Cembranoids from the Octocoral *Sarcophyton ehrenbergi*

Shi-Yie Cheng,[†,‡] Shang-Kwei Wang,*[,§] Shu-Fen Chiou,[†] Chi-Hsin Hsu,[†,‡] Chang-Feng Dai,[⊥] Michael Y. Chiang,[∥] and Chang-Yih Duh*[,†,‡]

Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China, Department of Microbiology, Kaohsiung Medical University, Kaohsiung, Taiwan, Republic of China, Institute of Oceanography, National Taiwan University, Taipei, Taiwan, Republic of China, Department of Chemistry, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China, and Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China

Received October 29, 2009

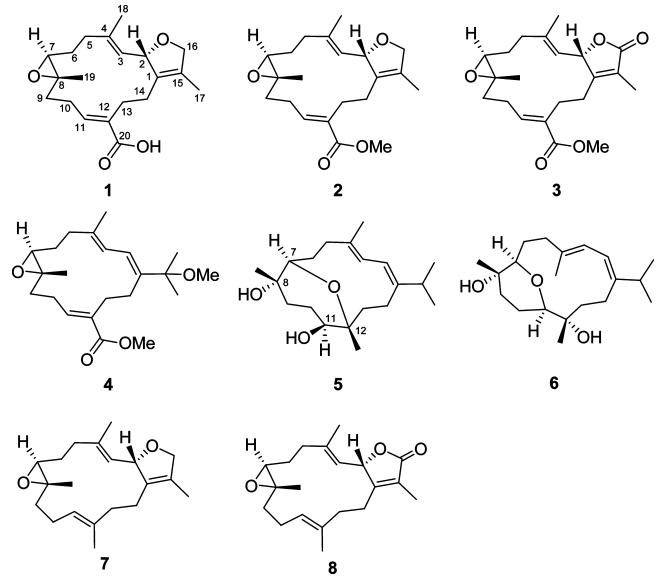
Chemical investigation of the octocoral *Sarcophyton ehrenbergi* led to the isolation of six new cembranoids, (+)-12-carboxy-11*Z*-sarcophytoxide (**1**), (+)-12-methoxycarbonyl-11*Z*-sarcophine (**3**), ehrenberoxides A−C (**4**−**6**), and lobophynin C (**2**), along with two known compounds, (+)-sarcophytoxide (**7**) and (+)-sarcophine (**8**). The structures of these isolated metabolites were elucidated through extensive spectroscopic analyses, while the relative configuration of **1** was confirmed by X-ray diffraction analyses. The chemical evidence combined with spectroscopic and physical data suggested that the locations of the epoxide and the methyl carboxylate for lobophynin C should be exchanged. Moreover, metabolites **1**−**6** were evaluated in vitro for their cytotoxicity against selected cancer and normal cells lines, antiviral activity against human cytomegalovirus, and antibacterial activity against *Salmonella enteritidis*.

Marine organisms, which have developed unique metabolic and physiological capabilities to ensure survival in extreme ocean habitats, offer the potential to produce new bioactive constituents that would not be observed from terrestrial organisms.[1] Soft corals belonging to the genus *Sarcophyton* (Alcyoniidae) have been well recognized as a rich source of terpenoids. These constituents, mainly macrocyclic cembrane-type diterpenoids and their derivatives, represent important chemical defense tools for the animals against their natural predators.[2] Previous bioassay results of some cembranoid analogues have been proven to exhibit a wide range of biological activities including antitumor,[3−9] ichthyotoxic,[10] anti-inflammatory,[11] neuroprotective,[12] antibacterial,[13] antiangiogenic,[14] antimetastatic,[14] and antiosteoporotic properties.[15]

Previous investigations of Australian soft coral samples of *Sarcophyton ehrenbergi* (von Marenzeller, 1886) resulted in the isolation of (−)-sarcophytoxide,[16a] isoneocembrene A,[16a,b] sarcophytol T,[16b] (1*E*,3*E*,7*E*,11*R**12,*R**)-15-(acetoxymethyl)cembra-11,12-epoxy-1,3,7-triene,[16b] (1*E*,3*R**,4*R**,7*E*)-15-(acetoxymethyl)-cembra-3,4:11,12-diepoxy-1,7-diene,[16b] and (2*S**,11*R**, 12*R**)-isosarcophytoxide.[16b] As part of our continuing search for bioactive secondary metabolites from a Taiwanese soft coral sample of *S. ehrenbergi*,[16c] six new cembranoids, designated as (+)-12-carboxy-11*Z*-sarcophytoxide (**1**), (+)-12-methoxycarbonyl-11*Z*-sarcophine (**3**), ehrenberoxides A−C (**4**−**6**), and lobophynin C (**2**), along with two known compounds, (+)-sarcophytoxide (**7**) and (+)-sarcophine (**8**), were isolated from the acetone extract of the organism. Herein, we describe the purification, structure elucidation, structural revision of lobophynin C,[17] and antiviral evaluation of these metabolites in detail.

Results and Discussion

The acetone extract of the octocoral *S. ehrenbergi* was partitioned between EtOAc and H_2O to afford the EtOAc-soluble fraction, which was subjected to silica gel column chromatography using a *n*-hexane−EtOAc−MeOH mixture as eluent. The fractions containing terpenoids were selected on the basis of characteristic peaks in the ^{1}H NMR spectra. These fractions were subsequently subjected to a series of chromatographic separations to result in the purification of metabolites **1**−**6**.



Metabolite **1**, colorless needles, was isolated as the most polar metabolite. The HRESIMS of **1** exhibited a pseudomolecular ion peak at *m*/*z* 355.1883 $[M + Na]^+$, consistent with the molecular formula $C_{20}H_{28}O_4$, requiring seven degrees of unsaturation. The IR spectrum of **1** showed a broad absorption band at 3266 cm^{-1} (OH stretching) and a strong absorption band at 1686 cm^{-1}, implying the presence of an α,β-unsaturated carboxylic acid carbonyl group, as well as a UV absorption (MeOH) λ_{max} (log ε) at 229 (3.89) nm.[18] This moiety was further identified by the ^{1}H NMR signals (Table 1) at δ_H 6.85 (1H, br t, J = 7.3 Hz) and ^{13}C NMR signals (Table 2) at δ_C 172.5 (C, C-20), 131.3 (C, C-12), and 144.0 (CH, C-11). In addition, a trisubstituted epoxide was recognized as being present in **1** from its ^{1}H NMR signals at δ_H 2.59 (1H, br d, J = 8.1 Hz) and ^{13}C NMR signals at δ_C 61.4 (C, C-8) and 62.9 (CH, C-7). Moreover, the ^{13}C NMR signals at δ_C 132.1 (C, C-1), 129.3 (C, C-15), 127.3 (CH, C-3), and 139.6 (C, C-4) assigned a trisubstituted double bond and a tetrasubstituted double bond in **1**, respectively.

