Briaviodiol A, a New Cembranoid from a Soft Coral *Briareum violacea*

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A new cembrane diterpenoid, briaviodiol A (1), has been isolated from a soft coral *Briareum violacea*. The structure of 1 was determined by spectroscopic methods and further confirmed by a single-crystal X-ray diffraction analysis.

Key words *Briareum violacea*; cembrane; briaviodiol; diterpenoid

Previous studies on the chemical constituents from the octocoral *Briareum violacea* (Quoy and Gaimard 1883, family Briareidae) (formerly known as *Pachyclavularia violacea*) have yielded a series of interesting natural products including cembrane,[1—5] briarane,[3,6,7] briarellin,[8—10] and secosterol analogues.[10] Recently, our chemical examination on the soft coral *B. violacea* has resulted in the isolation of a new cembranoid designated as briaviodiol A (**1**). The structure of **1** was elucidated by spectroscopic methods and further confirmed by a single-crystal X-ray diffraction analysis.

Results and Discussion

Briaviodiol A (**1**) was obtained as a white powder and could be recrystallized from methanol to form colorless prisms. The molecular formula of **1** was established as $C_{21}H_{32}O_6$ (six degrees of unsaturation) from a sodiated molecule at *m/z* 403 in the electronspray ionization (ESI)-MS spectrum and further supported by high resolution (HR)-ESI-MS (*m/z* 403.2093, Calcd 403.2096, $[C_{21}H_{32}O_6Na]^+$). The IR spectrum of **1** showed bands at 3411 and 1752 cm^{-1}, consistently with the presence of hydroxy and ester groups. From the ^{13}C-NMR data of **1** (Table 1), a suite of resonances at δ_C 171.3 (s, C-17), 158.1 (s, C-1), 130.6 (s, C-15), 108.5 (s, C-2), and 10.4 (q, C-16), could be assigned to the α-methyl-γ-butenolide moiety by comparison with the ^{13}C-NMR data of a known metabolite pachyclavulariolide F.[3] An additional unsaturated functionality was indicated by ^{13}C-NMR resonances at δ_C 131.5 (d, C-5) and 129.9 (s, C-4), suggesting the presence of a trisubstituted olefin. On the basis of overall unsaturation data, **1** was concluded to be a cembrane-type diterpenoid molecule possessing three rings.

The ^{1}H-NMR spectrum of **1** showed the presence of four methyl groups: a doublet (δ_H 0.86, couple to a methine proton at δ_H 1.26), a singlet at δ_H 1.38 representing a methyl group on an oxygenated quaternary carbon, a vinyl methyl (δ_H 1.76, s), a methyl on the butenolide moiety (δ_H 2.11, s). The ^{1}H-NMR coupling information in the ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum of **1** enabled identification of the C-5/-6/-7/-8/-9/-10/-11, C-13/-14, C-8/-19, and C-5/-18 (by allylic coupling) units (Fig. 1), which were assembled the assistance of a heteronuclear multiple bond coherence (HMBC) experiment (Fig. 1). The HMBC correlations between protons and quaternary carbons of **1**, such as H_2-3, H-14, H_3-16/C-1; H_2-3, H-14/C-2; H_2-3, H_3-18/C-4; H_2-11, H-13, H_3-20/C-12; H-14, H_3-16/C-15; and H_3-16/C-17, permitted elucidation of the carbon skeleton. A vinyl methyl at C-4 was confirmed by the allylic coupling between H-5/H_3-18 in the ^{1}H–^{1}H COSY spectrum and by the HMBC correlations between H_3-18/C-3, -4, -5; H-5/C-18; and H_2-3/C-18. Furthermore, the presence of hydroxy groups at C-13 and C-14 were deduced from the ^{1}H–^{1}H COSY correlations between the hydoxy protons (δ_H 2.25, 1H, d, J=10.0 Hz; 2.38, 1H, d, J=4.8 Hz) and the oxymethine protons at δ_H 3.41 (1H, dd, J=10.0, 1.2 Hz, H-13) and 5.20 (1H, dd, J=4.8, 1.2 Hz, H-14). The 2-methoxy group was indicated by an HMBC correlation between the proton signal of a methoxy group (δ_H 3.23, 3H, s) and an oxygenated quaternary carbon (δ_C 108.5,

