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Excavatoids A–D, new polyoxygenated briaranes from the octocoral *Briareum excavatum*

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ABSTRACT

Three polyoxygenated briaranes, including two new compounds, excavatoids A (**1**) and B (**2**), and a known metabolite, briaexcavatin I (**3**), were isolated from the cultured octocoral *Briareum excavatum*. Moreover, the wild type *B. excavatum*, collected off southern Taiwan coast, yielded two new 5,6-epoxy-briaranes, excavatoids C (**4**) and D (**5**). The structures of new compounds **1**, **2**, **4**, and **5** were determined by spectroscopic methods and the structure of **1** was further confirmed by X-ray diffraction data analysis. The X-ray structure for briaexcavatin I (**3**) was also reported for the first time. Excavatoid A (**1**) is the first briarane which possesses six hydroxy groups and a 17-methoxy group. Excavatoid C (**4**) is the first 12,13-secobriarane which possesses a novel pentacyclic skeleton with an ε-lactone. Excavatoid D (**5**) displayed moderate inhibitory effects on superoxide anion generation and elastase release by human neutrophils.

1. Introduction

Gorgonian corals belonging to the genus *Briareum* (Briareidae) were proven to be a rich source of marine diterpenoid derivatives, such as briarane, asbestinane, and eunicellin-type natural products,[1–4] and most compounds of these classes were found to possess complex structure.[5] In our continuing research on novel substances from the marine invertebrates originally distributed in tropical Taiwanese waters as part of the NSTPBP, Taiwan,[6] the octocoral *Briareum excavatum* was transplanted to the National Museum of Marine Biology & Aquarium, Taiwan (NMMBA), for its interesting chemical constituents. In this paper, we report on two new briaranes, excavatoids A (**1**) and B (**2**), and a known metabolite, briaexcavatin I (**3**), from the cultured *B. excavatum*; and two new briaranes, excavatoids C (**4**) and D (**5**), from the wild type *B. excavatum*.

2. Results and discussion

2.1. Isolation and structure determination of excavatoids A and B from cultured *B. excavatum*

In previous studies, a series of briarane-type diterpenoids, including 18 new briaranes, briaexcavatins I–Z,[7–11] were isolated and reported from the cultured *B. excavatum*. Excavatoid A (**1**) was obtained as a white powder and the molecular formula for **1** was determined to be $C_{21}H_{34}O_9$ (five degrees of unsaturation) by HRESIMS (*m*/*z* 453.2097, calcd for $C_{21}H_{34}O_9$+Na, 453.2100). Comparison of the DEPT data with the molecular formula indicated that there must be six exchangeable protons, requiring the presence of six hydroxy groups, and this deduction was supported by a broad absorption in the IR spectrum at 3447 cm^{-1}. The IR spectrum also showed a strong band at 1766 cm^{-1}, consistent with the presence of γ-lactone moiety. From the ^1H and ^{13}C NMR spectra (Table 1), **1** was found to possess a γ-lactone (δ_C 175.1, s, C-19) and a trisubstituted olefin [δ_H 5.12 (1H, d, *J*=7.2 Hz, H-6); δ_C 144.5 (s, C-5), 120.4 (d, CH-6)]. Thus, from the above data, two degrees of unsaturation were accounted for, and **1** must be tricyclic.

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Table 1
^{1}H and ^{13}C NMR data, ^{1}H–^{1}H COSY, and HMBC correlations for diterpenoid **1**

C/H	δ_H[a]	δ_C[b]	^{1}H–^{1}H COSY	HMBC (H→C)
1		46.3 (s)[d]		
2	3.45 s	88.8 (d)	H-3	C-3, -4, -10, -14
3	4.40 dd (3.6, 3.2)[c]	75.5 (d)	H-2, H_2-4	C-5
4α	1.64 dd (10.4, 3.6)	35.7 (t)	H-3, H-4β	C-3, -5, -6, -16
β	3.31 dd (10.4, 3.2)		H-3, H-4α	C-2, -3, -5, -6, -16
5		144.5 (s)		
6	5.12 d (7.2)	120.4 (d)	H-7, H_3-16	C-4, -5, -16
7	5.89 d (7.2)	80.9 (d)	H-6	C-5, -6
8		84.3 (s)		
9	4.32 d (9.2)	69.9 (d)	H-10	C-7, -8, -10, -11, -17
10	2.25 dd (9.2, 4.8)	40.5 (d)	H-9, H-11	C-1, -2, -8, -9, -11, -15, -20
11	2.62 m	38.2 (d)	H-10, H-12, H_3-20	C-1, -10, -12, -20
12	4.08 m	68.0 (d)	H-11, H_2-13	C-10, -13, -20
13α	1.60 m	34.7 (t)	H-12, H-13β, H-14	n.o.[e]
β	2.05 ddd (9.2, 9.2, 2.8)		H-12, H-13α, H-14	C-11, -12
14	3.60 dd (2.8, 2.8)	80.4 (d)	H_2-13	C-1, -10, -12, -15
15	1.09 s	21.2 (q)		C-1, -2, -10, -14
16	1.92 s	23.3 (q)	H-6	C-4, -5, -6
17		86.5 (s)		
18	1.35 s	10.9 (q)		C-8, -17, -19
19		175.1 (s)		
20	1.05 d (7.2)	9.3 (q)	H-11	C-10, -11, -12
17-OCH_3	3.37 s	52.7 (q)		C-17

[a] Spectra measured at 400 MHz in CD_3OD at 25 °C.
[b] Spectra measured at 100 MHz in CD_3OD at 25 °C.
[c] *J* values (in hertz) in parentheses.
[d] Attached protons were deduced by DEPT and HMQC experiments.
[e] n.o.=not observed.

