New Polyoxygenated Briarane Diterpenoids, Briaexcavatolides O−R, from the Gorgonian *Briareum excavatum*

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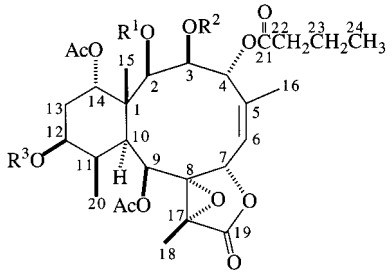
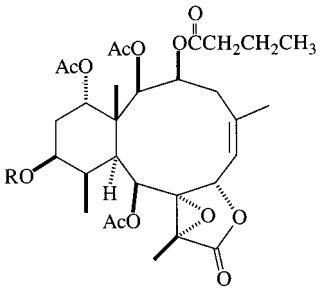
Four new polyoxygenated briarane-type diterpenoids, briaexcavatolides O−R (**1**−**4**), have been isolated from a gorgonian octocoral *Briareum excavatum*. Their structures were determined using spectroscopic and chemical methods. Metabolites **1**−**3** were found to contain oxygenated substituents at C-2, C-3, and C-4, and the relative configurations were assigned as 2*R**,3*R**,4*R** at these three positions. Briaranes containing this type of stereochemistry are reported for the first time. The structures of metabolites **1** and **2** were further confirmed by single-crystal X-ray analyses. Compound **2** has been shown to exhibit significant cytotoxicity toward P-388 and HT-29 cancer cells.

In the past 24 years, more than 290 metabolites featuring the briarane skeleton had been reported from a variety of marine organisms including Gorgonacea, Alcyonacea, Stolonifera, Pennatulacea, and Nudibranch.[1] The structural novelty and biological properties of these compounds have prompted continued attention since the first briarane-type product, briarein A, was described in 1977 by Burks et al.[2] In our screening for biologically active metabolites from the Taiwanese gorgonians, we have reported a series of novel metabolites from the gorgonian corals including *Briareum* sp.,[3] *Briareum excavatum*,[4−8] *Junceella fragilis*,[9] and *Isis hippuris*.[10−12] In this paper, we describe the isolation, structure elucidation, and cytotoxicity of four new polyoxygenated briarane-type diterpenoids, briaexcavatolides O−R (**1**−**4**), from the further study of *B. excavatum* (Nutting) (phylum Cnidaria, order Gorgonacea, family Briareidae).[13] The structures of metabolites **1**−**4** were determined by spectroscopic and chemical methods, and the structures of **1** and **2** were further confirmed by single-crystal X-ray analyses. Briaranes **1**−**3** were found to contain oxygenated substituents at C-2, C-3, and C-4, and the relative configurations were assigned as 2*R**,3*R**,4*R** at these three positions, respectively. Briaranes containing this type of stereochemistry are being reported for the first time.



1: $R^1 = R^3 = H$, $R^2 = Ac$
2: $R^1 = Ac$, $R^2 = R^3 = H$
3: $R^1 = R^3 = Ac$, $R^2 = H$



4: $R = C(O)(CH_2)_6CH_3$
5: R = H

Results and Discussion

The freeze-dried organisms were extracted with EtOAc at room temperature. The EtOAc extract was fractionated by extensive SiO_2 gel column chromatography to give the new metabolites **1**−**4**. Their structures were elucidated as described below.

Briaexcavatolide O (**1**) was obtained as a white solid. The HRFABMS of **1** provided a pseudomolecular ion $[M + H]^+$ at *m*/*z* 611.2704, indicating the molecular formula $C_{30}H_{42}O_{13}$ and 10 degrees of unsaturation for this metabolite. The IR spectrum revealed absorption bands for hydroxy (3428 cm^{-1}), γ-lactone (1772 cm^{-1}), and ester carbonyl (1734 cm^{-1}) moieties. The FABMS of **1** exhibited peaks at *m*/*z* 611 $[M + H]^+$, 551 $[M + H - HOAc]^+$, 523 $[M + H - C_3H_7CO_2H]^+$, 463 $[M + H - HOAc - C_3H_7CO_2H]^+$, 403 $[M + H - 2HOAc - C_3H_7CO_2H]^+$, 343 $[M + H - 3HOAc - C_3H_7CO_2H]^+$, and 307 $[M + H - 3HOAc - C_3H_7CO_2H - 2H_2O]^+$, suggesting the presence of a butyryloxy, two hydroxy, and three acetoxy groups in **1**. It was found that the ^1H NMR spectrum of **1** in $CDCl_3$ revealed many broad and overlapped signals between δ_H 3.5 and 6.0 ppm, suggesting the existence of slowly interconverting conformations for **1** in $CDCl_3$. Fortunately, we found that both ^1H and ^{13}C NMR spectra of **1** could be sharpened in C_5D_5N at 25 °C (Tables 1 and 2). Resonances in the ^{13}C NMR of **1** at δ 172.2 (s), 172.1 (s), 171.6 (s), 171.0 (s), and 170.8 (s) supported the presence of a γ-lactone and four additional esters. Three of the esters were identified as acetates by the presence of methyl resonances in the ^1H NMR spectrum at δ 2.35 (3H,

