Briarane Diterpenoids from the Formosan Gorgonian Coral *Junceella fragilis*

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A new trihydroxy briarane-related diterpenoid, junceellolide I (1), along with a known metabolite, (1*R*,2*R*,5*Z*,7*R*,8*S*,9*R*,10*R*,12*R*,14*R*,17*S*)-2,14-diacetoxy-8,17-epoxy-9,12-dihydroxybriara-5,11(20)-dien-19-one (2), have been isolated from the gorgonian coral *Junceella fragilis*, collected off the southern Taiwan coast. The structure, including the relative configuration of the new compound 1, was elucidated by the combination of spectral data, particularly in high-resolution ^{1}H- and ^{13}C-NMR spectroscopy utilizing COSY, HMBC, HMQC, and NOESY experiments.

Key words *Junceella fragilis*; junceellolide; gorgonian; briarane

Previous chemical investigations of the gorgonian coral *Junceella fragilis* (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Gorgonacea, family Ellisellidae)[1—3] have yielded 15 new diterpenoids possessing the briarane skeleton. These metabolites are junceellolides A—H,[4—6] (−)-4-deacetyljunceellolide D, (+)-11α,20α-epoxyjunceellolide D, (−)-11α,20α-epoxy-4-deacetyljunceellolide D, (−)-11α,20α-epoxy-4-deacetoxyjunceellolide D, (+)-junceellolide A,[7] 9-*O*-deacetylumbraculolide A,[8] and fragilide A.[9] In addition to species of the genus *Junceella*,[10] briarane-type metabolites have also been isolated from a variety of marine organisms, and the compounds of this type were found to possess extensive biological activity,[11] and could be originally synthesized by the corals.[12,13] In this paper, we report the isolation and structure determination of two briarane derivatives, including a new briarane, junceellolide I (**1**), together with a known metabolite, (1*R*,2*R*,5*Z*,7*R*,8*S*,9*R*,10*R*,12*R*,14*R*,17*S*)-2,14-diacetoxy-8,17-epoxy-9,12-dihydroxybriara-5,11(20)-dien-19-one (**2**),[14] from the gorgonian coral *Junceella fragilis* collected off southern Taiwan coast. The structure of the new diterpenoid **1** was elucidated by combined analysis of spectral data.

Junceellolide I (**1**) was obtained as white powder, $[\alpha]_D^{25}$ $-77°$ ($c=0.7$, $CHCl_3$). This metabolite has a molecular formula of $C_{24}H_{36}O_9$ as established by FAB-MS and NMR data, which indicates seven degrees of unsaturation. The IR absorptions of **1** showed the presence of hydroxy (3352 cm^{-1}), γ-lactone (1775 cm^{-1}), and ester (1736 cm^{-1}) groups. The FAB-MS of **1** exhibited peaks at *m/z* 451 $(M+H-H_2O)^+$, 433 $(M+H-2H_2O)^+$, 391 $(M+H-AcOH-H_2O)^+$, 373 $(M+H-AcOH-2H_2O)^+$, 349 $(M+H-2AcOH)^+$, 331 $(M+H-2AcOH-H_2O)^+$, 313 $(M+H-2AcOH-2H_2O)^+$, and 295 $(M+H-2AcOH-3H_2O)^+$, also indicating the presence of two acetoxy and three hydroxy groups in **1**. From the ^{13}C-NMR data of **1** (Table 1), the presence of a trisubstituted olefin was deduced from the signals of two carbons resonating at δ_C 146.2 (s) and 119.0 (d). Furthermore, in the ^{13}C-NMR spectrum, three carbonyl resonances appeared at δ_C 176.2 (s), 170.3 (s), and 169.4 (s), confirming the presence of a γ-lactone and two esters in **1**. In the ^{1}H-NMR spectrum of **1** (Table 1), two acetyl methyl groups (δ_H 2.18, 3H, s; 2.06, 3H, s) were observed. Thus, the NMR data accounted for four degrees of unsaturation and requiring **1** to be tricyclic. The ^{1}H-NMR spectrum also showed the presence of four methyl groups, including a methyl (δ_H 1.16, 3H, d, $J=7.5$ Hz, H$_3$-18) attached to a methine carbon, a methyl (δ_H 0.97, 3H, s, H$_3$-15) attached to a tertiary carbon, a methyl (δ_H 1.43, 3H, s, H$_3$-20) attached to an oxygen-bearing quaternary carbon, and a vinyl methyl (δ_H 2.03, 3H, s, H$_3$-16).

