

New β-Caryophyllene-Derived Terpenoids from the Formosan Soft Coral *Sinularia gibberosa*

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Seven new β-caryophyllene-derived terpenoids (**1**–**7**) were isolated from EtOAc extracts of the Formosan soft coral *Sinularia gibberosa*. The structures of compounds **1**–**7** were elucidated on the basis of extensive spectroscopic analyses and by comparison with the spectral data of related metabolites. Cytotoxicity evaluation of the above metabolites towards a limited panel of cancer cell lines also will be described.

Soft corals belonging to the genus *Sinularia* (Alcyoniidae) have been found to be a rich source of structurally unique and biologically active diterpenoids and norditerpenoids.[1] During the course of our investigation on bioactive natural products from marine invertebrates, a series of metabolites including steroids, sesquiterpenoids, diterpenoids, and norditerpenoids have been discovered.[2–11] Our present study on the chemical constituents of a soft coral *Sinularia gibberosa*, which was collected off the coast of northeastern Taiwan, has led to the isolation of seven new β-caryophyllene-derived terpenoids (**1**–**7**) (Chart 1). We describe herein the isolation, structure elucidation, and biological activity of these compounds.

Compound **1** was isolated as a colorless oil, $[\alpha]_D^{25}$ +42.8 (*c* 0.16, $CHCl_3$). Its HR-ESI-MS had a pseudomolecular ion peak at *m*/*z* 287.1622 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_3Na$, 287.1623), corresponding to the molecular formula of $C_{16}H_{24}O_3$. Thus, the compound was determined to have five degrees of unsaturation. The characteristic NMR signals [δ_H 3.69 (3H, s, OMe); δ_C 178.3 (C-13), 51.9 (OMe)] and an IR absorption at 1728 cm^{-1} indicated the presence of a methyl ester functional group. Furthermore, two methyls (δ_C 16.8, CH_3 and 17.0, CH_3), one trisubstituted epoxide (δ_C 59.4, C and 63.4, CH), and one exocyclic double bond (δ_C 114.0, CH_2 and 150.6, C) were assigned from the ^{13}C NMR and DEPT spectra of compound **1**. The above functionalities revealed that compound **1** is a tricyclic compound, including the presence of an epoxide. Using 2D NMR spectroscopic analysis (COSY, HMQC, and HMBC), the caryophyllene-based skeleton of compound **1** was elucidated (Fig. 1).

The relative stereochemistry of compound **1** (Fig. 2) was established from NOE correlations observed in a NOESY experiment. In the NOESY spectrum of compound **1**, H-1 interacted

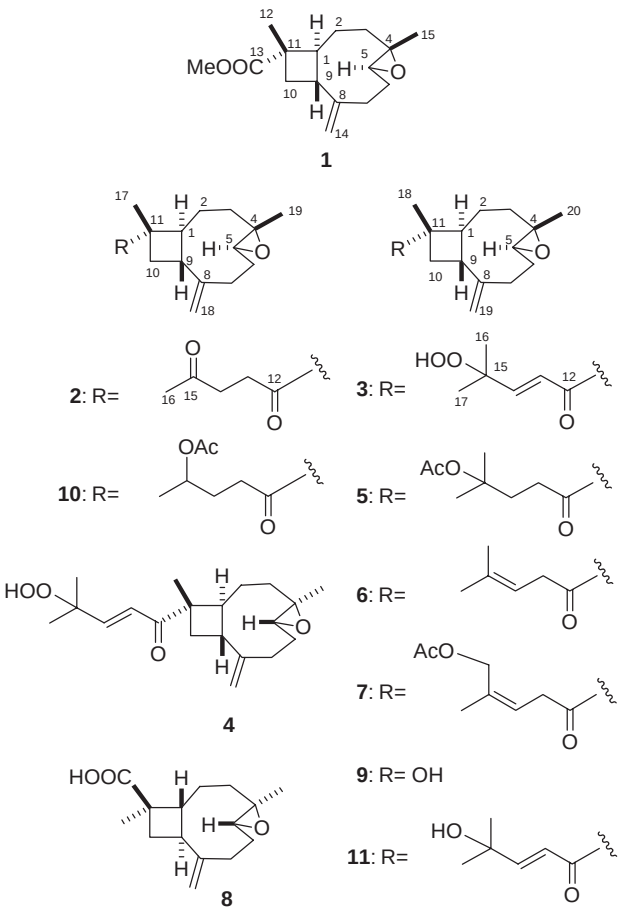

Chart 1.

