

Article

8-Hydroxybriaranes from Octocoral *Briareum stechei* (Briareidae) (Kükenthal, 1908)

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Abstract: Chemical investigation of the octocoral *Briareum stechei*, collected in the Ie Island, Okinawa, Japan, resulted in the isolation of a new briarane-type diterpenoid, briastecholide A (**1**), as well as the previously reported metabolites, solenolide C (**2**) and briarenolide S (**3**). The structures of briaranes **1–3** were characterized through spectroscopic analysis, and the absolute configuration of **2** was corroborated by a single-crystal X-ray diffraction analysis. Briarane **3** exhibited bioactivity against the protein expression of inducible nitric oxide synthase (iNOS).

Keywords: *Briareum stechei*; briarane; briastecholide; solenolide; anti-inflammation; iNOS

1. Introduction

Octocorals of the genus *Briareum* (family Briareidae), flourishing in the tropical and subtropical Indo-Pacific Ocean, are proven to be the most important sources to yield the 3,8-cyclized cembranoids (briarane), and compounds of this type were found to possess complex structures and extensive bioactivities, especially in anti-inflammatory activity [1,2]. *Briareum stechei* (Kükenthal, 1908) [3], one of the widely studied species of octocoral, is considered a rich source of briarane-type compound. In continuation of our interest in the comparative chemistry of *Briareum* species collected at diverse geographical locations, we obtained a specimen of *B. stechei* from Ie Island, Okinawa, Japan [3,4]. We report herein the isolation, structure determination and antiinflammatory activity of one new (briastecholide A (**1**)), one revised (solenolide C (**2**)) [5,6] and one known (briarenolide S (**3**)) [7] briarane (Figure 1).

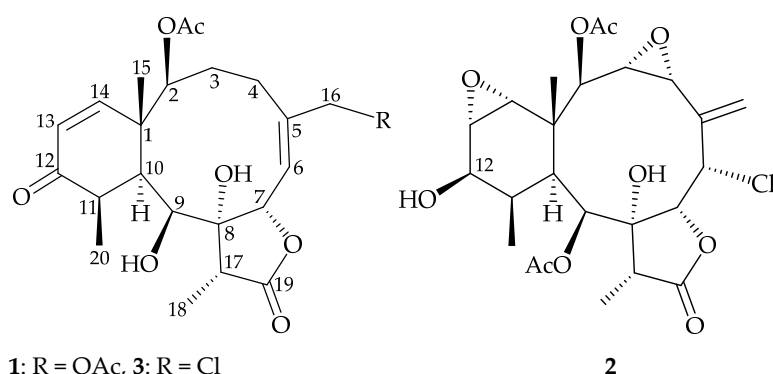


Figure 1. Structures of briastecholide A (1), solenolide C (2) and briarenolide S (3).

2. Results and Discussion

Freshly collected *B. stechei* was frozen and subsequently freeze-dried, powdered and extracted with a mixture of methanol/dichloromethane (MeOH/CH₂Cl₂) at a 1:1 ratio to produce an extract that was separated by ethyl acetate (EtOAc)–water partitioning. The EtOAc layer was collected and loaded onto a column chromatograph with silica gel, and separated using high performance liquid chromatography (HPLC), yielding briaranes 1–3.

Solenolide C (2) was first isolated from octocoral *Solenopodium* sp. [5], and the stereochemistry of C-12 in this compound was revised in a later study by NMR data analysis [6]. The structure of 2 was determined directly in this study for the first time by a single-crystal X-ray analysis, and the Oak Ridge Thermal Ellipsoid Plot (ORTEP) diagram (Figure 2) showed that the absolute configurations of stereogenic carbons of 2 are 1*R*,2*R*,3*R*,4*R*,6*S*,7*R*,8*R*,9*S*,10*S*,11*R*,12*R*,13*S*,14*R* and 17*R*. Analyzing the X-ray structure of 2 confirmed the β-orientation of the 12-hydroxy group, rather than an α-orientation, as stated in a previous study [5] (Supplementary Materials, Figures S12–S16).