From the ^{1}H–^{1}H COSY spectrum of **1** (Table 1), it was possible to identify the separate spin systems between H-2/H-3/H_2-4, H-6/H-7, and H-9/H-10. These data, together with the HMBC correlations between H-2/C-3, -4, -10; H-3/C-5; H_2-4/C-2, -3, -5, -6; H-6/C-4, -5; H-7/C-5, -6; H-9/C-7, -8, -10; and H-10/C-1, -2, -8, -9, established the connectivity from C-1 to C-10 within the ten-membered ring (Table 1). The vinyl methyl attached at C-5 was confirmed by the HMBC correlations between H_3-16/C-4, -5, -6; and H-6/C-16; and further supported by the allylic coupling between H-6 and H_3-16. The methylcyclohexane ring, which is fused to the ten-membered ring at C-1 and C-10, was elucidated by the ^{1}H–^{1}H COSY correlations between H-10/H-11/H-12/H_2-13/H-14 and H-11/H_3-20; and supported by the HMBC correlations between H-2/C-14; H-9/C-11; H-10/C-11, -20; H-11/C-1, -10, -20; H-12/C-10, -20; H-14/C-1, -10; and H_3-20/C-10, -11, -12. The ring junction C-15 methyl was positioned at C-1 from the HMBC correlations between H_3-15/C-1, -2, -10, -14 and H-10, H-14/C-15. The 17-methoxy group was indicated by an HMBC correlation between the proton signal of methoxy group (δ_H 3.37, 3H, s) and an oxygenated quaternary carbon (δ 86.5, s, C-17). The hydroxy groups had to be positioned at C-2, C-3, C-9, C-12, and C-14, as indicated by analysis of the ^{1}H–^{1}H COSY correlations and characteristic NMR signals analysis. Thus, the remaining hydroxy group is positioned at C-8, an oxygen-bearing quaternary carbon at δ 84.3 (s). These data, together with the HMBC correlations between H-9/C-17 and H_3-18/C-8, -17, -19, were used to establish the molecular framework of **1**.

Based on previous surveys, all the naturally occurring briaranes have the H-10 is trans to the C-15 methyl group, and these two groups are assigned as α and β-oriented in most briarane derivatives.[2–4] The relative configuration of **1** was elucidated from the interactions observed in a NOESY experiment and from vicinal protons coupling constant analysis. In the NOESY experiment of **1** (Fig. 1), the correlations of H-10 with H-3, H-11, and H-12, but not

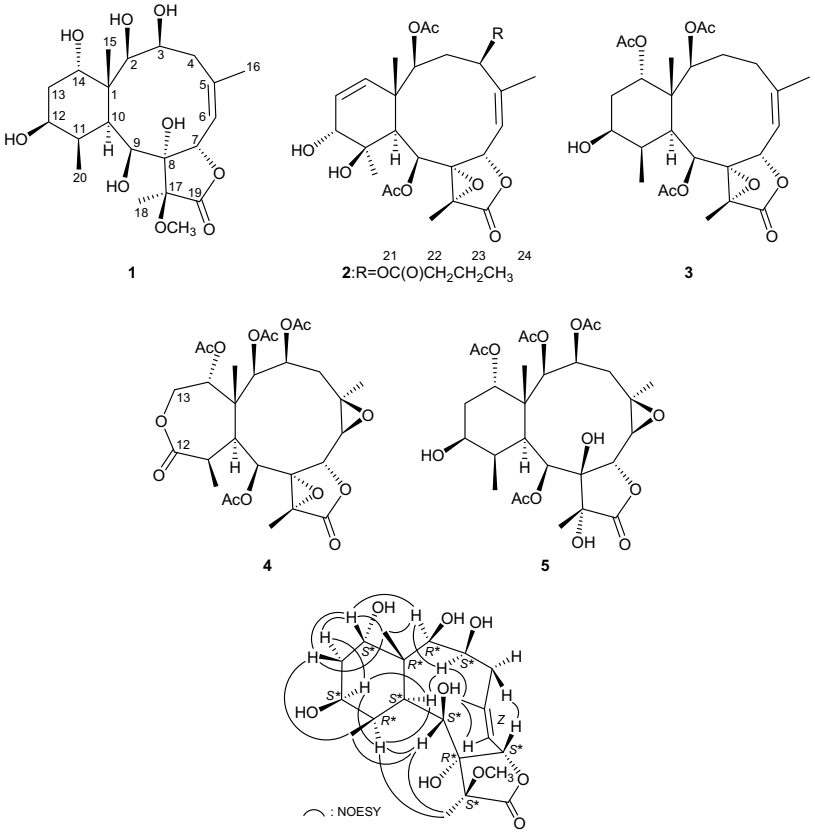

Figure 1. Selective NOESY correlations of **1**.

