Briarenols I—K, New Anti-inflammatory 8,17-Epoxybriaranes from the Octocoral Briareum excavatum (Briareidae)

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Abstract: Five 8,17-epoxybriaranes, including three new compounds—briarenols I–K (1–3), along with two known analogues, briaexcavatolide P (4) and briaexcavatin P (5), were isolated from the octocoral Briareum excavatum. The structures of briaranes 1–3 were elucidated by spectroscopic methods, including 1D and 2D NMR studies and (+)-HRESIMS. Briarane 4 exerted inhibition effects on inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) release from RAW 264.7.

Keywords: Briareum excavatum; briarenol; briarane; anti-inflammatory; iNOS; COX-2

1. Introduction

Octocorals of the genus Briareum (family Briareidae) [1–4] are proven to be the most important source to produce briarane-type diterpenoids [5]. The compounds of this type are only found in marine invertebrates, particularly in octocorals and demonstrated a wide spectrum of bioactivities, such as anti-inflammatory activity [6] and cytotoxicity [7]. In our continuing research into the chemical constituents of an octocoral B. excavatum (Nutting 1911), which was distributed extensively in the coral reefs of Taiwan, have resulted in isolation of three previously unreported 8,17-epoxybriaranes—briarenols I–K (1–3) along with two known analogues, briaexcavatolide P (4) [8] and briaexcavatin P (5) [9].
In the current study, the comprehensive workflow of isolation, structure determination, and anti-inflammatory activity evaluation, was implemented on briaranes 1–5.

![Diagram of briarenol structure]

### 2. Results and Discussion

Briarenol I (1) was isolated as an amorphous powder and displayed a sodiated adduct ion at m/z 649.24677 in the (+)-HRESIMS, which indicated its molecular formula was C_{30}H_{42}O_{14} (calculated for C_{30}H_{42}O_{14} + Na, 649.24668; unsaturation degrees = 10). The IR spectrum revealed absorptions for hydroxy (ν_{max} 3524 cm^{-1}), γ-lactone (ν_{max} 1783 cm^{-1}), and ester carbonyl (ν_{max} 1736 cm^{-1}) moieties. Resonances in the ^{13}C NMR of 1 at δC 172.9, 172.3, 170.5, 170.0, and 170.0 (5 × C) supported the presence of a γ-lactone and four esters (Table 1). Three of the esters were identified as acetates by the presence of three methyl singlet resonances in the ^1H NMR spectrum at δH 2.34, 2.15, and 2.08 (Table 2) and the remaining ester was found to be an n-butyroxy group based on ^1H NMR studies, including a correlation spectroscopy (COSY) experiment, which revealed seven contiguous protons (δH 2.30, 2H, t, J = 7.2 Hz; 1.63, 2H, tq, J = 7.2 Hz; 0.95, 3H, t, J = 7.2 Hz). From the COSY spectrum (Figure 2), the proton sequences from H-1 to C-14 was established. A hydroxymethyl group at C-5 was revealed by the HMBC between C-16 oxymethylene protons to C-4, C-5, and C-6. The C-15 methyl group at C-1 was confirmed by the HMBC between H_{2}-15/C-1, C-2, C-10, C-14, and H-10/C-15. The n-butyrate positions at C-4 was confirmed from the connectivity between H-4 (δH 6.14) and the carbonyl carbon of n-butyrate group (δC 172.3). HMBC from the oxymethine protons at δH 4.53 (H-2), 5.32 (H-9), and 4.88 (H-14) to the acetoxy carbons at δC 172.9, 170.0, and 170.0, placed the acetoxy groups on C-2, C-9, and C-14, respectively. Thirteen of the fourteen oxygen atoms in the molecular formula of 1 could be accounted for from the presence of a γ-lactone, four esters, and three hydroxy groups. The remaining oxygen atom had to be placed between C-8 and C-17 to form a tetrasubstituted epoxide based on the ^13C NMR evidences at δC 70.8 (C-8) and 62.5 (C-17) and the ^1H NMR chemical shift of a tertiary methyl at δH 1.66 (3H, s, H_{3}-18).
Table 1. $^{13}$C NMR ($\delta_{C}$ 100 MHz, CDCl$_3$) data for briaranes 1–3.

