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JUNCIN N, A NEW BRIARANE-TYPE DITERPENOID FROM THE GORGONIAN CORAL *JUNCEELLA JUNCEA*

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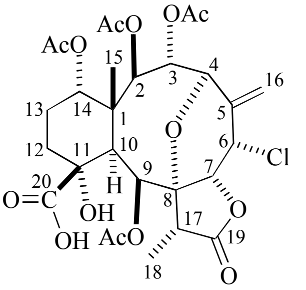
Abstract – A new chlorinated briarane-type diterpenoid, juncin N (**1**), has been isolated from the gorgonian coral *Junceella juncea*. The structure, including the relative configuration of the new compound (**1**), was elucidated by the combination of extensive spectral data analysis, especially in 1D and 2D NMR.

In the previous studies by others, a series of briaranes, including juncins A–M, have been isolated from the gorgonian coral *Junceella juncea* (Pallas) (phylum Coelenterta, class Anthozoa, subclass Octocorallia, order Gorgonacea, family Ellisellidae) within the past 14 years.[1–8] Compounds of this type continue to intrigue investigators because of the structural novelty and various biological activities.[9] In this paper, we report here a new chlorine-containing polyoxygenated briarane, juncin N (**1**), from the gorgonian *J. juncea*, collected off Southern Taiwan coast.

The molecular formula of juncin N (**1**) was determined as $C_{28}H_{35}O_{14}Cl$ by its HRFABMS. Thus, 11 degrees of unsaturation were determined for **1**. The IR spectrum showed bands at 3520, 3418, 1792, and 1744 cm^{-1}, consistent with the presence of carboxyl, hydroxy, γ-lactone, and ester carbonyl groups in **1**. The FABMS of **1** exhibited peaks at *m/z* 633 (0.3, M + H)$^+$, 631 (1, M + H)$^+$, 615, 613 (M + H – H_2O)$^+$, 573, 571 (M + H – HOAc)$^+$, 513, 511 (M + H – 2HOAc)$^+$, 453, 451 (M + H – 3HOAc)$^+$, 393, 391 (M + H – 4HOAc)$^+$, 375, and 373 (M + H – 4HOAc – H_2O)$^+$, which suggested the presence of a chlorine atom, a hydroxy, and four acetoxy groups in **1**. From the ^{13}C NMR spectral data of **1** (Table 1), the presence of an exocyclic carbon-carbon double bond was deduced from the signals of two carbons resonating at δ 133.8 (s) and 119.9 (t). Furthermore, in the ^{13}C NMR spectrum, six carbonyl resonances appeared at δ 191.2 (s), 174.5 (s), 170.2 (s), 169.7 (s), 169.7 (s), and 169.6 (s), confirming the presence of a carboxyl, a γ-lactone, and four esters in **1**. In the ^{1}H NMR spectrum (Table 1), a carboxylic acid proton (δ 9.25, s) and four acetate methyls (δ 2.38, 3H, s; 2.08, 3H, s; 2.05, 3H, s; 2.00, 3H, s) were observed. Thus, the ^{13}C NMR spectral data accounted for seven degrees of unsaturation and required **1** to be tetracyclic. The ^{1}H NMR spectrum also showed the presence of two methyl groups, including a methyl attached to a methine carbon (δ 1.46, d, *J* = 7.0 Hz, H_3-18) and a methyl attached to a tertiary carbon (δ 1.15, s, H_3-15).



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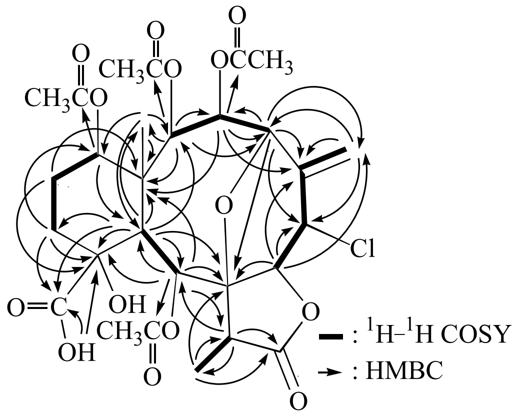

Figure 1. ^{1}H–^{1}H COSY and HMBC Correlations for **1**.

