

Oxygenated Cembranoids from a Formosan Soft Coral *Sinularia gibberosa*

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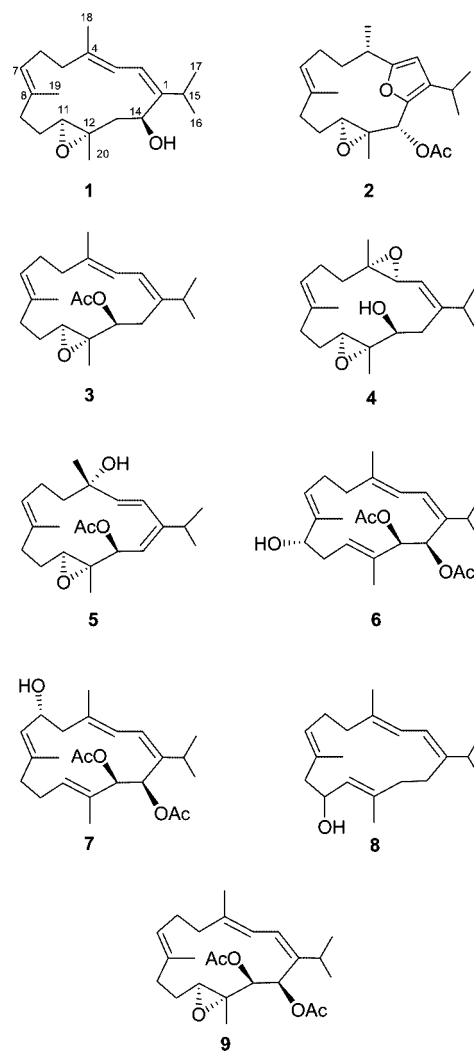
Chemical investigation of a Formosan soft coral, *Sinularia gibberosa*, has led to the isolation of eight oxygenated cembranoids, **1–8**, including seven new compounds, gibberosenes A–G (**2–8**). None of these compounds were found to be cytotoxic toward a limited panel of cancer cell lines. Compound **1** significantly inhibited the accumulation of the pro-inflammatory COX-2 protein of the LPS-stimulated RAW264.7 macrophage cells.

Marine terpenoids are considered to be metabolites of great interest due to their unique structures and wide range of biological activities.¹ Cembranoids^{1–7} and norcembranoids^{1,8–11} were found to comprise the main terpenoidal content in octocorals. Our previous chemical investigations on soft corals of the genus *Sinularia* have afforded several cembrane-,¹² norcembrane-,^{8–11} and xeniaphyllane-based diterpenoids.^{13,14} Some of these metabolites exhibit cytotoxic activity against the growth of various cancer cell lines.^{9–13} We have previously isolated three polyoxygenated sterols from a Formosan soft coral, *Sinularia gibberosa*, Tixier-Durivault (*Alcyoniidea*).¹⁵ Our further chemical examination of this soft coral has led to the isolation of seven new oxygenated cembranoids, gibberosenes A–G (**2–8**), along with a known metabolite, (+)-11,12-epoxysarcophytol A (**1**).^{16–18} The structures of the new metabolites were determined on the basis of extensive spectroscopic analysis, including 2D NMR (¹H–¹H COSY, HMQC, HMBC, and NOESY) spectroscopy. The macrocyclic rings of all structures, except that of **4**, were found to possess a conjugated diene. None of the metabolites (**1–8**) were found to be cytotoxic (IC₅₀'s > 20 μg/mL) to human tumor cell lines, including liver (Hep G2), breast (MCF-7 and MDA-MB-23), and lung (A-549) carcinoma cells. At a concentration of 10 μM, only compound **1** demonstrated an ability to inhibit the accumulation of the pro-inflammatory proteins inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophage cells.

Results and Discussion

The sliced bodies of the soft coral *S. gibberosa* were extracted exhaustively with EtOH, and then the concentrated EtOH extract was partitioned between CH₂Cl₂ and H₂O. The combined CH₂Cl₂-soluble fraction was concentrated under reduced pressure, and the residue was repeatedly chromatographed to yield diterpenoids **1–8**. All compounds were obtained as colorless oils. Compound **1** was found to be the known compound (+)-11,12-epoxysarcophytol A.^{16–18}

The new metabolite gibberosene A (**2**) exhibited a pseudomolecular ion peak in the HRESIMS at *m/z* 383.2196 [M + Na]⁺, establishing the molecular formula C₂₂H₃₂O₄ and seven degrees of



unsaturation. An acetoxy group (IR 1748 cm⁻¹; δ_H 2.08, 3H, s; δ_C 170.5, qC and 20.8, CH₃) and a trisubstituted epoxide (δ_H 3.08, 1H, dd, *J* = 9.5, 4.0 Hz; δ_C 60.0, qC and 57.6, CH) were revealed in **2**. Comparison of the NMR data (Tables 1 and 2) of **2** with those of **1** suggested that both compounds are related epoxycembranoids. One proton (δ 5.91, s) attached to an olefinic carbon (δ 103.6, CH) was found to exhibit HMBC correlations with three sp² quaternary carbons (δ 159.8, 140.5, and 131.0), indicating the

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Table 1. ^1H NMR Data for **1** and Gibberosenes A–E (**2–5**)

H#	1 ^a	2 ^a	3 ^b	4 ^a	5 ^a
2	5.99 d (10.5) ^c	5.91 s	6.02 d (11.0)	5.24 d (5.0)	6.08 d (16.5)
3	5.76 d (10.5)		5.81 d (11.0)	3.55 d (5.0)	5.80 d (15.5)
4		2.77 m			
5 α	2.18 2H, m	1.76 2H, m	2.21 m	2.01 m	1.82 2H, m
5 β			2.05 m	1.49 m	
6 α	2.22 2H, m	2.36 m	2.25 m	2.18 m	2.21 2H, m
6 β		1.76 m	2.12 m	1.99 m	
7	5.11 br dd (5.5, 5.5)	4.79 m	5.21 dd (8.6, 5.2)	5.27 dd (7.0, 5.5)	5.05 m
9 α	2.26 m	2.10 m	2.25 m	2.31 m	2.15 dd (10.0, 7.5)
9 β	2.09 m	1.89 ddd (14.0, 10.0, 3.5)	2.19 m	2.07 m	2.21 m
10 α	1.86 dddd (13.5, 13.5, 7.5, 3.5)	2.17 dd (14.5, 4.0)	1.63 m	2.20 m	1.62 m
10 β	1.50 dddd (13.5, 13.5, 6.0, 3.5)	1.36 m	1.52 m	1.39 m	1.91 m
11	3.19 dd (7.0, 7.0)	3.08 dd (9.5, 4.0)	3.01 dd (9.0, 4.2)	3.03 dd (11.0, 3.5)	2.63 d (9.5)
13 α	1.98 dd (10.5, 5.0)				5.80 d (9.5)
13 β	2.11 dd (10.5, 7.0)	5.91 s	5.29 dd (9.0, 4.2)	3.88 dd (11.0, 3.5)	
14 α	4.73 dd (7.0, 5.0)		2.70 dd (14.4, 4.2)	2.45 dd (14.0, 11.0)	5.06 d (9.0)
14 β			1.95 dd (14.4, 9.0)	2.26 dd (14.0, 3.5)	
15	2.67 septet (6.5)	2.92 septet (6.5)	2.45 septet (6.5)	2.37 septet (7.0)	2.42 septet (7.0)
16	1.07 3H, d (6.5)	1.15 3H, d (6.5)	1.03 3H, d (6.5)	1.07 3H, d (7.0)	1.04 3H, d (7.0)
17	1.09 3H, d (6.5)	1.19 3H, d (6.5)	1.05 3H, d (6.5)	1.11 3H, d (7.0)	1.01 3H, d (7.0)
18	1.74 3H, s	1.20 3H, d (7.5)	1.74 3H, s	1.21 3H, s	1.37 3H, s
19	1.59 3H, s	1.62 3H, s	1.48 3H, s	1.65 3H, s	1.59 3H, s
20	1.30 3H, s	1.32 3H, s	1.27 3H, s	1.34 3H, s	1.27 3H, s
OAc		2.08 3H, s	2.10 3H, s		2.09 3H, s