* To whom correspondence should be addressed. Tel: 886-7-5252000, ext. 5036. Fax: 886-7-5255020. E-mail: yihduh@nsysu.edu.tw or kwang@cc.kmu.edu.tw.
† Department of Marine Biotechnology and Resources, National Sun Yat-sen University.
‡ Asia-Pacific Ocean Research Center.
§ Kaohsiung Medical University.
⊥ National Taiwan University.
∥ Department of Chemistry, National Sun Yat-sen University.

10.1021/np900693r
Published on Web 01/25/2010

Table 1. ^{1}H NMR Spectroscopic Data of Metabolites **1**−**3**[a]

position	**1**, δ_H (*J* in Hz)	**2**, δ_H (*J* in Hz)	**3**, δ_H (*J* in Hz)
2	5.53, m	5.53, m	5.57, d (9.4)
3	5.28, d (9.9)	5.28, d (10.1)	5.08, d (9.4)
5	a: 2.38, m; b:1.73, m	a: 2.37, m; b: 1.73, m	a: 2.40, m; b: 1.73, m
6	a: 1.89, dt (14.5, 4.2); b: 1.70, m	a: 1.87, dt (14.5, 4.2); b: 1.70, m	a: 1.90, m; b: 1.74, m
7	2.59, br d (8.1)	2.59, br d (8.2)	2.56, br d (9.8)
9	a: 2.21, m; b: 0.92, br d (12.7)	a: 2.19, m; b: 0.89, br d (12.7)	a: 2.22, m; b: 0.92, dd (8.7, 5.8)
10	a: 2.18, m; b: 1.74, m	a: 2.24, m; b: 1.75, m	a: 2.24, m; b: 1.76, m
11	6.85, br t (7.3)	6.73, br t (7.3)	6.80, dd (10.3, 4.5)
13	a: 2.29, m; b: 1.85, m	a: 2.27, m; b: 1.85, m	a: 2.17, m; b: 1.79, m
14	a: 2.34, m; b: 1.74, m	a: 2.35, m; b: 1.73, m	a: 2.40, m; b: 1.70, m
16	a: 4.56, dd (11.7, 4.3) b: 4.47, br d (11.7)	a: 4.57, dd (11.7, 4.3) b: 4.47, br d (11.7)	
17	1.69, s	1.70, s	1.90, s
18	1.79, s	1.79, s	1.87, s
19	1.28, s	1.28, s	1.30, s
20-OMe		3.76, s	3.78, s

[a] Spectra were measured in $CDCl_3$ (300 MHz).

Table 2. ^{13}C NMR Spectroscopic Data of Metabolites **1**−**6**

position	**1**[a], δ_C, mult.	**2**[a], δ_C, mult.	**3**[a], δ_C, mult.	**4**[b], δ_C, mult.	**5**[c], δ_C, mult.	**6**[c], δ_C, mult.
1	132.1, C	132.3, C	160.7, C	144.8, C	151.5, C	150.5, C
2	83.2, CH	83.3, CH	78.2, CH	122.2, CH	118.1, CH	120.1, CH
3	127.3, CH	127.4, CH	121.0, CH	124.3, CH	123.7, CH	122.5, CH
4	139.6, C	139.5, C	144.6, C	136.7, C	132.9, C	137.8, C
5	37.8, CH_2	37.9, CH_2	37.6, CH_2	38.3, CH_2	39.5, CH_2	40.4, CH_2
6	22.8, CH_2	22.8, CH_2	22.8, CH_2	26.0, CH_2	31.1, CH_2	26.7, CH_2
7	62.9, CH	62.9, CH	62.3, CH	62.5, CH	79.2, CH	88.2, CH
8	61.4, C	61.4, C	61.3, C	61.2, C	75.3, C	69.8, C
9	38.3, CH_2	38.5, CH_2	38.1, CH_2	40.4, CH_2	43.9, CH_2	41.0, CH_2
10	26.0, CH_2	25.8, CH_2	25.8, CH_2	27.4, CH_2	29.9, CH_2	24.2, CH_2
11	144.0, CH	141.6, CH	142.4, CH	141.3, CH	79.4, CH	80.4, CH
12	131.3, C	131.7, C	130.8, C	134.7, C	80.5, C	73.5, C
13	25.1, CH_2	25.4, CH_2	26.9, CH_2	29.0, CH_2	38.2, CH_2	41.6, CH_2
14	25.4, CH_2	25.5, CH_2	25.2, CH_2	28.1, CH_2	24.6, CH_2	24.4, CH_2
15	129.3, C	129.3, C	124.2, C	78.4, C	37.3, CH	35.6, CH
16	78.0, CH_2	78.1, CH_2	174.4, C	24.5, CH_3	21.9, CH_3	22.4, CH_3
17	9.9, CH_3	9.9, CH_3	8.6, CH_3	27.9, CH_3	22.2, CH_3	23.0, CH_3
18	14.7, CH_3	14.7, CH_3	15.3, CH_3	16.1, CH_3	18.3, CH_3	17.6, CH_3
19	16.6, CH_3	16.6, CH_3	16.7, CH_3	16.0, CH_3	21.4, CH_3	20.4, CH_3
20	172.5, C	167.9, C	167.4, C	168.0, C	18.6, CH_3	24.3, CH_3
15-OMe				50.5, CH_3		
20-OMe		51.8, CH_3	52.0, CH_3	51.7, CH_3		

[a] Spectra were measured in $CDCl_3$ (75 MHz). [b] Spectra were measured in C_6D_6 (75 MHz). [c] Spectra were measured in C_6D_6 (125 MHz).

The above functionalities account for five of the seven degrees of unsaturation, suggesting a bicyclic structure in **1**.

By interpretation of ^{1}H−^{1}H COSY correlations, it was possible to establish three partial structures of consecutive proton systems extending from H-2 to H-3, from H_2-5 to H-7 through H_2-6, from H_2-9 to H-11 through H_2-10, and from H_2-13 to H_2-14, as well as long-range COSY correlations between H-2/H_2-14, H-2/H_2-16, Me-18/H-3, and Me-17/H_2-16. The long-range COSY correlations between H-2/H_2-16 and Me-17/H_2-16 also exhibited the presence of a 2,5-dihydrofuran ring in **1**. Subsequently, the connectivities of these partial structures were further established by the HMBC correlations (Figure 1). The long-range ^{1}H−^{13}C correlations observed from H_3-19 to C-7, C-8, and C-9 indicated the position of the epoxide at C-7 and C-8. Moreover, the HMBC correlations from H-11 to C-9, C-10, C-12, C-13, and C-20 and from H-13 to C-20 led to the assignment of the carboxylic acid group at C-12. The locations of the two double bonds at C-3/C-4 and C-1/C-15 were clarified by analysis of the HMBC correlations from Me-18 to C-3, C-4, and C-5 and from Me-17 to C-1, C-15, and C-16. Hence, the planar structure of **1** possesses a 2,5-dihydrofuran ring fused to a 14-membered ring at C-1 and C-2, an epoxide group at C-7 and C-8, and a carboxylic acid group at C-12.

