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Fragilide B: A Novel Briarane-Type Diterpenoid with a *S-cis* Diene Moiety

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A novel chlorinated briarane derivative, fragilide B (**1**), was isolated from the Formosan gorgonian coral *Junceella fragilis*. Fragilide B (**1**) possesses a *s-cis* diene moiety in the structure. The structure of **1** was elucidated by interpretation of spectral data and further supported by molecular mechanics calculations.

Previous studies on the gorgonian *J. fragilis* have resulted in the isolation of a series of novel metabolites, including fragilide A,[1] with a briarane carbon skeleton. In continuing research on the chemical constituents of *J. fragilis*, a novel chlorinated briarane, fragilide B (**1**), which has a *s-cis* diene moiety, was isolated. The isolation, structure determination, and bioactivity of **1** are reported in this paper.

The organism *J. fragilis* (wet wt, 424 g), collected off the southern coast of Taiwan in Aug. 2005, was minced and extracted with a mixture of MeOH and CH_2Cl_2 (1:1). The extract was partitioned between hexane and 9:1 MeOH–H_2O; the MeOH–H_2O phase was diluted with 1:1 MeOH–H_2O and partitioned against CH_2Cl_2. The CH_2Cl_2 layer (1.59 g) was separated by silica-gel column chromatography using CH_2Cl_2 and CH_2Cl_2/EtOAc mixtures of increasing polarity. A fraction eluted with CH_2Cl_2/EtOAc (8/1) was purified by normal-phase HPLC to yield **1** (hexane/EtOAc, 3/2, 6.0 mg).

Fragilide B (**1**), mp 123–125 °C; $[\alpha]_D^{25}$ +13 (*c* 0.3, $CHCl_3$), was obtained as a white powder. From HR-ESI-MS, the molecular formula was determined to be $C_{27}H_{35}ClO_{10}$ with *m/z* 577.1815 [$(M + Na)^+$, calcd 577.1816], indicating ten degrees of unsaturation. The IR spectrum of **1** showed the presence of hydroxy, γ-lactone, and ester (3742, 1782, and 1740 cm^{-1}) groups. From the ^{13}C NMR data of **1** (Table 1), a disubstituted and an exocyclic olefins were deduced from the signals of carbons resonating at δ 141.6 (s), 132.7 (d), 130.3 (d), and 116.3 (t) and further supported by four olefin protons signals at δ 6.81 (1H, d, $J = 16.0$ Hz), 6.09 (1H, dd, $J = 16.0$, 9.2 Hz), 5.34 (1H, br s), and 5.32 (1H, br s) in the ^1H NMR spectra of **1**. Moreover, four carbonyl resonances appeared at δ 174.9 (s), 173.3 (s), 170.3 (s), and 170.2 (s), confirming the presence of a γ-lactone and three esters in **1**. In the ^1H NMR spectrum of **1** (Table 1), a propionyloxy (δ 2.31, 2H, q, $J = 7.6$ Hz; 1.13, 3H, t, $J = 7.6$ Hz) and two acetoxy (δ 2.14, 3H, s; 2.12, 3H, s) groups were observed. Thus, from the NMR data, six degrees of unsaturation were accounted for, and **1** must be tetracyclic. An exocyclic epoxy group was confirmed from the signals of two oxygenated carbons at δ 57.4 (s, C-11) and 50.4 (t, CH_2-20). The proton chemical shifts of H_2-20 (δ 2.62, 1H, br d, $J = 3.2$ Hz, H-20a; 2.58, 1H, br d, $J = 3.2$ Hz, H-20b) confirmed the presence of this group. In addition, a methyl singlet (δ 1.15, 3H, s, H_3-15), a methyl doublet (δ 1.26, 3H, d, $J = 6.8$ Hz, H_3-18), two aliphatic methine protons (δ 3.23, 1H, br s, H-10; 2.86, 1H, q, $J = 6.8$ Hz, H-17), two pairs of methylene protons (δ 2.15, 1H, m; 1.20, 1H, ddd, $J = 12.8$, 3.2, 2.4 Hz, H_2-12; 1.92, 1H, m; 1.86, 1H, ddd, $J = 14.4$, 4.4, 2.4 Hz, H_2-13), four oxymethine protons (δ 5.63, 1H, d,