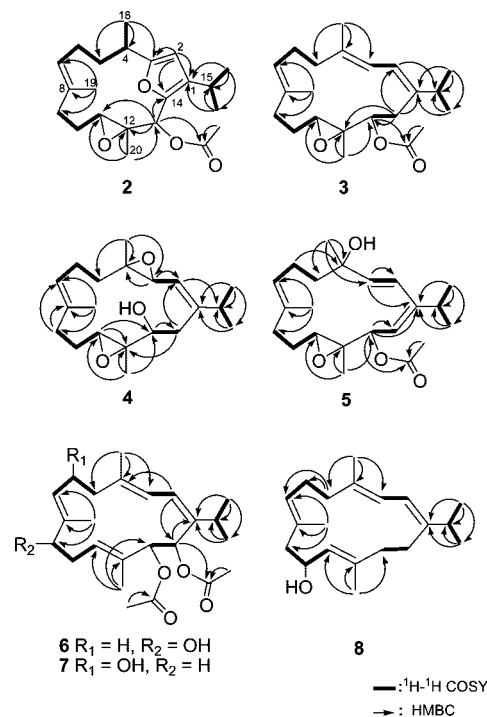
^a Spectra recorded at 500 MHz. ^b Spectra recorded at 300 MHz in CDCl_3 at 25 °C. ^c The J values are in Hz in parentheses.

Table 2. ^{13}C NMR Data for **1** and Gibberosenes A–D (**2–5**)

C#	1 ^a	2 ^b	3 ^a	4 ^b	5 ^a
1	148.5 (qC) ^c	131.0 (qC)	142.3 (qC)	146.0 (qC)	149.1 (qC)
2	118.4 (CH)	103.6 (CH)	120.4 (CH)	123.1 (CH)	122.6 (CH)
3	119.6 (CH)	159.8 (qC)	121.8 (CH)	60.9 (CH)	141.6 (CH)
4	136.8 (qC)	34.7 (CH)	136.6 (qC)	61.3 (qC)	73.9 (qC)
5	38.4 (CH ₂)	35.5 (CH ₂)	39.5 (CH ₂)	37.2 (CH ₂)	42.3 (CH ₂)
6	25.0 (CH ₂)	26.6 (CH ₂)	25.9 (CH ₂)	22.6 (CH ₂)	23.8 (CH ₂)
7	127.0 (CH)	126.6 (CH)	126.6 (CH)	126.4 (CH)	130.4 (CH)
8	133.7 (qC)	132.1 (qC)	133.9 (qC)	134.4 (qC)	132.2 (qC)
9	36.5 (CH ₂)	36.7 (CH ₂)	36.7 (CH ₂)	36.7 (CH ₂)	37.2 (CH ₂)
10	24.2 (CH ₂)	25.9 (CH ₂)	24.4 (CH ₂)	23.8 (CH ₂)	24.3 (CH ₂)
11	58.7 (CH)	57.6 (CH)	57.7 (CH)	57.1 (CH)	60.7 (CH)
12	60.0 (qC)	60.0 (qC)	60.9 (qC)	63.8 (qC)	62.5 (qC)
13	42.2 (CH ₂)	67.8 (CH)	74.2 (CH)	68.6 (CH)	73.9 (CH)
14	65.8 (CH)	140.5 (qC)	30.4 (CH ₂)	34.7 (CH ₂)	117.9 (CH)
15	27.7 (CH)	24.4 (CH)	32.5 (CH)	32.6 (CH)	33.9 (CH)
16	24.3 (CH ₃)	23.9 (CH ₃)	23.1 (CH ₃)	22.8 (CH ₃)	22.2 (CH ₃)
17	23.9 (CH ₃)	23.9 (CH ₃)	22.2 (CH ₃)	21.6 (CH ₃)	21.8 (CH ₃)
18	17.3 (CH ₃)	21.3 (CH ₃)	16.7 (CH ₃)	18.2 (CH ₃)	28.2 (CH ₃)
19	15.1 (CH ₃)	14.8 (CH ₃)	14.9 (CH ₃)	14.7 (CH ₃)	15.1 (CH ₃)
20	19.5 (CH ₃)	15.6 (CH ₃)	16.0 (CH ₃)	14.7 (CH ₃)	15.3 (CH ₃)
OAc		20.8 (CH ₃)	21.2 (CH ₃)	21.8 (CH ₃)	
		170.5 (qC)	170.7 (qC)		170.5 (qC)

^a Spectra recorded at 75 MHz. ^b Spectra recorded at 125 MHz in CDCl_3 at 25 °C. ^c Attached protons were determined by DEPT experiments. The values are in ppm downfield from TMS.

presence of a trisubstituted furan ring in **2**. The presence of a trisubstituted double bond was elucidated from the two sp^2 carbon signals at δ 132.1 (qC) and 126.6 (CH) and the corresponding proton signal at δ 4.79. Therefore, **2** was defined as a cembranoid with an acetoxy group, an epoxide, a trisubstituted olefin, and a trisubstituted furan moiety. From the ^1H – ^1H COSY spectrum (Figure 1), a partial structure of a proton spin system extending from H-7 (δ 4.79, 1H, m) to H₃-18 (δ 1.20, 3H, d, $J = 7.5$ Hz) through H-4 (δ 2.77, 1H, m) could be established, assigning a secondary methyl group at C-4. Furthermore, it was also found that the chemical shifts of C-7 to C-12 were quite similar to those of **1**, implying the location of an epoxide at C-11 and C-12 in **2**. Moreover, the HMBC correlations found from the oxymethine proton at δ 5.91 (1H, s) to the ester carbonyl carbon (δ 170.5, qC), epoxide carbons (δ 57.6, CH, C-11 and 60.0, qC, C-12), and one of the oxygenated furanoid carbons (δ 140.5, qC), and from H₃-18 to the other oxygenated furanoid carbon (δ 159.8, qC), revealed

**Figure 1.** ^1H – ^1H COSY and HMBC correlations for **2–8**.

the C-13 position of the acetoxy group and the presence of the 3,14-oxygen-bound furan. On the basis of the above findings and other detailed correlations in the COSY and HMBC spectra, the planar structure of **2** was established, as illustrated in Figure 1.

