Cyclopentenone and Furan Derivative from the Mycelia of *Cordyceps cicadae*

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Abstract A new cyclopentenone derivative, named as 2-(3-carboxy-2-hydroxypropyl)-3-methyl-2-cyclopentenone, and a new furan derivative, named as 5-(2-hydroxyethyl)-2-furanacetic acid, were isolated from the cultivated *Cordyceps cicadae* mycelia. Their structures were determined by spectroscopic methods, including 2D NMR analyses.

Keywords cyclopentenone derivative, furan derivative, structure elucidation, *Cordyceps cicadae*

Cordyceps cicadae, belonging to the genus *Cordyceps* (family Clavicipitaceae, Ascomycotina), is a major parasitic fungus growing on the larvae of *Cicada flammata*, *Platyleura kaempferi*, *Cryptotympana pustulata*, or *Platylomia pili*. Some bioactive contents, for example, polysaccharides [1], galactomannan [2], cordycepin, adenosine [3], and ISP-1 (myriocin) [4] have been isolated from *Cordyceps cicadae*. Because the natural resource of *Cordyceps cicadae* is very scarce, it is important to research on the cultivation of fungi, pharmacological function and chemical contents of the cultivated mycelia of *Cordyceps cicadae*. Chen [5] found that *Paecilomyces cicadae* could grow on the wheat culture medium well and the cultivated mycelia show the same pharmacological function as the natural fungi. Jin [6] found that the cultivated mycelia of *Cordyceps cicadae* could prevent the progression of chronic renal failure (CRF) in the rat

glomerulosclerosis model.

In order to investigate the chemical contents of cultivated mycelia of *Cordyceps cicadae*, we isolated the extract of 70% aqueous ethanol. In the preceding paper, we have reported five new aromatic 4-*O*-methylglucosides from the cultivated mycelia of *Cordyceps cicadae* [7]. In this paper, we report the isolation and structure elucidation of a new cyclopentenone derivative and a new furan derivative, from the cultivated *Cordyceps cicadae* mycelia. They were characterized as 2-(3-carboxy-2-hydroxypropyl)-3-methyl-2-cyclopentenone (**1**) and 5-(2-hydroxyethyl)-2-furanacetic acid (**2**), respectively, by spectroscopic methods.

The method of the cultivation of fungi was described in the preceding paper [5, 7].

The cultivated mycelia of *Cordyceps cicadae* (4.0 kg) were extracted three times with 24 liters 70% (v/v) aqueous EtOH at room temperature. The combined extracts were concentrated to give deep-brown syrup. Then, the syrup was suspended in water and extracted with CHCl₃ and BuOH orderly. The aqueous fraction after extraction by CHCl₃ and BuOH was concentrated to one liter. Then, this aqueous solution was subjected to column chromatography packed with MCI gel and eluted with MeOH:H₂O (0:1~4:6, step gradient) collecting 50 ml fractions to give Fractions B1~B5. Fraction B2 (eluted by 2:8 MeOH-H₂O) was rechromatographed by column over MCI gel and eluted with 20% aqueous methanol collecting 10 ml fractions. Fractions 28~43 afforded partially pure **1** and fractions 49~60 afforded partially pure **2**. Final purification was performed by HW-40F column chromatography eluted with H₂O to provide purified samples of **1** (15 mg) and **2** (8.0 mg).

1 was obtained as light-yellow gum. It was shown to have a molecular formula C₁₀H₁₄O₄ by HR-ESI-MS (*m/z* 221.0794, [M+Na]⁺, calcd 221.0790), which was consistent with ¹H- and ¹³C-NMR spectra and DEPT

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Table 1 NMR spectral data for 2-(3-carboxy-2-hydroxypropyl)-3-methyl-2-cyclopentenone (**1**) in D₂O^a

No.	¹ H (δ)	¹³ C (δ)	HMBC
1		217.8; s	
2		137.9; s	
3		183.6; s	
4	2.66; 2H, m	34.6; t	C-1, C-2, C-3, C-5
5	2.45; 2H, m	36.9; t	C-1, C-2, C-3, C-4
6	2.43; 2H, m	32.7; t	C-1, C-2, C-3, C-7, C-8
7	4.19; 1H, m	69.6; d	C-2, C-6, C-8, C-9
8	2.55; 1H, dd, <i>J</i> =4.8, 15.6 2.48; 1H, dd, <i>J</i> =8.2, 15.5	43.8; t	C-6, C-7, C-9
9		178.4; s	
10	2.18; 3H, s	19.9; q	C-2, C-3, C-4

^a Recorded on a Varian mercury NMR spectrometer at 400 MHz for ¹H and 100 MHz for ¹³C. δ in ppm; *J* in Hz. The multiplicities of the carbon signals were determined indirectly from HMQC experiments.

experiment. The IR spectrum revealed the presence of OH (3411 cm⁻¹), carboxyl (~1720 cm⁻¹, shoulder, overlapped with carbonyl absorption band at 1697 cm⁻¹), α,β-unsaturated carbonyl (1697 cm⁻¹, br s and 1635 cm⁻¹), whereas the UV spectrum showed an absorption maximum at 236 nm (ε 8395), which was suggestive of the presence of an enone.

