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New 8-Hydroxybriaranes from the Gorgonian Coral *Junceella fragilis* (Ellisellidae)

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Abstract: Three new 8-hydroxybriaranes—fragilides R–T (**1**–**3**) were obtained from a sea whip gorgonian coral *Junceella fragilis*. The structures of briaranes **1**–**3** were elucidated by using spectroscopic methods, including 1D (^{1}H and ^{13}C NMR), 2D (COSY, HSQC, HMBC, and NOESY experiments) NMR studies, and (+)-HRESIMS. Fragilides S and T (**2** and **3**) are the only briaranes known to possess 8α-hydroxy and 17β-methyl groups, respectively. Briarane **2** exerted an inhibition effect on iNOS release from RAW264.7; a macrophage cell line that originated from a mouse monocyte macrophage, stimulated with lipopolysaccharides.

Keywords: *Junceella fragilis*; fragilide; briarane; anti-inflammatory; iNOS

1. Introduction

Gorgonian corals belonging to the genus *Junceella* (family Ellisellidae) [1–3] have been found to produce marine origin briarane-type diterpenoids in abundance [4]. Our recent research into the chemical constituents and properties of a gorgonian coral, *Junceella fragilis* (Ridley 1884) (Figure 1), which was distributed extensively in the waters of Orchid Island (= Lanyu Island), intersection of Kuroshio current and South China Sea surface current, has resulted in the isolation of three new 8- hydroxybriaranes–fragilides R–T (**1**–**3**) (Figure 1). A pro-inflammatory suppression assay was employed to assess the activity of these isolates against the release of inducible nitric oxide synthase (iNOS) from macrophage cells.

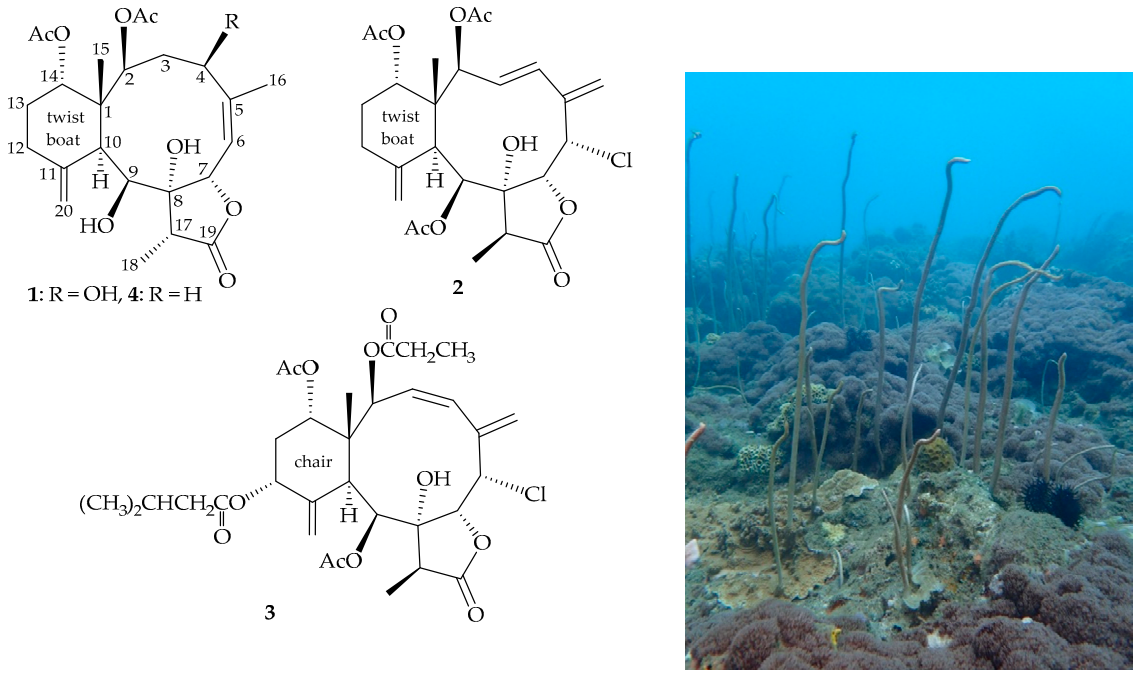

Figure 1. Structures of fragilides R–T (**1**–**3**), 9-*O*-deacetylumbraculolide A (**4**), and a picture of the gorgonian coral *Junceella fragilis*.

