New Briaranes from the South China Sea Gorgonian *Junceella fragilis*

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Three new briarane diterpenes, junceellonoids C–E (**1**–**3**), along with six known briaranes, junceellin A (**4**), praelolide (**5**), and junceellolides A-D (**6**–**9**), were isolated from the EtOH/CH_2Cl_2 extracts of the South China Sea gorgonian coral *Junceella fragilis*. The structures of **1**–**3** were established by extensive spectroscopic analysis, including 1D- and 2D-NMR data. Compounds **1** and **2** exhibited mild cytotoxicity against human galactophore carcinoma (MDA-mB-231 and MCF-7) cells at the concentration of 100 μM.

Introduction. – *Junceella fragilis* belonging to the genus *Junceella* was known to produce highly oxidized diterpenoids of the briarane class (3,8-cyclized cembranoids). About 18 briaranes, namely, junceellolides A–H [1–3], (−)-4-*O*-deacetyljunceellolide D, (+)-11α,20α-epoxyjunceellolide D [4], (−)-4-*O*-deacetyl-11α,20α-epoxyjunceellolide D [4], (−)-4-de(acetyloxy)-11α,20α-epoxyjunceellolide D [4], (+)-junceellolide A [4], 9-de(acetyl)oxyjunceellolide A [5], fragilide A [6], junceellolide I [7], and junceellonoids A and B [8] were first obtained from *J. fragilis*. Many briarane-type diterpenoids were found to possess extensive biological activities [9]. During the course of further searching for novel active compounds from gorgonians [10][11], we undertook the investigation of the South China Sea gorgonian coral *J. fragilis*. Three new briarane diterpenes, junceellonoids C–E[1])(**1**–**3**), along with six known briaranes, junceellin A (**4**) [4], praelolide (**5**) [12], and junceellolides A–D (**6**–**9**) [1], were isolated from the EtOH/CH_2Cl_2 extracts of *J. fragilis*. In the cytotoxicity testing of a number of compounds (including compounds **1**, **2**, and other compounds that are not reported herein), we observed that **1** and **2** exhibited mild cytotoxicity against human galactophore carcinoma (MDA-mB-231 and MCF-7) cells at a concentration of 100 μM, but almost no activity at the concentration of 33.3 μM. This paper deals with the isolation and structural elucidation of compounds **1**–**3**.

Results and Discussion. – The residue from the EtOH/CH_2Cl_2 extracts of *J. fragilis* was partitioned in H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was chromatographed over silica gel, and selected fractions were rechromatographed on *Sephadex LH-20* and silica gel to yield compounds **1**–**9**. All of the compounds possessed a briarane-type skeleton, and compounds **4**–**9** were identified as junceellin A (**4**) [4],

[1]) Arbitrary numbering; for systematic names, see *Exper. Part.*

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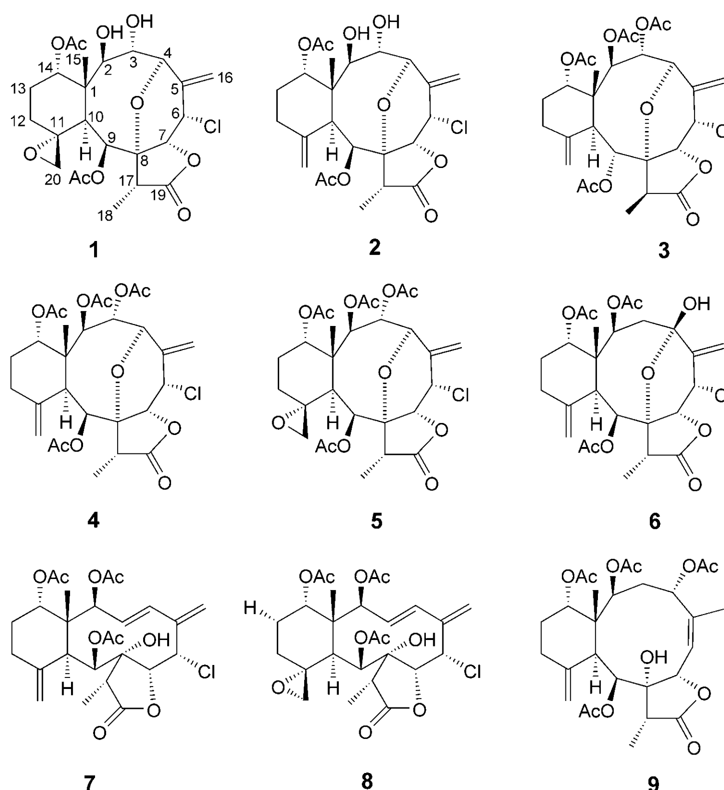

