Briaexcavatolides K−N, New Briarane Diterpenes from the Gorgonian *Briareum excavatum*

Ping-Jyun Sung,[†] Jui-Hsin Su,[†] Chang-Yih Duh,[†] Michael Y. Chiang,[‡] and Jyh-Horng Sheu*,[†]

Department of Marine Resources and Department of Chemistry, National Sun Yat-Sen University, Kaohsiung, 804, Taiwan, Republic of China

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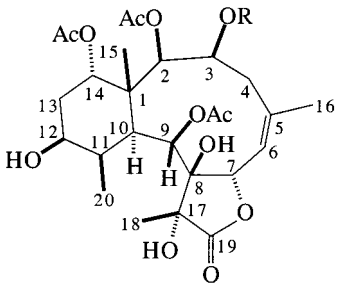
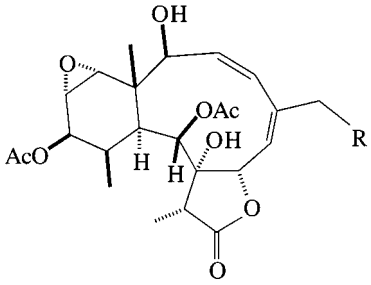
Four new briarane diterpenes, briaexcavatolides K−N (**1**−**4**), along with a known diterpene, **5**, have been isolated from the Taiwanese gorgonian *Briareum excavatum*. The structures of the new metabolites were established by extensive spectral analyses. Furthermore, the structure, including the relative configuration of briaexcavatolide K (**1**), was confirmed by a single-crystal X-ray analysis. Briaexcavatolides K and L (**1** and **2**) are the only briarane diterpenes known to possess hydroxyl groups at the C-8β and C-17α positions, respectively. Cytotoxicity of these metabolites toward various cancer cell lines also is described.

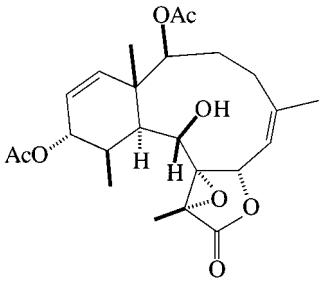
In the past 24 years, many diterpenes possessing the briarane carbon skeleton, which contain a γ-lactone in a bicyclo[8.4.0] system, have been isolated from marine coelenterates mainly from the subclass Octocorallia. The briaranes raised interest not only due to their novel structures but also for their extensive biological activities.[1] In a continuing survey of Taiwanese marine organisms with promising bioactive substances, the gorgonian *Briareum excavatum* (Nutting) (phylum Cnidaria, order Gorgonacea, family Briareidae) has been investigated. Previous studies on the chemical constituents of *B. excavatum* had led to the isolation of 36 briarane derivatives, excavatolides A−Z[1−4] and briaexcavatolides A−J.[5] In this paper, we report the isolation, structure determination, and cytotoxicity of four new briarane diterpenes, briaexcavatolides K−N (**1**−**4**), together with a known diterpene, (1*S**,2*S**,5*Z*,7*S**,8*S**,9*S**,10*S**,11*R**,12*R**,13*Z*,17*R**)-2,12-diacetoxy-8,17-epoxy-9-hydroxybriara-5,13-dien-18-one (**5**)[6] (asterisk denotes relative configuration), from the gorgonian *B. excavatum*. The structures of these compounds were established by spectroscopic methods. The relative configuration of briaexcavatolide K (**1**) was determined by a single-crystal X-ray analysis.



1 : R = Ac
2 : R = *n*-PrCO



3 : R = Cl
4 : R = OH



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Results and Discussion

Specimens of *B. excavatum* were frozen immediately after collection and subsequently freeze-dried. The freeze-dried organism was extracted with EtOAc to afford a crude extract. The extract was separated by column chromatography on silica gel and afforded diterpenes **1**−**5**.

