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Briaexcavatolides A–J, New Diterpenes From The Gorgonian *Briareum excavatum*

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Abstract: Ten new briarane-type diterpenes have been isolated from the gorgonian octocoral *Briareum excavatum*. The structures of these secondary metabolites, named briaexcavatolides A–J (**1–10**), were established by spectroscopic and chemical methods. The structure, including the relative configuration of briaexcavatolide B (**2**), was further confirmed by a single-crystal X-ray structure analysis. Cytotoxicity of these compounds toward various cancer cell lines also is described.

Keywords: *Briareum excavatum*, briarane diterpene, briaexcavatolide, gorgonian, cytotoxicity

Introduction

Gorgonian octocorals (phylum Cnidaria, order Gorgonacea) are recognized as rich sources of biological active and structurally interesting terpenoids.[1] As part of our investigations of the Taiwanese marine invertebrates, the gorgonian *Briareum excavatum* Nutting (family Briareidae) has been the subject of an investigation. The previous studies on the chemical constituents of the West Pacific Ocean gorgonian *B. excavatum* have led to the isolation of twenty-six highly oxygenated briarane-type diterpenes, excavatolides A–Z,[2–5] which containing a γ-lactone in a bicyclo [8, 4, 0] system. Briarane diterpenes continue to attract our attention as several briaranes have been shown to exhibit cytotoxicity toward various cancer cell lines.[2–4, 6–11] In this paper, we report the isolation of the additional nine new briarane-type diterpenes, briaexcavatolides A–D and F–J (**1–4** and **6–10**), from the further investigation of the organic extract of the gorgonian *B. excavatum*. Besides, briaexcavatolide E (**5**) was isolated for the first time as a natural product. The structures of metabolites **1–10** were elucidated by extensive spectral analyses and chemical methods and by comparison with the physical and spectral data from other known briarane-type compounds.

Results and Discussion

Specimens were frozen immediately after collection and subsequently freeze-dried. The minced organisms

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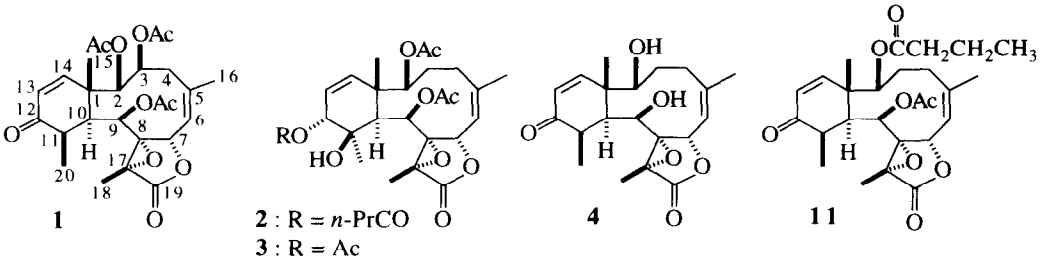
were extracted successively with EtOAc, and the extract was fractionated extensively using normal-phase absorbent (SiO_2 gel) to give the ten new metabolites, briaexcavatolides A–J (**1–10**), see Experimental Section.



Briaexcavatolide A (**1**) was obtained as a white powder. Its HRFABMS established the molecular formula $C_{26}H_{32}O_{10}$. The IR spectrum of **1** showed the presence of a carbonyl group of γ-lactone (υ_{max} 1786 cm^{-1}), ester carbonyl groups (υ_{max} 1740 cm^{-1}) and an α,β-unsaturated ketone carbonyl (υ_{max} 1700 cm^{-1}). The latter was confirmed by an UV absorption at 222 nm. The gross structure of **1** and all of the ^1H and ^{13}C chemical shifts (Table 1) associated with the molecule were determined by a series of 2D NMR experiments (^1H–^1H COSY, HETCOR, HMBC, and NOESY). From the ^1H–^1H COSY spectrum of **1**, it was possible to establish the proton sequences from H-2 to H_2-4; H-6 to H-7; H-9 to H-11; and H-13 to H-14. These data, together with the ^1H–^{13}C long-range correlations observed in an HMBC experiment, established the connectivities from C-1 to C-14. A vinyl methyl group attached at the C-5 position was confirmed by the HMBC correlations between H_3-16 and C-4, C-5, and C-6. The cyclohexenone ring which is fused to the ten-membered ring at C-1 and C-10, was elucidated by the key HMBC correlations between H-2 and C-14; H-9 and C-11; H-10 and C-12, C-14, and C-20; and H-11 and C-1, C-9. The ring juncture C-15 methyl group was positioned at C-1 from the key correlations between H_3-15 and C-1, C-2, C-10, and C-14. Furthermore, the HMBC correlations also revealed the positions of three acetoxyl groups attached to C-2, C-3, and C-9. These data, together with the HMBC correlations between H-9 and C-17; H_3-18 and C-8, C-17, and C-19, unambiguously established the molecular framework of **1**. The relative stereochemistry of **1** was deduced from vicinal ^1H–^1H coupling constants and from a NOESY experiment (Figure 1). The *cis* geometry of the C-13/C-14 double bond was indicated by a 10.4 Hz coupling constant between H-13 (δ 6.00) and H-14 (δ 6.48). Furthermore, the NOE correlations of H-10 with H-2, H-3, and H-11 indicated that these four protons are situated on the same face of the structure and were assigned as the α protons since the C-15 methyl is the β-substituent at C-1. The signal of H_3-18 showed NOE correlation with H_3-20, indicating the β-orientation of H_3-18. Also, H-9 was found to exhibit correlations with H-7 and H_3-18. From consideration of molecular models, H-9 was found to be reasonably close to H-7 and H_3-18 when H-7 was β-oriented and H-9 was placed on the α face. Based on the above observations, the structure of **1**, including the relative stereochemistry were elucidated unambiguously.

