HETEROCYCLES, Vol. 104, No. 8, 2022, pp. 1423 - 1434. © 2022 The Japan Institute of Heterocyclic Chemistry
Received, 10th May, 2022, Accepted, 7th June, 2022, Published online, 10th June, 2022
DOI: 10.3987/COM-22-14680

BRIARANE-TYPE DITERPENOIDS FROM THE CULTURED OCTOCORAL *BRIAREUM STECHEI* (KÜKENTHAL, 1908)

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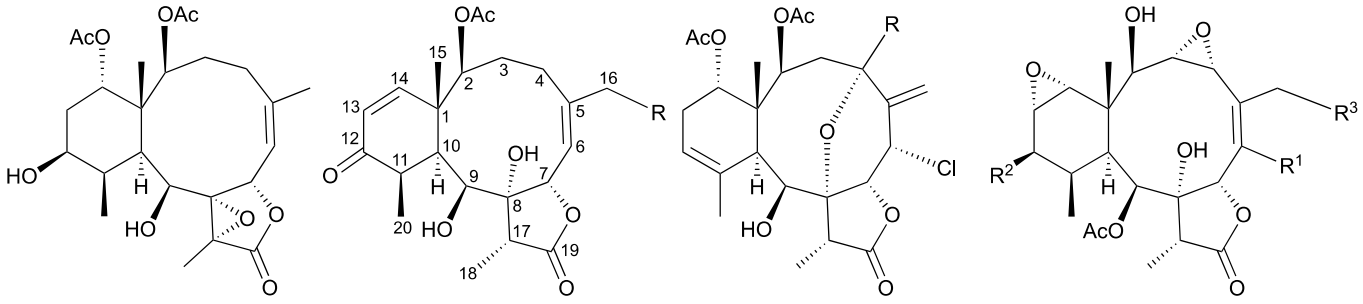
Abstract – A known briarane, excavatolide E (**1**), along with three previously unreported briaranes, briastecholides D–F (**2**–**4**), were purified from an aquacultured form of octocoral *Briareum stechei*. Spectroscopic approaches were first used to reveal the structures of newly-identified briaranes **2**–**4**; single-crystal X-ray analysis was then performed to identify the absolute configuration of **1**. To characterize their biological functions, an anti-inflammatory assay using

macrophage cell line RAW264.7 as an *in vitro* model was employed. The results of the assay showed that briarane **1** prevented the protein production of pro-inflammatory inducible nitric oxide synthase (iNOS) in macrophages caused by lipopolysaccharide stimulation.

INTRODUCTION

Soft corals are invertebrates extensively distributed in coastal waters, especially the coasts of equatorial Indo-West Pacific Asia regions. The creatures have been shown to be rich sources of an array of diterpenoid derivatives with uncommon or unique carbon skeletons that have various medicinal and bioactive properties.[1–6] The genus *Briareum* (Blainville, 1834, phylum: Cnidaria, class: Anthozoa, subclass: Octocorallia, order: Alcyonacea, suborder: Scleraxonia, family: Briareidae),[7–10] distributed in the Indo-Pacific Ocean, is comprised of four characterized species, *B. cylindrum*, *B. hamrum*, *B. stechei*, and *B. violaceum*,[10] and different groups of diterpenoids, including briarane (3,8-cyclized cembranoid) and eunicellin (2,11-cyclized cembranoid) diterpenoids,[11–13] have been obtained from these fascinating marine invertebrates of potential medicinal use.

We have conducted extensive investigations in previous years on cultured *Briareum stechei* (Kükenthal, 1908), from which a known briarane, excavatolide E (**1**),[14,15] and three new analogues, briastecholides D–F (**2**–**4**), were isolated (Figure 1). In this article, we summarized the processes of purification and structural identification of **1**–**4**, and their activities against inflammation.



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2: R = OH, **5**: R = Cl, **6**: R= OAc

3: R = OH, **7**: R = H

4: R^1 = OH, R^2 = $OC(O)(CH_2)_2Me$, R^3 = Cl
8: R^1 = H, R^2 = R^3 = OAc

Figure 1. Structures of excavatolide E (**1**), briastecholides D–F (**2**–**4**), briarenolide S (**5**), briastecholide A (**6**), excavatolide A (**7**), and briarenolide K (**8**)

RESULTS AND DISCUSSION

Excavatolide E (**1**) was a compound originally prepared from Taiwanese octocoral *Briareum excavatum*,[14] and its structure was revealed by spectroscopic approaches and X-ray crystal diffraction.[14,15] The previous study established the absolute configuration of excavatolide E by Cu Kα type X-ray diffraction and an Oak

Ridge Thermal Ellipsoid Plot (ORTEP). In the current study, compound **1** was verified by single-crystal X-ray diffraction with Mo Kα radiation to obtain an additional ORTPE result (Figure 2), which showed the configurations of stereocarbons of **1** to be (1*S*,2*S*,7*S*,8*R*,9*S*,10*S*,11*R*,12*S*,14*S*,17*R*),

