

Briaexcavatins G and H, Two New Briaranes from the Octocoral *Briareum excavatum*

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Two new briarane-related diterpenoids, designated as briaexcavatins G (**1**) and H (**2**), were isolated from the octocoral *Briareum excavatum*, collected off southern Taiwan coast. The structures, including the absolute stereochemistry of these two new metabolites, were established by spectroscopy and chemical conversion. The configuration of briarane **2** was further supported by molecular mechanics calculations. Briarane **1** showed weak inhibitory effects on human neutrophil elastase release.

Octocorals belonging to the *Briareum* genus in Briareidae family, which includes four known species distributed in Caribbean Sea and Indo-Pacific Ocean. These octocorals are *B. asbestinum*, *B. excavatum*, *B. polyanthes*, and *B. stechei*. *B. excavatum* (Nutting, 1911) is an octocoral with encrusting, purplish, and sheet-forming colonies that overgrow and cover substratum, most of which were dead hard corals. Previous studies on the chemical constituents of *B. excavatum* reported the isolation and structure determination of sixty-eight new briarane-type diterpenoids, including stecholides I–N and 16-hydroxystecholide C acetate,[1] excavatolides A–Z,[2–5] briaexcavatolides A–Z,[6–11] briaexcavatins A–F,[12,13] and briantheins A–C,[14] and the compounds of this type were proven to possess extensive biological activity.[15,16] Further investigation on *B. excavatum* led us to isolate two new briarane-type diterpenoids, briaexcavatins G (**1**) and H (**2**) (Chart 1). This paper deals with the isolation, structure determination, and biological activity of the new briaranes **1** and **2**.

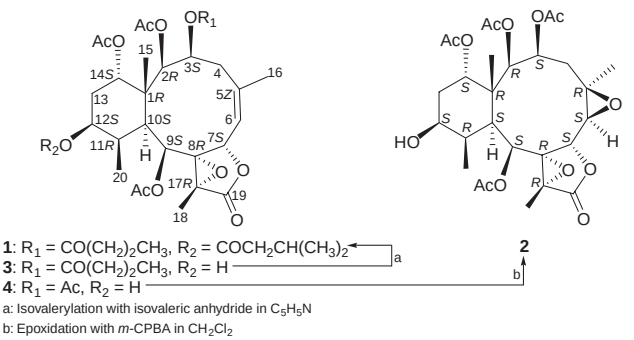

Chart 1.

Experimental

General Experimental Procedures. Melting points were determined on a FARGO apparatus and were uncorrected. Optical rotation values were measured with a JASCO P-1010 digital polarimeter at 25 °C. Infrared spectra were obtained on a VARIAN DIGLAB FTS 1000 FT-IR spectrometer. Low-resolution mass data were obtained by FAB with a VG QUATTRO GC/MS spectrometer. High-resolution mass data were recorded by ESI FT-MS on a BRUKER APEX II mass spectrometer. NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR at 400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively, in $CDCl_3$ or Me_2CO-d_6 using TMS as an internal standard. Column chromatography was performed on silica gel 60 (230–400 mesh) (Merck, Darmstadt, Germany). TLC spots (Si gel 60 F_{254}, Merck) were detected with an UV_{254} lamp and by 10% H_2SO_4 followed by heating at 120 °C for 3 min. All solvents and reagents used were analytical grade.

