HETEROCYCLES, Vol. 106, No. 2, 2023, pp.358 - 365. © 2023 The Japan Institute of Heterocyclic Chemistry
Received, 5th December, 2022, Accepted, 26th December, 2022, Published online 26th December, 2022
DOI: 10.3987/COM-22-14792

12-*EPI*-BRIACAVATOLIDE B, A NEW BRIARANE DITERPENOID FROM THE OCTOCORAL *BRIAREUM STECHEI*

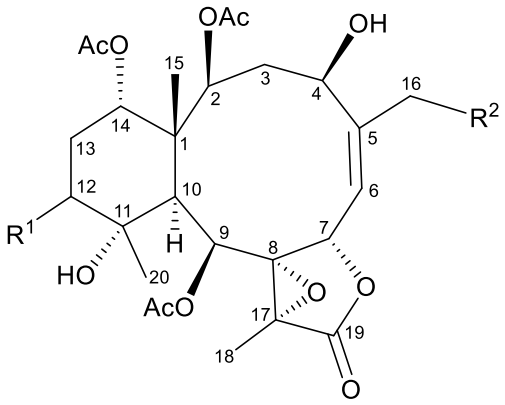
Chia-Jung Liu,[a,b] Hsin-Tzu Liu,[c] Thanh Hao Huynh,[a,b] Zhi-Hong Wen,[a,d] Chia-Ching Liaw,[e,f] Jih-Jung Chen,[g] Jui-Hsin Su,[a,b] and Ping-Jyun Sung[a,b,h,i,j]*

[a] Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804201, Taiwan. [b] National Museum of Marine Biology and Aquarium, Pingtung 944401, Taiwan. [c] Department of Medical Research, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien 970473, Taiwan. [d] Institute of BioPharmaceutical Sciences, National Sun Yat-sen University, Kaohsiung 804201, Taiwan. [e] Division of Chinese Materia Medica Development, National Research Institute of Chinese Medicine, Ministry of Health and Welfare, Taipei 112304, Taiwan. [f] Department of Biochemical Science and Technology, National Chiayi University, Chiayi 600355, Taiwan. [g] Department of Pharmacy, School of Pharmaceutical Sciences, National Yang Ming Chiao Tung University, Taipei 112304, Taiwan. [h] Chinese Medicine Research and Development Center, China Medical University Hospital, Taichung 404394, Taiwan. [i] Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807378, Taiwan. [j] Ph.D. Program in Pharmaceutical Biotechnology, Fu Jen Catholic University, New Taipei City 242062, Taiwan.
E-mail: pjsung@nmmba.gov.tw

Abstract – A new briarane-type diterpenoid, 12-*epi*-briacavatolide B (**1**), along with its known epimer, briacavatolide B (**2**), were purified from an octocoral identified as *Briareum stechei*. Spectroscopic approaches were used to reveal the structure of new briarane **1**.

Octocorals are invertebrates extensively distributed in coastal waters, especially the coasts of tropical and subtropical Indo-Pacific Ocean regions. The creatures have been shown to be rich sources of an array of diterpenoid derivatives with characteristic carbon skeletons that have various bioactivities.[1,2] The octocorals belonging to genus *Briareum* (phylum: Cnidaria, sub-phylum: Anthozoa, class: Octocorallia,

order: Scleralcyonacea, family: Briareidae),[3] distributed in the Indo-Pacific Ocean, are comprised of four characterized species, *B. cylindrum*, *B. hamrum*, *B. stechei*, and *B. violaceum*,[4] and different groups of diterpenoids, including briarane (3,8-cyclized cembranoid) and eunicellin (2,11-cyclized cembranoid) diterpenoids, have been obtained from these fascinating marine invertebrates of potential medicinal use.[5,6] We have conducted extensive investigations in previous years on *Briareum stechei* (Kükenthal, 1908) (synonyms *Briareum excavatum*),[4] from which a new briarane, 12-*epi*-briacavatolide B (**1**) and its known epimer briacavatolide B (**2**),[7] were isolated (Figure 1). In this article, we summarized the processes of purification and structural identification of **1** and **2**.