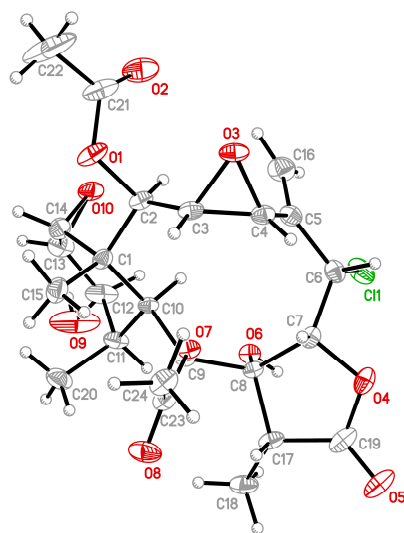


Figure 2. Crystal structure and absolute configuration of solenolide C (2) by X-ray diffraction.

Briastecholide A (1) was obtained as an amorphous powder. The positive mode high resolution electrospray ionization mass spectrum [(+)-HRESIMS] showed a peak at m/z 487.19356, suggesting a molecular formula C₂₄H₃₂O₉ (calcd. for C₂₄H₃₂O₉ + Na, 487.19385) (nine degrees of unsaturation). The IR spectrum indicated the presence of hydroxy (ν_{\max} 3462 cm⁻¹), γ-lactone (ν_{\max} 1771 cm⁻¹), ester carbonyl (ν_{\max} 1740 cm⁻¹) and α,β-unsaturated ketonic (ν_{\max} 1675 cm⁻¹) groups. The ¹³C NMR spectrum of 1 (Table 1) showed signals of 24 carbons. The multiplicity of carbon signals was determined from distortionless

enhancement by polarization transfer (DEPT) and heteronuclear single quantum coherence (HSQC) spectra: five methyls, three methylenes (one bearing a heteroatom), nine methines (three bearing a heteroatom and three olefins) and seven non-protonated carbons (four carbonyls, one olefin and one bearing a heteroatom). From ^{13}C and ^1H NMR spectra (Table 1), **1** was found to possess a γ -lactone (δ_{C} 177.6, C-19), two acetoxy (δ_{H} 2.29, 2.05, each $3\text{H} \times \text{s}$; δ_{C} 20.8, $2 \times$ acetate methyls; δ_{C} 170.6, 167.7, $2 \times$ acetate carbonyls), an α,β -unsaturated ketonic (δ_{H} 6.29, 1H, d, $J = 10.4$ Hz, H-14; 5.98, 1H, d, $J = 10.4$ Hz, H-13; δ_{C} 202.3, ketonic carbonyl, C-12; 154.0, CH-14; 126.3, CH-13) and an trisubstituted olefin (δ_{H} 5.73, 1H, d, $J = 9.6$ Hz, H-6; δ_{C} 140.5, C-5; 124.4, CH-6) moiety. Six double bonds accounted for six unsaturated degrees. The remaining three degrees of unsaturation defined **1** as a tricyclic molecule.

Table 1. ^1H and ^{13}C NMR data for briarane **1**.

Position.	δ_{H} ^a (J in Hz)	δ_{C} ^b , Mult.
1		45.1, C ^c
2	4.91 d (9.6)	81.3, CH
3 α / β	1.66 dd (15.6, 7.2); 2.35 m	24.3, CH ₂
4 α / β	1.96 m; 2.43 m	20.8, CH ₂
5		140.5, C
6	5.73 d (9.6)	124.4, CH
7	5.27 d (9.6)	76.1, CH
8		84.8, C
9	3.75 dd (9.2, 7.2)	69.3, CH
10	2.95 dd (7.2, 4.0)	38.7, CH
11	2.98 qd (7.2, 4.0)	44.4, CH
12		202.3, C
13	5.98 d (10.4)	126.3, CH
14	6.29 d (10.4)	154.0, CH
15	1.44 s	20.1, CH ₃
16	4.46 br s	67.1, CH ₂
17	3.43 q (7.2)	41.9, CH
18	1.21 d (7.2)	6.4, CH ₃
19		177.6, C
20	1.25 d (7.2)	14.5, CH ₃
OH-8	3.24 s	
OH-9	4.54 d (9.2)	
OAc-2		167.7, C
	2.29 s	20.8, CH ₃
OAc-16		170.6, C
	2.05 s	20.8, CH ₃

^a 400 MHz, CDCl₃, ^b 100 MHz, CDCl₃, ^c multiplicity deduced by ^{13}C and DEPT spectra.

