Four New Alkaloids from the Fermentation Broth of *Armillaria mellea*

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Four compounds with similar structures and UV spectra were isolated from the fermentation broth of *Armillaria mellea* by means of preparative HPLC. Their structures were established as methyl (2*S*)-1-[2-(furan-2-yl)-2-oxoethyl]-5-oxopyrrolidine-2-carboxylate (**1**), (2*S*)-1-[2-(furan-2-yl)-2-oxoethyl]-5-oxopyrrolidine-2-carboxylic acid (**2**), 1-[2-(furan-2-yl)-2-oxoethyl]pyrrolidin-2-one (**3**) and 1-[2-(furan-2-yl)-2-oxoethyl]piperidin-2-one (**4**) on the basis of their 1D- and 2D-NMR, and HR-MS data. The absolute configurations of compounds **1** and **2** were determined by comparison of the experimental and calculated electronic circular dichroism (ECD) data. Additionally, four known compounds, **5**–**8**, were also isolated.

Introduction. – *Armillaria mellea* (Ticholomataceae) is a well-known edible and medicinal fungus, symbiotic with the famous Chinese medicinal herb *Gastrodia elata* (Orchidaceae). Its fruit bodies (called *'Zhenmo'* in Chinese) are widely used for the treatment of epilepsy, pain on haunch, and stroke in Chinese folk medicine. Studies have shown that the mycelium and broth from liquid fermentation of *A. mellea* had similar pharmacological activities as *G. elata* itself [1]. As *A. mellea* can be mass-produced by industrial liquid fermentation, it has been used as an alternative to *G. elata*. In China, *A. mellea* is added to many formulations used clinically for the treatment of geriatric patients with palsy, dizziness, headache, neurasthenia, insomnia, numbness in limbs, and infantile convulsion [2].

Many studies about the chemical constituents of *A. mellea* have been performed, and a range of compounds has been reported [2][3].

In this study, four new alkaloids with similar structures were isolated from the AcOEt extract of *A. mellea* fermentation broth using preparative HPLC. In addition, four known compounds were also isolated. Their structures were deduced from NMR and HR-MS data. The absolute configurations of the chiral compounds were determined by comparison of the experimental electronic circular dichroism (ECD) spectra with the quantum-theory calculated ones.

Results and Discussion. – *A. mellea* fermentation broth was diluted with three volumes of 95% EtOH to remove the macromolecular impurities through precipitation. The supernatant was concentrated by evaporation and extracted with AcOEt. The extract was then purified by a combination of *AB-8* macroporous resin, *LH-20*, and silica-gel column chromatography, and preparative HPLC. Eight compounds were isolated (*Fig. 1*); their structures were established on the basis of their NMR and MS spectra.

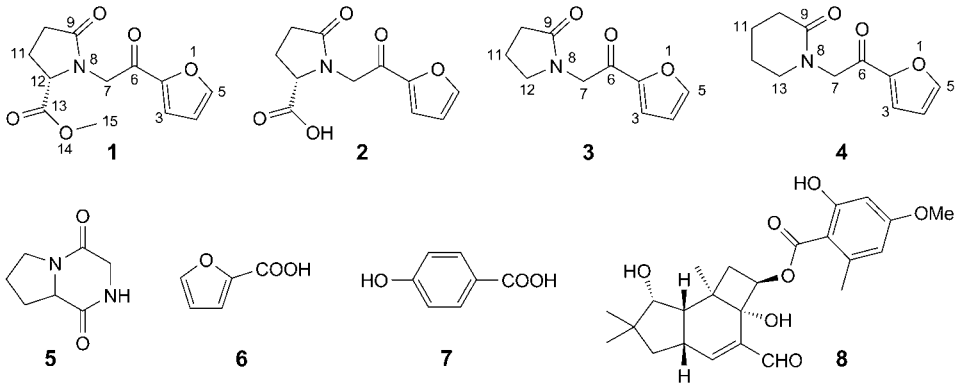

Fig. 1. *Structures of compounds* **1**–**8**

According to the ^{1}H- and ^{13}C-NMR data, compounds **1**–**4** all have a 2-acyl-substituted furan ring. The assignments based on HMQC and HMBC, and data are compiled in *Tables 1* and *2* [4].