1: R^1 = α-OH, R^2 = OAc
2: R^1 = β-OH, R^2 = OAc
3: R^1 = β-OH, R^2 = OH

Figure 1. Structures of 12-*epi*-briacavatolide B (**1**), briacavatolide B (**2**), and briavioid B (**3**)

12-*epi*-Briacavatolide B (**1**) was isolated as colorless oil that gave an $[M + N]^+$ ion peak at *m/z* 621.21529 in the positive mode HRESIMS indicating the molecular formula $C_{28}H_{38}O_{14}$ (calcd for $C_{28}H_{38}O_{14}$ + Na, 621.21538) requiring 10 sites of unsaturation. The IR absorptions were observed at ν_{max} 3500, 1779, and 1732 cm^{-1}, suggesting the presence of hydroxy, γ-lactone, and ester groups in **1**, respectively. From the ^{13}C NMR data (Table 1), together with DEPT and HSQC spectra, illustrated the presence of 28 carbons in **1**, including five ester carbonyls (δ_C 171.6, OAc-16; 170.6, OAc-2; 170.5, C-19; 169.6, OAc-14; 168.4, OAc-9), two olefin carbons (δ_C 144.5, C-5; 126.4, CH-6), and ten oxygen-bearing carbons (δ_C 75.6, CH-14; 74.6, C-11; 74.2, CH-2; 73.8, CH-12; 73.4, CH-7; 70.3, C-8; 69.2, CH-4; 68.6, CH_2-16; 67.4, CH-9; 66.0, C-17), as well as seven methyls, two aliphatic sp^3 methylenes, one aliphatic sp^3 methine, and one aliphatic sp^3 non-protonated carbon. Analysis of the ^1H (Table 1), ^{13}C, and HSQC spectra confirmed that **1** possessed a tetrasubstituted epoxide (δ_C 70.3, C-8; 66.0, C-17), a trisubstituted carbon-carbon double bond (δ_C 144.5, C-5; δ_H 5.55, 1H, br d, J = 8.4 Hz/δ_C 126.4, CH-6), four acetoxy groups (δ_H 2.25, 2.09, 2.03, 1.97, each 3H × s/δ_C 21.3, 21.0, 21.5, 21.2, 4 × acetate methyls; δ_C 168.4, 171.6, 169.6, 170.6, 4 × acetate carbonyls), and three hydroxy protons (δ_H 3.13, 1H, d, J = 0.4 Hz, OH-11; 2.85, 1H, d, J = 9.6 Hz, OH-12; 2.70, 1H, br s, OH-4), which in conjunction with carbons presented in the molecular formula, suggesting a diterpenoid molecule possessing four rings.

Table 1. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data for briaranes **1**, **2**, and briacavatolide B

