Chlorinated Briarane Diterpenoids from the Sea Whip Gorgonian Corals *Junceella fragilis* and *Ellisella robusta* (Ellisellidae)

Su-Hui Wang,[a,b] Yu-Chia Chang,[a,b] Michael Y. Chiang,[c] Yung-Husan Chen,[b] Tsong-Long Hwang,[d] Ching-Feng Weng,[a] and Ping-Jyun Sung*[,a,b,e]

[a] *Graduate Institute of Marine Biotechnology, Department of Life Science and Graduate Institute of Biotechnology, National Dong Hwa University; Checheng, Pingtung 944, Taiwan:* [b] *National Museum of Marine Biology and Aquarium; Checheng, Pingtung 944, Taiwan:* [c] *Department of Chemistry, National Sun Yat-sen University;* [e] *Department of Marine Biotechnology and Resources, Asia-Pacific Ocean Research Center, National Sun Yat-sen University; Kaohsiung 804, Taiwan: and* [d] *Graduate Institute of Natural Products, Chang Gung University; Taoyuan 333, Taiwan.*
Received March 9, 2010; accepted April 1, 2010; published online April 26, 2010

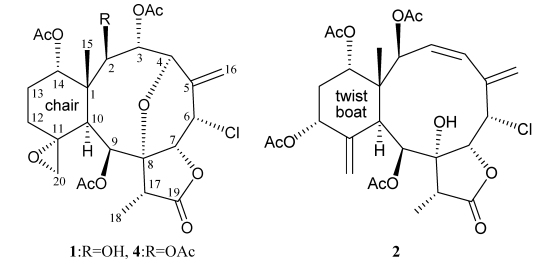
A new chlorinated briarane, fragilide J (1), has been isolated from the sea whip gorgonian coral *Junceella fragilis*. In addition, the sea whip gorgonian coral *Ellisella robusta* yielded two chlorinated briaranes, including a new compound, robustolide L (2), and a known metabolite, robustolide H (3). The structures of these compounds were determined using spectroscopic methods. The structure, including the absolute configuration of 3, was further confirmed by X-ray data analysis for the first time.

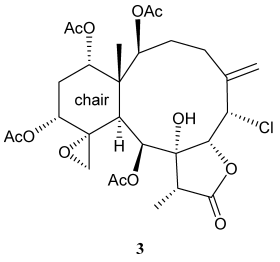
Key words *Junceella fragilis*; fragilide; *Ellisella robusta*; robustolide; briarane

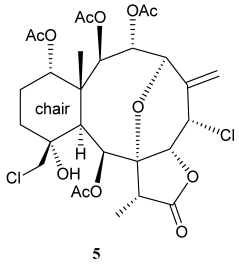
Previous studies on the chemical constituents of sea whip gorgonian corals belonging to the genus *Junceella* and *Ellisella* (family Ellisellidae), collected in waters off Taiwan, yielded a series of interesting briarane diterpenoids.[1—26] Briarane-type natural products are found only in marine organisms and mainly from octocorals.[27—29] Compounds of this type are suggested to be originally synthesized by host corals,[4,30] and confirmed to possess various bioactivities.[27—29] We have further isolated a new chlorinated briarane, fragilide J (**1**), from the sea whip gorgonian coral *Junceella fragilis*. Furthermore, two chlorinated briaranes, including a new compound, robustolide L (**2**), and a known metabolite, robustolide H (**3**),[24] were obtained from the sea whip gorgonian coral *Ellisella robusta*. In this paper, we describe the isolation, structure determination, and bioactivity of the above briaranes **1**—**3**.

Results and Discussion

Fragilide J from *J. fragilis* Fragilide J (**1**) was isolated as a white powder. The molecular formula of **1** was established as $C_{26}H_{33}ClO_{11}$ (10 degrees of unsaturation) from a sodiated molecule at *m*/*z* 579 in the electrospray ionization (ESI)-MS spectrum and further supported by the HR-ESI-MS (*m*/*z* 579.1604, Calcd 579.1609, $[C_{26}H_{33}{}^{35}ClO_{11}Na]^+$). The IR spectrum of **1** showed bands at 3491, 1790, and 1741 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester carbonyl groups. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of **1** showed that this compound has 26 carbons (Table 1), including five methyls, three sp^3 methylenes, nine sp^3 methines, three sp^3 quaternary carbons, an sp^2 methylene, and five sp^2 quaternary carbons. From the ^{1}H- and ^{13}C-NMR spectra (Table 1), **1** was found to possess three acetoxy groups (δ_H 2.30, 2.14, 2.07, each 3H×s; δ_C 169.8, 169.7, 169.4, each s; 21.2, 21.1, 20.6, each q), a γ-lactone moiety (δ_C 174.4, s, C-19), and an exocyclic carbon–carbon double bond (δ_C 134.9, s, C-5; 119.0, t, CH_2-16; δ_H 5.32, 1H, d, J=2.0 Hz, H-16a; 5.56, 1H, d, J=2.0 Hz, H-16b). The presence of an exocyclic epoxy group was confirmed from the signals of an oxygenated quaternary carbon at δ_C 56.4 (s, C-11) and an oxymethine at δ_C 51.6 (t, CH_2-20). The chemical shifts of C-20 methylene protons (δ_H 2.65, 1H, dd, J=3.2, 1.2 Hz, H-20a; 2.46, 1H, dd, J=3.2, 2.4 Hz, H-20b) confirmed the presence of this group. Thus, from the above data, five degrees of unsaturation were accounted for, and **1** was identified as a pentacyclic compound.







