Article

Excavatoids O and P, New 12-Hydroxybriaranes from the Octocoral *Briareum excavatum*

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Abstract: Two new 12-hydroxybriarane diterpenoids, designated as excavatoids O (**1**) and P (**2**), were isolated from the octocoral *Briareum excavatum*. The structures of briaranes **1** and **2** were established on the basis of extensive spectral data analysis. Excavatoid P (**2**) is the first metabolite which possesses a 6β-chlorine atom in briarane analogues.

Keywords: excavatoid; briarane; octocoral; *Briareum excavatum*

1. Introduction

In our research on the chemical constituents of the marine invertebrates collected in Taiwan waters, a series of briarane-type diterpenoid derivatives had been isolated from various octocorals belonging to the genus *Briareum* (family Briareidae), *Ellisella*, and *Junceella* (family Ellisellidae), and the compounds of this type were proven to possess various interesting bioactivities [1–3]. Recently, our further chemical examination of *Briareum excavatum* has resulted in the isolation of two new highly oxidized briarane-type diterpenoids, excavatoids O (**1**) and P (**2**) (Scheme 1). The structures of compounds **1** and **2** were established by spectroscopic methods.

Scheme 1. The Structures of Excavatoids O (**1**) and P (**2**).
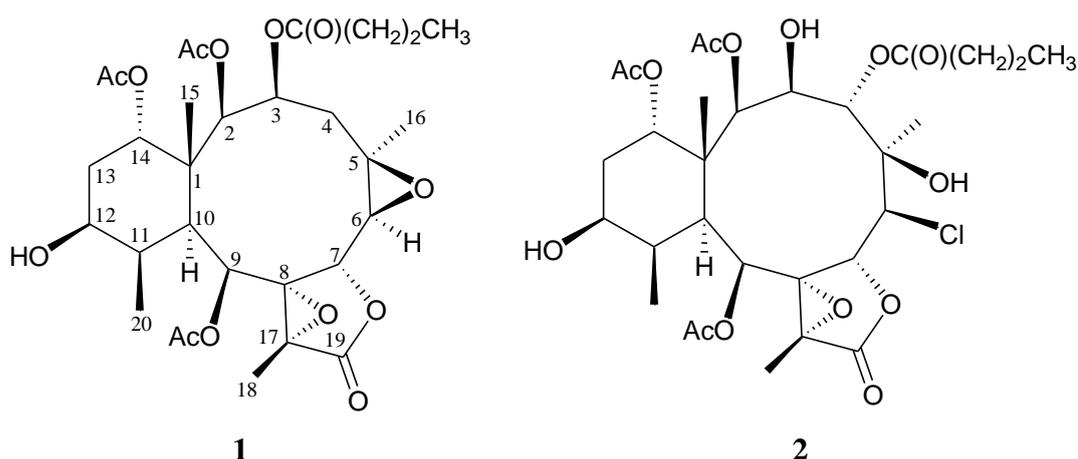
2. Results and Discussion

