Excavatolides U−Z, New Briarane Diterpenes from the Gorgonian *Briareum excavatum*

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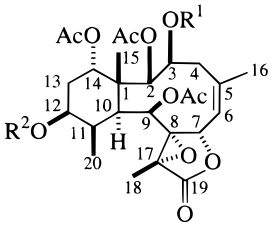
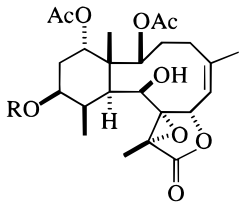
A recent study of the EtOAc extract of the gorgonian *Briareum excavatum* afforded six new diterpenes of the briarane skeleton, excavatolides U−Z (**1**−**6**). The structures and relative stereochemistry of metabolites **1**−**6** were assigned on the basis of NMR studies and chemical methods. The structure, including the relative configuration of excavatolide U (**1**), was further confirmed by a single-crystal X-ray analysis. Some of the excavatolides have displayed significant cytotoxicity toward P-388 and HT-29 tumor cells.

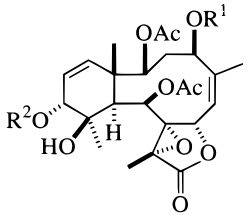
In the past decade, a large number of highly oxygenated diterpenoids, such as briaranes and asbestinins, have been discovered from the gorgonian corals of the genus *Briareum* (family Briareidae, order Gorgonacea, phylum Cnidaria),[1] which is placed taxonomically within the order Alcyonacea or Gorgonacea,[2−4] and has been reported under the synonym Solenopodium.[5] In the course of our search for new bioactive substances for potential biomedical uses from the Formosan marine invertebrates, the briarane-type diterpenes continue to be the target of our investigations because of the interesting biological activities (e.g., toxic,[6−8] cytotoxic,[4,9−15] antiinflammatory,[16−18] antiviral,[9,16] insecticidal,[19,20] antifouling,[21] immunodulatory,[22] and antibacterial[23]) associated with the compounds of this type. Previous investigations on the chemical constituents on *B. excavatum* (Nutting) have resulted in the isolation of 20 diterpenes, excavatolides A−T, possessing the briarane carbon skeleton.[14,15,24] Our continuing investigation of the secondary metabolites of *B. excavatum* has led to the isolation and structure elucidation of six new briarane diterpenes, excavatolides U−Z (**1**−**6**). The structures and relative stereochemistry of compounds **1**−**6** were established by the use of a series of 2D NMR experiments (^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY) and chemical transformations. The structure and relative configuration of excavatolide U (**1**) were further confirmed by a single-crystal X-ray diffraction analysis.



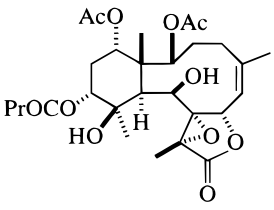
1 : R^1 = *n*-PrCO, R^2 = EtCO
2 : R^1 = Ac, R^2 = EtCO
7 : R^1 = *n*-PrCO, R^2 = H
8 : R^1 = Ac, R^2 = H



3 : R = EtCO
9 : R = H



4 : R^1 = Ac, R^2 = *n*-PrCO
5 : R^1 = EtCO, R^2 = Ac
10 : R^1 = R^2 = Ac



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Results and Discussion

Specimens were frozen immediately after collection and subsequently freeze-dried. The minced organisms were extracted successively with EtOAc, and the organic extract was fractionated extensively using a normal-phase absorbent (Si gel) to yield the six new diterpenes, excavatolides U−Z (**1**−**6**), see Experimental Section.

Excavatolide U (**1**) was crystallized as colorless prisms during slow evaporation of a Si gel chromatography fraction (hexanes−EtOAc 6:1). HRFABMS established a molecular formula, $C_{33}H_{46}O_{13}$, for this compound. The IR spectrum of **1** showed absorptions of a carbonyl group of a γ-lactone (ν_{max} 1798 cm^{-1}) and ester carbonyls (ν_{max} 1746 cm^{-1}). The FABMS of **1** exhibited peaks at *m*/*z* 651 $[M + H]^+$, 591 $[M + H - HOAc]^+$, 563 $[M + H - C_3H_7CO_2H]^+$, 517 $[M + H - C_2H_5CO_2H - HOAc]^+$, 503 $[M + H - C_3H_7CO_2H - HOAc]^+$, 489 $[M + H - C_3H_7CO_2H - C_2H_5CO_2H]^+$, 443 $[M + H - C_3H_7CO_2H - 2HOAc]^+$, 429 $[M + H - C_3H_7CO_2H - C_2H_5CO_2H - HOAc]^+$, 369 $[M + H - C_3H_7CO_2H - C_2H_5CO_2H - 2HOAc]^+$, and 309 $[M + H - C_3H_7CO_2H - C_2H_5CO_2H - 3HOAc]^+$, also suggesting the presence of an *n*-butyryloxyl, a propionyloxyl, and three acetoxyl groups in the molecule of **1**. It was found that the ^{1}H and ^{13}C NMR spectra of **1** in $CDCl_3$ revealed mostly broad peaks when measured at room temperature. However, the signals for each proton and carbon of the molecule were sharpened and could be assigned unambiguously by the assistance of 2D NMR (^{1}H−^{1}H COSY and HMQC) spectral analyses in cases that the NMR spectra were measured at −70 °C in Me_2CO-d_6. From the NMR (^{1}H and ^{13}C) spectral data (Tables 1 and 2), a trisubstituted olefin was deduced from the signals of two carbons at δ 122.5 (d) and 140.0 (s). An 8,17-epoxide group