praelolide (**5**) [12], and junceellolides A – D (**6** – **9**) [1] by comparison of their spectral data with literature values. The structures of **1** – **3** are described below.

Junceellonoid C (**1**) had the molecular formula $C_{24}H_{33}ClO_{11}$, as deduced from NMR spectra and the HR-ESI-MS. It showed IR absorptions at 3354, 1778, and 1736 cm^{-1}, which indicated the presence of OH groups, a γ-lactone, and ester groups. The ^{13}C-NMR and ^{1}H-NMR data (*Tables 1* and *2*) showed that **1** was a briarane-type diterpene and similar in structure to praelolide (**5**) [12]. Comparison of the ^{1}H- and ^{13}C-NMR spectra data of **1** and **5** revealed that the only difference between them was the lack of two acetate groups in **1**.

The HMBC and $^{1}H,^{1}H$-COSY (*Fig.*) and NOESY correlations established the structure of **1**, with the relative configuration *rel*-(1*R*,2*R*,3*R*,4*S*,6*R*,7*S*,8*S*,9*R*,10*R*,11*S*,14*R*,17*R*). The ^{13}C-DEPT- and ^{1}H-NMR spectra of **1** showed signals for two acetate esters (δ(C) 169.6 (*s*), 169.8 (*s*), 20.7 (*q*), 20.8 (*q*)), a tertiary Me group (δ(H) 1.02, *s*), a secondary Me group (δ(H) 1.23, *d*, $J = 7.1$ Hz), a γ-lactone (δ(C) 175.3), a spirocyclic oxirane moiety (δ(H) 2.67, 2.63 (*d*, $J = 3.2$ Hz, each 1 H), δ(C) 51.7 (*t*), 57.0 (*s*)] [12] [13], an exocyclic CH_2 group (δ(H) 5.63, 4.50 (each br. *s*); δ(C) 118.0 (*t*)), an oxygenated quaternary C-atom and six oxygenated CH groups (*Tables 1* and *2*). The lack of two AcO groups in **1** as compared to **5** was supported by the HMBC and $^{1}H,^{1}H$-COSY data. The HMBC correlations (*Fig.*) δ(H) 1.02 (*s*, Me(15))/δ(C) 72.5 (*d*), 72.0 (*d*), 40.9 (*d*, C(10)) and 48.2 (*s*, C(1)), δ(H) 2.93 (br. *s*, H–C(10))/δ(C) 72.5 (*d*), 72.0 (*d*), 15.2 (*q*, C(15)), δ(H) 3.72 (*d*, $J = 6.8$ Hz, 1 H) and 5.23 (br. *s*, 1 H)/δ(C) 40.9 (*d*, C(10)) indicated the oxygenation of C(2) (δ(C) 72.0, *d*) and C(14) (δ(C) 72.5, *d*), and correspondingly the presence of H–C(2) (δ(H) 3.72, *d*, $J = 6.8$ Hz) and H–C(14) (δ(H) 5.23, br. *s*).

Table 1. *^{13}C-NMR Data* (125 MHz) *for Compounds* **1**–**4**[1]). δ in ppm rel. to internal Me_4Si.

