

Juncenolides F and G, Two New Briarane Diterpenoids from Taiwanese Gorgonian *Junceella juncea*

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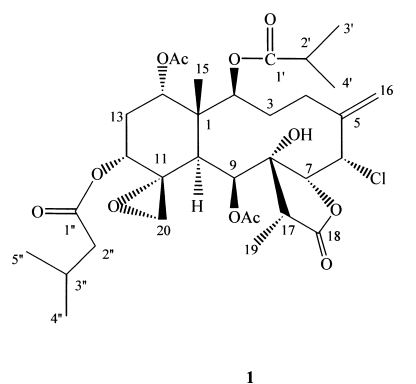
Chemical investigation of the gorgonian coral *Junceella juncea* collected in Taiwan has resulted in the isolation of the two novel briarane-type diterpenoid compounds, juncenolides F (1) and G (2). The structures were determined on the basis of spectral studies, especially 1D and 2D NMR.

Key words juncenolide F; juncenolide G; *Junceella juncea*

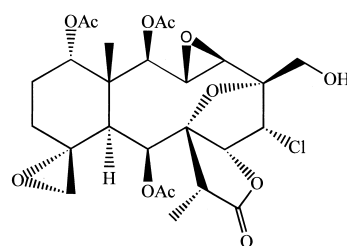
Briaranes are diterpenoid γ -lactones of highly substituted bicyclic six- and ten-membered rings that are extensively produced by marine octocorals including gorgonians.^{1,2)} The majority of these compounds are endowed with biological activities that ranges from cytotoxic^{3–5)} to anti-inflammatory,^{6,7)} immunomodulatory,⁸⁾ antiviral,⁹⁾ and insecticidal^{10,11)} actions. In continuation of our interest in the chemistry and potential medicinal importance of briaranes,^{12–14)} we investigated the gorgonian octocoral *Junceella juncea* collected in Taiwan. Several juncins,^{15,16)} gemmacolides,¹⁶⁾ juncelolides¹³⁾ and juncenolides^{12,14)} have been reported from this species. The current study resulted in the isolation of the two novel briarane-type diterpenoidal compounds, juncenolides F (1) and G (2) from the acetone extract of *Junceella juncea*. The structures were defined on the basis of spectral studies, especially 1D and 2D NMR.

The HR-ESI-MS of 1 revealed a quasi-molecular ion peak at m/z 693 $[M+Na]^+$ consistent with the molecular formula $C_{33}H_{47}O_{12}Cl$ and ten degrees of unsaturation. The IR spectrum displayed absorption bands diagnostic of hydroxyl, 5-membered lactone and ester groups. Both 1H - and ^{13}C -NMR spectral data (Table 1 and Experimental) indicated the presence of two acetate at δ_H 2.22, 1.96 (each 3H, s) and δ_C 21.2 (q, double intensity) and δ_C 169.3, 170.2. An isobutyrate ester was indicated by a septet at δ_H 2.50 which was spin-coupled with two CH_3 doublets at δ_H 1.12 and 1.17 along with carbon signals at δ_C 19.2 (q), 18.2 (q), 34.0 (d) and a carbonyl at δ_C 176.7 (s). Furthermore, an isovalerate ester was revealed by δ_H 0.92 (6H, d, $J=6$ Hz), 2.08 (m), 2.10 (m) and δ_C 22.3 (q), 22.4 (q), 25.5 (d), 43.5 (t) and a carbonyl at δ_C 171.6 (s), that was confirmed by a COSY experiment.^{15,16)} Besides the previous four ester carbonyls, the carbon signal at δ 174.5 was assigned to a 5-membered lactone ring together with the oxygenated methine at δ 81.2 (C-7). The two proton singlets at δ 5.81 and 5.52 along with the CH_2 signal at δ 121.2 were ascribed to an exocyclic methylene group. The 1H -NMR spectrum contained two mutually coupled signals at δ 2.82, 2.35 (each 1H, d, $J=4.0$ Hz) together with the corresponding δ_C 50.5 (t) and δ_C 57.4 (s) that were appropriate for an exocyclic epoxide. The two remaining degrees of unsaturation required a bicyclic carbon skeleton which fits a briarane diterpene.^{8,17)} The tertiary methyl singlet at δ 1.16 was assigned to C-15 while the secondary methyl doublet at δ 1.27 (d, $J=7$ Hz) was assigned to H-19. The OH singlet at δ 3.43 revealed by its HMBC corre-