The new briarane briaexcavatolide B (**2**) was isolated as a white solid. The molecular formula of $C_{28}H_{38}O_{10}$ was established by HRFABMS. Thus, metabolite **2** contained ten degrees of unsaturation. The IR absorptions of **2** showed the presence of a hydroxyl group (υ_{max} 3572 cm^{-1}), a carbonyl group of γ-lactone (υ_{max} 1790 cm^{-1}), and ester carbonyls (υ_{max} 1734 cm^{-1}) in the structure of **2**. The ^1H and ^{13}C NMR spectra of **2** measured at room temperature in $CDCl_3$ or acetone-d_6 revealed mostly broad signals, revealing the existence of slowly interconverting conformers of **2**. As increase of temperature was expected to speed up the rate of interconversion to the point where the NMR spectrometer could record an "average" conformation, both ^1H and ^{13}C NMR spectra of **2** were measured at elevated temperature (70 °C) in pyridine-d_5 (Table 1). Fortunately,

Table 1. The ^1H and ^{13}C NMR Chemical Shifts of Diterpenes **1–4**

	compound							
	1		**2**		**3**		**4**	
C/H no.	^1H[a]	^{13}C[b]	^1H[c]	^{13}C[d]	^1H[c]	^{13}C[d]	^1H[e]	^{13}C[f]
1		43.0 (s)[h]		46.4 (s)		46.5 (s)		46.3 (s)
2	5.28 br s	81.5 (d)	5.04 br s	80.1 (d)	5.05 br s	80.2 (d)	3.90 br s	79.3 (d)
3	4.76 br s	71.9 (d)	1.91 br d (13.2)	32.3 (t)	1.90 br d (13.2)	32.3 (t)	1.60m	27.4 (t)
			2.52 br d (13.2)		2.49 br d (13.2)		2.15 m	
4	2.02 br d (15.6)[g]	33.3 (t)	2.02 m	26.3 (t)	1.97 m	26.5 (t)	1.91 br t (13.8)	25.2 (t)
	3.41 dd (15.6; 4.8)		2.69 br d (10.0)		2.68 br d (10.0)		3.01 m	
5		139.3 (s)		145.8 (s)		145.9 (s)		145.4 (s)
6	5.28 d (7.6)	123.0 (d)	5.65 d (9.6)	120.2 (d)	5.62 d (9.6)	120.6 (d)	5.26 d (8.7)	122.1 (d)
7	5.34 d (7.6)	73.6 (d)	6.02 d (9.6)	74.9 (d)	6.03 d (9.6)	74.8 (d)	5.70 d (8.7)	74.2 (d)
8		69.1 (s)		71.0 (s)		71.1 (s)		71.6 (s)
9	5.13 d (10.4)	66.7 (d)	6.29 d (4.8)	68.6 (d)	6.27 d (4.8)	69.0 (d)	3.93 d (11.2)	67.5 (d)
10	3.23 dd (10.4; 4.4)	38.6 (d)	3.14 br s	42.5 (d)	3.32 br s	42.7 (d)	3.45 dd (11.2; 4.8)	39.7 (d)
11	2.78 m	41.3 (s)		72.9 (s)		72.9 (s)	2.61 m	41.7 (d)
12		200.9 (d)	5.35 d (5.6)	74.1 (d)	5.32 d (5.6)	74.8 (d)		202.4 (s)
13	6.00 d (10.4)	126.1 (d)	5.97 dd (10.0; 5.6)	120.7 (d)	5.93 dd (10.0; 5.6)	121.0 (d)	5.86 d (10.5)	126.1 (d)
14	6.48 d (10.4)	155.2 (d)	5.70 d (10.0)	142.2 (d)	5.64 d (10.0)	142.4 (d)	6.54 d (10.5)	157.4 (d)
15	0.99 s	17.0 (q)	1.54 s	20.2 (q)	1.50 s	20.3 (q)	1.50 s	19.2 (q)
16	1.66 s	21.7 (q)	1.98 s	21.0 (q)	1.95 s	21.2 (q)	1.67 s	23.1 (q)
17		60.0 (s)		63.2 (s)		63.3 (s)		59.6 (s)
18	1.51 s	10.1 (q)	1.78 s	10.4 (q)	1.74 s	10.7 (q)	1.56 s	9.5 (q)
19		171.1 (s)		170.1 (s)		170.3 (s)		173.1 (q)
20	1.14 d (11.2)	14.2 (q)	1.69 s	28.1 (q)	1.65 s	28.2 (q)	1.14 d (7.5)	14.4 (q)
acetate	1.96 s	21.2 (q)	2.21 s	21.1 (q)	2.05 s	21.3 (q)		
methyls	2.20 s	21.7 (q)	2.25 s	21.6 (q)	2.19 s	21.4 (q)		
	2.25 s	22.6 (q)			2.26 s	21.9 (q)		
ester		169.0 (s)		169.3 (s)		169.6 (s)		
carbonyls		169.8 (s)		171.8 (s)		170.2 (s)		
		170.4 (s)				172.1 (s)		
n-butyrate				172.6 (s)				
			1.00 t (7.2)	13.7 (q)				
			1.75 m	18.8 (t)				
			2.38 t (7.2)	36.7 (t)				