Position	**1** ((D_5)pyridine)	**2** ($CDCl_3$)	**3** ($CDCl_3$)	**4** ($CDCl_3$)
C(1)	48.2 (*s*)	49.1 (*s*)	47.6 (*s*)	47.5 (*s*)
C(2)	72.0 (*d*)	72.0 (*d*)	73.1 (*d*)	72.9 (*d*)
C(3)	65.0 (*d*)	64.6 (*d*)	64.1 (*d*)	63.9 (*d*)
C(4)	82.8 (*d*)	82.2 (*d*)	79.0 (*d*)	78.9 (*d*)
C(5)	137.4 (*s*)	135.2 (*s*)	134.2 (*s*)	134.4 (*s*)
C(6)	56.2 (*d*)	54.7 (*d*)	53.8 (*d*)	53.9 (*d*)
C(7)	79.8 (*d*)	79.3 (*d*)	78.9 (*d*)	79.2 (*d*)
C(8)	82.9 (*s*)	82.5 (*s*)	84.9 (*s*)	82.8 (*s*)
C(9)	76.0 (*d*)	78.2 (*d*)	73.9 (*d*)	77.6 (*d*)
C(10)	40.9 (*d*)	43.6 (*d*)	43.9 (*d*)	44.1 (*d*)
C(11)	57.0 (*s*)	147.8 (*s*)	147.1 (*s*)	147.3 (*s*)
C(12)	24.5 (*t*)	28.0 (*t*)	32.5 (*t*)	32.6 (*t*)
C(13)	30.3 (*t*)	32.7 (*t*)	27.6 (*t*)	27.6 (*t*)
C(14)	72.5 (*d*)	76.7 (*d*)	74.5 (*d*)	74.5 (*d*)
C(15)	15.2 (*q*)	14.3 (*q*)	15.3 (*q*)	15.0 (*q*)
C(16)	118.0 (*t*)	118.8 (*t*)	119.6 (*t*)	119.5 (*t*)
C(17)	49.3 (*d*)	49.6 (*d*)	50.6 (*d*)	49.9 (*d*)
C(18)	7.8 (*q*)	7.4 (*q*)	12.7 (*q*)	7.1 (*q*)
C(19)	175.3 (*s*)	174.4 (*s*)	175.5 (*s*)	174.0 (*s*)
C(20)	51.7 (*t*)	111.5 (*t*)	111.4 (*t*)	111.8 (*t*)
*Ac*O–C(14)	169.6 (*s*), 20.7 (*q*)	172.4 (*s*), 21.2 (*q*)	169.98 (*s*), 20.3 (*q*)	169.98 (*s*), 20.3 (*q*)
*Ac*O–C(9)	169.8 (*s*), 20.8 (*q*)	169.7 (*s*), 21.4 (*q*)	170.18 (*s*), 20.4 (*q*)	169.76 (*s*), 20.4 (*q*)
*Ac*O–C(3)			169.81 (*s*), 21.0 (*q*)	169.78 (*s*), 20.9 (*q*)
*Ac*O–C(2)			170.34 (*s*), 21.1 (*q*)	170.40 (*s*), 21.0 (*q*)

Table 2. *^{1}H-NMR Data* (500 MHz) *for Compounds* **1**–**3**[1]). δ in ppm, *J* in Hz.

Position	**1** ((D_5)pyridine)	**2** ($CDCl_3$)	**3** ($CDCl_3$)
H–C(2)	3.72 (*d*, *J* = 6.8)	3.91 (*d*, *J* = 7.1)	5.44 (*d*, *J* = 6.3)
H–C(3)	4.33 (*dd*, *J* = 6.8, 9.8)	4.33 (*dd*, *J* = 7.2, 9.8)	6.17 (*dd*, *J* = 6.3, 11)
H–C(4)	4.06 (*d*, *J* = 9.8)	4.06 (*d*, *J* = 9.8)	4.44 (*d*, *J* = 11)
H–C(6)	4.95 (br. *s*)	5.21 (*d*, *J* = 2.7)	4.96 (br. *s*)
H–C(7)	5.46 (br. *s*)	4.39 (*d*, *J* = 3.1)	4.60 (*d*, *J* = 2.5)
H–C(9)	5.85 (br. *s*)	5.50 (br. *s*)	6.09 (br. *s*)
H–C(10)	2.93 (br. *s*)	3.08 (br. *s*)	2.85 (br. *s*)
CH_2(12)	2.44, 2.28 (2*m*, each 1 H)	2.31, 1.22 (2*m*, each 1 H)	2.28, 2.24 (2*m*, each 1 H)
CH_2(13)	1.79 (*m*)	2.12, 1.82 (2*m*, each 1 H)	1.81, 1.70 (2*m*, each 1 H)
H–C(14)	5.23 (br. *s*)	5.25 (br. *s*)	4.96 (br. *s*)
Me(15)	1.02 (*s*)	1.23 (*s*)	1.12 (*s*)
CH_2(16)	5.63, 4.50 (2 br. *s*, each 1 H)	5.60, 5.45 (2 br. *s*, each 1 H)	5.57, 5.35 (2 br. *s*, each 1 H)
H–C(17)	2.72 (*q*, *J* = 7.1)	3.20 (*q*, *J* = 7.0)	2.68 (*q*, *J* = 7.0)
Me(18)	1.23 (*d*, *J* = 7.1)	1.47 (*d*, *J* = 7.0)	1.28 (*d*, *J* = 7.0)
CH_2(20)	2.67, 2.63 (2*d*, *J* = 3.2, each 1 H)	5.05, 4.69 (2 br. *s*, each 1 H)	5.09, 4.76 (2 br. *s*, each 1 H)
AcO	2.18, 2.20 (2*s*, each 3 H)	1.88, 2.10 (2*s*, each 3 H)	1.99, 2.05, 2.06, 2.32 (4*s*, each 3 H)