| Position | 1         | 2         | 3         |
|----------|-----------|-----------|-----------|
| 1        | 43.3, C   | 43.7, C   | 47.7, C   |
| 2        | 87.4, CH  | 85.6, CH  | 74.9, CH  |
| 3        | 73.1, CH  | 73.6, CH  | 31.6, CH$_2$ |
| 4        | 66.0, CH  | 65.9, CH  | 28.4, CH$_2$ |
| 5        | 142.0, C  | 139.3, C  | 144.8, C  |
| 6        | 125.5, CH | 124.3, CH | 118.4, CH |
| 7        | 74.1, CH  | 74.0, CH  | 74.9, CH  |
| 8        | 70.8, C   | 69.9, C   | 70.8, C   |
| 9        | 66.2, CH  | 67.1, CH  | 67.4, CH  |
| 10       | 40.5, CH  | 41.4, CH  | 49.0, CH  |
| 11       | 37.2, CH  | 36.4, CH  | 78.2, C   |
| 12       | 66.6, CH  | 67.0, CH  | 73.4, CH  |
| 13       | 30.2, CH$_2$ | 30.4, CH$_2$ | 30.2, CH$_2$ |
| 14       | 80.5, CH  | 80.1, CH  | 74.8, CH  |
| 15       | 18.6, CH$_3$ | 19.1, CH$_3$ | 14.3, CH$_3$ |
| 16       | 62.5, CH$_2$ | 16.8, CH$_3$ | 27.2, CH$_3$ |
| 17       | 62.5, C   | 61.8, C   | 66.5, C   |
| 18       | 10.3, CH$_3$ | 10.3, CH$_3$ | 10.4, CH$_3$ |
| 19       | 170.5, C  | 170.9, C  | 170.4, C  |
| 20       | 8.9, CH$_3$ | 8.7, CH$_3$ | 16.9, CH$_3$ |
| OAc-2    | 172.9, C  | 170.2, C  |         |
|          | 21.2, CH$_3$ | 21.2, CH$_3$ |         |
| OAc-4    |           | 169.5, C  | 21.0, CH$_3$ |
| OAc-9    | 170.0, C  | 169.2, C  | 168.1, C  |
|          | 21.5, CH$_3$ | 21.1, CH$_3$ | 21.5, CH$_3$ |
| OAc-14   | 170.0, C  | 170.0, C  | 170.4, C  |
|          | 21.2, CH$_3$ | 21.0, CH$_3$ | 21.3, CH$_3$ |
| n-OC(O)Pr$_4$ | 172.3, C  |         |         |
|          | 35.9, CH$_2$ |         |         |
|          | 18.2, CH$_2$ |         |         |
|          | 13.7, CH$_3$ |         |         |

* Multiplicity deduced by DEPT and HSQC spectra.

Figure 2. The COSY ( — ) correlations, selective HMBC (–), and protons with key NOESY correlations ( — ) of 1.
Table 2. $^1$H NMR ($\delta_H$, 400 MHz in CDCl$_3$) data (J in Hz) for briaranes 1–3.

