Novel Norhumulene and Xeniaphyllane-Derived Terpenoids from a Formosan Soft Coral *Sinularia gibberosa*

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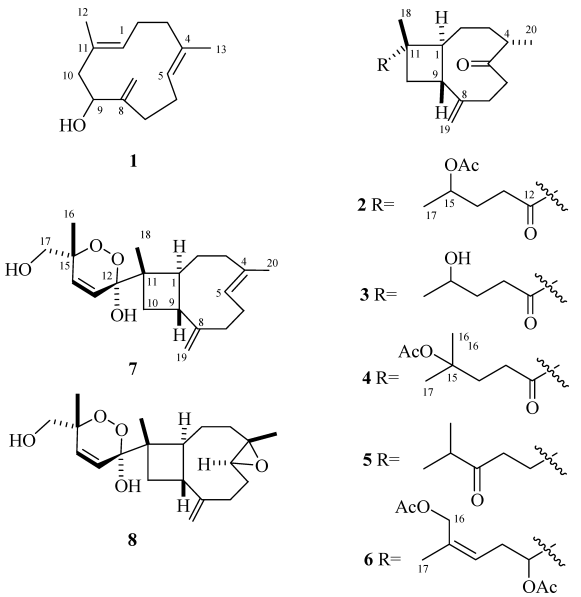
A novel skeletal norhumulene (1) and six xeniaphyllane-derived compounds, including norditerpenoids (2, 3) and diterpenoids (4—7), were isolated from the EtOAc extract of the Formosan soft coral *Sinularia gibberosa*. Their structures were elucidated by spectroscopic analysis. *In vitro* cytotoxic evaluation of the above metabolites revealed that 2 and 4 showed weak cytotoxicity toward a few cancer cell lines.

Key words diterpenoid; xeniaphyllane; *Sinularia gibberosa*; cytotoxic

Our previous study on the secondary metabolites of a Formosan soft coral *Sinularia gibberosa* TIXIER-DURIVAULT (Alcyoniidae) has resulted in the isolation of a series of β-caryophyllene-related metabolites and xeniaphyllane-related metabolites.[1—3] Some of these metabolites have been shown to exhibit cytotoxicity toward several caner cell lines.[1,3] Our continuing investigation on the chemical constituents of this soft coral has again led to the isolation of one novel norhumulene **1**, two new norxeniaphyllanes **2** and **3**, three new xeniaphyllanes **4**—**6**, and a new xeniaphyllane-derived diterpenoid **7** possessing a rare cyclic peroxyhemiketal (3,6-dihydro-1,2-dioxin-3-ol).[2,4] We 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 silica gel. The terpenoid-containing fractions (characterized by ^{1}H-NMR measurement in $CDCl_3$) were selected for further purification, using open column chromatography on silica gel and normal phase HPLC to afford terpenoids **1**—**7**.



Gibberosin N (**1**) displayed fourteen carbon signals in the ^{13}C-NMR spectrum (Table 1), attributable to two methyls, five sp^3 methylenes, one sp^3 methine, one sp^2 methylene, two sp^2 methines, and three sp^2 quaternary carbons. The IR spectrum displayed absorptions at 3423 and 1653 cm^{-1} for hydroxy group and carbon-carbon double bond, respectively. The NMR signals at δ_H 4.14 (dd, J=10.4, 4.4 Hz) and δ_C 74.8 (CH) revealed the presence of a secondary hydroxy group in the molecule. From NMR data (Tables 1, 2) and the presence of hydroxy group, the molecular formula of **1** was suggested to be $C_{14}H_{22}O$, implying four degrees of unsaturation. This was further confirmed from the HR-ESI-MS which showed a ion peak at m/z 189.1641 ($[M-H_2O+H]^+$). Moreover, two trisubstituted double bonds (δ_C 132.8, C, 130.4, C, 129.2, CH, and 127.1, CH and δ_H 5.18, dd, J=8.4, 7.2 Hz and 4.84, dd, J=10.0, 4.4 Hz) and one exocyclic double bond (δ_C 153.2, C and 109.5, CH_2 and δ_H 5.29, s and 5.09, s) were assigned in the structure. From the above data, **1** was suggested to possess a ring. 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. The olefinic methyls (δ_H 1.49, s and 1.59, s) attached at C-4 and C-11 were confirmed by HMBC correlations from H_3-13 to C-3, C-4, and C-5 and H_3-12 to C-1, C-10, and C-11, respectively. The hydroxy group was determined to be located at C-9 due to the HMBC correlations observed from H_2-14 (δ 5.09, s and 5.29, s) and H_2-10 (δ 2.02, m and 2.56, dd, J=12.0, 4.0) to C-9 (δ 74.8, CH). Thus, two methyl-containing double bonds and a hydroxy group were positioned at C-1/C-11, C-4/C-5 and C-9, respectively. The structure of **1** was thus established as a norhumulene. On the basis of above results, the structure of **1** was established as 9-hydroxy-13-norhumulene-1*E*,4*E*,8(14)-triene.

