Novel Cytotoxic Diterpenes, Excavatolides A−E, Isolated from the Formosan Gorgonian *Briareum excavatum*

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The chemistry of *Briareum excavatum*, a Formosan gorgonian coral, was investigated. This study has led to the isolation of five novel marine natural products, excavatolides A−E **(1−5)**, together with brianolide **(6)**. The structures of the above compounds were established by spectral and chemical methods. The relative configuration of excavatolide 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.

Gorgonians (order Gorgonacea, phylum Cnidaria) and soft corals (Alcyonacea, phylum Cnidaria) have been proven to be rich sources of structurally novel secondary metabolites. Previous studies on gorgonian corals of the genus *Briareum* resulted in the isolation of a series of novel diterpenoids, represented by briaranes,[1−11] and asbestinins.[12−16] Diterpenoids of the briarane class have also been isolated from other coelenterates such as a true soft coral,[17] a sea pansy,[18] and sea pens,[19−25] and continue to attract the attention of investigators because of the structural complexity and interesting biological activity (e. g., cytotoxic,[6,26,27] antiinflammatory,[9,28,29] antiviral,[6,29] and insecticidal[3]) associated with several compounds of this type. We have also previously isolated five cytotoxic briaran diterpenes from a Formosan gorgonian *Briareum* sp.[30] In connection with our continuing studies of the biomedical potential of marine invertebrates, the chemistry of a Formosan gorgonian *Briareum excavatum* Nutting (family Briareidae) was investigated. This study has led to the isolation of five novel briaranes, excavatolides A−E **(1−5)**, together with brianolide **(6)**. The structures of these compounds were established by extensive spectral experiments (IR, MS, ^{1}H NMR, and ^{13}C NMR).

Results and Discussion

Specimens were frozen immediately after collection and subsequently freeze-dried. Conventional extraction procedures were used, and the extracts were fractionated extensively using normal-phase absorbent (Si gel) to yield the six briaran diterpenoids, see Experimental Section. The structures of compounds **2**, **3**, and **5** were determined by ^{1}H and ^{13}C NMR spectra recorded in Me_2-CO-d_6 at −70 °C. Compounds **2** and **3** were distinctively major diterpene metabolites, while all others were minor components.

The new metabolite excavatolide A **(1)** had a molecular formula of $C_{24}H_{31}O_8Cl$ as determined by HR-FABMS. Thus, **1** contained nine degrees of unsaturation. The spectral data (^{1}H NMR and ^{13}C NMR, see Tables 1 and 2) of **1** were very similar to those of a known compound **7**.[8] It was found, however, that a propionate at C-2 of **7** was replaced by an acetate by comparing the ^{1}H NMR and ^{13}C NMR spectral data of compound **1** with those of **7**. Also, the hydroxyl group at C-17 in **7** was replaced by a hydrogen atom, as shown by the fact that the proton signal of H_3-18 (δ 1.47 ppm in pyridine-d_5) was split into a doublet (J = 6.8 Hz), and the signal of H-17 (δ 3.19 ppm) appeared as a quartet (J = 6.8 Hz) in compound **1**. The assignments of proton and carbon shifts of metabolite **1** were further confirmed by 2D NMR (^{1}H−^{1}H and ^{13}C−^{1}H COSY) spectra. Based on the above analysis, the structure of **1** was established as described by formula **1**.

The new briaran diterpene excavatolide B **(2)** was the most abundant metabolite. Its HRFABMS spectrum established the molecular formula $C_{30}H_{42}O_{12}$. Thus, 10 degrees of unsaturation were determined for the molecule of **2**. The IR spectrum of **2** showed the presence of a hydroxyl group (ν_{max} 3580 cm^{-1}), a carbonyl group of a γ-lactone (ν_{max} 1787 cm^{-1}), and ester carbonyl groups (ν_{max} 1749 cm^{-1}). The FABMS of **2** exhibited peaks at m/z 595 [M^+ + H], 577 [M^+ + H − H_2O], 535 [M^+ + H − AcOH], 517 [M^+ + H − H_2O − AcOH], 507 [M^+ + H − $C_3H_7CO_2H$], 475 [M^+ + H − 2AcOH], and 415 [M^+ + H − 3AcOH], also suggesting the presence of a hydroxyl, a butyryloxyl, and three acetoxyl groups. The ^{13}C NMR of **2** in $CDCl_3$ measured at room temperature gave 11 sharp signals and a set of very broad and weak signals for other carbons. The ^{1}H NMR spectra of **2** in $CDCl_3$ and C_6H_6-d_6 also revealed mostly broad peaks when it was measured at room temperature. These broad signals could not be sharpened considerably at elevated temperature (60 °C). These observations suggested the existence of slowly interconverting conformations of this compound in solution in the above temperature range. It was expected that at lower temperature the rate for the conversion of various conformers could be reduced effectively, making the assignments of NMR signals of the stablized conformers more likely. Thus, to obtain well-resolved spectra, both ^{1}H and ^{13}C NMR were measured at −70 °C in Me_2CO-