with H_3-15 and H_3-20, indicated that these protons (H-3, H-10, H-11, and H-12) were situated on the same face and were assigned as α protons since the C-15 and C-20 methyls are β-substituents at C-1 and C-11, respectively. H-2 exhibited an interaction with H-3, and no coupling was found between H-2 and H-3, indicating that the dihedral angle between these two protons is approximately 90° and the hydroxy group at C-2 should be β-oriented by modeling analysis. H-14 was found to exhibit a response with H_3-15, but not with H-10, showing that this proton has a β-orientation. H-9 was found to show responses with H-11, H_3-18, and H_3-20, but not with H_3-15. From modeling analysis, H-9 was found to be close to H-11, H_3-18, and H_3-20 when H-9 was α-oriented. Moreover, H_3-16 exhibited correlations with H-3 and H-6, and a doublet coupling constant (J=7.2 Hz) was detected between H-6 and H-7, suggesting the *Z*-configuration of C-5/6 double bond and β-orientation of H-7. H-11 showed a response with H_3-18, confirming the β-orientation for 17-methoxy group. However, due to all the hydroxy protons that were not detected in the NOESY spectrum of **1**, the stereochemistry of the tertiary hydroxy group cannot be determined by this method.

A single-crystal X-ray diffraction analysis was carried out in order to determine the structure of **1** (Fig. 2). The X-ray structure demonstrates the C-8 hydroxy group was α-oriented. Based on the X-ray diffraction analysis, the chiral centers in **1** were assigned as 1*R**, 2*R**, 3*S**, 7*S**, 8*R**, 9*S**, 10*S**, 11*R**, 12*S**, 14*S**, and 17*S**. From the above findings, the structure of **1** was elucidated unambiguously. By detailed analysis, the structure of excavatoid A (**1**) is not similar with those of the known briarane analogues. Except for the γ-lactone moiety, there is no acyloxy group was found in the structure of **1**. To the best of our knowledge, excavatoid A (**1**) is the most polar briarane that has been discovered and this compound also is the first briarane which possesses six hydroxy groups and a 17-methoxy group.

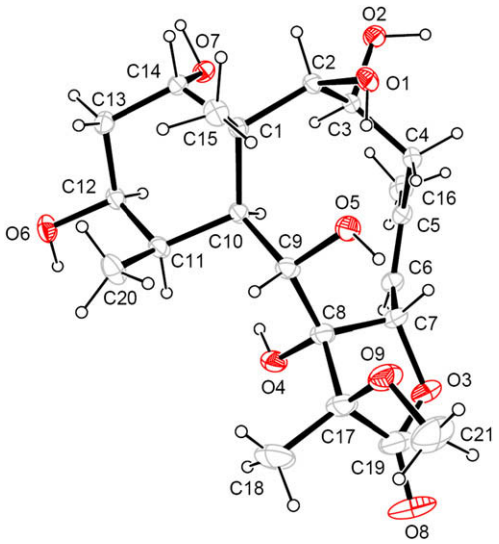

Figure 2. Computer-generated ORTEP plot of **1** showing the relative configuration.

Excavatoid B (**2**) had a molecular $C_{28}H_{38}O_{11}$ as deduced from HRESIMS (*m*/*z* 573.2314, calcd for $C_{28}H_{38}O_{11}$+Na, 573.2312). Its IR spectrum exhibited a broad OH stretch at 3443 cm^{-1}, γ-lactone at 1774 cm^{-1}, and ester carbonyls at 1728 cm^{-1}. Carbonyl resonances in the ^{13}C NMR spectrum of **2** at δ 172.7 (s), 171.0 (s, C-19), 170.4 (s), and 169.3 (s) revealed the presence of a γ-lactone and three additional esters in **2** (Table 2). Two of the esters were identified as acetates by the presence of methyl resonances in the ^1H NMR spectrum at δ 2.24 (3H, s) and 2.12 (3H, s). The other ester was found to be an *n*-butyryloxy group based on NMR studies, including

Table 2
^1H and ^{13}C NMR data, ^1H–^1H COSY, and HMBC correlations for diterpenoid **2**

C/H	δ_H[a]	δ_C[b]	^1H–^1H COSY	HMBC (H→C)
1		45.4 (s)[d]		
2	4.62 d (6.4)[c]	76.8 (d)	H-3α/β	C-4, -10, -15, acetate carbonyl
3α	2.12 ddd (14.6, 6.4, 5.6)	38.8 (t)	H-2, H-3β, H-4	C-2, -4
β	2.83 dd (14.6, 13.2)		H-2, H-3α, H-4	C-1, -4
4	5.07 ddd (13.2, 5.6, 1.2)	72.1 (d)	H-3α/β, H-6	C-5, -6, -16
5		145.0 (s)		
6	5.49 ddd (10.0, 1.2, 1.2)	122.6 (d)	H-4, H-7, H_3-16	C-4
7	5.84 d (10.0)	73.7 (d)	H-6	C-5, -8, -17
8		70.5 (s)		
9	5.81 d (4.4)	67.1 (d)	H-10	C-7, -8, -10, -11, -17, acetate carbonyl
10	2.55 d (4.4)	43.8 (d)	H-9	C-1, -2, -8, -9, -12, -15
11		75.3 (s)		
12	3.75 br s	71.8 (d)	H-13	n.o.[e]
13	5.80 dd (10.4, 5.2)	124.1 (d)	H-12, H-14	C-11, -12
14	5.52 d (10.4)	138.8 (d)	H-13	C-2, -10, -12, -15
15	1.23 s	17.8 (q)		C-1, -2, -10, -14
16	2.10 d (1.2)	25.6 (q)	H-6	C-4, -5, -6
17		62.7 (s)		
18	1.62 s	9.7 (q)		C-8, -17
19		171.0 (s)		
20	1.40 s	28.8 (q)		C-10, -11
2-OAc		170.4 (s)		
	2.12 s	21.1 (q)		Acetate carbonyl
9-OAc		169.3 (s)		
	2.24 s	21.7 (q)		Acetate carbonyl
4-OC(O)Pr		172.7 (s)		
	2.30 t (7.6) (H_2-22)	36.1 (t)	H_2-23	C-21, -23, -24
	1.65 sext (7.6) (H_2-23)	18.4 (t)	H_2-22, H_3-24	C-21, -22, -24
	0.95 t (7.6) (H_3-24)	13.6 (q)	H_2-23	C-22, -23

