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Briaexcavatins C–F, four new briarane-related diterpenoids from the Formosan octocoral *Briareum excavatum* (Briareidae)

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Abstract—Four new briarane-related diterpenoids, designated as briaexcavatins C–F (**1**–**4**), were isolated from the Formosan octocoral *Briareum excavatum*, collected off southern Taiwan coast. The structures of these new metabolites were elucidated by the interpretation of spectroscopic and chemical methods. The configuration of **1** was further supported by molecular mechanics calculations. Briarane **1** has been shown to exhibit mild cytotoxicity toward MDA-MB-231 human breast tumor cells and briarane **3** was found to show activity to inhibit neutrophil elastase release in humans.
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1. Introduction

Octocorals belonging to the genus *Briareum* (=*Solenopodium*) (Briareidae) are organisms taxonomically placed within the order Alcyonacea and Gorgonacea.[1,2] These organisms inhabit abundantly in the coral reefs of tropical and subtropical Indo-Pacific Ocean and Caribbean Sea and have been recognized as rich sources for marine natural products with novel structural features.[3–5] Since the first briarane-type natural product, briarein A, was obtained from the Caribbean octocoral *Briareum asbestinum* in 1977,[6] a number of briarane derivatives have been isolated from various marine organisms.[3,5] Briarane-related natural products continue to attract the attentions of investigators because of the structural complexity and interesting biological activity associated with numerous compounds of this type.[3,5] In previous studies, 64 new briaranes, including stecholides I–N and 16-hydroxy-stecholide C acetate,[7] excavatolides A–Z,[8] briaexcavatolides A–Z,[9] briantheins A–C,[10] and briaexcavatins A and B,[11] have been isolated from the octocoral *Briareum excavatum* (Nutting, 1911). During our continuing studies on the chemical constituents of *B. excavatum*, four new diterpenoids, briaexcavatins C–F (**1**–**4**), were isolated. We describe herein the isolation, structure elucidation, and biological activity of these new metabolites.

2. Results and discussion

Specimens of the octocoral *B. excavatum*, collected at southern Taiwan coast, were minced and extracted with EtOAc. The extract was separated on silica gel column chromatography to afford briaranes **1**–**4**. Briaexcavatin C (**1**) was obtained as a white powder. The HRESIMS data recorded at *m/z* 549.2340 established the molecular formula of **1** as $C_{28}H_{36}O_{11}$ (calcd for $C_{28}H_{36}O_{11}$+H, 549.2336). Thus, 11 degrees of unsaturation were determined for **1**. The IR spectrum showed bands at 1792, 1744, and 1688 cm^{-1}, consistent with the presence of γ-lactone, ester, and conjugated ketone groups in **1**. The conjugated ketone group was further confirmed by ^{13}C NMR signals at δ 200.7 (s), 154.5 (d), and 126.4 (d) (Table 1). The FABMS of **1** exhibited peaks at *m/z* 549 $(M+H)^+$, 489 $(M+H-AcOH)^+$, 429 $(M+H-2AcOH)^+$, and 341 $(M+H-C_3H_7CO_2H-2AcOH)^+$, which suggested the presence of a butyryloxy and two acetoxy groups. In the ^{13}C NMR spectrum, five carbonyl resonances appeared at δ 200.7 (s), 172.4 (s), 170.3 (2×s), and 169.0 (s),

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Table 1. ^{13}C NMR data for diterpenoids **1**–**4**