The stereochemistry of **2** was determined on the basis of NOE correlations and by comparison of NMR data with **1**. The similarity in chemical shifts (Tables 1 and 2) of the epoxy carbons and protons (C-11, C-12, H-11, and H₃-20) in **1** and **2**, and the biosynthetic relation of these two metabolites, suggested an α -orientation of the epoxide oxygen in **2**. Thus, a β -orientation of H₃-20 and an α -orientation of H-11 were proposed. Due to the overlapping of H-13 and H-2 signals (δ 5.91, 2H, s) on measuring the ^1H NMR in CDCl_3 , we also measured the NOESY spectrum of **2** in C_6D_6 , which showed signals of both H-13 and H-2 at δ 6.31 (s) and 5.78 (s), respectively. Therefore, the NOE interaction (measured in C_6D_6)

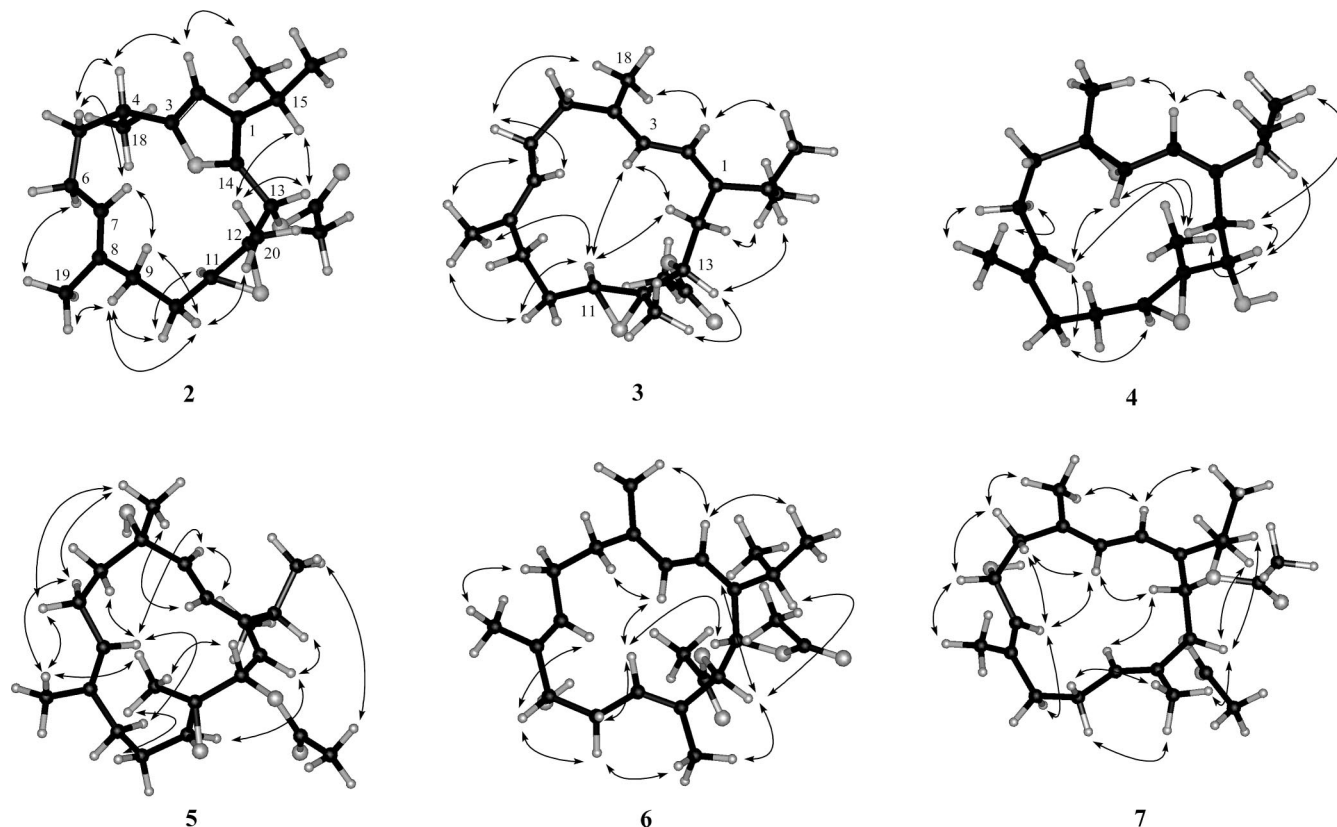


Figure 2. Computer-generated model for 2–7 using MM2 force field calculations and key NOE correlations.

displayed by the β -oriented methyl at C-12 (δ 1.28, s) with the acetoxymethine proton H-13 reflected the α -orientation of the acetoxy group at C-13. Moreover, the consecutive NOE correlations (measured in CDCl_3) of H-11 (δ_{H} 3.08, 1H, dd, $J = 9.5, 4.0$ Hz) with H-10 α (δ 2.17, dd, $J = 14.5, 4.0$ Hz), H-10 α with H-9 α (δ 2.10, m) and not with H-9 β (δ 1.89, ddd, $J = 14.0, 10.0, 3.5$ Hz), and H-9 α with H₃-19 (δ 1.62, s), H₃-19 with H-6 α (δ 2.36, m) and the correlations (measured in C_6D_6) of H-4 (δ 2.55, ddq, $J = 14.0, 7.0, 3.5$ Hz) with H-5 β (δ 1.59, m), H-5 β with H-7 (δ 4.88, dd, $J = 7.0, 7.0$ Hz), H-7 with H-9 β (δ 1.81, ddd, $J = 14.0, 11.0, 3.0$ Hz), H-9 β with H-10 β (δ 1.32, m), and H-10 β with H₃-20 (δ 1.28, s) indicate the α -orientation of H₃-18. The *E* geometry of the trisubstituted C-7/C-8 double bond was also assigned from the NOE correlation (measured in C_6D_6) of H₃-19 (δ 1.43, s) with H-6 (δ 2.27, ddd, $J = 14.0, 8.0, 8.0$ Hz) and from the higher field chemical shift of C-19 (δ 14.8, in CDCl_3). On the basis of the above findings and other detailed NOE correlations (Figure 2), the structure of gibberosene A (**2**) was deduced as (4*S**,11*S**,12*R**,13*R**,7*E*)-13-acetoxy-3(14),11(12)-diepoxycembra-2,7,14-triene.

Gibberosene B (**3**) was found to have the molecular formula $\text{C}_{22}\text{H}_{34}\text{O}_3$, as indicated from the HRESIMS (m/z 369.2405 [$\text{M} + \text{Na}]^+$) and NMR data (Tables 1 and 2). The IR (1740 cm^{-1}), EIMS (m/z 286 [$\text{M} - \text{AcOH}]^+$), and NMR data (δ_{H} 2.10, 3H, s; δ_{C} 170.7, qC and 21.2, CH_3) suggested the presence of an acetoxy group. A tetrasubstituted diene (δ_{H} 6.02 and 5.81, each 1H, d, $J = 11.0$ Hz; δ_{C} 142.3, qC, 136.6, qC, 121.8, CH, and 120.4, CH), a trisubstituted double bond (δ_{H} 5.21, 1H, dd, $J = 8.6, 5.2$ Hz; δ_{C} 133.9, qC and 126.6, CH), and a trisubstituted epoxide (δ_{H} 3.01, 1H, dd, $J = 9.0, 4.2$ Hz; δ_{C} 60.9, qC and 57.7, CH) were also evident. The HMBC correlations (Figure 1) between H₃-20 (δ 1.27, s) and epoxy carbons C-11, C-12, and the oxymethine carbon at δ 74.2 (C-13), and from H-13 (δ 5.29, dd, $J = 9.0, 4.2$ Hz) to a carbonyl carbon (δ 170.7), confirmed the C-13 location of the acetoxy group. These findings and other detailed COSY and HMBC correlations observed for **3** established the gross structure of **3**, as shown in Figure 1. The NOE correlations displayed by the β -oriented methyl on the epoxide (H₃-

20) with H-13 disclosed the α -orientation of the 13-OAc, as shown in Figure 2. Moreover, the strong NOE interactions exhibited by H-2 with both methyls at C-15 and C-4, and H₃-19 with H-6 (Figure 2), revealed the *E* geometries of the double bonds at C-1/C-2, C-3/C-4, and C-7/C-8. Thus, gibberosene B (**3**) was unambiguously identified as (11*S**,12*R**,13*S**,1*E*,3*E*,7*E*)-13-acetoxy-11,12-epoxycembra-1,3,7-triene.