2. Results and Discussion

Fragilide R (**1**) was isolated as an amorphous powder and displayed a pseudomolecular ion at *m/z* 489.20971 in the (+)-HRESIMS, which indicated its molecular formula was $C_{24}H_{34}O_9$ (calcd. for $C_{24}H_{34}O_9$ + Na, 489.20950) (Ω = 8). Both the ^{1}H and ^{13}C NMR data (Tables 1 and 2) indicated two acetates (δ_H 1.99, 1.94, each 3H × s; δ_C 21.3, 21.0, 2 × CH_3; δ_C 170.8, 170.8, 2 × C). Besides the above ester carbonyls, the carbon signal at δ_C 176.7 (C) was assigned to a γ-lactone ring along with an oxymethine (δ_H 5.97, 1H, d, J = 10.2 Hz; δ_C 76.4, CH-7). The spectroscopic data, including 1D and 2D NMR experiments (Figure 2), were similar to those of a known metabolite, 9-*O*-deacetyl- umbraculolide A (**4**) [5] (Figure 1), except that the hydroxy group at C-4 in **1** was replaced by a proton in **4**. It is interesting to note that an allylic coupling was observed between H-6 and H_3-16 (J = 1.2 Hz) in the COSY spectrum (Figure 2). In the NOESY spectrum (Figure 2), one of the C-3 methylene protons (δ_H 2.96) exhibited a correlation to H-7 and not with H-2, suggesting the β- orientation of this proton. A correlation from H-4 to H-3α (δ_H 1.91) as well as the coupling constants between H-4 and H-3α/β (J = 5.4, 12.6 Hz), suggested that H-4 was α-oriented according to modeling study. Based on the above findings, the structure, including the relative configuration of stereogenic centers of **1** were assigned as 1*R**,2*S**,4*R**,7*S**,8*R**,9*S**,10*S**,14*S**, and 17*R**, as those of **4** by correlations observed in a NOESY experiment.

One of the C-20 methylene protons (δ_H 4.88) showed an NOE correlation to H-9, demonstrating that this olefin proton was H-20b, and the other was assigned as H-20a (δ_H 4.97). The proton chemical shifts of the briarane derivatives containing an 11-methylidene group were summarized, and the difference between these two olefin protons (H-20a/b) was smaller than 0.2 ppm, whereas the methylidene-containing six-membered rings exhibited a twisted boat conformation [6]. Owing to the chemical shifts of C-20 methylene protons (δ_H 4.97 and 4.88), the conformation of six- membered ring in **1** was concluded to be twisted boat. In a previous study, the absolute configuration of a known chlorinated briarane, junceellin, was established by a single-crystal X-ray diffraction analysis [7].

As briaranes **1**–**3** were isolated along with junceellin from the same organism [7], it is reasonable on biogenetic grounds to assume that **1**–**3** have the same absolute configuration as that of junceellin. Therefore, the configuration of the stereogenic centers of **1** were elucidated as 1*R*,2*S*,4*R*,7*S*,8*R*,9*S*,10*S*,14*S*, and 17*R* (Figure 2), and this compound was found to be the 4β-hydroxy derivative of **4** (Supplementary Materials, Figures S1–S9).

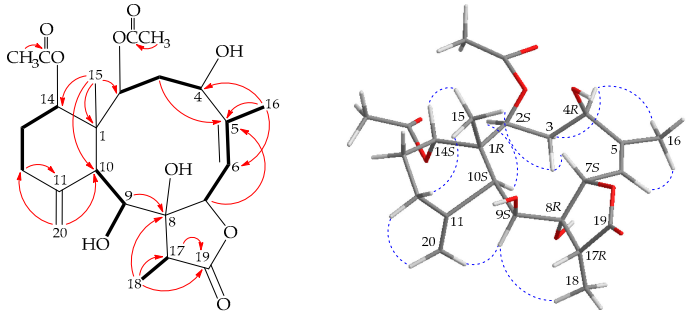

Figure 2. The COSY (▬) correlations, selective HMBC (↷), and selective protons with key NOESY correlations (⋯) of **1**.

