

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

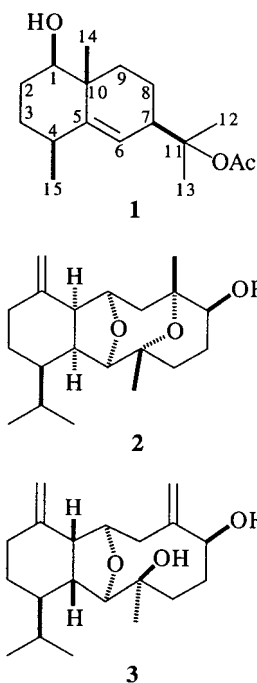
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A new sesquiterpenoid, junceol A (**1**), as well as two known diterpenoids, sclerophytin A (**2**) and cladiellisin (**3**), have been isolated from the sea pen octocoral *Virgularia juncea*. The structure of metabolite **1** was determined by extensive 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) ($ED_{50} = 7.7 \mu\text{g/mL}$). 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}



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 [$M - \text{HOAc}$]⁺, and 202 [$M - \text{HOAc} - \text{H}_2\text{O}$]⁺, suggesting the presence of

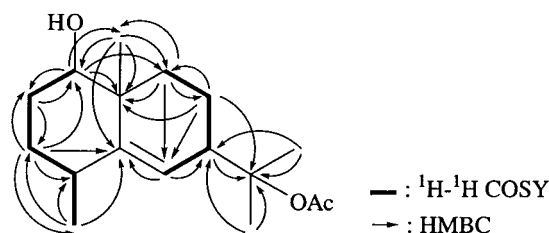


Figure 1. ^1H – ^1H COSY and HMBC correlations for **1**.

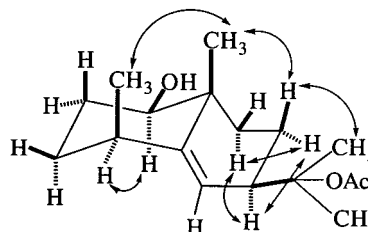


Figure 2. Selective NOESY correlations for **1**.

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 H₃-15; and H-6 to H₂-9. 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 H₃-14 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 H₃-12, H₃-13 (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.

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 H₃-14, 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. ^1H and ^{13}C NMR Chemical Shifts and HMBC and ^1H – ^1H COSY Correlations for **1**

C/H	$^1\text{H}^a$	$^{13}\text{C}^b$	HMBC	^1H – ^1H COSY
1	3.34 dd (11.5, 4.0) ^c	78.0 (d) ^d	H-2 α / β , H ₂ -3 H ₃ -14	H-2 α / β
2 α	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-2 α , H ₂ -3
3/3'	1.58 m	30.9 (t)	H-2 α / β , H ₃ -15	H-2 α / 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-8 α / β , H-9 α / β	H-7
7	2.70 m	42.5 (d)	H-6, H ₃ -12, H ₃ -13	H-6, H-8 α / β
8 α	1.50 m	34.7 (t)	H-9 α / β	H-7, H-8 β , H-9 α / β
β	1.63 m			H-7, H-8 α , H-9 α / β
9 α	1.52 m	19.7 (t)	H-1, H-8 α / β , H ₃ -14	H-9 β , H-8 α / β
β	1.67 m			H-9 α , H-8 α / β
10		40.0 (s)	H-1, H-8 α / β , H-9 α / β , H ₃ -14	
11		85.4 (s)	H-7, H-8 α / 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-9 α / 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 H₃-14 and H₃-12/13. From consideration of molecular models, H₃-12/13 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 *Sclerophyllum 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 ED₅₀'s of 5.1, 2.3, and 2.0 $\mu\text{g/mL}$, 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 ^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, 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 \times 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); $[\alpha]_{\text{D}}^{25} -1^\circ$ (c 0.1, CHCl_3); IR (KBr) ν_{max} 3437, 2938, 1726, 1458, 1372, 1258, 1148, and 1019 cm^{-1} ; ^1H 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 $\text{C}_{17}\text{H}_{28}\text{O}_3$, 280.2031).

Sclerophytin A (2): white powder (20 mg); mp 186–188 °C (lit.³ 187 °C); $[\alpha]_{\text{D}}^{24} -3^\circ$ (c 0.4, CHCl_3); spectral data of **2** (MS, IR, ^1H 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); $[\alpha]_{\text{D}}^{24} -11^\circ$ (c 1.1, CHCl_3); spectral data of **3** (MS, IR, ^1H 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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