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New Furanocembranoids from *Briareum violaceum*

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Abstract: Three new furanocembranoids—briaviodiol F (**1**) and briaviotriols A (**2**) and B (**3**)—along with a known analogue, briaviodiol A (**4**), were obtained from a cultured-type octocoral *Briareum violaceum*. The structures of cembranoids **1**–**3** were elucidated by using spectroscopic methods. In vitro study demonstrated that compounds **2** and **4** exerted inhibition effects on inducible nitric oxide synthase (iNOS) release from RAW 264.7, a macrophage cell line that originated from a mouse monocyte macrophage, stimulated with lipopolysaccharides.

Keywords: *Briareum violaceum*; briaviodiol; briaviotriol; anti-inflammatory; iNOS

1. Introduction

Briareum violaceum (Quoy and Gaimard, 1883) is a soft coral of the family Briareidae [1,2], which has been found to contain cembrane-type diterpenoids in abundance [3–10]. Diterpenoids of this type have been reported to have complicated structures and possess a variety of bioactivities [3–10]. Recently, in our research into the chemical constituents and properties of a cultured octocoral *B. violaceum*, we have isolated three previously unreported furanocembranoids— briaviodiol F (**1**), and briaviotriols A (**2**) and B (**3**)—along with a known analogue, briaviodiol A (**4**) [9] (Figure 1). A pro-inflammatory suppression assay was employed to assess the activities of these isolated compounds against the release of inducible nitric oxide synthase (iNOS) from macrophage cells.
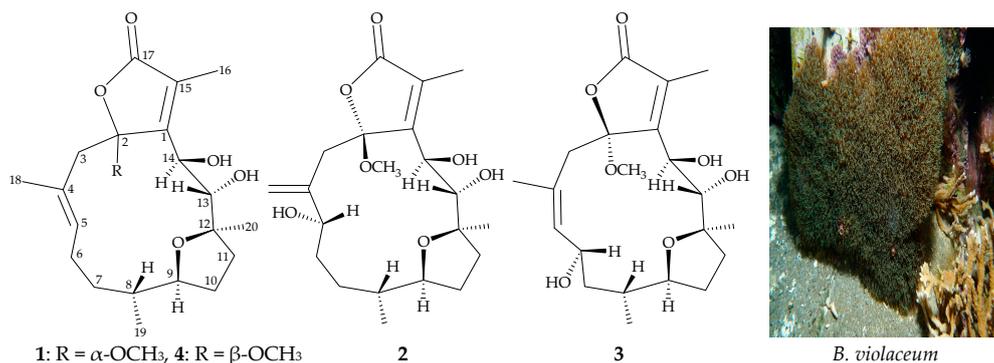

Figure 1. Structures of briaviodiol F (**1**), briaviotriols A (**2**) and B (**3**), and briaviodiol A (**4**), and a picture of the octocoral *B. violaceum*.

2. Results and Discussion

Briaviodiol F (**1**) was isolated as a colorless oil. Compound **1** displayed a pseudomolecular ion at *m/z* 403.20886 in the (+)-HRESIMS, which indicated its molecular formula was $C_{21}H_{32}O_6$ (calcd. for $C_{21}H_{32}O_6$ + Na, 403.20911), suggesting six degrees of unsaturation. Additionally, IR absorptions at 3497 and 1754 cm^{-1} indicated that **1** contained hydroxy and ester groups. As shown in Table 1, DEPT and ^{13}C NMR spectra indicated that a suite of ^{13}C resonances at δ_C 172.1 (C-17), 154.3 (C-1), 127.2 (C-15), 109.5 (C-2), and 9.2 (CH$_3$-16) were due to an α-methyl-γ-butenolide moiety by comparison with the data of known cembranoids briaviodiol A (**4**) [9] and pachyclavulariolide F [6]. Moreover, resonances at δ_C 127.1 (C-4) and 135.1 (CH-5), and the olefinic proton at δ_H 5.30 (1H, dd, *J* = 8.0, 5.6 Hz, H-5) (Table 1), indicated an additional unsaturated functionality, suggesting the presence of a trisubstituted olefin. In the HSQC spectrum, an sp^2 carbon (δ_C 135.1) correlated with the methine proton (δ_H 5.30). This proton had ^{3}*J*-correlations with H$_2$-6 (δ_H 1.93–1.99, 2H, m) in the ^{1}H–^{1}H COSY spectrum, and had ^{3}*J*-correlations with C-3 and C-18 in the HMBC spectrum (Table 1), further confirming the existence of a trisubstituted olefin. In light of the ^{1}H and ^{13}C NMR data, together with the degrees of unsaturation, **1** was determined as a tricyclic cembrane diterpene.