Compound **1**, obtained as an optically active, light-yellow semisolid, readily turned dark brown at room temperature. Its molecular formula was established as $C_{12}H_{13}NO_5$ from the HR-ESI-MS (*m*/*z* 274.0683 ($[M+Na]^+$; calc. 274.0686)). The HMQC spectrum revealed that the resonances at δ(H) 5.19 (*d*, *J* = 18.4, 1 H) and 4.27 (*d*, *J* = 18.4, 1 H) were due to the CH_2(7)[1]) group (δ(C) 47.4). The signal at δ(H) 4.50–4.53 (*m*, 1 H) was ascribed to the CH(12) group (δ(C) 59.6); the resonances at δ(H) 2.15–2.19 (*m*, 1 H) and 2.54–2.58 (*m*, 1 H) were attributed to the CH_2(11) group (δ(C) 22.9), and those at δ(H) 2.50–2.53 (*m*, 2 H) to CH_2(10) group (δ(C) 29.0). The large chemical-shift values of C(7) (δ(C) 47.4) and C(12) (δ(C) 59.6) evidenced that C(7) and C(12) were connected to the N-atom, and the HMBC between H–C(7) and C(12) (*Fig. 2*) confirmed this connection. H–C(7) also showed HMBCs with C(9) (δ(C) 175.7) and C(6) (183.1) (*Fig. 2*), so C(7) was further connected to the furan-2-carbonyl moiety, and C(9) (δ(C) 175.7) was part of a lactam group.

In the ^{1}H-NMR spectrum, the shape of the H-atom resonances (*Table 1*) indicated that C(11) and C(10) were connected to C(12) and C(11), respectively. The HMBCs of H–C(11) to C(9), and of H–C(10) to C(9) (*Fig. 2*) indicated that C(10) was further connected to C(9) to form another ring. The resonance at δ(H) 3.75 (*s*, Me(15)) was ascribed to a MeO group. The HMBC between δ(H) 3.75 (H–C(15)) and δ(C) 172.2

[1]) Arbitrary atom numbering as indicated in *Fig. 1*. For systematic names, see the *Exper. Part.*

Table 1. *^{1}H- and ^{13}C-NMR Data for Compounds* **1** *and* **2**. δ in ppm, J in Hz. Arbitrary atom numbering as indicated in *Fig. 1*.

Position	**1**[a])		**2**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
C(2)		151.0		149.3
H–C(3)	7.30 (*d*, J = 3.6)	118.0	7.48 (*d*, J = 3.6)	120.6
H–C(4)	6.56 – 6.57 (*m*)	112.5	6.64 – 6.66 (*m*)	112.4
H–C(5)	7.61 (*s*)	146.9	7.78 (*s*)	148.6
C(6)		183.1		184.3
CH_2(7)	5.19 (*d*, J = 18.4, H_a), 4.27 (*d*, J = 18.4, H_b)	47.4	4.91 (*d*, J = 18.0, H_a), 4.39 (*d*, J = 18.0, H_b)	47.3
C(9)		175.7		179.2
CH_2(10)	2.50 – 2.53 (*m*)	29.0	2.47 – 2.51 (*m*)	28.9
CH_2(11)	2.15 – 2.19 (*m*, H_a), 2.54 – 2.58 (*m*, H_b)	22.9	1.96 – 2.02 (*m*, H_a), 2.46 – 2.54 (*m*, H_b)	22.6
H–C(12)	4.50 – 4.53 (*m*)	59.6	4.13 – 4.17 (*m*)	62.9
C(13)		172.2		178.2
Me(15)	3.75 (*s*)	52.5		

[a]) Recorded in $CDCl_3$. [b]) Recorded in D_2O.

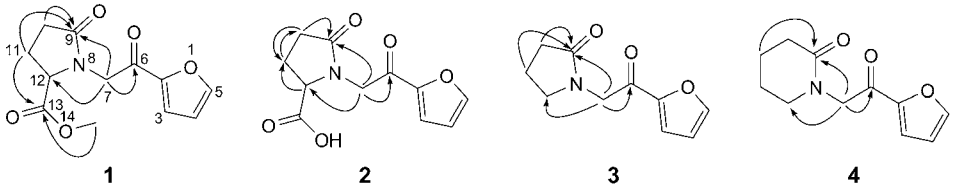

Fig. 2. *Main HMBCs* (H → C) *of compounds* **1** – **4**

(C(13)) (*Fig. 2*) indicated that this group was connected to C(13). C(13)=O was further connected to C(12), and this was confirmed by the HMBC between H–C(11) and C(13). Thus, compound **1** was determined as methyl 1-[2-(furan-2-yl)-2-oxoethyl]-5-oxopyrrolidine-2-carboxylate.