The gross structure of **1** and all of the ^{1}H and ^{13}C NMR spectral data associated with the molecule were determined by the assistance of 2D NMR experiments. From the ^{1}H–^{1}H COSY spectra of **1** (Figure 1), it was possible to establish the proton sequences form H-2/H-3; H-3/H-4; H-6/H-7; and H-9/H-10. These data, together with the HMBC correlations between H-2/C-1, C-3, C-4; H-3/C-1, C-4, C-5; H-4/C-3, C-5, C-6; H-7/C-5, C-6, C-8; H-9/C-1, C-8, C-10; and H-10/C-1, C-2, C-8, C-9 (Figure 1), established the connectivity from C-1 to C-10 within the ten-membered ring. An exocyclic carbon-carbon double bond attached at C-5 was confirmed by the HMBC correlations between H-4/C-16; H-7/C-16; and H_2-16/C-4, C-5, C-6. The cyclohexane ring, which is fused to the ten-membered ring at C-1 and C-10, was elucidated by the key HMBC correlations between H-9/C-11; H-10/C-11, C-12; H_2-13/C-1; and H-14/ C-10. The ring juncture C-15 methyl group was positioned at C-1 from the HMBC correlations between H-2/C-15; H-10/C-15; and H_3-15/C-1, C-2, C-10, C-14. The carboxyl and hydroxy groups positioned at C-11 were determined by the connectivity between the carboxylic acid proton (δ_H 9.25, 1H, s) and a tertiary oxygen-bearing carbon (δ_C 77.7, s, C-11) and further confirmed by the HMBC correlations between H-9/C-11; H-10/C-11, C-20; H_2-12/C-11, C-20; and H_2-13/C-11. The ether bridge between C-4 and C-8 was identified by an HMBC correlation between H-4 (δ_H 4.47, d, *J* = 11.0 Hz) and C-8 (δ_C 82.9, s). Furthermore, the HMBC correlations also revealed the positions of four acetoxy groups attached at

C-2, C-3, C-9, and C-14. These data, together with the HMBC correlations between H-9/C-17; H-17/C-9, C-18, C-19; and H_3-18/C-8, C-17, C-19, unambiguously established the molecular framework of **1**.

Table 1. ^{1}H and ^{13}C NMR Spectral Data for Juncin N (**1**).

Position	$^1H^a$	$^{13}C^b$
1		47.5 (s)[d]
2	5.35 (1H, d, J = 6.0 Hz)[c]	72.6 (d)
3	6.21 (1H, dd, J = 11.0, 6.0 Hz)	63.7 (d)
4	4.47 (1H, d, J = 11.0 Hz)	78.4 (d)
5		133.8 (s)
6	5.06 (1H, d, J = 2.5 Hz)	53.7 (d)
7	4.45 (1H, d, J = 2.5 Hz)	78.9 (d)
8		82.9 (s)
9	6.61 (1H, s)	74.3 (d)
10	3.01 (1H, s)	46.4 (d)
11		77.7 (s)
12α/β	2.28 (1H, m); 2.52 (1H, dt, J = 13.5, 3.0 Hz)	31.0 (t)
13	1.85 (2H, m)	24.0 (t)
14	4.99 (1H, br s)	73.1 (d)
15	1.15 (3H, s)	15.5 (q)
16a/b	5.59 (1H, d, J = 2.0 Hz); 5.37 (1H, d, J = 2.0 Hz)	119.9 (t)
17	2.94 (1H, q, J = 7.0 Hz)	49.2 (d)
18	1.46 (3H, d, J = 7.0 Hz)	8.5 (q)
19		174.5 (s)
20		191.2 (s)
acid proton	9.25 (1H, s)	
acetate methyls		
2-OCOCH_3	2.38 (3H, s)	21.1 (q)
3-OCOCH_3	2.08 (3H, s)	20.9 (q)
9-OCOCH_3	2.05 (3H, s)	20.4 (q)
14-OCOCH_3	2.00 (3H, s)	20.4 (q)
acetate carbonyls		
2-OCOCH$_3$		169.7 (s)
3-OCOCH$_3$		169.7 (s)
9-OCOCH$_3$		170.2 (s)
14-OCOCH$_3$		169.6 (s)

[a]Spectra recorded at 500 MHz in $CDCl_3$ at 25 °C. [b]125 MHz in $CDCl_3$ at 25 °C. [c]J values (in Hz) in parentheses. [d]Multiplicity deduced by DEPT and indicated by usual symbols. The values are in ppm downfield from TMS.

