Two New Metabolites from Basidiomycete *Sparassis crispa*

Meng-Yuan Jiang[a,b], Ling Zhang[a,b], Ze-Jun Dong[a], and Ji-Kai Liu[a]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China
[b] Graduate University of the Chinese Academy of Sciences, Beijing 100049, P. R. China

Reprint requests to Prof. Dr. Ji-Kai Liu. Fax: +86-871-5150227. E-mail: jkliu@mail.kib.ac.cn

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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

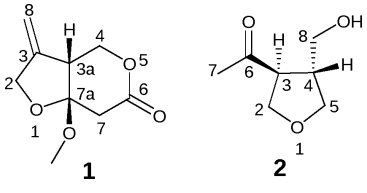
The fungus *Sparassis crispa* (Wulf.) Fr. (cauliflower mushroom), which belongs to the family of sparassidaceae, is a culinary-medicinal mushroom found through the temperate regions of Europe, Asia and North America. The fruiting bodies of *S. crispa* have been reported to exhibit an excellent effect 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 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 $C_9H_{12}O_4$ 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 δ = 169.6 (C-6), one terminal double bond at δ = 145.7 (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-OCH$_3$), and a quaternary carbon at δ = 107.5 (C-7a). Signals for one methine, four methylenes, and one oxymethyl group were observed in the ^{1}H 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

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)	44.6, d	2.69, m
		4.19, dd (11.8, 6.0)		
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)	29.3, q	2.22, s
		2.89, d (14.8)		
7a	107.5, s			
8	107.6, t	5.17, d (2.0)	64.0, t	3.68, dd (10.8, 6.4)
		5.13, d (2.0)		3.60, dd (10.8, 5.9)
O*Me*	48.9, q	3.31, s		

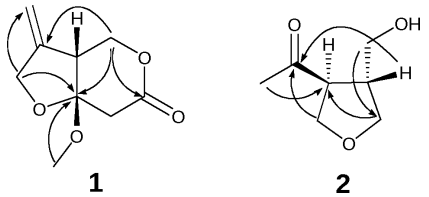


1 **2**

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

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 OCH_3 to C-7a. The relative configuration of **1** was determined by a ROESY spectrum. The ROESY cross peaks of H-3a/OCH_3 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 $C_7H_{12}O_3$ assigned by HRESIMS (*m/z* = 167.0689; calcd. 167.0684 for $C_7H_{12}O_3Na$). The IR spectrum showed absorptions at 3445 and 1709 cm^{-1}, revealing the presence of hydroxyl and carbonyl groups. The ^{13}C 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 CH_2-CH-CH(CH_2)-CH_2. 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.



1 **2**

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

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 $CHCl_3$-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, $[\alpha]_D^{26}$ = 0.00 (*c* = 0.15, $CHCl_3$). – IR (KBr): ν = 3471, 2998, 2916, 2862, 1748, 1673, 1468, 1426, 1387, 1322, 1284, 1184 cm^{-1}. – NMR ($CDCl_3$, 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 $C_9H_{12}O_4Na$, $[M+Na]^+$).

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

Colorless oil, $[\alpha]_D^{25}$ = +35.1 (*c* = 0.65, $CHCl_3$). – IR (KBr): ν = 3445, 2938, 2875, 1709, 1478, 1362, 1176, 1068, 924 cm^{-1}. – NMR ($CDCl_3$, 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 $C_7H_{12}O_3Na$, $[M+Na]^+$).

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