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Table 1. ^{1}H-NMR Chemical Shifts of Diterpenes **1-5** and **13**

	compound					
H	**1**[a]	**2**[b]	**3**[b]	**4**[c]	**5**[b]	**13**[d]
2	5.41 d; 6.4	5.13 br s	5.14 br s	3.50 br s	4.85 d; 8.4	5.01 d; 8.1
3	3.82 ddd; 15.6; 13.2; 6.4	5.80 d; 6.9	5.78 d; 5.1	5.76 br d; 5.2	3.00 dt; 15.0; 3.6	2.81 dt; 15.0; 3.6
	1.58 dd; 15.6; 5.2				1.41 m	1.60 m
4α	5.14 dd; 13.2; 5.2	2.05 m	2.07 m	1.95 m	1.68 m	2.0 m
β		3.98 dd; 15.9; 7.8	3.98 dd; 15.4; 6.8	3.99 dd; 15.4; 6.8	2.48 br d; 14.1	2.46 br d; 13.6
6	6.14 d; 2.8	5.36 d; 7.5	5.37 d; 7.5	5.32 d; 7.6	5.10 d; 8.4	5.25 d; 8.4
7	5.16 d; 2.8	5.67 d; 7.5	5.68 d; 7.5	5.66 d; 7.6	5.55 d; 8.4	5.50 d; 8.4
9	5.32 d; 6.8	5.55 d; 10.5	5.56 d; 10.5	5.51 d; 9.6	3.89 d; 11.7	3.80 br s
10	3.12 br s	3.06 dd; 10.5; 4.8	3.07 dd; 10.5; 5.4	3.05 dd; 9.6; 5.2	2.03 dd; 11.7; 5.2	2.28 br s
11		2.46 m	2.46 m	2.49 m	1.84 m	2.11 m
12	5.60 br s	3.88 m	3.89 m	3.91 m	3.89 m	5.07 m
13α	2.47 br d; 17.6	1.86 br d; 13.2	1.85 br d; 13.5	1.90 ddd; 14.1; 12.0; 2.8	1.79 m	1.84 m
β	2.14 br d; 17.6	1.67 br d; 13.2	1.67 br d; 13.5	1.67 ddt; 14.1; 3.8; 1.6	1.56 br d; 13.8	1.55 m
14	5.34 d; 4.0	4.61 br s	4.61 br s	4.67 br s	4.63 br s	4.80 br s
15	1.49 s	0.78 s	0.78 s	1.00 s	1.26 s	1.29 s
16	5.55 d; 2.0	1.92 s	1.85 s	2.00 s	1.91 s	1.98 s
	5.26 d; 2.0					
17	3.19 q; 6.8					
18	1.47 d; 6.8	1.47 s	1.48 s	1.53 s	1.48 s	1.55 s
20	1.98 s	1.00 d; 6.9	1.01 d; 6.9	1.05 d; 7.2	1.11 d; 7.2	1.16 d; 6.6
2-OH				4.38 d; 6.0		
9-OH	8.02 d; 6.8				6.07 br s	
12-OH		4.50 d; 3.3	4.51 d; 3.0	3.76 d; 4.0	4.46 br s	
acetate	2.03 s	2.37 s	2.38 s	2.24 s	1.91 s	2.02 s
methyls	2.10 s	2.22 s	2.23 s	2.14 s	1.90 s	2.04 s
		2.21 s	2.20 s	2.00 s		2.05 s
			1.93 s			
butyrate		2.08 t; 7.2				
		1.58 m				
		0.82 t; 7.2				

[a] Spectra recorded at 400 MHz in pyridine-d_5. [b] 300 MHz in Me_2CO-d_6 at −70 °C. [c] 400 MHz in Me_2CO-d_6 at 25 °C. [d] 300 MHz in $CDCl_3$ at 25 °C. The values are in ppm downfield from TMS.