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Table 1. ^{1}H NMR Chemical Shifts of Diterpenes **1**−**6**

	compound					
H	**1**[a]	**2**[a]	**3**[b]	**4**[c]	**5**[c]	**6**[c]
2	5.20 br s	5.11 br s	4.99 d (8.1)	4.51 d (6.0)	4.51 d (6.0)	5.12 d (8.0)
3/3′	5.89 d (5.7)[d]	5.77 br d (6.0)	1.58 m	2.05 m	2.06 m	1.59 m
			2.83 br t (15.0)	2.83 br t (16.0)	2.89 br t (16.0)	2.86 dt (15.6; 5.2)
4/4′	2.13 m	2.07 m	1.86 m	5.13 dd (12.8; 5.6)	5.18 dd (12.8; 5.6)	1.89 m
	4.05 dd (15.6; 6.9)	3.95 dd (15.3; 6.9)	2.43 br d (14.1)			2.47 br d (15.2)
6	5.43 d (7.5)	5.34 d (7.2)	5.23 d (7.8)	5.45 d (10.4)	5.51 d (10.4)	5.27 d (9.6)
7	5.73 d (7.5)	5.64 d (7.2)	5.50 d (7.8)	5.81 d (10.4)	5.88 d (10.4)	5.52 d (9.6)
9	5.59 d (10.5)	5.53 d (10.2)	3.78 br s	5.73 d (4.4)	5.80 d (4.4)	4.60 br s
10	3.21 dd (10.5; 5.1)	3.12 dd (10.2; 4.5)	2.27 m	2.55 d (4.4)	2.62 d (4.4)	2.17 m
11	2.65 m	2.57 m	2.12 m			
12	5.00 m	4.91 m	5.07 m	4.72 d (6.0)	4.78 d (5.6)	4.87 br s
13/13′	1.87 br d (13.5)	1.85 br d (13.5)	1.85 m	5.78 dd (10.4; 6.0)	5.84 dd (10.4; 5.6)	2.01 m
	2.06 m	2.05 m				2.27 m
14	4.71 br s	4.61 br s	4.78 br s	5.61 d (10.4)	5.67 d (10.4)	4.68 br s
15	0.89 s	0.79 s	1.28 s	1.21 s	1.28 s	1.40 s
16	2.00 s	1.82 s	1.97 s	2.11 s	2.17 s	2.01 s
18	1.54 s	1.45 s	1.53 s	1.56 s	1.62 s	1.66 s
20	1.12 d (7.2)	1.03 d (6.3)	1.15 d (6.9)	1.28 s	1.34 s	1.18 s
acetate methyls	2.29 s	1.91 s	1.98 s	2.00 s	2.05 s	1.97 s
	2.30 s	2.18 s	2.03 s	2.05 s	2.10 s	1.99 s
	2.44 s	2.20 s		2.19 s	2.25 s	
		2.36 s				
propionate	1.02 t (7.5)	0.93 t (7.5)	1.11 t (7.8)		1.15 t (7.6)	
	2.38 q (7.5)	2.28 q (7.5)	2.29 q (7.8)		2.35 q (7.6)	
n-butyrate	0.89 t (7.5)			0.90 t (7.6)		0.93 t (7.2)
	1.53 m			1.60 m		1.62 m
	2.31 t (7.5)			2.25 t (7.6)		2.23 t (7.2)

[a] Spectra recorded at 300 MHz in Me_2CO-d_6 at −70 °C. [b] 300 MHz in $CDCl_3$ at 25 °C. [c] 400 MHz in $CDCl_3$ at 25 °C. [d] *J* values (in Hz) in parentheses. The values are ppm downfield from TMS.

