

Excavatoids G–K, New 8,17-Epoxybriaranes from the Cultured Octocoral *Briareum excavatum* (Briareidae)

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Chemical investigations on the cultured octocoral *Briareum excavatum* have led to the isolation of five new 8,17-epoxybriarane diterpenoids, excavatoids G–K (**1**–**5**). The structures of briaranes **1**–**5** were determined on the basis of spectroscopic methods and the structure of **1** was further confirmed by a single-crystal X-ray diffraction analysis. Cytotoxicity of these metabolites toward various tumor cell lines is described. Excavatoid I (**3**) was found to show mild inhibitory effects on elastase release and superoxide anion generation by human neutrophils.

Previous studies on the chemical constituents of octocorals belonging to the genus *Briareum* (Briareidae) have yielded over 273 briarane-type diterpenoid derivatives.[1–3] In continuation of our search for new substances from the invertebrates originally collected off Taiwan waters, we have further isolated five new 8,17-epoxybriaranes, excavatoids G–K (**1**–**5**) (Chart 1), from the cultured octocoral *B. excavatum*. Briarane-type natural products are found only in marine organisms and mainly from octocorals.[1–3] The compounds of this type are suggested to be originally synthesized by host corals,[4,5] and proven to possess various interesting bioactivities.[1–3] In this paper, we describe the isolation, structure determination, and bioactivity of above five new briaranes **1**–**5**.

Results and Discussion

In our previous studies, 22 new briarane-type natural products, including briaexcavatins I–Z and excavatoids A, B, E, F,[6–11] had been isolated from this cultured species. Excavatoid G (**1**) was isolated as a white powder. The molecular formula of **1** was established as $C_{26}H_{36}O_{11}$ (nine degrees of unsaturation) from a sodiated molecule at m/z 547 in the ESI-MS spectrum and further supported by the HR-ESI-MS (m/z calcd: 547.2155; found: 547.2156, $[C_{26}H_{36}O_{11} + Na]^+$). The IR spectrum of **1** showed bands at 3450, 1761, and 1736 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester carbonyl groups. From the ^1H and ^{13}C NMR spectra (Table 1), **1** was found to possess three acetoxy groups (δ_H 2.09, 2.04, 2.00, each 3H × s; δ_C 170.7, 170.5, 169.2, each s; 21.3, 21.3, 21.1, each q), a γ-lactone moiety (δ_C 171.6, s, C-19), and a trisubstituted olefin (δ_C 146.0, s, C-5; 117.7, d, CH-6; δ_H 5.27, 1H, d, $J = 9.2$ Hz, H-6). The presence of a tetrasubstituted epoxide, containing a methyl substituent was established from the signals of two quaternary oxygenated carbons at δ_C 71.0 (s, C-8) and 63.3 (s, C-17), and confirmed by the proton signals of a methyl singlet at δ_H 1.66 (3H, s, H_3-18). Thus, from the NMR data, five degrees of unsaturation were accounted for, and **1** was identified as a tetracyclic compound.

From the ^1H–^1H COSY spectrum of **1** (Table 1), five different structural units, including C-2/-3/-4, C-4/-6 (by allylic coupling), C-6/-7, C-9/-10, and C-12/-13/-14, were identified. From these data and the HMBC correlations (Table 1), the connectivity from C-1 to C-14 could be established. A methyl attached at C-5 was confirmed by an allylic coupling between H_3-16/H-6 and by the HMBC correlations between H_3-16/C-4, -5, -6. The 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 acetate esters positioned at C-2 and C-12 were established by correlations between δ_H 4.98 (H-2) and 4.91 (H-12) and the acetate carbonyls at δ_C 170.5 and 169.2, respectively. The hydroxy proton signal at δ_H 2.44 was revealed by its ^1H–^1H COSY and HMBC correlations to H-9 (δ_H 4.65) and C-9 (δ_C 69.5), indicating its attachment to C-9. These data, together with the HMBC correlations between H_3-18/C-8, -17, -19, established the main molecular framework of **1**. The remaining hydroxy and acetoxy groups should