	1		**2**		briacavatolide B[d]		
Position	δ_H (J in Hz)	δ_C (mult.)	δ_H (J in Hz)	δ_C (mult.)	δ_H (J in Hz)	δ_C (mult.)	$\Delta\delta_C$[e]
1		47.2, C		47.4, C		47.4, C	(0.0)
2	4.93 d (9.2)	74.2, CH	4.87 d (8.8)	74.2, CH	4.88 d (8.4)	74.2, CH	(0.0)
3α	2.05 m	39.2, CH_2	2.05 m	39.4, CH_2	2.02 m	39.3, CH_2	(0.1)
β	2.80 dd (15.2, 12.0)		2.81 dd (15.6, 12.0)		2.81 dd (15.2, 12.0)		
4	4.10 dd (12.0, 5.6)	69.2, CH	4.10 dd (12.0, 5.2)	68.8, CH	4.15 dd (12.0, 5.2)	69.2, CH	(-0.4)
5		144.5, C		145.0, C		145.3, C	(-0.3)
6	5.55 br d (8.4)	126.4, CH	5.47 d (8.8)	124.7, CH	5.53 d (8.8)	125.4, CH	(-0.7)
7	5.75 d (8.4)	73.4, CH	5.81 d (8.8)	73.4, CH	5.81 d (8.8)	73.3, CH	(0.1)
8		70.3, C		70.5, C		70.5, C	(0.0)
9	5.86 s	67.4, CH	5.78 d (1.2)	67.0, CH	5.79 d (3.6)	67.0, CH	(0.0)
10	2.21 br s	44.5, CH	2.09 d (1.2)[b]	48.9, CH	2.10 m	49.0, CH	(-0.1)
11		74.6, C		78.1, C		78.1, C	(0.0)
12	3.57 ddd (9.6, 2.8, 2.8)	73.8, CH	3.71 dd (12.8, 4.0)	73.2, CH	3.72 dd (12.4, 4.0)	73.3, CH	(-0.1)
13α	2.00~2.19 m[a]	27.5, CH_2	2.01 m	30.2, CH_2	2.04 td (12.4, 2.0)	30.2, CH_2	(0.0)
β			1.66 ddd (12.4, 12.4, 2.0)		1.68 m		
14	4.93 dd (3.2, 3.2)	75.6, CH	4,81 dd (2.8, 2.8)	74.8, CH	4.83 dd (2.4, 2.0)	74.7, CH	(0.1)
15	1.28 s	14.6, Me	1.24 s	14.4, Me	1.26 s	14.5, Me	(-0.1)
16a	4.56 d (13.6)	68.6, CH_2	4.63 d (14.0)	67.9, CH_2	4.61 d (10.0)	68.3, CH_2	(-0.4)
b	4.70 dd (13.6, 1.6)		4.78 dd (14.0, 1.2)		4.75 dd (10.0, 1.6)		
17		66.0, C		66.3, C		66.3, C	(0.0)
18	1.82 s	10.3, Me	1.77 s	10.3, Me	1.79 s	10.3, Me	(0.0)
19		170.5, C		170.3, C		170.2, C	(0.1)
20	1.17 s	22.5, Me	1.14 s	17.0, Me	1.16 s	17.0, Me	(0.0)
OH-4	2.70 br s		n. o.[c]				
OH-11	3.13 d (0.4)		n. o.[c]				
OH-12	2.85 d (9.6)		n. o.[c]				
OAc-2		170.6, C		170.3, C		170.4, C	(-0.1)
	1.97 s	21.2, Me	1.98 s	21.3, Me	2.00 s	21.4, Me	(-0.1)
OAc-9		168.4, C		168.4, C		168.3, C	(0.1)
	2.25 s	21.3, Me	2.24 s	21.3, Me	2.26 s	21.3, Me	(0.0)
OAc-14		169.6, C		170.6, C		170.6, C	(0.0)
	2.03 s	21.5, Me	1.97 s	21.1, Me	1.98 s	21.1, Me	(0.0)
OAc-16		171.6, C		171.5, C		171.5, C	(0.0)
	2.09 s	21.0, Me	2.09 s[b]	21.0, Me	2.10 s	21.0, Me	(0.0)

[a] The signals for α- and β-protons are overlapped. [b] Signals overlapped, the coupling constant (J = 1.2 Hz) for H-10 in **2** was assigned by its vicinal coupling with H-9. [c] n. o. = not observed. [d] Data were reported by Yeh et al., please see ref. 7. [e] $\Delta\delta_C = \delta_C$ (**2**) – δ_C (briacavatolide B).

The gross structure of **1** was determined by 2D NMR studies including COSY, HSQC, and HMBC experiments. From the ^{1}H NMR coupling information contained in the COSY spectrum of **1** enabled identification of the separate spin systems from H-2/H_2-3/H-4, H-6/H-7, H-9/H-10, and H-12/H_2-13/H-14 (Figure 2a). These data, together with the key HMBC correlations between protons and non-protonated carbons were observed in the HMBC spectrum, such as H-2, H-3β, H-9, H-10, H-14, H_3-15/C-1; H_2-3, H-4, H-6, H-7, H_2-16/C-5; H-9, H-10, H_3-18/C-8; H-9, H-10, OH-11, H_2-13, H_3-20/C-11; H-9, H_3-18/C-17; and H-7, H_3-18/C-19 (Figure 2a), permitted elucidation of the 6/10/5 tricyclic ring system of a briarane-type diterpenoid. The HMBC correlations from H_3-15/C-1, C-2, C-10, C-14; H_3-18/C-8, C-17, C-19; and H_3-20/C-10, C-11, C-12, indicated that Me-15, Me-18, and Me-20 were placed at C-1, C-17, and C-11, respectively. An acetoxymethyl at C-5 was confirmed by the HMBC correlations from H_2-16/C-4, C-5, C-

6. The hydroxy groups at C-4 and C-12 were substantiated by COSY correlations between H-4 (δ_H 4.10)/OH-4 (δ_H 2.70); and H-12 (δ_H 3.57)/OH-12 (δ_H 2.85) (Figure 2a). Furthermore, in the HMBC spectrum, H-2 (δ_H 4.93), H-9 (δ_H 5.86), H-14 (δ_H 4.93), and H_2-16 (δ_H 4.70 and 4.56) were correlated with the acetate carbonyls at δ_C 170.6, 168.4, 169.6, and 171.6, indicating that these four acetoxy groups were placed at C-2, C-9, C-14, and C-16, respectively.

