Junceol A, a New Sesquiterpenoid from the Sea Pen *Virgularia juncea*

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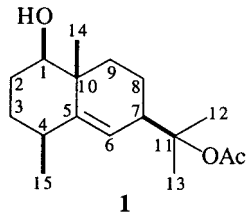
A new sesquiterpenoid, junceol A(**1**), as well as two known diterpenoids, sclerophytin A (**2**) and cladiellisin (**3**), have been isolated from the sea pen octocoral *Virgularia juncea*. The structure of metabolite **1** was determined by extensive spectral analysis. Compounds **1**−**3** have been shown to exhibit cytotoxicity toward P-388 cancer cells.

Previous studies on the chemical constituents of the sea pen octocorals of the genus *Virgularia* have led to isolation of several sterols and fatty acid derivatives.[1,2] In our continuing search for the bioactive substances from Taiwanese marine organisms, we examined an EtOAc extract of the sea pen octocoral *Virgularia juncea* (Pallas) (phylum Cnidaria, class Octocorallia, order Pennatulacea, family Virgulariidae), which was found to exhibit cytotoxicity against P-388 cells (mouse lymphocytic leukemia) (ED_{50} = 7.7 μg/mL). Initial study on the crude extract of this organism has led to the isolation of a new sesquiterpenoid, junceol A (**1**), and two known diterpenoids, sclerophytin A (**2**)[3,4] and cladiellisin (**3**).[5,6]



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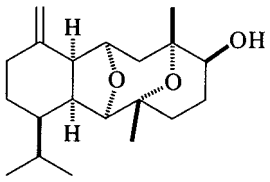

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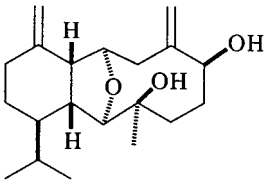

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Junceol A (**1**) was obtained as a colorless oil. The HREIMS of **1** indicated the molecular formula $C_{17}H_{28}O_3$ and four degrees of unsaturation for this metabolite. The IR spectrum revealed absorption bands for hydroxyl (3437 cm^{-1}) and ester carbonyl (1726 cm^{-1}) moieties. The EIMS of **1** exhibited peaks at *m*/*z* 280 [M^+], 220 [M − HOAc]$^+$, and 202 [M − HOAc − H_2O]$^+$, suggesting the presence of hydroxy and acetoxy groups in **1**. Resonances in the ^{13}C NMR spectrum of **1** at δ 170.5 (s) supported the presence of an ester which was identified as an acetate by the presence of a methyl resonance in the ^1H NMR spectrum at δ 1.99 (3H, s) (Table 1). From the $^1H-^1H$ COSY spectrum of **1** (Figure 1 and Table 1), it was possible to establish the proton sequences from H-1 to H-4; H-4 to H_3-15; and H-6 to H_2-9. The resonances in the ^{13}C NMR of **1** at δ 148.3 (s) and 123.0 (d) indicated the presence of a trisubstituted double bond. On the basis of these data and the $^1H-^{13}C$ long-range correlations observed in an HMBC experiment, the connectivities from C-1 to C-10 (Figure 1 and Table 1) could be further established. In the HMBC experiment of **1**, the ring juncture C-14 methyl group was positioned at C-10 from the key correlations between H_3-14 and C-1, C-5, C-9, and C-10. The acetoxy-bearing isopropyl group positioned at C-7 was confirmed from the HMBC correlation between H-7 (δ 2.70) and the quaternary oxygenated carbon C-11 (δ 85.4) and from the correlations between H_3-12, H_3-13 (δ 1.42, s, 6H) and C-7 (δ 42.5, d), respectively. Furthermore, analysis of the NMR (^1H and ^{13}C) chemical shifts and HMBC correlations also revealed the hydroxy and acetoxy groups should be positioned at C-1 and C-11, respectively.

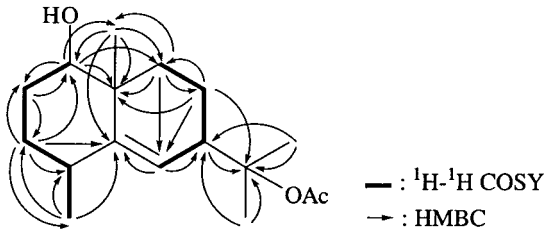

Figure 1. $^1H-^1H$ COSY and HMBC correlations for **1**.

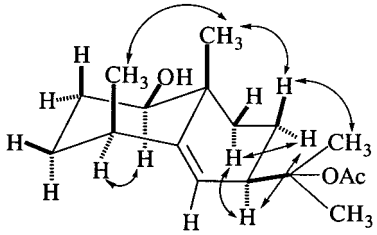

Figure 2. Selective NOESY correlations for **1**.

