Article

Fragilides U–W: New 11,20-Epoxybriaranes from the Sea Whip Gorgonian Coral *Junceella fragilis*

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Abstract: Three new 11,20-epoxybriaranes—fragilides U–W (**1**–**3**), as well as two known metabolites, junceellonoid D (**4**) and junceellin (**5**), were obtained from the octocoral *Junceella fragilis*. The structures of briaranes **1**–**3** were elucidated by spectroscopic methods and briaranes **3** and **5** displayed inhibition effects on inducible nitric oxide synthase (iNOS) release from RAW264.7.

Keywords: *Junceella fragilis*; fragilide; briarane; gorgonian; junceellonoid; junceellin; iNOS

1. Introduction

Gorgonian corals belonging to the genus *Junceella* (family Ellisellidae) [1–3], distributed abundantly in the coral reefs of tropical Indo-Pacific Ocean have been found to produce briarane diterpenoids, natural products of a marine origin, in abundance [4]. In our further research into the natural products of *Junceella fragilis* (Ridley 1884) (Figure 1), which was distributed extensively in the waters of Southern Taiwan, have resulted in the isolation of three new 11,20-epoxybriaranes– fragilides U–W (**1**–**3**) along with two know compounds junceellonoid D (**4**) [5] and junceellin (**5**) [6–15] (Figure 1). An anti-inflammatory assay was employed to evaluate the activity of these isolates against the release of inducible nitric oxide synthase (iNOS) from macrophage cells RAW264.7.

1

2: R = OH, 6: R = H

3

4: R1 = R2 = OH, 5: R1 = R2 = OAc

7

Junceella fragilis

Figure 1. Structures of fragilides U–W (**1**–**3**), junceellonoid D (**4**), junceellin (**5**), robustolide F (**6**), juncenolide M (= frajunolide S) (**7**), and a picture of *Junceella fragilis*.

2. Results and Discussion

Fragilide U (**1**) was isolated as an amorphous powder and displayed a sodiated pseudomolecular ion at *m/z* 589.22583 in the (+)-HRESIMS, which suggested that the molecular formula of **1** was $C_{28}H_{38}O_{12}$ (calcd. for $C_{28}H_{38}O_{12}$ + Na, 589.22555) (Ω = 10). The IR spectrum of **1** showed the presence of hydorxy (ν_{max} 3445 cm^{-1}), γ-lactone (ν_{max} 1780 cm^{-1}), and ester (ν_{max} 1733 cm^{-1}) groups. Analysis of the ^{1}H, ^{13}C NMR, and distortionless enhancement by polarization transfer (DEPT) spectra together with the molecular formula, suggested that there must be an exchangeable proton. The ^{13}C NMR spectrum (Table 1), in combination with DEPT and heteronuclear single quantum coherence (HSQC) spectra, revealed the presence of four acetoxy groups (δ_C 21.7, 21.2, 20.9, 20.8, 4 × CH_3; δ_C 170.1, 169.8, 169.4, 169.0, 4 × C), a γ-lactone moiety (δ_C 175.9), and a trisubstituted olefin (δ_C 142.0, C; 120.6, CH). Base on the ^{13}C NMR data and numbers of unsaturation, **1** was established as a diterpenoid featuring with four rings. An exocyclic epoxy group was deduced from the signals of an oxygenated quaternary carbon and an oxymethylene at δ_C 62.5 and 59.1, respectively, and further supported by the chemical shifts of oxymethylene protons at δ_H 2.85 (1H, d, *J* = 4.4 Hz) and 2.98 (1H, dd, *J* = 4.4, 1.6 Hz). Moreover, a methyl singlet (δ_H 1.09, 3H, s), a methyl doublet (δ_H 1.15, 3H, d, *J* = 7.6 Hz), a vinyl methyl (δ_H 2.00, 3H, d, *J* = 1.6 Hz), three pairs of aliphatic methylene protons (δ_H 2.04, 1H, m; 2.62, 1H, ddd, *J* = 18.4, 4.0, 2.4 Hz; 1.13, 1H, m; 2.30, 1H, m; 2.11, 1H, m; 1.81, 1H, m), two aliphatic methine protons (δ_H 2.36, 1H, dd, *J* = 5.6, 1.6 Hz; 2.33, 1H, q, *J* = 7.6 Hz), five oxymethine protons (δ_H 5.91, 1H, br s; 5.65, 1H, d, *J* = 5.6 Hz; 5.04, 1H, d, *J* = 8.8 Hz; 5.03, 1H, br s; 4.71, 1H, d, *J* = 4.8 Hz), an olefin proton (δ_H 5.67, 1H, dq, *J* = 8.8, 1.6 Hz), four acetate methyls (δ_H 2.24, 2.01, 1.99, 1.98, each 3H × s), and a hydroxy proton (δ_H 4.85, 1H, br s) were observed in the ^{1}H NMR spectrum (Table 2).