Excavatoid O (**1**) was obtained as a white powder and had a molecular formula $C_{30}H_{42}O_{13}$, as determined by HRESIMS ($C_{30}H_{42}O_{13}$ + Na, *m/z* found 633.2519; calculated 633.2523) indicating 10 degrees of unsaturation. The presence of hydroxy, lactone, and ester groups in **1** were evidenced by the IR absorptions at 3512, 1793, and 1741 cm^{-1}. It was found that the ^{1}H and ^{13}C spectra of **1** in CDCl$_3$ revealed mostly broad peaks when measured at 25 ℃. In order to make more reliable assignments of NMR signals of the stabilized conformers, the ^{1}H and ^{13}C NMR spectra of **1** were measured at 0 ℃ in CDCl$_3$. In the ^{13}C spectrum of **1**, five ester carbonyl resonances appeared at δ_C 173.6, 170.8, 170.1, 169.5, and 169.3 (5 × s) (Table 1). In the above carbonyl carbons, three were identified as acetate carbonyls by the presence of three methyl resonances in the ^{1}H NMR spectrum at δ_H 2.18, 2.12, and 1.96 (each 3H × s) and one was identified as *n*-butyrate carbonyl by the presence of seven contiguous protons at δ_H 0.95 (3H, t, *J* = 7.2 Hz), 1.64 (2H, m), and 2.23 (2H, m) (Table 1). On the basis of the unsaturation data overall, **1** was concluded to be a briarane diterpenoid molecule possessing five rings. A tetra-substituted epoxide containing a methyl substituent was elucidated from the signals of two oxygenated quaternary carbons at δ_C 72.6 (s, C-8) and 63.3 (s, C-17); and further confirmed by the proton signal of a methyl singlet at δ_H 1.57 (3H, s, H$_3$-18). In addition, a tri-substituted epoxide containing a methyl substituent was deduced from the signals of an oxymethine (δ_H 3.11, 1H, d, *J* = 8.8 Hz, H-6; δ_C 63.0, d, C-6), a quaternary oxygen-bearing carbon (δ_C 62.1, s, C-5), and a methyl singlet at δ_H 1.35 (3H, s, H$_3$-16).

Table 1. ^{1}H and ^{13}C NMR data for diterpenoids **1** and **2**.

Position	1		2	
	[1]H [a]	[13]C [b]	[1]H [c]	[13]C [d]
1		43.3 (s) [f]		44.0 (s)
2	5.81 d (2.0) [e]	69.3 (d)	4.62 s	88.2 (d)
3	5.13 br s	69.9 (d)	5.07 d (11.6)	68.8 (d)
4	2.25 m (2H)	33.7 (t)	5.82 s	70.9 (d)
5		62.1 (s)		77.8 (s)
6	3.11 d (8.8)	63.0 (d)	4.29 s	65.9 (d)
7	4.69 d (8.8)	78.3 (d)	5.26 s	75.7 (d)
8		72.6 (s)		67.5 (s)
9	5.76 s	68.5 (d)	5.36 d (8.4)	66.0 (d)
10	2.18 br s	45.3 (d)	3.52 dd (8.4, 4.4)	39.5 (d)
11	2.32 br s	34.5 (d)	2.54 m	37.3 (d)
12	3.96 br s	69.3 (d)	4.13 m	66.9 (d)
13	1.92 m (2H)	34.8 (t)	1.83 m (α)	30.3 (t)
			1.96 m (β)	
14	5.16 br s	73.0 (d)	4.84 br s	80.8 (d)
15	1.52 s	18.2 (q)	0.86 s	18.2 (q)
16	1.35 s	21.1 (q)	1.55 s	22.3 (q)
17		63.3 (s)		60.6 (s)
18	1.57 s	11.1 (q)	1.63 s	10.0 (q)
19		170.8 (s)		170.0 (s)
20	1.19 d (7.2)	16.3 (q)	1.11 d (7.6)	9.0 (q)
OH-3			3.18 d (11.6)	
OH-5			2.36 s	
OH-12	n.o. [g]		2.17 br s	
2-OAc		169.5 (s)		172.0 (s)
	2.12 s	21.1 (q)	2.03 s	21.1 (q)
9-OAc		169.3 (s)		170.4 (s)
	2.18 s	21.2 (q)	2.43 s	21.4 (q)
14-OAc		170.1 (s)		170.3 (s)
	1.96 s	21.1 (q)	2.17 s	21.3 (q)
3-OCOPr		173.6 (s)		
	2.23 m (2H)	35.6 (t)		
	1.64 m (2H)	17.8 (t)		
	0.95 t (7.2)	13.6 (q)		
4-OCOPr				173.9 (s)
			2.33 t (7.6) (2H)	36.3 (t)
			1.66 m (2H)	18.4 (t)
			0.98 t (7.6)	13.7 (q)

[a]: Spectra were recorded at 400 MHz at 0 °C; [b]: Spectra were recorded at 100 MHz at 0 °C; [c]: Spectra were recorded at 400 MHz at 25 °C; [d]: Spectra were recorded at 100 MHz at 25 °C; [e]: *J* values (in Hz) in parentheses; [f]: Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols; [g]: n.o. = not observed.