lation to the quaternary carbon signal at δ 81.3 (C-8) indicating its attachment to C-8. The COSY spectrum exhibited connectivities of H/2/H-3/H/4, H/6/H-7, H-12/H-13/H-14 and H-17/H-19. The intensity of (M+2) isotope peak observed in the low resolution FAB-MS $[(M^++H+2)/(M^++H)=0.39]$ and the occurrence of a fragment ion corresponding to $[M-35]^+$ were strong evidence of the presence of a chlorine atom in 1.⁷⁾ Consequently, the CH signal at δ 51.7 was confidently assigned to a chlorinated carbon (C-6) that was correlated to δ_H 4.63 (brs) in the HMQC spectrum. Besides CH signal at C-7 of the lactone ring (δ_C 81.2 and δ_H 4.46), additional four oxygenated methines were observed at δ_H 5.98, 5.74, 4.60, 4.84 were J^1 -correlated to carbons at δ_C 72.5, 72.2, 73.0, 73.3 and assigned to C-2, C-9, C-12 and C-14 respectively. At the same time, H-2, H-9, H-12 and H-14 showed HMBC correlations with ester carbonyls at δ 176.7 (isobutyrate), 169.3 (acetate), 171.6 (isovalerate) and 170.2 (acetate) respectively (Fig. 1). On the other hand, the HMBC displayed correlations between H-10/C-8, C-20; H-7/C-5; H-

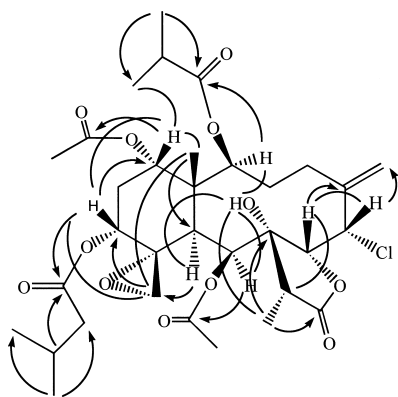


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Fig. 1. Selected HMBC (Arrow) and NOESY (Curve) Correlations for **1**

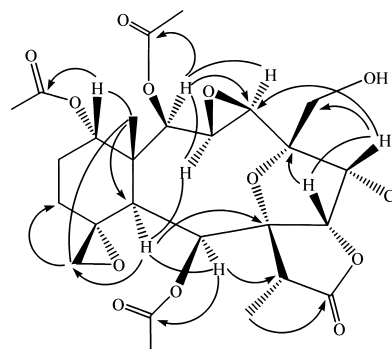
19/C-8; OH-8/C-8; H-6/C-16; H-14/C-12; H-20/C-12; H-15/C-2 and H-19/C-18. The relative stereochemistry of **1** was determined through analogy with naturally occurring briarane diterpenes and from its NOESY spectrum (Fig. 1). Naturally occurring briaranes have the C-15 methyl group in the β -orientation and H-10 in the α -orientation.⁹⁾ The correlations between H-15/H-14, H-20; H-20/H-12 implied the β -orientation of H-12, H-14 and H-20. In addition, the correlation between OH-8/H-10, H-2, H-9, H-19 indicated the α -orientation of OH-8, H-2, H-9 and H-19 while correlations between H-7/H-17, H-6 is consistent with the β -orientation of H-17, H-7 and H-6. Hence, The structure of juncenolide F (**1**) was determined as (1*R**,2*S**,6*S**,7*R**,8*R**,9*S**,10*S**,11*S**,12*R**,14*S**,17*R**)-6-chloro-11,20-epoxy-2-isobutoxyloxy-9,14-diacetoxy-12-isovaleroyloxy-8-hydroxybriaran-5(16)-en-18,7-olide.

The HR-ESI-MS data of **2** agreed with the molecular formula C₂₆H₃₃O₁₂Cl and 10 degrees of unsaturation. The IR revealed very close data to those of **1** showing the same functionalities. Detailed inspection of ¹H- and ¹³C-NMR spectral data (Experimental and Table 1) indicated the presence of the key structural features of a briarane skeleton with a lactone ring and exocyclic epoxide. Three acetate substituents were displayed at δ_{H} 2.04, 2.21 and 2.26 (each 3H, s), δ_{C} 21.1, 21.2, 21.4 and three carbonyls at δ_{C} 169.2, 169.4 and 171.1. The HMBC spectrum (Fig. 2) determined the attachment of the three acetates to C-2, C-9 and C-14 through revealing correlations of the carbonyl with the corresponding methane protons. Besides the exocyclic epoxide signals (δ_{H} 2.70, 3.06, δ_{C} 49.9), the NMR data revealed the existence of another epoxide ring from the two CH at δ 56.8 and 63.5 and CH singlets at δ 3.83 and 3.58. Both carbons at δ 56.8 and 63.5 had HMBC correlation to δ 5.53 (H-2) while the carbon at δ 63.5 was correlated to δ 4.68 (H-6), hence the epoxide was located at C-3/C-4. The Chemical shifts of C-3 and C-4 favored the β -orientation of the 3,4-epoxide.¹⁸⁾ The two *gem* protons at 3.87 and 3.71 (each 1H, br d, $J=12$ Hz) together with oxygenated CH_2 at δ_{C} 62.8 were assigned to a hydroxymethyl group attached to a quaternary carbon. The HMBC correlation of the CH_2 at δ_{C} 62.8 (C-16) to δ 4.68 (H-6) located the hydroxymethyl at C-5. On the other hand, the HMBC correlations between δ 4.54 (H-7) and each of the two oxygenated low field quaternaries δ_{C} 91.9 (C-8) and 85.0 (C-5) suggested the presence a C-5/C-8 ether linkage, thus satisfying 10 degrees of unsaturation. The NOESY correla-

