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New 8-hydroxybriarane diterpenoids from the gorgonians *Junceella juncea* and *Junceella fragilis* (Ellisellidae)

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Abstract

Four new 8-hydroxybriarane diterpenoids, including junceols A–C (**1**–**3**) and fragilide D (**4**), have been isolated from the gorgonian corals *Junceella juncea* and *Junceella fragilis*, respectively. The structures of briaranes **1**–**4** were elucidated by the interpretations of spectral data analysis. Briaranes **1**–**3** have displayed inhibitory effects on superoxide anion generation by human neutrophils.

Keywords: *Junceella*; Junceol; Fragilide; Briarane; Superoxide anion

1. Introduction

We recently reported a series of novel terpenoid derivatives from the Formosan octocorals, including the briarane diterpenoids from *Briareum* sp.,[1] *Briareum excavatum*,[2–4] *Ellisella robusta*,[5–8] *Junceella fragilis*,[4,9–15] *Junceella juncea*;[11,16] and the caryophyllane sesquiterpenoids from *Rumphella antipathies*.[17–22] In continuation of our search for bioactive natural substances from the invertebrates collected off Taiwanese waters, we have further isolated four new 8-hydroxybriarane diterpenoids, junceols A–C (**1**–**3**) and fragilide D (**4**), from the gorgonians *J. juncea* and *J. fragilis* (Ellisellidae), respectively. Briarane-type natural products are suggested to be originally synthesized by host corals,[11,23] and the compounds of this type were proven to possess various biological activity.[24,25] In this paper, we described the isolation, structure determination, and biological activity of above new metabolites. The structures, including the relative configurations of briaranes **1**–**4**, were elucidated by spectroscopic methods. Briaranes **1**–**3** showed inhibitory effects on superoxide anion generation by human neutrophils.

2. Results and discussion

2.1. Isolation and structure determination of junceols from *J. juncea*

Previous chemical investigations of the gorgonian coral *J. juncea* have yielded a series of interesting new natural products including 37 briaranes, juncins A–Z,[16,26–30] (+)-gemmacolides A and B,[27] juncin ZI,[30] and juncenolides A–G;[31–35] five

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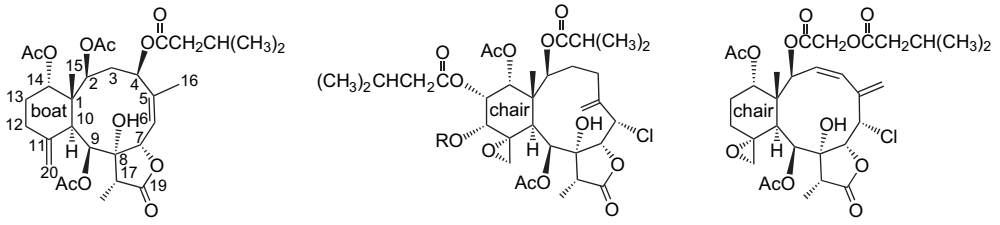

1 **2**:R= Ac , **3**:R=C(O)$CH_2CH(CH_3)_2$ **4**

steroid derivatives, junceellosides A–D[36] and 4′-*O*-acetyl-3-*O*-[β-D-arabinopyranosyloxy]-cholest-5-ene-3β,19-diol;[37] a glycerol derivative, 1,2-*O*-[2′-hydroxyoctadecyl]-glycerol;[37] and a sphingolipid, (2*S*,3*R*,4*E*)-1,3-dihydroxy-2-[(nonadecanoyl)-amino]-octadec-4-ene.[38]

Junceol A (**1**) was obtained as a white powder. The HRESIMS data recorded at 615.2780 established the molecular formula of **1** as $C_{31}H_{44}O_{11}$ (calcd for $C_{31}H_{44}O_{11}$+Na, 615.2781). The IR absorptions of **1** showed the presence of 3470, 1786, and 1733 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester groups. From the ^{13}C NMR data of **1** (Table 1), the presence of a trisubstituted olefin and an exocyclic carbon–carbon double bond were deduced from the signals of four carbons resonating at δ 151.1 (s, C-11), 144.5 (s, C-5), 123.8 (d, CH-6), and 113.0 (t, CH_2-20), and further supported by three olefin proton signals at δ 5.79 (1H, dd, *J*=10.4, 1.2 Hz, H-6), 5.03 (1H, s, H-20a), and 4.88 (1H, s, H-20b) in the ^1H NMR spectrum of **1** (Table 2). Furthermore, in the ^{13}C NMR spectrum, five carbonyl resonances appeared at δ 175.9 (s, C-19), 172.2 (s, ester carbonyl), 170.5 (s, ester carbonyl), 170.3 (s, ester carbonyl), and 169.3 (s, ester carbonyl), confirming the presence of a γ-lactone and four esters in **1**. In the ^1H NMR spectrum, three acetate methyls (δ 1.90, 3H, s; 2.00, 3H, s; 2.22, 3H, s) and an isovaleroxy group (δ 0.95, 2×3H, d, *J*=6.8 Hz; 2.12, 1H, m; 2.17, 2H, d, *J*=7.6 Hz) were observed. The ^1H NMR spectrum of **1** also showed the presence of a vinyl methyl (δ 2.23, 3H, d, *J*=1.2 Hz, H_3-16), a methyl doublet (δ 1.12, 3H, d, *J*=7.2 Hz, H_3-18), a methyl singlet (δ 1.10, 3H, s, H_3-15), two aliphatic methine protons (δ 3.31, 1H, d, *J*=5.6 Hz, H-10; 2.47, 1H, q, *J*=7.2 Hz, H-17), five oxymethine protons (δ 5.59, 1H, d, *J*=10.4 Hz, H-7; 5.26, 1H, d, *J*=5.6 Hz, H-9; 5.22, 1H, dd, *J*=13.6, 5.2 Hz, H-4; 4.77, 1H, d, *J*=4.0 Hz, H-2; 4.70, 1H, d, *J*=4.0 Hz, H-14), and three pairs of aliphatic methylene protons (δ 2.75, 1H, dd, *J*=13.6, 13.6 Hz; 1.95, 1H, m, H_2-3; 2.27, 1H, m; 2.09, 1H, br t, *J*=6.8 Hz, H_2-12; 2.04, 1H, m; 1.77, 1H, m, H_2-13) were observed in the ^1H NMR spectrum of **1**.

