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Two New Halogenated Briarane Diterpenes from the Papuan Gorgonian Coral *Junceella fragilis*

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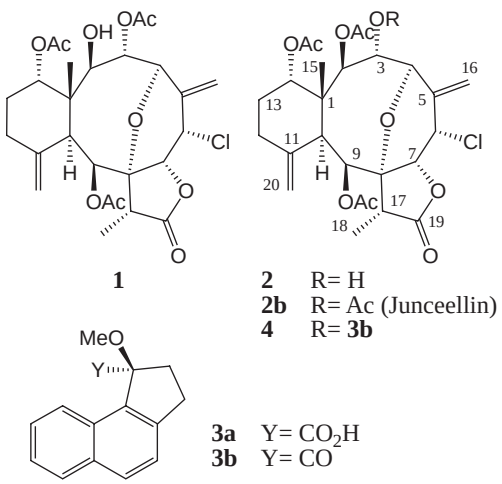
Two new halogenated briarane diterpenes, (−)-2-deacetyljunceellin (**1**) and (−)-3-deacetyljunceellin (**2**), and five known diterpenoids, junceellin, praelolide, junceellolide A, B, and D were isolated from the Papuan gorgonian coral *Junceella fragilis*. The structures of the new metabolites were determined by extensive 2D NMR experiments and by chemical conversion. The absolute configuration of **2** was determined through the ^{1}H NMR study of the corresponding ester with a newly developed chiral auxiliary **3a**.

In the course of our search for metabolites from marine organisms, we investigated the gorgonian coral *Junceella fragilis* Ridley from Papua New Guinea, and isolated two new halogenated briarane diterpenes **1** and **2** (Chart 1) and five known briaranes, junceellin, praelolide, junceellolide A, B, and D, which were identified by comparison of their spectroscopic properties with literature data.[1] In this paper, we describe the isolation and structure elucidation of the new briaranes.

The HRFABMS spectrum of **1** determined a molecular formula of $C_{26}H_{33}ClO_{10}$ with ten degrees of unsaturation. The IR spectrum showed the presence of hydroxy, γ-lactone, and acetate groups. The 1D and 2D NMR spectra of **1** showed signals for the presence of three acetyl methyls (δ_H 2.07, 2.13, and 2.31, each 3H, s) along with a lactone carbonyl (δ_C 174.3), two exocyclic double bonds (δ_H 5.33, 1H, d, $J = 1.8$ Hz, H-16a and δ_H 5.57, 1H, d, $J = 1.8$ Hz, H-16b; δ_H 4.75, 1H, brs, H-20a and δ_H 5.06, 1H, brs, H-20b), an oxygenated quaternary carbon (δ_C 82.8, C-8), six-oxygenated methines (δ_H 6.11, 1H, dd, $J = 11.0$, 7.3 Hz, H-3; δ_H 5.92, 1H, brs, H-9; δ_H 5.29, 1H, brt, $J = 2.7$ Hz, H-14; δ_H 4.51, 1H, d, $J = 3.1$ Hz, H-7; δ_H 4.23, 1H, d, $J = 11.0$ Hz, H-4; δ_H 4.11, 1H, dd, $J = 7.3$, 3.7 Hz, H-2), a chlorinated methine[2] (δ_C 54.0, C-6, δ_H 5.03, 1H, brq, $J = 2.5$ Hz, H-6), two methines (δ_H 3.07, 1H, brs, H-10; δ_H 2.76, 1H, q, $J = 7.4$ Hz, H-17), two methylenes (δ_H 2.33, 1H, m, H-12*eq* and δ_H 2.23, 1H, m, H-12*ax*; δ_H 1.87, 1H, m, H-13*eq* and δ_H 1.70, 1H, m, H-13*ax*), a tertiary methyl (δ_H 1.02, 3H, s, H-15), and a secondary methyl (δ_H 1.25, 3H, d, $J = 7.4$ Hz, H-18). These NMR signals were very similar to those of co-occurring junceellin (**2b**) with the exception of the absence of one acetyl group. Each of the three acetate moieties of **1** were assigned to be at C-3, C-9, and C-14 positions from the HMBC correlations between the acetate carbonyl carbons and the corresponding methine protons. A D_2O exchange experiment led to the elimination of the small coupling (3.7 Hz) from the methine proton at δ 4.11 (H-2), which was coupled to the adjacent methine proton at δ 6.11 (H-3) in a COSY spectrum. Furthermore, the methine at δ 4.11 showed HMBC correlations to three carbons at δ 48.9 (C-1), 65.6 (C-3), and 14.6 (C-15). From the above data, a secondary hydroxy group was placed at C-2. Thus, compound **1** was elucidated as a 2-deacetyl analogue of junceellin (**2b**). The relative stereochemistry of **1** was deduced from the observed NOESY correlations (Fig. 1) and vicinal coupling constant analysis. Finally, the structure of **1** was confirmed by the fact that the acetylation product of **1** showed identical ^{1}H and ^{13}C NMR properties with those of junceellin (**2b**).

