Xeniaphyllane-Derived Terpenoids from the Formosan Soft Coral *Sinularia gibberosa*

Shin-Pin CHEN,[a] Jui-Hsin SU,[a] Atallah Fouad AHMED,[a,b] Chang-Feng DAI,[c] Yang-Chang WU,[d] and Jyh-Horng SHEU*,[a,e]

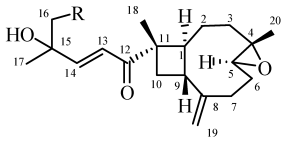
[a] *Department of Marine Biotechnology and Resources, National Sun Yat-sen University;* [e] *Asian Pacific Ocean Research Center, National Sun Yat-sen University; Kaohsiung 804, Taiwan, R.O.C.:* [b] *Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University; Mansoura 35516, Egypt:* [c] *Institute of Oceanography, National Taiwan University; Taipei 112, Taiwan, R.O.C.: and* [d] *Graduate Institute of Natural Products, Kaohsiung Medical University; Kaohsiung 807, Taiwan, R.O.C.* Received May 10, 2007; accepted July 12, 2007

New xeniaphyllane-derived metabolites (1—7) were isolated from the EtOAc extract of the Formosan soft coral *Sinularia gibberosa*. The structures and relative configurations of these compounds were elucidated on the basis of extensive spectroscopic analysis (including 2D NMR) and by comparison of their spectral data with those of related compounds. *In vitro* cytotoxic evaluation of the above metabolites towards a limited panel of cancer cell lines is also described.

Key words xeniaphyllane-derived; soft coral; *Sinularia gibberosa*

Soft corals belonging to genus *Sinularia* (Alcyoniidae) have been well recognized as a rich source of structurally unique and biologically active metabolites.[1)] During the course of our screening of bioactive metabolites from marine organisms,[2—10)] we have reported the isolation of β-caryophyllene-based sesquiterpenoids and diterpenoids (xeniaphyllanes) from the genus *Sinularia*.[9—11)] Some of these metabolites showed selective *in vitro* cytotoxicity.[9)] In addition to gibberosins A—H,[11)] a continuous chemical investigation on the chemical constituents of the soft coral *Sinularia gibberosa* TIXIER-DURIVAULT (Alcyoniidae) has again led to the isolation of seven new xeniaphyllanes, gibberosins G—M (**1**—**7**). We will describe herein the isolation, structure elucidation, and biological activity of these compounds.

The minced bodies of *S. gibberosa* were extracted exhaustively with EtOAc. The combined extract was concentrated under reduced pressure, and the residue was fractionated by open column chromatography on Si gel. The terpenoid-containing fractions (recognized by ^{1}H-NMR measurement in $CDCl_3$) were selected for further purification, using a column of the Sephadex LH-20 and the normal phase HPLC to afford terpenoids **1**—**7**. All isolated compounds were obtained as colorless oil.



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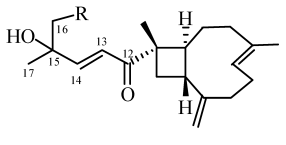

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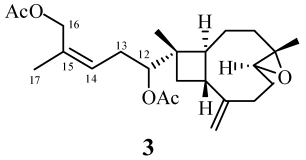

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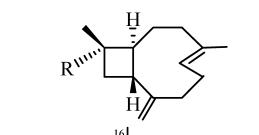

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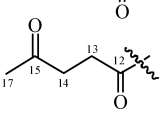