From the ^1H–^1H COSY spectrum of **1** (Fig. 1), it was possible to establish the separate spin systems from H-2/3/4; H-6/7; and H-9/10. These data, together with the HMBC correlations between H-2/C-1, -3, -4, -10; H_2-3/C-1, -2, -4, -5; H-4/C-3, -5, -6; H-6/C-4, -7; H-7/C-5, -6, -8; H-9/C-7, -8, -10; and H-10/C-1, -2, -8, -9 (Fig. 1 and Table 3), established the connectivity from C-1 to C-10 within the 10-membered ring. A vinyl methyl attached at C-5 was confirmed by the allylic coupling between H_3-16 and H-6 and by the HMBC correlations between H-4/C-16; H-6/C-16; and H_3-16/C-4, -5, -6. The methylenecyclohexane ring, which is fused to the 10-membered ring at C-1 and C-10, was established by the ^1H–^1H COSY correlations between H_2-12/H_2-13 and H_2-13/H-14; and by the HMBC correlations between H-2/C-14; H-9/C-11; H-10/C-11, -12, -14, -20; H_2-12/C-10, -11, -13, -20; H_2-13/C-1, -12, -14; and H-14/C-1, -10, -12. The exocyclic carbon–carbon bond, which is attached to the six-membered

Table 1
^{13}C NMR data for diterpenoids **1**–**3**[a]

Position	1	2	3
1	47.4 (s)[b]	47.3 (s)	47.3 (s)
2	72.0 (d)	72.4 (d)	72.2 (d)
3	38.0 (t)	28.3 (t)	28.4 (t)
4	72.4 (d)	33.9 (t)	34.1 (t)
5	144.5 (s)	146.7 (s)	146.5 (s)
6	123.8 (d)	53.9 (d)	54.1 (d)
7	77.2 (d)	81.2 (d)	80.9 (d)
8	82.9 (s)	81.3 (s)	81.2 (s)
9	71.2 (d)	72.2 (d)	71.6 (d)
10	42.3 (d)	35.3 (d)	35.6 (d)
11	151.1 (s)	56.9 (s)	56.7 (s)[c]
12	25.7 (t)	73.6 (d)	73.1 (d)
13	27.6 (t)	66.5 (d)	66.5 (d)
14	73.7 (d)	73.0 (d)	72.9 (d)
15	15.1 (q)	14.3 (q)	14.3 (q)
16	26.1 (q)	121.2 (t)	121.1 (t)
17	42.5 (d)	51.4 (d)	51.4 (d)
18	6.4 (q)	5.9 (q)	5.7 (q)
19	175.9 (s)	174.3 (s)	174.3 (s)
20	113.0 (t)	50.4 (t)	50.6 (t)
Acetates	169.3 (s)	169.9 (s)	169.9 (s)
	21.8 (q)	20.8 (q)	20.9 (q)
	170.5 (s)	169.3 (s)	169.3 (s)
	20.9 (q)	21.2 (q)	21.2(q)
	170.3 (s)	169.2 (s)	
	21.2 (q)	20.6 (q)	
Isovalerates	172.2 (s)	171.8 (s)	172.1 (s)
	43.3 (t)	42.6 (t)	171.8 (s)
	25.9 (d)	24.9 (d)	43.5 (t)
	22.5 (q)	22.3 (q)	42.6 (t)
	22.3 (q)	22.3 (q)	25.6 (d)
			25.0 (d)
			22.3 (4×q)
Isobutyrates		177.1 (s)	177.4 (s)
		34.0 (d)	34.1 (d)
		19.1 (q)	19.1 (q)
		17.9 (q)	17.9 (q)

[a] Spectra measured at 100 MHz in $CDCl_3$ at 25 °C.
[b] Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols.
[c] Due to the broad signal, the ^{13}C NMR chemical shift for C-11 in **3** was assigned by the assistances of HMBC correlations.

Table 2
^{1}H NMR data for diterpenoids **1**–**3**[a]

Position	1	2	3
2	4.77 d (4.0)[b]	5.91 d (8.0)	5.89 d (8.4)
3α	1.95 m	1.71 m	1.62 m
3β	2.75 dd (13.6, 13.6)	2.72 m	2.71 m
4α	5.22 dd (13.6, 5.2)	2.32 m	2.36 m
4β		2.45 m	2.43 m
6	5.79 dd (10.4, 1.2)	4.61 d (3.2)	4.61 d (2.4)
7	5.59 d (10.4)	4.45 br s	4.46 br s
9	5.26 d (5.6)	5.70 s	5.68 s
10	3.31 d (5.6)	3.69 s	3.67 s
12α	2.09 br t (6.8)	4.86 dd (3.6, 1.2)	4.89 dd (3.6, 1.2)
12β	2.27 m		
13α	1.77 m		
13β	2.04 m	5.23 dd (3.6, 3.6)	5.25 dd (3.6, 3.2)
14	4.70 d (4.0)	5.21 dd (3.6, 1.2)	5.21 dd (3.2, 1.2)
15	1.10 s	1.26 s	1.26 s
16a	2.23 d (1.2)	5.79 s	5.79 s
16b		5.50 s	5.50 s
17	2.47 q (7.2)	2.96 q (6.8)	2.97 q (7.2)
18	1.12 d (7.2)	1.26 d (6.8)	1.26 d (7.2)
20a	5.03 s	2.95 dd (3.6, 1.2)	2.94 dd (3.6, 1.2)
20b	4.88 s	2.39 d (3.6)	2.38 d (3.6)
OH-8	n.o.[c]	3.43 s	3.42 s
Acetate	1.90 s	1.99 s	1.99 s
Methyls	2.00 s	2.06 s	2.22 s
	2.22 s	2.22 s	
Isovalerates	0.95 d (6.8) (2×3H)	0.91 d (6.4)	0.95 d (6.8) (2×3H)
	2.12 m	0.92 d (6.4)	0.91 d (6.8)
	2.17 d (7.6)	1.98 m	0.90 d (6.8)
		2.08 d (6.8)	1.99 m
			2.12 m
			2.04 d (6.8)
			2.16 m[d]
Isobutyrates		1.11 d (6.8)	1.11 d (7.2)
		1.16 d (6.8)	1.16 d (7.2)
		2.51 Septet (6.8)	2.50 Septet (7.2)

[a] Spectra measured at 400 MHz in $CDCl_3$ at 25 °C.
[b] *J* values (in hertz) in parentheses.
[c] n.o.=not observed.
[d] Signals overlapping.

Table 3
HMBC correlations (H→C) for diterpenoids **1**–**3**

Position	1	2	3
H-2	C-1, -3, -4, -10, -14, -15, Acetate carbonyl	C-1, -4, -15, Isobutyrate carbonyl	C-1, -15, Isobutyrate carbonyl
H-3	C-1, -2, -4, -5	C-2, -4, -5	C-2
H-4	C-3, -5, -6, -16, Isovalerate carbonyl	C-2, -3, -5, -16	C-2, -3, -5, -16
H-6	C-4, -7, -16	C-4, -16	C-16
H-7	C-5, -6, -8	n.o.	n.o.
H-9	C-7, -8, -10, -11, -17, Acetate carbonyl	C-1,- 7, -8, -10, -11, -17, Acetate carbonyl	C-1, -8, -10, -11, -17, Acetate carbonyl
H-10	C-1, -2, -8, -9, -11, -12, -14, -15, -20	C-1, -2, -8, -11, -15, -20	C-1, -2, -8, -9, -11, -14, -15, -20
H-12	C-10, -11, -13, -20	C-10, -11, -13, -14, Acetate carbonyl	C-10, -11, -13, -14, Isovalerate carbonyl
H-13	C-1, -12, -14	C-1, -12, Isovalerate carbonyl	C-14
H-14	C-1, -10, -12, -15, Acetate carbonyl	C-1,-10, -12, -13, Acetate carbonyl	C-1, -10, -12, Acetate carbonyl
H-15	C-1, -2, -10, -14	C-1, -2, -10, -14	C-1, -2, -10, -14
H-16	C-4, -5, -6	C-4, -5	C-4
H-17	C-8, -9, -18, -19	C-7, -8, -18, -19	C-7, -18, -19
H-18	C-8, -17, -19	C-8, -17, -19	C-8, -17, -19
H-20	C-10, -11, -12	C-11, -12	n.o.
OH-8	n.o.[a]	C-7, -8, -9	C-7, -8, -9