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Table 1. ^{1}H and ^{13}C Chemical Shifts for Diterpenoids **1**−**4**

position	**1**[a]	**2**[a]	**3**[b]	**4**[c]
2	3.95 d (6.4)[d]	5.41 s	5.46 s	5.22 br s
3	6.18 d (6.0)	4.73 d (10.8)	4.75 d (10.5)	5.90 d (6.0)
4/4′	7.18 d (6.0)	6.62 s	6.66 s	2.14 m; 4.01 dd (15.5, 7.0)
6	5.82 d (7.2)	5.52 d (6.0)	5.54 d (6.0)	5.45 d (7.5)
7	6.65 d (7.2)	6.40 d (6.0)	6.43 d (6.0)	5.77 d (7.5)
9	5.90 d (10.0)	5.70 d (8.8)	5.70 d (9.0)	5.60 d (10.0)
10	3.43 dd (10.0, 5.2)	3.06 dd (8.8, 5.2)	3.14 dd (9.0, 5.0)	3.30 dd (10.0; 5.0)
11	3.09 m	2.92 m	3.04 m	2.67 m
12	4.44 m	4.44 m	5.43 m	4.98 m
13/13′	2.16 m	2.18 m	2.10 m; 1.80 m	1.88 br d (14.0); 2.05 m
14	5.09 br s	5.34 br s	5.37 br s	4.69 br s
15	1.24 s	1.08 s	1.09 s	0.90 s
16	2.16 s	2.01 s	2.00 s	2.02 s
18	1.88 s	1.85 s	1.87 s	1.58 s
20	1.41 d (7.2)	1.38 d (7.2)	1.11 d (7.5)	1.15 d (7.5)
2-OH	6.89 d (6.4)			
3-OH		5.86 d (10.8)	6.01 d (10.5)	
12-OH	6.31 br s	6.39 br s		
acetate methyls	2.35 s	2.36 s	2.39 s	2.41 s
	2.28 s	2.28 s	2.33 s	2.33 s
	1.99 s	2.11 s	2.13 s	2.28 s
			2.01 s	
n-butyrate	CH_2 2.22 t (7.2)	CH_2 2.22 t (7.6)	CH_2 2.22 t (7.5)	CH_2 2.31 t (7.5)
	CH_2 1.56 m	CH_2 1.56 m	CH_2 1.58 m	CH_2 1.53 m
	CH_3 0.80 t (7.2)	CH_3 0.79 t (7.6)	CH_3 0.81 t (7.5)	CH_3 0.89 t (7.5)
n-octanoate				CH_2 2.10 t (7.5)
				CH_2 1.50 m
				$(CH_2)_4$ 1.22 br s
				CH_3 0.91 t (7.5)

[a] Spectra recorded at 400 MHz in C_5D_5N at 25 °C. [b]500 MHz in C_5D_5N at 25 °C. [c]500 MHz in acetone-d_6 at −70 °C. [d]*J* values (in Hz) in parentheses.

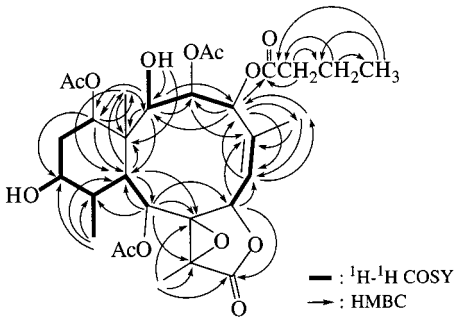

Figure 1. ^{1}H−^{1}H COSY and HMBC correlations for **1**.

s), 2.28 (3H, s), and 1.99 (3H, s). The other ester was found to be an *n*-butyryloxy group based on ^{1}H NMR studies, including an ^{1}H−^{1}H COSY spectrum, which revealed seven contiguous protons (δ 2.22, 2H, t, J = 7.2 Hz; 1.56, 2H, m; 0.80, 3H, t, J = 7.2 Hz). From the ^{1}H−^{1}H COSY spectrum of **1** (Figure 1), it was possible to establish the proton sequences from H-2 to H-4; H_3-16 to H-6; H-6 to H-7; H-9 to H-11; H-11 to H_3-20; and H-11 to H-14. Two hydroxy proton signals occurring at δ 6.89 (1H, d, J = 6.4 Hz) and 6.31 (1H, br s) were found to correlate in the ^{1}H−^{1}H COSY spectrum with H-2 (δ 3.95, 1H, d, J = 6.4 Hz) and H-12 (δ 4.44, 1H, m), respectively. Thus, the two hydroxy groups could be positioned at C-2 and C-12. Twelve of the thirteen oxygen atoms in the molecular formula of compound **1** could be accounted for from the presence of a γ-lactone, four esters, and two hydroxy groups. The remaining oxygen atom had to be placed between C-8 and C-17 to form a tetrasubstituted epoxide based on the ^{13}C NMR evidence (two quaternary oxygenated carbons at δ 69.8 and 60.4 ppm) and the chemical shift of the tertiary methyl H_3-18 (δ 1.88, 3H, s). Olefinic resonances in the ^{13}C NMR of **1** at δ 139.4 (s) and 127.3 (d) indicated the presence of a trisubstituted double bond. On the basis of these data and the ^{1}H−^{13}C long-range correlations observed in an HMBC experiment, the connectivities from C-1 to C-14 (Figure 1 and Table 3) could be further established. 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 methylcyclohexane ring which is fused to the 10-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; and H-14 and C-10. 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. In addition, the carbon signal at δ 172.2 (s) was correlated with the signal of the methylene protons at δ 2.22 in the HMBC spectrum and was consequently assigned as the carbon atom of the *n*-butyrate carbonyl. Therefore, the *n*-butyrate positioned at C-4 was confirmed by the connectivity between H-4 (δ 7.18) and the carbonyl carbon (δ 172.2, s) of the *n*-butyryloxy group. Furthermore, the HMBC correlations also revealed the positions of three acetoxy groups at C-3, C-9, and C-14, respectively. 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 determined by the correlations observed in the NOESY spectrum (Figure 2 and Table 4). In the NOESY experiment of **1**, H-10 gives NOESY correlations to H-2, H-3, H-11, and H-12, but not to H_3-15. This indicated that H-2, H-3, H-10, H-11, and H-12 are situated on the same face of the molecule and must be assigned as the α protons, since the C-15 methyl is the β-substituent at C-1. H-14 gave NOESY correlations to H-2 and H_3-15, but not to H-10, confirming the β-orientation for this proton. The signal of H_3-18 showed NOESY correlation with H_3-20, indicating the β-orientation of H_3-