Gibberosin O (**2**) was found to possess a molecular formula $C_{21}H_{32}O_4$ from the pseudomolecular ion peak appearing at m/z 371.2197 $[M+Na]^+$ in the HR-ESI-MS, corresponding to six degrees of unsaturation. The IR spectrum of

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

C#	**1**[a]	**2**[b]	**3**[c]	**4**[b]	**5**[b]	**6**[a]	**7**[b]
1	129.2 (CH)[d]	47.0 (CH)	47.4 (CH)	46.9 (CH)	49.9 (CH)	44.5 (CH)	44.5 (CH)
2	25.4 (CH_2)	27.5 (CH_2)	27.4 (CH_2)	27.4 (CH_2)	27.5 (CH_2)	26.8 (CH_2)	30.5 (CH_2)
3	39.0 (CH_2)	30.2 (CH_2)	30.0 (CH_2)	30.1 (CH_2)	30.5 (CH_2)	29.8 (CH_2)	39.6 (CH_2)
4	132.8 (C)	48.3 (CH)	48.7 (CH)	48.2 (CH)	47.8 (CH)	47.5 (CH)	135.3 (C)
5	127.1 (CH)	216.8 (C)	217.4 (C)	216.8 (C)	217.1 (C)	217.2 (C)	124.5 (CH)
6	25.0 (CH_2)	41.6 (CH_2)	41.9 (CH_2)	41.7 (CH_2)	42.3 (CH_2)	42.8 (CH_2)	28.3 (CH_2)
7	34.7 (CH_2)	32.3 (CH_2)	32.2 (CH_2)	31.3 (CH_2)	32.1 (CH_2)	31.7 (CH_2)	34.7 (CH_2)
8	153.2 (C)	151.9 (C)	152.0 (C)	151.9 (C)	152.8 (C)	152.6 (C)	154.2 (C)
9	74.8 (CH)	41.7 (CH)	42.0 (CH)	41.6 (CH)	43.3 (CH)	43.0 (CH)	47.4 (CH)
10	49.9 (CH_2)	34.0 (CH_2)	34.2 (CH_2)	34.1 (CH_2)	36.9 (CH_2)	33.4 (CH_2)	34.0 (CH_2)
11	130.4 (C)	48.1 (C)	48.2 (C)	48.2 (C)	36.6 (C)	40.5 (C)	41.2 (C)
12	16.7 (CH_3)	213.3 (C)	213.3 (C)	213.6 (C)	36.9 (CH_2)	77.6 (CH)	99.9 (C)
13	15.3 (CH_3)	32.5 (CH_2)	32.7 (CH_2)	32.2 (CH_2)	35.3 (CH_2)	28.0 (CH_2)	126.8 (CH)
14	109.5 (CH_2)	29.5 (CH_2)	29.3 (CH_2)	34.8 (CH_2)	215.1 (C)	126.1 (CH)	131.5 (CH)
15		70.3 (CH)	67.6 (CH)	81.4 (C)	40.8 (CH)	132.2 (C)	80.4 (C)
16		—	—	25.7 (CH_3)	18.4 (CH_3)	63.0 (CH_2)	19.4 (CH_3)
17		20.1 (CH_3)	23.9 (CH_3)	25.7 (CH_3)	18.4 (CH_3)	21.5 (CH_3)	66.8 (CH_2)
18		17.0 (CH_3)	17.1 (CH_3)	17.0 (CH_3)	19.3 (CH_3)	16.3 (CH_3)	16.8 (CH_3)
19		112.5 (CH_2)	112.7 (CH_2)	112.5 (CH_2)	111.6 (CH_2)	112.1 (CH_2)	112.6 (CH_2)
20		16.4 (CH_3)	16.5 (CH_3)	16.3 (CH_3)	16.5 (CH_3)	16.0 (CH_3)	16.3 (CH_3)
OAc		170.8 (C)		170.4 (C)		170.8 (C)	
		21.3 (CH_3)		22.4 (CH_3)		21.0 (CH_3)	
OAc						171.1 (C)	
						21.1 (CH_3)	

a) Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C. *b*) Spectra recorded at 125 MHz in $CDCl_3$ at 25 °C. *c*) Spectra recorded at 75 MHz in $CDCl_3$ at 25 °C. *d*) Attached protons were deduced by DEPT spectra.

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

H#	**1**[a]	**2**[b]	**3**[c]	**4**[b]	**5**[b]	**6**[a]	**7**[b]
1	4.84 dd (10.0, 4.4)[d]	1.98 m	1.99 m	2.00 m	1.47 m	1.62 m	2.58 dd (9.5, 9.5)
2α	2.02 m—2.14 m	1.73 m	1.76 m	1.78 m	1.60 m	1.56 m	1.63 m
2β		1.38 m	1.40 m	1.40 m	1.28 m	1.25 m	1.50 m
3α	1.98 m—2.03 m	1.65 m	1.66 m	1.66 m	1.66 m	1.63 m	1.98 m
3β		1.83 m	1.84 m	1.84 m	1.80 m	1.80 m	2.05 m
4		2.52 m	2.52 m	2.52 m	2.52 m	2.51 m	
5	5.18 dd (8.4, 7.2)						5.37 dd (10.0, 9.5)
6α	2.24 m	2.50 m	2.50 m	2.48 m	2.50 m	2.49 m	2.02 m
6β							2.35 m
7α	1.76 m—2.35 m	2.44 m	2.45 m	2.46 m	2.47 m	2.46 m	2.25 m
7β							2.01 m
9	4.14 dd (10.4, 4.4)	2.41 m	2.42 m	2.42 m	2.40 m	2.41 m	2.32 q (9.5)
10α	2.02 m	2.03 m	2.05 m	1.95 m	1.55 m	1.49 m	2.23 dd (10.0, 9.5)
10β	2.56 dd (12.0, 4.0)	1.68 m	1.66 m	1.72 m		1.78 m	1.53 m
12	1.59 s				1.52 m	4.68 dd (9.2, 3.6)	
13	1.49 s	2.35 m	2.35 m	2.38 m	2.31 m	2.16 m	5.87 d (10.5)
14	5.09 s; 5.29 s	1.77 m	1.76 m	1.94 m		5.32 dd (7.2, 6.8)	5.95 d (10.5)
15		4.88 m	3.79 m		2.63 septet (7.0)		
16				1.45 s	1.10 d (7.0)	4.51 d (12.0); 4.60 d (12.0)	1.35 s
17		1.23 d (6.0)	1.25 d (6.3)	1.45 s	1.10 d (7.0)	1.73 s	3.59 d (12.5); 3.64 d (12.5)
18		1.18 s	1.20 s	1.21 s	0.95 s	1.01 s	1.11 s
19		4.91 s; 4.92 s	4.91 s; 4.92 s	4.91 s; 4.92 s	4.88 s; 4.89 s	4.88 s; 4.91s	4.86 s; 5.06 s
20		1.05 d (7.0)	1.05 d (6.9)	1.05 d (7.0)	1.03 d (7.0)	1.00 d (6.8)	1.60 s
OAc		2.05 s		1.99 s		2.07 s	
OAc						2.13 s	

a) Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. *b*) Spectra recorded at 500 MHz in $CDCl_3$ at 25 °C. *c*) Spectra recorded at 300 MHz in $CDCl_3$ at 25 °C. *d*) The *J* values (in Hz) in parentheses.

