Juncenolide A, a New Briarane from the Taiwanese Gorgonian *Junceella juncea*

Ya-Ching Shen,*,† Yu-Chi Lin,† and Michael Y. Chiang‡

Institute of Marine Resources, National Sun Yat-sen University, 70 Lien-Hai Road, Kaohsiung, Taiwan, Republic of China, and Department of Chemistry, National Sun Yat-sen University, Taiwan, Republic of China

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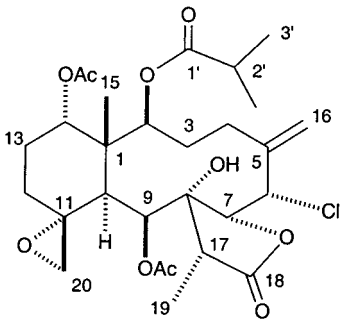
Fractionation of the acetone extract of *Junceella juncea* collected in Taiwan resulted in the isolation of a new briarane, designated juncenolide A (**1**). The structure of **1** was determined on the basis of 2D NMR techniques, including COSY, HSQC, HMBC, and NOESY experiments, and was also confirmed by X-ray crystallographic analysis.

The chemistry of Gorgonians has been extensively investigated worldwide in the past decades.[1−4] Studies of marine gorgonians as sources of new natural products with potential antitumor, antiinflammatory, and antibacterial activities have afforded a variety of novel compounds, such as polyhydroxysteroids and diterpenoids.[5−8] Although many new briareins and juncins were recently isolated and their structures charaterized, the stereochemistry of some juncins was not fully determined.[9−11] Also, the taxonomy of gorgonians is sometimes ambiguous, and it is of significance to identify them from the viewpoint of chemotaxonomy. During the course of searching for bioactive natural products from marine resources, we have surveyed a series of crude extracts of marine invertebrates by applying cytotoxicity tests. Among them, a lipophilic extract of *Junceella juncea* (Pallas) (Ellisellidae) was found to possess significant cytotoxicity against human NUGC and HONE-1 tumor cells (12% and 3%, respectively). To explore the origin of bioactivity, chemical investigation to obtain the active component from this species was initiated. Extensive fractionation by solvent partition and chromatography resulted in the isolation of a new compound, juncenolide A (**1**), from *Junceella juncea*. Herein we wish to report the isolation and structural elucidation of compound **1**.



Juncenolide A (**1**), [α] −25.5° (*c* 0.05, CH_2Cl_2), had a molecular formula of $C_{28}H_{39}ClO_{10}$, as derived from a molecular ion at *m*/*z* 571 $[M]^+$ in the EIMS and DEPT spectra. Its IR bands indicated the presence of a tertiary hydroxyl (3400 cm^{-1}, sharp), a five-membered-ring lactone (1778 cm^{-1}), and ester (1738, 1732, and 1720 cm^{-1}) groups.

Table 1. ^1H and ^{13}C NMR ($CDCl_3$) Spectral Data of Juncenolide A (**1**)

position	$^{13}C^a$	$^1H^b$	COSY	HMBC
1	47.9 s			H-9, H-10, H-14, Me-15
2	72.9 d	5.83 (d, 8.4)	H-3	H-14
3	28.6 t	1.60 m, 2.63 m	H-2, H-4,	
4	33.1 t	2.35 m	H-3	H-6
5	146.0 s			
6	51.2 d	4.65 (d, 3.2)	H-7	
7	81.1 d	4.48 (brs)	H-6	H-6
8	81.2 s			H-9, H-10, H-17, Me19
9	72.5 d	5.68 s	H-10	H-10
10	51.4 d	3.18 s	H-9	H-14
11	57.1 s			H-9, H-10, H-20
12	24.3 t	1.90 m		H-14
13	30.3 t	1.85 m	H-14	H-14
		1.98 m		
14	74.1 t	4.84 (brs)	H-13	
15	14.5 q	1.13 s		H-10, H-14
16	120.7 t	5.71 s, 5.43 s		H-6
17	34.0 d	2.97 (q, 7.1)	Me19	Me19, H-9
18	174.9 s			Me19, H-17
19	6.6 q	1.23 (d, 7.1)	H-17	H-17
20 A	51.4 t	2.64 (d, 3.4)	H-20 B	H-10
20 B		2.22 (d, 3.4)	H-20 A	
9-OAc	169.5 s	2.18 s[c]		H-9
	21.1 q			
14-OAc	170.1 s	1.97 s[c]		H-14
	21.1 q			
1′	176.5 s			H-2, H-2′, H-3′, H-4′
2′	40.5 d	2.48 (hep, 7.0)	H-3′, H-4′	
3′	19.2 q	1.14 (d, 7.0)	H-2′	H-4′
4′	18.1 q	1.10 (d, 6.9)	H-2′	H-3′
OH		3.44 s		

[a] Assignments made using the HMQC and HMBC techniques. [b] Multiplicities and coupling constants in Hz in parentheses. [c] Data interchangeable.