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

Position	**1** [a]	**2** [a]	**3** [b]
1	47.0, C [c]	46.1, C	49.1, C
2	72.2, CH	72.1, CH	75.4, CH
3	37.5, CH_2	37.6, CH_2	136.4, CH
4	70.6, CH	73.4, CH	128.2, CH
5	142.0, C	144.1, C	135.9, C
6	120.6, CH	58.0, CH	128.8, CH
7	76.5, CH	79.0, CH	80.0, CH
8	80.4, C	81.9, C	80.9, C
9	67.4, CH	68.9, CH	67.1, CH
10	39.9, CH	39.8, CH	39.5, CH
11	62.5, C	62.6, C	60.8, C
12	23.6, CH_2	23.5, CH_2	29.4, CH_2
13	24.2, CH_2	24.0, CH_2	25.5, CH_2
14	73.2, CH	73.5, CH	78.9, CH
15	14.6, CH_3	15.4, CH_3	16.0, CH_3
16	21.4, CH_3	121.0, CH_2	48.0, CH_2
17	42.4, CH	43.8, CH	45.2, CH
18	6.6, CH_3	7.1, CH_3	6.9, CH_3
19	175.9, C	175.2, C	174.8, C
20	59.1, CH_2	58.2, CH_2	50.4, CH_2
Acetate methyls	21.7, CH_3	21.9, CH_3	21.7, CH_3
	21.2, CH_3	21.0, CH_3	21.4, CH_3
	20.9, CH_3	21.0, CH_3	21.3, CH_3
	20.8, CH_3		
Acetate carbonyls	170.1, C	171.0, C	170.4, C
	169.8, C	170.1, C	169.7, C
	169.4, C	169.7, C	169.3, C
	169.0, C		

[a] Spectra measured at 100 MHz in $CDCl_3$. [b] Spectra measured at 150 MHz in $CDCl_3$. [c] Multiplicity deduced by distortionless enhancement by polarization transfer (DEPT) and heteronuclear single quantum coherence (HSQC) spectra.

Table 2. ^{1}H NMR data (*J* in Hz) for briaranes **1**–**3**.

Position	**1** [a]	**2** [a]	**3** [b]
2	5.03 br s	4.96 dd (2.8, 2.8)	5.63 d (6.0)
3α/β	2.04 m; 2.62 ddd (18.4, 4.0, 2.4)	1.75 m; 2.38 m	6.04 dd (17.4, 6.0)
4	5.91 br s	4.85 m	6.42 d (17.4)
6	5.67 dq (8.8, 1.6)	5.49 br s	5.66 d (3.6)
7	5.04 d (8.8)	5.15 d (2.4)	5.62 d (3.6)
9	5.65 d (5.6)	5.52 d (6.4)	4.77 d (6.6)
10	2.36 dd (5.6, 1.6)	2.31 d (6.4)	3.32 d (6.6)
12α/β	1.13 m; 2.30 m	1.15 m; 2.30 m	1.29 m; 2.18 m
13α/β	2.11 m; 1.81 m	2.14 m; 1.78 m	2.08 m; 1.85 dddd (12.0, 12.0, 3.6, 1.8)
14	4.71 d (4.8)	4.86 d (4.4)	4.94 dd (3.6, 3.0)
15	1.09 s	1.13 s	0.87 s
16a/b	2.00 d (1.6)	5.74 s; 5.97 s	4.13 d (12.0); 4.19 d (12.0)
17	2.33 q (7.6)	2.29 q (7.6)	2.36 q (7.2)
18	1.15 d (7.6)	1.22 d (7.6)	1.18 d (7.2)
20a/b	2.85 d (4.4); 2.98 dd (4.4, 1.6)	2.83 d (4.0); 2.85 br d (4.0)	2.76 d (2.4); 3.40 dd (2.4, 2.4)
OH-8	4.85 br s	4.63 br s	3.13 d (1.2)
Acetate methyls	2.24 s	2.23 s	2.27 s
	2.01 s	2.03 s	2.25 s
	1.99 s	1.99 s	2.11 s
	1.98 s		