The H-2/H₂-3/H₂-4, H-6/H-7, H-9/H-10/H-11/H₃-20, H-13/H-14 and H-17/H₃-18 spin systems, measured in the ^1H - ^1H correlation spectroscopy (COSY) (Figure 3), were fit to the regiochemistry of vicinal proton couplings in **1**. The tricyclic network was established by heteronuclear multiple bond coherence (HMBC) experiments, especially by 2J - and 3J - ^1H - ^{13}C long-range correlations between protons and non-protonated carbons, such as H-2, H-9, H-10, H-13, H-14, H₃-15/C-1; H₂-3, H₂-4, H-7, H₂-16/C-5; H-9, H-10, H-17, H₃-18/C-8; H-11, H-14, H₃-20/C-12; and H-17, H₃-18/C-19, thus permitting the elucidation of the carbon skeleton of **1** (Figure 3). Methyl groups Me-15, Me-18 and Me-20 at C-1, C-17 and C-11 were confirmed by the HMBC correlations between H₃-15/C-1, C-2, C-10, C-14; H₃-18/C-8, C-17 and C-19 and H₃-20/C-10, C-11 and C-12, respectively. Acetate esters at C-2 and C-16 were established from the correlations between oxymethine proton H-2 (δ_{H} 4.91) and oxymethylene protons H₂-16 (δ_{H} 4.46) and acetate carbonyls resonating at δ_{C} 167.7 and 170.6, respectively, observed in the HMBC spectrum of **1**. The hydroxy proton signal at δ_{H} 4.54 (1H, d, $J = 9.2$ Hz, OH-9) was revealed by its ^1H - ^1H COSY and HMBC correlations to H-9 (δ_{H} 3.75, 1H, dd, $J = 9.2, 7.2$ Hz) and δ_{C} 69.3 (CH-9), respectively, indicating its attachment to C-9. Eight of the nine oxygen atoms in the molecular formula

of **1** could be accounted for by the presence of a γ -lactone, two esters, an α,β -unsaturated ketonic group and a hydroxy group. Thus, the remaining oxygen atom had to be positioned at C-8, an oxygen-bearing, non-protonated carbon, as a hydroxy group, as indicated by its ^{13}C NMR chemical shifts resonating at δ_{C} 84.8. Based on the above findings, the planar structure of **1** was established.

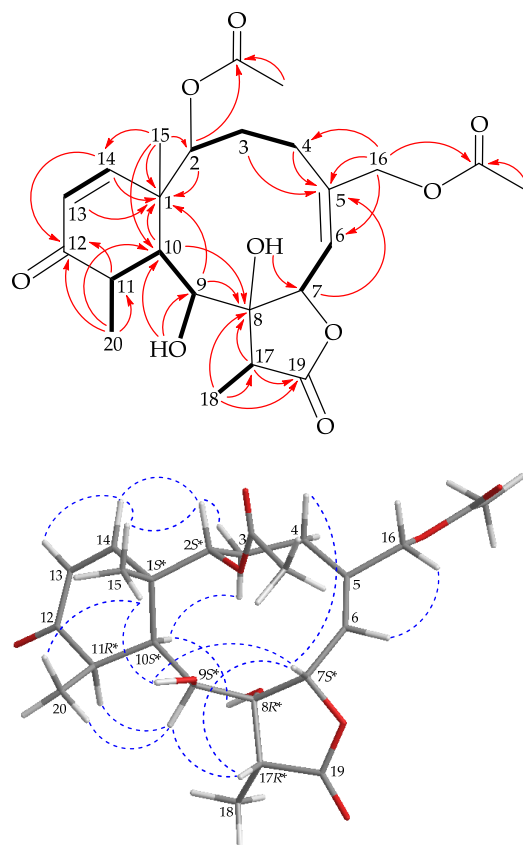


Figure 3. Key COSY (—), HMBC (---) and protons with NOESY (⋯) correlations of **1**.