7 R=

Gibberosin G (**1**) was found to possess a molecular formula $C_{20}H_{30}O_4$ from the quasimolecular ion peak appearing at *m/z* 357.2040 $[M+Na]^+$ in the HR-ESI-MS, corresponding to six degrees of unsaturation. The IR spectrum of **1** showed the presence of hydroxy (3421 cm^{-1}) and keto-carbonyl (1684 cm^{-1}) functionalities. The ^{13}C-NMR spectrum of **1** showed signals of twenty carbons (Table 1) among which eleven have very similar chemical shifts to those of the bicyclic structure (C-1 to C-11) of the known β-caryophyllene type metabolites,[11)] suggesting that **1** could be a β-caryophyllene-related diterpenoid. This could be further confirmed by the very similar ^{1}H-NMR data (Table 2) of **1** with those of these known metabolites. The gross structure of **1** was successively established by the assistance of ^{1}H–^{1}H COSY and HMBC correlations as shown in Fig. 1. Therefore, the C-12 position of the keto-carbonyl in the molecule was interpreted by the HMBC correlations observed from H-13 (δ 6.52, d, J=15.5 Hz), and H_3-18 (δ 1.32, s), to C-12 (δ 203.2, C). The hydroxy groups were also determined to be located at C-15 and C-16 due to the HMBC correlations observed from H-14 (δ 6.87, d, J=15.5 Hz), H_3-17 (δ 1.32, s), and H_2-16 (δ 3.55, dd, J=11.0, 11.0 Hz), to C-15 (δ 73.7, C). The NOE correlations (Fig. 2) observed by H_3-20 with H-3β (δ 2.09, m), H-3β with H-2β (δ 1.57, m), H-2β with H_3-18 (δ 1.32, s), H_3-18 with H-9 (δ 2.72, q, J=10.0 Hz), and that displayed by H-5 (δ 2.89, dd, J=10.0, 3.5 Hz) with H-1, but not with H-9, indicated the 1*S**, 4*S**, 5*S**, 9*R**, and 11*S** configurations in **1**. The double bond between C-13 and C-14 was determined to have a *trans* geometry based on the coupling constant (J=15.5 Hz) between H-13 (δ 6.52, d) and H-14 (δ 6.87, d). Further NOE analysis revealed that **1** possessed the same configurations at C-1, C-4, C-5, C-9, and C-11 as those of the known metabolite (Fig. 1).[11)] Based on the above results, the structure of **1** was established as (1*S**,4*S**,5*S**,9*R**,11*S**,13*E*)-15,16-dihydroxy-4,5-epoxy-xeniaphylla-8(19),13-dien-12-one.

Gibberosin H (**2**) had a molecular formula $C_{22}H_{32}O_5$ as established from its HR-ESI-MS (*m/z* 399.2148, $[M+Na]^+$).

* To whom correspondence should be addressed. e-mail: sheu@mail.nsysu.edu.tw

Table 1. ^{13}C-NMR Spectral Data of Compounds **1**—**7**

C #	**1**[a)]	**2**[b)]	**3**[a)]	**4**[b)]	**5**[a)]	**6**[b)]	**7**[a)]
1	45.2 (CH)[c)]	45.3 (CH)	44.9 (CH)	48.5 (CH)	48.4 (CH)	48.6 (CH)	48.6 (CH)
2	28.1 (CH_2)	28.2 (CH_2)	27.6 (CH_2)	30.2 (CH_2)	30.1 (CH_2)	30.0 (CH_2)	29.9 (CH_2)
3	38.5 (CH_2)	38.6 (CH_2)	38.7 (CH_2)	39.5 (CH_2)	39.5 (CH_2)	39.5 (CH_2)	39.4 (CH_2)
4	59.6 (C)	59.6 (C)	59.6 (C)	135.2 (C)	135.2 (C)	135.1 (C)	135.0 (C)
5	63.6 (CH)	63.6 (CH)	63.9 (CH)	124.7 (CH)	124.6 (CH)	124.8 (CH)	124.7 (CH)
6	30.0 (CH_2)	30.0 (CH_2)	30.2 (CH_2)	28.1 (CH_2)	28.1 (CH_2)	28.3 (CH_2)	28.2 (CH_2)
7	29.6 (CH_2)	29.7 (CH_2)	29.4 (CH_2)	34.7 (CH_2)	34.6 (CH_2)	34.6 (CH_2)	34.5 (CH_2)
8	150.6 (C)	150.7 (C)	151.3 (C)	153.6 (C)	153.6 (C)	153.5 (C)	153.4 (C)
9	47.3 (CH)	47.3 (CH)	48.0 (CH)	47.2 (CH)	47.1 (CH)	47.2 (CH)	47.1 (CH)
10	35.1 (CH_2)	35.2 (CH_2)	34.8 (CH_2)	35.6 (CH_2)	35.5 (CH_2)	35.6 (CH_2)	35.4 (CH_2)
11	47.7 (C)	47.8 (C)	40.2 (C)	47.1 (C)	47.0 (C)	47.8 (C)	47.4 (C)
12	203.2 (C)	203.1 (C)	78.7 (CH)	203.9 (C)	203.9 (C)	213.6 (C)	213.2 (C)
13	123.3 (CH)	123.1 (CH)	27.8 (CH_2)	123.7 (CH)	120.4 (CH)	36.6 (CH)	30.6 (CH_2)
14	149.9 (CH)	148.8 (CH)	125.9 (CH)	149.6 (CH)	153.1 (CH)	116.5 (CH)	36.8 (CH_2)
15	73.7 (C)	72.6 (C)	132.4 (C)	73.8 (C)	71.2 (C)	134.9 (C)	207.4 (C)
16	69.2 (CH_2)	70.5 (CH_2)	62.9 (CH_2)	69.4 (CH_2)	29.5 (CH_3)	18.2 (CH_3)	
17	23.9 (CH_3)	24.7 (CH_3)	21.5 (CH_3)	23.9 (CH_3)	29.5 (CH_3)	25.8 (CH_3)	30.0 (CH_3)
18	17.0 (CH_3)	17.1 (CH_3)	15.7 (CH_3)	17.9 (CH_3)	17.9 (CH_3)	17.8 (CH_3)	17.8 (CH_3)
19	114.1 (CH_2)	114.1 (CH_2)	113.6 (CH_2)	112.9 (CH_2)	112.8 (CH_2)	113.0 (CH_2)	112.9 (CH_2)
20	16.8 (CH_3)	16.9 (CH_3)	17.1 (CH_3)	16.4 (CH_3)	16.3 (CH_3)	16.4 (CH_3)	16.3 (CH_3)
OAc		171.1 (C)	170.6 (C)				
		20.9 (CH_3)	20.9 (CH_3)				
OAc			171.0 (C)				
			21.0 (CH_3)				