The relative configuration of **1** assigned by a NOESY spectrum was compatible with that of **1** suggested by computer modeling, in which the close contacts of atoms calculated in space were

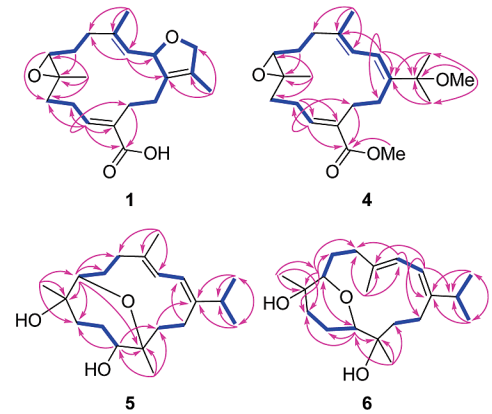


Figure 1. ^{1}H−^{1}H COSY (—) and key HMBC (→) correlations of **1** and **4**−**6**.

consistent with the NOESY correlations (Figure 2). The absence of a NOESY cross-peak between the vinylic H-11 and H_2-13 suggested the *E* geometry for the C-11/C-12 double bond, which was also identified by the chemical shift of H-11 at δ_H 6.85.[19] The geometry of the trisubstituted olefin at C-3/C-4 was assigned as *E* on the basis of the higher field chemical shift of the olefinic methyl signal for C-18 (δ_C 14.7). Furthermore, the crucial NOE correlations between H-2/Me-18, H-2/H-13a (δ_H 2.29), Me-18/H-6b (δ_H 1.70),

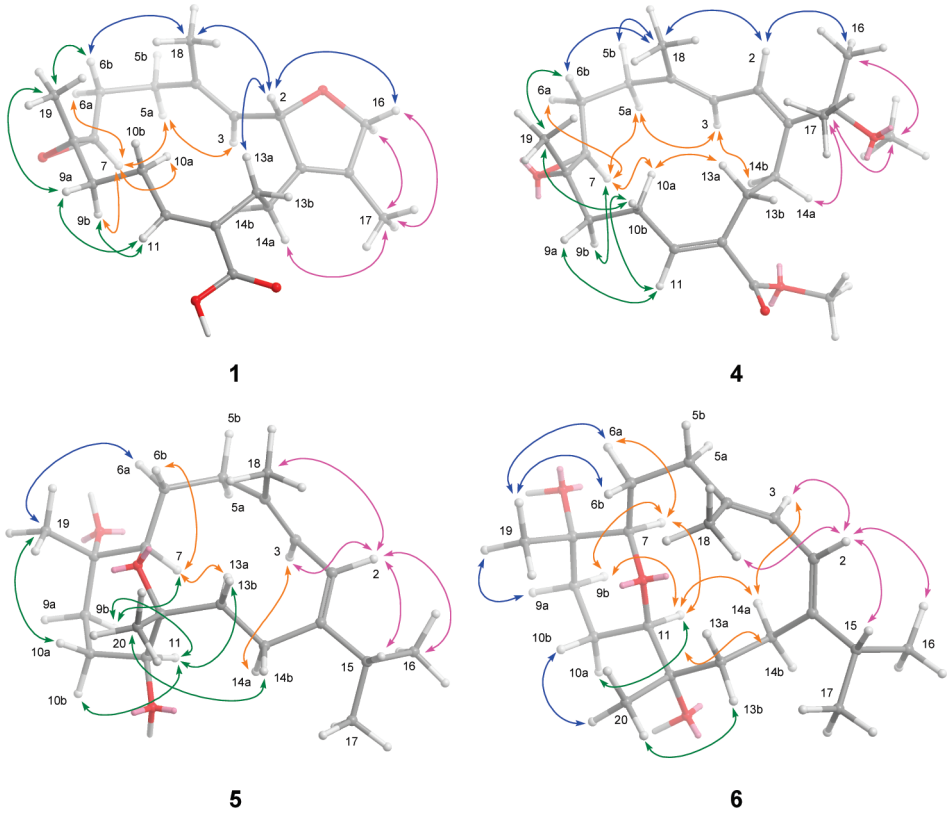


Figure 2. Key NOE correlations and computer-generated perspective model using MM2 force field calculations for **1** and **4**−**6**.

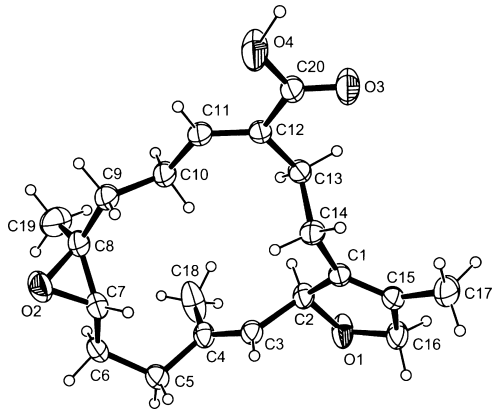


Figure 3. X-ray ORTEP diagram of **1**.

Me-19/H-6b, Me-19/H-9a (δ_H 2.21), H-7/H-6a (δ_H 1.89), H-7/H-9b, H-7/H-5a (δ_H 2.38), and H-3/H-5a demonstrated the 2*S**, 7*S**, and 8*S** configurations as depicted in Figure 2. Definitive support for the proposed structure of **1** was further provided by X-ray crystallographic analysis (Figure 3),[20] and consequently the relative structure of **1** was unambiguously established. A careful analysis of the NMR spectroscopic data coupled with COSY, HSQC, HMBC, and NOESY correlations proved that **1** is actually the 12-carboxy derivative of (+)-11*Z*-sarcophytoxide.[21] All of the NMR spectroscopic data of **1** were satisfactorily consistent with the structure shown as (+)-12-carboxy-11*Z*-sarcophytoxide. The absolute configuration for **1** is proposed as 2*S*, 7*S*, and 8*S* on the basis of the co-isolation of (+)-sarcophine ($[\alpha]^{24}_D$ +93) (lit. $[\alpha]^{25}_D$ + 92),[22a] whose absolute configuration has been unambiguously determined as 2*S*, 7*S*, and 8*S*.[22a] A shared biogenesis for these related cembranoid metabolites suggests the configurations at key asymmetric centers are the same.