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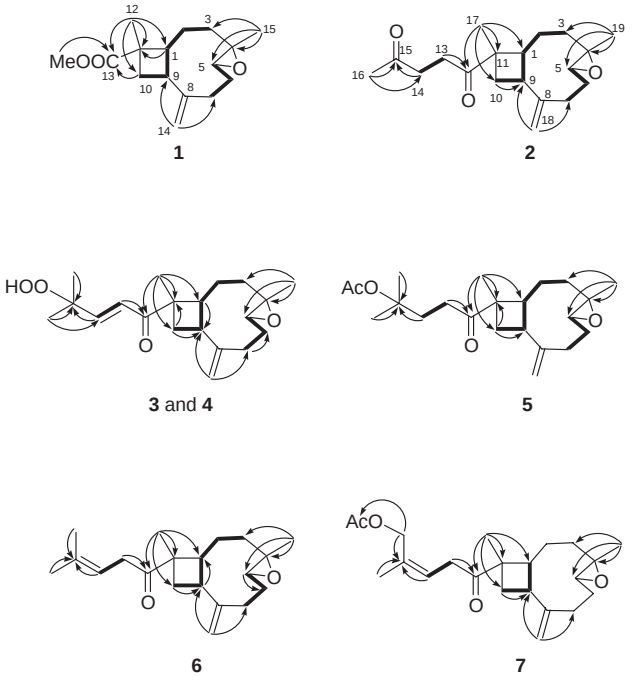

Fig. 1. Selective ^{1}H–^{1}H COSY and HMBC correlations of compounds **1**–**7**.

with H-5 but not with H-9, H_3-12, and H_3-15, suggesting that H-1 is situated on the opposite face of the compound from H-9, H_3-12, and H_3-15. It was found that the relative stereochemistry of compound **1** is the same as that of a known metabolite **8**.[12,13] Also, we have previously isolated a related norcaryophyllene, compound **9**, from a soft coral of the same genus *Sinularia*.[6] Thus, a (1*S**,4*S**,5*S**,9*R**,11*S**)-configuration for compound **1** was suggested.

Gibberosin A (**2**) was obtained as a colorless oil, $[\alpha]_D^{25}$ +43.2 (*c* 1.04, $CHCl_3$). The molecular formula of compound **2**, $C_{19}H_{28}O_3$, was established by HR-ESI-MS, and it was determined to have six degrees of unsaturation. The ^{13}C NMR spectrum of compound **2** had signals for 19 carbons (Table 1), including those of two ketones (δ_C 213.4 and 207.6), one trisubstituted epoxide (δ_C 59.5, C and 63.7, CH), and one exocyclic double bond (δ_C 114.2, CH_2 and 150.8, C). From a comparison of ^{1}H and ^{13}C NMR spectral data of compound **2** (Tables 1 and 2) with those of compound **1** and nanolobatin C (**10**),[6] it was found that compound **2** contains the same ring structure as that of compounds **1** and **10**, and the acetoxy-bearing methine at C-15 in compound **10** was replaced by a ketone in compound **2**, which was also confirmed by the HMBC and COSY correlations as shown in Fig. 1. Thus, the planar structure of compound **2** was fully established. From the NOE correlations observed in the NOESY spectrum of **2** (Fig. 2), H-1 interacted with H-5, but not with H-9, H_3-17, and H_3-19. Also, H-5 did not interact with H_3-19. This suggests that H-5 has an α-orientation and H-9, H_3-17, and H_3-19 have β-orientation. Therefore, the relative configurations on C-1, C-4, C-5, C-9, and C-11 in compound **2** were found to be the same as those of compound **1**.