a) Spectra recorded at 125 MHz in $CDCl_3$. *b*) 75 MHz in $CDCl_3$ at 25 °C. *c*) Deduced by distortionless enhancement by polarization transfer (DEPT).

Table 2. ^{1}H-NMR Spectral Data of Compounds **1**—**7**

H #		**1**[a)]	**2**[b)]	**3**[a)]	**4**[b)]	**5**[a)]	**6**[b)]	**7**[a)]
1		2.47 dd (10.0, 10.0)[c)]	2.47 dd (9.6, 9.6)	1.96 dd (10.0, 10.0)	2.32 dd (9.6, 9.6)	2.32 dd (10.0, 10.0)	2.32 dd (9.6, 9.6)	2.32 dd (9.5, 9.5)
2	α	1.87 m	1.87 m	1.63 m	1.74 m	1.74 m	1.69 m	1.69 m
	β	1.57 m	1.57 m	1.45 m	1.63 m	1.62 m	1.57 m	1.59 m
3	α	1.10 m	1.09 m	0.92 ddd (13.0, 13.0, 5.0)	2.03 m	2.03 m	2.02 m	2.03 m
	β	2.09 m	2.10 m	2.08 m	2.13 m	2.12 m	2.08 m	2.10 m
5		2.89 dd (10.0, 3.5)	2.86 dd (10.5, 3.6)	2.82 dd (10.0, 3.5)	5.31 m	5.33 dd (9.5, 9.0)	5.31 m	5.35 dd (9.5, 9.5)
6	α	2.24 m	2.23 m	2.25 m	2.00 m	2.00 m	1.97 m	2.00 m
	β	1.34 m	1.34 m	1.33 m	2.35 m	2.35 m	2.35 m	2.35 m
7	α	2.28 m	2.28 m	2.31 m	2.18 m	2.17 m	2.16 m	2.17 m
	β	2.12 m	2.12 m	2.16 m	1.99 m	2.00 m	1.99 m	2.02 m
9		2.72 q (10.0)	2.70 q (9.6)	2.63 q (9.0)	2.40 q (9.0)	2.40 q (9.0)	2.37 q (9.0)	2.38 q (10.5)
10	α	2.16 m	2.16 m	1.59 m	2.22 m	2.22 m	2.26 m	2.27 t (10.5)
	β	1.86 m	1.87 m	1.86 t (10.0)	1.79 m	1.81 m	1.82 m	1.82 m
12				4.75 dd (9.5, 4.0)				
13		6.52 d (15.5)	6.50 d (15.3)	2.19 m	6.88 d (15.6)	6.97 d (15.0)	3.12 m	2.56 m
14		6.87 d (15.5)	6.87 d (15.3)	5.31 dd (7.5, 7.0)	6.56 d (15.6)	6.43 d (15.0)	5.31 m	2.71 m
16		3.55 dd (11.0, 11.0)	4.07 dd (9.0, 9.0)	4.49 d (12.0) 4.61 d (12.0)	3.56 dd (8.1, 8.1)	1.39 s	1.62 s	
17		1.32 s	1.34 s	1.73 s	1.32 s	1.39 s	1.75 s	2.21 s
18		1.32 s	1.30 s	1.09 s	1.30 s	1.28 s	1.27 s	1.29 s
19		4.91 s	4.90 s	4.88 s	4.86 s	4.86 s	4.85 s	4.86 s
		5.00 s	4.99 s	5.00 s	4.93 s	4.93 s	4.94 s	4.96 s
20		1.21 s	1.20 s	1.18 s	1.63 s	1.63 s	1.62 s	1.62 s
OAc			2.08 s	2.05 s				
OAc				2.07 s				