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, including ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H-detected heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) experiments. From the ^{1}H–^{1}H COSY spectrum of **1** (Fig. 1), it was possible to establish the separate spin systems that map out the proton sequences from H-2/H$_2$-3; H$_2$-3/H$_2$-4; H-6/H-7; and H-9/H-10. These data, together with the HMBC correlations between H-2/C-1, C-3, C-4, C-10; H$_2$-3/C-1, C-2, C-4, C-5; H$_2$-4/C-3, C-5, C-6; H-7/C-5, C-6, C-8; H-9/C-7, C-8, C-10; and H-10/C-1, C-2, C-8, C-9 (Fig. 1, Table 1), established the connectivity from C-1 to C-10 within the ten-membered ring. A vinyl methyl attached at C-5 was confirmed by the long-range ^{1}H–^{1}H COSY correlations between H$_3$-16 and H-6 and the HMBC correlations between H$_3$-16/C-4, C-5, and C-6. The methylcyclohexane ring was elucidated by the combination of ^{1}H–^{1}H COSY correlations between H$_2$-12/H$_2$-13 and H$_2$-13/H-14 and the HMBC correlations between H$_2$-12/C-11, C-20; H$_2$-13/C-1, C-11, C-12, C-14; H-14/C-10, C-12, C-13; and H$_3$-20/C-10, C-11, C-12. The methylcyclohexane ring, which is fused to the ten-membered ring at C-1 and C-10, was elucidated by the long-range *W*-coupling between H-10 and H-12α and by the key HMBC correlations between H-2/C-14; H-9/C-11; H-10/C-11; and H-14/C-2. 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. Furthermore, the acetoxy groups positioned at C-2 and C-9 were confirmed from the HMBC correlations between δ_H 4.51 (H-2), 5.18 (H-9) and the ester carbonyl carbons appeared at δ_C 170.3 (s), 169.4 (s), respectively. In addition, the proton of the hydroxy-bearing methine showed the signal at δ_H 4.12 (1H, d, $J=5.0$ Hz) was assigned to H-14. The 11-hydroxy group was confirmed from the signal of a quaternary oxygen-bearing carbon at δ_C 89.1 (s) and from the chemical shift of the tertiary methyl H$_3$-20 (δ_H 1.43, 3H, s). Thus, the remaining

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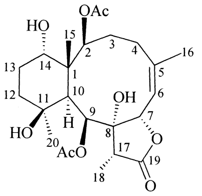
Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts and HMBC and ^{1}H–^{1}H COSY Correlations for **1**

