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Briarenolide D, a New Hydroperoxybriarane Diterpenoid from a Cultured Octocoral *Briareum* sp.

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Chemical investigation on a cultured octocoral identified as *Briareum* sp. has led to the isolation of a new 12-hydroperoxybriarane, briarenolide D (**1**) and a known briarane, 2β-acetoxy-2-(debutyryloxy)stecholide E (**2**). The structure of briarane **1** was established by spectroscopic methods and the structure of **2** was further confirmed by X-ray data analysis for the first time. Cytotoxicity of hydroperoxybriarane **1** toward various tumor cell lines is also described.

In continuation of our search for new substances from invertebrates originally collected off Taiwan waters, we have isolated a series of new briarane-type natural products from cultured and wild type octocorals belonging to the genus *Briareum*, including briarenolides A–C.[1–11] In this paper, we describe the isolation, structure determination, and bioactivity of a new hydroperoxybriarane, briarenolide D (**1**), along with a known briarane, 2β-acetoxy-2-(debutyryloxy)stecholide E (**2**) from an octocoral *Briareum* sp. (Chart 1).[12] The structure of briarane **1** was established by spectroscopic methods and the structure of **2** was further confirmed by X-ray data analysis.

Specimens of *Briareum* sp. (wet weight 125 g, dry weight 40 g) were collected by hand from an 18-ton exhibition tank equipped with a flow-through water system. The organisms were extracted with a mixture of MeOH and CH_2Cl_2 (1:1). The extract was partitioned between EtOAc and H_2O. The EtOAc layer (1.8 g) was separated by silica gel and eluted using hexane/EtOAc (stepwise, 100:1–pure EtOAc) to yield 13 fractions. Fraction 7 was separated by Sephadex LH-20 and eluted using MeOH to yield the 4 fractions, 7A–7D. Fraction 7C was repurified by reverse phase HPLC, using a mixture of MeOH and H_2O (55:45) to afford **1**.

Briarenolide D (**1**), 3.0 mg; mp 140–142 °C; $[\alpha]_D^{22}$ −27 (*c* 0.14, $CHCl_3$), was isolated as a white powder. The molecular formula of **1** was established as $C_{24}H_{32}O_{10}$ (nine degrees of unsaturation) from a sodiated molecule at *m*/*z* 503 in the ESIMS and further supported by HRESIMS at *m*/*z* 503.1892 (calcd 503.1893, $[C_{24}H_{32}O_{10} + Na]^+$). The IR spectrum of **1** showed bands at 3409, 1771, and 1730 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester groups. From the ^{13}C NMR data of **1** (Table 1), the presence of a trisubstituted olefin and an exocyclic carbon–carbon double bond were deduced from the signals at δ_C 144.7 (s, C-5), 121.0 (d, CH-6), 142.6 (s, C-11), and 120.0 (t, CH_2-20) and further supported by three olefin proton signals at δ_H 5.21 (1H, s, H-20a), 5.24 (1H, s, H-20b), and 5.55 (1H, s, H-6) in the ^1H NMR spectrum of **1** (Table 1). Three carbonyl resonances at δ_C 172.2 (s, C-19), 170.6 and 170.3 (2 × s, ester carbonyls) confirmed the presence of a γ-lactone and two ester groups in **1**; two acetyl methyls (δ_H 1.98 and 1.90, each 3H × s) were also observed. On the basis of overall unsaturation data, **1** was concluded to be a briarane-type diterpenoid molecule possessing four rings. The presence of a tetrasubstituted epoxide containing a methyl substituent was elucidated from the signals of two oxygenated carbons at δ_C 71.3 (s, C-8) and 59.8 (s, C-17), and further confirmed by the proton signal of a methyl singlet at δ_H 1.54 (3H, s, H_3-18).

The ^1H NMR coupling information in the ^1H–^1H COSY spectrum of **1** enabled identification of the C-2/-3/-4, C-6/-7, C-9/-10, C-12/-13/-14, C-6/-16 (by allylic coupling), and C-10/-20 (by allylic coupling) units (Figure 1), which were assembled with the assistance of an HMBC experiment. The HMBC correlations between protons and quaternary carbons of

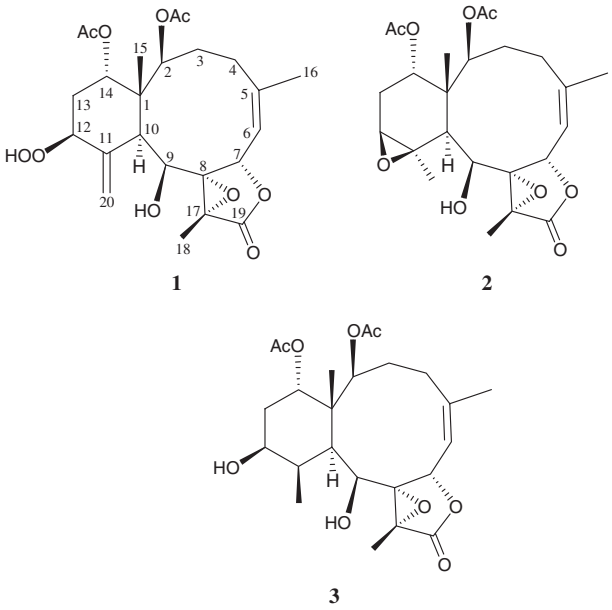

Chart 1.