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

C/H	^1H[a)]	^{13}C[b)]
1		49.3 (s)
2	5.63 d (9.2)	75.5 (d)
3	6.09 dd (16.0, 9.2)	130.3 (d)
4	6.81 d (16.0)	132.7 (d)
5		141.6 (s)
6	5.07 d (3.6)	64.0 (d)
7	4.16 d (3.6)	81.0 (d)
8		82.8 (s)
9	5.17 d (2.0)	72.3 (d)
10	3.23 br s	39.1 (d)
11		57.4 (s)
12α/β	2.15 m; 1.20 ddd (12.8, 3.2, 2.4)	30.2 (t)
13α/β	1.92 m; 1.86 ddd (14.4, 4.4, 2.4)	25.0 (t)
14	4.94 br s	74.3 (d)
15	1.15 s	15.2 (q)
16a/b	5.34 br s; 5.32 br s	116.3 (t)
17	2.86 q (6.8)	50.3 (d)
18	1.26 d (6.8)	7.5 (q)
19		174.9 (s)
20a/b	2.62 br d (3.2); 2.58 br d (3.2)	50.4 (t)
OH-8	3.03 br s	
Acetates	2.14 s	21.2 (q)
		170.3 (s)
	2.12 s	21.4 (q)
		170.2 (s)
Propionate	1.13 t (7.6)	8.9 (q)
	2.31 q (7.6)	27.7 (t)
		173.3 (s)

a) Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. b) Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C.

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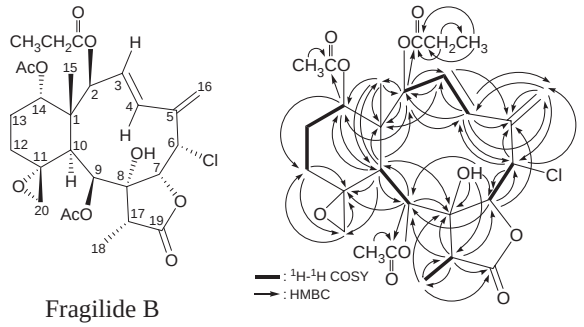

Fig. 1. ^{1}H–^{1}H COSY and HMBC correlations for **1**.

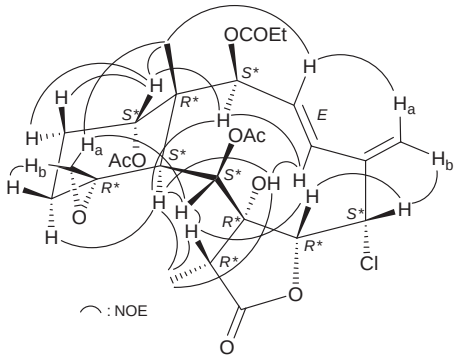

Fig. 2. Selective key NOESY correlations for **1**.

$J = 9.2$ Hz, H-2; 5.17, 1H, d, $J = 2.0$ Hz, H-9; 4.94, 1H, br s, H-14; 4.16, 1H, d, $J = 3.6$ Hz, H-7), four olefin protons (δ 6.81, 1H, d, $J = 16.0$ Hz, H-4; 6.09, 1H, dd, $J = 16.0$, 9.2 Hz, H-3; 5.34, 1H, br s, H-16a; 5.32, 1H, br s, H-16b), a chlorinated methine proton (δ 5.07, 1H, d, $J = 3.6$ Hz, H-6), and a hydroxy proton (δ 3.03, 1H, br s, OH-8) were observed in the ^{1}H NMR spectrum of **1**.

The gross structure of **1** and all of the ^{1}H and ^{13}C NMR data associated with the molecule were determined by 2D NMR studies. From the ^{1}H–^{1}H COSY spectrum of **1** (Fig. 1), it was possible to establish the proton sequences from H-2/H-3, H-3/H-4, H-6/H-7, H-9/H-10, H_2-12/H_2-13, and H_2-13/H-14. Based on these data and the HMBC correlations (Fig. 1), the connectivity from C-1 to C-14 could be established. An exocyclic double bond attached at C-5 was confirmed by the HMBC correlations between H_2-16/C-4, C-5, C-6; H-4/C-16; and H-6/C-16. The epoxy group positioned at C-11/C-20 was confirmed by the connectivity between H_2-12/C-11, C-20, and H_2-20/C-11, C-12. The ring-juncture C-15 methyl group was positioned at C-1 from the key HMBC correlations between H_3-15/C-1, C-2, C-10, C-14; H-2/C-15; and H-10/C-15. In the HMBC spectrum of **1**, the carbon signal at δ 173.3 (s) was correlated with the signal of the methylene protons at δ 2.31 and was assigned to be the carbon atom of the propionate carbonyl. The propionate ester positioned at C-2 was confirmed from the connectivity between H-2 (δ 5.63) and carbonyl carbon (δ 173.3) of the propionate. In addition, the HMBC correlations also indicated that two acetoxy groups should be attach at C-9 and C-14, respectively. Thus, the remaining hydroxy group would be at C-8, an oxygen-bearing quaternary carbon resonating at δ 82.8. This observation was further confirmed from the HMBC correlations observed between OH-8/C-7, C-8, C-9, C-17. These data, together with the ^{1}H–^{1}H COSY correlation between H-17/H_3-18 and the HMBC correlations among H-17/C-7, C-8, C-9, C-18, C-19 and H_3-18/C-8, C-17, C-19, were used to establish unambiguously the molecular framework of **1**.