Briaexcavatolide K (**1**) was isolated as colorless prisms, and a molecular formula $C_{28}H_{40}O_{13}$ was established by HRFABMS. Accordingly, nine degrees of unsaturation were determined for the molecule of **1**. The IR absorptions of **1** showed the presence of hydroxyl (ν_{max} 3428 cm^{-1}), γ-lactone (ν_{max} 1772 cm^{-1}), and ester carbonyl (ν_{max} 1734 cm^{-1}) groups in **1**. The FABMS of **1** exhibited peaks at *m*/*z* 585 $[M + H]^+$, 567 $[M + H - H_2O]^+$, 525 $[M + H - HOAc]^+$, 507 $[M + H - H_2O - HOAc]^+$, 465 $[M + H - 2HOAc]^+$, 447 $[M + H - H_2O - 2HOAc]^+$, 405 $[M + H - 3HOAc]^+$, 345 $[M + H - 4HOAc]^+$, 327 $[M + H - H_2O - 4HOAc]^+$, 309 $[M + H - 2H_2O - 4HOAc]^+$, and 291 $[M + H - 3H_2O - 4HOAc]^+$, also suggesting the presence of three hydroxyl and four acetoxyl groups in the molecule of **1**. From the ^{13}C NMR spectral data of **1** (Table 2), a trisubstituted olefin was deduced from the signals of two carbons at δ 141.9 (s) and 125.1 (d). Furthermore, in the ^{13}C NMR spectrum of **1**, five carbonyl resonances appear at δ 176.1 (s), 170.7 (s), 170.6 (s), 170.1 (s), and 169.4 (s), confirming the presence

* To whom correspondence should be addressed. Tel: 886-7-5252000 ext. 5030. Fax: 886-7-5255020. E-mail: sheu@mail.nsysu.edu.tw.
[†] Department of Marine Resources, National Sun Yat-Sen University.
[‡] Department of Chemistry, National Sun Yat-Sen University.

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Table 1. ^{1}H Chemical Shifts for Diterpenes **1**−**4**

	1[a]	**2**[b]	**3**[b]	**4**[b]
2	5.83 br s	5.83 br s	4.18 d (9.9)	4.13 d (9.9)
3	4.88 br s	4.89 br s	5.87 br t (10.8)	5.82 br t (10.5)
4			6.22 d (10.8)	6.27 d (10.5)
4β	3.21 dd (15.6; 4.2)[c]	3.21 dd (15.3; 3.9)		
α	2.02 br d (15.6)	2.02 br d (15.3)		
6	5.55 d (9.6)	5.55 d (8.7)	5.81 d (8.7)	5.75 d (8.7)
7	5.40 d (9.6)	5.40 d (8.7)	5.08 d (8.7)	5.20 d (8.7)
9	6.10 br s	6.09 br s	5.14 d (6.4)	5.16 d (6.9)
10	2.99 d (6.4)	2.98 d (6.6)	1.94 m	1.95 m
11	2.16 m	2.14 m	2.00 m	2.04 m
12	3.88 br s	3.88 br s	4.81 d (4.8)	4.75 d (4.8)
13β	1.98 m	1.99 m	3.23 br s	3.23 br s
α	1.90 m	1.91 m		
14	5.24 dd (10.8; 6.4)	5.24 dd (10.8, 6.3)	3.23 br s	3.23 br s
15	1.53 s	1.52 s	1.10 s	1.14 s
16	1.79 s	1.78 s	4.21 br s	4.30 br s
17			2.33 q (6.9)	2.32 q (7.2)
18	1.26 s	1.25 s	1.10 q (6.9)	1.16 q (7.2)
20	1.09 d (7.2)	1.08 d (7.2)	1.04 d (6.9)	1.06 d (7.2)
acetate	2.20 s	2.22 s	2.19 s	2.18 s
methyls	2.13 s	2.11 s	2.13 s	2.12 s
	2.02 s	1.94 s		
	1.95 s			
n-butyrate		CH_2 2.26 t (7.5)		
		CH_2 1.66 m		
		CH_3 0.95 t (7.5)		

[a] Spectra recorded at 400 MHz in $CDCl_3$. [b] 300 MHz in $CDCl_3$. [c] *J* values (in Hz) in parentheses. The values are ppm downfield from TMS.

