HETEROCYCLES, Vol. 60, No. 5, 2003, pp. 1199 - 1202
Received, 14th January, 2003, Accepted, 5th March, Published online, 10th March, 2003

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.

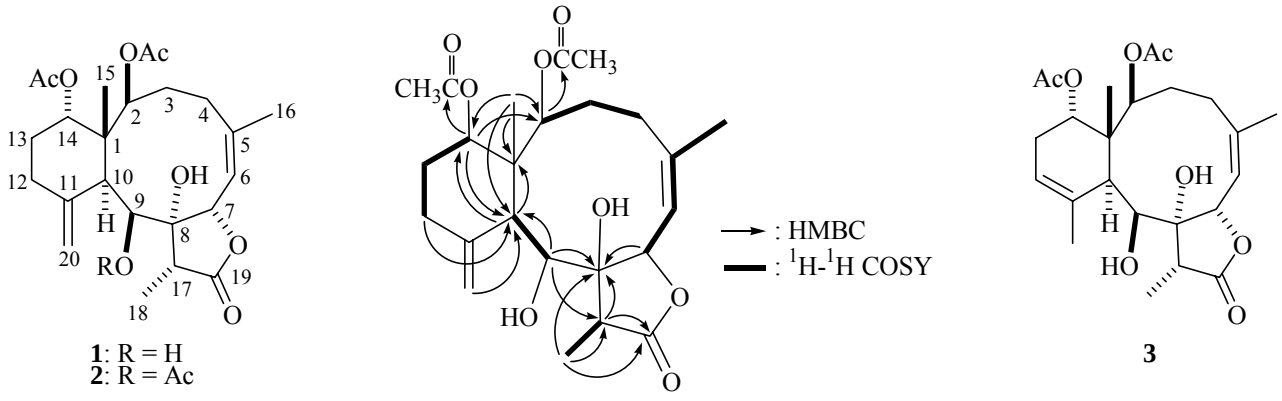

Figure 1. Selective ^{1}H–^{1}H COSY and HMBC Correlations of **1**.

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 ^1H 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}; ^1H 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]

ACKNOWLEDGMENT

The authors would like to thank Dr. Lee-Shing Fang for his contribution to establish the marine natural products chemistry research programs in the National Museum of Marine Biology and Aquarium of the Republic of China. We appreciate Ms. Chao-Lein Ho and Shiu-Ching Yu of the NSC Southern Instrument Center in National Sun Yat-Sen University for measurements of NMR and MS spectral data. This research work was supported by a grant from the National Science Council of the Republic of China (Contract no. NSC 91-2320-B-291-001) awarded to P.-J. Sung.

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.