Fragilide S (**2**) had a molecular formula $C_{26}H_{33}ClO_9$ as deduced by (+)-ESIMS, which showed a pair of peaks at *m*/*z* 547/549 $[M + Na]^+$:$[M + 2 + Na]^+$ (3:1), suggesting a chlorine atom, and further confirmed by (+)-HRESIMS at *m*/*z* 547.17055 (calcd. for $C_{26}H_{33}{}^{35}ClO_9$ + Na, 547.17053). The IR spectrum indicated the presence of hydroxy (3447 cm^{-1}), γ-lactone (1785 cm^{-1}), and ester carbonyl (1733 cm^{-1}) groups. The ^{13}C NMR data (Table 2), showed the presence of a disubstituted olefin (δ_C 135.4, CH-4; 129.7, CH-3) and two methylidene groups (δ_C 142.6, C-5; 118.8, CH_2-16; 149.3, C-11; 111.4, CH_2-20). Moreover, four carbonyl resonances at δ_C 174.1, 170.4, 170.1, and 169.2 in the ^{13}C spectrum confirmed the presence of a γ-lactone and three other ester groups. In the ^1H NMR spectrum (Table 1), three acetate methyls (δ_H 2.09, 2.04, 2.01, each 3H × s) were observed. Moreover, a methyl singlet, a methyl doublet, two aliphatic methines, two pair of aliphatic methylenes, four oxymethines, a chlorinated methine, and a hydroxy proton were observed (Table 1).

Table 1. ^1H NMR data for briaranes **1**–**3**.

Position	1 [a]	2 [b]	3 [a]
2	4.83 d (7.2) [c]	5.47 d (9.5)	6.33 d (10.8)
3α/β	1.91 ddd (15.6, 7.2, 5.4); 2.96 dd (15.6, 12.6)	5.67 dd (16.0, 9.5)	5.78 dd (12.0, 10.8)
4	4.18 dd (12.6, 5.4)	6.72 d (16.0)	5.96 d (12.0)
6	5.64 d (10.2, 1.2)	5.20 s	5.12 br s
7	5.97 d (10.2)	4.97 br s	4.88 d (4.2)
9	4.22 dd (5.4, 3.6)	5.53 s	5.59 s
10	3.20 d (3.6)	3.47 s	4.08 s
12α/β	2.22 m	2.24 m; 2.38 m	5.37 dd (5.4, 3.0)
13α/β	1.96 m; 1.81 m	1.84 m; 1.74 m	2.06 m
14	4.74 dd (4.2, 1.8)	4.84 dd (2.5, 2.0)	4.94 dd (4.2, 3.0)
15	1.25 s	1.18 s	1.16 s
16a/b	2.09 d (1.2)	5.49 s; 5.32 s	5.66 s; 5.48 s
17	3.11 q (7.2)	2.93 q (7.5)	2.90 q (7.2)
18	1.14 d (7.2)	1.18 d (7.5)	1.22 d (7.2)
20a/b	4.97 s; 4.88 s	4.93 s; 4.76 s	5.33 s; 4.72 s
OH-8	-	3.11 s	2.70 s
OH-9	2.09 d (5.4)	-	-
Acetoxy groups	1.94 s	2.01 s	2.00 s
	1.99 s	2.04 s	2.16 s
		2.09 s	
Propionoxy group	-	-	1.08 t (7.2)
			2.26 m
Isovaleroxy group	-	-	0.96 d (6.6)
			0.98 d (6.6)
			2.10 m
			2.15 m

[a] Spectra measured at 600 MHz in $CDCl_3$. [b] Spectra measured at 500 MHz in $CDCl_3$. [c] *J* values (in Hz) in parentheses.

Table 2. ^{13}C NMR data for briaranes **1**–**3**.

