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Article

Four New Briarane Diterpenoids from Taiwanese Gorgonian *Junceella fragilis*

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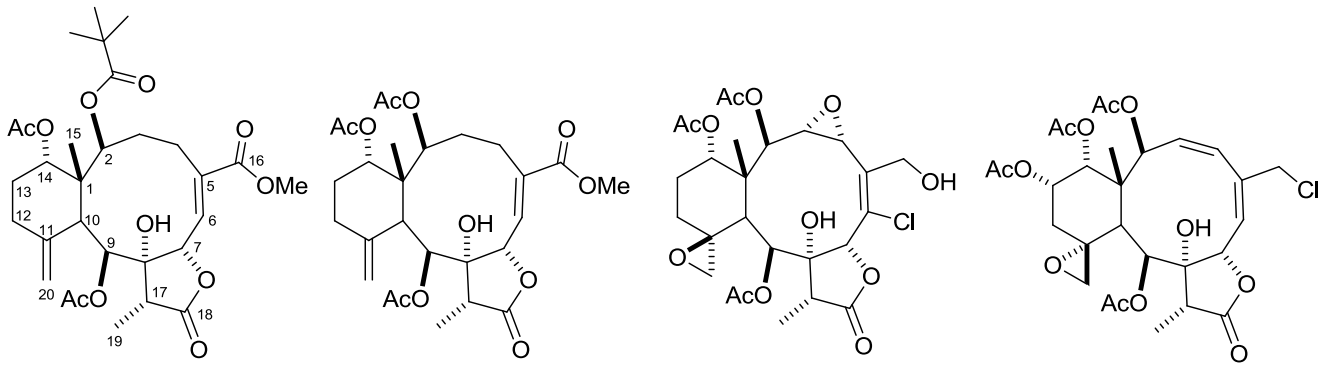
Abstract: Four new 8-hydroxybriarane diterpenoids, frajunolides P–S (**1**–**4**), together with umbraculolide A, juncenolide C, junceellonoid A and juncin R, were isolated from the acetone extract of the gorgonian *Junceella fragilis*, collected from the southeast coast of Taiwan. Compound **1** contains an unusual pivaloyloxy group at C-2, while **3** is a rare compound having a chlorine atom on the olefinic carbon (C-6). The structures of the isolated compounds were established by extensive spectroscopic analysis, including 1D- and 2D-NMR, as well as HRMS data. Compound **1** was further confirmed by X-ray crystallographic analysis. In the anti-inflammatory test, compounds **1** and **2** exhibited moderate inhibition on superoxide anion generation and elastase release by human neutrophils in response to formylmethionylleucyl-phenylalanine/dihydrocytochalasin B (fMLP/CB).

Keywords: *Junceella fragilis*; briarane-type diterpenoids; frajunolides; anti-inflammatory

1. Introduction

Marine invertebrates, especially gorgonian octocorals, have been proven to be rich and important sources of natural products as lead compounds in drug discovery. Members of the gorgonians, *Junceella* and *Briareum*, have yielded numerous and highly oxygenated briarane-type diterpenes with a γ-lactone ring, produced from 3,8-cyclized cembranoids [1–3]. Many of the briarane diterpenoids have been reported to exhibit interesting biological activities, such as cytotoxic [4–6], anti-inflammatory [7,8], antiviral [8], insecticidal [9] and immunomodulatory [10] activities. Our previous chemical investigation of the genus *Junceella* has resulted in the isolation of over 20 briaranes, including frajunolides A–O and juncenolides A–O [11–18]. As part of our continuing search for bioactive natural products, the chemical constituents from other chromatographic fractions of *J. fragilis* were investigated. Herein, we report the isolation and structural elucidation of four additional new 8-hydroxybriarane diterpenoids, frajunolides P–S (Figure 1, **1**–**4**), from the acetone extract of this source, collected from the southeast coast of Taiwan. Their anti-inflammatory activities were tested and evaluated by superoxide anion generation and elastase release by human neutrophils in response to formylmethionylleucyl-phenylalanine/dihydrocytochalasin B (fMLP/CB).

Figure 1. Frajunolides P–S (**1**–**4**) isolated from gorgonian *J. fragilis*.