[a] Spectra measured at 400 MHz in $CDCl_3$ at 25 °C.
[b] Spectra measured at 100 MHz in $CDCl_3$ at 25 °C.
[c] J values (in hertz) in parentheses.
[d] Attached protons were deduced by DEPT and HMQC experiments.
[e] n.o.=not observed.

an ^1H–^1H COSY spectrum (Table 2), which revealed seven contiguous protons (δ 2.30, 2H, t, J=7.6 Hz; 1.65, 2H, sext, J=7.6 Hz; 0.95, 3H, t, J=7.6 Hz). The carbon signal at δ 172.7 (s) revealed correlation with the signals of methylene protons at δ 2.30 and 1.65 in the HMBC spectrum and was assigned as the carbonyl carbon of *n*-butyrate (Table 2). It was found that the NMR data of **2** were similar with those of a known briarane analogue, briaexcavatin K (**6**),[7] except that the signals corresponding to the 4-hydroxy group in **6** were not present, and had been replaced by those of an *n*-butyryloxy group in **2**. In addition, the HMBC correlations also revealed that two acetates should attach at C-2 and C-9, respectively. The remaining *n*-butyryloxy group was positioned at C-4 as indicated by analysis of ^1H–^1H COSY correlations and characteristic NMR signals analysis, although no HMBC correlation was observed between H-4 and the *n*-butyrate carbonyl. The correlations from a NOESY experiment of **2** (Fig. 3) also showed that the relative configurations of most chiral centers of **2** is similar to those of **6**. H-4 exhibited an interaction with H_3-16 and a doublet of doublets with the coupling constants (J=13.2, 5.6 Hz) was found between H-4 and C-3 methylene protons, indicating that the *n*-butyryloxy group at C-4 was β-oriented and the chiral centers in **2** were assigned as 1*S**, 2*S**, 4*R**, 7*S**, 8*R**, 9*S**, 10*S**, 11*R**, 12*R**, and 17*S**.

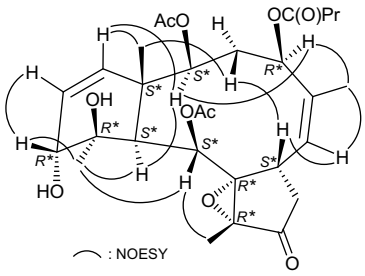

Figure 3. Selective NOESY correlations of **2**.

The known briarane, briaexcavatin I (**3**), was described in our previous report, which was also isolated from this cultured species.[7] In this study, we reported the X-ray structure of briaexcavatin I (**3**) for the first time (Fig. 4).

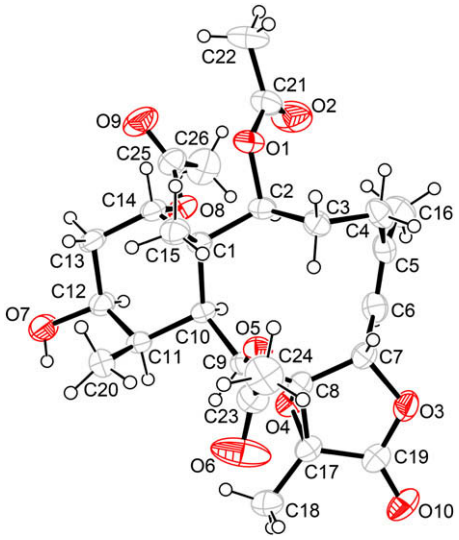

Figure 4. Computer-generated ORTEP plot of **3** showing the relative configuration.