2 showed the presence of ester (1734 cm^{-1}) and ketone (1716 cm^{-1}) functionalities. This was further supported by the ^{13}C-NMR signals appearing at δ 170.8 (C), 213.3 (C), 216.8 (C), respectively. In the ^{1}H-NMR spectrum, the signals at δ 1.98 (1H, m), 2.41 (1H, m), 4.91 (1H, s) and 4.92 (1H, s) were found to be HMQC correlated with the carbon signals at δ 47.0 (CH), 41.7 (CH) and 112.5 (CH_2), respectively, indicating that **2** might be a β-caryophyllene-derived compound.[1,2,5] The gross structure of **2** was successively established by the assistance of ^{1}H–^{1}H COSY and HMBC correlations as shown in Fig. 1. Accordingly, the C-5 and C-12 positions of two keto-carbonyls **2** were interpreted by the HMBC

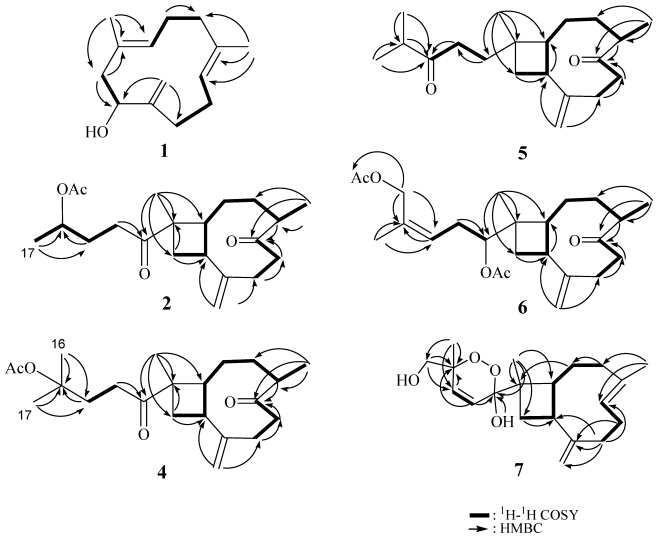

Fig. 1. Key ^{1}H–^{1}H COSY and HMBC Correlations for **1**, **2** and **4**—**7**

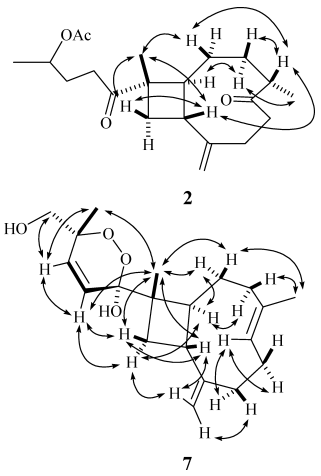

Fig. 2. Selective NOESY Correlations of **2** and **7**

correlations observed from H-4 (δ 2.52, m), H_2-6 (δ 2.50, m) and H_3-20 (δ 1.05, d, J=7.0 Hz) to C-5 (δ 216.8, C), and from H_2-13 (δ 2.35, m) and H_3-18 (δ 1.18, s) to C-12 (δ 213.3, C). The acetoxy group was also determined to be located at C-15 due to the HMBC correlations observed from H_2-14 (1.77, m) and H_3-17 (δ 1.23, d, J=6.0 Hz) to C-15 (δ 70.3, CH). Thus, the norxeniaphyllane framework of metabolite **2** and the lack of one methyl group at C-15 relative to other xeniaphyllane diterpenoids[1,2,5] were suggested.

The relative stereochemistry of the four chiral centers at C-1, C-4, C-9, and C-11 in **2** was established on the basis of the NOE correlations (Fig. 2) observed in the NOESY spectrum. Assuming the β orientation of H-9,[1,2] it was found that H-9 exhibited NOE correlations with H-4, H-10β (δ 1.68, m) and H_3-18, but not with H-1 (δ 1.98, m), and H_3-20, while H-3α (δ 1.65, m) displayed NOE interaction with H-1 and H_3-20. These observations suggested that H-1, and H_3-20 should be α-oriented. On the basis of the above findings and other detailed NOE interactions (Fig. 2), the relative structure of compound **2** was unambiguously established as (1*S**,4*S**,9*R**,11*S**)-15-acetoxy-16-norxeniaphylla-8(19)-en-5,12-dione.