Gibberosin B (**3**) was obtained as a colorless oil, $[\alpha]_D^{25}$ +32.1 (*c* 0.28, $CHCl_3$). According to the HR-ESI-MS (*m*/*z* 357.2041, $[M + Na]^+$) and ^{13}C NMR data, its molecular formula is $C_{20}H_{30}O_4Na$. Therefore, compound **3** was determined to have six degrees of unsaturation. The ^{1}H and ^{13}C NMR spectral data were very similar to those of a known compound **11**[6] (Tables 1 and 2) except that a carbon signal at δ 71.2 (C-15) in compound **11** was shifted to δ 82.2 in compound **3**. Furthermore, the ^{1}H NMR spectrum of compound **3** showed an additional signal at δ 7.51 (br s) which was considered to be a hydroperoxy proton.[14] In other words, the hydroxy group in compound **11** was replaced by a hydroperoxy group in compound **3**. The double bond between C-13 and C-14 was determined to have a *trans* geometry based on the coupling constant (J = 15.9 Hz) between H-13 (δ 6.34, d) and H-14 (δ 6.92, d). The relative configurations on C-1, C-4, C-5, C-9, and C-11 in compound **3** were found to be the same as those of compounds **2** and **11**, on the basis of the NOE correlations observed in a NOESY experiment as shown in Fig. 2.

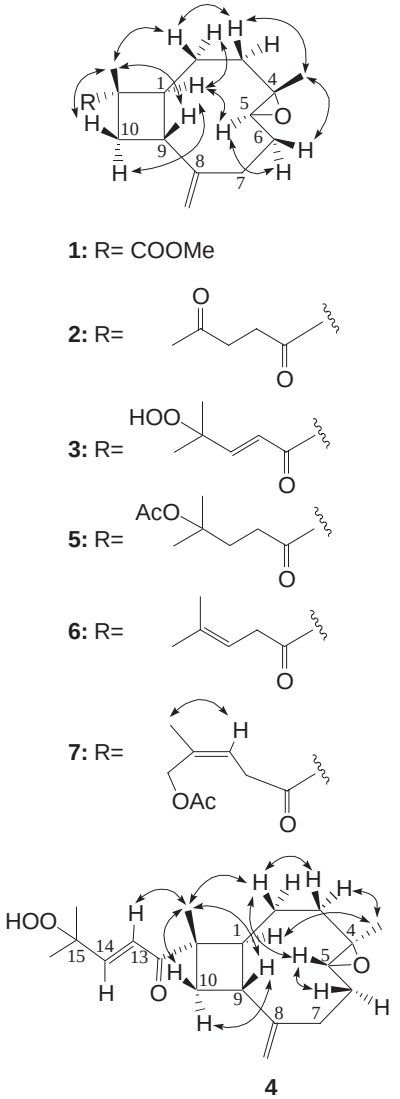

Fig. 2. Key NOESY correlations of compounds **1**–**7**.

Gibberosin C (**4**) was isolated as a colorless oil, $[\alpha]_D^{25}$ +13.3 (*c* 0.32, $CHCl_3$). Its HR-ESI-MS established the same molecular formula, $C_{20}H_{30}O_4$, as that of compound **3**. The ^{1}H and ^{13}C NMR data of compound **4** (Tables 1 and 2, respectively) are very similar to those of compound **3**. Careful inspection