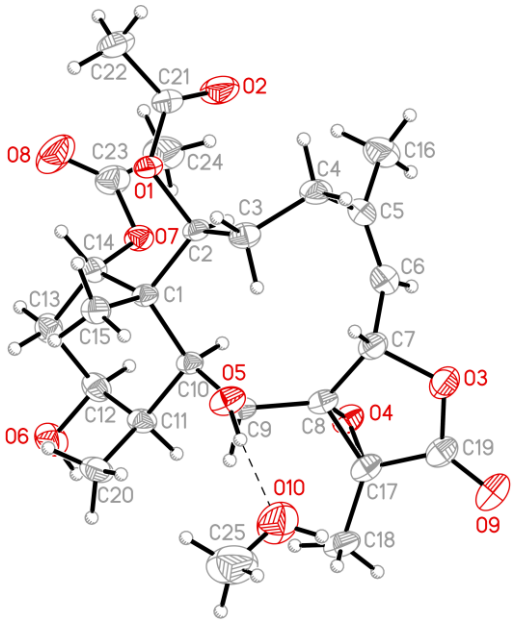


Figure 2. Computer-generated ORTEP diagram of excavatolide E (**1**)

Briastecholide D (**2**) was obtained as colorless oil and had the molecular formula $C_{22}H_{30}O_8$ (8° of unsaturation) as determined by ESIMS, with a sodiated molecular ion peak at *m/z* 445; the finding was further confirmed by identification of a peak at *m/z* 445.18353 (calcd for $C_{22}H_{30}O_8$ + Na, 445.18329) from HRESIMS. From the ^{1}H and ^{13}C NMR spectra (Table 1), HSQC, and HMBC spectra, **2** was found to have a structure that included an α,β-unsaturated ketone (δ_C 203.8, C-12; δ_H 5.97, 1H, d, J = 10.2 Hz/δ_C 126.0, CH-13; δ_H 6.31, 1H, d, J = 10.2 Hz/δ_C 154.7, CH-14), a γ-lactone (δ_C 177.6, C-19), an acetoxy group (δ_C 167.5, acetate carbonyl; δ_H 2.30, 3H, s/δ_C 20.8, acetate methyl), and one trisubstituted olefin (δ_C 145.5, C-5; δ_H 5.76, 1H, d, J = 9.6 Hz/δ_C 120.9, CH-6). Based on the aforementioned unsaturation data, it was determined that five double bonds represented five unsaturated degrees, and **2** was identified as a tricyclic molecule based on the remaining three degrees of unsaturation.

From ^{1}H–^{1}H COSY analysis of **2**, the spin systems of H-2/H_2-3/H_2-4, H-6/H-7, H-9/H-10/H-11/H_3-20, H-13/H-14, and H-17/H_3-18 were differentiated (Figure 3a). According to this information, in combination with data from HMBC correlations between H-7/C-5 and H-14/C-12 (Figure 3a), the major carbon skeleton of **2** was determined. Methyl groups Me-15, Me-20, and Me-18 at C-1, C-11, and C-17 were established from HMBC correlations between H_3-15/C-1, C-2, C-10, C-14; H_3-20/C-10, C-11, C-12; and H_3-18/C-8, C-17, C-19, respectively. An acetate ester at C-2 was identified based on results showing a correlation between the oxymethine proton H-2 (δ_H 4.91) and an acetate carbonyl resonating at δ_C 167.5, detected

Table 1. ^{1}H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR data for isolated briaranes **2**–**4**