Position	1 [a]	2 [b]	3 [a]
1	48.2, C [c]	47.5, C	47.7, C
2	73.3, CH	76.9, CH	70.4, CH
3	39.7, CH_2	129.7, CH	130.4, CH
4	71.4, CH	135.4, CH	128.7, CH
5	146.9, C	142.6, C [d]	137.5, C
6	123.1, CH	66.0, CH [d]	64.5, CH
7	76.4, CH	79.5, CH	76.9, CH
8	83.4, C	81.8, C	80.3, C
9	74.1, CH	76.3, CH	74.8, CH
10	43.0, CH	41.8, CH	37.0, CH
11	152.3, C	149.3, C	146.3, C
12	29.0, CH_2	29.1, CH_2	74.3, CH
13	27.8, CH_2	26.0, CH_2	31.4, CH_2
14	74.2, CH	74.4, CH	73.0, CH
15	15.6, CH_3	16.4, CH_3	14.7, CH_3
16	26.2, CH_3	118.8, CH_2 [d]	116.4, CH_2
17	43.8, CH	49.3, CH	50.7, CH
18	6.6, CH_3	9.1, CH_3	8.7, CH_3
19	176.7, C	174.1, C [d]	173.9, C
20	111.3, CH_2	111.4, CH_2	115.2, CH_2
Acetoxy groups	21.0, CH_3	21.2, CH_3	20.7, CH_3
	170.8, C	170.4, C	169.7, C
	21.3, CH_3	21.2, CH_3	21.3, CH_3
	170.8, C	170.1, C	169.6, C
		21.2, CH_3	
		169.2, C	
Propionoxy group	-	-	8.8, CH_3
			27.8, CH_2
			172.0, C
Isovaleroxy group	-	-	22.6, CH_3
			22.8, CH_3
			25.0, CH
			43.5, CH_2
			172.6, C

[a] Spectra measured at 150 MHz in $CDCl_3$. [b] Spectra measured at 125 MHz in $CDCl_3$. [c] Multiplicity deduced by DEPT and HSQC spectra. [d] Chemical shfits were assigned by HSQC or HMBC experiments.

Analyses of 2D NMR data established a tricyclic nucleus. This assignment was evident from the spin systems from H-2 to H-3, H-3 to H-4, H-6 to H-7, H-9 to H-10, H_2-12 to H_2-13, H_2-13 to H-14, and H-17 to H_3-18 (Figure 3), while the HMBC between protons and quaternary carbons such as H-2, H-9, H-10, H_3-15/C-1; H-6, H-16a/C-5; H-6, H-9, H-10, H-17, H_3-18, OH-8/C-8; H-9, H-10, H-20b/C-11; and H-17, H_3-18/C-19, revealed the carbon skeleton (Figure 3). The methylidene groups at C-5 and C-11 were confirmed by the HMBC between H_2-16 to C-4 and C-5, H_2-20 to C-10, C-11, and C-12, respectively. The C-15 methyl group at C-1 was confirmed by the HMBC between H_3-15 to C-1, C-2, C-10, and C-14. HMBC spectrum also revealed that the carbon signal at δ_C 170.4, 170.1, and 169.2 correlated with the signals of the methyl protons at δ_H 2.04, 2.01, and 2.09, respectively, and were assigned as the carbon atom of acetate carbonyl groups. The acetates at C-2, C-9, and C-14 were confirmed from the connectivity between H-2 (δ_H 5.47), H-9 (δ_H 5.53), and H-14 (δ_H 4.84) to the carbonyl carbons of the acetate groups at δ_C 170.1, 169.2, and 170.4, respectively. The hydroxy group at C-8 was deduced from the HMBC of a hydroxy proton (δ_C 3.11) to C-8 and C-9.

In the NOESY spectrum of **2** (Figure 3), one of the C-16 methylene protons (δ_H 5.49) showed a correlation to H-4, demonstrating that this olefinic proton was H-16a and the other was assigned as H-16b (δ_H 5.32). Moreover, one of the C-20 methylene protons (δ_H 4.76) correlated to H-10, indicating that this proton was H-20b and the other was assigned as H-20a. According to a summary for the chemical shifts of 11-methylidene groups, the configuration of six-membered ring was in a twisted boat conformation [6]. The *E*-geometry of C-3/4 double bond was determined by a large proton coupling constant (J = 16.0 Hz) between H-3 and H-4. Correlations between H-10 with H-2 and H-9, while no correlation was seen with Me-15, suggested that H-2, H-9, and H-10 were all in α-oriented. Meanwhile, a correlation of Me-15 with H-14 indicated that H-14 was β-oriented. Furthermore, OH-8 showed correlations with H-4 and H-17, indicating that the hydroxy group at C-8 and proton at C-17 were

α-oriented. In addition, H-7 exhibited correlations with H-6 and H_3-18 but not with OH-8, suggesting that H-7 was β-oriented. Based on above findings, the configuration of stereogenic carbons was determined as 1*R*,2*S*,6*S*,7*R*,8*R*,9*S*,10*S*,14*S* and 17*S* (see Figures S10–S18 in the Supplementary Materials).