Carbon	**1**[a]	**2**[b]	**3**[c]	**4**[b]
1	43.3 (s)[d]	43.7 (s)	44.1 (s)	43.6 (s)
2	80.7 (d)	81.0 (d)	81.4 (d)	81.3 (d)
3	70.5 (d)	73.2 (d)	73.5 (d)	72.9 (d)
4	33.3 (t)	33.6 (t)	33.9 (t)	33.9 (t)
5	59.5 (s)	138.9 (s)	139.2 (s)	139.1 (s)
6	62.8 (d)	121.6 (d)	122.0 (d)	121.6 (d)
7	76.5 (d)	73.7 (d)	74.0 (d)	73.7 (d)
8	68.8 (s)	68.6 (s)	69.0 (s)	68.7 (s)
9	66.2 (d)	65.8 (d)	66.2 (d)	65.9 (d)
10	41.8 (d)	39.7 (d)	40.1 (d)	39.7 (d)
11	40.0 (d)	32.2 (d)	32.6 (d)	32.1 (d)
12	200.7 (s)	69.2 (d)	69.2 (d)	69.2 (d)
13	126.4 (d)	26.9 (t)	27.2 (t)	26.9 (t)
14	154.5 (d)	80.6 (d)	80.9 (d)	80.4 (d)
15	17.5 (q)	18.0 (q)	18.3 (q)	17.9 (q)
16	21.5 (q)	22.2 (q)	22.5 (q)	22.3 (q)
17	59.9 (s)	60.0 (s)	60.3 (s)	60.0 (s)
18	10.4 (q)	10.0 (q)	10.3 (q)	10.0 (q)
19	170.3 (s)	171.7 (s)	172.0 (s)	171.6 (s)
20	14.5 (q)	10.2 (q)	10.5 (q)	10.2 (q)
Acetate methyls	21.8 (q)	22.2 (q)	22.6 (q)	22.2 (q)
	21.7 (q)	22.2 (q)	22.5 (q)	22.1 (q)
		21.6 (q)	21.9 (q)	21.6 (q)
		21.0 (q)	21.3 (q)	
Acetate carbonyls	170.3 (s)	171.0 (s)	172.7 (s)	171.3 (s)
	169.0 (s)	170.3 (s)	170.7 (s)	170.9 (s)
		169.5 (s)	169.8 (s)	169.4 (s)
		169.4 (s)	169.7 (s)	
n-Butyrate	13.6 (q)			13.6 (q)
	18.1 (t)			18.1 (t)
	36.0 (t)			35.8 (t)
	172.4 (s)			171.7 (s)
3-Vinylpropionate		115.6 (t)		115.6 (t)
		136.4 (d)		136.4 (d)
		28.5 (t)		28.5 (t)
		33.2 (t)		33.3 (t)
		172.3 (s)		172.3 (s)
Isovalerate			22.7 (2×q)	
			25.8 (d)	
			43.7 (t)	
			171.3 (s)	

[a] Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C.
[b] Spectra recorded at 150 MHz in $CDCl_3$ at −20 °C.
[c] Spectra recorded at 100 MHz in $CDCl_3$ at −20 °C.
[d] Multiplicity deduced by DEPT and indicated by usual symbols. The values are downfield in parts per million from TMS.

supporting the presence of a ketone, a lactone, and three esters. In the ^{1}H NMR spectrum (Table 2), an *n*-butyryloxy group (δ 2.24, 2H, t, J=7.2 Hz; 1.66, 2H, m; 0.95, 3H, t, J=7.2 Hz) and two acetate methyls (δ 2.25, 3H, s; 2.23, 3H, s) were further observed. Thus, the ^{13}C NMR data accounted for six degrees of unsaturation and required **1** to be pentacyclic. The presence of a tetrasubstituted epoxide containing a methyl substituent was elucidated from the signals of two oxygen-bearing quaternary carbons at δ 68.8 (s, C-8) and 59.9 (s, C-17), and further confirmed from the proton signal of a methyl singlet resonating at δ 1.64 (3H, s, H_3-18). In addition, a trisubstituted epoxide containing a methyl substituent was deduced from the signals of an oxymethine (δ_H 3.06, 1H, d, J=8.0 Hz, H-6; δ_C 62.8, d, C-6), an oxygen-bearing quaternary carbon (δ 59.5, s, C-5), and a methyl singlet resonating at δ 1.34 (3H, s, H_3-16). Moreover, a methyl doublet (δ 1.27, 3H, d, J=7.2 Hz, H_3-20), a methyl singlet (δ 1.08, 3H, s, H_3-15), two conjugated olefin protons (δ 6.62, 1H, d, J=10.4 Hz, H-14; 6.19, 1H, d, J=10.4 Hz, H-13), two aliphatic methine protons (δ 2.94, 1H, dd, J=9.6, 4.8 Hz, H-10; 2.93, 1H, m, H-11), a pair of methylene protons (δ 2.48, 1H, dd, J=16.0, 6.4 Hz; 2.11, 1H, d, J=16.0 Hz, H_2-4), and five oxygenated methine protons (δ 5.27, 1H, br s, H-2; 5.24, 1H, d, J=9.6 Hz, H-9; 5.08, 1H, d, J=6.4 Hz, H-3; 4.43, 1H, d, J=8.0 Hz, H-7; 3.06, 1H, d, J=8.0 Hz, H-6) were further assigned by the assistance of ^{1}H–^{1}H COSY and HSQC spectrum. From the ^{1}H–^{1}H COSY and HMBC correlations, the epoxy groups positioned at C-5/C-6 and C-8/C-17, acetoxy groups positioned at C-2 and C-9, and an *n*-butyryloxy group positioned at C-3, were established (Fig. 1).