The HRESIMS and NMR data (Tables 1 and 2) gave the molecular formula of **2** as $C_{21}H_{30}O_4$, which was 14 amu greater than that of **1**. A comparison of the NMR and IR data revealed that **2** differed from **1** solely due to the substitution at C-20, where a methyl ester of **2** replaced the carboxylic acid of the latter. This assignment was supported by the presence of a methoxy group [δ_H 3.76 (3H, s) and δ_C 51.8 (C)] and the HMBC correlation of its protons with the carbonyl carbon C-20. In order to further prove the structure of **2**, metabolite **1** was dissolved in CH_2Cl_2 in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 4-(dimethylamino)pyridine, and 4-(dimethylamino)pyridine hydrochloride, followed by anhydrous MeOH, to produce the corresponding methyl ester.[23] The ^{1}H and ^{13}C NMR spectroscopic data of the product were in good agreement with those of **2**. The structure of **2** indicated that it should be an isomer of lobophynin C.[17] However, by carefully comparing physical and spectroscopic data of metabolite **2** with those of lobophynin C, it is apparent that **2** and lobophynin C are the same compounds. The locations of the epoxide group at C-11/C-12 and the methyl ester group at C-8 of lobophynin C, therefore, should be changed to the C-7/C-8 and C-12 positions, respectively. In addition, the specific rotation of **2** ($[\alpha]^{24}_D$ +110) was consistent with that of the methyl ester ($[\alpha]^{24}_D$ +115) prepared from **1**, suggesting that the relative configurations at C-2, C-7, and C-8 of **2** should be the same as those of **1**. On the basis of the above findings, the structure of **2** was determined unambiguously. In order to rule out the possibility of **2** as an isolation artifact, an EtOAc−MeOH solution of **1** was kept at room temperature with silica gel for three days. However, the formation of **2** was not observed.

Metabolite **3** gave a formula of $C_{21}H_{28}O_5$, from the interpretation of its HRESIMS and ^{13}C NMR spectroscopic data (Table 2). The NMR features (Tables 1 and 2) of **3** were analogous to those of sarcophine[22] with the exception that the resonances for the methyl at C-20 were replaced by those of a methoxycarbonyl group. The complete analysis of the correlations observed in the COSY, HMBC, and HSC spectra permitted us to assign all of the spectroscopic signals and to propose the planar structure for **3**. On the basis of biogenic considerations and the similarity of key

Table 3. ^{1}H NMR Spectroscopic Data of Metabolites **4**−**6**

position	**4**[a], δ_H (*J* in Hz)	**5**[b], δ_H (*J* in Hz)	**6**[b], δ_H (*J* in Hz)
2	6.32, d (10.6)	6.09, d (8.5)	5.92, br d (4.5)
3	6.10, d (10.6)	6.39, d (8.5)	6.02, br d (4.5)
5	a: 2.03, m; b: 1.97, m	a: 2.37, m; b: 2.05, m	a: 2.16, m; b: 2.10, td (13.0, 3.0)
6	a: 1.97, m	a: 2.17, ddd (13.0, 6.5, 3.0)	a: 1.80, dt (13.0, 3.0)
	b: 1.14, m	b: 1.80, br d (13.0)	b: 1.40, m
7	2.67, d (9.7)	3.09, dd (3.5, 3.0)	2.93, d (8.5)
9	a: 1.93, m	a: 1.48, ddd (12.0, 5.5, 2.0)	a: 1.64, m
	b: 0.84, m	b: 1.18, br d (12.0)	b: 1.36, m
10	a: 2.16, m; b: 1.77, m	a: 1.72, br d (10.0); b: 1.03, m	a: 1.60, m; b: 1.38, m
11	6.94, dd (10.2, 6.6)	3.03, d (10.0)	3.26, dd (10.5, 2.0)
13	a: 2.52, dd (13.8, 4.9)	a: 2.00, dt (12.0, 3.5)	a: 1.82, m
	b: 1.14, m	b: 1.18, br d (12.0)	b: 1.48, m
14	a: 2.61, m; b: 2.08, m	a: 2.69, br t (12.0); b: 1.83, m	a: 2.30, m; b: 1.84, m
15		2.33, m	2.31, m
16	1.37, s	1.06, d (7.0)	1.11, d (7.0)
17	1.51, s	1.05, d (7.0)	1.09, d (7.0)
18	1.54, s	1.76, s	1.66, s
19	1.04, s	1.14, s	1.03, s
20		1.11, s	1.05, s
15-OMe	3.02, s		
20-OMe	3.42, s		

[a] Spectra were measured in C_6D_6 (300 MHz). [b] Spectra were measured in C_6D_6 (500 MHz).

NOESY correlations, the relative configurations at C-2, C-7, and C-8 of **3** were assumed to be identical with those of **1**. All the NMR spectroscopic data (Tables 1 and 2) of **3**, assigned by COSY, HSC, HMBC, and NOESY correlations, were satisfactorily consistent with the structure for metabolite **3** and designated as (+)-12-methoxycarbonyl-11*Z*-sarcophine.

Ehrenberoxide A (**4**) was assigned a molecular formula of $C_{22}H_{34}O_4$, according to its HRESIMS and NMR spectroscopic data (Tables 2 and 3). The IR absorptions of **4** at 1717 cm^{-1} revealed the presence of an α,β-unsaturated methyl ester functionality, which was confirmed by its NMR spectroscopic data [δ_H 6.94 (1H, dd, *J* = 10.2, 6.7 Hz) and 3.42 (3H, s); δ_C 168.0 (C, C-20), 134.7 (C, C-12), 141.3 (CH, C-11), and 51.7 (CH_3, COOMe)]. The NMR spectroscopic data also indicated that **4** possesses a trisubstituted epoxide [δ_H 2.67 (1H, br d, *J* = 9.7 Hz); δ_C 61.2 (C, C-8) and 62.5 (CH, C-7)] and two trisubstituted olefins [δ_H 6.32 (1H, d, *J* = 10.6 Hz) and 6.10 (1H, d, *J* = 10.6 Hz); δ_C 144.8 (C, C-1), 122.2 (CH, C-2), 124.3 (CH, C-3), and 136.7 (C, C-4)]. The above functionalities account for five of the six degrees of unsaturation, suggesting that **4** must consist of a 14-membered ring diterpenoid skeleton.

The structure of **4** was established through COSY and HMBC experiments. Crucial HMBC correlations from Me-18 to C-3, C-4, and C-5, from Me-19 to C-7, C-8, and C-9, from H-11 to C-10, C-11, C-13, and C-20, and from Me-16/Me-17 to C-15 and C-1 confirmed the connectivity among these partial structures (Figure 1). The position of the methyl carboxylate at C-12 was established by the HMBC correlations from H-11 and H-13 to C-20. The HMBC correlations from 15-OMe to C-15 and from Me-16/Me-17 to C-15 and C-1 also located the methoxy group at C-15. A COSY experiment established a correlation between the two vinylic protons at δ_H 6.32 (H-2) and 6.10 (H-3). These results allowed the assignment of the planar structure of **4** as shown.

The configurations of all double bonds were determined from a NOESY experiment on **4**. The crucial NOE correlations (Figure 2) between H-2 (δ_H 6.32)/Me-16 (δ_H 1.37), H-2/Me-18 (δ_H 1.54), and H-3 (δ_H 5.90)/H-5a (δ_H 1.97) indicated that the geometries of the conjugated diene at C-1/C-2 and C-3/C-4 were both *E*. The absence of a NOE correlation between H-2 and H-3 and the large coupling constant ($J_{2,3}$ = 10.6 Hz)[18,19,23] further suggested the *s-trans* geometry of the conjugated double bonds. The presence of a NOESY cross-peak between the vinylic H-11 and H-9a (δ_H 2.16) made it possible to identify the configuration of the olefin at C-11/C-12 as the *E* geometry, which was also confirmed by the chemical shift of H-11 at δ_H 6.94.[19] Moreover, the crucial NOESY correlations between Me-19/H-6b (δ_H 1.14), Me-19/H-9a (δ_H 1.93), H-7/H-6a (δ_H 1.97), H-7/H-9b (δ_H 0.84), and H-7/H-5a (δ_H 2.03) demonstrated the configurations of C-7 and C-8 as 7*S** and 8*S**, respectively. On the basis of the aforementioned observations and other detailed NOESY correlations (Figure 4), the structure of ehrenberoxide A (**4**) was established unambiguously.