2. Results and Discussion

Compound **1**, $[\alpha]_D^{25}$ +4 (*c* 0.5 CH_2Cl_2), was isolated as colorless prisms and had a molecular formula of $C_{30}H_{42}O_{11}$ deduced from HRESIMS (*m/z* 601.2620 $[M + Na]^+$, calcd. for $C_{30}H_{42}O_{11}Na$, 601.2625), indicating ten degrees of unsaturation. The IR spectrum of compound **1** exhibited diagnostic absorption bands of hydroxyl (3443 cm^{-1}), γ-lactone (1776 cm^{-1}), ester carbonyl (1722 cm^{-1}) and conjugated ketone (1655 cm^{-1}) functionalities. The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) indicated the presence of a methyl singlet (δ_H 1.08; δ_C 16.5, C-15), a methyl doublet (δ_H 1.19, J = 7.0 Hz; δ_C 8.5, C-19), one exocyclic double bond (δ_H 5.02, 4.98, each s, H_2-20; δ_C 112.5, C-20; δ_C 149.7, C-11), one trisubstituted double bond (δ_H 6.89, d, J = 9.6 Hz, H-6; δ_C 134.5, C-5; 136.8, C-6), four oxygenated methine protons and carbons, (δ_H 5.11, d, J = 7.6 Hz; δ_C 75.6, C-2; δ_H 5.31, d, J = 9.6 Hz; δ_C 78.3, C-7; δ_H 5.60, d, J = 2.8 Hz; δ_C 72.8, C-9; δ_H 4.64, t, J = 2.8 Hz; δ_C 74.1, C-14), an oxygenated quaternary carbon (δ_C 83.5, C-8), four methylene carbons (δ_C 32.3, 24.1, 31.4, 28.9) and two methine carbons (δ_C 43.9 and 44.7), together with a conjugated ester carbonyl (δ_C 166.8, C-16)

and γ-lactone carbonyl carbon (δ_C 174.4, C-19). Detailed analysis of spectroscopic data of **1** and comparison with the related structures of the genus *Junceella* suggested that compound **1** is a highly oxygenated briarane-type diterpenoid with a fused γ-lactone ring similar to juncenolide O, previously isolated from *J. juncea* [18]. In addition, the remaining NMR spectroscopic data contained a methoxy group (δ_H 3.81), two acetate groups (δ_H 2.20, 1.92, each 3H) and a pivaloyloxy group (δ_H 1.38 × 3, 9H). Furthermore, the HMBC correlation showed that the latter was located at C-2, while the acetyl groups were located at C-9 and C-14, and the methoxy group was attached at C-16. The complete planar structure of **1** was further confirmed by the ^{1}H–^{1}H COSY and HMBC correlations (Figure 2).

Table 1. ^{1}H-NMR spectroscopic data for compounds **1**–**4**. (δ in ppm, *J* in Hz).

