Briaexcavatins M—P, Four New Briarane-Related Diterpenoids from Cultured Octocoral *Briareum excavatum* (Briareidae)

Ping-Jyun Sung,*[,a,b,c] Mei-Ru Lin,[a,d] Tsong-Long Hwang,[e] Tung-Yung Fan,[f,g] Wei-Chun Su,[f] Chiu-Chin Ho,[f] Lee-Shing Fang,[g,h] and Wei-Hsien Wang[a,d]

[a] *Taiwan Coral Research Center and Department of Planning and Research, National Museum of Marine Biology & Aquarium;* [f] *Taiwan Coral Research Center and Department of Biology, National Museum of Marine Biology & Aquarium; Checheng, Pingtung 994, Taiwan:* [b] *Institute of Marine Biotechnology, National Dong Hwa University;* [g] *Institute of Marine Biodiversity and Evolution, National Dong Hwa University; Checheng, Pingtung 944, Taiwan:* [c] *Asia-Pacific Ocean Research Center, National Sun Yat-sen University;* [d] *Department of Marine Biotechnology and Resources, National Sun Yat-sen University; Kaohsiung 804, Taiwan:* [e] *Graduate Institute of Natural Products, Chang Gung University; Taoyuan 333, Taiwan: and* [h] *Department of Sport, Health, and Leisure, Cheng Shiu University; Niaosong, Kaohsiung County 833, Taiwan.* Received February 15, 2008; accepted March 17, 2008; published online April 14, 2008

Four new briarane-related diterpenoids, designated as briaexcavatins M—P (1—4), were isolated from the cultured octocoral *Briareum excavatum*. The structures, including the relative configurations of natural products 1—4 were established on the basis of extensive spectral data analysis and by comparison with the spectral data from other known metabolites featuring a briarane carbon skeleton.

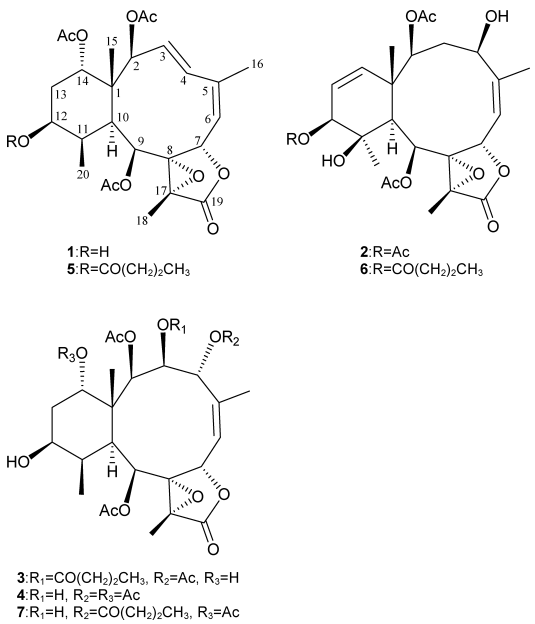
Key words *Briareum excavatum*; briaexcavatin; briarane; cultured octocoral

Gorgonian corals are recognized as rich sources of diterpenoid derivatives with the well-known briarane carbon skeleton, a group of diterpenoids having a highly oxidized bicyclo[8.4.0] system with a γ-lactone group. Compounds of this type exist only in marine organisms, particularly in octocorals.[1,2] In our screening for novel natural products from the marine invertebrates collected in Taiwanese waters, we have discovered a series of terpenoid and steroid metabolites from the octocorals *Alcyonium* sp.,[3,4] *Briareum* sp.,[5] *Briareum excavatum*,[6—10] *Ellisella robusta*,[11—14] *Junceella fragilis*,[10,15—22] *Junceella juncea*,[17,23] *Rumphella antipathies*,[24—28] and the tunicate *Eudistoma* sp.[29]; and some of these metabolites have been reported to exhibit interesting biological activity such as anti-inflammatory,[8,9,20,22] cytotoxicity,[8,29] and antibacterial activity.[11,24,26,27] In this paper, we describe the isolation and structure determination of four new briarane-type diterpenoids, briaexcavatins M—P (**1**—**4**), from the cultured octocoral *Briareum excavatum* (Briareidae). The structures of briaranes **1**—**4** were established by spectroscopic methods.

Results and Discussion

Specimens of the octocoral *B. excavatum*, collected from the culturing tank in the National Museum of Marine Biology and Aquarium (NMMBA), Taiwan, were minced and extracted with a mixture of MeOH and CH_2Cl_2 (1 : 1). The extract was further partitioned with EtOAc and H_2O. The EtOAc layer was successively subjected to silica gel gravity column chromatography and re-purified by normal phase HPLC guided by the signals for the interesting structures found in the proton NMR experiments to afford briaranes **1**—**4** (see Experimental).