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

C#	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[c]	**6**[a]	**7**[b]
1	47.2 (CH)[d]	45.4 (CH)	45.1 (CH)	48.3 (CH)	45.5 (CH)	45.3 (CH)	45.5 (CH)
2	27.6 (CH_2)	28.1 (CH_2)	28.1 (CH_2)	28.6 (CH_2)	28.1 (CH_2)	28.0 (CH_2)	28.2 (CH_2)
3	38.6 (CH_2)	38.5 (CH_2)	38.5 (CH_2)	35.8 (CH_2)	38.6 (CH_2)	38.5 (CH_2)	38.7 (CH_2)
4	59.4 (C)	59.5 (C)	59.6 (C)	60.7 (C)	59.1 (C)	59.5 (C)	59.7 (C)
5	63.4 (CH)	63.7 (CH)	63.6 (CH)	61.0 (CH)	63.7 (CH)	63.6 (CH)	63.8 (CH)
6	30.0 (CH_2)	30.2 (CH_2)	30.1 (CH_2)	36.3 (CH_2)	30.1 (CH_2)	30.1 (CH_2)	30.3 (CH_2)
7	29.7 (CH_2)	29.4 (CH_2)	29.5 (CH_2)	29.5 (CH_2)	29.6 (CH_2)	29.4 CH_2)	29.7 (CH_2)
8	150.6 (C)	150.8 (C)	150.6 (C)	151.6 (C)	151.6 (C)	150.1 (C)	150.8 (C)
9	47.8 (CH)	47.4 (CH)	47.3 (CH)	45.6 (CH)	48.5 (CH)	47.2 (CH)	47.4 (CH)
10	35.4 (CH_2)	35.0 (CH_2)	35.1 (CH_2)	37.6 (CH_2)	35.2 (CH_2)	35.2 (CH_2)	35.3 (CH_2)
11	42.0 (C)	48.2 (C)	47.7 (C)	46.8 (C)	47.3 (C)	48.4 (C)	48.7 (C)
12	16.8 (CH_3)	213.4 (C)	203.3 (C)	203.3 (C)	213.4 (C)	212.6 (C)	211.8 (C)
13	178.3 (C)	30.6 (CH_2)	123.0 (CH)	123.0 (CH)	31.3 (CH_2)	36.5 (CH_2)	35.8 (CH_2)
14	114.0 (CH_2)	36.8 (CH_2)	149.3 (CH)	149.1 (CH)	34.9 (CH_2)	116.1 (CH)	122.2 (CH)
15	17.0 (CH_3)	207.6 (C)	82.2 (C)	82.2 (C)	81.5 (C)	134.5 (C)	133.6 (C)
16		30.1 (CH_3)	24.2 (CH_3)	24.2 (CH_3)	25.9 (CH_3)	18.1 (CH_3)	63.3 (CH_2)
17		16.9 (CH_3)	24.2 (CH_3)	24.2 (CH_3)	25.9 (CH_3)	25.7 (CH_3)	21.8 (CH_3)
18		114.2 (CH_2)	16.9 (CH_3)	16.8 (CH_3)	17.1 (CH_3)	17.0 (CH_3)	17.0 (CH_3)
19		17.2 (CH_3)	114.1 (CH_2)	113.3 (CH_2)	114.2 (CH_2)	114.0 (CH_2)	114.4 (CH_2)
20			17.1 (CH_3)	22.4 (CH_3)	16.9 (CH_3)	16.8 (CH_3)	17.3 (CH_3)
OAc					170.7 (C)		171.2 (C)
					22.3 (CH_3)		21.1 (CH_3)
OMe	51.9 (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. The values are in ppm downfield from TMS.

of the ^{1}H–^{1}H COSY and HMBC spectral data of compound **4** showed that compound **4** had a planar structure similar to that of compound **3** (Fig. 1). The relative stereochemistry of compound **4** was elucidated by analysis of the vicinal ^{1}H–^{1}H coupling constants and NOE correlations observed in a NOESY experiment. The double bond between C-13 and C-14 was also determined to have a *trans* geometry based on the coupling constant ($J = 15.5$ Hz) between H-13 (δ 6.35, d) and H-14 (δ 6.94, d). In the NOESY spectrum of compound **4**, H-1 interacted with H_3-20 but not with H-5, H-9, and H_3-18, suggesting that H-1 and H_3-20 have an α-orientation and H-5, H-9, and H_3-18 have a β-orientation. Therefore, compound **4** was determined to be a C-4, C-5 diastereomer of compound **3**.