[a]Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. [b]100 MHz in $CDCl_3$ at 25 °C. [c]400 MHz in pyridine-d_5 at 70 °C. [d]100 MHz in pyridine-d_5 at 70 °C. [e]300 MHz in acetone-d_6 at 25 °C. [f]75 MHz in acetone-d_6 at 25 °C. [g] *J* values (in Hz) in parentheses. [h]Multiplicity deduced by DEPT and indicating by usual symbols. The values are ppm downfield from TMS.

it was found that at this temperature the signals for each proton and carbon were sharpened and could be assigned by the assistance of DEPT and 2D NMR spectra. From the above spectral data, a trisubstituted olefin was deduced from the signals of two carbons at δ 120.2 (d) and 145.8 (s), and a disubstituted olefin was found from the signals of carbons at δ 120.7 (d) and 142.2 (d). An 8,17-epoxide was confirmed from the signals of two quaternary oxygenated carbons at δ 63.2 (s) and 71.0 (s), and from the chemical shift of H_3-18 (δ 1.78, 3H, s). In the ^{13}C NMR spectrum of **2**, four carbonyl resonances were appeared at δ 169.3 (s), 170.1 (s), 171.8 (s), and 172.6 (s), and further confirmed the presence of a γ-lactone and three other ester groups in **2**. Based on the above observations, diterpene **2** was found to be a tetracyclic compound. In the ^1H NMR spectrum of **2**, two acetate methyls were observed (δ 2.21, 3H, s; 2.25, 3H, s). The additional acyl group was found to be an *n*-

butyryloxyl group based on the ^1H NMR studies, including an ^1H–^1H COSY experiment, which revealed seven contiguous protons (δ 1.00, 3H, t, J = 7.2 Hz; 1.75, 2H, m; 2.38, 2H, t, J = 7.2 Hz). The carbon signal at δ 172.6 (s) was correlated with the signals of the methylene protons of the *n*-butyrate at δ 2.38 in the HMBC spectrum of **2**, and was consequently assigned as the carbon atom of the *n*-butyrate carbonyl. From the ^1H–^1H COSY experiment of **2**, it was possible to establish the separate spin systems that map out the proton sequences from H-2 to H_2-4; H-6 to H-7; H-9 to H-10; and H-12 to H-14. These data, together with the ^1H–^{13}C long-range correlations observed in the HMBC experiment of **2**, suggested the briarane-type molecular framework of **2**. Furthermore, the *n*-butyrate ester was positioned at C-12 from the connectivity between H-12 (δ 5.35) and carbonyl carbon (δ 172.6) of the *n*-butyrate. By above spectral analyses, the structure of **2** were determined unambiguously. The X-ray structure of **2** (Figure 2) also demonstrates the location of the *n*-butyrate at C-12 and confirms the relative, not the absolute configuration of **2**.

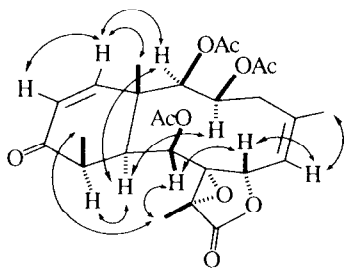


Figure 1. Selective NOE Correlations of **1**

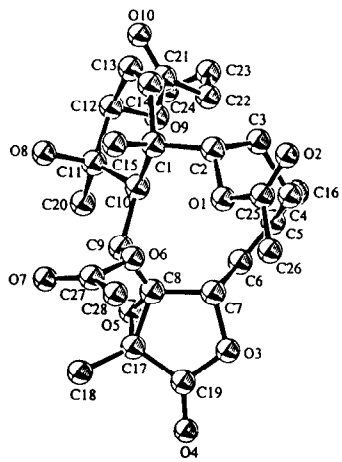
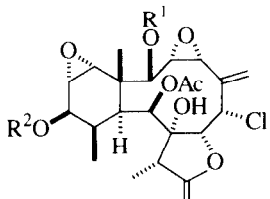


Figure 2. A computer-generated ORTEP plot of **2** showing relative configuration. Hydrogen atoms have been omitted for calrity.

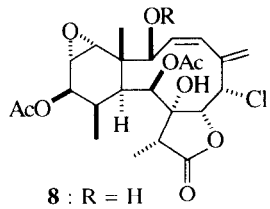
Briaexcavatolide C (**3**), was isolated as an amorphous solid and had the molecular formula $C_{26}H_{34}O_{10}$ on the basis of HRFABMS. Its IR spectrum exhibited a broad OH stretch at 3528 cm^{-1}, a γ-lactone carbonyl at 1786 cm^{-1}, and ester carbonyls at 1734 cm^{-1}. The broad NMR signals (^1H and ^{13}C) measured at room temperature and the sharpened NMR (^1H and ^{13}C) signals of diterpene **3** measured in pyridine-d_5 at 70 °C were found to be very close to those of **2**. Furthermore, by comparison of the ^1H and ^{13}C NMR spectral data of **3** with **2**, it was revealed that the signals corresponding to the *n*-butyryloxyl group in **2** were replaced by an acetoxyl group in **3**. Based on the above data, briaexcavatolide C (**3**) was found to be the 12-O-debutyryl-12-O-acetyl derivative of **2**.