The second compound **2** was considered to be an isomer of **1** from the molecular formula, $C_{26}H_{33}ClO_{10}$, determined by a HRFABMS spectrum. The ^{1}H and ^{13}C NMR spectra of **2**



1

2 R= H
2b R= Ac (Junceellin)
4 R= **3b**

3a Y= CO_2H
3b Y= CO

Chart 1.

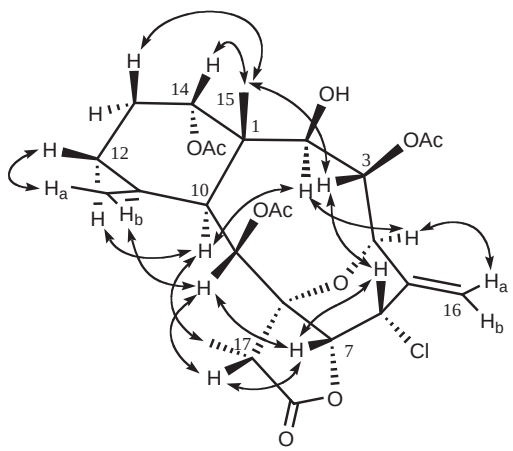


Fig. 1. Key NOESY correlations observed for **1**.

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showed a marked structural resemblance to compound **1**. The ^{1}H NMR spectrum of **2** showed H-2 and H-3 signals at δ 5.32 (1H, d, $J = 6.7$ Hz) and 4.45 (1H, ddd, $J = 10.4$, 6.7, 3.2 Hz), respectively. The small coupling (3.2 Hz) of the latter proton disappeared in a D_2O added NMR spectrum. The change in chemical shift and pattern of these signals accounted for the presence of an OH group at C-3 in **2** by comparison of the above NMR with that of **1**. Thus, the structure of **2** was assigned as an isomer of **1** differing in the acetylated positions only. The full and unambiguous ^{1}H and ^{13}C NMR assignments for **2** were confirmed using a combination of DEPT, COSY, NOESY, HMBC, and HMQC experiments. The relative stereochemistry of **2** was shown to be the same as for **1** from the NOESY spectral data. In addition, acetylation of **2** afforded the same product **2b** as obtained from **1**.

The absolute configuration of the secondary alcohol of **2** was determined by the application of a new method using a combination of chemical shift simulation and molecular dynamic (MD) calculation.[3] Compound **2** was esterified with a new chiral auxiliary **3a** to give the corresponding ester **4**. Esterification shifts of **2** can be estimated from comparison of the chemical shifts of **4** and a reference compound **2b**.

At room temperature many conformers of the ester are in dynamic equilibrium. To simulate the conformational equilibrium of **4**, molecular dynamic (MD) calculation with the Metropolis Monte Carlo (MC) algorithm and stochastic dynamic (SD) simulation was applied for 1000 ps.[4] A snapshot of the structure was monitored at every 0.5 ps. A total of 2000 structures were obtained. Esterification shifts in each structure can be calculated by using the naphthalene ring current[5] and a magnetic shielding parameter of the OMe group.[6] Averaging these 2000 esterification shifts gave the predicted value of each proton of **2**. The predicted esterification shifts are very close to the observed values (Fig. 2), giving a high correlation coefficient ($R^2 = 0.9489$; Fig. 3a). Therefore, the absolute configuration at C-3 was assigned as *S*. A similar calculation for the enantiomer of **2** gave a poor correlation ($R^2 = 0.0023$; Fig. 3b). Thus, the absolute configuration of **2** was confirmed unambiguously by this calculation. Furthermore, the absolute configuration of **2b** turned out to be identical to that of junceellin, which had been confirmed by X-ray crystallography.[1e]