[a] Spectra measured at 400 MHz in $CDCl_3$. [b] Spectra measured at 600 MHz in $CDCl_3$.

Coupling information in the correlation spectroscopy (COSY) analysis enabled the proton sequences from H-2/H_2-3/H-4, H-6/H-7, H-9/H-10, H_2-12/H_2-13/H-14, and H-17/H_3-18 (Figure 2), which was assembled with a heteronuclear multiple bond correlation (HMBC) experiment (Figure 2). The HMBC between protons and quaternary carbons, such as H-2, H-10, H-13β, H-14, H_3-15/C-1; H-7, H_3-16/C-5; H-9, H-10, H_3-18/C-8; H-9, H-10, H_2-12, H-13α, H_2-20/C-11; and H-17, H_3-18/C-19, permitted elucidation of the carbon skeleton of **1**. A vinyl methyl at C-5 was confirmed by the HMBC between H_3-16/C-4, C-5, C-6 and H-6/C-16; and further supporting by an allylic coupling between H-6 and H_3-16 (J = 1.6 Hz). The methyl group on C-1 (Me-15) was substantiated by the HMBC from H_3-15/C-1, C-2, C-10, C-14; and H-2, H-10, H-14/C-15. The epoxy group at C-11/20 was confirmed by the HMBC between H_2-20/C-10, C-11; and H-20a/C-12; and further supporting by a long range ^4J-^{1}H–^{1}H correlation between H-10 (δ_H 2.36) and H-20b (δ_H 2.98) (J = 1.6 Hz). A hydroxy group attaching at C-8 was to infer that an HMBC of a hydroxy proton at δ_H 4.85 to C-7, C-8, and C-9. Moreover, HMBC from the oxymethine protons at δ_H 5.03 (H-2), 5.65 (H-9), and 4.71 (H-14) to the acetate carbonyls at δ_C 169.0, 169.4, and 170.1, placed the acetoxy groups on C-2, C-9, and C-14, respectively. Ten of the 12 oxygen atoms in the molecular formula of **1** could be accounted for the presence of a γ-lactone, three esters, an epoxide, and a hydroxy group. Thus, the remaining two oxygen atoms had to be positioned at C-4 as an acetoxy group, as indicated by its ^{1}H and ^{13}C NMR chemical shifts (δ_H 5.91, 1H, br s; δ_C 70.6, CH), although no HMBC was observed from H-4 to any acetate carbonyl.