Diterpene **4** (briaexcavatolide D), $C_{20}H_{26}O_6$ by HRFABMS, was isolated as a white powder. The IR spectrum of **4** showed absorptions that indicated the presence of two different carbonyl types: γ-lactone (υ_{max} 1784 cm^{-1}), and α,β-unsaturated ketone (υ_{max} 1690 cm^{-1}). The latter structural feature was confirmed by an UV absorption at 224 nm, the presence of signals at δ 202.4 (s), 126.1 (d), and 157.4 (d) in the ^{13}C NMR spectrum, and the presence of a mutually coupled pair of doublet signals (J = 10.5 Hz) in the ^1H NMR spectrum of **4** at δ 5.86 (H-13) and 6.54 (H-14) corresponding to the α and β olefinic protons, respectively. It was observed that the spectral data (^1H and ^{13}C NMR) of **4** were similar to those of a known diterpene,

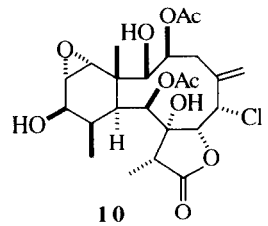
briareolide I (**11**).[12] However, it was found that the acyloxyl groups at C-2 and C-9 of compound **11** were replaced by hydroxyl groups by comparing the related spectral data of **4** with those of **11**. Based on the above observations, briaexcavatolide D (**4**) was assigned as the 2-O-debutyryl-9-O-deacetyl derivative of briareolide I (**11**).



5 : $R^1 = R^2 = Ac$
6 : $R^1 = H$, $R^2 = n$-PrCO
7 : $R^1 = R^2 = H$
12 : $R^1 = H$, $R^2 = Ac$



8 : R = H
9 : R = Ac



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Briaexcavatolide E (**5**) was isolated as a white powder. HRFABMS established the molecular formula, $C_{26}H_{33}O_{11}Cl$, for this compound. The IR spectrum of **5** showed absorptions of a hydroxyl group (υ_{max} 3484 cm^{-1}), a carbonyl group of γ-lactone (υ_{max} 1781 cm^{-1}), and ester carbonyls (υ_{max} 1740 cm^{-1}). Carbonyl resonances in the ^{13}C NMR spectrum of **5** (Table 3) at δ 168.8 (s), 169.0 (s), 169.5 (s), and 174.0 (s) confirmed the presence of a γ-lactone and three other esters in **5**. In the ^1H NMR spectrum of **5** (Table 2), three acetate methyls were observed at δ 2.10 (3H, s), 2.14 (3H, s), and 2.22 (3H, s). Comparison of the physical (mp and optical rotation) and spectral (IR, MS, ^1H, and ^{13}C NMR) data found for **5** with those of other briarane-type diterpenes reported in the literatures indicated that diterpene **5** is the acetate derivative of brianolide (**12**), which was isolated previously from an Okinawa gorgonian coral *Briareum* sp.[13] Diterpene **5** has been obtained by acetylation of **12** in the same work, however, this is the first time that **5** (briaexcavatolide E) was isolated from natural source.

The new briarane diterpenes briaexcavatolide F (**6**) and briaexcavatolide G (**7**) had the molecular formulas of $C_{26}H_{35}O_{10}Cl$ and $C_{22}H_{29}O_9Cl$, respectively, as determined by HRFABMS. It was found that the spectral data (IR, ^1H, and ^{13}C NMR) of **6** and **7** were very similar to those of brianolide (**12**).[13] However, the ^1H and ^{13}C NMR spectra (Tables 2 and 3) revealed that the signals corresponding to an acetoxyl group in **12** were disappeared, and were replaced by those of an *n*-butyryloxyl and a hydroxyl groups in **6** and **7**, respectively. In the HMBC experiment of **6**, the carbon signal at δ 173.0 (s) which showed a correlation with H-12 (δ 5.17) was found to be correlated with the signal of the methylene protons at δ 2.30, and was consequently assigned as the carbon atom of the *n*-butyrate carbonyl. Thus, the *n*-butyrate ester should be positioned at C-12 in **6** and the hydroxyl group was positioned at C-12 in **7**. Furthermore, acetylation of **7** gave a less polar product, which was found to be identical with briaexcavatolide E (**5**) by comparison of the physical and spectral data, and confirmed the structure of diterpene **7**. On the basis of the above observations, the structures of **6** and **7** were found to be the 12-O-deacetyl-12-O-*n*-butyryl and 12-O-deacetyl derivatives of **12**, respectively.

