

New Briarane-Related Diterpenoids from the Sea Whip Gorgonian Coral *Junceella fragilis* (Ellisellidae)

Ping-Jyun Sung,*[1,2,3] Su-Hui Wang,[1,2] Yu-Chia Chang,[1,2] Yung-Husan Chen,[2] Mei-Ru Lin,[2] I-Chu Huang,[2] Jih-Jung Chen,[4] Jan-Jung Li,[2] Ting-Hsuan Kung,[2] Lee-Shing Fang,[5] Wei-Hsien Wang,[2,3] and Ching-Feng Weng[1]

[1]Graduate Institute of Marine Biotechnology, Department of Life Science and Graduate Institute of Biotechnology, National Dong Hwa University (NDHU), Checheng, Pingtung 944, Taiwan

[2]National Museum of Marine Biology & Aquarium (NMMBA), Checheng, Pingtung 944, Taiwan

[3]Department of Marine Biotechnology and Resources, Asia-Pacific Ocean Research Center (APORC), National Sun Yat-sen University (NSYSU), Kaohsiung 804, Taiwan

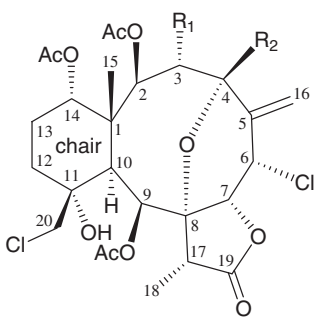
[4]Department of Pharmacy, Tajen University, Pingtung 907, Taiwan

[5]Department of Sport, Health, and Leisure, Cheng Shiu University, Kaohsiung 833, Taiwan

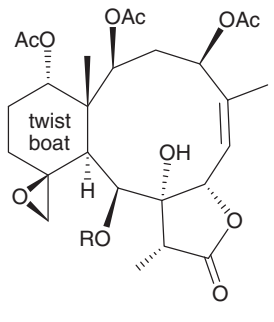
Received March 10, 2010; E-mail: pjsung@nmmba.gov.tw

Two new briarane-type diterpenoids, fragilides H (**1**) and I (**2**), along with a known metabolite, (+)-11β,20β-epoxyjunceellolide D (**3**), were isolated from the sea whip gorgonian coral *Junceella fragilis*. The structures of briaranes **1**–**3** were elucidated by spectroscopic methods and by comparison with the spectral data from other known metabolites featuring a briarane carbon skeleton.

Our research of natural products from marine invertebrates collected off Taiwan waters has revealed that gorgonian corals are important sources of various diterpenoids. In previous studies, a series of interesting briarane-type natural products (3,8-cyclized cembranoid, bicyclo[8.4.0] system) had been isolated from the sea whip gorgonian coral *Junceella fragilis* (family Ellisellidae), studied by Taiwan research groups.[1–14] Recently, our further chemical examination of *J. fragilis* has resulted in the isolation of two new highly oxidized briarane diterpenoids, fragilides H (**1**), I (**2**), and a known briarane (+)-11β,20β-epoxyjunceellolide D (**3**)[7,15] (Chart 1). The structures of new briaranes **1** and **2** were elucidated by extensive spectroscopic methods, particularly with 1D and 2D NMR experiments and by comparison with the spectral data from other known briarane analogs.



1 : R_1 = H, R_2 = OH
4 : R_1 = OAc, R_2 = H



2 : R = C(O)CH$_2$CH(CH$_3$)$_2$
3 : R = Ac

Chart 1.