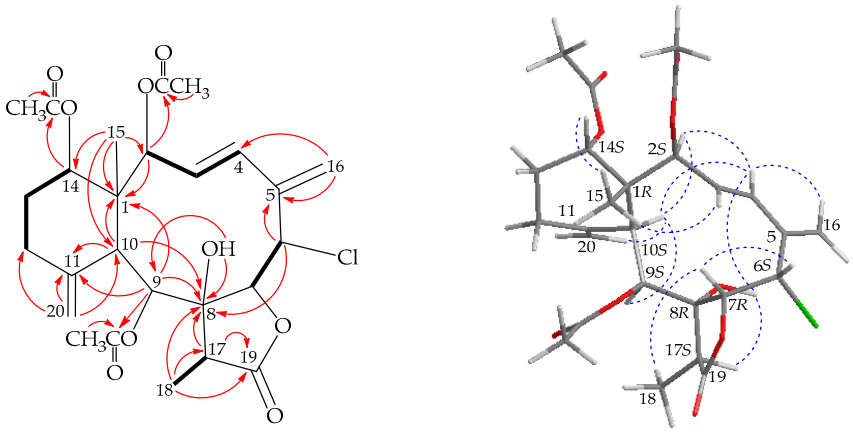

Figure 3. The COSY (▬) correlations, selective HMBC (↷), and selective protons with key NOESY correlations (⋯) of **2**.

Compound **3** (fragilide T) has a molecular formula $C_{32}H_{43}ClO_{11}$ according to its (+)-HRESIMS at *m*/*z* 661.23866 (calcd. for $C_{32}H_{43}{}^{35}ClO_{11}$ + Na, 661.23861). Both the ^{1}H and ^{13}C NMR (Tables 1 and 2) indicated two acetates (δ_H 2.16, 2.00, each 3H × s; δ_C 21.3, 20.7, 2 × acetate methyls; 169.7, 169.6, 2 × acetate carbonyls), a propionate (δ_H 1.08, 3H, t, *J* = 7.2 Hz; 2.26, 2H, m; δ_C 8.8, CH_3; 27.8, CH_2; 172.0, propionate carbonyl), and an isovalerate (δ_H 0.96, 0.98, each 3H, d, *J* = 6.6 Hz; 2.10, 1H, m; 2.15, 2H, m; δ_C 22.6, 22.8, 2 × CH_3; 25.0, CH; 43.5, CH_2; 172.6, isovalerate carbonyl). Besides the above ester carbonyls, the carbon signal at δ_C 173.9 was assigned to a γ-lactone ring along with an oxymethine (δ_H 4.88, 1H, d, *J* = 4.2 Hz; δ_C 76.9, CH-7). Two pairs of proton signals at δ_H 5.66 and 5.48, and 5.33 and 4.72, correlating to the methylidene signals at δ_C 116.4 and 115.2 respectively, were ascribed to two methylidene groups. The tertiary methyl singlet at δ_H 1.16 (3H, s) was assigned to H_3-15 while the secondary methyl doublet at δ_H 1.22 (3H, d, *J* = 7.2 Hz) was assigned to H_3-18. In the HMBC spectrum (Figure 4), the propionoxy group at C-2 was confirmed by the connectivity between H-2 (δ_H 6.33) with the carbonyl carbon (δ_C 172.0) of propionoxy group. The HMBC also revealed that an acetoxy group at C-9 (Figure 4) and the remaining isovaleroxy and acetoxy groups should be positioned at C-12 or C-14, oxygen-bearing methines, by analysis of characteristic NMR signals (δ_H 5.37, 1H, dd, *J* = 5.4, 3.0 Hz; δ_C 74.3, CH-12; δ_H 4.94, 1H, dd, *J* = 4.2, 3.0 Hz; δ_C 73.0, CH-14). However, due to no HMBC detected between H-12 and H-14 and ester carbonyl, the positions of the isovalerate and remaining acetoxy group cannot be determined by HMBC.

