

Briaexcavatins A and B, novel briaranes from the octocoral *Briareum excavatum*

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Abstract—Two novel briaranoid derivatives, designated as briaexcavatins A (**1**) and B (**2**), along with two known metabolites, excavatolides B and C (**3** and **4**), were isolated from the octocoral *Briareum excavatum*. Both compounds **1** and **2** possess unprecedented 5,6-epoxy moiety in the 10-membered ring. Diterpenoid **1** possesses a novel pentacyclic skeleton with a ϵ -lactone. The structures of diterpenoids **1** and **2** were elucidated by interpretation of spectral data and the absolute stereochemistry was established by application of modified Mosher's method on **3**.

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Previous studies on the octocoral *Briareum excavatum* have resulted in the isolation of a series of novel metabolites with briarane carbon skeleton, including stecholides I–N and 16-hydroxystecholide C acetate,¹ excavatolides A–Z,^{2–5} briaexcavatolides A–Z,⁶ and briartheins A–C.⁷ Some of these metabolites have been reported to exhibit significant cytotoxicity. Recently, our continuing investigation on the chemical constituents of *B. excavatum* has afforded four metabolites including two novel diterpenoids, briaexcavatins A (**1**) and B (**2**), and two known metabolites, excavatolides B and C (**3** and **4**).² We describe herein the isolation, structure determination, and biogenetic relationships of these marine metabolites.

The organism of *B. excavatum* (wet wt, 1.0 kg), collected off the coast of the southern most tip of Taiwan in October 2003, was minced and extracted with EtOAc. The EtOAc-soluble portion was subjected to column chromatography. A fraction eluted with *n*-hexane/EtOAc (2:1) was purified by normal-phase HPLC (*n*-hexane/

EtOAc 4:1 to 2:1) to yield **1** (1.2 mg), **2** (1.5 mg), **3** (47.2 mg), and **4** (40.2 mg).

The absolute configuration of known metabolite **3** was determined by a modified Mosher method.⁸ Comparison of ¹H NMR chemical shifts between the (*R*)- and (*S*)-MTPA esters of **3** (**3a** and **b**) (Δ values shown in Fig. 1) led to the assignment of the *S*-configuration at C-12. Therefore, the absolute structure of **3** was determined as shown in formula **3**. Due to biogenetic considerations, the absolute configurations of C-1, C-2, C-3, C-7, C-8, C-9, C-10, C-11, C-12, C-14, and C-17 of other briaranes isolated from the same organism, reported previously or discovered by the present study, are suggested to be the same as those of **3**, in cases that they have the similar substitution pattern at each of the above carbons.

Briaexcavatin A (**1**), $[\alpha]_D^{25} -6$ (*c* 0.12, CHCl₃), was isolated as a colorless gum and had a molecular formula C₂₈H₃₆O₁₄, as determined by HRESIMS (*m/z* Calcd: 597.2183. Found: 597.2178 [M+H]⁺) indicating eleven degrees of unsaturation. The presence of lactone and ester groups in **1** were evidenced by the IR absorptions at 1770, 1760, and 1734 cm⁻¹. The gross structure of **1** was established by NMR experiments and ¹³C resonances at δ 171.4 (s), 170.1 (s), 169.4 (s), 168.9 (s),

Keywords: Briarane; Briaexcavatin A; Briaexcavatin B; *Briareum excavatum*; Octocoral.

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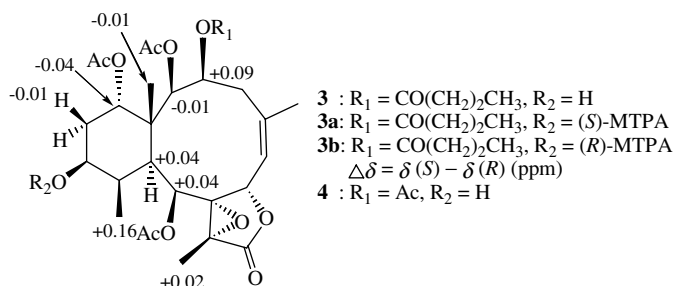


Figure 1. The structures of briaranes **3** and **4** and the ^1H NMR chemical shifts differences [$\delta(S)\text{-MTPA} - \delta(R)\text{-MTPA}$] of the MTPA esters of briarane **3**.

