Two New Metabolites from Basidiomycete *Sparassis crispa*

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Two new metabolites, named crispacolide (1) and 3-acetyl-4-hydroxymethyl-tetrahydrofuran (2), were isolated from the fruiting bodies of basidiomycete *Sparassis crispa* (Wulf.) Fr. The structures and stereochemistry were established on the basis of spectroscopic means.

**Key words:** Crispacolide, 3-Acetyl-4-hydroxymethyl-tetrahydrofuran, *Sparassis crispa*, Basidiomycete

**Introduction**

The fungus *Sparassis crispa* (Wulf.) Fr. (cauliflower mushroom), which belongs to the family of sparsisidaceae, is a culinary-medicinal mushroom found through the temperate regions of Europe, Asia and North America. The fruiting bodies of *S. crispa* have been reported to exhibit an excellent effect for curing human diseases such as gastric ulcer, oesophageal cancer, hypertension, and diabetes in China [1 – 3]. There are some reports on the isolation of bioactive β-glucan [2, 4 – 11], phenyl derivatives [12, 13], chalcones [14], and sesquiterpenenoids [15]. As a part of our search for naturally occurring bioactive metabolites from higher fungi in China [16 – 18], we have carried out the chemical investigation on the fruiting bodies of *S. crispa* and isolated two new metabolites, crispacolide (1) and 3-acetyl-4-hydroxymethyl-tetrahydrofuran (2). This paper describes the isolation and the structure elucidation of these two new compounds.

**Result and Discussion**

Compound 1 was obtained as a colorless oil. Its molecular formula was established as C9H12O4 on the basis of positive ESIMS, 13C NMR and DEPT spectra and further confirmed by HRESIMS at *m/z* = 207.0635 (calcd. 207.0633 for C9H12O4Na). The IR spectrum showed the absorption of a carbonyl group at 1748 cm⁻¹. The 13C NMR and DEPT spectra (Table 1) revealed nine carbon resonances for one carbonyl at δ = 169.6 (C-3) and 107.6 (C-8), three methylenes at δ = 70.9 (C-2), 68.0 (C-4) and 37.9 (C-7), one methine at δ = 48.5 (C-3a), one oxymethyl at δ = 48.9 (C-OCH3), and a quaternary carbon at δ = 107.5 (C-7a). Signals for one methine, four methylenes, and one oxymethyl group were observed in the 1H NMR spectrum of 1 (Table 1). Its HMBC spectrum (Fig. 2) exhibited the following key correlations: from H-2 to C-3, C-3a and

![Fig. 1. Structures of 1 and 2.](image_url)

**Table 1. NMR spectroscopic data (CDCl₃) for compounds 1 and 2.**

<table>
<thead>
<tr>
<th></th>
<th>δC (mult., J in Hz)</th>
<th>δH (mult., J in Hz)</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>70.9, t 4.43, d (2.0)</td>
<td>69.6, t 4.05, dd (8.8, 8.3)</td>
</tr>
<tr>
<td>3</td>
<td>145.7, s</td>
<td>3.06, ddd (8.8, 6.8, 6.8)</td>
</tr>
<tr>
<td>4</td>
<td>68.0, t 4.39, dd (11.8, 5.0)</td>
<td>4.46, d 2.69, m</td>
</tr>
<tr>
<td></td>
<td>4.19, dd (11.8, 6.0)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>70.7, t</td>
<td>3.91, dd (8.8, 7.8)</td>
</tr>
<tr>
<td>6</td>
<td>169.6, s</td>
<td>207.9, s</td>
</tr>
<tr>
<td>7</td>
<td>37.9, t 2.99, d (14.8)</td>
<td>2.89, d (14.8)</td>
</tr>
<tr>
<td>8</td>
<td>107.5, s</td>
<td>29.3, q 2.22, s</td>
</tr>
<tr>
<td>9</td>
<td>107.6, t 5.17, d (2.0)</td>
<td>5.13, d (2.0)</td>
</tr>
<tr>
<td>OMe</td>
<td>48.9, q 3.31, s</td>
<td>64.0, t 3.68, dd (10.8, 6.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.13, d (2.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.60, dd (10.8, 5.9)</td>
</tr>
</tbody>
</table>

(C-3) and 107.6 (C-8), three methylenes at δ = 70.9 (C-2), 68.0 (C-4) and 37.9 (C-7), one methine at δ = 48.5 (C-3a), one oxymethyl at δ = 48.9 (C-OCH3), and a quaternary carbon at δ = 107.5 (C-7a). Signals for one methine, four methylenes, and one oxymethyl group were observed in the 1H NMR spectrum of 1 (Table 1). Its HMBC spectrum (Fig. 2) exhibited the following key correlations: from H-2 to C-3, C-3a and
Experimental Section

as shown in Fig. 1. The ROESY cross peaks of H-3a/OCH₃ indicated that H-3a and the oxymethyl group are on the same side. Therefore, the structure of 1 was assigned as shown in Fig. 1, and named as crispacolide. Compound 1 is a methyl ketal. It cannot be excluded that it was formed during the extraction and isolation procedures (see Experimental Section below) from the hemiketal which likewise would be a new natural product. Surprisingly, compound 1 is optically inactive. This also may point at the possible cyclization of an optically inactive precursor during the extraction procedure.

