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Tetrahedron 64 (2008) 2596–2604
Tetrahedron
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New briaranes from the octocorals *Briareum excavatum* (Briareidae) and *Junceella fragilis* (Ellisellidae)

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Received 14 December 2007; received in revised form 7 January 2008; accepted 7 January 2008
Available online 11 January 2008

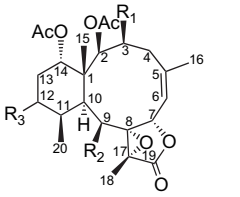
Abstract

Six 12-hydroxybriaranes, including four new diterpenoids, briaexcavatins I–L (**1**–**4**), and two known metabolites, excavatolides C (**5**) and E (**6**), have been isolated from the cultured scleraxonia *Briareum excavatum*. In addition, the gorgonian coral *Junceella fragilis* yielded a new chlorinated briarane, fragilide C (**10**). The structures of above compounds were determined by spectroscopic methods and the structures of **5** and **6** were further confirmed by X-ray data analysis for the first time. The absolute configuration of **6** was elucidated by chemical conversion. Some of these briaranes have displayed inhibitory effects on superoxide anion generation by human neutrophils.

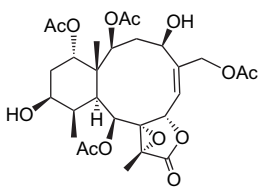
Keywords: Briarane; *Briareum excavatum*; Briaexcavatin; *Junceella fragilis*; Fragilide; Superoxide anion

1. Introduction

In our continuing research on novel natural substances obtained from the marine invertebrates of Taiwanese waters, a series of interesting terpenoids and steroids have been isolated from the octocorals *Alcyonium* sp.,[1,2] *Briareum* sp.,[3] *Briareum excavatum*,[4–7] *Ellisella robusta*,[8–11] *Junceella fragilis*,[12–19] *Junceella juncea*,[14,20] *Rumphella antipathies*,[21–26] and the tunicate *Eudistoma* sp.[27] In this paper, we report the isolation, structure determination, and biological activity of four new briaranes, briaexcavatins I–L (**1**–**4**), along with two known compounds, excavatolides C (**5**) and E (**6**),[28] from the cultured scleraxonia *B. excavatum* (Briareidae). In addition, a new chlorinated briarane metabolite, fragilide C (**10**), was obtained from the gorgonian *J. fragilis* (Ellisellidae). The structures of compounds **1**–**6** and **10** were established by extensive spectral data analysis; the structures of briaranes **5** and **6** were further determined by X-ray analysis and the absolute configuration of **6** was established by chemical methods. Some of these



1: R_1 = H, R_2 = OAc, R_3 = β-OH
5: R_1 = R_2 = OAc, R_3 = β-OH
6: R_1 = H, R_2 = OH, R_3 = β-OH
7: R_1 = H, R_2 = OAc, R_3 = α-OH



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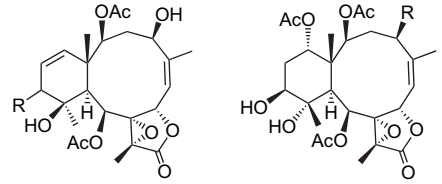
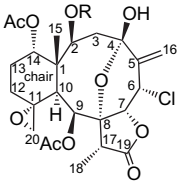
0040-4020/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2008.01.023



3: R = α-OH
8: R = β-OCO(CH$_2$)$_2$CH$_3$

4: R = OH
9: R = OAc



10: R = COCH$_2$CH$_3$
11: R = Ac

briaranes exhibited inhibitory effects on superoxide anion generation by human neutrophils.

2. Results and discussion

2.1. Isolation and structure determination of briaranes from *B. excavatum*

The briaexcavatins and excavatolides were isolated by conventional methods as outlined in Section 3. Briaexcavatin I (**1**) was obtained as a white powder and the molecular formula of **1** was determined to be $C_{26}H_{36}O_{10}$ (nine degrees of unsaturation) by analysis of ^{13}C and ^{1}H NMR data (Tables 1 and 2) in conjunction with DEPT results; this conclusion was further confirmed by HRESIMS (*m/z* calcd: 531.2206; found: 531.2209 $[M+Na]^+$). Comparison of the ^{1}H NMR and DEPT data with the molecular formula indicated that there must be an exchangeable proton, requiring the presence of a hydroxy group, and this deduction was supported by a broad absorption in the IR spectrum at 3497 cm^{-1}. The IR spectrum of **1** also showed strong bands at 1775 and 1742 cm^{-1}, consistent with the presence of γ-lactone and ester groups, respectively. From the ^{13}C NMR data of **1** (Table 1), the presence of a trisubstituted olefin group was deduced from the signals of two carbons resonating at δ 145.1 (s, C-5) and 118.3 (d, CH-6), and was further supported by an olefin proton signal at δ 5.23 (1H, d, *J*=8.4 Hz, H-6) in the ^{1}H NMR spectrum of **1** (Table 2). An 8,17-epoxide group was confirmed from the signals of two quaternary oxygenated carbons at δ 71.0 (s, C-8) and 65.1 (s, C-17), and from the chemical shift of the C-18 tertiary methyl (δ_H 1.63, 3H, s; δ_C 11.0, q). Moreover, four carbonyl resonances appeared at δ 170.6 (s, C-19), 170.5 (s, ester carbonyl), 170.4 (s, ester carbonyl), and 168.2 (s, ester carbonyl), confirming the presence of a γ-lactone and three ester groups in **1**. In the ^{1}H NMR spectrum of **1**, three acetate methyls (δ 2.22, 3H, s; 2.01, 3H, s; 1.99, 3H, s) were observed. Thus, from the NMR data, five degrees of unsaturation were accounted for and **1** must be tetracyclic. In addition, a vinyl methyl (δ 2.00, 3H, s, H_3-16), a methyl singlet (δ 1.19, 3H, s, H_3-15), a methyl doublet (δ 1.04, 3H, d, *J*=7.2 Hz, H_3-20), two aliphatic protons (δ 2.40, 1H, d, *J*=4.0 Hz, H-10; 2.05, 1H, m, H-11), five oxymethine protons (δ 5.17, 1H, d, *J*=8.4 Hz, H-7; 5.05, 1H, d, *J*=7.6 Hz, H-2; 4.97, 1H, br s, H-9; 4.79, 1H, dd, *J*=3.2, 2.8 Hz, H-14; 4.06, 1H, m, H-12), and three pairs of aliphatic methylene protons (δ 2.58, 1H, td, *J*=14.8, 5.6 Hz, H-3β; 1.60, 1H, m, H-3α; 2.47, 1H, br d, *J*=14.0 Hz, H-4β; 1.94, 1H, td, *J*=14.8, 4.4 Hz, H-4α; 1.82, 2H, m, H_2-13) were observed in the ^{1}H NMR spectrum of **1**.