Briaexcavatolide H (**8**) was obtained as a white solid. The molecular formula $C_{24}H_{31}O_9Cl$, was established by HRFABMS. A strong UV absorption at 228 nm suggested the presence of a conjugated diene system in diterpene **8**. The IR spectrum showed bands at 3396, 1778, and 1727 cm^{-1}, consistent with the presence of hydroxyl, γ-lactone, and ester carbonyl groups. In the ^{13}C NMR spectrum of **8** (Table 3), a disubstituted and an exocyclic olefins were deduced from the signals of four carbons at δ 118.3 (t), 126.1 (d), 135.1 (d), and 137.0

(s). An 13,14-epoxide group was confirmed from the two tertiary oxygenated carbons at δ 58.0 (d) and 62.5 (d), and from the chemical shifts of H-13 (δ 3.23, d, J = 3.6 Hz) and H-14 (δ 3.16, d, J = 3.6 Hz). From the ^{13}C NMR spectrum of **8**, three carbonyl resonances appeared at δ 170.1 (s), 170.2 (s), and 174.4 (s) and confirmed the presence of a carbon resonance of γ-lactone and two other ester groups. In the ^1H NMR of **8**, two acetate methyls (δ 2.15, 3H, s; 2.17, 3H, s) were observed. The *cis* geometry of the C-3/C-4 double bond was indicated by a 12.0 Hz coupling constant between H-3 (δ 5.83) and H-4 (δ 5.84). Based on the above findings, it was suggested that metabolite **8** is probably a deoxygenated derivative of **12** and should possess a structure as represented by formula **8**. The molecular structure of **8** was further confirmed by 2D NMR experiments (^1H–^1H COSY, HMBC, and NOESY, see figures 3 and 4).

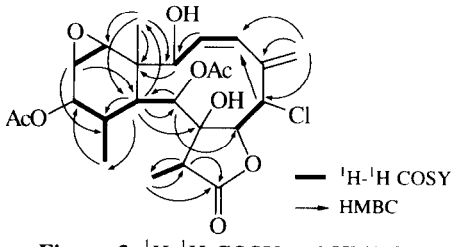

Figure 3. ^1H-^1H COSY and HMBC Correlations for **8**

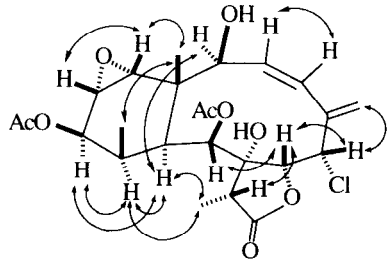

Figure 4. Selective NOE Correlations of **8**

Table 2. The ^1H NMR Chemical Shifts of Diterpenes **5**–**10**

H	compound					
	5[a]	**6**[b]	**7**[b]	**8**[a]	**9**[a]	**10**[c]
2	5.13 d (9.2)[d]	4.30 d (9.0)	4.29 d (9.0)	5.15 d (6.4)	6.13 d (9.2)	3.62 d (3.3)
3	3.40 dd (9.2; 4.0)	3.98 dd (9.0; 3.9)	4.01 dd (9.0; 3.9)	5.83 dd (12.0; 6.4)	5.62 dd (12.0; 9.2)	5.17 d (3.3)
4	3.62 d (4.0)	4.29 d (3.9)	4.28 d (3.9)	5.84 d (12.0)	5.90 d (12.0)	1.99 m 2.86 m
6	5.37 d (3.2)	5.83 d (3.3)	5.86 d (3.6)	5.20 d (4.0)	5.22 d (2.8)	5.20 d (3.0)
7	5.04 d (3.2)	5.73 d (3.3)	5.73 d (3.6)	4.95 d (4.0)	4.94 d (2.8)	4.92 d (3.0)
9	5.34 d (8.8)	5.80 d (8.7)	5.89 d (9.0)	5.24 d (7.6)	5.25 d (7.6)	5.22 d (4.2)
10	1.75 dd (8,8; 2.4)	2.43 dd (8.7; 1.8)	2.40 dd (9.0; 1.8)	1.93 dd (7.6; 2.0)	2.02 dd (7.6; 2.8)	1.95 d (4.2)
11	2.24 m	3.04 m	3.05 m	2.13 m	2.17 m	1.99 m
12	4.61 d (4.8)	5.17 d (5.1)	4.33 d (5.4)	4.60 d (4.8)	4.62 d (4.4)	4.27 dd (6.9; 3.6)
13	3.13 d (3.2)	3.77 d (3.9)	3.80 d (3.9)	3.23 d (3.6)	3.16 d (3.2)	3.20 d (3.6)
14	2.89 d (3.2)	3.48 d (3.9)	3.71 d (3.9)	3.16 d (3.6)	2.98 d (3.2)	2.92 d (3.6)
15	1.22 s	1.50 s	1.57 s	1.10 s	1.17 s	1.21 s
16	6.03 d (2.4) 6.12 d (2.4)	5.57 d (1.5) 5.99 d (1.5)	5.59 d (1.5) 6.04 d (1.5)	5.58 d (2.8) 5.93 d (2.8)	6.01 d (2.4) 6.29 d (2.4)	6.03 d (1.8) 6.06 d (1.8)
17	2.45 q (7.2)	3.20 q (6.9)	3.18 q (7.2)	2.35 q (7.2)	2.34 q (7.2)	2.36 q (7.2)
18	1.17 d (7.2)	1.41 d (6.9)	1.53 d (7.2)	1.15 d (7.2)	1.15 d (7.2)	1.15 d (7.2)
20	1.04 d (7.2)	1.34 d (6.9)	1.42 d (6.9)	1.06 d (7.2)	1.04 d (6.8)	1.07 d (7.2)
acetate methyls	2.10 s 2.14 s 2.22 s	2.32 s	2.30 s	2.15 s 2.17 s	2.04 s 2.10 s 2.17 s	2.15 s 2.17 s
n-butyrate		0.85 t (7.5) 1.59 m 2.30 t (7.5)				

[a]Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. [b]300 MHz in pyridine-d_5 at 25 °C. [c]300 MHz in $CDCl_3$ at 25 °C. [d]J values (in Hz) in parentheses. The values are ppm downfield from TMS.

