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12-*epi*-FRAGILIDE G, A NEW BRIARANE-TYPE DITERPENOID FROM THE GORGONIAN CORAL *ELLISELLA ROBUSTA*

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Abstract – A new chlorinated briarane-type diterpenoid, 12-*epi*-fragilide G (**1**), was isolated from the gorgonian coral *Ellisella robusta*. The structure of **1** was elucidated by the interpretations of spectral data analysis and this compound was found to possess an *s-cis* diene moiety in its structure. Briarane **1** displayed inhibitory effects on elastase release by human neutrophils.

Previous studies on the gorgonians belonging to the genus *Ellisella* (family Ellisellidae), have resulted in the isolation of a series of novel natural products, including robustolides A–K, featuring with briarane carbon skeleton (3,8-cyclized cembranoid).^{1–6} During our further studies on the chemical constituents of a gorgonian coral *Ellisella robusta*, collected off Taiwan waters, a new chlorinated briarane, 12-*epi*-fragilide G (**1**) (Chart 1), which was found to possess an *s-cis* diene moiety, was isolated. In this paper, we reported the isolation, structure determination, and bioactivity of above new briarane **1**.

12-*epi*-Fragilide G (**1**) was obtained as a white powder. The HRESIMS data established the molecular formula of **1** as C₂₈H₃₅ClO₁₂, with *m/z* 621.1718 [(M+Na)⁺, calcd. 621.1715], indicating 11 degrees of unsaturation. The IR spectrum of **1** showed the presence of hydroxy (3464 cm⁻¹), γ -lactone (1783 cm⁻¹), and ester (1737 cm⁻¹) groups. From the ¹³C NMR data of **1** (Table 1), a disubstituted olefin and an

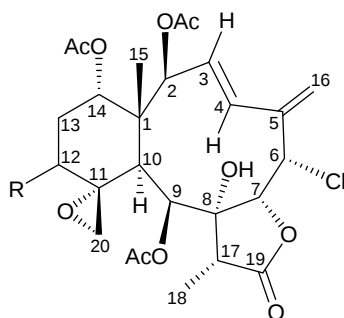


Chart 1. 12-*epi*-Fragilide G (**1**):R= α -OAc
Fragilide G (**2**):R= β -OAc

Table 1. ^1H and ^{13}C NMR Chemical Shifts and ^1H - ^1H COSY and HMBC Correlations for **1**

C/H	$^1\text{H}^a$	$^{13}\text{C}^b$	^1H - ^1H COSY	HMBC (H $\rightarrow$ C)
1		49.1 (s) ^d		
2	5.70 d (9.6) ^c	75.7 (d)	H-3	C-1, -3, -4, -14, -15, acetate carbonyl
3	6.01 dd (15.6, 9.6)	130.4 (d)	H-2, H-4	C-5
4	6.88 d (15.6)	132.6 (d)	H-3, H-16a	C-2, -3, -6, -16
5		142.1 (s)		
6	5.07 d (4.0)	65.1 (d)	H-7	C-4, -5, -7, -8, -16
7	4.16 d (4.0)	80.6 (d)	H-6	C-6, -9, -17
8		82.8 (s)		
9	5.18 d (2.4)	72.1 (d)	H-10	C-1, -8, -10, -11, -17, acetate carbonyl
10	3.84 br s	33.8 (d)	H-9	C-1, -8, -9, -11, -15, -20
11		57.3 (s)		
12	4.52 dd (3.2, 2.8)	73.7 (d)	H ₂ -13	C-11, -14, acetate carbonyl
13 α	2.27 m	29.0 (t)	H-12, H-13 β , H-14	C-1, -11, -14
β	2.01 m		H-12, H-13 α , H-14	n.o. ^f
14	4.98 dd (2.8, 2.8)	73.1 (d)	H ₂ -13	C-12
15	1.17 s	14.4 (q)		C-1, -2, -10, -14
16a	5.34 s	115.2 (t)	H-4, H-16b	C-4, -6
b	5.26 s		H-16a	C-4, -5, -6
17	2.85 q (7.2)	50.0 (d)	H ₃ -18	C-8, -9, -18, -19
18	1.25 d (7.2)	6.9 (q)	H-17	C-8, -17, -19
19		174.6 (s)		
20a	2.77 dd (4.0, 1.2)	49.2 (t)	H-20b	n.o.
b	2.65 d (4.0)		H-20a	C-11, -12
OH-8	3.08 br s			C-7, -8, -9
Acetate	2.11 s	21.4 (q) ^e		Acetate carbonyl
methylys	2.10 s	21.1 (q) ^e		Acetate carbonyl
	2.05 s	21.1 (q) ^e		Acetate carbonyl
	2.02 s	21.0 (q) ^e		Acetate carbonyl
Acetate		170.3 (s)		
carbonyls		170.0 (s)		
		169.9 (s)		
		169.3 (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. ^c *J* values (in Hz) in parentheses. ^d Multiplicity deduced by DEPT and HMQC spectra. ^e Data exchangeable. ^f n.o.=not observed.