Results and Discussion

Fragilide H (**1**) was obtained as a white powder and its molecular formula was established as $C_{26}H_{34}Cl_2O_{11}$ (nine degrees of unsaturation) from a sodiated molecule at *m/z* 615 in the ESI-MS and further supported by the HR-ESI-MS (*m/z* 615.1373, calcd. 615.1376, $[C_{26}H_{34}{}^{35}Cl_2O_{11} + Na]^+$). The IR spectrum of **1** showed bands at 3378, 1789, and 1740 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester groups. From the ^{13}C NMR data of **1** (Table 1), an exocyclic carbon–carbon double bond was deduced from the signals at δ_C 137.7 (s, C-5) and 117.9 (t, CH$_2$-16), and confirmed by two olefin proton signals at δ_H 5.92 (1H, d, J = 2.0 Hz, H-16a) and 5.65 (1H, d, J = 2.0 Hz, H-16b) in the ^{1}H NMR spectrum of **1** (Table 1). Four carbonyl resonances at δ_C 175.0 (s, C-19), 173.4, 169.4, and 169.4 (3 × s, ester carbonyls) confirmed the presence of a γ-lactone and three ester groups in **1**; three acetyl methyls (δ_H 2.28, 2.07, 2.07, each 3H × s) were also observed. On the basis of overall unsaturation data, **1** was concluded to be a briarane diterpenoid molecule possessing four rings.

^{1}H NMR coupling information in the ^{1}H–^{1}H COSY spectrum of **1** enabled identification of the C-2/-3, C-6/-7, C-9/-10, C-12/-13/-14, C-6/-16 (by allylic coupling), and C-17/-18 units, which were assembled with the assistance of an HMBC experiment (Table 1). The HMBC correlations between protons and quaternary carbons of **1**, such as H-3β, H-9, H-10, H$_3$-15/C-1; H-2, H-3α, H$_2$-16, OH-4/C-4; H-16b, OH-4/C-5; H-10, H$_3$-18/C-8; H-9, OH-11/C-11; and H-17, H$_3$-18/C-19, permitted elucidation of the carbon skeleton. An exocyclic double bond at C-5 was established by the HMBC correlations

Published on the web August 26, 2010; doi:10.1246/bcsj.20100067

Table 1. ^{1}H and ^{13}C NMR Data, ^{1}H–^{1}H COSY, and HMBC Correlations for Diterpenoid **1**

Position	δ_H[a)]	δ_C[b)]	^{1}H–^{1}H COSY	HMBC (H → C)
1		45.6 (s)[d)]		
2	5.26 d (7.2)[c)]	73.4 (d)	H_2-3	C-4, -10, -15, acetate carbonyl
3α	1.57 d (12.4)	41.0 (t)	H-2, H-3β	C-2, -4
β	3.33 dd (12.4, 7.2)		H-2, H-3α	C-1, -2
4		96.7 (s)		
5		137.7 (s)		
6	4.98 ddd (2.8, 2.0, 2.0)	55.4 (d)	H-7, H_2-16	n.o.[e)]
7	4.30 d (2.8)	78.6 (d)	H-6	n.o.
8		81.8 (s)		
9	6.42 s	74.6 (d)	H-10	C-1, -10, -11, -17, acetate carbonyl
10	2.68 s	45.9 (d)	H-9	C-1, -8, -9, -15, -20
11		73.8 (s)		
12α	1.65 m	29.5 (t)	H-12β, H_2-13	n.o.
β	2.20 m		H-12α, H_2-13	n.o.
13α	1.82 m	22.9 (t)	H_2-12, H-13β, H-14	n.o.
β	1.66 m		H_2-12, H-13α, H-14	n.o.
14	4.94 d (2.4)	73.5 (d)	H_2-13	n.o.
15	1.26 s	16.1 (q)		C-1, -2, -10, -14
16a	5.92 d (2.0)	117.9 (t)	H-6, H-16b	C-4, -6
b	5.65 d (2.0)		H-6, H-16a	C-4, -5, -6
17	2.82 q (6.8)	50.2 (d)	H_3-18	C-18, -19
18	1.41 d (6.8)	7.5 (q)	H-17	C-8, -17, -19
19		175.0 (s)		
20a	3.88 d (11.2)	48.8 (t)	H-20b	n.o.
b	3.46 d (11.2)		H-20a	n.o.
OH-4	6.60 s			C-4, -5
OH-11	2.82 s			C-10, -11
2-OAc	2.07 s	21.1 (q)		Acetate carbonyl
		173.4 (s)		
9-OAc	2.28 s	21.7 (q)		Acetate carbonyl
		169.4 (s)		
14-OAc	2.07 s	20.9 (q)		Acetate carbonyl
		169.4 (s)		

a) Spectra measured at 400 MHz in $CDCl_3$ at 25 °C. b) Spectra measured at 100 MHz in $CDCl_3$ at 25 °C. c) *J* values (in hertz) in parentheses. d) Attached protons were deduced by DEPT and HMQC spectra. e) n.o.: not observed.