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

position	**1**[a]	**2**[a]	**3**[b]	**4**[c]
1	44.8 (s)[d]	44.0 (s)	44.4 (s)	44.3 (s)
2	83.1 (d)	87.2 (d)	87.3 (d)	81.2 (d)
3	75.0 (d)	74.1 (d)	74.5 (d)	73.5 (d)
4	65.8 (d)	67.9 (d)	68.3 (d)	34.4 (t)
5	139.4 (s)	141.0 (s)	141.8 (s)	140.0 (s)
6	127.3 (d)	124.1 (d)	123.5 (d)	122.3 (d)
7	74.4 (d)	75.2 (d)	75.5 (d)	74.1 (d)
8	69.8 (s)	71.6 (s)	70.9 (s)	68.8 (s)
9	65.4 (d)	66.8 (d)	66.8 (d)	64.8 (d)
10	41.7 (d)	41.4 (d)	41.7 (d)	40.3 (d)
11	36.3 (d)	38.0 (d)	35.0 (d)	33.0 (d)
12	66.3 (d)	66.1 (d)	71.8 (d)	70.0 (d)
13	31.3 (t)	31.2 (t)	30.4 (t)	27.2 (t)
14	82.3 (d)	81.6 (d)	81.2 (d)	81.5 (d)
15	20.1 (q)	18.3 (q)	18.4 (q)	18.1 (q)
16	18.7 (q)	17.2 (q)	17.6 (q)	22.1 (q)
17	60.4 (s)	62.8 (s)	63.2 (s)	60.5 (s)
18	10.0 (q)	10.7 (q)	11.1 (q)	9.9 (q)
19	172.1 (s)	171.6 (s)	171.8 (s)	172.3 (s)
20	9.5 (q)	9.6 (q)	10.8 (q)	10.4 (q)
acetate	21.7 (q)	21.8 (q)	22.2 (q)	22.1 (q)
methyls	21.6 (q)	21.3 (q)	21.7 (q)	21.9 (q)
	20.7 (q)	20.3 (q)	21.7 (q)	21.8 (q)
			21.4 (q)	
acetate	171.6 (s)	172.1 (s)	172.4 (s)	171.7 (s)
carbonyls	171.0 (s)	170.9 (s)	171.3 (s)	170.7 (s)
	170.8 (s)	170.1 (s)	170.5 (s)	170.2 (s)
			170.4 (s)	
n-butyrate	172.2 (s)	173.0 (s)	173.5 (s)	172.6 (s)
	13.4 (q)	13.7 (q)	14.1 (q)	14.3 (q)
	18.6 (t)	18.7 (t)	19.1 (t)	19.2 (t)
	36.1 (t)	35.9 (t)	36.3 (t)	36.5 (t)
n-octanoate				174.3 (s)
				34.5 (t)
				31.9 (t)
				31.6 (t)
				31.5 (t)
				25.1 (t)
				19.1 (t)
				14.1 (q)

[a] Spectra recorded at 100 MHz in C_5D_5N at 25 °C. [b]125 MHz in C_5D_5N at 25 °C. [c]125 MHz in $CDCl_3$ at −75 °C. [d]Multiplicity deduced by DEPT and indicated by usual symbols. The values are in ppm downfield from TMS.

18. Also, H-7 was found to exhibit correlations with H-4, H-9, and H_3-18. From consideration of molecular models,

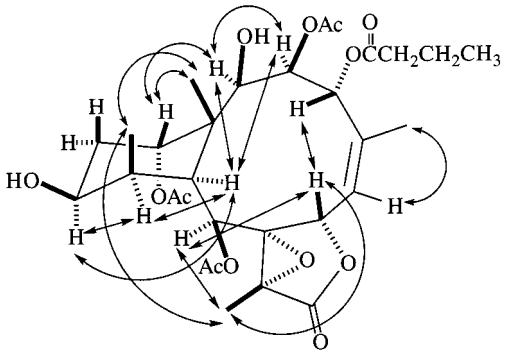

Figure 2. Selective NOESY correlations of **1**.

H-7 was found to be reasonably close to H-4, H-9, and H_3-18, while H-7 and H-4 were β-oriented and H-9 was placed on the α face. Thus, the relative configurations of all centers are assigned as 1*S**,2*R**,3*R**,4*R**,5*Z*,7*S**,8*S**,9*S**,10*S**,11*R**,12*S**,14*S**,17*R** (asterisk indicates relative configuration), respectively. As the slow evaporation of the pyridine solution of **1** afforded colorless prisms, a single-crystal X-ray structure analysis was carried out in order to confirm the molecular structure of **1**. The X-ray structure (Figure 3 and Table 5) further unambiguously established the relative, not the absolute, configuration of **1**.