2.2. Isolation and structure determination of excavatoids C and D from the wild type *B. excavatum*

Excavatoid C (**4**) was isolated as a white powder and had a molecular formula $C_{28}H_{36}O_{14}$, as determined by HRESIMS ($C_{28}H_{36}O_{14}$+Na *m/z* found 619.2006; calcd 619.2003) indicating eleven degrees of unsaturation. The presences of lactone and ester groups in **4** were evidenced by the IR absorptions at 1793 and 1739 cm^{-1}. In the ^{13}C spectrum of **4**, six ester carbonyls resonances appeared at δ 172.0 (s, C-12), 171.3 (s), 169.9 (s, C-19), 169.2 (s), 168.8 (s), and 167.8 (s) (Table 3). In the above carbonyl carbons, four were identified as acetate carbonyls by the presence of four methyl resonances in the ^1H NMR spectrum at δ 2.21, 2.14, 2.06, and 2.01 (each 3H×s) (Table 3). According to the above data, briarane **4** was found to be a pentacyclic compound with two lactones, as no other unsaturated functional group could be found. A tetrasubstituted epoxide containing a methyl substituent was elucidated from the signals of two oxygenated quaternary carbons at δ 71.7 (s, C-8) and 63.2 (s, C-17); and further confirmed by the proton signal of a methyl singlet at δ 1.51 (3H, s, H_3-18). In addition, a trisubstituted epoxide containing a methyl substituent was deduced from the signals of an oxymethine (δ_H 3.09, 1H, d, J=8.4 Hz, H-6; δ_C 63.3, d, CH-6), a quaternary oxygen-bearing carbon (δ 61.7, s, C-5), and a methyl singlet at δ 1.38 (3H, s, H_3-16). From the ^1H–^1H COSY and HMBC correlations (Table 3), the epoxy groups at C-5/6 and C-8/17; and the acetoxy groups at C-2, C-3, C-9, and C-14 were established, respectively. Furthermore, the ε-lactone formed by insertion of an oxygen atom between C-12 and C-13 was confirmed by an HMBC correlation between one proton of C-13 methylene (δ 3.86, H-13b) and C-12 carbonyl (δ 172.0, s). Thus, the planar structure of **4** was established.

Table 3
^1H and ^{13}C NMR data, ^1H–^1H COSY, and HMBC correlations for diterpenoid **4**

C/H	δ_H[a]	δ_C[b]	^1H–^1H COSY	HMBC (H→C)
1		44.6 (s)[d]		
2	5.90 d (2.4)[c]	69.3 (d)	H-3	C-3, -4, -14, acetate carbonyl
3	5.05 ddd (4.0, 2.4, 2.4)	70.3 (d)	H-2, H_2-4	n.o.[e]
4α	2.33 dd (16.8, 4.0)	33.8 (t)	H-3, H-4β	C-6, -16
β	2.18 dd (16.8, 2.4)		H-3, H-4α	C-2, -16
5		61.7 (s)		
6	3.09 d (8.4)	63.3 (d)	H-7	C-5
7	4.60 d (8.4)	78.3 (d)	H-6	C-6
8		71.7 (s)		
9	5.44 d (2.4)	69.1 (d)	H-10	C-7, -8, -10, -11, acetate carbonyl
10	2.42 br s	46.4 (d)	H-9, H-11	C-1, -2, -8, -9, -11, -12, -14
11	3.42 qd (6.8, 1.2)	36.4 (d)	H-10, H_3-20	C-1, -9, -10, -12, -20
12		172.0 (s)		
13a	4.57 dd (13.6, 6.4)	64.2 (t)	H-13b, H-14	n.o.
b	3.86 dd (13.6, 2.4)		H-13a, H-14	C-1, -12, -14
14	4.86 dd (6.4, 2.4)	74.9 (d)	H_2-13	C-2, acetate carbonyl
15	1.54 s	18.7 (q)		C-1, -2, -10, -14
16	1.38 s	21.4 (q)		C-4, -5, -6
17		63.2 (s)		
18	1.51 s	10.4 (q)		C-8, -17, -19
19		169.9 (s)		
20	1.39 d (6.8)	17.1 (q)	H-11	C-1, -10, -11, -12
2-OAc		168.8 (s)		
	2.01 s	20.5 (q)		Acetate carbonyl
3-OAc		169.2 (s)		
	2.14 s	20.9 (q)		Acetate carbonyl
9-OAc		167.8 (s)		
	2.21 s	20.6 (q)		Acetate carbonyl
14-OAc		171.3 (s)		
	2.06 s	20.6 (q)		Acetate carbonyl

[a] Spectra measured at 400 MHz in $CDCl_3$ at 25 °C.
[b] Spectra measured at 100 MHz in $CDCl_3$ at 25 °C.
[c] *J* values (in hertz) in parentheses.
[d] Attached protons were deduced by DEPT and HMQC experiments.
[e] n.o.=not observed.

The relative stereochemistry of **4** was elucidated from the interactions observed in a NOESY experiment (Fig. 5). Due to the α orientation of H-10, the ring junction C-15 methyl should be β-oriented as no correlation was observed between H-10 and H_3-15. In the NOESY spectrum of **4**, H-10 displayed correlations with H-3, H-9, and H-11; and H-3 correlated with H-2 and H_3-16. Thus, H-2, H-3, H-9, H-10, H-11, and H_3-16 were 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 H_3-20, but instead exhibited a correlation with H-11. The oxygen of 5,6-epoxy group was positioned on the β face by a correlation between H_3-16 and H-6, but not with H-7. H-14 was found to exhibit responses with H-2 and H_3-15, but not with H-10, revealing the α-orientation of 14-acetoxy group. Furthermore, the signal of H_3-18 showed correlations with H-9 and H-11. By detailed analysis of molecular models, H_3-18 was found to be reasonably close to H-9 and H-11, when H_3-18 was placed on the β face in the γ-lactone moiety. Based on the above findings, the structure of **1** was elucidated and the configurations of all chiral centers of **4** were assigned as 1*R**, 2*R**, 3*S**, 5*R**, 6*S**, 7*S**, 8*R**, 9*S**, 10*S**, 11*R**, 14*R**, and 17*R**.