between H-16a/C-4, -6 and H-16b/C-4, -5, -6; and further confirmed by the allylic coupling between H_2-16/H-6. The ring junction C-15 methyl group was positioned at C-1 from the HMBC correlations between H_3-15/C-1, -2, -10, -14; H-2/C-15; and H-10/C-15. Furthermore, the acetate esters at C-2 and C-9 were established by correlations between H-2 (δ_H 5.26), H-9 (δ_H 6.42) and the acetate carbonyls observed in the HMBC spectrum of **1**. The presence of hydroxy groups at C-4 and C-11 were deduced from the HMBC correlations between the hydroxy protons (δ_H 6.60, OH-4 and 2.82, OH-11) with C-4 (δ_C 96.7) and C-11 (δ_C 73.8) oxygenated quaternary carbons, respectively. The C-4 hydroxy group was concluded to be a part of a hemiketal constellation on the basis of a characteristic carbon signal at δ_C 96.7 (a quaternary hemiketal carbon, C-4). A carbon signal at δ_C 81.8 (s, C-8) showed ^{3}J-coupling with protons at δ_H 2.68 (H-10) and 1.41 (H_3-18). Thus, the remaining acetoxy group was positioned at C-14 as indicated by analysis of ^{1}H–^{1}H COSY correlations and characteristic NMR signals analysis, although no HMBC correlation was observed between H-14 (δ_H 4.94) and the acetate carbonyl.

The intensity of sodiated molecules $(M + 2 + Na)^+$ and $(M + 4 + Na)^+$ isotope peaks observed in the ESI-MS and HR-ESI-MS spectrum $[(M + Na)^+:(M + 2 + Na)^+:(M + 4 + Na)^+ = 9:6:1]$ was evidence of the presence of two chlorine atoms in **1**. The methine unit at δ_C 55.4 (d) was more shielded than expected for an oxygenated carbon and was correlated to the methine proton at δ_H 4.98 (H-6) in the HMQC spectrum and this proton signal was ^{3}J-correlated with H-7 (δ_H 4.30) in the ^{1}H–^{1}H COSY spectrum, proving the attachment of a chlorine atom at C-6. Moreover, the methylene unit at δ_C 48.8 (t, CH_2-20) was also more shielded than expected for an oxygenated C-atom and was correlated to the methylene protons at δ_H 3.88 (H-20a) and 3.46 (H-20b) in the HMQC spectrum. These two protons showed a typical geminal coupling pattern with each other ($J = 11.2$ Hz) only, proving the attachment of a chlorinated methyl group at C-11. These data, together with the HMBC correlations between H-17/C-18, C-19 and H_3-18/C-8, C-17, C-19, established the molecular framework of **1**.

The relative configuration of **1** was elucidated on the basis of a NOESY experiment and by vicinal ^{1}H–^{1}H proton coupling constants analysis. Most naturally occurring briarane natural

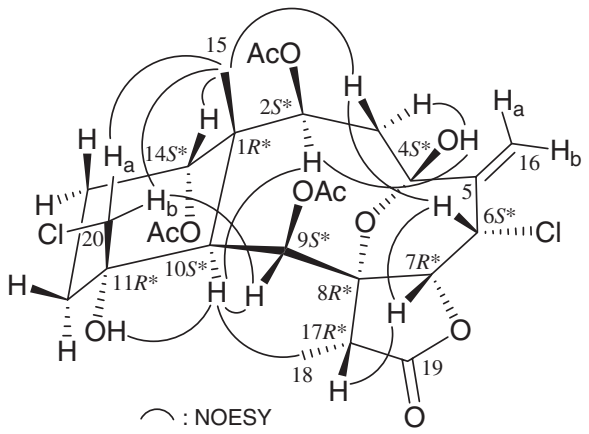


Figure 1. Selective NOESY correlations of **1**.