| Position | 1          | 2          | 3          |
|----------|------------|------------|------------|
| 2        | 4.53 s     | 3.45 d (10.4) | 5.13 d (8.4) |
| 3α/β     | 4.59 d (12.0) | 4.27 d (10.4) | 1.67 m; 2.60 ddd (16.0, 14.8, 6.0) |
| 4/4'     | 6.14 s     | 6.05 d (1.2)  | 2.48 br d (16.0); 1.90 m  |
| 6        | 5.51 d (6.0) | 5.29 dq (6.4, 1.6) | 5.19 s  |
| 7        | 5.62 d (6.0) | 5.71 d (6.4)  | 5.19 s  |
| 9        | 5.32 d (8.8) | 5.26 d (9.2)  | 5.78 d (1.2) |
| 10       | 2.64 dd (8.8, 4.8) | 2.55 dd (9.2, 5.2) | 2.14 br s  |
| 11       | 2.41 m     | 2.47 m      | 3.72 dd (12.4, 4.8) |
| 12       | 4.10 m     | 4.05 m      | 3.72 dd (12.4, 4.8) |
| 13α/β    | 1.75 m; 2.01 m | 1.69 m; 2.00 m | 1.67 m; 2.04 m |
| 14       | 4.88 dd (2.8, 2.8) | 4.92 dd (2.8, 2.8) | 4.79 dd (2.8, 2.0) |
| 15       | 0.99 s     | 0.99 br s   | 1.21 s    |
| 16a/b    | 4.35 dd (13.6, 4.4); 4.04 dd (13.6, 9.6) | 1.89 br s | 1.99 s |
| 18       | 1.66 s     | 1.66 s      | 1.77 s    |
| 20       | 1.12 d (6.8) | 1.07 d (7.2) | 1.15 s    |
| OH-2     | 2.79 d (10.4) |             |          |
| OH-3     | 4.30 d (12.0) | 2.87 d (10.4) |          |
| OH-12    | 1.49 d (4.0) | 1.43 d (4.0) |          |
| OH-16    | 3.49 dd (9.6, 4.4) |             |          |
| OAc-2    | 2.08 s     |             | 2.00 s    |
| OAc-4    |              | 2.14 s     |          |
| OAc-9    | 2.34 s     | 2.32 s      | 2.22 s    |
| OAc-14   | 2.15 s     | 2.16 s      | 2.03 s    |
| n-OC(O)Pr-4 | 0.95 t (7.2) | 1.63 tq (7.2) | 2.30 t (7.2) |

The stereochemistry of 1 was deduced from an NOESY experiment (Figure 2) and biogenetic considerations. The NOE correlations of H-10/H-11, H-10/H-12, and H-11/H-12 indicated that these protons were situated on the same face of the structure and were assigned as the α protons since the C-15 methyl is the β-substituent at C-1. The NOE correlation between H$_3$-15 and H-14 implied that H-14 had a β-orientation. H-3 exhibited a correlation with H-10, and, as well as a lack of coupling constants were detected between H-2/H-3 and H-3/H-4, indicating the dihedral angles between H-2/H-3 and H-3/H-4 were approximately 90$^\circ$ and the 2-acetoxy, 3-hydroxy, and 4-$n$-butyroxy groups were β-, β-, and α-oriented, respectively. A correlation from H-4 to H-7, suggested that H-7 was β-oriented. The Z-configuration of C-5/C-6 double bond was confirmed based on the fact that the C-6 olefinic proton ($\delta_H$ 5.53) correlated to one of the C-16 hydroxymethyl protons ($\delta_H$ 4.04). H-9 was found to correlate with H-11, H$_3$-18, and H$_3$-20. From a consideration of molecular model, H-9 was found to be reasonably close to H-11, H$_3$-18, and H$_3$-20, thus, H-9 should be placed on the α face, and Me-18 was β-oriented in the γ-lactone moiety, and the 8,17-epoxy group should be α-oriented. It was found that the NMR signals of 1 were similar to those of a known briarane, briaexcavatolide P (4) (Figure 1) [8], except that the signals corresponding to the Me-16 vinyl methyl in 4 were replaced by signals for a hydroxymethyl group in 1. Additionally, as briaranes 1–5 were isolated along with a known briarane, excavatolide B (6) [6,10,11] from the same target organism, B. excavatum, and the absolute configuration of 6 was determined by a single-crystal X-ray diffraction analysis [6,11]. Therefore, on biogenetic grounds to assume that briaranes 1–5 had the same absolute stereochemistry as that of 6, tentatively, and the configurations of stereogenic carbons of 1 were determined as 1R,2R,3S,4R,7S,8S,9S,10S,11R,12S,14S, and 17R (Supplementary Materials, Figures S1–S10).