To determine the absolute configuration at C(12), the (*S*)-enantiomer was first analyzed for conformer distribution at molecular-mechanics level mmff 94s_NoEstat force field by using the Omega 2.4.3 program [5]. Twelve conformers with relative energy within 3 kcal/mol were generated and further geometry-optimized in MeOH (with PCM model) at B3LYP/6-31G(d) level using Gaussian 09 program [6]. Six stable conformers were obtained, and frequency analyses were performed at the same level to show no imaginary frequencies. ECD Spectra for the six conformers were calculated using TD-DFT method at B3LYP/6-31 + + G(d,p) level. The final *Boltzmann* factor-weighted theoretical ECD spectrum (*Fig. 3*) showed a positive *Cotton* effect at *ca.* 210 nm and a negative *Cotton* effect at *ca.* 280 nm, which were similar to the experimental ECD spectrum (*Fig. 3*). Thus, compound **1** was identified as (2*S*)-configured.

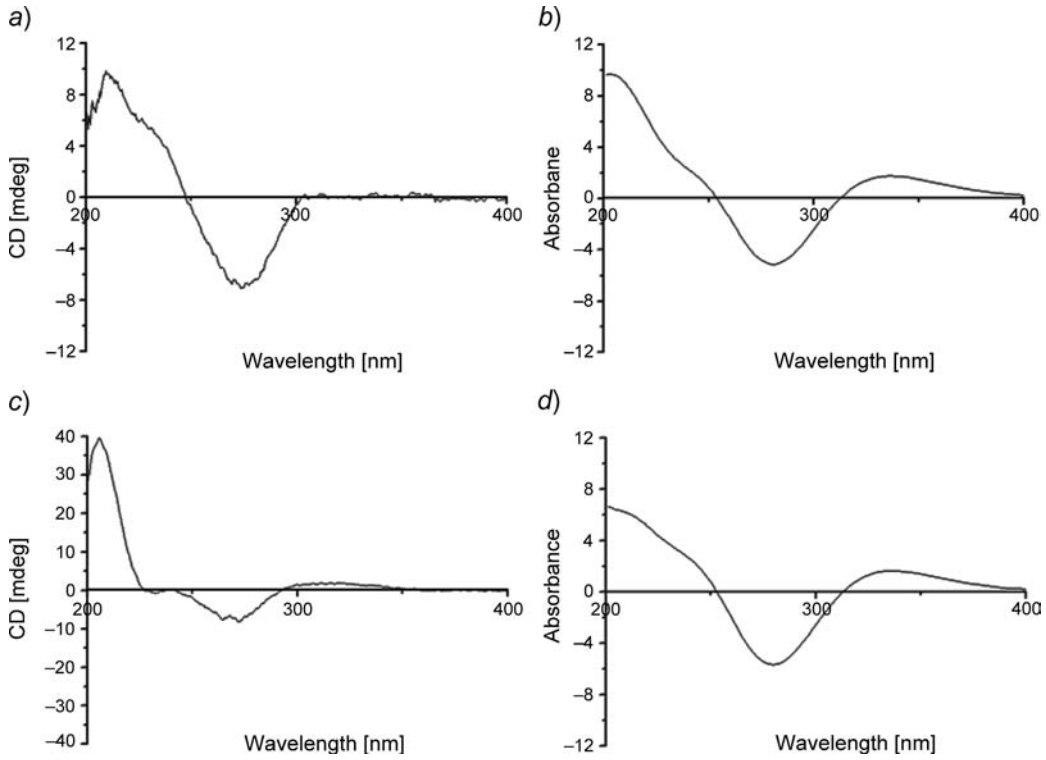

Fig. 3. a) *Experimental ECD spectrum of compound* **1** *in* MeOH. b) *Calculated ECD spectrum for compound* **1** *at B3LYP/6-31G*(*d*) *level* (averaged by *Boltzmann* factors). c) *Experimental ECD spectrum of compound* **2** *in* MeOH. d) *Calculated ECD spectrum for compound* **2** *at B3LYP/6-31G*(*d*) *level* (averaged by *Boltzmann* factors).