The relative stereochemistry of **1** was elucidated from the NOE interactions observed in an NOESY experiment (Fig. 2) and by the vicinal ^{1}H–^{1}H coupling constants. As per convention while analyzing the stereochemistry of briarane-type diterpenoids, H-10 and H_3-15 were assigned to the α and β face, anchoring the stereochemical analysis because no NOE correlation was found between H-10 and H_3-15. In the NOESY experiment of **1**, H-10 gives NOE correlations to H-3 and H-11, and H-3 was found to show responses with H-2 and one proton of the C-4 methylene (δ 2.11), indicating that these protons (H-2, H-3, H-4α, H-10, and H-11) are located on the same face of the molecule and therefore are assigned as α protons, as the C-15 methyl is the β-substituent at C-1. Furthermore, H_3-16 exhibited NOE correlations with H-4α and H-6, but not with H-7, suggesting that H_3-16 and H-6 were positioned on the α face in the epoxy group and H-7 was β-oriented in the 10-membered ring. H-9 was found to show NOE correlations with H-10, H-11, and H_3-18. From the detailed consideration of molecular models, H-9 was found to be reasonably close to H-10, H-11, and H_3-18, while H-9 should be placed on the α face in **1**, and H_3-18 was β-oriented in the γ-lactone ring. The cis geometry of the C-13/C-14 double bond was indicated by a 10.4 Hz coupling constant between H-13 (δ 6.19) and H-14 (δ 6.62) and by the NOE correlation between H-13 and H-14. Based on above observations, the configurations of all chiral centers of **1** are assigned as 1*R**,2*R**,3*S**,5*R**,6*S**,7*S**,8*R**,9*S**,10*S**,11*R**,13*Z*,17*R**. Geometrical optimization of **1** was performed with DISCOVER utilizing the consistent valence force field (CVFF) calculations for energy minimization. The calculated results were visualized using INSIGHT II, running on a Silicon Graphics IRIS (SGI) Indigo XS24/4000 workstation. The conformation search suggested that the most stable conformation and the calculated distances of selected key protons of **1** are shown in Table 3.

The new briarane diterpene, briaexcavatin D (**2**), had the molecular formula of $C_{33}H_{44}O_{13}$, as determined by HRESIMS. It was found that the ^{13}C and ^{1}H NMR spectra of **2** in $CDCl_3$ revealed mostly broad peaks when measured at room temperature (25 °C). In order to make more reliable assignments of the NMR signals of briarane **2**, the ^{13}C and ^{1}H NMR spectra of **2** were measured at −20 °C in $CDCl_3$ (Tables 1 and 2). It was found that at this temperature the signals for each proton and carbon of the molecule were sharpened and could be assigned by the assistance of 1D and 2D NMR experiments. By detailed analysis, the NMR data of **2** were very similar to those of a known metabolite, excavatolide C (**5**).[8a] However, the ^{13}C and ^{1}H NMR spectra revealed that the signals corresponding to the hydroxy group in **5** (δ_C 65.8, d, C-12; δ_H

