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9-*O*-DEACETYLUMBRACULOLIDE A, A NEW DITERPENOID FROM THE GORGONIAN *JUNCEELLA FRAGILIS*

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Abstract – A new briarane-type diterpenoid, 9-*O*-deacetylumbraculolide A (**1**), has been isolated from a Formosan gorgonian *Junceella fragilis*. The structure of this metabolite was established by spectroscopic and chemical methods.

In the previous studies, the gorgonian coral *Junceella fragilis* (Ridley) (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Gorgonacea, family Ellisellidae)[1] are known to produce several diterpenoids with briarane carbon skeleton,[2–4] and the compounds of this type were found to possess extensive biological activities.[5] In this paper, we report the isolation and structure determination of a new briarane, 9-*O*-deacetylumbraculolide A (**1**), from *J. fragilis*. The structure, including the relative configuration of metabolite (**1**), was elucidated by the combination of spectral data analyses and chemical evidences.

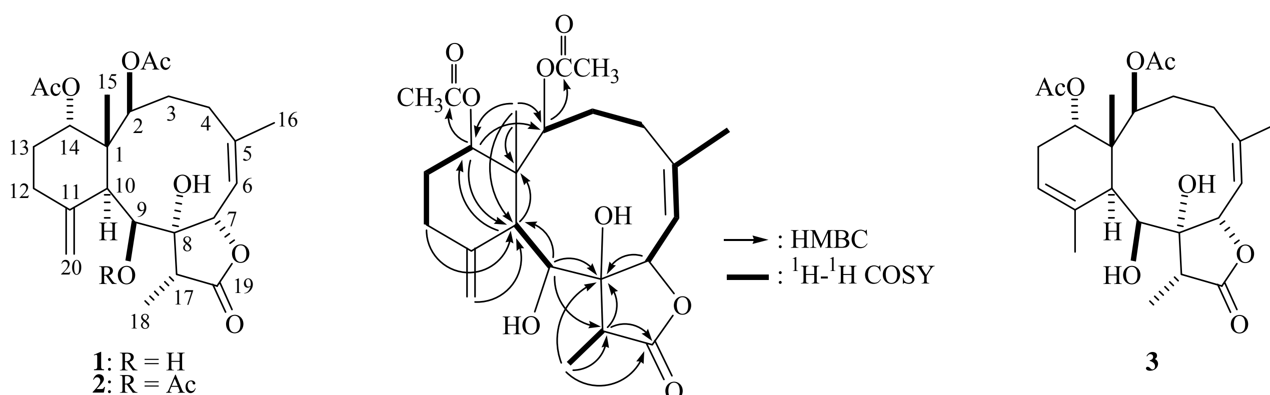

Figure 1. Selective ^{1}H–^{1}H COSY and HMBC Correlations of **1**.

9-*O*-deacetylumbraculolide A (**1**) was obtained as a white powder. The HRFABMS of **1** established a molecular formula of $C_{24}H_{34}O_8$ (M^+ *m/z* 450.2250), implying eight degrees of unsaturation. The IR absorptions of **1** showed the presence of hydroxyl (ν_{max} 3457 cm^{-1}), γ-lactone (ν_{max} 1788 cm^{-1}), and ester carbonyl (ν_{max} 1736 cm^{-1}) groups. The FABMS spectrum of **1** exhibited peaks at *m/z* 450 [M^+], 391 [M – HOAc + H]$^+$, 373 [M – HOAc – H_2O + H]$^+$, 331 [M – 2HOAc + H]$^+$, 313 [M – 2HOAc – H_2O + H]$^+$, and 295 [M – 2HOAc – 2H_2O + H]$^+$, suggesting the presence of two acetoxyl and two hydroxyl groups in **1**. The 1D (^{1}H and ^{13}C) and 2D (^{1}H–^{1}H COSY and HETCOR) NMR spectra showed that **1** possesses a

lactone carbonyl (δ_C 177.3); two acetate groups (δ_C 170.5, s; 170.4, s; 21.4, q; 21.0, q; δ_H 2.07, 3H, s; 2.04, 3H, s); an exocyclic carbon-carbon double bond (δ_C 151.2, s; 111.1, t; δ_H 5.10, 1H, s; 4.90, 1H, s); a methyl substituted (*Z*)-trisubstituted olefin (δ_C 145.9, s; 120.4, d; 25.3, q; δ_H 5.47, 1H, d, J = 9.0 Hz; 1.92, 3H, s); a tertiary hydroxyl group (δ_C 83.5, s); four oxymethine carbons (δ_C 78.2, d; 75.8, d; 75.2, d; 71.6, d; δ_H 5.56, 1H, d, J = 9.0 Hz; 5.22, 1H, br s; 4.69 br s; 4.31, br s); an aliphatic quaternary carbon (δ_C 47.3, s); two aliphatic methine carbons (δ_C 43.6, d; 42.7, d; δ_H 3.35, 1H, br s; 3.09, 1H, q, J = 7.0 Hz); a secondary methyl group (δ_C 6.6, q; δ_H 1.17, 3H, d, J = 7.0 Hz); and tertiary methyl group (δ_C 14.1, q; δ_H 1.12, 3H, s). By careful analyses, these data indicated that **1** is a briarane-type metabolite.