Gibberosene C (**4**) showed the pseudomolecular ion peak [$\text{M} + \text{Na}]^+$ at m/z 343.2250 in the HRESIMS, corresponding to the molecular formula $\text{C}_{20}\text{H}_{32}\text{O}_3$ and five degrees of unsaturation. The IR absorption at 3350 cm^{-1} indicated the presence of a hydroxy group in **4**. Comparison of the NMR data of **4** with those of **3** revealed the replacement of one double bond (δ_{H} 5.81, 1H, d, $J = 11.0$ Hz; δ_{C} 136.6, qC and 121.8, CH) in **3** by a trisubstituted epoxide moiety in **4** (δ_{H} 3.55, 1H, d, $J = 5.0$ Hz; δ_{C} 61.3, qC and 60.9, CH). The proton of this epoxide was found to exhibit a $^1\text{H}-^1\text{H}$ COSY correlation with an olefinic proton (δ 5.24, d, $J = 5.0$ Hz, H-2), which in turn was found to be HMBC-correlated to the isopropyl methine carbon (δ 32.6, CH, C-15). Therefore, the second epoxide of **4** should be positioned at C-3/C-4. Moreover, the HMBC correlation found from the epoxide-bound H₃-20 to the oxymethine carbon at δ 68.6 assigned the C-13 location of the hydroxy group. These and other detailed COSY and HMBC correlations (Figure 1) were employed to establish the planar structure of **4**. The *E* geometries of the two trisubstituted double bonds at C-1/C-2 and C-7/C-8 were indicated by the NOE interactions of H-2 with the methyls at C-15 and H₃-19 with H₂-6, respectively. The similar splitting patterns and *J* values of H-13 in both **3** and **4**, and the NOE interaction of H₃-20 with H-13, assigned the α -orientation of the 13-OH. Moreover, the epoxide at C-3/C-4 was established as a *trans*-oxacyclopropane from the strong NOE correlations displayed by H-2 with the methyls at C-4 and C-15, and those observed for H-3 with H-14 α and H-7, but not with H₃-18. These results, together with other detailed analysis of NOE correlations of **4** (Figure 2), unambiguously established the structure of

Table 3. ^1H and ^{13}C NMR Data for Gibberosenes E–G (6–8)

C/H	6		7		8	
	$^1\text{H}^a$	$^{13}\text{C}^b$	$^1\text{H}^a$	$^{13}\text{C}^b$	$^1\text{H}^a$	$^{13}\text{C}^b$
1		139.2 (qC)		139.2 (qC)		146.9 (qC)
2	6.21 d (11.6) ^c	124.1 (CH) ^d	6.21 s	124.2 (CH)	6.03 d (11.1)	118.9 (CH)
3	6.07 d (11.6)	121.9 (CH)	6.21 s	123.8 (CH)	5.81 d (11.1)	121.4 (CH)
4		137.8 (qC)		134.7 (qC)		135.0 (qC)
5	2.17 m 2.24 m	39.7 (CH ₂)	2.26 dd (11.7, 3.0) 2.43 dd (11.7, 11.7)	49.2 (CH ₂)	2.09–2.16 2H, m	38.8 (CH ₂)
6	2.22–2.26 2H, m	25.3 (CH ₂)	4.49 m	67.7 (CH)	2.15–2.22 2H, m	25.0 (CH ₂)
7	5.34 br dd (6.0, 6.0)	124.2 (CH)	5.12 d (8.5)	128.9 (CH)	5.00 br dd (5.0, 5.0)	127.1 (CH)
8		136.9 (qC)		137.4 (qC)		131.3 (qC)
9	4.17 br s	74.7 (CH)	2.16 m; 2.24 m	39.0 (CH ₂)	2.13 m; 2.46 br d (12.5)	47.9 (CH ₂)
10	2.39 m; 2.42 m	32.2 (CH ₂)	2.10 m; 2.16 m	24.8 (CH ₂)	4.54 ddd (12.5, 9.0, 3.7)	66.6 (CH)
11	5.53 br dd (6.6, 6.6)	124.2 (CH)	5.44 m	128.7 (CH)	5.15 d (9.0)	128.4 (CH)
12		133.4 (qC)		131.3 (qC)		140.7 (qC)
13	5.39 d (9.2)	77.3 (CH)	5.34 d (10.0)	76.0 (CH)	2.05 m; 2.23 m	40.0 (CH ₂)
14	6.14 d (9.2)	72.8 (CH)	6.16 d (10.0)	72.7 (CH)	2.23 m; 2.36 m	28.5 (CH ₂)
15	2.52 septet (6.6)	29.8 (CH)	2.53 septet (6.9)	28.8 (CH)	2.34 m	34.4 (CH)
16	1.04 3H, d (6.6)	25.3 (CH ₃)	1.01 3H, d (6.9)	25.8 (CH ₃)	1.05 3H, d (6.3)	22.6 (CH ₃)
17	1.05 3H, d (6.6)	23.7 (CH ₃)	1.07 3H, d (6.9)	23.3 (CH ₃)	1.07 3H, d (6.3)	21.9 (CH ₃)
18	1.75 3H, s	16.3 (CH ₃)	1.78 3H, s	17.2 (CH ₃)	1.74 3H, s	17.3 (CH ₃)
19	1.44 3H, s	13.4 (CH ₃)	1.41 3H, s	15.6 (CH ₃)	1.57 3H, s	16.8 (CH ₃)
20	1.65 3H, s	16.3 (CH ₃)	1.63 3H, s	17.5 (CH ₃)	1.69 3H, s	17.1 (CH ₃)
OAc	2.01 3H, s	21.1 (CH ₃)	2.01 3H, s	21.1 (CH ₃)		
		169.9 (qC)		170.0 (qC)		
	2.05 3H, s	21.1 (CH ₃)	2.05 3H, s	21.2 (CH ₃)		
		170.5 (qC)		170.5 (qC)		

^a Spectra recorded at 300 MHz in CDCl₃. ^b Spectra recorded at 75 MHz in CDCl₃. ^c *J* values (in Hz) parentheses. ^d Attached protons were deduced by DEPT experiments.

gibberosene C (**4**) as (3*S**,4*S**,11*S**,12*S**,1*E*,7*E*)-3(4),11(12)-diepoxy-13-hydroxycembra-1,7-diene.

Gibberosene D (**5**) was found to have the molecular formula C₂₂H₃₄O₄, as revealed from the HRESIMS (*m/z* 385.2353 [M + Na]⁺) and NMR data (Tables 1 and 2), implying six degrees of unsaturation. The IR absorptions at 1734 and 3350 cm⁻¹ and the ion peaks at *m/z* 303 [M – AcOH + H]⁺ and 285 [M – AcOH – H₂O + H]⁺ indicated the presence of one acetoxy and one hydroxy group. Moreover, the NMR data (Tables 1 and 2) designated one trisubstituted epoxide (δ_{H} 2.63, 1 H, d, *J* = 9.5 Hz; δ_{C} 62.5, qC and 60.7, CH) and three olefins, including one *trans* 1,2-disubstituted double bond (δ_{H} 6.08 and 5.80, each 1H, d, *J* = 16.5 and 15.5 Hz; δ_{C} 122.6, CH, 141.6, CH). As in the case of **3**, similar HMBC correlations from H₃-20 (δ 1.27, 3H, s) to the oxymethine carbon (δ 73.9, CH, C-13) and from H-13 (δ 5.80, 1H, d, *J* = 9.5 Hz) to the carbonyl carbon (δ 170.5, qC) and C-1 (δ 149.1) assigned the C-13 position of the acetoxy group. One olefinic double bond was found to be located at C-14/C-1 from the ^1H – ^1H COSY correlations between H-13 and the olefinic proton at δ 5.06 (1H, d, *J* = 9.0 Hz, H-14), while the other (a 1,2-disubstituted double bond) was found to be positioned at C-2/C-3, as H-3 (δ 5.80) exhibited HMBC correlations with both C-1 and C-2 (δ 122.6, CH). Thus, these two double bonds constitute a 1,3-butadiene moiety from C-3 (δ 141.6, CH) to C-2 and then to C-1 and C-14. The HMBC correlations from methyl protons resonating at δ 1.37 (s) to an oxygenated carbon C-4 (δ 73.9, qC) and C-3 revealed the C-4 position of this methyl group. The epoxide should be located at C-11/C-12, as H₃-20 showed HMBC correlations not only to C-13 but also to the epoxy carbons C-11 and C-12 (Figure 1). Thus, the gross structure of **5** was established as 11,12-epoxy-4-hydroxycembra-2,7,14-triene.