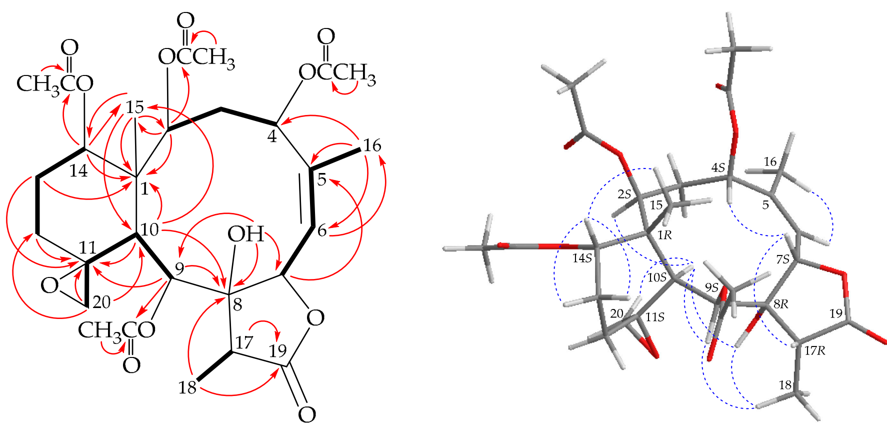

Figure 2. The correlation spectroscopy (COSY) (▬) correlations, selective heteronuclear multiple bond correlation (HMBC) experiment (⌒), and protons with key nuclear Overhauser effect spectroscopy (NOESY) (⌒) correlations of **1**.

Based on a summary of the ^{13}C chemical shifts of 11,20-epoxy group in naturally occurring briarane analogues, with ^{13}C NMR data for C-11 and C-20 at δ_C 62–63 and 58–60 ppm, the epoxide group was β-oriented and the cyclohexane ring existed in a twist boat conformation [16]; thus, the configuration of the 11,20-epoxy group in **1** should be β-oriented and the cyclohexane ring was found to be in a twist boat conformation for the ^{13}C chemical shifts at δ_C 62.5 (C-11) and 59.1 (CH_2-20). The relative stereochemistry of **1** was established by the analysis of correlations observed in a nuclear Overhauser effect spectroscopy (NOESY) experiment (Figure 2). In the NOESY spectrum of **1**, NOE correlations between H-10/H-2, H-10/H-9, and H-10/OH-8, while no correlation was seen between H-10 and H_3-15, suggesting that these protons H-2, H-9, and H-10, and the hydroxy group at C-8 were α-oriented; meanwhile, an NOE correlation of H_3-15 with H-14 indicated that H-14 was β-oriented. The NOESY spectrum showed a correlation from H-6 to H_3-16, revealing the *Z* geometry of C-5/6 double bond. H_3-18 exhibited NOE correlations to OH-8 and H-9, suggesting the α-orientation of Me-18 at C-17. H-7 displayed NOE correlations with H-4 and H-17, which further confirmed that these three protons were in β-orientation at C-7, C-4, and C-17. Based on the above findings, the relative configurations of stereogenic carbons of **1** were elucidated as 1*R**,2*S**,4*S**,7*S**, 8*R**,9*S**,10*S**,11*S**,14*S** and 17*R**. However, as briaranes **1–4** were isolated along with the known chlorinated briarane,

junceellin (**5**) [6], and the structure, including the absolute configuration of junceellin (**5**) was further confirmed by a single-crystal X-ray diffraction analysis [7,15]. It is reasonable, therefore, on biogenetic grounds to assume that briaranes **1–4** have the same absolute configuration as that of **5**. Therefore, the configurations of the stereogenic carbons of **1** should be elucidated as 1*R*,2*S*,4*S*,7*S*,8*R*,9*S*,10*S*,11*S*,14*S* and 17*R* (Supplementary Materials, Figures S1–S8).

Our present study has also led to the isolation of a new briarane, fragilide V (**2**). The molecular formula of $C_{26}H_{35}ClO_{11}$ was deduced from (+)-HRESIMS at *m/z* 581.17589 (calcd. for $C_{26}H_{35}{}^{35}ClO_{11}$ + Na, 581.17601). Carbonyl resonances in the ^{13}C NMR spectrum of **2** (Table 1) at δ_C 175.2, 171.0, 170.1, and 169.7 revealed the presence of a γ-lactone and three esters. In the ^{1}H NMR spectrum of **2** (Table 2), the signals for three acetate methyls were observed at δ_H 2.23, 2.03, and 1.99. It was found that the 1D (Tables 1 and 2) and 2D NMR (Figure 3) data of **2** were similar to those of a known briarane, robustolide F (**6**) [17,18] (Figure 1), except that the signals corresponding to a hydroxy group in **2** were replaced by signals for a proton in **6**. In the NOESY spectrum, one of the C-3 methylene protons (δ_H 2.38) showed a correlation to H-7 and not with H-2, suggesting the β-orientation of this proton by modeling study and the other was assigned as H-3α (δ_H 1.75). A correlation from H-4 to H-3α, suggested that H-4 was α-oriented according to modeling analysis. Therefore, the configuration of the stereogenic carbons of **2** were elucidated as 1*R*,2*S*,4*R*,6*S*,7*R*,8*R*,9*S*,10*S*,11*S*,14*S*, and 17*R* (Figure 3) (Supplementary Materials, Figures S9–S16).

