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Briarenolide E: the first 2-ketobriarane diterpenoid from an octocoral *Briareum* sp. (Briareidae)

Pei-Han Hong [a,b], Yin-Di Su [b,c], Nai-Cheng Lin [b], Yung-Husan Chen [b], Yueh-Hsiung Kuo [d,e], Tsong-Long Hwang [f], Wei-Hsien Wang [b,c,g], Jih-Jung Chen [h], Jyh-Horng Sheu [c,g,*], Ping-Jyun Sung [a,b,c,g,i,*]

[a] *Graduate Institute of Marine Biotechnology, National Dong Hwa University, Pingtung 944, Taiwan*
[b] *National Museum of Marine Biology and Aquarium, Pingtung 944, Taiwan*
[c] *Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan*
[d] *School of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, College of Pharmacy, China Medical University, Taichung 404, Taiwan*
[e] *Tsuzuki Institute for Traditional Medicine, College of Pharmacy, China Medical University, Taichung 404, Taiwan*
[f] *Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan*
[g] *Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 804, Taiwan*
[h] *Department of Pharmacy, Tajen University, Pingtung 907, Taiwan*
[i] *Department of Life Science and Institute of Biotechnology, National Dong Hwa University, Hualien 974, Taiwan*

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ABSTRACT

A novel 2-ketobriarane diterpenoid, briarenolide E (**1**), was isolated from an octocoral *Briareum* sp. The structure of briarane **1** was elucidated by interpretations of spectral data. Compound **1** displayed modestly inhibitory effects on the generation of superoxide anions and the release of elastase by human neutrophils.

Previous chemical investigations of the chemical constituents of octocorals belonging to the genus *Briareum* (family Briareidae)[1–3] collected off the waters of Taiwan have yielded a series of interesting new briarane-related diterpenoids (3,8-cyclized cembranoid), which possess a bicyclo[8.4.0] carbon skeleton. Over 500 naturally-occurring briarane-type metabolites have been isolated from various marine organisms, and all compounds of this type are recognized as being of marine origin, and in particular are produced by various octocorals.[4–7] Owing to their interesting chemical constituents and potential medicinal usage, octocorals belonging to the genus *Briareum* have been proven to be important target organisms.[8,9] In our continuing studies of the chemical constituents of an octocoral identified as *Briareum* sp., a novel 2-ketobriarane derivative, briarenolide E (**1**) was isolated. In this Letter, we describe the isolation, structural characterization, and bioactivity of briarane **1**.

Sliced bodies of *Briareum* sp. (wet weight 6.32 kg, dry weight 2.78 kg) were extracted with a mixture of MeOH and DCM (1:1). The extract was partitioned between EtOAc and H_2O. The EtOAc layer was separated on silica gel and eluted using *n*-hexane/EtOAc (stepwise, 100:1–pure EtOAc) to yield 18 fractions. Fraction 8 was purified by normal-phase HPLC, using a mixture of *n*-hexane and acetone (3:1) as the mobile phase to afford compound **1** (1.2 mg).

Briarenolide E (**1**), $[\alpha]_D^{25}$ +28 (*c* 0.06, $CHCl_3$); mp 117–118 °C, was isolated as a white powder that gave a pseudomolecular ion $(M+Na)^+$ at *m/z* 487.1946 in the HRESIMS, indicating the molecular formula $C_{24}H_{32}O_9$ (calcd for $C_{24}H_{32}O_9$+Na, 487.1944) and implying nine degrees of unsaturation. IR absorptions were observed at 3456, 1776, 1745, and 1710 cm^{-1}, suggesting the presence of hydroxy, γ-lactone, ester, and ketone groups in **1**. From the ^1H and ^{13}C NMR spectra (Table 1), **1** was found to possess a ketone (δ_C 211.7, C-2), a γ-lactone moiety (δ_C 176.0, C-19), two acetoxy groups (δ_H 2.14, 2.13, each 3H × s; δ_C 21.0, 20.9, 2 × acetate methyls; δ_C 170.2, 170.2, 2 × ester carbonyls), and a trisubstituted olefin (δ_C 145.5, C-5; 118.7, CH-6; δ_H 5.35, 1H, dq, *J* = 9.2, 0.8 Hz, H-6). On the basis of the above unsaturation data, **1** was concluded to be a diterpenoid molecule possessing four rings. A disubstituted epoxide was elucidated from the signals of two oxymethines at δ_C

* Corresponding authors. Tel.: +886 7 5252000x5030; fax: +886 7 5255020 (J.-H.S.); tel.: +886 8 8825037; fax: +886 8 8825087 (P.-J.S.).
E-mail addresses: sheu@mail.nsysu.edu.tw (J.-H. Sheu), pjsung@nmmba.gov.tw (P.-J. Sung).