[a] n.o.=not observed.

ring at C-11, was elucidated by allylic coupling between H-10 and H-20a; and one proton of C-12 methylene (*δ* 2.27) and H-20b; and by the HMBC correlations between H-10/C-20; H_2-12/C-20; and H_2-20/C-10, -11, -12. The ring junction C-15 methyl group was positioned at C-1 from the HMBC correlations between H-2/C-15; H-10/C-15; H-14/C-15; and H_3-15/C-1, -2, -10, -14. In addition, the carbon signal appeared at *δ* 172.2 (s) was correlated with the signals of the methylene and methine protons at *δ* 2.17 and 2.12 in the HMBC spectrum and was assigned as the carbon atom of the isovalerate carbonyl. The isovalerate positioned at C-4 was confirmed by the connectivity between H-4 (*δ* 5.22) and the carbonyl carbon (*δ* 172.2) of the isovaleroxy group. Furthermore, the acetoxy groups positioned at C-2, -9, and C-14 were confirmed from the HMBC correlations between *δ* 4.77 (H-2), 5.26 (H-9), 4.70 (H-14) and the acetate carbonyls appeared at *δ* 170.5 (s), 169.3 (s), 170.3 (s), respectively. Thus, the remaining hydroxy group had to be positioned at C-8, an oxygen-bearing quaternary carbon resonating at *δ* 82.9 (s). These data, together with the HMBC correlations between H-9/C-17; H_3-18/C-8, -17, -19; and H-17/C-8, -9, -18, -19, unambiguously established the molecular framework of **1**.

Based on previous reviews, all the naturally occurring briarane-type natural products have the C-15 methyl group trans to H-10, and these two groups are assigned as β- and α-oriented in most briarane derivatives.[24,25] The relative stereochemistry

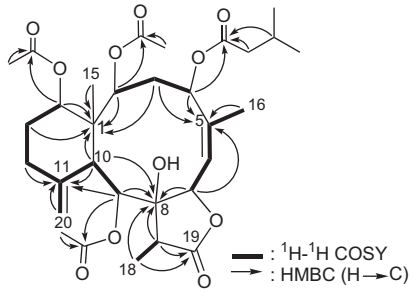

Figure 1. The ^{1}H–^{1}H COSY and selective HMBC correlations (protons and quaternary carbons) of **1**.

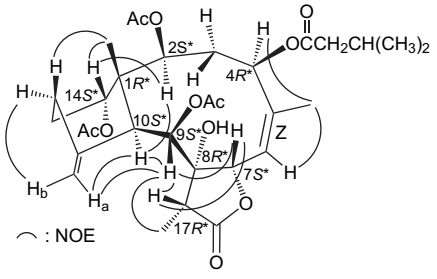

Figure 2. Selective NOESY correlations of **1**.

of **1** was elucidated from the NOE interactions observed in an NOESY experiment (Fig. 2). In the NOESY experiment of **1**, H-10 gives NOE correlations to H-2 and H-9, but not to H_3-15, indicating that these protons (H-2, H-9, and H-10) are located on the same face of the molecule and assigned as α protons, since C-15 methyl group is the β-substituent at C-1. H-14 was found to exhibit NOE correlations with H-2 and H_3-15, showing that this proton was positioned on the equatorial direction and has a β-orientation at C-14. H-9 was found to exhibit NOE responses with H-17 and H_3-18; and H-9 exhibited a light NOE correlation with H-7. From consideration of molecular models, H-9 was found to be reasonably close to H-7, H-17, and H_3-18, thus being concluded that H-9 and H_3-18 should be placed on the α face; and H-7 and H-17 are β-oriented. The latter was supported by an NOE response between H-7 and H-17. The *Z*-configuration of C-5/6 double bond was elucidated by an NOE response between C-6 olefin proton and C-16 vinyl methyl. Due to H-4 exhibited an NOE interaction with H_3-16, and a doublet doublet coupling was found between H-4 and C-3 methylene protons (J=13.6, 5.2 Hz), indicating that the isovaleroxy group attaching at C-4 was β-oriented. However, due to the signal of hydroxy proton was not observed in the ^{1}H NMR spectrum of **1**, so the stereochemistry of C-8 hydroxy group cannot be determined by this way.

In previous studies,[24,25] when the briarane derivatives possessing the β-hydroxy-γ-lactone system, the C-8 hydroxy groups in these metabolites are almost α-oriented and only three 8β-hydroxy-briaranes, briaexcavatolides K, L, and Z were found.[39,40] By comparing the ^{13}C NMR chemical shifts for C-8 of **1** (δ 82.9, s) with those of briaexcavatolides K (δ 81.1, s), L (δ 81.2, s), Z (δ 81.7, s); and a briarane, junceellolide E, which possessed an 8α-hydroxy group (δ 83.1, s),[41] the 8-hydroxy group in **1** should be α-oriented. In the configuration of methylenecyclohexane ring of **1**, a proton of C-20 methylene (δ 5.03, H-20a) was found to exhibit NOE correlations with H-9 and H-10; H_3-15 showed an NOE correlation with one proton of C-12 methylene (δ 2.27, H-12β); and H-20b correlated with H-12α (δ 2.09), indicating that the methylenecyclohexane ring of **1** should be presented as a boat rather than a chair conformation for **1**, and the chiral centers for **1** are assigned as 1*R**, 2*S**, 4*R**, 5*Z*, 7*S**, 8*R**, 9*S**, 10*S**, 14*S**, and 17*R**.

