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; pseuboydone F.

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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 \(^1^H\) or \(^{13}\)C 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 \(^1^H\) 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 apiöspерnum* 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 \(^1^H\) NMR spectroscopy. At the high field area, two sets of signals at \(\delta_0^{1\ H}\) 0.50 (dd, 9.6, 9.6, 13.2 Hz) and \(\delta_0^{1\ H}\) 0.60 (dd, 9.6, 9.6, 13.2 Hz) were observed. By comparing similar signals, we found that the spectrum of F44-1 is identical to that of the known fungus *P. ellipsoidea* [3].

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9.6) and δH 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 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 scedogines 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 × 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: δH 7.26 and δC 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 °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 °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 (3×10 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, tR = 30 min)
to obtain compound 1 (1.2 mg). Further HPLC purification of Fr.6-8-6 with CH$_3$OH-H$_2$O (75:25, v/v, t$_R$ = 37.5 min) gave compound 2 (3.9 mg).

3. Results and Discussion

3.1. Structure Elucidation

Pseudboydone F (1) was obtained as a pale yellow oil. The molecular formula was determined to be C$_{15}$H$_{25}$O$_3$ by the HR-APCI-MS peak at m/z 249.14906 [M-H$^-$] (calcd C$_{15}$H$_{25}$O$_3$, 249.14962) indicating five degrees of unsaturation. The $^{13}$C NMR and DEPT spectra displayed two sp$^2$ quaternary carbons, one sp$^3$ quaternary carbon, one sp$^2$ methine, five sp$^3$ methines, four sp$^3$ methylenes and two methyls (Table 1). The $^1$H-$^1$H COSY correlations of H$_2$-15/H-4/H-5/H-6/H-7/H$_2$-8/H$_2$-9/H-10/H$_2$-14 revealed the fragment –CH$_2$–CH–CH–CH–CH–CH$_2$–CH–CH–CH$_3$ (Figure 2). In the $^1$H 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 H$_2$-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 H$_2$-12 to C-6, C-7 and C-11, from H$_3$-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 H$_2$-15 to C-4 and C-5.

<table>
<thead>
<tr>
<th>No.</th>
<th>δ$_C$ (C)</th>
<th>δ$_H$ (H, J Hz)</th>
<th>δ$_H$ (H, J Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>190.3, C</td>
<td>187.7, C</td>
<td>5.86 (d, 1.2)</td>
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<tr>
<td>2</td>
<td>126.6, CH</td>
<td>125.2, CH</td>
<td>5.81 (s)</td>
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<td>3</td>
<td>210.7, C</td>
<td>211.5, C</td>
<td>2.50 (dd, 6.6, 6.6)</td>
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<tr>
<td>4</td>
<td>57.9, CH</td>
<td>46.7, CH</td>
<td>2.45 (d, 9.6)</td>
</tr>
<tr>
<td>5</td>
<td>44.0, CH</td>
<td>44.3, CH</td>
<td>0.50 (dd, 9.6, 9.6)</td>
</tr>
<tr>
<td>6</td>
<td>28.5, CH</td>
<td>25.2, CH</td>
<td>0.87 (ddd, 11.4, 9.6, 6.2)</td>
</tr>
<tr>
<td>7</td>
<td>25.7, CH</td>
<td>25.0, CH</td>
<td>1.27 (m)</td>
</tr>
<tr>
<td>8</td>
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<td>2.00 (m)</td>
</tr>
<tr>
<td>9</td>
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<td>40.2, CH</td>
<td>1.44 (ddd, 12.6, 12.6, 12.0)</td>
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<tr>
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<td>27.4, C</td>
<td>26.7, C</td>
<td>1.97 (ddd, 12.6, 6.6, 6.6)</td>
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<tr>
<td>11</td>
<td>72.6, CH$_2$</td>
<td>72.3, CH$_2$</td>
<td>2.35 (ddq, 12.6, 6.6, 6.6)</td>
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<tr>
<td>12</td>
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<td>11.24 (s)</td>
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<tr>
<td>13</td>
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<td>19.7, CH$_3$</td>
<td>126 (d, 6.6)</td>
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<tr>
<td>14</td>
<td>63.4, CH$_2$</td>
<td>10.0, CH$_3$</td>
<td>3.69 (dd, 10.2, 6.6)</td>
</tr>
</tbody>
</table>

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

The relative configuration of compound 1 was established by analysis the NOESY spectrum. The cross peaks of H-6/H-7, H-6/H$_2$-12, H-6/ H$_2$-15, and H-7/H$_2$-12, implied that H-6, H-7, H$_2$-12 and
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H$_2$-15 having the same $\beta$-oriented. The NOE interactions of H-5/H$_3$-13 and H-5/H$_3$-14 suggested H-5, H$_3$-13 and H$_3$-14 had an $\alpha$-orientation.

![Diagram](image)

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

Followed the triplet at $\delta_H$ 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 $\mu$M).

Acknowledgments

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Supporting Information


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References


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