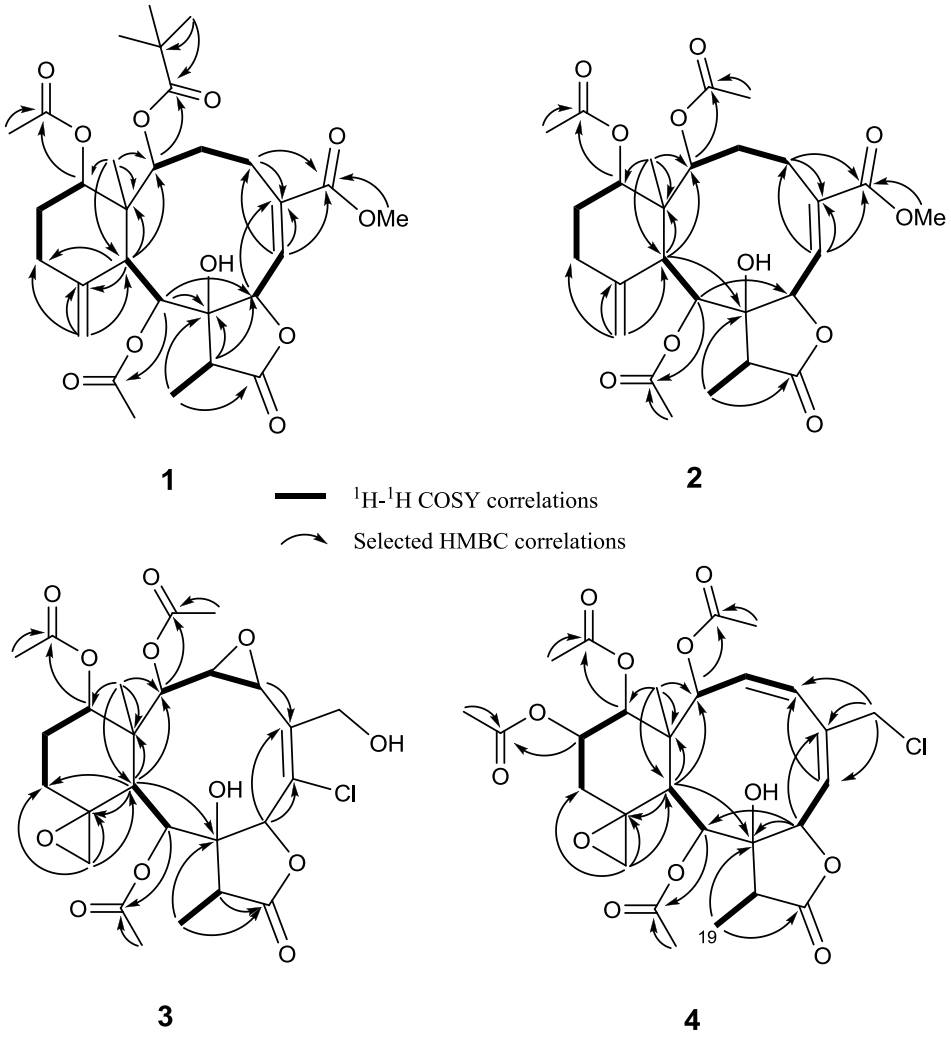
No.	1	2	3	4
2	5.11 (d, *J* = 7.6)	5.00 (m)	4.83 (d, *J* = 9.2)	5.33 (d, *J* = 9.2)
3	2.53 (m)	2.48 (m)	3.40 (dd, *J* = 9.6, 3.6)	5.57 (dd, *J* = 10.4, 9.6)
	1.80 (m)	1.81 (m)		
4	2.52 (m)	2.81 (m)	4.08 (d, *J* = 3.6)	6.30 (d, *J* = 10.4)
	2.74 (m)	2.51 (m)		
6	6.89 (d, *J* = 9.6)	6.84 (d, *J* = 10.0)	-	5.96 (d, *J* = 8.8)
7	5.31 (d, *J* = 9.6)	5.32 (d, *J* = 10.0)	5.41 (s)	4.91 (d, *J* = 8.8)
9	5.60 (d, *J* = 2.8)	5.56 (d, *J* = 3.5)	5.64 (d, *J* = 8.0)	4.68 (d, *J* = 5.2)
10	3.38 (d, *J* = 2.8)	3.27 (d, *J* = 3.5)	2.49 (d, *J* = 8.0)	3.02 (d, *J* = 5.2)
12	2.23 (m)	2.25 (m, 2H)	2.18 (m)	2.47 (td, *J* = 12.4, 1.6)
	1.80 (m)	-	1.78 (m)	1.32 (dd, *J* = 13.2, 3.6)
13	1.80 (m)	1.81 (m, 2H)	2.30 (m)	4.95 (ddd, *J* = 12.8, 4.0, 2.8)
	1.40 (m)		1.12 (m)	
14	4.64 (t, *J* = 2.8)	4.71 (t, *J* = 3.5)	4.88 (d, *J* = 5.2)	5.18 (br s)
15	1.08 (s)	1.25 (s)	1.24 (s)	1.09 (s)
16	-	-	4.57 (dd, *J* = 12.4, 8.8)	4.56 (s, 2H)
	-	-	4.31 (dd, *J* = 12.4, 6.0)	-
17	2.63 (q, *J* = 7.2)	2.60 (q, *J* = 7.0)	2.26 (q, *J* = 7.2)	2.26 (q, *J* = 6.8)
19	1.19 (d, *J* = 7.2)	1.19 (d, *J* = 7.0)	1.22 (d, *J* = 7.2)	1.12 (d, *J* = 6.8)
20	5.02 (s)	5.04 (s)	2.98 (d, *J* = 4.4)	3.52 (br s)
	4.98 (s)	4.99 (s)	2.80 (d, *J* = 4.0)	2.72 (d, *J* = 2.4)
2-OCOC$\underline{H}_3$	1.92 (s)	1.97 (s)	2.10 (s)	1.93 (s)
9-OCOC$\underline{H}_3$	2.20 (s)	2.23 (s)	2.24 (s)	2.16 (s)
13-OCOC$\underline{H}_3$	-	-	-	2.07 (s)
14-OCOC$\underline{H}_3$	-	1.93 (s)	1.96 (s)	1.95 (s)
2-OCOC(C$\underline{H}_3$)$_3$	1.38 (s, 9H)	-	-	-
16-OC$\underline{H}_3$	3.81 (s)	3.82 (s)	-	-
8-OH	-	-	5.86 br s	-
16-OH	-	-	3.72 (dd, *J* = 8.0, 6.0)	-

Table 2. ^{13}C-NMR spectroscopic data for compounds **1**–**4** (δ in ppm, mult).