Table 2. ^{13}C Chemical Shifts for Diterpenes **1**−**4**

	1[a]	**2**[b]	**3**[b]	**4**[b]
1	42.7 (s)[c]	42.8 (s)	40.3 (s)	40.4 (s)
2	72.8 (d)	72.9 (d)	75.8 (d)	75.6 (d)
3	73.4 (d)	73.2 (d)	136.8 (d)	136.0 (d)
4	32.9 (t)	33.0 (t)	126.2 (d)	125.1 (d)
5	141.9 (s)	141.7 (s)	140.8 (s)	145.4 (s)
6	125.1 (d)	125.2 (d)	124.8 (d)	120.0 (d)
7	85.4 (d)	85.5 (d)	79.3 (d)	79.9 (d)
8	81.1 (s)	81.2 (s)	81.8 (s)	81.5 (s)
9	65.1 (d)	65.2 (d)	70.0 (d)	70.0 (d)
10	43.8 (d)	43.8 (d)	36.8 (d)	37.2 (d)
11	37.0 (d)	37.0 (d)	38.0 (d)	37.7 (d)
12	69.8 (d)	69.6 (d)	71.9 (d)	72.0 (d)
13	34.5 (t)	34.6 (t)	57.3 (d)	57.8 (d)
14	74.3 (d)	74.5 (d)	62.4 (d)	63.1 (d)
15	20.0 (q)	19.9 (q)	15.0 (q)	15.0 (q)
16	22.7 (q)	22.6 (q)	46.5 (t)	63.8 (t)
17	76.9 (s)	76.8 (s)	43.2 (d)	43.5 (d)
18	16.6 (q)	16.2 (q)	6.3 (q)	6.4 (q)
19	176.1 (s)	176.7 (s)	175.7 (s)	177.1 (s)
20	15.5 (q)	15.5 (q)	9.7 (q)	9.6 (q)
acetate	22.0 (q)	21.8 (q)	21.8 (q)	21.8 (q)
methyls	21.0 (q)	21.0 (q)	21.0 (q)	21.1 (q)
	21.0 (q)	21.0 (q)		
	20.8 (q)			
acetate carbonyls	170.7 (s)	171.0 (s)	170.5 (s)	171.1 (s)
	170.6 (s)	170.4 (s)	169.8 (s)	170.1 (s)
	170.1 (s)	169.3 (s)		
	169.4 (s)			
n-butyrate		173.2 (s)		
		CH_3 13.6 (q)		
		CH_2 17.9 (t)		
		CH_2 35.8 (t)		

[a] Spectra recorded at 100 MHz in $CDCl_3$. [b] 75 MHz in $CDCl_3$. [c] Multiplicity deduced by DEPT and indicated by usual symbols. The values are ppm downfield from TMS.

of a γ-lactone and four other ester groups. In the ^{1}H NMR spectrum of **1** (Table 1), signals for four acetate methyls are observed at δ 2.20 (3H, s), 2.13 (3H, s), 2.02 (3H, s), and 1.95 (3H, s). On the basis of the above data, diterpene **1** was found to be a tricyclic compound. The structure and all of the ^{1}H and ^{13}C chemical shifts of **1** were determined by the assistance of 2D NMR studies, including an HMBC experiment (Table 3). Four acetoxyl groups were shown to be attached at C-2, C-3, C-9, and C-14 positions, respectively, based on the observed HMBC correlations. Thus, the remaining hydroxyl groups had to be attached at C-8, C-12, and C-17 positions.

The relative stereochemistry of **1** was deduced from a NOESY experiment (Figure 1 and Table 4). The NOE correlations of H-10 with H-3 and H-11 indicated that these protons are situated on the same face and were assigned as the α protons since the C-15 methyl is β-oriented and H_3-15 did not show a correlation with H-10. H-14 was found to exhibit NOE responses with H-13β and H_3-15, but not with H-10, revealing the β-orientation of this proton. Also, the hydroxyl group at C-12 was found to be in the β face by the NOE correlations between H-12 with H-11 and H-13α. H-7 showed NOE correlations with H-4β and H_3-15, confirming the β-orientation for this proton. H-9 was found to exhibit correlation with H_3-20. From consideration of molecular models, H-9 was found to be reasonably close to H_3-20 when H-9 was α-oriented and H_3-20 was placed on the β face. The acetoxyl group at the β face of C-2 was confirmed by the NOE responses between H-2 with H-3 and H-13α. However, the stereochemistry of hydroxyl groups at the C-8 and C-17 positions cannot be fully determined by this way.

A single-crystal X-ray structure analysis was carried out in order to confirm the molecular structure of **1**. The X-ray structure (Figure 2) demonstrates the locations of two hydroxyl groups on the β-orientation of C-8 and the α-orientation of C-17, respectively. The structure, including the relative stereochemistry of diterpene **1**, was thus elucidated unambiguously.

Briaexcavatolide L (**2**) was isolated as a white powder and had the molecular formula $C_{30}H_{44}O_{13}$ on the basis of EIMS and ^{13}C NMR spectral data. Carbonyl resonances in the ^{13}C NMR spectrum of **2** (Table 2) at δ 176.7 (s), 173.2 (s), 171.0 (s), 170.4 (s), and 169.3 (s) revealed the presence of a γ-lactone and four other esters in **2**. In the ^{1}H NMR