Table 1
^{13}C NMR data for diterpenoids **1**–**4**[a]

Position	1	2	3	4
1	45.7 (s)[b]	45.6 (s)	45.4 (s)	47.9 (s)
2	75.1 (d)	74.3 (d)	77.8 (d)	74.2 (d)
3	31.7 (t)	39.5 (t)	41.4 (t)	40.3 (t)
4	28.5 (t)	69.2 (d)	71.2 (d)	70.8 (d)
5	145.1 (s)	145.5 (s)	147.3 (s)	146.2 (s)
6	118.3 (d)	125.0 (d)	122.2 (d)	122.2 (d)
7	74.8 (d)	73.3 (d)	73.5 (d)	73.6 (d)
8	71.0 (s)	70.7 (s)	70.3 (s)	70.6 (s)
9	74.1 (d)	73.6 (d)	67.1 (d)	67.2 (d)
10	41.3 (d)	41.5 (d)	43.8 (d)	48.9 (d)
11	44.5 (d)	44.6 (d)	75.3 (s)	78.2 (s)
12	66.8 (d)	66.8 (d)	71.8 (d)	73.3 (d)
13	28.9 (t)	28.9 (t)	124.0 (d)	30.2 (d)
14	76.1 (d)	76.0 (d)	139.0 (d)	74.9 (d)
15	15.4 (q)	15.5 (q)	17.8 (q)	14.4 (q)
16	27.2 (q)	68.2 (t)	25.8 (q)	25.3 (q)
17	65.1 (s)	65.1 (s)	62.7 (s)	66.4 (s)
18	11.0 (q)	11.0 (q)	9.6 (q)	10.3 (q)
19	170.6 (s)	170.3 (s)	171.1 (s)	170.3 (s)
20	9.0 (q)	9.0 (q)	28.1 (q)	17.0 (q)
Acetate methyls	21.5 (q)	21.4 (q)	21.7 (q)	21.4 (q)
	21.4 (q)	21.4 (q)	21.1 (q)	21.3 (q)
	21.2 (q)	21.1 (q)		21.2 (q)
		21.0 (q)		
Acetate carbonyls	170.5 (s)	171.4 (s)	170.5 (s)	170.2 (s)
	170.4 (s)	170.7 (s)	169.6 (s)	170.2 (s)
	168.2 (s)	170.7 (s)		168.2 (s)
		168.4 (s)		

[a] Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C.
[b] Multiplicity deduced by DEPT and HMQC spectra and indicated by the usual symbol.

The structure and all of the assignments made from the ^{1}H and ^{13}C NMR data of **1** were determined with the assistance of 2D NMR studies. From the $^{1}H-^{1}H$ COSY spectrum of **1** (Fig. 1), it was possible to establish the proton sequences from H-2/H_2-3, H_2-3/H_2-4, H_2-4/H-6 (by allylic coupling), H-6/H-7, and H-9/H-10. These data, together with the HMBC correlations between H-2/C-1, 4, 10; H_2-3/C-1, 4, 5; H_2-4/C-2, 3, 5, 6; H-6/C-4; H-7/C-5, 6; H-9/C-8; and H-10/C-1, 8, 9 (Fig. 1), established the connectivity from C-1 to C-10 within the 10-membered ring. The vinyl methyl attached at C-5 was confirmed by the HMBC correlations between H_3-16/C-4, 5; H_2-4/C-16; and H-6/C-16, and was further confirmed by the allylic coupling between H_3-16/H-6. The methylcyclohexane ring, which is fused to the 10-membered ring at C-1 and C-10, was elucidated by $^{1}H-^{1}H$ COSY correlations from H-10/H-11, H-11/H_3-20, H-11/H-12, H-12/H_2-13, and H_2-13/H-14; and by the HMBC correlations between

Table 2
^{1}H NMR data for diterpenoids **1**–**4**[a]