Briaexcavatolide P (**2**) had the same molecular formula as that of **1**, $C_{30}H_{42}O_{13}$, as determined by HRFABMS, with 10 degrees of unsaturation, indicating that compounds **1** and **2** are isomers. By detailed ^1H, ^{13}C (Tables 1 and 2), and 2D NMR spectral analysis, compound **2** has the same substituents as that of **1** (an *n*-butyryloxy, three acetoxy, and two hydroxy groups). On the basis of the $^1H-^1H$ COSY spectrum of **2**, it was possible to establish the sequences of the protons attached to the carbon skeleton of **2**. Furthermore, two hydroxy proton signals appeared at δ 5.86 (1H, d, J = 10.8 Hz) and 6.39 (1H, br s). These were correlated in the $^1H-^1H$ COSY spectrum with H-3 (δ 4.73, 1H, d, J = 10.8 Hz) and H-12 (δ 4.44, 1H, m), respectively, indicating that these two hydroxy groups were positioned at C-3 and C-12, respectively. In the HMBC experiment of **2** (Table 3), the ^{13}C NMR signal at δ 173.0 (s) was correlated with the signal for the methylene protons at δ 2.22 in the spectrum and was assigned as the carbon atom

Table 3. HMBC Correlations for Diterpenoids **1**−**3**

carbon	**1**	**2**	**3**
1	H-2, OH-2, H-10, H_3-15	H-2, H-10, H_3-15	H-2, H-10
2	OH-2, H-4, H_3-15	H-4, H_3−15	H-4, H_3-15
3	H-2, H-4	H-2, OH-3, H-4	H-2
4	H-2, H-6, H_3-16	H-2, H-6, H_3−16	H-2, H_3-16
5	H-3, H-7, H_3-16	H-3, H-7, H_3−16	H-7, H_3-16
6	H-4, H-7, H_3-16	H-4, H-7, H_3−16	H-4, H-7, H_3-16
7	H-9	H-9	H-9
8	H-9, H-10, H_3-18	H-9, H-10, H_3-18	H-9, H_3-18
9	H-10	H-10	n.o.[a]
10	H-2, H-9, H-14, H_3-15, H_3-20	H-2, H-9, H-14, H_3-15, H_3-20	H-2, H_3-15, H_3-20
11	H-9, H_3-20	H-9, H_3-20	H-9, H_3-20
12	H-14, H_3-20	H-14, H_3-20	H_3-20
14	H-2, H_3-15	H-2, H_3−15	H-2, H_3−15
15	H-10, H-14	H-10, H-14	n.o.[a]
16	H-4, H-6	H-4, H-6	H-6
17	H-9, H_3-18	H-9, H_3-18	H-9, H_3-18
19	H-7, H_3-18	H-7, H_3-18	H-7, H_3-18
2-O*C*OMe		H-2	H-2
3-O*C*OMe	H-3		
9-O*C*OMe	H-9	H-9	H-9
12-O*C*OMe			H-12
14-O*C*OMe	H-14	H-14	H-14
4-O*C*OPr	H-4, H_2-22	H-4, H_2-22	H-4, H_2-22

[a] n.o. = not observed.

Table 4. Key NOESY Correlations for Diterpenoids **1**−**3**

	1	2	3
H-2	H-3, H-10, H-14	H-3, H-10, H-14	H-3, H-10, H-14
H-3	H-2, H-10	H-2, H-10	H-2, H-10
H-4	H-7	H-7	H-7
H-6	H_3-16	H_3-16	H_3-16
H-7	H-4, H-9, H_3-18	H-4, H-9, H_3-18	H-4, H-9, H_3-18
H-9	H-7, H_3-18	H-7, H_3-18	H-7, H_3-18
H-10	H-2, H-3, H-11, H-12	H-2, H-3, H-11, H-12	H-2, H-3, H-11, H-12
H-11	H-10, H-12	H-10, H-12	H-10, H-12
H-12	H-10, H-11	H-10, H-11	H-10, H-11
H-14	H-2, H_3-15	H-2, H_3-15	H-2, H_3-15
H_3-15	H-14, H_3-20	H-14, H_3-20	H-14, H_3-20
H_3-16	H-6	H-6	H-6
H_3-18	H-7, H-9, H_3-20	H-7, H-9, H_3-20	H-7, H-9, H_3-20
H_3-20	H_3-15, H_3-18	H_3-15, H_3-18	H_3-15, H_3-18