Table 3. HMBC Correlations for Diterpenes **1**−**4**

carbon	**1**	**2**	**3**	**4**
1	H-2, H-10, H-12, H-14, H_3-15	H-2, H-10, H-12, H-14, H_3-15	H-2, H_3-15	H-2, H_3-15
2	H-10, H_3-15	H-10, H_3-15	H-3, H_3-15	H-3, H_3-15
3	H-2	H-2		
4	H-2, H-6, H_3-16	H-2, H-6, H_3-16	H-3, H_2-16	H-3, H_2-16
5	H_2-4, H-7, H_3-16	H_2-4, H-7, H_3-16	H_2-16	H_2-16
6	H-7, H_3-16	H-7, H_3-16	H_2-16	H_2-16
7	H-6	H-6	H-6, H-9	H-6, H-9
8	H-7, H-9,	H-7, H-9,	H-7, H-9, H-10	H-7, H-9, H-10
9			H-10	H-10
10	H-9, H-11, H-12, H_3-15, H_3-20	H-9, H-11, H-12, H_3-15, H_3-20	H-9, H-14, H_3-15	H-9, H-14, H_3-15
11	H-9, H-10, H_3-20	H-9, H-10, H_3-20	H-10, H-13	H-10, H-13
12	H-10, H_3-20	H-10, H_3-20	H_3-20	H_3-20
14	H-2, H-10, H-12, H_2-13, H_3-15	H-2, H-10, H-12, H_2-13, H_3-15	H_3-15	H_3-15
15			H-10	H-10
16	H-6	H-6		
17	H-9, H_3-18	H-9, H_3-18	H_3-18	H_3-18
18			H-17	H-17
19	H-7, H_3-18	H-7, H_3-18	H-17, H_3-18	H-17, H_3-18
20	H-12	H-12	H-10	H-10
2-OCOMe	H-2	H-2		
3-OCOMe	H-3			
9-OCOMe	H-9	H-9	H-9	H-9
12-OCOMe			H-12	H-12
14-OCOMe	H-14	H-14		
3-OCOPr		H-3		

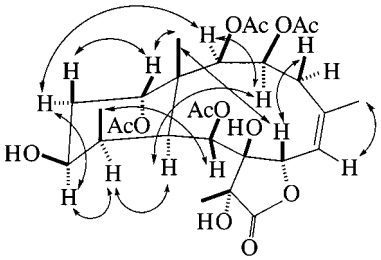


Figure 1. Selected NOE correlations of **1**.

spectrum of **2** (Table 1), signals for three acetate methyls were observed at δ 2.22 (3H, s), 2.11 (3H, s), and 1.94 (3H, s). The additional acyl group was found to be an *n*-butanoyl group, which showed seven contiguous protons (δ 0.95, 3H, t, $J = 7.5$ Hz; 1.66, 2H, m; 2.26, 2H, t, $J = 7.5$ Hz). The ^{13}C NMR signal at δ 173.2 (s) correlated with the methylene protons at δ 2.26 in the HMBC spectrum and was consequently assigned as the carbon atom of the *n*-butyrate carbonyl. It was found that the NMR signals (^{1}H and ^{13}C) of **2** were similar to those of **1**, except that the signals corresponding to an acetoxyl group in **1** were replaced by signals for an *n*-butyryloxyl group in **2**. The *n*-butyrate ester was positioned at C-3 from the ^{1}H−^{13}C long-range correlation between H-3 (δ 4.89) and the carbonyl carbon of the *n*-butyrate (δ 173.2). The correlations from a NOESY experiment of **2** (Table 4) also showed that the stereochemistry of this metabolite is identical with that of **1**. Thus, briaexcavatolide L (**2**) was found to be the 3-deacetyl-3-*n*-butyryl derivative of **1**.