Briaexcavatolide I (**9**) had the molecular formula $C_{26}H_{33}O_{10}Cl$, as determined by HRFABMS. It was found that the NMR spectra (^1H and ^{13}C) of **9** were very similar to those of **8**. However, the ^1H NMR spectrum of **9** showed the presence of three acetate methyls which were observed at δ 2.04 (3H, s), 2.10 (3H, s), and 2.17 (3H, s). The positions of the three acetoxyl groups at C-2, C-9, and C-12 were confirmed by the connectivities between the three methine protons at δ 6.13 (H-2), 5.25 (H-9), and 4.62 (H-12) and the ester carbonyls at 169.7 (s), 170.0 (s), and 170.1 (s), respectively, in the HMBC spectrum of **9**. Thus, the structure of briaexcavatolide I (**9**) was established as the 2-acetyl derivative of **8**.

Table 3. The ^{13}C NMR Chemical Shifts of Diterpenes **5–10**

	compound					
position	**5**[a]	**6**[b]	**7**[b]	**8**[a]	**9**[a]	**10**[c]
C-1	38.3 (s)[d]	40.0 (s)	40.9 (s)	41.3 (s)	40.6 (s)	40.2 (s)
C-2	74.9 (d)	73.3 (d)	73.3 (d)	71.7 (d)	75.2 (d)	70.6 (d)
C-3	60.0 (d)	64.3 (d)	64.4 (d)	135.1 (d)	130.8 (d)	64.7 (d)
C-4	57.1 (d)	58.8 (d)	58.7 (d)	126.1 (d)	128.0 (d)	35.4 (d)
C-5	133.4 (s)	138.7 (s)	138.8 (s)	137.0 (s)	136.0 (s)	144.3 (s)
C-6	61.1 (d)	61.8 (d)	61.8 (d)	62.4 (d)	62.4 (d)	74.5 (d)
C-7	76.5 (d)	79.8 (d)	79.8 (d)	77.7 (d)	77.7 (d)	77.0 (d)
C-8	83.9 (s)	84.5 (s)	84.8 (s)	83.9 (s)	83.9 (s)	84.4 (s)
C-9	69.3 (d)	70.6 (d)	70.9 (d)	69.9 (d)	69.8 (d)	71.4 (d)
C-10	37.4 (d)	38.0 (d)	38.6 (d)	37.6 (d)	37.6 (d)	37.5 (d)
C-11	35.9 (d)	37.5 (d)	40.0 (d)	37.2 (d)	37.1 (d)	42.0 (d)
C-12	71.7 (d)	73.8 (d)	70.9 (d)	72.6 (d)	72.2 (d)	70.1 (d)
C-13	56.6 (d)	64.2 (d)	64.4 (d)	58.0 (d)	57.3 (d)	59.3 (d)
C-14	61.4 (d)	58.3 (d)	60.8 (d)	62.5 (d)	62.0 (d)	63.3 (d)
C-15	16.5 (q)	17.0 (q)	17.2 (q)	15.1 (q)	15.9 (q)	17.6 (q)
C-16	120.9 (t)	117.5 (t)	117.3 (t)	118.3 (t)	119.4 (t)	121.2 (t)
C-17	45.5 (d)	45.8 (d)	45.8 (d)	45.1 (d)	45.1 (d)	43.7 (d)
C-18	6.2 (q)	7.1 (q)	7.1 (q)	6.3 (q)	6.3 (q)	6.9 (q)
C-19	174.0 (s)	175.9 (s)	175.9 (s)	174.4 (s)	174.3 (s)	174.5 (s)
C-20	9.7 (q)	10.7 (q)	10.6 (q)	9.5 (q)	9.4 (q)	8.6 (q)
acetate methyls	21.0 (q) 21.0 (q) 22.0 (q)	22.4 (q)	22.3 (q)	21.1 (q) 21.1 (q)	21.0 (q) 21.0 (q) 22.0 (q)	21.2 (q) 22.0 (q)
ester carbonyls	168.8 (s) 169.0 (s) 169.5 (s)	171.6 (s)	171.6 (s)	170.1 (s) 170.2 (s)	169.7 (s) 170.0 (s) 170.1 (s)	169.5 (s) 173.1 (s)
n-butyrate		173.0 (s) CH_3 14.1 (q) CH_2 19.2 (t) CH_2 36.6 (t)				

[a]Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C. [b]75 MHz in pyridine-d_5 at 25 °C. [c]75 MHz in $CDCl_3$ at 25 °C. [d]Multiplicity deduced by DEPT and indicating by usual symbols. The values are in ppm downfield from TMS.