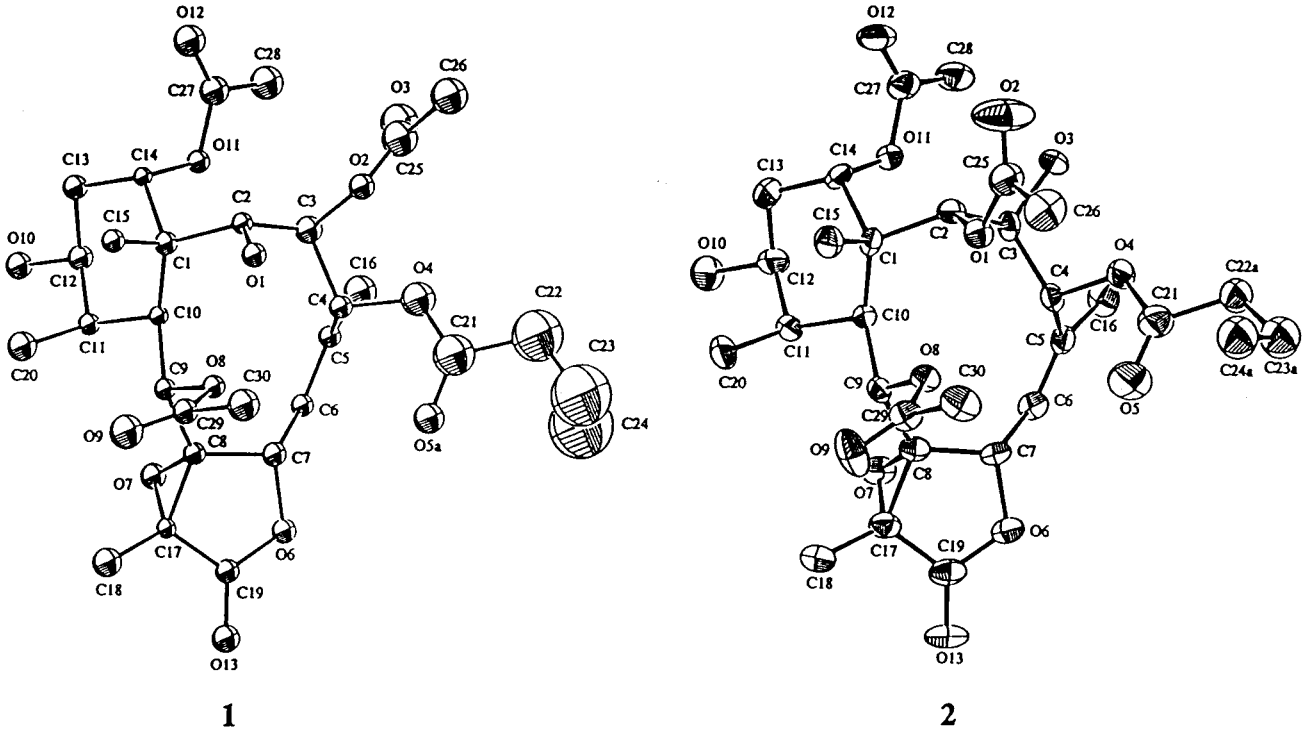

Figure 3. Computer-generated ORTEP plots of metabolites **1** and **2** showing the relative configurations of **1** and **2**. Hydrogen atoms have been omitted for clarity.

of the *n*-butyrate carbonyl. The HMBC correlations of **2** further revealed the connectivity between H-4 (δ 6.62) and the carbonyl carbon (δ 173.0) of the *n*-butyrate unit and demonstrated the location of the *n*-butyrate to be at C-4. In an HMBC experiment on **2**, the positions of the three acetoxy groups at C-2, C-9, and C-14 were also confirmed by the connectivities between the three methine protons at δ 5.41 (H-2), 5.34 (H-14), 5.70 (H-9), and δ 170.1 (s), 170.9 (s), 172.1 (s), respectively. The relative stereochemistry of **2** was determined by a NOESY experiment (Table 4). The NOESY interactions of **2** were found to be very similar with those of **1**, and the relative configurations of all centers of **2** were established as 1*R**,2*R**,3*R**,4*R**,5*Z*,7*S**,8*S**,9*S**,10*S**,11*R**,12*S**,14*S**,-17*R**, respectively. Furthermore, as with **1**, the structure, including the relative configuration of **2**, was also confirmed by a single-crystal X-ray analysis (Figure 3 and Table 6).

Briaexcavatolide Q (**3**) had the molecular formula $C_{32}H_{44}O_{14}$, as determined by HRFABMS. It was found that the spectral data (IR, ^{1}H, and ^{13}C NMR) of **3** were very close to those of **2**. Furthermore, by comparison of the ^{1}H, ^{13}C NMR, ^{1}H−^{1}H COSY, and HMBC spectral data of **3** with those of **2**, it was revealed that the signals corresponding to the hydroxy-bearing C-12 methine group in **2** (δ_H 4.44, m; δ_C 66.1, d) were shifted downfield in **3** (δ_H 5.43, m; δ_C 71.8, d). On the basis of the above observations, metabolite **3** was suggested to be the 12-acetyl derivative of **2**. The relative stereochemistry of **3** was elucidated by a NOESY experiment (Table 4), and the results revealed that metabolite **3** possesses the same relative stereochemistry as that of **2**, and all centers were elucidated as 1*R**,2*R**,3*R**,4*R**,5*Z*,7*S**,8*S**,9*S**,10*S**,11*R**,12*S**,14*S**,-17*R**, respectively.

Briaexcavatolide R (**4**) was obtained as a white powder. HRFABMS established a molecular formula of $C_{38}H_{56}O_{13}$ for this compound. The IR spectrum of **4** showed absorptions of a carbonyl group of a γ-lactone (ν_{max}1788 cm^{-1}) and ester carbonyls (ν_{max}1740 cm^{-1}). The FABMS of **4** exhibited peaks at *m/z* 721 $[M + H]^+$, 661 $[M + H - AcOH]^+$, 633 $[M + H - C_3H_7CO_2H]^+$, 573 $[M + H - C_3H_7CO_2H - AcOH]^+$, 517 $[M + H - C_7H_{15}CO_2H - AcOH]^+$, 513 $[M + H - C_3H_7CO_2H - 2AcOH]^+$, 489 $[M + H - C_3H_7CO_2H - C_7H_{15}CO_2H]^+$, 429 $[M + H - C_3H_7CO_2H - C_7H_{15}CO_2H - AcOH]^+$, 369 $[M + H - C_3H_7CO_2H - C_7H_{15}CO_2H - 2AcOH]^+$, and 309 $[M + H - C_3H_7CO_2H - C_7H_{15}CO_2H - 3AcOH]^+$, also suggesting the presence of an octanoyloxy,