The ^{1}H NMR coupling information in the COSY spectrum of **1** enabled the determination of the proton sequences between H-5/H$_2$-6/H$_2$-7/H-8/H-9/H$_2$-10/H$_2$-11 and H-8/H$_3$-19 (Table 1). The carbon skeleton of **1** was elucidated based on the key HMBC from H-3β, H-13, H-14, H$_3$-16 to C-1; H$_2$-3, H-14 to C-2; H$_2$-3, H$_3$-18 to C-4; H-10β, H-11α, H-13, H-14, H$_3$-20 to C-12; H-14, H$_3$-16 to C-15; and H$_3$-16 to C-17. The presence of a vinyl methyl group on C-4 was supported by HMBC from H$_3$-18 to C-3, C-4, C-5; H-3α (δ_H 2.78) to C-18 and H-5 to C-18. Furthermore, HMBC from OH-13 to C-13, C-14 and OH-14 to C-1, C-13, C-14 suggested the existence of hydroxy groups at C-13 and C-14, respectively. Therefore, the methoxy group was on C-2, since the HMBC spectrum exhibited a correlation between the singlet at δ_H 3.39 (OMe) and C-2 (δ_C 109.5). Taking into account the molecular formula, the remaining oxygen atom must be part of the tetrahydrofuran ring located between C-9 and C-12.

Based on NOESY correlations and further information provided by MM2 forcefield calculations [11], the relative stereochemistry of **1** with the stable conformation is shown in Figure 2 (Supplementary Figures S1–10). When H-9 was α-oriented in **1**, a correlation between H-9 and H$_3$-19 was observed, suggesting that these protons were on the α-face, and H-8 was β-oriented. H-8 correlated with H-13, and the hydroxy proton OH-13 correlated with H$_3$-20, suggesting that the hydroxy group at C-13 and the Me-20 at C-12 were α-oriented. H-14 exhibited a NOESY correlation with H-13, and no coupling constant was detected between H-13 and H-14 in the ^{1}H NMR spectrum, implying that the dihedral angle located between H-13 and H-14 was about 90°, and the 14-hydroxy group was β-oriented. Correlations between H-5 and H-3, and H-14 and H-3 (δ_H 2.78) suggested that this proton is α, and the proton at δ_H 3.04 is 3β. Additionally, the proton signal of a methoxy group displayed NOESY correlations with both H-3α/β, which indicated that the methoxy group at C-2 was α-oriented. H$_3$-18 was found to show a NOESY correlation with H-3β, but not with H-5, and H-5 was shown to be correlated with H-3α and H-14, which suggested an *E*-configuration of the C-4/5 double bond. The aforementioned results enabled establishment of the relative configuration of **1**, and therefore its stereogenic carbons were assigned as 2*R**,8*R**,9*S**,12*R**,13*S**, 14*R**.

Table 1. ^{1}H (400 MHz, CDCl$_3$) and ^{13}C (100 MHz, CDCl$_3$) NMR, COSY, HMBC data for **1**.

