

Cordyceamides A and B from the Culture Liquid of *Cordyceps sinensis* (BERK.) SACC.

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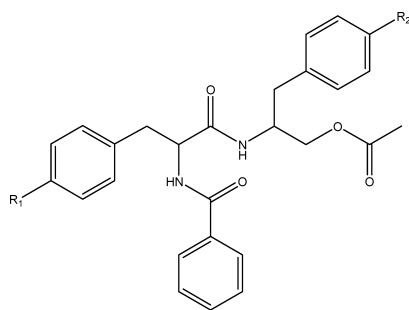
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Two new aurantiamides named as cordyceamides A and B were isolated from the culture liquid of *Cordyceps sinensis* (BERK.) SACC., along with one known compound, aurantiamide acetate. Their structures were elucidated as *N*-benzoyl-L-tyrosinyl-L-phenylalaninol acetate and *N*-benzoyl-L-tyrosinyl-L-*p*-hydroxyphenylalaninol acetate by 1D, 2D-NMR techniques and comparison with literatures.

Key words *Cordyceps sinensis*; culture liquid; aurantiamide

Cordyceps sinensis (BERK.) SACC. belongs to the class *Ascomycetes*, mainly distributing in Qinghai, Xizang, Sichuan provinces, China. It has been regarded as a popular and effective folk medicine for treating various human diseases, such as hepatitis, hypertension, hypercholesterolaemia, and gastric cancer, *etc.*¹ It has been used for medicinal purposes due to its various physiological activities including immunostimulating and anti-tumor activities.² But the natural resources of *Cordyceps sinensis* (BERK.) SACC. are very limited. So *Cordyceps sinensis* (BERK.) SACC. has been cultured to fulfill the medicinal need.³ Meanwhile it is necessary to study the chemical constituents of the culture liquid of *Cordyceps sinensis* (BERK.) SACC. In our previous study, a new cyclodipeptide had been isolated from the butanol fraction and it showed better effect on several tumor cell lines.⁴ In this paper, we report the isolation and identification of two new amides and their cytotoxic activities.

Compound **1** was obtained as white needles (MeOH), mp 210–211 °C, $[\alpha]_D^{20}$ –40.35° (*c*=0.8, MeOH). Its molecular formula was determined as C₂₇H₂₈N₂O₅ by high-resolution EI mass (HR-EI-MS), exhibiting an ion peak at *m/z* 460.2010 [M] (Calcd 460.1998). The IR spectrum showed absorptions at 3350 (phenyl OH), 3133 (NH), 1730 (COOR), 1662, 1635 (CONH), 1605, 1536 (benzene) cm⁻¹. ¹H-NMR (CD₃COCD₃) showed one 1,4-disubstituted phenyl groups [7.15 (2H, d, *J*=8.2 Hz, H-2'', 6''), 7.01 (2H, d, *J*=8.2 Hz, H-3'', 5'')] and two single substituted phenyl group [7.46 (2H, t, *J*=7.9 Hz, H-3', 5'), 7.53 (1H, t, *J*=7.9 Hz, H-4'), 7.82 (2H,



- 1 R₁ = OH, R₂ = H
2 R₁ = OH, R₂ = OH
3 R₁ = R₂ = H

Fig. 1. Structures of **1**–**3**

d, *J*=7.9 Hz, H-2', 6'); 7.30 (2H, d, *J*=8.0 Hz, H-2''', 6'''), 7.25 (3H, m, H-3''', 4''', 5'''). Three methylenes [4.07 (1H, dd, *J*=11.1, 5.9 Hz, H-3), 3.96 (1H, dd, *J*=11.1, 4.7 Hz, H-3); 3.22 (1H, dd, *J*=13.9, 7.8 Hz, H-10), 3.08 (1H, dd, *J*=13.9, 4.3 Hz, H-10); 2.90 (1H, dd, *J*=14.0, 6.7 Hz, H-11), 2.87 (1H, dd, *J*=14.0, 7.5 Hz, H-11)], two methines [4.82 (1H, m, H-7) and 4.35 (1H, m, H-4)], one methyl [1.96 (3H, s, 1-CH₃)] and three active hydrogens [9.50 (1H, s, 4''-OH), 7.60 (1H, d, *J*=7.5 Hz, 8-NH) and 7.35 (1H, d, *J*=8.0 Hz, 5-NH)] were also observed in ¹H-NMR spectrum. These were further confirmed by ¹³C-NMR (Table 1) and heteronuclear single quantum coherence (HSQC). ¹³C-NMR also suggested the presence of three carbonyls [171.6 (C-2), 170.9 (C-6) and 167.2 (C-9)] besides the above groups. The presence of fragments of a tyrosine, a phenylpropanolamine, a benzoyl and an acetyl were deduced by heteronuclear multiple bond connectivity (HMBC) correlations (Fig. 2). An amide bond was formed between the benzoyl group and the tyrosine fragment by the correlation between the amino hydrogen signal (δ_H 7.60) and the carbonyl signal (δ_C 167.2) in HMBC. Correlation between the amino hydrogen signal (δ_H 7.35) and the carbonyl signal (δ_C 170.9) in HMBC also suggested another amide bond was formed between the phenylpropanolamine and the tyrosine fragments. The phenylpropanolamine was acetylated by the HMBC correlation between 3-CH₂- and the carbonyl of the acetyl. Therefore the structure of **1** was established as *N*-benzoyl-L-tyrosinyl-L-phenylalaninol acetate. The configurations of the tyrosine and the phenylpropanolamine were both determined as L by comparison the $[\alpha]_D^{20}$ of the HCl hydrolysate with standards.