exocyclic carbon-carbon double bond were deduced from the signals of four carbons at δ_{C} 142.1 (s, C-5), 132.6 (d, CH-4), 130.4 (d, CH-3), and 115.2 (t, CH₂-16). Five carbonyl resonances at δ_{C} 174.6 (s, C-19), 170.3, 170.0, 169.9, and 169.3 (4 $\times$ s, ester carbonyls), confirmed the presence of a γ -lactone and four esters in **1**. In the ^1H NMR spectrum of **1** (Table 1), four acetyl methyls (δ_{H} 2.11, 2.10, 2.05, and 2.02,

each 3H×s) were observed. Thus, the NMR data accounted for seven degrees of unsaturation and requiring **1** to be tetracyclic. An exocyclic epoxy group was confirmed from the signals of two oxygenated carbons at δ_C 57.3 (s, C-11) and 49.2 (t, CH₂-20), and further supported by the proton chemical shifts of H₂-20 (δ_H 2.77, 1H, dd, J = 4.0, 1.2 Hz, H-20a; 2.65, 1H, d, J = 4.0 Hz, H-20b). Moreover, a methyl singlet (δ_H 1.17, 3H, s, H₃-15), a methyl doublet (δ_H 1.25, 3H, d, J = 7.2 Hz, H₃-18), two aliphatic methine protons (δ_H 3.84, 1H, br s, H-10; 2.85, 1H, q, J = 7.2 Hz, H-17), a pair of aliphatic methylene protons (δ_H 2.27, 1H, m; 2.01, 1H, m; H₂-13), five oxymethine protons (δ_H 5.70, 1H, d, J = 9.6 Hz, H-2; 5.18, 1H, d, J = 2.4 Hz, H-9; 4.98, 1H, dd, J = 2.8, 2.8 Hz, H-14; 4.52, 1H, dd, J = 3.2, 2.8 Hz, H-12; 4.16, 1H, d, J = 4.0 Hz, H-7), four olefin protons (δ_H 6.88, 1H, d, J = 15.6 Hz, H-4; 6.01, 1H, dd, J = 15.6, 9.6 Hz, H-3; 5.34, 1H, s, H-16a; 5.26, 1H, s, H-16b), a chlorinated methine proton (δ_H 5.07, 1H, d, J = 4.0 Hz, H-6), and a hydroxy proton (δ_H 3.08, 1H, br s, OH-8) were observed in the ¹H NMR spectrum of **1**.