Table 2. ^{13}C NMR Chemical Shifts of Diterpenes **1**−**5** and **13**

	compound					
position	**1**[a]	**2**[b]	**3**[b]	**4**[c]	**5**[b]	**13**[d]
C-1	44.61 (s)[e]	43.93 (s)	43.97 (s)	45.79 (s)	46.25 (s)	45.99 (s)
C-2	74.27 (d)	81.21 (d)	81.05 (d)	84.00 (d)	75.57 (d)	75.28 (d)
C-3	35.96 (t)	73.46 (d)	73.62 (d)	77.51 (d)	29.27 (t)	28.56 (t)
C-4	77.36 (d)	34.22 (t)	34.02 (t)	35.14 (t)	28.23 (t)	26.05 (t)
C-5	140.30 (s)	139.96 (s)	139.96 (s)	141.87 (s)	144.94 (s)	145.57 (s)
C-6	56.74 (d)	122.50 (d)	122.52 (d)	123.31 (d)	119.12 (d)	117.80 (d)
C-7	81.55 (d)	74.29 (d)	74.29 (d)	75.47 (d)	75.69 (d)	75.75 (d)
C-8	84.46 (s)	69.11 (s)	69.14 (s)	70.79 (s)	72.85 (s)	71.90 (s)
C-9	74.02 (d)	65.24 (d)	65.26 (d)	66.80 (d)	74.95 (d)	75.95 (d)
C-10	41.72 (d)	39.98 (d)	39.99 (d)	42.26 (d)	41.68 (d)	41.43 (d)
C-11	133.39 (s)	35.71 (d)	35.72 (d)	36.95 (d)	46.07 (d)	29.68 (d)
C-12	122.24 (d)	65.81 (d)	65.81 (d)	67.58 (d)	66.57 (d)	70.14 (d)
C-13	28.93 (t)	30.28 (t)	30.22 (t)	31.98 (t)	31.88 (t)	31.78 (t)
C-14	72.69 (d)	81.99 (d)	82.04 (d)	82.65 (d)	76.44 (d)	75.16 (d)
C-15	14.94 (q)	18.25 (q)	18.31 (q)	20.79 (q)	15.27 (q)	14.17 (q)
C-16	114.53 (t)	22.20 (q)	20.85 (q)	23.59 (q)	27.18 (q)	27.07 (q)
C-17	50.26 (d)	60.36 (s)	60.37 (s)	61.17 (s)	64.64 (s)	63.44 (s)
C-18	8.12 (q)	10.09 (q)	10.10 (q)	10.72 (q)	10.47 (q)	10.26 (q)
C-19	176.68 (s)	172.59 (s)	172.61 (s)	173.22 (s)	172.18 (s)	172.41 (s)
C-20	24.57 (q)	9.28 (q)	9.28 (q)	10.07 (q)	9.76 (q)	15.40 (q)
acetate	21.11 (q)	22.78 (q)	22.81 (q)	22.76 (q)	21.70 (q)	21.05 (q)
methyls	20.94 (q)	22.30 (q)	22.42 (q)	22.51 (q)	21.30 (q)	21.26 (q)
		21.48 (q)	22.13 (q)	22.09 (q)		21.50 (q)
			21.45 (q)			
ester	170.79 (s)	172.32 (s)	172.41 (s)	171.56 (s)	170.79 (s)	170.21 (s)
carbonyls	170.34 (s)	170.82 (s)	170.84 (s)	171.03 (s)	170.64 (s)	171.36 (s)
		170.23 (s)	170.26 (s)	170.83 (s)		170.84 (s)
			169.81 (s)			
butyrate		171.99 (s)				
		CH_3 13.70 (q)				
		CH_2 18.31 (t)				
		CH_2 35.75 (t)				

[a] Spectra recorded at 100 MHz in pyridine-d_5. [b] 75 MHz in Me_2CO-d_6 at −70 °C. [c] 100 MHz in Me_2CO-d_6 at 25 °C. [d] 75 MHz in $CDCl_3$ at 25 °C. [e] Multiplicity deduced by DEPT and indicated by usual symbols. The values are in ppm downfield from TMS.

Table 3. Protons to Which Long-Range Correlations Were Observed in the HMBC Experiments, $^1H-^1H$ COSY, and NOESY of Diterpene **2**

C/H no.	HMBC	$^1H-^1H$ COSY	NOESY
1	H-2, H-3, H-10, H-13 α/*β*,H_3−15		
2	H-4*β*, H_3−15	H-3	H-3, H-14, H_3−15
3	H-2, H-4α/*β*, H_2−24	H-2, H-4*β*	H-2, H-4α, H-10,H_3−16
4α	H-2, H-3, H-6, H_3−16	H-4*β*	H-3, H-4*β*
β		H-3, H-4α	H-4α, H-7
5	H-3, H-4α/*β*, H−7, H_3−16		
6	H-4α/*β*, H-7, H_3−16	H-7, H_3−16	H_3−16
7	H-9	H-6	H-4*β*, H-9
8	H-9, H-10, H_3−18		
9	H-6, H-7, H-14, H_3−20	H-10	H-7, H-11, H_3-15, H_{3-18}, H_3−20
10	H-2, H-9, H-14, H_3-15, H_3−20	H-9, H-11	H-3, H-11, H-12
11	H-9, H_3−20	H-10, H_3−20	H-9, H-10, H-12, H_3−20
12	H-10, H-14, H_3−20	H-13α	H-10, H-11, H-13*β*
13α		H-12, H-13*β*	H-13*β*
β		H-13α, H-14	H-12, H-13α, H-14
14	H-2, H_3−15	H-13*β*	H-2, H-13*β*, H_3−15
15	H-10, H-14		H-2, H-9, H-14
16	H-4α/*β*, H-6	H-6	H-3, H-6
17	H-9, H_3−18		
18			H-9
19	H_3−18		
20	H-10, H_3−15	H-11	H-9, H-11
n-PrCO	H-3, H_2−24		

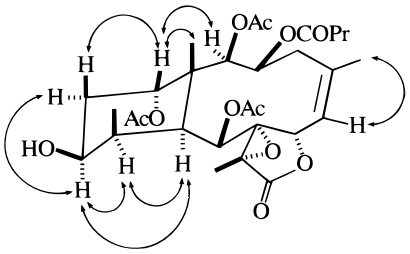


Figure 1. Selective NOE correlation of **2**.