Animal Material. Specimen of the octocoral *B. excavatum* was collected by hand using scuba off the southern Taiwan coast in October 2003 at a depth of −10 m. Living reference specimens are being maintained in the authors' cultivating tanks, and a voucher specimen was deposited in the National Museum of Marine Biology and Aquarium (NMMBA). This organism was identified from descriptions.[17,18]

Extraction and Isolation. The organism (wet weight 1.0 kg) was collected and freeze-dried. The freeze-dried material (0.57 kg) was minced and extracted with EtOAc. The extract was separated by silica-gel column chromatography, using hexane and

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Table 1. ^{1}H and ^{13}C NMR Data for Briaranes **1** and **2**

	1		**2**	
Position	^{1}H[a)]	^{13}C[b)]	^{1}H[c)]	^{13}C[d)]
1		43.7 (s)[f)]		43.6 (s)
2	5.15 br s	81.7 (d)	5.10 br s	79.6 (d)
3	5.74 d (6.4)[e)]	73.2 (d)	5.81 br d (6.0)	73.2 (d)
4α	1.95 m	34.3 (t)	1.95 m	32.7 (t)
β	3.73 dd (15.2, 6.4)		2.94 dd (16.0, 9.2)	
5		139.4 (s)		60.0 (s)
6	5.36 d (6.0)	121.9 (d)	3.11 d (8.8)	62.1 (d)
7	5.29 d (6.0)	74.0 (d)	4.61 d (8.8)	77.4 (d)
8		69.0 (s)		67.8 (s)
9	5.31 d (10.0)	66.2 (d)	5.54 d (10.4)	64.6 (d)
10	3.00 dd (10.0, 5.2)	40.0 (d)	2.78 dd (10.4, 5.2)	41.2 (d)
11	2.58 m	32.5 (d)	2.55 m	35.5 (d)
12	5.03 dt (12.0, 4.8)	69.3 (d)	4.01 m	65.8 (d)
13	1.86 br t (14.0)	27.3 (t)	1.71 m	29.2 (t)
13′	1.97 m		2.04 m	
14	4.84 br s	80.7 (d)	4.69 br s	81.7 (d)
15	0.82 s	18.2 (q)	0.81 s	18.4 (q)
16	1.92 s	22.6 (q)	1.68 s	22.8 (q)
17		60.3 (s)		59.6 (s)
18	1.58 s	10.3 (q)	1.52 s	9.8 (q)
19		172.0 (s)		172.2 (s)
20	1.04 d (7.2)	10.5 (q)	1.02 d (6.8)	9.2 (q)
OH-12			4.41 d (3.6)	
Acetate	2.36 s	21.8 (q)	2.33 s	22.8 (q)
methyls	2.35 s	22.4 (q)	2.19 s	22.1 (q)
	2.16 s	22.4 (q)	2.18 s	21.3 (q)
			1.84 s	20.7 (q)
Acetate		169.8 (s)		172.0 (s)
carbonyls		170.6 (s)		170.6 (s)
		171.2 (s)		170.1 (s)
				169.6 (s)
n-Butyrate	0.87 t (7.6)	13.9 (q)		
	1.55 m	18.4 (t)		
	2.13 t (6.8)	36.2 (t)		
		172.7 (s)		
Isovalerate	0.89 d (6.4, 2 × 3H)	22.6 (q)		
		22.7 (q)		
	2.03 m	25.8 (d)		
	2.11 d (6.8)	44.0 (t)		
		171.9 (s)		

a) Spectra recorded at 400 MHz in $CDCl_3$ at −20 °C. b) Spectra recorded at 100 MHz in $CDCl_3$ at −20 °C. c) Spectra recorded at 400 MHz in Me_2CO-d_6 at −50 °C. d) Spectra recorded at 100 MHz in Me_2CO-d_6 at −50 °C. e) *J* Value (in Hz) in Parentheses. f) Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols. The values are downfield in ppm from TMS.

hexane–EtOAc mixtures of increased polarity. Briarane **1** was eluted with hexane–EtOAc (5:1) and **2** with hexane–EtOAc (2:1).