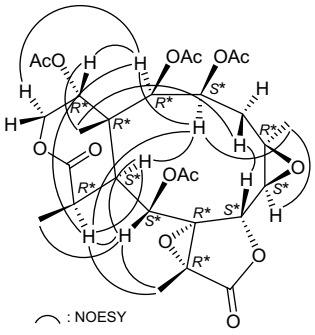

Figure 5. Selective NOESY correlations of **4**.

In our previous study, an 11,12-secobriarane, briaexcavatin A, which possesses an ε-lactone moiety was also obtained from this organism.[12] By detailed analysis, the structure of excavatoid C (**4**) was found to be similar with that of briaexcavatin A for their ε-lactone moieties. However, these two compounds possess different carbon skeletons. It has to be noted that **4** is the first 12,13-secobriarane possessing an ε-lactone.

The new briarane, excavatoid D (**5**), was isolated as a white powder. The molecular formula $C_{28}H_{40}O_{14}$ (nine degrees of unsaturation) was established by HRESIMS ($C_{28}H_{40}O_{14}$+Na *m*/*z* found 623.2318; calcd 623.2316). The IR spectrum of **5** also showed strong bands at 3468, 1776, and 1734 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester groups. From the ^{13}C NMR data of **5** (Table 4), the presence of five carbonyl resonances at δ 175.4 (s, C-19), 170.9, 170.5, 170.0, and 169.4 (4×s, ester carbonyls), confirmed the presence of a γ-lactone and four ester groups in **5**. In the ^{1}H NMR spectrum of **5** (Table 4), four acetate methyl signals were observed (δ 2.21, 2.10, 2.02, and 1.94, each 3H×s). The presence of a methyl containing trisubstituted epoxide was elucidated from the signals of an oxygenated quaternary carbon (δ_C 62.8, s, C-5) and an oxymethine (δ_H 3.40, 1H, d, *J*=9.6 Hz, H-6; δ_C 63.1, d, CH-6), and further confirmed by a methyl singlet (δ_H 1.37, 3H, s, H_3-16; δ_C 21.4, q, CH_3-16).

The gross structure of **5** was determined using 2D NMR studies. From the ^{1}H–^{1}H COSY spectrum of **5**, five different structural units H-2/H-3/H_2-4, H-6/H-7, H-9/H-10/H-11/H-12/H_2-13/H-14, and H-11/H_3-20 were identified (Table 4), which were assembled with the assistance of an HMBC experiment (Table 4). The HMBC correlations between protons and quaternary carbons of **1**, such as H-2, H-9, H-10, H_2-13, H_3-15/C-1; H-4, H-7, H_3-16/C-5; H-7, H-9, H-10, H_3-18/C-8; H-7, H-9, H_3-18/C-17; and H-7, H_3-18/C-19, permitted elucidation of the carbon skeleton. The ring junction C-15 methyl group was positioned at C-1 from the HMBC correlations between H_3-15/C-1, -2, -10, -14. A methyl attached at C-5 was confirmed by the HMBC correlations between H_3-16/C-4, -5, -6; and H-4/C-16. The presence of acetate esters positioned at C-2, C-9, and C-14 were established by the HMBC correlations between protons H-2 (δ 5.76), H-9 (δ 6.19), H-14 (δ 5.27) and acetate carbonyls (δ 169.4, 170.5, 170.0). The presences of 8-hydroxy group and 17-hydroxy group were deduced from the HMBC correlations between the hydroxy protons at δ 2.90 (s, OH-8) with C-7, -8, -9; and δ 3.00 (s, OH-17) with C-8, -17, -19, respectively. The remaining acetoxy and hydroxy groups were positioned at C-3 and C-12, respectively, as indicated by analysis of ^{1}H–^{1}H COSY correlations and characteristic NMR signal analysis. Thus, the planar structure of **5** was fully established. The relative stereochemistry of **5** was deduced from a NOESY experiment and the chiral centers for this compound were assigned as 1*R**, 2*R**, 3*S**, 5*R**, 6*S**, 7*S**, 8*S**, 9*S**, 10*S**, 11*R**, 12*S**, 14*S**, and 17*R**, by its NOESY correlations (Fig. 6).

By detailed analysis, the structure of **5** was similar to that of a known 5,6-epoxybriarane, briaexcavatin H,[13] and this compound

Table 4
^{1}H and ^{13}C NMR data, ^{1}H–^{1}H COSY, and HMBC correlations for diterpenoid 5