Junceol B (**2**) had the molecular formula $C_{35}H_{49}ClO_{14}$ (HRESIMS, *m/z* 751.2704, calcd for $C_{35}H_{49}ClO_{14}$+Na, 751.2709), and its IR absorptions at 3486, 1785, and 1740 cm^{-1}, typical for hydroxy, γ-lactone, and ester functionalities. Both the ^{1}H and ^{13}C NMR data (Tables 1 and 2) indicated of three acetates at δ 1.99 (3H, s), 2.06 (3H, s), 2.22 (3H, s); δ 20.8 (q), 20.6 (q), 21.2 (q); and δ 169.9 (s), 169.2 (s), 169.3 (s). An isobutyrate ester was indicated by a methine septet at δ 2.51 (1H, septet, J=6.8 Hz), which was spin-coupling with two methyl doublets at δ 1.16 (3H, d, J=6.8 Hz) and 1.11 (3H, d, J=6.8 Hz) along with the carbon signals at δ 34.0 (d), 19.1 (q), 17.9 (q), and an ester carbonyl at δ 177.1 (s). Furthermore, an isovalerate ester was revealed by contiguous proton chemical shifts at δ 0.91 (3H, d, J=6.4 Hz), 0.92 (3H, d, J=6.4 Hz), 1.98 (1H, m), 2.08 (2H, d, J=6.8 Hz), and by the five carbon signals at δ 22.3 (2×q), 24.9 (d), 42.6 (t), 171.8 (s). Besides above ester carbonyls, the carbon signal at δ 174.3 (s) was assigned to a γ-lactone ring along with the oxygen-bearing methine (δ 4.45, 1H, br s; δ 81.2, d, CH-7). The two proton singlets at δ 5.79 and 5.50 correlated with the methylene signal at δ 121.2 (t) were ascribed to an exocyclic double bond. The ^{1}H NMR spectrum contained two mutually coupled signals at δ 2.95 (1H, dd, J=3.6, 1.2 Hz) and 2.39 (1H, d, J=3.6 Hz) along with the corresponding carbon signals at δ 56.9 (s) and 50.4 (t) that were appropriate for an exocyclic epoxide group. The tertiary methyl singlet at δ 1.26 was assigned to C-15 while the secondary methyl doublet at δ 1.26 (3H, d, J=6.8 Hz) was assigned to H_3-18.

The planar structure of **2** was also determined by 2D NMR studies. The ^{1}H–^{1}H COSY experiment of **2** established the following correlations: H-2/3/4; H-6/7; H-9/10; H-12/13/14; and H-17/18 (Fig. 3). These observations together with the HMBC correlations between H-2/C-1, -4; H_2-3/C-2, -4, -5; H_2-4/C-2, -3, -5; H-6/C-4; OH-8/C-7, -8, -9; H-9/C-1, -7, -8, -10, -11; H-10/C-1, -2, -8, -11; H-12/C-10, -11, -13, -14; H-13/C-1, -12; and H-14/C-1, -10, -12, -13, established the connectivity from C-1 to C-14 (Fig. 3 and Table 3). The exocyclic double bond attached at C-5, was elucidated by the HMBC correlations between H-4/C-16; H-6/C-16; and H_2-16/C-4, -5. The C-11/20 epoxide group was confirmed by the HMBC correlations between H_2-20/C-11, -12; and H-10/C-20. The intensity of (M+Na+2) isotope peak observed in the ESIMS [(M+Na)$^+$/(M+2+Na)$^+$=3:1] was strong evidence of the presence of a chlorine atom in **2**. Consequently, the methine proton signal at δ 4.61 (1H, d, J=3.2 Hz) was confidently assigned to H-6, which attached at a chlorinated carbon (δ 53.9, d, CH-6), was confirmed by the ^{1}H–^{1}H COSY correlations between H-6/7 and H-6/16 (by allylic coupling); and by the HMBC correlations between H-6/C-4, -16. C-15 methyl group was positioned at C-1 from the HMBC correlations between H_3-15/C-1, -2, -10, -14; H-10/C-15; and H-2/C-15. Furthermore, six oxygenated methine protons were observed at δ 5.91, 5.70, 5.23, 5.21, 4.86, 4.45, were ^1J-correlated to the carbons δ 72.4, 72.2, 66.5, 73.0, 73.6, 81.2, and assigned to C-2, -9, -13, -14, -12, -7, respectively. The carbon signal appeared at δ 177.1 (s) was correlated with the signal of the methine proton at δ 2.51 (1H, septet, J=6.8 Hz) in the HMBC spectrum and was assigned as the carbon atom of the isobutyrate carbonyl. The isobutyrate ester positioned at C-2 was confirmed from the connectivity between

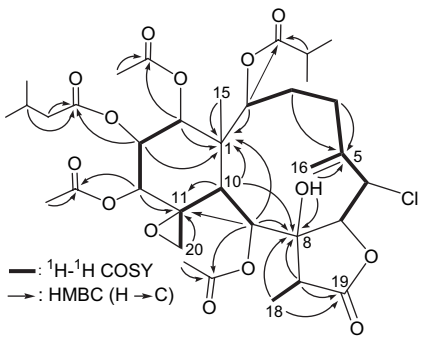

Figure 3. The ^{1}H–^{1}H COSY and selective HMBC correlations (protons and quaternary carbons) of **2**.

H-2 (δ 5.91) and the carbonyl carbon of the isobutyrate (δ 177.1). At the same time, H-9, -12, -13, and H-14 showed HMBC correlations with the ester carbonyl at δ 169.2 (acetate carbonyl), 169.3 (acetate carbonyl), 171.8 (isovalerate carbonyl), and 169.9 (acetate carbonyl). The hydroxy proton signal at δ 3.43 (1H, s) was revealed by its HMBC correlations to the quaternary oxygenated carbon (δ 81.3, s, C-8) and two oxymethines (δ 81.2, d, CH-7; 72.2, d, CH-9), indicating its attachment to C-8. These data, together with the observations of HMBC correlations between H-17/C-7, -8, -18, -19; and H_3-18/C-8, -17, -19, the molecular framework of **2** could be established.

The chemical shifts of exocyclic 11,20-epoxy groups in briarane derivatives have been summarized, and although the ^{13}C NMR peaks for C-11 and C-20 appeared at δ 55–61 ppm and 47–52 ppm, respectively, the epoxy group is α-oriented (11*R**), and the cyclohexane ring is of a chair conformation.[14] Based on above observations, the configuration of 11,20-epoxy group in **2** (δ 56.9, s, C-11; 50.4, t, CH_2-20) should be α-oriented and the cyclohexane ring in **2** should be in a chair conformation. The relative stereochemistry of **2** was elucidated from the NOE interactions observed in an NOESY experiment (Fig. 4). Due to the α-orientation of H-10, the ring junction C-15 methyl group should be β-oriented as no NOE correlation was observed between H-10 and H_3-15. The correlations between H_3-15/H-13; H_3-15/H-14; H_3-15/H_2-20; H-12/H-20b; and H-12/H-13, indicated the β-orientation of H-12, H-13, H-14, and H_2-20. In addition, the NOE correlations between H-10/H-2, OH-8, H-9, H_3-18; and OH-8/H-2, H_3-18, suggested the α-orientation of these protons (H-2, H-9, H-10, OH-8, H_3-18) and H-17 is β-oriented in the γ-lactone ring. Furthermore, H-7 showed NOE correlations with H-17 and H-6, suggesting that these protons are on the β face of **2**. On the other hand, the strong NOE correlations observed between OH-8/H_2-16; and H-2/H-16a, suggested the $\Delta^{5(16)}$ exocyclic double bond should be close to H-2 and OH-8 and should be presented as shown in Figure 4. Based on above findings, the configurations of all chiral centers of **2** were assigned as 1*S**, 2*S**, 6*S**, 7*R**, 8*R**, 9*S**, 10*S**, 11*R**, 12*R**, 13*S**, 14*S**, and 17*R**.