Table 2. ^{13}C NMR Chemical Shifts of Diterpenes **1**−**6**

	compound					
position	**1**[a]	**2**[a]	**3**[b]	**4**[c]	**5**[c]	**6**[c]
C-1	44.0 (s)[d]	44.0 (s)	46.0 (s)	45.4 (s)	45.4 (s)	46.2 (s)
C-2	81.1 (d)	80.9 (d)	75.4 (d)	76.7 (d)	76.8 (d)	75.8 (d)
C-3	73.4 (d)	73.6 (d)	31.7 (d)	39.1 (t)	39.1 (t)	31.9 (t)
C-4	34.1 (t)	33.9 (t)	28.5 (t)	72.1 (d)	72.2 (d)	28.4 (t)
C-5	140.0 (s)	139.9 (s)	145.6 (s)	145.4 (s)	145.5 (s)	145.9 (s)
C-6	122.5 (d)	122.5 (d)	117.7 (d)	122.4 (d)	122.4 (d)	117.6 (d)
C-7	74.2 (d)	74.2 (d)	76.0 (d)	73.5 (d)	73.5 (d)	75.4 (d)
C-8	69.0 (s)	69.1 (s)	72.0 (s)	70.1 (s)	70.1 (s)	71.2 (s)
C-9	64.9 (d)	64.8 (d)	74.9 (d)	66.8 (d)	66.9 (d)	69.4 (d)
C-10	40.1 (d)	40.1 (d)	41.4 (d)	44.1 (d)	44.1 (d)	45.3 (d)
C-11	32.9 (d)	32.9 (d)	27.2 (d)	73.4 (s)	73.4 (s)	76.8 (s)
C-12	69.9 (d)	69.9 (d)	70.0 (d)	73.3 (d)	73.3 (d)	75.1 (d)
C-13	27.0 (t)	27.0 (t)	26.0 (t)	120.6 (d)	120.6 (d)	24.5 (t)
C-14	81.5 (d)	81.5 (d)	75.8 (d)	141.7 (d)	141.8 (d)	74.9 (d)
C-15	18.0 (q)	18.1 (q)	15.4 (q)	18.3 (q)	18.3 (q)	15.6 (q)
C-16	22.2 (q)	20.8 (q)	27.1 (q)	25.6 (q)	25.6 (q)	26.9 (q)
C-17	60.5 (s)	60.5 (s)	63.6 (s)	62.3 (s)	62.3 (s)	63.6 (s)
C-18	10.1 (q)	10.1 (q)	10.2 (q)	9.6 (q)	9.7 (q)	9.7 (q)
C-19	172.3 (s)	172.4 (s)	172.9 (s)	171.2 (s)	171.1 (s)	171.9 (s)
C-20	10.3 (q)	10.3 (q)	10.3 (q)	27.8 (q)	27.8 (q)	29.4 (q)
acetate methyls	21.5 (q)	21.4 (q)	21.3 (q)	20.9 (q)	21.0 (q)	21.3 (q)
	22.3 (q)	22.2 (q)	21.5 (q)	21.0 (q)	21.0 (q)	21.6 (q)
	22.7 (q)	22.3 (q)		21.7 (q)	21.7 (q)	
		22.8 (q)				
acetate carbonyls	170.2 (s)	169.7 (s)	170.3 (s)	168.9 (s)	168.9 (s)	170.4 (s)
	170.7 (s)	170.1 (s)	170.9 (s)	169.8 (s)	169.7 (s)	170.8 (s)
	172.0 (s)	170.7 (s)		170.1 (s)	170.1 (s)	
		172.2 (s)				
propionate	173.6 (s)	173.6 (s)	173.6 (s)		173.6 (s)	
	CH_3 9.0 (q)	CH_3 9.0 (q)	CH_3 9.1 (q)		CH_3 9.0 (q)	
	CH_2 27.3 (t)	CH_2 27.3 (t)	CH_2 27.7 (t)		CH_2 27.3 (t)	
n-butyrate	172.4 (s)			172.7 (s)		173.0 (s)
	CH_3 13.7 (q)			CH_3 13.6 (q)		CH_3 14.1 (q)
	CH_2 18.3 (t)			CH_2 18.3 (t)		CH2 19.2 (t)
	CH_2 35.7 (t)			CH_2 36.1 (t)		CH_2 36.4 (t)

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

was confirmed from the signals of two quaternary oxygenated carbons at δ 60.5 (s) and 69.0 (s), and from the chemical shift of the teritary methyl H_3-18 (δ 1.54, 3H, s). In the ^{13}C NMR spectrum of **1**, six carbonyl resonances appeared at δ 170.2 (s), 170.7 (s), 172.0 (s), 172.3 (s), 172.4 (s), and 173.6 (s) and confirmed the presence of a γ-lactone and five other ester groups. In the ^1H NMR spectrum of **1**, three acetate methyls (δ 2.29, 3H, s; 2.30, 3H, s; 2.44, 3H, s), a propionyloxyl [δ 1.02 (3H, t, J = 7.5 Hz) and 2.38 (2H, q, J = 7.5 Hz)], and an *n*-butyryloxyl [δ 0.89 (3H, t, J = 7.5 Hz), 1.53 (2H, m), and 2.31 (2H, t, J = 7.5 Hz)] groups were further observed. It was found that the spectral data (^1H and ^{13}C NMR) of **1** were very similar to those of excavatolide B (**7**).[14] However, the chemical shifts for H-12 and C-12 of **1** (δ_H 5.00, m; δ_C 69.9, d) were found to be shifted downfield, in comparison with the analogous data of **7** (δ_H 3.88, m; δ_C 65.8, d), suggesting that the 12β-hydroxyl group of **7** was acylated in diterpene **1**. The main problem was to locate the *n*-butyrate and propionate groups at two of the positions of C-2, C-3, C-9, C-12, or C-14 and the three acetates at the remaining three positions. It was found that propionylation of excavatolide B (**7**) gave a less polar product, which was identical with diterpene **1** by comparison of the physical (mp and optical rotation) and spectral (IR, MS, ^1H and ^{13}C NMR) data. Thus, the *n*-butyrate and propionate should be located at C-3 and C-12, respectively. On the basis of the above observations, excavatolide U (**1**) was assumed to be the 12-propionyl derivative of excavatolide B (**7**), with the structure as described by formula **1**.