Table 1. ¹H- (CDCl₃, 500 MHz) and ¹³C-NMR (125 MHz) Data of **1** and **2**^{a)}

Position	1	2
1	47.8 s	48.1 s
2	5.98 d (8)	72.5 d
3	2.73 m	28.3 t
	1.62 m	3.73 br s
4	2.45 m	33.4 t
	2.35 m	3.58 br s
5		146.6 s
6	4.63 br s	51.7 d
7	4.46 br s	81.2 d
8		81.3 s
9	5.74 s	72.2 d
10	3.70 s	35.6 d
11		57.4 s
12	4.60 br s	73.0 d
13	2.25 m	29.2 t
	2.02 m	1.90 m
14	4.84 br s	73.3 d
15	1.16 s	14.0 q
16	5.81 s	121.2 t
	5.52 s	3.71 brd (12)
17	2.97 q (7)	51.7 d
18		174.5 s
19	1.27 d (7)	6.6 q
20	2.82 d (4)	50.5 t
	2.35 d (4)	3.06 d (3)
1'		176.7 s
2'	2.50 septet (7)	34.0 d
3'	1.12 d (7)	19.2 q
4'	1.17 d (7)	18.2 q
1''		171.6 s
2''	2.10 d (7)	43.5 t
3''	2.08 m	25.5 d
4''	0.92 d (6)	22.3 q
5''	0.92 d (6)	22.4 q
Ac-2 C=O		169.4 s
CH ₃		2.21 s
Ac-9 C=O	169.3 s	169.2 s
CH ₃	2.22	2.26
Ac-14 C=O	170.2 s	171.1 s
CH ₃	2.12 q	2.04 s

a) Assignments of protons were made using HMQC and HMBC techniques, coupling constants in Hz in parentheses. Multiplicities of carbon were determined by DEPT experiments.

Fig. 2. Selected HMBC (Arrow) and NOESY (Curve) Correlations for **2**

tions between H-4/H-2, H-2/H-3, H-10/H-3, H-9, H-19 implied the α -orientation of H-2, H-3, H-4, H-9 and H-19. In addition, the correlations between H-6/H-7; H-15/H-14, H-20 indicated the β -orientation of the H-6, H-7, H-14 and H-20. It was concluded that **2** had the structure of (1*R**,2*S**,3*R**,4*R**,5*R**,6*S**,7*R**,8*R**,9*S**,10*S**,11*S**,14*S**,17*R**)-6-chloro-3,4-epoxy-11,20-epoxy-2,9,14-triacetoxy-5(8)ether-

briaran-18,7-olide, and it was designated juncenolide G.

Reviewing the available literature, It was noted that compound **2** is the first report of a naturally-occurring briarane with an ether linkage between C-8/C-5 and not between C-8/C-4 and that the exocyclic epoxy ring, present in **1** and **2**, is not of common occurrence in related compounds.²⁾

Experimental

General Experimental Procedures Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on Hitachi T-2001 and Hitachi U-3210 spectrophotometers, respectively. Low-resolution EI-MS and FAB-MS spectra were recorded on a VG Quattro 5022 mass spectrometer. High-resolution ESI-MS spectra were measured on a JEOL HX 110 mass spectrometer. The ¹H-, ¹³C-NMR, COSY, HMQC, HMBC, and NOESY spectra were recorded on a Bruker FT-300 spectrometer or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ¹H and 125 MHz for ¹³C, respectively, using TMS as internal standard. The chemical shifts are given in δ (ppm) and coupling constants in Hz. Silica gel 60 (Merck) was used for column chromatography (CC), and pre-coated silica gel plates (Merck, Kieselgel 60 F-254, 1 mm) were used for preparative TLC. Sephadex LH-20 (Amersham Pharmacia Biotech AB, Sweden) was used for either purification or separation.