Compound **2**, obtained as a light-yellow semisolid, which readily turned dark brown at room temperature. Its molecular formula was established as $C_{11}H_{11}NO_5$ from the HR-ESI-MS (*m*/*z* 260.0526 ($[M+Na]^+$; calc. 260.0529)). The ^{1}H- and ^{13}C-NMR spectra indicated a structure very similar to that of compound **1**, except for the missing MeO group. Thus, compound **2** was identified as 1-[2-(furan-2-yl)-2-oxoethyl]-5-oxopyrrolidine-2-carboxylic acid. This proposal was confirmed by pertinent HMQC and HMBC data (*Fig. 2*).

The absolute configuration of compound **2** was determined in the same way as in for compound **1** above. Sixteen conformers with relative energies within 3 kcal/mol were generated for further geometry optimization at B3LYP/6-31G(d) level, and six stable conformers were obtained. The final *Boltzmann* factor-weighted spectrum, similar to the experimental ECD data, showed a positive *Cotton* effect at *ca.* 210 nm and a negative *Cotton* effect at *ca.* 280 nm (*Fig. 3*). Thus, compound **2** was determined as (2*S*)-configured.

Compound **3** was obtained as a pale brown semisolid, which readily turned dark brown at room temperature. Its molecular formula, $C_{10}H_{11}NO_3$, was established by HR-ESI-MS (*m*/*z* 194.0825 ($[M+H]^+$; calc. 194.0812)). The H-atom resonances at δ(H)

3.52 (*t*, *J* = 7.0, 2 H), 2.48 (*t*, *J* = 8.0, 2 H), and 2.07–2.15 (*m*, 2 H) (*Table 2*) indicated the presence of three CH_2 groups, and there was no other H-atom resonance to be coupled with, so a $CH_2CH_2CH_2$ group (C(12), C(10), C(11)) was established. Based on its large chemical-shift value, the CH_2 group with the resonance at δ(H) 3.52 (CH_2(12)) was determined to be connected to the N-atom. The resonance at δ(H) 4.59 (CH_2(7)) was assigned to another CH_2 group which was connected to the same N-atom based on the HMBC between H–C(7) and C(12) (*Fig. 2*). H–C(7) also showed HMBCs with the C=O resonance at δ(C) 183.0 (C(6)) and 175.8 (C(9)), so C(7) was connected to the furan-2-carbonyl moiety, and C(9) was connected to the N-atom. The C(9)-atom was further connected to C(10) which was supported by HMBCs between H–C(10) and C(9), and H–C(11) and C(9) (*Fig. 2*). Therefore, compound **3** was identified as 1-[2-(furan-2-yl)-2-oxoethyl]pyrrolidin-2-one.

Table 2. *^{1}H- and ^{13}C-NMR Spectroscopic Data for Compounds* **3** *and* **4** *in $CDCl_3$.* δ in ppm, *J* in Hz. Arbitrary atom numbering as indicated in *Fig. 1*.

Position	**3**		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)
C(2)		151.2		151.4
H–C(3)	7.30 (*d*, *J* = 3.6)	117.9	7.28 (*d*, *J* = 3.6)	117.7
H–C(4)	6.55–6.57 (*m*)	112.4	6.55–6.56 (*m*)	112.4
H–C(5)	7.61 (*s*)	146.8	7.59 (br. *s*)	146.6
C(6)		183.0		183.2
CH_2(7)	4.59 (*s*)	48.6	4.66 (*s*)	53.1
C(9)		175.8		170.9
CH_2(10)	2.48 (*t*, *J* = 8.0)	30.3	2.47 (br. *s*)	32.0
CH_2(11)	2.07–2.15 (*m*)	18.0	1.88 (br. *s*)	21.3/23.1
CH_2(12)	3.52 (*t*, *J* = 7.0)	47.9	1.88 (br. *s*)	23.1/21.3
CH_2(13)			3.37 (br. *s*)	49.6