| Position	δ_H (*J* in Hz)	δ_C, type	COSY	HMBC
1		154.3, C		
2		109.5, C		
3α/β	2.78 d (14.0); 3.04 d (14.0)	44.8, CH$_2$		C-1, C-2, C-4, C-5, C-18
4		127.1, C		
5	5.30 dd (8.0, 5.6)	135.1, CH	H$_2$-6	C-3, C-18
6	1.93–1.99 m	26.3, CH$_2$	H-5, H$_2$-7	C-4, C-5, C-7, C-8
7α/β	1.80 m; 1.26 m	34.9, CH$_2$	H$_2$-6, H-8	C-5, C-6, C-8, C-9, C-19
8	0.68 m	41.4, CH	H$_2$-7, H-9, H$_3$-19	C-9
9	3.68 ddd (9.6, 9.6, 6.0)	85.2, CH	H-8, H$_2$-10	C-7
10α/β	1.51 m; 1.98 m	30.3, CH$_2$	H-9, H$_2$-11	C-11, C-12
11α/β	1.56 m; 2.38 dd (12.0, 6.4)	36.9, CH$_2$	H$_2$-10	C-9, C-10, C-12, C-13, C-20
12		84.1, C		
13	3.60 d (10.0)	70.7, CH	OH-13	C-1, C-11, C-12, C-20
14	5.06 s	63.9, CH	-	C-1, C-2, C-12, C-15
15		127.2, C		
16	2.11 s	9.2, CH$_3$		C-1, C-15, C-17
17		172.1, C		
18	1.44 s	15.3, CH$_3$		C-3, C-4, C-5
19	0.78 d (6.4)	17.3, CH$_3$	H-8	C-7, C-8, C-9
20	1.30 s	20.9, CH$_3$		C-11, C-12, C-13
OMe-2	3.39 s	51.0, CH$_3$		C-2
OH-13	3.14 d (10.0)		H-13	C-13, C-14
OH-14	3.34 s		-	C-1, C-13, C-14
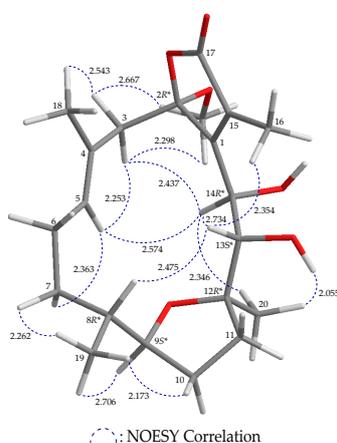
Figure 2. Computer-depicted model drawing of **1** and calculated distances (unit = Å) between protons with main NOESY correlations.

Briaviotriol A (**2**) was found to have the molecular formula $C_{21}H_{32}O_7$, as established by (+)-HRESIMS at *m/z* 419.20377 (calcd. for $C_{21}H_{32}O_7$ + Na, 419.20402). The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**. Comparison between the ^{1}H and ^{13}C NMR data of **2** (Table 2) and those of **1** suggested that the double bond is located between C-4 and C-18 in **2** instead of C-4 and C-15 in **1**. HMBC from H_2-18 to C-3, C-4, C-5; and from H_2-3 and H-5 to C-18, corroborated the existence of an exocyclic double bond at C-4. In the HSQC spectrum, an oxymethine carbon (δ_C 69.1) correlated with the methine proton (δ_H 4.57), and this proton had ^3J-correlations with H_2-6 (δ_H 1.81, 1H, m and 1.93, 1H, m) in the ^{1}H–^{1}H COSY spectrum, demonstrating that a hydroxy group was attached to C-5.

Table 2. ^{1}H (400 MHz, CDCl$_3$) and ^{13}C (100 MHz, CDCl$_3$) NMR, COSY, HMBC data for **2**.

Position	δ_H (*J* in Hz)	δ_C, type	COSY	HMBC
1		157.9, C		
2		107.8, C		
3α/β	2.67 d (14.4); 3.15 d (14.4)	42.2, CH$_2$		C-1, C-2, C-4, C-5, C-18
4		146.4, C		
5	4.57 dd (7.2, 6.0)	69.1, CH	H$_2$-6	C-4, C-6, C-18
6/6′	1.81m; 1.93 m	32.2, CH$_2$	H-5, H$_2$-7	-
7/7′	1.33 m; 1.89 ddd (14.4, 4.8, 4.4)	30.6, CH$_2$	H$_2$-6, H-8	C-5
8	1.53 m	37.4, CH	H$_2$-7, H-9, H$_3$-19	-
9	3.65 ddd (8.4, 8.4, 6.0)	85.4, CH	H-8, H$_2$-10	-
10/10′	1.52 m; 2.10 m	31.5, CH$_2$	H-9, H$_2$-11	-
11/11′	1.65 m; 2.16 m	37.2, CH$_2$	H$_2$-10	C-9, C-10, C-12, C-13, C-20
12		84.4, C		
13	3.50 d (5.6)	75.2, CH	OH-13	-
14	5.18 br s	67.7, CH	OH-14	-
15		130.0, C		
16	2.11 s	10.1, CH$_3$		C-1, C-15, C-17
17		171.1, C		
18a/b	5.13 s; 5.30 s	115.6, CH$_2$		C-3, C-4, C-5
19	0.85 d (6.4)	16.7, CH$_3$	H-8	C-7, C-8, C-9
20	1.29 s	21.8, CH$_3$		C-11, C-12, C-13
OMe-2	3.26 s	50.7, CH$_3$		C-2
OH-13	2.42 br d (5.6)		H-13	-
OH-14	2.29 br d (4.4)		H-14	-