Briaexcavatolide M (**3**) was obtained as a white powder. The molecular formula $C_{24}H_{31}O_9Cl$ was established by HRFABMS. The IR spectrum showed bands at 3433, 1780, and 1739 cm^{-1}, consistent with the presence of hydroxyl, γ-lactone, and ester carbonyl groups. The FABMS of **3** showed peaks at *m*/*z* 501 $[M + H + 2]^+$, 499 $[M + H]^+$, 481 $[M + H - H_2O]^+$, 439 $[M + H - HOAc]^+$, 379 $[M + H - 2HOAc]^+$, and 307 $[M + H - 2H_2O - 2HOAc - HCl]^+$, indicating the presence of two hydroxyl groups, two acetoxyl groups, and a chlorine atom in the molecule of **3**. A strong UV absorption at 234 nm suggested the presence of a conjugated diene system in **3**. In the ^{13}C NMR of **3** (Table 2), four olefin carbons were deduced from the signals at δ 140.8 (s), 136.8 (d), 126.2 (d), and 124.8 (d). A 13,14-epoxide group was confirmed from the two tertiary oxygenated carbons at δ 62.4 (d) and 57.3 (d) and from the chemical shifts of H-13 and H-14 (δ 3.23, 2H, br s). From the ^{13}C NMR spectrum of **3**, three carbonyl resonances appeared at δ 175.7 (s), 170.5 (s), and 169.8 (s) and confirmed the presence of a γ-lactone and two other ester groups. In the ^{1}H NMR of **3** (Table 1), two acetate methyls (δ 2.19, 3H, s; 2.13, 3H, s) also were observed. From an ^{1}H−^{1}H COSY experiment of **3** (Figure 3), it was possible to establish the separate spin systems that map out the proton sequences from H-2 to H-4, H-6 to H-7, H-9 to H-12, H-11 to H_3-20, H-13 to H-14, and H-17 to H_3-18. The HMBC data for **3** (Figure 3 and Table 3) revealed that two acetoxyl groups are attached to the C-9 and C-14 positions, respectively. Thus, the remaining hydroxyl group had to be positioned at C-8, an oxygen-bearing quarternary carbon resonating at δ 83.1 ppm.

The relative stereochemistry of **3** was deduced from a NOESY experiment (Figure 4 and Table 4). The NOE correlations of H-10 with H-11 and H-2 indicated that these protons are situated on the same face and were assigned as the α protons since the C-15 methyl is β-orientated and H_3-15 did not show correlation with H-10. The epoxy protons, H-13 and H-14 (δ 3.23), were found to exhibit NOE responses with H_3-15, but not with H-10, revealing the β-orientations of these two protons. Furthermore, H-12 correlated with H-11, but not with the epoxy protons, indicating that the acetoxyl group at C-12 should be positioned on the β face. Additionally, it was found that H-7, H-9, and H-17 showed NOE correlations with each other. Consideration of molecular models revealed that H-9 is reasonably close to H-7 and H-17 when H-7 and H-17 are β-oriented, and H-9 is placed on the α face. On the basis

Table 4. Key NOESY Correlations for Diterpenes **1**−**4**

	1	2	3	4
H-2	H-3, H-13α	H-3, H-13α	H-10	H-10
H-3	H-2, H-10, H-11	H-2, H-10, H-11	H-4, H_3-15	H-4, H_3-15
H-4			H-3	H-3
H-4β	H-7, H-10	H-7, H-10		
H-6	H_3-16	H_3-16	H_2-16	H_2-16
H-7	H-4β, H_3-15	H-4β, H_3-15	H-9, H-17	H-9, H-17
H-9	H_3-20	H_3-20	H-7, H-17, H_3-20	H-7, H-17, H_3-20
H-10	H-3, H-4β, H-11	H-3, H-4β, H-11	H-2, H-11	H-2, H-11
H-11	H-3, H-10, H-12	H-3, H-10, H-12	H-10, H-12, H_3-18	H-10, H-12, H_3-18
H-12	H-11, H-13α	H-11, H-13α	H-11	H-11
H-13			H-14	H-14
H-13α	H-2, H-12	H-2, H-12		
H-13β	H-14	H-14		
H-14	H-13β, H_3-15	H-13β, H_3-15	H-13, H_3-15	H-13, H_3-15
H_3-15	H-7, H-14	H-7, H-14	H-14, H_3-20	H-14, H_3-20
H_2-16			H-6	H-6
H_3-16	H-6	H-6		
H-17			H-7, H-9	H-7, H-9
H_3-18			H-11	H-11
H_3-20	H-9	H-9	H-9, H_3-15	H-9, H_3-15

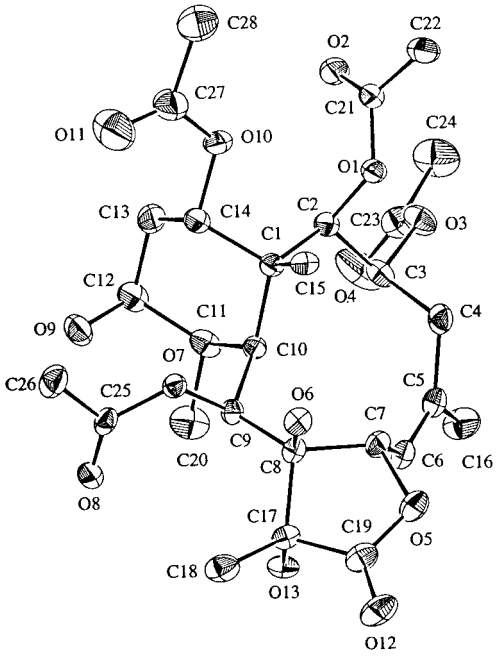


Figure 2. Computer-generated ORTEP of diterpene **1** showing relative configuration. Hydrogen atoms have been omitted for clarity.