Briaexcavatin G (1). White powder (54.2 mg); mp 188–189 °C; $[\alpha]_D^{25}$ +26° (*c* 0.60, $CHCl_3$); IR (neat) ν_{max} 1786, 1735 cm^{-1}; ^{1}H ($CDCl_3$, 400 MHz, −20 °C) and ^{13}C ($CDCl_3$, 100 MHz, −20 °C) NMR data, see Table 1; FAB-MS *m*/*z* 701 $(M + Na)^+$, 679 $(M + H)^+$, 619, 591, 577, 559, 531, 517, 489, 471, 457, 429, 369, 309; HR-ESI-MS *m*/*z* 701.3146 (calcd for $C_{35}H_{50}O_{13}$ + Na, 701.3149).

Briaexcavatin H (2). White powder (11.2 mg); mp 164–165 °C; $[\alpha]_D^{25}$ −31° (*c* 0.45, $CHCl_3$); IR (neat) ν_{max} 3477, 1790, 1740 cm^{-1}; ^{1}H (Me_2CO-d_6, 400 MHz, −50 °C) and ^{13}C (Me_2CO-d_6, 100 MHz, −50 °C) NMR data, see Table 1; FAB-MS *m*/*z* 605 $(M + Na)^+$, 583 $(M + H)^+$, 565, 523, 505, 463, 445, 403, 385, 343, 325; HR-ESI-MS *m*/*z* 605.2208 (calcd for $C_{28}H_{38}O_{13}$ + Na, 605.2210).

Isovalerylation of Excavatolide B (3). Excavatolide B (**3**) (5.0 mg) was stirred with isovaleric anhydride (2 mL) in pyridine (2 mL) for 96 h at room temperature. After evaporation of excess reagent, the residue was separated by column chromatography on silica gel to give pure briaexcavatin G (**1**) (hexane/EtOAc, 5:1, 4.1 mg, 72%); physical and spectral data were in full agreement with those of natural product **1**.

Reaction of Excavatolide C (4) with *m*-CPBA. Excavatolide

C (**4**) (22.0 mg) was stirred with 3-chloroperbenzoic acid (*m*-CPBA, 10.0 mg) in CH_2Cl_2 (3 mL) for 48 h at room temperature, and the resultant mixture was separated by column chromatography on silica gel to give pure briaexcavatin H (**2**) (hexane/EtOAc, 2:1, 19 mg, 83%). Physical and spectral data were full agreement with those of natural product **2**.

(*S*)- and (*R*)-MTPA Esters of 4. To a solution of compound **4** (5.0 mg) in pyridine (1.0 mL) was added (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride (2.3 μL) at room temperature for 4–5 h. The reaction mixture was concentrated to dryness under reduced pressure and purified by a short silica-gel column with hexane/EtOAc (3:1) to give the (*S*)-MTPA ester **4a** (4.5 mg). The (*R*)-MTPA ester **4b** (2.5 mg) was prepared from (+)-MTPA chloride by the same method. Selected $\Delta\delta$ values were shown in Fig. 4.

Molecular Mechanics Calculations. The minimum energy conformations of briaexcavatin H (**2**) calculated at various temperatures (25, 0, −25, and −50 °C) were determined using the MSI Insight II/DISCOVER version 95 molecular modeling package, incorporating a consistent valence force field (CVFF),[19] on Silicon Graphic IRIS (SGI) Indigo XS24/4000 workstation. All force field calculations were carried out in vacuo (dielectric constant = 1). The conformational space of **2** was scanned using the high-temperature molecular dynamics simulation technique, followed by energy minimization. A 100 ps molecular dynamics simulation at 1000 K provided a set of 500 conformations of **2**. Each conformation was used as a starting structure for the subsequent energy minimization (1000 steps, conjugated gradient algorithm). The conformational search suggested that the most stable conformations of briarane **2** shown in Fig. 1 are the lowest energy conformations of **2** calculated at 25, 0, −25, and −50 °C, respectively.

Human Neutrophil Superoxide Generation and Elastase Release. Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Superoxide generation and elastase release were carried out according to the procedures described previously.[20,21] Briefly, superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*. Elastase release experiments were performed using MeO–Suc–Ala–Ala–Pro–Valp–nitroanilide as the elastase substrate.