Compound 2 was also obtained as a colorless oil with a molecular formula of C₇H₁₂O₃ assigned by HRESIMS (m/z = 167.0689; calcld. 167.0684 for C₇H₁₂O₃Na). The IR spectrum showed absorptions at 3445 and 1709 cm⁻¹, revealing the presence of hydroxyl and carbonyl groups. The ¹³C NMR and DEPT spectra displayed seven signals, including a carbonyl group [δ = 207.9 (C-6)], two methines [δ = 55.3 (C-3), 44.6 (C-4)], three methylenes [δ = 70.7 (C-5), 69.6 (C-2), 64.0 (C-8)], and a methyl group [δ = 29.3 (C-7)]. The ¹H NMR spectrum exhibited resonances at δ = 2.22 (3H, s, H-7) for a methyl, δ = 2.69 (1H, m, H-4) and 3.06 (1H, ddd, J = 8.3, 6.8, 6.8 Hz, H-3) for two methines, and δ = 3.60–4.05 (6H) for three methylenes. Interpretation of the ¹H-¹H COSY and HSQC spectra provided evidence for a partial structure CH₂-CH-CH(CH₂)-CH₂. The HMBC correlations of H-2 with C-4, C-5 and C-6, H-3 with C-5, C-7 and C-8, H-4 with C-2 and C-6, H-7 with C-3 and C-6 confirmed the presence of the functional groups noted above and allowed the assignment of the gross structure. The relative configuration of H-3, H-4-trans was deduced from the ROESY correlations of H-3/H-8a, while no ROESY correlation of H-3/H-4 was observed. Accordingly, the structure of 2 was elucidated as shown in Fig. 1.

Experimental Section

General experimental procedures

Optical rotations were measured on a Horiba SEPA-300 polarimeter. IR spectra were obtained using a Bruker Tensor 27 FT IR spectrometer with KBr pellets. NMR spectra were acquired with Bruker DRX-500 and AV-400 instruments at r.t. Mass spectra were recorded with a VG Autospec-3000 spectrometer and an API QSTAR Pulsar i spectrometer. Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography.

Fungal material

The fungus *S. crispa* was collected at Gaoligong Mountains, Yunnan Province, People’s Republic of China, in July 2007, and identified by Prof. Mu Zang, Kunming Institute of Botany. A voucher specimen (HFG 07058) was deposited at the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The fresh fruiting bodies of *S. crispa* (5.0 kg) were extracted with 95 % EtOH (10 L) at r.t. to obtain 285 g of crude extract, which was submitted to silica gel column chromatography (CC), eluting with a CHCl₃-MeOH gradient, to afford fractions A–D. Fraction B was subjected to repeated silica gel and Sephadex LH-20 CC to afford compounds 1 (5.0 mg) and 2 (9.0 mg).

Crispacolide (1)

Colorless oil, [α]D²⁶ = 0.00 (c = 0.15, CHCl₃). – IR (KBr): ν = 3471, 2998, 2916, 2862, 1748, 1673, 1468, 1426, 1387, 1322, 1284, 1184 cm⁻¹. – NMR (CDCl₃, 500 MHz) see Table 1. – MS ((+)-ESI): m/z = 185 [M+H]⁺, 207 [M+Na]⁺. – HRMS ((+)-ESI): m/z = 207.0635 (calcld. 207.0633 for C₇H₁₂O₃Na, [M+Na]⁺).

3-Acetyl-4-hydroxymethyl-tetrahydrofuran (2)

Colorless oil, [α]D²⁶ = +35.1 (c = 0.65, CHCl₃). – IR (KBr): ν = 3445, 2938, 2875, 1709, 1478, 1362, 1176, 1068, 924 cm⁻¹. – NMR (CDCl₃, 500 MHz) see Table 1. – MS (EI, 70 eV): m/z (%) = 143 ([M–H]⁻, 5), 129 ([M–Me]⁻, 25), 113 (100). – HRMS (+-ESI): m/z = 167.0689 (calcld. 167.0684 for C₇H₁₂O₃Na, [M+Na]⁺).

Acknowledgement

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