From the ^{1}H-^{1}H COSY experiment of **1** (Table 2), it was possible to establish the separate spin systems that map out the proton sequences from H-2/H-3/H$_2$-4, H-6/H-7, and H-9/H-10. These data, together with the HMBC correlations between H-2/C-1, -4; H-3/C-4; H$_2$-4/C-3, -5, -6; H-7/C-5, -6; H-9/C-1, -7, -8, -10; and H-10/C-1, -2, established the connectivity from C-1 to C-10 in the 10-membered ring (Table 2). The methyl group at C-5 was confirmed by the HMBC correlations between H$_3$-16/C-4, -5, -6. The methylcyclohexane ring, which is fused to the 10-membered ring at C-1 and C-10, was elucidated by the ^{1}H-^{1}H COSY correlations between H-10/H-11/H-12/H$_2$-13/H-14 and H-11/H$_3$-20 and by the HMBC correlations between H-2/C-14; H-9/C-11; H-10/C-11, -12, -14; H-11/C-10, -20; H$_2$-13/C-1; H-14/C-1, -2; 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-2/C-15; and H$_3$-15/C-1, -2, -10, -14. In addition, the HMBC correlations also revealed that three acetates should be attached at C-2, C-9, and C-14, respectively. The remaining *n*-butyrate ester and hydroxy groups were positioned at C-3 and C-12 as indicated by analysis of ^{1}H-^{1}H COSY correlations and characteristic NMR signals analysis (δ_H 5.13, 1H, br s, H-3; δ_C 69.9, d, C-3; δ_H 3.96, 1H, br s, H-12; δ_C 69.3, d, C-12). These data, together with the HMBC correlations between H-7/C-17, -19 and H$_3$-18/C-8, -17, -19, were used to establish the molecular framework of **1**.

Table 2. The ^{1}H-^{1}H COSY and HMBC (H→C) correlations for diterpenoids **1** and **2**.

Position	**1** ^{1}H-^{1}H COSY	HMBC	**2** ^{1}H-^{1}H COSY	HMBC
H-2	H-3	C-1, -4, -14, -15, acetate carbonyl	H-3	C-1, -3, -4, -10, -14, acetate carbonyl
H-3	H-2, H$_2$-4	C-4	H-2, H-4, OH-3	C-1, -5
H-4	H-3	C-3, -5, -6	H-3	C-2, -5, -16, *n*-butyrate carbonyl
H-6	H-7	n.o.	H-7	C-4, -5, -7, -8, -16
H-7	H-6	C-5, -6, -17, -19	H-6	C-5, -6, -9, -19
H-9	H-10	C-1, -7, -8, -10, -11, acetate carbonyl	H-10	C-7, -8, -10, -11, -17, acetate carbonyl
H-10	H-9, H-11	C-1, -2, -11, -12, -14	H-9, H-11	C-1, -8, -9, -11, -12, -15, -20
H-11	H-10, H-12, H$_3$-20	C-10, -20	H-10, H-12, H$_3$-20	C-1, -10, -12, -20
H-12	H-11, H$_2$-13	n.o.	H-11, H$_2$-13	C-20
H-13	H-12, H-14	C-1, -14	H-12, H-14	C-12
H-14	H$_2$-13	C-1, -2, -12, -13, acetate carbonyl	H$_2$-13	C-10, -12
H-15		C-1, -2, -10, -14		C-1, -2, -10, -14
H-16		C-4, -5, -6		C-4, -5, -6
H-18		C-8, -17, -19		C-8, -17, -19
H-20	H-11	C-10, -11, -12	H-11	C-10, -11, -12
OH-3			H-3	C-3
OH-5				C-4, -5, -16
OH-12	n.o. ^{a}	n.o.	H-12	C-11

^{a}: n.o. = not observed.