Results and Discussion

Specimens of the octocoral *B. excavatum*, collected off southern Taiwan coast, were minced and extracted with EtOAc. The extract was separated on silica-gel column chromatography to afford briaranes **1** and **2**. Briaexcavatin G (**1**) was obtained as a white powder. The molecular formula of $C_{35}H_{50}O_{13}$ was deduced from HR-ESI-MS with *m/z* 701.3146 (calcd for $C_{35}H_{50}O_{13}$ + Na, 701.3149). This showed that briarane **1** contained eleven degrees of unsaturation. The IR absorptions of **1** showed the presences of carbonyl groups of γ-lactone (ν_{max} 1786 cm^{-1}) and ester (ν_{max} 1735 cm^{-1}). The FAB-MS of **1** exhibited peaks at *m/z* 701 $(M + Na)^+$, 679 $(M + H)^+$, 619 $(M + H - AcOH)^+$, 591 $(M + H - C_3H_7CO_2H)^+$, 577 $(M + H - C_4H_9CO_2H)^+$, 559 $(M + H - 2AcOH)^+$, 531 $(M + H - C_3H_7CO_2H - AcOH)^+$, 517 $(M + H - C_4H_9CO_2H - AcOH)^+$, 489 $(M + H - C_3H_7CO_2H - C_4H_9CO_2H)^+$, 471 $(M + H - C_3H_7CO_2H - 2AcOH)^+$, 457 $(M + H - C_4H_9CO_2H - 2AcOH)^+$, 429 $(M + H - C_3H_7CO_2H - C_4H_9CO_2H - AcOH)^+$, 369 $(M + H - C_3H_7CO_2H - C_4H_9CO_2H - 2AcOH)^+$, and 309 $(M + H - C_3H_7CO_2H - C_4H_9CO_2H - 3AcOH)^+$, which suggested the presence of a butyryloxy, a valeryloxy, and three acetoxy groups in the molecule. It was found that the ^1H and ^{13}C spectra of **1** in $CDCl_3$ revealed mostly broad peaks when measured at room temperature (25 °C). In order to make more reliable assignments of the NMR signals of the stabilized conformers, the ^1H and ^{13}C NMR spectra of **1** were measured at −20 °C in $CDCl_3$. It was found that at this temperature mainly one conformer existed, and the signals for each proton and carbon of the molecule were sharpened and could be assigned (Table 1).

By detailed analysis, the NMR data of **1** were similar to those of a known metabolite, excavatolide B (**3**).[2] However, the NMR spectra revealed that the signals corresponding to the hydroxy group in **3** (δ_C 65.8, d, CH-12; δ_H 3.88, 1H, m, H-12) was replaced by those of an isovaleryloxy group (δ_C 171.9, s; 44.0, t; 25.8, d; 22.7, q; 22.6, q; δ_H 2.11, 2H, d, J = 6.8 Hz; 2.03, 1H, m; 0.89, 2 × 3H, d, J = 6.4 Hz) in **1**. In the ^1H-detected multiple-bond heteronuclear multiple-quantum coherence (HMBC) experiment of **1**, the carbon signal at δ 171.9 (s) which showed a correlation with H-12 (δ 5.03) was found to be correlated with the signal of the methylene protons at δ 2.11 and was consequently assigned as the carbon atom of the isovalerate carbonyl. Thus, the isovalerate ester should be positioned at C-12 in **1**. Furthermore, the isovalerylation of **3** gave a less polar product, which was found to be identical with natural product **1** by comparison of the physical and spectral data and confirmed the structure of diterpenoid **1**. Since the absolute configuration of the known briarane, excavatolide B (**3**), had been determined by modified Mosher's method,[12] we were able to assign the absolute configurations of all the chiral centers of **1** as 1*R*,2*R*,3*S*,5*Z*,7*S*,8*R*,9*S*,10*S*,11*R*,12*S*,14*S*,17*R*. Based on above findings, the structure of **1** was established unambiguously.