The stereochemistry of **2** was established from the correlations observed in the NOESY spectrum (Figure 3 and Supplementary Figures S2–10). In addition, in the NOESY spectrum H-9 was correlated with H$_3$-19, which suggested that these protons were positioned on the same face and were assigned as α protons, as H-8 was β-oriented. H-13 correlated with H-8 and H-14, but no coupling between H-13 and H-14 was observed, demonstrating that the hydroxy groups at C-13 and C-14 were α- and β-oriented, respectively. Correlations between an oxygen-bearing methyl (δ_H 3.26) and H-13 suggested that the C-2 methoxy group was situated on the β face. Additionally, correlation between H-5 and H-8 supported a β-orientation of H-5. Based on the aforementioned results, the relative configurations of the stereogenic carbons of **2** were determined as 2*S**,5*S**,8*R**, 9*S**,12*R**,13*S**,14*R**.
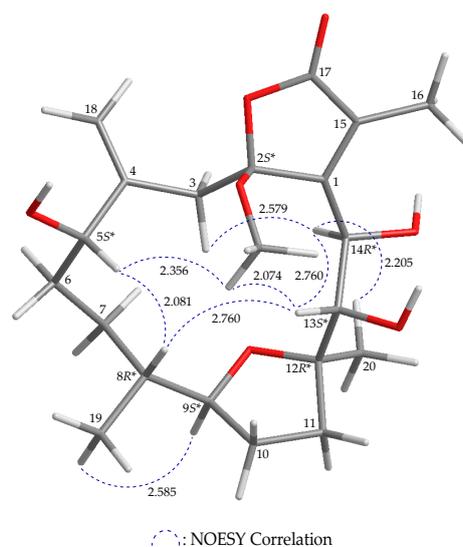
: NOESY Correlation

Figure 3. Computer-depicted model drawing of **2** and calculated distances (unit = Å) between protons with main NOESY correlations.

Compound **3** has a molecular formula $C_{21}H_{32}O_7$ according to its (+)-HRESIMS *m/z* 419.20399 (calcd. for $C_{21}H_{32}O_8$ + Na, 419.20402). The ^1H and ^{13}C NMR features of **3** resemble those of **1**; comparison of the ^1H and ^{13}C NMR chemical shifts of the sp^2 methine proton and its respective carbon (δ_H 5.29, 1H, d, *J* = 8.4 Hz; δ_C 133.5, CH-5), and the sp^2 quaternary carbon (δ_C 131.0, C-4) of **3** (Table 3) with those of **1** (δ_H 5.30, 1H, dd, *J* = 8.0, 5.6 Hz; δ_C 135.1, CH-5; δ_C 127.1, C-4) (Table 1), as well as a NOESY correlation between H-5 and H_3-18, indicated the *Z*-configuration of the C-4/5 double bond (Figure 4 and Supplementary Figure S3–10). Furthermore, the HSQC spectrum showed that an oxymethine carbon (δ_C 68.7) was correlated with a methine proton (δ_H 4.86; H-6), and this proton exhibited ^3J-correlations with the olefinic proton H-5 (δ_H 5.29) and H_2-7 (δ_H 1.34, 1H, m; 1.80, 1H, m) in the COSY spectrum, which confirmed a hydroxy group at C-6. As H-6 showed a NOESY correlation with H-3β, this suggested that the C-6 hydroxy group was α-oriented. Based on a NOESY experiment (Figure 4 and Supplementary Figures S3–10), **3** was identified to have the stereogenic centers 2R*,6S*,8R*,9S*,12R*,13S*,14R*. Since **3** has never been previously reported, it was named briaviotriol B.

Table 3. ^1H (400 MHz, CDCl$_3$) and ^{13}C (100 MHz, CDCl$_3$) NMR, and COSY, HMBC data for **3**.