From the ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum of **1**, five different structural units, C-2/-3/-4, C-6/-7, C-9/-10, C-12/-13/-14, and C-17/-18, were identified (Table 1), which were assembled with the assistance of a heteronuclear multiple-bond coherence (HMBC) experiment (Table 1). The HMBC correlations between protons and quaternary carbons of **1**, such as H-9, H-10, H_3-15/C-1; H-4/C-5; H-4, H-17, H_3-18/C-8; H-9, H-10, H_2-12/C-11; and H-17, H_3-18/C-19, permitted the elucidation of the carbon skeleton of **1**. An exocyclic double bond at C-5 was confirmed by the allylic coupling between H-6 and H_2-16 in the ^{1}H–^{1}H COSY experiment and by the HMBC correlations between H_2-16/C-4, -6. The ring junction C-15 methyl group was positioned at C-1

* To whom correspondence should be addressed. e-mail: pjsung@nmmba.gov.tw

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

C/H	δ_H [a]	δ_C [b]	^{1}H–^{1}H COSY	HMBC (H→C)
1		48.1 (s)[d]		
2	4.06 dd (7.2, 3.6)[c]	72.2 (d)	H-3	n.o.[e]
3	6.16 dd (10.8, 7.2)	65.9 (d)	H-2, H-4	Acetate carbonyl
4	4.23 d (10.8)	79.5 (d)	H-3	C-3, -5, -6, -8
5		134.9 (s)		
6	4.99 ddd (3.2, 2.0, 2.0)	54.0 (d)	H-7, H_2-16	n.o.
7	4.40 d (3.2)	79.1 (d)	H-6	n.o.
8		82.9 (s)		
9	5.53 s	71.0 (d)	H-10	C-1, -10, -11, acetate carbonyl
10	2.79 s	40.4 (d)	H-9	C-1, -11, -15, -18, -20
11		56.4 (s)		
12α	2.21 m	29.7 (t)	H-12β, H_2-13	C-11
β	1.24 m		H-12α, H_2-13	C-11
13α	1.99 m	24.4 (t)	H_2-12, H-13β, H-14	n.o.
β	1.85 dddd (14.4, 14.4, 4.4, 2.0)		H_2-12, H-13α, H-14	n.o.
14	5.31 br s	75.0 (d)	H_2-13	n.o.
15	1.13 s	15.4 (q)		C-1, -2, -10
16a	5.32 d (2.0)	119.0 (t)	H-6, H-16b	C-4, -6
b	5.56 d (2.0)		H-6, H-16a	C-4, -6
17	2.78 q (6.8)	49.3 (d)	H_3-18	C-8, -19
18	1.29 d (6.8)	7.3 (q)	H-17	C-8, -17, -19
19		174.4 (s)		
20a	2.65 dd (3.2, 1.2)	51.6 (t)	H-20b	n.o.
b	2.46 dd (3.2, 2.4)		H-20a	n.o.
OH-2	2.40 d (3.6)		H-2	n.o.
3-OAc	2.14 s	21.1 (q)		Acetate carbonyl
		169.8 (s)		
9-OAc	2.07 s	20.6 (q)		Acetate carbonyl
		169.7 (s)		
14-OAc	2.30 s	21.2 (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.

from the HMBC correlations between H_3-15/C-1, -2, -10 and H-10/C-15. The acetate esters at C-3 and C-9 were established by correlations between H-3 (δ_H 6.16), H-9 (δ_H 5.53) and the acetate carbonyls observed in the HMBC spectrum of **1**. The hydroxy proton signal at δ_H 2.40 was revealed by its ^{1}H–^{1}H COSY correlation to H-2 (δ_H 4.06), indicating its attachment to C-2. The remaining acetate ester was at C-14, as indicated by analysis of the ^{1}H–^{1}H COSY correlations and characteristic NMR signal analysis.

