS *m/z* 343 [100, (M + Na)⁺]; HRESIMS *m/z* 343.2246 (calcd for C₂₀H₃₂O₃Na, 343.2249).

Lobophylin E (**5**): colorless oil; $[\alpha]_D^{25} = +19$ (*c* 0.2, CHCl₃); IR (neat) ν_{\max} 3444, 2929, 1715, 1640, 1454, 1374 and 1214 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS *m/z* 373 [100, (M + Na)⁺]; HRESIMS *m/z* 373.2356 (calcd for C₂₁H₃₄O₄Na, 373.2355).

3.4. Cytotoxicity Testing

Cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays of compounds **1–5** were performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method [22].

3.5. Molecular Mechanics Calculations

Implementation of the MM2 force field in Chem3D Pro software from Cambridge Soft Corporation, Cambridge, MA, USA (ver. 9.0, 2005), was used to calculate molecular models.

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