Briarenol J (2) had a molecular formula C$_{26}$H$_{36}$O$_{12}$ by its (+)-HRESIMS at m/z 563.21007 (calculated for C$_{26}$H$_{36}$O$_{12}$ + Na, 563.20990). The IR spectrum showed bands at 3483, 1779, and 1727 cm$^{-1}$, consistent with the presence of hydroxy, γ-lactone, and ester groups, respectively, in 2. From the $^{13}$C and DEPT data (Table 2), one trisubstituted double bond was deduced from the signals of two carbons at $\delta_C$ 139.3 (C-5) and 124.3 (CH-6). A methyl-containing tetrasubstituted epoxy group was confirmed from the signals of two oxygenated quaternary carbons at $\delta_C$ 69.9 (C-8) and 61.8 (C-17), and from the
chemical shift of a tertiary methyl ($\delta_H$ 1.66, 3H, s; $\delta_C$ 10.3, CH$_3$-18; Tables 1 and 2). Four carbonyl resonances at $\delta_C$ 170.9, 170.0, 169.5, and 169.2 in the $^{13}$C spectrum confirmed the presence of a $\gamma$-lactone and three esters. All the esters were identified as acetates by the presence of three methyl singlet resonances in the $^1$H NMR spectrum at $\delta_H$ 2.32, 2.16, and 2.14, respectively.

Coupling constants information in the COSY spectrum of 2 enabled identification of H-6/H-7, H-9/H-10/H-11/H-12/H$_2$-13/H-14, H-11/H$_3$-20, and H-6/H$_3$-16 (by allylic coupling; Figure 3), these data, together with the HMBC experiment (Figure 3), the molecular framework of 2 could be established. The HMBC also indicated that the acetoxy groups should be attached at C-4, C-9, and C-14, respectively. Thus, the remaining hydroxy groups have to be positioned at C-2, C-3, and C-12, as indicated by the COSY correlations between H-2/OH-2, H-3/OH-3, and H-12/OH-12.

![Figure 3. The COSY (---) correlations, selective HMBC (--), and protons with key NOESY correlations (-----) of 2.](image)

The stereochimistry of 2 was elucidated from the NOE interactions observed in a NOESY experiment (Figure 3) and by the vicinal $^1$H–$^1$H coupling constant analysis. In the NOESY spectrum, correlations were observed between H-10 with H-3 and H-12; and H-12 correlated with H-11, indicating that these protons should be $\alpha$-oriented. H-14 gave a correlation with H$_2$-15, confirming the $\beta$-orientation for this proton. H-2 showed a correlation with H-14, and a lack of coupling constant was detected between H-2/H-3, indicating the dihedral angle between H-2/H-3 is approximately 90° and the 2-hydroxy group was $\beta$-oriented. H-4 exhibited correlations with H-7 and 2-hydroxy proton, confirming the $\beta$-orientations for H-4 and H-7. H-9 was found to show correlations with H-11, H$_3$-18, and H$_3$-20, and from molecular models, H-9 and H$_3$-18 should be placed on the $\alpha$- and $\beta$-face, respectively. The $Z$-configuration of C-5/C-6 double bond was elucidated by a correlation between H-6 and H$_3$-16. The NMR data of 2 were found to be similar to those of a known briarane, briaexavatin P (5) [9]. It was found that the 2-acetoxy substituent in 5 was replaced by a hydroxy group in 2. By comparison of the proton and carbon chemical shifts, coupling constants, NOESY correlations, and rotation value of 2 with those of 5, the stereochimistry of 2 was confirmed to be the same as that of 5, and the configurations of the stereogenic centers of 2 were assigned as 1S,2R,3R,4R, 7S,8S,9S,10S,11R,12S,14S, and 17R (Supplementary Materials, Figures S11–S20).

