

New Aromadendrane Sesquiterpenoid Pseuboydone F from the Marine-derived Fungus *Pseudallescheria boydii* F44-1

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Abstract: A new aromadendrane sesquiterpenoid pseuboydone F (**1**), along with a known pseuboydone A (**2**), were isolated from the marine-derived fungus *Pseudallescheria boydii* F44-1 associated with the soft coral *Sarcophyton* sp.. The structures were elucidated by HRMS, 1D and 2D NMR spectroscopic data.

Keywords: Marine fungus; *Pseudallescheria boydii*; aromadendrane; sesquiterpenoid; pseudboydone F. © 2020 ACG Publications. All rights reserved.

1. Introduction

Marine-derived fungi are considered as a promising source of novel natural products with biodiversity and chemical diversity. Although numerous novel metabolites have been obtained from a large number of marine-derived fungi, actually the discovering rate is still not high. It's challengeable to develop efficient dereplication techniques based on NMR or MS analysis [1,2]. In our previous studies, we reported the strategies to increase the discovery rate of new compounds by tracking the diagnostic ¹H or ¹³C NMR resonance signals. For example, a pair of unprecedented epimonothiodiketopiperazine diastereomers, pseudellones A and B were isolated from the marine fungus *Pseudallescheria ellipsoidea* by tracking the relatively rare ¹H NMR resonated signals in the range of 8.00-8.50 ppm [3]. Following with the rich aromatic proton signals in aromatic range of 6.5-8.5 ppm, 14 new alkaloids were obtained from marine-derived fungus *Scedosporium apiospermum* F41-1 [4].

Recently, we have isolated a fungal strain *Pseudallescheria boydii* (collection No. F44-1) from the soft coral *Sarcophyton* sp. collected in the Hainan Sanya National Coral Reef Reserve, China. This fungus was cultured in glucose-peptone-yeast extract (GPY) media and prescreened the metabolites extract using the ¹H NMR spectroscopy. At the high field area, two sets of signals at δ_{H} 0.50 (dd, 9.6, 9.6) and δ_{H} 0.17 (dd, 9.6, 9.6) attracted our attention. To the best of our knowledge, the aromadendrane sesquiterpenoids showed the signals in the range of 0-0.6 ppm due to the shielding

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effect of the cyclopropane ring. Previously, by tracking the proton resonance signals in this region, the aromadendrane sesquiterpenoids pseuboydones A and B were separated from marine-derived fungus *Pseudallescheria boydii* F19-1[5], and scedogiines A-F were isolated from the marine-derived fungus *Scedosporium dehoogii* F41-4 [6]. Using the same strategy, a new aromadendrane sesquiterpenoid pseuboydone F (**1**) and a known pseuboydone A (**2**) (Figure 1) were isolated from the fungus *Pseudallescheria boydii* F44-1. Herein we report the isolation and structural elucidation of these compounds.

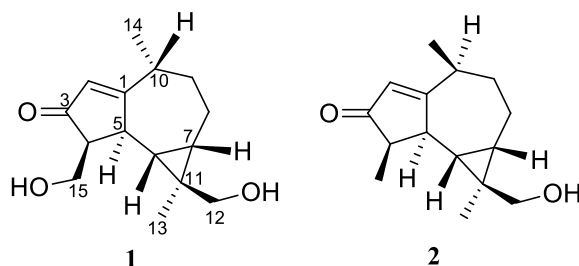


Figure 1. The chemical structures of compounds **1** and **2**

2. Materials and Methods

2.1. General Experimental Procedures

Preparative HPLC was performed using a Shimadzu LC-20AT HPLC pump (Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan) and installed with an SPD-20A dual λ absorbance detector (Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan) and a Capcell-Pak C18 UG80 HPLC column (250 mm \times 20 mm, Shiseido, Japan). 1D and 2D NMR experiments were measured with Bruker Avance 400 spectrometers and Bruker Avance 600 spectrometers. The chemical shifts are relative to the residual solvent signal (CDCl₃: δ_{H} 7.26 and δ_{C} 77.0). The HR-APCI-MS spectrum was measured with Thermo Orbitrap Fusion Lumos liquid chromatography-mass spectrometry.