| Position	δ_H (*J* in Hz)	δ_C, type	COSY	HMBC
1		159.8, C		
2		107.7, C		
3α/β	1.76 d (14.4); 3.56 d (14.4)	39.6, CH$_2$		C-1, C-2, C-4, C-5, C-18
4		131.0, C		
5	5.29 d (8.4)	133.5, CH	H-6	C-3, C-18
6	4.86 ddd (12.0, 8.4, 3.2)	68.7, CH	H-5, H$_2$-7	-
7/7′	1.34 m; 1.80 m	44.9, CH$_2$	H-6, H-8	C-5, C-6, C-8, C-9, C-19
8	1.21 m	35.3, CH	H$_2$-7, H-9, H$_3$-19	-
9	3.65 ddd (10.0, 10.0, 4.4)	87.3, CH	H-8, H$_2$-10	-
10/10′	1.38 m; 2.05 m	32.8, CH$_2$	H-9, H$_2$-11	C-9, C-11, C-12
11/11′	1.61 m; 2.22 dd (13.2, 8.0)	35.5, CH$_2$	H$_2$-10	C-9, C-12, C-13, C-20
12		85.1, C		
13	3.40 d (8.4)	76.7, CH	OH-13	C-1, C-12, C-20
14	4.75 d (5.2)	63.5, CH	OH-14	C-1, C-2, C-12, C-13, C-15
15		128.5, C		
16	2.08 s	9.6, CH$_3$		C-1, C-15, C-17
17		171.9, C		
18	1.83 s	24.0, CH$_3$		C-3, C-4, C-5
19	0.84 d (6.8)	20.1, CH$_3$	H-8	C-7, C-8, C-9
20	1.28 s	21.1, CH$_3$		C-11, C-12, C-13
OMe-2	3.20 s	51.1, CH$_3$		C-2
OH-13	3.05 d (8.4)		H-13	C-13, C-14
OH-14	2.70 d (5.2)		H-14	C-13, C-14
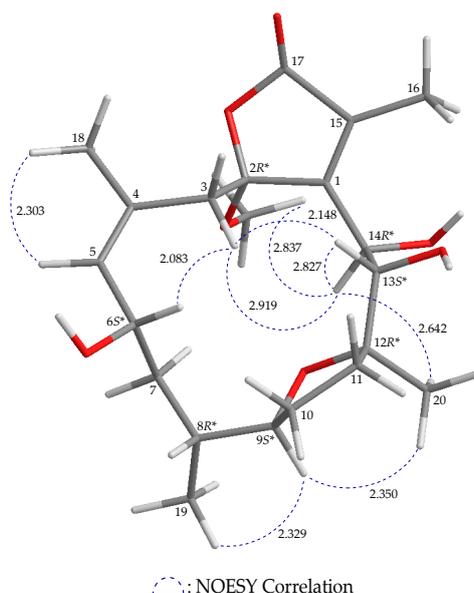
Figure 4. Computer-depicted model drawing of **3** and calculated distances (unit = Å) between protons with main NOESY correlations.

Compound **4** was identified as briaviodiol A (Figure 1), by comparison of its ^{1}H and ^{13}C NMR data with those in the literature [9].

Using an in vitro pro-inflammatory suppression assay, the effects of **1–4** on the release of iNOS protein from lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells were assessed. First, alamar blue cell viability assessment revealed that **1–4** did not have significant cytotoxic effects in RAW 264.7 cells. The results of the in vitro pro-inflammatory suppression assay showed that **2** and **4** at 10 μM suppressed the release of iNOS to 67.7 and 61.9%, respectively, when compared with results of the cells stimulated with only LPS (Table 4). Compound **1** showed no suppression effect on iNOS release.

Table 4. Effects of **1–4** on LPS-induced pro-inflammatory iNOS release in RAW 264.7 cells at a concentration of 10 μM. The data presented are the relative intensity normalized to the LPS- stimulated group. Compounds **2** and **4** were found to have the higher inhibition effects on LPS- induced iNOS expression in macrophages expression.

	iNOS
	Expression (% of LPS)
LPS	100.0 ± 7.0
1	109.0 ± 19.2
2	67.7 ± 2.4
3	79.5 ± 9.4
4	61.9 ± 7.3

3. Experimental Section

3.1. General Experimental Procedures

The JEOL NMR spectrometer (model ECZ400S, Tokyo, Japan) was used to record the spectra with the solvent peak of CHCl$_3$ (δ_H 7.26 ppm) and CDCl$_3$ (δ_C 77.1 ppm) as internal references for ^{1}H NMR 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, IR spectra and optical rotation were performed according to our earlier research [10].

3.2. Animal Material

Specimens of *B. violaceum* used for this study were collected in December 2016 from the cultivation tank (capacity = 270 tons) at the National Museum of Marine Biology and Aquarium (NMMBA) in Southern Taiwan. For its identification, this coral species was compared to reliable sources published earlier [1,2]. A voucher specimen was deposited in the NMMBA (voucher no.: NMMBA-CSC-005).