Junceol C (**3**), $C_{38}H_{55}ClO_{14}$ (HRESIMS, *m/z* 793.3174, calcd for $C_{38}H_{55}ClO_{14}$+Na, 793.3178), was recognized as a 6-chlorinated briarane diterpenoid closely related to junceol B (**2**) from their ^{13}C and ^1H NMR data (Tables 1 and 2). Both briaranes **2** and **3** have identical substituents: a chloride atom at C-6; an exocyclic methylene at C-5; a tertiary hydroxy group at C-8; secondary acetates at C-9 and C-14; and a secondary isobutyrate at C-2. They also have the C-11/20 exocyclic epoxy group in common. While briarane **2** showed the presence of an isovalerate and two secondary acetates in the methylenecyclohexane ring, **3** showed an acetate and two isovalerates in the methylenecyclohexane ring. The ^1H and ^{13}C NMR data assignments of junceol C (**3**) were made in comparison with the values of **2**. An acetate group and two isovalerate groups have to be located in the methylenecyclohexane ring of **3** at the positions C-12, -13, and -14. By detailed analysis, the chemical shift of H-12 (δ 4.89) appears at higher field when compared to the values of H-13 (δ 5.25) and H-14 (δ 5.21). Considering the coupling constants of the methine protons deshielded by the ester groups, the values at δ 4.89 (1H, dd, J=3.6, 1.2 Hz), 5.25 (1H, dd, J=3.6, 3.2 Hz), and 5.21 (1H, dd, J=3.2, 1.2 Hz) could be assigned to H-12, -13, and H-14, respectively. From the HMBC correlations noticed between the acetate and isovalerate carbonyl carbons at δ 169.9 (s) and 171.8 (s), and the proton at δ 5.21 (H-14) and 4.89 (H-12), the acetoxy and isovaleroxy groups should be attached at C-14 and C-12, respectively (Table 3). Thus, the remaining isovalerate group should be positioned at C-13, as indicated by the ^1H–^1H COSY correlations and characteristic NMR signal analysis, although no HMBC correlation was observed between H-13 and the isovalerate carbonyl.

The other HMBC correlations observed fully supported the location of functional groups, and hence junceol C (**3**) was assigned as the structure **3** with the same relative stereochemistry as in briarane **2** because for the chiral centers that **3** has in common with **2**, the ^1H and ^{13}C NMR chemical shifts and proton coupling constants match well. Based on above findings, the chiral centers of **3** were assigned as 1*S**, 2*S**, 6*S**, 7*R**, 8*R**, 9*S**, 10*S**, 11*R**, 12*R**, 13*S**, 14*S**, and 17*R**. To the best of our knowledge, junceol C (**3**) is the briarane-type natural product, which possesses the highest molecular weight.

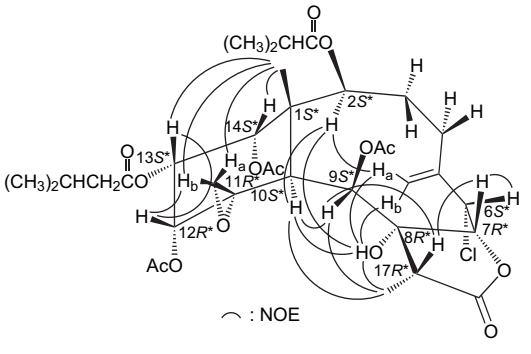

Figure 4. Selective NOESY correlations of **2**.

2.2. Isolation and structure determination of fragilide D from *J. fragilis*

Compound **4**, fragilide D, was isolated as a white powder and the molecular formula was determined by HRESIMS (*m/z* 663.2179, calcd for $C_{31}H_{41}ClO_{12}$+Na, 663.2184). The IR spectrum indicated absorptions due to hydroxy group (3467 cm^{-1}), γ-lactone (1782 cm^{-1}), and ester carbonyl (1741 cm^{-1}) functionalities. In the ^1H NMR spectrum of **4** (Table 4), a tertiary methyl (δ 1.06, 3H, s, H_3-15), a secondary methyl (δ 1.21, d, J=7.2 Hz, H_3-18), an exocyclic double bond (δ 5.94, 1H, br s; 5.91, 1H, d, J=2.8 Hz H_2-16), an exocyclic epoxide (δ 3.05, 1H, dd, J=2.8, 2.4 Hz; 2.62, 1H, d, J=2.4 Hz, H_2-20), two acetate methyls (δ 2.15, 3H, s; 2.06, 3H, s), and a 2-(3-methylbutanoyloxy)acetoxy (δ 4.59, 1H, d, J=16.0 Hz; 4.49, 1H, d, J=16.0 Hz; 2.29, 2H, dd, J=7.6, 6.8 Hz; 2.12, 1H, m; 0.99, 2×3H, J=6.4 Hz, –OC(O)-$CH_2OC(O)CH_2CH(CH_3)_2$) groups were observed. The above data, together with the characteristic NMR signals that were found in the spectrum of briarenes, which have been isolated so far in the species,[24,25,42] implies that fragilide D (**4**) was a briarane-type diterpenoid. The ^1H–^1H COSY spectrum

Table 4
^{1}H and ^{13}C NMR data and HMBC correlations for diterpenoid **4**

Position	^{1}H[a]	^{13}C[b]	HMBC (H→C)
1		47.7 (s)[d]	
2	6.51 d (9.2)[c]	72.8 (d)	C-1, -3, -4, -14, -15, -1′
3	5.68 dd (11.6, 9.2)	129.6 (d)	C-5
4	5.96 d (11.6)	128.9 (d)	C-2, -5, -6, -16
5		136.9 (s)	
6	5.10 m	62.2 (d)	n.o.[e]
7	4.77 d (3.6)	78.6 (d)	C-6, -8
8		81.2 (s)	
9	4.85 d (3.6)	70.8 (d)	C-7, -8, -10, -11, -17, Acetate carbonyl
10	3.25 d (3.6)	38.2 (d)	C-1, -2, -8, -9, -11, -15, -20
11		59.2 (s)	
12α	2.24 m	29.6 (t)	n.o.
12β	1.14 d (7.2)		
13α	1.93 m	25.0 (t)	n.o.
13β	1.80 m		
14	4.88 br s	73.9 (d)	Acetate carbonyl
15	1.06 s	14.5 (q)	C-1, -2, -10, -14
16a	5.94 br s	117.1 (t)	C-4, -5, -6
16b	5.91 d (2.8)		
17	2.62 q (7.2)	47.1 (d)	C-8, -9, -18, -19
18	1.21 d (7.2)	7.6 (q)	C-8, -17, -19
19		175.0 (s)	
20a	3.05 dd (2.8, 2.4)	50.5 (t)	n.o.
20b	2.62 d (2.4)		
OH-8	3.15 s		C-7, -8, -9, -17
Acetates	2.15 s	21.4 (q)	Acetate carbonyl
		170.1 (s)	
	2.06 s	21.2 (q)	Acetate carbonyl
		170.3 (s)	
$OC(O)CH_2OC(O)CH_2CH(CH_3)_2$		166.7 (s)	
$OC(O)CH_2OC(O)CH_2CH(CH_3)_2$	4.59 d (16.0)	60.7 (t)	C-1′, -3′
	4.49 d (16.0)		
$OC(O)CH_2OC(O)CH_2CH(CH_3)_2$		172.3 (s)	
$OC(O)CH_2OC(O)CH_2CH(CH_3)_2$	2.29 dd (7.6, 6.8)	42.7 (t)	C-3′, -5′, -6′, -7′
$OC(O)CH_2OC(O)CH_2CH(CH_3)_2$	2.12 m	25.6 (d)	C-3′, -4′, -6′, -7′
$OC(O)CH_2OC(O)CH_2CH(CH_3)_2$	0.99 d (2×3H, 6.4)	22.4 (2×q)	C-4′, -5′

[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] Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols.
[e] n.o.=not observed.