The intensity of the sodiated molecule isotope peak $(M+2+Na)^+$ observed in the ESI-MS spectrum $[(M+Na)^+ : (M+2+Na)^+ = 3 : 1]$ was evidence of the presence of a chlorine atom in **1**. The methine unit at δ_C 54.0 (d) was more shielded than that expected for an oxygenated C-atom and correlated with the methine proton at δ_H 4.99 in the heteronuclear multiple-quantum coherence (HMQC) spectrum, and this proton showed ^3J- and ^4J-correlations with H-7 and H_2-16, respectively, in the ^{1}H–^{1}H COSY spectrum, confirming the attachment of a chlorine atom at C-6. In addition, the methylene unit at δ_C 51.6 (t) was correlated with the methylene protons at δ_H 2.65 and 2.46 in the HMQC spectrum and the HMBC correlations between H-9, H-10, H_2-12/C-11 (an oxygenated quaternary carbon, δ_C 56.4, s), and H-10/C-20, confirming the attachment of an epoxy group at C-11/20. Furthermore, an HMBC correlation between H-4 (δ_H 4.23) and an oxygenated quaternary carbon at δ_C 82.9 (s, C-8) suggested the presence of a C-4/8 ether linkage in **1**.

The chemical shifts of exocyclic 11,20-epoxy groups in briarane analogues have been summarized, and although the

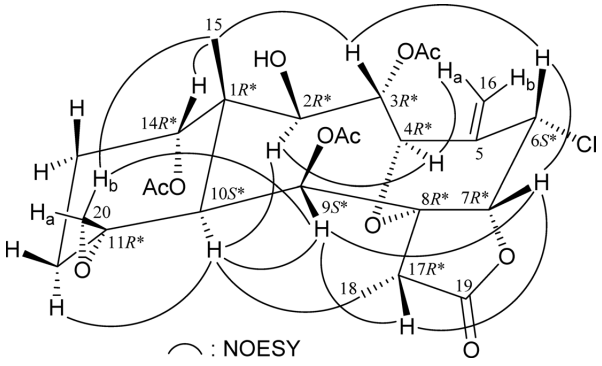


Fig. 1. Selective NOESY Correlations of **1**

^{13}C-NMR peaks for C-11 and C-20 appear at δ_C 55—61 and 47—52 ppm, respectively, the epoxy group is α-oriented (11*R**) and the cyclohexane ring is of a chair conformation.[7] Based on the above findings, the configuration of 11,20-epoxy group in **1** (δ_C 56.4, s, C-11; 51,6, t, CH_2-20) was α-oriented and the cyclohexane ring in **1** should be in a chair conformation. Based on previous surveys, all the briaranes have the H-10 *trans* to C-15 methyl, and these two groups are assigned as α- and β-oriented in most briarane analogues.[27—29] The relative stereochemistry of **1** was established from the interactions observed in a nuclear Overhauser effect spectroscopy (NOESY) experiment and by vicinal ^{1}H–^{1}H coupling constant analysis. In the NOESY experiment of **1** (Fig. 1), the correlations between H-10 and H-2, H-9, one proton

Table 2. ^{1}H- and ^{13}C-NMR Data, ^{1}H–^{1}H COSY, and HMBC Correlations for Diterpenoid **2**

C/H	δ_H [a]	δ_C [b]	^{1}H–^{1}H COSY	HMBC (H→C)
1		47.7 (s) [d]		
2	6.25 d (8.0) [c]	71.2 (d)	H-3	Acetate carbonyl
3	5.62 dd (12.0, 8.0)	130.6 (d)	H-2, H-4	C-5
4	5.97 d (12.0)	129.0 (d)	H-3	C-2, -16
5		138.3 (s)		
6	5.19 d (3.6)	62.4 (d)	H-7, H_2-16	C-5, -7
7	5.01 d (3.6)	78.5 (d)	H-6	C-6
8		84.8 (s)		
9	5.45 br s	71.0 (d)	H-10	Acetate carbonyl
10	3.67 br s	42.8 (d)	H-9	n.o. [e]
11		146.3 (s)		
12	5.34 dd (8.0, 7.6)	68.1 (d)	H_2-13	C-11, -13, -20, acetate carbonyl
13α	1.78 ddd (15.2, 8.0, 2.4)	34.2 (t)	H-12, H-13β, H-14	C-1, -12, -14
β	2.46 m		H-12, H-13α, H-14	n.o.
14	4.75 br s	73.3 (d)	H_2-13	Acetate carbonyl
15	1.11 s	15.1 (q)		C-1, -2, -10
16a	6.05 br s	118.1 (t)	H-6, H-16b	C-4, -6
b	6.01 d (2.4)		H-6, H-16a	C-4, -5, -6
17	2.43 q (7.2)	44.9 (d)	H_3-18	C-8, -18, -19
18	1.16 d (7.2)	6.7 (q)	H-17	C-8, -17, -19
19		175.0 (s)		
20	5.13 br s (2H)	112.3 (t)		n.o.
OH-8	2.65 s			C-7, -8, -9
2-OAc	2.08 s	21.0 (q)		Acetate carbonyl
		169.8 (s)		
9-OAc	2.01 s	21.0 (q)		Acetate carbonyl
		170.0 (s)		
12-OAc	2.15 s	21.7 (q)		Acetate carbonyl
		169.8 (s)		
14-OAc	1.94 s	21.1 (q)		Acetate carbonyl
		170.1 (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.