No.	1	2	3	4
1	48.8 (s)	47.4 (s)	44.7 (s)	46.4 (s)
2	75.6(d)	73.6 (d)	75.9 (d)	74.1 (d)
3	32.3 (t)	30.9 (t)	58.7 (d)	131.7 (d)
4	24.1 (t)	22.8 (t)	59.0 (d)	128.0 (d)
5	134.5 (s)	134.2 (s)	135.7 (s)	139.9 (s)
6	136.8 (d)	138.1 (d)	132.4 (d)	126.0 (d)
7	78.3 (d)	77.5 (d)	75.7 (d)	78.4 (d)
8	83.5 (s)	83.5 (s)	81.2 (s)	80.8 (s)
9	72.8 (d)	72.8 (d)	66.8 (d)	64.2 (d)
10	43.9 (d)	43.2 (d)	40.2 (d)	37.4 (d)
11	149.7 (s)	150.5 (s)	61.2 (s)	58.1 (s)
12	31.4 (t)	29.4 (t)	24.1 (t)	34.2 (t)
13	28.9 (t)	27.3 (t)	24.3 (t)	67.6 (d)
14	74.1 (d)	74.8 (d)	72.7 (d)	73.7 (d)
15	16.5 (q)	15.0 (q)	14.7 (q)	14.3 (q)
16	166.8 (s)	168.0 (s)	58.8 (s)	44.6 (s)
17	44.7 (d)	42.9 (d)	42.9 (d)	43.8 (d)
18	174.4 (s)	175.4 (s)	174.8 (s)	175.2 (s)
19	8.5 (q)	6.6 (q)	6.4 (q)	6.3 (q)
20	112.5 (d)	112.7 (d)	58.4 (t)	50.1 (t)
2-O$\underline{C}$OCH$_3$	-	170.0 (s)	171.2 (s)	170.0 (s)
2-OCO$\underline{C}$H$_3$	-	20.9 (q)	20.9 (q)	20.8 (q)
9-O$\underline{C}$OCH$_3$	168.3 (s)	169.3 (s)	169.2 (s)	170.2 (s)
9-OCO$\underline{C}$H$_3$	23.3 (q)	21.7 (q)	21.9 (q)	21.5 (q)
13-O$\underline{C}$OCH$_3$	-	-	-	170.2 (s)
13-OCO$\underline{C}$H$_3$	-	-	-	21.0 (q)
14-O$\underline{C}$OCH$_3$	169.6 (s)	170.5 (s)	170.2 (s)	170.0 (s)
14-OCO$\underline{C}$H$_3$	23.0 (q)	21.2 (q)	21.0 (q)	21.3 (q)
2-O$\underline{C}$OC(CH$_3$)$_3$	174.7 (s)	-	-	-
2-OCO$\underline{C}$(CH$_3$)$_3$	- [a]	-	-	-
2-OCOC(C$\underline{H}_3$)$_3$	28.0 (q)	-	-	-
16-O$\underline{C}$H$_3$	53.6 (q)	52.5 (q)	-	-

[a] Signal not observed.

Figure 2. ^{1}H–^{1}H COSY and HMBC correlations of compounds **1**–**4**.