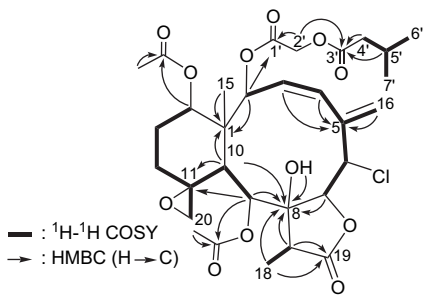

Figure 5. The $^{1}H-^{1}H$ COSY and selective HMBC correlations (protons and quaternary carbons) of **4**.

revealed sequences of the correlations from H-2/3/4; H-4/16 (by allylic coupling); H-16/6 (by allylic coupling); H-6/7; H-9/10; H-10/20b (by long-range *w* coupling); H-12/13/14; and H-17/18 (Fig. 5).

The downfield chemical shifts of H-2 (δ 6.51, 1H, d, *J*=9.2 Hz), H-9 (δ 4.85, 1H, d, *J*=3.6 Hz), and H-14 (δ 4.88, 1H, br s), suggested that three acyl groups were positioned at C-2, -9, and -14. In the HMBC spectrum of **4** (Fig. 5 and Table 4), the oxygenated methylene protons at δ 4.59 and 4.49 were correlated with the carbon signals observed at δ 166.7 (s, C-1′) and 172.3 (s, C-3′) and these two carbons were thus assigned as the ester carbonyls of the 2-(3-methylbutanoyloxy)acetate. The main problem was to locate the 2-(3-methylbutanoyloxy)acetate group at C-2, -9, or -14, and the acetates at the remaining two positions. The 2-(3-methylbutanoyloxy)acetate ester was positioned at C-2 was from the $^{1}H-^{13}C$ long-range correlations between H-2 (δ 6.51) and the carbonyl carbon (δ 166.7, s, C-1′) of the 2-(3-methylbutanoyloxy)acetate in the HMBC spectrum. The position of the secondary methyl group at δ 1.21 was assigned as C-18 methyl by a correlation of H-17 (δ 2.62, 1H, q, *J*=7.2 Hz) to the

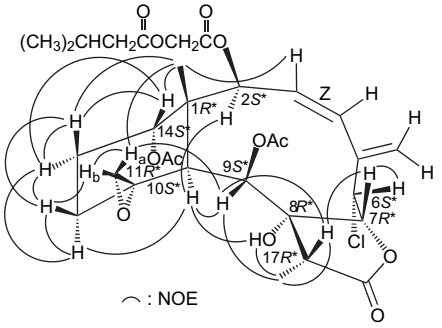

Figure 6. Selective NOESY correlations of **4**.

C-19 γ-lactone carbonyl (δ 175.0, s) in the HMBC spectrum. The presence of the tertiary hydroxy group at C-8 was defined by the HMBC correlations of H-7/C-8; H-9/C-8; H-10/C-8; H-17/C-8; H_3-18/C-8; and OH-8/C-7, -8, -9, -17. From above observations, the gross structure of **4** was readily established.

The relative stereochemistry of **4** was elucidated by the coupling patterns and NOESY spectrum (Fig. 6). The NOEs between H-10 and H-2, -9, OH-8, and one proton of C-12 methylene (δ 2.24, H-12α), supported that these protons were situated on the same face and assigned as α protons. The trans ring junction between the 6-membered and 10-membered rings was assigned by the lack of NOE correlation between H-10 and H_3-15. One proton of the C-20 epoxy methylene (δ 3.05) was found to exhibit NOE responses with H_3-15 and H-9, but not with H-10, showing that the C-20 epoxy group in **4** was α-oriented and the cyclohexane ring of **4** was proven to be existing in a chair form by comparing the ^{13}C NMR chemical shifts of C-11 and C-20 with those of junceols B and C, and the related compounds.[14] Furthermore, H-14 was found to exhibit an NOE response with H_3-15, showing that this proton is of β-orientation. H-9 was found to show NOE correlations with H-10, H-17, and H_3-18, and, from molecular models, was found to be reasonably close to H-10, H-17, and H_3-18; therefore, H-9 should be placed on the α face in **4**, and H-17 and H_3-18 are β- and α-oriented in the γ-lactone ring, respectively. Moreover, H-7 exhibited NOE correlations with H-17 and H-6, suggesting that these protons are on the β face of **4**. The cis geometry of the C-3/4 double bond was indicated by an 11.6 Hz coupling constant between H-3 (δ 5.68) and H-4 (δ 5.96). Based on above findings, the configurations of all chiral centers of **4** were assigned to be 1*R**, 2*S**, 3*Z*, 6*S**, 7*R**, 8*R**, 9*S**, 10*S**, 11*R**, 14*S**, and 17*R**. It is worthwhile to note that a briarane diterpene containing a 2-(3-methylbutanoyloxy)acetate ester substituent, like **4**, was observed for the first time. In addition, the 11,20-epoxybriarane derivatives were also proven to be a chemical marker for the gorgonian corals belong to family Ellisellidae.[6]

2.3. Biological activity

In biological activity experiments, junceols A–C (**1**–**3**) were found to show 45.64, 159.60, and 124.14% inhibitory effects on superoxide anion generation by human neutrophils at 10 μg/mL, respectively (Table 5).

Table 5
Inhibitory effects of briaranes **1**–**3** on superoxide anion generation by human neutrophils in response to fMet-Leu-Phe/cytochalastin B

Compound	Superoxide generation inhibition (%)[a]
1	45.64±7.44[b]
2	159.60±13.08[c]
3	124.14±0.85[b]

[a] Percentage of inhibition (Inh%) at 10 μg/mL. Results are presented as mean±SEM (n=4∼5).
[b] P<0.001.
[c] P<0.01.

3. Experimental

3.1. General experimental procedures

Melting points were determined on a FARGO apparatus and were uncorrected. Optical rotation values were measured with a JASCO P-1010 digital polarimeter at 25 °C. Infrared spectra were obtained on a VARIAN DIGLAB FTS 1000 FT-IR spectrophotometer. The NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Proton chemical shifts were referenced to the residual $CHCl_3$ signal (δ 7.26 ppm). ^{13}C NMR spectra were referenced to the center peaks of $CDCl_3$ at δ 77.0 ppm. ESIMS and HRESIMS data were recorded on a BRUKER APEX II mass spectrometer. 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, Darmstadt, Germany) and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. HPLC was performed using a system comprising of a HITACHI L-7100 pump, a HITACHI photo diode array detector L-7455, and a RHEODYNE 7725 injection port. A normal phase semi-preparative column (Hibar 250–25 mm, LiChrospher Si 60, 5 μm) and a semi-preparative reverse phase column (Hibar 250–10 mm, Purospher STAR RP-18e, 5 μm) were used for HPLC. All solvents used were either freshly distilled or of analytical grade.