of the C-12 methylene (δ_H 2.21), and H_3-18, indicate that these protons were situated on the same face; they were assigned as α protons, as the C-15 methyl was β-oriented and H_3-15 did not show a correlation with H-10. The oxymethine protons H-3, H-14, and one proton of the C-20 methylene (δ_H 2.46, H-20b) were found to exhibit interactions with H_3-15, but not with H-10, revealing that H-3 and H-14 were β-oriented and the epoxy group between C-11/20 was α-oriented. H-9 was found to show correlations with H-7, H-17, and H-20b. From modeling analysis, H-9 was found to be reasonably close to H-7, H-17, and H-20b and can therefore be placed on the α face in **1**, and the methine protons H-7 and H-17 are β-oriented. H-7 exhibited correlations with H-6, H-9, and H-17 and H-6 correlated with H-3, indicating that these protons were on the β face of **1**. Moreover, H-4 showed correlations with H-2 and one proton of the C-16 methylene (δ_H 5.32, H-16a) and a large coupling constant was found between H-4 and H-3 (J=10.8 Hz), indicating that the dihedral angle between H-3 and H-4 is approximately 180° and H-4 has an α-orientation at C-4. It was found that the structure of **1** was similar to that of a known briarane, praelolide (**4**),[3,31,32] which was first isolated from the gorgonian coral *Plexaureides praelonga* collected off the South China Sea,[31] except that the signals corresponding to a 2β-acetoxy group in **4** was replaced by a 2β-hydroxy group in **1**. Based on the above findings, the structure of **1** was established and the chiral centers of **1** were assigned as 1*R**, 2*R**, 3*R**, 4*R**, 6*S**, 7*R**, 8*R**, 9*S**, 10*S**, 11*R**, 14*R**, and 17*R**.

In our previous study, a chlorinated briarane derivative, fragilide F (**5**), was isolated from the same marine organism (*J. fragilis*) as **1**, and the absolute stereochemistry of **5** was determined directly by X-ray diffraction analysis.[13] Based on biosynthetic derivation, the new briarane **1** (fragilide J) is assumed to have the same absolute configuration as **5**, because these two compounds were isolated from the same organism. Therefore, fragilide J should possess the absolute configuration as represented by the structure of **1**.

Robustolides L and H from *E. robusta* Robustolide L (**2**) was obtained as a white powder. The HR-ESI-MS of **2** revealed a sodiated molecular ion peak at *m*/*z* 605.1769 $(M+Na)^+$ consistent with the molecular formula $C_{28}H_{35}ClO_{11}$ (Calcd for $C_{28}H_{35}{}^{35}ClO_{11}Na$, 605.1765) and 11 degrees of unsaturation. The IR spectrum of **2** showed bands at 3471, 1783, and 1737 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester groups. From the ^{13}C-NMR data of **2** (Table 2), the presence of a disubstituted olefin and two exocyclic carbon–carbon double bonds was deduced from the signals of six carbons at δ_C 146.3 (s, C-11), 138.3 (s, C-5), 130.6 (d, CH-3), 129.0 (d, CH-4), 118.1 (t, CH_2-16), and 112.3 (t, CH_2-20). This was further supported by six olefin proton signals at δ_H 6.05 (1H, br s, H-16a), 6.01 (1H, d, J= 2.4 Hz, H-16b), 5.97 (1H, d, J=12.0 Hz, H-4), 5.62 (1H, dd, J=12.0, 8.0 Hz, H-3), and 5.13 (2H, br s, H_2-20) in the ^{1}H-NMR spectrum of **2** (Table 2). In the ^{13}C-NMR spectrum, five ester carbonyl resonances were detected at δ_C 175.0 (s, C-19), 170.1, 170.0, 169.8, and 169.8 (4×s), confirming the presence of a γ-lactone and four other ester groups. In the ^{1}H-NMR spectrum of **2**, four acetyl methyls (δ_H 2.15, 2.08,

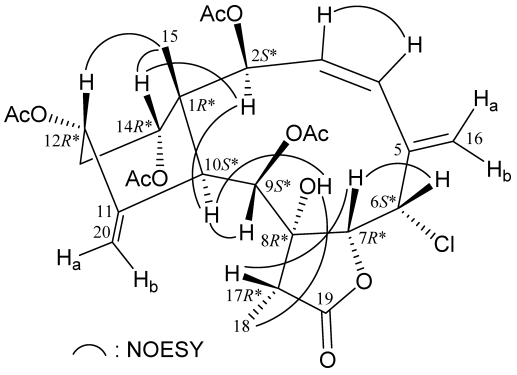


Fig. 2. Selective NOESY Correlations of **2**

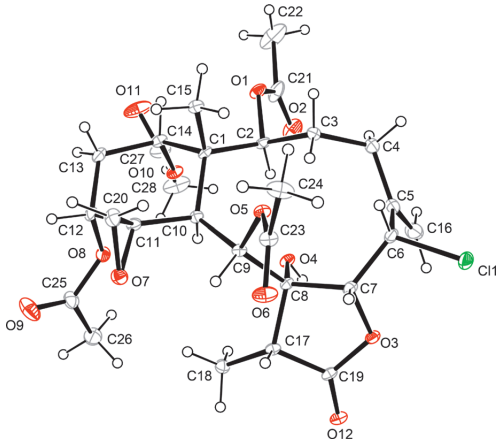


Fig. 3. Computer-generated ORTEP Plot of **3** Showing the Absolute Configuration