	2		**3**		**4**	
Position	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)	δ_C, type
1		45.1, C[a]		44.2, C[a]		39.0, C[a]
2	4.91 d (10.2)	81.2, CH	5.07 d (6.6)	72.5, CH	3.30 d (9.0)	75.9, CH
3	2.36 m	20.4, CH_2	1.53 m	40.9, CH_2	3.44 dd (9.0, 4.2)	60.9, CH
3′	2.00 dd (13.8, 13.8)		3.62 dd (16.2, 6.6)			
4/4′	1.57 m; 2.41 m	24.6, CH_2		96.8, C	4.14 d (4.2)	58.5, CH
5		145.5, C		138.4, C		134.8, C
6	5.76 d (9.6)	120.9, CH	5.54 ddd (2.4, 2.4, 2.4)	55.9, CH		---[d]
7	5.30 d (9.6)	76.3, CH	4.67 d (2.4)	79.8, CH	5.42 s	74.4, CH
8		84.4, C		82.1, C		82.9, C
9	3.76 dd (9.6, 7.2)	69.5, CH	4.98 d (5.4)	76.3, CH	5.33 d (7.8)	68.8, CH
10	2.98 d (7.2)[b]	38.6, CH	2.94 br s	41.2, CH	1.74 dd (7.8, 3.0)	36.6, CH
11	2.98 m[b]	44.6, CH		130.8, C	2.28 m	36.4, CH
12		203.8, C	5.65 br s[c]	123.1, CH	4.68 d (4.8)	71.4, CH
13/13′	5.97 d (10.2)	126.0, CH	2.05 m; 2.40 m	28.3, CH_2	3.21 d (3.0)	57.2, CH
14	6.31 d (10.2)	154.7, CH	5.09 d (4.2)	71.8, CH	3.30 d (3.0)	61.9, CH
15	1.45 s	19.9, Me	1.19 s	14.4, Me	1.20 s	15.1, Me
16a/b	4.05 s; 4.05 s	65.9, CH_2	5.65 d (2.4);[c] 5.90 d (2.4)	116.8, CH_2	4.32 d (12.6); 4.64 d (12.6)	60.2, CH_2
17	3.42 q (7.2)	41.8, CH	2.59 q (7.2)	50.8, CH	2.36 q (7.2)	43.1, CH
18	1.23 d (7.2)	6.3, Me	1.32 d (7.2)	7.6, Me	1.21 d (7.2)	6.3, Me
19		177.6, C		174.7, C		173.9, C
20	1.26 d (7.2)	14.3, Me	1.89 s	24.3, Me	1.05 d (7.2)	9.6, Me
4-OH			6.68 d (1.2)			
9-OH	4.52 d (9.2)		2.14 d (5.4)			
2-OAc		167.5, C		173.3, C		
	2.30 s	20.8, Me	2.11 s	21.2, Me		
9-OAc						169.0, C
					2.23 s	21.7, Me
14-OAc				170.1, C		
			2.02 s	20.9, Me		
12-*n*-OC(O)Pr						172.5, C
					2.35 t (7.2)	36.1, CH_2
					1.69 sext (7.2)	18.1, CH_2
					0.97 t (7.2)	13.6, Me

[a] Multiplicity deduced from HSQC and HMBC experiments. [b,c] Signals overlapped. [d] not observed.

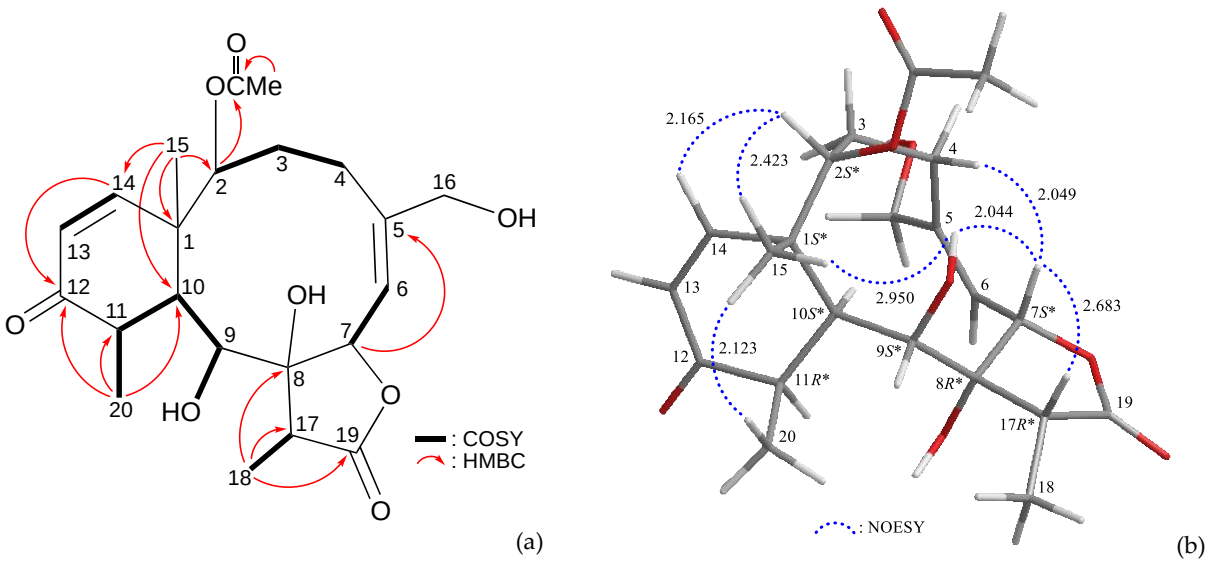


Figure 3. (a) Key COSY and HMBC correlations and (b) stereoview of **2** and calculated distances (Å) between particular protons that have crucial NOESY correlations