Since compounds **1** and **2** may be formed from co-occurring junceellin, we were led to examine the possibility that these are artifacts produced during the isolation process. Junceellin did not give any deacetyl derivative when it was allowed to stand in a CH_2Cl_2/MeOH (1:1) solution for 2 months at room temperature. Furthermore, when treated with 0.5 M HCl in MeOH, junceellin gave a mixture of several deacetoxy derivatives in which no trace amount of **1** or **2** was detected. From these experiments, we conclude that these compounds are truly present in the coral.

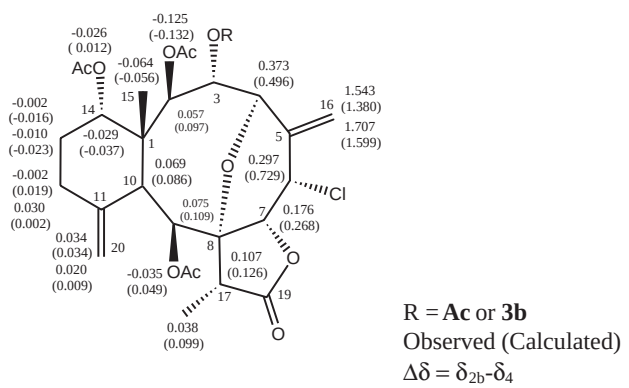

Fig. 2. Observed and calculated esterification shift differences ($\Delta\delta$, ppm).

Experimental

General. Melting points were determined on a Yanagimoto micro melting point apparatus. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a JASCO FT/IR-420S infrared spectrometer. NMR spectra were recorded on a JEOL GSX-500 spectrometer at 25 °C in $CDCl_3$. Chemical shifts were referenced to the residual $CHCl_3$ at δ_H 7.26 and $CDCl_3$ at δ_C 77.0. FABMS spectra were measured on a JEOL SX102A spectrometer. Column chromatography was carried out by flash technique using 40–63 μm silica gel 60 N (Kanto Chemical, Tokyo).

Isolation and Purification. The gorgonian coral *Junceella fragilis* Ridley was collected at the Pass Reef of Madang, Papua New Guinea, on November 14th, 1991 at a depth of 25 m. Intact specimens were immediately frozen and kept at −20 °C until extraction. The freeze-dried organism (380 g) was crushed and extracted with CH_2Cl_2/MeOH (1:1) three times. The extract (1.5 L) was concentrated under vacuum and partitioned between CH_2Cl_2 and water. The CH_2Cl_2-soluble material (21 g) was fractionated by rapid chromatography over a SiO_2 column using a hexane/EtOAc garadient. The resultant fractions containing briarane diterpenoids were monitored by ^{1}H NMR and chromatographed repeat-

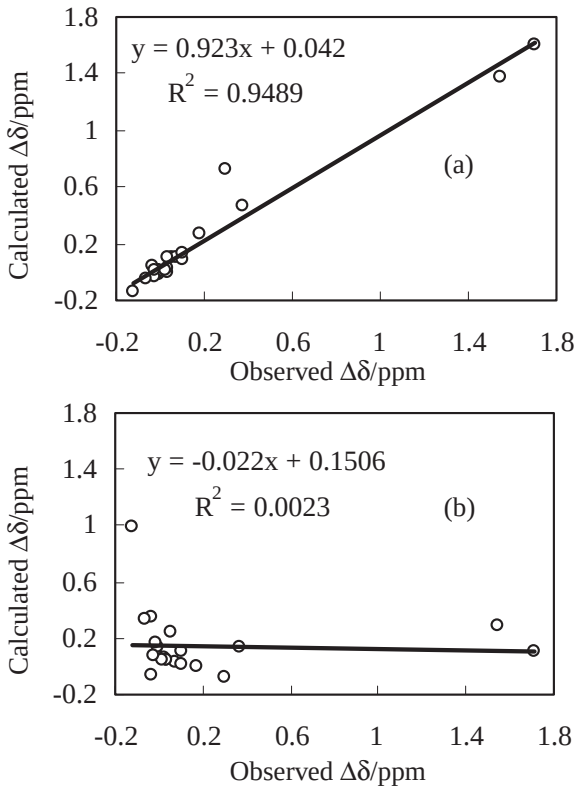

Fig. 3. (a) Plot of observed and calculated values of **4** and (b) the ester of **3a** and enantiomer of **2**.

edly over a SiO_2 column (hexane/EtOAc, 2:1) to afford **1** (31.4 mg), **2** (22.1 mg), junceellin (173.9 mg), praelolide (53.2 mg), junceellolide A (42.7 mg), junceellolide B (54.9 mg), and junceellolide D (56.4 mg).