The relative stereochemistry of **1** was determined by correlations observed in the NOESY spectrum (Figure 2). In the NOESY experiment of **1**, H-1 gives NOESY correlations to H-4, not to H_3-14, indicating that H-1 and H-4 are situated on the same face of the structure and are assigned as the α-protons since the C-14 methyl is arbitrarily

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Table 1. ^{1}H and ^{13}C NMR Chemical Shifts and HMBC and ^{1}H−^{1}H COSY Correlations for **1**

C/H	^{1}H[a]	^{13}C[b]	HMBC	^{1}H−^{1}H COSY
1	3.34 dd (11.5, 4.0)[c]	78.0 (d)[d]	H-2α/*β*, H_2-3 H_3-14	H-2α/*β*
2α	1.68 m	26.5 (t)	H-1, H_2−3	H-1, H-2*β*, H_2-3
β	1.82 m			H-1, H-2α, H_2-3
3/3′	1.58 m	30.9 (t)	H-2α/*β*, H_3-15	H-2α/*β*, H-4
4	2.44 m	38.6 (d)	H_2-3, H_3-15	H_2-3, H_3-15
5		148.3 (s)	H_2-3, H-6, H_3-14, H_3-15	
6	5.43 d (3.0)	123.0 (d)	H-8α/*β*, H-9α/*β*	H-7
7	2.70 m	42.5 (d)	H-6, H_3-12, H_3-13	H-6, H-8α/*β*
8α	1.50 m	34.7 (t)	H-9α/*β*	H-7, H-8*β*, H-9α/*β*
β	1.63 m			H-7, H-8α, H-9α/*β*
9α	1.52 m	19.7 (t)	H-1, H-8α/*β*, H_3-14	H-9*β*, H-8α/*β*
β	1.67 m			H-9α, H-8α/*β*
10		40.0 (s)	H-1, H-8α/*β*, H-9α/*β*, H_3-14	
11		85.4 (s)	H-7, H-8α/*β*, H_3-12, H_3-13	
12	1.42 s	23.8 (q)		
13	1.42 s	23.8 (q)		
14	1.08 s	20.7 (q)	H-1, H-9α/*β*	
15	1.15 d (7.0)	22.1 (q)	H_2-3	H-4
acetate methyl	1.99 s	22.6 (q)		
acetate carbonyl		170.5 (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.

assigned as the *β*-substituent at C-10. H-8*β* was found to exhibit correlations with H_3-14 and H_3-12/13. From consideration of molecular models, H_3-12/13 was found to be reasonably close to H-8*β*, when C-11 was *β*-oriented, and H-7 should be placed on the α-face. On the basis of the above observations, the structure of **1**, including the relative stereochemistry, was elucidated unambiguously.

The previously known compounds sclerophytin A (**2**) and cladiellisin (**3**) were identified by their physical and spectral data. Sclerophytin A (**2**) was isolated previously from the West Pacific Ocean soft coral *Sclerophytum capitalis*.[3] The occurrence of cladiellisin (**3**) in the soft corals *Cladiella similis* and *Cladiella sphaeroides* have been reported;[5,6] however, this is the first observation of the existence of compounds **2** and **3** in the sea pen octocoral.

The cytotoxicity of metabolites **1**−**3** against the growth of P-388 cancer cells was studied, and the results showed that compounds **1**−**3** exhibited cytotoxicity against P-388 cancer cells with ED_{50}'s of 5.1, 2.3, and 2.0 *μ*g/mL, respectively.[7]

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus and were uncorrected. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. IR spectra were recorded on a Hitachi I-2001 infrared spectrophotometer. EIMS were obtained with a VG Quattro GC/MS spectrometer at 70 eV. HREIMS were recorded on a JEOL JMX-HX 110 mass spectrometer. The NMR spectra were recorded on 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, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC.

Animal Material. The sea pen *V. juncea* was collected by hand at the Penghu Islands located on the west coast of Taiwan, in August 2000, at a depth of 0.3−0.5 m and was immediately stored in a freezer until extraction. A voucher specimen was deposited in the Department of Marine Resources, National Sun Yat-Sen University (specimen no. PHSP-101).

Extraction and Separation. The sea pen (0.8 kg fresh wt) was collected and freeze-dried. The freeze-dried material was minced and extracted exhaustively with EtOAc (3 L × 5). The organic extract was evaporated to dryness and separated by Si gel column chromatography. Metabolite **1** was eluted with hexanes−EtOAc (7:1), **2** with hexanes−EtOAc (5:1), and **3** with hexanes−EtOAc (3:1).

Junceol A (1): colorless oil (7 mg); $[\alpha]_D^{25}$ −1° (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3437, 2938, 1726, 1458, 1372, 1258, 1148, and 1019 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS (70 eV) *m*/*z* (rel int) 280 (0.1, M^+), 220 (10), 202 (2), 187 (3), 179 (10), 161 (7), 43 (100); HREIMS *m*/*z* 280.2034 (calcd for $C_{17}H_{28}O_3$, 280.2031).

Sclerophytin A (2): white powder (20 mg); mp 186−188 °C (lit.[3] 187 °C); $[\alpha]_D^{24}$ −3° (*c* 0.4, $CHCl_3$); spectral data of **2** (MS, IR, ^{1}H and ^{13}C NMR) in full agreement with those reported previously.[3,4]

Cladiellisin (3): white powder (12 mg); mp 180−181 °C (lit.[5] 181−182 °C); $[\alpha]_D^{24}$ −11° (*c* 1.1, $CHCl_3$); spectral data of **3** (MS, IR, ^{1}H and ^{13}C NMR) in full agreement with those reported previously, although the optical rotation value was different from those reported.[5,6]

Cytotoxicity Testing. The P-388 cell line was kindly provided by Prof. J. M. Pezzuto, University of Illinois at Chicago. The cytotoxicity of tested compounds **1**−**3** against the P-388 cancer cells 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.[8,9]

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