Briaexcavatin M (**1**) was obtained as a white powder. The molecular formula of $C_{26}H_{34}O_{10}$ was deduced from HR-ESI-MS with *m/z* 529.2052 (Calcd for $C_{26}H_{34}O_{10}Na$, 529.2050). This showed that **1** contained 10 degrees of unsaturation. The IR absorptions of **1** showed the presence of hydroxy (3468 cm^{-1}), γ-lactone (1768 cm^{-1}), and ester (1740 cm^{-1}) groups. From the ^{13}C-NMR spectrum (Table 1), compound **1** was found to possess three acetoxy groups (δ_C 21.3, 2×q; 21.2, q; 170.1, 2×s; 169.9, s), a γ-lactone moiety (δ_C 170.2, s, C-19), a trisubstituted olefin (δ_C 141.9, s, C-5; 116.9, d, CH-6), and a disubstituted olefin (δ_C 137.4, d, CH-4; 126.8, d, CH-3). Thus, from the above NMR data, six degrees of unsaturation were accounted for, and compound **1** must be tetracyclic. The presence of a tetrasubstituted epoxide containing a methyl substituent was elucidated from the signals of two oxygenated quaternary carbons at δ_C 68.2 (s, C-8) and 63.1 (s, C-17), and further confirmed by the proton signal of a methyl singlet resonating at δ_H 1.57 (3H, s, H_3-18) (Table 2). Moreover, three acetoxy methyls (δ_H 1.98, 3H, s; 2.08, 3H, s; 2.19, 3H, s), a methyl singlet (δ_H 1.37, 3H, s, H_3-15), a methyl doublet (δ_H 1.00, 3H, d, J=7.6 Hz, H_3-20), a vinyl methyl (δ_H 1.87, 3H, s, H_3-16), a pair of methylene protons



* To whom correspondence should be addressed. e-mail: pjsung@nmmba.gov.tw

Table 1. ^{13}C-NMR Data for Diterpenoids **1**—**4**[a)]

Position	**1**	**2**	**3**	**4**
1	45.4 (s)[b)]	45.4 (s)	44.8 (s)	43.2 (s)
2	75.6 (d)	77.8 (d)	81.9 (d)	87.9 (d)
3	126.8 (d)	41.7 (t)	74.5 (d)	73.6 (d)
4	137.4 (d)	71.1 (d)	64.8 (d)	66.9 (d)
5	141.9 (s)	147.8 (s)	137.7 (s)	139.9 (s)
6	116.9 (d)	122.1 (d)	126.2 (d)	123.3 (d)
7	76.7 (d)	73.4 (d)	73.7 (d)	74.2 (d)
8	68.2 (s)	70.1 (s)	69.5 (s)	70.8 (s)
9	73.6 (d)	66.9 (d)	66.5 (d)	66.2 (d)
10	37.9 (d)	44.0 (d)	39.5 (d)	40.5 (d)
11	42.6 (d)	73.4 (s)	36.2 (d)	37.0 (d)
12	67.3 (d)	73.4 (d)	66.0 (d)	66.7 (d)
13	29.5 (t)	120.4 (d)	33.4 (t)	30.3 (t)
14	74.6 (d)	142.0 (d)	79.4 (d)	80.5 (d)
15	16.3 (q)	18.3 (q)	17.8 (q)	18.6 (q)
16	23.6 (q)	25.7 (q)	16.8 (q)	17.1 (q)
17	63.1 (s)	62.3 (s)	61.0 (s)	62.4 (s)
18	10.1 (q)	9.7 (q)	10.0 (q)	10.3 (q)
19	170.2 (s)	170.3 (s)	171.3 (s)	170.8 (s)
20	9.2 (q)	27.8 (q)	9.2 (q)	8.9 (q)
2-OAc	21.2 (q)	21.0 (q)	21.2 (q)	21.1 (q)
	169.9 (s)	169.8 (s)	171.2 (s)	172.5 (s)
4-OAc			21.0 (q)	20.7 (q)
			171.8 (s)	169.8 (s)
9-OAc	21.3 (q)	21.7 (q)	21.7 (q)	21.5 (q)
	170.1 (s)	169.2 (s)	170.3 (s)	170.1 (s)
12-OAc		21.1 (q)		
		169.3 (s)		
14-OAc	21.3 (q)			21.2 (q)
	170.1 (s)			170.0 (s)
3-OCOPr			13.7 (q)	
			18.5 (t)	
			36.1 (t)	
			171.7 (s)	

a) Spectra measured at 100 MHz in $CDCl_3$ at 25 °C. *b*) Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols. The values are downfield in ppm from TMS.