The HMBC correlations δ(H) 5.85 (br. *s*, H–C(9))/δ(C) 169.6 (*s*), and δ(H) 5.23 (br. *s*, H–C(14))/δ(C) 169.8 (*s*) suggested the presence of two acetate groups placed at C(9) and C(14). The ^{1}H,^{1}H-COSY correlations (*Fig.*) δ(H) 4.33 (*dd*, *J* = 6.8, 9.8 Hz, 1 H)/δ(H) 3.72 (*d*, *J* = 6.8 Hz, H–C(2)) and 4.06 (*d*, *J* = 9.8 Hz, 1 H) allowed the assignment of C(3) (δ(C) 65.0, *d*) and C(4) (δ(C) 82.8, *d*). The NOE correlations Me(15)/H–C(3), H–C(4), H–C(14), and AcO–C(9), and H–C(3))/H–C(4) and H–C(6) suggested that CH_2(20), H–C(14),

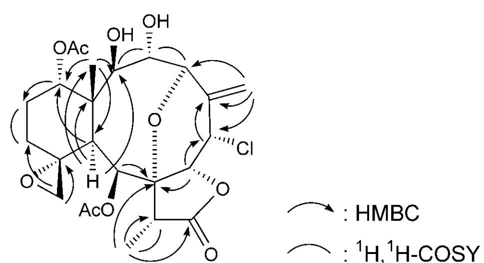

Figure. *Key HMBC and ^{1}H,^{1}H-COSY correlations for compound* **1**

H−C(6), H−C(3), H−C(4) and Me(15) were all β-positioned. The NOE correlations H−C(2), H−C(10) and H−C(9), and Me(18)/H−C(9) and H−C(10) indicated that H−C(2), H−C(9), H−C(10), and Me(18) were all α-positioned, with the corresponding correlation of H−C(17)/H−C(7) suggesting the β-orientation of H−C(17) and H−C(7).

Junceellonoid D (**2**) had the molecular formula $C_{24}H_{33}ClO_{10}$ by HR-ESI-MS and ^{13}C-NMR spectrometry. The ^{13}C- and ^{1}H-NMR data of **2** (*Tables 1* and *2*) were very similar to those of **1**. However, the ^{13}C- and ^{1}H-NMR spectra of **2** showed that the spirocyclic oxirane moiety of **1** was converted to a C=C bond (δ(C) 147.8 (*s*) and 111.5 (*t*); δ(H) 5.05, 4.69 (2 br. *s*, each 1 H)). This was supported by the HMBC spectrum (correlations δ(H) 5.05 and 4.69/δ(C) 147.8 (*s*, C(11)) and 43.6 (*d*, C(10)). Based on the 1D- and 2D-NMR studies, including HSQC, HMBC, and NOESY experiments, the structure of junceellonoid D (**2**) was elucidated as shown, and its relative configuration was determined as *rel*-(1*R*,2*R*,3*R*,4*S*,6*R*,7*S*,8*S*,9*R*,10*R*,14*R*,17*R*).