Published on the web April 29, 2010; doi:10.1246/bcsj.20090306

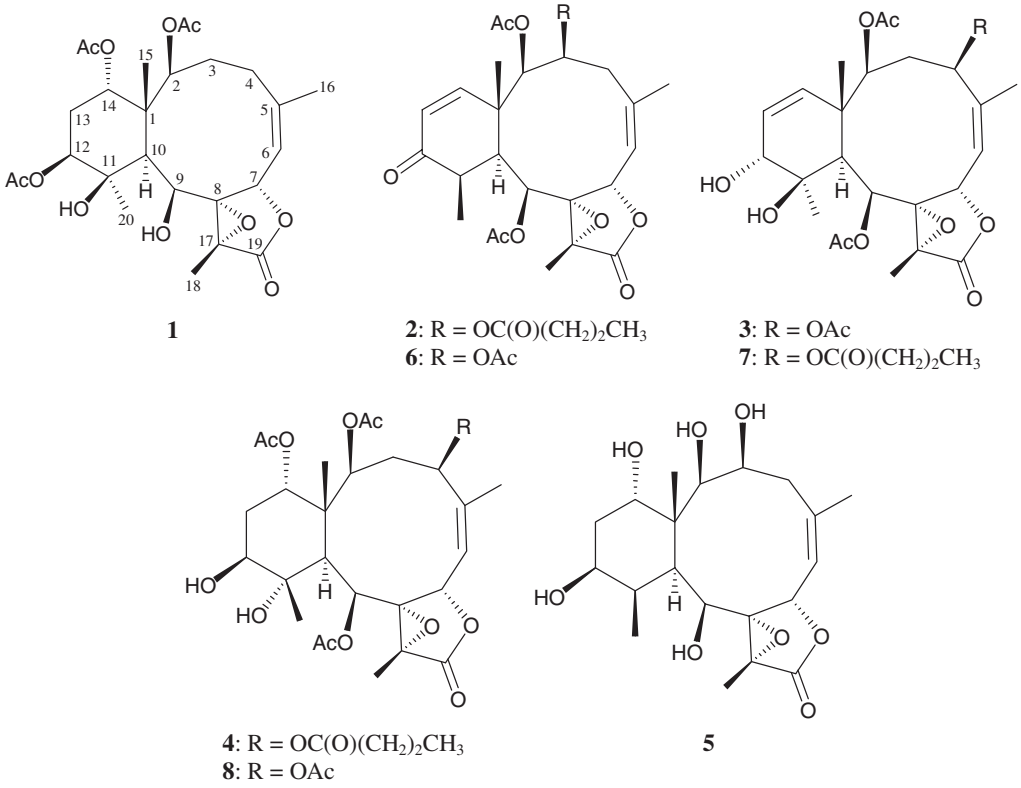

Chart 1.

be positioned at C-11 or C-14, but because the proton signal for this hydroxy group and the key HMBC correlations were not observed, the accurate positions for these two groups cannot be determined by this method.

Based on previous surveys, all the briaranes have the H-10 *trans* to the C-15 methyl, and these two groups are assigned as α- and β-oriented in most briarane analogs.[1–3] The relative stereochemistry of **1** was established by a NOESY experiment (Figure 1). The correlations between H-10 and H-2, H-9, and H_3-20; and H_3-20 exhibited a correlation with H-12, indicated that these protons were situated on the same face; they were assigned as α protons, as C-15 methyl was β-oriented and H_3-15 did not show correlation with H-10. H-14 was found to exhibit an interaction with H_3-15, but not with H-10, revealing the β-orientation of this proton. One of the methylene protons at C-3 (δ_H 2.79) exhibited a correlation with H_3-15 and was assigned as H-3β, while the other one was denoted as H-3α (δ_H 1.56). A correlation observed between H-3β and H-7, reflected the β-orientation of H-7. Furthermore, H-9 was found to show responses with H-10, 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-10, H_3-18, and H_3-20 when H-9 was α-oriented and H_3-18 should be β-oriented. Moreover, H_3-16 exhibited a correlation with H-6, suggesting the *Z*-configuration of C-5/6 double bond.

A single-crystal X-ray diffraction analysis was carried out in order to determine the structure of **1** (Figure 2). The X-ray structure demonstrates that the remaining hydroxy and acetoxy groups were positioned at C-11 and C-14, respectively. From the above findings, the structure of **1** was elucidated unambiguously.

From HR-ESI-MS, the molecular formula of **2** (excavatoid H) was determined to be $C_{28}H_{36}O_{10}$ with m/z 555.2210 (calcd for $C_{28}H_{36}O_{10}$ + Na, 555.2206), indicating 11 degrees of unsaturation. The IR absorptions of **2** showed the presence of 1788, 1743, and 1686 cm^{-1}, consistent with the presence of γ-lactone, ester, and α,β-unsaturated ketone groups. From the ^{13}C NMR data of **2** (Table 2), the presence of a trisubstituted olefin and an α,β-unsaturated ketone group were deduced from the signals of five carbons at δ_C 139.4 (s, C-5), 123.1 (d, CH-6), 200.9 (s, C-12), 126.4 (d, CH-13), and 155.1 (d, CH-14), and further supported by three olefin proton signals at δ_H 5.37 (1H, d, J = 7.2 Hz, H-6), 6.33 (1H, d, J = 10.4 Hz, H-13), and 6.56 (1H, d, J = 10.4 Hz, H-14) in the ^1H NMR spectrum of **2** (Table 3). Furthermore, in the ^{13}C NMR spectrum, four carbonyl resonances at δ_C 173.0 (s), 171.2 (s, C-19), 170.4 (s), and 169.0 (s), confirming the presence of a γ-lactone and three esters in **2**. In the ^1H NMR spectrum, two acetyl methyls (δ_H 2.31, 2.26, each 3H × s) and an *n*-butyryl group (δ_H 0.95, 3H, t, J = 7.2 Hz; 1.62, 2H, sext, J = 7.2 Hz; 2.25, 2H, t, J = 7.2 Hz) were observed.