Our present study has also led to the isolation of the new briarane, briaexcavatin H (**2**). Briarane **2** was obtained as a white powder. The HR-ESI-MS data recorded at *m/z* 605.2208, established the molecular formula of **2** as $C_{28}H_{38}O_{13}$ (calcd $C_{28}H_{38}O_{13}$ + Na, 605.2210). Thus, ten degrees of unsaturation were determined for **2**. The IR spectrum showed bands at 3477, 1790, and 1740 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester groups in **2**. The FAB-MS of **2** exhibited peaks at *m/z* 605 $(M + Na)^+$, 583 $(M + H)^+$, 565 $(M + H - H_2O)^+$, 523 $(M + H - AcOH)^+$, 505 $(M + H - H_2O - AcOH)^+$, 463 $(M + H - 2AcOH)^+$, 445 $(M + H - H_2O - 2AcOH)^+$, 403 $(M + H - 3AcOH)^+$, 385 $(M + H - H_2O - 3AcOH)^+$, 343 $(M + H - 4AcOH)^+$, and 325 $(M + H - H_2O - 4AcOH)^+$, which suggested the presence of a hydroxy and four acetoxy groups. Like as those of **1**, the NMR spectrum of **2** in $CDCl_3$ measured at room temperature revealed mostly broad peaks. Thus, in order to obtain well-resolved NMR spectra, all 1D and 2D NMR spectra of **2** were measured at −50 °C in Me_2CO-d_6 (Table 1). The ^1H NMR spectra recorded at various temperatures (25, 0, −25, and −50 °C) are shown in Fig. 1.[22]

In the ^{13}C NMR spectrum, five carbonyl resonances appeared at δ 172.2 (s), 172.0 (s), 170.6 (s), 170.1 (s), and 169.6 (s), supporting the presence of a lactone and four esters. In the ^1H NMR spectrum, four acetate methyls (δ 2.33, 3H, s; 2.19, 3H, s; 2.18, 3H, s; 1.84, 3H, s) were further observed. Thus,