Compound **4** was isolated as a pale-brown semisolid, which readily turned to dark brown at room temperature. The molecular formula was established as $C_{11}H_{13}NO_3$ from the HR-ESI-MS (*m*/*z* 230.0796 ($[M+Na]^+$; calc. 230.0788)). In the HMBC spectrum, H–C(7) (δ(H) 4.66) showed correlations with C(6) (δ(C) 183.2), C(9) (170.9), and C(13) (49.6) (*Fig. 2*), so as in compounds **1**–**3**, C(7) was connected to C(6) and to the N-atom, and C(13) and C(9)=O group were also connected to the N-atom. According to DEPT 135° data, the C-atom resonances at δ(C) 53.1 (C(7)), 32.0 (C(10)), 21.3 (C(11,12), 23.1 (C(12,11), and 49.6 (C(13)) were attributed to four CH_2 groups. Based on HMQC, resonances at δ(H) 1.88 (*s*, 4 H) were assigned to two CH_2 groups (C(11) and C(12)). The COSY spectrum showed that the resonances at δ(H) 3.37 (H–C(13)) and 2.47 (H–C(10)) were both coupled with resonances at δ(H) 1.88 (*Fig. 4*), since the signal at δ(H) 1.88 was due to the two CH_2 groups, the connections of C(10) to C(11), and of C(12) to C(13) were established. H–C(11) showed HMBC with C(9), so C(10) was connected to C(9). According to the molecular formula $C_{11}H_{13}NO_3$, C(11) was connected to C(12) to form another ring. Together with furan-2-carbonyl moiety, compound **4** was elucidated as 1-[2-(furan-2-yl)-2-oxoethyl]piperidin-2-one.

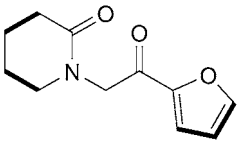

Fig. 4. *Key* $^{1}H,^{1}H$*-COSY* (—) *correlations of compound* **4**

Four known compounds were also isolated and identified, by means of their ^{1}H- and ^{13}C-NMR data, as hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (**5**), furan-2-carboxylic acid (**6**), 4-hydroxybenzoic acid (**7**), and armillarigin (**8**) [3m] (*Fig. 1*).

This work was supported by the *National Natural Science Foundation of China* (Grant Nos. 30873409, 30670220, and 31070318), the *National Key Technologies R&D Program of China* (Grant No. 2007BA138B06), the *Cultivation Fund of the Scientific and Technical Innovation Project of Northeast Normal University* (Grant NENU-STB07008), the *Research Foundation of Jilin Provincial Science & Technology Development* (Grant No. 20102203, YYZX201106), and the *Fundamental Research Funds for the Central Universities*. The calculations using Gaussian 09 were carried out at the School of Chemistry, Northeast Normal University, Renmin Street 5268, Changchun 130024, P. R. China.

Experimental Part

General. HPLC Analysis: *Agilent 1100* apparatus equipped with a DAD detector and *ZORBAX Eclipse XDB-C*$_{18}$ 4.6 mm × 150 mm column. Prep. HPLC: *Waters PrepLC* System with *Waters 2487* UV detector and *Delta-Pak C*$_{18}$ 25 mm × 100 mm column. Optical rotations: *Autopol IV* polarimeter. UV Spectra: recorded online when analyzed using HPLC-DAD method; λ_{max} (log ε) in nm. ECD Spectra: *JESCO-J810* spectrophotometor. NMR Spectra: *Brucker AV 400* NMR spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Brucker micrOTOF* mass spectrometer; in *m*/*z*.

The chromatography fractions were analyzed using HPLC-DAD method. The UV spectra of the compounds were compared with the target UV spectrum by the *Agilent* ChemStation for LC 3D. The fractions containing compounds with relevant UV spectra were further isolated using column chromatography (CC) and finally purified by prep. HPLC.

Material. Armillaria mellea strain was obtained from Institute of Microbiology Chinese Academy of Sciences. The strain was first cultured by shaking in 500 ml of medium (glucose (12 g), soy bean (12 g), cane sugar (24 g), silkworm chrysalis (6 g), $MgSO_4$ (0.9 g), and KH_2PO_4 (1.8 g) per liter) using five 250-ml flasks at 25°, dark, 180 rpm for 5 d. Then, the liquid seed culture was further cultured in a 10-l liquid fermentation tank using the same medium for 6 d. At the end of the fermentation, the culture was filtered to obtain the fermentation broth.

The voucher specimen (No. 2008016) was deposited with the National Engineering Laboratory for Druggable Gene and Protein Screening, Northeast Normal University, China.