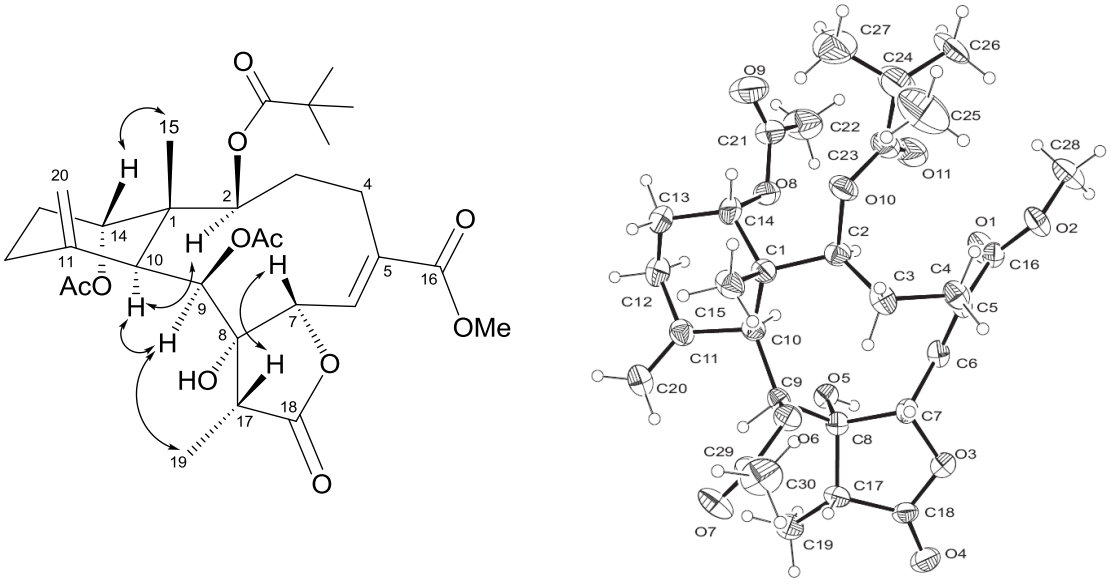


The configuration of the Me-15 in naturally occurring briaranes was previously assigned β-orientation, and H-10 was in the α-orientation. In the NOESY spectrum of **1** (Figure 3), correlations of Me-15/H-14, H-10/H-2, H-10/H-9, H-9/Me-19 and H-7/H-17 suggested that H-7, Me-15 and H-17 were all β-oriented, while H-2, H-9 and H-14 were α-disposition. Finally, the absolute configuration of compound **1** was unambiguously established by a single-crystal X-ray diffraction, as illustrated in Figure 3. Hence, compound **1** was determined as (1*S*,2*S*,6*Z*,7*S*,8*R*,9*S*,10*S*,14*S*,17*R*)-2-pivaloyloxy-9, 14-diacetoxy-8-hydroxybriaran-5(6)*Z*-dien-18,7-olide, and the name frajunolide P was given.

Compound **2** was isolated as a colorless amorphous gum and had the molecular formula $C_{27}H_{36}O_{11}$, as determined by HRESIMS and distortionless enhancement by polarization transfer (DEPT) NMR analysis. The presence of a hydroxyl, an ester group and a γ-lactone were consistent with IR absorption bands at 3443, 1736 and 1780 cm^{-1}, respectively. It was found that the ^{1}H- and ^{13}C NMR spectroscopic data (Tables 1 and 2) were similar to those of compound **1**, except that the pivaloyloxy group at C-2 was replaced by an acetate group (δ_H 1.97; δ_C 170.0, 20.9). This was confirmed by the HMBC correlation (Figure 2) between H-2 (δ_H 5.00) and the carbonyl carbon at δ_C 170.0. The planar structure and NMR assignments for **2** were established by detailed analysis of 2D NMR, including ^{1}H–^{1}H COSY, HMQC and HMBC correlations. The configurations of compound **2** were determined

by observation of NOESY correlations and on the basis of biogenetic consideration similar to compound **1**. Therefore, compound **2** was identified as (1*S*,2*S*,6*Z*,7*S*,8*R*,9*S*,10*S*,14*S*,17*R*)-2,9, 14-triacetoxy-8-hydroxybriaran-5(6)-dien-18,7-olide and named frajunolide Q.

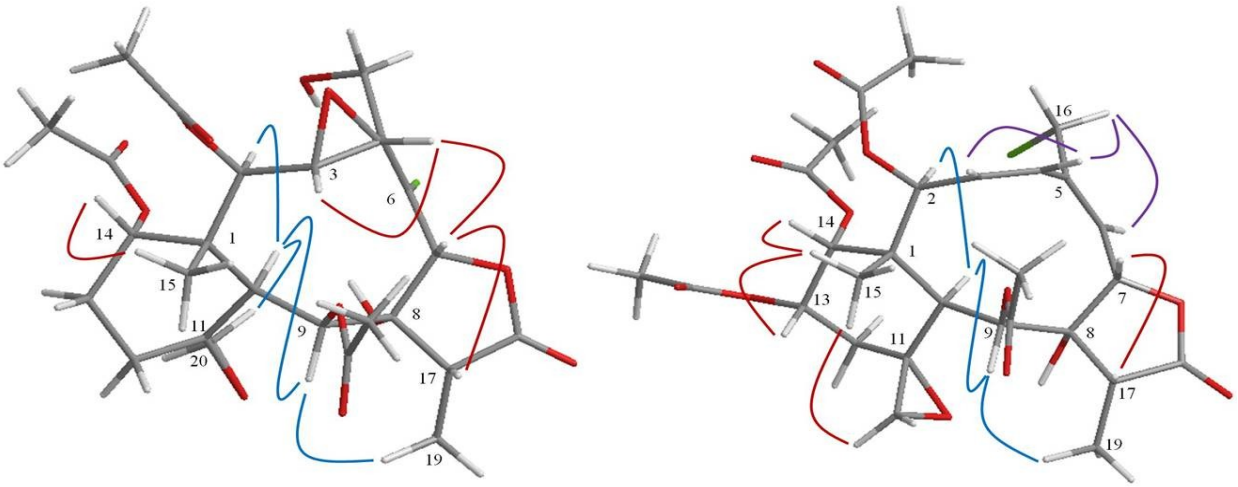
Figure 3. Key NOESY correlations and X-ray crystallographic diagram of compound **1**.