Briarane 3 (briarenol K) was found to have a molecular formula of C$_{26}$H$_{36}$O$_{11}$ based on its (+)-HRESIMS peak at $m/z$ 547.21514 (calculated for C$_{26}$H$_{36}$O$_{11}$ + Na, 547.21498). Its absorption peaks in the IR spectrum showed ester carbonyl, $\gamma$-lactone, and broad OH stretching at 1739, 1780, and 3468 cm$^{-1}$, respectively. The $^{13}$C NMR spectrum indicated that three esters and a $\gamma$-lactone were present, as carbonyl resonances were observed at $\delta_C$ 168.1, 170.2, 170.4, and 170.4, respectively (Table 1). The $^1$H NMR data also indicated that presence of three acetate methyls at $\delta_H$ 2.22, 2.03, and 2.00 (each 3H × s; Table 2). It was found that the spectroscopic data of 3 were similar to those of a known briarane, briareolide B (7) [12]; however, by comparison of the $^1$H and $^{13}$C NMR chemical shifts of CH-12 oxymethine ($\delta_H$ 3.72, 1H, dd, $J = 12.4, 4.8$ Hz; $\delta_C$ 73.4), CH$_2$-13 sp$^3$ methylene ($\delta_H$ 1.67, 1H,
m; 2.04, 1H, m; δC 30.2), C-11 oxygenated quaternary carbon (δC 78.2), and Me-20 tertiary methyl (δH 1.15, 3H, s; δC 16.9) of 3 with those of 7 (δH 3.56, 1H, m; δC 73.9), CH-12; δH 2.03, 1H, m; 2.12, 1H, m; δC 27.6, CH2-13; δC 74.7, C-11; δH 1.16, 3H, s; δC 22.5, Me-20) [12] showed that the hydroxy group at C-12 in 3 was β-oriented. The locations of the functional groups were further confirmed by other HMBC and COSY correlations (Figure 4), and hence briarenol K was assigned the structure of 3. The NOESY spectrum exhibited a correlation from H-10 to H-12, further supporting that H-12 was α-oriented and the stereogenic centers of 3 were assigned as 15, 25, 7S, 8S, 9S, 10S, 11S, 12S, 14S, and 17R, by the correlations observed in a NOESY spectrum (Figure 4) and this compound was found to be the 12-epimer of briareolide B (7) [12] (Supplementary Materials, Figures S21–S30).

![Figure 4](image)

**Figure 4.** The COSY (—) correlations, selective HMBC (→), and protons with key NOESY correlations (••••) of 3.

The inhibition effects of briaranes 1–5 on the release of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein from lipopolysaccharides (LPS)-stimulated RAW 264.7 were assessed. The results showed that briarane 4 reduced the release of iNOS and COX-2 to 35.37% and 54.61% at a concentration of 10 μM, respectively (Figure 5 and Table 3). Briarane 1 was found to be weaker than those of 4 in term of reducing the expression of iNOS and COX-2, indicating that the hydroxy group at C-16 in 1 reduced the activity.

![Figure 5](image)

**Figure 5.** Western blotting showed that briarane 4 downregulated the expression of iNOS and COX-2. Data were normalized to the cells treated with LPS only, and cells treated with dexamethasone (Dex; 10 μM) were used as a positive control. Data are expressed as the mean ± SEM (n = 2–4), *Significantly different from cells treated with LPS (p < 0.05).
Table 3. Effects of briaranes 1–5 on LPS-induced pro-inflammatory iNOS and COX-2 protein expression in macrophages.

