Junceellonoids A and B, Two New Briarane Diterpenoids from the Chinese Gorgonian *Junceella fragilis* RIDLEY

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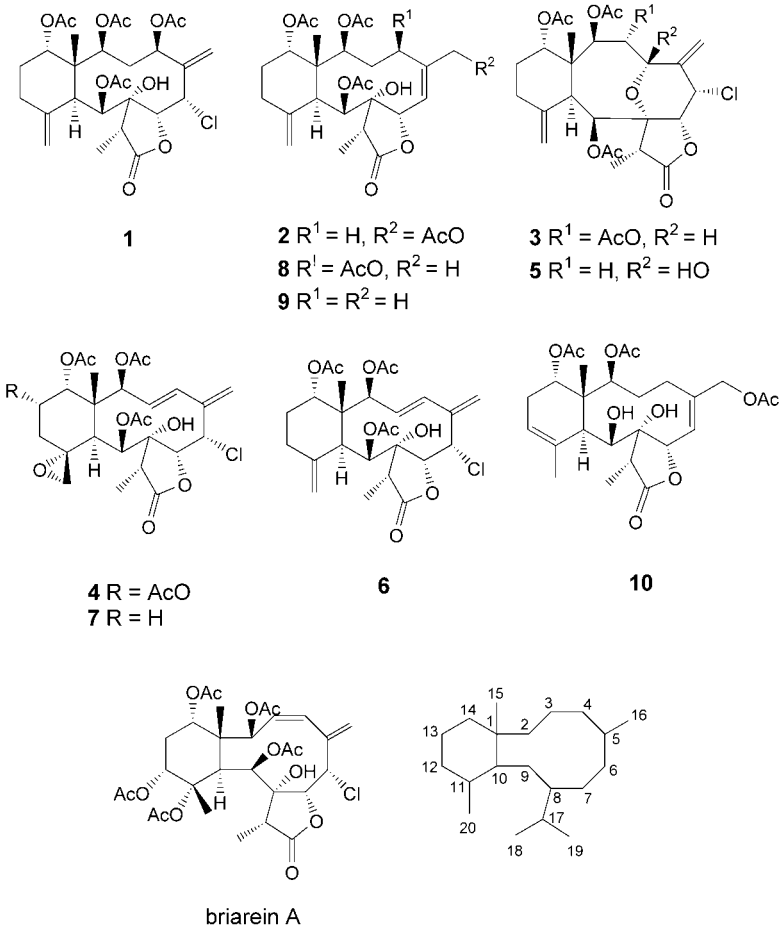
Two new diterpenoids, junceellonoids A and B (**1** and **2**, resp.), together with the seven known diterpenoids **3**–**9**, all possessing the briarane carbon skeleton, were isolated from the gorgonian *Junceella fragilis* RIDLEY, collected in the South Sea of China. The structures and relative configurations of the new metabolites were elucidated based on extensive spectral analysis and by comparison with known spectral data.

Introduction. – Gorgonian is a rich source of diterpenoids with the well-known briarane skeleton. Briarein A, isolated from the gorgonian *Briareum asbestinum*, was the first reported example of these novel bicarbocyclic diterpenes [1]. Subsequently, more than twenty-one dozen diterpenes with the same skeleton have been reported from octocorals belonging to the orders Pennatulacea and Gorgonacea, and from a Mediterranean nudibranch and its octocoral prey as well.

Previous studies on four species of the genus *Junceella* (Gorgonaceae) have resulted in the isolation of forty structurally novel and highly oxygenated briarane-type diterpenes [2–13], eight of which were isolated from the species of *Junceella fragilis* RIDLEY.

Our investigation of gorgonian *Junceella fragilis* RIDLEY collected from the South Sea of China resulted in the discovery of two new briarane diterpenes, junceellonoids A (**1**) and B (**2**), which were isolated along with seven known diterpenes, junceellin (**3**) [2], junceellin B (**4**) [3], junceelloides A–D (**5**–**8**) [4], and an unnamed known diterpene **9** [14]. The structures of these compounds were determined on the basis of a series of spectral analyses and by comparison with reported spectral and physical data from other known compounds. Herein, we wish to report the isolation and structure elucidation of these metabolites.