through analysis of the HMBC spectrum. Additionally, the hydroxy proton signal at δ_H 4.52 (1H, d, J = 9.6 Hz) was identified based on its correlation with H-9 (δ_H 3.76, 1H, dd, J = 9.6, 7.2 Hz) from ^{1}H–^{1}H COSY analysis. This suggested its connection with C-9. Among the eight oxygen atoms of the compound, six can be interpreted through the existence of one γ-lactone, one ester, one α,β-unsaturated ketone, and one hydroxy group. Hence, the remaining two oxygen atoms must be located at C-8, an oxygen-containing non-protonated carbon, and C-16, an oxymethylene group, as hydroxy groups, as designated by their ^{13}C NMR chemical shifts resonating at δ_C 84.4 and 65.9, respectively. Therefore, although a few correlations were absent in the HMBC spectrum of **2** to connect the moiety fragments, according to the integrated analysis of the aforementioned data and comparison of the NMR data between **2** and known briaranes briarenolide S (**5**)[16] and briastecholide A (**6**) (Figure 1),[17] the planar structure of **2** could be elucidated.

Stereochemical analysis of **2** was performed via a 2D NMR spectroscopic method using a NOESY experiment (Figure 3b). The naturally-occurring briaranes were found to have Me-15 in the β-orientation and proton H-10 in the α-orientation, as proved by the absence of a NOESY correlation between these two groups. Also, in the NOESY study, H_3-15 was found to be associated with OH-9 and H_3-20, while OH-9 was associated with H-7, suggesting that these protons were on the same side and enabling allocation as β protons. By modeling analysis from NOESY data, the correlation between H-7 and H-17 showed that H-17 and the 8-hydroxy group were β- and α-oriented in the γ-lactone group, respectively. The coupling constant of 10.2 Hz between H-13 (δ_H 5.97) and H-14 (δ_H 6.31) specified the *cis* geometry of the C-13/14 double bond. Additionally, H-2 showed NOESY associations with H-14 and H_3-15, revealing the *S** configuration of the stereocenter at C-2. H-7 exhibited a cross peak with one of the methylene protons (δ_H 2.41) at C-4, but not with H-6, and a greater coupling constant (J) of 9.6 Hz was noted between H-6 and H-7, showing that the planes between H-6 and H-7 have a dihedral angle of about 180° and demonstrating the *E*-geometry of the C-5/6 double bond. By comparison with NMR data of other compounds obtained in prior studies, investigation of current NMR data revealed that **2** was akin to known briaranes briarenolide S (**5**)[16] and briastecholide A (**6**),[17] with the exception that the signals related to the 16-chlorine group in **5** and the 16-acetoxy group in **6** were replaced by a hydroxy group in **2**. Therefore, as the stereocarbons of **2** appeared to be the same as those of **5** and **6**, briastecholide D (**2**) was considered to have the same relative stereochemical structure as **5** and **6**, and the stereocenter configuration of **2** was elucidated to be (1*S**,2*S**,7*S**,8*R**,9*S**,10*S**,11*R**,17*R**).

Briarane **3** (briastecholide E) showed the signals of its sodiated molecule isotope peaks at *m/z* 521.15494 $(M + Na)^+$ and 523.15402 $(M + 2 + Na)^+$ with a ratio of 3:1 in the HRESIMS spectrum, providing evidence of the existence of a chlorine atom in **3**, and found to possess a molecular formula of $C_{24}H_{31}ClO_9$ (calcd for $C_{24}H_{31}ClO_9$ + Na, 521.15488). The ^{13}C NMR spectrum showed the existence of two esters and one γ-lactone, as carbonyl resonances were detected at δ_C 173.3, 170.1, and 174.7 (Table 1). The methine unit at

δ_C 55.9 (CH-6) was more shielded than predicted for an oxygenated carbon and was connected to the methine proton at δ_H 5.54 (H-6) in the HSQC spectrum, and this proton signal was ^3J-correlated with H-7 (δ_H 4.67) in the ^{1}H–^{1}H COSY spectrum (Figure 4a), proving the existence of a chlorine atom attached to C-6. The spectroscopic data of **3** showed the compound to be similar to an identified briarane, excavatolide A (**7**) (Figure 1),[14] except for the existence of a hydroxy group in **3** rather than a proton at C-4 in **7**. The planar structure of **3** was finally identified by confirming the experimental HMBC and COSY correlations (Figure 4a) and comparing the NMR data between **3** and **7**. Besides, in the NOESY experiment of **3**, H-2 showed NOESY correlations with H-10 and the C-4 hydroxy proton (δ_H 6.68), revealing the *S** configuration of the stereogenic center at C-4. Hence, briastecholide E (**3**) was found to be the 4-hydroxy derivative of **7**. The stereochemistry of **3** was further concluded by NOESY analysis (Figure 4b) and comparison between the NOE data and those of fragilisinin A[18] to be (1*R**,2*S**,4*S**,6*S**,7*R**,8*R**,9*S**,10*S**, 14*S**,17*R**).