Junceellonoid E (**3**) was assigned the molecular formula of $C_{28}H_{35}ClO_{11}$ on the basis of its ESI-MS and NMR spectra. The ^{13}C- and ^{1}H-NMR data of **3** (*Tables 1* and *2*) were almost identical with those of junceellin A [4] (**4**; $CDCl_3$ as solvent). However, the chemical shifts of C(18), C(9), and C(8) in **3** were obviously different from those in **4** (δ(C) 12.7 *vs.* 7.1, 73.9 *vs.* 77.6, and 84.9 *vs.* 82.8, resp.). With the assistance of 2D-NMR studies, including ^{1}H,^{1}H-COSY, HSQC, HMBC, and NOESY experiments, the structure of **3** was inferred as 9,17-diepijunceellin A. The relative configuration of **3** was determined as *rel*-(1*R*,2*R*,3*R*,4*S*,6*R*,7*S*,8*S*,9*S*,10*R*,14*R*,17*R*).

The NOE cross-peaks of **3** for Me(15)/H−C(3) and H−C(14), H−C(3)/H−C(4) and H−C(6) suggested the β-configuration of H−C(14), H−C(6), H−C(4), H−C(3), and Me(15), while the NOE correlations H−C(10)/H−C(2) and H−C(17) indicated the α-configuration of H−C(2), H−C(10), and H−C(17), and the corresponding correlation H−C(7)/H−C(9) and Me(18), and H−C(9)/Me(18) suggested the β-orientation of Me(18), H−C(9), and H−C(7).

Compounds **1** and **2** showed mild cytotoxicity against human breast carcinoma MDA-MB-231 and MCF cell lines at a concentration of 100 μM, but almost no activity was detected at a concentration of 33.3 μM, so their IC_{50} values were not calculated.

Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh), from *Qindao Marine Chemical Factory*, Qindao, People's Republic of China. TLC: GF_{254} silica gel, from *Qindao Marine Chemical Factory.* $[\alpha]_D$: *Horiba SEAP-300* spectropolarimeter. UV Spectra: *Shimadzu* double-beam *210A* spectrophotometer;

MeOH soln. IR Spectra (KBr): *Bio-Rad FTS-135* IR spectrophotometer. NMR Spectra: *Bruker AV-500-MHz* spectrometer; δ in ppm rel. to $SiMe_4$ as internal standard, *J* in Hz. MS: *Finnigan LCQ Deca-XP HPLC/MS^n*; spectrometer for ESI-MS; in *m*/*z* (rel. %).

Animal Material. The South China Sea gorgonian coral *Junceella fragilis* (8 kg, wet weight) was collected in Sanya, Hainan province, China, in October 2003 and identified by Prof. *R. L. Zou*, South China Sea Institute of Oceanology, *Academia Sinica*. A voucher specimen (No. 0311) was deposited in the South China Sea Institute of Oceanology, *Academia Sinica*, Guangzhou, China.

Extraction and Isolation. The frozen specimen was extracted with $EtOH/CH_2Cl_2$ 2:1 (3 ×) at r.t., and the solvent was evaporated. The residue was partitioned in H_2O and extracted with $CHCl_3$ (3 ×). The $CHCl_3$ extract was evaporated: 66 g of residue. The $CHCl_3$ portion was subjected to CC (silica gel, petroleum ether/AcOEt 100:10 → 0:100; TLC (GF_{254}) monitoring): *Fractions 1–8*. The fractions were purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1) and repeated normal-phase CC. *Fr. 2* afforded junceellin A (**4**; 28 mg), praelolide (**5**; 34 mg), junceellolide B (**7**; 20 mg), and junceellolide C (**8**; 21 mg). *Fr. 3* gave junceellolide A (**6**; 30 mg) and junceellolide D (**9**; 18 mg). *Fr. 4* yielded junceellonoid E (**3**; 3.4 mg), and from *Fr. 5*, junceellonoid C (**1**; 12 mg) and junceellonoid D (**2**; 20 mg) were obtained.