13 of the 14 oxygen atoms in the molecular formula accounted for the presence of one γ-lactone, four esters, one epoxy, and two hydroxy groups. Thus, the remaining one oxygen atom had to be positioned at C-11 as a hydroxy group and this finding was supported by the HMBC correlations of a hydroxy proton resonating at δ_H 3.13 (1H, d, J = 0.4 Hz) with C-11 and C-20, respectively.

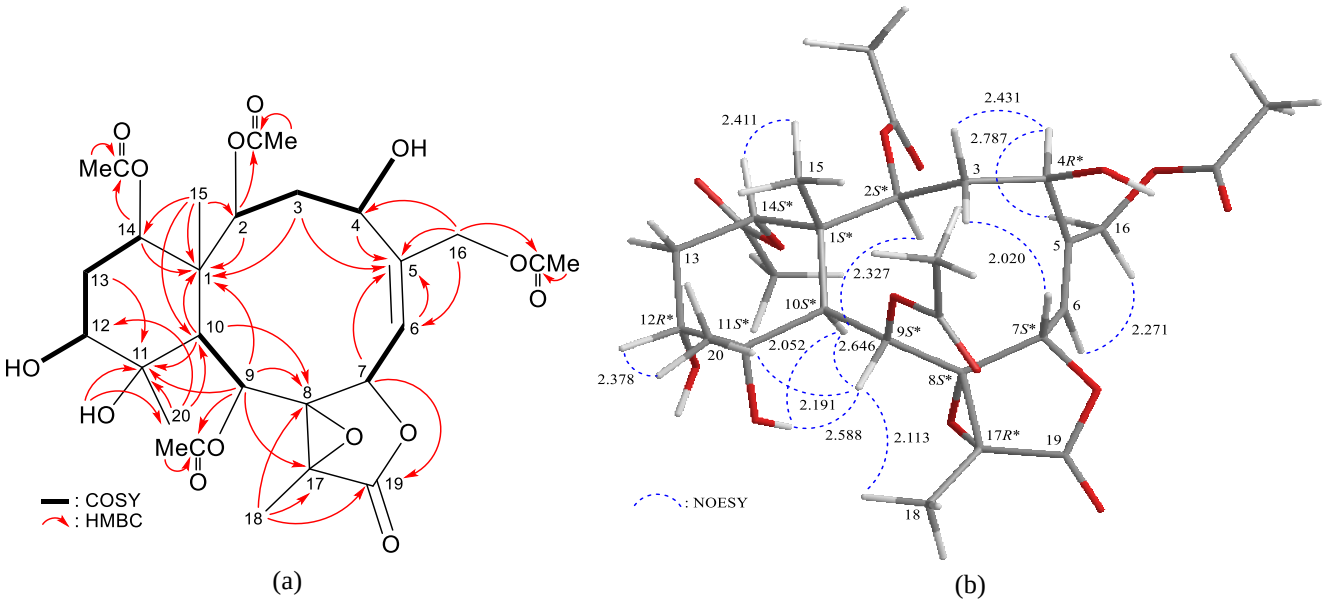


Figure 2. (a) Key COSY and HMBC correlations and (b) stereoview of **1** and calculated distances (Å) between particular protons that have crucial NOESY correlations