Table 2. ^{1}H NMR data for diterpenoids **1**–**4**

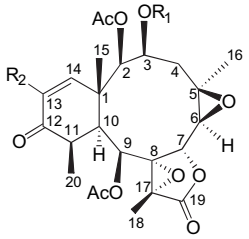
Proton	**1**[a]	**2**[b]	**3**[b]	**4**[b]
2	5.27 br s	5.13 br s	5.17 br s	5.16 br s
3	5.08 d (6.4)[c]	5.69 m	5.72 d (6.8)	5.74 m
4α	2.11 d (16.0)	1.96 m	1.96 m	1.97 m
4β	2.48 dd (16.0, 6.4)	3.67 dd (16.0, 7.2)	3.72 dd (16.0, 6.8)	3.73 dd (15.2, 6.8)
6	3.06 d (8.0)	5.33 d (6.8)	5.37 d (6.4)	5.37 d (6.8)
7	4.43 d (8.0)	5.25 d (6.8)	5.30 d (6.4)	5.29 d (6.8)
9	5.24 d (9.6)	5.28 d (10.4)	5.32 d (10.4)	5.31 d (10.0)
10	2.94 dd (9.6, 4.8)	2.97 dd (10.4, 4.8)	3.01 dd (10.4, 5.2)	3.00 dd (10.0, 6.8)
11	2.93 m	2.57 m	2.59 m	2.60 m
12		5.02 m	5.04 m	5.03 m
13/13′	6.19 d (10.4)	2.29 m; 1.86 m	2.01 m; 1.83 m	2.32 m; 1.87 m
14	6.62 d (10.4)	4.80 br s	4.82 br s	4.85 br s
15	1.08 s	0.78 s	0.82 s	0.83 s
16	1.34 s	1.88 s	1.92 s	1.93 s
18	1.64 s	1.55 s	1.58 s	1.59 s
20	1.27 d (7.2)	1.00 d (6.8)	1.04 d (7.2)	1.95 d (7.2)
Acetate methyls	2.25 s	2.43 s	2.37 s	2.37 s
	2.23 s	2.21 s	2.24 s	2.25 s
		2.13 s	2.16 s	2.17 s
		1.90 s	1.93 s	
n-Butyrate	2.24 t (7.2)			2.33 t (7.2)
	1.66 m			1.57 m
	0.95 t (7.2)			0.88 t (7.2)
3-Vinylpropionate		5.77 m		5.78 m
		5.02 m		5.03 m
		2.35 m		2.36 m
		2.30 t (7.2)		2.36 t (7.2)
Isovalerate			0.89 d (2×3H, 6.8)	
			2.03 m	
			2.12 d (7.2)	

[a] Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C.
[b] Spectra recorded at 400 MHz in $CDCl_3$ at −20 °C.
[c] *J* value (in Hz) in parentheses. The values are downfield in parts per million from TMS.

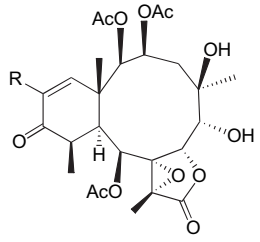
3.89, 1H, m, H-12) were replaced by those of a 3-vinylpropionyloxy group (δ_C 172.3, s; 136.4, d; 115.6, t; 33.2, t; 28.5, t; δ_H 5.77, 1H, m; 5.02, 2H, m; 2.35, 2H, m; 2.30, 2H, t, *J*=7.2 Hz) in **2**. In the HMBC experiment of **2**, the carbon signal at δ 172.3 (s), which showed a correlation with H-12 (δ 5.02) was found to be correlated with the signal of the methylene protons at δ 2.30, and was consequently assigned as the carbon atom of the 3-vinylpropionate carbonyl. Thus, the 3-vinylpropionate ester should be positioned at C-12 in **2**. Furthermore, vinylpropionylation of **5** gave a less polar product, which was found to be identical with natural product **2** by comparison of the physical and spectral data, and confirmed the structure of diterpenoid **2**.