A single-crystal X-ray structure analysis was carried out in order to confirm the molecular structure of **1**. The final atomic parameters of the non-hydrogen atoms are listed in Table 3. The X-ray structure (Figure 1) demonstrates the location of *n*-butyrate and propionate at C-3 and C-12 positions, respectively, and unambiguously confirms the relative, not the absolute, configuration of **1**.

The new briarane diterpene, excavatolide V (**2**), had a molecular formula of $C_{31}H_{42}O_{13}$, as established by HR-FABMS. Absorptions in the IR spectrum indicated the presence of a carbonyl group of a γ-lactone (ν_{max} 1784 cm^{-1}) and ester carbonyl carbons (ν_{max} 1736 cm^{-1}) in the structure of **2**. The FABMS of **2** exhibited peaks at *m/z* 623 $[M + H]^+$, 563 $[M + H - HOAc]^+$, 549 $[M + H - C_2H_5CO_2H]^+$, 503 $[M + H - 2HOAc]^+$, 489 $[M + H - HOAc - C_2H_5CO_2H]^+$, 443 $[M + H - 3HOAc]^+$, 429 $[M + H - 2HOAc - C_2H_5CO_2H]^+$, 369 $[M + H - 3HOAc - C_2H_5CO_2H]^+$, and 309 $[M + H - 4HOAc - C_2H_5CO_2H]^+$, suggesting the presence of a propionyloxyl and four acetoxyl groups in **2**. The well-resolved ^1H and ^{13}C NMR spectra of **2** were also recorded at −70 °C in Me_2CO-d_6. The NMR data (^1H and ^{13}C) of **2** were very similar to those of diterpene **1**. However, the signals for the *n*-butyryloxyl group of **1** were found to be replaced by an acetoxyl group. Furthermore, propionylation of a known diterpene, excavatolide C (**8**),[14] gave a less polar product identical with **2** by comparison of the physical (mp and optical rotation) and spectral (MS, IR, ^1H and ^{13}C NMR) data. Thus, excavatolide V (**2**) was found to be the 12-propionyl derivative of excavatolide C (**8**), with the structure as described by formula **2**.

Excavatolide W (**3**) was isolated as an amorphous solid and had the molecular formula $C_{27}H_{38}O_{10}$, as determined by HRFABMS. Its IR spectrum exhibited a broad OH stretch at 3448 cm^{-1}, a γ-lactone carbonyl at 1770 cm^{-1}, and ester carbonyls at 1732 cm^{-1}. In the FABMS spectrum of **3**, the peaks at *m/z* 523 $[M + H]^+$, 505 $[M + H - H_2O]^+$, 463 $[M + H - HOAc]^+$, 449 $[M + H - C_2H_5CO_2H]^+$, 403 $[M + H - 2HOAc]^+$, and 329 $[M + H - 2HOAc - C_2H_5CO_2H]^+$ were observed and suggested the presence of a

Table 3. Atomic Coordinates and B_{eq} of Excavatolide U (**1**)