Position	**1**	**2**	**3**	**4**
2	5.05 d (7.6)[b]	4.80 d (8.0)	4.53 d (6.4)	4.95 d (10.4)
3α	1.60 m	2.02 m	2.08 m	1.98 m
3β	2.58 td (14.8, 5.6)	2.80 dd (15.2, 12.0)	2.77 dd (15.2, 12.4)	2.89 dd (15.6, 12.0)
4α	1.94 td (14.8, 4.4)	4.19 dd (12.0, 4.8)	4.24 dd (12.4, 4.8)	4.13 dd (12.0, 5.2)
4β	2.47 br d (14.0)			
6	5.23 d (8.4)	5.55 d (8.0)	5.50 d (9.6)	5.33 ddd (8.8, 1.6, 1.2)
7	5.17 d (8.4)	5.81 d (8.0)	6.15 d (9.6)	5.81 d (8.8)
9	4.97 br s	4.96 br s	5.80 d (4.4)	5.77 d (1.2)
10	2.40 d (4.0)	2.33 d (3.6)	2.55 d (4.4)	2.12 d (1.2)
11	2.05 m	2.04 m		
12	4.06 m	4.06 m	3.75 d (5.6)	3.71 ddd (11.2, 2.8, 2.8)
13α	1.82 m (2H)	1.82 m (2H)	5.79 dd (10.0, 5.6)	1.67 ddd (14.6, 12.8, 2.0)
13β				2.03 m
14	4.79 dd (3.2, 2.8)	4.83 dd (3.2, 3.2)	5.53 d (10.0)	4.81 dd (2.2, 2.0)
15	1.19 s	1.24 s	1.23 s	1.24 s
16a	2.00 s	4.65 d (14.8)	2.02 s	2.08 d (1.2)
16b		4.75 d (14.8)		
18	1.63 s	1.66 s	1.61 s	1.77 s
20	1.04 d (7.2)	1.04 d (7.2)	1.39 s	1.17 s
Acetate methyls	2.22 s	2.25 s	2.23 s	2.25 s
	2.01 s	2.09 s	2.11 s	2.01 s
	1.99 s	1.98 s		1.99 s
		1.97 s		

[a] Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C.
[b] *J* values (in Hz) in parentheses.

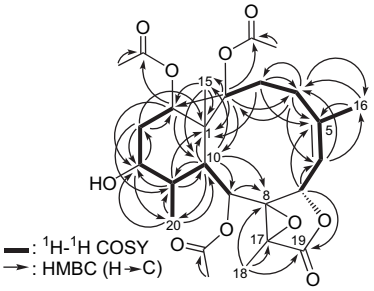


Figure 1. The ^{1}H–^{1}H COSY and HMBC correlations of **1**.

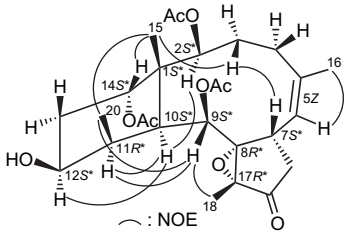


Figure 2. Selective NOESY correlations of **1**.

H-2/C-14; H-10/C-11, 20; H-11/C-1, 10, 12; H-12/C-13, 20; H_2-13/C-1, 11, 12, 14; H-14/C-10, 12; and H_3-20/C-10, 11, 12. The ring junction C-15 methyl group was positioned at C-1 from the HMBC correlations between H_3-15/C-1, 10, 14; H-2/C-15; and H-10/C-15. The HMBC correlations also indicated that the acetoxy groups are attached at C-2 and C-14. The remaining acetoxy and hydroxy groups were positioned at C-9 and C-12, as indicated by analysis of key ^{1}H–^{1}H COSY correlations and characteristic NMR signals. These data, together with the HMBC correlations between H-7/C-19 and H_3-18/C-8, 17, 19, were used to establish the molecular framework of **1**.

Based on previous surveys, all the naturally occurring briarane-type metabolites have the C-15 methyl group that is *trans* to H-10, and these two groups are assigned as β- and α-oriented in most briarane derivatives.[29,30] The relative configuration of **1** was elucidated from the NOE interactions observed in an NOESY experiment (Fig. 2). In the NOESY experiment of **1**, H-10 gives strong NOE responses with H-2, H-11, and H-12, indicating that these protons are situated on same face of the structure; these were assigned as the α protons, as the C-15 methyl is the β-substituent at C-1. Thus, the hydroxy group at C-12 is at the β face and is *cis* to the C-20 methyl group. H-9 was found to exhibit strong NOE responses with H-11, H_3-18, and H_3-20. From the consideration of molecular models, H-9 was found to be reasonably close to H-11, H_3-18, and H_3-20 when H-9 was α-oriented in the 10-membered ring and H_3-18 was placed on the β face in the γ-lactone moiety. H-14 showed an NOE response with H_3-15 but not with H-10, showing that this proton was positioned on the equatorial direction and has a β-orientation at C-14. Furthermore, one proton of the C-3 methylene (δ 2.58, H-3β) showed strong NOE correlations with H_3-15 and H-7, suggesting that these protons are on the β face of **1**. The NOE correlation between H-6 and H_3-16 suggested that the Δ^5 double bond exists in the *Z* form. Based on the above findings, the structure, including the relative stereochemistry of **1**, was elucidated and the configurations of all chiral centers of **1** were assigned as 1*S**, 2*S**, 5*Z*, 7*S**, 8*R**, 9*S**, 10*S**, 11*R**, 12*S**, 14*S**, 17*R**. By detailed analysis, the spectral data of **1** were found to be very similar to those of a known briarane metabolite, briareolide F (**7**).[31] However, by comparison of the ^{1}H

and ^{13}C NMR chemical shifts of the C-12 methine of **1** (δ_H 4.06, 1H, m; δ_C 66.8, d) with those of **7** (δ_H 3.70, 1H, m; δ_C 71.2, d), it was shown that the hydroxy group in **1** attached at C-12 is β-oriented.