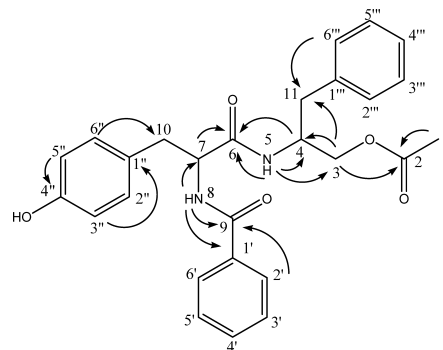


Fig. 2. The Key HMBC Correlations of **1**

Table 1. ¹H- and ¹³C-NMR Data of **1** (CD₃COCD₃) and **2** (CD₃OD) (δ in ppm, J in Hz)

No.	δ _H of 1	δ _C of 1	δ _H of 2	δ _C of 2
1-CH ₃	1.96 (3H, s)	20.7	2.01 (3H, s)	21.2
2-CO		171.6		172.1
3-CH ₂	4.07 (1H, dd, J=11.1, 5.9 Hz)	65.4	4.10 (1H, dd, J=11.2, 6.0 Hz)	66.4
	3.96 (1H, dd, J=11.1, 4.7 Hz)		3.98 (1H, dd, J=11.2, 4.5 Hz)	
4-CH	4.35 (1H, m)	50.6	4.39 (1H, m)	50.9
5-NH	7.35 (1H, d, J=8.0 Hz)		7.64 (1H, d, J=8.0 Hz)	
6-CO		170.9		170.8
7-CH	4.82 (1H, m)	55.7	4.86 (1H, m)	56.0
8-NH	7.60 (1H, d, J=7.5 Hz)		7.80 (1H, d, J=7.5 Hz)	
9-CO		167.2		167.9
10-CH ₂	3.22 (1H, dd, J=13.9, 7.8 Hz)	38.3	3.22 (1H, dd, J=14.0, 7.9 Hz)	39.1
	3.08 (1H, dd, J=13.9, 4.3 Hz)		3.08 (1H, dd, J=14.0, 4.2 Hz)	
11-CH ₂	2.90 (1H, dd, J=14.0, 6.7 Hz)	37.9	2.90 (1H, dd, J=14.0, 6.9 Hz)	38.2
	2.87 (1H, dd, J=14.0, 7.5 Hz)		2.87 (1H, dd, J=14.0, 7.3 Hz)	
1'		135.3		135.3
2'	7.82 (1H, d, J=7.9 Hz)	129.0	7.83 (1H, d, J=7.9 Hz)	129.2
3'	7.46 (1H, t, J=7.9 Hz)	127.2	7.48 (1H, t, J=7.9 Hz)	127.5
4'	7.53 (1H, t, J=7.9 Hz)	132.1	7.54 (1H, t, J=7.9 Hz)	132.3
5'	7.46 (1H, t, J=7.9 Hz)	127.2	7.48 (1H, t, J=7.9 Hz)	127.5
6'	7.82 (1H, d, J=7.9 Hz)	129.0	7.83 (1H, d, J=7.9 Hz)	129.2
1''		132.9		132.9
2''	7.15 (1H, d, J=8.2 Hz)	126.7	7.18 (1H, d, J=8.3 Hz)	127.0
3''	7.01 (1H, d, J=8.2 Hz)	115.5	7.03 (1H, d, J=8.2 Hz)	115.8
4''		159.2		160.4
5''	7.01 (1H, d, J=8.2 Hz)	115.5	7.03 (1H, d, J=8.2 Hz)	115.8
6''	7.15 (1H, d, J=8.2 Hz)	126.7	7.18 (1H, d, J=8.3 Hz)	127.0
4'''-OH	9.50 (1H, s)		9.30 (1H, s)	
1'''		138.5		132.8
2'''	7.30 (1H, d, J=8.0 Hz)	129.8	7.20 (1H, d, J=8.0 Hz)	126.8
3'''	7.25 (1H, m)	126.8	7.06 (1H, d, J=8.0 Hz)	116.8
4'''	7.25 (1H, m)	134.3		159.3
5'''	7.25 (1H, m)	126.8	7.06 (1H, d, J=8.0 Hz)	116.8
6'''	7.30 (1H, d, J=8.0 Hz)	129.8	7.20 (1H, d, J=8.0 Hz)	126.8
4'''-OH			9.12 (1H, s)	