Stereochemical analysis of **1** was performed via a 2D NMR spectroscopic method using a NOESY experiment (Figure 2b) and by vicinal ^1H–^1H coupling constant analysis. In the NOESY experiment, H-2 and H-10 were concluded to be located on the same face of the molecule, as these two protons were correlated together, while not correlated with H_3-15. As a result, they were assigned as α protons, since Me-15 was a β-substituent at C-1. Due to H-14 being correlated with H_3-15, this proton was of a β-orientation at C-14. The C-13 methylene protons displayed coupling with H-12 (J = 2.8, 2.8 Hz) and H-14 (J = 3.2, 3.2 Hz), respectively, indicating that both H-12 and H-14 should be positioned on the β-equatorial direction in the six-membered ring of **1**. Furthermore, H_3-20 showed a correlation to H-12 and the absence of a cross-peak with H-10, suggesting a β-oriented methyl group at C-11. As one of the C-3 methylene protons (δ_H 2.80) exhibited a correlation to H-7, and not to H-2, suggesting the β-orientations of this proton and H-7 by modeling study, and the other was assigned as H-3α (δ_H 2.05). A correlation was found between H-4 and

H-3α, a greater coupling constant (*J*) of 12.0 Hz was noted between H-4 and H-3β, showing that the plane between H-4 and H-3β have a dihedral angle of about 180° and demonstrating the α-orientation of H-4. A correlation between H-6 and a proton of the C-16 methylene (δ_H 4.70), but not with H-7, as well as a large coupling constant between H-7 and H-6 (*J* = 8.4 Hz) suggested that the dihedral angle between H-7 and H-6 was nearly 130°, revealing the *Z*-geometry of Δ^5. Also, no coupling was found between H-9 and H-10, indicating that the dihedral angle between these two protons was 90°, suggested that H-9 had an α-orientation. H_3-18 was found to be associated with H-9, suggesting that the C-18 methyl in the γ-lactone moiety had a β-orientation. Therefore, based on the above findings the relative configurations of the stereogenic centers of **1** were elucidated as 1*S**,2*S**,4*R**,7*S**,8*S**,9*S**,10*S**,11*S**,12*R**,14*S**,17*R**.

Compound **1** seemed to be very similar to briacavatolide B, which was previously isolated from the octocoral *Briareum excavatum*,[7] and was also obtained in this study and presented as compound **2** (Figure 1). It was clear that **1** was found to be the 12-*epi*-compound of **2**. However, we found that the ^{13}C NMR data and specific rotation value for briacavatolide B differed slightly from those of **2** reported in this article. The ^{13}C NMR data of C-4 (δ_C 69.2), C-5 (δ_C 145.3), C-6 (δ_C 125.4), and C-16 (δ_C 68.3) (Table 1) and rotation value ($[\alpha]_D^{25}$ –57.8 (*c* 0.1, $CHCl_3$)) for briacavatolide B[7] were different by comparison with those of **2** (δ_C 68.8, C-4; 145.0, C-5; 124.7, C-6; 67.9, C-16) ($[\alpha]_D^{24}$ –6.1 (*c* 0.09, $CHCl_3$)). The structure of briacavatolide B is not in question; we suggested that the differences in the ^{13}C NMR data and rotation values were arose from conformational flexibility.[8–11]

Additionally, as briaranes **1** and **2** were isolated along with a known briarane, excavatolide M,[12] from the same target organism, *B. stechei*, and the absolute configuration of excavatolide M was determined by a single-crystal X-ray diffraction analysis.[13] Therefore, it is reasonable on biogenetic grounds to assume that briaranes **1** and **2** had the same absolute stereochemistry as that of excavatolide M. Based on the above findings, the configurations of the stereogenic centers of **1** and **2** were elucidated as 1*S*,2*S*,4*R*,7*S*,8*S*,9*S*,10*S*,11*S*,12*R*,14*S*,17*R* and 1*S*,2*S*,4*R*,7*S*,8*S*,9*S*,10*S*,11*S*,12*S*,14*S*,17*R*, respectively.

Based on the past reports, *Briareum* spp. showed a promising anti-inflammatory effect.[5,14] Therefore, in *in vitro* study of anti-inflammatory activity, upregulation of pro-inflammatory inducible nitric oxide synthase (iNOS) protein expression in LPS-stimulated RAW 264.7 macrophage cells were evaluated using immunoblot analysis. Unfortunately, at a concentration of 10 μM, briaranes **1** and **2** were found to be inactive to reduce the level of iNOS in related to control cells stimulated with LPS only (Table 2). Using trypan blue staining to measure the cytotoxic effects of the compounds, it was observed that **1** and **2** did not induce cytotoxicity in RAW 264.7 macrophage cells. However, we observed that a known briarane, briavioid B (**3**) (Figure 1),[15] which was found to be the 16-deacetyl derivative of **2**, had higher activity than **2** in terms of reducing iNOS production from RAW 264.7 cells, indicating that the anti-inflammatory activity of these two compounds is largely dependent on the functional groups at C-16; the hydroxy group

at C-16 might enhance the activity.