2.01, 1.94, each 3H×s) were observed. Thus, from the above NMR data, eight degrees of unsaturation were accounted for, and **2** was identified as a tricyclic compound.

From the ^{1}H–^{1}H COSY spectrum of **2** (Table 2), it was possible to establish the separate spin systems that map out the proton sequences from H-2/H-3/H-4, H-6/H-7, H-6/H_2-16 (by allylic coupling), H-9/H-10, H-12/H_2-13/H-14, and H-17/H_3-18. Based on these data and HMBC correlations (Table 2), the carbon skeleton of **2** was established. The exocyclic double bonds attached at C-5 and C-11, respectively, were confirmed by the HMBC correlations between H_2-16/C-4, -5, -6; H-4/C-16; and H-12/C-11, -20. The C-15 methyl group was positioned at C-1 from the HMBC correlations between H_3-15/C-1, -2, -10. The HMQC and ^{1}H–^{1}H COSY correlations also revealed that the chlorine atom was at the C-6 methine (δ_H 5.19, δ_C 62.4). The presence of acetate esters at C-2, C-9, C-12, and C-14 was established by the HMBC correlations between H-2 (δ_H 6.25), H-9 (δ_H 5.45), H-12 (δ_H 5.34), H-14 (δ_H 4.75) and the acetate carbonyls at δ_C 169.8 (s), 170.0 (s), 169.8 (s), 170.1 (s), respectively. The 8-hydroxy group was confirmed from the HMBC correlations between a hydroxy proton (δ_H 2.65, 1H, br s) and an oxygenated quaternary carbon (δ_C 84.8, s, C-8). The methine unit at δ_C 62.4 (d, CH-6) was more shielded than would be expected for an oxygenated C-atom and was correlated with the methine proton at δ_H 5.19 in the HMQC spectrum. The latter methine proton signal was ^{3}J- and ^{4}J-correlated with H-7 and H_2-16, respectively, confirming the attachment of a chlorine atom at C-6. These data, together with the HMBC correlations between H-17/C-8, -18, -19 and H_3-18/C-8, -17, -19, unambiguously established the molecular framework of **2**.

In a previous study, the proton chemical shifts for the briarane derivatives contained an 11,20-exocyclic carbon–carbon double bond were summarized: while the chemical shifts for the olefin protons H_2-20 appeared at δ_H 4.95—5.30 and 4.85—5.15 ppm, respectively, and the cyclohexane rings show a twist boat conformation.[13] Due to the chemical shifts of C-20 methylene protons (δ_H 5.13, 2H), the configuration of the cyclohexane ring in **2** should exist in a twist boat conformation. The relative stereochemistry of **2** was elucidated mainly from the interactions observed in a NOESY experiment (Fig. 2) and by the vicinal ^{1}H–^{1}H coupling constants. In the NOESY experiment of **2**, H-10 gave correlations to H-2, H-9, and OH-8, while OH-8 correlated with H_3-18, suggesting that these protons were located on the same face and assigned as α protons, since the C-15 methyl is the β-substituent at C-1. H-14 was found to exhibit a correlation with H_3-15, revealing the β-orientation of this proton. In addition, H-12 was found to correlate with H_3-15, indicating that the C-12 acetoxy group was α-oriented. H-7 exhibited correlations with H-6 and H-17, suggesting that these protons were positioned on the β face in **2**. The *cis* geometry of the C-3/4 double bond was indicated by a 12.0 Hz coupling constant between H-3 (δ_H 5.62) and H-4 (δ_H 5.97), and further supported by a NOESY correlation between these two protons. Based on the above findings, the structure of **2** was established, and the configurations of all chiral centers of **1** are assigned as 1*R**, 2*S**, 6*S**, 7*R**, 8*R**, 9*S**, 10*S**, 12*R**, 14*R**, and 17*R**.

The known briarane, robustolide H (**3**), was first isolated from *E. robusta* in our previous study.[24] Its structure, including the absolute configuration of this metabolite was determined using X-ray diffraction analysis for the first time in this study (Fig. 3), and the chiral centers of this compound were assigned as 1*S*, 2*S*, 6*S*, 7*R*, 8*R*, 9*S*, 10*S*, 11*R*, 12*R*, 14*S*, and 17*R*. Furthermore, because the absolute configuration of **3** was determined based on its X-ray structure, the new briarane **2** (robustolide L) is assumed to have the same absolute configuration as **3** because these compounds were isolated from the same organism.