Table 5. Atomic Coordinates and B_{eq} of Diterpenoid **1**

atom	*x*	*y*	*z*	B_{eq}[a]	occ
C (1)	−0.543(2)	−0.3765(9)	−0.7139(9)	2.5(4)	
C (2)	−0.383(2)	−0.3914(8)	−0.7543(8)	2.0(4)	
C (3)	−0.395(2)	−0.421(1)	−0.827(1)	3.4(5)	
C (4)	−0.426(2)	−0.4998(9)	−0.8350(9)	2.5(4)	
C (5)	−0.590(2)	−0.5169(9)	−0.8615(9)	2.2(4)	
C (6)	−0.686(2)	−0.5652(9)	−0.8319(9)	2.3(4)	
C (7)	−0.653(2)	−0.5995(9)	−0.7695(9)	2.7(4)	
C (8)	−0.714(2)	−0.5680(9)	−0.7034(9)	2.3(4)	
C (9)	−0.633(2)	−0.5033(9)	−0.6753(8)	2.2(4)	
C (10)	−0.670(2)	−0.4343(8)	−0.7129(9)	1.8(4)	
C (11)	−0.837(2)	−0.4109(9)	−0.6860(9)	2.2(4)	
C (12)	−0.890(2)	−0.3466(9)	−0.7238(9)	2.9(4)	
C (13)	−0.772(2)	−0.2864(9)	−0.7123(9)	2.8(4)	
C (14)	−0.613(2)	−0.3067(8)	−0.7405(8)	1.6(4)	
C (15)	−0.489(2)	−0.3572(9)	−0.6400(9)	2.5(4)	
C (16)	−0.636(3)	−0.486(1)	−0.929(1)	5.1(6)	
C (17)	−0.793(2)	−0.6221(8)	−0.6640(8)	1.8(4)	
C (18)	−0.812(3)	−0.631(1)	−0.590(1)	3.9(5)	
C (19)	−0.797(2)	−0.6820(9)	−0.7113(9)	2.6(4)	
C (20)	−0.860(2)	−0.406(1)	−0.611(1)	4.0(5)	
C (21)	−0.261(4)	−0.587(2)	−0.882(1)	7.8(8)	
C (22)	−0.149(4)	−0.602(2)	−0.945(2)	12(1)	
C (23)	−0.082(5)	−0.673(2)	−0.944(2)	18(2)	
C (24)	−0.244(6)	−0.694(2)	−0.982(2)	20(2)	
C (25)	−0.220(3)	−0.377(1)	−0.910(1)	5.9(7)	
C (26)	−0.048(3)	−0.362(1)	−0.928(1)	6.0(7)	
C (27)	−0.602(2)	−0.256(1)	−0.853(1)	3.8(5)	
C (28)	−0.641(3)	−0.266(1)	−0.925(1)	5.1(6)	
C (29)	−0.401(2)	−0.538(1)	−0.614(1)	2.9(5)	
C (30)	−0.235(2)	−0.559(1)	−0.627(1)	4.7(6)	
O (1)	−0.271(1)	−0.4277(6)	−0.7177(6)	2.7(3)	
O (2)	−0.235(2)	−0.4065(6)	−0.8515(6)	3.1(3)	
O (3)	−0.321(2)	−0.3593(9)	−0.9486(8)	6.7(5)	
O (4)	−0.318(2)	−0.5232(7)	−0.8879(7)	5.3(4)	
O (5b)	−0.250(5)	−0.615(2)	−0.819(2)	6(1)	0.450
O (5a)	−0.322(3)	−0.633(1)	−0.846(1)	4.1(7)	0.550
O (6)	−0.731(1)	−0.6688(6)	−0.7699(6)	2.8(3)	
O (7)	−0.879(1)	−0.5678(6)	−0.7007(6)	3.1(3)	
O (8)	−0.468(1)	−0.5241(5)	−0.6766(6)	2.3(3)	
O (9)	−0.464(2)	−0.5329(7)	−0.5606(7)	5.3(4)	
O (10)	−1.044(1)	−0.3266(6)	−0.7020(6)	3.3(3)	
O (11)	−0.634(1)	−0.3124(6)	−0.8143(6)	2.4(3)	
O (12)	−0.542(2)	−0.2044(7)	−0.8306(7)	4.6(3)	
O (13)	−0.853(1)	−0.7400(6)	−0.6966(6)	3.6(3)	