Gibberosin D (**5**) has the molecular formula $C_{22}H_{34}O_4$ based on its HR-ESI-MS and NMR spectral data (Tables 1 and 2, respectively). Comparison of the ^{13}C NMR spectral data of compound **5** with those of compound **3** (Table 1) suggested that compound **5** is another caryophyllene-derived diterpeneoid. From careful inspection on the ^{1}H and ^{13}C NMR spectral data of compound **5**, C-12 was assigned to have a saturated prenyl substituent. Also, two methyl groups (δ_H 1.44 (6H, s) and δ_C 25.9) were attached to C-15, and an acetoxy group (δ_C 22.3 and 170.7; IR absorption at 1734 cm^{-1}) was found to be attached to C-15 (δ_C 81.5; δ_H (H_3-16 and H_3-17) 1.44, 6H, s). Based on the data, the molecular framework of compound **5** was determined. In addition, the stereochemistry of compound **5** was established based on key NOE correlations (Fig. 2).

Gibberosin E (**6**) was isolated as a colorless oil, $[\alpha]_D^{25}$ +25.0 (*c* 0.44, $CHCl_3$). The molecular formula of compound **6** was determined to be $C_{20}H_{30}O_2$ based on HR-ESI-MS, and it has six degrees of unsaturation. The ^{1}H and ^{13}C NMR data of compound **6** revealed that this compound has the same ring skeleton as those of compounds **1–5**. An IR absorption at 1670 cm^{-1} corresponded to a trisubsituted double bond. In the ^{1}H NMR spectrum, no signal was observed for an acetoxy group; however, an additional olefinic proton (δ 5.28, $J = 7.8$, 7.8, 1.2 Hz), as compared to that of compound **5** was observed. Based on these findings, together with the correlations observed in the ^{1}H–^{1}H COSY and HMBC spectra (Fig. 1), compound **6** was determined to have a planar structure. The relative stereochemistry of caryophyllene-based moiety for compound **6** was found to be the same as that of compound **5**, determined from the NOE correlations observed in a NOESY spectrum (Fig. 2).

Gibberosin F (**7**) was obtained as a colorless oil, $[\alpha]_D^{25}$ +68.7 (*c* 0.16, $CHCl_3$). The molecular formula of compound **7** was determined to be $C_{22}H_{32}O_4$ based on HR-ESI-MS. The ^{1}H and ^{13}C NMR data are very similar to those of compound **6**. By comparison of the NMR spectral data of compound **6** with those of compound **7**, it was found that a vinyl methyl attached to C-15 in compound **6** was replaced by an acetoxy-bearing methylene in compound **7**. The HMBC cross peaks from H_3-17 to C-15, and H_2-16 to C-15 and the acetoxy carbonyl carbon confirmed the above observation. The double bound between C-14 and C-15 was determined to have a *cis* geometry based on the NOE interaction between H-14 and H_3-17.

The caryophyllene moiety in compounds **2**, **3**, and **5–7** now are known to have the same relative stereochemistry at the asymmetric carbons, C-1, C-4, C-5, C-9, and C-11 as that in compound **1**. Thus, the relative configurations of these five compounds were assigned to be 1*S**,4*S**,5*S**,9*R**,11*S**. How-