Briaexcavatolide J (**10**) had the molecular formula $C_{24}H_{33}O_{10}Cl$ as determined by HRFABMS. The IR spectrum of **10** showed the presence of hydroxyl groups (υ_{max} 3424 cm^{-1}), carbonyl group of γ-lactone (υ_{max} 1780 cm^{-1}), and ester carbonyls (υ_{max} 1733 cm^{-1}). The gross structure of **10** and all of the ^1H and ^{13}C chemical shifts associated with the molecule were also determined by the assistance of DEPT and 2D NMR experiments (^1H–^1H COSY, HETCOR, HMBC, and NOESY). From the above spectral data, an exocyclic olefin was deduced from the signals of two carbons at δ 121.2 (t) and 144.3 (s). An 13,14-epoxide group was confirmed from the signals of two oxygenated methine carbons at δ 59.3 (d) and 63.3 (d), and from the chemical shifts of H-13 (δ 3.20, d, J = 3.6 Hz) and H-14 (δ 2.92, d, J = 3.6 Hz). In the ^{13}C NMR spectrum of **10**, three carbonyl resonances appeared at δ 169.5 (s), 173.1 (s), and 174.5 (s), and confirmed the presence of a

γ-lactone and two other ester groups in **10**. In the ^{1}H NMR spectrum of **10**, two acetate methyls (δ 2.15, 3H, s; 2.17, 3H, s) were also observed. From the ^{1}H–^{1}H COSY spectrum, it was possible to establish the protons sequences from H-2 to H_2-4; H-6 to H-7; and H-9 to H-14. The positions of the two acetoxyl groups at the C-3 and C-9 were confirmed by the ^{1}H NMR chemical shifts of H-3 (δ 5.17) and H-9 (δ 5.22) and by the connectivities between the two methine protons and the ester carbonyls at δ 169.5 (s) and 173.1 (d), respectively, in the HMBC spectrum of **10**. The relative stereochemistry of **10** was established by a NOESY experiment (Figure 5). On the basis of the above analyses, the structure of diterpene **10** were established. It is noted that the briarane-type diterpenes known possess three hydroxyl groups in structures are rarely found.[3]

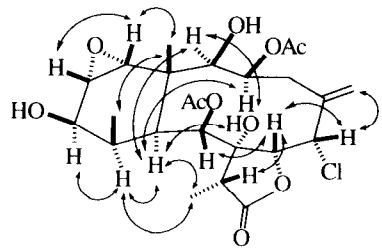

Figure 5. Selective NOE Correlations of **10**

The absolute configuration of brianolide (**12**) has been determined previously by X-ray analysis, as shown by structure **12**.[13] As briaexcavatolides A–J (**1**–**10**) were isolated along with brianolide from the same organism,[2] it is reasonable on biogenetic grounds to assume that diterpenes **1**–**10** have the same absolute configurations as **12**. The acetylation of briaexcavatolide G (**7**) and brianolide (**12**) gave the same compound which was found to be identical with briaexcavatolide E (**5**), and further confirmed the above assumption. Therefore, briaexcavatolides A–J were assumed to possess the absolute configurations as represented by structures **1**–**10**.

The cytotoxicity of the new diterpenes **1**–**10** against the growth of P-388, KB, A549, and HT-29 tumor cells were studied, and the results showed that diterpenes **1**, **3**, **4**, **5**, and **7**–**10** were found to be not cytotoxic toward the above cells. Compound **2** exhibited significant cytotoxicity against P-388 and KB tumor cells with ED_{50} of 1.3 and 1.5 μg/mL, respectively, and compound **6** exhibited significant cytotoxicity against A549 tumor cells with ED_{50} of 1.3 μg/mL.[14]

Experimental Section

General Experimental Procedures. Ultraviolet spectra were recorded on a Hitachi U-3210 UV spectrophotometer. 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, respectively, in $CDCl_3$ or pyridine-d_5 using TMS as internal standard, unless otherwise indicated. Other general experimental procedures followed those reported previously.[2,3]

Collection, Extraction, and Separation. The organism *B. excavatum* (3.6 kg wt) 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 is stored at the Department of Marine Resources, National Sun Yat-Sen University (specimen no. KTSC-103). The extraction and isolation schemes followed the standard procedures that have been reported by our group previously.[2,3] The organic extract was chromatographed on SiO_2 gel CC, using hexanes and EtOAc mixtures of increasing polarity, yielded diterpenes **1**–**10**. Diterpene **1** was eluted with hexanes–EtOAc (5:1), **2** with hexanes–EtOAc (5:1–4:1), **3** with hexanes–

EtOAc (4:1), **5** with hexanes–EtOAc (4:1–7:2), **9** with hexanes–EtOAc (3:1), **4** with hexanes–EtOAc (3:1–2:1), **8** with hexanes–EtOAc (2:1), **6** with hexanes–EtOAc (2:1–3:2), **7** with hexanes–EtOAc (3:2–1:1), **10** with hexanes–EtOAc (1:1).

Briaexcavatolide A (1): white solid (4.1 mg); mp 85–87 °C; $[\alpha]^{25}_D$ −10° (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} 222 nm (ε 5524); IR (KBr) υ_{max} 1786, 1740, and 1700 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; FABMS *m/z* 505 [11.0, (M + H)$^+$]; HRFABMS *m/z* 505.2071 (calcd for $C_{26}H_{33}O_{10}$ 505.2064).

Briaexcavatolide B (2): white powder (60.1 mg); mp 219–221 °C; $[\alpha]^{25}_D$ −111° (*c* 1.0, $CHCl_3$); IR (KBr) υ_{max} 3572, 1790, and 1734 cm^{-1}; ^1H and ^{13}C NMR data, See Table 1; FABMS *m/z* 535 [0.5, (M + H)$^+$]; HRFABMS *m/z* 535.2573 (calcd for $C_{28}H_{39}O_{10}$, 535.2544).

Briaexcavatolide C (3): white powder (9.8 mg); mp 226–228 °C; $[\alpha]^{25}_D$ −96° (*c* 0.4, $CHCl_3$); IR (KBr) υ_{max} 3528, 1786, and 1734 cm^{-1}; ^1H and ^{13}C NMR data, See Table 1; FABMS *m/z* 507 [2, (M + H)$^+$]; HRFABMS *m/z* 507.2235 (calcd for $C_{26}H_{35}O_{10}$, 507.2220).