The ^1H NMR data of **1** (Table 1) showed two acetyl singlets (δ 2.18, 1.97), a typical methyl doublet (δ 1.23, J = 7.1 Hz), and a methyl singlet (δ 1.13), a pair of doublets with small coupling constant (J = 3.4 Hz) at δ 2.64 and 2.22 (H-20), a methine singlet at δ 3.18 (H-10), two olefinic methylene singlets (δ 5.71 and 5.43), and four oxygenated methine protons (δ 5.83, d, J = 8.4 Hz, H-2; 5.68, s, H-9; 4.48, brs, H-7; 4.84, brs, H-14) in addition to a chlorinated methine doublet at δ 4.65 (J = 3.2 Hz). To assign the methine protons and overlapping signals between 1 and 3 ppm of **1**, a COSY spectrum was obtained to reveal the connectivities of H-2/H-3/H-4, H-6/H-7, H-9/H-10, H-13/H-14, and

* To whom correspondence should be addressed. Tel: (886) 7-525-2000, ext. 5058. Fax: (886) 7-525-5020. E-mail: ycshen@mail.nsysu.edu.tw.
† Institute of Marine Resources.
‡ Department of Chemistry.

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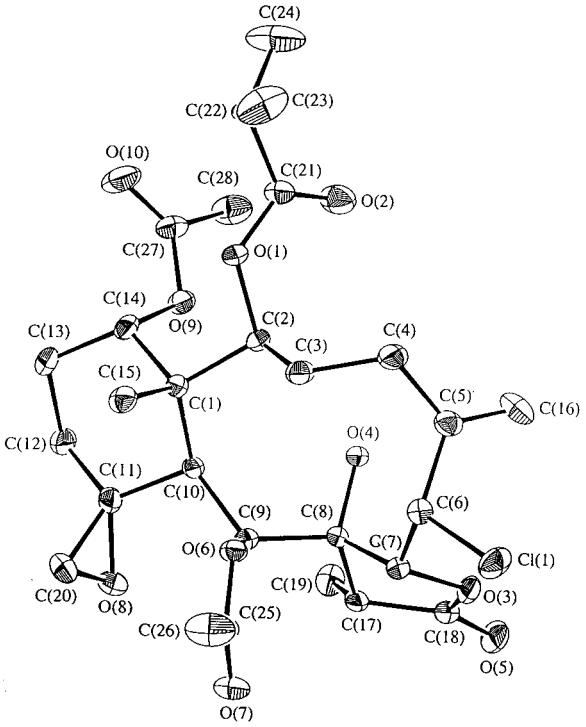

Figure 1. ORTEP diagram showing the crystallographic atom-numbering scheme and solid state confirmation of juncenolide A (**1**). The hydrogen atoms have been omitted for clarity.