The gross structure of **1** was determined by 2D NMR studies, including ¹H–¹H COSY, HMQC, and HMBC experiments. From the ¹H–¹H COSY spectrum of **1** (Table 1), it was possible to establish the separate spin systems that map out the proton sequences from H-2/H-3, H-3/H-4, H-6/H-7, and H-9/H-10. These data, together with the HMBC correlations between H-2/C-1, -3, -4; H-3/C-5; H-4/C-2, -3, -6; H-6/C-4, -5, -7, -8; H-7/C-6, -9; H-9/C-1, -8, -10; and H-10/C-1, -8, -9 (Table 1), established the connectivity from C-1 to C-10 in a ten-membered ring. An exocyclic double bond at C-5 was confirmed by the HMBC correlations between H-16a/C-4, -6; H-16b/C-4, -5, -6; H-4/C-16; and H-6/C-16; and further confirmed by the ¹H–¹H COSY correlation between H-4 and H-16a (by allylic coupling). The cyclohexane ring, which is fused to the ten-membered ring at C-1 and C-10, was elucidated by the HMBC correlations between H-2/C-14; H-9/C-11; H-10/C-11, -20; and one proton of C-13 methylene (H-13 α)/C-1. The epoxy group positioned at C-11/20 was confirmed by the connectivity between H-20b and C-11, -12. The C-15 methyl group was positioned at C-1 from the HMBC correlations between H₃-15/C-1, -2, -10, -14; H-2/C-15; and H-10/C-15. In addition, the HMBC correlations also revealed that three acetoxy groups should attach at C-2, C-9, and C-12, respectively (Table 1). The hydroxy proton signal at δ_H 3.08 was revealed by its HMBC correlations to C-7, -8, and C-9, indicating its attachment to C-8, an oxygen-bearing quaternary carbon at δ_C 82.8. Thus, the remaining acetoxy group should be positioned at C-14, as indicated by analysis of the ¹H–¹H COSY correlations and characteristic NMR signals analysis. These data, together with the ¹H–¹H COSY correlation between H-17 and H₃-18 and the HMBC correlations between H-17/C-8, -9, -18, -19 and H₃-18/C-8, -17, -19, unambiguously established the molecular framework of **1**.

In a previous study, the ¹³C chemical shifts of exocyclic 11,20-epoxy groups in briarane derivatives were

summarized, that while the ^{13}C NMR data for C-11 and C-20 were appeared at δ_{C} 55–61 and 47–52 ppm, respectively, the epoxy group was α -oriented (11 R^*) and the cyclohexane ring should be existed in chair conformation.⁷ Based on the above observations, the configuration of 11,20-epoxy group in **1** (δ_{C} 57.3, s, C-11; 49.2, t, CH₂-20) should be α -oriented and the cyclohexane ring was existed in a chair conformation.

The relative stereochemistry of **1** was elucidated mainly from the interactions observed in a NOESY experiment (Figure 1) and by the vicinal ^1H – ^1H coupling constants. As per convention while analyzing the stereochemistry of briarane-type natural products, H-10 and H₃-15 were assigned to the α and β face, anchoring the stereochemical analysis because no correlation was observed between H-10 and H₃-15. In the NOESY experiment of **1**, H-10 gave correlations to H-2, H-9, OH-8, and H₃-18, suggesting that these protons were located on the same face and assigned as α protons, since C-15 methyl is the β -substituent at C-1. H-14 was found to exhibit responses with H-2, H-13 α/β , and H₃-15, but not with H-10, revealing the β -orientation of this proton. In addition, H-12 was found to correlate with H-13 α/β and one proton of C-20 methylene (δ_{H} 2.27, H-20a), indicating the C-12 acetoxy group was α -oriented. H-7 exhibited correlations with H-6 and H-17, suggesting that these protons were positioned on the β face in **1**. The *trans* geometry of C-3/4 double bond was indicated by a 15.6 Hz coupling constant between H-3 (δ_{H} 6.01) and H-4 (δ_{H} 6.88). Moreover, the olefin proton H-3 showed a correlation with H₃-15, but not with H-2; and H-4 showed responses with H-2 and OH-8, demonstrating the *E*-configuration of $\Delta^{3,4}$ and established the conjugated *s-cis* diene moiety in **1**. Based on the above findings, the structure of **1** was established, and the configurations of all chiral centers of **1** are assigned as 1 R^* , 2 S^* , 6 S^* , 7 R^* , 8 R^* , 9 S^* , 10 S^* , 11 R^* , 12 R^* , 14 S^* , and 17 R^* . By comparison the related spectral data with those of a known briarane analogue, fragilide G (**2**) (Chart 1), which was isolated from a Taiwanese gorgonian coral *Junceella fragilis*.⁸ The structure of **1** was found to be the 12-*epi*-compound of fragilide G (**2**) and should be named as 12-*epi*-fragilide G.