C/H	δ_H[a]	δ_C[b]	^{1}H–^{1}H COSY	HMBC (H→C)
1		42.8 (s)[d]		
2	5.76 d (3.2)[c]	70.4 (d)	H-3	C-1, -3, -4, -14, acetate carbonyl
3	5.21 br s	70.6 (d)	H-2, H_2-4	n.o.[e]
4	2.17 m (2H)	33.0 (t)	H-3	C-2, -5, -6, -16
5		62.8 (s)		
6	3.40 d (9.6)	63.1 (d)	H-7	n.o.
7	4.41 d (9.6)	87.9 (d)	H-6	C-5, -6, -8, -9, -17, -19
8		81.7 (s)		
9	6.19 s	64.3 (d)	H-10	C-1, -8, -10, -11, -17, acetate carbonyl
10	2.18 br s	46.4 (d)	H-9, H-11	C-1, -3, -8, -9, -11, -12, -14, -20
11	2.37 m	37.0 (d)	H-10, H-12, H_3-20	C-9, -10, -12, -20
12	3.95 br s	69.4 (d)	H-11, H_2-13	C-10, -14
13	1.98 m (2H)	34.4 (t)	H-12, H-14	C-1, -11, -12, -14
14	5.27 dd (10.4, 6.0)	73.8 (d)	H_2-13	C-2, -12, -13, acetate carbonyl
15	1.38 s	20.0 (q)		C-1, -2, -10, -14
16	1.37 s	21.4 (q)		C-4, -5, -6
17		76.9 (s)		
18	1.39 s	16.4 (q)		C-8, -17, -19
19		175.4 (s)		
20	1.20 d (7.2)	16.0 (q)	H-11	C-10, -11, -12
OH-8	2.90 s			C-7, -8, -9
OH-17	3.00 s			C-8, -17, -19
2-OAc		169.4 (s)		
	2.10 s	20.9 (q)		Acetate carbonyl
3-OAc		170.9 (s)		
	2.02 s	20.8 (q)		Acetate carbonyl
9-OAc		170.5 (s)		
	2.21 s	21.8 (q)		Acetate carbonyl
14-OAc		170.0 (s)		
	1.94 s	20.9 (q)		Acetate carbonyl

[a] Spectra measured at 400 MHz in $CDCl_3$ at 25 °C.
[b] Spectra measured at 100 MHz in $CDCl_3$ at 25 °C.
[c] *J* values (in hertz) in parentheses.
[d] Attached protons were deduced by DEPT and HMQC experiments.
[e] n.o.=not observed.

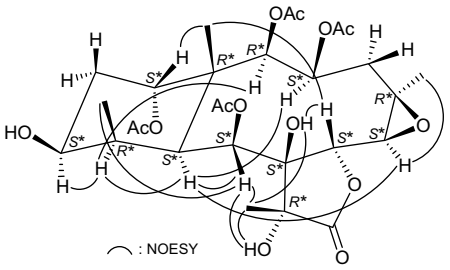

Figure 6. Selective NOESY correlations of **5**.

(briarane **5**) was found to be the 8,17-deoxy-8β,17α-dihydroxy derivative of briaexcavatin H.[13]

When the compounds **1** and **5** were obtained, we considered that these two metabolites could be artifacts. We dissolved the briaranes which possessing an 8,17-epoxide group, such as briaexcavatin I (**3**) in methanol with silica gel. After two weeks, we analyzed the solution by TLC and NMR, and there is no side product was found in the solution. Based on these observations, the authors suggested that excavatoids A (**1**) and D (**5**) are originally produced by corals and are not artifacts.

All the corals are claimed to be threatened species. We want to keep and culture these interesting specimens as the sources of potential natural products. In our continuing research for novel

substances from marine invertebrates collected in Taiwanese waters, we analyzed the organic extracts from the octocoral *B. excavatum* (including wild type and cultured *B. excavatum*), in the hope of identifying extracts that exhibit bioactivity. Briarane **5** (excavatoid D) displayed 23.8% inhibitory effect on superoxide anion generation and 39.4% inhibitory effect on elastase release by human neutrophils at 10 μg/mL, respectively, and the new compounds **1**, **2**, and **4** are not active in anti-inflammatory testing. Unfortunately, all the new compounds described herein are not active in cytotoxicity testing with DLD-1 (human colon adenocarcinoma) and CCRF-CEM (human T-cell acute lymphoblastic leukemia) cells. Due to the screening platforms are limited; and lots of material were consumed in physical and spectral experiments. The other possible biological activities for these interesting substances will not be assayed at this stage. The extensive assay platforms for the natural products will be set up by the National Science and Technology Program for Biotechnology and Pharmaceuticals (NSTPBP), Taiwan. The possible bioactivity for these compounds will be studied if we can get enough material from the cultured *B. excavatum* in the future.

3. Experimental

3.1. General experimental procedures

Melting points were determined using FARGO apparatus and were uncorrected. Optical rotation values were measured with a JASCO P-1010 digital polarimeter. Infrared spectra were obtained on a VARIAN DIGLAB FTS 1000 FT-IR 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, in CD_3OD or $CDCl_3$ using TMS as an internal standard. ESIMS and HRESIMS data were recorded on a BRUKER APEX II mass spectrometer. Column chromatography was performed on silica gel (230–400 mesh, Merck, Darmstadt, Germany), and 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 composed of a HITACHI L-7100 pump, a HITACHI photo diode array detector L-7455, and a RHEODYNE 7725 injection port. A normal phase semi-preparative column (Hibar 250×25 mm, LiChrospher Si 60, 5 μm) and a reverse phase semi-preparative column (Hibar 250×10 mm, Purospher STAR RP-18e, 5 μm) were used for HPLC.