atom	*x*	*y*	*z*	B_{eq}[a]
C (1)	0.8178 (7)	0.5290 (5)	0.8408 (4)	3.4 (2)
C (2)	0.9402 (7)	0.4805 (6)	0.8604 (4)	3.7 (2)
C (3)	0.9401 (7)	0.3838 (6)	0.8865 (4)	3.8 (3)
C (4)	0.9096 (7)	0.3688 (5)	0.9612 (4)	4.2 (2)
C (5)	0.7991 (9)	0.3138 (6)	0.9717 (4)	4.6 (3)
C (6)	0.7076 (8)	0.3374 (6)	1.0080 (4)	4.4 (3)
C (7)	0.6951 (8)	0.4244 (6)	1.0452 (4)	3.9 (2)
C (8)	0.6309 (8)	0.4983 (6)	1.0092 (4)	3.4 (2)
C (9)	0.6961 (8)	0.5542 (5)	0.9586 (4)	3.5 (2)
C (10)	0.7029 (7)	0.5129 (5)	0.8875 (4)	3.4 (2)
C (11)	0.5782 (7)	0.5385 (6)	0.8552 (4)	4.4 (3)
C (12)	0.5737 (8)	0.4964 (6)	0.7843 (4)	4.8 (3)
C (13)	0.6715 (8)	0.5277 (6)	0.7411 (4)	5.2 (3)
C (14)	0.7895 (8)	0.4953 (7)	0.7691 (4)	4.7 (3)
C (15)	0.8501 (8)	0.6288 (6)	0.8345 (4)	5.2 (3)
C (16)	0.8042 (9)	0.2213 (5)	0.9398 (4)	7.1 (3)
C (17)	0.5383 (8)	0.5312 (6)	1.0528 (4)	4.3 (3)
C (18)	0.4825 (8)	0.6219 (7)	1.0575 (5)	6.8 (3)
C (19)	0.5363 (8)	0.4722 (7)	1.1125 (5)	4.5 (3)
C (20)	0.5464 (8)	0.6379 (7)	0.8513 (4)	7.2 (3)
C (21)	1.1273 (10)	0.5508 (7)	0.8814 (6)	5.3 (3)
C (22)	1.2025 (9)	0.5871 (7)	0.9368 (5)	8.4 (4)
C (23)	1.0989 (9)	0.3230 (7)	0.8195 (5)	4.8 (3)
C (24)	1.228 (1)	0.2856 (7)	0.8218 (5)	7.0 (4)
C (25)	1.3077 (9)	0.3320 (7)	0.8659 (5)	7.1 (3)
C (26)	1.4358 (8)	0.2801 (7)	0.8665 (5)	9.8 (4)
C (27)	0.8344 (9)	0.6533 (6)	1.0145 (5)	4.9 (3)
C (28)	0.9506 (9)	0.6545 (6)	1.0522 (5)	7.5 (3)
C (29)	0.412 (1)	0.453 (1)	0.7142 (6)	8.3 (5)
C (30)	0.300 (1)	0.494 (1)	0.6825 (5)	14.6 (6)
C (31)	0.311 (1)	0.514 (1)	0.6185 (7)	15.7 (6)
C (32)	0.792 (1)	0.3574 (8)	0.7115 (5)	6.0 (3)
C (33)	0.7751 (9)	0.2607 (7)	0.7234 (5)	8.8 (4)
O (1)	1.0133 (5)	0.5321 (4)	0.9060 (3)	4.2 (2)
O (2)	1.1579 (7)	0.5417 (5)	0.8261 (3)	7.3 (2)
O (3)	1.0661 (5)	0.3550 (4)	0.8805 (3)	4.9 (2)
O (4)	1.0361 (6)	0.3180 (6)	0.7721 (3)	9.6 (3)
O (5)	0.6220 (5)	0.4085 (4)	1.1029 (3)	4.8 (2)
O (6)	0.4733 (5)	0.4722 (5)	1.1609 (3)	6.9 (2)
O (7)	0.5087 (5)	0.4775 (4)	0.9945 (3)	4.9 (2)
O (8)	0.8152 (5)	0.5714 (3)	0.9874 (2)	3.8 (2)
O (9)	0.7682 (6)	0.7136 (4)	1.0074 (4)	7.5 (2)
O (10)	0.4580 (5)	0.5167 (5)	0.7554 (3)	6.7 (2)
O (11)	0.4606 (8)	0.3858 (7)	0.7018 (4)	10.3 (4)
O (12)	0.7837 (5)	0.3982 (4)	0.7714 (3)	4.7 (2)
O (13)	0.8025 (8)	0.3939 (5)	0.6596 (3)	8.1 (2)

[a] B_{eq} is the mean of the principal axes of the thermal ellipsoid.

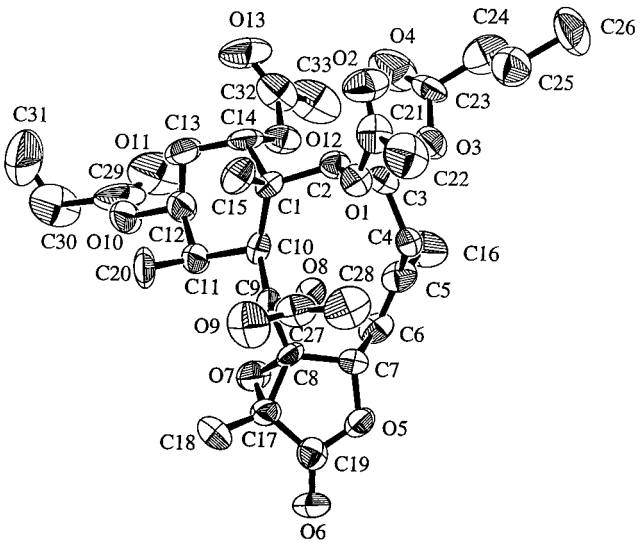

Figure 1. A computer-generated ORTEP plot of **1** showing relative configuration. Hydrogen atoms have been omitted for clarity.

Table 4. Protons to Which Long-Range Correlations Were Observed in the HMBC Experiments of Diterpenes **4**−**6**

carbon	**4**	**5**	**6**
1	H-10, H-14, H3−15	H-10, H-14, H_3-15	H-2, H-9, H-10, H-14, H_3-15
2	H-10, H_3-15	H-10, H_3-15	H_2-4, H-10, H-14, H_3-15
3			H_2-4
4	H-6, H_3-16	H-2, H-3, H-6, H_3-16	H-2, H-6, H_3-16
5	H_3-16	H-7, H_3-16	H_2-4, H-7, H_3-16
6	H_3-16	H_3-16	H_2-4, H-7, H_3-16
7	H-6, H-9	H-6, H-9	H-6, H-9
8	H-9, H_3-18	H-9, H-10, H_3-18	H-9, H-10, H_3-18
9	H-10	H-10	H-10
10	H-14, H_3-15, H_3-20	H-14, H_3-15, H_3-20	H-9, H-12, H-14, H_3-15, H_3-20
11	H-13, H_3-20	H-9, H-13, H_3-20	H-9, H-10, H-12, H_3-20
12	H-13, H-14, H_3-20	H-13, H-14, H_3-20	H-10, H_2-13, H-14, H_3-20
13	H-12, H-14	H-12, H-14	
14	H_3-15	H-12, H-13, H_3-15	H-2, H-12, H_2-13, H_3-15
15	H-14	H-14	H-2, H-10
16	H-6	H-6	H-6, H_2-4
17	H_3-18	H_3-18	H-9, H_3-18
19	H_3-18	H_3-18	H_3-18
20			H-10
4-EtCO		H-4	
12-*n*-PrCO	H-12		H-12