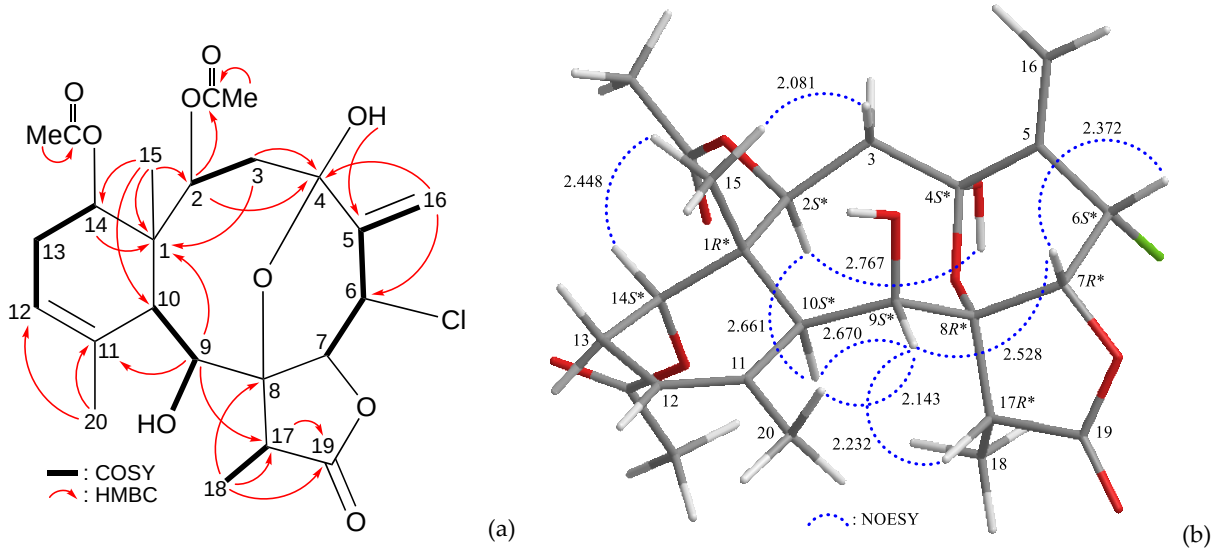


Figure 4. (a) Key COSY and HMBC correlations and (b) stereoview of **3** and calculated distances (Å) between particular protons that have crucial NOESY correlations.

ESIMS of briastecholide F (**4**) showed sodium adduct ions at *m/z* 581 and 583 with a 3:1 ratio, while the pseudo molecular ion peaks in the HRESIMS spectrum exhibited the same ratio of 3:1 at *m/z* 581.17585 $(M + Na)^+$ and 583.17373 $(M + 2 + Na)^+$, demonstrating the existence of a chlorine atom in **4**, and its molecular formula was determined as $C_{26}H_{35}ClO_{11}$ (calcd for $C_{26}H_{35}ClO_{11}$ + Na, 581.17601). Detailed inspection of ^{1}H NMR, HSQC, and HMBC spectroscopic data (Table 1 and Figure 5a) of **4** indicated the presence of an 8-hydroxybriarane skeleton with two epoxides, one acetate ester, and one *n*-butyrate ester. Although a few cross-peaks were absent in the HMBC spectrum of **4**, our analyses revealed that the critical spectroscopic data of **4** were akin to those of a known briarane, briarenolide K (**8**) (Figure 1).[19] The epoxy

groups' locations were verified by comparing the similarity of the ^{1}H and ^{13}C NMR spectra of **4** and **8**. The acetate ester group (δ_H 2.23, s, 3H; δ_C 169.0, C; 21.7, CH_3) was assigned at C-9 based on the clear HMBC correlation between H-9 (δ_H 5.33) and δ_C 169.0 (Figure 5a). The 16-acetoxy and 6-olefinic proton signals in **8**[19] replaced by a chlorine atom (δ_H 4.32, 1H, d, J = 12.6 Hz; 4.64, 1H, d, J = 12.6 Hz/δ_C 60.2, CH_2-16) and a hydroxy group in **4**, respectively, were also confirmed according to their 1D NMR data comparison. Moreover, the *n*-butyrate ester (δ_H 2.35, 2H, t, J = 7.2 Hz; 1.69, 2H, sext, J = 7.2 Hz; 0.97, 3H, t, J = 7.2 Hz; δ_C 172.5, C; 36.1, CH_2; 18.1, CH_2; 13.6 CH_3) was further assumed at C-12 due to analysis the deshielded proton signal at δ_H 4.68 (d, J = 4.8 Hz, H-12) in **4** and a known briarane with hydroxy-substituted C-12, briarenolide P (δ_H 3.87, H-12).[16]

In order to identify the relative configuration of **4**, NOESY analysis was performed. The results (Figure 5b) of NOESY correlations and the corresponding protons were identical to those of **8**.[19] This finding indicated that **4** had 1*S**,10*S** configurations within the ring junction. Accordingly, **4** was identified as briastecholide F, and the configurations of stereocarbons were revealed to be (1*S**,2*R**,3S*,4*R**,7*S**,8*R**,9*S**,10*S**,11*R**, 12*R**,13*S**,14*R**,17*R**).