2.2. Fungal Identification and Culture Method

The marine fungus *Pseudallescheria boydii* (collection number F44-1) was isolated from the inner tissue of the soft coral *Sarcophyton* sp. collected from Hainan Sanya National Coral Reef Reserve, P. R. China. This fungal strain was conserved in 15% (v/v) glycerol aqueous solution at -80 $^{\circ}\text{C}$. A voucher specimen was deposited in the School of Chemistry, Sun Yat-sen University, Guangzhou, P. R. China. Analysis of the ITS rDNA by BLAST database screening provided 99.9% match to *Pseudallescheria boydii*. The marine fungus *Pseudallescheria boydii* F44-1, was cultured in the GPY medium which included 15 g/L glucose, 5 g/L peptone, 2 g/L yeast extract, 25 g/L sea salt, and 1 L H₂O at pH 7.0. Fungal mycelia were cut and transferred aseptically to 1000 mL conical flasks each containing 600 mL sterilized liquid medium. The fungus was incubated at 28 $^{\circ}\text{C}$ for 20 days.

2.3. Extraction and Isolation

10 liters culture broth was filtered through cheesecloth. The liquid was successively extracted three times with EtOAc (3 \times 10 L). Finally, the extract was concentrated by low-temperature rotary evaporation to get a crude extract (2.8 g). The extract was chromatographed on a silica gel column (diameter: 4 cm, length: 50 cm, silica gel, 35 g) with a gradient of petroleum Ether-EtOAc (30:0-0:30, v/v) followed by EtOAc-MeOH (30:0-0:30, v/v) to yield ten fractions (Fr.1-Fr.10). The fractions were monitored by TLC and similar fractions, Fr.6-Fr.8 were combined and concentrated in vacuo, and then, the constituents was purified by silica gel column using a step gradient elution with ether-EtOAc (10:0-0:10, v/v) to get 10 subfractions (Fr.6-8-1 to Fr.6-8-10). Then Fr.6-8-5 was further purified using reversed phase preparative HPLC with a mobile phase of CH₃CN-H₂O (60:40, v/v, t_{R} = 30 min) to obtain compound **1** (1.2 mg). Further HPLC purification of Fr.6-8-6 with CH₃OH-H₂O (75:25, v/v, t_{R} = 37.5 min) gave compound **2** (3.9 mg).

3. Results and Discussion

3.1. Structure Elucidation

Pseudoboydone F (**1**) was obtained as a pale yellow oil. The molecular formula was determined to be C₁₅H₂₂O₃ by the HR-APCI-MS peak at *m/z* 249.14906 [M-H]⁻ (calcd C₁₅H₂₁O₃, 249.14962) indicating five degrees of unsaturation. The ¹³C NMR and DEPT spectra displayed two sp² quaternary carbons, one sp³ quaternary carbon, one sp² methine, five sp³ methines, four sp³ methylenes and two methyls (Table 1). The ¹H-¹H COSY correlations of H₂-15/H-4/H-5/H-6/H-7/H₂-8/H₂-9/H-10/H₃-14 revealed the fragment -CH₂-CH-CH-CH-CH-CH₂-CH₂-CH-CH₃ (Figure 2). In the ¹H NMR spectrum, two characteristic signals at δ_H 0.50 (dd, 9.6, 9.6, H-6) and 0.87 (ddd, 11.4, 9.6, 6.2, H-7) (Table 1) displayed the existence of a cyclopropane ring. One carbonyl group (δ_C 210.7, C-3) and a trisubstituted double bond (δ_C 190.3, C-1; 126.6, C-2) illustrated two degrees of unsaturation. So, compound **1** had to contain another two rings. Further analysis of the HMBC correlations from H-2 to C-4 and C-5, from H₃-14 to C-1, the olefinic quaternary carbon C-1 was connected to C-5 (δ_C 44.0) forming a bridge. A five-membered ring system was constructed by quaternary carbon C-1 and methine C-4 (δ_C 57.9) via carbonyl group C-3. Therefore, compound **1** contains a five- and seven-membered rings and cyclopropane fused ring system, which belong to aromadendrane sesquiterpenoid. In addition, one oxygenated methylene and one methyl connected with C-11 (δ_C 27.4), which was confirmed by the HMBC correlations from H₂-12 to C-6, C-7 and C-11, from H₃-13 to C-11 and C-12. The remaining one oxygenated methylene C-15 was attached to the C-4 position based on the HMBC correlations from H₂-15 to C-4 and C-5.