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

Table 6. Atomic Coordinates and B_{eq} of Diterpenoid **2**

atom	*x*	*y*	*z*	B_{eq}[a]	occ
C (1)	0.3291(4)	0.1316(4)	0.7517(8)	3.1(2)	
C (2)	0.3882(4)	0.0698(4)	0.7077(8)	3.5(2)	
C (3)	0.4174(4)	0.0138(4)	0.8052(8)	3.2(2)	
C (4)	0.4952(4)	0.0351(4)	0.8710(8)	3.3(2)	
C (5)	0.4914(5)	0.0200(4)	1.0122(8)	3.4(2)	
C (6)	0.4941(5)	0.0730(4)	1.0977(8)	3.7(2)	
C (7)	0.5124(5)	0.1527(4)	1.0722(8)	3.4(2)	
C (8)	0.4447(5)	0.2051(4)	1.0499(8)	3.2(2)	
C (9)	0.4080(5)	0.2141(4)	0.9205(8)	3.1(2)	
C (10)	0.3393(4)	0.1594(4)	0.8939(7)	2.9(2)	
C (11)	0.2636(5)	0.1952(4)	0.9522(9)	3.8(2)	
C (12)	0.1955(5)	0.1430(5)	0.938(1)	4.8(3)	
C (13)	0.1796(5)	0.1290(5)	0.800(1)	4.9(3)	
C (14)	0.2486(5)	0.0909(5)	0.7381(8)	3.9(2)	
C (15)	0.3281(5)	0.1940(5)	0.6506(9)	4.5(2)	
C (16)	0.4772(5)	−0.0592(4)	1.0488(9)	4.8(2)	
C (17)	0.4527(5)	0.2651(5)	1.1465(9)	4.1(2)	
C (18)	0.4278(6)	0.3433(5)	1.135(1)	6.2(3)	
C (19)	0.5181(5)	0.2435(5)	1.232(1)	4.5(3)	
C (20)	0.2423(5)	0.2736(5)	0.912(1)	5.7(3)	
C (21)	0.6280(6)	0.0199(6)	0.805(1)	5.6(3)	
C (22a)	0.686(1)	−0.045(1)	0.773(2)	5.3(5)	0.550
C (22b)	0.679(1)	−0.030(1)	0.714(2)	5.0(6)	0.450
C (23a)	0.771(2)	−0.022(2)	0.747(3)	7.8(7)	0.550
C (23b)	0.770(2)	0.017(3)	0.729(4)	4.1(9)	1/4
C (23c)	0.759(3)	0.002(3)	0.636(5)	8(1)	0.300
C (24a)	0.774(2)	0.047(2)	0.632(3)	8.1(8)	0.450
C (24b)	0.811(3)	−0.015(3)	0.676(6)	9(1)	1/4
C (25)	0.4736(6)	0.0699(7)	0.525(1)	5.6(3)	
C (26)	0.5515(6)	0.0939(6)	0.478(1)	6.9(3)	
C (27)	0.2239(5)	−0.0395(6)	0.738(1)	4.4(3)	
C (28)	0.2337(6)	−0.1060(5)	0.814(1)	5.8(3)	
C (29)	0.5016(5)	0.2723(5)	0.7865(9)	4.1(2)	
C (30)	0.5688(6)	0.2581(5)	0.701(1)	5.9(3)	
O (1)	0.4563(3)	0.1000(3)	0.6396(5)	3.9(1)	
O (2)	0.4330(5)	0.0273(6)	0.4695(9)	12.3(3)	
O (3)	0.4198(3)	−0.0573(3)	0.7497(7)	5.7(2)	
O (4)	0.5552(3)	−0.0082(3)	0.8093(6)	4.2(1)	
O (5)	0.6436(4)	0.0796(4)	0.8468(8)	8.1(2)	
O (6)	0.5490(3)	0.1792(3)	1.1918(6)	4.4(2)	
O (7)	0.3931(3)	0.2085(3)	1.1607(6)	4.5(2)	
O (8)	0.4702(3)	0.2079(3)	0.8286(5)	3.4(1)	
O (9)	0.4768(4)	0.3308(3)	0.8161(8)	7.3(2)	
O (10)	0.1267(3)	0.1720(4)	1.0012(7)	6.7(2)	
O (11)	0.2543(3)	0.0191(3)	0.7991(6)	3.8(1)	
O (12)	0.1941(4)	−0.0344(4)	0.6349(7)	6.4(2)	
O (13)	0.5405(4)	0.2745(3)	1.3273(6)	6.0(2)	

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

an butyryloxy, and three acetoxy groups in the molecule of **4**. Also, it was found that the ^{1}H and ^{13}C NMR spectra of **4** 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 when 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 140.0 (s) and 122.3 (d). An 8,17-epoxide group was confirmed from the signals of two quaternary oxygenated carbons at δ 68.8 (s) and 60.5 (s) and from the chemical shift of the teritary methyl H_3-18 (δ 1.58, 3H, s). In the ^{13}C NMR spectrum of **4**, six carbonyl resonances appeared at δ 174.3 (s), 172.6 (s), 172.3 (s), 171.7 (s), 170.7 (s), and 170.2 (s) and confirmed the presence of a γ-lactone and five other ester groups. In the ^{1}H NMR spectrum of **4**, three acetate methyls (δ 2.41, 3H, s; 2.33, 3H, s; 2.28, 3H, s), an *n*-octanoyloxy (δ 2.10, 2H, t, J = 7.5 Hz; 1.50, 2H, m; 1.22, 8H, br s; 0.91, 3H, t, J = 7.5 Hz), and an *n*-butyryloxy (δ 2.31, 2H, t, J = 7.5 Hz; 1.53, 2H, m; 0.89, 3H, t, J = 7.5 Hz) group were further observed. It was found that the spectral data (^{1}H and ^{13}C NMR) of **4** were very similar to those of a known metabolite, excavatolide B (**5**).[4] However, the chemical shifts for H-12 and C-12 of **4** (δ_H 4.98, m; δ_C 70.0, d) were found to be shifted downfield, in comparison with the analogous data of **5** (δ_H 3.88, m; δ_C 65.8, d), suggesting that the 12-hydroxy group in **5** had to be replaced by an ester group in **4**. The main problem was positional assignments of the *n*-butyrate and *n*-octanoate 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 the *n*-octanoylation of excavatolide B (**5**) gave a less polar product which was found to be identical with metabolite **4** by comparison of the physical (mp and optical rotation) and spectral (IR, MS, ^{1}H and ^{13}C NMR) data. Thus, the *n*-butyrate and *n*-octanoate in **4** should be located at C-3 and C-12, respectively. On the basis of the above observations, briaexcavatolide R (**4**) was found to be the 12-*n*-octanoyl derivative of excavatolide B (**5**), with the structure as described by formula **4**.

The cytotoxic activities of the new metabolites **1**−**4** against the growth of P-388 (mouse lymphocytic leukemia), A549 (human lung adenocarcinoma), and HT-29 (human colon adenocarcinoma) cancer cells were studied, and the results indicated that diterpenoids **1**, **3**, and **4** were inactive in the above cell lines. Diterpenoid **2** exhibited cytotoxicity

against P-388, A549, and HT-29 cancer cells with ED_{50}'s of 0.9, 4.8, and 3.1 μg/mL, respectively.[14]

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. IR spectra were recorded on a Hitachi I-2001 infrared spectrophotometer. FABMS were obtained with a VG Quattro GC/MS spectrometer. HRFABMS were recorded on a JEOL JMX-HX 110 mass spectrometer. The NMR spectra were recorded on a Bruker AMX-400 FT-NMR at 400 MHz for ^{1}H and 100 MHz for ^{13}C or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively, in C_5D_5N using TMS as an internal standard, unless otherwise indicated. Si gel (Merck, 230−400 mesh) was used for column chromatography. Precoated Si gel plates (Merck, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC.