It was observed that the NMR data of **2** were similar to those of a known briarane analog, briaexcavatolide A (**6**).[12] However, it was found that an acetoxy group in **6** was replaced by an *n*-butyroxy group in **2**. The HMBC correlations revealed that two acetoxy groups should attach at C-2 and C-9, respectively (Table 4). The remaining *n*-butyroxy group was positioned at C-3 as indicated by analysis of ^1H–^1H COSY correlations (Table 5) and characteristic NMR signals analysis, although no HMBC correlation was observed between H-3 and the *n*-butyrate carbonyl. By comparing the related NMR data,

Table 1. ^{1}H and ^{13}C NMR Data and ^{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		46.2 (s)[d)]		
2	4.98 d (7.6)[c)]	75.5 (d)	H_2-3	C-1, -3, -4, -15, acetate carbonyl
3α	1.56 m	32.6 (t)	H-2, H-3β, H_2-4	C-4
β	2.79 dt (14.8, 4.8)		H-2, H-3α, H_2-4	C-4
4α	1.94 m	28.7 (t)	H_2-3, H-4β, H-6	C-5, -6, -16
β	2.50 m		H_2-3, H-4α, H-6	C-5, -6, -16
5		146.0 (s)		
6	5.27 d (9.2)	117.7 (d)	H_2-4, H-7, H_3-16	C-4
7	5.49 d (9.2)	75.1 (d)	H-6	C-5
8		71.0 (s)		
9	4.65 dd (6.0, 2.0)	69.5 (d)	H-10, OH-9	C-1, -8, -10, -11, -17
10	2.07 d (2.0)	48.1 (d)	H-9	C-1, -8, -9, -15
11		76.2 (s)		
12	4.91 dd (9.2, 8.0)	73.7 (d)	H_2-13	C-13, acetate carbonyl
13	1.92–2.00 m (2H)	25.7 (t)	H-12, H-14	C-11, -12
14	4.85 dd (3.6, 2.8)	75.7 (d)	H_2-13	n.o.[e)]
15	1.41 s	15.9 (q)		C-1, -2, -10, -14
16	2.00 s	26.9 (q)	H-6	C-4, -5, -6
17		63.3 (s)		
18	1.66 s	9.5 (q)		C-8, -17, -19
19		171.6 (s)		
20	1.71 s	28.1 (q)		C-10, -11, -12
OH-9	2.44 d (6.0)		H-9	C-8, -9
Acetate	2.09 s	21.3 (q)		Acetate carbonyl
methyls	2.04 s	21.3 (q)		Acetate carbonyl
	2.00 s	21.1 (q)		Acetate carbonyl
Acetate		170.7 (s)		
carbonyls		170.5 (s)		
		169.2 (s)		

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 spectra. e) n.o.: not observed.

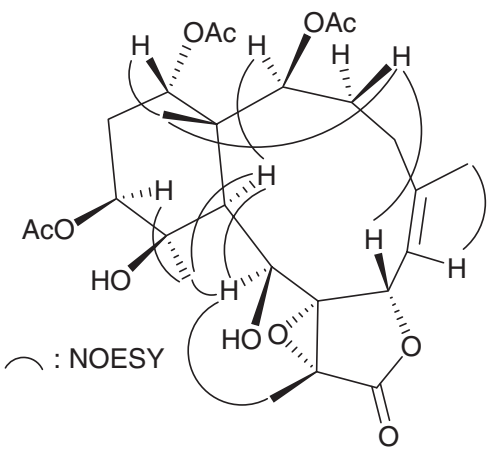

Figure 1. Selective NOESY correlations of **1**.