products have the Me-15 in the β-orientation and H-10 in the α-orientation,[16–18] which were verified by the absence of correlation between these two groups. In the NOESY experiment of **1** (Figure 1), H-10 correlated with H-2, H-9, H_3-18, and OH-11, indicating that these protons were situated on the same face; they were assigned as α protons, as C-15 methyl was β-oriented at C-1. The oxymethine proton H-14 and the chlorinated C-20 methylene protons (H-20a/b) were found to exhibit responses with H_3-15 but not with H-10, revealing that H-14 and C-20 chlorinated methyl were β-oriented at C-14 and C-11, respectively. H-9 was found to show correlations with H-17 and one proton of C-20 methylene (δ_H 3.46, H-20b). From modeling analysis, H-9 was found to be reasonably close to H-17 and H-20b and can therefore be placed on the α face in the 10-membered ring of **1** and H-17 was β-oriented in the γ-lactone moiety. One proton of C-3 methylene (δ_H 3.33) was found to exhibit a correlation with H_3-15 and assigned as H-3β proton and the other one was assigned as H-3α (δ_H 1.57). H-6 showed a correlation with H-3β, confirming the β-orientation for this proton. Furthermore, H-7 showed correlations with H-6 and H-17; and a small coupling constant was found between H-6 and H-7 ($J = 2.8$ Hz), indicating that the dihedral angle between H-6 and H-7 is approximately 50° and H-7 has a β-orientation at C-7.[19] The hydroxy proton OH-4 (δ_H 6.60) exhibited correlations with H-2 and H-3α, indicating that the C-4 chiral center exists in S^* configuration. Based on the above findings, the structure of **1** was elucidated and the chiral centers for **1** were assigned as 1R^*, 2S^*, 4S^*, 6S^*, 7R^*, 8R^*, 9S^*, 10S^*, 11R^*, 14S^*, 17R^*. By comparing the spectral data of **1** with those of a known briarane, fragilide F (**4**),[12] which also possesses two chlorine atoms in its structure, compound **1** was found to be the 3-deacetoxy-4-hydroxy derivative of **4**.

To the best of our knowledge, only two briarane natural products, robustolide D and fragilide F, possessing two chlorine atoms in their structures, were obtained from the gorgonian corals *Ellisella robusta* and *Junceella fragilis* belonging to family Ellisellidae, respectively.[12,20] Fragilide H (**1**) is the third example which possesses two chlorine atoms in its structure and also the first dichlorinated briarane possessing a hemiketal group.

The new briarane, fragilide I (**2**), was isolated as a white powder and its molecular formula $C_{31}H_{44}O_{12}$ was established by HR-ESI-MS (m/z 631.2734, calcd. 631.2730, $[C_{31}H_{44}O_{12} + Na]^+$). The IR spectrum of **2** showed absorptions at 3361, 1775, and 1740 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester groups. In the ^{1}H NMR spectrum of **2** (Table 2), three acetyl methyls (δ_H 2.26, 2.04, 2.01, each 3H × s) and an isovaleryl group (δ_H 0.94, 3H, d, $J = 6.0$ Hz; 0.95, 3H, d, $J = 6.0$ Hz; 2.05, 1H, m; 2.11, 2H, d, $J = 8.0$ Hz) were observed. It was found that the ^{1}H NMR data of **2** were similar to those of a known briarane derivative, (+)-11β,20β-epoxyjunceellolide D (**3**),[7,15] that was also isolated in this study, except that the signals corresponding to an acetoxy group in **3** were replaced by an isovaleroxy group in **2** (Table 2). Because a trace amount of **2** was obtained, it is difficult to get enough valuable information in the ^{13}C and HMBC spectra of **2**, the location of isovaleroxy group in **2** cannot be determined directly by spectroscopic methods. Fortunately, the 1D and 2D NMR data for the known briarane **3** were assigned in this study (Table 2).[21] It was found that the 9-acetoxy group in **3** should be replaced by an isovaleroxy group in **2**. On the basis of the above observations, briarane **2** was found to be the 9-deacetoxy-9-isovaleroxy derivative of **3**. It is noteworthy to mention that metabolite **2** represents the first example of a briarane possessing a 9-isovaleroxy group.