Based on previous studies, all naturally occurring briarane-type diterpenoids have the C-15 methyl group as *trans* to H-10, and these two groups are assigned as *β*- and *α*-oriented, respectively, as shown in most briarane derivatives [1–3]. The relative stereochemistry of **1** was established from a NOESY experiment (Figure 1). In the NOESY experiment of **1**, the correlations of H-10 with H-2, H-3, H-6, H-9, and H-11; and H-11 correlated with H-12, indicated that these protons are situated on the same face and were assigned as *α* protons since the C-15 methyl is the *β*-substituent at C-1. H-14 was found to exhibit a correlation with H$_3$-15, revealing the *β*-orientation of this proton. The correlations between H$_3$-16 and H-3, H-6, reflected the *α*-orientation of H$_3$-16. H-7 correlated with H$_3$-15, indicating this proton should be *β*-oriented. Furthermore, H$_3$-18 showed a correlation with H-9. By detailed analysis of molecular models, H$_3$-18 was found to be reasonably close to H-9 when H$_3$-18 was placed on the *β* face in the *γ*-lactone moiety. Based on the above findings, the structure of **1** was elucidated unambiguously.

**Figure 1.** Selective NOESY correlations of **1**.
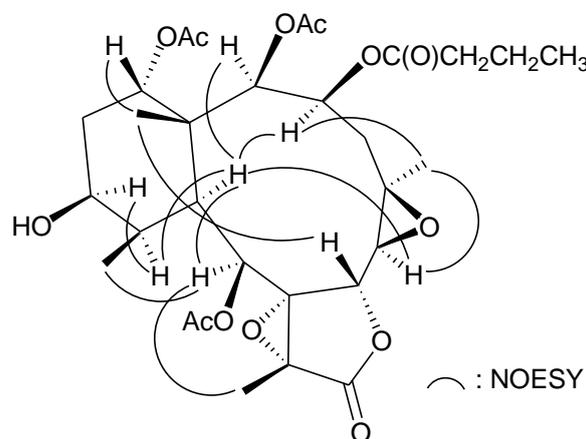
The molecular formula of excavatoid P (**2**) was determined as $C_{30}H_{43}ClO_{14}$ by its HRESIMS (*m/z* 685.2235, calculated for $C_{30}H_{43}ClO_{14}$ + Na, 685.2239). The IR spectrum showed bands at 3472, 1784, and 1734 cm^{-1}, consistent with the presence of hydroxy, *γ*-lactone, and ester groups in **2**. From the ^{13}C NMR data of **2** (Table 1), five carbonyl resonances appeared at δ_C 173.9, 172.0, 170.4, 170.3, and 170.0 (5 × s), confirming the presence of a *γ*-lactone and four esters in **2**; three acetyl methyls (δ_H 2.43, 2.17, 2.03, each 3H × s) and an *n*-butyryl group (δ_H 0.98, 3H, t, *J* = 7.6 Hz; 1.66, 2H, m; 2.33, 2H, t, *J* = 7.6 Hz) were also observed. According to the above data, briarane **2** was found to be a tetracyclic compound with a *γ*-lactone, as no other unsaturated functional group could be found.