The *Z* geometry of the trisubstituted double bond at C-14/C-1 in **5** was established from the NOE interactions of H-14 with the isopropyl proton H-15. As in the cases of **2**–**4**, the NOE interaction observed for H-13 with H₃-20 but not with the epoxide H-11 indicated the α -orientation of the C-13 acetoxy group, as shown in Figure 2. Moreover, the NOESY spectrum showed correlations between H-3 and H-7, H-2 and H₃-18, H₃-18 and H₂-6, H₂-6 and H₃-19, and H₃-19 and H₃-20, revealing the β -orientation of the

methyl substitution at C-4. The above observations established the structure of gibberosene D (**5**) as (4*S**,11*S**,12*R**,13*S**,2*E*,7*E*,14*Z*)-13-acetoxy-11,12-epoxy-4-hydroxycembra-2,7,14-triene.

The related metabolite gibberosene E (**6**) has the molecular formula C₂₄H₃₆O₅, as indicated by the HRESIMS (*m/z* 427.2462, [M + Na]⁺) and NMR data (Table 3). It was found that **6** differs from **1**–**5** by the presence of an additional acetoxy group (IR 1743 cm⁻¹ and ESIMS *m/z* 307 [M – 2 AcOH + Na]⁺) in **6**. Moreover, the NMR data revealed the presence of four trisubstituted double bonds and the absence of an epoxide group. Again, two of these double bonds represented a conjugated diene moiety, as shown in the structure of **6** (δ_{H} 6.21 and 6.07, each 1H, d, *J* = 11.6 Hz; δ_{C} 139.2, qC, 137.8, qC, 124.2, CH, and 121.9, CH). The interpretation of the ^1H – ^1H COSY and HMBC correlations (Figure 1) were successively used to determine the positions of the hydroxy group, double bonds, and the acetoxy groups and resulted in the establishment of the planar structure of **6** as 13,14-diacetoxy-9-hydroxycembra-1,3,7,11-tetraene.

On the basis of the biogenetic relationship of **6** with **2**–**5**, it was assumed that in **6** the acetoxy group at C-13 has the same orientation as those of **2**–**5**, as shown in Figure 2. It was found that H-13 exhibited NOE correlations with H-15 and H₃-20, but not with H-14, which further correlated with H-11 but not with H₃-20. Thus, both H-13 and H₃-20 should be positioned on the β -face and H-14 should be situated on the α -face. This was further supported by comparison of the splitting patterns of H-13 and H-14 of **6** (d, *J* = 9.2 Hz) with those of the known cembranoid **9** (d, *J* = 9.7 Hz, for both H-13 and H-14), isolated from the soft coral *Cladiella kashmani* (Alcyoniidea).¹⁹ Moreover, the NOE correlations (Figure 2) observed from H₃-20 to H-10 β , H-10 β to H-9, and the olefinic H-11 (δ 5.53, 1H, br dd, *J* = 6.6, 6.6 Hz) to H-14 (δ 6.14, 1H, d, *J* = 9.2 Hz) but not to H-9 indicated the α -orientation of the hydroxy group at C-9 and the *E*-configuration of the double bond at C-11/C-12. Therefore, the structure of gibberosene E was unequivocally established as (9*S**,13*R**,14*R**,1*Z*,3*E*,7*E*,11*E*)-13,14-diacetoxy-9-hydroxycembra-1,3,7,11-tetraene.

Gibberosene F (**7**) was found to have the molecular formula C₂₄H₃₆O₅, as indicated by the HRESIMS (*m/z* 427.2463, [M + Na]⁺), suggesting **7** to be an isomer of **6**. The IR, UV, MS, and

NMR (Table 3) spectra of **7** also resembled those of **6** and indicated the presence of two acetoxy groups, one hydroxy group, and four trisubstituted double bonds, including a conjugated diene. Owing to the complete overlap of the two olefinic proton signals (δ 6.21, 2H, s, in CDCl_3 , H-2 and H-3), the ^1H NMR spectrum of **7** was also measured in C_6D_6 , where H-2 and H-3 appeared as two separated doublets ($J = 11.5$ Hz) at δ 6.24 and 6.42, respectively. The chemical shifts of CH_2 -5 were unveiled primarily by a HMBC correlation of H_3 -18 to C-5 (measured in CDCl_3). As C-5 (δ 49.2) of **7** was found to be significantly downfield-shifted relative to that of **6**, the hydroxy group should be located at C-6 (δ 67.7) of **7**. This was further supported by the ^1H - ^1H COSY correlations (measured in CDCl_3 , Figure 1) observed between the hydroxymethine proton (δ 4.49, m, H-6) and both H_2 -5 (δ 2.43, dd, $J = 11.7$, 11.7 Hz and 2.26, dd, $J = 11.7$, 3.0 Hz) and H-7 (δ 5.12, d, $J = 8.5$ Hz). These findings and other detailed HMBC correlations (Figure 1) confirmed the planar structure of **7**. The *R* configurations at both C-13 and C-14 in **7** might have been the same as those of **6**, on the basis of the quite similar chemical shifts, splitting patterns, and *J* values for H-13 and H-14 of both compounds. NOE interactions (measured in C_6D_6) between one of the C-5 methylene protons (δ 2.14, dd, $J = 11.0$, 11.0 Hz) and both H-3 and H-7, H-7 and H-3, and H-3 and H-14 revealed that this C-5 proton should be assigned as H-5 α . Furthermore, H-5 β (δ 2.31, d, $J = 11.0$ Hz) showed NOE correlation with H-6, reflecting the α -orientation of the 6-OH. The above findings, together with other detailed correlations (measured in C_6D_6) in the NOESY spectrum of **7** (Figure 2), established the structure of gibberosene F (**7**) as (6*R**,13*R**,14*R**,1*Z*,3*E*,7*E*,11*E*)-13,14-diacetoxy-6-hydroxycembra-1,3,7,11-tetraene.

Gibberosene G (**8**), $[\alpha]_D^{25} = -159$ (CHCl_3), exhibited a quasi-molecular ion peak at m/z 311.2348 $[\text{M} + \text{Na}]^+$ in the HRESIMS, appropriate for the molecular formula $\text{C}_{20}\text{H}_{32}\text{O}$. The oxygen atom in the molecule was attributable to the presence of one hydroxy group (IR 3373 cm^{-1} ; EIMS m/z 270 $[\text{M} - \text{H}_2\text{O}]^+$). The hydroxymethine proton (δ 4.54, ddd, $J = 12.5$, 9.0, 3.7 Hz) showed COSY correlations with a set of methylene protons (δ 2.13, m and 2.46, br d, $J = 12.5$ Hz, H_2 -9) and an olefinic proton (δ 5.15, d, $J = 9.0$ Hz, H-11), while C-9 was HMBC correlated with H_3 -19 (δ 1.57, 3H, s). Thus, the hydroxy group in **8** should be located at C-10. The *E*-geometries of the four double bonds at C-1/C-2, C-3/C-4, C-7/C-8, and C-11/C-12 were determined by the NOE interactions displayed by the methyl protons at C-15 with H-2, H-2 with H_3 -18, H_2 -6 with H_3 -19, and H_3 -20 with H-10, respectively. The structure of gibberosene G (**8**) was established as (–)-(1*E*,3*E*,7*E*,11*E*)-10-hydroxycembra-1,3,7,11-tetraene.