d_6. Fortunately, it was found that at this lower temperature mainly one conformation existed, and the signals for each proton (Table 1) and carbon (Table 2) of the molecule were sharpened and could be assigned unambiguously by the assistance of DEPT and 2D NMR ($^1H-^1H$ and $^{13}C-^1H$ COSY) spectra. From the above data, a trisubstituted olefin could be identified from signals of two carbons that appeared at δ 122.50 (d) and 139.96 (s). The 8,17-epoxide was confirmed from signals of two oxygenated carbons appearing at δ 69.11 (s) and 60.36 (s) and from the chemical shift of H_3-18 (δ 1.47, 3H, s). Carbonyl resonances in the ^{13}C NMR spectrum of **2** at δ 170.23, 170.82, 171.99, 172.32, and 172.59 further confirmed the presence of a γ-lactone and four other esters. In the ^1H NMR spectrum of **1**, three acetate methyl signals were observed at δ 2.21 (3H, s), 2.22 (3H, s), and 2.37 (3H, s). The additional acyl group was found to be an *n*-butanoyl group based on ^1H NMR studies, including a $^1H-^1H$ COSY spectrum, which revealed seven contiguous protons [δ 0.82 (3H, t, J = 7.2 Hz), 1.58 (2H, m), and 2.08 (2H, t, J = 7.2 Hz)]. The carbon signal at δ 171.99 was correlated with the signal of the methylene protons at δ 2.08 in the HMBC spectrum and was consequently assigned as the carbon atom of the butyrate carbonyl. From a $^1H-^1H$ COSY experiment it was possible to determine the separate spin systems that map out the proton sequences H-2 to H-3 to H-4; H-6 to H-7, H-16; and H-9 to H-10. These data, together with the $^1H-^{13}C$ long-range correlations observed in an HMBC experiment (Table 3), suggested the molecular framework of structure **2**. Furthermore, the butyrate ester was positioned at C-3 from the

Table 4. Atomic Coordinates and B_{eq} of Excavatolide B (**2**)

atom	*x*	*y*	*z*	B_{eq}[a]
C(1)	−0.0581(5)	0.9126(6)	0.2953(3)	2.7(1)
C(2)	0.0141(4)	0.7665(6)	0.3037(3)	2.6(1)
C(3)	0.1056(4)	0.7108(6)	0.2357(3)	2.8(1)
C(4)	0.0497(4)	0.6620(6)	0.1465(3)	2.8(1)
C(5)	0.0716(5)	0.7563(6)	0.0686(3)	2.8(1)
C(6)	−0.0212(5)	0.7925(6)	0.0114(3)	2.8(1)
C(7)	−0.1616(5)	0.7617(6)	0.0178(3)	3.0(1)
C(8)	−0.2363(5)	0.8738(6)	0.0616(3)	2.75(10)
C(9)	−0.2318(4)	0.8851(6)	0.1594(3)	2.47(9)
C(10)	−0.1087(4)	0.9583(6)	0.2006(3)	2.34(10)
C(11)	−0.1383(5)	1.1188(6)	0.1922(3)	3.0(1)
C(12)	−0.0180(5)	1.2005(6)	0.2254(3)	2.90(10)
C(13)	0.0095(5)	1.1671(6)	0.3215(3)	3.2(1)
C(14)	0.0492(5)	1.0418(6)	0.3307(3)	2.8(1)
C(15)	−0.1668(5)	0.9127(6)	0.3621(3)	3.1(1)
C(16)	0.2088(5)	0.8080(7)	0.0611(3)	4.1(1)
C(17)	−0.3459(5)	0.9193(6)	0.0015(3)	3.4(1)
C(18)	−0.4697(5)	0.9850(7)	0.0202(3)	4.2(1)
C(19)	−0.3337(5)	0.8303(6)	−0.0793(3)	4.0(1)
C(20)	−0.2646(5)	1.1707(6)	0.2308(3)	4.3(1)
C(21)	−0.0527(5)	0.5927(7)	0.4048(3)	3.7(1)
C(22)	−0.1513(5)	0.4800(7)	0.4161(3)	4.5(2)
C(23)	0.2684(6)	0.6056(7)	0.3288(4)	4.6(1)
C(24)	0.3168(6)	0.4686(7)	0.3679(4)	5.6(2)
C(25)	0.4227(6)	0.4832(7)	0.4371(4)	7.3(2)
C(26)	0.4778(8)	0.3484(7)	0.4692(5)	10.9(3)
C(27)	−0.3628(5)	0.6901(7)	0.1867(4)	4.0(1)
C(28)	−0.3591(6)	0.5448(7)	0.2220(4)	6.8(2)
C(29)	0.2786(5)	1.0315(7)	0.3273(4)	4.0(1)
C(30)	0.3937(5)	1.0147(7)	0.2717(3)	5.0(2)
O(1)	−0.0762(3)	0.6557(5)	0.3243(2)	2.89(8)
O(2)	0.0279(4)	0.6299(5)	0.4573(2)	6.0(1)
O(3)	0.1625(3)	0.5860(4)	0.2767(2)	3.31(9)
O(4)	0.3200(4)	0.7124(5)	0.3435(3)	7.2(1)
O(5)	−0.2230(3)	0.7522(5)	−0.0725(2)	4.23(10)
O(6)	−0.4046(4)	0.8243(5)	−0.1437(2)	6.3(1)
O(7)	−0.2255(3)	1.0000[b]	0.0111(2)	3.15(8)
O(8)	−0.2402(3)	0.7450(4)	0.1924(2)	2.74(8)
O(9)	−0.4541(3)	0.7443(5)	0.1548(3)	6.0(1)
O(10)	−0.0504(3)	1.3448(5)	0.2136(2)	4.34(9)
O(11)	0.1665(3)	0.9959(4)	0.2832(2)	2.79(8)
O(12)	0.2820(4)	1.0671(6)	0.4028(3)	6.3(1)