The new briarane diterpene, briaexcavatin J (**2**), had a molecular formula of $C_{28}H_{38}O_{13}$ as deduced by HRESIMS (*m/z* calcd: 605.2210; found: 605.2209 $[M+Na]^+$). The IR spectrum of **2** indicated the presence of hydroxy (3439 cm^{-1}), γ-lactone (1775 cm^{-1}), and ester (1735 cm^{-1}) groups. From the ^{13}C NMR data of **2** (Table 1), a trisubstituted olefin (δ 145.5, s, C-5; 125.0, d, CH-6) and five carbonyl resonances (δ 171.4, 170.7, 170.7, 168.4, 4×s, ester carbonyls; 170.3, s, C-19) were observed. The four esters were identified as acetates by the presence of four methyl resonances in the ^1H NMR spectrum of **2** at δ 2.25 (3H, s), 2.09 (3H, s), 1.98 (3H, s), and 1.97 (3H, s) (Table 2). The planar structure of **2** was determined by 2D NMR experiments. The coupling information in the $^1H-^1H$ COSY experiment of **2** enabled identification of the C-2/3/4, C-4/6 (by allylic coupling), C-6/7, C-6/16 (by allylic coupling), C-9/10/11/12/13/14, and C-11/20 units. From these data, together with the results of an HMBC experiment of **2**, the molecular framework of **2** could be further established. The HMBC correlations also revealed that the acetate groups are attached at C-2, C-9, C-14, and C-16; thus, the remaining hydroxy groups should be positioned at C-4 and C-12.

The relative stereochemistry of **2** was elucidated from the NOE interactions observed in an NOESY experiment (Fig. 3) and the configurations of all chiral centers except C-1 and C-4 of **2** were confirmed as being the same as those of **1** by comparison of the proton chemical shifts, coupling constants, and NOE correlations. The hydroxy group at C-4 was assigned the β-configuration primarily due to the NOE correlation between H-4 and H-3α, and this led to the assignment of the *R**-configuration at C-1. The relative configurations of all chiral centers of **2** were assigned as 1*R**, 2*S**, 4*R**, 5*Z*, 7*S**, 8*R**, 9*S**, 10*S**, 11*R**, 12*S**, 14*S**, 17*R**.

Briaexcavatin K (**3**) had the molecular formula $C_{24}H_{32}O_{10}$, as established by HRESIMS (*m/z* calcd: 503.1893; found: 503.1895 $[M+Na]^+$). Its IR spectrum exhibited a broad OH stretch at 3438 cm^{-1}, a γ-lactone carbonyl group at 1773 cm^{-1}, and ester carbonyl groups at 1728 cm^{-1}. Carbonyl resonances in the ^{13}C NMR spectrum of **3** confirmed the presence of a γ-lactone and two other esters (Table 1). In the ^1H NMR spectrum of **3** (Table 2), two acetate methyls were observed at δ 2.23 (3H, s) and 2.11 (3H, s). The planar structure of **3** was proposed with the assistance of 2D NMR studies. The

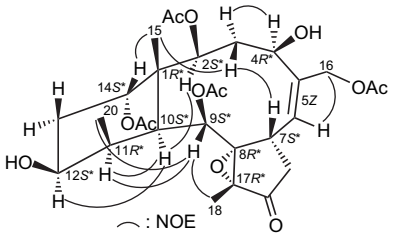


Figure 3. Selective NOESY correlations of **2**.

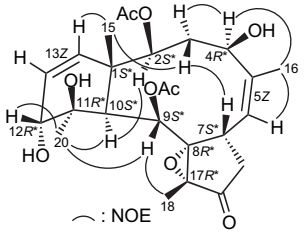


Figure 4. Selective NOESY correlations of **3**.

positions of two acetoxy groups at C-2 and C-9 were confirmed by the correlations between the oxymethine protons at δ_H 4.53 (H-2) and 5.80 (H-9) with the acetate carbonyls at δ_C 170.5 (s) and 169.6 (s), respectively, in the HMBC spectrum of **3**. The relative configuration of **3** was confirmed as being similar to that of a known metabolite, briaexcavatolide W (**8**),[32] by comparison of the NMR chemical shifts and coupling constants analysis for the chiral centers C-1, -2, -4, -7, -8, -9, -10, and -17. In the NOESY experiment of **3** (Fig. 4), H_3-20 was found to exhibit NOE correlations with H-9, H-10, and H-12, but not with H_3-15, indicating that the C-20 methyl was α-oriented in **3**. However, H-12 was assigned on the β face by a strong NOE correlation between H-12 and H_3-20, but not between H-10 and H_3-15. By consideration of molecular models, H-12 was found to be reasonably close to H_3-20, but not to H-10 and H_3-15, when H-12 and CH_3-20 were β- and α-oriented, respectively, and these two groups should be positioned on the equatorial direction in the cyclohexene ring. The *cis* geometry of the C-13/C-14 double bond was indicated by a 10.0 Hz coupling constant between H-13 (δ 5.79, 1H, dd, J=10.0, 5.6 Hz) and H-14 (δ 5.53, 1H, d, J=10.0 Hz). Based on the above findings, the structure of **3** was established and the chiral centers for this compound were assigned as 1*S**, 2*S**, 4*R**, 5*Z*, 7*S**, 8*R**, 9*S**, 10*S**, 11*R**, 12*R**, 13*Z*, 17*R**.

Briaexcavatin L (**4**), was isolated as a white powder, and had the molecular formula $C_{26}H_{36}O_{12}$ on the basis of HRESIMS. The IR spectrum of **4** showed bands at 3428, 1759, and 1728 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester carbonyl groups, respectively. Based on detailed spectral data analysis and by comparison of the ^1H and ^{13}C NMR data of **4** with those of other briarane diterpenoids reported previously, it was found that diterpenoid **4** is the 4-*O*-deacetyl derivative of a known briarane metabolite, briaexcavatolide U (**9**),[33] and should possess a structure as represented by formula **4**. The structure of **4** was further confirmed by 2D NMR experiments and the chiral centers for this compound were assigned

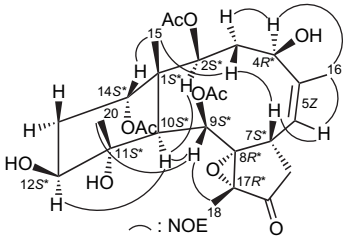


Figure 5. Selective NOESY correlations of **4**.