The stereochemical evaluation of **1** was established using a nuclear Overhauser effect spectroscopy (NOESY) experiment (Figure 3). In naturally occurring briaranes, proton H-10 and Me-15 at C-1 are α - and β -oriented, respectively. In the NOESY experiment, H₃-15 correlated with OH-9 and H₃-20, while OH-9 correlated with H-7, indicating that these protons were situated on the same face, and were assigned as β protons. A NOESY correlation observed between H-7 and H-17 reflected that H-17 and 8-hydroxy groups were β - and α -oriented in the γ -lactone moiety, respectively, by modeling analysis. The *cis* geometry of the C-13/14 double bond was indicated by a 10.4 Hz coupling constant between H-13 (δ_{H} 5.98) and H-14 (δ_{H} 6.29), and further confirmed by a NOESY correlation between these two olefin protons. Furthermore, H-2 showed NOESY correlations with H-14 and H₃-15, demonstrating the *S*^{*}-configuration of the stereogenic center C-2. H-6 exhibited a cross peak with H₂-16, but not with H-7, and a large coupling constant ($J = 9.6$ Hz) was detected between H-6 and H-7, indicating that the dihedral angle between H-6 and H-7 was approximately 180° , and the *E*-geometry of C-5/6 double bond was elucidated. It was found that the NMR data of **1** were similar to those of a known briarane, briarenolide S (**3**) [7] (Figure 1), which was also obtained in this study, except that the signals corresponding to the 16-chlorine group in **3** were replaced by those of an acetoxy group in **1**. Therefore, briastecholide A (**1**) was assigned as having a structure with the same relative stereochemistry as briarenolide S (**3**) because of the stereogenic carbons that **1** has in common with **3**, and the configurations of the stereogenic centers of **1** were elucidated as (1*S*^{*},2*S*^{*},7*S*^{*},8*R*^{*},9*S*^{*},10*S*^{*},11*R*^{*} and 17*R*^{*}) (Supplementary Materials, Figures S1–S11).

Briarane **3** was obtained as an amorphous powder. The (+)-ESIMS mass showed a pair of peaks at m/z 463/465 ($[M + Na]^+ / [M + 2 + Na]^+$) (3:1), with a relative intensity suggestive of a chlorine atom, and was found to have the molecular formula $C_{22}H_{29}ClO_7$ by analysis of ^{13}C and 1H NMR data. The result revealed that this compound had eight degrees of unsaturation. Strong bands at 3459, 1753 and 1735 cm^{-1} in the IR spectrum indicated the presence of hydroxy, γ -lactone and ester groups. The ^{13}C NMR and DEPT spectra revealed that **3** had 22 carbons, including four methyls, three sp^3 methylenes, six sp^3 methines, three sp^2 methines, two sp^3 non-protonated carbons and four sp^2 non-protonated carbons. Therefore, **3** was identified as having briarane. It was found that the spectroscopic data of **3** were identical to those of a known briarane, briarenolide S [7], and these two compounds possessed negative optical rotation values, suggesting that compound **3** is briarenolide S (Supplementary Materials, Figures S17–S21).

The effects of briaranes **1–3** on the release of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) from lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells were assessed (Table 2). Briarane **3** at $10\ \mu\text{M}$ suppressed the release of iNOS to $66.5 \pm 3.4\%$, as compared to the results of the cells stimulated with LPS only, and by comparison of the activities of **1** with those of **3**, it was found that **3** was more active in terms of reducing the expression of iNOS, indicating that the activity of **1** and **3** is largely dependent on the functional group at C-16.

Table 2. Suppression effects of cembranoids **1–3** on iNOS and COX-2 protein/enzyme expressions in LPS-induced macrophages.

