HETEROCYCLES, Vol. 81, No. 4, 2010, pp. 991 - 996. © The Japan Institute of Heterocyclic Chemistry
Received, 29th December, 2009, Accepted, 9th February, 2010, Published online, 10th February, 2010
DOI: 10.3987/COM-09-11899

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

Yu-Chia Chang,[a,b] Tsong-Long Hwang,[c] Sheng-Kai Huang,[b,d] Li-Wan Huang,[b,d] Mei-Ru Lin,[b] and Ping-Jyun Sung[a,b,e,*]

[a] Graduate Institute of Marine Biotechnology and Department of Life Science and Graduate Institute of Biotechnology, National Dong Hwa University, Checheng, Pingtung 944, Taiwan
[b] National Museum of Marine Biology & Aquarium, Checheng, Pingtung 944, Taiwan. E-mail: pjsung@nmmba.gov.tw
[c] Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan
[d] Department of Pharmacy, Tajen University, Pingtung 907, Taiwan
[e] Department of Marine Biotechnology and Resources and Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 804, Taiwan

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 $C_{28}H_{35}ClO_{12}$, 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 ^{13}C NMR data of **1** (Table 1), a disubstituted olefin and an

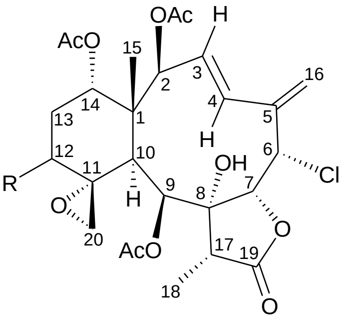

Chart 1. 12-*epi*-Fragilide G (**1**):R=α-OAc
Fragilide G (**2**):R=β-OAc

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

C/H	^{1}H[a]	^{13}C[b]	^{1}H–^{1}H COSY	HMBC (H→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
13α	2.27 m	29.0 (t)	H-12, H-13β, H-14	C-1, -11, -14
β	2.01 m		H-12, H-13α, 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
methyls	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, CH_2-16). Five carbonyl resonances at δ_C 174.6 (s, C-19), 170.3, 170.0, 169.9, and 169.3 (4×s, ester carbonyls), confirmed the presence of a γ-lactone and four esters in **1**. In the ^{1}H 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, CH_2-20), and further supported by the proton chemical shifts of H_2-20 (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, H_3-15), a methyl doublet (δ_H 1.25, 3H, d, J = 7.2 Hz, H_3-18), 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; H_2-13), 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 ^{1}H NMR spectrum of **1**.

The gross structure of **1** was determined by 2D NMR studies, including ^{1}H–^{1}H COSY, HMQC, and HMBC experiments. From the ^{1}H–^{1}H 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 ^{1}H–^{1}H 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-13α)/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 H_3-15/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 ^{1}H–^{1}H COSY correlations and characteristic NMR signals analysis. These data, together with the ^{1}H–^{1}H COSY correlation between H-17 and H_3-18 and the HMBC correlations between H-17/C-8, -9, -18, -19 and H_3-18/C-8, -17, -19, unambiguously established the molecular framework of **1**.

In a previous study, the ^{13}C 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, CH_2-20) 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 ^{1}H–^{1}H coupling constants. As per convention while analyzing the stereochemistry of briarane-type natural products, H-10 and H_3-15 were assigned to the α and β face, anchoring the stereochemical analysis because no correlation was observed between H-10 and H_3-15. In the NOESY experiment of **1**, H-10 gave correlations to H-2, H-9, OH-8, and H_3-18, 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-13α/β, and H_3-15, but not with H-10, revealing the β-orientation of this proton. In addition, H-12 was found to correlate with H-13α/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 H_3-15, 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.

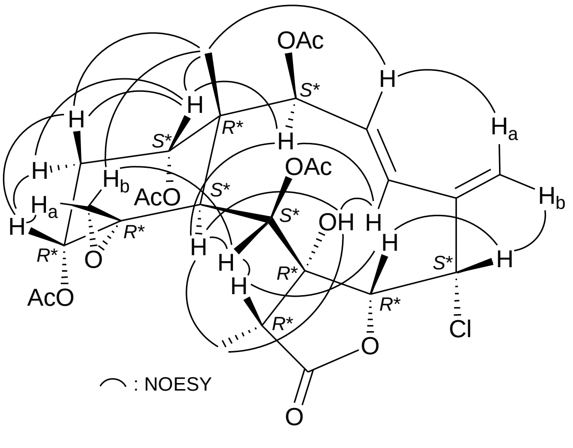

Figure 1. Selective Key NOESY Correlations of **1**

It has to be noted here that a briarane containing an *s-cis* diene moiety as presented in **1**, is unprecedential. 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. Brarane **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 ^{1}H and 100 MHz for ^{13}C, in $CDCl_3$, respectively. 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. 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 F_{254} (0.25 mm, Merck) and spots were visualized by spraying with 10% H_2SO_4 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 CH_2Cl_2 (1:1). The residue was partitioned between EtOAc and H_2O. 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 CH_2Cl_2/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 CH_2Cl_2/acetone to afford **1** (1.2 mg, 13:1).

12-*epi*-Fragilide G (1): white powder; mp 238–240 °C; $[\alpha]_D^{23}$ –102 (*c* 0.05, $CHCl_3$); IR (neat) ν_{max} 3464, 1783, 1737 cm^{-1}; ^{13}C NMR ($CDCl_3$, 100 MHz) and ^{1}H NMR ($CDCl_3$, 400 MHz) data, see Table 1; ESIMS *m/z* 621 $(M+Na)^+$; HRESIMS *m/z* 621.1718 (Calcd for $C_{28}H_{35}{}^{35}ClO_{12}+Na$, 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 previoulsy.[13,14] Briefly, the elastase release experiment was performed using MeO-Suc-Ala-Ala-Pro-Valp-nitroanilide as the elastase substrate.

ACKNOWLEDGEMENTS

This research was supported by grants from the NMMBA (981001101, 99200321, and 99200322); APORC, NSYSU (96C031702); NDHU; and NSTPBP, National Science Council (NSC 98-2323-B-291-001 and NSC 98-2320-B-291-001-MY3), Taiwan, awarded to P.-J.S.

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