

Three New Keto Esters from Cultures of the Basidiomycete *Craterellus cornucopioides*

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[ABSTRACT] AIM: To investigate the bioactive constituents from cultures of the basidiomycete *Craterellus cornucopioides*. **METHODS:** Compounds were separated and purified by silica gel, Rp-18, Sephadex LH-20 column chromatography. Their structures were elucidated on the basis of extensive spectroscopic analysis including IR, MS, 1D and 2D NMR experiments. **RESULTS:** Three new keto esters, 4-oxohex-1,6-diyl diacetate (**1**), 4-oxohex-5-enyl acetate (**2**) and 6-hydroxy-4-oxohexyl acetate (**3**), were isolated from cultures of the basidiomycete *C. cornucopioides*. **CONCLUSION:** Compounds **1-3** are new keto esters.

[KEY WORDS] *Craterellus cornucopioides*; Cantharellaceae; Basidiomycete; Keto ester

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1 Introduction

Keto esters and their derivatives have been reported to show antitumor activities against Ehrlich cells and HeLa cells [1], and they could regulate flowering times of some plants [2]. In our previous studies, ketols and their esters have also been found to show inhibitory activities against two isozymes of 11 β -hydroxysteroid dehydrogenases (11 β -HSD1 and 11 β -HSD2), which catalyse the interconversion of active cortisol and inactive cortisone [3]. The basidiomycete *Craterellus cornucopioides* (L. Fr.) Pers. (Cantharellaceae) is an edible fungus with a wide distribution in most parts of China, especially in Southwestern China. During our continuing research for naturally occurring bioactive secondary metabolites from higher fungi in China [4-8], we investigated the cultures of *C. cornucopioides*, which led to the isolation of three new keto esters, 4-oxohex-1,6-diyl diacetate (**1**), 4-oxohex-5-enyl acetate (**2**) and 6-hydroxy-4-oxohexyl acetate (**3**) (Fig. 1). Details of the isolation and structural elucidation of **1-3** are described herein. Although these metabolites are structurally simple, there are few reports about them as natural products.

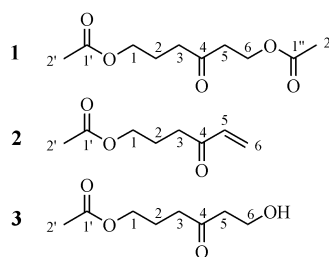


Fig. 1 Structures of compounds **1-3**

2 Results and Discussion

4-Oxohex-1,6-diyl diacetate (**1**) was obtained as a colorless oil. The positive-ion FAB-MS showed a quasi-molecular ion peak $[M + H]^+$ at m/z 217. The molecular formula, $C_{10}H_{16}O_5$, was established by HR-ESI-MS (found $[M + H]^+$ 217.1057, calcd. for 217.1075), indicating three degrees of unsaturation. The IR spectrum of **1** displayed absorption at 1739 cm^{-1} ascribable to carbonyl functional group. The 1H NMR spectrum (Table 1) displayed signals due to two tertiary methyls δ_H 1.98 (s, H-2') and 2.00 (s, H-2''), two oxygenated methylenes δ_H 4.03 (t, $J = 6.3$ Hz, H-1) and 4.28 (t, $J = 6.2$ Hz, H-6) and three methylenes δ_H 1.89 (m, H-2), 2.49 (t, $J = 7.2$ Hz, H-3) and 2.71 (t, $J = 6.2$ Hz, H-5). The ^{13}C NMR and DEPT spectra (Table 1) revealed 10 carbon resonances, including two oxymethylene carbons at δ_C 63.4 (C-1) and 59.2 (C-6), as well as two methyl carbons at δ_C 20.9 (C-2') and 20.8 (C-2''), three methylene carbons at

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Table 1 ^1H and ^{13}C NMR data for compounds 1-3 (CDCl_3 , 500 and 125 MHz) (δ , J in Hz)

| Position | 1 | | 2 | | 3 | |
|----------|---------------------|---------------------|---------------------|--|---------------------|---------------------|
| | δ_{C} | δ_{H} | δ_{C} | δ_{H} | δ_{C} | δ_{H} |
| 1 | 63.4 (t) | 4.03 (t, 6.3) | 63.6 (t) | 4.10 (t, 6.4) | 63.5 (t) | 4.05 (t, 6.4) |
| 2 | 22.4 (t) | 1.89 (m) | 22.8 (t) | 1.98 (m) | 22.4 (t) | 1.91 (m) |
| 3 | 39.3 (t) | 2.49 (t, 7.2) | 35.8 (t) | 2.68 (t, 7.3) | 39.5 (t) | 2.52 (t, 7.2) |
| 4 | 206.6 (s) | | 199.6 (s) | | 210.3 (s) | |
| 5 | 41.3 (t) | 2.71 (t, 6.2) | 136.4 (d) | 6.37 (dd, 17.0, 10.5) | 44.5 (t) | 2.66 (t, 6.2) |
| 6 | 59.2 (t) | 4.28 (t, 6.2) | 128.3 (t) | 6.25 (dd, 17.0, 0.9) 5.85 (dd, 10.5, 0.9) | 57.7 (t) | 3.84 (t, 6.2) |
| 1' | 171.0 (s) | | 171.1 (s) | | 171.1 (s) | |
| 2' | 20.9 (q) | 1.98 (s) | 20.9 (q) | 2.05 (s) | 20.9 (q) | 2.02 (s) |
| 1'' | 170.9 (s) | | | | | |
| 2'' | 20.8 (q) | 2.00 (s) | | | | |