[a] B_{eq} is the mean of the principal axes of the thermal ellipsoid. [b] The *y* coordinate of O (7) is set to be 1.00 for origin fixation.

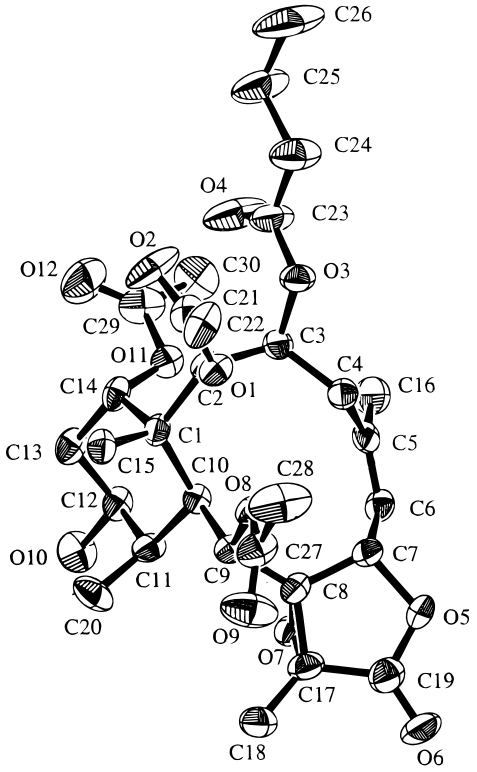


Figure 2. A computer-generated ORTEP plot of **2** showing relative configuration. Hydrogen atoms have been omitted for clarity.

connectivities between H-3 (δ 5.80 ppm) and carbonyl carbon (δ 171.99 ppm) of the butyrate. By comparison of the ^{1}H and ^{13}C NMR spectra data of **2** with those of briareolides E **(8)** and F **(9),**[9] which contain 11β-methyl and 12α-hydroxyl groups, it was found that the chemical shifts for C-11, C-12, C-14, C-20, and H_3-20 of **2** were significantly different from those of **8** and **9**, indicating that the relative stereochemistry of **2** in the six-membered ring is possibly not the same as that of **8** and **9**. The relative stereochemistry of **2** was determined further by a NOESY experiment (Table 3 and Figure 1). H-14 was found to exhibit NOE responses with H-2 and Me-15, but not with H-10, confirming the β-orientation for this proton. Also, the hydroxyl group at C-12 was found to be in the β face and is cis to Me-20. Based on these findings and by comparison of the NMR data with those of 3-acetoxystecholide E **(10)** and 11,12-deoxy-11-H,12-acetoxystecholide E acetate **(11)**,[27] the structure of **2** was established. Briaran diterpenes which possessing a 12β-hydroxyl group and a 11β-methyl group in the same six-membered ring are rarely found.

A single-crystal X-ray structure analysis was carried out to confirm the molecular structure of **2**. The final atomic parameters of the nonhydrogen atoms are listed in Table 4. The X-ray structure (Figure 2) demonstrates the location of the butyrate at C-3 and unambiguously confirms the relative, not the absolute, configuration of **2**.

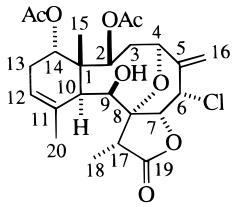
The new briaran diterpene excavatolide C **(3)** had a molecular formula of $C_{28}H_{38}O_{12}$ as determined by HR-FABMS. Thus, 10 degrees of unsaturation were determined for the molecule of **3**. The sharpened NMR (^{1}H and ^{13}C) signals of **3** were also obtained in Me_2CO-d_6 at −70 °C. It was found that the spectral data (IR, ^{1}H NMR and ^{13}C NMR) of **3** were very similar to those of **2**. However, the ^{1}H NMR and ^{13}C NMR spectra revealed that the signals corresponding to the butyryloxy group in **2** disappeared and were replaced by those of an additional acetoxy group. Thus, compound **3** was found to be the 3-(debutyryloxy)-3β-acetoxy derivative of **2** with the structure as described by formula **3**.