C/H	^{1}H$^{a)}$	^{13}C$^{b)}$	HMBC (H→C)	^{1}H–^{1}H COSY
1		51.6 (s)$^{d)}$	H-2, H_2-3, H-10, H_2-13, H_3-15	
2	4.51 (1H, t, J=5.0 Hz)$^{c)}$	77.4 (d)	H_2-3, H-4α, H-10, H-14, H_3-15	H-3α/β
3α	1.91 m	32.6 (t)	H-2, H_2-4	H-2, H-3β, H-4α/β
β	2.04 m			H-2, H-3α, H-4α/β
4α	2.29 (1H, ddd , J=15.0, 10.5, 5.0 Hz)	28.9 (t)	H-2, H_2-3, H_3-16	H-3α/β; H-4β
β	2.59 (1H, dt, J=15.0, 5.0 Hz)			H-3α/β; H-4α
5		146.2 (s)	H_2-3, H_2-4, H-7, H_3-16	
6	5.46 (1H, d, J=9.5 Hz)	119.0 (d)	H_2-4, H-7, H_3-16	H-7, H_3-16
7	5.27 (1H, d, J=9.5 Hz)	77.6 (d)	H-9, OH-8	H-6
8		80.9 (s)	H-7, H-9, H-10, H-17, H_3-18, OH-8	
9	5.18 (1H, d, J=6.0 Hz)	68.3 (d)	H-10, H-17, OH-8	H-10
10	2.13 (1H, dd, J=6.0, 2.0 Hz)	49.9 (d)	H-2, H-9, H_2-12, H-14, H_3-15, H_3-20	H-9, H-12α
11		89.1 (s)	H-9, H-10, H_2-12, H_2-13, H_3-20	
12α	1.30 (1H, m)	29.2 (t)	H-10, H_2-13, H-14, H_3-20	H-10, H-12β, H-13α/β
β	2.00 (1H, m)			H-12α, H-13α/β
13α	2.88 (1H, m)	27.9 (t)	H-14	H-12α/β, H-13β, H-14
β	1.68 (1H, m)			H-12α/β, H-13α, H-14
14	4.12 (1H, d, J=5.0 Hz)	82.1 (d)	H-2, H_2-13, H_3-15	H-13α/β
15	0.97 (3H, s)	15.4 (q)	H-2, H-10	
16	2.03 (3H, s)	26.5 (q)	H-4α, H-6	H-6
17	2.43 (1H, q, J=7.5 Hz)	42.0 (d)	H-9, H_3-18, OH-8	H_3-18
18	1.16 (3H, d, J=7.5 Hz)	6.6 (q)	H-17	H-17
19		176.2 (s)	H-17, H_3-18	
20	1.43 (3H, s)	23.2 (q)	H-10, H_2-12	
OH-8	1.97 (1H, s)			
Acetates	2.18 (3H, s)	21.4 (q)		
		169.4 (s)	H-9, acetate methyl	
	2.06 (3H, s)	21.1 (q)		
		170.3 (s)	H-2, acetate methyl	

a) Measured at 500 MHz in $CDCl_3$ at 25 °C. *b*) Measured at 125 MHz in $CDCl_3$ at 25 °C. *c*) *J* values (in Hz) in parentheses. *d*) Multiplicity deduced by DEPT and indicated by usual symbols.



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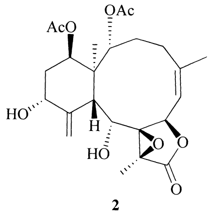

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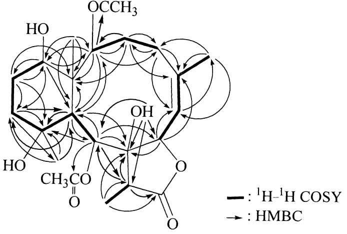

Fig. 1. The ^{1}H–^{1}H COSY and HMBC Correlations of **1**

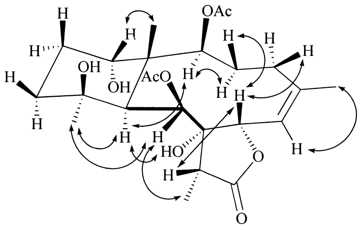

Fig. 2. Selective NOE Correlations of **1**

hydroxy group had to be positioned at C-8, an oxygen-bearing quaternary carbon resonating at δ_C 80.9. The latter was further confirmed from the HMBC correlations observed between OH-8/C-7, C-8, C-9, C-17. These data, together with the HMBC correlations between 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 a nuclear overhauser effect spectroscopy (NOESY) experiment (Fig. 2). In the NOESY experiment of **1**, H-10 gives NOE correlations to H-2, OH-8, and H_3-20, but not to H_3-15, and H-2 was found to show NOE responses with one proton of the C-3 methylene (δ_H 1.91, m, H-3α), indicating that these protons are located on the same face of the molecule and assigned as α-protons, since C-15 methyl and 11-hydroxy groups are the β-substituents at C-1 and C-11, respectively, and 8-hydroxy group was α-oriented. H-14 was found to exhibit NOE responses with H_3-15, but not with H-10, revealing the β-orientation of this proton. Furthermore, H-7 was found to exhibit NOE correlations with H-3β, one proton of the C-4 methylene (δ_H 2.59, 1H, dt, J=15.0, 5.0 Hz, H-4β), and H-17, but not with OH-8, indicating H-7 and H-17 should be placed on the β face and H_3-18 was α-oriented in **1**. H-9 was found to show NOE responses with H_3-18 and H_3-20. From the consideration of molecular models, H-9 was found to be reasonably close to H_3-18 and H_3-20, thus being concluded that H-9 should be placed on the α face in **1**. From the above results, the structure, including the relative configuration of **1**, was established unambiguously.