The trisubstituted double bond in **1** could be present at Δ^5 or Δ^{11}. In the former case, the olefinic proton (H-6) would be coupled to an oxymethine proton (H-7), but in the latter case the olefin (H-12) would be coupled to an aliphatic proton (H-13). In the ^{1}H–^{1}H COSY spectrum of **1** (Figure 1), a correlation between the olefinic proton (δ_H 5.47, d, J = 9.0 Hz) and an oxymethine proton (δ_H 5.56, d, J = 9.0 Hz) was observed. Therefore, the trisubstituted double bond in **1** was located at Δ^5; consequently, the exocyclic double bond was at $\Delta^{11(20)}$. The ^{1}H–^{1}H COSY spectrum of **1** also showed connectivity between H-10 (δ_H 3.35, br s) and an oxymethine proton (δ_H 4.31, br s). Because C-1 and C-11 carbons do not have hydrogens, the oxymethine proton signal of δ 4.31 must be due to H-9 proton. Thus, one of the two hydroxyl groups could be assigned to C-9 in **1**. As mentioned above, the H-7 and H-9 protons were only found to show correlations with H-6 and H-10, respectively. If there were any hydrogen at C-8, the above signals would have been further split. Thus, the C-8 carbon does not have any hydrogen but possesses a substituent. Therefore, the tertiary hydroxyl group could be assigned to the C-8 position. Moreover, from the ^{1}H–^{1}H COSY experiment of **1**, it was possible to establish the proton sequences from H-2 to H_2-3; H_2-3 to H_2-4; H_3-16 to H-6; H-6 to H-7; H-9 to H-10; H_2-12 to H_2-13; H_2-13 to H-14; and H-17 to H_3-18. On the basis of these data and the key ^{1}H–^{13}C long-range correlations observed in the HMBC experiment of **1** (Figure 1), the carbon skeleton of **1** could be further established. In addition, the acetoxyl groups positioned at C-2 and C-14 were confirmed by the correlations between H-2 (δ 5.22) and the acetate carbonyl (δ 170.4); and H-14 (δ 4.69) and the acetate carbonyl (δ 170.5) of the acetoxyl groups. Based on above observations, the molecular framework of **1** was elucidated.

The relative stereochemistry of **1** was determined by the NOE correlations observed in the NOESY spectrum of **1**. The NOE correlations of H-10 with H-2 and H_3-18 indicated that these protons are situated on the same face and were assigned as α protons because the C-15 methyl is β-oriented and H_3-15 did not show correlation with H-10. H-14 was found to exhibit NOE responses with H_3-15, but not with H-10, revealing the β-orientation of this proton. It was found that H-17 showed NOE correlations with H-7 and H-9. Consideration of molecular models revealed that H-17 is reasonably close to H-7 and H-9 when H-7

and H-17 are β-oriented, and H-9 is placed on the α face. However, the stereochemistry of C-8 hydroxyl group cannot be determined by this way. Based on above observation, the structure of **1** could be very similar to those of two known metabolites, umbraculolide A (**2**)[6,7] and 9-deacetylstylatulide lactone (**3**).[8] By comparison of the ^{13}C NMR spectral signal of C-8 of **1** (δ_C 83.5, s) with those of **3** (δ_C 83.00, s), it was revealed that the C-8 hydroxyl group in **1** should be α-oriented. Furthermore, acetylation of the new metabolite (**1**) gave a less polar product, which was identical with umbraculolide A (**2**), by comparison of the related physical and spectral data. Thus, the C-8 hydroxyl group in **1** was positioned at α phase and the structure of **1** was established unambiguously.

EXPERIMENTAL

General Experimental Procedures. Melting point was determined using a Fargo apparatus and was uncorrected. Optical rotation was measured on a JASCO D-370 digital polarimeter. IR spectrum was recorded on a JASCO 5300 FT-IR. FABMS was obtained with a VG QUATTRO GC/MS spectrometer. HRFABMS was recorded on a JEOL JMS SX/SX 102A mass 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. Precoated silica gel plates (Merck, Kiselgel 60 F_{254}, 0.2 mm) were used for analytical TLC.

Animal Material. Specimen of *Junceella fragilis* was collected in June 2002, at Southern Taiwan coast, at a depth of 15 m. A voucher specimen is deposited in the National Museum of Marine Biology and Aquarium (specimen no. TWGC–001).