hydroxyl, a propionyloxyl, and two acetoxyl groups in the molecule. Unlike **1** and **2**, the well-resolved NMR spectra (^{1}H and ^{13}C) of **3** could be obtained in $CDCl_3$ at 25 °C. In the ^{1}H NMR spectrum of **3**, two acetate methyl signals were observed (δ 1.98, 3H, s; 2.03, 3H, s). The additional acyl group was confirmed as a propionyloxyl group based on the ^{1}H NMR studies, including an ^{1}H−^{1}H COSY experiment, which revealed five contiguous protons [δ 1.11 (3H, t, J = 7.8 Hz) and 2.29 (2H, q, J = 7.8 Hz)]. The carbon signal at δ 173.6 was correlated with the signal of the methylene protons at δ 2.29 in the HMBC spectrum and was thus assigned as the carbon atom of the propionate carbonyl. Also, it was found that the spectral data (IR, ^{1}H and ^{13}C NMR) of **3** were very similar to those of a known diterpene, excavatolide E (**9**),[14] except that **3** showed signals corresponding to an additional propionyloxyl substitution. The propionate ester was positioned at C-12 from the ^{1}H−^{13}C long-range correlations between H-12 (δ 5.07) and carbonyl carbon (δ 173.6) of the propionate, suggesting that excavatolide W (**3**) is the 12-propionyl derivative of compound **9**. Furthermore, propionylation of **9** also gave a less polar product, which was found to be identical to **3** by comparison of the physical (mp and optical rotation) and spectral (MS, IR, ^{1}H and ^{13}C NMR) data. Thus, the structure of **3**, including the relative configuration, was established unambiguously.

Excavatolide X (**4**) had the composition $C_{30}H_{40}O_{12}$, as determined by HRFABMS. The IR spectrum of **4** showed the presence of a hydroxyl (ν_{max} 3436 cm^{-1}), a γ-lactone (ν_{max} 1776 cm^{-1}), and ester carbonyls (ν_{max} 1738 cm^{-1}). The FABMS exhibited peaks at *m/z* 593 $[M + H]^+$, 533 $[M + H - HOAc]^+$, 505 $[M + H - C_3H_7CO_2H]^+$, 473 $[M + H - 2HOAc]^+$, and 413 $[M + H - 3HOAc]^+$, indicating the presence of an *n*-butyryloxyl and three acetoxyl groups in the molecule of **4**. The NMR spectral data (^{1}H and ^{13}C) of **4** were very similar to those of known compound **10**, which had been isolated previously from an Australian gorgonian coral *Briareum* sp.[2] However, it was found that the acetoxyl group at the C-12 position of compound **10** was replaced by an *n*-butyryloxyl group by comparison of the related spectral data of compound **4** with those of compound **10**. The *n*-butyrate was located at C-12 on the basis of the data from an HMBC experiment (Table 4). The relative stereochemistry of **4** was further confirmed by a NOESY experiment (Figure 2). By above observations, diterpene **4** was found to be the 12-deacetyl-12-*n*-butyryl derivative of diterpene **10**.

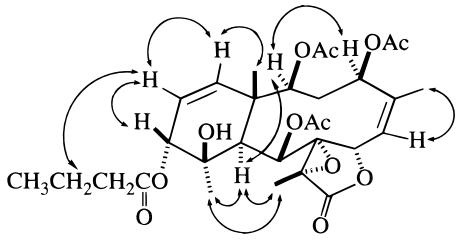

Figure 2. Selective NOE correlations of **4**.

The new briarane diterpene excavatolide Y (**5**) had a molecular formula of $C_{29}H_{38}O_{12}$ as determined by HR-FABMS. The FABMS exhibited peaks at *m/z* 579 $[M + H]^+$, 519 $[M + H - HOAc]^+$, 505 $[M + H - C_2H_5CO_2H]^+$, 445 $[M + H - C_2H_5CO_2H - HOAc]^+$, 385 $[M + H - C_2H_5CO_2H - 2HOAc]^+$, and 325 $[M + H - C_2H_5CO_2H - 3HOAc]^+$, indicating the presence of a propionate and three acetates in **5**. It was found that the spectral data (IR, ^{1}H and ^{13}C NMR) of **5** were very similar to those of diterpene **4**. The carbon signal at δ 173.6, which showed correlations with H-4 (δ 5.18), was also found to be correlated with the signal of the methylene protons at δ 2.35 in the HMBC spectrum of **5** (Table 4) and was consequently assigned as the carbon atom of the propionate carbonyl. Thus, the propionate ester should be positioned at C-4 in the molecule of **5**. On the basis of the above observations, the structure of **5** was established as described by formula **5**.