In biological activity testing, fragilide J (**1**) exhibited an 11.5% inhibitory effect on elastase release by human neutrophils at 10 μg/ml; robustolide L (**2**) displayed a 13.9% inhibitory effect on superoxide anion generation by human neutrophils at 10 μg/ml and this compound showed weak cytotoxicity toward IMR-32 (human neuroblastoma) tumor cells (ED_{50}=33.8 μg/ml).

Experimental

General Melting points were measured on a FARGO apparatus and are uncorrected. Optical rotation values were measured with a JASCO P-1010 digital polarimeter. 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 $CDCl_3$. 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. 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, Hitachi L-7455 photodiode array detector, and Rheodyne 7725 injection port. A semi-preparative normal-phase column (Hibar 250–10 mm, LiChrospher Si 60, 5 μm) was used for HPLC. Preparative TLC was carried out on precoated Kieselgel 60 F_{254} (layer thickness 210—270 mm).

Animal Material Specimens of the sea whip gorgonian corals *J. fragilis* and *E. robusta* were collected off the coast of Pingtung, southern Taiwan. A voucher specimen were deposited in the National Museum of Marine Biology and Aquarium, Taiwan. These two organisms were identified by comparison with previous descriptions.[33—36)]

Extraction and Isolation. *J. fragilis* 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 extract was partitioned between EtOAc and H_2O. The EtOAc layer was separated on silica gel and eluted using hexane/EtOAc (stepwise, 20 : 1–pure EtOAc) to yield the 19 fractions A—S. Fraction R was separated on a gravity column with silica gel and eluted using hexane/acetone (stepwise, 50 : 1—1 : 2) to afford the 14 fractions R1—R14. Fraction R10 was repurified with preparative TLC and eluted using hexane/EtOAc (2 : 1) to afford the nine fractions R10-1—10-9. Fraction R10-9 was separated with normal-phase HPLC using hexane/acetone (4 : 1) to afford **1** (1.5 mg).

Fragilide J (**1**): White powder; mp 153—156 °C; $[\alpha]_D^{22}$ −153° (c=0.005, $CHCl_3$); IR (neat) ν_{max} 3491, 1790, 1741 cm^{-1}; ^{13}C-NMR ($CDCl_3$, 100 MHz) and ^{1}H-NMR ($CDCl_3$, 400 MHz) data: see Table 1; ESI-MS m/z: 579 $(M+Na)^+$, 581 $(M+2+Na)^+$; HR-ESI-MS m/z: 579.1604 (Calcd for $C_{26}H_{33}{}^{35}ClO_{11}Na$, 579.1609).

E. robusta The freeze-dried and minced material of *E. robusta* (dry weight 830 g) was extracted with a mixture of MeOH and CH_2Cl_2 (1 : 1). The residue was partitioned between EtOAc and H_2O. The EtOAc layer was separated on silica gel and eluted using hexane/EtOAc to yield 28 fractions. Fraction 10 was separated on silica gel and eluted using CH_2Cl_2/EtOAc (10 : 1–pure EtOAc) to yield the 14 fractions 10A—10N. Fraction 10D was purified with normal phase HPLC using CH_2Cl_2/acetone (50 : 1–pure acetone) to afford the 15 fractions 10D-1—10D-15. Fraction 10D-5 was repurified with normal-phase HPLC using CH_2Cl_2/EtOAc (14 : 1) to afford **2** (3.5 mg). Fractions 10F and 10G were combined and reseparated on silica gel and eluted using hexane/acetone (5 : 1–pure acetone) to afford **3** (2 : 1, 37.4 mg).

Robustolide L (**2**): White powder; mp 169—171 °C; $[\alpha]_D^{23}$ −125° (c= 0.002, $CHCl_3$); IR (neat) ν_{max} 3471, 1783, 1737 cm^{-1}; ^{13}C-NMR ($CDCl_3$, 100 MHz) and ^{1}H-NMR ($CDCl_3$, 400 MHz) data: see Table 2; ESI-MS m/z: 605 $(M+Na)^+$, 607 $(M+2+Na)^+$; HR-ESI-MS m/z: 605.1769 (Calcd for $C_{28}H_{35}{}^{35}ClO_{11}Na$, 605.1765).