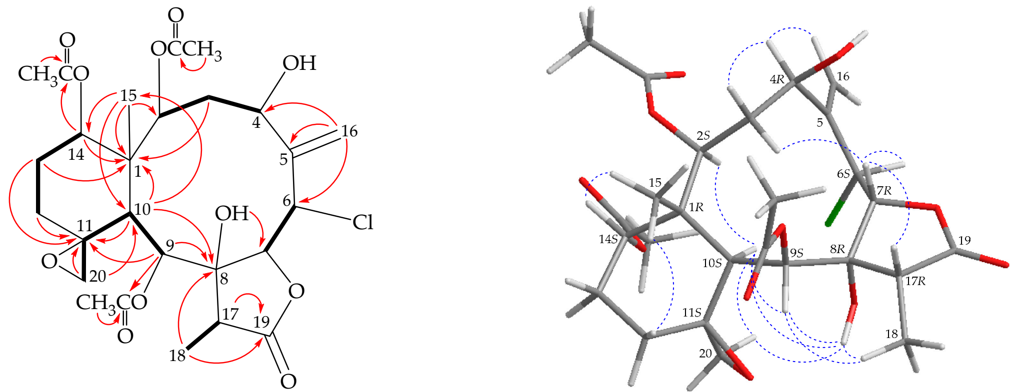

Figure 3. The COSY (▬) correlations, selective HMBC (⌒), and protons with key NOESY (⋯) correlations of **2**.

Briarane **3** (fragilide W) was found to have a molecular formula of $C_{26}H_{33}ClO_{10}$ based on its (+)-HRESIMS at *m/z* 563.16554 (calcd. for $C_{26}H_{33}{}^{35}ClO_{10}$ + Na, 563.16545). Its absorption peaks in the IR spectrum showed ester, γ-lactone, and broad OH stretching at 1738, 1777, and 3459 cm^{-1}, respectively. The ^{13}C NMR spectrum indicated that three esters and a γ-lactone were present, as carbonyl resonances were observed at δ_C 174.8, 170.4, 169.7, 169.3 (Table 1). The ^{1}H NMR spectrum indicated the presence of three acetate methyls (δ_H 2.27, 2.25, 2.11, each 3H × s) (Table 2). The ^{1}H and ^{13}C NMR spectra of **3** was found to be similar with those of a known briarane, juncenolide M (= frajunolide S) (**7**) (Figure 1), isolated from *J. juncea* and *J. fragilis* [19,20], except that the signals corresponding to the 13-acetoxy and 3(*Z*)-ene moieties in **7** were disappeared and replaced by a proton and an (*E*)-ene moieties in **3**, respectively. The locations of the functional groups were confirmed by 2D-NMR correlations (Figure 4), and hence the structure of fragilide W was assigned as **3**, and the configurations of the stereogenic carbons were elucidated as 1*R*,2*S*,7*S*,8*R*,9*S*,10*S*,11*R*, 14*S*, and 17*R* (Figure 4) (Supplementary Materials, Figures S17–S24).

The known compound **4** was found to be identical with the known junceellonoid D, on the basis of the comparison of its physical and spectroscopic data with those of reported previously [5,21] (Supplementary Materials, Figures S25–S32).