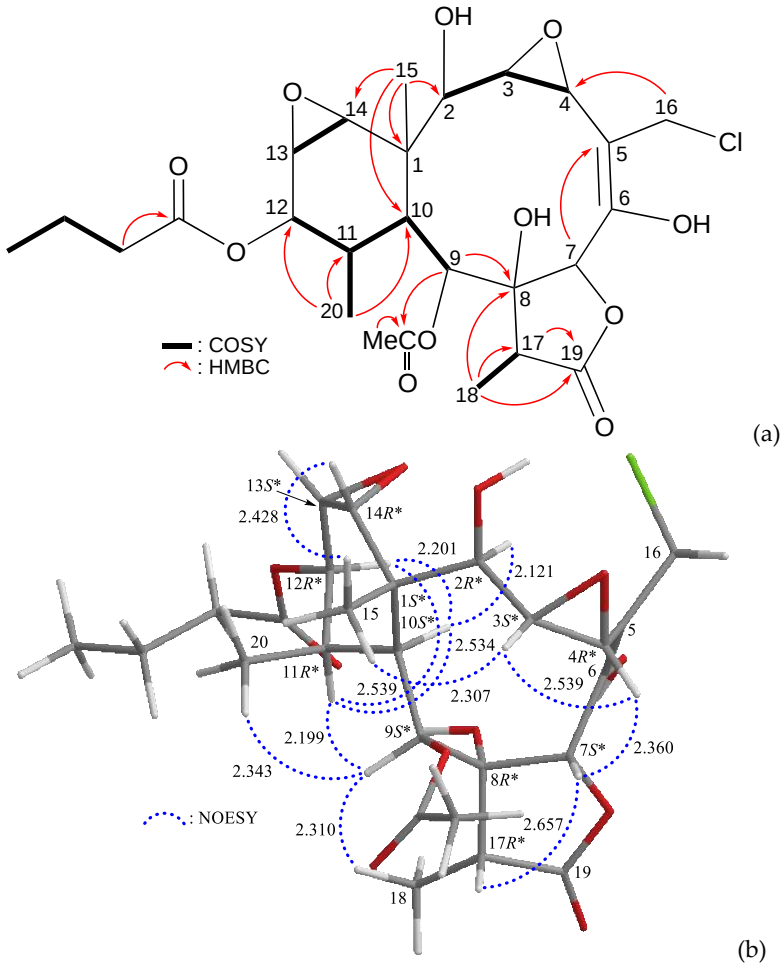
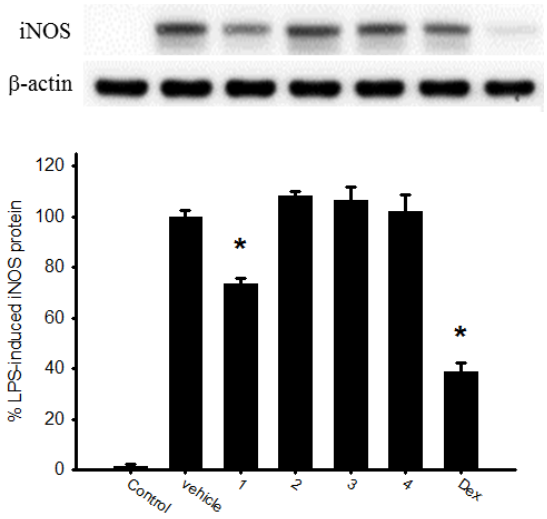


Figure 5. (a) Key COSY and HMBC correlations and (b) stereoview of **4** and calculated distances (Å) between particular protons that have crucial NOESY correlations

As briaranes **2**–**4** were purified together with briarane **1** from the same organism, according to the principles of biogenesis, it is reasonable to speculate that briaranes **2**–**4** may have the same absolute configuration as that of **1**. Therefore, the configurations of the stereocarbons of **2**–**4** should be assigned as (1*S*,2*S*,7*S*,8*R*, 9*S*,10*S*,11*R*,17*R*), (1*R*,2*S*,4*S*,6*S*,7*R*,8*R*,9*S*,10*S*,14*S*,17*R*), and (1*S*,2*R*,3*S*,4*R*,7*S*,8*R*,9*S*,10*S*,11*R*,12*R*,13*S*,14*R*, 17*R*), respectively.