Robustolide H (**3**): The related physical (mp, optical rotation value) and spectral (IR, ^{1}H-, ^{13}C-NMR) data of **3** are in full agreement with those reported previously.[24)]

X-ray Diffraction Analysis of Robustolide H (3)[37)] Suitable colorless prisms of **3** were obtained from a solution of MeOH. The crystal (0.42×0.38×0.20 mm) belongs to the monoclinic system, space group C2 (# 5), with a=17.4792(8) Å, b=18.9594(8) Å, c=9.9193(4) Å, β=104.639(2)°, V=3180.5(2) Å^3, Z=4, D_{Calcd}=1.255 g/cm^3, and λ (Mo$K\alpha$)=0.71073 Å. Intensity data were measured on a Bruker Smart diffractometer up to $2\theta_{max}$ of 50.12°. All 5467 reflections were collected. The structure was solved using direct methods and refined using a full-matrix least-squares procedure. The refined structural model converged to a final $R1$=0.033 and $wR2$=0.0942 for 5175 observed reflections [$I>2\sigma(I)$] and 380 variable parameters. The absolute configuration of **3** was determined using Flack's method in which the fractional contribution of the inverted component of its racemic twin structure, expressed as Flack's parameter (zero for correct absolute configuration), was refined against data with Bijvoet pairs. In this case, Flack's parameter was determined to be 0.00(5).[38)]

Human Neutrophil Superoxide Anion Generation and Elastase Release Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Superoxide anion generation was carried out according to the procedures described previously.[39,40)] Briefly, superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*. Elastase release experiments were performed using MeO–Suc–Ala–Ala–Pro–Val–*p*-nitroanilide as the elastase substrate.

Cytotoxicity Testing The cytotoxicity of tested compounds was assayed with a modification of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. Cytotoxicity assays were carried out according to the procedures described previously.[41,42)]

Acknowledgments This research work was supported by grants from the National Museum of Marine Biology and Aquarium (99200321 and 99200322); National Dong Hwa University; Asia-Pacific Ocean Research Center, National Sun Yat-sen University (96C031702); and National Science and Technology Program for Biotechnology and Pharmaceuticals, National Science Council (NSC 98-2323-B-291-001 and 98-2320-B-291-001-MY3), Taiwan, awarded to P.-J.S.