Ehrenberoxide B (**5**) analyzed for $C_{20}H_{34}O_3$ from HRESIMS and ^{13}C NMR spectroscopic data (Table 2), corresponding to four degrees of unsaturation. The IR spectrum of **5** at 3266 cm^{-1} demonstrated a broad absorption band diagnostic of hydroxy groups. The presence of one oxygenated methine [δ_H 3.09 (dd, 1H, *J* = 3.5, 3.0 Hz) and δ_C 79.2 (C-7)] and an oxygenated quaternary carbon [δ_C 80.5 (C-12)] implied that an oxygen bridge is probably present between C-7 and C-12, which was supported by the HMBC correlations from H-7 to C-12. The NMR spectroscopic data (Tables 2 and 3) indicated that **5** possesses a conjugated diene [δ_H 6.09 (1H, d, *J* = 8.5 Hz) and 6.39 (1H, d, *J* = 8.5 Hz); δ_C 151.5 (C, C-1), 118.1 (CH, C-2), 123.7 (CH, C-3), and 132.9 (C, C-4)]. The above functionalities account for three of the four degrees of unsaturation, suggesting that metabolite **5** must consist of a 14-membered ring diterpenoid incorporating an unprecedented oxepane ring, two hydroxy groups, and a conjugated diene.

Correlations deduced from extensive analyses of the ^{1}H−^{1}H COSY correlations of **5** in C_6D_6 enabled initially the establishment of five partial structures. The structural fragments were subsequently interconnected by the HMBC correlations (Figure 1). Two oxygen-bearing carbons at δ_C 75.3 (C) and 79.4 (CH) were ascribable to C-8 and C-11 on the basis of the HMBC correlations from Me-19 to C-7, C-8, and C-9 and from Me-20 to C-11, C-12, and C-13. The attachment of isopropyl to C-1 was established on the grounds of HMBC correlations from Me-16/Me-17 to C-15 and C-1. The positions of the conjugated double bonds at C-1/C-2 and C-3/C-4 were confirmed by the HMBC cross-peaks from Me-18 to C-3, C-4, and C-5, as well as a COSY correlation between H-2 and H-3. The planar structure of ehrenberoxide B (**5**) was thus elucidated.

The relative configuration and the detailed ^{1}H NMR spectroscopic data assignments of **5** were determined mainly by the assistance of the NOESY experiment (Figure 2). The crucial NOE correlations between H-2/H-3, H-2/Me-16, H-2/Me-18, H-2/H-15, H-3/H-6a (δ_H 1.80), and H-3/H-14b (δ_H 2.69) indicated that the geometries of the two olefins at C-1/C-2 and C-3/C-4 were assigned as both *E*. The coupling constant between H-2 and H-3 ($J_{2,3}$ = 8.5 Hz)[18,19,23] further suggested the *s-trans* geometry of the conjugated double bonds. Furthermore, the crucial NOE correlations between H-7/H-9b (δ_H 1.18), H-11/H-9b, H-11/H-10b (δ_H 1.03), H-11/H-13b (δ_H

1.18), Me-19/H-6a (δ_H 2.17), Me-19/H-10a (δ_H 1.72), Me-20/H-13b, Me-20/H-14b (δ_H 1.83), and H-3/H-14a (δ_H 2.69) demonstrated the 7*R**, 8*S**, 11*S**, and 12*R** configurations as depicted in Figure 2. Accordingly, the structure of **5** was determined as (7*R**,8*S**,11*S**,-12*R**,1*Z*,3*E*)-8,11-dihydroxy-7,12-epoxycembra-1(2),3-diene.

The positive HRESIMS spectrum of ehrenberoxide C (**6**) exhibited a pseudomolecular ion peak at *m/z* 345.2402 $[M + Na]^+$, consistent with the molecular formula of $C_{20}H_{34}O_3$, implying four degrees of unsaturation. The presence of two oxygenated methines [δ_H 2.93 (d, 1H, $J = 8.5$ Hz) and δ_C 88.2 (C-7); δ_H 3.26 (dd, 1H, $J = 10.5$, 2.0 Hz) and δ_C 80.4 (C-11)] implied that an ether linkage is probably present between C-7 and C-11, which was confirmed by the HMBC correlations from H-7 to C-11 and from H-11 to C-7. The NMR spectroscopic data (Tables 2 and 3) indicated that **6** possesses a conjugated diene [δ_H 5.92 (1H, d, $J = 4.5$ Hz) and 6.02 (1H, d, $J = 4.5$ Hz); δ_C 150.5 (C, C-1), 120.1 (CH, C-2), 122.5 (CH, C-3), and 137.8 (C, C-4)]. The above functionalities account for three of the four degrees of unsaturation, implying that **6** is a cembranoid characterized by the presence of an ether linkage between C-7 and C-11.

The final assembly of **6** was determined by the information from COSY and HMBC experiments. The $^1H-^{13}C$ long-range correlations as determined from the HMBC spectrum allowed the connectivity of the structural fragments around each methyl group to be deduced (Figure 1). The crucial NOESY correlations (Figure 2) proved that the geometries of the conjugated diene at C-1/C-2 and C-3/C-4 were both *E*. The coupling constant ($J_{2,3} = 4.5$ Hz)[18,19] further suggested the *s-cis* geometry of the above functionality. The key NOESY correlations between H-3/H-14a (δ_H 2.30), H-14a/H-11, H-11/H-7, H-11/H-9b (δ_H 1.36), H-11/H-10a (δ_H 1.60), H-7/H-9b, H-7/H-3, H-7/H-6a (δ_H 1.80), Me-19/H-9a (δ_H 1.64), Me-19/H-6b (δ_H 1.40), Me-20/H-10b (δ_H 1.38), and Me-20/H-13b (δ_H 1.48) suggested that H-7 and H-11 are on the same face (β), whereas Me-19 and Me-20 are oriented toward the other face (α), as shown in a computer-generated 3D drawing. The above findings indicated the 7*R**, 8*S**, 11*R**, and 12*S** configurations as depicted in Figure 2. Therefore, the structure of **6** was elucidated as (7*R**,8*S**,11*R**,-12*S**,1*Z*,3*E*)-8,12-dihydroxy-7,11-epoxycembra-1(2),3-diene. Comparison of the ^1H NMR data (recorded in CD_3OD, see the Supporting Information) of **6** with those of the diastereomeric cembradiene[23] of undetermined relative configuration isolated from a soft coral of the genus *Eunicea* by Fenical's group showed that they differ in the configuration of the 1,3-diene due to the distinctive chemical shifts and coupling constants of H-2 [δ_H 5.67 (d, 1H, $J = 4.8$ Hz)] and H-3 [δ_H 5.87 (d, 1H, $J = 4.4$ Hz)]; [lit. δ_H 5.82 (d, 1H, $J = 8.6$ Hz, H-2) and 6.05 (d, 1H, $J = 8.6$ Hz, H-3)].[18,19] In addition, the distinct chemical shift differences of H-7 [δ_H 3.11 (d, 1H, $J = 8.0$ Hz)] and H-11 [δ_H 3.39 (dd, 1H, $J = 11.2$, 2.4 Hz)]; [lit. δ_H 3.45 (br d, 1H, $J = 9.3$ Hz, H-7) and 3.90 (dd, 1H, $J = 11.6$, 2.8 Hz, H-11)] demonstrated that these two cembradienes might possess different configurations at C-7, C-8, C-11, or C-12.