Briaexcavatin E (**3**) had the molecular formula of $C_{33}H_{46}O_{13}$ as determined by HRESIMS, with 11 degrees of unsaturation thereby being determined for the molecule. The IR absorptions of **3** showed the presence of γ-lactone (ν_{max} 1787 cm^{-1}) and ester carbonyl groups (ν_{max} 1742 cm^{-1}). Like as those of **2**, the sharpened ^{13}C and ^{1}H NMR signals of **3** (Tables 1 and 2) were also obtained in $CDCl_3$ at −20 °C. Carbonyl resonances in the ^{13}C NMR spectrum of **3** at δ 172.7 (s), 172.0 (s), 171.3 (s), 170.7 (s), 169.8 (s), and 169.7 (s) confirmed the presence of a γ-lactone and five other esters in the molecule. In the ^{1}H NMR spectrum, four acetate methyls were observed at δ 2.37 (3H, s), 2.24 (3H, s), 2.16 (3H, s), and 1.93 (3H, s). The additional acyl group was found to be an isovaleryl group, which showed nine contiguous protons (δ 2.12, 2H, t, *J*=7.2 Hz; 2.03, 1H, m; 0.89, 2×3H, d, *J*=6.8 Hz) were observed. The ^{13}C NMR signal appeared at δ 171.3 (s) correlated with the signal of the methylene protons at δ_H 2.12 in the HMBC spectrum and was consequently assigned as the carbon atom of the isovalerate carbonyl. The ^{1}H–^{13}C long-range correlations observed in an HMBC experiment of **3** further revealed the connectivity between H-12 (δ 5.04) and the carbonyl carbon (δ 171.3) of the isovalerate unit and demonstrated the location of the isovalerate to be at C-12. The positions of the other four acetoxy groups at C-2, C-3, C-9, and C-14 were also confirmed by the connectivities between the four oxymethine protons at δ 5.17 (H-2), 5.72 (H-3), 5.32 (H-9), 4.82 (H-14) and the four ester carbonyls (δ 172.7, s; 170.7, s; 169.8, s; 169.7, s) in the HMBC spectrum of **3**. Moreover, isovalerylation of excavatolide C (**5**)[8a] yielded a compound that was found to be identical with diterpenoid **3** by physical and spectral data comparison.

Our present study has also led to the isolation of the new briarane, briaexcavatin F (**4**). The molecular formula of $C_{35}H_{48}O_{13}$ was deduced from HRESIMS with *m/z* 699.2996 (calcd for $C_{35}H_{48}O_{13}$+Na, 699.2993). This showed that briarane **4** contained 12 degrees of unsaturation. From detailed analysis, the NMR data of **4** (measured in $CDCl_3$ at −20 °C) (Tables 1 and 2) were found to be close to those of **2** and the known metabolite, excavatolide B (**6**),[8a] and showed the presence of a γ-lactone, an *n*-butyryloxy, a 3-vinylpropionyloxy, and three acetoxy groups. Comparison of the ^{1}H and ^{13}C NMR spectral data of **4** with those of



1: $R_1 = CO(CH_2)_2CH_3$, $R_2 = H$
10: $R_1 = Ac$, $R_2 = OH$ (revised)
11: $R_1 = Ac$, $R_2 = H$ (revised)



7: R = OH
8: R = H

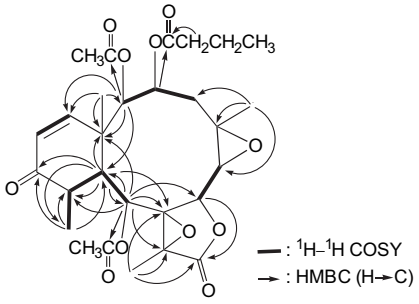
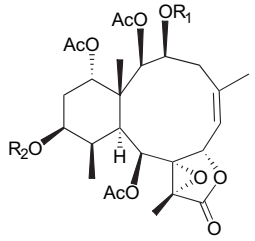
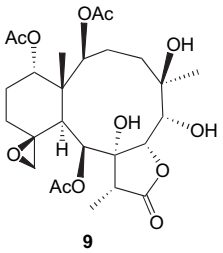


Figure 1. The ^{1}H–^{1}H COSY and selective HMBC correlations of **1**.



2: $R_1 = Ac$, $R_2 = CO(CH_2)_2CH{=}CH_2$
3: $R_1 = Ac$, $R_2 = COCH_2CH(CH_3)_2$
4: $R_1 = CO(CH_2)_2CH_3$, $R_2 = CO(CH_2)_2CH{=}CH_2$
5: $R_1 = Ac$, $R_2 = H$
6: $R_1 = CO(CH_2)_2CH_3$, $R_2 = H$



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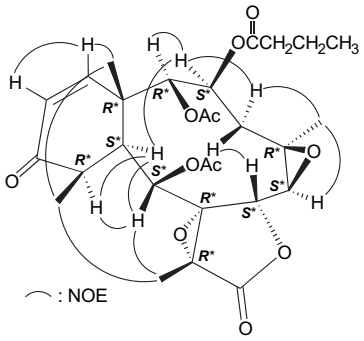


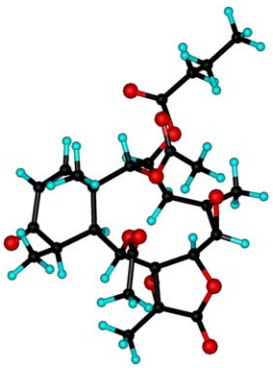
Figure 2. Selective NOESY correlations of **1**.