3.3. Extraction and Isolation

Sliced bodies (wet/dry weight = 358.7/144.5 g) of the coral specimen were prepared and extracted with a 1:1 mixture of MeOH and CH_2Cl_2 to give 17.2 g of crude extract which was partitioned between EtOAc and H_2O to obtain 6.3 g of the EtOAc extract. The EtOAc extract was then applied onto a silica gel column and eluted with gradients of *n*-hexane/EtOAc (100% *n*-hexane−100% EtOAc, stepwise), to furnish 14 fractions (fractions: A−N). Fraction G was further chromatographed on a silica gel column and eluted with gradients of *n*-hexane/Me_2CO (20:1−100% Me_2CO, stepwise) to afford 11 subfractions (fractions: G1−G11). Fraction G4 was applied onto a silica gel column and eluted with gradients of *n*-hexane and Me_2CO (20:1−100% Me_2CO, stepwise) to give 12 subfractions (fractions: G4A−G4L). Afterwards, fraction G4E was then separated by normal-phase HPLC (NP-HPLC) using a mixture of *n*-hexane and Me_2CO (5:1) as solvent to obtain 5 subfractions (fractions: G4E1−G4E5). Then, fraction G4E1 was separated by NP-HPLC using a mixture of CH_2Cl_2 and Me_2CO (with volume: volume = 80:1; at a flow rate = 3.0 mL/min) to afford **1** (62.7 mg). Fraction G4H was separated by NP-HPLC using a mixture of *n*-hexane and Me_2CO (with volume: volume = 4:1; at a flow rate = 2.0 mL/min) to afford **4** (17.0 mg). Fraction G4J was repurified by NP-HPLC using a mixture of *n*-hexane and Me_2CO (with volume: volume = 3:1; at a flow rate = 2.0 mL/min) to afford **3** (0.9 mg). Fraction G8 was separated by NP-HPLC using a mixture of *n*-hexane and Me_2CO (3:1) to obtain 6 subfractions G8A−G8F. Fraction G8F was repurified by reverse-phase HPLC (RP-HPLC) using a mixture of MeCN and H_2O (with volume: volume = 1:1; at a flow rate = 1.0 mL/min) to yield **2** (1.2 mg).

Briaviodiol F (**1**): Colorless oil; $[\alpha]_D^{21}$ +223 (*c* 1.48, CHCl$_3$); IR (neat) ν_{max} 3497, 1754 cm^{-1}; ^{1}H and ^{13}C NMR data (see Table 1); ESIMS: *m/z* 403 [M + Na]$^+$; HRESIMS: *m/z* 403.20886 (calcd. for $C_{21}H_{32}O_6$ + Na, 403.20911).

Briaviotriol A (**2**): Colorless oil; $[\alpha]_D^{22}$ −68 (*c* 0.06, CHCl$_3$); IR (neat) ν_{max} 3424, 1749 cm^{-1}; ^{1}H and ^{13}C NMR data (see Table 2); ESIMS: *m/z* 419 [M + Na]$^+$; HRESIMS: *m/z* 419.20377 (calcd. for $C_{21}H_{32}O_7$ + Na, 419.20402).

Briaviotriol B (**3**): Colorless oil; $[\alpha]_D^{23}$ −39 (*c* 0.04, CHCl$_3$); IR (neat) ν_{max} 3424, 1749 cm^{-1}; ^{1}H and ^{13}C NMR data (see Table 3); ESIMS: *m/z* 419 [M + Na]$^+$; HRESIMS: *m/z* 419.20399 (calcd. for $C_{21}H_{32}O_7$ + Na, 419.20402).

Briaviodiol A (**4**): Colorless crystal; $[\alpha]_D^{21}$ −52 (*c* 0.85, CHCl$_3$) (Reference [9] $[\alpha]_D^{23}$ −31 (*c* 0.14, CHCl$_3$)); IR (neat) ν_{max} 3467, 1747 cm^{-1}; ^{1}H and ^{13}C NMR data were found to be in absolute agreement with previous study [9]; ESIMS: *m/z* 403 [M + Na]$^+$.

3.4. Molecular Mechanics Calculations

The molecular models were generated by implementing the MM2 force field [11] in ChemBio 3D Ultra software (ver. 12.0) which was created by CambridgeSoft (PerkinElmer, Cambridge, MA, USA).