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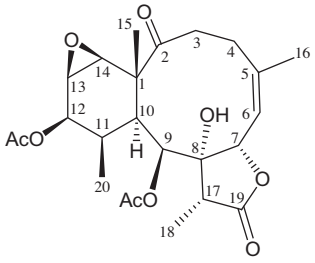
Table 1
^{1}H and ^{13}C NMR data, ^{1}H–^{1}H COSY, and HMBC correlations for **1**

Position	δ_H[a]	δ_H[b]	^{1}H–^{1}H COSY	HMBC (^{1}H→^{13}C)
1		48.6 (qC)		
2		211.7 (qC)		
3α	3.15 m	36.1 (CH_2)	H-3β, H_2-4	n.o.[c]
β	2.57 m		H-3α, H_2-4	n.o.
4α	2.41 dd (14.0, 2.0)[d]	28.2 (CH_2)	H_2-3, H-4β	C-2, C-3, C-5, C-6, C-16
β	2.54 m		H_2-3, H-4α	C-2, C-3
5		145.5 (qC)		
6	5.35 dq (9.2, 0.8)	118.7 (CH)	H-7, H_3-16	C-4, C-7, C-16
7	5.15 d (9.2)	78.1 (CH)	H-6	C-5, C-6, C-8, C-17
8		81.1 (qC)		
9	5.15 d (9.2)	67.8 (CH)	H-10	C-7, C-8, C-10, C-11 C-17, acetate carbonyl
10	2.68 dd (9.2, 2.8)	33.6 (CH)	H-9, H-11	C-1, C-2, C-8, C-9, C-11, C-15, C-20
11	2.72 m	32.0 (CH)	H-10, H-12, H_3-20	C-1, C-10, C-12, C-13
12	4.90 d (5.6)	72.0 (CH)	H-11	C-10, C-11, C-13, C-14, C-20, acetate carbonyl
13	3.07 d (3.6)	52.4 (CH)	H-14	n.o.
14	2.76 d (3.6)	58.6 (CH)	H-13	C-1, C-10
15	1.17 s	15.8 (CH_3)		C-1, C-2, C-14
16	1.92 d (0.8)	23.8 (CH_3)	H-6	C-4, C-5, C-6
17	2.35 q (7.2)	43.2 (CH)	H_3-18	C-8, C-18, C-19
18	1.18 d (7.2)	6.3 (CH_3)	H-17	C-8, C-17, C-19
19		176.0 (qC)		
20	1.02 d (7.2)	10.5 (CH_3)	H-11	C-10, C-11, C-12
9-OAc		170.2 (qC)		
	2.14 s	21.0 (CH_3)		Acetate carbonyl
12-OAc		170.2 (qC)		
	2.13 s	20.9 (CH_3)		Acetate carbonyl

[a] Spectra measured at 400 MHz in $CDCl_3$ at 25 °C.
[b] Spectra measured at 100 MHz in $CDCl_3$ at 25 °C.
[c] n.o. = not observed.
[d] J values (in hertz) in parentheses.

58.6 (CH-14) and 52.4 (CH-13) and further confirmed by proton signals at δ_H 2.76 (1H, d, J = 3.6 Hz, H-14) and 3.07 (1H, d, J = 3.6 Hz, H-13).



Briarenolide E (**1**)

From the ^{1}H–^{1}H COSY spectrum of **1** (Fig. 1 and Table 1), it was possible to establish the separate spin systems that map out the proton sequences from H_2-3/H_2-4, H-6/H-7, and H-9/H-10. These data, together with the HMBC correlations between H-4α/C-2, C-3, C-5, C-6; H-4β/C-2, C-3; H-6/C-4, C-7; H-7/C-5, C-6, C-8; H-9/C-7, C-8, C-10; and H-10/C-1, C-2, C-8, C-9, established the connectivity from C-1 to C-10 in the 10-membered ring (Table 1 and Fig. 1). The methyl group at C-5 was confirmed by the HMBC correlations between H_3-16/C-4, C-5, C-6; H-4α/C-16; and H-6/C-16; and further confirmed by an allylic coupling between H-6 and H_3-16 (J = 0.8 Hz). 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-13/H-14, and H-11/H_3-20 and by the HMBC correlations between H-9, H-10/C-11; H-10/C-20; H-11/C-1, C-10, C-12, C-13; H-12/C-10, C-11, C-13, C-14, C-20; H-14/C-1, C-10; and H_3-20/C-10, C-11, C-12. The ring junction C-15 methyl group was positioned at C-1 from the HMBC correlations between H_3-15/C-1, C-2, C-14; and H-10/C-15. The 2-ketone group was elucidated by the HMBC correlations between H_2-4, H-10, H_3-15 and the ketone carbonyl (δ_C 211.7, C-2).