a) Spectra recorded at 500 MHz in $CDCl_3$. *b*) 300 MHz in $CDCl_3$. *c*) *J* value (in Hz) in parentheses.

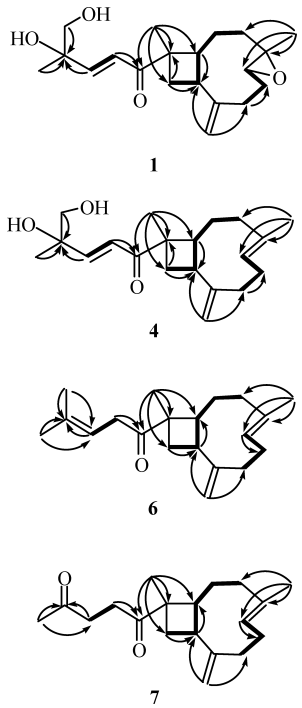

Fig. 1. Selective ^{1}H–^{1}H COSY and HMBC Correlations of **1**, **4**, **6**, and **7**

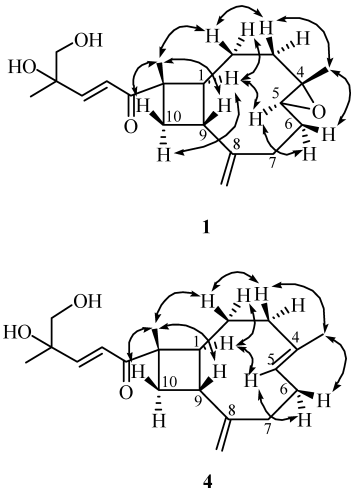

Fig. 2. Key NOESY Correlations of **1** and **4**

The IR spectrum indicated the presence of hydroxy (3443 cm^{-1}), ester carbonyl (1734 cm^{-1}), and keto-carbonyl (1685 cm^{-1}) moieties. It was found that the ^{1}H- and ^{13}C-NMR spectral data of **2** (Tables 1, 2) were very similar to those of **1**, however, the NMR chemical shifts for H_2-16 and C-16 of **2** (δ 4.07, 2H, dd, J=9.0, 9.0 Hz; δ 70.5) were found to be shifted to a lower field, in comparison with the analogous data of **1** (δ 3.55, 2H, dd, J=11.0, 11.0 Hz; δ 69.2), suggesting that the hydroxy group of **1** was replaced by an acetoxy group in **2**. The above observations revealed that **2** is simply the 16-*O*-acetyl derivative of **1**.