(δ_H 1.85, 1H, m; 1.25, 1H, m, H_2-13), two aliphatic methine protons (δ_H 2.18, 1H, d, J=4.0 Hz, H-10; 2.04, 1H, m, H-11), five oxymethine protons (δ_H 5.61, 1H, s, H-9; 5.43, 1H, d, J=10.0 Hz, H-2; 4.93, 1H, dd, J=3.2, 3.2 Hz, H-14; 4.63, 1H, d, J=4.8 Hz, H-7; 4.00, 1H, m, H-12), two conjugated olefin protons (δ_H 6.64, 1H, d, J=15.2 Hz, H-4; 5.80, 1H, dd, J=15.2, 10.0 Hz, H-3), and an olefin proton (δ_H 5.31, 1H, m, H-6), were observed in the ^{1}H-NMR spectrum of **1**.

The gross structure of **1** was elucidated with the assistance of 2D-NMR studies. From the ^{1}H–^{1}H COSY experiment of **1** (Fig. 1), it was possible to establish the separate spin system that maps out the proton sequences from H-2/3/4, H-4/6 (by allylic coupling), H-6/7, and H-9/10. These data, together with the HMBC correlations between H-2/C-1, -3, -4; H-3/C-1, -2, -5; H-4/C-2; H-6/C-8; H-7/C-5, -6; H-9/C-1, -7, -8, -10; and H-10/C-1, -2, -8, -9, established the connectivity from C-1 to C-10 in a 10-membered ring (Fig. 1, Table 3). A vinyl methyl group attached at C-5 was confirmed by the HMBC correlations between H_3-16/C-4, -5, -6, and by the ^{1}H–^{1}H allylic coupling between H-6 and H_3-16. The methylcyclohexane ring, which is fused to the 10-membered ring at C-1 and C-10, was elucidated by the ^{1}H–^{1}H correlations from H-10/11/12/13/14 and H-11/H_3-20; and by the HMBC correlations between H-2/C-14; H-9/C-11; H-10/C-11, -12, -20; H-11/C-1, -10; H-12/C-20; H_2-13/C-12; H-14/C-12; and H_3-

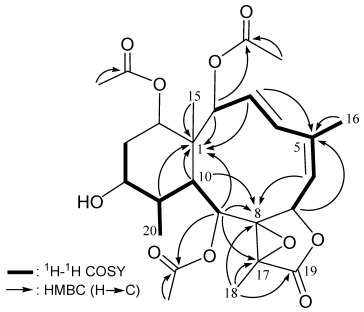


Fig. 1. ^{1}H–^{1}H COSY and Selective HMBC Correlations (Protons and Quaternary Carbons) of **1**

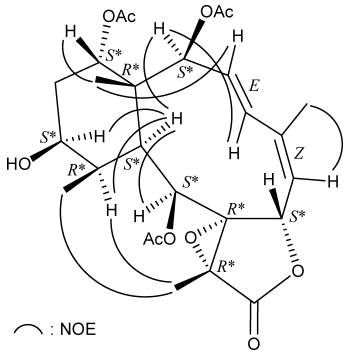


Fig. 2. Selective NOE Correlations of **1**

20/C-10, -11, -12. The ring junction C-15 methyl group was positioned at C-1 from the HMBC correlations between H-2/C-15; H-10/C-15; and H_3-15/C-1, -2, -10, -14. Furthermore, the HMBC correlations also revealed the positions of two acetoxy groups attached to C-2 and C-9, respectively. These data, together with the HMBC correlations between H-9/C-17 and H_3-18/C-8, -17, -19, unambiguously established the molecular framework of **1**, except the remaining acetoxy and hydroxy groups, which had to be positioned at C-12 or C-14.

Based on previous studies, all the naturally occurring briarane-type natural products have the C-15 methyl group as *trans* to H-10, and these two groups are assigned as β- and α-oriented, respectively, as shown in most briarane derivatives.[1,2)] The relative stereochemistry of **1** was deduced from a NOESY experiment (Fig. 2) and from vicinal ^{1}H–^{1}H coupling constants analysis. In the NOESY experiment of **1**, the NOE correlations of H-10 with H-2/9/11 and H-12 indicated that these protons are situated on the same face and were assigned as α protons since the C-15 methyl is the β-substituent at C-1. H-14 was found to exhibit a strong NOE response with H_3-15, revealing the β-orientation of this proton. Furthermore, H_3-18 was found to exhibit NOE responses with H-11 and H_3-20, indicating the β-orientation of C-18 methyl in the γ-lactone moiety. However, due to there being no NOE response observed between H-7 and the other protons in the NOESY experiment of **1**, the configuration of C-7 in **1** could not be determined in this way. The *trans* geometry of the C-3/C-4 double bond was indicated by a 15.2 Hz coupling constant between H-3 (δ_H 5.80) and H-4 (δ_H 6.64). The *Z*-configuration of the C-5/C-6 double bond was elucidated by a strong NOE response between the C-6 olefin proton (δ_H 5.31) and the C-16 vinyl methyl (δ_H 1.87). Moreover, the

Table 2. ^{1}H-NMR Chemical Shifts for Diterpenoids **1**—**4**[a]