Gibberosin P (**3**) had a molecular formula $C_{19}H_{30}O_3$ as established from its HR-ESI-MS (*m/z* 329.2093, $[M+Na]^+$). The IR spectrum indicated the presence of hydroxy (3420 cm^{-1}) and carbonyl (1716 cm^{-1}) groups. The ^{1}H- and ^{13}C-NMR spectroscopic data of **3** were quite similar to those of **2** except that the oxymethine carbon signal at δ 70.3 (C-15) in **2** was found to be upfield shifted to 67.6 ppm in compound **3**. This was associated by the disappearance of the NMR signals of acetate and the IR absorption band of the ester group in **3**. Thus, compound **3** was elucidated as the deacetyl derivative of **2**. The relative configurations at C-1, C-4, C-9, and C-11 in compound **3** were found to be identical to those of **2** due to the same NOE correlations displayed in NOESY spectra of **2** and **3**. Compound **3** was thus identified as (1*S**,4*S**,9*R**,11*S**)-15-hydroxy-16-norxeniaphylla-8(19)-en-5,12-dione.

The molecular formula of gibberosin Q (**4**) was established as $C_{22}H_{34}O_4$ by HR-ESI-MS (*m/z* 385.2353 $[M+Na]^+$) and NMR data (Tables 1, 2). As in the case of **2**, the IR absorptions of **4** at 1734, and 1716 cm^{-1} also suggested the presence of ester and ketone moieties, respectively. The ester function was also represented by one acetoxy group from the NMR signals at δ_C 170.4 (C) and 22.4 (CH_3); δ_H 1.99 (s). Comparison of the ^{13}C-NMR spectroscopic data of compound **4** with those of compound **2** (Table 1) indicated that compound **4** has the same carbon skeleton from C-1 to C-14, while two tertiary methyl groups (δ_H 1.45, s, 6H; δ_C 25.7, CH_3, 2C) were found to be attached at C-15 in **4** by showing HMBC correlations from protons of these two methyls to C-14 and C-15. Thus, gibberosin Q (**4**) was identified as (1*S**,4*S**,9*R**,11*S**)-15-acetoxyxeniaphylla-8(9)-en-5,12-dione.

Gibberosin R (**5**) exhibited a pseudomolecular ion peak in the HR-ESI-MS at *m/z* 327.2301, appropriate for a molecular formula of $C_{20}H_{32}O_2$. It differs from **4** in the absence of an acetoxy group as revealed from the disappearance of IR absorption band of ester function and NMR signals of acetate group. The carbon of the side chain carbonyl carbon (δ 215.1, C) was found to be C-14 due to the HMBC correlations observed from H_2-13 (δ 2.31, m), H-15 (δ 2.63, septet, J=7.0 Hz), and H_3-16 and H_3-17 (δ 1.10, d, J=7.0 Hz) to the carbon signal at δ 215.1. On the basis of the above findings, the molecular framework of compound **5** was determined as shown in Fig. 1. Furthermore, the analysis of the NOESY spectrum of **5** revealed **5** possesses the same relative configurations at C-1, C-4, C-5, C-9, and C-11 as those in **2**—**4**. Thus, the relative structure of metabolite **5** was determined as (1*S**,4*S**,9*R**,11*S**)-xeniaphylla-8(19)-en-5,14-dione.

Gibberosin S (**6**) was found to possess a molecular formula $C_{24}H_{36}O_5$, as indicated from its HR-ESI-MS at *m/z* 427.2462 $[M+Na]^+$. It also exhibited IR absorptions at 1736 and 1717 cm^{-1}, suggesting the presence of ester and ketone moieties. Comparison of the ^{1}H- and ^{13}C-NMR data of compound **6** with those of **2**—**5** revealed that **6** is also a 5-oxoxeniaphyllane with a trisubstituted double bond and two acetoxy groups in the side chain. The HMBC correlations (Fig. 1) observed from H_3-18 (δ 1.01, s) to C-1 (δ 44.5, CH), C-10 (δ 33.4, CH_2), C-11 (δ 40.5, C) and an oxymethine carbon at δ 77.6 (CH) assigned this oxymethine carbon as C-12. This finding together with the upfield shift of C-11 relative to