Gibberosin I (**3**) exhibited a quasimolecular ion peak in the HR-ESI-MS at *m/z* 427.2461 $[M+Na]^+$ and showed NMR spectroscopic data (Tables 1, 2) consistent with a molecular formula $C_{24}H_{36}O_5$. Comparison of the ^{1}H- and ^{13}C-NMR data of compound **3** with those of **1** and **2** revealed that **3** has the same ring structure but with a different side chain. The HMBC correlations observed from H_3-18 to C-1, C-10, C-11, and an oxymethine carbon at δ 78.7, assigned the latter oxygenated carbon to be C-12. This finding and the upfield chemical shift of C-11 (δ 40.2), relative to those of metabolites **1** (δ 47.7) and **2** (δ 47.8), indicated the attachment of one acetoxy group at C-12. Furthermore, the protons of the oxymethylene group in **3** (δ 4.49 and 4.61, each d, J=12.0 Hz) were found to exhibit HMBC correlations with the carbonyl carbon of the other acetoxy group and an olefinic carbon (δ 132.4, C, C-15), while the latter carbon was found to be correlated with the proton of an olefinic methyl (δ 1.73, 3H, s, H_3-17). Therefore, the trisubstituted double bond was positioned between C-14 and C-15 where an acetoxymethyl and a methyl should be located at C-15. The ^{1}H–^{1}H COSY correlations found from H-12 (δ 4.75, dd, J=9.5, 4.0 Hz) to H_2-13 (δ 2.19 m) and from H_2-13 to the olefinic proton at δ 5.31 (dd, J=7.5, 7.0 Hz, H-14) further supported the C-14/C-15 position of the double bond. This double bond was determined to have a *cis* geometry on the basis of the NOE interaction found between H-14 and H_3-17. Furthermore, the analysis of the NOESY spectrum of **3** revealed the same relative configurations at C-1, C-4, C-5, C-9, and C-11 as in **1** and **2**. Therefore, compound **3** was established as (1*S**,4*S**,5*S**,9*R**,11*S**,14*Z*)-12,16-diacetoxy-4,5-epoxyxeniaphylla-8(19),14-dien.

Gibberosin J (**4**) exhibited a quasimolecular ion peak at *m/z* 341.2092 $[M+Na]^+$ in the HR-ESI-MS, appropriate for a molecular formula $C_{20}H_{30}O_3$ and six degrees of unsaturation. The ^{13}C-NMR spectrum of **4** also revealed the presence of 20 carbon signals, characteristic for a xeniaphyllane-derived terpenoids (Table 1). Through extensive NMR experiments (^{1}H-, ^{13}C-NMR, COSY, HMQC, and HMBC), the structure of **4** was found to be close to that of **1** except that a trisubstituted epoxide at C-4/C-5 (δ_H 2.89, 1H, dd, J=10.0, 3.5 Hz, H-5; δ_C 59.6, C, C-4; δ_C 63.6, CH, C-5) in **1** was replaced by a trisubstituted double bond (δ_H 5.31, 1H, m, H-5; δ_C 135.2, C, C-4; δ_C 124.7, CH, C-5) in **4**. The molecular framework of **4** was further established by the ^{1}H–^{1}H COSY and HMBC correlations as illustrated in Fig. 1. The relative configurations at C-1, C-9, and C-11 in **4** were found to be the same as those of **1**—**3** on the basis of the NOE correlations (Fig. 2). The *E* geometry of the 4,5-endocyclic double bond in **4** was indicated by the lack of NOE correlation between the olefinic methyl protons (δ 1.63, s) attached at C-4 and H-5 (δ 5.31, m) and the upfield shift of C-20 (δ <20 ppm). Compound **4** was thus identified as (1*S**,9*R**,11*S**,4*E*,13*E*)-15,16-dihydroxyxeniaphylla-4,8(19),13-trien-12-one.

On the basis of its HR-ESI-MS (*m/z* 325.2146, $[M+Na]^+$), the molecular formula of gibberosin K (**5**) was established as $C_{20}H_{30}O_2$. The IR spectrum indicated the presence of hydroxy (3566 cm^{-1}) and carbonyl (1684 cm^{-1}) functionalities. The ^{1}H- and ^{13}C-NMR data were very similar to those of compound **4**. By comparison of the NMR spectral data of compound **4** with those of compound **5**, it was found that a hydroxy-bearing methylene (δ_H 3.56, dd, J=8.1, 8.1 Hz; δ_C 69.4) at C-15 in **4** was replaced by a methyl in **5**. Compound **5** was thus identified as (1*S**,9*R**,11*S**,4*E*,13*E*)-15-hydroxyxeniaphylla-4,8(19),13-trien-12-one.

Gibberosin L (**6**) was found to have a molecular formula

$C_{20}H_{30}O$ from the HR-ESI-MS (*m/z* 309.2192 $[M+Na]^+$) and showed an IR absorption band of carbonyl (1717 cm^{-1}) functionality. The ^{13}C-NMR data (Table 1) of **6** were found to be very similar to those of compound **5** from C-1 to C-11 and C-18 to C-20. The side-chain structure was elucidated by the ^1H–^1H COSY and HMBC correlations of **6** as shown in Fig. 1. Moreover, the NOE correlations for protons of the bicycle moiety were found to be the same as those of **5** and thus led to the determination of the 1*S**, 9*R**, and 11*S** configurations of this compound. Also, the lack of NOE correlation between the H_3-20 and H-5 and the upfield shift of C-20 (δ 16.4) confirmed the *E* geometry of the 4,5-endocyclic double bond. The structure of **6** was then established as (1*S**,9*R**,11*S**,4*E*)-xeniaphylla-4,8(19),14-trien-12-one.