H-17/H-19. Detailed analysis of the ^{1}H and ^{13}C NMR and HSQC spectra (Table 1) revealed that **1** is a 6/10-membered ring with a δ-lactone at the C-7 and C-8 positions.[10] The ^{13}C NMR and DEPT spectra of **1** showed signals for six methyl carbons, six methylene carbons, eight methine carbons, and eight quarternary carbons including four ester carbons (δ 169.5, 170.1, 174.9, and 176.5). Considering the molecular formula of **1** and the aformentioned partial structures, there remained an isobutyl group, and this was also observed in the NMR spectra (δ_H 1.10, 1.14, 2.48; δ_C 18.1, 19.2, and 40.5). This type of skeleton was confirmed from the observation of long-range correlations of C-1/H-9, H-10, H-14, Me-15; C-8/H-9, H-10, H-17, Me-19; and C-11/H-9, H-10, H-20, and cross-peaks from H-9 and Me-19 to C-17 and H-17 and Me-19 to C-18 in the HMBC data (Table 1). The two acetoxyl groups were assigned at C-9 and C-14 because their carbonyl carbons were correlated with corresponding methine protons and the observation of H-14/C-2, C-15 and H-10/C-9 in the HMBC of **1**. The correlations of the carbonyl carbon of the isobutanoyl group with H-2, H-2′, H-3′, and H-4′ confirmed that an isobutanoyl group was present and was attached at C-2. The relative stereochemistry of **1** was determined from NOESY experiments and X-ray diffraction analysis of **1**. The NOESY correlations of H-6/H-7, H-17 and H-14/Me-15 in **1** suggested that H-6, H-7, H-14, H-17, and Me-15 were in β-orientation. Correlations of H-2, H-10 and Me-19 agreed with an α-configuration of H-2, H-10, and Me-19. An ORTEP stereodrawing of **1** (Figure 1) from X-ray crystallographic analysis established the complete structure and stereochemistry of **1**. Thus, the structure of juncenolide A (**1**) was determined as (1*R*,2*S*,6*S*,7*R*,8*R*,9*S*,10*S*,11*S*,14*S*,-17*R*)-6-chloro-11,20-epoxy-2-isobutyl-9,14-diacetoxy-8-hydroxybriaran-5(16)-en-18,7-olide. General biological study revealed that compound **1** exhibited moderate cytotoxicity against human colon adenocarcinoma (DLD) and oral epidermoid carcinoma (KB-16) cells at a concentration of 3.4 and 5.9 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Melting points were measured on a Buchi melting point B-540 apparatus and are uncorrected. Optical rotations were taken on a JASCO DIP-1000 polarimeter. IR spectra were recorded with a HORIBA FT-720 spectrophotometer. EIMS and HREIMS were measured with a VG Quettro 5022 and JEOL JMS-SX 102 mass spectrometers. ^{1}H, ^{13}C NMR, DEPT, COSY, HSQC, HMBC, and NOESY spectra were recorded using a Bruker FT-300 (AVANCE) or a Varian FT-500 (INOVA) NMR instrument.

Animal Material. *Junceella juncea* (Pallas) was collected in Nan-wan in February 2000. A voucher specimen (WSG-4) was deposited in the Institute of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan.

Extraction and Isolation. The outer red layer of *J. juncea* (wet, 0.8 kg) was ground and extracted with acetone (3 L) to afford a crude extract, which was partitioned between H_2O (0.3 L) and EtOAc (0.3 L) to yield an EtOAc-soluble fraction (3.24 g). This residue was chromatographed on a Si gel column (70 g) and eluted with *n*-hexane (1 L) and a solvent mixture of *n*-hexane/CH_2Cl_2/MeOH (250:20:1 to 30:20:1, each 1 L) to afford 5 fractions, A (570 mg), B (244 mg), C (201 mg), D (0.84 g), and E (0.2 g). Fraction B was crystallized to yield juncenolide A (**1**) (93 mg).

Juncenolide A (**1**): colorless prisms; mp 203−205° C; $[\alpha]^{25}_D$ −25.5° (*c* 0.05, CH_2Cl_2); IR (neat) ν_{max} 3400, 2987, 2933, 1778, 1738, 1732, 1720, 1383, 1252,1200, 1045, 1001 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$) in Table 1; EIMS *m*/*z* (rel int) 573 ([M + 2]$^+$, 0.5), 571 ([M]$^+$, 1.5), 513 ([M − AcOH + 2]$^+$, 0.5), 511 ([M − AcOH]$^+$, 1.5), 485 ([M − $C_4H_6O_2$ − H_2O + 2]$^+$, 3), 483 ([M − $C_4H_6O_2$ − H_2O]$^+$, 9), 447 ([M − $C_4H_6O_2$ − Cl]$^+$, 2), 424 (6), 422 (7), 407 (8), 387 (26), 327 (19), 136 (20), 135 (29), 107 (31), 105 (33), 91 (30), 81 (35), 71 (89), 57 (41), 55 (46).