δ_{C} 22.4 (C-2), 39.3 (C-3) and 41.3 (C-5), two ester carbonyls at δ_{C} 171.0 (C-1') and 170.9 (C-1'') and one keto carbonyl at δ_{C} 206.6 (C-4). The above spectral data suggested that **1** might be a keto ester. This was confirmed by careful analysis of ^1H - ^1H COSY, HSQC, and HMBC spectra.

Analysis of ^1H - ^1H COSY and HSQC spectra led to the identification of two spin systems $\text{CH}_2(1)\text{--CH}_2(2)\text{--CH}_2(3)$ and $\text{CH}_2(5)\text{--CH}_2(6)$ (see **1** for numbering system). The HMBC correlations from H-1 and H-2' to C-1' (δ_{C} 171.0), and from H-6 and H-2'' to C-1'' (δ_{C} 170.9) exhibited that the two acetate groups were located at C-1 and C-6, respectively. Furthermore, the HMBC correlations from H-2, H-3, H-5 and H-6 to C-4 (δ_{C} 206.6) established the direct linkage of C-4 (keto carbonyl) with C-3 and C-5. On the basis of the above evidence, compound **1** was determined as 4-oxohex-1, 6-diyl diacetate (Fig. 1).

4-Oxohex-5-enyl acetate (**2**) was isolated as a colorless oil and gave a quasi-molecular ion peak at m/z 179 $[\text{M} + \text{Na}]^+$ in its positive-ion ESI-MS spectrum and was assigned a molecular formula of $\text{C}_8\text{H}_{12}\text{O}_3$, as determined by HR-ESI-MS (found $[\text{M} + \text{Na}]^+$ 179.167 2, calcd. for 179.167 8) and NMR data. The IR spectrum showed absorptions for carbonyl groups (1 732 and 1 715 cm^{-1}) and double bond (1 634 cm^{-1}). The ^1H and ^{13}C NMR spectra of **2** (Table 1) showed features similar to those of **1**, which suggested that they were closely related. The key differences were that one of two acetate groups and two methylenes in **1** were absent and an additional terminal double bond (δ_{C} 136.4, d, C-5; 128.3, t, C-6) was observed in **2**. The HMBC correlations from olefinic protons δ_{H} 6.37 (dd, $J = 17.0, 10.5$ Hz, H-5), 6.25 (dd, $J = 17.0, 0.9$ Hz, H-6a), 5.85 (dd, $J = 10.5, 0.9$ Hz, H-6b) to the carbonyl signal at δ_{C} 199.6 (C-4) indicated the connectivity of C-4 and C-5. From the above evidence, compound **2** was determined as 4-oxohex-5-enyl acetate (Fig. 1).

6-Hydroxy-4-oxohexyl acetate (**3**) was obtained as colorless oil. The positive-ion ESI-MS showed a quasi-molecular ion peak $[\text{M} + \text{Na}]^+$ at m/z 197, and the HR-ESI-MS allowed the molecular formula of $\text{C}_8\text{H}_{14}\text{O}_4$ to be determined (found $[\text{M} + \text{Na}]^+$ 197.079 8, calcd. for 197.078 9). The IR

absorptions at 3 435, 1 737 and 1 719 cm^{-1} indicated the presence of hydroxyl and carbonyl groups. The ^1H and ^{13}C NMR spectroscopic data of **3** (Table 1) were closely similar to those of **1**, except for the absence of one of two acetate groups in **1**. The ^1H NMR signal at δ_{H} 3.84 (t, $J = 6.2$ Hz) for H-6 occurred upfield shifts about 0.44 comparing to that of **1**, which indicated that C-6 was substituted by a hydroxyl group rather than an acetyloxy group as in **1**. The remaining structure of **3** was identical to that of **1**, as determined by ^1H - ^1H COSY, HSQC, and HMBC experiments. Hence, compound **3** was determined as 6-hydroxy-4-oxo- hexyl acetate (Fig. 1).