Position	**1**	**2**	**3**	**4**
2	5.43 d (10.0)[b]	4.48 d (5.2)	5.36 s	4.58 s
3α	5.80 dd (15.2, 10.0)	2.12 m	5.81 d (4.4)	4.29 d (10.8)
β		2.81 dd (15.2, 12.0)		
4α	6.64 d (15.2)	4.32 ddd (12.0, 4.4, 1.2)		
β			6.65 d (4.4)	6.06 s
6	5.31 m	5.53 ddd (10.0, 1.2, 1.2)	5.42 dd (6.8, 1.6)	5.22 dd (6.0, 1.2)
7	4.63 d (4.8)	6.18 d (10.0)	5.76 d (6.8)	5.63 d (6.0)
9	5.61 s	5.77 d (4.4)	5.35 d (9.6)	5.31 d (8.8)
10	2.18 d (4.0)	2.60 d (4.4)	2.80 dd (9.6, 5.2)	2.61 dd (8.8, 4.8)
11	2.04 m		2.46 m	2.43 m
12	4.00 m	4.77 d (6.0)	4.29 dt (8.0, 4.0)	4.10 dt (12.4, 4.4)
13α	1.25 m	5.82 dd (10.4, 6.0)	1.75 m	1.74 m
β	1.85 m		1.95 m	2.00 m
14	4.93 dd (3.2, 3.2)	5.68 d (10.4)	3.75 br s	4.90 br t (2.8)
15	1.37 s	1.27 s	0.77 s	0.96 s
16	1.87 s	2.07 d (1.2)	1.80 d (1.6)	1.87 d (1.2)
18	1.57 s	1.60 s	1.63 s	1.65 s
20	1.00 d (7.6)	1.33 s	1.06 d (7.2)	
OH-3				3.79 d (10.8)
2-OAc	1.98 s	2.09 s	2.10 s	2.09 s
4-OAc			2.08 s	2.08 s
9-OAc	2.08 s	2.22 s	2.42 s	2.34 s
12-OAc		2.04 s		
14-OAc	2.19 s			2.18 s
3-OCOPr			0.96 t (7.2) 1.66 sext (7.2) 2.32 t (7.2)	

a) Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. *b*) *J* values (in Hz) in parentheses. The values are downfield in ppm from TMS.

Table 3. The HMBC Correlations for Diterpenoids **1**—**4** (H→C)

Position	**1**	**2**	**3**	**4**
H-2	C-1, 3, 4, 14, 15, acetate carbonyl	C-1, 3, 4, 10, 14, 15, acetate carbonyl	C-1, 3, 4, 10, 14, acetate carbonyl	C-1, 3, 4, 10, 14, acetate carbonyl
H-3	C-1, 2, 5	C-1, 2, 4, 5	C-2, 5, butyrate carbonyl	C-5
H-4	C-2	C-3, 5, 6, 16	C-2, 3, 6, 16, acetate carbonyl	C-2, 3, 5, 6, 16, acetate carbonyl
H-6	C-8	C-4, 16	C-4, 16	C-4, 16
H-7	C-5, 6	C-5, 6	C-5, 6	C-5, 6, 19
H-9	C-1, 7, 8, 10, 11, 17, acetate carbonyl	C-7, 8, 10, 11, 17, acetate carbonyl	C-7, 8, 10, 11, 17, acetate carbonyl	C-7, 8, 10, 11, 17, acetate carbonyl
H-10	C-1, 2, 8, 9, 11, 12, 15, 20	C-1, 2, 8, 9, 15	C-1, 2, 8, 9, 11, 15, 20	C-1, 8, 9, 11, 12, 15, 20
H-11	C-1, 10, 12, 13		C-1, 10, 12, 13, 20	C-1, 10, 12, 13, 20
H-12	C-20	C-10, 11, 13, 14, 20, acetate carbonyl	C-20	C-20
H-13	C-12	C-1, 12	C-11, 12	C-11, 12
H-14	C-12	C-1, 2, 10, 12, 13, 15	n.o.[a]	C-10, 12, 15, acetate carbonyl
H-15	C-1, 2, 10, 14	C-1, 2, 14	C-1, 2, 10, 14	C-1, 2, 10, 14
H-16	C-4, 5, 6	C-4, 5, 6	C-4, 5, 6	C-4, 5, 6
H-18	C-8, 17, 19	C-8, 17, 19	C-8, 17, 19	C-8, 17, 19
H-20	C-10, 11, 12	C-10, 11, 12	C-10, 11, 12	C-10, 11, 12

a) n.o.=not observed.