Compound/Treatment (10 μM)	iNOS	COX-2 Production Level	β -Actin	<i>n</i>
Control	2.2 ± 0.9	1.0 ± 0.1	106.1 ± 4.2	4
Vehicle	100.0 ± 4.3	100.0 ± 2.6	100.0 ± 0.7	4
1	88.3 ± 0.3	101.8 ± 3.1	100.4 ± 5.3	4
2	88.9 ± 2.9	94.7 ± 3.2	101.1 ± 4.4	4
3	66.5 ± 3.4	112.3 ± 5.8	99.3 ± 4.4	4
Dexamethasone	54.5 ± 3.6	17.7 ± 1.8	103.1 ± 2.5	4

Values of cells treated with LPS alone were set to 100% as the reference for normalization. Dexamethasone at $10\ \mu\text{M}$ was used as a positive reference to treat cells. Experimental results are shown as the mean \pm S.E.M.

3. Materials and Methods

3.1. General Experimental Procedures

For IR spectra, a Nicolet iS5 FT-IR spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used. Optical rotation values were measured using a Jasco P-1010 digital polarimeter (Jasco, Tokyo, Japan). NMR spectra were measured with a Jeol ECZ 400 MHz spectrometer (Jeol, Tokyo, Japan). ESIMS and HRESIMS analyses were conducted using the Bruker 7 Tesla solarix FTMS system (Bruker, Bremen, Germany). Column chromatography was carried out with silica gel (230–400 mesh, Merck, Darmstadt, Germany). Thin layer chromatography (TLC) was performed on plates precoated with Kieselgel 60 F₂₅₄ (0.25 mm, Merck), then sprayed with 10% H_2SO_4 solution followed by heating to visualize the spots. Normal-phase high performance liquid chromatography (NP-HPLC) was performed using a system comprising a pump (L-7110, Hitachi, Tokyo, Japan), an injection port (Rheodyne 7725i; Rohnert Park, CA, USA) and a semi-preparative normal-phase column (YMC-Pack SIL, $250 \times 20\text{ mm}$, $5\ \mu\text{m}$; Sigma-Aldrich, St. Louis, MO, USA). Reverse-phase HPLC (RP-HPLC) was performed using a system comprising a Hitachi L-2130 pump, a Hitachi L-2455 photodiode array detector, a Rheodyne 7725i injection port and a semi-preparative reverse-phase column (Luna, $5\ \mu\text{m}$, C18(2) 100 \AA , AXIA, $250 \times 21.2\text{ mm}$; Phenomenex, Torrance, CA, USA).

3.2. Animal Material

Specimens of *Briareum stechei* used for this study were collected in the Ie Island, Okinawa, Japan (N26.44.21.8, E127.48.33.8) in 2019. The coral specimen was identified as *Briareum stechei* (Kükenthal, 1908) based on its morphology and micrographs of the coral sclerites [3,4]. The samples were then stored at $-20\text{ }^{\circ}\text{C}$ until extraction.

3.3. Extraction and Isolation

Freeze-dried and sliced bodies (wet/dry weight = 618/305 g) of the coral specimen were extracted at room temperature with MeOH/CH₂Cl₂ (1:1). The extract was concentrated under reduced pressure to produce 42.7 g crude extract, which was partitioned between EtOAc and H₂O. The EtOAc layer (15.1 g) was applied to column chromatography on silica gel and eluted with gradients of hexanes/EtOAc. By TLC monitoring and by using sulfuric acid spray reagent, 11 fractions A–K were obtained. Fraction F was separated by NP-HPLC using a mixture of *n*-hexane/EtOAc (3:2) to yield fractions F1–F10. Fraction F8 was re-purified by RP-HPLC using a mixture of MeOH/H₂O (60:40) (flow rate = 5.0 mL/min) to afford **3** (1.5 mg). Purification of fraction G was performed by NP-HPLC using a mixture of *n*-hexane/acetone (3:1) to yield fractions G1–G10. Fraction G7 and G8 were re-purified by RP-HPLC using a mixture of MeOH/H₂O (60:40 for G7, 55:45 for G8; flow rate = 5 mL/min) to afford **1** (1.2 mg) and **2** (6.0 mg), respectively.