The new briaran diterpene excavatolide D **(4)** had the composition $C_{26}H_{36}O_{11}$ as determined by HRFABMS. This showed that compound **4** contained nine degrees of unsaturation. The spectral data (IR, ^{1}H NMR, and ^{13}C NMR) of **4** were very similar to those of **3** and showed the presence of three acetoxyl groups, a trisubstituted double bond, and a carbonyl group of a γ-lactone. Thus, **4** was found to be a tetracyclic compound. Based on the above data, compound **4** is a deacetyl derivative of **3**. By comparison of the ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) spectral data of **4** with those of **3**, it was found that the acetoxyl group attached to C-2 in compound **3** was replaced by a hydroxyl group in the molecule of **4**. This could be further confirmed by the results of 2D NMR (^{1}H−^{1}H and ^{13}C−^{1}H COSY) experiments. Thus, compound **4** was found to be a 2-deacetyl analogue of **3** with the structure as described by formula **4**. It is worthwhile to mention here that, although **4** is analogous to **2** and **3**, its NMR (^{1}H and ^{13}C) spectra in Me_2CO-d_6 exhibited sharpened peaks at 25 °C, indicating that replacement of a small group at C-2 in compounds of this type could increase the rates of interconversion around different conformers and lead to the sharpening of the NMR signals.

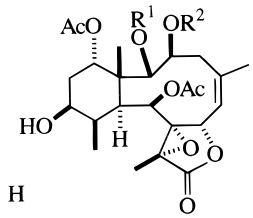
The new briaran diterpene excavatolide E **(5)** had a molecular formula of $C_{24}H_{34}O_9$ as determined by HR-FABMS, indicating that metabolite **5** contained eight degrees of unsaturation. The well-resolved spectra (^{1}H and ^{13}C NMR) of **5** were obtained in Me_2CO-d_6 at −70 °C. On the basis of its spectral data (IR, ^{1}H NMR, and ^{13}C NMR) and by comparison of these data with briareolide H **(12)**[9] and **11**, the structure of **5** was established as 11α-H-12β-hydroxy-briareolide H, described by formula **5**. Furthermore, acetylation of **5** yielded a compound that was found to be 12-acetylexcavatolide E **(13)** by comparing its ^{1}H and ^{13}C NMR (Tables 1 and 2) spectral data with those of **11**. Thus, the molecule structure, including the relative configuration of **5** (and also **2**−**4**), was further confirmed.

Compound **6** had a molecular composition of $C_{24}H_{31}O_{10}$-Cl as determined by spectral data (^{1}H NMR, ^{13}C NMR, and FABMS). The physical data (melting point, optical rotation) and the spectral data (IR, ^{1}H NMR, ^{13}C NMR, and MS) were found to be in full agreement with those reported for a known diterpene, brianolide.[28] This chlorodiepoxyl diterpene has been reported to exhibit modest antiinflammatory activity in a mouse-ear assay.[28]

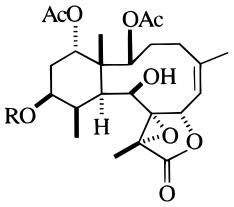
The absolute configuration of brianolide **(6)** has been determined previously, as shown by structure **6**.[28] Based on biosynthetic derivation, the new briaran excavatolides A−E are assumed to have the same absolute configurations as **6,** because these compounds were isolated from the same organism. Therefore, excavatolides A−E should possess the absolute configurations as represented by structures **1**−**5**.



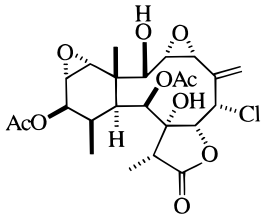
1



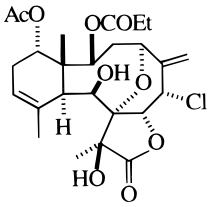
2 : R^1 = Ac, R^2 = n-PrCO
3 : R^1, R^2 = Ac
4 : R^1 = H, R^2 = Ac



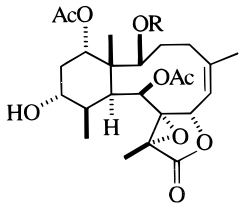
5 : R = H
13 : R = Ac



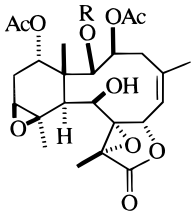
6



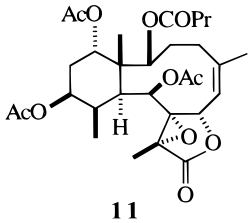
7



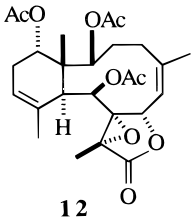
8 : R = n-PrCO
9 : R = Ac



10 : R = n-PrCO



11



12

The cytotoxicities of the new briareins **1**−**5** against the growth of P-388, KB, A-549, and HT-29 cancer cells were studied, and the results are shown in Table 5. These data show that the diterpenes **3**−**5** exhibited significant cytotoxicity toward P-388 cancer cells. Both compounds **3** and **5** were found to exhibit significant activity against the growth of the above four cancer cells. Compound **4** exhibited selective cytotoxicity toward P-388 and HT-29 cells. Metabolite **1** also showed selective cytotoxicity against the growth of KB cells. Compound **2** was found to be inactive against the above four cancer cell lines, indicating that the bulky butyrate at C-3 could significantly reduce cytotoxicity. Although compound **6** has been reported to possess antiinflammatory activity,[28] it was found to be inactive against the growth of the above four tested cancer cells. Compound **13**, a 12-acetyl derivative of **5**, was found to exhibit much weakened cytotoxicity against the test cancer cells in comparison with **5**. All of these results suggest that small structural variations could influence the biological activities of the compounds of this type (see Table 5) and may warrant further studies in the future.