Excavatolide Z (**6**) was isolated as a white solid and had the molecular formula $C_{28}H_{40}O_{11}$, as determined by HR-FABMS. The presence of hydroxyl, γ-lactone, and ester groups were evident from IR absorptions at 3472, 1786, and 1736 cm^{-1}, respectively. The FABMS of **5** exhibited peaks at *m/z* 553 $[M + H]^+$, 535 $[M + H - H_2O]^+$, 493 $[M + H - HOAc]^+$, 475 $[M + H - H_2O - HOAc]^+$, 447 $[M + H - C_3H_7CO_2H - H_2O]^+$, 433 $[M + H - 2HOAc]^+$, 415 $[M + H - H_2O - 2HOAc]^+$, 387 $[M + H - C_3H_7CO_2H - HOAc - H_2O]^+$, 327 $[M + H - C_3H_7CO_2H - 2HOAc - H_2O]^+$, and 309 $[M + H - C_3H_7CO_2H - 2HOAc - 2H_2O]^+$, suggesting the presence of an *n*-butyryloxyl, two acetoxyl, and two hydroxyl groups in the molecule of **6**. ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) revealed that **6** contains a trisubstituted double bond. The gross structure of **6** and all of the ^{1}H and ^{13}C chemical shifts associated

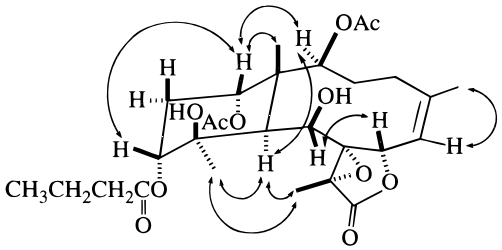

Figure 3. Selective NOE correlations of **6**.

Table 5. Cytotoxic Data of Diterpenes **1**−**6**[a]

	cell lines ED_{50} (μg/mL)			
compound	P-388	KB	A549	HT-29
1	>50	>50	>50	>50
2	3.9	7.0	19.1	20.4
3	19.4	>50	>50	>50
4	>50	>50	>50	>50
5	9.5	>50	>50	15.1
6	1.3	6.5	11.2	2.8

[a] For significant activity of pure compounds, an ED_{50} value of ≤4.0 μg/mL is required. See Geran et al.[28]

with the molecule were determined by a series of 2D NMR experiments. In the HMBC spectrum of **6**, the *n*-butyrate positioned at C-12 was confirmed from the connectivity between H-12 (δ 4.87) with the carbonyl carbon (δ 173.0) of the *n*-butyryloxyl group. Furthermore, the HMBC correlations also revealed that two acetates were attached to C-2 and C-14. These data, together with the other $^1H-^{13}C$ long-range correlations (Table 4), unambiguously established the molecular framework of **6**. The relative stereochemistry of **6** was deduced from a NOESY experiment (Figure 3). H-10 exhibited NOE correlations with H_3-20, but not with H_3-15, indicating that H-10 and C-20 methyl are situated on the α face of the six-membered ring, since the C-15 methyl is assigned as the β-substituent at C-1. H-14 was found to exhibit NOE responses with H-2, H-12, and H_3-15, but not with H-10, revealing the β-orientation of H-12 and H-14 and the α-orientation of H-2, based on a molecular model. This could be further supported, as the NMR signal of H-12 appeared as broad singlet suggesting that H-12 is an equatorial proton and should be placed on the β face as indicated in Figure 3. Thus, the *n*-butyrate at C-12 was on the α face and is cis to C-20 methyl. It was found that H-7 showed NOE correlations with H-9. Consideration of molecular models showed that H-7 should be placed on the β face. Furthermore, H_3-18 was found to exhibit NOE responses with H_3-20, indicating the β-orientation of with the C-18 methyl. Based on the above observations, the structure of **6**, including the relative stereochemistry, was elucidated unambiguously.

The cytotoxicity of metabolites **1**−**6** against the growth of P-388 (mouse lymphocytic leukemia), KB (human oral epidermoid carcinoma), A549 (human lung adenocarcinoma), and HT-29 (human colon adenocarcinoma) cancer cell lines was studied, and the results are shown in Table 5. Both diterpenes **2** and **6** exhibited significant cytotoxicity toward P-388 tumor cells. Diterpene **6** was also found to exhibit significant cytotoxicity toward HT-29 tumor cells. Compound **1** did not show cytotoxicity toward the four cancer cell lines, implying that the presence of a large substituent at C-3 would weaken the activity in comparison with the structure and cytotoxicity of **2**.

Experimental Section

General Experimental Procedures. FABMS were obtained with a VG QUATTRO GC−MS spectrometer. HR-FABMS were recorded on a JEOL JMS SX/SX 102A mass spectrometer. The NMR spectra were recorded on a Varian VXR-300/5 FT−NMR at 300 MHz for ^1H and 75 MHz for ^{13}C or on a Bruker AMX-400 FT−NMR at 400 MHz for ^1H and 100 MHz for ^{13}C, respectively, in $CDCl_3$ using TMS as internal standard, unless otherwise indicated. Other general experimental procedures followed those reported previously.[14,15]

Animal Material. The gorgonian coral *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. A voucher specimen is stored at 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.[14,15] 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. Diterpene **1** was eluted with hexanes−EtOAc (6:1), **2** with hexanes−EtOAc (5:1), **3** with hexanes−EtOAc (3:1), **4** with hexanes−EtOAc (3:1−5:2), **5** with hexanes−EtOAc (2:1), and **6** with hexanes−EtOAc (3:2).