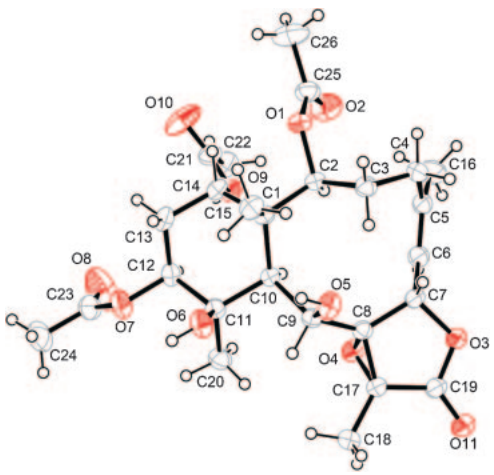

Figure 2. Computer-generated ORTEP of **1** showing the relative configuration.

including the NOESY spectrum of **2** (Figure 3), with those of **6**, excavatoid H (**2**) was assigned as the 3-*O*-deacetyl-3-*O*-*n*-butyryl derivative of briaexcavatolide A (**6**).

Excavatoid I (**3**) was isolated as a white powder and had the molecular formula $C_{26}H_{34}O_{11}$ on the basis of HR-ESI-MS (*m*/*z* 545.2002, calcd for $C_{26}H_{34}O_{11}$ + Na, 545.1999). Its IR spectrum exhibited a broad OH stretch at 3464 cm^{-1}, a γ-lactone carbonyl at 1780 cm^{-1}, and ester carbonyls at 1734 cm^{-1}. It was found that the NMR data of **3** (Tables 2 and 3) were similar to those of a known compound, excavatoid B (**7**).[10] However, it was found that the *n*-butyroxy group in **7** was replaced by an acetoxy group in **3** by comparison of the related NMR data of **3** with those of **7**. The relative configuration of **3** was also deduced from the interactions

Table 2. ^{13}C NMR Data for Diterpenoids **2**–**5**

Position	**2**[a)]	**3**[a)]	**4**[a)]	**5**[b)]
1	43.2 (s)[c)]	45.4 (s)	47.9 (s)	45.8 (s)
2	81.9 (d)	76.8 (d)	73.2 (d)	87.8 (d)
3	71.8 (d)	38.7 (t)	37.7 (t)	74.2 (d)
4	33.5 (t)	72.4 (d)	72.0 (d)	36.9 (t)
5	139.4 (s)	144.8 (s)	143.6 (s)	143.3 (s)
6	123.1 (d)	122.6 (d)	123.3 (d)	121.4 (d)
7	73.7 (d)	73.7 (d)	73.7 (d)	75.7 (d)
8	69.3 (s)	70.2 (s)	70.5 (s)	71.9 (s)
9	66.9 (d)	67.1 (d)	67.2 (d)	67.1 (d)
10	38.7 (d)	43.8 (d)	48.9 (d)	42.1 (d)
11	41.4 (d)	75.3 (s)	78.2 (s)	36.1 (d)
12	200.9 (s)	71.8 (d)	73.3 (d)	68.0 (d)
13	126.4 (d)	124.1 (d)	30.2 (t)	34.7 (t)
14	155.1 (d)	138.8 (d)	74.8 (d)	78.8 (d)
15	17.1 (q)	17.8 (q)	14.4 (q)	21.5 (q)
16	21.8 (q)	25.6 (q)	25.3 (q)	22.6 (q)
17	60.0 (s)	62.6 (s)	66.3 (s)	60.2 (s)
18	10.2 (q)	9.7 (q)	10.2 (q)	9.4 (q)
19	171.2 (s)	171.1 (s)	170.5 (s)	174.6 (s)
20	14.4 (q)	28.1 (q)	17.0 (q)	9.4 (q)
2-OAc	170.4 (s)	170.4 (s)	170.1 (s)	
	21.5 (q)	21.1 (q)	21.3 (q)	
4-OAc		170.1 (s)		
		21.1 (q)		
9-OAc	169.0 (s)	169.4 (s)	167.9 (s)	
	21.9 (q)	21.7 (q)	21.4 (q)	
14-OAc			170.2 (s)	
			21.1 (q)	
3-OC(O)$(CH_2)_2CH_3$	173.0 (s)			
	36.1 (t)			
	18.1 (t)			
	13.6 (q)			
4-OC(O)$(CH_2)_2CH_3$			170.7 (s)	
			36.2 (t)	
			18.4 (t)	
			13.6 (q)	

a) Spectra measured at 100 MHz in $CDCl_3$ at 25 °C. b) Spectra measured at 100 MHz in CD_3OD at 25 °C. c) Attached protons were deduced by DEPT and HMQC spectra.

observed in a NOESY experiment (Figure 4). All of the interactions of **3** were found to be similar with those of **7**, and this compound was found to be the 4-*O*-debutyryl-4-*O*-acetyl derivative of excavatoid B (**7**).