^{1}H NMR coupling information in the ^{1}H-^{1}H COSY spectrum of **2** enabled identification of the C-2/-3/-4, C-6/-7, C-9/-10/-11/-12/-13/-14, and C-11/-20 units (Table 2), which were assembled with the assistance of an HMBC experiment (Table 2). The HMBC correlations between protons and quaternary carbons, such as H-2, H-3, H-10, H-11, H$_3$-15/C-1; H-3, H-4, H-6, H-7, H$_3$-16, OH-5/C-5; H-6, H-9, H-10, H$_3$-18/C-8; H-9, H$_3$-18/C-17; and H-7, H$_3$-18/C-19, permitted elucidation of the carbon skeleton. A methyl at C-5 was established by the HMBC correlations between H$_3$-16/C-4, -5, -6 and H-4, H-6, OH-5/C-16. The ring junction C-15 methyl group was positioned at C-1 from the HMBC correlations between H$_3$-15/C-1, -2, -10, -14; and H-10/C-15. The acetate esters at C-2 and C-9 were established by correlations between H-2 (δ_H 4.62), H-9 (δ_H 5.36) and the acetate carbonyls

observed in the HMBC spectrum of **2**. The *n*-butyrate ester positioned at C-4 was confirmed from the HMBC correlation between H-4 (δ_H 5.82) and the carbonyl carbon (δ_C 173.9) of *n*-butyrate ester. Thus, the remaining acetoxy group was positioned at C-14 as indicated by analysis of ^{1}H-^{1}H COSY correlations and characteristic NMR signals (δ_H 4.84, 1H, br s, H-14; δ_C 80.8, d, C-14). The presence of hydroxy groups at C-3 and C-12 was deduced from the ^{1}H-^{1}H COSY correlations between the hydroxy protons (δ_H 3.18, OH-3 and δ_H 2.17, OH-12) and H-3 (δ_H 5.07) and H-12 (δ_H 4.13), respectively. The C-5 hydroxy group was also confirmed by the HMBC correlations between the hydroxy proton (δ_H 2.36, OH-5) and C-4, -5, -16. Thus, the remaining chlorine atom in **2** should be attached C-6 by the ^{1}H-^{1}H COSY correlation between H-6 (δ_H 4.29) and H-7 (δ_H 5.26) and further supported by the HMBC correlations between H-6/C-4, -5, -7, -8, -16 and H-7, H$_3$-16/C-6.

The relative configuration of **2** was elucidated from the interactions observed in a NOESY experiment and from vicinal proton coupling constant analysis. In the NOESY experiment of **2** (Figure 2), the correlations of H-10 with H-3, H-11, and H-12, indicated that these protons were situated on the same face and were assigned as α protons since the C-15 and C-20 methyls are β-substituents at C-1 and C-11, respectively. H-2 exhibited an interaction with H-3, and no coupling was found between H-2 and H-3, indicating that the dihedral angle between H-2/H-3 is approximately 90° and the acetoxy group at C-2 should be β-oriented. H-14 was found to exhibit a response with H$_3$-15, showing that H-14 has a β-orientation. H-9 was found to show responses with H-11, H$_3$-18, and H$_3$-20. From modeling analysis, H-9 was found to be close to H-11, H$_3$-18, and H$_3$-20 when H-9 was α-oriented. Moreover, H$_3$-16 exhibited correlations with H-3 and H-6, and no coupling constant was detected between H-6 and H-7, suggesting the α-orientation of H$_3$-16 and H-6; and β-orientation of H-7. H-7 exhibited a correlation with H-4, and no coupling was found between H-3 and H-4, indicating that the dihedral angle between H-3 and H-4 is also approximately 90° and the *n*-butyrate ester group at C-4 was α-oriented. On the basis of the above results, the structure of **2** was elucidated. To the best of our knowledge, briarane **2** is the first briarane which possesses a 6β-chlorine atom.