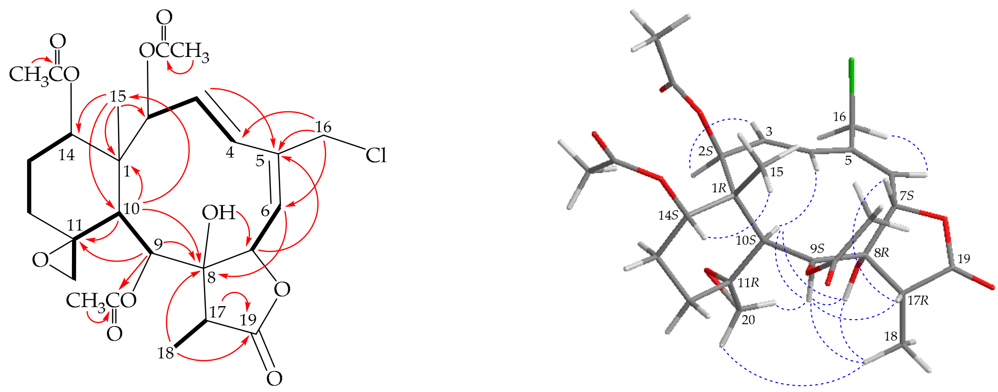

Figure 4. The COSY (▬) correlations, selective HMBC (⌒), and protons with key NOESY (⋰⋱) correlations of **3**.

Using an *in vitro* pro-inflammatory suppression assay, the activities of briaranes **1**–**5** on the release of iNOS and cyclooxygenase-2 (COX-2) protein from lipopolysaccharides (LPS)-stimulated RAW264.7 were assayed (Figure 5 and Table 3). The results showed that briaranes **3** and **5** reduced the release of iNOS to 28.55 and 33.72% at a concentration of 10 μM. Briarane **4** was found to be more weak in terms of reducing the expression of iNOS, indicating that the activity of briaranes **4** and **5** is largely dependent on the functional groups at C-2 and C-3. It is interesting to note that briarane **4** was found to enhance the expression of COX-2 to 130.88%, at a concentration of 10 μM.

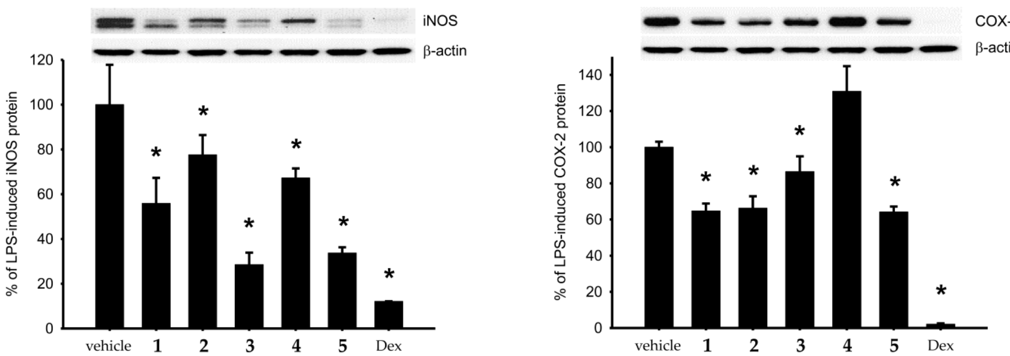

Figure 5. Activities of briaranes **1**–**5** on the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) proteins in LPS-treated murine RAW264.7 macrophage cells. Western blotting showed that briaranes **3** and **5** reduced the expression of iNOS. Data were normalized to the cells treated with LPS only, and cells treated with dexamethasone were used as a positive control. Data are expressed as the mean ± SEM ($n = 3$). * Significantly different from cells treated with LPS ($p < 0.05$).