The molecular formula, $C_{26}H_{33}O_{12}Cl$, of compound **3** was obtained from the HRESIMS, which showed a *quasi*-molecular ion peak at *m/z* 595.1559 $[M + Na]^+$. The presence of a chlorine atom was suggested from an isotope ion at *m/z* 597.1536 $[M + Na]^+$, which exhibited one-third of the relative intensity of the normal ion peak. The IR spectrum showed absorption bands at 3480, 3241, 1782 and 1742 cm^{-1}, indicating the presence of two hydroxyl, γ-lactone and ester carbonyl functionalities. The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) further supported the existence of three acetate groups (δ_C 171.2, 170.2, 169.2, 21.9, 21.0, 20.9), assigned to C-2 (δ_C 75.9), C-9 (δ_C 66.8) and C-14 (δ_C 72.7) with the aid of HMBC correlations between H-2 (δ_H 4.83, d, J = 9.2 Hz), H-9 (δ_H 5.64, d, J = 8.0 Hz), H-14 (δ_H 4.88, d, J = 5.2 Hz) and acetate carbonyls, respectively. The remaining ^1H and ^{13}C NMR signals revealed that compound **3** possessed a methyl singlet (δ_H 1.24, Me-15), a methyl doublet (δ_H 1.22, d, J = 7.2 Hz, Me-19), one epoxy ring (δ_C 58.7, 59.0; δ_H 3.40, dd, J = 9.6, 3.6 Hz; 4.08, d, J = 3.6 Hz), one spirocyclic oxirane ring (δ_C 61.2, 58.4; δ_H 2.98, d, J = 4.4 Hz), 2.80, d, J = 4.0 Hz), one tetrasubstituted double bond (δ_C 135.7, 132.4), two methylene carbons (δ_C 24.3, 24.1), one oxymethylene (δ_C 58.8; δ_H 4.57, dd, J = 12.4, 8.8 Hz; 4.31, dd, J = 12.4, 6.0 Hz), one oxymethine (δ_C 75.7; δ_H 5.41, s), two methine protons (δ_H 2.49, d, J = 8.0 Hz; 2.26, q, J = 7.2 Hz) and two quaternary carbons (δ_C 44.7,C-1; 81.2, C-8), together with γ-lactone carbonyl carbon at δ_C 174.4 (C-18). The above observation agreed with a 8-hydroxybriarane with γ-lactone in **3**. The structure was further established by detailed analysis of 2D NMR. The signals of the H-20 showed HMBC correlations (Figure 2) with C-11, C-12 and C-10, indicating an epoxy ring at C-11 (δ_C 61.2)/C-20 (δ_C 58.4). The other epoxy ring was located at C-3/C-4 by observation of COSY (H-2/H-3/H-4) and HMBC correlations between H-4 and C-5. Finally, the chlorine atom has to be attached to C-6 (δ_C 132.4) of the tetrasubstituted double bond. This was confirmed by comparison with the NMR data of briarein F [19]. The configuration of compound **3** (Figure 4) was determined by a NOESY

experiment and coupled with molecular model MM2 minimized energy calculation [20]. The NOESY spectrum showed correlations of H-3/H-4, H-7/H-4, H-17 and Me-15/H-14 indicated that H-3, H-4, H-7, H-14, H-17 and Me-15 are all β-orientation, while H-2, H-9 and Me-19 favored α disposition, due to correlations of H-10/H-2, H-9 and H-9/Me-19. Moreover, the configuration at C-11 was assigned as *S*, which was determined by the NOESY correlation of H-10/H-20 and comparison with ^{13}C NMR data of the related literature [21]. The above interpretation suggested that compound **3** was a novel 8-hydroxybriarane possessing a chlorine atom at C-6, and thus, the name frajunolide R was given.

Figure 4. Key NOESY correlations and computer-generated perspective model of compounds **3** and **4**.



3 **4**

The HRESIMS of **4** exhibited two *pseudo*-molecular ion peaks at *m/z* 621.1716 $[M + Na]^+$ and 623.1690 $[M + Na + 2]^+$, accounting for a chlorine atom in the molecular formula, $C_{28}H_{35}O_{12}Cl$. The IR spectrum showed absorption bands of a hydroxyl (3467 cm^{-1}), a γ-lactone (1780 cm^{-1}) and an ester carbonyl (1739 cm^{-1}) group. The ^{1}H- and ^{13}C-NMR spectroscopic data (Tables 1 and 2) of **4** resembled those of juncenolide B, previously isolated from *J. juncea* [15], suggesting that they were analogs. Detailed analysis of NMR and MS data concluded that the only difference between them was the presence of a chlorine atom at C-16 in **4**, replacing the original hydroxyl group in juncenolide B. This finding was supported by observation of the chemical shift of C-6 at δ_C 44.6. The relative configuration of **4** was determined by comparing the proton coupling constants of **4** with those of juncenolide B and NOESY studies. Molecular modeling based on MM2 minimized energy was calculated to confirm the structure as illustrated in Figure 4. Thus, compound **4** was elucidated as a 16-chlorinated derivative of juncenolide B, and the name frajunolide S was given.