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

C/H	^{1}H[a] (δ)	^{13}C[b] (δ)
1		45.7 (C)
2	4.78 dd (4.8, 4.4)[c]	74.8 (CH)
3α	1.82 m	30.8 (CH_2)
β	2.58 m	
4α	2.18 ddd (14.4, 10.8, 4.8)	29.5 (CH_2)
β	2.70 ddd (14.4, 5.2, 5.2)	
5		144.7 (C)
6	5.55 s	121.0 (CH)
7	5.55 s	73.8 (CH)
8		71.3 (C)
9	4.37 dd (7.2, 3.2)	71.7 (CH)
10	3.53 d (7.2)	42.2 (CH)
11		142.6 (C)
12	4.48 dd (8.8, 3.6)	83.6 (CH)
13α	2.25 ddd (16.0, 8.8, 2.4)	30.4 (CH_2)
β	1.93 ddd (16.0, 3.6, 3.6)	
14	4.71 dd (3.6, 2.4)	73.4 (CH)
15	1.40 s	16.3 (CH_3)
16	2.04 s	26.3 (CH_3)
17		59.8 (C)
18	1.54 s	9.3 (CH_3)
19		172.2 (C)
20a	5.21 s	120.0 (CH_2)
b	5.24 s	
9-OH	2.64 d (3.2)	
12-OOH	8.20 br s	
2-OAc	1.98 s	21.0 (CH_3)
		170.6 (C)
14-OAc	1.90 s	21.0 (CH_3)
		170.3 (C)

[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.

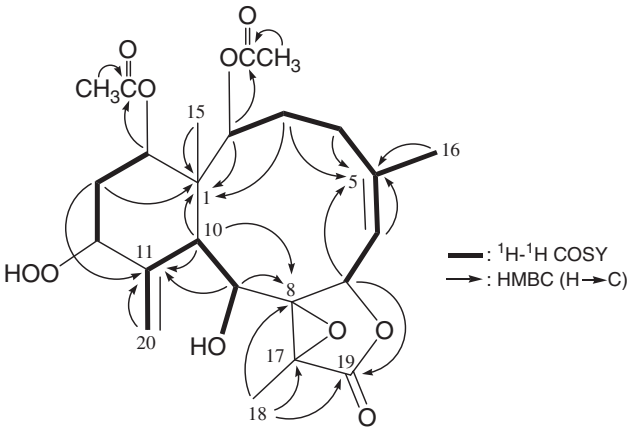

Figure 1. The ^{1}H–^{1}H COSY and selective HMBC correlations (protons → quaternary carbons) of **1**.

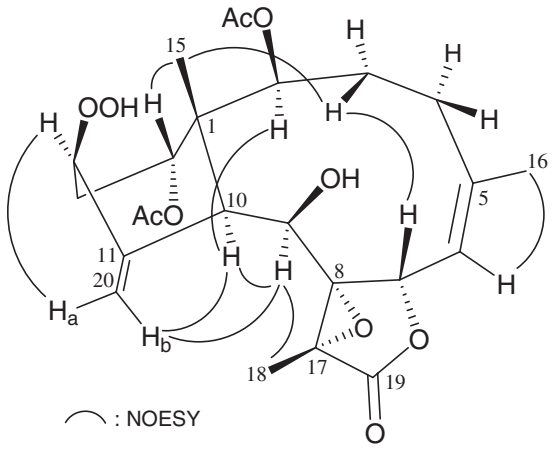

Figure 2. Selective NOESY correlations of **1**.

1, such as H-2, H_2-3, H-10, H-13α, H_3-15/C-1; H_2-3, H_2-4, H-6, H-7, H_3-16/C-5; H-9, H-10, H_3-18/C-8; H-9, H-10, H_2-13, H_2-20/C-11; H_3-18/C-17; and H-7, H_3-18/C-19, permitted elucidation of the carbon skeleton (Figure 1). An exocyclic double bond at C-11 was established by the HMBC correlations between H_2-20/C-10, -11, -12; and confirmed by the allylic coupling between H-10/H_2-20. A methyl at C-5 was confirmed by the allylic coupling between H-6/H_3-16 in the ^{1}H–^{1}H COSY spectrum and by the HMBC correlations between H_3-16/C-4, -5, -6; H_2-4/C-6; and H-6/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; H-2/C-15; and H-10/C-15. Furthermore, the acetate esters at C-2 and C-14 were established by correlations between H-2 (δ_H 4.78), H-14 (δ_H 4.71) and the acetate carbonyls observed in the HMBC spectrum of **1**. The presence of hydroxy group at C-9 was deduced from the ^{1}H–^{1}H COSY correlation between a hydroxy proton (δ_H 2.64) and H-9 (δ_H 4.37). The presence of a hydroperoxy group in **1** was supported by a hydroperoxy proton signal observed at δ_H 8.20 as a broad signlet,[11,13] and this group should be attached at C-12 as indicated by analysis of the ^{1}H–^{1}H COSY correlations and characteristic NMR signal analysis.