Metabolites **1**−**6** were not cytotoxic against P-388 (mouse lymphocytic leukemia), HT-29 (human colon adenocarcinoma) tumor cells, and human embryonic lung (HEL) cells with IC_{50} values greater than 50 μg/mL. However, metabolites **1**−**6** displayed antiviral activity against human cytomegalovirus, with IC_{50s} of 180.7, 5.8, 27.2, 24.8, 4.7, and 16.1 μM, respectively. In addition, the results for inhibition of antibacterial activity assay against *Salmonella enteritidis* (ATCC13076) are all negative at a concentration of 250 μg/mL.

(+)-Sarcophytoxide was reported by Kashman's,[22b] Faulkner's,[22c] and Kobayashi's[22d] groups. Its enantiomer, (−)-sarcophytoxide, was published by Coll's[16] and Tursch's groups.[22e] Judging from the positive specific rotation and X-ray structure of **1**, it is possible that **1** arises from oxidation of the methyl group at C-12 of an undiscovered (+)-11*Z*-sarcophytoxide or from the oxidation of (+)-sarcophytoxide with an 11*E* to 11*Z* configuration change during the oxidation process. Noteworthy is the geometry of the α,β-unsaturated acid/ester in **1**−**4**, as the *cis* geometry of the functionality was provided by X-ray crystallographic analysis of **1**. In addition, it is possible that both **5** and **6** arise from the same precursor with a 7,8-epoxide. Transannular opening of the 7,8-epoxide by either end of an 11,12-diol would give rise to **5** and **6**. It is worthwhile to mention that **5**, with an unprecedented oxepane ring, has not previously been observed for cembranoids.

Experimental Section

General Experimental Procedures. The melting point was determined using a Fisher-Johns melting point apparatus. Optical rotations were determined with a JASCO P1020 digital polarimeter. Ultraviolet (UV) and infrared (IR) spectra were obtained on a JASCO V-650 and a JASCO FT/IR-4100 spectrophotometer, respectively. The NMR spectra were recorded on Bruker Avance 300 NMR/Varian 400 MR NMR/Varian Unity INOVA 500 FT-NMR spectrometers (300/400/500 MHz for ^1H and 75/100/125 MHz for ^{13}C, respectively). Chemical shifts are expressed in δ (ppm) referring to the solvent peaks δ_H 7.15 and δ_C 128.5 for C_6D_6 and δ_H 7.27 and δ_C 77.0 for $CDCl_3$, respectively, and coupling constants are expressed in Hz. ESIMS were recorded by ESI FT-MS on a Bruker APEX II mass spectrometer. The crystallographic data were collected on a Rigaku AFC7S diffractometer using graphite-monochromated Mo Kα radiation. Silica gel 60 (Merck, 230−400 mesh), Sephadex LH-20 (Pharmacia), and LiChroprep RP-18 (Merck, 40−63 μm) were used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) and precoated RP-18 F_{254s} plates (Merck, 1.05560) were used for TLC analyses. High-performance liquid chromatography (HPLC) was performed on a Hitachi L-7100 pump equipped with a Hitachi L-7400 UV detector at 220 nm and a semipreparative reversed-phase column (Merck, Hibar Purospher RP-18e, 5 μm, 250 × 10 mm).

Animal Material. The octocoral *Sarcophyton ehrenbergi* was collected by hand using scuba at the Dongsha Atoll off Taiwan, in October 2006, at a depth of 10 m, and was stored in a freezer for five weeks until extraction. This soft coral was identified by one of the authors (C.-F.D.). A voucher specimen (TS-01) was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

Extraction and Isolation. The frozen soft coral was chopped into small pieces and extracted with acetone in a percolator at room temperature. The combined acetone extracts were concentrated to a brown gum, which was partitioned between H_2O and EtOAc. The dried EtOAc-soluble material (30.0 g) was subjected to gravity chromatography on silica gel, using *n*-hexane with increasing amounts of EtOAc, followed by EtOAc with increasing amounts of MeOH as eluents, to obtain 40 fractions. Fraction 13 (1.2 g) eluted with *n*-hexane−EtOAc (8:1) was subjected to a silica gel column to give (+)-sarcophine (637 mg). Similarly, fraction 15 (0.2 g), eluted with *n*-hexane−EtOAc (4:1), was chromatographed over silica gel, eluted stepwise with *n*-hexane−EtOAc (1:0 to 0:1), to afford (+)-sarcophytoxide (78 mg). Fraction 20 (1.2 g), eluted with *n*-hexane−EtOAc (1:4), was chromatographed over silica gel, eluted stepwise with CH_2Cl_2−MeOH (1:0 to 0:1), to afford six subfractions. Subfraction 20-2 (349 mg) was subsequently fractionated over Sephadex LH-20 (MeOH) to afford **1** (254 mg). Fraction 19 (332 mg), eluted with *n*-hexane−EtOAc (1:2), was subjected to a RP-18 gravity column by eluting with 50% MeOH in H_2O, 70% MeOH in H_2O, 90% MeOH in H_2O, and 100% MeOH. Altogether, six fractions were obtained, from which fraction 1 (90 mg) was further purified by RP-18 HPLC using 60% MeOH in H_2O as eluent (flow rate 5.0 mL/min) to give **5** (1 mg) and **6** (1 mg). Fraction 18 (365 mg), eluted with *n*-hexane−EtOAc (1:1), was submitted to repeated chromatography over silica gel using *n*-hexane−EtOAc mixtures of increasing polarity as eluent, to give 10 subfractions. Subsequently, subfraction 18-6 (136 mg), eluted with *n*-hexane−EtOAc (1:2), was subjected to a RP-18 gravity column eluting with 65% MeOH in H_2O to yield a mixture (64 mg), which was further purified by RP-18 HPLC eluting with 70% MeOH in H_2O (flow rate 5.0 mL/min) to afford **4** (2 mg). Fraction 17 (531 mg), eluted with *n*-hexane−EtOAc (2:1), was subjected to a silica gel column (*n*-hexane−EtOAc, from 10:1 to 1:10) to obtain 10 subfractions. Subfraction 17-1 (202 mg), eluted with *n*-hexane−EtOAc (1:3), was subjected to column chromatography on a RP-18 gravity column eluting with 65% MeOH in H_2O to afford a mixture (138 mg), which was further purified by RP-18

HPLC eluting with 70% MeOH in H_2O (flow rate 5.0 mL/min) to give **2** (3 mg) and **3** (2 mg).