Table 1. ^{1}H- and ^{13}C-NMR Data for Cembranoid **1**

C/H	δ_H [a)]	δ_C [b)]
1		158.1 (s)[d)]
2		108.5 (s)
3a/b	3.02 d (14.0)[c)]; 2.35 d (14.0)	47.2 (t)
4		129.9 (s)
5	5.53 t (8.0)	131.5 (d)
6a/b	2.14 m; 1.54 m	30.5 (t)
7a/b	1.16 m; 1.80 m	32.5 (t)
8	1.26 m	38.3 (d)
9	3.70 dt (10.0, 6.8)	84.7 (d)
10a/b	1.67 m; 2.00 m	38.2 (t)
11	2.11 m (2H)	24.6 (t)
12		85.4 (s)
13	3.41 dd (10.0, 1.2)	76.2 (d)
14	5.20 dd (4.8, 1.2)	68.4 (d)
15		130.6 (s)
16	2.11 s	10.4 (q)
17		171.3 (s)
18	1.76 s	18.9 (q)
19	0.86 d (6.4)	16.3 (q)
20	1.38 s	23.0 (q)
2-OCH_3	3.23 s	50.8 (q)
OH-13	2.25 d (10.0)	
OH-14	2.38 d (4.8)	

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 indicated by usual symbols.

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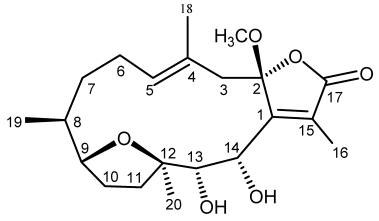

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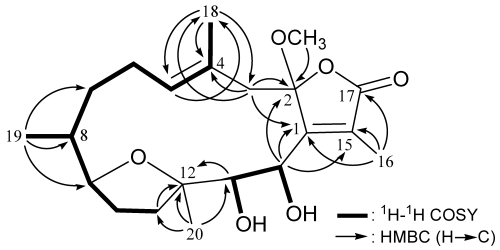

Fig. 1. ^{1}H–^{1}H COSY and Selective HMBC Correlations of **1**

s, C-2). Based on the consideration of molecular formula, a more oxygen atom had to be placed between C-9 and C-12 to form a tetrahydrofuran ring.

Due to the conformational mobility of the 14-membered ring, the stereochemistry for the chiral centers of **1** could not be fully determined by a nuclear Overhauser effect spectroscopy (NOESY) experiment. Fortunately, the single crystals of **1** could be obtained from the slow evaporation of methanol solution of **1**. A single crystal X-ray diffraction analysis was then carried out and the ORTEP drawing of the structure of **1** is shown in Fig. 2, which unambiguously confirmed the structure of **1**, and the relative configurations of chiral centers of **1** were assigned as 2*S**, 8*R**, 9*S**, 12*R**, 13*S**, and 14*R**. The geometry of the C-4/5 double bond in **1** was also found to exist in *trans* configuration.

In the cytotoxicity testing, briaviodiol A (**1**) was not active toward the DLD-1 (human colon adenocarcinoma), CCRF-CEM (human T-cell acute lymphoblastic leukemia), HL-60 (human promyelocytic leukemia), and P388D1 (murine macrophage cell) tumor cells (ED_{50}>40 mg/ml). Due to the screening platforms are limited; and lots of material were consumed in physical and spectral experiments. The other possible biological activities for this interesting substance will not be assayed at this stage. The extensive assay platforms for the natural products will be set up by the National Science and Technology Program of Biotechnology and Pharmaceuticals (NSTPBP), Taiwan.[11] The other possible bioactivities for this compound will be studied if we can get enough material in the future.

Experimental

General Melting points were measured on a FARGO apparatus and were uncorrected. Optical rotation values were measured with a JASCO P-1010 digital polarimeter. IR spectra were obtained on a VARIAN DIGLAB FTS 1000 Fourier transform-infrared (FT-IR) spectrophotometer. NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR, respectively, in $CDCl_3$. 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. ESI-MS and HR-ESI-MS data were recorded on a BRUKER APEX II mass spectrometer. Gravity column chromatography was performed on silica gel (230—400 mesh, Merck, Darmstadt, Germany). TLC was carried out on precoated Kieselgel 60 F_{254} (0.2 mm, Merck) and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. HPLC was performed using a system comprised of a HITACHI L-7100 pump, a HITACHI L-7455 photodiode array detector, and a RHEODYNE 7725 injection port. A normal phase column (Hibar 250×10 mm, LiChrospher Si 60, 5 μm) was used for HPLC.