|          | iNOS     | COX-2     | β-Actin   | n  |
|----------|----------|-----------|-----------|----|
|          | Expression (% of LPS) | Expression (% of LPS) | Expression (% of LPS) |    |
| Negative Control | 1.71 ± 0.13 | 0.62 ± 0.09 | 120.48 ± 1.28 | 2  |
| LPS      | 100.00 ± 4.53 | 100.00 ± 6.05 | 100.00 ± 3.09 | 4  |
| 1        | 60.27 ± 7.05 | 80.63 ± 2.32 | 100.29 ± 2.46 | 4  |
| 2        | 60.94 ± 4.89 | 79.65 ± 4.27 | 98.29 ± 3.35 | 4  |
| 3        | 66.64 ± 4.79 | 97.28 ± 5.66 | 100.49 ± 6.44 | 4  |
| 4        | 35.37 ± 4.94 | 54.61 ± 4.03 | 105.56 ± 2.83 | 4  |
| 5        | 62.36 ± 5.42 | 72.63 ± 2.6  | 104.79 ± 2.76 | 4  |
| Dexamethasone | 41.00 ± 2.63 | 3.73 ± 0.35 | 104.24 ± 5.82 | 2  |

Data were normalized to those of cells treated with LPS alone, and cells treated with dexamethasone were used as a positive control. Data are expressed as the mean ± SEM (n = 2–4).

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotation values were measured using a Jasco P-1010 digital polarimeter (Japan Spectroscopic, Tokyo, Japan). IR spectra were measured on a Thermo Scientific Nicolet iS5 FT-IR spectrophotometer (Waltham, MA, USA). NMR spectra were taken on a Jeol Resonance ECZ 400 S NMR spectrometer (Tokyo, Japan), using the residual CHCl$_3$ signal ($\delta_H$ 7.26 ppm) and CDCl$_3$ ($\delta_C$ 77.1 ppm) as the internal standard for $^1$H and $^{13}$C NMR, respectively; coupling constants ($J$) are presented in Hz. ESIMS and HRESIMS were recorded using a Bruker 7 Tesla solarix FTMS system. Column chromatography was carried out with silica gel (230–400 mesh, Merck, Darmstadt, Germany). TLC was performed on plates precoated with Kieselgel 60 F$_{254}$ (0.25-mm-thick, Merck, Darmstadt, Germany), then sprayed with 10% H$_2$SO$_4$ solution followed by heating to visualize the spots. Normal-phase HPLC (NP-HPLC) was performed using a system comprised of a Hitachi L-7100 pump (Tokyo, Japan) and a Rheodyne 7725i injection port (Rohnert Park, CA, USA). Reverse-phase HPLC (RP-HPLC) was performed using a system comprised of a Hitachi L-2130 pump (Tokyo, Japan), a Hitachi L-2455 photodiode array detector (Tokyo, Japan), and a Rheodyne 7725i injection port (Rohnert Park, CA, USA). A semipreparative normal-phase column (YMC-Pack SIL, S-5 µm, 250 mm × 20 mm, Sigma-Aldrich, St. Louis, MO, USA) was used for NP-HPLC. A semipreparative reverse-phase column (Luna, 5 µm, C18(2) 100 Å, AXIA Packed, 250 mm × 21.2 mm; Phenomenex, Torrance, CA, USA) was used for RP-HPLC.

3.2. Animal Material

Specimens of *B. excavatum* were collected in June 2017 by hand with self-contained underwater breathing apparatus (SCUBA) divers off the coast of Lanyu Island (Orchid Island), Taiwan. The samples were then stored in a −20 °C freezer until extraction. A voucher specimen was deposited in the National Museum of Marine Biology and Aquarium, Taiwan (NMMB-TW-SC-2017-418). Identification of the species of this organism was performed by comparison as described in previous publications [1–4].