Table 2. Effect of briaranes **1**–**3** (10 μM) on LPS-induced pro-inflammatory iNOS protein expression in macrophages

Compounds	iNOS Expression (% of LPS)	
control	1.66 ± 0.48	2.40 ± 0.47[a]
vehicle	100.00 ± 0.80	100.00 ± 0.00[a]
1	96.25 ± 1.72	
2	97.31 ± 3.92	
3		80.24 ± 1.92[a]
Dexamethasone	48.05 ± 2.46	41.36 ± 2.13[a]

Cells treated with LPS only were used as the reference to normalize the briarane-treated cells. Dexamethasone-treated (10 μM) cells were employed as a positive control. Data are shown as the mean ± SEM (n = 3~6). [a] Data were reported by Huynh et al., please see ref.[15]

EXPERIMENTAL

General Experimental Procedures. Optical rotations were determined with JASCO P-2000 digital polarimeter. IR were obtained with a Thermo Scientific Nicolet iS5 FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a spectrometer (Jeol, model #: ECZ-400) 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, L7110) 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, L 2130) with a photo-diode array detector (Hitachi, L2455), equipped with a reverse-phase column (Luna, 5 μm, C18(2) 100Å, 250 × 21.2 mm) was used.

Animal Materials. Specimens of *Briareum stechei* investigated in this study were collected by hand using SCUBA apparatus equipment off the coast of Haikou, Pingtung County, Taiwan in June 2020 and stored at –20 °C until extraction. Identification of the species was performed based on the comparison of its morphology and micrographs of the coral sclerites with those described in previous descriptions.[3,4,16–18]

Extraction and Isolation. Freeze-dried and sliced bodies (wet/dry weight = 7.30/2.92 kg) of the coral specimens were extracted with EtOAc to give 231 g of crude extract. The EtOAc extract was applied in a

silica gel column chromatography (Si C.C.) and eluted with gradients of *n*-hexane/EtOAc (100% *n*-hexane –100% EtOAc, stepwise) to furnish 11 sub-fractions A~K. Fraction F was chromatographed by Si C.C. and eluted with a mixture of *n*-hexane/acetone (3:2) to obtain 8 sub-fractions F1~F8. Fraction F5 was re-purified by RP-HPLC using a mixture of MeCN/H_2O (40:60; flow rate = 5.0 mL/min) to afford **1** (2.3 mg). Fraction F8 was separated by NP-HPLC using a mixture of CH_2Cl_2/EtOAc (3:2; flow rate = 3.0 mL/min) to furnish sub-fractions F8A~F8C. Fraction F8A was re-purified by RP-HPLC using a mixture of MeCN/H_2O (30:70; flow rate = 5.0 mL/min) to afford **2** (16.7 mg).

12-*epi*-Briacavatolide B (1): colorless oil; $[\alpha]_D^{24}$ –38.9 (*c* 0.09, $CHCl_3$); IR (ATR) ν_{max} 3500, 1779, 1732 cm^{-1}; ^1H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data, see Table 1; ESIMS *m/z* 621 $(M + Na)^+$; HRESIMS *m/z* 621.21529 (Calcd for $C_{28}H_{38}O_{14}$ + Na, 621.21538).

Briacavatolide B (2): colorless oil; $[\alpha]_D^{24}$ –6.1 (*c* 0.09, $CHCl_3$) (ref. 7, $[\alpha]_D^{25}$ –57.8 (*c* 0.1, $CHCl_3$)); IR (ATR) ν_{max} 3455, 1778, 1732 cm^{-1}; ^1H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data, see Table 1; ESIMS *m/z* 621 $(M + Na)^+$.

***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** and **2** in relation to the secretion of iNOS from cells, as reported in previous research.[19,20]

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). This work was supported by funding from the National Museum of Marine Biology and Aquarium and the National Science and Technology Council (funding nos: MOST-109-2320-B-291-001-MY3, 111-2320-B-291-001, and 111-2320-B-291-002), Taiwan, awarded to Jui-Hsin Su and Ping-Jyun Sung.