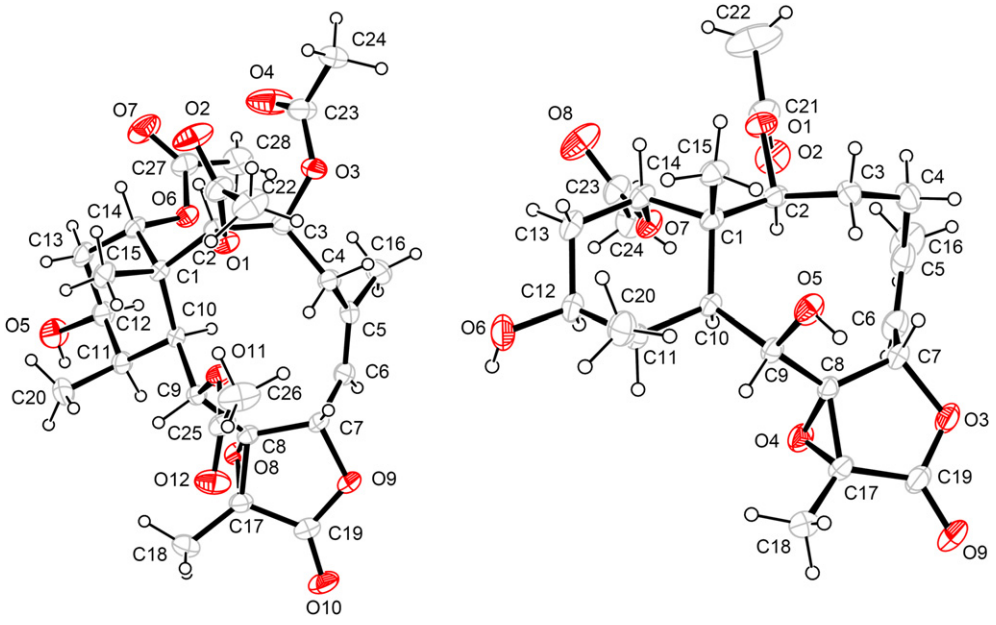
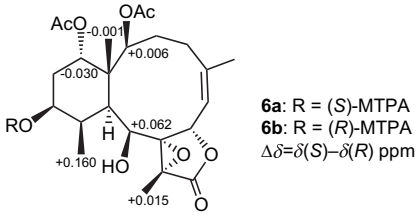


Figure 6. Computer-generated ORTEP plots of **5** and **6** showing the relative configurations.



6a: R = (*S*)-MTPA
6b: R = (*R*)-MTPA
$\Delta\delta=\delta(S)-\delta(R)$ ppm

Figure 7. ^{1}H NMR chemical shift difference [$\delta(S)$-MTPA−$\delta(R)$-MTPA] of the MTPA esters of **6**.

as 1*S**, 2*S**, 4*R**, 5*Z*, 7*S**, 8*R**, 9*S**, 10*S**, 11*S**, 12*S**, 14*S**, 17*R** by its NOESY experiment (Fig. 5).

The known briaranes, excavatolides C (**5**) and E (**6**), were first isolated from a wild-type Taiwanese octocoral *B. excavatum* and their structures were elucidated by spectral data analysis.[28] The absolute configuration of **5** was then determined by chemical methods in a later study.[7] In this study, we report the X-ray structures of excavatolides C (**5**) and E (**6**) for the first time (Fig. 6). In order to determine the absolute configuration of briarane **6**, this compound was treated with (−) or (+)-MTPA chloride to yield the (*S*)- and (*R*)-MTPA esters **6a** and **6b**, respectively. Comparison of the ^{1}H NMR chemical shifts for **6a** and **6b** (Δ values shown in Fig. 7) led to the assignment of the *S*-configuration at C-12. Therefore, the absolute configurations of all chiral centers of **6** were assigned as 1*S*, 2*S*, 5*Z*, 7*S*, 8*R*, 9*S*, 10*S*, 11*R*, 12*S*, 14*S*, 17*R*.

2.2. Isolation and structure determination of fragilide C from *J. fragilis*

The new chlorinated briarane, fragilide C (**10**), was isolated as a white solid. The molecular formula of $C_{27}H_{35}ClO_{11}$ (10 degrees of unsaturation) was established from the mass ions at *m/z* 593 $(M+Na)^+$ and 595 $(M+2+Na)^+$ in the ESIMS spectrum and was further supported by HRESIMS (*m/z* calcd: 593.1765; found: 593.1767 $[M+Na]^+$). The IR spectrum of **10** also showed strong bands at 3385, 1789, and 1740 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester

Table 3
^{1}H and ^{13}C NMR data for diterpenoid **10**

Position	^{1}H NMR[a]	^{13}C NMR[b]
1		47.1 (s)[d]
2	5.32 d (7.6)[c]	72.5 (d)
3α/β	1.53 d (16.0); 3.38 dd (16.0, 7.6)	40.5 (t)
4		97.2 (s)
5		137.9 (s)
6	4.90 m	55.3 (d)
7	4.33 d (2.8)	78.6 (d)
8		81.4 (s)
9	5.64 d (2.0)	71.7 (d)
10	2.80 br s	40.7 (d)
11		56.2 (s)
12α/β	2.22 m; 1.25 m	29.7 (t)
13/13′	1.92 m; 1.84 m	24.7 (t)
14	5.02 dd (3.2, 3.2)	73.9 (d)
15	1.23 s	15.5 (q)
16a/b	5.94 d (2.0); 5.64 dd (2.0, 1.6)	117.9 (t)
17	2.76 q (6.8)	50.0 (d)
18	1.32 d (6.8)	7.2 (q)
19		174.2 (s)
20a/b	2.42 dd (3.2, 2.4); 2.64 d (2.4)	51.2 (t)
OH-4	6.61 br s	
Acetates	2.23 s	21.6 (q)
		169.3 (s)
	2.09 s	20.8 (q)
		169.9 (s)
Propionate	1.14 t (7.6)	8.6 (q)
	2.36 m	27.8 (t)
		176.6 (s)

[a] Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C.
[b] Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C.
[c] *J* values (in Hz) in parentheses.
[d] Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbol.