By detailed comparison of the ^{1}H NMR data of **3** with those of (+)-11β,20β-epoxyjunceellolide D,[7,15,21] we found that the ^{1}H NMR data for (+)-11β,20β-epoxyjunceellolide D differ from those of **3** that we reported herein. For example, the chemical shifts, including the coupling patterns and coupling constants of H-10, H-12, and H_2-13 for (+)-11β,20β-epoxyjunceellolide D are different from those of **3**, but the ^{13}C NMR data for these two briaranes are almost identical (Table 2). We suggest that the ^{1}H NMR data for (+)-11β,20β-epoxyjunceellolide D be reexamined.

All the corals are claimed to be threatened species. We want to keep and culture these interesting marine organisms with our advanced flow-through sea water system located in the National Museum of Marine Biology & Aquarium, Taiwan, in the hope of identifying extracts that exhibit chemical meanings. Unfortunately, the new compounds **1** and **2** described herein are not active in cytotoxicity testing with DLD-1 (human colon adenocarcinoma), CCRF-CEM (human T cell acute lymphoblastic leukemia), HL-60 (human promyelocytic leukemia), and P388D1 (mouse lymphoid neoplasm) cells. Due to the screening platforms are limited and lots of compound is consumed in physical and spectral experiments. The other possible biological activities for these interesting natural products will be assayed in the future if we can get enough material.

Experimental

General Experimental Procedures. IR spectra were obtained on a VARIAN DIGLAB FTS 1000 FT-IR spectrophotometer. NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR in $CDCl_3$, respectively. Proton chemical shifts were referenced to the residual $CHCl_3$ signal (δ_H 7.26 ppm). ^{13}C NMR spectra were referenced to the center peak of $CDCl_3$ at δ_C 77.1 ppm. ESI-MS and HR-ESI-MS data were recorded on a BRUKER APEX II mass spectrometer.

Table 2. ^{1}H NMR Data for Diterpenoid **2** and ^{1}H and ^{13}C NMR Data and HMBC Correlations for **3**[21]

Position	**2**	**3**			(+)-11β,20β-Epoxyjunceellolide D	
	δ_H[a)]	δ_H[a)]	δ_C[b)]	HMBC (H → C)	δ_H[g)]	δ_C[g)]
1			47.1 (s)[d)]			47.1 (s)
2	4.61 d (4.4)[c)]	4.67 d (4.4)	71.8 (d)	C-1, -4, -15, Acetate carbonyl	4.67 br d (4.7)	71.8 (d)
3	2.77 ddd (13.6, 13.6, 1.2)	2.75 ddd (13.6, 13.6, 1.2)	37.8 (t)	C-1, -4	2.75 t (14)	37.7 (t)
3′	1.92 m	1.93 m		C-2, -4, -5	1.93 m	
4	5.17 dd (13.6, 6.0)	5.13 dd (13.6, 5.6)	72.4 (d)	C-5, -6, -16, Acetate carbonyl	5.12 dd (12.7, 5.7)	72.4 (d)
5			143.1 (s)			143.1 (s)
6	5.71 dd (10.4, 1.2)	5.71 dd (10.4, 1.2)	124.8 (d)	C-7, -8	5.70 d (10.3)	124.8 (d)
7	5.49 dd (10.4, 1.2)	5.49 dd (10.4, 1.2)	77.0 (d)	C-5, -6	5.48 d (10.3)	77.1 (d)
8			80.1 (s)			80.1 (s)
9	4.92 d (4.8)	4.86 d (4.8)	73.2 (d)	C-10, acetate carbonyl	4.85 d (5.1)	73.2 (d)
10	2.17 d (4.8)[e)]	2.38 d (4.8)	39.8 (d)	C-1, -2, -11, -12, -15	2.38 s	39.8 (d)
11			62.3 (s)			62.3 (s)
12	2.32 m	2.30 m	23.7 (t)	n.o.[f)]	1.90 m	23.6 (t)
12′	1.13 m	1.14 m		n.o.	1.14 m	
13	2.14 m	2.13 m	24.3 (t)	n.o.	2.40 m	24.3 (t)
13′	1.74 m	1.78 m		C-1	2.24 m	
14	5.67 d (5.6)	5.66 d (5.6)	67.4 (d)	C-10, acetate carbonyl	5.65 d (5.6)	67.4 (d)
15	1.13 s	1.13 s	14.7 (q)	C-1, -2, -10	1.12 s	14.7 (q)
16	2.17 d (1.2)[e)]	2.17 d (1.2)	25.9 (q)	C-4, -5, -6	2.17 s	25.9 (q)
17	2.34 q (7.2)	2.34 q (7.2)	42.2 (d)	C-8, -18, -19	2.36 q (7.0)	42.2 (d)
18	1.16 d (7.2)	1.16 d (7.2)	6.6 (q)	C-8, -17, -19	1.15 d (7.0)	6.6 (q)
19			176.4 (s)			176.5 (s)
20a	2.96 dd (4.0, 1.2)	2.94 dd (4.0, 1.2)	59.1 (t)	C-11	2.94 d (3.4)	59.1 (t)
b	2.84 d (4.0)	2.86 d (4.0)		C-11	2.85 d (3.4)	
OH-8	4.81 d (1.2)	4.82 d (1.2)		C-7, -8	4.82 s	
2-OAc	2.01 s	2.02 s	20.9 (q)	Acetate carbonyl	2.07 s	20.7 (q)
			170.5 (s)			170.4 (s)
4-OAc	2.04 s	2.04 s	21.1 (q)	Acetate carbonyl	2.13 s	21.1 (q)
			170.1 (s)			170.1 (s)
9-OAc		1.96 s	21.0 (q)	Acetate carbonyl	1.98 s	20.9 (q)
			169.8 (s)			169.8 (s)
14-OAc	2.26 s	2.26 s	21.8 (q)	Acetate carbonyl	2.25 s	21.8 (q)
			169.5 (s)			169.5 (s)
9-Isovaleryl group	0.94 d (6.0)					
	0.95 d (6.0)					
	2.05 m					
	2.11 d (8.0)					