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

Extraction and Isolation. The extraction scheme followed the standard procedures of our group.[5] The freeze-dried animal material (1.9 kg) was minced and extracted exhaustively with EtOAc. The extract was separated on Si gel column chromatography, using hexanes and hexanes−EtOAc mixtures of increasing polarity. Diterpenoid **4** was eluted with hexanes−EtOAc (8:1), **3** with hexanes−EtOAc (5:2), **1** with hexanes−EtOAc (2:1−3:2), and **2** with hexanes−EtOAc (3:2).

Briaexcavatolide O (1): colorless prisms (29 mg); mp 281−284 °C; $[\alpha]^{27}_D$ +165° (*c* 0.9, $CHCl_3$); IR (KBr) ν_{max}3428, 1772, and 1734 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 611 [0.1, $(M + H)^+$], 551 (3), 523 (3), 463 (0.9), 403 (0.9), 343 (0.9), and 307 (16); HRFABMS *m/z* 611.2704 (calcd for $C_{30}H_{43}O_{13}$, 611.2691).

Briaexcavatolide P (2): colorless prisms (44 mg); mp 248−251 °C; $[\alpha]^{27}_D$ +167° (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max}3676, 1790, and 1734 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 611 [0.1, $(M + H)^+$], 593 (5), 533 (2), 523 (0.2), 491 (0.6), 463 (0.9), 403 (1), 343 (1), 325 (0.6), and 307 (0.5); HRFABMS *m/z* 611.2697 (calcd for $C_{30}H_{43}O_{13}$, 611.2691).

Briaexcavatolide Q (3): white powder (2.2 mg); mp 204−206 °C; $[\alpha]^{27}_D$ +253° (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3536, 1786, and 1732 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 675 [0.1, $(M + Na)^+$], 653 [0.2, $(M + H)^+$], 635 (1), 593 (1), 575 (0.6), 533 (0.3), 413 (2), and 391 (10); HRFABMS *m/z* 675.2603 (calcd for $C_{32}H_{44}O_{14}Na$, 675.2616).

Briaexcavatolide R (4): white powder (14.4 mg); mp 150−151 °C; $[\alpha]^{25}_D$ −39° (*c* 0.8, $CHCl_3$); IR (KBr) ν_{max}1788 and 1740 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 721 [1, $(M + H)^+$], 661 (0.3), 633 (0.6), 573 (0.4), 517 (0.7), 513 (0.5), 489 (0.4), 429 (0.5), 369 (1), 309 (1); HRFABMS *m/z* 721.3790 (calcd for $C_{38}H_{57}O_{13}$, 721.3783).

***n*-Octanoylation of Excavatolide B (5).** Excavatolide B (**5**) (30 mg) was stirred with 4 mL of *n*-octanoic anhydride in 4 mL of pyridine for 96 h at room temperature. After evaporation of excess reagent, the residue was separated by column chromatography on Si gel to give pure briaexcavatolide R (**4**) (hexanes−EtOAc, 8:1, 25.1 mg, 69%) as white powder; mp 149−151 °C; $[\alpha]^{25}_D$ −38° (*c* 0.5, $CHCl_3$); physical and spectral data were in full agreement with those of the natural product **4**.

Single-Crystal X-ray Crystallography of 1.[15] Suitable colorless prisms of **1** were obtained from a pyridine solution. The crystal (0.40 × 0.50 × 0.55 mm) belongs to the orthorhombic system, space group $P2_12_12_1$ (No. 19) with a = 8.549(4) Å, b = 19.241(4) Å, c = 19.674(5) Å, V = 3236(1) Å^3, Z = 4, D_{calcd} = 1.253 g/cm^3, λ(Mo Kα) = 0.71073 Å. Intensity data were measured on a Rigaku AFC6S diffractometer up to 2θ of 50.0°. All 3268 unique reflections were collected. The structure was solved by direct methods and refined by a full-matrix least-squares procedure. All atoms were given isotropic thermal parameters. The *n*-butyryloxy group at the C-4 position was disordered (C21−C24 and O5, see details in Table 5) and was only partially resolved. The refinement converged to a final R = 0.108, R_w = 0.081 for 1338 observed reflections [$I > 3.00\sigma(I)$] and 177 variable parameters.

Single-Crystal X-ray Crystallography of 2.[15] Suitable colorless prisms of **2** were obtained from a pyridine solution. The crystal (0.24 × 0.30 × 0.58 mm) belongs to the orthorhombic system, space group $P2_12_12_1$ (No. 19) with a = 17.145(3) Å, b = 18.173(5) Å, c = 10.423(3) Å, V = 3248(1) Å^3, Z = 4, D_{calcd} = 1.249 g/cm^3, λ(Mo Kα) = 0.71073 Å. Intensity data were measured on a Rigaku AFC6S diffractometer up to 2θ of 50.0°. All 3246 unique reflections were collected. The structure was solved by direct methods and refined by a full-matrix least-squares procedure. The non-hydrogen atoms were given anisotropic thermal parameters, except for the *n*-butyryloxy group at C-4 position that is slightly disordered (C22−C24, see details in Table 6). The refinement converged to a final R = 0.050, R_w = 0.050 for 1631 observed reflections [$I > 3.00\sigma(I)$] and 389 variable parameters.

Cytotoxicity Testing. The P-388 cell line was kindly provided by Prof. J. M. Pezzuto, University of Illinois at Chicago; A549 and HT-29 cells were purchased from the American Type Culture Collection. The cytotoxic activities of compounds **1**−**4** against the above three cancer cells were measured via a modification of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. Cytotoxicity assays were carried out by Dr. C.-Y. Duh according to the procedures described previously.[16,17]

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Supporting Information Available: X-ray structure report of briaexcavatolide P. This material is available free of charge via the Internet at http://pubs.acs.org.

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