REFERENCES

1. J. Rocha, L. Peixe, N. C. M. Gomes, and R. Calado, *Mar. Drugs*, 2011, **9**, 1860.
2. N. B. A. Nguyen, L.-Y. Chen, M. El-Shazly, B.-R. Peng, J.-H. Su, H.-C. Wu, I.-T. Lee, and K.-H. Lai, *Mar. Drugs*, 2022, **20**, 640.
3. C. S. McFadden, L. P. van Ofwegen, and A. M. Quattrini, *Bull. Soc. Syst. Biol.*, 2022, **1**, 8735.
4. K. Samimi-Namin and L. P. van Ofwegen, *ZooKeys*, 2016, **557**, 1.
5. Y.-H. Chen, H.-K. Chin, B.-R. Peng, Y.-Y. Chen, C.-C. Hu, L.-G. Zheng, T.-H. Huynh, T.-P. Su, Y.-L. Zhang, Z.-H. Wen, T.-L. Hwang, Y.-C. Wu, and P.-J. Sung, *Heterocycles*, 2020, **100**, 857.
6. P.-J. Sung and M.-C. Chen, *Heterocycles*, 2002, **57**, 1705.

7. T.-Y. Yeh, S.-K. Wang, C.-F. Dai, and C.-Y. Duh, *Mar. Drugs*, 2012, **10**, 1019.
8. G. H. Phan, H.-C. Hu, F.-R. Chang, Z.-H. Wen, J.-J. Chen, H.-M. Chung, Y.-C. Tsai, and P.-J. Sung, *RSC Adv.*, 2022, **12**, 27970.
9. K. B. Wiberg, P. H. Vaccaro, and J. R. Cheeseman, *J. Am. Chem. Soc.*, 2003, **125**, 1888.
10. K. B. Wiberg, Y.-G. W, P. H. Vaccaro, J. R. Cheeseman, and M. R. Luderer, *J. Phys. Chem. A*, 2005, **109**, 3405.
11. M. Caricato, *J. Phys. Chem. A*, 2015, **119**, 8303.
P.-J. Sung, J.-H. Su, G.-H. Wang, S.-F. Lin, C.-Y. Duh, and J.-H. Sheu, *J. Nat. Prod.*, 1999, **62**, 457.
12. Y.-T. Yeh, C.-Y. Chen, P.-J. Chen, Y.-H. Hsieh, S.-Y. Chien, C.-J. Liu, Z.-H. Wen, T.-L. Hwang, N.-F. Chen, and P.-J. Sung, *Tetrahedron Lett.*, 2022, **103**, 153997.
13. W.-C. Wei, P.-J. Sung, C.-Y. Duh, B.-W. Chen, J.-H. Sheu, and N.-S. Yang, *Mar. Drugs*, 2013, **11**, 4083.
14. T. H. Huynh, Z.-H. Wen, S.-Y. Chien, H.-M. Chung, J.-H. Su, L.-S. Fang, Y.-J. Wu, S.-H. Lin, and P.-J. Sung, *Tetrahedron*, 2022, **125**, 133037.
15. F. M. Bayer, *Proc. Biol. Soc. Wash.*, 1981, **94**, 902.
16. Y. Benayahu, M.-S. Jeng, S. Perkol-Finkel, and C.-F. Dai, *Zool. Stud.*, 2004, **43**, 548.
17. Y. Miyazaki and J. D. Reimer, *Zool. Sci.*, 2014, **31**, 692.
18. Y.-H. Jean, W.-F. Chen, C.-C. Sung, C.-Y. Duh, S.-Y. Huang, C.-S. Lin, M.-H. Tai, S.-F. Tzeng, and Z.-H. Wen, *Br. J. Pharmacol.*, 2009, **158**, 713.
19. L.-C. Chen, Y.-Y. Lin, Y.-H. Jean, Y. Lu, W.-F. Chen, S.-N. Yang, H.-M. D. Wang, I.-Y. Jang, I.-M. Chen, J.-H. Su, P.-J. Sung, J.-H. Sheu, and Z.-H. Wen, *Molecules*, 2014, **19**, 14667.