Compound 1: White powder; $[\alpha]^{25}_{D}$ −5.9° (*c* 0.44, MeOH); IR (KBr) ν_{max} 3487, 1792, 1741, 1374, and 1236 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 174.3 (C-19), 170.2 and 21.0 (Ac at C-14), 169.8 and 20.6 (Ac at C-9), 169.7 and 21.1 (Ac at C-3), 147.9 (C-11), 134.9 (C-5), 119.0 (C-16), 111.4 (C-20), 82.8 (C-8), 79.6 (C-4), 79.2 (C-7), 77.5 (C-9), 75.6 (C-14), 72.1 (C-2), 65.6 (C-3), 54.0 (C-6), 49.7 (C-17), 48.9 (C-1), 43.6 (C-10), 32.6 (C-12), 27.3 (C-13), 14.6 (C-15), 7.1 (C-18); HRMS (FAB) $[M+H]^+$, Found: *m/z* 541.1860. Calcd for $C_{26}H_{34}{}^{35}ClO_{10}$: 541.1841.

Acetylation of 1: **1** (2 mg) in a 1:1 mixture of Ac_2O/pyridine (0.5 mL) was left at room temperature for 24 h. The reagents were removed in vacuo, and the residue was passed through a SiO_2 short column eluted with hexane/EtOAc (2:1) to obtain the acetate derivative (ca. 0.9 mg): $[\alpha]^{25}_{D}$ −11.7° (*c* 0.1, $CHCl_3$). The ^1H and ^{13}C NMR spectral properties were identical in all respects with natural junceellin.

Compound 2: Colorless plates (CH_2Cl_2); mp 181–182 °C; $[\alpha]^{25}_{D}$ −17.4° (*c* 0.63, MeOH); IR (KBr) ν_{max} 3450, 1792, 1741, and 1375 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.89 (1H, brs, H-9), 5.64 (1H, brs, H-16b), 5.46 (1H, brs, H-16a), 5.32 (1H, d, J = 6.7 Hz, H-2), 5.07 (1H, brs, H-20a), 5.02 (1H, brt, J = 2.7 Hz, H-14), 4.90 (1H, brq, J = 2.5 Hz, H-6), 4.68 (1H, brs, H-20b), 4.49 (1H, d, J = 3.1 Hz, H-7), 4.45 (1H, ddd, J = 10.4, 6.7, 3.2 Hz, H-3), 4.34 (1H, d, J = 10.4 Hz, H-4), 3.08 (1H, brs, H-10), 2.74 (1H, q, J = 7.4 Hz, H-17), 2.30 (1H, m, H-12*ax*), 2.25 (1H, m, H-12*eq*), 2.22 (3H, s, Ac at C-9), 2.10 (3H, s, Ac at C-2), 2.06 (3H, s, Ac at C-14), 1.84 (1H, m, H-13*eq*), 1.70 (1H, m, H-13*ax*), 1.28 (3H, d, J = 7.4 Hz, H-18), and 1.13 (3H, s, H-15); ^{13}C NMR ($CDCl_3$) δ 174.3 (C-19), 171.9 and 20.7 (Ac at C-2), 170.0 and 21.0 (Ac at C-14), 169.5 and 21.4 (Ac at C-9), 147.5 (C-11), 134.7 (C-5), 119.6 (C-16), 111.7 (C-20), 82.3 (C-8), 81.1 (C-4), 79.1 (C-7), 78.1 (C-9), 74.8 (C-2), 74.7 (C-14), 64.9 (C-3), 54.4 (C-6), 49.7 (C-17), 47.5 (C-1), 44.0 (C-10), 32.6 (C-12), 27.5 (C-13), 14.8 (C-15), 7.3 (C-18); HRMS (FAB) $[M+H]^+$: Found: *m/z* 541.1849. Calcd for $C_{26}H_{34}{}^{35}ClO_{10}$: 541.1841.