In the *in vitro* study of anti-inflammatory properties, lipopolysaccharide (LPS) treatment is known to cause an increase in the protein expression of inducible nitric oxide synthase (iNOS) in RAW264.7 macrophages. Using western blotting analysis, when RAW264.7 cells were treated with 10 μM of compound **1**, the cells showed a significantly reduced pro-inflammatory iNOS expression caused by LPS stimulation, which was reduced to 73.50% (Figure 6). However, new briaranes **2**–**4** (at a concentration of 10 μM) were inactive in inhibiting iNOS production. Furthermore, we also observed that briaranes **5** (57.4%)[16] and **6** (88.3%)[17] had higher activity than **2** in terms of reducing iNOS production from RAW264.7 cells, suggesting that the functional groups at C-16 in **2**, **5**, and **6** influence the activity of the compounds.



	iNOS	*n*
control	1.26 ± 0.95	4
vehicle	100.00 ± 2.50	4
1	73.50 ± 1.95	4
2	108.32 ± 1.69	4
3	106.55 ± 5.01	4
4	101.94 ± 6.79	4
Dex.	38.76 ± 3.58	4

Figure 6. Western blotting of the bioactivities of compounds **1–4** in terms of affecting the protein expression of pro-inflammatory iNOS in an LPS-stimulated RAW264.7 macrophage cell line. Signals of immunoreactibity indicated the amounts of protein expression in the samples, measured by quantifying the band intensities. Cells treated with LPS alone were used as the reference (set to 100%) to normalize the results of cells treated with compounds and the positive control (dexamethasone; Dex). Compound **1** induced significant inhibition of iNOS expression in RAW264.7 cells caused by LPS stimulation. The results shown are the data from three individual experiments (*****$p < 0.05$, significant difference as compared with the LPS-stimulated group).

EXPERIMENTAL

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a spectrometer (Jeol,

model #: ECZ-600) in solution in $CDCl_3$ [residual $CHCl_3$ (δ_H = 7.26 ppm) and $CDCl_3$ (δ_C = 77.0 ppm) were used as internal standards]. For coupling constants (*J*), the results were presented in frequency units (Hz). For positive-mode ESIMS and HRESIMS, the results were obtained using a Bruker SolariX FT mass spectrometer. Column chromatography was carried out with silica gel (230–400 mesh particle size, Merck). TLC was performed on plates precoated with silica gel 60 F_{254} (layer thickness 0.25 mm, Merck) and RP-18W/UV_{254} (layer thickness 0.15 mm, Macherey-Nagel), and visualization of the TCL plates was performed using an aqueous solution of 10% H_2SO_4, consequently heated to show the spots of signals. For normal-phase HPLC (NP-HPLC) separation, a HPLC system consisting of a pump (Hitachi, L 711 0) coupled with an injection port (Rheodyne, model: 7725) was used; the system was equipped with a normal-phase column (YMC-Pack SIL, 250 × 20 mm, 5 μm; Sigma-Aldrich). For reverse-phase HPLC (RP-HPLC) separation, a system that consisted of a pump (Hitachi, L2130) with a diode array detector (HITACHI, L 2455), equipped with a reverse-phase column (Luna, 5 μm, C18(2) 100Å, 250 × 21.2 mm) was used. ACS grade *n*-hexane, dichloromethane, ethyl acetate, acetone, methanol, 95% sulfuric acid, and HPLC grade methanol were purchased from Macron Fine Chemicals™, Avantor Performance Materials, LLC. (Radnor, PA). Deuterated solvents, $CDCl_3$ and CD_3OD, were obtained from Sigma-Aldrich. Water for chromatographic separation was purified using a Elga PURELAB® Ultra water system (ELGA LabWater, High Wycombe, UK).

Animal Materials. Specimens used in this study were of *Briareum stechei*, originally collected in April 2016 from the wild and from culture in an 80-ton culture reservoir containing a flowing seawater filtration system at the National Museum of Marine Biology & Aquarium (NMMBA). By comparison with published reports in the literature,[7–10] the species of the coral was identified. Sample organisms for use as a living reference are kept in our marine coral culture reservoirs, and specimens prepared as sample vouchers have been stored at the NMMBA, Taiwan (voucher number: NMMBA-TW-GC-2016-031).

Extraction and Isolation. *Briareum stechei* specimens (wet weight = 3.98 kg) were harvested and cut into small pieces. After freeze-drying, the lyophilized sample (1.86 kg, dry weight) was crushed to powder and extracted with a solvent mixture of MeOH and CH_2Cl_2 (1:1, v/v) to give a crude extract (104 g). The crude extract was then applied on silica column chromatography (Si C.C.) and eluted with a gradient solvent system of *n*-hexane/EtOAc mixtures (pure *n*-hexane, *n*-hexane/EtOAc 95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 (v/v), and pure EtOAc) to give fractions A–L. Fractions H and I were pooled together (total 19.0 g) and further purified by Si C.C. with a gradient solvent system of *n*-hexane/EtOAc mixtures (from 50:1 to pure EtOAc, v/v) to give subfractions H1–H8. Fraction H6 (1.2 g) was then separated by Si C.C. with a mixture solvent system of *n*-hexane/EtOAc/acetone (*n*-hexane/EtOAc 2:1, 1:1 (v/v), pure EtOAc, and pure acetone) to give subfractions H6A–H6K based on the TLC monitoring. Moreover, H6E (614.0 mg) was further subjected to Si C.C. with the eluent of CH_2Cl_2/acetone (4:1, v/v) to