Excavatoid J (**4**) had the composition $C_{30}H_{42}O_{13}$, as determined by HR-ESI-MS (*m*/*z* 633.2527, calcd for $C_{30}H_{42}O_{13}$ + Na, 633.2523). The IR spectrum of **4** showed the presence of hydroxy (ν_{max} 3463 cm^{-1}), γ-lactone (ν_{max} 1781 cm^{-1}), and ester carbonyls (ν_{max} 1739 cm^{-1}). The NMR data of **4** (Tables 2 and 3) were found to be close to those of a known briarane, briaexcavatolide U (**8**).[13] It was found that one of the four acetoxy groups in **8** was replaced by an *n*-butyroxy group in **4** by comparison of the related NMR data of **4** with those of **8**. The location of this *n*-butyroxy group in **4** was confirmed by an HMBC correlation between H-4 (δ_H 5.03) and the *n*-butyrate carbonyl (δ_C 170.7) (Table 4). On the basis of the above findings and by the correlations observed in the NOESY experiment of **4** (Figure 5), excavatoid J (**4**) was found to be the 4-*O*-deacetyl-4-*O*-*n*-butyryl derivative of briaexcavatolide U (**8**).

The molecular formula for **5** (excavatoid K) was determined to be $C_{20}H_{30}O_8$ (six degrees of unsaturation) by HR-ESI-MS (*m*/*z* 421.1835, calcd for $C_{20}H_{30}O_8$ + Na, 421.1838). Comparison of the DEPT and the molecular formula indicated that there must be five hydroxy groups in **5**, and this deduction was supported by a broad absorption in the IR spectrum at 3373 cm^{-1}. The IR spectrum also showed a band at 1771 cm^{-1}, consistent with the presence of a γ-lactone moiety. From the ^{13}C NMR spectrum (Table 2), **5** was found to possess a γ-lactone (δ_C 174.6, s, C-19) and a trisubstituted olefin (δ_C 143.3, s, C-5; 121.4, d, CH-6). Thus, **5** must be a tetracyclic compound.

From the ^{1}H–^{1}H COSY spectrum of **5** (Table 5), six structural units H-2/H-3/H_2-4, H_2-4/H-6 (by allylic coupling), H-6/H-7, H-9/H-10/H-11/H-12/H_2-13/H-14, H-6/H_3-16 (by allylic coupling), and H-11/H_3-20 were identified, which were assembled with the assistance of an HMBC experiment (Table 4). The HMBC correlations between protons and quaternary carbons of **5** permitted elucidation of the carbon skeleton. The C-15 methyl group was positioned at C-1 from the HMBC correlations between H_3-15/C-1, -2, -10, -14 and H-10/C-15. The vinyl methyl at C-5 was confirmed by the HMBC correlations between H_3-16/C-4, -5, -6; H_2-4/C-16; and H-6/C-16. The presence of hydroxy groups positioned at C-2, C-3, C-9, C-12, and C-14, as indicated by analysis of ^{1}H–^{1}H COSY correlations. The relative stereochemistry of **5** was also determined by a NOESY experiment. In the NOESY experiment of **5** (Figure 6), H-10 gave correlations to H-3, H-11, and H-12; and H-3 was found to show responses with H-2 and one proton of the C-4 methylene (δ_H 1.89), indicating that these protons were located on the same face and, therefore, were assigned as α protons, as the C-15 methyl was a β-substituent at C-1. H-14 gave a correlation to H_3-15, 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 a correlation between H-11 and H-12. Furthermore, H_3-16 exhibited correlations with H-3 and H-6, but not with H-7, suggesting that the configuration of the C-5/6 double bond exists in *Z* form and H-7 is β-oriented. This observation was further supported by a correlation between H-4β and H-7. H_3-18 was found to show a correlation with H-9. From the detailed consideration of molecular models, H_3-18 is β-oriented in the γ-lactone unit.

The cytotoxicity of briaranes **1**–**5** toward CCRF-CEM (human T cell acute lymphoblastic leukemia), HL-60 (human promyelocytic leukemia), DLD-1 (human colon adenocarcinoma), and IMR-32 (human neuroblastoma) tumor cells were studied and the results are shown in Table 6. These data showed that excavatoid H (**2**) exhibited modest cytotoxicity against CCRF-CEM cells. In addition, excavatoid I (**3**) was found to display 38.3 and 21.8% inhibitory effects on human elastase release and superoxide anion generation by human neutrophils at 10 μg/mL, respectively.

Experimental

General Experimental Procedures. Melting points were measured on a FARGO apparatus and were uncorrected.