The ^{13}C chemical shifts of exocyclic 11,20-epoxy groups in briarane derivatives have been summarized, and although the ^{13}C NMR peaks for C-11 and C-20 appear at δ 55–61 and 47–52 ppm, respectively, the epoxy group is α-oriented (11*R**), and the cyclohexane ring should be in a chair conformation.[2] Based on above observations, the configuration of 11,20-epoxy group in **1** (δ 57.4, s, C-11; 50.4, t, CH_2-20) should be α-oriented, and the cyclohexane ring should exists in a chair conformation. The relative stereochemistry of **1** was elucidated mainly from the NOE interactions observed in an NOESY experiment (Fig. 2) and by the vicinal ^{1}H–^{1}H coupling constants. Due to the α orientation of H-10, the ring juncture C-15 methyl should be β-oriented as no NOE correlations were observed between H-10 and H_3-15. In the NOESY spectrum of **1**, H-10 gives NOE correlations to H-2, H-9, OH-8, and one proton of the C-12 methylene (δ 2.15), and H_3-18 showed NOE correlations with H-10 and OH-8, suggesting that these protons (H-2, OH-8, H-9, H-10, H-12α, H_3-18) are located on the same face. They were assigned as α protons, since C-15 methyl is β-oriented and H-10 did not show correlation with H_3-15. H-14 was found to exhibit NOE responses with H-2, H_2-13, and H_3-15, but not with H-10, showing that this proton has a β-orientation. H-7 exhibited strong NOE correlations with H-6 and H-17, suggesting that these protons are on the β face of **1**. The *trans* geometry of C-3/C-4 double bond is indicated by a 16.0 Hz coupling constant between H-3 (δ 6.09) and H-4 (δ 6.81). Moreover, the olefin proton H-3 showed strong NOE correlations with H_3-15 and H-16a, but not with H-2, and H-4 showed strong responses with H-2 and OH-8, demonstrating the *E*-configuration of $\Delta^{3,4}$. Therefore, *s-cis* diene moiety in **1** was elucidated. Based on above findings, the configurations of all chiral centers of **1** were assigned to be 1*R**,2*S**,3*E*,6*S**,7*R**,8*R**,9*S**,10*S**,11*R**,14*S**,17*R**.

By detailed analysis, the NMR data (^{1}H and ^{13}C) of **1** were found to be very close with those of the chlorinated briarane derivatives with a $\Delta^{3,5(16)}$-butadiene system, such as junceellolides B and C,[3] umbraculolide D,[4] juncin O,[5] and an unnamed briarane isolated from a Japanese octocoral *Pteroeides* sp.[6] The *E* configuration between the olefinic protons H-3/H-4 in these metabolites is supported by a large coupling constant (16.0 Hz). However, the geometry for the $\Delta^{3,5(16)}$-butadiene in these metabolites which exists in a *s-trans* or *s-cis* configuration has not been discussed previously. Based on the NOESY experiment analysis described above, the authors suggest that the $\Delta^{3,5(16)}$-butadiene system in these metabolites (junceellolides B and C, umbraculolide D, juncin O, etc.) should be the *s-cis* form as found in **1**. In a review article for the marine natural products, the $\Delta^{3,5(16)}$-butadiene moiety in the unnamed briarane isolated from a Japanese octocoral *Pteroeides* sp. also has been revised to be a *s-cis* diene system.[7]

Furthermore, a geometrical optimization of **1** was performed with DISCOVER utilizing the consistent valence force field (CVFF) calculations for energy minimization.[8] The calcu-

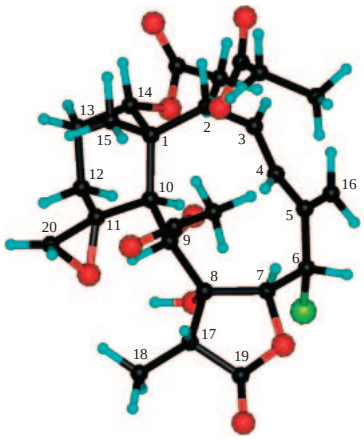

Fig. 3. Stereoview of **1** generated from computer modeling.

Table 2. Inhibitory Effects of Briarane **1** on Superoxide Anion Generation and Elastase Release by Human Neutrophil in Response to fMet-Leu-Phe/Cytochalastin B

Compound	Superoxide anion generation/%[a)]	HNE release/%[a)]
1	17.55 ± 0.07	20.67 ± 0.34
Genistein[b)]	94.69 ± 5.08	78.26 ± 6.26

a) Percentage of inhibition (%) at $10\,\mu g\,mL^{-1}$. b) Genistein: a tyrosine kinase inhibitor, was used as positive control in anti-inflammatory assay.

lated results were visualized using INSIGHT II, running on a Silicon Graphics IRIS (SGI) Indigo XS24/4000 workstation. The conformation search suggested that the most stable conformation of **1** is shown in Fig. 3.

Fragilide B (**1**) exhibited weak activity towards the inhibition of human neutrophil elastase (HNE) release (Table 2). Superoxide generation and elastase release were carried out according to the procedures described previously.[9,10]

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