Excavatolide U (1): colorless prisms (47.2 mg); mp 169−170 °C; $[\alpha]^{25}_D$ +34° (*c* 0.8, $CHCl_3$); IR (KBr) ν_{max} 1798, 1746, 1456, 1368, 1218, 1010 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m*/*z* 651 [0.3, $(M + H)^+$], 591 (1), 563 (0.4), 517 (1), 503 (0.4), 489 (0.3), 443 (0.4), 429 (0.4), 369 (0.9), 309 (2); HRFABMS *m*/*z* 651.3011 (calcd for $C_{33}H_{47}O_{13}$, 651.3003).

Excavatolide V (2): white powder (27.2 mg); mp 183−185 °C; $[\alpha]^{25}_D$ +40° (*c* 0.6, $CHCl_3$); IR (KBr) ν_{max} 1784, 1736, 1452, 1368, 1218, 1014 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m*/*z* 623 [1, $(M + H)^+$], 563 (3), 549 (0.3), 503 (0.6), 489 (2), 443 (0.7), 429 (0.7), 369 (1), 309 (3); HRFABMS *m*/*z* 623.2680 (calcd for $C_{31}H_{43}O_{13}$, 623.2691).

Excavatolide W (3): white powder (45.9 mg); mp 222−224 °C; $[\alpha]^{25}_D$ +53° (*c* 0.8, $CHCl_3$); IR (KBr) ν_{max} 3448, 1770, 1732, 1442, 1372, 1244, 1022 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m*/*z* 523 [0.6, $(M + H)^+$], 505 (0.3), 463 (2), 449 (0.3), 403 (0.8), 329 (4); HRFABMS *m*/*z* 523.2549 (calcd for $C_{27}H_{39}O_{10}$, 523.2532).

Excavatolide X (4): white solid (6.3 mg); mp 189−190 °C; $[\alpha]^{27}_D$ −61° (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3436, 1776, 1738, 1376, 1212, 1030 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m*/*z* 593 [1, $(M + H)^+$], 533 (2), 505 (0.9), 473 (0.3), 413 (0.3); HRFABMS *m*/*z* 593.2569 (calcd for $C_{30}H_{41}O_{12}$, 593.2586).

Excavatolide Y (5): white solid (22.4 mg); mp 147−148 °C; $[\alpha]^{27}_D$ −71° (*c* 0.4, $CHCl_3$); IR (KBr) ν_{max} 3516, 1778, 1736, 1374, 1214, 1026 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m*/*z* 579 [2, $(M + H)^+$], 519 (3), 505 (1), 445 (0.5), 385 (2), 325 (1); HRFABMS *m*/*z* 579.2446 (calcd for $C_{29}H_{39}O_{12}$, 579.2430).

Excavatolide Z (6): white powder (10.9 mg); mp 257−259 °C; $[\alpha]^{25}_D$ +22° (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max} 3472, 1786, 1736, 1368, 1266, 1030 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m*/*z* 553 [0.5, $(M + H)^+$], 535 (0.3), 493 (0.6), 475 (0.4), 447 (0.5), 433 (0.7), 415 (0.4), 387 (0.5), 327 (1), 309 (1); HRFABMS *m*/*z* 553.2629 (calcd for $C_{28}H_{41}O_{11}$, 553.2637).

Propionylation of Excavatolide B (7). Excavatolide B (**7**) (30.7 mg) was stirred with 3 mL of propionic anhydride 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 to give pure excavatolide U (**1**) (hexanes−EtOAc, 6:1, 27.9 mg, 83%); physical and spectral data were in full agreement with those of the natural product **1**.

Propionylation of Excavatolide C (8). According to the above procedure, excavatolide C (**8**) (22.9 mg) was propionylated to the product excavatolide V (**2**) (hexanes−EtOAc, 5:1, 20.4 mg, 81%); physical and spectral data were in full agreement with those of the natural product **2**.

Propionylation of Excavatolide E (9). According to the above procedure, excavatolide E (**9**) (27.8 mg) was propiony-

lated to the product excavatolide W (**3**) (hexanes−EtOAc, 3:1, 25.8 mg, 83%); physical and spectral data were in full agreement with those of the natural product **3**.

Single-Crystal X-ray Crystallography of 1.[25] Suitable colorless prisms of **1** were obtained from a solution in EtOAc. The crystal (0.48 × 0.68 × 0.72 mm) belongs to the monoclinic system, space group $P2_12_12_1$ with a = 11.169(1) Å, b = 15.072(2) Å, c = 20.123(2) Å, V = 3387.5(6) Å^3, Z = 4, D_{calcd} = 1.276 g/cm^3, λ (Mo Kα) = 0.71069 Å. Intensity data were measured on Rigaku AFC6S diffractometer up to 2θ of 25.0°. All 3376 unique reflections were collected. The structure was solved by direct method 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 = 0.056, R_w = 0.027 for 1655 observed reflections [$I > 3.00\sigma(I)$] and 415 variable parameters.

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. Cytotoxicity assays were carried out according to the procedure described previously.[26,27]

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