Table 3. Effects of briaranes **1**–**5** on LPS-induced pro-inflammatory inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein expression in macrophages at a concentration of 10 μM.

	iNOS	**COX-2**	**β-Actin**
Compound	**Expression (% of LPS)**		
Lipopolysaccharides	100.01 ± 17.81	100.00 ± 3.04	100.00 ± 6.05
1	55.88 ± 11.42	64.73 ± 4.07	90.05 ± 7.11
2	77.58 ± 8.82	66.23 ± 6.59	99.29 ± 5.24
3	28.55 ± 5.35	86.46 ± 8.46	101.69 ± 6.46
4	67.25 ± 4.27	130.88 ± 13.94	103.20 ± 2.56
5	33.72 ± 2.57	64.15 ± 3.03	84.52 ± 7.78
Dexamethasone	12.11 ± 0.03	2.11 ± 0.44	123.86 ± 2.99

Data were normalized to those of cells treated with LPS alone, and cells treated with dexamethasone were used as a positive control (10 μM). Data are expressed as the mean ± SEM ($n = 3$).

3. Materials and Methods

3.1. General Experimental Procedures

Melting points were determined using a Fargo apparatus and the values were uncorrected. 1D and 2D NMR spectra were recorded on a 600 MHz Jeol NMR (model ECZ600R, Tokyo, Japan) or on a 400 MHz Jeol NMR (model ECZ400S) spectrometers using the residual $CHCl_3$ signal (δ_H 7.26 ppm) and $CDCl_3$ (δ_C 77.1 ppm) as internal standards for ^{1}H 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, HPLC, IR, and optical rotation were performed according to our earlier research [15].

3.2. Animal Material

Specimens of *J. fragilis* used for this study were collected in April 2017 by self-contained underwater breathing apparatus (SCUBA) at depths of 10–15 m off the coast of South Bay, Kenting, 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-2017-022). 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/dry weight = 795/313 g) of the coral specimen were prepared and extracted with a 1:1 mixture of methanol (MeOH) and dichloromethane (CH_2Cl_2) (1:1) to give a crude extract (19.0 g) which was partitioned between ethyl acetate (EtOAc) and H_2O. The EtOAc extract (8.0 g) was then applied to a silica gel column chromatograph (C.C.) 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 chromatographed on silica gel C.C. and eluted with gradients of *n*-hexane/EtOAc (2:1 to 1:2, stepwise) to furnish 4 fractions (fractions F1−F4). Fraction F3 was washed with a mixture of *n*-hexane/acetone (30:1) and the undissolved **5** (23.5 mg) was obtained. Fraction G was purified by normal-phase HPLC (NP-HPLC) using a mixture of *n*-hexane and EtOAc (4:1 to 1:1, stepwise) to afford 16 fractions (fractions G1−G16). Afterward, fraction G15 was separated by NP-HPLC using a mixture of CH_2Cl_2 and acetone (v:v = 10:1; at a flow rate = 2.0 mL/min) to yield **1** (1.7 mg). Fraction G14 was separated by NP-HPLC using a mixture of *n*-hexane/acetone (2:1; volume ratio) to yield 6 fractions (fractions: G14A−G14F). Fractions G14C and G14D were combined and purified by reverse-phase HPLC (RP-HPLC) using a mixture of MeOH and H_2O (v:v = 65:35; at a flow rate = 4.0 mL/min) to afford **2** (0.8 mg). Fraction G9 was purified by RP-HPLC using a mixture of MeOH and H_2O (v:v = 60:40; at a flow rate = 4.0 mL/min) to yield 16 fractions (fractions G9A−G9P), including compound **3** (0.8 mg, G9D). Fraction G9M was separated by RP-HPLC using a mixture of acetonitrile and H_2O (v:v = 55:45; at a flow rate = 4.0 mL/min) to obtain **4** (0.8 mg).

Fragilide U (**1**): Amorphous powder; $[\alpha]_D^{24}$ −12 (*c* 0.09, $CHCl_3$); IR (KBr) ν_{max} 3445, 1780, 1733 cm^{-1}; ^{13}C (100 MHz, $CDCl_3$) and ^{1}H (400 MHz, $CDCl_3$) NMR data, see Table 1; Table 2; ESIMS: *m/z* 589 $[M + Na]^+$; HRESIMS: *m/z* 589.22583 (calcd. for $C_{28}H_{38}O_{12}$ + Na, 589.22555).