olefin proton H-3 showed a strong NOE response with H_3-15, but not with H-2 or H-4, and H-4 showed an NOE response with H-2, but not with H_3-16, demonstrating the 3(*E*),5(*Z*)-configuration of $\Delta^{3,5}$. Therefore, the *s-trans* diene moiety in **1** was elucidated. By detailed analysis, the NMR data of **1** were found to be similar to those of the briarane derivatives with a 3(*E*),5(*Z*)-diene system, such as excavatolide F (**5**),[30] briaranolides I and J,[31] which all possess a β-proton at C-7 position, respectively. By comparing the ^{1}H- and ^{13}C-NMR chemical shifts for C-7 oxymethine of **1** (δ_H 4.63, d, J=4.8 Hz; δ_C 76.7, d) with those of the known briaranes, excavatolide F (**5**) (δ_H 4.58, d, J=4.4 Hz; δ_C 76.6, d),[30] and briaranolides I (δ_H 4.66, d, J=4.4 Hz; δ_C 77.0, d), and J (δ_H 4.71, d, J=4.7 Hz; δ_C 76.6, d),[31] the authors suggested the configuration of H-7 in **1** should be assigned as β-orientation like the known briaranes reported previously.[30,31] Furthermore, comparison of the ^{1}H- and ^{13}C-NMR chemical shifts for the C-12 (δ_H 4.00, 1H, m; δ_C 67.3, d) and C-14 (δ_H 4.93, 1H, dd, J=3.2, 3.2 Hz; δ_C 74.6, d) oxymethines of **1** with those of the known briarane excavatolide F (**5**) (δ_H 4.97, 1H, m, H-12; δ_C 69.9, d, CH-12; δ_H 4.94, 1H, br s, H-14; δ_C 74.1, d, CH-14),[30] confirmed that the remaining acetoxy group should be positioned at C-14 in **1** although no HMBC correlation was observed between H-14 and the acetate carbonyl, and the hydroxy group had to be positioned at C-12. Based on the above findings, the structure, including the relative stereochemistry of **1**, was elucidated and this compound was found to be the 12-debutyryloxy-12-hydroxy derivative

of **5**. The configurations of all chiral centers of **1** were assigned as 1*R**, 2*S**, 3*E*, 5*Z*, 7*S**, 8*R**, 9*S**, 10*S**, 11*R**, 12*S**, 14*S**, and 17*R**.

The molecular formula of briaexcavatin N (**2**) was determined as $C_{26}H_{34}O_{11}$ by its HR-ESI-MS (*m/z* 545.1998, Calcd for $C_{26}H_{34}O_{11}Na$, 545.1999). The IR spectrum showed bands at 3461, 1775, and 1728 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester groups in **2**. From the ^{13}C-NMR data of **2** (Table 1), trisubstituted and disubstituted double bonds were deduced from the signals of four carbons resonating at δ_C 147.8 (s, C-5), 142.0 (d, CH-14), 122.1 (d, CH-6), and 120.4 (d, CH-13). A methyl-containing tetrasubstituted epoxy group was confirmed from the signals of two quaternary oxygenated carbons at δ_C 70.1 (s, C-8) and 62.3 (s, C-17), and from the chemical shift of tertiary methyl protons (δ_H 1.60, 3H, s, H_3-18; δ_C 9.7, q, CH_3-18) (Tables 1, 2). Moreover, four carbonyl resonances appeared at δ_C 170.3 (s, C-19), 169.8 (s, ester carbonyl), 169.3 (s, ester carbonyl), and 169.2 (s, ester carbonyl), confirming the presence of a γ-lactone and three esters in **2**. All the esters were identified as acetates by the presence of methyl resonances in the ^1H-NMR spectrum at δ_H 2.04 (3H, s), 2.09 (3H, s), and 2.22 (3H, s).

The planar structure of **2** was determined by 2D-NMR studies. The coupling information in the ^1H–^1H COSY spectrum of **2** enabled identification of the H-2/3/4, H-4/6 (by allylic coupling), and H-6/7, H-9/10, and H-12/13/14, were established (Fig. 3). With these data, together with the correlations observed in an HMBC experiment of **2** (Fig. 3, Table 3), the molecular framework of **2** could be further established. The HMBC correlations also indicated that the acetoxy groups should attach at C-2, C-9, and C-12. Thus, the remaining hydroxy groups have to be positioned at C-4 and

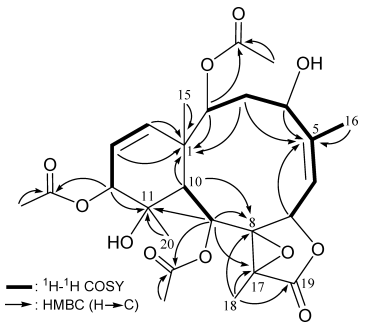


Fig. 3. ^1H–^1H COSY and Selective HMBC Correlations (Protons and Quaternary Carbons) of **2**

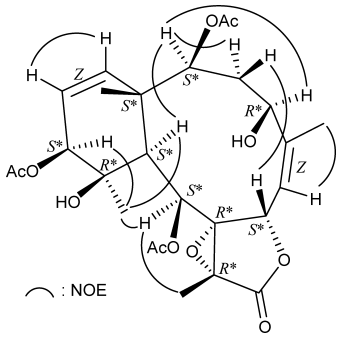


Fig. 4. Selective NOE Correlations of **2**

C-11, as indicated by the key ^1H–^1H COSY correlations and characteristic NMR signal analysis.