Results and Discussion. – The collected gorgonian *Junceella fragilis* RIDLEY samples were immediately cooled to $-20°$ and kept frozen until the extraction. Frozen organisms were cut into small pieces and subsequently exhaustively extracted with acetone to give a crude extract. The crude extract was partitioned between H_2O and Et_2O to yield a Et_2O-soluble fraction. The latter was subjected to repeated column chromatography (CC) (silica gel, Et_2O/petroleum ether mixtures; *Sephadex LH-20*, petroleum ether/$CHCl_3$/MeOH 2:1:1) and reversed-phase HPLC (MeCN/H_2O 2:3) to afford nine pure compounds **1**–**9**.



Junceellonoid A (**1**) was obtained as colorless plates. Its ESI-MS (positive ion) displayed two pseudo-molecular ions at *m/z* 607 ($[M+Na]^+$) and 1191 ($[2\,M+Na]^+$). The HR-ESI-MS experiment established the molecular formula $C_{28}H_{37}ClO_{11}$, with *m/z* 607.1924 ($[M+Na]^+$, $C_{28}H_{37}ClNaO_{11}^+$; calc. 607.1922, indicating ten degrees of unsaturation. The structure of **1** was elucidated on the basis of extensive spectroscopic analysis.

The IR spectrum of **1** showed bands at 3431, 1778 and 1739 cm^{-1}, suggesting the presence of hydroxy, γ-lactone, and ester carbonyl groups in the structure. Analysis of ^{13}C-NMR and DEPT spectra allowed us to assign seven of the ten unsaturation degrees to two exocyclic C=C bonds resonating at δ 150.8 (*s*), 143.4 (*s*), 125.0(*t*), and 110.1(*s*), and five carbonyl resonances appearing at δ 175.6 (*s*), 171.9 (*s*), 170.6 (*s*), 170.4 (*s*), and 169.6 (*s*), which confirmed the presence of a γ-lactone and four other ester groups (*Table*). The remaining unsaturations were due to three rings. In addition, the ^{13}C-NMR spectrum showed six O-bearing C-atoms with signals at δ 84.4 (*d*), 82.2 (*s*), 79.7 (*d*), 77.0 (*d*), 75.0 (*d*), and 72.6 (*d*), and one typical Cl-bearing C-atom at δ 53.8 (*d*). The remaining signals observed between δ 51.4 and 6.5 were attributed to twelve sp^3 C-atoms (six Me, three CH_2, two CH, and one C). The ^{1}H-NMR spectrum (*Table*) showed ten downfield signals between δ 6.73 and 4.81, assigned to olefinic and CH–O protons. Four of the six Me groups were attributed to the AcO groups (δ 2.25,

Table. *[1]H- and [13]C-NMR Data*[a]) *for Junceellonoids A* (**1**)[b]) *and B* (**2**)[c]). Trivial numbering.