Extraction and Isolation. The *Armillaria mellea* liquid fermentation culture was filtered, and then the fermentation broth (100 l) was precipitated by three volumes of 95% EtOH to remove the macromolecular constituents. The supernatant was condensed and extracted with AcOEt. The AcOEt extract was then fractionated by *AB-8* macroporous resin column with H_2O, 20% EtOH, and 95% EtOH to give three fractions, *Frs. 1–3*.

Fr. 1 (5.3 g) was subjected to CC (*Sephadex LH-20*; MeOH) to give four fractions, *Frs. 1.1–1.4*. *Fr. 1.2* was further purified by CC (*LH-20*; gradient H_2O/MeOH) to afford five fractions, *Frs. 1.2.1–1.2.5*. Further purified by CC (silica gel; $CHCl_3$/MeOH), *Fr. 1.2.1* gave compound **5** (8.8 mg) and *Fr. 1.2.4* compound **6** (12.0 mg). *Fr. 1.2.2* was first isolated by CC (silica gel) to give compound **1** and several other subfractions. Since **1** was identified as a so far unknown compound, the other subfractions were analyzed by using HPLC-DAD method. The subfraction which contained a compound with a UV spectrum similar to that of **1** was further separated by prep. HPLC to furnish compound **2** (2.1 mg). *Fr. 1.3* was also

purified by CC (*LH-20*; gradient H_2O/MeOH) to give three fractions, *Frs. 1.3.1–1.3.3*. *Fr. 1.3.2* was separated by CC (silica gel) to furnish compound **7** (6.6 mg).

Fr. 2 (8.9 g) was separated by CC (*Sephadex LH-20*; gradient H_2O/MeOH) to give four fractions, *Frs. 2.1–2.4*. *Fr. 2.2* was further separated by CC (polyamide; H_2O/MeOH from 0 to 100%) to afford four fractions, *Frs. 2.2.1–2.2.4*. *Fr. 2.2.1* was analyzed by HPLC-DAD method to find compounds with UV spectra similar to that of **1**, so it was further subjected to CC (silica gel) to give five subfractions. The second subfraction was analyzed by HPLC-DAD to yield the target compounds, and it was further separated by prep. TLC, and then purified by prep. HPLC to give compounds **3** (3.1 mg) and **4** (2.4 mg).

Fr. 3 (5.7 g) was separated by CC (*Sephadex LH-20*; MeOH) to give four fractions, and the 3rd fraction was subjected to CC (silica gel; $CHCl_3$/MeOH from 0 to 50%) to give three fractions, *Frs. 3.1–3.3*. *Fr. 3.3* was purified by CC (*LH-20*) and then by prep. TLC to give compound **8** (2.0 mg).

Methyl (2S)-1-[2-(Furan-2-yl)-2-oxoethyl]-5-oxopyrrolidine-2-carboxylate (**1**). Light-yellow semisolid. $[\alpha]_D^{25} = -16.9$ ($c = 0.0011$, MeOH). UV: 195, 225, 275. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 274.0683 ($[M+Na]^+$, $C_{12}H_{13}NNaO_5^+$; calc. 274.0686).

(2S)-1-[2-(Furan-2-yl)-2-oxoethyl]-5-oxopyrrolidine-2-carboxylic Acid (**2**). Light yellow semisolid. $[\alpha]_D^{25} = -14.2$ ($c = 0.0017$, MeOH). UV: 195, 225, 275. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 260.0526 ($[M+Na]^+$, $C_{11}H_{11}NNaO_5^+$; calc. 260.0529).

1-[2-(Furan-2-yl)-2-oxoethyl]pyrrolidin-2-one (**3**). Pale-brown semisolid. UV: 195, 225, 275. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 194.0825 ($[M+H]^+$, $C_{10}H_{12}NO_3^+$; calc. 194.0812).

1-[2-(Furan-2-yl)-2-oxoethyl]piperidin-2-one (**4**). Pale-brown semisolid. UV: 195, 225, 275. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 230.0796 ($[M+Na]^+$, $C_{11}H_{13}NNaO_3^+$; calc. 230.0788).