Based on previous studies, while the difference between the two olefin protons (H-20a/b) was bigger than 0.3 ppm, the six-membered rings showed a chair conformation [6]. Owing to the chemical shifts of the C-20 methylene protons (δ_H 5.33 and 4.72 ppm), the configuration of the methylidene-containing six-membered ring was concluded to exist in a chair conformation. In the NOESY experiment (Figure 4), H-10 correlated to H-2, H-9, and OH-8, but not to H_3-15, indicating that these protons are located on the same face and can be assigned as α-protons, as C-15 methyl group is a β-substituent at C-1. H-14 was found to exhibit a correlation with H_3-15, showing that this proton is positioned on the equatorial direction and has a β-orientation at C-14. The *cis* geometry of the C-3/4 double bond was indicated by a 12.0 Hz coupling constant between H-3 (δ_H 5.78) and H-4 (δ_H 5.96). Moreover, a correlation between H-3 and H_3-15, and there are correlations which were observed among H-4, H-6, and H-7, further supported the *Z*-form of C-3/4 double bond and indicated that H-6 and H-7 were on the β face. A correlation between OH-8 and H-17 showed that Me-18 at C-17 was β-oriented. The C-12 oxymethine proton (δ_H 5.37) was found to couple C-13 methylene protons with coupling constants

J = 5.4, 3.0 Hz, showing that this proton should be positioned on the equatorial direction and has a β-orientation. Fortunately, a correlation between the methyl protons of acetoxy group at C-9 and methyl protons of isovaleroxy group, indicated that the isovaleroxy group should be located at C-12 by modeling analysis. Thus, based on the above findings, the stereogenic centers were assigned as 1*R*,2*S*,6*S*,7*R*,8*R*,9*S*,10*R*,12*R*,14*S*, and 17*S* (see Figures S19–S27 in the Supplementary Materials).

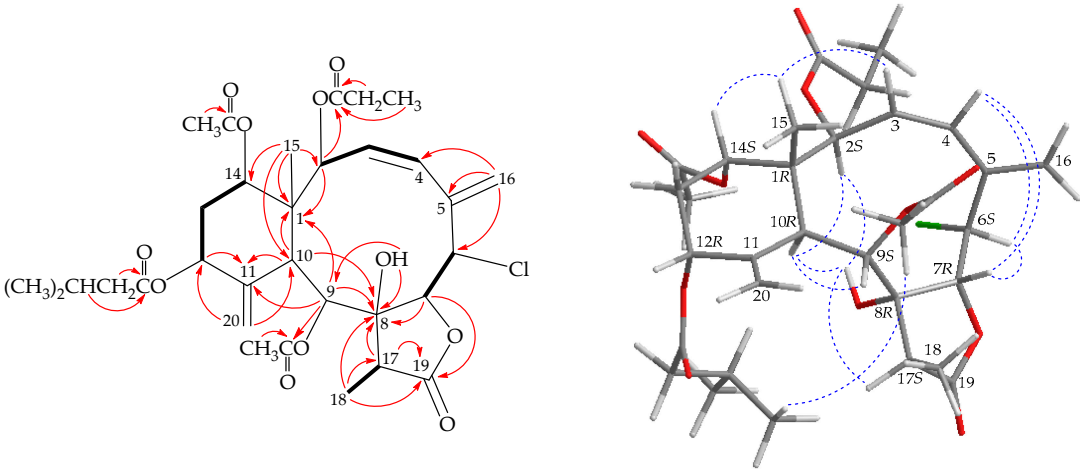

Figure 4. The COSY (▬) correlations, selective HMBC (↷), and selective protons with key NOESY correlations (⤳) of **3**.

Using an *in vitro* pro-inflammatory suppression assay, the effects of briaranes **1**–**3** on the release of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein from lipopolysaccharides (LPS)-stimulated RAW264.7 macrophage cells were assessed. The results of pro-inflammatory suppression assay showed that briarane **2** at 10 μM suppressed the release of iNOS to 61.21 ± 9.61% as compared with results of the cells stimulated with LPS only (Table 3).