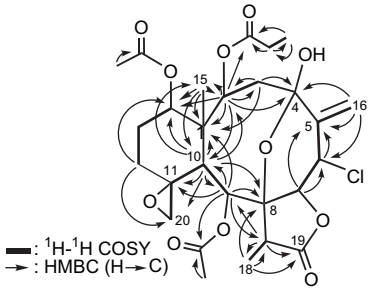


Figure 8. The ^{1}H–^{1}H COSY and HMBC correlations of **10**.

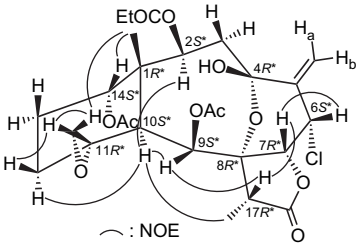


Figure 9. Selective NOESY correlations of **10**.

groups. From the ^{13}C NMR data of **10** (Table 3), the presence of an exocyclic carbon–carbon double bond was deduced from the signals of two carbons resonating at δ 137.9 (s, C-5) and 117.9 (t, CH_2-16), and further supported by two olefin proton signals at δ 5.94 (1H, d, J=2.0 Hz, H-16a) and 5.64 (1H, dd, J=2.0, 1.6 Hz, H-16b) in the ^{1}H NMR spectrum of **10** (Table 3). Moreover, four carbonyl resonances appeared at δ 176.6 (s), 174.2 (s), 169.9 (s), and 169.3 (s), confirming the presence of a γ-lactone and three ester groups in **10**. In the ^{1}H NMR spectrum of **10**, two acetate methyl signals were observed (δ 2.23, 3H, s; 2.09, 3H, s). The additional acyl group was confirmed as a propionyloxy group based on ^{1}H NMR studies, including a ^{1}H–^{1}H COSY experiment (Fig. 8), which revealed five contiguous protons (δ 1.14, 3H, t, J=7.6 Hz; 2.36, 2H, m). The carbon signal observed at δ 176.6 (s) was correlated with the signal of the methylene protons at δ 2.36 in the HMBC spectrum and was thus assigned as the carbon atom of the propionate carbonyl (Fig. 8). Also, it was found that the NMR data of **10** were similar to those of a known diterpene, juncin ZI (**11**), which was isolated previously from a South China Sea gorgonian coral *J. juncea*,[34] except that an acetoxy group of compound **11** was replaced by a propionyloxy group in **10**. The main problem was to locate the propionate group at C-2, -9, or -14, and the two acetates at the remaining two positions. The propionate ester was positioned at C-2 from the ^{1}H–^{13}C long-range correlations observed between H-2 (δ 5.32) and the carbonyl carbon (δ 176.6) of the propionate in the HMBC spectrum of **10** (Fig. 8), suggesting that fragilide C (**10**) is the 2-deacetoxy-2-propionyloxy derivative of compound **11**.

The chemical shifts of exocyclic 11,20-epoxy groups in briarane derivatives have been summarized, and although the ^{13}C NMR peaks for C-11 and C-20 appear at δ 55–61 and 47–52 ppm, respectively, the epoxy group is α-oriented (11*R**), and the cyclohexane ring is of a chair conformation.[17] Based on the above observations, the configuration of the 11,20-epoxy group in **10** (δ 56.2, s, C-11; 51.2, t, CH_2-20) should be α-oriented and the cyclohexane ring in **10** should be of a chair conformation. The relative stereochemistry of **10** was elucidated from the NOE interactions observed in an NOESY experiment (Fig. 9). Due to the α orientation of H-10, the ring junction C-15 methyl group should be β-oriented, as no NOE correlation was observed between H-10 and H_3-15. In the NOESY spectrum of **10**, H-10 gives NOE correlations with H-2, H-9, H_3-18, and one proton of the C-12 methylene (δ 2.22), suggesting that these protons (H-2, H-9, H-10, H-12α, and H_3-18) are located on the same face and can be assigned as α protons, as the C-15 methyl group is β-oriented. H-14 was found to exhibit a strong NOE response with H_3-15, but not with H-10, showing that this proton is of β-orientation. H-9 was found to show NOE correlations with H-10 and H-17, and, from molecular models, was found to be reasonably close to H-10 and H-17; therefore, H-9 should be placed on the α face in **10**, and H-17 is β-oriented in the γ-lactone moiety. However, no NOE response was observed between OH-4 and any proton in the NOESY experiment of **10**, so the stereochemistry of the hydroxy group at C-4 cannot be determined by this way. Fortunately, by comparing the ^{13}C NMR chemical shifts for C-4 (δ 97.2, s) and C-8 (δ 81.4, s) of **10** with those of the known briarane **11** (δ 97.2, s, C-4; 81.4, s, C-8), the 4-hydroxy group in **10** should be β-oriented. Furthermore, H-7 exhibited strong NOE correlations with H-17 and H-6, suggesting that these protons are on the β face of **7**. Based on the above findings, the configurations of all chiral centers of **10** were assigned to be 1*R**, 2*S**, 4*R**, 6*S**, 7*R**, 8*R**, 9*S**, 10*S**, 11*R**, 14*S** and 17*R**.