Compound **2** was identified as a known diterpene, (1*R*,2*R*,5*Z*,7*R*,8*S*,9*R*,10*R*,12*R*,14*R*,17*S*)-2,14-diacetoxy-8,17-epoxy-9,12-dihydroxybriara-5,11(20)-dien-19-one, which had been isolated from an Australian gorgonian coral *Junceella gemmacea*. Its physical (optical rotation value) and spectral (^{1}H- and ^{13}C-NMR) data are in full agreement with those reported previously.[14)]

Experimental

Melting points were determined using a FARGO apparatus and were uncorrected. Optical rotation values were measured in $CHCl_3$ using a JASCO D-370 digital polarimeter at 25 °C. Infrared spectra were recorded on a JASCO 5300 FT-IR. FAB-MS was obtained with a VG QUATTRO GC/MS spectrometer. HR-FAB-MS was recorded on a VG 70—250S GC/MS spectrometer. NMR spectra were recorded 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. TLC spots (Si gel 60 F_{254}, 0.2 mm, Merck) were detected with an UV_{254} lamp and by 20% H_2SO_4 followed by heating at 120 °C for 5 min. All solvents used were either freshly distilled or of analytical grade.

Animal Material The gorgonian coral *Junceella fragilis* was collected by hand using scuba gear off the southern Taiwan coast on Dec. 12, 2002, at a depth of −10 m. Taxonomic identification was provided by Dr. Tung-Yung Fan from the National Museum of Marine Biology and Aquarium (NMMBA), R.O.C. The living reference specimen was deposited in the NMMBA (TWGC-003). This organism was identified from descriptions.[1—3)]

Extraction and Isolation 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 **2** was eluted with *n*-hexane–EtOAc (5 : 2) and **1** with *n*-hexane–EtOAc (1 : 1).

Junceellolide I (**1**): White powder (7.8 mg); mp 210—212 °C; $[\alpha]_D^{25}$ −77° (c=0.7, $CHCl_3$); IR (neat) cm^{-1} 3352, 1775, 1736; ^{1}H- and ^{13}C-NMR data, see Table 1; FAB-MS *m/z*: 451, 433, 391, 373, 349, 331, 313, 295. HR-FAB-MS: *m/z* 451.2332 (Calcd for $C_{24}H_{36}O_9+H-H_2O$, 451.2333).

(1*R*,2*R*,5*Z*,7*R*,8*S*,9*R*,10*R*,12*R*,14*R*,17*S*)-2,14-diacetoxy-8,17-epoxy-9,12-dihydroxybriara-5,11(20)-dien-19-one (**2**): Colorless oil (1.0 mg); $[\alpha]_D^{25}$ +113° (c=0.1, $CHCl_3$) (lit.[14)] $[\alpha]_D$ +115.1° (c=0.08)); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 5.53 (1H, d, J=9.0 Hz), 5.34 (1H, d, J=9.0 Hz), 5.31 (1H, s), 5.14 (1H, d, J=7.0 Hz), 5.04 (1H, s), 4.77 (1H, br s), 4.35 (1H, br s), 4.31 (1H, t, J=7.0 Hz), 3.04 (1H, br s), 2.65 (1H, br t, J=15.5 Hz), 2.57 (1H, br d, J=15.5 Hz), 2.18 (1H, m), 2.00 (3H, s), 1.98 (3H, s), 1.96 (3H, s), 1.88 (1H, m), 1.78 (1H, m), 1.70 (1H, m), 1.53 (3H, s), 1.26 (3H, s); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 171.8 (s), 170.6 (s), 170.2 (s), 151.7 (s), 144.5 (s), 119.3 (d), 110.5 (t), 75.0 (d), 74.3 (d), 74.2 (d), 73.6 (d), 71.4 (s), 69.7 (d), 62.2 (s), 47.2 (s), 44.0 (d), 36.4 (t), 31.2 (t), 28.5 (t), 26.9 (q), 21.4 (q), 21.1 (q), 15.0 (q), 10.0 (q). The related physical (optical rotation value) and spectral (^{1}H- and ^{13}C-NMR) data of **2** are in full agreement with those reported previously.[14)]

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