2 showed that the acetoxy group attached to C-3 in **2** could be replaced by an *n*-butyryloxy group in **4**. These observations were further confirmed by the correlations observed in the 2D NMR experiments of **4** including ^{1}H–^{1}H COSY, HSQC, and HMBC spectra. Similar to that of briarane **2**, vinylpropionylation of **6** gave a less polar product, which was found to be identical with natural product **4** by comparison of the physical and spectral data. Since the absolute configuration of the known briarane, excavatolide B (**6**), had been determined by modified Mosher's method,[11] we were able to assign the absolute configurations of all the chiral centers of **4** as 1*R*,2*R*,3*S*,5*Z*,7*S*,8*S*,9*S*,10*S*,11*R*,12*S*,14*S*,17*R*. Based on above findings, the structure of **4** was established unambiguously.

In a previous study, we reported the isolation and structure elucidation of two 5,6-dihydroxybriarane metabolites, briaexcavatolides X (**7**) and Y (**8**).[9f] However, based on the detailed spectral data analysis and by comparing the ^{1}H and ^{13}C NMR chemical shifts of C-5 and C-6 in briaranes **7** (δ_C 59.3, s, C-5; 62.7, d, C-6; δ_H 3.05, 1H, d, J=8.5 Hz, H-6) and **8** (δ_C 59.4, s, C-5; 62.7, d, C-6; δ_H 3.06, 1H, d, J=8.5 Hz, H-6) with those of briarane **1** (δ_C 59.5, s, C-5; 62.8, d, C-6; δ_H 3.06, 1H, d, J=8.0 Hz, H-6) and a known 5,6-dihydroxybriarane, junceellolide L (**9**) (δ_C 89.6, s, C-5; 82.7, d, C-6; δ_H 4.22, 1H, br s, H-6),[12] the 5,6-dihydroxy groups in briaranes **7** and **8** should be revised as 5β,6β-epoxy groups as presented in briaranes **10** and **11**, respectively. The spectral data (IR and MS) for briaranes **10** (briaexcavatolide X) and **11** (briaexcavatolide Y) are reassigned in this study (see Section 3).

In the biological activity testing, briaexcavatin C (**1**) exhibited mild cytotoxicity toward MDA-MB-231 human breast tumor cells (IC_{50}=17.50 μg/mL) and briaexcavatin

Table 3. The stereoview of **1** (generated from computer modeling) and the calculated distances (Å) between selected protons having key NOE correlations[a]

Briaexcavatin C (**1**)	H/H	(Å)
	H-2/H-3	2.44
	H-3/H-10	3.09
	H-3/H-4α	2.24
	H-4α/H_3-16	2.57
	H-6/H_3-16	2.40
	H-9/H-10	2.73
	H-9/H-11	2.41
	H-9/H_3-18	2.37
	H-10/H-11	2.28
	H-13/H-14	2.38

[a] The calculated distance between H-10 (α) and H_3-15 (β) is 3.91 Å.

Table 4. Inhibitory effects of briarane **3** on superoxide generation and elastase release by human neutrophil in response to fMet-Leu-Phe/cytochalasin B[a]

Compound	Concn (μM)	Superoxide generation (%)	Elastase release (%)
3	3	—	87.77±5.86
	5	—	65.96±9.94
	10	101.19±4.15	37.89±13.53

[a] Data obtained without any drugs were set to 100%. Means±SEM of three separate experiments are shown.

E (**3**) was found to show the activity to inhibit human neutrophil elastase (HNE) release but not superoxide anion generation (Table 4). To the best of our knowledge, briaexcavatin E (**3**) is the first briarane-type natural product reported to possess the activity to inhibit HNE release.