There have been several reports concerning the chemical constituents of *S. gibberosa*,^{7,14,15,20–26} and the diterpenoids identified have shown significant variation among different sampling times and/or locations. A specimen collected from Kenting, the same location where *S. gibberosa* was obtained for analysis in this study, contained cembranoids with molecular structures very similar to those in our present work.²¹ The results suggest that the chemical composition of this coral may be affected by geographic and/or time variations.

The cytotoxicity study of diterpenoids **1–8** against the growth of Hep G2, MCF-7, MDA-MB-23, and A-549 cancer cell lines showed that none of these compounds have cytotoxicity against cell growth (IC_{50} 's > 20 $\mu\text{g}/\text{mL}$). As **1** and **3** were obtained in larger quantities than the other metabolites, the *in vitro* anti-inflammatory effect of diterpenoids **1** and **3** was tested. In this assay, the accumulation of the pro-inflammatory iNOS and COX-2 proteins of LPS-stimulated RAW 264.7 macrophage cells was evaluated using the immunoblot analysis. It was found that compound **1** at 10 μM reduced the levels of the iNOS and COX-2 proteins to $76.2 \pm 6.7\%$ and $54.0 \pm 6.2\%$, respectively, relative to the control cells stimulated with LPS alone. However, the same

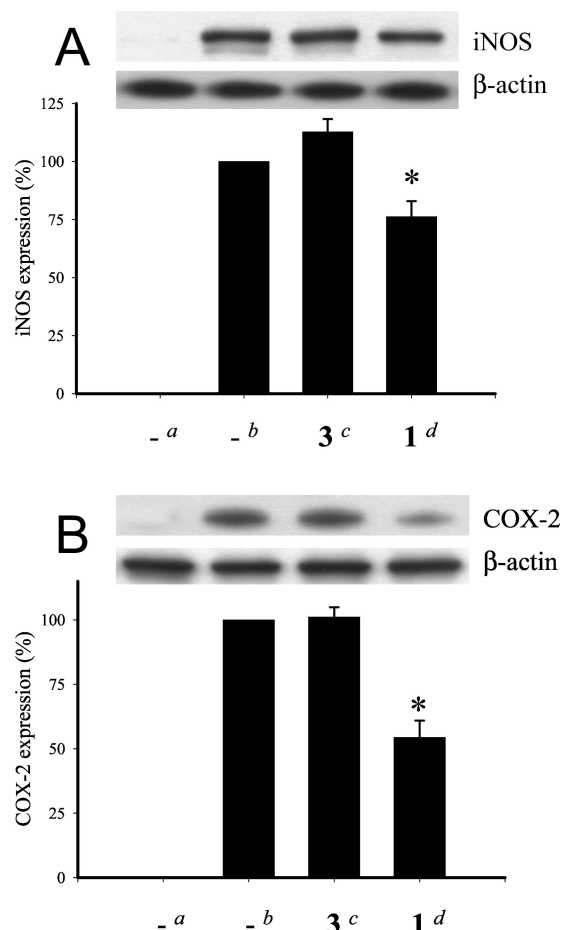


Figure 3. Effect of compounds **1** and **3** on iNOS and COX-2 protein expression of RAW264.7 macrophage cells by immunoblot analysis. (A) Immunoblots of iNOS and β -actin; (B) immunoblots of COX-2 and β -actin; values are mean \pm SEM ($n = 6$). Relative intensity of the LPS alone stimulated group was taken as 100%. *Significantly different from LPS alone stimulated group (* $P < 0.05$). ^aCells not stimulated, ^bstimulated with LPS, ^cstimulated with LPS in the presence of **3** (10 μM), ^dstimulated with LPS in the presence of **1** (10 μM).

concentration of the related compound **3** did not produce any inhibition of LPS-induced iNOS and COX-2 expression (Figure 3). The toxicity of **1** and **3** to RAW264.7 cells was also assessed by trypan blue staining and expression of β -actin. The results indicated that RAW264.7 cell survival and β -actin expression were not affected by the presence of **1** and **3** at 10 μM .

Experimental Section

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-DPX 300 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 , respectively, in CDCl_3 or C_6D_6 using TMS as internal standard. Low-resolution MS data were obtained by EI on a VG Quattro GC/MS spectrometer or by ESI on a Bruker APEX II mass spectrometer. HRMS data were recorded by ESI FT-MS on a Bruker APEX II mass spectrometer. Si gel 60 (Merck, 230–400 mesh) and Sephadex LH-20 (Pharmacia) were used for open CC. Precoated Si plates (Merck, Kieselgel 60 F₂₅₄, 0.2 mm) were used for analytical TLC analyses. Isolation by HPLC was performed by a Shimadzu SPD-10A instrument equipped with a normal-phase column (Hibar Lichrosorb Si-60, 7 μm , 250 \times 25 mm) or a reversed-phase column (Hibar Purospher RP-18e, 5 μm , 250 \times 10 mm).

Animal Material. The soft coral *S. gibberosa* was collected by hand using scuba off the coast of Kenting, Taiwan, in June 2004, at

depths of 15 to 20 m and stored in a freezer until extraction. A voucher sample (SC-61) was deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

Extraction and Separation. The sliced bodies of the soft coral *S. gibberosa* (1.3 kg, wet wt) were exhaustively extracted with EtOH (1 L \times 4). The organic layer was filtered and concentrated under vacuum, and the residue of aqueous suspension was partitioned between CH₂Cl₂ and H₂O. The solvent-free CH₂Cl₂ extract (10 g) was subjected to CC on Si gel and eluted with EtOAc in *n*-hexane (0–100%, gradient) to yield 23 fractions. ¹H NMR spectroscopy was employed to detect the terpenoid-rich fractions. Fraction 4, eluted with EtOAc–*n*-hexane (1:9), was further isolated over Si gel using EtOAc–*n*-hexane (1:20) followed by reversed-phase HPLC, using acetone–H₂O (4:1) to yield **3** (5.5 mg). Fraction 6, eluted with EtOAc–*n*-hexane (1:4), was rechromatographed over a Sephadex LH-20 column, using acetone as the mobile phase to afford 10 subfractions (F1–F10). Subfraction F7 was separated by normal-phase HPLC, using EtOAc–*n*-hexane (1:20) to afford **1** (9.7 mg) and a crude mixture of terpenoids. The latter was further isolated by reversed-phase HPLC, using MeOH–H₂O (6:1), to afford **4** (1.4 mg) and **8** (2.7 mg), respectively. Fraction 8, eluted with EtOAc–*n*-hexane (1:2), was rechromatographed over Si gel using EtOAc–*n*-hexane (1:11) followed by normal-phase HPLC, using EtOAc–*n*-hexane (1:7), to afford **2** (1.5 mg). Fraction 9, eluted with EtOAc–*n*-hexane (1:1), was purified on a Sephadex LH-20 column, using acetone as the mobile phase, and then further purified by normal-phase HPLC, using EtOAc–*n*-hexane (1:5), to give **5** (2.0 mg). Fraction 10, eluted with EtOAc–*n*-hexane (1:1), was rechromatographed on a column of Sephadex LH-20 column, using acetone as the mobile phase, and then further separated by normal-phase HPLC, using EtOAc–*n*-hexane (1:3), to afford **6** (3.0 mg) and **7** (2.8 mg).