Table 1. ^1H and ^{13}C NMR chemical shifts for diterpenoids **1** and **2**

C/H	1		2	
	$^1\text{H}^a$	$^{13}\text{C}^b$	$^1\text{H}^a$	$^{13}\text{C}^b$
1		45.6 (s) ^c		43.4 (s)
2	5.92 br s	69.1 (d)	5.79 d (2.4)	74.6 (d)
3	4.93 br s	70.5 (d)	5.41 ddd (4.8, 2.4, 2.0)	71.9 (d)
4	2.20 m (ca.) ^c	33.9 (t)	2.20 dd (16.8, 2.0)	33.6 (t)
4'	2.33 br d (16.4) ^d		2.54 dd (16.8, 4.8)	
5		61.8 (s)		60.8 (s)
6	3.07 d (8.0)	63.4 (d)	3.06 d (8.0)	62.8 (d)
7	4.58 d (8.0)	78.4 (d)	4.45 d (8.0)	77.6 (d)
8		72.0 (s)		69.5 (s)
9	5.80 br s	67.7 (d)	5.30 d (7.2)	66.9 (d)
10	2.49 br s	47.9 (d)	2.81 m	44.0 (d)
11	4.99 br q (5.6)	71.5 (d)	3.09 m	44.3 (d)
12		168.1 (s)		206.1 (s)
13	2.51 d (13.6)	37.2 (t)	2.80 m	41.1 (t)
13'	3.41 dd (13.6, 13.6)			
14	4.91 br d (13.6)	72.3 (d)	5.10 t (7.2)	77.2 (d)
15	1.53 s (ca.) ^c	19.3 (q)	1.28 s	18.3 (q)
16	1.36 s	21.7 (q)	1.44 s	22.2 (q)
17		63.5 (s)		61.4 (s)
18	1.54 s (ca.) ^c	10.6 (q)	1.58 s	10.5 (q)
19		170.1 (s)		170.4 (s)
20	1.54 d (ca. 5.6) ^c	20.5 (q)	1.27 d (6.8)	13.8 (q)
Acetate	2.20 s	21.1 (q)	2.24 s	21.5 (q)
methyls	2.14 s	20.9 (q)	2.16 s	21.3 (q)
	2.07 s	20.9 (q)	2.00 s	21.3 (q)
	1.98 s	20.9 (q)	2.03 s	21.0 (q)
Acetate		171.4 (s)		170.3 (s)
carbonyls		169.4 (s)		170.1 (s)
		168.9 (s)		169.6 (s)
		167.9 (s)		168.9 (s)

^a Spectra recorded at 400 MHz in CDCl_3 .

^b Spectra recorded at 100 MHz in CDCl_3 .

^c The proton signals of H-4, H₃-15, H₃-18, H₃-20, and H₂O are overlapping.

^d J value (in Hz) in parentheses were measured by ^1H NMR and $^1\text{H}-^1\text{H}$ COSY spectra. Thus, the δ and J values of these protons were deduced by the correlations of HSQC and $^1\text{H}-^1\text{H}$ COSY spectra.

^e Multiplicity were deduced by HSQC and DEPT spectra and indicated by usual symbol.

168.1 (s), and 167.9 (s) (Table 1). Among the above carbons, four were identified as acetate carbonyls by the presence of four methyl resonances in the ^1H NMR

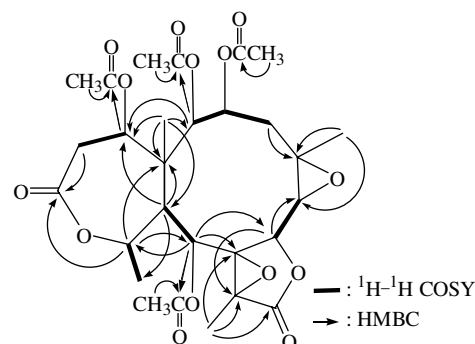


Figure 2. $^1\text{H}-^1\text{H}$ COSY and selective HMBC correlations of **1**.

spectrum at δ 2.20 (3H, s), 2.14 (3H, s), 2.07 (3H, s), and 1.98 (3H, s) (Table 1). According to the above data, briarane **1** was found to be a pentacyclic compound with two lactones, as no any other unsaturated functional group could be found. A tetrasubstituted epoxide containing a methyl substituent was elucidated from the signals of two oxygen-bearing quaternary carbons at δ 72.0 (s, C-8) and 63.5 (s, C-17), and was further confirmed from the proton signal of a methyl singlet resonating at δ 1.54 (3H, s, H₃-18). In addition, a trisubstituted epoxide containing a methyl substituent was deduced from the signals of an oxymethine (δ_{H} 3.07, 1H, d, $J = 8.0$ Hz, H-6; δ_{C} 63.4, d, C-6), a quaternary oxygen-bearing carbon (δ_{C} 61.8, s, C-5), and a methyl singlet resonating at δ 1.36 (3H, s, H₃-16). Moreover, proton signals for a tertiary methyl, a secondary methyl, two methylenes, and seven methines including six oxygenated ones were further assigned (Table 1) by the assistance of HSQC spectrum. From the $^1\text{H}-^1\text{H}$ COSY and HMBC correlations (Fig. 2), the epoxy groups positioned at C-5/C-6 and C-8/C-17, and acetoxy groups positioned at C-2, C-3, C-9, and C-14 were established. Furthermore, the ϵ -lactone formed by insertion of an oxygen between C-11 and C-12 was confirmed by an HMBC correlation between H-11 (δ 4.99) and C-12 (δ 168.1). Thus, the planar structure of **1** was fully established.