References and Notes

1) Sung P.-J., Wu S.-L., Fang H.-J., Chiang M. Y., Wu J.-Y., Fang L.-S., Sheu J.-H., *J. Nat. Prod.*, **63**, 1483—1487 (2000).
2) Sung P.-J., Fan T.-Y., *Heterocycles*, **60**, 1199—1202 (2003).
3) Sung P.-J., Fan T.-Y., Fang L.-S., Wu S.-L., Li J.-J., Chen M.-C., Cheng, Y.-M., Wang G.-H., *Chem. Pharm. Bull.*, **51**, 1429—1431 (2003).
4) Sung P.-J., Fan T.-Y., Chen M.-C., Fang L.-S., Lin M.-R., Chang P.-C., *Biochem. Syst. Ecol.*, **32**, 111—113 (2004).
5) Sung P.-J., Lin M.-R., Fang L.-S., *Chem. Pharm. Bull.*, **52**, 1504—1506 (2004).
6) Sung P.-J., Lin M.-R., Chen W.-C., Fang L.-S., Lu C.-K., Sheu J.-H., *Bull. Chem. Soc. Jpn.*, **77**, 1229—1230 (2004).
7) Sheu J.-H., Chen Y.-P., Hwang T.-L., Chiang M. Y., Fang L.-S., Sung P.-J., *J. Nat. Prod.*, **69**, 269—273 (2006).
8) Sung P.-J., Chen Y.-P., Su Y.-M., Hwang T.-L., Hu W.-P., Fan T.-Y., Wang W.-H., *Bull. Chem. Soc. Jpn.*, **80**, 1205—1207 (2007).
9) Sung P.-J., Lin M.-R., Su Y.-D., Chiang M. Y., Hu W.-P., Su J.-H., Cheng M.-C., Hwang T.-L., Sheu J.-H., *Tetrahedron*, **64**, 2596—2604 (2008).
10) Sung P.-J., Pai C.-H., Su Y.-D., Hwang T.-L., Kuo F.-W., Fan T.-Y., Li J.-J., *Tetrahedron*, **64**, 4224—4232 (2008).
11) Sung P.-J., Pai C.-H., Hwang T.-L., Fan T.-Y., Su J.-H., Chen J.-J., Fang L.-S., Wang W.-H., Sheu J.-H., *Chem. Pharm. Bull.*, **56**, 1276—1281 (2008).
12) Sung P.-J., Li G.-Y., Chen Y.-P., Huang I-C., Chen B.-Y., Wang S.-H., Huang S.-K., *Chem. Lett.*, **38**, 454—455 (2009).
13) Sung P.-J., Wang S.-H., Chiang M. Y., Su Y.-D., Chang Y.-C., Hu W.-P., Tai C.-Y., Liu C.-Y., *Bull. Chem. Soc. Jpn.*, **82**, 1426—1432 (2009).
14) Shen Y.-C., Lin Y.-C., Chiang M. Y., *J. Nat. Prod.*, **65**, 54—56 (2002).
15) Shen Y.-C., Lin Y.-C., Huang Y.-L., *J. Chin. Chem. Soc.*, **50**, 1267—1270 (2003).
16) Shen Y.-C., Lin Y.-C., Ko C.-L., Wang L.-T., *J. Nat. Prod.*, **66**, 302—305 (2003).
17) Lin Y.-C., Huang Y.-L., Khalil A. T., Chen M.-H., Shen Y.-C., *Chem. Pharm. Bull.*, **53**, 128—130 (2005).
18) Shen Y.-C., Chen Y.-H., Hwang T.-L., Guh J.-H., Khalil A. T., *Helv. Chim. Acta*, **90**, 1391—1398 (2007).
19) Liaw C.-C., Shen Y.-C., Lin Y.-S., Hwang T.-L., Kuo Y.-H., Khalil A. T., *J. Nat. Prod.*, **71**, 1551—1556 (2008).
20) Wang S.-S., Chen Y.-H., Chang J.-Y., Hwang T.-L., Chen C.-H., Khalil A. T., Shen Y.-C., *Helv. Chim. Acta*, **92**, 2092—2100 (2009).
21) Sung P.-J., Tsai W.-T., Chiang M. Y., Su Y.-M., Kuo J., *Tetrahedron*, **63**, 7582—7588 (2007).
22) Su Y.-M., Fan T.-Y., Sung P.-J., *Nat. Prod. Res.*, **21**, 1085—1090 (2008).
23) Sung P.-J., Chiang M. Y., Tsai W.-T., Su J.-H., Su Y.-M., Wu Y.-C., *Tetrahedron*, **63**, 12860—12865 (2007).
24) Sung P.-J., Tsai W.-T., Lin M.-R., Su Y.-D., Pai C.-H., Chung H.-M., Su J.-H., Chiang M. Y., *Chem. Lett.*, **37**, 88—89 (2008).
25) Hwang T.-L., Lin M.-R., Tsai W.-T., Yeh H.-C., Hu W.-P., Sheu J.-H., Sung P.-J., *Bull. Chem. Soc. Jpn.*, **81**, 1638—1646 (2008).
26) Chang Y.-C., Hwang T.-L., Huang S.-K., Huang L.-W., Lin M.-R., Sung P.-J., *Heterocycles*, **81**, 991—996 (2010).
27) Sung P.-J., Sheu J.-H., Xu J.-P., *Heterocycles*, **57**, 535—579 (2002).
28) Sung P.-J., Chang P.-C., Fang L.-S., Sheu J.-H., Chen W.-C., Chen Y.-P., Lin M.-R., *Heterocycles*, **65**, 195—204 (2005).
29) Sung P.-J., Sheu J.-H., Wang W.-H., Fang L.-S., Chung H.-M., Pai C.-H., Su Y.-D., Tsai W.-T., Chen B.-Y., Lin M.-R., Li G.-Y., *Heterocycles*, **75**, 2627—2648 (2008).
30) Kokke W. C. M. C., Epstein S., Look S. A., Rau G. H., Fenical W., Djerassi C., *J. Biol. Chem.*, **259**, 8168—8173 (1984).
31) Luo Y., Long K., Fang Z., *Zhongshan Daxue Xuebao, Ziran Kexueban*, **1**, 83—92 (1983).
32) Shin J., Park M., Fenical W., *Tetrahedron*, **45**, 1633—1638 (1989).
33) Bayer F. M., *Proc. Biol. Soc. Wash.*, **94**, 902—947 (1981).
34) Chen C.-C., Chang K.-H., *Bull. Inst. Zool. Acad. Sin.*, **30**, 149—182

(1991).
35) Bayer F. M., Grasshoff M., *Senckenbergiana Biol.*, **74**, 21—45 (1994).
36) Fabricus K., Alderslade P., "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, 2001, pp. 224—225, 230—231.
37) Crystallographic data for the structure of robustolide H (**3**) were deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 770689. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].
38) Flack H. D., *Acta Crystallogr.*, **A39**, 876—881 (1983).
39) Hwang T.-L., Su Y.-C., Chang H.-L., Leu Y.-L., Chung P.-J., Kuo L.-M., Chang Y.-J., *J. Lipid Res.*, **50**, 1395—1408 (2009).
40) Hwang T.-L., Li G.-L., Lan Y.-H., Chia Y.-C., Hsieh P.-W., Wu Y.-H., Wu Y.-C., *Free Radic. Biol. Med.*, **46**, 520—528 (2009).
41) Alley M. C., Scudiero D. A., Monks A., Hursey M. L., Czerwinski M. J., Fine D. L., Abbott B. J., Mayo J. G., Shoemark R. H., Boyd M. R., *Cancer Res.*, **48**, 589—601 (1988).
42) Scudiero D. A., Shoemark R. H., Paull K. D., Monks A., Tierney S., Nofziger T. H., Currens M. J., Seniff D., Boyd M. R., *Cancer Res.*, **48**, 4827—4833 (1988).