Briastecholide A (**1**): Amorphous powder; $[\alpha]_{\text{D}}^{22} -30$ (c 0.1, CHCl₃); IR (KBr) ν_{max} 3462, 1771, 1740, 1675 cm⁻¹; ¹H (400 MHz, CDCl₃) and ¹³C (100 MHz, CDCl₃) NMR data, see Table 1; ESIMS: *m/z* 487 [M + Na]⁺; HRESIMS: *m/z* 487.19356 (calcd. for C₂₄H₃₂O₉ + Na, 487.19385).

Solenolide C (**2**): Colorless prisms; $[\alpha]_{\text{D}}^{20} -40$ (c 0.3, MeOH) ([5] $[\alpha]_{\text{D}}^{20} -25$ (c 0.76, MeOH)); IR (ATR) ν_{max} 3538, 1764, 1741 cm⁻¹; the ¹H and ¹³C NMR data of **2** are in full agreement with those reported previously [5]; ESIMS: *m/z* 537 [M + Na]⁺, 539 [M + 2 + Na]⁺.

Briarenolide S (**3**): Amorphous powder; $[\alpha]_{\text{D}}^{20} -8$ (c 0.1, CHCl₃) ([7] $[\alpha]_{\text{D}}^{20} -4$ (c 0.2, CHCl₃)); IR (ATR) ν_{max} 3459, 1753, 1735 cm⁻¹; the ¹H and ¹³C NMR data of **3** are in full agreement with those reported previously [7]; ESIMS: *m/z* 463 [M + Na]⁺, 465 [M + 2 + Na]⁺.

3.4. Single-Crystal X-ray Crystallography of Solenolide C (2)

The structures of **2** were determined by single-crystal X-ray diffraction on two dual source equipped Bruker D8 Venture diffractometers. Solenolide C (**2**) was crystallized from methanol as colorless prisms in the monoclinic crystal system in the *P*2₁ (#4) space group—the crystal (0.211 × 0.174 × 0.163 mm³) with *a* = 10.07780(10) Å, *b* = 14.4322(2) Å, *c* = 19.5756(2) Å, *V* = 2761.45(6) Å³, *Z* = 2, *D*_{calcd} = 1.277 Mg/m³, λ (Mo Kα) = 0.71073 Å. All 33,970 reflections were collected. The structure was solved by direct methods and refined by a full-matrix least-squares procedure. The refined structural model converged to a final *R*1 = 0.0556; *wR*2 = 0.1579 for 16054 observed reflections [*I* > 2σ(*I*)] and 671 variable parameters. Absolute configuration could be determined reliably for compound **2** with Flack's parameter of 0.02(2). Full crystallographic data can be retrieved from the CIF (crystallographic information files) file CCDC (Cambridge Crystallographic Data Centre) 2056551 containing the supplementary crystallographic data for this paper [8]. These data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk/structures/ (accessed on 16 January 2021).

3.5. In Vitro Inflammatory Assay

Pro-inflammatory proteins inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in macrophages were induced by incubating them for 16 h in a medium containing LPS (0.01 µg/mL) without compounds. For the anti-inflammatory activity assay, compounds or positive control (dexamethasone) were added to the cells 5 min before the lipopolysaccharides (LPS) administrate. After exposure to the compounds or dexamethasone, the macrophages were washed with ice-cold phosphate-buffered saline (PBS), lysed in ice-cold lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, 100 µg/mL

phenylmethylsulfonyl fluoride and 1 µg/mL aprotinin) and centrifuged at 20,000 × *g* for 30 min at 4 °C. The supernatants were decanted and reserved for Western blotting. Protein concentrations were measured using a protein assay kit (Bio-Rad, Hercules, CA, USA). The method of Western blotting was similar to that in our previous study [8]. Anti-β-actin antibody was obtained from Sigma Chemical (St. Louis, MO, USA). Anti-iNOS and anti-COX-2 antibodies were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Horseradish peroxidase-conjugated secondary antibodies were obtained from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). The images of Western blotting were obtained using the UVP BioChem Imaging System (UVP, Upland, CA, USA). Relative densitometric quantification of the Western blotting band was performed using LabWorks 4.0 software (UVP LLC, Upland, CA, USA). The intensity of the LPS only group was set at 100%. The β-actin was used as the loading/internal control.