The relative stereochemistry of **1** was elucidated from the NOE interactions observed in an NOESY experiment (Fig. 3). Due to the α orientation of H-10, the ring juncture C-15 methyl should be β -oriented as no NOE

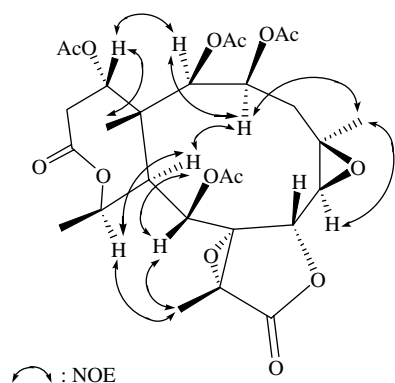


Figure 3. Selective NOESY correlations of **1**.

correlation was observed between H-10 and H₃-15. In the NOESY spectrum of **1**, H-10 displayed correlations with H-3, H-9, and H-11, and H-3 displayed correlations with H-2 and H₃-16. Thus, H-2, H-3, H-9, H-10, H-11, and H₃-16 are located on the same face of the molecule and assigned as α protons. C-20 methyl was found to be β -substituted at C-11, as the α -oriented H-10 did not show correlation with H₃-20, but instead exhibited an NOE correlation with H-11. The oxygen of 5,6-epoxy group was positioned on the β face by a strong NOE correlation observed between H₃-16 and H-6, but not between H-6 and H-7. H-14 was found to exhibit NOE responses with H-2 and H₃-15, but not with H-10, revealing the α -orientation of 14-OAc. Furthermore, the signal of H₃-18 showed NOE correlations with H-9 and H-11. From a detailed consideration of molecular models, H₃-18 was found to be reasonably close to H-9 and H-11, when H₃-18 was placed on the β face in the γ -lactone moiety. Furthermore, the molecular mechanics calculations were performed to study the conformational behavior of briarane **1**. The conformational search suggested the most stable conformation as shown in Figure 4. It was found that the calculated distances between those protons having key NOE correlations of **1** are all shorter than 3 Å as shown in Table 2.

Briaexcavatin B (**2**), isolated as a colorless gum, $[\alpha]_D^{25} +15$ (c 0.08, CHCl₃), has a molecular formula C₂₈H₃₆O₁₃ from its HRESIMS (m/z Calcd: 603.2054. Found: 603.2053, [M+Na]⁺), indicating eleven degrees of unsaturation. The IR spectrum of **2** showed the presence of γ -lactone, ester, and ketone functionalities (ν_{\max} 1792, 1748, and 1717 cm⁻¹). The ¹³C and ¹H NMR spectrum revealed the presence of a ketone (δ_C 206.1, s, C-12), a γ -lactone (δ_C 170.4, s, C-19), four acetates (δ_C 170.3, s; 170.1, s; 169.6, s; 168.9, s; δ_H 2.24, 3H, s; 2.16, 3H, s; 2.03, 3H, s; 2.00, 3H, s), a tetrasubstituted epoxide (δ_C 69.5, s, C-8; 61.4, s, C-17) containing a methyl substituent (δ_H 1.58, 3H, s, H₃-18; δ_C 10.5, q, C-18) and a trisubstituted epoxide (δ_H 3.06, 1H, d, J = 8.0 Hz, H-6; δ_C 62.8, d, C-6; 60.8, s, C-5) containing a methyl substituent (δ_H 1.44, 3H, s, H₃-16; δ_C 22.2, q, C-16) (Table 1). The gross structure of **2** was established by the assistance of ¹H–¹H COSY and HMBC spectra (Fig. 5). The structure of **2** with a 5,6-epoxy group was deduced from the HMBC correlations from H₂-4 to C-5, H-7 to C-6 and H₃-16 to C-4, C-5, C-6. The

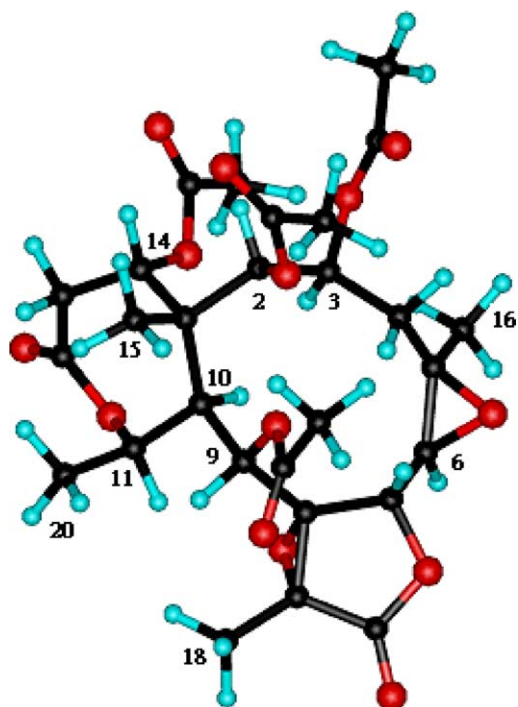


Figure 4. Stereoview of **1** generated from computer modeling.

