Fragilide A, a Novel Diterpenoid from *Junceella fragilis*

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A novel briarane-type diterpenoid, fragilide A (**1**), has been isolated from the Taiwanese gorgonian coral *Junceella fragilis*. The structure, including the relative configuration of this new metabolite, was elucidated by spectroscopic methods.

In connection with our long-standing interest in the chemistry of marine invertebrates, we investigated the Indo-Pacific gorgonian corals collected off the Southern coast of Taiwan. From one of the gorgonian corals, *Junceella fragilis* (Cnidaria, Gorgonacea, Ellisellidae),[1–3] we isolated a novel briarane-related derivative **1**, designated as fragilide A. The gorgonians of the genus *Junceella* are well-known for containing briarane-type metabolites.[4–6] The freshly collected gorgonian coral was kept frozen until needed. Fragilide A (**1**) was obtained from the ethyl acetate extract, after chromatography on silica gel, as a white powder.

Specimens of the gorgonian *J. fragilis* was minced and extracted with EtOAc. The extract was separated by Si-gel column chromatography. Fragilide A (**1**) was obtained as a white powder. The FABMS and NMR data established the molecular formula of **1** as being $C_{28}H_{38}O_{13}$. Thus, ten degrees of unsaturation were determined for **1**. The IR spectrum showed bands at 3468, 1784, and 1732 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester carbonyl groups. The FABMS of **1** exhibited peaks at m/z 565 ($M^+ + H - H_2O$), 523 ($M^+ + H -$ AcOH), 505 ($M^+ + H -$ AcOH $- H_2O$), 463 ($M^+ + H -$ 2AcOH), 403 ($M^+ + H -$ 3AcOH), 367 ($M^+ + H -$ 3AcOH $- 2H_2O$), and 343 ($M^+ + H -$ 4AcOH), which suggested the presence of two hydroxy and four acetoxy groups. From the ^{13}C spectral data of **1** (Table 1), a trisubstituted olefin was deduced from the signals of two carbons resonating at δ 139.2 (s) and 124.4 (d). An 8,17-epoxide group was confirmed from the signals of two quaternary oxygenated carbons at δ 68.8 (s) and 62.8 (s), and from the chemical shift of the tertiary methyl H_3-18 (δ 1.63, 3H, s). Furthermore, in the ^{13}C NMR spectrum, five carbonyl resonances appeared at δ 170.9 (s), 170.1 (2 × s), 169.7 (s), and 169.6 (s), confirming the presence of a γ-lactone and four esters in **1**. In the ^1H NMR spectrum (Table 1), four acetate methyls (δ 2.36, 3H, s; 2.28, 3H, s; 2.18, 3H, s; 1.94, 3H, s) were observed. Thus, the ^{13}C NMR data accounted for six degrees of unsaturation, and required **1** to be tetracyclic.

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

Position	^1H	^{13}C
1		44.3 (s)
2	5.16 (1H, d, J = 3.0 Hz)	78.7 (d)
3	6.33 (1H, dd, J = 11.0, 3.0 Hz)	70.3 (d)
4	6.15 (1H, dq, J = 11.0, 1.5 Hz)	124.4 (d)
5		139.2 (s)
6	4.01 (1H, d, J = 8.0 Hz)	75.3 (d)
7	4.59 (1H, d, J = 8.0 Hz)	81.8 (d)
8		68.8 (s)
9	5.23 (1H, d, J = 8.5 Hz)	66.5 (d)
10	2.64 (1H, dd, J = 8.5, 5.0 Hz)	40.5 (d)
11	2.22 (1H, m)	37.4 (d)
12	3.95 (1H, m)	66.9 (d)
13	1.96 (2H, m)	30.0 (t)
14	4.87 (1H, br s)	81.1 (d)
15	0.86 (3H, s)	18.9 (q)
16	2.14 (3H, d, J = 1.5 Hz)	19.5 (q)
17		62.8 (s)
18	1.63 (3H, s)	10.3 (q)
19		170.1 (s)
20	1.08 (3H, d, J = 7.0 Hz)	9.7 (q)
Acetate	2.36 (3H, s)	21.8 (q)
methyls	2.28 (3H, s)	21.8 (q)
	2.18 (3H, s)	21.4 (q)
	1.94 (3H, s)	20.8 (q)
Acetate		170.9 (s)
carbonyls		170.1 (s)
		169.7 (s)
		169.6 (s)

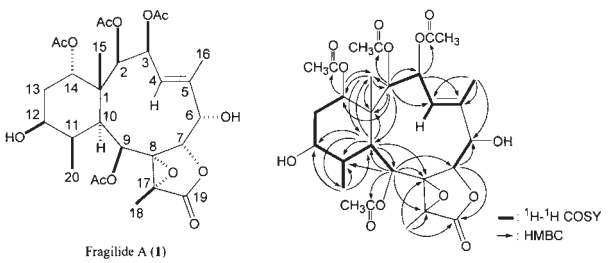

Fig. 1. ^1H–^1H COSY and HMBC correlations for **1**.