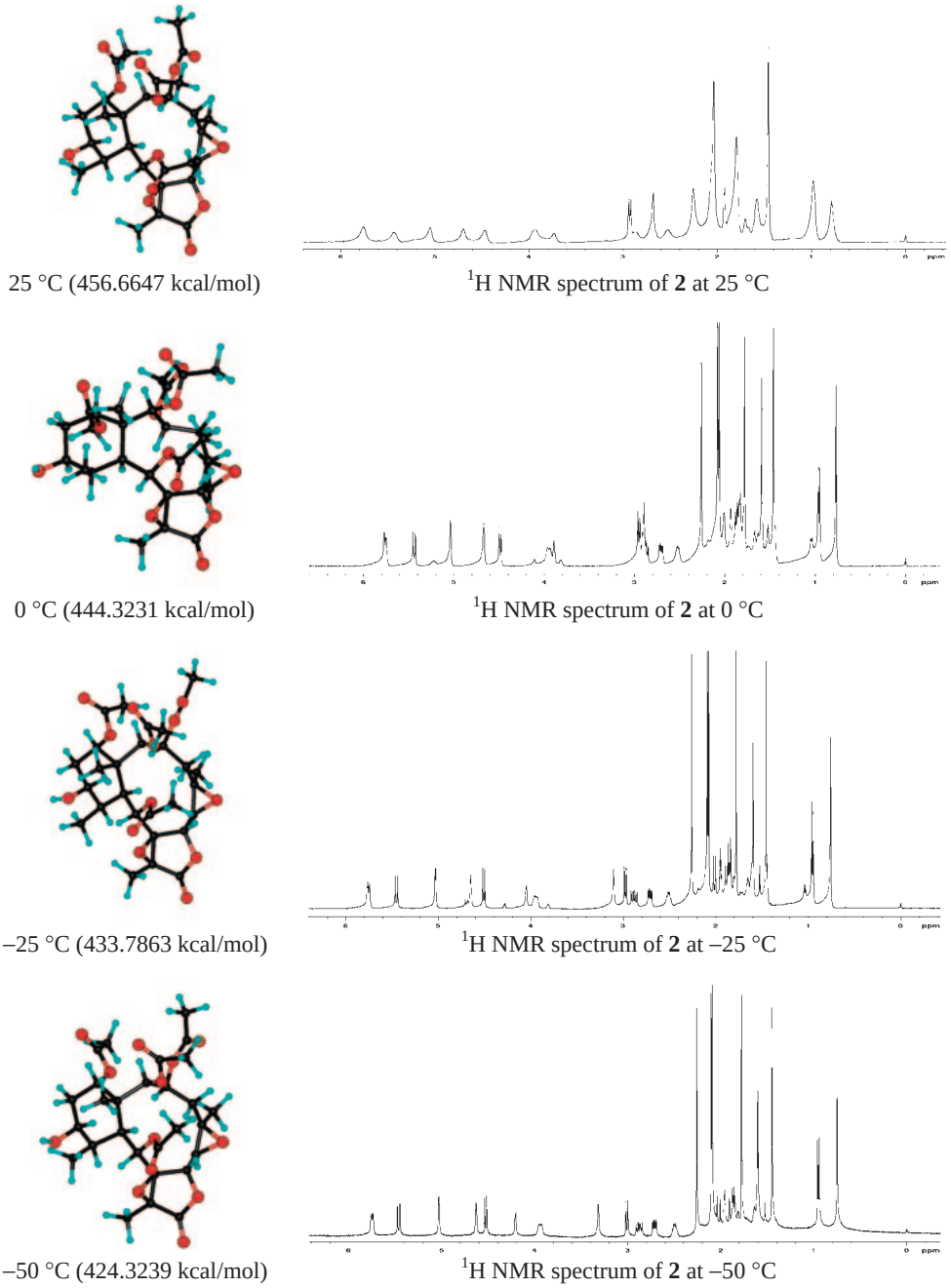


Fig. 1. The stereoviews, minimum energy, and ^{1}H NMR spectra measured at various temperatures (25, 0, −25, −50 °C, Me_2CO-d_6) of **2**.

the ^{13}C NMR data accounted for five degrees of unsaturation and required **2** to be pentacyclic. The presence of a tetrasubstituted epoxide containing a methyl substituent was elucidated from the signals of two oxygen-bearing quaternary carbons at δ 67.8 (s, C-8) and 59.6 (s, C-17) and further confirmed from the proton signal of a methyl singlet resonating at δ 1.52 (3H, s, H_3-18). In addition, a trisubstituted epoxide containing a methyl substituent was deduced from the signals of an oxymethine (δ 62.1, d, CH-6), an oxygen-bearing quaternary carbon (δ 60.0, s, C-5), and a methyl singlet resonating at δ 1.68 (3H, s, H_3-16). Moreover, a methyl doublet (δ 1.02, 3H, d, J = 6.8 Hz, H_3-20), a methyl singlet (δ 0.81, 3H, s, H_3-15), two aliphatic methine protons (δ 2.78, 1H, dd, J = 10.4, 5.2 Hz, H-10; 2.55, 1H, m, H-11), two pair of methylene protons (δ 2.94, 1H, dd, J = 16.0, 9.2 Hz; 1.95, 1H, m, H_2-4; 2.04, 1H, m; 1.71, 1H, m, H_2-13), seven-oxygenated methine protons (δ 5.81, 1H, br d, J = 6.0 Hz, H-3; 5.54, 1H, d, J = 10.4 Hz, H-9; 5.10, 1H, br s, H-2; 4.69, 1H, br s, H-14; 4.61, 1H, d, J = 8.8 Hz, H-7; 4.01, 1H, m, H-12; 3.11, 1H, d, J = 8.8 Hz, H-6), and a hydroxy proton (δ 4.41, 1H, d, J = 3.6 Hz, OH-12) were further assigned by the assistance of ^{1}H–^{1}H COSY and HMQC spectrum.