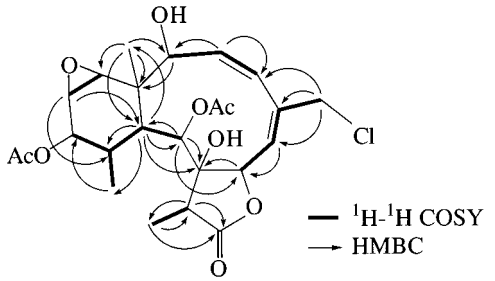


Figure 3. ^{1}H−^{1}H COSY and HMBC correlations for **3**.

of the above findings, the molecular structure, including the relative stereochemistry of **3**, was fully determined.

Briaexcavatolide N (**4**) has the molecular formula $C_{24}H_{32}O_{10}$, as determined by HRFABMS. The FABMS of **4** showed peaks at *m*/*z* 481 $[M + H]^+$, 463 $[M + H - H_2O]^+$, 445 $[M + H - 2H_2O]^+$, 403 $[M + H - H_2O - HOAc]^+$, 385 $[M + H - 2H_2O - HOAc]^+$, 343 $[M + H - H_2O - 2HOAc]^+$, 325 $[M + H - 2H_2O - 2HOAc]^+$, and 307 $[M + H - 3H_2O - 2HOAc]^+$, suggesting the presence of three hydroxyl and

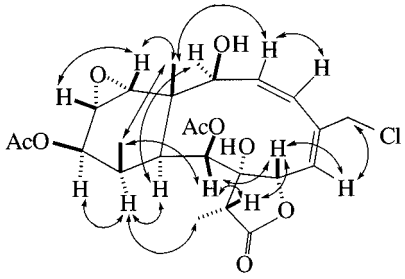


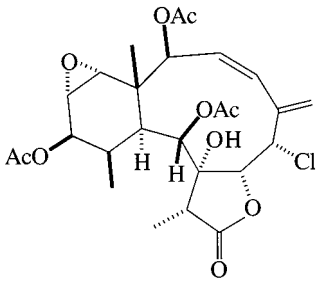
Figure 4. Selected NOE correlations of **3**.

two acetoxyl groups in the molecule of **4**. It was found that the ^{1}H and ^{13}C NMR spectral data of **4** (Tables 1 and 2) were very similar to those of **3**. However, the NMR chemical shifts for H_2-16 and C-16 of **4** (δ_H 4.30, 2H, br s; δ_C 63.8, t) were found to be shifted downfield, in comparison with the analogous data of **3** (δ_H 4.21, 2H, br s; δ_C 46.5, t), suggesting that the 16-chloro group of **3** was replaced by a hydroxyl group in diterpene **4**. The relative configuration of **4** was also confirmed by a NOESY experiment (Table 4). Thus, the structure of **4** was clearly elucidated.

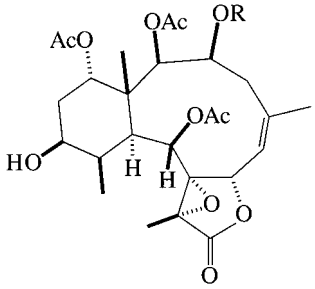
Diterpene **5** was identified as a diterpene, (1*S**,2*S**,5*Z*,7*S**,8*S**,9*S**,10*S**,11*R**,12*R**,13*Z*,17*R**)-2,12-diacetoxy-8,-17-epoxy-9-hydroxybriara-5,13-dien-18-one, which had been isolated from an Australian gorgonian. Its physical (optical rotation) and spectral (IR, MS, ^{1}H and ^{13}C NMR) data are in full agreement with those previously reported.[6]

Previous studies revealed that most chloro-containing briarane diterpenes have the chlorine atom located at C-6.[7−13] The allylic chloromethyl group in **3**, however, may come from the allylic rearrangement of a previously isolated compound, briaexcavatolide H (**6**).[5] Also, it is worthwhile to note that briarane diterpenes containing both hydroxyl groups at the β face of C-8 and the α face of C-17 in the lactone ring, like **1** and **2**, were observed for the first time. As it was observed that excavatolides C (**7**),[2] B (**8**),[2] and briaexcavatolide H (**6**)[5] were stable and could not be converted into **1**, **2**, and **3**, respectively, in solvents such as EtOAc, $CHCl_3$, and EtOAc−hexanes (1:1) at room temperature, and because treatment of **7** with silica gel in EtOAc or with a trace amount of TFA in acetone also could not yield **1**, the possibilities that **1**−**4** are artifacts arising from the corresponding hydrolysis of **7** and **8** and the allylic rearrangement of **6** during the extraction and the purification process could be ruled out. Thus, compounds **1**−**4** are

regarded as the natural products of *B. excavatum*.