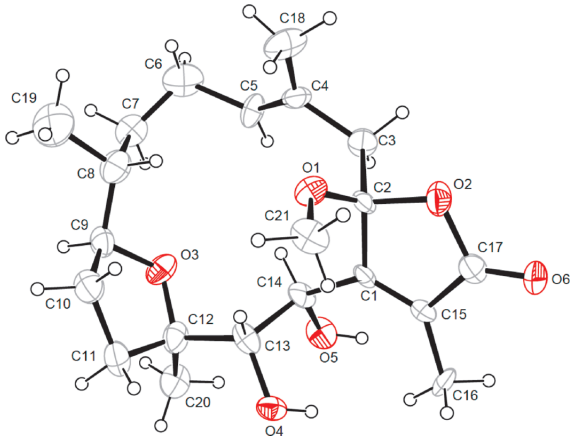

Fig. 2. Computer-Generated ORTEP Plot of **1** Showing the Relative Configuration

Animal Material Specimen of the octocoral *Briareum violacea* was collected off the coast of Pingtung, southern Taiwan, in March 2008, and this organism was identified by comparison with previous descriptions.[12] The voucher specimen was deposited in the National Museum of Marine Biology and Aquarium, Taiwan.

Extraction and Isolation The freeze-dried and sliced bodies of *B. violacea* (wet weight 1015 g, dry weight 395 g) were minced and extracted with a mixture of MeOH and CH_2Cl_2 (1 : 1) at room temperature. The extract was partitioned between EtOAc and H_2O. The EtOAc layer (12.2 g) was separated on silica gel and eluted using *n*-hexane/EtOAc (stepwise, 100 : 1–pure EtOAc) to yield 20 fractions. Fraction 7 was separated by silica gel and eluted using *n*-hexane/acetone (stepwise, 6 : 1–pure acetone) to yield the 13 fractions 7A—M. Fraction 7F was repurified by normal phase HPLC using a mixture of *n*-hexane and acetone to yield **1** (2.7 mg, 5/1).

Briaviodiol A (**1**): Colorless prisms; mp 162—164 °C; $[\alpha]_D^{23}$ −31° (*c*= 0.14, $CHCl_3$); IR (neat) ν_{max} 3411, 1752 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) data see Table 1; ESI-MS *m/z* 403 $(M+Na)^+$; HR-ESI-MS *m/z* 403.2093 (Calcd for $C_{21}H_{32}O_6Na$, 403.2096).

X-Ray Diffraction Analysis of Briaviodiol A (1) Suitable colorless prisms of **1** were obtained from a solution of MeOH. The crystal (0.50×0.30×0.20 mm) belongs to the monoclinic system, space group $P2_12_12_1$ (#19), with a=7.3813(19) Å, b=9.700(3) Å, c=31.818(11) Å, V= 2278.1(12) Å^3, Z=4, D_{calcd}=1.162 g/cm^3, and λ (Mo$K\alpha$)=0.71073 Å. Intensity data were measured on a Rigaku AFC7S diffractometer up to $2\theta_{max}$ of 52.00°. All 2571 reflections were collected. The structure was solved using direct methods and refined using a full-matrix least-squares procedure. The refined structural model converged to a final $R1$=0.0458 and $wR2$=0.1086 for 1255 observed reflections [$I>2\sigma(I)$] and 264 variable parameters.[13]

Cytotoxicity Testing The cytotoxicity of tested compound **1** was assayed with a modification of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. Cytotoxicity assays were carried out according to the procedures described previously.[14,15]

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13) Crystallographic data of the structure of briaviodiol A (**1**) have been deposited with Cambridge Crystallographic Data Center as supplementary publication number CCDC 784285. Cpoies of the data can be obtained, free of charge, on application the CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44 (0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].
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