3.5. In Vitro Anti-Inflammatory Assay

The pro-inflammatory suppression assay was performed using a murine macrophage cell line, RAW 264.7, which was purchased from the American Type Culture Collection (ATCC cell line no. TIB-71; Manassas, VA, USA). Untreated or LPS-induced RAW 264.7 cells were used to determine the anti-inflammatory activities of cembranoids **1–4** by assessing the inhibition of pro-inflammatory iNOS release from macrophage cells. The iNOS protein levels were measured by using western blotting

analysis [12–14]. Briefly, in the control group, macrophages were incubated in compound-free medium with LPS (10 μM) alone for 16 h; and in the cembranoid-treated groups, the cells were pre-treated with cembranoids **1**–**4** (10 μM) for 10 min followed by an LPS challenge for 16 h. After the incubation, cell lysates were collected, and equal amounts of the total protein samples were subjected to western blot analysis. The immunoreactivities were caculated based on the optical densities of the corresponding iNOS bands of each group on the membrane, and the cells with LPS treatment alone were set to be 100%. Viability of macrophage cells of different groups was determined after treatment with alamar blue (Invitrogen, Carlsbad, CA, USA), a chemical of tetrazolium dye that is reduced by living cells to a fluorescent substance. The assay has been shown to have accurate measurement in determining the survival of RAW 264.7 cells [15,16], which is based on a mechanism similar to that of an assay using 3-(4,5-dimethyldiazol-2-yl)-2,5- diphenyltetrazolium bromide. Data analyses were firstly performed using one-way analysis of variance (ANOVA), and further analyzed by the Student-Newman-Keuls post hoc test for multiple comparison. All the data with a *p*-value of < 0.05 were considered as a significant difference.

4. Conclusions

B. violaceum has been demonstrated to have a wide structural diversity of interesting diterpenoids that possess various pharmacological properties [17]. This specimen was encrusted on different species of scleractinian hard corals in the Indo-Pacific coral reef system [18]. In our continued study of *B. violaceum*, three previously unreported furanocembranoids **1**–**3** were isolated, together with the previously described briaviodiol A (**4**). In the present study, the anti- inflammatory activities of **1**–**4** were assessed using inhibition of pro-inflammatory iNOS release from macrophages. The results indicated that briaviotriol A (**2**) and briaviodiol A (**4**) showed the most potent suppressive effects on iNOS release.

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References

1. Samimi-Namin, K.; van Ofwegen, L.P. Overview of the genus *Briareum* (Cnidaria, Octocorallia, Briareidae) in the Indo-Pacific, with the description of a new species. *ZooKeys* **2016**, *557*, 1–44. [CrossRef] [PubMed]
2. Bayer, F.M. Key to the genera of octocorallia exclusive of Pennatulacea (Coelenterata: Anthozoa), with diagnoses of new taxa. *Proc. Biol. Soc. Wash.* **1981**, *94*, 902–947.
3. Bowden, B.F.; Coll, J.C.; Mitchell, S.J.; Raston, C.L.; Stokie, G.J.; White, A.H. Studies of Australian soft corals XV. The structure of pachyclavulariadiol, a novel furano-diterpene from *Pachyclavularia violacea*. *Aust. J. Chem.* **1979**, *32*, 2265–2274. [CrossRef]
4. Inman, W.; Crews, P. The structure and conformational properties of a cembranolide diterpene from *Clavularia violacea*. *J. Org. Chem.* **1989**, *54*, 2526–2529. [CrossRef]
5. Sheu, J.-H.; Wang, G.-H.; Sung, P.-J.; Duh, C.-Y.; Chiang, M.Y. Pachyclavulariolides G–L and seco-pachyclavulariaenone A, seven novel diterpenoids from the soft coral *Pachyclavularia violacea*. *Tetrahedron* **2001**, *57*, 7639–7648. [CrossRef]
6. Xu, L.; Patrick, B.O.; Roberge, M.; Allen, T.; van Ofwegen, L.; Andersen, R.J. New diterpenoids from the octocoral *Pachyclavularia violacea* collected in Papua New Guinea. *Tetrahedron* **2000**, *56*, 9031–9037. [CrossRef]

7. Sheu, J.-H.; Wang, G.-H.; Duh, C.-Y.; Soong, K. Pachyclavulariolides M–R, six novel diterpenoids from a Taiwanese soft coral *Pachyclavularia violacea*. *J. Nat. Prod.* **2003**, *66*, 662–666. [CrossRef] [PubMed]

