The Structure and Absolute Stereochemistry of Briaexcavatin U, a New Chlorinated Briarane from a Cultured Octocoral *Briareum excavatum*

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A new chlorinated briarane, briaexcavatin U (**1**), has been isolated from a cultured octocoral *Briareum excavatum*. The structure, including the absolute stereochemistry of **1** was elucidated by spectroscopic methods and further confirmed by X-ray data analysis.

In our continuing search for novel substances from cultured marine invertebrates originally distributed in Taiwanese waters, we studied the extract from a cultured octocoral *B. excavatum* (Briareidae),[1] which is originally distributed in the tropical waters of the Indo-Pacific Ocean. We describe herein the isolation and structure determination of a new chlorinated briarane derivative, briaexcavatin U (**1**) (Chart 1), from *B. excavatum*. The structure of **1** was established by spectroscopic methods and supported by X-ray data analysis.

Specimens of *B. excavatum* were collected from cultivating tanks located in the NMMBA, Taiwan, in December 2006, and a series of briarane analogues, including briaexcavatins I–T, had been isolated from this cultured organism.[2–4]

The freeze-dried and minced material of *B. excavatum* (wet weight 672 g, dry weight 270 g) was extracted with a mixture of MeOH and CH_2Cl_2 (1:1) and 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 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 C9 was repurified by reverse phase HPLC, eluted using a mixture of MeOH, CH_3CN, and H_2O to afford **1** (1.9 mg, 49:1:50).

Briaexcavatin U (**1**) was obtained as a white powder and recrystallized as colorless prisms in a mixture of MeOH and acetone (3:1); mp 260–262 °C; $[\alpha]_D^{25}$ −136 (*c* 0.05, $CHCl_3$). The molecular formula for **1** was determined to be $C_{26}H_{33}ClO_{11}$ (10 degrees of unsaturation) was confirmed by HRESIMS data ($m/z[C_{26}H_{33}{}^{35}ClO_{11} + Na]^+$: found, 579.1604; calcd, 579.1609). Comparison of the ^{1}H and DEPT spectra with the molecular formula indicated that there must be two exchangeable protons, requiring the presence of two hydroxy groups. The IR spectrum of **1** also showed strong bands at 3277, 1774, and 1737 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 disubstituted olefin and an exocyclic olefin were deduced from the signals resonating at δ 129.9, 128.2, 135.9, 117.8, and further supported by four olefin proton signals resonating at δ 5.65, 5.92, 5.95, and 6.40 in the ^{1}H NMR spectrum of **1** (Table 1). Four carbonyl resonances appeared at δ 174.3, 170.4, 169.9, and 169.6 confirming the presence of a γ-lactone and three ester groups in **1**; three acetate methyls (δ 2.18, 2.12, and 2.07, each 3H × s) were also observed. So from the NMR data, six degrees of unsaturation were accounted for, and **1** must be tetracyclic. The presence of an epoxide was elucidated from the signals of two oxymethines at δ 61.9 and 54.1 and further confirmed by the proton signals resonating at

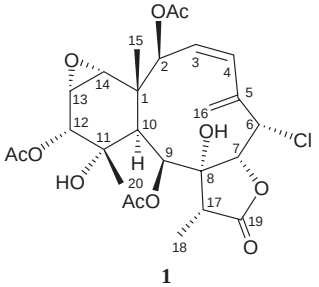

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Chart 1.

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

C/H	$^1H^a/\delta$	$^{13}C^b/\delta$	HMBC (H → C)
1		43.1 (s)[d]	
2	6.26 d (9.2)[c]	75.9 (d)	C-1, -4, -15, acetate carbonyl
3	5.65 dd (11.6, 9.2)	129.9 (d)	n.o.[f]
4	5.92 d (11.6)	128.2 (d)	C-2, -6, -16
5		135.9 (s)	
6	5.20 m	63.0 (d)	n.o.
7	5.05 d (4.0)	77.8 (d)	n.o.
8		83.7 (s)	
9	5.79 d (6.0)	68.4 (d)	C-7, -8, -10, -11, -17, acetate carbonyl
10	2.66 d (6.0)	36.2 (d)	C-1, -8, -9, -11, -12, -15, -20
11		73.3 (s)	
12	4.55 d (5.6)	73.0 (d)	C-10, -11, -20, acetate carbonyl
13	3.60 dd (5.6, 3.2)	54.1 (d)	C-11, -12
14	2.95 d (3.2)	61.9 (d)	C-1, -10, -15
15	1.20 s	15.6 (q)	C-1, -2, -10, -14
16/16′	5.95 d (2.4); 6.40 br s	117.8 (t)	C-4, -5, -6
17	2.41 q (6.8)	45.7 (d)	C-8, -9, -18, -19
18	1.26 d (6.8)	7.0 (q)	C-8, -17, -19
19		174.3 (s)	
20	1.30 s	22.3 (q)	C-10, -11, -12
OH-8	4.31 br s[e]		n.o.
OH-11	3.79 br s[e]		n.o.
2-OAc		169.6 (s)	
	2.07 s	21.0 (q)	acetate carbonyl
9-OAc		169.9 (s)	
	2.18 s	22.0 (q)	acetate carbonyl
12-OAc		170.4 (s)	
	2.12 s	20.4 (q)	acetate carbonyl

Spectra recorded at [a]400 and [b]100 MHz in $CDCl_3$ at 25 °C, respectively. [c]*J* values (in Hz) in parentheses. [d]Multiplicity deduced by DEPT and indicated by usual symbols. [e]Data exchangeable. [f]n.o. = not observed.