(+)-12-Carboxy-11*Z*-sarcophytoxide (1): colorless needles; mp 192−193 °C, $[\alpha]^{24}_D$ +100 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 229 (3.76) nm; IR (KBr) ν_{max} 3266, 3053, 2948, 1686, 1639, 1438, 1387, 1272, 1242, 1035, 930, 737 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 355 $[M + Na]^+$; HRESIMS *m/z* 355.1883 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_4Na$, 355.1885).

Lobophynin C (2):[17] colorless, viscous oil; $[\alpha]^{24}_D$ +110 (*c* 0.3, $CHCl_3$) (lit. $[\alpha]^{25}_D$ +109 (*c* 0.3, $CHCl_3$)); UV (MeOH) λ_{max} (log ε) 227 (3.64) nm; IR (KBr) ν_{max} 2952, 1714, 1634, 1436, 1385, 1239, 1036, 927, 734 cm^{-1}; ^1H NMR and ^{13}C NMR data, Tables 1 and 2 ESIMS *m/z* 369 $[M + Na]^+$; HRESIMS *m/z* 369.2403 $[M + Na]^+$ (calcd for $C_{21}H_{30}O_4Na$, 369.2402).

(+)-12-Methoxycarbonyl-11*Z*-sarcophine (3): colorless, viscous oil; $[\alpha]^{24}_D$ +120 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 226 (3.82) nm; IR (KBr) ν_{max} 2952, 1716, 1708, 1637, 1454, 1386, 1242, 1035, 930, 737 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 383 $[M + Na]^+$; HRESIMS *m/z* 383.1835 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_5Na$, 383.1834).

Ehrenberoxide A (4): colorless, viscous oil; $[\alpha]^{24}_D$ +88 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 252 (3.69), 226 (3.75) nm; IR (KBr) ν_{max} 2959, 1717, 1637, 1437, 1385, 1242, 1200, 1034, 930, 738 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 385 $[M + Na]^+$; HRESIMS *m/z* 385.2358 $[M + Na]^+$ (calcd for $C_{22}H_{34}O_4Na$, 385.2355).

Ehrenberoxide B (5): colorless, viscous oil; $[\alpha]^{24}_D$ +110 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 248 (3.75) nm; IR (KBr) ν_{max} 3266, 2948, 1634, 1438, 1386, 1240, 1176, 1132, 1034, 928, 736 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 345 $[M + Na]^+$; HRESIMS *m/z* 345.2403 $[M + Na]^+$ (calcd for $C_{20}H_{34}O_3Na$, 345.2406).

Ehrenberoxide C (6): colorless, viscous oil; $[\alpha]^{24}_D$ +10 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 246 (3.77) nm; IR (KBr) ν_{max} 3249, 2937, 1634, 1447, 1385, 1243, 1177, 1131, 1036, 930, 738 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 345 $[M + Na]^+$; HRESIMS *m/z* 345.2402 $[M + Na]^+$ (calcd for $C_{20}H_{34}O_3Na$, 345.2406).

(+)-Sarcophytoxide (7): $[\alpha]^{24}_D$ +42 (*c* 1.4, MeOH); lit. $[\alpha]^{25}_D$ +40 (*c* 2.2, MeOH).[22b]

(+)-Sarcophine (8): $[\alpha]^{24}_D$ +93 (*c* 0.6, $CHCl_3$); lit. $[\alpha]^{25}_D$ +92 (*c* 1.0, $CHCl_3$).[22a]

Crystallographic Data and X-ray Structure Analysis of 1.[20] A suitable colorless crystal (0.5 × 0.5 × 0.2 mm^3) of **1** was obtained by slow evaporation from the CH_2Cl_2−MeOH (1:1) solution. Crystal data: $C_{20}H_{28}O_4$, orthorhombic, M_r = 332.42 g/mol; a = 6.3119(13) Å, b = 8.0445(16) Å, c = 38.236(8) Å, V = 1941.5(7) $Å^3$, space group $P2_12_12_1$, Z = 4, D_{calc} = 1.137 g/cm^3, λ = 0.71073 Å, μ(Mo Kα) = 0.078 mm^{-1}, $F(000)$ = 720, T = 298(2) K. A total of 4018 reflections were collected in the range $2.13° < \theta < 25.98°$, of which 3269 unique reflections with $I > 2\sigma(I)$ were used for the analysis. The data were solved using the direct method, and the structure was refined by full-matrix least-squares procedure on F^2 values. All non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms. The final indices were R_1 = 0.0403, wR_2 = 0.1013 with goodness-of-fit = 1.021. Both current and inverted configurations of **1** were refined to give similar Flack parameter [0.6(1.7) and 0.4(1.7)], R values (R_1 = 0.0403 and 0.0403), and goodness-of-fit (1.021 and 1.020). Thus the absolute configuration of **1** remains undetermined.

Chemical Transformation of 1 to 2. Duplicate (3.0 mg, 0.009 mmol) samples of **1** in CH_2Cl_2 (1.0 mL) were successively treated with DMAP (1.3 mg, 0.011 mmol), 4-DMAP·HCl (1.7 mg, 0.011 mmol), and EDC·HCl (2.1 mg, 0.011 mmol). After the mixture was stirred at 0 °C for 3 h, anhydrous MeOH (0.2 mL) was added. The mixture was then warmed to room temperature and allowed to react overnight. The reaction was quenched by water, followed by extraction with EtOAc (3 × 1.5 mL). The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$ and evaporated to give a residue, which was subjected to a short silica gel column eluting with *n*-hexane−EtOAc (3:1) to yield the corresponding methyl ester (2.8 mg, 0.008 mmol), which was identified to be **2**.

Cytotoxicity Testing. Cytotoxicity was determined against P-388 (mouse lymphocytic leukemia), HT-29 (human colon adenocarcinoma) tumor cells, and HEL (human embryonic lung) cells using the MTT assay method. The experimental details of this assay were carried out according to a previously described procedure.[24]

Anti-cytomegalovirus Assay. To determine the effects of **1**−**6** upon human cytomegalovirus (HCMV) cytopathic effect (CPE), confluent human embryonic lung (HEL) cells grown in 24-well plates were incubated for 1 h in the presence or absence of various concentrations of tested compounds. Metabolites **1**−**6** were not cytotoxic against HEL cells, with ED_{50} values greater than 50 μg/mL. Then, HEL cells were infected with HCMV at an input of 1000 pfu (plaque forming units) per well of the 24-well dish. Antiviral activity is expressed as the IC_{50} value (50% inhibitory concentration), or the concentration required to reduce virus-induced CPE by 50% after 7 days as compared with the untreated control. To monitor the cell growth upon treating with natural products, an MTT-colorimetric assay was employed.[25]

In Vitro Antibacterial Assay. The antibiotic activity evaluation method was conducted based on previous reports.[26−28]

Acknowledgment. Financial support was provided by National Science Council (NSC96-2320-B-110-003-MY3) of Taiwan awarded to C.-Y.D.