The proton chemical shifts for the briarane derivatives contained an 11,20-exocyclic carbon–carbon double bond are summarized: the differences between these two olefin protons (H-20a/b) are smaller than 0.2 ppm, while the cyclohexane rings show a twist boat conformation.[14] Due to the chemical shifts of C-20 methylene protons (δ_H 5.21, 1H, s; 5.24, 1H, s), the configuration of cyclohexane ring in **1** should exist in a twist boat conformation. The relative stereochemistry of **1** was elucidated mainly from the interactions observed in a NOESY experiment (Figure 2). In the NOESY experiment of **1**, H-10 gave a correlation to H-2, suggesting that these two protons were located on the same face and assigned as α protons, since the C-15 methyl is the β-substituent at C-1 and H_3-15 did not show correlation with H-10. H-14 exhibited a correlation with H_3-15, revealing the β-orientation of this proton. H-12 was found to correlate with one proton of C-20 methylene (δ_H 5.21, H-20a), but not with H-10 and H_3-15; and H-10 correlated with H-20b (δ_H 5.24), indicating that the C-12 hydroperoxy group was β-oriented and further supported that the methylene–cyclohexane ring in **1** existed in a twist boat conformation. The Z-configuration of C-5/6 double bond was elucidated by a correlation between C-6 olefin proton (δ_H 5.55) and C-16 vinyl

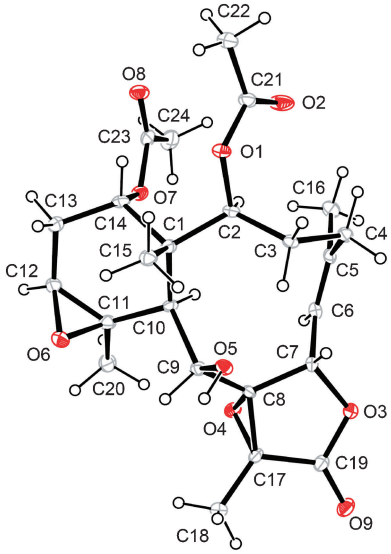

Figure 3. Computer-generated ORTEP plot of **2** showing the relative configuration.

methyl (δ_H 2.04). One proton of C-3 methylene (δ_H 2.58) correlated with H_3-15, but not with H-2, and was assigned as H-3β proton. H-7 showed a correlation with H-3β, confirming the β-orientation for H-7. Furthermore, H-9 showed correlations with H-10, H_3-18, and H-20b, and, from molecular models, was found to be reasonably close to H-10, H_3-18, and H-20b; therefore, H-9 should be placed on the α face in **1**, and H_3-18 is β-oriented in the γ-lactone moiety. On the basis of the above findings, the structure of **1** was elucidated.[15]

Since 1977, when the first briarane-type natural product, briarein A, was obtained from the Caribbean octocoral *Briareum asbestinum*,[16] over 500 3,8-cyclized cembranoid (briarane) compounds have been isolated and reported from various marine organisms, and the compounds of this type are only found in marine organisms, particularly from octocorals.[17–19] In these briarane metabolites, only two hydroperoxybriaranes, brianthein B and briarenolide B, were obtained from octocorals belonging to the genus *Briaruem*.[11,13] Briarenolide D (**1**) is the third briarane which possesses a hydroperoxy group in structure. The natural products of this type (12-hydroperoxybriarane) could be a chemical marker for the octocorals belonging to the genus *Briareum* (family Briareidae). A known briarane, excavatolide E (**3**) (Chart 1), was also isolated from a cultured octocoral belonging to genus *Briareum* in our previous study.[1] Its structure, including the absolute configuration of this metabolite was determined by chemical method.[1] Based on biosynthetic derivation, the new briarane **1** is assumed to have the same absolute configuration as **3**, because these compounds were isolated from the marine organisms belonging to the same genus.

Briarane **2** [2β-acetoxy-2-(debutyryloxy)stecholide E] was first isolated from a Taiwan octocoral identified as *Briareum* sp.,[12] and its structure was determined by X-ray diffraction analysis for the first time in this study (Figure 3).[20]

In cytotoxicity testing, hydroperoxybriarane **1** showed moderate cytotoxicity toward DLD-1 (human colon adenocarcinoma) and CCRF-CEM (human T cell acute lymphoblastic leukemia) cells (ED_{50} = 9.6, 6.9 μg mL^{-1}, respectively), but not active toward HL-60 (human promyelocytic leukemia) and P388D1 (murine macrophage cell) cells (ED_{50} > 40.0 μg mL^{-1}, respectively). Briarane **2** was also reported to show cytotoxicity toward P388 and HT-29 (human colon adenocarcinoma) tumor cells.[12]

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20 Crystallographic data of 2β-acetoxy-2-(debutyryloxy)stecholide E (**2**) have been deposited with Cambridge Crystallographic Data Center as a supplementary publication number CCDC-761299. Copies of the data can be obtained, free of charge, on application to CCDC, 12, Union Road, Cambridge, CB2 1EZ, U.K. (fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