	1		**2**	
	δ(H)	δ(C)[d])	δ(H)	δ(C)[d])
C(1)		48.7 (*s*)		47.2 (*s*)
H–C(2)	6.73 (*d*, *J* = 8.7)	72.6 (*d*)	4.84 (*m*)	74.7 (*d*)
H_α–C(3)	1.99 (*dd*, *J* = 8.7, 16.1)	37.0 (*t*)	1.76 (*m*)	31.8 (*t*)
H_β–C(3)	3.46 (*dd*, *J* = 11.3, 16.1)		2.45 (*m*)	
H_α–C(4)	5.89 (*d*, *J* = 11.3)	77.0 (*d*)	2.13 (*m*)	25.7 (*d*)
H_β–C(4)			2.55 (*m*)	
C(5)		143.4 (*s*)		142.2 (*s*)
H–C(6)	5.42 (*d*, *J* = 3.6)	53.8 (*d*)	5.76 (*d*, *J* = 10.2)	120.4 (*d*)
H–C(7)	4.97 (*d*, *J* = 3.6)	84.8 (*d*)	5.32 (*d*, *J* = 10.2)	77.3 (*d*)
C(8)		82.2 (*s*)		83.4 (*s*)
H–C(9)	6.28 (*s*)	79.7 (*d*)	5.30 (*d*, *J* = 5.3)	71.3 (*d*)
H–C(10)	3.76 (*s*)	45.2 (*d*)	3.28 (*d*, *J* = 5.3)	42.4 (*d*)
C(11)		150.8 (*s*)		151.1 (*s*)
H_α–C(12)	2.32 (*dt*, *J* = 4.6, 13.3)	33.6 (*t*)	2.30 (*m*)	26.3 (*t*)
H_β–C(12)	2.15 (*dd*, *J* = 4.6, 13.3)		2.16 (*m*)	
H_α–C(13)	1.85 (*m*)	27.9 (*t*)	2.01 (*m*)	27.4 (*t*)
H_β–C(13)	1.72 (*m*)		1.77 (*m*)	
H–C(14)	5.19 (*s*)	75.0 (*d*)	4.70 (*m*)	74.0 (*d*)
Me(15)	1.15 (*s*)	14.6 (*q*)	1.10 (*s*)	15.4 (*q*)
H_a–C(16)	5.80 (*s*)	125.0 (*t*)	5.06 (*d*, *J* = 15.4)	67.5 (*t*)
H_b–C(16)	5.37 (*s*)		4.52 (*d*, *J* = 15.4)	
H–C(17)	3.44 (*q*, *J* = 7.1)	51.4 (*d*)	2.46 (*q*, *J* = 7.1)	42.6 (*d*)
Me(18)	1.23 (*d*, *J* = 7.1)	6.5 (*q*)	1.13 (*d*, *J* = 7.1)	6.7 (*q*)
C(19)		175.6 (*s*)		175.8 (*s*)
H_a–C(20)	5.06 (*s*)	110.1 (*t*)	5.03 (*s*)	113.2 (*t*)
H_b–C(20)	4.81 (*s*)		4.91 (*s*)	
*Me*COO–C(2)	2.24 (*s*)	21.6 (*q*)	1.98 (*s*)[e])	21.1 (*q*)[e])
Me*C*OO–C(2)		171.9 (*s*)		170.8 (*s*)
*Me*COO–C(4)	1.93 (*s*)	21.3 (*q*)		
Me*C*OO–C(4)		169.6 (*s*)		
*Me*COO–C(9)	2.25 (*s*)	20.9 (*q*)	2.20 (*s*)	21.8 (*q*)
Me*C*OO–C(9)		170.6 (*s*)		169.4 (*s*)
*Me*COO–C(14)	2.04 (*s*)	21.3 (*q*)	1.94 (*s*)[e])	21.2 (*q*)[e])
Me*C*OO–C(14)		170.4 (*s*)		170.8 (*s*)
*Me*COO–C(16)			2.14 (*s*)	20.9 (*q*)
Me*C*OO–C(16)				170.2 (*s*)
OH–C(8)	8.28 (*s*)			

[a]) *Bruker DRX-400* spectrometers; assignments made by ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY experiments. [b]) In C_5D_5N, δ in ppm referred to C_5H_5N (δ(H) 7.20, 7.57, 8.73) and C_5D_5N (δ(C) 123.6, 135.8, 150.0). [c]) In $CDCl_3$, δ in ppm referred to $CHCl_3$ (δ(H) 7.26) and $CDCl_3$ (δ(C) 77.0). [d]) By DEPT sequence. [e]) Signals may be interchanged.

2.24, 2.04, 1.93; *s*, each 3 H), corresponding to the four ester groups displayed in the ^{13}C-NMR spectrum. The *m*s integrating for eight H between δ 3.76 and 1.72 were due to three CH_2 and two CH groups by HMQC experiment. The remaining resonance at δ(H) 8.28 (*s*) was assigned to the OH group. These data suggested the presence of a highly oxygenated tricyclic diterpenoid framework with a γ-lactone group.

The briarane skeleton of **1** was established by detailed analysis of the 2D-NMR spectra (^{1}H,^{1}H-COSY, HMQC, and HMBC experiments). The ^{1}H,^{1}H-COSY experiment revealed the connectivities H–C(2)/CH_2(3)/H–C(4), H–C(6)/H–C(7), H–C(9)/H–C(10), CH_2(12)/CH_2(13)/H–C(14), and H–C(17)/Me(18) (trivial numbering). The molecular framework of **1** was further deduced from the HMBC data, *i.e.*, from the long-range

correlations H–C(10)/C(1), C(8), C(9), C(10), C(11), C(12), and C(20), Me(15)/C(1), C(2), C(10), and C(14), CH_2(16)/C(4), C(5), and C(6), H–C(17)/C(8), C(18), and C(19), Me(18)/C(8), C(17), and C(19), and CH_2(20)/C(10), and C(12). The HMBC correlations H–C(2)/C(1), C(4), C(5), and C(δ171.9), H–C(4)/C(6) and C(δ169.6), H–C(9)/C(1), C(8), C(10), C(11), C(17), and C(δ170.6), and OH/C(9) not only further confirmed the established molecular framework but also revealed that the three AcO groups (with C=O at δ 171.9, 169.6, and 170.6) were attached to C(2), C(4), and C(9), respectively, while the OH group should be positioned at C(8), and O-bearing tertiary C-atom resonating at δ 82.2 (*s*). The remaining AcO group had to be linked to C(14) in turn.