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

H#		**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[c]	**6**[a]	**7**[b]
1		2.46 dd (9.6, 9.6)[d]	2.48 dd (10.0, 10.0)	2.46 dd (10.0, 10.0)	2.43 dd (10.0, 10.0)	2.42 m	2.43 dd (9.6, 9.6)	2.45 m
2	α	1.82 m	1.81 m	1.83 m	1.74 m	1.78 m	1.80 m	1.85 m
	β	1.51 m	1.54 m	1.59 m	1.57 m	1.57 m	1.53 m	1.57m
3	α	1.03 m	1.10 ddd (12.8, 12.8, 3.6)	1.09 m	1.90 m	1.09 m	1.07 ddd (12.8, 12.8, 4.8)	1.07 ddd (13.0, 13.0, 5.0)
	β	2.09 m	2.09 ddd (12.8, 12.8, 3.6)	2.14 m	1.72 m	2.10 m	2.09 ddd (12.4, 12.4, 3.6)	2.08 m
5		2.89 dd (10.4, 4.4)	2.93 dd (10.4, 3.6)	2.89 dd (10.4, 3.6)	3.00 dd (11.5, 2.5)	2.89 dd (10.5, 3.6)	2.88 dd (10.4, 4.0)	2.89 dd (10.0, 3.5)
6	α	2.27 m	2.31 m	2.28 m	2.57 m	2.21 m	2.27 m	2.29 m
	β	1.37 m	1.32 m	1.30 m		1.29 m	1.30 m	1.29 m
7	α	2.38 m	2.34 m	2.30 m	2.24 m	2.20 m	2.32 m	2.30 m
	β	2.12 m	2.17 m	2.16 m	2.14 m	2.15 m	2.16 m	2.15 m
9		2.68 q (10.0)	2.69 m	2.73 q (9.0)	2.4 q (9.0)	2.68 q (9.6)	2.69 q (10.0)	2.70 m
10	α	2.31 m	2.24 m	2.17 m	2.17 m	2.17 m	2.22 m	2.14 m
	β	1.76 m	1.77 m	1.87 m	2.04 m	1.77 m	1.77 m	1.83 m
12		1.32 s						
13			2.66 m	6.34 d (15.9)	6.35 d (15.5)	2.45 m	3.06 dd (18.0, 7.8)	3.21 dd (18.0, 6.5)
							3.13 dd (18.0, 7.8)	3.28 dd (18.0, 6.5)
14		4.93 s	2.72 m	6.92 d (15.9)	6.94 d (15.5)	2.02 m	5.28 dd m[e] (7.8, 7.8, 1.2)	5.58 dd (6.5, 6.5)
		5.05 s						
15		1.21 s						
16			2.19 s	1.38 s	1.39 s	1.44 s	1.61 s	4.52 s
17			1.33 s	1.39 s	1.40 s	1.44 s	1.75 s	1.81 s
18			4.90 s	1.31 s	1.28 s	1.31 s	1.32 s	1.32 s
			5.01 s					
19			1.20 s	4.91 s	5.03 s	4.91 s	4.90 s	4.90 s
				5.00 s	5.08 s	5.00 s	5.00 s	5.00 s
20				1.21 s	1.25 s	1.18 s	1.20 s	1.20 s
OAc						1.97 s		2.06 s
OMe		3.69 s						
OOH				7.51 s	7.51 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. The values are in ppm downfield from TMS. d) *J* value (in Hz) in parentheses. e) m is equal to septet.

ever, compound **4** was assumed to possess a (1*S**,4*R**,5*R**,9*R**,11*S**) configuration as H-1 and H_3-20 were found to be positioned on the α face.

Cytotoxicity of metabolites **1–7** toward a limited panel of cancer cell lines was evaluated. Compound **3** had weak cytotoxicity towards Hep G2 (Human hepatocellular carcinoma), A549 (human lung carcinoma), and MDA-MB-231 (human breast carcinoma) cell lines with IC_{50}'s of 18.7, 18.5, and 15.2 μg mL^{-1}, respectively. Also, metabolite **6** had weak cytotoxicity (IC_{50} 16.3 μg mL^{-1}) towards MDA-MB-231. The other metabolites were inactive toward the growth of the above three cancer cell lines.

Experimental

General Experimental Procedures. 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 ^{1}H and 75 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. 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) 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, or a Hitachi L-7400 UV detector and with the Merck Hibar Si-60 column (250 × 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 (specimen No. 20040621-5).