a) Spectra measured at 400 MHz in $CDCl_3$ at 25 °C. b) Spectra measured at 100 MHz in $CDCl_3$ at 25 °C. c) *J* values (in hertz) in parentheses. d) Attached protons were deduced by DEPT and HMQC spectra. e) Due to the signals for H_3-16 and H-10 were overlapped, the coupling constant for H-10 in **2** was deduced from the coupling patterns and correlations observed between H-9 and H-10. f) n.o.: not observed. g) Data were reported by García et al. Please see Ref. 15.

Gravity column chromatography was performed on silica gel (230–400 mesh, Merck, Darmstadt, Germany). TLC was carried out on precoated Kieselgel 60 F_{254} (0.2 mm, Merck) and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. HPLC was performed using a system comprised of a HITACHI L-7100 pump, a HITACHI L-7455 photodiode array detector, and a RHEODYNE 7725 injection port. A semi-preparative reverse phase column (Hibar 250–10 mm, Purospher Star RP-18e, 5 μm) was used for HPLC.

Animal Material. Specimens of *J. fragilis* were collected by divers equipped with SCUBA off the coast of southern Taiwan in August 2006, at a depth of −20 m. Living reference specimens are being maintained in the authors' marine organisms cultivating tanks and a voucher specimen was deposited in the NMMBA, Taiwan. This organism was identified by comparison with previous descriptions.[22,23]

Extraction and Isolation. The freeze-dried and minced material of *J. fragilis* (dry weight 74 g) was extracted with a mixture of MeOH and CH_2Cl_2 (1:1) at room temperature. The residue was partitioned between EtOAc and H_2O. The EtOAc layer was separated on silica gel and eluted using hexane/EtOAc (20:1–pure EtOAc) to yield 19 fractions A–S. Fraction Q was separated on silica gel and eluted using hexane/EtOAc to afford 14 fractions, Q1–Q14. Fractions Q9 and Q11 were repurified by reverse phase HPLC (RP-HPLC) and eluted with MeOH/H_2O to afford **2** (0.3 mg, 60:40) and **1** (0.5 mg, 50:50),

respectively. Fraction R was separated on gravity column with silica gel and eluted using hexane/acetone to afford 12 fractions, R1–R12. Fraction R10 was reseparated by RP-HPLC and eluted with MeOH/acetonitrile/H_2O to obtain **3** (0.3 mg, 49:1:50).