6



7 : R = Ac
8 : R = *n*-PrCO

The cytotoxicity of the metabolites **1**−**5** against the growth of P-388 (mouse lymphocytic leukemia), A549 (human lung adenocarcinoma), and HT-29 (human colon adenocarcinoma) tumor cells was studied, and the results showed that the diterpenes **1**, **3**, and **4** were not cytotoxic toward the above cells. Compound **2** exhibited significant cytotoxicity against P-388 tumor cells with an ED_{50} of 0.5 μg/mL, and compound **5** exhibited significant cytotoxicity against P-388 and HT-29 tumor cells with ED_{50}'s of 0.4 and 1.1 μg/mL.[14]

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus and were uncorrected. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. IR spectra were recorded on a Hitachi I-2001 infrared spectrophotometer. UV spectra (in MeOH) were recorded on a Hitachi U-3210 UV spectrophotometer. FABMS were obtained with a VG Quattro GC/MS spectrometer. HRFABMS were recorded on JEOL JMS SX/SX 102A mass spectrometer. The NMR spectra were recorded on a Varian VXR-300/5 FT-NMR at 300 MHz for ^{1}H and 75 MHz for ^{13}C or on a Bruker AMX-400 FT-NMR at 400 MHz for ^{1}H and 100 MHz for ^{13}C or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively, in $CDCl_3$ using TMS as an internal standard. Si gel (Merck, 230−400 mesh) was used for column chromatography. Precoated Si gel plates (Merck, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC.

Animal Material. The gorgonian *B. excavatum* was collected by hand using scuba at South Bay, Kenting, located in the southernmost tip of Taiwan, in July 1995, at depths of 4−5 m, and was stored in a freezer until extraction. A voucher specimen was deposited in the Department of Marine Resources, National Sun Yat-Sen University (specimen no. KTSC-103).

Extraction and Isolation. The extraction scheme followed the standard procedures of our group.[2,3] The freeze-dried animal material (1.9 kg) was minced and extracted exhaustively with EtOAc. The EtOAc extract was chromatographed on Si gel column chromatography, using hexanes and hexanes−EtOAc mixtures of increasing polarity. Compound **5** was eluted with hexanes−EtOAc (4:1), compound **3** with hexanes−EtOAc (2:1), compound **4** with hexanes−EtOAc (3:2), compound **2** with hexanes−EtOAc (3:2−1:1), and compound **1** with hexanes−EtOAc (1:1).

Briaexcavatolide K (1): colorless prisms (26 mg); mp 270−273 °C; $[\alpha]^{27}_D$ −25° (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3428, 1772, and 1734 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m*/*z* 585 [0.2, $(M + H)^+$], 567 (0.6), 525 (0.6), 507 (0.3), 465 (0.3), 447 (0.3), 405 (0.5), 345 (0.8), 327 (0.8), 309 (1), and 291 (2); HRFABMS *m*/*z* 585.2541 (calcd for $C_{28}H_{41}O_{13}$, 585.2535).

Briaexcavatolide L (2): white powder (22 mg); mp 164−166 °C; $[\alpha]^{27}_D$ −37° (*c* 0.8, $CHCl_3$); IR (KBr) ν_{max} 3430, 1778, and 1730 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 552 [0.1 $(M - AcOH)^+$], 534 (0.2), 524 (0.2), 506 (0.3), 492 (0.3), 464 (0.4), 446 (0.3), 404 (0.4), 386 (0.3), 344 (0.3), 326 (0.3), and 290 (0.3); HREIMS *m*/*z* 552.2554 [calcd for $C_{28}H_{40}O_{11}$, $(M - AcOH)^+$, 552.2559].

Briaexcavatolide M (3): white powder (6 mg); mp 211−213 °C; $[\alpha]^{26}_D$ −29° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} 234 (log ϵ = 3.97); IR (KBr) ν_{max} 3433, 1780, and 1739 cm^{-1}. ^{1}H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m*/*z* 501 [0.1, $(M + H + 2)^+$], 499 [0.3, $(M + H)^+$], 481 (0.3), 439 (0.3), 379 (0.3), and 307 (12); HRFABMS *m*/*z* 499.1740 (calcd for $C_{24}H_{32}O_9Cl$, 499.1726).