Extraction and Isolation. The soft coral *S. gibberosa* (1.3 kg fresh wt) was collected and freeze dried. The freeze-dried material was minced and extracted exhaustively with EtOAc. The EtOAc extract (15.4 g) was fractionated by using open column chromatography on silica gel using hexane and hexane–EtOAc mixtures of increasing polarity to yield 32 fractions. Fraction 6, eluted with hexane/EtOAc (8:1), was subjected to Sephadex LH-20 column (2×90 cm) using Acetone, which was followed by normal phase HPLC eluted with hexane/acetone (9:1 to 7:1) to afford compounds **1** (0.7 mg) and **6** (1.1 mg). Fraction 15, eluted with hexane/EtOAc (5:1), was further purified by silica gel column, using hexane/EtOAc (5:1) as eluent to afford compound **2** (10.4 mg). A combined mixture of fractions 17–19 was purified by normal phase HPLC by using hexane/EtOAc (4:1) and then hexane/acetone (5:1) as eluents to afford compound **5** (3.5 mg). Fraction 20, eluted with hexane/EtOAc (3:1), was further purified by normal phase HPLC using hexane/acetone (5:1 to 3:1) to give compounds **7** (1.0 mg), **4** (0.8 mg), and **3** (1.1 mg).

Methyl-(1*S,4*S**,5*S**,9*R**,11*S**)-4,5-epoxycaryophyllen-13-oate (1):** Colorless oil; $[\alpha]_D^{25}$ +42.8 (*c* 0.16, $CHCl_3$); IR (KBr) ν_{max} 1728, 1653 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; ESI-MS *m/z* 287 ($[M + Na]^+$); HR-ESI-MS *m/z* 287.1622 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_3Na$, 287.1623).

Gibberosin A (2): Colorless oil; $[\alpha]_D^{25}$ +43.2 (*c* 1.04, $CHCl_3$); IR (KBr) ν_{max} 1716, 1701, 1653 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; ESI-MS *m/z* 327 ($[M + Na]^+$); HR-ESI-MS *m/z* 327.1934 $[M + Na]^+$ (calcd for $C_{19}H_{28}O_3Na$, 327.1936).

Gibberosin B (3): Colorless oil; $[\alpha]_D^{25}$ +32.1 (*c* 0.28, $CHCl_3$); IR (KBr) ν_{max} 3564, 1716, 1684, 1649 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; ESI-MS *m/z* 357 ($[M + Na]^+$); HR-ESI-MS *m/z* 357.2041 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_4Na$, 357.2042).

Gibberosin C (4): Colorless oil; $[\alpha]_D^{25}$ +13.3 (*c* 0.32, $CHCl_3$); IR (KBr) ν_{max} 3568, 1716, 1684, 1653 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; ESI-MS *m/z* 357 ($[M + Na]^+$); HR-ESI-MS *m/z* 357.2041 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_4Na$, 357.2042).

Gibberosin D (5): Colorless oil; $[\alpha]_D^{25}$ +64.3 (*c* 0.28, $CHCl_3$); IR (KBr) ν_{max} 1734, 1701, 1653 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; ESI-MS *m/z* 385 ($[M + Na]^+$); HR-ESI-MS *m/z* 385.2352 $[M + Na]^+$ (calcd for $C_{22}H_{34}O_4Na$, 385.2355).

Gibberosin E (6): Colorless oil; $[\alpha]_D^{25}$ +25.0 (*c* 0.44, $CHCl_3$); IR (KBr) ν_{max} 1716, 1670, 1653 cm^{-1}; ^1H NMR 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 F (7): Colorless oil; $[\alpha]_D^{25}$ +68.7 (*c* 0.16, $CHCl_3$); IR (KBr) ν_{max} 1734, 1716, 1670, 1653 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; ESI-MS *m/z* 383 ($[M + Na]^+$); HR-ESI-MS *m/z* 383.2197 $[M + Na]^+$ (calcd for $C_{22}H_{32}O_4Na$, 383.2198).

Cytotoxicity Testing. Compounds were assayed for cytotoxicity against Hep G2, A549, and MDA-MB-231 cancer cells using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method.[15] 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 days in culture, attached cells were incubated with MTT (0.5 mg mL^{-1}, 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.

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