3.2. Animal material

Specimens of the gorgonian corals *J. juncea* and *J. fragilis* were collected by hand using scuba gear off the southern Taiwan coast in Sep. 2006. These two organisms were identified by comparison with previous descriptions.[43–45] The living reference specimens are being maintained in the authors' marine organisms cultivating tank and the voucher specimen were deposited in the National Museum of Marine Biology & Aquarium (NMMBA), Taiwan.

3.3. Extraction and isolation

3.3.1. J. juncea

The freeze-dried and minced material of the gorgonian coral *J. juncea* (wet weight 369 g, dry weight 108 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 (2.57 g) was separated on silica gel and eluted using hexane/EtOAc (stepwise, 10:1–pure EtOAc) to yield 22 fractions (Fr. 1–22). Fr. 16 was separated on silica gel and eluted using hexane/EtOAc (stepwise, 5:1–pure EtOAc) to yield Fr. 16A–I. Fr. 16C was repurified by normal phase HPLC, using the mixtures of CH_2Cl_2 and EtOAc to afford briarane **1** (6.0 mg, 10:1). Fr. 17 and 18 were combined and separated by normal phase HPLC, using the mixtures of CH_2Cl_2 and acetone to yield 13 fractions Fr. 17A–M. Fr. 17B and 17F were further repurified by reverse phase HPLC, using the mixtures of MeOH and H_2O to afford briaranes **2** (0.6 mg, 9:1) and **3** (0.9 mg, 7:3), respectively.

*3.3.1.1. Junceol A (**1**).* White powder; mp 105–108 °C; $[\alpha]_D^{23}$ +1.3 (*c* 0.3, $CHCl_3$); IR (neat) ν_{max} 3470, 1786, 1733 cm^{-1}; ^{13}C ($CDCl_3$, 100 MHz) and ^1H ($CDCl_3$, 400 MHz) NMR data, see Tables 1 and 2; ESIMS *m/z* 615 $(M+Na)^+$; HRESIMS *m/z* 615.2780 (calcd for $C_{31}H_{44}O_{11}$+Na, 615.2781).

*3.3.1.2. Junceol B (**2**).* White powder; mp 223–226 °C; $[\alpha]_D^{23}$ −24 (*c* 0.02, $CHCl_3$); IR (neat) ν_{max} 3486, 1785, 1740 cm^{-1}; ^{13}C ($CDCl_3$, 100 MHz) and ^1H ($CDCl_3$, 400 MHz) NMR data, see Tables 1 and 2; ESIMS *m/z* 751 $(M+Na)^+$, 753 $(M+2+Na)^+$; HRESIMS *m/z* 751.2704 (calcd for $C_{35}H_{49}ClO_{14}$+Na, 751.2709).

*3.3.1.3. Junceol C (**3**).* White powder; mp 260–262 °C; $[\alpha]_D^{23}$ +21 (*c* 0.02, $CHCl_3$); IR (neat) ν_{max} 3447, 1783, 1725 cm^{-1}; ^{13}C ($CDCl_3$, 100 MHz) and ^1H ($CDCl_3$, 400 MHz) NMR data, see Tables 1 and 2; ESIMS *m/z* 793 $(M+Na)^+$, 795 $(M+2+Na)^+$; HRESIMS *m/z* 793.3174 (calcd for $C_{38}H_{55}ClO_{14}$+Na, 793.3178).

3.3.2. *J. fragilis*

The freeze-dried and minced material of *J. fragilis* (wet weight 628 g, dry weight 206 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 (4.77 g) was separated on silica gel and eluted using hexane/EtOAc (stepwise, 50:1–pure EtOAc) to yield 17 fractions (Fr. A–Q) and one of these fraction (fraction K) was further separated by gravity column with silica gel and eluted using CH_2Cl_2/EtOAc (stepwise, 20:1–pure EtOAc) to afford briarane **4** (0.7 mg, 7:1).

*3.3.2.1. Fragilide D (**4**).* White powder; mp 102–103 °C; $[\alpha]_D^{25}$ −81 (*c* 0.04, $CHCl_3$); IR (neat) ν_{max} 3467, 1782, 1741 cm^{-1}; ^{13}C ($CDCl_3$, 100 MHz) and ^1H ($CDCl_3$, 400 MHz) NMR data, see Table 4; ESIMS *m/z* 663 $(M+Na)^+$, 665 $(M+2+Na)^+$; HRESIMS *m/z* 663.2179 (calcd for $C_{31}H_{40}ClO_{12}$+Na, 663.2184).

3.4. Human neutrophil superoxide generation

Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Superoxide generation was carried out according to the procedures described previously.[46,47] Briefly, superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*.