Comparison of the NMR spectral data of **2** with those of the known metabolite, excavatolide C (**4**),[2] showed that the carbon–carbon double bond between C-5 and C-6 in **4** (δ_H 5.37, 1H, d, J = 7.5 Hz, H-6; δ_C 140.0, s, C-5; 122.5, d,

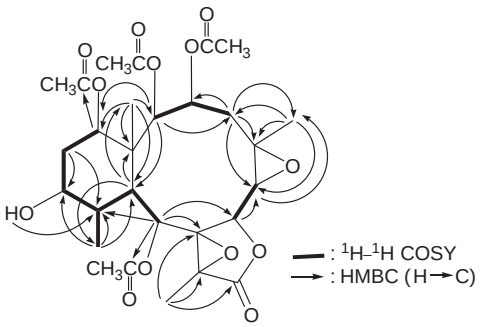


Fig. 2. The ^{1}H–^{1}H COSY and selective HMBC correlations of **2**.

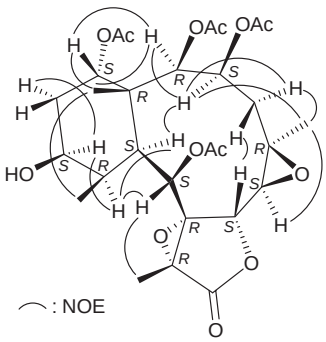


Fig. 3. Selective NOESY correlations of **2**.

CH-6) was replaced by an epoxy group in **2** (δ_H 3.11, 1H, d, $J = 8.8$ Hz, H-6; δ_C 60.0, s, C-5; 62.1, d, CH-6). This observation and the planar structure of **2** could be determined essentially from the ^{1}H–^{1}H COSY and HMBC correlations are shown in Fig. 2.

The stereochemistry of **2** was elucidated from the NOE interactions observed in an NOESY experiment (Fig. 3). As per convention, when analyzing the stereochemistry of briarane-type diterpenoids, H-10 and H_3-15 were assigned to the α and β face, anchoring the stereochemical analysis because no NOE correlation was found between H-10 and H_3-15. In the NOESY experiment of **2**, H-10 gives NOE correlations to H-3 and H-11, and H-3 was found to show responses with H-2 and one proton of the C-4 methylene (δ 1.95), indicating that these protons (H-2, H-3, H-4α, H-10, and H-11) are located on the same face of the molecule and, therefore, are assigned as α protons, as the C-15 methyl is the β-substituent at C-1. H-14 gives NOE correlations to H-2 and H_3-15, but not to H-10, confirming the β-orientation for this proton. Also, the hydroxy group at C-12 was found to be in the β face and is *cis* to Me-20 by the NOE correlation between H-11 and H-12. Furthermore, H_3-16 exhibited NOE correlations with H-3, H-4α, and H-6, but not with H-7, suggesting that H_3-16 and H-6 were positioned on the α face in the epoxy group and H-7 was β-oriented in the ten-membered ring. This observation was further supported by a strong NOE correlation observed between H-4β and H-7. H-9 was found to show NOE correlations with H-11 and H_3-18. From the detailed consideration of molecular models, H-9 was found to be reasonably close to H-11 and H_3-18, while H-9 should be placed on the α face in **2**, and H_3-18 was β-oriented in the γ-lactone unit.