Table 3. ^{1}H NMR Data for Diterpenoids **2–5**

Position	**2**[a)]	**3**[a)]	**4**[a)]	**5**[b)]
2	5.35 s	4.61 d (6.0)	5.01 d (8.0)	3.62 s
3α	4.84 dd (4.4, 2.4)[c)]	2.05 ddd (14.0, 6.0, 4.8)	2.04 ddd (15.6, 8.0, 5.6)	4.80 dd (10.4, 6.4)
β		2.83 dd (14.0, 12.8)	2.91 dd (15.6, 12.8)	
4α	2.07 br d (15.6)	5.06 ddd (12.8, 4.8, 0.8)	5.03 dd (12.8, 5.6)	1.89 dd (15.2, 10.4)
β	3.47 dd (15.6, 4.4)			3.40 dd (15.2, 6.4)
6	5.37 d (7.2)	5.50 ddd (10.0, 1.6, 0.8)	5.35 ddd (8.8, 1.6, 1.2)	5.20 d (7.2)
7	5.39 d (7.2)	5.84 d (10.0)	5.56 d (8.8)	5.71 d (7.2)
9	5.20 d (10.4)	5.82 d (4.4)	5.79 d (1.2)	4.06 d (10.0)
10	3.31 dd (10.4, 4.4)	2.55 d (4.4)	2.14 d (1.2)	2.78 dd (10.0, 4.8)
11	2.86 qd (7.6, 4.4)			2.33 m
12		3.75 br s	3.72 dd (8.8, 4.4)	3.99 ddd (12.0, 4.0, 4.0)
13α	6.33 d (10.4)	5.79 dd (10.0, 5.6)	2.00 ddd (10.8, 4.4, 1.6)	1.58 ddd (12.4, 4.0, 3.2)
β			1.67 ddd (10.8, 8.8, 2.0)	1.97 ddd (12.4, 12.0, 2.8)
14	6.56 d (10.4)	5.52 d (10.0)	4.81 dd (2.0, 1.6)	3.65 dd (3.2, 2.8)
15	1.06 s	1.23 s	1.23 s	1.10 s
16	1.73 s	2.10 d (1.6)	2.15 d (1.6)	1.91 s
18	1.58 s	1.62 s	1.78 s	1.52 s
20	1.21 d (7.6)	1.40 s	1.17 s	0.93 d (7.2)
2-OAc	2.26 s	2.07 s	2.02 s	
4-OAc		2.12 s		
9-OAc	2.31 s	2.25 s	2.23 s	
14-OAc			2.01 s	
3-OC(O)$(CH_2)_2CH_3$	2.25 t (7.2)			
	1.62 sext (7.2)			
	0.95 t (7.2)			
4-OC(O)$(CH_2)_2CH_3$			2.26 t (7.2)	
			1.63 sext (7.2)	
			0.93 t (7.2)	

a) Spectra measured at 400 MHz in $CDCl_3$ at 25 °C. b) Spectra measured at 400 MHz in CD_3OD at 25 °C. c) *J* values (in hertz) in parentheses.

Table 4. HMBC Correlations (H → C) for Diterpenoids **2–5**

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

Optical rotation values were measured with a JASCO P-1010 digital polarimeter. IR spectra were obtained on a VARIAN DIGLAB FTS 1000 FT-IR spectrophotometer. NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, respectively, in $CDCl_3$ or CD_3OD using TMS as an internal standard. ESI-MS and HR-ESI-MS data were recorded on a BRUKER APEX II mass spectrometer. Gravity column chromatography

Table 5. ^{1}H–^{1}H COSY Correlations for Diterpenoids **2**–**5**

Position	**2**	**3**	**4**	**5**
H-2	H-3	H_2-3	H_2-3	H-3
H-3	H-2, H_2-4	H-2, H-4	H-2, H-4	H-2, H_2-4
H-4	H-3, H-6	H_2-3, H-6	H_2-3, H-6	H-3, H-6
H-6	H_2-4, H-7, H_3-16	H-4, H-7, H_3-16	H-4, H-7, H_3-16	H_2-4, H-7, H_3-16
H-7	H-6	H-6	H-6	H-6
H-9	H-10	H-10	H-10	H-10
H-10	H-9, H-11	H-9	H-9	H-9, H-11
H-11	H-10, H_3-20			H-10, H-12, H_3-20
H-12		H-13	H_2-13	H-11, H_2-13
H-13	H-14	H-12, H-14	H-12, H-14	H-12, H-14
H-14	H-13	H-13	H_2-13	H_2-13
H-16	H-6	H-6	H-6	H-6
H-20	H-11			H-11

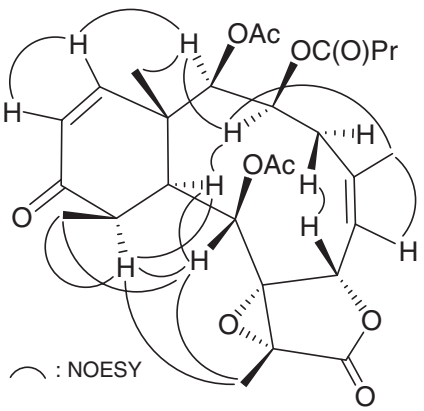

Figure 3. Selective NOESY correlations of **2**.