A molecular formula $C_{19}H_{28}O_2$ for gibberosin M (**7**) was established by the HR-ESI-MS (*m/z* 311.1986 $[M+Na]^+$) and NMR data (Tables 1, 2). The ^{13}C-NMR spectrum exhibited nineteen carbon signals, including those of three methyls (δ 30.0, 17.8, 16.3, each CH_3), two keto-carbonyls (δ 213.2, 207.4, each C), one exomethylene (δ 153.4, C, and 112.9, CH_2), one trisubstituted double bond (δ 135.0, C, and 124.7, CH), and two ring-junctured methines (δ 48.6, 47.1, CH) which identified compound **7** as a norxeniaphyllane. The ^{13}C-NMR spectroscopic data of compound **7** were found to be nearly identical to those of compounds **5** and **6** from C-1 to C-12 and C-18 to C-20, revealing the same bicyclic structure of **7** as those of **5** and **6**. However, one methyl displayed a 3H singlet at δ 2.21 in the ^1H-NMR spectrum and showed an HMBC correlation to one of the keto-carbonyl carbons (δ 207.4, C), indicating the presence of a terminal acetyl group in the side chain of **7** instead of two methyls at C-15 in **5** and **6**. This was further supported by the ^1H–^1H COSY and HMBC correlations as illustrated in Fig. 1. These findings together with the analysis of NOE correlations for **7** determined gibberosin M to be (1*S**,9*R**,11*S**,4*E*)-16-norxeniaphylla-4,8(19)-dien-12,15-dione.

Cytotoxicity of metabolites **1**—**7** toward a limited panel of cancer cell lines was evaluated. Compounds **5** and **6** have been shown to exhibit moderate activity against A-549 (human lung carcinoma), Hep G2 (human hepatocellular carcinoma), and MDA-MB-231 (human breast carcinoma) cell lines with IC_{50}'s of 7.1, 12.4, and 5.5 μg/ml, IC_{50}'s of 11.3, 14.5, and 7.7 μg/ml, respectively. Other metabolites were found to be inactive against the growth of the above three cancer cell lines (>20.0 μg/ml).

Experimental

Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were recorded on a Jasco FT-5300 infrared spectrophotometer. NMR spectra were recorded on a Bruker AVANCE DPX300 FT-NMR at 300 MHz for ^1H and 75 MHz for ^{13}C or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^1H and 125 MHz for ^{13}C, in $CDCl_3$ using TMS as an internal standard. Low-resolution mass data and HR-MS data were recorded by ESI FT-MS on a Bruker APEX II mass spectrometer. Silica gel (Merck, 230—400 mesh) and Sephadex LH-20 (Amersham Biosciences) were 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 Hitachi L-7100 apparatus equipped with a Bischoff refractive index detector and the Merck Hibar Si-60 column (250 mm×21 mm, 7 μm).

Animal Material The soft coral *Sinularia gibberosa* was collected by hand using scuba equipment off the coast of northeastern Taiwan in May 2004, at a depth of 15—20 m, and was stored in a freezer until extraction. A voucher specimen was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University (voucher no. SC-20040621-5).

Extraction and Isolation The bodies of *S. gibberosa* (1.3 kg fresh weight) were minced and extracted exhaustively with EtOAc, and the extract was concentrated under reduced pressure to give a dark brown viscous residue (15.4 g). The residue was fractionated by open column chromatography on silica gel using an *n*-hexane and *n*-hexane–EtOAc mixture of increasing polarity to yield 32 fractions. Fraction 6, eluted with *n*-hexane/EtOAc (8 : 1), was subjected to Sephadex LH-20 column using acetone and followed by normal phase HPLC eluted with *n*-hexane/acetone (7 : 1), to afford compounds **3** (2.1 mg), and **6** (14.6 mg). Fraction 15, eluted with *n*-hexane/EtOAc (5 : 1), was further purified by silica gel column using *n*-hexane/EtOAc (5 : 1) as eluent to afford compound **7** (5.8 mg). Fraction 20, eluted with *n*-hexane/EtOAc (3 : 1), was further purified by normal phase HPLC using *n*-hexane/acetone (5 : 1 to 3 : 1) to give compound **5** (3.8 mg). Fraction 22, eluted with *n*-hexane/EtOAc (1 : 1), was further purified by normal phase HPLC using *n*-hexane/acetone (2 : 1), to give compounds **1** (3.0 mg), **2** (1.2 mg), and **4** (2.4 mg).