3 Experimental

3.1 General

UV spectra were recorded on a Shimadzu UV-210 spectrometer. IR spectra were obtained on a Bruker Tensor 27 instrument with KBr pellets. Both 1D and 2D NMR experiments were performed on Bruker AM-400 and Bruker DRX-500 spectrometers in CDCl_3 with TMS as an internal standard. FAB-MS was taken on a VG Auto Spec-3000 spectrometer, and HR-ESI-MS was recorded with an API QSTAR Pulsar 1 spectrometer. Silica gel (200-300 mesh, Qingdao Marine Chemical Inc., China), Lichroprep Rp-18 gel (40-63 μm , Merck, Germany) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

3.2 Fungal material

The fungus *C. cornucopioides* was isolated from the tissue culture of its fruiting bodies collected at Ruili City, Yunnan province, China, in August 2007, and identified by Prof. Mu Zang, Kunming Institute of Botany, Chinese Academy of Sciences (CAS). The voucher specimen was deposited in the Herbarium of Kunming Institute of Botany, CAS.

3.3 Cultivation

The culture medium consisted of potato (peeled off) (200 g), glucose (20 g), KH_2PO_4 (3 g), MgSO_4 (1.5 g), citric acid (0.1 g) and thiamine hydrochloride (10 mg) in 1 L of deionized H_2O . The fungus was grown in reagent bottles (500 mL;

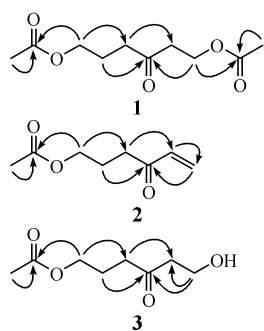


Fig. 2 Key HMBC correlations of compounds 1-3

media of 300 mL). The pH was adjusted to 6.5 before autoclaving, and the fermentation was carried out on a shaker at 25 °C and 150 r·min⁻¹ for 25 days.

3.4 Extraction and isolation

The whole culture broth of *C. cornucopioides* (20 L) was filtered, and the filtrate was extracted four times with EtOAc. The organic layer was concentrated *in vacuo* to give a crude extract (15.0 g), and the residue was subjected to column chromatography over silica gel (200-300 mesh) eluted with CHCl₃-MeOH (100 : 0 : 100 (V/V)) to afford fractions A-G. Fraction B (2.1 g) was subjected to Sephadex LH-20 column chromatography using CHCl₃-MeOH (1 : 1 (V/V)) as eluent, and R_p-18 column chromatography eluted with MeOH-H₂O from 0 : 100 (V/V) to 100 : 0 (V/V) to produce nine subfractions B₁-B₉. Subfraction B₂ was further purified by a Sephadex LH-20 column chromatography to give compound 3 (17 mg). Subfraction B₉ was further separated by column chromatography on silica gel eluted with petroleum ether-acetone (15 : 1 (V/V)) and on Sephadex LH-20 to afford compounds 1 (37 mg) and 2 (3 mg).

4 Structural Identification

4-Oxohex-1,6-diyl diacetate (1): Colorless oil; *R_f* (petroleum ether-acetone 2 : 1) 0.40; UV (MeOH, λ_{max}, nm, log ε): 194 (2.6), 223 (2.8); IR (KBr, cm⁻¹): 2 965, 2 940, 1 739, 1 386, 1 369, 1 247, 1 043, 974; NMR: see Table 1;

FAB-MS(pos.) *m/z* 217 [M + H]⁺; HR-ESI-MS *m/z* 217.105 7 (calcd. 217.107 5 for C₁₀H₁₇O₅, [M + H]⁺).

4-Oxohex-5-enyl acetate (2): Colorless oil; *R_f* (petroleum ether-acetone 2 : 1) 0.60; UV (MeOH, λ_{max}, nm, log ε): 220 (3.6); IR (KBr, cm⁻¹): 2 922, 2 852, 1 732, 1 715, 1 634, 1 464, 1 376, 1 251, 1 040; NMR: see Table 1; ESI-MS (pos.) *m/z* 179 [M + Na]⁺; HR-ESI-MS *m/z* 179.167 2 (calcd. 179.167 8 for C₈H₁₂O₃Na, [M + Na]⁺).

6-Hydroxy-4-oxohexyl acetate (3): Colorless oil; *R_f* (petroleum ether-acetone 3 : 2) 0.50; IR (KBr, cm⁻¹): 3 435, 2 962, 2 899, 1 737, 1 719, 1 369, 1 248, 1 044, 869; NMR: see Table 1; ESI-MS (pos.) *m/z* 197 [M + Na]⁺; HR-ESI-MS *m/z* 197.079 8 (calcd. 197.078 9 for C₈H₁₂O₃Na, [M + Na]⁺).

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担子菌灰号角发酵液中三个新的酮酯

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【摘要】 目的: 研究担子菌灰号角(*Craterellus cornucopioides*)的活性成分。方法: 运用硅胶层析柱、反相 R_p-18、半制备薄层层析和凝胶柱层析对担子菌灰号角发酵液进行分离纯化, 借助光谱学手段鉴定其结构。结果: 从担子菌灰号角发酵液中分离得到 3 个新的酮酯, 鉴定为 4-oxohex-1,6-diyl diacetate (1), 4-oxohex-5-enyl acetate (2), 6-hydroxy-4-oxohexyl acetate (3)。结论: 化合物 1~3 为新化合物。

【关键词】 灰号角菌; 鸡油菌科; 担子菌; 酮酯

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