4. Conclusions

A new briarane, briastecholide A (**1**), and two known briarane analogues, solenolide C (**2**) and briarenolide S (**3**), were identified from octocoral *B. stechei*, collected in Ie Island, Okinawa, Japan, where the Kuroshio current and South China Sea surface current converge to provide high biodiversity. Briaranes **1** and **3** were isolated along with **2** from the same target organism, *B. stechei*, and the absolute configuration of **2** was determined by X-ray diffraction analysis. Therefore, it is biogenetically reasonable to conclude that **1** and **3** have the same absolute configuration as **2**, and the configurations of the stereogenic centers of **1** were elucidated as 1*S*,2*S*,7*S*,8*R*,9*S*,10*S*,11*R* and 17*R*. Briarane **3** displayed reducing effects on the production of iNOS at a concentration of 10 µM.

Supplementary Materials: Supplementary Materials are available online at <http://www.mdpi.com/1660-3397/18/x/xxx/s1>: ESIMS, IR, 1D (¹H and ¹³C) and DEPT NMR spectra of briaranes **1–3**; HRESIMS and 2D (HSQC, HMBC, COSY and NOESY) NMR spectra of **1**.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Chen, Y.-H.; Chin, H.-K.; Peng, B.-R.; Chen, Y.-Y.; Hu, C.-C.; Zheng, L.-G.; Huynh, T.-H.; Su, T.-P.; Zhang, Y.-L.; Wen, Z.-H.; et al. Survey of briarane-type diterpenoids—Part VII. *Heterocycles* **2020**, *100*, 857–870.
2. Wei, W.-C.; Sung, P.-J.; Duh, C.-Y.; Chen, B.-W.; Sheu, J.-H.; Yang, N.-S. Anti-inflammatory activities of natural products isolated from soft corals of Taiwan between 2008 and 2012. *Mar. Drugs* **2013**, *11*, 4083–4126. [[CrossRef](#)] [[PubMed](#)]
3. 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](#)]

4. Miyazaki, Y.; Reimer, J.D. Morphological and genetic diversity of *Briareum* (Anthozoa: Octocorallia) from the Ryukyu Archipelago, Japan. *Zool. Sci.* **2014**, *31*, 692–702. [[CrossRef](#)] [[PubMed](#)]
5. Groweiss, A.; Look, S.A.; Fenical, W. Solenolides, new antiinflammatory and antiviral diterpenoids from a marine octocoral of the genus *Solenopodium*. *J. Org. Chem.* **1988**, *53*, 2401–2406. [[CrossRef](#)]
6. Kwak, J.H.; Schmitz, F.J.; Williams, G.C. Miolides, new briarane diterpenoids from the Western Pacific octocoral *Briareum stechei*. *J. Nat. Prod.* **2001**, *64*, 754–760. [[CrossRef](#)] [[PubMed](#)]
7. Su, Y.-D.; Wen, Z.-H.; Wu, Y.-C.; Fang, L.-S.; Chen, Y.-H.; Chang, Y.-C.; Sheu, J.-H.; Sung, P.-J. Briarenolides M–T, new briarane diterpenoids from a Formosan octocoral *Briareum* sp. *Tetrahedron* **2016**, *72*, 944–951. [[CrossRef](#)]
8. Chen, C.-H.; Chen, N.-F.; Feng, C.-W.; Cheng, S.-Y.; Hung, H.-C.; Tsui, K.-H.; Hsu, C.-H.; Sung, P.-J.; Chen, W.-F.; Wen, Z.-H. A coral-derived compound improves functional recovery after spinal cord injury through its antiapoptotic and anti-inflammatory effects. *Mar. Drugs* **2016**, *14*, 160. [[CrossRef](#)] [[PubMed](#)]