The relative configuration of **1** was deduced from the NOESY experiment. The NOE correlation Me(15)/H_β–C(3), H_β–C(13), and H–C(14), H_β–C(3)/H–C(6) and H–C(17), H_β–C(13)/H_β–(12) suggested the β-orientation of these protons. On the other hand, the NOE between H–C(10)/H–C(2), H–C(9), H–C(12), Me(18), and OH, H–C(2)/H_α–C(3), H–C(4), and H–C(10), H_α–C(12)/H_α–C(13), and OH/H–C(2), H–C(4), H–C(10), and Me(18) indicated that all these protons were on the opposite side of the cyclodecane moiety and were assigned to α-positions because no NOE correlation was found between H–C(10) and Me(15).

Junceellonoid B (**2**) was isolated as a colorless solid. Its molecular formula $C_{28}H_{38}O_{11}$ was deduced from the HR-EI-MS (*m/z* 550.2432 (M^+, $C_{28}H_{38}O_{11}^+$; calc. 550.2414). The structure of **2** was readily determined by comparison of its ^{1}H- and ^{13}C-NMR data with those of the known compound **9**, and further confirmed by its MS and 2D-NMR experiments.

The IR spectrum of **2** suggested the presence of OH, γ-lactone, and ester carbonyl groups, with bands of 3448, 1780, and 1736 cm^{-1}, respectively. The EI-MS displayed peaks at *m/z* 550 (M^+), 533 ([M – OH]$^+$), 490 ([M – AcOH]$^+$), 430 ([M – 2 AcOH]$^+$), 370 ([M – 3 AcOH]$^+$), 310 ([M – 4 AcOH]$^+$), indicating the presence of an OH and four AcO groups in **2**. A comparison of the overall ^{1}H- and ^{13}C-NMR data (*Table*) revealed the similarities between **2** and **9**. However, the conversion of one of the Me groups of **9** to a CH_2 group (δ 67.5, *t*) in the ^{13}C-NMR spectrum of **2**, along with the presence of one more AcO group (δ 20.9 (*q*), and 170.2 (*s*)), suggested that the Me group of **9** was substituted by an AcO group in **2**. This conclusion was consistent with the enhancement by 58 mass units of M^+ in the MS. The replacement of the Me signal at relatively high δ(H) of **9** by the Ac *s* and the signals of a CH_2O group of **2** (δ(H) 1.94 (MeCO), 5.06 and 4.52 (2*d*, J = 15.4, each 1 H)) suggested that acetylation had occurred at C(16). This conclusion was strongly supported by the HMBC experiment with **2**, which demonstrated the long-range correlations between CH_2(16) and H–C(5), H–C(6), and C (δ 170.2). The assignment of the other three AcO groups was performed by the long-range correlations between the C=O signals and their corresponding AcO–C*H* protons (*Table*). Furthermore, NOESY experiments of **2** suggested the same configuration as that of **9**. Thus, the structure of junceellonoid B (**2**) was unambiguously determined to be the 16-acetate of **9**. The assignment of the NMR data and the configuration of the cyclodecane-ring of **2** was also supported by the data of milolide K (**10**), a briarane-type diterpene found in *Briareum stechei* [15].

Compounds **3**–**9** were characterized as junceelin (**3**) [2], junceelin B (**4**) [3], junceellolides A–D (**5**–**8**) [4], and the unnamed known diterpene **9** [14] by comparing their spectral data with those reported in the literature.

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 and 400–600 mesh) from *Qing Dao Hai Yang Chemical Group Co*. Anal. TLC: precoated silica gel *G60 F-254* plates from *Yan Tai Zi Fu Chemical Group Co*.