Table 1. ¹H and ¹³C NMR data of compounds **1** and **2** in CDCl₃ (δ in ppm, *J* in Hz).

No.	1 ^a		2 ^b	
	δ _C	δ _H	δ _C	δ _H
1	190.3, C		187.7, C	
2	126.6, CH	5.86 (d, 1.2)	125.2, CH	5.81 (s)
3	210.7, C		211.5, C	
4	57.9, CH	2.50 (dd, 6.6, 6.6)	46.7, CH	2.60 (m)
5	44.0, CH	2.45 (d, 9.6)	44.3, CH	2.60 (m)
6	28.5, CH	0.50 (dd, 9.6, 9.6)	25.2, CH	0.17 (dd, 9.6, 9.6)
7	25.7, CH	0.87 (ddd, 11.4, 9.6, 6.2)	25.0, CH	0.83 (m)
8	23.5, CH ₂	1.27 (m)	23.2, CH	1.24 (m)
		2.07 (ddd, 14.4, 6.6, 6.2)		2.06 (m)
9	35.1, CH ₂	1.44 (ddd, 12.6, 12.6, 12.0)	35.8, CH ₂	1.36 (m)
		1.97 (ddd, 12.6, 6.6, 6.6)		2.00 (m)
10	40.7, CH	2.35 (ddq, 12.6, 6.6, 6.6)	40.2, CH	2.34 (m)
11	27.4, C		26.7, C	
12	72.6, CH ₂	3.29 (d, 10.8)	72.3, CH ₂	3.24 (d, 10.8)
		3.39 (d, 10.8)		3.43 (d, 10.8)
13	11.4, CH ₃	1.24 (s)	11.5, CH ₃	1.20 (s)
14	20.1, CH ₃	1.26 (d, 6.6)	19.7, CH ₃	1.24 (d, 6.8)
15	63.4, CH ₂	3.69 (dd, 10.2, 6.6)	10.0, CH ₃	1.13 (d, 6.4)
		3.76 (dd, 10.2, 6.6)		

^a ¹³C NMR data were recorded at 150 MHz and ¹H NMR data were recorded at 600 MHz.

^b ¹³C NMR data were recorded at 100 MHz and ¹H NMR data were recorded at 400 MHz.

The relative configuration of compound **1** was established by analysis the NOESY spectrum. The cross peaks of H-6/H-7, H-6/H₂-12, H-6/H₂-15, and H-7/H₂-12, implied that H-6, H-7, H₂-12 and H₂-15 having the same β-oriented. The NOE interactions of H-5/H₃-13 and H-5/H₃-14 suggested H-5, H₃-13 and H₃-14 had an α-orientation.

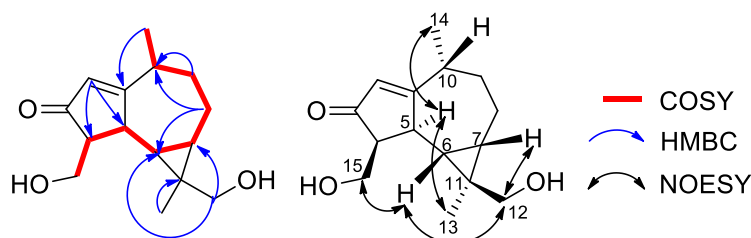


Figure 2. ^1H - ^1H COSY, key HMBC and key NOESY correlations of **1**

Followed the triplet at δ_{H} 0.17 (dd, 9.6, 9.6), compound **2** was purified. By comparing its NMR data with the literature values, compound **2** was identified as pseuboydone A [5].

3.2. Cytotoxicity

Seven cancer cell lines, including CNE1, CNE2, HONE1, SUNE1, A549, GLC82 and HL7702 were used to examine the cytotoxic activities of compounds **1** and **2** *in vitro*. This assay revealed that **1** and **2** were apparently inactive (IC_{50} values > 100 μM).

Acknowledgments

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Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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