δ 3.60 and 2.95. In addition, two methyl singlets, a methyl doublet, two aliphatic protons, four oxymethine protons, a chlorinated methine proton, and two hydroxy protons were observed in the ^{1}H NMR spectrum of **1**.

The gross structure of **1** was determined by 2D NMR studies. ^{1}H NMR coupling information in the ^{1}H–^{1}H COSY spectrum of **1** enabled identification of the C-2/-3/-4, C-6/-7, C-9/-10, and C-12/-13/-14 units. From these data and the HMBC correlations (Table 1), the connectivity from C-1 to C-14 could be established. An exocyclic double bond attached at C-5 was confirmed by the allylic coupling between H-4/H_2-16 and H-6/H_2-16 in the ^{1}H–^{1}H COSY spectrum of **1** and by the HMBC correlations between H_2-16/C-5 and H-4/C-16. The ring junction C-15 methyl group was positioned at C-1 from the HMBC correlations between H-2/C-15, H-10/C-15, H-14/C-15, and H_3-15/C-1, C-2, C-10, C-14. Furthermore, the acetate esters positioned at C-2, C-9, and C-14 were established by the correlations between δ 6.26 (H-2), 5.79 (H-9), 4.55 (H-12) and the acetate carbonyls appeared at δ 169.6, 169.9, and 170.4, respectively. The 11-hydroxy group was confirmed from the signal of an oxygenated quaternary carbon resonating at δ 73.3 and from the proton chemical shift of C-20 tertiary methyl (δ 1.30) and by the HMBC correlations between H_3-20 and C-10, C-11, and C-12. The methine unit at δ 63.0 was more shielded than would be expected for an oxygenated C-atom and was correlated to the methine proton at δ 5.20 in the HMQC spectrum. The latter methine signal was ^3J-correlated with H-7 (δ 5.05), proving the attachment of a chloride atom at C-6. Thus, the remaining hydroxy group had to be positioned at C-8. These data, together with the HMBC correlations between H-9/C-17; H-17/C-8, C-9, C-18, C-19; and H_3-18/C-8, C-17, C-19, unambiguously established the molecular framework of **1**.

The relative stereochemistry of **1** was elucidated from the NOESY interactions observed in a NOESY experiment (Figure 1) and by the vicinal ^{1}H–^{1}H coupling constants analysis. In the NOESY experiment of **1**, H-10 gives correlations to H-2 and H-9, but not with H_3-15 and H_3-20, indicating that H-2, -9, and H-10 are located on the same face of the molecule and assigned as α-protons, since C-15 and C-20 methyls are β-substituents at C-1 and C-11, respectively. The C-20 methyl protons were found to exhibit responses with H-12, H-13, H-14, and H_3-15 showing that the 11-hydroxy, 12-acetoxy, and C-13/14 epoxy groups all were α-oriented. H-9 was found to be reasonably close to H-17, H_3-18, and H_3-20 and can be therefore be placed on the α face in the 10-membered ring of **1**. The cis geometry of C-3/4 double bond was indicated by a strong correlation between H-3 (δ 5.65) and H-4 (δ 5.92) and confirmed by an

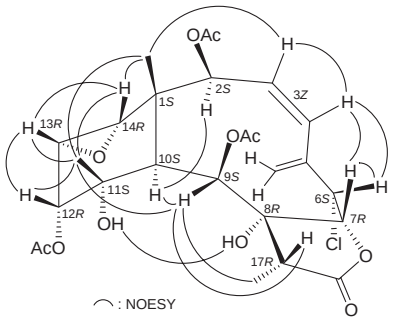

Figure 1. Selective NOESY correlations of **1**.

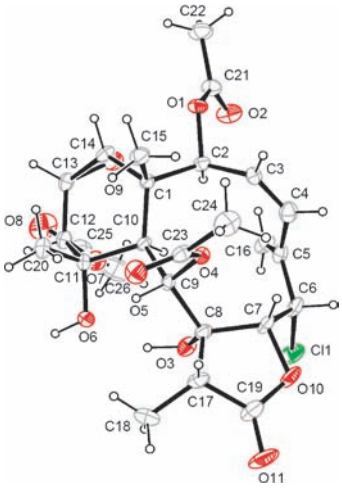

Figure 2. Computer-generated ORTEP plot of **1** showing the absolute configuration.

11.6 Hz coupling constant between these two olefin protons. Furthermore, a strong interaction was observed between H-3 and H_3-15; and there are responses which were observed among H-4, H-6, and H-7, indicating that H-6 and H-7 are on the β face of **1**. Moreover, a correlation observed between the two hydroxy protons showed that the 8-hydroxy group was α-oriented. However, no response was observed between H-17 and any proton in the NOESY experiment of **1**, except for H-9, so the stereochemistry of the C-17 methine cannot be determined by this method.

A single-crystal X-ray diffraction analysis was carried out in order to determine the structure of **1**. The X-ray structure (Figure 2) demonstrates the C-17 methine proton was β-oriented and the $\Delta^{3,5(16)}$-butadiene system was found to be present in an s-cis system. On the basis of the X-ray diffraction analysis, the chiral centers in **1** were assigned as 1S, 2S, 6S, 7R, 8R, 9S, 10S, 11S, 12R, 13R, 14R, and 17R.[5] From the above findings, the structure, including the absolute configuration of **1**, was therefore elucidated unambiguously.

The cytotoxicity of **1** toward CCRF-CEM (human T-cell acute lymphoblastic leukemia) and DLD-1 (human colon adenocarcinoma) tumor cells was assayed, and it was found that compound **1** was inactive (ED_{50} > 50 µg/mL) toward these two tumor cell lines. Other possible biological activities of **1** will be assayed in the future.

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5 Crystallographic data for the structure of briaexcavatin U (**1**) has been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 700216. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

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