The relative stereochemistry of **2** was elucidated from the NOE interactions observed in a NOESY experiment (Fig. 4) and by the vicinal ^1H–^1H coupling constant analysis. The *cis* geometry of the C-13/C-14 double bond was indicated by a 10.4 Hz coupling constant between H-13 (δ_H 5.82) and H-14 (δ_H 5.68) and further confirmed by a strong NOE correlation between these two olefin protons. In the NOESY spectrum of **2**, strong NOE correlations were observed between H-10 with H-2 and H_3-20, indicating that these protons should be positioned on the α face in **2**. One proton attaching at C-3 and resonating at δ_H 2.12 (m) was found to exhibit an NOE correlation with H-2, and was assigned as H-3α proton. Since H-4 exhibited an NOE interaction with H-2, the C-4 hydroxy group should attach on the β face. H-7 showed a strong NOE correlation with H-3β, confirming the β-orientation for H-7. Furthermore, H-9 was found to show NOE correlations with H_3-18 and H_3-20, and, from molecular models, was found to be reasonably close to H_3-18 and H_3-20; therefore, H-9 should be placed on the α face in **2**, and H_3-18 is β-oriented in the γ-lactone ring. The acetoxy group attached at C-12 was placed on the β face by an NOE correlation between H-12 and H_3-20. Moreover, the *Z*-configuration of the C-5/C-6 double bond was elucidated by a strong NOE response between H-6 (δ_H 5.53) and the H_3-16 (δ_H 2.07). On the basis of the above results, the structure of **2**, including the relative configuration, was elucidated, and all the chiral centers of **2** were assigned as 1*S**, 2*S**, 4*R**, 5*Z*, 7*S**, 8*R**, 9*S**, 10*S**, 11*R**, 12*S**, 13*Z*, and 17*R**. By detailed analysis, it was found that the spectral data of **2** were similar to those of a known briarane derivative, briaexcavatolide W (**6**),[32] except that the signals corresponding to a butyryloxy group in **6** were replaced by signals for an acetoxy group in **2**. Thus, briaexcavatin N (**2**) was found to be the 12-debutyryloxy-12-acetoxy derivative of **6**.

Briaexcavatin O (**3**) had a molecular formula of $C_{30}H_{42}O_{13}$ as deduced from HR-ESI-MS (*m/z* 633.2520, Calcd for $C_{30}H_{42}O_{13}Na$, 633.2522). Its IR spectrum exhibited a broad OH stretch at 3476 cm^{-1}, a γ-lactone carbonyl group at 1782 cm^{-1}, and ester carbonyl groups at 1733 cm^{-1}. Carbonyl resonances in the ^{13}C-NMR spectrum of **3** confirmed the presence of a γ-lactone and four ester groups (Table 1). Three of the esters were identified as acetates by the presence of three methyl resonances in the ^1H-NMR spectrum at δ_H 2.42 (3H, s), 2.10 (3H, s), and 2.08 (3H, s) (Table 2). The other ester was found to be a butyryloxy group based on ^1H-NMR studies, which revealed seven contiguous protons (δ_H 2.32, 2H, t, J=7.2 Hz; 1.66, 2H, sext, J=7.2 Hz; 0.96, 3H, t, J=7.2 Hz). However, due to their slight distortion, a set of smaller, extraneous ^1H-NMR peaks were found for the methylene protons attaching at butyrate carbonyl. From the data of an HMBC experiment of **3** (Fig. 5, Table 3), the molecular framework of **3** could be further established. These data also revealed that the carbon signal at δ_C 171.7 (s) was correlated with the signals of the methylene protons of the butyrate at δ_H 2.32 and 1.66 in the HMBC spectrum of **3** and was consequently assigned as the carbon atom of the butyrate carbonyl group. The butyrate positioned at C-3 was confirmed by the connectivity between H-3 (δ_H 5.81) and the carbonyl carbon (δ_C 171.7) of the butyryloxy group. Furthermore, the HMBC

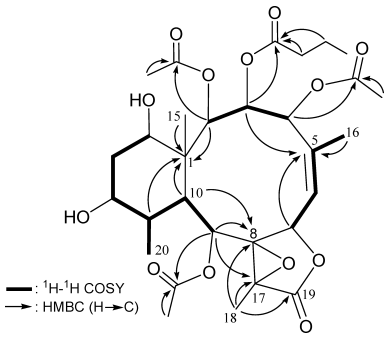


Fig. 5. ^{1}H–^{1}H COSY and Selective HMBC Correlations (Protons and Quaternary Carbons) of **3**