Compound **2** was obtained as white amorphous powder, $[\alpha]_D^{20} -60.28^\circ$ ($c=0.8$, MeOH). Its molecular formula was determined as C₂₇H₂₈N₂O₆ by HR-EI-MS, exhibiting an ion peak at m/z 476.1962 [M] (Calcd 476.1947). The IR spectrum showed absorptions at 3350 (phenyl OH), 3130 (NH), 1735 (COOR), 1665, 1630 (CONH), 1608, 1535 (benzene) cm⁻¹. ¹H- and ¹³C-NMR showed similar data with compound **1**. The difference between **1** and **2** was that two 1,4-disubstituted phenyl groups [7.18 (2H, d, J=8.3 Hz, H-2'', 6''), 7.03 (2H, d, J=8.2 Hz, H-3'', 5''); 7.20 (2H, d, J=8.0 Hz, H-2''', 6'''), 7.06 (2H, d, J=8.0 Hz, H-3''', 5''')] and four active hydrogens [9.30 (1H, s, 4'''-OH), 9.12 (1H, s, 4'''-OH), 7.80 (1H, d, J=7.5 Hz, 8-NH) and 7.64 (1H, d, J=8.0 Hz, 5-NH)] was found. This suggested the presence of two 4-hydroxyphenyl groups, which belonged to a tyrosine and a *p*-hydroxyphenylalaninol respectively by HMBC analysis (Fig. 2) and *N*-benzoyl-L-tyrosinyl-L-*p*-hydroxyphenylalaninol acetate. The configurations of the tyrosine and the *p*-hydroxyphenylalaninol were both determined as L by comparison the $[\alpha]_D^{20}$ of the HCl hydrolysate with standards.

Compound **3** was determined as aurantiamide acetate by comparison physicochemical properties and NMR data with those of references.⁵⁻⁷⁾

Experimental

General Procedures Melting points were measured with a Yanaco melting apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 281 spectrophotometer. Optical rotations were on a HITACHI

U-3210 polarimeter. The HR-EI-MS was measured on Zabspec (England) spectrometer. The NMR spectra were recorded at 500 MHz for ¹H- and 125 MHz for ¹³C-NMR spectra on a Bruker ARX-500 spectrometer, and chemical shifts were given on a δ (ppm) scale with tetramethylsilane as an internal standard. Standard pulse sequences were employed for the DEPT, HMQC and HMBC experiments. TLC were performed on precoated Kieselgel 60 F254 plates (Merck). Column chromatography was carried out on silica gel (200–300 mesh), Sephadex LH-20 (Pharmacia).

Plant Material *Cordyceps sinensis* (BERK.) SACC. was obtained as whole herbs from Ganzi in Sichuan Province of China and identified by Professor Qi-shi Sun, Department of Pharmacognosy of Shenyang Pharmaceutical University. The voucher specimen was deposited at the same department.

Liquid Culture Material The liquid medium of *Cordyceps sinensis* include: sugar (1%), yeast extract (0.4%), CaCl₂ (0.01%), MgSO₄·7H₂O (0.04%), KH₂PO₄ (0.01%). The culture conditions were as follow: temperature: 28±1 °C, light intensity: 58.4 μmol·m⁻²·s⁻¹, light period: 12 h/d, culture time: 72 h 150 rpm, inoculated density: 10% (150 ml medium in 500 ml flask).

Extraction and Isolation The culture liquid of *Cordyceps sinensis* (BERK.) SACC. (201) were dried under vacuum. The dried powder was extracted with methanol under reflux. The methanol extract was subjected to silical gel column chromatography with chloroform and methanol (1:0—0:1) as eluent to afford 15 fractions. The fraction (CHCl₃:CH₃OH 5:1) was subjected to Sephadex LH-20 (CH₃OH:H₂O 8:2) to afford **1** (9 mg), **2** (11 mg) and **3** (59 mg).

Cytotoxic Assay Three kinds of cell lines L929, A375 and HeLa were selected in this experiment. Present results suggested that both compounds **1** and **2** had cytotoxic effects on these three cell lines. On L929 cell and A375 cell compound **1** showed better effect than **2**, but on HeLa cell compound **2** showed better effect.

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