Table 4
Inhibitory effects of briaranes **1**, **4**–**6**, and **10** on superoxide anion generation by human neutrophils in response to fMet-Leu-Phe/cytochalastin B

Compound	Superoxide generation inhibition[a] (%)
1	2.38±1.06
4	3.04±3.30
5	15.47±2.92
6	4.88±5.09
10	11.61±2.80

[a] Percentage of inhibition (Inh %) at 10 μg/mL concentration. Results are presented as means±SEM (n=3).

2.3. Biological activity

In biological activity experiments, excavatolide C (**5**) and fragilide C (**10**) were found to show 15.47 and 11.61% inhibitory effects on superoxide anion generation by human neutrophils at 10 μg/mL, respectively (Table 4).

3. Experimental

3.1. General experimental procedures

Melting points were determined on FARGO apparatus and were uncorrected. 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 FT-IR spectrophotometer. NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR at 400 MHz for ^{1}H and 100 MHz for ^{13}C, in $CDCl_3$. Proton chemical shifts were referenced to the residual $CHCl_3$ signal (δ 7.26 ppm). ^{13}C NMR spectra were referenced to the center peak of $CDCl_3$ at δ 77.1 ppm. ESIMS and HRESIMS data were recorded on a BRUKER APEX II mass spectrometer. Column chromatography was performed on silica gel (230–400 mesh, Merck, Darmstadt, Germany). TLC was carried out on precoated Kieselgel $60F_{254}$ (0.25 mm, Merck, Darmstadt, Germany) and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. HPLC was performed using a system comprising a HITACHI L-7100 pump, a HITACHI photo diode array detector L-7455, and a RHEODYNE 7725 injection port. A semi-preparative normal phase column (Hibar 250–25 mm, LiChrospher Si 60, 5 μm) and a semi-preparative reverse phase column (Hibar 250–10 mm, Purospher STAR RP-18e, 5 μm) were used for HPLC.

3.2. Animal material

3.2.1. B. excavatum

Specimens of the cultured octocoral *B. excavatum* were collected by hand in a 0.6-ton cultivating tank located in the National Museum of Marine Biology and Aquarium (NMMBA), Taiwan, in December 2006. This organism was identified by comparison with previous descriptions.[35–37] Living reference specimens are being maintained in the authors' marine organism cultivating tank and a voucher specimen was deposited in the NMMBA, Taiwan.

3.2.2. J. fragilis

Specimens of the gorgonian coral *J. fragilis* were collected by hand using SCUBA off the coast of southern Taiwan in August 2006, at a depth of 20 m. This organism was identified by comparison with previous descriptions.[36,38] Living reference specimens are being maintained in the authors' marine organism cultivating tank and a voucher specimen was deposited in the NMMBA, Taiwan.

3.3. Extraction and isolation

3.3.1. B. excavatum

The freeze-dried and minced material of the octocoral *B. excavatum* (wet weight 672 g, dry weight 270 g) was extracted with a mixture of MeOH and CH_2Cl_2 (1:1) at room temperature. The residue was partitioned between EtOAc and H_2O. The EtOAc layer was separated on Sephadex LH-20 and eluted using MeOH/CH_2Cl_2 (2:1) to yield three fractions, A–C. Fraction C was separated on silica gel and eluted using hexane/EtOAc (stepwise, 20:1—pure EtOAc) to yield fractions 1–9. Fraction C8 was separated by gravity column with silica gel and eluted using hexane/EtOAc to afford briaranes **5** (35 mg, 3:1) and **6** (130 mg, 2:1). A mixture from C8 was purified by normal phase HPLC, using a mixture of CH_2Cl_2 and acetone to afford briarane **1** (3.4 mg, 20:1). Fraction C9 was separated by normal phase HPLC, using mixtures of CH_2Cl_2 and acetone to afford fractions from C9-1 to C9-8. Fraction C9-6 was repurified by reverse phase HPLC, using mixtures of CH_3CN and H_2O to afford briaranes **4** (4.3 mg, 4:1), **3** (2.1 mg, 1:2), and **2** (2.6 mg, 1:3).

3.3.2. Briaexcavatin I (*1*)

White powder; mp 273–275 °C; $[\alpha]_D^{25}$ +50 (*c* 0.15, $CHCl_3$); IR (neat) ν_{max} 3497, 1775, 1742 cm^{-1}; for ^{13}C ($CDCl_3$, 100 MHz) and ^{1}H ($CDCl_3$, 400 MHz) NMR data, see Tables 1 and 2; ESIMS *m/z* 531 $(M+Na)^+$; HRESIMS *m/z* 531.2209 (calcd for $C_{26}H_{36}O_{10}$+Na, 531.2206).

3.3.3. Briaexcavatin J (*2*)

White powder; mp 130–132 °C; $[\alpha]_D^{25}$ +21 (*c* 0.13, $CHCl_3$); IR (neat) ν_{max} 3439, 1775, 1735 cm^{-1}; for ^{13}C ($CDCl_3$, 100 MHz) and ^{1}H ($CDCl_3$, 400 MHz) NMR data, see Tables 1 and 2; ESIMS *m/z* 605 $(M+Na)^+$; HRESIMS *m/z* 605.2209 (calcd for $C_{28}H_{38}O_{13}$+Na, 605.2210).