3. Experimental

3.1. General experimental procedures

Optical rotation values were measured with a JASCO P-1010 digital polarimeter at 25 °C. Infrared spectra were obtained on a VARIAN DIGLAB FTS 1000 FTIR spectrometer. EIMS and FABMS were obtained with a VG QUATTRO GC/MS spectrometer. HRMS data were recorded by ESI FT-MS on a BRUKER APEX II mass spectrometer or by EI on a JEOL JMS-700 mass spectrometer. ^{1}H NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR at 400 MHz and ^{13}C NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR at 100 MHz or on a VARIAN UNITY INOVA 600 FT-NMR at 150 MHz, in $CDCl_3$ using TMS as an internal standard. Column chromatography was performed on silica gel 60 (230–400 mesh) (Merck, Darmstadt, Germany). TLC spots (silica gel 60 F_{254}, Merck) were detected with an UV_{254} lamp and by 10% H_2SO_4 followed by heating at 120 °C for 5 min. All solvents and reagents used were analytical grade.

3.2. Animal material

Specimen of the octocoral *B. excavatum* was collected by hand using scuba off the coast of southern Taiwan in October 2003, at a depth of −10 m. Live reference specimens are being maintained in the authors' marine organism cultivating tanks and a voucher specimen was deposited in the National Museum of Marine Biology and Aquarium (NMMBA).

3.3. Extraction and isolation

The organism (wet weight 1.0 kg) was collected and freeze-dried. The freeze-dried material (0.57 kg) was minced and extracted with EtOAc. The extract was separated by silica gel column chromatography, using *n*-hexane and *n*-hexane–EtOAc mixtures of increased polarity. Briarane **3** was eluted with *n*-hexane–EtOAc (6:1→5:1), **4** with *n*-hexane–EtOAc (5:1), **2** with *n*-hexane–EtOAc (4:1), and **1** with *n*-hexane–EtOAc (5:2).

3.3.1. Briaexcavatin C (1). White powder (2.1 mg); 79–81 °C; $[\alpha]_D^{25}$ −25 (*c* 0.40, $CHCl_3$); IR (neat) ν_{max} 1792, 1744, 1688 cm^{-1}; ^{13}C ($CDCl_3$, 100 MHz) and ^{1}H ($CDCl_3$, 400 MHz) NMR data, see Tables 1 and 2; FABMS *m/z* 549 $(M+H)^+$, 489, 429, 341; HRESIMS *m/z* 549.2340 (calcd for $C_{28}H_{36}O_{11}$+H, 549.2336).

3.3.2. Briaexcavatin D (2). Colorless gum (2.4 mg); $[\alpha]_D^{25}$ +32 (*c* 0.12, CH_2Cl_2); IR (neat) ν_{max} 1792, 1737 cm^{-1}; ^{13}C ($CDCl_3$, 150 MHz) and ^{1}H ($CDCl_3$, 400 MHz) NMR data, see Tables 1 and 2; FABMS *m/z* 671 $(M+Na)^+$, 649, 589, 529, 489, 469, 429, 369, 309; HRESIMS *m/z* 671.2684 (calcd for $C_{33}H_{44}O_{13}$+Na, 671.2680).

3.3.3. Briaexcavatin E (3). White powder (2.2 mg); 251–252 °C; $[\alpha]_D^{29}$ +42 (*c* 0.40, $CHCl_3$); IR (neat) ν_{max} 1787, 1742 cm^{-1}; ^{13}C ($CDCl_3$, 100 MHz) and ^{1}H ($CDCl_3$, 400 MHz) NMR data, see Tables 1 and 2; FABMS *m/z* 673 $(M+Na)^+$, 651, 591, 549, 489, 471, 429, 369, 309; HRESIMS *m/z* 673.2834 (calcd for $C_{33}H_{46}O_{13}$+Na, 673.2836).

3.3.4. Briaexcavatin F (4). Colorless gum (1.7 mg); $[\alpha]_D^{25}$ +46 (*c* 0.09, CH_2Cl_2); IR (neat) ν_{max} 1787, 1735 cm^{-1}; ^{13}C ($CDCl_3$, 150 MHz) and ^{1}H ($CDCl_3$, 400 MHz) NMR data, see Tables 1 and 2; FABMS *m/z* 699 $(M+Na)^+$, 677 $(M+H)^+$, 617, 589, 557, 529, 517, 469, 409, 369, 309; HRESIMS *m/z* 699.2996 (calcd for $C_{35}H_{48}O_{13}$+Na, 699.2993).