Table 3. Effects of briaranes **1**–**3** on LPS-induced pro-inflammatory iNOS and COX-2 protein expression in macrophages.

Compound	iNOS	COX-2	β-Actin	*n*
	Expression (% of LPS)			
Negative Control	1.80 ± 0.21	1.04 ± 0.35	110.02 ± 5.23	2
LPS	100.01 ± 5.06	100.06 ± 0.43	100.07 ± 8.4	4
1	104.11 ± 16.63	100.51 ± 6.11	105.70 ± 7.05	4
2	61.21 ± 9.61	100.01 ± 5.11	99.29 ± 11.29	3
3	100.91 ± 24.08	96.36 ± 21.31	115.29 ± 3.4	4
Dexamethasone	5.54 ± 1.72	8.15 ± 5.13	105.21 ± 15.57	4

Data were normalized to those of cells treated with LPS alone, and cells treated with dexamethasone were used as a positive control. Data are expressed as the mean ± SEM (n = 2–4).

3. Materials and Methods

3.1. General Experimental Procedures

NMR spectra were recorded on a 600 MHz Jeol NMR (model ECZ600R, Tokyo, Japan) or on 500 MHz Varian NMR (model Unity Inova-500, Palo Alto, CA, USA) spectrometers using the residual $CHCl_3$ signal (δ_H 7.26 ppm) and $CDCl_3$ (δ_C 77.1 ppm) as internal references for ^1H and ^{13}C NMR, respectively. ESIMS and HRESIMS were obtained from the Bruker mass spectrometer with 7 Tesla magnets (model: SolariX FTMS system, Bremen, Germany). Column chromatography, high-performance liquid chromatography (HPLC), IR spectra, and optical rotation were performed according to our earlier research [7].

3.2. Animal Material

Specimens of *J. fragilis* used for this study were collected in June 2017 by self-contained underwater breathing apparatus (SCUBA) divers off the coast of Orchid Island, Taiwan. The samples were stored in a −20 °C freezer until extraction. A voucher specimen was deposited in the NMMBA (voucher no.: NMMBA-TW-GC-2017-08). Identification of the species of this organism was performed by comparison as described in previous studies [1–3].

3.3. Extraction and Isolation

Sliced bodies (wet weight = 423 g) of the coral specimen were prepared and extracted with a 1:1 mixture of methanol (MeOH) and dichloromethane (CH_2Cl_2) to give 5.53 g of crude extract, which was partitioned between ethyl acetate (EtOAc) and H_2O. The EtOAc extract (2.50 g) was then applied to a silica gel column and eluted with gradients of *n*-hexane/acetone (stepwise from 50:1 to 1:2; volume ratio) to furnish 8 fractions (fractions: A−H). Fraction F was purified by normal-phase HPLC (NP-HPLC) using a mixture of *n*-hexane and EtOAc (3.5:1 of volume ratio) as solvent to obtain 14 subfractions (fractions: F1−F14). Fraction F10 was repurified by reverse-phase HPLC (RP-HPLC) using a mixture of MeOH and H_2O (with volume:volume = 80:20; at a flow rate = 4.0 mL/min) to yield **2** (0.2 mg) and **3** (0.5 mg). Fraction G was separated by NP-HPLC using a mixture of *n*-hexane/EtOAc (1:1; volume ratio) to yield 9 fractions (fractions: G1−G9). Fraction G2 was purified by RP-HPLC using a mixture of MeOH and H_2O (with volume:volume = 65:35; at a flow rate = 4.0 mL/min) to afford **1** (0.2 mg).

Fragilide R (**1**): Amorphous powder; $[\alpha]_D^{28}$ −288 (*c* 0.07, $CHCl_3$); IR (ATR) ν_{max} 3391, 1779, 1736 cm^{-1}; ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR data, see Tables 1 and 2; ESIMS: *m/z* 489 $[M + Na]^+$; HRESIMS: *m/z* 489.20971 (calcd. for $C_{24}H_{34}O_9$ + Na, 489.20950).