3.3.4. Briaexcavatin K (*3*)

White powder; mp 154–156 °C; $[\alpha]_D^{25}$ +67 (*c* 0.11, $CHCl_3$); IR (neat) ν_{max} 3438, 1773, 1728 cm^{-1}; ^{13}C ($CDCl_3$, 100 MHz) and ^{1}H ($CDCl_3$, 400 MHz) NMR data, see Tables 1 and 2; ESIMS *m/z* 503 $(M+Na)^+$; HRESIMS *m/z* 503.1895 (calcd for $C_{24}H_{32}O_{10}$+Na, 503.1893).

3.3.5. Briaexcavatin L (*4*)

White powder; mp 180–182 °C; $[\alpha]_D^{25}$ +72 (*c* 0.22, $CHCl_3$); IR (neat) ν_{max} 3428, 1759, 1728 cm^{-1}; ^{13}C ($CDCl_3$, 100 MHz) and ^{1}H ($CDCl_3$, 400 MHz) NMR data, see Tables 1 and 2; ESIMS *m/z* 563 $(M+Na)^+$; HRESIMS *m/z* 563.2101 (calcd for $C_{26}H_{36}O_{12}$+Na, 563.2104).

3.3.6. Excavatolide C (*5*)

The related physical (mp, optical rotation value) and spectral (IR, ^{1}H, and ^{13}C NMR) data of **5** are in full agreement with those reported previously.[28]

3.3.7. Excavatolide E (*6*)

The related physical (mp, optical rotation value) and spectral (IR, ^{1}H, and ^{13}C NMR) data of **6** are in full agreement with those reported previously.[28]

3.3.8. J. fragilis

The freeze-dried and minced material of the gorgonian coral *J. fragilis* (wet weight 628 g, dry weight 206 g) was extracted with a mixture of MeOH and CH_2Cl_2 (1:1) at room temperature. The residue was partitioned between EtOAc and H_2O. The EtOAc layer was separated on silica gel and eluted using hexane/EtOAc (stepwise, 50:1—pure EtOAc) to yield 17 fractions A–Q, and one of these fractions (fraction K) was further separated by gravity column with silica gel and eluted using CH_2Cl_2/EtOAc (stepwise, 20:1—pure EtOAc) to afford 23 fractions K1–K23. Fraction K7 was

purified by normal phase HPLC, using a mixture of CH_2Cl_2 and EtOAc to afford briarane **10** (0.9 mg, 15:1).

*3.3.9. Fragilide C (**10**)*

White powder; mp 274–275 °C; $[\alpha]_D^{25}$ +28 (*c* 0.05, $CHCl_3$); IR (neat) ν_{max} 3385, 1789, 1740 cm^{-1}; for ^{13}C ($CDCl_3$, 100 MHz) and ^1H ($CDCl_3$, 400 MHz) NMR data, see Table 3; ESIMS *m/z* 593 $(M+Na)^+$, 595 $(M+2+Na)^+$; HRESIMS *m/z* 593.1767 (calcd for $C_{27}H_{35}ClO_{11}$+Na, 593.1765).

*3.4. Single-crystal X-ray crystallography of excavatolide C (**5**)*[39]

Suitable colorless prisms of **5** were obtained from a solution of EtOAc. The crystal (0.2×0.5×0.8 mm) belongs to the monoclinic system, space group $P2_1$ (# 4), with a=8.999(2) Å, b=14.718(3) Å, c=10.882(2) Å, β=94.91(3)°, V=1435.9(5) $Å^3$, Z=2, D_{calcd}=1.310 g/cm^3, λ (Mo Kα)=0.71073 Å. Intensity data were measured on a Rigaku AFC7S diffractometer up to $2\theta_{max}$ of 52°. All 4363 reflections were collected. The structure was solved by direct methods and refined by a full-matrix least-squares procedure. The refined structural model converged to a final $R1$=0.0353; $wR2$=0.0854 for 2935 observed reflections [$I>2\sigma(I)$] and 369 variable parameters.

*3.5. Single-crystal X-ray crystallography of excavatolide E (**6**)*[39]

Suitable colorless prisms of **6** were obtained from a solution of EtOAc. The crystal (0.68×0.55×0.50 mm) belongs to the orthorhombic system, space group $P2_12_12_1$ (# 19), with a=6.799(3) Å, b=18.512(6) Å, c=19.594(6) Å, V=2466.4(14) $Å^3$, Z=4, D_{calcd}=1.256 g/cm^3, λ (Mo Kα)=0.71073 Å. Intensity data were measured on a Rigaku AFC7S diffractometer up to $2\theta_{max}$ of 52°. All 4132 reflections were collected. The structure was solved by direct methods and refined by a full-matrix least-squares procedure. The refined structural model converged to a final $R1$=0.0352; $wR2$=0.0885 for 3365 observed reflections [$I>2\sigma(I)$] and 310 variable parameters.

*3.6. (S)- and (R)-MTPA esters of excavatolide E (**6**)*

To a solution of briarane **6** (2.4 mg) in pyridine (2.0 mL) was added (−)-α-methoxy-α-(tri-fluoromethyl)phenylacetyl (MTPA) chloride at room temperature for 6 h. The reaction mixture was concentrated to dryness under reduced pressure and purified by a short silica gel column with hexane/EtOAc (4:1) to give (*S*)-MTPA ester **6a** (2.4 mg). The (*R*)-MTPA ester **6b** (1.8 mg) was prepared from (+)-MTPA chloride using the same method. Selected $\Delta\delta$ values are shown in Figure 7.

3.7. Human neutrophil superoxide generation

Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Superoxide generation was carried out according to the procedures described previously.[40,41] Briefly, superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*.

Acknowledgements

This research work was supported by grants from the National Science Council (NSC 95-2320-B-291-001-MY2) and by intramural funding from the NMMBA, Taiwan, awarded to P.-J.S.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2008.01.023.

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