The relative stereochemistry of **1** was elucidated from a NOESY experiment (Figure 2). Strong NOE correlations were observed between H-10 and H-2, H-9, and H_3-18; while H-2 showed a NOE correlation to H-4 suggesting that all these protons were on the same side of the molecule. This face was assigned as the α face since the C-15 methyl is normally assigned as a β-substituent at C-1. The H-3 and H-14 protons showed NOE correlations with H_3-15, but not with H-10, revealing the β-orientation of these protons. The H-7 signal showed NOE correlations with H-6, H-9, and H-17, and H-9 showed a NOE response with H-17. Consideration of molecular models revealed that H-7 is reasonably close to H-6, H-9, and H-17 when H-6, H-7, and H-17 are β-oriented and H-9 is placed on the α face. The acid group at C-11 was placed in the β face by the NOE correlations between the acid proton (δ_H 9.25) with H_3-15 and H-12β. Thus, the hydroxy group at C-11 must be α-oriented. Based on above results, the structure of **1**, including the relative configuration, was elucidated unambiguously. To the best of our knowledge, juncin N (**1**) is the first briarane derivative containing a carboxylic acid group.

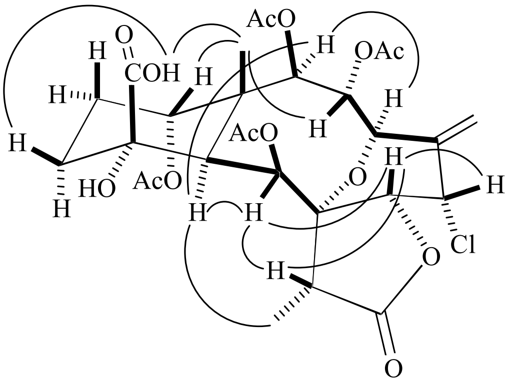

Figure 2. Key NOESY Correlations for **1**.

Although many briarane-based diterpenoids have been shown to exhibit various biological activities,[9] juncin N (**1**) was found not to be cytotoxic toward a limited panel of tumor cell lines. These cells are P-388D1 (mouse lymphoid neoplasm), DLD-1 (human colon adenocarcinoma), IMR-32 (human neuroblastoma), RPMI 7951 (human malignant melanoma), and CCRF-CEM (human T-cell acute lymphoblastic leukemia) cells.

EXPERIMENTAL

General Experimental Procedures. Melting points were determined using a Fargo melting point apparatus and was uncorrected. Optical rotation values were measured on a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a JASCO FT/IR-5300 infrared spectrophotometer. FABMS were obtained on a VG QUATTRO GC/MS spectrometer. HRFABMS were recorded on a JEOL JMS SX/SX 102A mass spectrometer. The NMR spectra were recorded with a VARIAN UNITY INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C, in $CDCl_3$, using TMS as an internal standard. Silica gel (Merck, 230–400 mesh) was used during column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F_{254}) were used for analytical TLC. All solvents used were either freshly distilled or of analytical grade.

Animal Material. The gorgonian *J. juncea* were collected by hand using scuba in Dec. 2002, off the Southern Taiwan coast at depths of 16 m. This organism was identified by comparison with descriptions.[10–12] Living reference specimens are being maintained in the authors' tanks and a voucher specimen was deposited at the National Museum of Marine Biology and Aquarium (specimen no. TWGC–003).

Extraction and Isolation. The gorgonian *J. juncea* (wet weight 0.70 kg) was freeze-dried soon after collection. The freeze-dried material (0.45 kg) was minced and extracted with EtOAc (6 × 500 mL) for 120 h at 25 °C. The EtOAc extract (7.2 g) was separated by silica gel column chromatography using hexanes and hexanes–EtOAc mixtures of increasing polarity. Juncin N (**1**) was eluted with hexanes–EtOAc (2:1).

Juncin N (1): white powder (0.7 mg); mp 214–216 °C (EtOAc); $[\alpha]_D^{25}$ +18° (*c* 0.3, $CHCl_3$); IR (neat, $CHCl_3$) ν_{max} 3520, 3418, 1792, and 1744 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; FABMS *m/z* 633 (0.3, M^+ + H), 631 (1, M^+ + H), 615, 613, 573, 571, 513, 511, 453, 451, 393, 391, 375, and 373; HRFABMS *m/z* 631.1799 (calcd for $C_{28}H_{35}O_{14}Cl$ + H, 631.1794).

Cytotoxicity Testing. The cytotoxicity of tested compound (**1**) was assayed with a modification of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. Cytotoxicity assays were carried out according to the procedures described previously.[13]

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