Fragilide H (1): White powder; mp 206–207 °C; $[\alpha]_D^{23}$ −230 (*c* 0.002, $CHCl_3$); IR (neat) ν_{max} 3378, 1789, 1740 cm^{-1}; ^{13}C NMR ($CDCl_3$, 100 MHz) and ^1H NMR ($CDCl_3$, 400 MHz) data, see Table 1; ESI-MS *m/z* 615 $(M + Na)^+$, 617 $(M + 2 + Na)^+$, 619 $(M + 4 + Na)^+$; HR-ESI-MS *m/z* 615.1373 (calcd for $C_{26}H_{34}{}^{35}Cl_2O_{11}$ + Na, 615.1376).

Fragilide I (2): White powder; mp 185–187 °C; $[\alpha]_D^{23}$ −374 (*c* 0.001, $CHCl_3$); IR (neat) ν_{max} 3361, 1775, 1740 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) data, see Table 2; ESI-MS *m/z* 631 $(M + Na)^+$; HR-ESI-MS *m/z* 631.2734 (calcd for $C_{31}H_{44}O_{12}$ + Na, 631.2730).

(+)-11β,20β-Epoxyjunceellolide D (3): White powder; mp 127–130 °C; $[\alpha]_D^{23}$ +2 (*c* 0.09, $CHCl_3$) [Ref. 15 $[\alpha]_D^{22}$ +5.3 (*c* 0.4, CH_2Cl_2)]; IR (neat) ν_{max} 3296, 1773, 1738 cm^{-1}; ^{13}C NMR ($CDCl_3$, 100 MHz) and ^1H NMR ($CDCl_3$, 400 MHz) data, see Table 2.

Cytotoxicity Assays. The cytotoxicity of compounds **1** and **2** were assayed with a modification of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. Cytotoxicity assays were carried out according to procedures described previously.[24,25]

This research work was supported by grants from the NMMBA (Grant No. 99200321 and 99200322); NDHU; APORC, NSYSU (96C031702); and National Science Council (NSC 98-2323-B-291-001 and 98-2320-B-291-001-MY3), Taiwan, awarded to P.-J.S.

Supporting Information

^1H NMR, ^1H–^1H COSY spectra, and HR-ESI-MS spectrum of the new compound fragilide I (**2**). This material is available free of charge on the web at http://www.csj.jp/journals/bcsj/.

References

1 P.-J. Sung, S.-L. Wu, H.-J. Fang, M. Y. Chiang, J.-Y. Wu, L.-S. Fang, J.-H. Sheu, *J. Nat. Prod.* **2000**, *63*, 1483.

2 P.-J. Sung, T.-Y. Fan, *Heterocycles* **2003**, *60*, 1199.

3 P.-J. Sung, T.-Y. Fan, L.-S. Fang, S.-L. Wu, J.-J. Li, M.-C. Chen, Y.-M. Cheng, G.-H. Wang, *Chem. Pharm. Bull.* **2003**, *51*, 1429.

4 P.-J. Sung, T.-Y. Fan, M.-C. Chen, L.-S. Fang, M.-R. Lin, P.-C. Chang, *Biochem. Syst. Ecol.* **2004**, *32*, 111.

5 P.-J. Sung, M.-R. Lin, L.-S. Fang, *Chem. Pharm. Bull.* **2004**, *52*, 1504.

6 P.-J. Sung, M.-R. Lin, W.-C. Chen, L.-S. Fang, C.-K. Lu, J.-H. Sheu, *Bull. Chem. Soc. Jpn.* **2004**, *77*, 1229.

7 J.-H. Sheu, Y.-P. Chen, T.-L. Hwang, M. Y. Chiang, L.-S. Fang, P.-J. Sung, *J. Nat. Prod.* **2006**, *69*, 269.

8 P.-J. Sung, Y.-P. Chen, Y.-M. Su, T.-L. Hwang, W.-P. Hu, T.-Y. Fan, W.-H. Wang, *Bull. Chem. Soc. Jpn.* **2007**, *80*, 1205.

