Fragilide E, a Novel Chlorinated 20-Acetoxybriarane from the Gorgonian Coral *Junceella fragilis*

Ping-Jyun Sung,*[1,2] Gung-Ying Li,[1] Yu-Pei Chen,[1,3] I-Chu Huang,[1] Bo-Yuan Chen,[1] Su-Hui Wang,[1] and Sheng-Kai Huang[1,4]
[1]*Taiwan Coral Research Center (TCRC), National Museum of Marine Biology & Aquarium (NMMBA), and Graduate Institute of Marine Biotechnology, National Dong Hwa Univeristy (NDHU), Checheng, Pingtung 944, Taiwan*
[2]*Asia-Pacific Ocean Research Center (APORC), National Sun Yat-sen University (NSYSU), Kaohsiung 804, Taiwan*
[3]*Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan*
[4]*Department of Pharmacy, Tajen University, Pingtung 907, Taiwan*

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A novel chlorinated briarane, fragilide E (**1**), which possesses an unprecedented 20-acetoxy group, was isolated from the gorgonian coral *Junceella fragilis*. The structure of **1** was elucidated by spectroscopic methods.

In our research on new substances from octocorals collected in Taiwanese waters, a series of interesting briarane derivatives, including fragilides A–D,[1–4] have been isolated from *Junceella fragilis*. In this paper, we report the isolation, structure determination, and bioactivity of a novel briarane, fragilide E (**1**) (Chart 1), from further studies on *J. fragilis*. The structure of **1** was established by spectroscopic methods.

Specimens of *J. fragilis* (wet weight 780 g, dry weight 570 g) were collected by hand using scuba at the southern Taiwan coast in December 2002, at a depth of 20 m, and a voucher specimen was deposited in the NMMBA. The organisms were extracted with EtOH, and the residue was partitioned between EtOAc and H_2O. The EtOAc layer was separated by silica gel column chromatography, using CH_2Cl_2 and CH_2Cl_2–acetone mixtures of increasing polarity. Briarane **1** was eluted with CH_2Cl_2–acetone (8:1).

Fragilide E (**1**), 1.1 mg; mp 143–144 °C; $[\alpha]_D^{25}$ +13° (*c* 0.05, $CHCl_3$), was isolated as a white solid. The molecular formula of **1** was established as $C_{28}H_{37}ClO_{13}$ (10 degrees of unsaturation) from a sodiated molecule at *m*/*z* 639 in the ESIMS and was further supported by HRESIMS (*m*/*z* calcd: 639.1820; found: 639.1824, $[C_{28}H_{37}{}^{35}ClO_{13} + Na]^+$). The IR spectrum of **1** showed bands at 3486, 1786, and 1742 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester carbonyl groups. The ^{13}C NMR and DEPT spectra of **1** showed that this compound has 28 carbons, including six methyls, three sp^3 methylenes, an sp^2 methylene, nine sp^3 methines, three sp^3 quaternary carbons, and six sp^2 quaternary carbons. From the ^1H and ^{13}C NMR spectra (Table 1), briarane **1** was found to possess four acetoxy groups (δ_H 2.15, 2.03, 2.01, 1.97, each 3H × s; δ_C 171.5, 170.3, 170.1, 169.3, each s), a γ-lactone moiety (δ 175.3, s), and an exocyclic carbon–carbon double bond (δ_C 135.2, s; 118.6, t; δ_H 5.54, 1H, d, J = 2.0 Hz; 5.29, 1H, d, J = 2.0 Hz). Thus, from the NMR data, six degrees of unsaturation were accounted for, and **1** was identified as a tetracyclic compound.

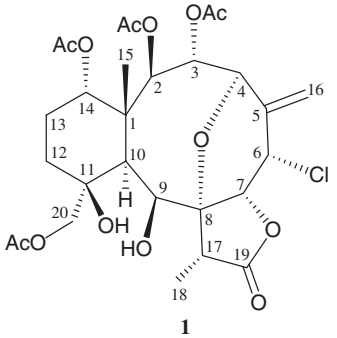

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Chart 1.