Gibberosene A (2): colorless oil; [α]_D²⁵ –52 (*c* 0.6, CHCl₃); IR (neat) ν_{\max} 2966, 2926, 2868, 1748, 1653, 1458, 1373, 1233 cm⁻¹; UV λ_{\max} MeOH nm (log ϵ) 225 (3.89); ¹H and ¹³C NMR data (CDCl₃), see Tables 1 and 2, respectively; ¹H NMR (C₆D₆, 500 MHz) δ 6.31 (1H, s, H-13), 5.78 (1H, s, H-2), 4.88 (1H, dd, *J* = 7.0, 7.0 Hz, H-7), 3.34 (1H, dd, *J* = 9.0, 4.0 Hz, H-11), 2.88 (1H, septet, *J* = 7.0 Hz, H-15), 2.55 (1H, ddq, *J* = 14.0, 7.0, 3.5 Hz, H-4), 2.27 (1H, ddd, *J* = 14.0, 8.0, 8.0 Hz, H-6 α), 2.09 (1H, m, H-10 α), 1.93 (1H, br d, *J* = 14.0 Hz, H-9 α), 1.81 (1H, ddd, *J* = 14.0, 11.0, 3.0 Hz, H-9 β), 1.75 (3H, s, OAc), 1.69 (2H, m, H-5 α and H-6 β), 1.59 (1H, m, H-5 β), 1.43 (3H, s, H₃-19), 1.32 (1H, m, H-10 β), 1.28 (3H, s, H₃-20), 1.11 (1H, d, *J* = 7.0 Hz, H₃-18), 1.10 (3H, d, *J* = 7.0 Hz, H₃-17), 1.09 (3H, d, *J* = 7.0 Hz, H₃-16); EIMS *m/z* 360 [8.6, (M)⁺], 318 (6.6), 300 [2.2, (M – AcOH)⁺], 179 (96.4); ESIMS *m/z* 399 [12, (M + K)⁺], 383 [100, (M + Na)⁺], 301 [61, (M – AcOH + H)⁺]; HRESIMS *m/z* 383.2196 (calcd for C₂₂H₃₂O₄Na, 383.2198).

Gibberosene B (3): colorless oil; [α]_D²⁵ +50 (*c* 0.9, CHCl₃); IR (neat) ν_{\max} 2961, 2921, 2860, 1740, 1655, 1456, 1373, 1236 cm⁻¹; UV λ_{\max} MeOH nm (log ϵ) 241 (3.97); ¹H and ¹³C NMR data (CDCl₃), see Tables 1 and 2, respectively; EIMS *m/z* 346 [0.8, (M)⁺], 286 [2.5, (M – AcOH)⁺], 259 (8.2), 243 (7.3), 191 (35.3), 175 (51.0); ESIMS *m/z* 385 [25, (M + K)⁺], 369 [100, (M + Na)⁺]; HRESIMS *m/z* 369.2405 (calcd for C₂₂H₃₄O₃Na, 369.2406).

Gibberosene C (4): colorless oil; [α]_D²⁵ –66 (*c* 0.6, CHCl₃); IR (neat) ν_{\max} 3350, 2960, 2920, 2858, 1458, 1387 cm⁻¹; ¹H and ¹³C NMR data (CDCl₃), see Tables 1 and 2, respectively; ESIMS *m/z* 343 [100, (M + Na)⁺]; HRESIMS *m/z* 343.2250 (calcd for C₂₀H₃₂O₃Na, 343.2249).

Gibberosene D (5): colorless oil; [α]_D²⁵ +49 (*c* 1.0, CHCl₃); IR (neat) ν_{\max} 3350, 2960, 2918, 2860, 1734, 1647, 1456, 1373, 1238 cm⁻¹; UV λ_{\max} MeOH nm (log ϵ) 239 (3.50); ¹H and ¹³C NMR data (CDCl₃), see Tables 1 and 2, respectively; ESIMS *m/z* 401 [15, (M + K)⁺], 385 [100, (M + Na)⁺], 325 [7, (M – AcOH + Na)⁺], 303 [23, (M – AcOH + H)⁺], 285 [23, (M – AcOH – H₂O + H)⁺]; HRESIMS *m/z* 385.2353 (calcd for C₂₂H₃₄O₄Na, 385.2355).

Gibberosene E (6): colorless oil; [α]_D²⁵ +79 (*c* 1.0, CHCl₃); IR (neat) ν_{\max} 3422, 2962, 2926, 2868, 1743, 1647, 1456, 1373, 1244 cm⁻¹; UV λ_{\max} MeOH nm (log ϵ) 248 (4.02); ¹H and ¹³C NMR data (CDCl₃), see Table 3; ESIMS *m/z* 443 [84, (M + K)⁺], 427 [100, (M + Na)⁺], 307 [5, (M – 2 AcOH + Na)⁺]; HRESIMS *m/z* 427.2462 (calcd for C₂₄H₃₆O₅Na, 427.2460).

Gibberosene F (7): colorless oil; [α]_D²⁵ +128 (*c* 1.0, CHCl₃); IR (neat) ν_{\max} 3422, 2959, 2926, 2857, 1743, 1649, 1456, 1373, 1244 cm⁻¹; UV λ_{\max} MeOH nm (log ϵ) 253 (4.01); ¹H and ¹³C NMR data (CDCl₃), see Table 3; ¹H NMR (C₆D₆, 300 MHz) δ 6.61 (1H, d, *J* = 10.0 Hz,

Table 4. Growth Inhibition (%) of Cancer Cells by Compounds **1–8** at 20 μ g/mL

	Hep G2	MCF-7	MDA-MB-231	A-549
1	45.5	33.4		27.2
2	– ^a	41.3		
3	24.0	21.5		
4				
5				
6	31.3	21.0		25.8
7				
8	23.3			
doxorubicin ^b	66.9	51.6	66.6	73.2

^a Growth inhibition is less than 20%. ^b Doxorubicin used at 0.5 μ g/mL.

H-14), 6.42 (1H, d, *J* = 11.5 Hz, H-3), 6.24 (1H, d, *J* = 11.5 Hz, H-2), 5.75 (1H, d, *J* = 10.0 Hz, H-13), 5.58 (1H, m, H-11), 4.78 (1H, d, *J* = 8.1 Hz, H-7), 4.25 (1H, br dd, *J* = 11.0, 8.1 Hz, H-6), 2.56 (1H, septet, *J* = 6.6 Hz, H-15), 2.31 (1H, d, *J* = 11.0 Hz, H-5 β), 2.14 (1H, dd, *J* = 11.0, 11.0 Hz, H-5 α), 1.95 (1H, m, H-9), 1.93 (1H, m, H-10), 1.91 (2H, m, H-9, H-10), 1.79 (3H, s, OAc), 1.72 (3H, s, OAc), 1.69 (1H, d, *J* = 7.0 Hz, H₃-20), 1.52 (3H, s, H₃-18), 1.15 (3H, s, H₃-19), 1.09 (3H, d, *J* = 6.6 Hz, H₃-17), 0.99 (3H, d, *J* = 6.6 Hz, H₃-16); ESIMS *m/z* 427 [100, (M + Na)⁺], 285 [55, (M – 2 AcOH + H)⁺]; HRESIMS *m/z* 427.2463 (calcd for C₂₄H₃₆O₅Na, 427.2460).

Gibberosene G (8): colorless oil; [α]_D²⁵ –159 (*c* 1.0, CHCl₃); IR (neat) ν_{\max} 3373, 2959, 2926, 2857, 1670, 1456, 1381, 1261 cm⁻¹; UV λ_{\max} MeOH nm (log ϵ) 250 (3.98); ¹H and ¹³C NMR data (CDCl₃), see Table 3; EIMS *m/z* 288 [9.9, (M)⁺], 270 (1.2, [M – H₂O]⁺); ESIMS *m/z* 311 [100, (M + Na)⁺]; HRESIMS *m/z* 311.2348 (calcd for C₂₀H₃₂O₄Na, 311.2350).