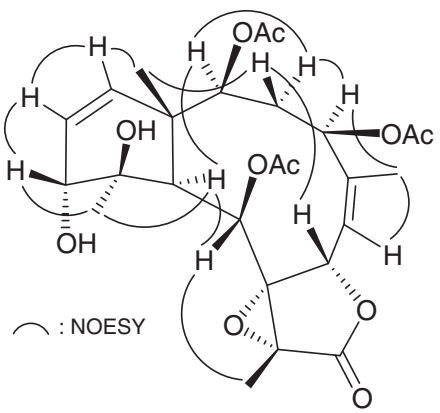

Figure 4. Selective NOESY correlations of **3**.

was performed on silica gel (230–400 mesh, Merck, Darmstadt, Germany). TLC was carried out on precoated Kieselgel 60 F_{254} (0.2 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 L-7455 photodiode array detector, and a RHEODYNE 7725 injection port. A semi-preparative reverse phase column (Hibar 250–10 mm, Purospher Star RP-18e, 5 μm) was used for HPLC.

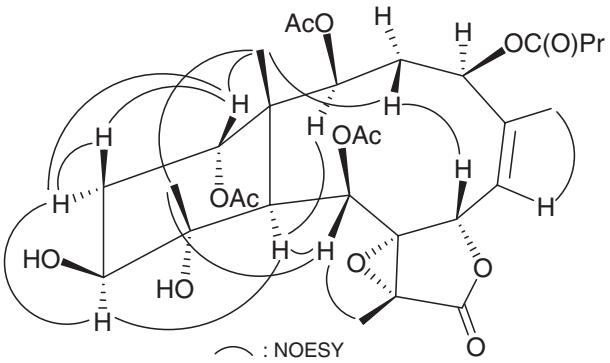

Figure 5. Selective NOESY correlations of **4**.

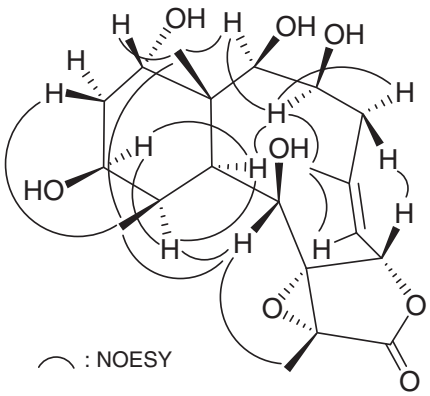

Figure 6. Selective NOESY correlations of **5**.

Table 6. Cytotoxic Data of Diterpenoids **1**–**5**

Compound	Cell lines ED_{50}/μg mL^{-1}			
	CCRF-CEM	HL-60	DLD-1	IMR-32
1	>40.0	>40.0	>40.0	>40.0
2	13.1	>40.0	21.4	>40.0
3	>40.0	>40.0	>40.0	31.1
4	>40.0	38.4	25.1	>40.0
5	>40.0	>40.0	>40.0	>40.0
Doxorubicin[a)]	0.18	0.49	0.60	0.017

a) Doxorubicin was used as a reference compound.

Animal Material. Specimen of the cultured octocoral *B. excavatum* were collected and transplanted to five 0.6-ton cultivating tanks located in the National Museum of Marine Biology and Aquarium (NMMBA), Taiwan, in December 2003, and the material for this research work were collected from the tanks in December 2006. This organism was identified by comparison with previous descriptions.[14–16] A voucher specimen was deposited in the NMMBA, Taiwan (NMMBA-CSC-001).

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 (1:1) to yield fractions A–C. Fr. C was separated on silica gel and eluted using hexane/EtOAc (stepwise, 20:1–pure EtOAc) to yield Fr. C1–9. Fr. C7

was repurified by reverse phase HPLC (RP-HPLC), using mixtures of MeOH, CH_3CN, and H_2O to afford briaranes **1** (0.5 mg, 62/1/37), **2** (0.6 mg, 62/1/37), and **3** (0.7 mg, 44/1/55). Fr. C9 was also separated by RP-HPLC, using mixtures of MeOH and H_2O to afford briaranes **4** (0.4 mg, 50/50) and **5** (5.8 mg, 50/50).