¹H-NMR spectrum showed 13 proton signals and ¹³C-NMR spectrum showed 10 carbon signals (Table 1). The 10 carbons were characterized by DEPT-135 spectrum as one methyl, four methylenes, one methine, two olefinic quaternary carbon, one carbonyl carbon, and one carboxyl carbon (Table 1).

The COSY experiment on **1** indicated the presence of a common partial structure shown as bold lines in Fig. 1, whereas the connectivities of the quaternary carbons were elucidated by an HMBC experiment. The HMBC correlations from H-4 to C-1 and C-3, from H-5 to C-1, and from H-CH₃ to C-2, C-3 and C-4 indicated the fragment of 3-methylcyclopent-2-enone. The HMBC correlations from H-7 and H-8 to the carboxyl carbon indicated the carboxyl group was connected to C-8 (δ 43.8). The HMBC correlations from H-6 to C-1 (δ 217.8), C-2 (δ 137.9) and C-3 (δ 183.6) and from H-7 to C-2 (δ 137.9) indicated that the C-6 atom was connected to C-2.

On the basis of these evidences, the structure of **1** were elucidated as 2-(3-carboxy-2-hydroxypropyl)-3-methyl-2-cyclopentenone (Fig. 1).

2 was isolated as light-yellow powder. HR-ESI-MS

Fig. 1 The COSY and key HMBC correlations of 2-(3-carboxy-2-hydroxypropyl)-3-methyl-2-cyclopentenone (**1**).**Table 2** NMR spectral data for 5-(2-hydroxyethyl)-2-furanacetic acid (**2**) in CD₃OD^a

No.	¹ H (δ)	¹³ C (δ)	HMBC
1		175.5; s	
2	3.61; 2H, s	35.6; t	C-1, C-3, C-4
3		148.8; s	
4	6.12; 1H, d, <i>J</i> =3.12	110.1; d	C-3, C-5, C-6
5	6.06; 1H, d, <i>J</i> =3.1	108.6; d	C-3, C-4, C-6
6		154.2; s	
7	2.80; 2H, t, <i>J</i> =6.7	32.6; t	C-5, C-6, C-8
8	3.77; 2H, t, <i>J</i> =6.7	61.6; t	C-7, C-6

^a Recorded on a Varian mercury NMR spectrometer at 400 MHz for ¹H and 100 MHz for ¹³C. δ in ppm; *J* in Hz. The multiplicities of the carbon signals were determined indirectly from HMQC experiments.

analysis revealed the molecular of **2** was C₈H₁₀O₄ (*m/z* 193.0479, [M+Na]⁺, calcd 193.0477). The IR spectrum showed the presence of hydroxyl (3427 cm⁻¹), carboxyl (1712 cm⁻¹) and olefinic (1635 cm⁻¹) absorption bands.

The ¹H-NMR spectrum showed eight proton signals, including an olefinic –CHCH– coupling system [δ 6.06 (1H, d, *J*=3.1 Hz, 5-H) and δ 6.12 (1H, d, *J*=3.1 Hz, 4-H)], a –CH₂CH₂– coupling system [δ 3.77 (2H, t, *J*=6.7 Hz, 8-H) and 2.80 (2H, t, *J*=6.7, 7-H)], and a singlet signal at δ 3.61 (2H, s, 2-H) (Table 2). The ¹³C-NMR and DEPT-135 spectra showed eight carbon signals, characterized as a COOH, two oxygenated quaternary *sp*² carbon atoms, two *sp*² methines, and three *sp*³ methylenes (Table 2). The ¹³C-NMR shifts of olefinic carbons [δ 148.8 (C-3), 110.1 (C-4), 108.6 (C-5), 154.2 (C-6)] and the coupling constant of two olefinic protons (*J*=3.1 Hz) indicated the present of furan ring.

The COSY experiment on **2** indicated the presence of two partial structures (=CH–CH= and –CH₂CH₂–) shown

Fig. 2 The COSY and key HMBC correlations of 5-(2-hydroxyethyl)-2-furanacetic acid (**2**).

as bold lines in Fig. 2. The HMBC experiment showed the singlet protons at δ 3.61 were correlated to the carboxyl carbon at δ 175.5 and two olefinic carbons at δ 148.8 and δ 110.1, and the triplet protons at δ 2.80 (2H, t, $J=6.7$ Hz, 7-H) were correlated to two olefinic carbons at δ 154.2 and δ 108.6. Other HMBC correlations were shown in Fig. 2 and Table 2.

Thus, **2** were characterized as 5-(2-hydroxyethyl)-2-furanacetic acid.

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