Extraction and Isolation. The gorgonian (1.0 kg) was collected and freeze-dried. The freeze-dried material (0.7 kg) was minced and extracted with EtOAc (5 × 500 mL) for 96 h at 25 °C. The organic extract (15.5 g) was separated by silica gel column chromatography using *n*-hexane and *n*-hexane–EtOAc mixtures of increasing polarity. Compound (**1**) was eluted with *n*-hexane–EtOAc (3:1).

9-*O*-Deacetylumbraculolide A (1): white powder (9.8 mg); mp 99–101 °C (EtOAc); $[\alpha]_D^{25}$ + 28° (*c* 0.8, $CHCl_3$); IR (net, $CHCl_3$) ν_{max} 3457, 1788, and 1736 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.56 (1H, d, *J* = 9.0 Hz, H-7), 5.47 (1H, d, *J* = 9.0 Hz, H-6), 5.22 (1H, br s, H-2), 5.10 (1H, s, H_a-20), 4.90 (1H, s, H_b-20), 4.69 (1H, br s, H-14), 4.31 (1H, br s, H-9), 3.35 (1H, br s, H-10), 3.09 (1H, q, *J* = 7.0 Hz, H-17), 2.67 (1H, m, H-4), 2.56 (1H, m, H-3), 2.28 (2H, m, H_2-12), 2.07 (3H, s, acetate methyl), 2.04 (3H, s, acetate methyl), 1.94 (1H, m, H-3'), 1.92 (3H, s, H_3-16), 1.86 (1H, m, H-4'), 1.85 (2H, m, H_2-13), 1.17 (3H, d, *J* = 7.0 Hz, H_3-18), and 1.12 (3H, s, H_3-15); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.3 (s, C-19), 170.5 (s, acetate carbonyl), 170.4 (s, acetate carbonyl), 151.2 (s, C-11), 145.9 (s, C-5), 120.4 (d, CH-6), 111.1 (t, CH_2-20), 83.5 (s, C-8), 78.2 (d, CH-7), 75.8 (d, CH-14), 75.2 (d, CH-2), 71.6 (d, CH-9), 47.3 (s, C-1), 43.6 (d, CH-17), 42.7 (d, CH-10), 31.6 (t, CH_2-4), 30.9 (t, CH_2-12), 29.7 (t, CH_2-3), 27.2 (t, CH_2-13), 25.3 (q,

CH_3-16), 21.4 (q, acetate methyl), 21.0 (q, acetate methyl), 14.1 (q, CH_3-15), and 6.6 (q, CH_3-18); FABMS *m/z* 450 (0.2), 391 (7.3), 373 (0.1), 331 (0.2), 313 (0.1), and 295 (0.1); HRFABMS *m/z* 450.2250 (calcd for $C_{24}H_{34}O_8$, M^+, 450.2254).

Acetylation of 9-*O*-deacetylumbraculolide A (1): 9-*O*-Deacetylumbraculolide A (**1**) (5.0 mg) was stirred with 1 mL of acetic anhydride in 1 mL of pyridine for 96 h at rt. After evaporation of excess reagent, the residue was separated by column chromatography on silica gel to give pure umbraculolide A (**2**) (*n*-hexane–EtOAc 4:1, 3.1 mg, 57%). The physical (mp and rotation value) and spectral (^{1}H and ^{13}C NMR) data of **2** were in full agreement with those of reported previously.[6]

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REFERENCES

1. F. M. Bayer and M. Grasshoff, *Senckenbergiana Biol.*, 1994, **74**, 21.
2. J. Shin, M. Park, and W. Fenical, *Tetrahedron*, 1989, **45**, 1633.
3. M. García, J. Rodríguez, and C. Jiménez, *J. Nat. Prod.*, 1999, **62**, 257.
4. P.-J. Sung, S.-L. Wu, H.-J. Fang, M. Y. Chiang, J.-Y. Wu, L.-S. Fang, and J.-H. Sheu, *J. Nat. Prod.*, 2000, **63**, 1483.
5. P.-J. Sung, J.-H. Sheu, and J.-P. Xu, *Heterocycles*, 2002, **57**, 535, and related references cited therein.
6. C. Subrahmanyam, R. Kulatheeswaran, and R. S. Ward, *J. Nat. Prod.*, 1998, **61**, 1120.
7. C. Subrahmanyam, S. Ratnakumar, and R. S. Ward, *Tetrahedron*, 2000, **56**, 4585.
8. J.-H. Sheu, P.-J. Sung, L.-H. Huang, S.-F. Lee, T. Wu, B.-Y. Chang, C.-Y. Duh, L.-S. Fang, K. Soong, and T.-J. Lee, *J. Nat. Prod.*, 1996, **59**, 935.