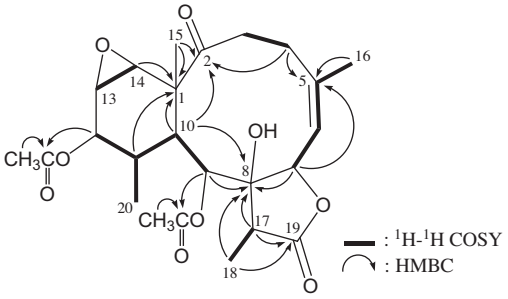
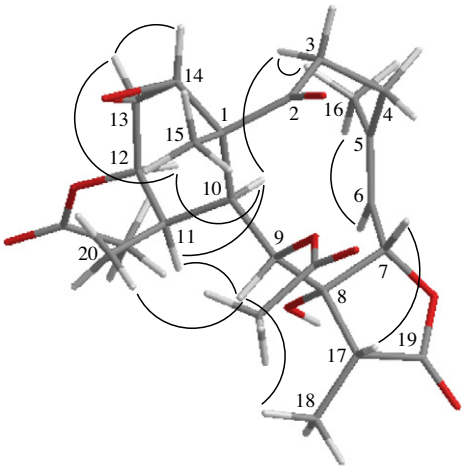


Figure 1. The ^{1}H–^{1}H COSY and selective key HMBC correlations (protons→quaternary carbons) of **1**.

Furthermore, the acetate esters at C-9 and C-12 were established by correlations between H-9 (δ_H 5.15), H-12 (δ_H 4.90) and the acetate carbonyls (δ_C 170.2, two ester carbonyls) observed in the HMBC spectrum of **1**. Thus, the remaining hydroxy group is positioned at C-8, an oxygen-bearing quaternary carbon at δ_C 81.1. These data, together with the ^{1}H–^{1}H COSY correlation between H-17 and H_3-18 and the HMBC correlations between H-7, H-9/C-17; H-17/C-8, C-18, C-19, and H_3-18/C-8, C-17, C-19, were used to establish the molecular framework of **1**.

In all naturally-occurring briaranes, H-10 is trans to the C-15 methyl group, and these two groups are assigned as α- and β-oriented in most briarane derivatives.[4–7] The relative configuration of **1** was elucidated from the interactions observed in a NOESY experiment and was found to be compatible with that of **1** offered by computer modeling (Table 2)[10] and that obtained from vicinal proton coupling constant analysis. In the NOESY experiment of **1**, the correlations of H-10 with H-3α, H-11, and H-12, but not with H_3-15 and H_3-20, indicated that these protons (H-3α, H-10, H-11, and H-12) were situated on the same face, and these were assigned as α protons, since the C-15 and C-20 methyls are β-substituents at

Table 2
The stereoview of **1** (generated from computer modeling) and the calculated distances (Å) between selected protons with key NOESY correlations

Briarenolide E (**1**)	H/H	(Å)
	H-3α/H-10	2.610
	H-3α/H_3-16	2.259
	H-6/H_3-16	2.285
	H-7/H-17	2.844
	H-9/H-11	2.555
	H-9/H_3-18	2.398
	H-9/H_3-20	2.147
	H-10/H-11	2.432
	H-10/H-12	2.371
	H-12/H-13	2.551
	H-13/H-14	2.544

C-1 and C-11, respectively. H-13 showed correlations with H-12 and H-14, as well as the lack of coupling was detected between H-12 and H-13, indicating the dihedral angle between H-12 and H-13 is approximately 90° and H-13 has an α-orientation at C-13. H-9 was found to show responses to 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. The C-16 vinyl methyl showed correlations with H-3α and H-6, and a large coupling constant was detected between H-6 and H-7 (J = 9.2 Hz), indicating the *Z*-configuration of the C-5/6 double bond; in addition, the dihedral angle between H-6 and H-7 is approximately 180°, and H-7 has a β-orientation at C-7.[11] Furthermore, H-7 exhibited a correlation with H-17, suggesting that H-17 and the 8-hydroxy group are β- and α-oriented in the γ-lactone moiety, respectively, by modeling analysis. Based on the above findings, the structure of **1** was established unambiguously.

It is worth noting that a briarane analogue possessing a 2-keto group, **1** (briarenolide E), was discovered for the first time in this study. The in vitro anti-inflammatory effects of **1** were tested. Briarenolide E (**1**) displayed modestly inhibitory effects on the generation of superoxide anion (inhibition rate 23.7%) and the release of elastase (inhibition rate 28.3%) by human neutrophils at a concentration of 10 μg/mL.[12–14] Owing to structural complexity, it is difficult to obtain sufficient amounts of the bioactive metabolite **1** for further study of its potential medicinal usage from natural sources. The octocoral *Briareum* sp. has begun to be transplanted into tanks using our highly-developed aquaculture technology for the extraction of natural products to establish a stable supply of bioactive materials.

Acknowledgments

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12. In the in vitro anti-inflammatory bioassay, the inhibitory effect on the generation of superoxide anions and the release of elastase by activated neutrophils were used as indicators. To indicate significant activity of pure compounds, an inhibition rate >40% is required (inhibition rate <10%, not active, 20% >inhibition rate >10%, weakly anti-inflammatory; 40% >inhibition rate >20%, modestly anti-inflammatory). Diphenyl indonium (DPI) and elastatinal were used as reference compounds in anti-inflammatory activity testing. DPI displayed an inhibitory effect on superoxide anion generation (IC_{50} = 0.9 μg/mL), and elastatinal exhibited an inhibitory effect on elastase release (IC_{50} = 30 μg/mL) by human neutrophils, respectively.
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