3.2. Animal material

3.2.1. Cultured B. excavatum

Specimens of the cultured octocoral *B. excavatum* were collected by hand in 0.6-ton cultivating tanks located in the NMMBA, Taiwan, in December 2006. This organism was identified by comparison with previous descriptions.[14–16]

3.2.2. Wild type B. excavatum

Specimens of the octocoral *B. excavatum* were collected by hand by divers equipped with SCUBA off the coast of southern Taiwan in October 2003 at a depth of −10 m. Living reference specimens are being maintained in the authors' marine organism cultivating tank and a voucher specimen was deposited in the NMMBA, Taiwan. This organism was identified from descriptions.[14–16]

3.3. Extraction and isolation

3.3.1. Cultured B. excavatum

The freeze-dried and minced material of the cultured octocoral *B. excavatum* (wet weight 672 g) were extracted with a mixture of MeOH and CH_2Cl_2 (1:1) and 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). Briarane **1** was obtained by reverse phase HPLC, using a mixture of MeOH and H_2O (6.3 mg, 30:70). Briarane **2** was also obtained by reverse phase HPLC, using a mixture of MeOH and H_2O (1.2 mg, 60:40).

*3.3.1.1. Excavatoid A (**1**).* White powder; mp 209–210 °C; $[\alpha]_D^{22}$ −19 (*c* 0.3, MeOH); IR (neat) ν_{max} 3447, 1766 cm^{-1}; ^{1}H (CD_3OD, 400 MHz) and ^{13}C (CD_3OD, 100 MHz) NMR data, see Table 1; ESIMS *m*/*z* 453 (M+Na)$^+$; HRESIMS *m*/*z* 453.2097 (calcd for $C_{21}H_{34}O_9$+Na, 453.2100).

*3.3.1.2. Excavatoid B (**2**).* White powder; mp 144–145 °C; $[\alpha]_D^{25}$ −9 (*c* 0.06, $CHCl_3$); IR (neat) ν_{max} 3443, 1774, 1728 cm^{-1}; ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 2; ESIMS *m*/*z* 573 (M+Na)$^+$; HRESIMS *m*/*z* 573.2314 (calcd for $C_{28}H_{38}O_{11}$+Na, 573.2312).

*3.3.1.3. Briaexcavatin I (**3**).* The related physical (mp and optical rotation value) and spectral data (IR, MS, ^{1}H, and ^{13}C NMR) data of **3** were reported previously.[7]

3.3.2. Wild type B. excavatum

The organism (wet weight 1.0 kg) was collected and freeze-dried. The freeze-dried material were minced and extracted with EtOAc. The extract was separated by silica gel column chromatography, using hexane and hexane/EtOAc mixtures of increased polarity. Briarane **4** was further obtained by reverse phase HPLC, using a mixture of MeOH, CH_3CN, and H_2O (0.4 mg, 55:1:44). Briarane **5** was obtained by normal phase HPLC, using a mixture of ethyl acetate and CH_2Cl_2 (1.0 mg, 1:3).

*3.3.2.1. Excavatoid C (**4**).* White powder; mp 159–160 °C; $[\alpha]_D^{22}$ −112 (*c* 0.02, $CHCl_3$); IR (neat) ν_{max} 1793, 1739 cm^{-1}; ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 3; ESIMS *m*/*z* 619 (M+Na)$^+$; HRESIMS *m*/*z* 619.2006 (calcd for $C_{28}H_{36}O_{14}$+Na, 619.2003).

*3.3.2.2. Excavatoid D (**5**).* White powder; mp 114–115 °C; $[\alpha]_D^{22}$ −12 (*c* 0.05, $CHCl_3$); IR (neat) ν_{max} 3468, 1776, 1734 cm^{-1}; ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 4; ESIMS *m*/*z* 623 (M+Na)$^+$; HRESIMS *m*/*z* 623.2318 (calcd for $C_{28}H_{40}O_{14}$+Na, 623.2316).

3.4. Single-crystal X-ray crystallography of excavatoid A (1)[17]

Suitable colorless crystals of **1** cocrystalized with one MeOH and 1.5H_2O were obtained from a solution of MeOH. The crystal (0.80×0.80×0.60 mm) belongs to the monoclinic system, space group $P2_12_12_1$ (#19), with a=8.358(3) Å, b=10.029(2) Å, c=29.977(7) Å, V=2513(1) Å^3, Z=4, D_{calcd}=1.294 g/cm^3, λ (Mo Kα)=0.71073 Å. Intensity data were measured on a Rigaku AFC7S diffractometer up to $2\theta_{max}$ of 26°. All 2839 reflections were collected. The structure was solved by direct methods and refined by a full-matrix least-squares procedure. The refined structural model converged to a final $R1$=0.0517; $wR2$=0.1547 for 2010 observed reflections [$I>2\sigma(I)$] and 308 variable parameters.

3.5. Single-crystal X-ray crystallography of briaexcavatin I (3)[17]

Suitable colorless crystals of **3** were obtained from a solution of MeOH. The crystal (0.60×0.40×0.40 mm) belongs to the monoclinic system, space group $P2_12_12_1$ (#19), with a=8.829(1) Å, b=13.921(2) Å, c=21.578(2) Å, V=2652.0(5) Å^3, Z=4, D_{calcd}=1.274 g/cm^3, λ (Mo Kα)=0.71073 Å. Intensity data were measured on a Rigaku AFC7S diffractometer up to $2\theta_{max}$ of 26°. All 2960 reflections were collected. The structure was solved by direct

methods and refined by a full-matrix least-squares procedure. The refined structural model converged to a final $R1=0.0371$; $wR2=0.0904$ for 1994 observed reflections [$I>2\sigma(I)$] and 332 variable parameters.

3.6. 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.[18,19] 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.

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