Four known briaranes were also isolated and identified as umbraculolide A [22], juncenolide C [15], junceellonoid A [23] and juncin R [24], respectively, by comparison with the spectroscopic data reported in the literature. The anti-inflammatory activities (Table 3) of briaranes **1**–**4** were tested and evaluated for their inhibition of elastase release and generation of superoxide anion by human neutrophils in response to fMet-Leu-Phe (fMLP)/cytochalasin B. Compounds **1** and **2** showed moderate inhibitory activities on both superoxide anion generation and elastase release at 10 μg/mL.

Table 3. Effects of compounds on superoxide anion generation and elastase release by human neutrophils in response to formylmethionylleucyl-phenylalanine/dihydrocytochalasin B (fMLP/CB).

Compound	Superoxide anion	Elastase release
	Inhibition (%)	Inhibition (%)
1	32.5 ± 1.5 ***	35.6 ± 3.2 *
2	28.7 ± 3.4 *	34.1 ± 2.9 **
3	9.70 ± 1.3 **	16.0 ± 5.3 *
4	5.80 ± 3.0	−4.5 ± 3.4

Percentage of inhibition (%) at 10 μg/mL concentration. Results are presented as the mean ± S.E.M. (n = 3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the control value.

3. Experimental Section

3.1. General Experimental Procedures

Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR spectra were measured on Hitachi T-2001 spectrophotometer. LRESIMS and HRESIMS were taken on a JEOL JMS-HX 110 mass spectrometer. The ^{1}H, ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY spectra were recorded on a Varian MR 400 and UNITY INOVA 500 spectrometers. The chemical shifts were given in δ (ppm) and coupling constants in Hz. Silica gel 60 (Merck) was used for column chromatography, and pre-coated silica gel plates (Merck, Kieselgel 60 F-254, 1 mm) were used for preparative TLC. Sephadex LH-20 (Amersham Pharmacia Biotech AB, Sweden) was used for separation. LiChrospher® Si 60 (5 μm, 250-10, Merck, Germany) and LiChrospher® 100 RP-18e (5 μm, 250-10, Merck, Germany) were used for NP-HPLC and RP-HPLC (Hitachi), respectively.

3.2. Animal Material

The gorgonian *Junceella fragilis* Ridley (Ellisellidae) was collected in Tai-Tong County, Taiwan, by scuba diving at a depth of 15 m, in February 2006. The fresh gorgonian was immediately frozen after collection and kept at −20 °C until processed. A voucher specimen (WSG-5) was deposited in the School of Pharmacy, College of Medicine, National Taiwan University, Taiwan.

3.3. Extraction and Isolation

The gorgonian *J. fragilis* (wet, 3.9 kg) was minced and extracted with acetone (3 × 5 L) at room temperature, and the acetone extract was concentrated under vacuum. The crude extract (33 g) was partitioned between EtOAc and H_2O (1:1). The EtOAc-soluble portion (24 g) was shaken with *n*-hexane-MeOH–H_2O (4:3:1), and the MeOH layer was evaporated and separated on Sephadex LH-20 to give eight fractions (L1 to L8). Fraction L3 (3 g) was subjected to column chromatography using silica gel and a gradient of *n*-hexane/CH_2Cl_2/MeOH to obtain 33 fractions (L3-1 to L3-33). Fraction L3-14 (111 mg) was separated on NP-HPLC using *n*-hexane/CH_2Cl_2/MeOH (40:20:1) to yield **1** (3.5 mg) and **2** (1.0 mg). Fraction L3-17 (104 mg) was subjected to RP-HPLC using MeOH/H_2O/CH_3CN (70:25:5) to give **4** (7.5 mg), umbraculolide A (18 mg) and junceellonoid A (16 mg). L3-20 (97 mg)

was separated on RP HPLC using MeOH/H_2O/CH_3CN (70:25:5) to obtain **3** (3.5 mg), junceellolide C (12 mg) and juncin R (2.8 mg).

Frajunolide P (**1**): colorless prisms; $[\alpha]_D^{24}$ +4 (*c* 0.5, CH_2Cl_2); IR ν_{max} 3443, 2934, 1776, 1722, 1655, 1379, 1267, 1220 cm^{-1}; ^1H NMR data (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR data (100 MHz, $CDCl_3$), see Table 2; ESIMS *m/z* 601 $[M + Na]^+$; HRESIMS *m/z* 601.2620 $[M + Na]^+$ (calcd. for $C_{30}H_{42}O_{11}Na$, 601.2625).