8. Duh, C.-Y.; El-Gamal, A.A.H.; Chu, C.-J.; Wang, S.-K.; Dai, C.-F. New cytotoxic constituents from the Formosan soft corals *Clavularia viridis* and *Clavularia violacea*. *J. Nat. Prod.* **2002**, *65*, 1535–1539. [CrossRef] [PubMed]

9. Chang, Y.-C.; Huang, I.-C.; Chiang, M.Y.-N.; Hwang, T.-L.; Kung, T.-H.; Lin, C.-S.; Sheu, J.-H.; Sung, P.-J. Briaviodiol A, a new cembranoid from a soft coral *Briareum violacea*. *Chem. Pharm. Bull.* **2010**, *58*, 1666–1668. [CrossRef] [PubMed]

10. Huang, P.-C.; Tseng, C.-C.; Peng, B.-R.; Hu, C.-C.; Lin, N.-C.; Chen, N.-F.; Chen, J.-J.; Wen, Z.-H.; Wu, Y.-C.; Sung, P.-J. Briaviodiols B–E, new anti-inflammatory hydroperoxyfurancembranoids from *Briareum violaceum*. *Tetrahedron* **2019**, *75*, 921–927. [CrossRef]

11. Allinger, N.L. Conformational analysis. 130. MM2. A hydrocarbon force field utilizing V_1 and V_2 torsional terms. *J. Am. Chem. Soc.* **1977**, *99*, 8127–8134. [CrossRef]

12. Huang, S.-Y.; Chen, N.-F.; Chen, W.-F.; Hung, H.-C.; Lee, H.-P.; Lin, Y.-Y.; Wang, H.-M.; Sung, P.-J.; Sheu, J.-H.; Wen, Z.-H. Sinularin from indigenous soft coral attenuates nociceptive responses and spinal neuroinflammation in carrageenan-induced inflammatory rat model. *Mar. Drugs* **2012**, *10*, 1899–1919. [CrossRef] [PubMed]

13. Jean, Y.-H.; Chen, W.-F.; Sung, C.-S.; Duh, C.-Y.; Huang, S.-Y.; Lin, C.-S.; Tai, M.-H.; Tzeng, S.-F.; Wen, Z.-H. Capnellene, a natural marine compound derived from soft coral, attenuates chronic constriction injury-induced neuropathic in rats. *Br. J. Pharmacol.* **2009**, *158*, 713–725. [CrossRef] [PubMed]

14. Jean, Y.-H.; Chen, W.-F.; Duh, C.-Y.; Huang, S.-Y.; Hsu, C.-H.; Lin, C.-S.; Sung, C.-S.; Chen, I.-M.; Wen, Z.-H. Inducible nitric oxide synthase and cyclooxygenase-2 participate in anti-inflammatory and analgesic effects of the natural marine compound lemnalol from Formosan soft coral *Lemnalia cervicorni*. *Eur. J. Pharmacol.* **2008**, *578*, 323–331. [CrossRef] [PubMed]

15. Chen, L.-C.; Lin, Y.-Y.; Jean, Y.-H.; Lu, Y.; Chen, W.-F.; Yang, S.-N.; Wang, H.-M.D.; Jang, I.-Y.; Chen, I.-M.; Su, J.-H.; et al. Anti-inflammatory and analgesic effects of the marine-derived compound comaparvin isolated from the crinoid *Comanthus bennetti*. *Molecules* **2014**, *19*, 14667–14686. [CrossRef] [PubMed]

16. Oliveira, T.; Figueiredo, C.A.; Brito, C.; Stavroullakis, A.; Prakki, A.; da Silva Velozo, E.; Nogueira-Filho, G. Effect of *Allium cepa* L. on lipopolysaccharide-stimulated osteoclast precursor cell viability, count, and morphology using 4′,6-diamidino-2-phenylindole-staining. *Int. J. Cell Biol.* **2014**, *2014*, 535789. [CrossRef] [PubMed]

17. Chang, Y.-C.; Sheu, J.-H.; Wu, Y.-C.; Sung, P.-J. Terpenoids from octocorals of the genus *Pachyclavularia*. *Mar. Drugs* **2017**, *15*, 382. [CrossRef] [PubMed]

18. Geetha, S.; Kumar, J.S.Y.; Raghunathan, C.; Sornaraj, R. Space competition studies between *Briareum violaceum* (Octocorallia; Alcyonacea) and scleractinian corals in Shark Island, North Andaman, India. *Indian J. Geo-Mar. Sci.* **2018**, *47*, 2390–2394.