Excavatoid G (1): White powder; mp 283–284 °C; $[\alpha]_D^{25}$ −28 (*c* 0.03, $CHCl_3$); IR (neat) ν_{max} 3450, 1761, 1736 cm^{-1}; ^{13}C NMR ($CDCl_3$, 100 MHz) and ^1H NMR ($CDCl_3$, 400 MHz) data, see Table 1; ESI-MS *m/z* 547 $(M + Na)^+$; HR-ESI-MS *m/z* 547.2156 (calcd for $C_{26}H_{36}O_{11}$ + Na, 547.2155).

Excavatoid H (2): White powder; mp 110–111 °C; $[\alpha]_D^{25}$ −24 (*c* 0.02, $CHCl_3$); IR (neat) ν_{max} 1788, 1743, 1686 cm^{-1}; ^{13}C NMR ($CDCl_3$, 100 MHz) and ^1H NMR ($CDCl_3$, 400 MHz) data, see Tables 2 and 3; ESI-MS *m/z* 555 $(M + Na)^+$; HR-ESI-MS *m/z* 555.2210 (calcd for $C_{28}H_{36}O_{10}$ + Na, 555.2206).

Excavatoid I (3): White powder; mp 134–135 °C; $[\alpha]_D^{25}$ −43 (*c* 0.04, $CHCl_3$); IR (neat) ν_{max} 3464, 1780, 1734 cm^{-1}; ^{13}C NMR ($CDCl_3$, 100 MHz) and ^1H NMR ($CDCl_3$, 400 MHz) data, see Tables 2 and 3; ESI-MS *m/z* 545 $(M + Na)^+$; HR-ESI-MS *m/z* 545.2002 (calcd for $C_{26}H_{34}O_{11}$ + Na, 545.1999).

Excavatoid J (4): White powder; mp 124–125 °C; $[\alpha]_D^{25}$ +93 (*c* 0.02, $CHCl_3$); IR (neat) ν_{max} 3463, 1781, 1739 cm^{-1}; ^{13}C NMR ($CDCl_3$, 100 MHz) and ^1H NMR ($CDCl_3$, 400 MHz) data, see Tables 2 and 3; ESI-MS *m/z* 633 $(M + Na)^+$; HR-ESI-MS *m/z* 633.2527 (calcd for $C_{30}H_{42}O_{13}$ + Na, 633.2523).

Excavatoid K (5): White powder; mp 224–225 °C; $[\alpha]_D^{25}$ +4 (*c* 0.30, MeOH); IR (neat) ν_{max} 3373, 1771 cm^{-1}; ^{13}C NMR (CD_3OD, 100 MHz) and ^1H NMR (CD_3OD, 400 MHz) data, see Tables 2 and 3; ESI-MS *m/z* 421 $(M + Na)^+$; HR-ESI-MS *m/z* 421.1835 (calcd for $C_{20}H_{30}O_8$ + Na, 421.1838).

Single-Crystal X-ray Crystallography of Excavatoid G (1). Suitable colorless prisms of **1** were obtained from a solution of MeOH. The crystal ($0.70 \times 0.65 \times 0.60$ mm^3) belongs to the monoclinic system, space group $P2_1$ (#4), with $a = 9.9764(14)$ Å, $b = 12.668(4)$ Å, $c = 10.2096(16)$ Å, $V = 1288.8(5)$ $Å^3$, $Z = 2$, $D_{calcd} = 1.352$ $g\,cm^{-3}$, λ(Mo Kα) = 0.71073 Å. Intensity data were measured on a Rigaku AFC7S diffractometer up to $2\theta_{max}$ of 26°. All 3423 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 $R1 = 0.0334$; $wR2 = 0.0866$ for 2715 observed reflections [$I > 2\sigma(I)$] and 341 variable parameters.

Crystallographic data for the structure of excavatoid G (**1**) have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 748527. Copies of the data can be obtained, free of charge, on application to CCDC, 12, Union Road, Cambridge, CB2 1EZ, U.K. (Fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

Cytotoxicity Assays. The cytotoxicity of tested compounds **1**–**5** was assayed with a modification of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. Cytotoxicity assays were carried out according to procedures described previously.[17]

Human Nuetrophil 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 procedures described previoulsy.[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.

This research work was supported by grants from the NMMBA (No. 981001101); APORC, NSYSU (No. 96C031702); and NSTPBP, National Science Council (No. NSC 98-2323-B-291-001 and 98-2320-B-291-001-MY3), Taiwan, awarded to P.-J.S.

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