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

C/H	$^1H^a/\delta$	$^{13}C^b/\delta$	HMBC (H → C)
1		45.0 (s)[d]	
2	5.20 d (6.4)[c]	72.9 (d)	C-1, -3, -4, -10, -14, -15, acetate carbonyl
3	6.17 dd (10.8, 6.4)	65.5 (d)	C-1, -2, -4, -5, acetate carbonyl
4	4.36 d (10.8)	78.3 (d)	C-2, -3, -5, -6, -8, -16
5		135.2 (s)	
6	5.58 d (2.8)	54.8 (d)	C-4
7	4.74 d (2.8)	80.3 (d)	C-5, -6, -8, -9
8		82.9 (s)	
9	4.75 d (3.2)	76.5 (d)	C-1, -11, -17
10	2.18 d (2.0)	43.1 (d)	C-1, -8, -9, -11, -15
11		75.8 (s)	
12/12′	1.76 m; 1.69 m	30.4 (t)	C-10, -11, -14
13	1.73 m (2H)	20.7 (t)	C-1, -11, -14
14	5.02 br s	74.3 (d)	C-2, -12
15	1.57 s	17.5 (q)	C-1, -2, -10, -14
16a	5.29 d (2.0)	118.6 (t)	C-4, -5, -6
b	5.54 d (2.0)		C-4, -5, -6
17	2.59 q (7.2)	49.7 (d)	C-9, -18, -19
18	1.31 d (7.2)	8.3 (q)	C-8, -17, -19
19		175.3 (s)	
20a	3.98 d (11.2)	72.3 (t)	C-10, -12, acetate carbonyl
b	4.24 d (11.2)		C-10, -11, -12, acetate carbonyl
OH-9	2.88 d (3.2)		C-8, -9
OH-11	2.56 d (2.0)		C-10, -11
2-OAc		170.3 (s)	
	2.03 s	20.5 (q)	Acetate carbonyl
3-OAc		169.3 (s)	
	1.97 s	20.5 (q)	Acetate carbonyl
14-OAc		170.1 (s)	
	2.01 s	20.9 (q)	Acetate carbonyl
20-OAc		171.5 (s)	
	2.15 s	20.8 (q)	Acetate carbonyl

Spectra recorded at [a]400 and [b]100 MHz in $CDCl_3$ at 25 °C, respectivley. [c]*J* values (in Hz) in parentheses. [d]Multiplicity deduced by DEPT and indicated by usual symbols.

The gross structure of **1** was determined using 2D NMR studies. From the ^1H–^1H COSY spectrum of **1**, five different structural units were identified (Figure 1), which were assembled with the assistance of an HMBC experiments (Table 1 and Figure 1). The HMBC correlations between protons and quater-

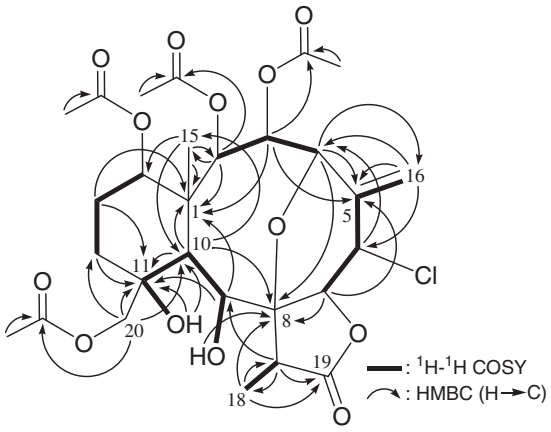

Figure 1. The ^{1}H–^{1}H COSY and selective key HMBC correlations of **1**.

nary carbons of **1**, such as H-2, H-3, H-9, H-10, H_3-15/C-1; H-3, H-4, H-7, H_2-16/C-5; H-4, H-7, H-10, H_3-18, OH-9/C-8; H-9, H-10, H_2-12, H_2-13, H-20b/C-11; and H-17, H_3-18/C-19, permitted elucidation of the carbon skeleton. An exocyclic double bond attached at C-5 was established by the HMBC correlations between H_2-16/C-4, -5, -6 and H-4/C-16; and further confirmed by allylic couplings between H_2-16 and H-6. The ring junction 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. The presence of acetate esters positioned at C-2, C-3, and C-20 were established by the HMBC correlations between protons H-2 (δ 5.20), H-3 (δ 6.17), H-20a/b (δ 3.98, 4.24) and acetate carbonyls (δ 170.3, 169.3, 171.5). The remaining acetoxy group was positioned at C-14 as indicated by analysis of ^{1}H–^{1}H COSY correlations and characteristic NMR signals analysis, although no HMBC correlation was observed between H-14 and acetate carbonyl. The hydroxy proton signal appearing at δ 2.88 (1H, d, $J = 3.2$ Hz) was revealed by its ^{1}H–^{1}H COSY and HMBC correlations to H-9 (δ 4.75, 1H, d, $J = 3.2$ Hz) and C-9 (δ 76.5, d), respectively, indicating its attachment to C-9. The presence of an 11-hydroxy group was deduced from the HMBC correlations between a hydroxy proton (δ 2.56) with an oxygenated quaternary carbon resonating at δ 75.8 (C-11) and C-10 methine (δ 43.1). This observation was also evidenced by the HMBC correlations between one proton of acetoxymethyl (δ 4.24, H-20b) and C-11 oxygenated quaternary carbon and C-12 methylene.