The structure of briarane **2** was further supported by chemical interconversion from the known metabolite **4**. Briarane **4** was epoxidized with *m*-CPBA in dichloromethane to give a compound whose physical and spectral data were coincident with those of natural product **2**. In order to determine the absolute configurations of briaranes **2** and **4**, the known briarane **4** was treated with (−) or (+)-MTPA chloride to yield the (*S*)- and (*R*)-MTPA esters **4a** and **4b**, respectively. Comparison of the ^{1}H NMR chemical shifts for **4a** and **4b** (Δ values shown in Fig. 4) led to the assignment of the *S*-configuration at C-12. Therefore, the absolute configurations of all chiral centers of **4** were determined as 1*R*,2*R*,3*S*,5*Z*,7*S*,8*R*,9*S*,10*S*,11*R*,12*S*,-14*S*,17*R*. Because of biogenic consideration and chemical conversion, the absolute configurations of all chiral centers of **2** was assigned as 1*R*,2*R*,3*S*,5*R*,6*S*,7*S*,8*R*,9*S*,10*S*,11*R*,12*S*,-14*S*,17*R*.

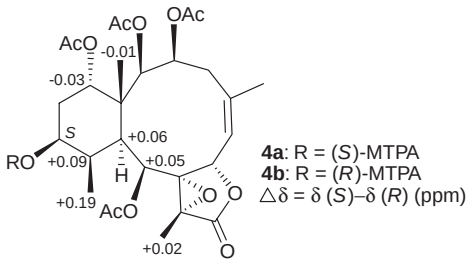
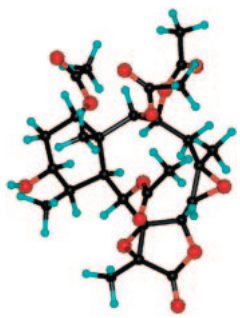


Fig. 4. ^{1}H NMR chemical shift differences [δ(*S*)-MPTA–δ(*R*)-MTPA] of the MTPA esters of **4**.

Table 2. The Stereoview of **1** (Generated from Computer Modeling under −50 °C) and the Calculated Distances (Å) between Selected Protons Having Key NOE Correlations[a)]

Briaexcavatin H (**2**)	H/H	(Å)
	H-2/H-3	2.64
	H-2/H-14	2.26
	H-3/H-10	2.10
	H-3/H-4α	2.81
	H-3/H_3-16	2.48
	H-4α/H_3-16	2.89
	H-4β/H-7	2.43
	H-6/H_3-16	2.48
	H-9/H-11	2.83
	H-9/H_3-18	2.49
	H-10/H-11	2.32
	H-11/H-12	2.33
	H-14/H_3-15	2.28
	H_3-15/H_3-20	2.03

a) The calculated distance between H-10 (α) and H_3-15 (β) is 3.89 Å.

Geometrical optimizations of **2** under various temperatures (25, 0, −25, and −50 °C) were performed with DISCOVER utilizing the consistent valence force field (CVFF) calculations for energy minimization. The calculated results were visualized using INSIGHT II, running on a Silicon Graphics IRIS (SGI) Indigo XS24/4000 workstation. The conformation search suggested that the most stable conformation and the calculated minimum energy for briarane **2** are shown in Fig. 1. It was found that the calculated distances between those protons having key NOESY correlations of **2** are all shorter than 3 Å as shown in Table 2.

Table 3. Inhibitory Effects of Briaranes **1** and **2** on Superoxide Anion Generation and Elastase Release by Human Neutrophils in Response to fMet–Leu–Phe/Cytochalasin B

	Superoxide generation	Elastase release
Compounds	Inh/%[a)]	Inh/%
1	-8.49 ± 2.70	36.44 ± 2.86
2	9.02 ± 7.31	5.19 ± 2.63

a) Percentage of inhibition (Inh %) at $10\,\mu g\,mL^{-1}$. Results are presented as mean $\pm$ SEM ($n = 4$).

In the biological activity testing, briarane **1** was found to inhibit weakly human neutrophil elastase release at $10\,\mu g\,mL^{-1}$ (Table 3).

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