Figure 2. Selective NOESY correlations of **2**.
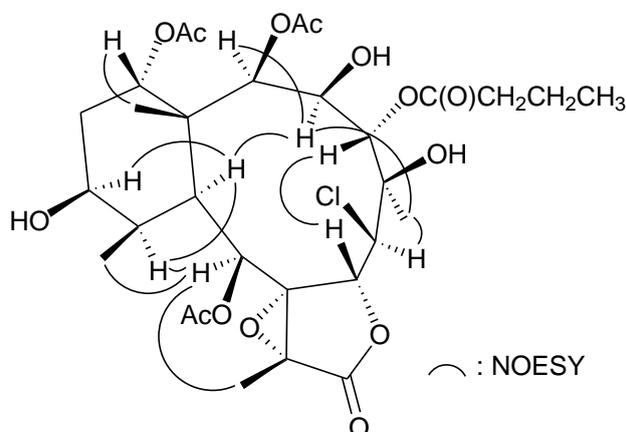
In the biological activity testing, briaranes **1** and **2** displayed 16.9 and 16.1% inhibitory effects on elastase release by human neutrophils at 10 μg/mL, resepectively [4].

3. Experimental

3.1. General Experimental Procedures

Melting points were determined on a 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 spectrometer. The 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$_3$, at 25 or 0 °C, respectively. Proton chemical shifts were referenced to the residual CHCl$_3$ signal (δ_H 7.26 ppm). ^{13}C NMR spectra were referenced to the center peak of CDCl$_3$ at δ_C 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 60 F$_{254}$ (0.25 mm, Merck) and spots were visualized by spraying with 10% H$_2$SO$_4$ solution followed by heating. HPLC was performed using a system comprised of a HITACHI L-7100 pump, a HITACHI photodiode array detector L-7455, and a RHEODYNE 7725 injection port. A normal phase column (Hibar 250 × 10 mm, Merck, silica gel 60, 5 μm,) was used for HPLC.

3.2. Animal Material

Specimens of the octocoral *Briareum excavatum* were collected and transplanted in 0.6-ton cultivating tanks located in the NMMBA, Taiwan, in December 2003. This organism was identified by comparison with previous descriptions [5–7]. A voucher specimen was deposited in the National Museum of Marine Biology and Aquarium, Taiwan.

3.3. Extraction and Isolation

The organism (wet weight 1.0 kg) was collected and freeze-dried. The freeze-dried material was minced and extracted with EtOAc. The extract was separated by silica gel column chromatography, using hexane and hexane/EtOAc mixtures of increased polarity to yield 12 fractions. Fraction 3 was separated by normal phase HPLC, using a mixture of dichloromethane and acetone to afford briarane **2** (0.9 mg, 9/1). Fraction 2 was separated by normal phase HPLC, using a mixture of hexane and EtOAc to afford briarane **1** (13.2 mg, 1/1).

Excavatoid O (**1**): white powder; mp 137–138 °C; $[\alpha]_D^{25}$ − 39 (*c* 0.4, CHCl$_3$); IR (neat) ν_{max} 3512, 1793, 1741 cm^{-1}; ^{13}C NMR (CDCl$_3$, 100 MHz) and ^{1}H NMR (CDCl$_3$, 400 MHz) data, see Table 1; ESIMS *m/z* 633 (M + Na)$^+$; HRESIMS *m/z* 633.2519 (Calcd for C$_{30}$H$_{42}$O$_{13}$Na, 633.2523).

Excavatoid P (**2**): white powder; mp 154–155 °C; $[\alpha]_D^{25}$ + 14 (*c* 0.05, CHCl$_3$); IR (neat) ν_{max} 3472, 1784, 1734 cm^{-1}; ^{13}C NMR (CDCl$_3$, 100 MHz) and ^{1}H NMR (CDCl$_3$, 400 MHz) data, see Table 1; ESIMS *m/z* 685 (M + Na)$^+$; HRESIMS *m/z* 685.2235 (Calcd for C$_{30}$H$_{43}$ClO$_{14}$Na, 685.2239).

3.4. Human Neutrophil Elastase Release

Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Elastase release experiments were performed using MeO-Suc-Ala-Ala-Pro-Valp-nitroanilide as the elastase substrate [8,9].

Acknowledgements

References and Notes

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Samples Availability: Not available.