Acetylation of 2 to 2b: **2** (3 mg) was acetylated by the same manner as for **1** to afford the 3-acetyl derivative **2b** (3.2 mg): $[\alpha]^{25}_{D}$ −13.5° (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 6.13 (1H, dd, J = 11.0, 6.7 Hz, H-3), 5.94 (1H, s, H-9), 5.57 (1H, d, J = 2.4 Hz, H-16b), 5.43 (1H, d, J = 6.7 Hz, H-2), 5.35 (1H, d, J = 2.5 Hz, H-16a), 5.09 (1H, brs, H-20a), 5.01 (1H, brq, J = 2.5 Hz, H-6), 4.97 (1H, brt, J = 2.7 Hz, H-14), 4.76 (1H, brs, H-20b), 4.51 (1H, d, J = 2.5 Hz, H-7), 4.48 (1H, d, J = 11.0 Hz, H-4), 3.11 (1H, brs, H-10), 2.77 (1H, q, J = 6.7 Hz, H-17), 2.32 (3H, s, Ac at C-9), 2.31 (1H, m, H-12*ax*), 2.25 (1H, m, H-12*eq*), 2.07 (3H, s, Ac at C-14), 2.05 (3H, s, Ac at C-2), 2.00 (3H, s, Ac at C-3), 1.82 (1H, m, H-13*eq*), 1.70 (1H, m, H-13*ax*), 1.29 (3H, d, J = 6.7 Hz, H-18), and 1.12 (3H, s, H-15). The ^{13}C NMR data also coincided with those of natural junceellin.

Preparation of 4: 2,4,6-Trichlorobenzoyl chloride (6.5 μL) was added to a mixture of **3a** (10 mg) and triethylamine (5.8 μL) in THF (2 mL), and the reaction mixture was stirred at room temperature for 45 min. After the removal of triethylamine hydrochloride by filtration with a short plug of Celite, the filtrate was evaporated in vacuo and the residue was dissolved in toluene (0.5 mL). To this solution was added a mixture of **2** (5 mg) and DMAP (5 mg) in toluene (1 mL), and the resulting mixture was stirred at room temperature overnight, while monitoring by TLC. The reaction mixture was then applied to a SiO_2 short column and eluted with hexane/ether (3:7). The solvent was removed in vacuo to obtain **4** (5.9 mg). ^1H NMR ($CDCl_3$) δ 8.13 (1H, d, J = 7.9 Hz, 9′), 7.84 (1H, d, J = 7.9 Hz, 6′), 7.81 (1H, d, J = 8.6 Hz, 5′), 7.48 (1H, t, J = 8.6 Hz, 8′), 7.45 (1H, t, J = 7.95 Hz, 7′), 7.35 (1H, d, J = 7.95 Hz, 4′), 3.18 (3H, s, 1′ OCH_3), 3.12 (2H, m, 3′), 2.52 (2H, m, 2′), 6.22 (1H, dd, J = 11.0, 6.7 Hz, H-3), 5.87 (1H, s, H-9), 5.37 (1H, d, J = 6.7 Hz, H-2), 5.07 (1H, brs, H-20a), 5.00 (1H, brt, J = 2.7 Hz, H-14), 4.73 (1H, brs, H-20b), 4.72 (1H, brq, J = 2.5 Hz, H-6), 4.34 (1H, d, J = 2.5 Hz, H-7), 4.11 (1H, d, J = 11.0 Hz, H-4), 3.86 (1H, d, J = 2.4 Hz, H-16b), 3.81 (1H, d, J = 2.5 Hz, H-16a), 3.04 (1H, brs, H-10), 2.67 (1H, q, J = 6.7 Hz, H-17), 2.34 (3H, s, Ac at C-14), 2.28 (1H, m, H-12*ax*), 2.25 (1H, m, H-12*eq*), 2.18 (3H, s, Ac at C-2), 2.03 (3H, s, Ac at C-9), 1.82 (1H, m, H-13*eq*), 1.71 (1H, m, H-13*ax*), 1.25 (3H, d, J = 6.7 Hz, H-18), and 1.18 (3H, s, H-15).

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