The gross structure of **1** and all of the ^1H and ^{13}C chemical shifts associated with the molecule were determined by 2D NMR experiments. From the ^1H–^1H COSY spectra of **1** (Fig. 1), it was possible to establish the proton sequences between H-2/H-3; H-3/H-4; H-6/H-7; H-9/H-10; H-10/H-11; H-11/H-12; H-12/H_2-13; H_2-13/H-14; and H-11/H_3-20. Based on these data and the HMBC correlations (Fig. 1), the connectivity from C-1 to C-14 could be further established. A vinyl methyl group attached at C-5 was confirmed by an ^1H–^1H COSY correlation between H_3-16 and H-4 and HMBC correlations between H_3-16/C-4, C-5, and C-6. The methylcyclo-

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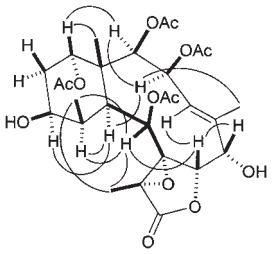

Fig. 2. Selective key NOESY correlations for **1**.

hexane ring, which is fused to the ten-membered ring at C-1 and C-10, was elucidated by key HMBC correlations between H-2/C-1, C-10, C-14; H-9/C-10, C-11; H-10/C-1, C-8, C-9, C-11, C-12, C-20; and H_3-20/C-10. The ring juncture C-15 methyl group was positioned at C-1 from the key 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. In addition, the acetoxy groups positioned at C-2, C-3, C-9, and C-14 were confirmed by the connectivity between H-2 (δ 5.16), H-3 (δ 6.33), H-9 (δ 5.23), H-14 (δ 4.87), and the ester carbonyl carbons δ 170.9 (s), 169.7 (s), 170.1 (s), and 169.6 (s). Furthermore, the HMBC and ^{1}H–^{1}H COSY correlations also revealed that the hydroxy groups could attach at C-6 and C-12, respectively. These data, together with the HMBC correlations between H-7/C-19; H-9/C-7, C-8, C-17; 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 NOE interactions observed in the NOESY experiment (Fig. 2) and by the vicinal ^{1}H–^{1}H coupling constants. In the NOESY experiment of **1**, H-10 exhibited correlations with H-3, H-11, and H-12; and H-3 showed a strong NOE correlation to H-2, suggesting that all of these protons are located on the same face, and were assigned as α protons, because the C-15 and C-20 methyl groups are β-oriented and H-10 did not show any correlation with H_3-15 and H_3-20. H-14 was found to exhibit NOE responses with H-2 and H_3-15, but not with H-10, revealing the β-orientation of this proton. The signal of H_3-18 showed NOE correlations with H_3-20, indicating the β-orientation of H_3-18. The configuration at C-9 is worthy of comment. H-9 was found to show strong NOE correlations with H-7, H-11, and H_3-18; also, this proton (H-9) exhibited a slight correlation with H_3-20. From a consideration of molecular models, H-9 was found to be reasonably close to H-7, H-11, H_3-18, and H_3-20, while H-9 was placed on the α face and H-7 was β-oriented. Moreover, H-6 showed a strong NOE interaction with H-7, suggesting that the hydroxy group attached at C-6 was α-oriented. The *cis* relationship between H-6 and H-7 was further established by the coupling constants ($J = 8.0$ Hz) for these two vicinal protons.[7] The geometry of the C-4/C-5 double bond in **1** was also determined by NOE correlations. The vinyl methyl H_3-16 exhibited NOE responses with H-3, but not with H-4; also, a strong correlation was observed for H-7 with H-4, demonstrating the *trans* configuration of Δ.[4,5] Based on the above results, the structure, including the relative configuration of **1**, was elucidated unambiguously. To the best of our knowledge, fragilide A (**1**) is the first briarane derivative possessing a 6-hydroxy group. The double bond attached at the C-4(5) position in briarane-type metabolites is also rarely found.[8,9]

Experimental

General Method. Melting points were determined using a FARGO apparatus, and were uncorrected. Optical rotations were measured in $CHCl_3$ with a JASCO D-370 digital polarimeter. Infrared spectra were measured on a JASCO 5300 FT-IR spectrometer. FABMS spectral data were obtained with a VG QUATTRO GC/MS spectrometer. ESI-FTMS were recorded on a BRUKER DALTONICS APEX II mass spectrometer. NMR spectra were recorded on a VARIAN UNITY INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively, in $CDCl_3$ using TMS as an internal standard. Silica gel (Merck, 230–400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F_{254}) were used for analytical TLC.

Isolation and Purification. Specimens of *J. fragilis* were collected by hand using scuba gear off the Southern Taiwan coast in Dec. 2002, at a depth of -10 m. Living reference specimens are being maintained in the authors' tanks and the voucher specimen was deposited in the National Museum of Marine Biology and Aquarium (specimen no. TWGC-003). This organism was identified from descriptions.[1–3] The organism (780 g) was collected and freeze-dried. The freeze-dried material (557 g) was minced and extracted with EtOAc (5×500 mL) for 120 h at 25 °C. The organic extract (11.1 g) was separated by silica-gel column chromatography using *n*-hexane and *n*-hexane–EtOAc mixtures of increasing polarity. Briarane **1** was eluted with *n*-hexane–EtOAc (5:2).

Fragilide A (1). 0.8 mg, white powder. mp > 300 °C. $[\alpha]_D^{25}$ +68.2° (*c* 0.4, $CHCl_3$); FABMS *m/z* 565 ($M^+ + H - H_2O$), 523, 505, 463, 403, 367, 343. ESI-FTMS *m/z* 523.2176 ($M^+ + H -$ AcOH, requires 523.2180). IR (neat, $CHCl_3$) ν_{max} 3468, 1784, 1732 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data are listed in Table 1.

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