Computation Methods. Conformation analyses were carried out using Omega 2.4.3 [5] program. The resulting conformers were further geometry-optimized at the B3LYP/6-31G(d) level using Gaussian 09 [6] program. Frequency analyses were also carried out at the same level to confirm their minima. *Boltzmann* factor for each conformer was calculated based on *Gibbs* free energy. The excitation energies and rotatory strengths of the obtained stable conformers were calculated using TD-DFT method at B3LYP/6-31++G(d, p) level. The ECD spectra were then simulated using GaussSum program [7]. The final theoretical spectra were generated by average the spectrum of each conformers weighted by their *Boltzmann* factors.

REFERENCES

[1] D.o.P. New Drug Group, Institute of Materia Medica of Chinese Academy of Medical Science, *Chin. J. Med.* **1977**, *8*, 470.

[2] J. S. Yang, Y. W. Chen, X. Z. Feng, D. Q. Yu, C. H. He, Q. T. Zheng, J. Yang, X. T. Liang, *Planta Med.* **1989**, *55*, 564.

[3] a) D. M. X. Donnelly, F. Abe, D. Coveney, N. Fukuda, J. O'Reilly, J. Polonsky, T. Prangé, *J. Nat. Prod.* **1985**, *48*, 10; b) D. M. X. Donnelly, D. J. Coveney, N. Fukuda, J. Polonsky, *J. Nat. Prod.* **1986**, *49*, 111; c) D. M. X. Donnelly, D. J. Coveney, J. Polonsky, *Tetrahedron Lett.* **1985**, *26*, 5343; d) D. M. X. Donnelly, R. M. Hutchinson, *Phytochemistry* **1990**, *29*, 179; e) D. M. X. Donnelly, R. M. Hutchinson, D. Coveney, M. Yonemitsu, *Phytochemistry* **1990**, *29*, 2569; f) D. M. X. Donnelly, P. F. Quigley, D. J. Coveney, J. Polonsky, *Phytochemistry* **1987**, *26*, 3075; g) J. M. Gao, Z. J. Dong, J. K. Liu, *Chin. Chem. Lett.* **2001**, *12*, 139; h) J. M. Gao, X. Yang, C. Y. Wang, J. K. Liu, *Fitoterapia* **2001**, *72*, 858; i) T. Obuchi, H. Kondoh, N. Watanabe, M. Tamai, S. Omura, J.-S. Yang, X.-T. Liang, *Planta Med.* **1990**, *56*, 198; j) N. Watanabe, T. Obuchi, M. Tamai, H. Araki, S. Omura, J.-S. Yang, D.-Q. Yu, X.-T. Liang, J.-H. Huan, *Planta Med.* **1990**, *56*, 48; k) J. S. Yang, Y. W. Chen, X. Z. Feng, D. Q. Yu, X. T. Liang, *Planta Med.* **1984**, *50*, 288; l) J. S. Yang, Y. L. Su, Y. L. Wang, X. Z. Feng, D. Q. Yu, X. T. Liang, *Planta Med.* **1991**, *57*, 478; m) J. S. Yang, Y. L. Su, Y. L. Wang, X. Z. Feng, D. Q. Yu, P. Z. Cong, M. Tamai, T. Obuchi, H. Kondoh, X.-T. Liang, *Planta Med.* **1989**, *55*, 479; n) M. Misiek, J. Williams, K. Schmich, W. Hüttel, I. Merfort, C. E. Salomon, C. C. Aldrich, D. Hoffmeister, *J. Nat. Prod.* **2009**, *72*, 1888; o) M. Bohnert, S. Miethbauer, H.-M. Dahse, J. Ziemen, M. Nett, D. Hoffmeister, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2003; p) M. Misiek, D. Hoffmeister, *Mycol. Prog.*

2011, 1; q) P. Cremin, D. M. X. Donnelly, J.-L. Wolfender, K. Hostettmann, *J. Chromatogr., A* **1995**, *710*, 273.

[4] S. Kametani, A. Kojima-Yuasa, H. Kikuzaki, D. O. Kennedy, M. Honzawa, I. Matsui-Yuasa, *Biosci., Biotechnol., Biochem.* **2007**, *71*, 1220.

[5] OMEGA, Program for conformation analyses, OpenEye Science Software, Santa Fe, New Mexico, USA, 2011.

[6] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, *Gaussian, Inc.,* Wallingford CT, 2009.

[7] N. M. O'Boyle, A. L. Tenderholt, K. M. Langner, *J. Comput. Chem.* **2008**, *29*, 839.

Received April 9, 2012