Supporting Information Available: Description of X-ray crystal data for **1** and ^1H and ^{13}C NMR spectra for **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Blunt, J. W.; Copp, B. R.; Hu, W.-P.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2009**, *26*, 170–244, and literature cited in previous reviews.
(2) Coll, J. C. *Chem. Rev.* **1992**, *92*, 613–631.
(3) Gross, H.; Wright, A. D.; Beil, W.; König, G. M. *Org. Biomol. Chem.* **2004**, *2*, 1133–1138.
(4) Huang, H.-C.; Ahmed, A. F.; Su, J.-H.; Chao, C.-H.; Wu, Y.-C.; Chiang, M. Y.; Sheu, J.-H. *J. Nat. Prod.* **2006**, *69*, 1554–1559.
(5) Zhang, C.; Li, J.; Su, J.; Liang, Y.; Yang, X.; Zheng, K.; Zeng, L. *J. Nat. Prod.* **2006**, *69*, 1476–1480.
(6) Feller, M.; Rudi, A.; Berer, N.; Goldberg, I.; Stein, Z.; Benayahu, Y.; Schleyer, M.; Kashman, Y. *J. Nat. Prod.* **2004**, *67*, 1303–1308.
(7) El Sayed, K. A.; Hamann, M. T.; Waddling, C. A.; Jensen, C.; Lee, S. K.; Dunstan, C. A.; Pezzuto, J. M. *J. Org. Chem.* **1998**, *63*, 7449–7455.
(8) Yan, X.-H.; Gavagnin, M.; Cimino, G.; Guo, Y.-W. *Tetrahedron Lett.* **2007**, *48*, 5313–5316.
(9) Cheng, Y.-B.; Shen, Y.-C.; Kuo, Y.-H.; Khalil, A. T. *J. Nat. Prod.* **2008**, *71*, 1141–1145.
(10) Iwagawa, T.; Nakamura, S.; Masuda, T.; Okamura, H.; Nakatani, M.; Siro, M. *Tetrahedron* **1995**, *51*, 5291–5298.
(11) Sawant, S.; Youssef, D.; Mayer, A.; Sylvester, P.; Wali, V.; Arant, M.; El Sayed, K. A. *Chem. Pharm. Bull.* **2006**, *54*, 1119–1123.
(12) Badria, F. A.; Guirguis, A. N.; Perovic, S.; Steffen, R.; Müller, W. E. G.; Schröder, H. C. *Toxicology* **1998**, *131*, 133–143.
(13) Bishara, A.; Rudi, A.; Benayahu, Y.; Kashman, Y. *J. Nat. Prod.* **2007**, *70*, 1951–1954.
(14) Sawant, S.; Youssef, D.; Reiland, J.; Ferniz, M.; Marchtti, D.; El Sayed, K. A. *J. Nat. Prod.* **2006**, *69*, 1010–1013.
(15) Cuong, N. X.; Tuan, T. A.; Kiem, P. V.; Minh, C. V.; Choi, E. M.; Kim, Y. H. *Chem. Pharm. Bull.* **2008**, *56*, 988–992.
(16) (a) Bowden, B. F.; Coll, J. C.; Hicks, W.; Kazlauskas, R.; Mitchell, S. *J. Aust. J. Chem.* **1978**, *31*, 2707–2712. (b) König, G. M.; Wright, A. D. *J. Nat. Prod.* **1998**, *61*, 494–496. (c) Cheng, S.-Y.; Wen, Z.-H.; Chiou, S.-F.; Tsai, C.-W.; Wang, S.-K.; Hsu, C.-H.; Dai, C.-F.; Chiang, M. Y.; Wang, W.-H.; Duh, C.-Y. *J. Nat. Prod.* **2009**, *72*, 465–468.
(17) Yamada, K.; Ryu, K.; Miyamoto, T.; Higuchi, R. *J. Nat. Prod.* **1997**, *60*, 798–801.
(18) (a) Tanaka, C. M. A.; Sarragiotto, M. H.; Zukerman-Schpector, J.; Marsaioli, A. J. *Phytochemistry* **1997**, *44*, 1547–1549. (b) del Carmen Ramírez, M.; Toscano, R. A.; Arnason, J.; Omar, S.; Cerda-García-Rojas, C. M.; Mata, R. *Tetrahedron* **2000**, *56*, 5085–5091.
(19) Roengsumran, S.; Achayindee, S.; Petsom, A.; Pudhom, K.; Singtothong, P.; Surachetapan, C.; Vilaivan, T. *J. Nat. Prod.* **1998**, *61*, 652–654.
(20) Crystallographic data for **1** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 734051). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
(21) Kashman, Y.; Zadock, E.; Néeman, I. *Tetrahedron* **1974**, *30*, 3615–3620.

(22) (a) Bernstein, J.; Shmeuli, U.; Zadock, E.; Kashman, Y.; Néeman, I. *Tetrahedron* **1974**, *30*, 2817–2824. (b) Kashman, Y.; Zadock, E.; Neeman, I. *Tetrahedron* **1974**, *30*, 3615–3620. (c) Frincke, J. M.; McIntyre, D. E.; Faulkner, D. J. *Tetrahedron Lett.* **1980**, *21*, 735–738. (d) Kobayashi, J.; Ohizumi, Y.; Nakamura, H.; Yamakado, T.; Matsuzaki, T.; Hirata, Y. *Experientia* **1983**, *39*, 67–68. (e) Tursch, B. *Pure Appl. Chem.* **1976**, *48*, 1–6.

(23) Shin, J.; Fenical, W.; Stout, T. J.; Clardy, J. *Tetrahedron* **1993**, *49*, 515–524.

(24) Hou, R.-S.; Duh, C.-Y.; Chiang, M. Y.; Lin, C.-N. *J. Nat. Prod.* **1995**, *58*, 1126–1130.

(25) Stevens, M.; Balzarini, J.; Tabarrini, O.; Andrei, G.; Snoeck, R.; Cecchetti, V.; Fravolini, A.; De Clercq, E.; Pannecouque, C. *J. Antimicrob. Chemother.* **2005**, *56*, 847–855.

(26) Hou, L.; Shi, Y.; Zhai, P.; Le, G. *J. Ethnopharmacol.* **2007**, *111*, 227–231.

(27) Arias, M. E.; Gomez, J. D.; Cudmani, N. M.; Vattuone, M. A.; Isla, M. I. *Life Sci.* **2004**, *75*, 191–202.

(28) Cheng, S.-Y.; Wen, Z.-H.; Chiou, S.-F.; Wang, S.-K.; Hsu, C.-H.; Dai, C.-F.; Chiang, M. Y.; Duh, C.-Y. *Tetrahedron* **2008**, *64*, 9698–9704.

NP900693R