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References and notes

1. Sung, P.-J.; Hu, W.-P.; Fang, L.-S.; Fan, T.-Y.; Wang, J.-J. *Nat. Prod. Res.* **2005**, *19*, 689–694.
2. Sung, P.-J.; Chen, Y.-P.; Hwang, T.-L.; Hu, W.-P.; Fang, L.-S.; Wu, Y.-C.; Li, J.-J.; Sheu, J.-H. *Tetrahedron* **2006**, *62*, 5686–5691.
3. Chen, Y.-P.; Wu, S.-L.; Su, J.-H.; Lin, M.-R.; Hu, W.-P.; Hwang, T.-L.; Sheu, J.-H.; Fan, T.-Y.; Fang, L.-S.; Sung, P.-J. *Bull. Chem. Soc. Jpn.* **2006**, *79*, 1900–1905.
4. 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* **2008**, *64*, 2596–2604.
5. Sung, P.-J.; Tsai, W.-T.; Chiang, M. Y.; Su, Y.-M.; Kuo, J. *Tetrahedron* **2007**, *63*, 7582–7588.
6. Su, Y.-M.; Fan, T.-Y.; Sung, P.-J. *Nat. Prod. Res.* **2007**, *21*, 1085–1090.
7. Sung, P.-J.; Chiang, M. Y.; Tsai, W.-T.; Su, J.-H.; Su, Y.-M.; Wu, Y.-C. *Tetrahedron* **2007**, *63*, 12860–12865.
8. 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.* **2008**, *37*, 88–89.
9. Sung, P.-J.; Fan, T.-Y. *Heterocycles* **2003**, *60*, 1199–1202.
10. 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.* **2003**, *51*, 1429–1431.
11. Sung, P.-J.; Fan, T.-Y.; Chen, M.-C.; Fang, L.-S.; Lin, M.-R.; Chang, P.-C. *Biochem. Syst. Ecol.* **2004**, *32*, 111–113.
12. Sung, P.-J.; Lin, M.-R.; Fang, L.-S. *Chem. Pharm. Bull.* **2004**, *52*, 1504–1506.
13. Sung, P.-J.; Lin, M.-R.; Chen, W.-C.; Fang, L.-S.; Lu, C.-K.; Sheu, J.-H. *Bull. Chem. Soc. Jpn.* **2004**, *77*, 1229–1230.
14. Sheu, J.-H.; Chen, Y.-P.; Hwang, T.-L.; Chiang, M. Y.; Fang, L.-S.; Sung, P.-J. *J. Nat. Prod.* **2006**, *69*, 269–273.
15. 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.* **2007**, *80*, 1205–1207.
16. Sung, P.-J.; Fan, T.-Y.; Fang, L.-S.; Sheu, J.-H.; Wu, S.-L.; Wang, G.-H.; Lin, M.-R. *Heterocycles* **2003**, *61*, 587–592.
17. Chuang, L.-F.; Fan, T.-Y.; Li, J.-J.; Kuo, J.; Fang, L.-S.; Wang, W.-H.; Sung, P.-J. *Platax* **2007**, *4*, 61–67.
18. Sung, P.-J.; Chuang, L.-F.; Kuo, J.; Fan, T.-Y.; Hu, W.-P. *Tetrahedron Lett.* **2007**, *48*, 3987–3989.
19. Chuang, L.-F.; Fan, T.-Y.; Li, J.-J.; Sung, P.-J. *Biochem. Syst. Ecol.* **2007**, *35*, 470–471.
20. Sung, P.-J.; Chuang, L.-F.; Kuo, J.; Chen, J.-J.; Fan, T.-Y.; Li, J.-J.; Fang, L.-S.; Wang, W.-H. *Chem. Pharm. Bull.* **2007**, *55*, 1296–1301.
21. Sung, P.-J.; Chuang, L.-F.; Fan, T.-Y.; Chou, H.-N.; Kuo, J.; Fang, L.-S.; Wang, W.-H. *Chem. Lett.* **2007**, *36*, 1322–1323.
22. Sung, P.-J.; Chuang, L.-F.; Hu, W.-P. *Bull. Chem. Soc. Jpn.* **2007**, *80*, 2395–2399.
23. Kokke, W. C. M. C.; Epstein, S.; Look, S. A.; Rau, G. H.; Fenical, W.; Djerassi, C. *J. Biol. Chem.* **1984**, *259*, 8168–8173.
24. Sung, P.-J.; Sheu, J.-H.; Xu, J.-P. *Heterocycles* **2002**, *57*, 535–579.
25. Sung, P.-J.; Chang, P.-C.; Fang, L.-S.; Sheu, J.-H.; Chen, W.-C.; Chen, Y.-P.; Lin, M.-R. *Heterocycles* **2005**, *65*, 195–204.
26. Isaacs, S.; Carmely, S.; Kashman, Y. *J. Nat. Prod.* **1990**, *53*, 596–602.
27. Anjaneyulu, A. S. R.; Rao, N. S. K. *J. Chem. Soc., Perkin Trans. 1* **1997**, 959–962.

28. Anjaneyulu, A. S. R.; Rao, V. L.; Sastry, V. G.; Venugopal, M. J. R. V.; Schmitz, F. J. *J. Nat. Prod.* **2003**, *66*, 507–510.
29. Qi, S.-H.; Zhang, S.; Huang, H.; Xiao, Z.-H.; Huang, J.-S.; Li, Q.-X. *J. Nat. Prod.* **2004**, *67*, 1907–1910.
30. Qi, S.-H.; Zhang, S.; Qian, P.-Y.; Xiao, Z.-H.; Li, M.-Y. *Tetrahedron* **2006**, *62*, 9123–9130.
31. Shen, Y.-C.; Lin, Y.-C.; Chiang, M. Y. *J. Nat. Prod.* **2002**, *65*, 54–56.
32. Shen, Y.-C.; Lin, Y.-C.; Ko, C.-L.; Wang, L.-T. *J. Nat. Prod.* **2003**, *66*, 302–305.
33. Krishna, N.; Muralidhar, P.; Kumar, M. M. K.; Rao, D. V.; Rao, C. H. B. *Asian J. Chem.* **2003**, *15*, 344–348. There are two different briaranes designated as juncenolide B by two research groups. Please see Refs. 32 and 33.
34. Shen, Y.-C.; Lin, Y.-C.; Huang, Y.-L. *J. Chin. Chem. Soc.* **2003**, *50*, 1267–1270.
35. Lin, Y.-C.; Huang, Y.-L.; Khalil, A. T.; Chen, M.-H.; Shen, Y.-C. *Chem. Pharm. Bull.* **2005**, *53*, 128–130.
36. Qi, S.; Zhang, S.; Huang, J.; Xiao, Z.; Wu, J.; Li, Q. *Magn. Reson. Chem.* **2005**, *43*, 266–268.
37. Qi, S.-H.; Zhang, S.; Xiao, Z.-H.; Huang, J.-S.; Wu, J.; Li, Q.-X. *Chem. Pharm. Bull.* **2004**, *52*, 1476–1478.
38. Krishna, N.; Muralidhar, P.; Kumar, M. M. K.; Rao, D. V.; Rao, C. H. B. *Nat. Prod. Res.* **2004**, *18*, 551–555.
39. Sung, P.-J.; Su, J.-H.; Duh, C.-Y.; Chaing, M. Y.; Sheu, J.-H. *J. Nat. Prod.* **2001**, *64*, 318–323.
40. Sung, P.-J.; Hu, W.-P.; Wu, S.-L.; Su, J.-H.; Fang, L.-S.; Wang, J.-J.; Sheu, J.-H. *Tetrahedron* **2004**, *60*, 8975–8979.
41. Sung, P.-J.; Wu, S.-L.; Fang, H.-J.; Chiang, M. Y.; Wu, J.-Y.; Fang, L.-S.; Sheu, J.-H. *J. Nat. Prod.* **2000**, *63*, 1483–1487.
42. Sung, P.-J.; Gwo, H.-H.; Fan, T.-Y.; Li, J.-J.; Dong, J.; Han, C.-C.; Wu, S.-L.; Fang, L.-S. *Biochem. Syst. Ecol.* **2004**, *32*, 185–196.
43. Bayer, F. M. *Proc. Biol. Soc., Washington* **1981**, *94*, 902–947.
44. Bayer, F. M.; Grasshoff, M. *Senckenberg. Biol.* **1994**, *74*, 21–45.
45. 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 Sciences: Queensland, Australia, 2001; pp 230–231.
46. Hwang, T.-L.; Hung, H.-W.; Kao, S.-H.; Teng, C.-M.; Wu, C.-C.; Cheng, S.-J. *Mol. Pharmacol.* **2003**, *64*, 1419–1427.
47. Yeh, S.-H.; Chang, F.-R.; Wu, Y.-C.; Yang, Y.-L.; Zhou, S.-K.; Hwang, T.-L. *Planta Med.* **2005**, *71*, 904–909.