Fragilide V (**2**): Amorphous powder; $[\alpha]_D^{23}$ −26 (*c* 0.04, $CHCl_3$); IR (KBr) ν_{max} 3444, 1779, 1738 cm^{-1}; ^{13}C (100 MHz, $CDCl_3$) and ^{1}H (400 MHz, $CDCl_3$) NMR data, see Table 1; Table 2; ESIMS: *m/z* 581 $[M + Na]^+$, 583 $[M + 2 + Na]^+$; HRESIMS: *m/z* 581.17589 (calcd. for $C_{26}H_{35}{}^{35}ClO_{11}$ + Na, 581.17601).

Fragilide W (**3**): Amorphous powder; $[\alpha]_D^{23}$ −21 (*c* 0.04, $CHCl_3$); IR (KBr) ν_{max} 3459, 1777, 1738 cm^{-1}; ^{13}C (150 MHz, $CDCl_3$) and ^{1}H (600 MHz, $CDCl_3$) NMR data, see Table 1; Table 2; ESIMS: *m/z* 563 $[M + Na]^+$, 565 $[M + 2 + Na]^+$; HRESIMS: *m/z* 563.16554 (calcd. for $C_{26}H_{33}{}^{35}ClO_{10}$ + Na, 563.16545).

Junceellonoid D (**4**): Amorphous powder; $[\alpha]_D^{24}$ −18 (*c* 0.04, $CHCl_3$) (ref. [5] $[\alpha]_D$ −44.8 (*c* 0.10, $CHCl_3$, MeOH); ref. [21] $[\alpha]_D^{23}$ −31 (*c* 0.05, $CHCl_3$)); IR (ATR) ν_{max} 3509, 1793, 1733 cm^{-1}; ^{13}C and ^{1}H

NMR data were found to be in full agreement with those reported previously [21]; ESIMS: *m/z* 521 $[M + Na]^+$, 523 $[M + 2 + Na]^+$; HRESIMS: *m/z* 521.15469 (calcd. for $C_{24}H_{31}{}^{35}ClO_9$ + Na, 521.15488).

Junceellin (**5**): Colorless crystals; mp 277–278 °C (ref. [15] mp 272–275 °C); $[\alpha]_D^{23}$ −3 (*c* 1.18, $CHCl_3$) (ref. [15] $[\alpha]_D^{25}$ −2 (*c* 0.89, $CHCl_3$)); IR (KBr) ν_{max} 1794, 1743 cm^{-1}; ^{13}C and ^{1}H NMR data were found to be in full agreement with those reported previously [6,8,9,12]; ESIMS: *m/z* 605 $[M + Na]^+$, 607 $[M + 2 + Na]^+$; HRESIMS: *m/z* 605.17612 (calcd. for $C_{28}H_{35}{}^{35}ClO_{11}$ + Na, 605.17601).

3.4. In Vitro Anti-Inflammatory Assay

The anti-inflammatory assay was employed to evaluate the activities of briaranes **1**–**5** reduce the release of iNOS and COX-2 from macrophage cells as the literature reported [22–25].

4. Conclusions

J. fragilis was proven to be a rich source to produce a wide structural diversity of briarane-type diterpenoids that possess various biomedical properties, particularly in anti-inflammatory activity [26,27]. In our continued study on *J. fragilis*, three previously unreported 11,20-epoxybriaranes, fragilides U–W (**1**–**3**), along with two known briaranes, junceellonoid D (**4**) and junceellin (**5**), were isolated. The exocyclic 11,20-epoxy group was proven to be a chemical marker for briarane-type natural products from the gorgonian corals belonging to the family Ellisellidae [28]. In the present study, the anti-inflammatory activity of **1**–**5** was assayed using inhibition of iNOS and COX-2 and the results indicated that fragilide W (**3**) and junceellin (**5**) showed the most potent suppressive effect on iNOS release.

Supplementary Materials: The Supplementary Materials are available online at http://www.mdpi.com/1660-3397/17/12/706/s1. HRESIMS, IR, 1D and 2D NMR spectra of compounds **1**–**4**.

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