Table 2. Calculated distances between selective protons having key NOE correlations of **1**^a

H/H	Distance (Å)
H-2/H-3	2.69
H-2/H-14	2.16
H-3/H-10	2.08
H-3/H ₃ -16	2.53
H-6/H ₃ -16	2.46
H-9/H-10	2.95
H-9/H ₃ -18	2.35
H-10/H-11	2.37
H-11/H ₃ -18	2.38
H-14/H ₃ -15	2.39
H ₃ -15/H ₃ -20	2.16
H ₃ -18/H ₃ -20	2.70

^a The calculated distance between H-10 (α) and H₃-15 (β) is 3.83 Å.

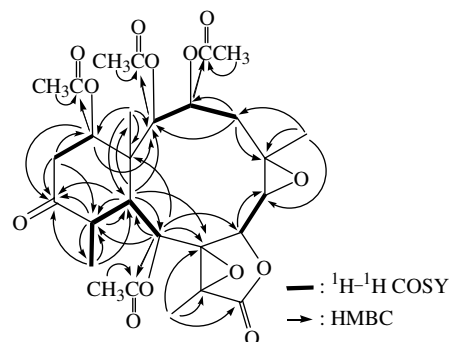
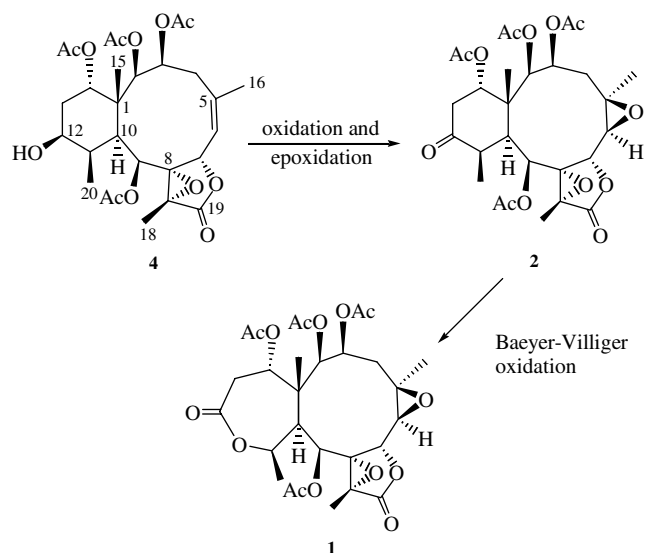


Figure 5. ¹H–¹H COSY and selective HMBC correlations of **1**.

ketone group attaching at C-12 was confirmed by the HMBC correlations from protons H-10, H-11, H₂-13, H-14, and H₃-20 to the carbonyl carbon appeared at δ



Scheme 1. Plausible biogenetic relationships for compounds **1**, **2**, and **4**.

206.1. The acetoxy groups positioned at C-2, C-3, C-9, and C-14 were also confirmed by HMBC correlations and by comparison of NMR data with those of **1**. The relative stereochemistry of **2** was deduced from a NOESY experiment (Supplementary data, Figure S1) and the configurations of the twelve chiral centers including C-1, C-2, C-3, C-5, C-6, C-7, C-8, C-9, C-10, C-11, C-14, and C-17 were found to be the same as those of **1**. Thus, by above findings, and by comparison of the NMR data with those of **1** and a known metabolite, excavatolide C (**4**),² established **2** to be the 12-keto-5 β ,6 β -epoxy derivative of excavatolide C.

A plausible biogenetic pathway for **1** and **2** from **4** was proposed as illustrated in Scheme 1. Oxidation of the secondary alcohol at C-12 and epoxidation of 5,6-double bond in **4** would yield metabolite **2**, which was further lactonized to **1** by the Baeyer–Villiger oxidation.

It has to be noted here that **1** is the first 11,12-secobriarane possessing a ϵ -lactone. Also, to the best of our knowledge, briaranes like **1** and **2** containing the 5,6-epoxide have not been found previously.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.tetlet.2005.10.160](https://doi.org/10.1016/j.tetlet.2005.10.160). ¹H and ¹³C NMR spectra for compounds **1** and **2** and selective NOESY correlations of **2**.

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