Gibberosin G (**1**): Colorless oil; $[\alpha]_D^{25}$ +24.6° (*c*=0.3, $CHCl_3$); IR (neat) ν_{max} 3421, 2924, 1684 cm^{-1}; ^1H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z* 357 ($[M+Na]^+$); HR-ESI-MS *m/z* 357.2040 $[M+Na]^+$ (Calcd for $C_{20}H_{30}O_4Na$, 357.2042).

Gibberosin H (**2**): Colorless oil; $[\alpha]_D^{25}$ +20.0° (*c*=0.24, $CHCl_3$); IR (neat) ν_{max} 3443, 2966, 1734, 1685 cm^{-1}; ^1H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z* 399 ($[M+Na]^+$); HR-ESI-MS *m/z* 399.2148 $[M+Na]^+$ (Calcd for $C_{22}H_{32}O_5Na$, 399.2147).

Gibberosin I (**3**): Colorless oil; $[\alpha]_D^{25}$ +75.0° (*c*=0.3, $CHCl_3$); IR (neat) ν_{max} 2926, 1734 cm^{-1}; ^1H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z* 427 ($[M+Na]^+$); HR-ESI-MS *m/z* 427.2461 $[M+Na]^+$ (Calcd for $C_{24}H_{36}O_5Na$, 427.2460).

Gibberosin J (**4**): Colorless oil; $[\alpha]_D^{25}$ +35.0° (*c*=0.16, $CHCl_3$); IR (neat) ν_{max} 3420, 2929, 1684 cm^{-1}; ^1H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z* 341 ($[M+Na]^+$); HR-ESI-MS *m/z* 341.2092 $[M+Na]^+$ (Calcd for $C_{20}H_{30}O_3Na$, 341.2093).

Gibberosin K (**5**): Colorless oil; $[\alpha]_D^{25}$ +20.0° (*c*=0.6, $CHCl_3$); IR (neat) ν_{max} 3566, 2926, 1684 cm^{-1}; ^1H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z* 325 ($[M+Na]^+$); HR-ESI-MS *m/z* 325.2146 $[M+Na]^+$ (Calcd for $C_{20}H_{30}O_2Na$, 325.2143).

Gibberosin L (**6**): Colorless oil; $[\alpha]_D^{25}$ +14.4° (*c*=1.5, $CHCl_3$); IR (neat) ν_{max} 2934, 1717 cm^{-1}; ^1H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z* 309 ($[M+Na]^+$); HR-ESI-MS *m/z* 309.2192 $[M+Na]^+$ (Calcd for $C_{20}H_{30}ONa$, 309.2194).

Gibberosin M (**7**): Colorless oil; $[\alpha]_D^{25}$ +13.8° (*c*=0.6, $CHCl_3$); IR (neat) ν_{max} 2945, 1717 cm^{-1}; ^1H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z* 311 ($[M+Na]^+$); HR-ESI-MS *m/z* 311.1986 $[M+Na]^+$ (Calcd for $C_{19}H_{28}O_2Na$, 311.1987).

Cytotoxicity Testing Compounds were assayed for cytotoxicity against A-549, Hep G2, and MDA-MB-231 cancer cells using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method.[12,13] Freshly trypsinized cell suspensions were seeded into a 96-well microtiter plate at densities of 5000—10000 cells per well and then the test compounds were added from DMSO-diluted stock solutions. After 3 d in culture, attached cells were incubated with MTT (0.5 mg/ml, 1 h) and subsequently dissolved in DMSO. The absorbency at 550 nm was then measured using a microplate reader. The IC_{50} is the concentration of agent that reduced cell growth by 50% under the experimental conditions.

Acknowledgments Financial support was provided by the Ministry of Education (C030313) and the National Science Council of Taiwan (NSC 95-2113-M-110-011-MY3) awarded to J.-H. Sheu.

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