3.3. Extraction and Isolation

The freeze-dried and sliced bodies (wet/dry weight = 1344/568 g) of the specimen were extracted with supercritical CO$_2$ to give 58.9 g of extract. Partial extract (36.4 g) was then applied on silica gel column and eluted with gradients of $n$-hexane/EtOAc to furnish fractions A–K. Fraction F was purified by NP-HPLC using a mixture of $n$-hexane/acetone (4:1) to yield fractions F1–F13. Fraction F6 was repurified by RP-HPLC, using a mixture of MeOH/H$_2$O (60:40; at a flow rate = 4 mL/min) to afford 4 (6.7 mg). Fraction G was separated by NP-HPLC, using a mixture of $n$-hexane/acetone (3:1) to yield fractions G1–G12. Fractions G6 and G7 were repurified by RP-HPLC using a mixture of MeOH/H$_2$O.
(60:40; at a flow rate = 4.0 mL/min) to afford 5 (1.3 mg) and 3 (1.0 mg), respectively. Fraction H was separated by NP-HPLC using a mixture of n-hexane and acetone (3:1) to yield fractions H1–H18. Fractions H12 and H15 were repurified by RP-HPLC, using a mixture of MeOH/H2O (60:40; at a flow rate = 4.0 mL/min) to afford 2 (2.1 mg) and 1 (0.6 mg), respectively.

Briarenol I (1): Amorphous powder; [α]D22° + 207 (c 0.03, CHCl3), IR (ATR) νmax 3524, 1783, 1736, 1222, 891 cm−1; 13C (100 MHz, CDCl3) and 1H (400 MHz, CDCl3) NMR data (see Tables 1 and 2); ESIMS: m/z 649 [M + Na]+; HRESIMS m/z 649.24677 (calculated for C30H42O14 + Na, 649.24668).

Briarenol J (2): Amorphous powder; [α]D26° + 140 (c 0.08, CHCl3), IR (ATR) νmax 3483, 1779, 1727, 1220, 890 cm−1; 13C (100 MHz, CDCl3) and 1H (400 MHz, CDCl3) NMR data (see Tables 1 and 2); ESIMS: m/z 563 [M + Na]+; HRESIMS m/z 563.21007 (calculated for C26H36O12 + Na, 563.20990).

Briarenol K (3): Amorphous powder; [α]D23° + 37 (c 0.06, CHCl3), IR (ATR) νmax 3468, 1780, 1739, 1255, 892 cm−1; 13C (100 MHz, CDCl3) and 1H (400 MHz, CDCl3) NMR data (see Tables 1 and 2); ESIMS: m/z 547 [M + Na]+; HRESIMS m/z 547.21514 (calculated for C26H36O11 + Na, 547.21498).

Briarcavatolide P (4): Amorphous powder; [α]D24° + 182 (c 0.3, CHCl3) (ref. [8], [α]D27D + 167 (c 1.0, CHCl3)), IR (ATR) νmax 3513, 1783, 1731, 1218, 889 cm−1; 1H and 13C NMR data were found to be in agreement with previous study [8]; ESIMS: m/z 635 [M + Na]+.

Briarcavatin P (5): Amorphous powder; [α]D25° + 134 (c 0.05, CHCl3) (ref. [9], [α]D25D + 198 (c 0.08, CHCl3)), IR (ATR) νmax 3503, 1785, 1735, 1240, 889 cm−1; 1H and 13C NMR data were found to be in agreement with previous study [9]; ESIMS: m/z 605 [M + Na]+.

3.4. In Vitro Anti-inflammatory Assay

The proinflammatory suppression assay was employed to assess the activities of the isolated compounds 1–5 against the release of iNOS and COX-2 from macrophage cells as the literature reported [13–15].

4. Conclusions

B. excavatum was demonstrated to have a wide structural diversity of briarane-type diterpenoids that possessed various pharmacological properties, especially in anti-inflammatory activity. In our continued study on B. excavatum, three previously unreported briaranes, briarenols I–K (1–3), along with the known analogues, briarcavatolide P (4) and briarcavatin P (5), were isolated. In the present study, the anti-inflammatory activity of 1–5 was assessed using inhibition of pro-inflammatory iNOS and COX-2 release from macrophages. The results indicated that briarcavatolide P (4) showed the most potent suppressive effect on iNOS release.

Supplementary Materials: The Supplementary Materials are available online. ESIMS, HRESIMS, IR, 1D and 2D NMR spectra of new compounds 1–3.

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