Junceellonoid C (= rel-*(1*R*,2*R*,3*R*,4*S*,6*R*,7*S*,8*S*,9*R*,10*R*,11*S*,14*R*,17*R*)-9,13-Bis(acetyloxy)-4-chloro-3a,4,5,6,7,8,8a,9,10,11,12a,13-dodecahydro-7,8-dihydroxy-1,8a-dimethyl-5-methylenespiro[12*H*-6,13a-epoxybenzo[4,5]cyclodeca[1,2-*b*]furan-12,2′-oxiran]-2(1*H*)-one*; **1**): White powder: $[\alpha]_D = -67.6$ ($c = 0.05$, pyridine). IR (KBr): 3354, 1778, 1736, 1220. ^{1}H-NMR: *Table 2*. ^{13}C-NMR: *Table 1.* ESI-MS (pos.): 533 ($[M+H]^+$). HR-ESI-MS: 533.1782 ($[M+H]^+$, $C_{24}H_{34}ClO_{11}^+$; calc. 533.1789).

Junceellonoid D (= rel-*(1*R*,2*R*,3*R*,4*S*,6*R*,7*S*,8*S*,9*R*,10*R*,14*R*,17*R*)-9,13-Bis(acetyloxy)-4-chloro-3a,4,6,7,8,8a,9,10,11,12,12a,13-dodecahydro-7,8-dihydroxy-1,8a-dimethyl-5,12-dimethylene-5*H*-6,13a-epoxybenzo[1,5]cyclodeca[1,2-*b*]furan-2(1*H*)-one*; **2**): white powder; $[\alpha]_D = -44.8$ ($c = 0.10$, $CHCl_3$ MeOH). IR (KBr). 3356, 1780, 1738, 1240. ^{1}H-NMR: *Table 2.* ^{13}C-NMR: *Table 1.* ESI-MS (pos.): 517 ($[M+H]^+$). HR-ESI-MS: 517.1836 ($[M+H]^+$, $C_{24}H_{34}ClO_{10}^+$; calc. 517.1840).

Junceellonoid E (= rel-*(1*R*,2*R*,3*R*,4*S*,6*R*,7*S*,8*S*,9*S*,10*R*,14*R*,17*R*)-7,8,9,13-Tetrakis(acetyloxy)-4-chloro-3a,4,6,7,8,8a,9,10,11,12,12a,13-dodecahydro-1,8a-dimethyl-5,12-dimethylene-5*H*-6,13a-epoxybenzo[4,5]cyclodeca[1,2-*b*]furan-2(1*H*)-one*; **3**): White powder: $[\alpha]_D = -18.0$ ($c = 0.05$, $CHCl_3$). IR (KBr): 3430, 1786, 1750, 1736, 1720, 1450, 1389, 1030. ^{1}H-NMR: *Table 2.* ^{13}C-NMR: *Table 1.* ESI-MS (pos.): 583 ($[M+H]^+$). HR-ESI-MS: 583.1942 ($[M+H]^+$, $C_{28}H_{36}ClO_{11}^+$; calc. 583.1946).

Cytotoxicity Testing. Cytotoxicity assays were carried out by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric method as described previously [14]. In brief, human galactophore carcinoma MDA-MB-231 and MCF cell lines were purchased from the *American Type Culture Collection* (*ATCC*, Rockville, MD). MDA-MB-231 cells and MCF cells were grown in RPMI1640 medium and *Dulbecco*'s modified *Eagle*'s medium (DMEM), respectively, supplemented with 10% fetal bovine serum, penicillin (1×10^5 U l^{-1}), streptomycin (100 mg l^{-1}), $NaHCO_3$ (2 g), and L-glutamine (0.3 g), at 37° in a humidified atmosphere containing 5% CO_2. MDA-MB-231 cells and MCF cells were plated (5×10^4/well/100 µl) in a 96-well plate and incubated for 24 h, and culture media was removed and replaced with 200 µl of fresh complete culture medium containing the appropriate tested compounds with concentrations of 100, 33.3, 11.1, 3.70, 1.24, 0.41, 0.137, and 0.046 µM. Control wells were replaced with fresh cell culture media only. The cells were then incubated for further 48 h, and the colorometric MTT dye reduction assay was used to assess cell viability. All assays were performed in triplicate.

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