Briaexcavatolide N (4): white powder (7 mg); mp 174−175 °C; $[\alpha]^{26}_D$ −23° (*c* 0.2 $CHCl_3$); UV (MeOH) λ_{max} 236 (log ϵ = 3.94); IR (KBr) ν_{max} 3421, 1770, and 1733 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m*/*z* 481 [0.5, $(M + H)^+$], 463 (0.8), 445 (0.6), 403 (0.8), 385 (0.6), 343 (0.6), 325 (0.7), and 307 (10); HRFABMS *m*/*z* 481.2071 (calcd for $C_{24}H_{33}O_{10}$, 481.2064).

Diterpene 5: white powder (10.9 mg); mp 67−68 °C; $[\alpha]^{25}_D$ −42° (*c* 0.3, $CHCl_3$) (lit.[6] $[\alpha]_D$ −44.9° (*c* 0.31)); IR (KBr) ν_{max} 3488, 1784, and 1728 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.87 (1H, dd, *J* = 10.0, 6.0 Hz, H-13), 5.65 (1H, d, *J* = 8.5 Hz, H-7), 5.52 (1H, d, *J* = 10.0 Hz, H-14), 5.32 (1H, d, *J* = 8.5 Hz, H-6), 4.91 (1H, dd, *J* = 6.0, 2.0 Hz, H-12), 4.85 (1H, d, *J* = 8.5 Hz, H-2), 3.73 (1H, dd, *J* = 10.0, 5.0 Hz, H-9), 3.20 (1H, dd, *J* = 10.0, 4.0 Hz, H-10), 2.58 (1H, m, H-3β), 2.55 (1H, dd, *J* = 12.0, 3.5 Hz, H-4β), 2.30 (1H, m, H-11), 2.23 (3H, s, acetate methyl), 2.07 (3H, s, acetate methyl), 1.93 (1H, br t, *J* = 12.0 Hz, H-4α), 1.86 (3H, s, H_3-16), 1.68 (1H, m, H-3α), 1.59 (3H, s, H_3-18), 1.21 (3H, s, H_3-15), and 0.98 (3H, d, *J* = 7.5 Hz, H_3-20); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4 (s, C-19), 170.4 (s, acetate carbonyl), 168.0 (s, acetate carbonyl), 142.1 (s, C-5), 140.3 (d, CH-14), 123.4 (d, CH-13), 121.0 (d, CH-6), 82.0 (d, CH-2), 73.7 (d, CH-7), 70.7 (s, C-8), 70.2 (d, CH-12), 68.4 (d, CH-9), 58.7 (s, C-17), 43.2 (s, C-1), 36.3 (d, CH-10), 32.6 (d, CH-11), 24.4 (t, CH_2-4), 23.9 (q, CH_3-16), 23.0 (t, CH_2-3), 21.3 (q, acetate methyl), 20.8 (q, acetate methyl), 19.1 (q, CH_3-15), 13.6 (q, CH_3-20), 9.4 (q, CH_3-18); FABMS *m*/*z* 449 [0.6, $(M + H)^+$], 389 (1), 329 (2), and 311 (2). The physical and spectral data of **5** are in full agreement with those reported previously.[6]

Single-Crystal X-ray Crystallography of Briaexcavatolide K (1).[15] Suitable colorless prisms of **1** were obtained from a solution in EtOH. The crystal (0.50 × 0.56 × 0.60 mm) belongs to the monoclinic system, space group $P2_12_12_1$ with *a* = 8.568(3) Å, *b* = 17.490(4) Å, *c* = 19.846(2) Å, *V* = 2974(1) Å^3, *Z* = 4, D_{calcd} = 1.306 g/cm^3, λ(Mo Kα) = 0.71069 Å. Intensity data were measured on Rigaku AFC7S diffractometer up to 2θ of 50.0°. All 3000 unique reflections were collected. The structure was solved by direct methods and refined by a full-matrix least-squares procedure. The non-hydrogen atoms were given anisotropic thermal parameters. The refinement converged to a final *R* = 0.044, R_w = 0.033 for 1987 observed reflections [$I > 3.00\sigma(I)$] and 370 variable parameters.

Cytotoxicity Testing. The P-388 cell line was kindly provided by Prof. J. M. Pezzuto, University of Illinois at Chicago; A549 and HT-29 cells were purchased from the American Type Culture Collection. The cytotoxicity of tested compounds **1**−**5** against the above three cancer cells was assayed with a modification of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[16]

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