Frajunolide Q (**2**): colorless amorphous gum; $[\alpha]_D^{24}$ +32 (*c* 0.1, CH_2Cl_2); IR ν_{max} 3443, 2923, 1780, 1736, 1645, 1375, 1264, 1219 cm^{-1}; ^1H NMR data (500 MHz, $CDCl_3$), see Table 1; ^{13}C NMR data (125 MHz, $CDCl_3$), see Table 2; ESIMS *m/z* 559 $[M + Na]^+$; HRESIMS *m/z* 559.2156 $[M + Na]^+$ (calcd. for $C_{27}H_{36}O_{11}Na$, 559.2155).

Frajunolide R (**3**): colorless amorphous gum; $[\alpha]_D^{24}$ +13 (*c* 0.3, CH_2Cl_2); IR ν_{max} 3480, 3241, 2926, 2856, 1782, 1742, 1373, 1252, 1212 cm^{-1}; ^1H NMR data (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR data (100 MHz, $CDCl_3$), see Table 2; ESIMS *m/z* 595 $[M + Na]^+$, *m/z* 597 $[M + Na + 2]^+$; HRESIMS *m/z* 595.1559 $[M + Na]^+$ (calcd. for $C_{26}H_{33}{}^{35}ClO_{12}Na$, 595.1558).

Frajunolide S (**4**): colorless amorphous gum; $[\alpha]_D^{24}$ −22.0 (*c* 0.2, CH_2Cl_2); IR ν_{max} 3476, 2947, 1780, 1739, 1372, 1250, 1223 cm^{-1}; ^1H NMR data (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR data (100 MHz, $CDCl_3$), see Table 2; ESIMS *m/z* 621 $[M + Na]^+$, *m/z* 623 $[M + Na + 2]^+$; HRESIMS *m/z* 621.1716 $[M + Na]^+$ (calcd. for $C_{28}H_{35}{}^{35}ClO_{12}Na$, 621.1715).

3.4. Single Crystal X-ray Structure Determination of Frajunolide P (**1**)

A suitable colorless crystal (0.37 × 0.14 × 0.08 mm^3) of **1** for diffraction was obtained by simple evaporation from methanol solution. Crystal data: $C_{30}H_{42}O_{11}$ orthorhombic, *a* = 10.1174(2) Å, *b* = 14.0223(3) Å, *c* =21.0529(5) Å, *V* = 2986.76(11) $Å^3$, space group $P22_12_1$, *Z* = 4, D_{calcd} 1.287 mg/m^3, λ = 0.71073 Å, μ(Mo Kα) 90.098 mm^{-1}, *F*(000) = 1240, *T* = 293(2) K. A total of 18,766 reflections collected, of, which 5275 unique reflections (R_{int} = 0.0770) with I > 2σ(*I*) were used for the analysis. The data was solved using the direct method, and the structure was refined by full-matrix least-squares procedure on F^2 values. The refined structural model converged to a final *R*1 0.0684, *wR*2 0.1808 with goodness-of-fit = 1.036. The final X-ray molecular model is shown in Figure 3.

3.5. Anti-Inflammatory Assays

3.5.1. Human Neutrophils Elastase Release

Degranulation of azurophilic granules was determined by elastase release, as described previously [25]. Experiments were performed using MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as the elastase substrate. After supplementation with MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (100 μM), neutrophils (6 × 10^5 cell/mL) were equilibrated at 37 °C for 2 min and incubated with each test compound for 5 min. Cells were activated by fMLP (100 nM)/CB (0.5 μg/mL), and changes in absorbance at 405 nm were monitored continuously for elastase release. The results are expressed as the percentage of the initial rate of elastase release in the fMLP/CB-activated, test compound-free (DMSO) control system.

3.5.2. Human Neutrophil Superoxide Generation

Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*. In brief, after supplementation with 0.5 mg/mL ferricytochrome *c* and 1.0 mM Ca^{2+}, neutrophils were equilibrated at 37 °C for 2 min and incubated with drugs for 5 min. Cells were activated with 100 nM fMLP for 10 min. When fMLP was used as a stimulant, CB (1 μg/mL) was incubated for 3 min before activation by the peptide (fMLP/CB). Changes in absorbance with the reduction of ferricytochrome *c* at 550 nm were continuously monitored in a double-beam, six-cell positioner spectrophotometer with constant stirring (Hitachi U-3010, Tokyo, Japan). Calculations were based on differences in the reactions with and without superoxide dismutase (SOD, 100 U/mL) divided by the extinction coefficient for the reduction of ferricytochrome *c*.

4. Conclusions

Our continuing investigation on constituents of Taiwanese gorgonian *Junceella fragilis* has resulted in the isolation of eight 8-hydroxybriarane diterpenoids, including four new ones, frajunolides P–S (**1**–**4**). In the anti-inflammatory effects on elastase release and generation of superoxide anion by human neutrophils, compounds **1** and **2** exhibited moderate inhibitory activities.

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