Cytotoxicity Assay. A bioassay against KB (oral epidermoid carcinoma) and DLD (colon adenocarcinoma) tumor cells was based on reported procedures.[12,13] The cytotoxicity assay depends on the binding of methylene blue to fixed monolayers of cells at pH 8.5, washing the monolayer, and releasing the dye by lowering the pH value. The 96-well plate was dipped into a 0.01 M borate-buffer solution four times in order to remove the dye. Then, 100 μL/well ethanol/0.1 M HCl (1:1) was added as a dye-eluting solvent, and the absorbance was measured on a microtiter plate reader (Dynatech, MR 7000) at a wavelength of 650 nm. The IC_{50} value was defined by a comparison with the untreated cells as the concentration of test sample resulting in 50% reduction of absorbance. Doxorubicine was used as the standard compound, exhibiting an IC_{50} value of 0.4 μg/mL under the above conditions.

Single-Crystal X-ray Structure Determination of Juncenolide A (1).[14] A single crystal of juncenolide A (**1**) suitable for diffraction study was obtained by simple evaporation from methanol solution. The crystal (0.40 × 0.80 × 0.82 mm) belongs to the monoclinic space group $P2_1$ (No. 4) with the following crystal data: a = 9.176(3) Å, b = 19.350(5) Å, c = 9.930 Å, β = 117.76(2)°, V = 1560(1) Å^3, Z = 2, D_{calc} = 1.284 g/cm^3. Intensity data were measured on a Rigaku AFC7S diffractometer up to 2θ of 52° using Mo radiation (Mo Kα λ = 0.71069 Å). Of the 3370 reflections collected, 3174 were unique (R_{int} = 0.051). The structure was solved by direct methods and refined by a full-matrix least-squares procedure using the *teXsan* package.[15] The non-hydrogen atoms were given anisotropic thermal parameters. The hydroxyl hydrogen atoms were located from a difference Fourier map. These hydroxyl hydrogen atoms and other hydrogen atoms with their calculated positions (d_{C-H} = 0.95 Å) were put in the final cycle of refinement but not refined. The refinement converged to a final R = 0.046, R_w = 0.066 for 2417 observed reflections [I > 3.00σ(I)] and 369 variables. The final X-ray model is shown in Figure 1.

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References and Notes

(1) Tanaka, J.; Higa, T.; Tachibana, K.; Iwashita, T. *Chem Lett.* **1982**, 1295−1296.
(2) Sheu, J. H.; Sung, P. J.; Su, J. H.; Wang, G. H.; Duh, C. Y.; Shen, Y. C.; Chiang, M. Y.; Chen, I. T. *J. Nat. Prod.* **1999**, *62*, 1415−1420, and references therein.
(3) Neve, J. E.; McCool, B. J.; Bowden, B. F. *Aust. J. Chem*. **1999**, *52*, 359−366.
(4) Bandurraga, M. M.; Fenical, W.; Donovan, S. F.; Clardy, J. *J. Am. Chem. Soc.* **1982**, *104*, 6463−6464.
(5) Shen, Y. C.; Prakash, C. V. S.; Chang, Y. T. *Steroids* **2001**, *66*, 721−725.
(6) Shen, Y. C.; Prakash, C. V. S.; Chang, Y. T. Hung, M. C.; Chen, S. J.; Chen, H. J.; Hsu, M. C. *Chin Pharm. J*. **2000**, *52*, 341−351.
(7) Rodriguez, J.; Nieto, R. M.; Jimenez, C. *J. Nat. Prod*. **1998**, *61*, 313−317.
(8) Kwak, J. H.; Schmitz, F. J.; Williams, G. C. *J. Nat. Prod*. **2001**, *64*, 754−760.
(9) Isaacs, S.; Carmely, S.; Kashman, Y. *J. Nat. Prod*. **1990**, *53*, 596−602.
(10) Hamann, M. T.; Harrison, K. N.; Carrol, A. R.; Sheuer, P. J. *Heterocycles* **1996**, *42*, 325−331.
(11) Anjaneyulu, A. S. R.; Rao, N. S. K. *J. Chem. Soc., Perkin Trans. 1* **1997**, 959−962.
(12) Elliott, W. M.; Auersperg, N. *Biotech. Histochem*. **1993**, *68*, 29−35.
(13) Finlay, G. J.; Baguley, B. C.; Wilson, W. R. *Anal. Biochem*. **1984**, *139*, 272−277.
(14) All data including atomic coordinates, thermal parameters, bond distances, angles, and observed and calculated structure factors have been deposited in the Cambridge Crystallographic Data Centre and can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223−336003 or e-mail: deposit@ccdc.cam.ac.uk).
(15) *teXsan*: Crystal Structure Analysis Package; Molecular Structure Corporation, 1985 and 1992.

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