9 P.-J. Sung, M.-R. Lin, Y.-D. Su, M. Y. Chiang, W.-P. Hu, J.-H. Su, M.-C. Cheng, T.-L. Hwang, J.-H. Sheu, *Tetrahedron* **2008**, *64*, 2596.

10 P.-J. Sung, C.-H. Pai, Y.-D. Su, T.-L. Hwang, F.-W. Kuo, T.-Y. Fan, J.-J. Li, *Tetrahedron* **2008**, *64*, 4224.

11 P.-J. Sung, G.-Y. Li, Y.-P. Chen, I.-C. Huang, B.-Y. Chen, S.-H. Wang, S.-K. Huang, *Chem. Lett.* **2009**, *38*, 454.

12 P.-J. Sung, S.-H. Wang, M. Y. Chiang, Y.-D. Su, Y.-C. Chang, W.-P. Hu, C.-Y. Tai, C.-Y. Liu, *Bull. Chem. Soc. Jpn.* **2009**, *82*, 1426.

13 Y.-C. Shen, Y.-H. Chen, T.-L. Hwang, J.-H. Guh, A. T. Khalil, *Helv. Chim. Acta* **2007**, *90*, 1391.

14 C.-C. Liaw, Y.-C. Shen, Y.-S. Lin, T.-L. Hwang, Y.-H. Kuo, A. T. Khalil, *J. Nat. Prod.* **2008**, *71*, 1551.

15 M. García, J. Rodríguez, C. Jiménez, *J. Nat. Prod.* **1999**, *62*, 257. The metabolite **3** was originally named (+)-11α,20α-epoxyjunceellolide D in this reference, but the stereochemistry of the 11,20-epoxy group in this compound had been revised as having a β-orientation and should be renamed (+)-11β,20β-epoxyjunceellolide D. Please see Ref. 7.

16 P.-J. Sung, J.-H. Sheu, J.-P. Xu, *Heterocycles* **2002**, *57*, 535.

17 P.-J. Sung, P.-C. Chang, L.-S. Fang, J.-H. Sheu, W.-C. Chen, Y.-P. Chen, M.-R. Lin, *Heterocycles* **2005**, *65*, 195.

18 P.-J. Sung, J.-H. Sheu, W.-H. Wang, L.-S. Fang, H.-M. Chung, C.-H. Pai, Y.-D. Su, W.-T. Tsai, B.-Y. Chen, M.-R. Lin, G.-Y. Li, *Heterocycles* **2008**, *75*, 2627.

19 R. M. Silverstein, F. X. Webster, D. J. Kiemle, *Spectrometric Identification of Organic Compounds*, 7th ed., John-Wiley & Sons, Inc., Hoboken, NJ, USA, **2005**, pp. 171–172.

20 P.-J. Sung, M. Y. Chiang, W.-T. Tsai, J.-H. Su, Y.-M. Su, Y.-C. Wu, *Tetrahedron* **2007**, *63*, 12860.

21 Because few compound **3** ((+)-11β,20β-epoxyjunceellolide D) was isolated in this study, the 1D and 2D NMR data for compound **3** (1.8 mg) assigned in Table 2 were measured with a sample that we obtained in a previous study.

22 F. M. Bayer, M. Grasshoff, *Senckenbergiana Biol.* **1994**, *74*, 21.

23 K. Fabricius, P. Alderslade, *Soft Corals and Sea Fans–A Comprehensive Guide to the Tropical Shallow-Water Genera of the Central-West Pacific, the Indian Ocean and the Red Sea*, Australian Institute of Marine Science, Queensland, Australia, **2001**, pp. 230–231.

24 M. C. Alley, D. A. Scudiero, A. Monks, M. L. Hursey, M. J. Czerwinski, D. L. Fine, B. J. Abbott, J. G. Mayo, R. H. Shoemarker, M. R. Boyd, *Cancer Res.* **1988**, *48*, 589.

25 D. A. Scudiero, R. H. Shoemarker, K. D. Paull, A. Monks, S. Tierney, T. H. Nofziger, M. J. Currens, D. Seniff, M. R. Boyd, *Cancer Res.* **1988**, *48*, 4827.