those of metabolites **2**—**4** (δ_C 48.1—48.2), and the downfield shift of C-11 relative to that of **5** (δ_C 36.6), indicated the C-12 attachment of an acetoxy group. Furthermore, the protons of an oxymethylene group in **6** (δ_H 4.51 and 4.60, each 1H, d, J=12.0 Hz) were found to exhibit HMBC correlations with the carbonyl carbon of another acetoxy group (δ_C 170.8, C) and an olefinic carbon (δ_C 132.2, C, C-15), while this olefinic carbon was found to be correlated with the protons of an olefinic methyl (δ_H 1.73, s,). Therefore, the trisubstituted double bond was positioned between C-14 and C-15 where an acetoxymethyl and a methyl were the substituents at C-15. The ^{1}H–^{1}H COSY correlations found from H-12 (δ 4.68, dd, J=9.2, 3.6 Hz) to H_2-13 (δ 2.16, m) and from H_2-13 to the olefinic proton (δ 5.32, dd, J=7.2, 6.8 Hz, H-14) further supported the C-14/C-15 position of the double bond. This double bond was determined to have a *Z* geometry on the basis of the NOE interaction found between H-14 and H_3-17. Furthermore, the analysis of the NOESY spectrum of **6** revealed the similar stereochemistries at C-1, C-9, C-10, and C-11 as those in **2**—**5**. Therefore, the relative structure of compound **6** was established as (1*S**,4*S**,9*R**,11*S**,14*Z*)-12,16-diacetoxyxeniaphylla-8(19),14-dien-5-one.

Sinugibberoside F (**7**) was found to possess a molecular formula $C_{20}H_{30}O_4$ as established from its HR-ESI-MS (*m/z* 357.2044, $[M+Na]^+$), implying six degrees of unsaturation. The IR spectrum indicated the presence of hydroxy (ν_{max} 3420 cm^{-1}) group in the molecule. The ^{1}H-NMR data (Table 2) revealed the presence of one upper field shifted methyl (δ 1.11, s), one olefinic methyl (δ 1.60, s) one trisubstituted double bond (δ 5.37, dd, J=10.0, 9.5 Hz), one 1,1-disubstituted double bond (δ 4.86, s and 5.06, s), and two ^{1}H–^{1}H COSY correlated ring-juncture methines (δ 2.58, dd, J=9.5, 9.5 Hz and 2.32, q, J=9.5 Hz), characteristic for a caryophyllene moiety in **7**.[3] The ^{1}H- and ^{13}C-NMR data of **7** spectra were very similar to those of a known compound sinugibberoside C (**8**),[2] except that a trisubstituted epoxide at C-4/C-5 in **8** was replaced by a trisubstituted double bond (δ_H 5.37, dd, J=10.0, 9.5 Hz, H-5; δ_C 135.3, C, C-4; δ_C 124.5, CH, C-5) in **7**. The molecular framework of **7** was further established by the ^{1}H–^{1}H COSY and HMBC correlations as illustrated in Fig. 1. The *E* geometry of the 4,5-endocyclic double bond in **7** was indicated by the absence of NOE correlation between the olefinic methyl protons (δ 1.60, s) attached at C-4 and H-5 (δ 5.37, dd) and the upfield shift of C-20 (δ 16.3). Further NOE analysis revealed that **7** possessed the same relative configurations at C-1, C-9, C-11, C-12, and C-15 as those of the known metabolite **8**.[2] Based on the above results, the relative structure of **7** was determined as (1*S**,9*R**,11*S**,12*R**,15*R**,4*E*)-12,15-epidioxy-xeniaphylla-4,8(19),13-trien-12,17-diol.

Cytotoxicity of metabolites **1**—**7** toward a limited panel of cancer cell lines was evaluated. Compound **2** was found to exhibit weak cytotoxicity toward Hep G2 (human hepatocellular carcinoma) and A549 (human lung carcinoma) cell lines with IC_{50}'s of 18.7 and 19.5 μg/ml, respectively. Also, metabolite **4** showed weak cytotoxicity (IC_{50} 11.0 μg/ml) toward A549 cells. Other metabolites were found to be inactive against the growth of the above three cancer cell lines.