Fragilide S (**2**): Amorphous powder; $[\alpha]_D^{28}$ +45 (*c* 0.16, $CHCl_3$); IR (ATR) ν_{max} 3447, 1785, 1733 cm^{-1}; ^1H (500 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) NMR data, see Tables 1 and 2; ESIMS: *m/z* 547 $[M + Na]^+$, 549 $[M + 2 + Na]^+$; HRESIMS: *m/z* 547.17055 (calcd. for $C_{26}H_{33}{}^{35}ClO_9$ + Na, 547.17053).

Fragilide T (**3**): Amorphous powder; $[\alpha]_D^{20}$ −168 (*c* 0.17, $CHCl_3$); IR (ATR) ν_{max} 1788, 1737 cm^{-1}; ^1H (600 MHz, $CDCl_3$), and ^{13}C (150 MHz, $CDCl_3$) NMR data, see Tables 1 and 2; ESIMS: *m/z* 661 $[M + Na]^+$, 663 $[M + 2 + Na]^+$; HRESIMS: *m/z* 661.23866 (calcd. for $C_{32}H_{43}{}^{35}ClO_{11}$ + Na, 661.23861).

3.4. In Vitro Anti-inflammatory Assay

The pro-inflammatory suppression assay was employed to assess the activities of the isolated compounds **1**–**3** against the release of iNOS and COX-2 from macrophage cells as the literature reported [8–11].

4. Conclusions

J. fragilis has been demonstrated to have a wide structural diversity of briarane-type diterpenoids that possess various pharmacological properties, particularly in anti-inflammatory activity [12,13]. In our continued study of *J. fragilis*, three previously unreported fragilides R–T (**1**–**3**) were isolated. In the previous studies [14], all the Me-18 attached at C-17 was *cis* to the hydroxy group at C-8, and most of these two groups were α-oriented in briarane derivatives, respectively. Fragilides S and T (**2** and **3**) were proved to be the only two briaranes known to possess hydroxy group at C-8α and methyl group at C-17β, respectively. In the present study, the anti-inflammatory activity of **1**–**3** was assessed using inhibition of pro-inflammatory iNOS and COX-2 release from macrophages. The results indicated that fragilide S (**2**) showed the most potent suppressive effect on iNOS release.

Supplementary Materials: The Supplementary Materials are available online at http://www.mdpi.com/1660-3397/17/9/534/s1. Figure S1: ESIMS spectrum of compound 1, Figure S2: HRESIMS spectrum of compound 1, Figure S3: IR spectrum of compound 1, Figure S4: 1H NMR spectrum (600 MHz) of compound 1 in CDCl3, Figure S5: 13C NMR spectrum (150 MHz) of compound 1 in CDCl3, Figure S6: HSQC spectrum of compound 1 in CDCl3, Figure S7: 1H-1H COSY spectrum of compound 1 in CDCl3, Figure S8: HMBC spectrum of compound 1 in CDCl3, Figure S9: NOESY spectrum of compound 1 in CDCl3, Figure S10: ESIMS spectrum of compound 2, Figure S11: HRESIMS spectrum of compound 2, Figure S12: IR spectrum of compound 2, Figure S13: 1H NMR spectrum (600 MHz) of compound 2 in CDCl3, Figure S14: 13C NMR spectrum (150 MHz) of compound 2 in CDCl3, Figure S15: HSQC spectrum of compound 2 in CDCl3, Figure S16: 1H-1H COSY spectrum of compound 2 in CDCl3, Figure S17: HMBC spectrum of compound 2 in CDCl3, Figure S18: NOESY spectrum of compound 2 in CDCl3, Figure S19: ESIMS spectrum of compound 3, Figure S20: HRESIMS spectrum of compound 3, Figure S21: IR spectrum of compound 3, Figure S22: 1H NMR spectrum (600 MHz) of compound 3 in CDCl3, Figure S23: 13C NMR spectrum (150 MHz) of compound 3 in CDCl3, Figure S24: HSQC spectrum of compound 3 in CDCl3, Figure S25: 1H-1H COSY spectrum of compound 2 in CDCl3, Figure S26: MBC spectrum of compound 3 in CDCl3, Figure S27: NOESY spectrum of compound 3 in CDCl3.

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