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 sparassidaceae, 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 sesquiterpenoids [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 $C_9H_{12}O_4$ on the basis of positive ESIMS, ^{13}C NMR and DEPT spectra and further confirmed by HRESIMS at $m/z = 207.0635$ (calcd. 207.0633 for $C_9H_{12}O_4Na$). The IR spectrum showed the absorption of a carbonyl group at 1748 cm^{-1} . The ^{13}C NMR and DEPT spectra (Table 1) revealed nine carbon resonances for one carbonyl at $\delta = 169.6$ (C-6), one terminal double bond at $\delta = 145.7$

Table 1. NMR spectroscopic data ($CDCl_3$) for compounds **1** and **2**.

	1		2	
	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)
2	70.9, t	4.43, d (2.0)	69.6, t	4.05, dd (8.8, 8.3) 3.87, dd (8.8, 6.8)
3	145.7, s		55.3, d	3.06, ddd (8.3, 6.8, 6.8)
3a	48.5, d	3.01, m		
4	68.0, t	4.39, dd (11.8, 5.0) 4.19, dd (11.8, 6.0)	44.6, d	2.69, m
5			70.7, t	3.91, dd (8.8, 7.8) 3.62, dd (8.8, 5.9)
6	169.6, s		207.9, s	
7	37.9, t	2.99, d (14.8) 2.89, d (14.8)	29.3, q	2.22, s
7a	107.5, s			
8	107.6, t	5.17, d (2.0) 5.13, d (2.0)	64.0, t	3.68, dd (10.8, 6.4) 3.60, dd (10.8, 5.9)
OMe	48.9, q	3.31, s

Fig. 1. Structures of **1** and **2**.

(C-3) and 107.6 (C-8), three methylenes at $\delta = 70.9$ (C-2), 68.0 (C-4) and 37.9 (C-7), one methine at $\delta = 48.5$ (C-3a), one oxymethyl at $\delta = 48.9$ (C-OCH₃), and a quaternary carbon at $\delta = 107.5$ (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

C-8, from H-4 to C-3, C-6 and C-7a, from H-7 to C-3a, C-6 and C-7a, and from OCH₃ to C-7a. The relative configuration of **1** was determined by a ROESY spectrum. 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; calcd. 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.,

Fig. 2. The key HMBC correlations of compounds **1** and **2**.

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, 400 MHz) see Table 1. – MS ((+)-ESI): m/z = 185 [M+H]⁺, 207 [M+Na]⁺. – HRMS ((+)-ESI): m/z = 207.0635 (calcd. 207.0633 for C₉H₁₂O₄Na, [M+Na]⁺).

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

Colorless oil, [α]_D²⁵ = +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 (calcd. 167.0684 for C₇H₁₂O₃Na, [M+Na]⁺).

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