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 DPX 300 FT-NMR at 300 MHz for ^{1}H and 75 MHz for ^{13}C, or on a Varian Mercury Plus 400 FT-NMR at 400 MHz for ^{1}H and 100 MHz for ^{13}C, or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C, in $CDCl_3$ using TMS as an internal standard. LR-MS and HR-MS were obtained by ESI on a Bruker APEX II mass spectrometer. Silica gel (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 Hitachi L-7100 apparatus equipped with a Bischoff refractive index detector and a Merck Hibar Si-60 column (250 mm×21 mm, 7 μm).

Animal Material The soft coral *Sinularia gibberosa* was collected by hand using scuba 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 *n*-hexane and *n*-hexane–EtOAc mixture of increasing polarity to yield 32 fractions. Fraction 15, eluting with *n*-hexane/EtOAc (5 : 1), was further purified by silica gel column using *n*-hexane/EtOAc (5 : 1) to give compound **5** (1.2 mg). A combined mixture of fractions 17—19, eluting with *n*-hexane/EtOAc (5 : 1→4 : 1), was purified by normal phase HPLC using *n*-hexane/EtOAc (4 : 1) to give compounds **2** (1.5 mg) and **4** (12.4 mg), and then using *n*-hexane/acetone (5 : 1) to give compounds **1** (1.0 mg) and **6** (1.1 mg). Fraction 20, eluting 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 **3** (3.8 mg). Fraction 22, eluting with *n*-hexane/EtOAc (1 : 1), was further purified by normal phase HPLC using *n*-hexane/acetone (2 : 1), to give compound **7** (1.9 mg).

Gibberosin N (**1**): Colorless oil; $[\alpha]_D^{25}$ +16.0 (c=0.3, $CHCl_3$); IR (KBr) ν_{max} 3423, 1653 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z* 189 ($[M-H_2O+H]^+$); HR-ESI-MS *m/z* 189.1641 $[M-H_2O+H]^+$ (Calcd for $C_{14}H_{21}$, 189.1643).

Gibberosin O (**2**): Colorless oil; $[\alpha]_D^{25}$ −9.8 (c=0.6, $CHCl_3$); IR (KBr) ν_{max} 1734, 1716 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z* 371 ($[M+Na]^+$); HR-ESI-MS *m/z* 371.2197 $[M+Na]^+$ (Calcd for $C_{21}H_{32}O_4Na$, 371.2198).

Gibberosin P (**3**): Colorless oil; $[\alpha]_D^{25}$ −5.0 (c=0.6, $CHCl_3$); IR (KBr) ν_{max} 3420, 1716 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z* 329 ($[M+Na]^+$); HR-ESI-MS *m/z* 329.2093 $[M+Na]^+$ (Calcd for $C_{19}H_{30}O_3Na$, 329.2092).

Gibberosin Q (**4**): Colorless oil; $[\alpha]_D^{25}$ −4.8 (c=1.2, $CHCl_3$); IR (KBr) ν_{max} 1734, 1716 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z* 385 ($[M+Na]^+$); HR-ESI-MS *m/z* 385.2353 $[M+Na]^+$ (Calcd for $C_{22}H_{34}O_4Na$, 385.2355).

Gibberosin R (**5**): Colorless oil; $[\alpha]_D^{25}$ −29.5 (c=0.4, $CHCl_3$); IR (KBr) ν_{max} 1717 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z* 327 ($[M+Na]^+$); HR-ESI-MS *m/z* 327.2301 $[M+Na]^+$ (Calcd for $C_{20}H_{32}O_2Na$, 327.2300).

Gibberosin S (**6**): Colorless oil; $[\alpha]_D^{25}$ −17.3 (c=0.7, $CHCl_3$); IR (KBr) ν_{max} 1736, 1717 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z* 427 ($[M+Na]^+$); HR-ESI-MS *m/z* 427.2462 $[M+Na]^+$ (Calcd for $C_{24}H_{36}O_5Na$, 427.2460).

Sinugibberoside F (**7**): Clorless oil, $[\alpha]_D^{25}$ +32.0 (c=0.6, $CHCl_3$); IR (neat) ν_{max} 3420, 1647 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS *m/z* 357 ($[M+Na]^+$); HR-ESI-MS *m/z* 357.2044 (Calcd for $C_{20}H_{30}O_4Na$, 357.2042).

Cytotoxicity Testing Cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays of the test compounds **1**—**7** were performed using the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[6,7]

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