give subfractions H6E1–H6E6. Subfraction H6E1 (26.1 mg) was further separated by RP-HPLC on Luna C18(2) column with an isocratic solvent system of MeOH-H_2O (70:30, v/v; 5.0 mL/min to give subfractions H6E1A–H6E1K. Subfraction H6E1K (14.0 mg) was further purified by RP-HPLC on Luna C18(2) column with an isocratic solvent system of MeOH-H_2O (80:20, v/v; 4.0 mL/min) to give **3** (0.7 mg). Subfraction H6H (11.0 mg) was separated by RP-HPLC on Luna C18(2) column with an isocratic solvent system of MeOH-H_2O (50:50, v/v; 5.0 mL/min) to give **2** (0.2 mg). Subfractons H6I (3.7 mg) and H6J (4.5 mg) were also purified by RP-HPLC on Luna C18(2) column with an isocratic solvent system of MeOH-H_2O (60:40, v/v; 5.0 mL/min) to give **4** (0.3 mg) and **1** (1.1 mg), respectively.

Excavatolide E (1): colorless prismatic crystal; ^{1}H and ^{13}C NMR data of **1** were in complete agreement with earlier studies.[14,15]

Briastecholide D (2): colorless oil; $[\alpha]_D$ +23 (*c* 0.13, $CHCl_3$); ^{1}H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR data, see Table 1; ESIMS *m/z* 445 $(M + Na)^+$; HRESIMS *m/z* 445.18353 (Calcd for $C_{22}H_{30}O_8$ + Na, 445.18329).

Briastecholide E (3): colorless oil; $[\alpha]_D$ –45 (*c* 0.23, $CHCl_3$); ^{1}H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR data, see Table 1; ESIMS *m/z* 521 $(M + Na)^+$, 523 $(M + 2 + Na)^+$; HRESIMS *m/z* 521.15494 (Calcd for $C_{24}H_{31}ClO_9$ + Na, 521.15488).

Briastecholide F (4): colorless oil; $[\alpha]_D$ +167 (*c* 0.03, $CHCl_3$); ^{1}H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR data, see Table 1; ESIMS *m/z* 581 $(M + Na)^+$, 583 $(M + 2 + Na)^+$; HRESIMS *m/z* 581.17585 (Calcd for $C_{26}H_{35}ClO_{11}$ + Na, 581.17601).

Single-Crystal X-Ray Crystallography of Excavatolide E (1). MeOH solution was used to generate crystalized compound **1** to prepare suitable prims for X-ray diffraction. The colorless crystal (0.222 × 0.197 × 0.085 mm^3) was found to be orthorhombic, and fit into the space group $P2_12_12_1$ (# 19), with a = 9.5838(2) Å, b = 14.4150(4) Å, c = 18.7890(4) Å, V = 2595.71(11) $Å^3$, Z = 4, D_{calcd} = 1.276 Mg/m^3, and λ (Mo Kα) = 0.71073 Å. A diffractometer measurement (Bruker D8 VENTURE) showed that the crystal had a θ_{max} of 27.498°. A total of 45,145 reflections were obtained. To solve the structure of compound **1**, direct methods were first used, and data were further refined using the full-matrix least-squares calculations approach. The advanced structural model resulted in a refinement converging to a final R1 = 0.0477; wR2 = 0.1381 for 5,578 observed reflections [$I > 2\sigma(I)$] and 326 variable parameters. The Flack parameter (x = 0.1(3))[20,21] was employed to establish the absolute configuration of the compound. Crystallographic data for the structure of **1** have been submitted to the Cambridge Crystallographic Data Centre with supplementary publication number CCDC 2113017, and are freely available through http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2, 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccde.cam.ac.uk).

***In Vitro* Anti-Inflammatory Assay.** An inflammatory assay that used the RAW264.7 macrophage cell line

as an *in vitro* model was employed to evaluate the biological activities of briaranes **1**−**4** in relation to the secretion of iNOS from cells, as reported in previous research.[22,23]

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to C.-L. Ho and H.-C. Yu from Sun Yat-sen University (the HVI Center) for the performance of NMR (NMR001100) and collection of mass spectrum data (MS000600) (grant no.: MOST-111-2731-M-110-001). We also acknowledge the Instrumentation Center, National Taiwan University, for the use of X-ray facilities (funding nos: MOST-111-2731-M-002-001, XRD 000200). This work was supported by funding from the National Museum of Marine Biology and Aquarium and the Ministry of Science and Technology (funding nos: MOST-107-2320-B-291-001-MY3 and 109-2320-B-291-001-MY3), awarded to Ping-Jyun Sung.

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