Table 5. Cytotoxicity of Diterpenes **1**−**6** and **13**[a]

	cell lines ED_{50} (μg/mL)			
compound	P-388	KB	A-549	HT-29
1	>50	2.5	21.9	>50
2	>50	>50	>50	>50
3	0.3	1.9	1.9	1.9
4	1.8	4.2	>50	1.3
5	1.6	0.8	1.2	1.6
6	>50	>50	>50	>50
13	5.8	>50	37.2	4.4

[a] For significant activity of pure compounds, an ED_{50} value of ≤ 4.0 μg/mL is required. See Geran et al.[31]

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 1000 digital polarimeter. IR spectra were recorded on a Hitachi I-2001 infrared spectrophotometer. The NMR spectra were recorded on VXR-300/5 FT-NMR at 300 MHz for ^{1}H and 75 MHz for ^{13}C or on a Varian Unity Plus 400 MHz FT-NMR for ^{1}H and 100 MHz for ^{13}C, respectively, in Me_2CO-d_6, using TMS as internal standard unless otherwise indicated. FABMS were obtained with a VG QUATTRO GC/MS spectrometer. HRFABMS were recorded on JMX-HX 110 mass spectrometer. 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.

Marine Organism. The organism *Briareum excavatum* was collected by hand using scuba at the South Bay, Kenting, located in the southernmost tip of Taiwan, in June 1993, at a depth of 6 m and was stored in a freezer until extraction. The *Briareum excavatum* is a species with encrusting, purplish, sheet-forming colonies that overgrow and cover substrata, most of which were dead corals. A voucher specimen was deposited in the Department of Marine Resources, National Sun Yat-Sen University (specimen no. KTSC-102).

Extraction and Separation. The marine organism (4.45 kg fresh wt) was collected and freeze-dried. The freeze-dried material (2.25 kg) was minced and extracted exhaustively with EtOAc (12 L × 5). The organic extract was evaporated to give a dark green residue (58.4 g). The EtOAc solution of the residue was stored at 0 °C to give a solid (5.1 g), which was found to be a mixture of long-chained esters arising from saturated fatty acids and alcohols, and was discarded. The remaining mixture was separated by Si gel column chromatography, using hexane and hexane−EtOAc mixtures of increasing polarity. Diterpene **1** was eluted with hexane−EtOAc (4:1), **2** with hexane−EtOAc (3:1),

3 with hexane−EtOAc (3:1−2:1), and **4** with hexane−EtOAc (2:1−3:2), and **5** with hexane−EtOAc (3:2), and **6** with hexane−EtOAc (3:2−1:1).

Excavatolide A (1): white powder (28 mg); mp >290 °C ; $[\alpha]^{28}_D$ +38° (*c* 0.05, pyridine); IR (KBr) ν_{max} 3516, 1768, 1726, 1374, 1254, 1088, 1064 cm^{-1}; ^1H and ^{13}C NMR data in Tables 1 and 2; FABMS *m/z* (rel int) 483 [6.9, $(M + H)^+$], 425 (2.3), 423 (5.9), 365 (0.9), 363 (2.4); HRFABMS *m/z* 483.1770 (calcd for $C_{24}H_{32}O_8Cl$, 483.1777.)

Excavatolide B (2): white solid (1.082 g); mp 224−225 °C; $[\alpha]^{26}_D$ −23° (*c* 0.09, $CHCl_3$); IR (KBr) ν_{max} 3580, 1787, 1749, 1638, 1378, 1254, 1030 cm^{-1}; ^1H and ^{13}C NMR data in Tables 1 and 2; FABMS *m/z* (rel int) 595 [1.4, $(M + H)^+$], 577 (0.2), 535 (1.7), 517 (1.1), 507 (0.4), 475 (0.5), 447 (0.3), 415 (0.4); HRFABMS *m/z* 595.2751 (calcd for $C_{30}H_{43}O_{12}$, 595.2742.)

Excavatolide C (3): white solid (260.5 mg); mp 134−135 °C; $[\alpha]^{30}_D$ −13° (*c* 0.18, $CHCl_3$); IR (KBr) ν_{max} 3592, 1788, 1746, 1606, 1378, 1274, 1216, 1160 cm^{-1}; ^1H and ^{13}C NMR data in Tables 1 and 2; FABMS *m/z* (rel int) 567 [2.7, $(M + H)^+$], 549 (0.3), 507 (3.3), 489 (1.8), 447 (1.3), 429 (0.8), 387 (1.6), 369 (1.0); HRFABMS *m/z* 567.2402 (calcd for $C_{28}H_{39}O_{12}$, 567.2382.)

Excavatolide D (4): white solid (163.7 mg); mp 235−237 °C; $[\alpha]^{24}_D$ +32° (*c* 0.38, $CHCl_3$); IR (KBr) ν_{max} 3472, 1770, 1732, 1374, 1262, 1228, 1082, 1038 cm^{-1}; ^1H and ^{13}C NMR data in Tables 1 and 2; FABMS *m/z* (rel int) 525 [0.5, $(M + H)^+$], 465 (0.9), 405 (0.3); HRFABMS *m/z* 525.2338 (calcd for $C_{26}H_{37}O_{11}$, 525.2335.)