3.3.5. Briaexcavatolide X (10). IR (neat) ν_{max} 3472, 1792, 1748, 1703 cm^{-1}; EIMS *m/z* 537 $(M+H)^+$, 459 $(M+H-H_2O-AcOH)^+$, 417 $(M+H-2AcOH)^+$, 399 $(M+H-H_2O-2AcOH)^+$, 357 $(M+H-3AcOH)^+$, 339 $(M+H-H_2O-3AcOH)^+$; HREIMS *m/z* 536.1894 (calcd for $C_{26}H_{32}O_{12}$, 536.1894).

3.3.6. Briaexcavatolide Y (11). IR (neat) ν_{max} 1792, 1744, 1686 cm^{-1}; FABMS *m/z* 521 $(M+H)^+$, 461 $(M+H-AcOH)^+$, 401 $(M+H-2AcOH)^+$, 341 $(M+H-3AcOH)^+$; HRESIMS *m/z* 543.1844 (calcd for $C_{26}H_{32}O_{11}$+Na, 543.1842).

3.4. Vinylpropionylation of excavatolide C (5)

Excavatolide C (**5**) (5.0 mg) was stirred with 2 mL of 3-vinylpropionic anhydride (pent-4-enoic anhydride) in 2 mL of pyridine for 96 h at room temperature. After evaporation of excess reagent, the residue was separated by column chromatography on silica gel to give pure briaexcavatin D (**2**) (*n*-hexane–EtOAc, 4:1, 4.0 mg, 70%); physical (R_f and optical rotation values) and NMR data were in full agreement with those of natural product **2**.

3.5. Isovalerylation of excavatolide C (5)

Excavatolide C (**5**) (5.0 mg) was stirred with 2 mL of isovaleric anhydride in 2 mL of pyridine for 96 h at room temperature. After evaporation of excess reagent, the residue was separated by column chromatography on silica gel to give pure briaexcavatin E (**3**) (*n*-hexane–EtOAc, 6:1→5:1, 3.9 mg, 68%); physical (mp, R_f, and optical rotation values) and NMR data were in full agreement with those of natural product **3**.

3.6. Vinylpropionylation of excavatolide B (6)

Excavatolide B (**6**) (8.0 mg) was stirred with 2.5 mL of 3-vinylpropionic anhydride (pent-4-enoic anhydride) in

2.5 mL of pyridine for 96 h at room temperature. After evaporation of excess reagent, the residue was separated by column chromatography on silica gel to give pure briaexcavatin F (**4**) (*n*-hexane–EtOAc, 5:1, 6.6 mg, 72%); physical (R_f and optical rotation values) and NMR data were in full agreement with those of natural product **4**.

3.7. Molecular mechanics calculations

The minimum energy conformation of briaexcavatin C (**1**) was determined using the MSI Insight II/DISCOVER version 95 molecular modeling package incorporating an empirical force field, the consistent valence force field (CVFF),[13] on Silicon Graphic IRIS (SGI) Indigo XS24/4000 workstation. All force field calculations were carried out in vacuo (dielectric constant=1). The conformational space of **1** was scanned using the high-temperature molecular dynamics simulation technique followed by energy minimization. A 100 ps molecular dynamics simulation at 1000 K provided a set of 500 conformations of **1**. Each conformation was used as a starting structure for the subsequent energy minimization (1000 steps, conjugated gradient algorithm). In the subsequent analysis, only 10 conformations with a reasonably low energy (at most 5 kcal/mol higher with respect to the lowest energy conformer) were used. The conformational search suggested that the most stable conformation of briarane **1** shown in Table 3 is the lowest.

3.8. Cytotoxicity assays

Compounds were assayed for cytotoxicity against MDA-MB-231 cells using the MTT method. Freshly trypsinized cell suspensions were seeded in 96-well microtitre plates at densities of 5000–10,000 cells per well with tested compounds added from DMSO-diluted stock. After 3 days in culture, attached cells were incubated with MTT (0.5 mg/mL, 1 h) and subsequently solubilized in DMSO. The absorbency at 550 nm was then measured using a microplate reader. The IC_{50} is the concentration of agent that reduced cell growth by 50% under the experimental conditions.

3.9. Human neutrophil superoxide generation and elastase release

Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Superoxide generation and elastase release were carried out according to the procedures described previously.[14,15] Briefly, superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*. Elastase release experiments were performed using MeO-Suc-Ala-Ala-Pro-Valp-nitroanilide as the elastase substrate.

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