Cytotoxicity Testing. Cell lines were purchased from the American type Culture Collection (ATCC). Cytotoxicity assays of compounds **1–8** were performed using the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.^{27,28}

In Vitro Anti-inflammatory Assay. The anti-inflammatory assay was modified from Ho et al.²⁹ and Park et al.³⁰ Murine RAW 264.7 macrophages were obtained from the American Type Culture Collection (ATCC, No TIB-71) and cultured in Dulbecco's modified essential medium (DMEM) containing 10% heat-inactivated fetal bovine serum, at 37 °C in a humidified 5% CO₂–95% air incubator under standard conditions. Inflammation in macrophages was induced by incubating them for 16 h in a medium containing only LPS (0.01 μ g/mL; Sigma) without the presence of test compounds. For the anti-inflammatory activity assay, compounds **1** and **3** were added to the cells 5 min before LPS challenge, respectively. Then, cells were washed with ice-cold PBS, lysed in ice cold lysis buffer, and then centrifuged at 20000g for 30 min at 4 °C. The supernatant was decanted from the pellet and retained for Western blot analysis. Protein concentrations were determined by the DC protein Assay kit (Bio-Rad) modified by the method of Lowry et al.³¹ Samples containing equal quantities of proteins were subjected to SDS-polyacrylamide gel electrophoresis, and the separated proteins were electrophoretically transferred to polyvinylidene difluoride membranes (PVDF; Immobilon-P, Millipore, 0.45 μ m pore size). The resultant PVDF membranes were incubated with blocking solution and incubated for 180 min at room temperature with antibody against inducible nitric oxide synthase (iNOS; 1:1000 dilution; Transduction Laboratories) and cyclooxygenase-2 (COX-2; 1:1000 dilution; Cayman Chemical) protein. The blots were detected using ECL detection reagents (Perkin-Elmer, Western Blot Chemiluminescence Reagent Plus) according to the manufacturer's instructions and finally exposed to X-ray film (Kodak X-OMAT LS, Kodak). The membranes were re-probed with a monoclonal mouse anti- β -actin antibody (1:2500, Sigma) as the loading control. After X-ray film scanning, the integrated optical density of the bands was estimated (Image-Pro plus 4.5 software) and normalized to the background values. Relative variations between the bands of the drug-treated samples and the samples treated with LPS alone were calculated in the same image.

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Supporting Information Available: ¹H and ¹³C NMR spectra of **2–8** are 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.* **2007**, *24*, 31–86.
- (2) Rodríguez, A. D. *Tetrahedron* **1995**, *51*, 4571–4618.
- (3) Iwashima, M.; Matsumoto, Y.; Takenaka, Y.; Iguchi, K.; Yamori, T. *J. Nat. Prod.* **2002**, *65*, 1441–1446.
- (4) Duh, C.-Y.; El-Gamal, A. A. H.; Chu, C.-J.; Wang, S. K.; Dai, C.-F. *J. Nat. Prod.* **2002**, *65*, 1535–1539.
- (5) Su, J.; Yang, R.; Kuang, Y.; Zeng, L. *J. Nat. Prod.* **2000**, *63*, 1543–1545.
- (6) Herin, M.; Tursch, B. *Bull. Soc. Chim. Belg.* **1976**, *85*, 707–719.
- (7) Li, G.; Zhang, Y.; Deng, Z.; van Ofwegen, L.; Proksch, P.; Lin, W. *J. Nat. Prod.* **2005**, *68*, 649–652.
- (8) Tseng, Y.-J.; Ahmed, A. F.; Dai, C.-F.; Chiang, M. Y.; Sheu, J.-H. *Org. Lett.* **2005**, *7*, 3813–3816.
- (9) Ahmed, A. F.; Su, J.-H.; Kuo, Y.-H.; Sheu, J.-H. *J. Nat. Prod.* **2004**, *67*, 2079–2082.
- (10) Ahmed, A. F.; Shiue, R.-T.; Wang, G.-H.; Dai, C.-F.; Kuo, Y.-H.; Sheu, J.-H. *Tetrahedron* **2003**, *59*, 7337–7344.
- (11) Sheu, J.-H.; Ahmed, A. F.; Shiue, R.-T.; Dai, C.-F.; Kuo, Y.-H. *J. Nat. Prod.* **2002**, *65*, 1904–1908.
- (12) Su, J.-H.; Ahmed, A. F.; Sung, P.-J.; Chao, C.-H.; Kuo, Y.-H.; Sheu, J.-H. *J. Nat. Prod.* **2006**, *69*, 1134–1139.
- (13) Ahmed, A. F.; Su, J.-H.; Shiue, R.-T.; Pan, X.-J.; Dai, C.-F.; Kuo, J.-H.; Sheu, J.-H. *Tetrahedron* **2003**, *59*, 7337–7344.
- (14) Chen, S.-P.; Ahmed, A. F.; Dai, C.-F.; Lu, C.-K.; Hu, W.-P.; Wang, J.-J.; Sheu, J.-H. *Tetrahedron* **2006**, *62*, 6802–6807.
- (15) Ahmed, A. F.; Hsieh, Y.-T.; Wen, Z.-H.; Wu, Y.-C.; Sheu, J.-H. *J. Nat. Prod.* **2006**, *69*, 1275–1279.
- (16) Bowden, B. F.; Coll, J. C.; Tapiolas, D. M. *Aust. J. Chem.* **1983**, *36*, 2289–2295.
- (17) Greenland, G. J.; Bowden, B. F. *Aust. J. Chem.* **1994**, *47*, 2013–2021.
- (18) Lan, J.; Liu, Z.; Yuan, H.; Peng, L.; Li, W.-D.; Li, Y.; Li, Y.; Chan, A. S. C. *Tetrahedron Lett.* **2000**, *41*, 2181–2184.
- (19) Gray, C. A.; Davies-Coleman, M. T.; Schleyer, M. H. *J. Nat. Prod.* **2000**, *63*, 1551–1553.
- (20) Hou, R.-S.; Duh, C.-Y.; Chang, M. Y.; Lin, C.-N. *J. Nat. Prod.* **1995**, *58*, 1126–1130.
- (21) Duh, C.-Y.; Hou, R.-S. *J. Nat. Prod.* **1996**, *59*, 595–598.
- (22) Ahmed, A. F.; Kuo, Y.-H.; Dai, C.-F.; Sheu, J.-H. *J. Nat. Prod.* **2005**, *68*, 1208–1212.
- (23) Ahmed, A. F.; Duh, C.-Y.; Kuo, Y.-H.; Sheu, J.-H. *Steroid* **2003**, *68*, 377–381.
- (24) Chen, S.-P.; Chao, C.-H.; Huang, H.-C.; Lu, C.-K.; Dai, C.-F.; Sheu, J.-H. *Bull. Chem. Soc. Jpn.* **2006**, *79*, 1547–1551.
- (25) Kobayashi, M.; Rao, K. M. C. A.; Anjaneyulu, V. *J. Chem. Res. (S)* **1994**, 140–141.
- (26) Anjaneyulu, A. S. R.; Sagar, K. S.; Prakash, C. V. S. *Indian J. Chem. B Org. Med. Chem.* **1996**, *35*, 819–825.
- (27) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589–601.
- (28) Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827–4833.
- (29) Ho, F. M.; Lai, C. C.; Huang, L. J.; Kuo, T. C.; Chao, C. M.; Lin, W. W. *Br. J. Pharmacol.* **2004**, *141*, 1037–1047.
- (30) Park, E. K.; Shin, Y. W.; Lee, H. U.; Kim, S. S.; Lee, Y. C.; Lee, B. Y.; Kim, D. H. *Biol. Pharm. Bull.* **2005**, *28*, 652–656.
- (31) Lowry, D. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265–275.

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