Excavatolide E (5): white solid (45.2 mg); mp 190−191 °C; $[\alpha]^{30}_D$ +53° (*c* 0.14, $CHCl_3$); IR (KBr) ν_{max} 3480, 1782, 1746, 1446, 1376, 1250, 1030 cm^{-1}; ^1H and ^{13}C NMR data in Tables 1and 2; FABMS *m/z* (rel int) 467 [0.8, $(M + H)^+$], 407 (1.3), 347 (0.6), 329 (1.1); HRFABMS *m/z* 467.2271 (calcd for $C_{24}H_{35}O_9$, 467.2261.)

Brianolide (6): white solid (85.0 mg); mp 224−226 °C; $[\alpha]^{24}_D$ −15° (*c* 0.42, MeOH); IR (KBr) ν_{max} 3508, 1780, 1738, 1712, 1232, 1038 cm^{-1}; ^1H NMR (pyridine-d_5, 300 MHz) δ 5.99 (1H, d, 1.8), 5.82 (1H, d, 3.6), 5.78 (1H, d, 8.4), 5.70 (1H, d, 3.6), 5.55 (1H, d, 1.8), 5.12 (1H, d, 4.5), 4.95 (1H, br s), 4.29 (1H, d, 9.0), 4.26 (1H, d, 3.9), 3.96 (1H, dd, 9.0, 3.9), 3.75 (1H, d, 3.6), 3.45 (1H, d, 3.6), 3.18 (1H, q, 7.2), 3.05 (1H, m), 2.40 (1H, dd, 8.4, 2.1), 2.31 (3H, s), 2.02 (3H, s), 1.52 (3H, s), 1.40 (3H, d, 7.2), 1.31 (3H, d, 7.2); ^{13}C NMR (pyridine-d_5, 75 MHz) δ 175.81 (s), 171.58 (s), 170.45 (s), 138.58 (s), 117.53 (t), 84.45 (s), 79.80 (d), 73.91 (d), 73.26 (d), 70.59 (d), 64.30 (d), 64.12 (d), 61.74 (d), 58.73 (d), 58.20 (d),45.82 (d), 39.97 (s), 37.92 (d), 37.38 (d), 22.33 (q), 21.31 (q), 16.96 (q), 10.68 (q), 7.13 (q); FABMS *m/z* (rel int) 517 [$(M + H + 2)^+$, 0.8], 515 [$(M + H)^+$, 2.2], 497 (0.2), 455 (0.2), 395 (0.3); physical and spectral data in full agreement with those reported previously.[28]

12-Acetylexcavatolide E (13). Excavatolide E **(5)** (12.1 mg) was stirred with 3 mL of Ac_2O in 3 mL of pyridine for 48 h at room temperature. After evaporation of excess reagent, the residue was separated by column chromatography on Si gel (hexane−EtOAc, 3:1) to give pure 12-acetylexcavatolide E **(13)** (8.3 mg, 75%) as white solid: mp 268−270 °C; $[\alpha]^{26}_D$ +28° (*c* 0.18, $CHCl_3$); IR (KBr) ν_{max} 3428, 1758, 1730, 1630, 1384, 1262, 1030 cm^{-1}; ^1H and ^{13}C NMR data in Tables 1 and 2; FABMS *m/z* (rel int) 509 [$(M + H)^+$, 1.4], 491 (0.6), 449 (3.7). 389 (1.7), 371 (0.7).

Single-Crystal X-ray Crystallography of 2.[32] Suitable colorless prisms of **2** were obtained from a solution in Me_2CO. The crystal (0.33 × 0.33 × 0.41 mm) belongs to the monoclinic system, space group $P2_1$ with a = 10.294 (3), b = 9.712 (3), c = 15.254 (3) Å, β = 92.47 (2)°, V = 1523.7 (7) $Å^3$, Z = 2, D_{clacd} = 1.296 g/cm^3, λ (Mo Kα) = 0.71069 Å. Intensity data were measured on a Rigaku AFC6S diffractometer up to 2θ of 47.1°. Of the 2574 reflections that were collected, 2426 were unique (R_{int} = 0.035). The structure was solved by direct 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 = 0.049, R_w = 0.034 for 1604 observed reflections [I > 2.0σ(I)] and 378 variable parameters.

Cytotoxicity Testing: KB and P-388 cells were kindly provided by Prof. J. M. Pezzuto, University of Illinois at Chicago; A-549 (human lung adenocarcinoma) and HT-29 (human colon adenocarcinoma) were purchased from the American Type Culture Collection. The cytotoxic activities of tested compounds **1**−**6** and **13** were assayed by a modification of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[33] The cultured cells were treated at eight concentrations of pure test compounds ranging from 0.00064 to 50 μg/mL. All assays were performed in triplicate. The results were expressed as a percentage, relative to control incubations, and the effective dose required to inhibit cell growth by 50% (ED_{50}) was determined.

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