The intensity of sodiated molecule $(M + 2 + Na)^+$ isotope peak observed in ESIMS $[(M + Na)^+:(M + Na + 2)^+ = 3:1]$ was strong evidence of the presence of a chlorine atom in **1**. The methine unit at δ 54.8 (d) was more shielded than that expected for an oxygenated C-atom, and was correlated to the methine proton appearing at δ 5.58 in the HMQC spectrum. The latter methine signal was ^3J-correlated with C-4 (δ 78.3, d), proving the attachment of a chlorine atom at C-6. Furthermore, an HMBC correlation between H-4 (δ 4.36) and an oxygenated quaternary carbon appearing at δ 82.9 (s, C-8) suggested the presence of C-4/8 ether linkage. These data, together with the HMBC correlations between H-17/C-9, -18, -19; and H_3-18/C-8, -17, -19, unambiguously established the molecular framework of **1**.

The relative stereochemistry of **1** was elucidated by analysis of NOESY correlations (Figure 2) and by coupling constant

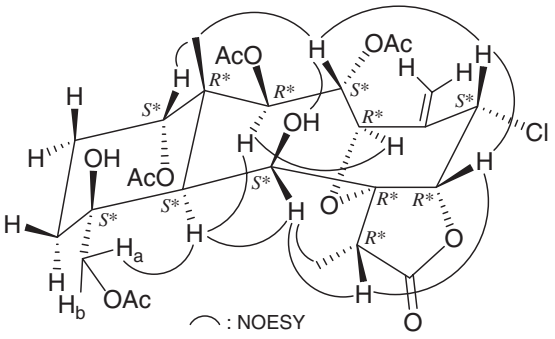

Figure 2. Selective NOESY correlations of **1**.

analysis. The NOESY correlations between H-10 and H-2, H-9, and one proton of C-20 methylene (δ 3.98, H-20a) indicated that these protons were situated on the same face; they were assigned as α protons, as C-15 methyl was β-oriented and H_3-15 did not show correlation with H-10. H-10 exhibited a small coupling constant with OH-11 by a long-range *w*-coupling ($J = 2.0$ Hz), indicating the 11-hydroxy group was β-oriented. H-14 was found to exhibit a response with H_3-15 but not with H-10, revealing the β-orientation of this proton. The correlations observed between H-3/H_3-15, H-3/OH-9, H-3/H-6, H-6/H-7, and H-7/H-17, reflected the β-orientation of protons attached at C-3, C-6, C-7, and C-17. Furthermore, H-4 was found to show a correlation with H-2; and a large coupling constant was found between H-4 and H-3 ($J = 10.8$ Hz), indicating the dihedral angle between H-4 and H-3 is approximately 180° and H-4 has an α-orientation at C-4. Based on the above findings, the chiral centers of **1** were assigned as 1R*, 2R*, 3S*, 4R*, 6S*, 7R*, 8R*, 9S*, 10S*, 11S*, 14S*, and 17R*.

It is worth noting that briarane metabolites possessing an oxygenated C-20 group, such as acetoxy,[5] hydroxy,[6] or carboxylic acid groups,[7] are rarely found. The structure of **1** was found to be similar with that of the first briarane containing a carboxylic acid group, juncin N,[7] however, fragilide E (**1**) is the second 20-acetoxybriarane ever discovered.[5] In biological activity experiment, **1** displayed 16.6% inhibitory effect on superoxide anion generation and 17.7% inhibitory effect on elastase release by human neutrophil at 10 μg/mL, respectively.

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