Animal Material *Junceella juncea* PALLAS (Ellisellidae) was collected in Ping-Tong County, Taiwan, by scuba diving at a depth of 15 m, in February 2000. The fresh gorgonian was immediately frozen after collection and kept at -20°C until processed. A voucher specimen (WSG-4) was deposited in the Institute of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan.

Extraction and Isolation The outer red layer of *J. juncea* (wet, 0.8 kg) was extracted with acetone (3 l) and the acetone extract was concentrated under vacuum and then partitioned between EtOAc and H₂O. The EtOAc-soluble residue (3.24 g) was chromatographed on silica gel column (70 g) using a gradient of *n*-hexane/CH₂Cl₂/MeOH (250:20:1 to 30:20:1, each 1 l) to give 11 fractions. Fraction-6 (164 mg) was subjected to NP-PTLC and developed with *n*-hexane/EtOAc (3:2) to yield juncenolide B (8 mg) and a solid mixture (76 mg). The latter mixture F-6-m was column chromatographed on silica gel using *n*-hexane/EtOAc (50:1 to 1:1) to give 9 fractions. Fraction-5 (F-6-m-5) was further fractionated by RP-PTLC using H₂O/MeOH (3:7) followed by NP-HPLC using *n*-hexane/CH₂Cl₂/MeOH (15:15:1) to afford **1** (1.7 mg). Part of F-7 (340 mg) was fractionated on Sephadex LH-20 using MeOH to produce 4 fractions. Fraction 2 (F-7-s-2, 210 mg) was separated on a silica gel column using a gradient of *n*-hexane/EtOAc (30:1 to 1:30) to give 3 fractions. Fraction 2 (F-7-s-2-2) purified using NP-HPLC using *n*-hexane/CH₂Cl₂/MeOH (12:12:1) to yield **2** (2 mg).

Juncenolide F (**1**): Colorless crystals; $[\alpha]_{\text{D}} +9.7^{\circ}$ ($c=0.2$, CH₂Cl₂); IR (CH₂Cl₂) ν_{max} 3480 br (OH), 2961 (CH), 1778 (C=O, lactone), 1732 (C=O, ester), 1235 (C–O st), 1047 cm⁻¹; ¹H-NMR (500 MHz, CDCl₃) and ¹³C-NMR (125 MHz, CDCl₃) spectral data, see Table 1; FAB-MS: m/z 693 [M+Na]⁺; 673 [M+H+2]⁺, 671 [M+H]⁺; EI-MS m/z (rel. int.): 672 [M+2]⁺ (0.01), 670 [M]⁺ (0.03), 610 [M–AcOH]⁺ (0.04), 582 [M–C₄H₈O₂]⁺ (1.2), 550 [M–2AcOH]⁺ (0.05), 533 [M–2AcOH–OH]⁺ (0.43), 522 [M–AcOH–C₄H₈O₂]⁺ (0.6), 154 (44), 137 (42), 85 [C₅H₉O]⁺ (29), 71

[C₄H₉O]⁺ (27); HR-ESI-MS m/z 693.2659 [M+Na]⁺ (Calcd for C₃₃H₄₇O₁₂ClNa, 693.2655).

Juncenolide G (**2**): Colorless crystals; $[\alpha]_{\text{D}} +6.5^{\circ}$ ($c=0.2$, CH₂Cl₂); IR (CH₂Cl₂) ν_{max} 3523 br (OH), 2922 (CH), 1773 (C=O, lactone), 1740 (C=O, ester), 1231 (C–O st), 1037 cm⁻¹; ¹H-NMR (500 MHz, CDCl₃) and ¹³C-NMR (125 MHz, CDCl₃) spectral data, see Table 1; FAB-MS: m/z 595 [M+Na]⁺; 575 [M+H+2]⁺, 573 [M+H]⁺; EI-MS m/z (rel. int.): 574 [M+2]⁺ (0.01), 572 [M]⁺ (0.03), 512 [M–AcOH]⁺ (0.03), 452 [M–2AcOH]⁺ (0.04), 392 [M–3AcOH]⁺ (0.07), 375 [M–3AcOH–OH]⁺ (0.29), 357 [M–3AcOH–OH–H₂O]⁺ (0.34), 177 (16), 137 (18), 135 (39), 107 (29), 95 (25); HR-ESI-MS m/z 595.1556 [M+Na]⁺ (Calcd for C₂₆H₃₃O₁₂ClNa, 595.1559).

Acknowledgments The authors are grateful to the National Science Council, Taipei, Taiwan, for financial support (Grant # NSC 91-2320-B-110-009). We thank Ms. Ho chao Lein and Yu shiu Ching, NSC Southern NMR and MS Instrument Center in the National Sun Yat-sen University, for measurement of NMR and MS spectra.

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