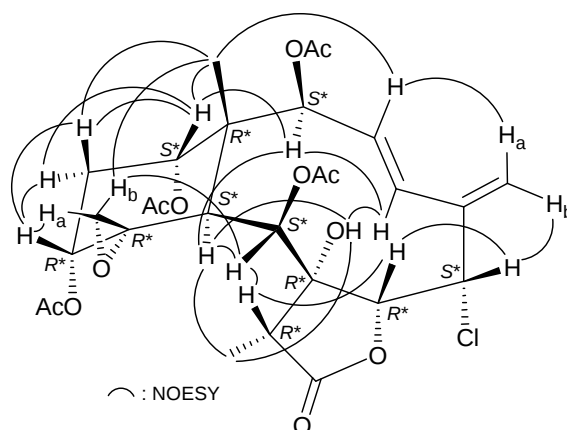


Figure 1. Selective Key NOESY Correlations of **1**

It has to be noted here that a briarane containing an *s-cis* diene moiety as presented in **1**, is unprecedented. To the best of knowledge, only three briarane metabolites, robustolide J, fragilides B, and fragilide G,^{6,8,9} were found to possess the functional group of this type. 12-*epi*-Fragilide G (**1**) is the fourth example which possessing an *s-cis* diene moiety in structure. Briarane **1** displayed 61.4% inhibitory effect on elastase release by human neutrophils at 10 µg/mL.

EXPERIMENTAL

General Experimental Procedures. Optical rotation values were measured with a JASCO P-1010 digital polarimeter. Infrared spectra were obtained on a VARIAN DIGLAB FTS 1000 FT-IR spectrophotometer. The NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR at 400 MHz for ¹H and 100 MHz for ¹³C, in CDCl₃, respectively. Proton chemical shifts were referenced to the residual CHCl₃ signal (δ_{H} 7.26 ppm). ¹³C NMR spectra were referenced to the center peak of CDCl₃ at δ_{C} 77.1 ppm. ESIMS and HRESIMS data were recorded on a BRUKER APEX II mass spectrometer. Column chromatography was performed on silica gel (230–400 mesh, Merck, Darmstadt, Germany). TLC was carried out on precoated Kieselgel 60 F₂₅₄ (0.25 mm, Merck) and spots were visualized by spraying with 10% H₂SO₄ solution followed by heating. HPLC was performed using a system composed of a HITACHI L-7100 pump, a HITACHI L-7455 photo diode array detector, a RHEODYNE 7725 injection port, and a normal phase semi-preparative column (Hibar 250×10 mm, LiChrospher Si 60, 5 µm).

Animal Material. Specimens of *Ellisella robusta* were collected by hand using scuba gear off the southern Taiwan coast. This organism was identified by comparison with previous descriptions.^{10–12} A voucher specimens was deposited in the National Museum of Marine Biology & Aquarium (NMMBA), Taiwan.

Extraction and Isolation. The freeze-dried and minced material of *E. robusta* (wet weight 1909 g, dry weight 830 g) was extracted with a mixture of MeOH and CH₂Cl₂ (1:1). The residue was partitioned between EtOAc and H₂O. The EtOAc layer was separated by silica gel and eluted using hexane/EtOAc to yield 28 fractions. Fraction 10 was separated on silica gel and eluted using CH₂Cl₂/EtOAc (10:1–pure EtOAc) to yield 14 fractions, 10A–10N. Fractions 10F and 10G were combined and purified by normal phase HPLC, using a mixture of CH₂Cl₂/acetone to afford **1** (1.2 mg, 13:1).

12-*epi*-Fragilide G (1**):** white powder; mp 238–240 °C; $[\alpha]_{\text{D}}^{23}$ –102 (c 0.05, CHCl₃); IR (neat) ν_{max} 3464, 1783, 1737 cm^{–1}; ¹³C NMR (CDCl₃, 100 MHz) and ¹H NMR (CDCl₃, 400 MHz) data, see Table 1; ESIMS *m/z* 621 (M+Na)⁺; HRESIMS *m/z* 621.1718 (Calcd for C₂₈H₃₅³⁵ClO₁₂+Na, 621.1715).

Human Neutrophil Elastase Release. Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Elastase release were carried out according to the procedures

described previously.^{13,14} Briefly, the elastase release experiment was performed using MeO-Suc-Ala-Ala-Pro-Valp-nitroanilide as the elastase substrate.

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