Briaexcavatolide D (4): white powder (5.6 mg); mp 217–219 °C; $[\alpha]^{25}_D$ −14° (*c* 0.2, $CHCl_3$); UV ($CHCl_3$) λ_{max} 224 nm (ε 6074); IR (KBr) υ_{max} 3330, 1784, and 1690 cm^{-1}; ^1H and ^{13}C NMR data, See Table 1; FABMS *m/z* 363 [0.3, (M + H)$^+$]; HRFABMS *m/z* 363.1811 (calcd for $C_{20}H_{27}O_6$, 363.1800).

Briaexcavatolide E (5): white powder (2.2 mg); mp 171–173 °C; $[\alpha]^{26}_D$ −27°(*c* 0.1, MeOH); IR (KBr) υ_{max} 3484, 1781, and 1740 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; FABMS *m/z* 559 [0.2, (M + H + 2)$^+$], 557 [0.4, (M + H)$^+$]; HRFABMS *m/z* 557.1795 (calcd for $C_{26}H_{34}O_{11}Cl$, 557.1780).

Briaexcavatolide F (6): white powder (9.7 mg); mp 184–186 °C; $[\alpha]^{25}_D$ −21° (*c* 0.1, MeOH); IR (KBr) υ_{max} 3400, 1765, and 1745 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; FABMS *m/z* 545 [0.4, (M + H + 2)$^+$], 543 [0.2, (M + H)$^+$]; HRFABMS *m/z* 543.1987 (calcd for $C_{26}H_{36}O_{10}Cl$, 543.1987).

Briaexcavatolide G (7): white powder (6.0 mg); mp 233–235 °C; $[\alpha]^{25}_D$ −18° (*c* 0.2, MeOH); IR (KBr) υ_{max} 3371, 1768, and 1740 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; FABMS *m/z* 475 [0.2, (M + H + 2)$^+$], 473 [0.4, (M + H)$^+$]; HRFABMS *m/z* 473.1577 (calcd for $C_{22}H_{30}O_9Cl$, 473.1570).

Briaexcavatolide H (8): white solid (3.5 mg); mp 201–203 °C; $[\alpha]^{25}_D$ −254° (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} 228 nm (ε 5426); IR (KBr) υ_{max} 3396, 1778, and 1727 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; FABMS *m/z* 501 [0.6, (M + H + 2)$^+$], 499 [1.4, (M + H)$^+$]; HRFABMS *m/z* 499.1727 (calcd for $C_{24}H_{32}O_9Cl$, 499.1726).

Briaexcavatolide I (9): white solid (2.7 mg); mp 224–226 °C; $[\alpha]^{25}_D$ −53° (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} 226 nm (ε 5568); IR (KBr) υ_{max} 3422, 1781, and 1734 cm^{-1}; ^1H and ^{13}C NMR data, See Tables 2 and 3; FABMS *m/z* 543 [0.3, (M + H + 2)$^+$], 541 [0.8, (M + H)$^+$]; HRFABMS *m/z* 541.1841 (calcd for $C_{26}H_{34}O_{10}Cl$, 541.1831).

Briaexcavatolide J (10): white powder (2.5 mg); mp 183–185 °C; $[\alpha]^{25}_D$ −26° (*c* 0.1, $CHCl_3$); IR (KBr) υ_{max} 3424, 1780, and 1733 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; FABMS *m/z* 519 [0.4, (M + H + 2)$^+$], 517 [0.8, (M + H)$^+$]; HRFABMS *m/z* 517.1837 (calcd for $C_{24}H_{34}O_{10}Cl$, 517.1831).

Acetylation of Briaexcavatolide G (7): Briaexcavatolide G (**7**) (2.0 mg) was stirred with 1 mL of Ac_2O in 1 mL of pyridine for 48 h at room temperature. After evaporation of excess reagent, the residue was separated by column chromatography on SiO_2 gel to give pure briaexcavatolide E (**5**) (hexanes : EtOAc = 7 : 2; 1.9 mg, 81%); physical and spectral data were in full agreement with those of the natural product **5**.

Cytotoxicity Testing. KB and P-388 cells were 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

cytotoxic activities of tested compounds against the above four cancer cells were assayed with a modification of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. Cytotoxicity assays were carried out according to the procedure described previously.[15,16]

Single-Crystal X-ray Crystallography of 2.[17] Suitable colorless prisms of **2** were otabined from a solution in EtOH. The crystal (0.33 × 0.50 × 0.50 mm) belongs to the monoclinic system, space group $P2_12_12_1$ with a = 14.785 (5), b = 21.551 (5), c = 8.773 (4) Å, V = 2795 (1) Å^3, Z = 4, D_{calc} = 1.268 g/cm^3, λ (MoKα) = 0.71069 Å. Intensity data were measured on Rigaku AFC6S diffractometer up to 2θ of 47.1°. All 2407 unique reflections were collected. The structure was solved by dirtect method and refined by a full-matrix least -squares procedure. The nonhydrogen atoms were given anisotropic thermal parameters. The refinement converged to a final R = 0.091, R_w = 0.057 for 1321 observed reflections [$I > 3.00\ \sigma\ (I)$] and 153 variable parameters.

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