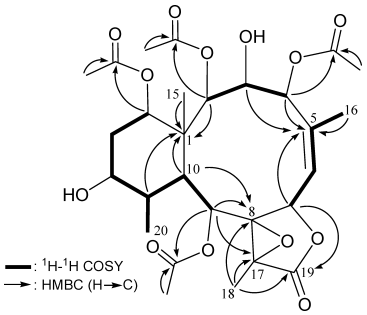


Fig. 7. ^{1}H–^{1}H COSY and Selective HMBC Correlations (Protons and Quaternary Carbons) of **4**

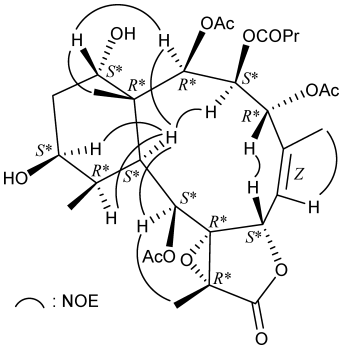


Fig. 6. Selective NOE Correlations of **3**

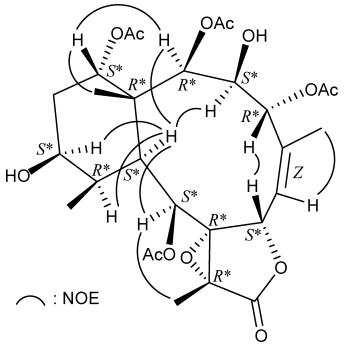


Fig. 8. Selective NOE Correlations of **4**

correlations revealed that the three acetoxy groups are attached to C-2, C-4, and C-9, respectively. Thus, the remaining hydroxy groups had to be positioned at C-12 and C-14 by the key ^{1}H–^{1}H COSY correlations and characteristic NMR signal analysis. The relative stereochemistry of **3** was confirmed as being similar to that of a known metabolite, briaexcavatolide P (**7**),[33] by comparison of the NMR chemical shifts and coupling constant analysis for the chiral centers. In the NOESY experiment of **3** (Fig. 6), H-10 gives NOE responses with H-2/3/9/11 and H-12. These indicated that H-2/3/9/10/11 and H-12 are situated on the same face of the molecule and are assigned as α protons, since the C-15 methyl is the β-substituent at C-1. H-14 gives NOE correlations with H-2 and H_3-15, confirming the β-orientation for this proton. The signal of H_3-18 showed an NOE correlation with H-9, indicating H_3-18 should be positioned on the β face of γ-lactone moiety in **3**. Also, H-4 was found to exhibit an NOE correlation with H-7, and from molecular models, was found to be reasonably close to H-7; therefore, H-4 and H-7 should be placed on the β face in **3**. Furthermore, the *Z*-configuration of the C-5/C-6 double bond was elucidated by a strong NOE response between the C-6 olefin proton (δ_H 5.42) and the C-16 vinyl methyl (δ_H 1.80). Thus, the relative configurations of all chiral centers of **3** were assigned as 1*R**, 2*R**, 3*S**, 4*R**, 5*Z*, 7*S**, 8*R**, 9*S**, 10*S**, 11*R**, 12*S**, 14*S**, and 17*R**.

Our present study has also led to the isolation of a new briarane, briaexcavatin P (**4**). The molecular formula of $C_{28}H_{38}O_{13}$ was deduced from HR-ESI-MS with *m/z* 605.2207 (Calcd for $C_{28}H_{38}O_{13}Na$, 605.2210). Carbonyl resonances in the ^{13}C-NMR spectrum of **4** (Table 1) at δ_C 172.5 (s), 170.8 (s), 170.1 (s), 170.0 (s), and 169.8 (s) revealed the presence of a γ-lactone and four esters in **4**. In the ^{1}H-NMR spectrum of **4** (Table 2), the signals for four acetate methyls were observed at δ_H 2.34 (3H, s), 2.18 (3H, s), 2.09 (3H, s), and 2.08 (3H, s). It was found that the 1D- (Tables 1, 2) and 2D-NMR (Fig. 7, Table 3) data of **4** were similar to those of a known briarane derivative, briaexcavatolide P (**7**),[33] except that the signals corresponding to a butyryloxy group in **7** were replaced by signals for an acetoxy group in **4**. The correlations from a NOESY experiment of **4** (Fig. 8) also showed that the stereochemistry of this metabolite is identical with those of **3** and **7**. Thus, briaexcavatin P (**4**) was found to be the 4-debutyryloxy-4-acetoxy derivative of **7**. The relative configurations of all centers of **4** were established as 1*R**, 2*R**, 3*S**, 4*R**, 5*Z*, 7*S**, 8*R**, 9*S**, 10*S**, 11*R**, 12*S**, 14*S**, and 17*R**.

In previous studies, Cardellina, Kashman, and their coworkers have pointed out the changes of the coupling constants between H-9 and H-10 with the substituent at C-11, and this conclusion can explain the differences of coupling constant between H-9 and H-10 in compound **2** (J= 4.4 Hz) with those of compounds **3** (J=9.6 Hz) and **4** (J= 8.8 Hz).[34—36] Our study also showed that the changes in configurations of the 10-membered ring unit will cause the coupling constant between H-9 and H-10 to change significantly, even if the C-11 remains unchanged as the present compound **1** (J=0 Hz).[36]

In the biological activity testing, briaexcavatin P (**4**) was found to show a 14.99% inhibitory effect on superoxide anion generation by human neutrophils at 10 μg/ml.