M.p.: *X-5 apparatus, uncorrected.* $[\alpha]_D$: *Perkin-Elmer-341* polarimeter. IR Spectra: *Nicolet-Magna-FT-IR-750* spectrometer; $\tilde{\nu}_{max}$ in cm^{-1}. NMR Spectra: *Bruker-DRX-400* spectrometer; at 400 (^1H) and 100 MHz (^{13}C); chemical shifts δ in ppm, with the residual $CHCl_3$ (δ(H) 7.26, δ(C) 77.0) or C_5H_5N (δ(H) 7.20, 7.57 and 8.73, δ(C) 123.6, 135.8, and 150.0) as an internal standard, coupling constants J in Hz; assignments supported by $^1H,^1H$-COSY, HMQC, HMBC, and NOESY experiments. ESI-MS and HR-EI-MS: *Finnigan-MAT-95* mass spectrometer.

Animal Material. Specimens of gorgonian *Junceella fragilis* RIDLEY were collected along the coast of the Xiaodong Sea, Hainan province, China, in December 2001, at a depth of 20 m, and frozen immediately after collection. The species of gorgonian was identified by Prof. *Ren-lin Zhou*. A voucher specimen is available for inspection at the Institute of Materia Medica, SIBS-CAS.

Extraction and Purification. The frozen animals (dried weight 102.6 g) were cut into pieces and extracted exhaustively with acetone (3×1.5 l) at r.t. The org. extract was evaporated and the residue (7.2 g) partitioned between Et_2O and H_2O. The Et_2O soln. was evaporated and the dark green residue (3.3 g) fractionated by CC (silica gel, 0–100% acetone/petroleum ether) and the fractions purified by CC (*Sephadex LH-20*, petroleum ether/$CHCl_3$/MeOH 2:1:1) and repeated normal-phase CC to afford junceelin (**3**, 11.8 mg) and *junceellolide A* (**5**, 4.5 mg) from *Fr. 4* (petroleum ether/Me_2CO 8:2), and *junceellonoid A* (**1**; 4.1 mg) from *Fr. 6* (petroleum ether/Me_2CO 7:3). *Fr. 5* (petroleum ether/Me_2CO 8:2) was subjected to reversed-phase HPLC (MeCN/H_2O 2:3) to yield the other six pure compounds: *junceellonoid B* (**2**; 1.3 mg), *junceellin B* (**4**, 1.9 mg), *junceellolide B* (**6**; 2.6 mg), *junceellolide C* (**7**, 1.1 mg), *junceellolide D* (**8**, 6.6 mg), and **9** (2.3 mg).

Junceellonoid A (= *(1*R*,3a*R*,4*S*,6*R*,8*S*,8a*R*,9*S*,12a*S*,13*S*,13a*R*)-6,8,9,13-Tetrakis(acetyloxy)-4-chlorotetradecahydro-13a-hydroxy-1,8a-dimethyl-5,12-bis(methylidene)benzo[4,5]cyclodeca[1,2-*b*]furan-2(1*H*)-one*; **1**): Colorless plates. M.p. 167–169°. $[\alpha]_D^{20} = 31.2$ ($c = 0.17$, $CHCl_3$). IR (KBr): 3431, 2929, 1778, 1739, 1240. ^1H-NMR (C_5D_5N, 400 MHz) and ^{13}C-NMR (C_5D_5N, 100 MHz): *Table*. ESI-MS: 607 ($[M+Na]^+$), 1191 ($[2M+Na]^+$). HR-ESI-MS: 607.1924 ($C_{28}H_{37}ClO_{11}Na^+$; calc. 607.1922).

Junceellonoid B (= *(1*R*,3a*S*,4*Z*,8*S*,8a*R*,9*S*,12a*S*,13*S*,13a*R*)-8,9,13-Tris(acetyloxy)-5-[(acetyloxy)methyl]-3a,6,7,8,8a,9,10,11,12,12a,13,13a-dodecahydro-13a-hydroxy-1,8a-dimethyl-12-methylidenebenzo[4,5]cyclodeca[1,2-*b*]furan-2(1*H*)-one*; **2**): Colorless solid. M.p. 135–136° (dec). $[\alpha]_D^{20} = -29.0$ ($c = 0.13$, $CHCl_3$). IR (neat): 3448, 2924, 1780, 1736, 1219. ^1H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz): *Table*. EI-MS: 550 (M^+), 533, 490, 462, 448, 430, 402, 388, 370, 342, 328, 310. HR-EI-MS: 550.2432 ($C_{28}H_{38}O_{11}^+$; calc. 550.2414).

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