Experimental

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. The NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR at 400 MHz for ^{1}H and 100 MHz for ^{13}C, in $CDCl_3$, respectively. Proton chemical shifts were referenced to the residual $CHCl_3$ signal (δ 7.26 ppm). ^{13}C-NMR spectra were referenced to the center peak of $CDCl_3$ at δ 77.1 ppm. ESI-MS and HR-ESI-MS data were recorded on a BRUKER APEX II mass spectrometer. Column chromatography was performed on silica gel (230—400 mesh, Merck, Darmstadt, Germany). TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck) and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. HPLC was performed using a system comprised of a HITACHI L-7100 pump, a HITACHI photodiode array detector L-7455, and a RHEODYNE 7725 injection port. A semi-preparative column (Hibar 250—25 mm, LiChrospher Si 60, 5 μm) was used for HPLC.

Animal Material Specimens of the octocoral *Briareum excavatum* were collected from the culturing tank in the NMMBA at March 2007. This organism was identified by comparison with previous descriptions.[37,38]

Extraction and Isolation The freeze-dried and minced material of *B. excavatum* (wet weight 672 g, dry weight 270 g) was extracted with a mixture of MeOH and CH_2Cl_2 (1 : 1) at room temperature. The residue was partitioned between EtOAc and H_2O. The EtOAc layer was separated on Sephadex LH-20 and eluted using MeOH/CH_2Cl_2 (2 : 1) to yield three fractions A—C. Fraction C was separated on silica gel and eluted using hexane/EtOAc (stepwise, 20 : 1—pure EtOAc) to yield fractions 1—9. Fraction 8 was separated by normal phase HPLC, using the mixtures of CH_2Cl_2 and EtOAc to afford briaranes **1** (1.3 mg, 3 : 1), **2** (1.4 mg, 3 : 1), and **3** (3.3 mg, 2 : 1). A mixture from fraction 8 was repurified by normal phase HPLC, using the mixtures of CH_2Cl_2 and acetone to afford briarane **4** (2.6 mg, 10 : 1).

Briaexcavatin M (**1**): White powder; mp 120—122 °C; $[\alpha]_D^{25}$ −7° (c=0.05, $CHCl_3$); IR (neat) ν_{max} 3468, 1768, 1740 cm^{-1}; ^{13}C-NMR ($CDCl_3$, 100 MHz) and ^{1}H-NMR ($CDCl_3$, 400 MHz) data, see Tables 1 and 2; ESI-MS m/z 529 $(M+Na)^+$; HR-ESI-MS m/z 529.2052 (Calcd for $C_{26}H_{34}O_{10}Na$, 529.2050).

Briaexcavatin N (**2**): White powder; mp 247—249 °C; $[\alpha]_D^{25}$ −94° (c=0.02, $CHCl_3$); IR (neat) ν_{max} 3461, 1775, 1728 cm^{-1}; ^{13}C-NMR ($CDCl_3$, 100 MHz) and ^{1}H-NMR ($CDCl_3$, 400 MHz) data, see Tables 1 and 2; ESI-MS m/z 545 $(M+Na)^+$; HR-ESI-MS m/z 545.1998 (Calcd for $C_{26}H_{34}O_{11}Na$, 545.1999).

Briaexcavatin O (**3**): White powder; mp 180—181 °C; $[\alpha]_D^{25}$ +95° (c=0.12, $CHCl_3$); IR (neat) ν_{max} 3476, 1782, 1733 cm^{-1}; ^{13}C-NMR ($CDCl_3$, 100 MHz) and ^{1}H-NMR ($CDCl_3$, 400 MHz) data, see Tables 1 and 2; ESI-MS m/z 633 $(M+Na)^+$; HR-ESI-MS m/z 633.2520 (Calcd for $C_{30}H_{42}O_{13}Na$, 633.2522).

Briaexcavatin P (**4**): White powder; mp 272—274 °C; $[\alpha]_D^{25}$ +198° (c=0.08, $CHCl_3$); IR (neat) ν_{max} 3521, 1774, 1742 cm^{-1}; ^{13}C-NMR ($CDCl_3$, 100 MHz) and ^{1}H-NMR ($CDCl_3$, 400 MHz) data, see Tables 1 and 2; ESI-MS m/z 605 $(M+Na)^+$; HR-ESI-MS m/z 605.2207 (Calcd for $C_{28}H_{38}O_{13}Na$, 605.2210).

Human Neutrophil Superoxide Anion Generation Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Superoxide generation was carried out according to the procedures described previously.[39,40] Briefly, superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*.

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