·化学与分析·

雷公藤内生真菌中的环肽及其乙酰胆碱酯酶抑制活性

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［摘要］ **目的**：内生菌作为一种良好的生物资源，已产生许多结构新颖、活性多样的代谢产物，在进行药用植物内生菌的研究中，分离得到了一株踝节菌属真菌，该属真菌是一个产多样代谢产物的真菌，从该内生菌中分离鉴定环肽类成分，并研究其乙酰胆碱酯酶抑制活性。**方法**：利用柱色谱法，包括硅胶柱色谱、大孔树脂柱色谱和小孔树脂（MCI）凝胶柱色谱等分离化合物；利用波谱方法，包括氢核磁共振、碳核磁共振、电喷雾质谱等鉴定化合物结构；采用改良的 Ellman 法测定化合物的乙酰胆碱酯酶抑制活性。**结果**：分离鉴定了 4 个环肽，它们分别鉴定为 discarine-M（**1**），fumitremorgin C（**2**），fructigenine B（**3**），spirotryprostatin A（**4**），化合物 **1** 显示了中等强度的抗乙酰胆碱酯酶活性，其半抑制浓度（IC_{50}）56 μmol·L^{-1}。**结论**：分离的 4 个化合物均为从踝节菌属（*Talaromyces*）真菌中首次分得。

［关键词］ 环肽；踝节菌属；雷公藤
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Cyclopeptides from Fungal Endophyte Isolated from *Tripterygiun wilfordii* and Their Acetylcholineterase Inhibitory Activity

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［**Abstract**］ **Objective**: Endophytes, as a kind of good biological resources, have already produced many metabolites with new structures and various activities. In this paper, during the study of medicinal plant's endophytes, the author isolated and obtained Talaromyceson fungi which could produce diverse metabolites. This paper is to isolate and identify cyclopeptides from this endophytic fungus and study on their acetylcholineterase inhibitory activity. **Method**: The compounds were isolated by column chromatography methods, including silica gel column chromatography, punching resin column chromatography, and MCI gel chromatography; the structures of compounds were identified using wave spectrum methods, including hydrogen nuclear magnetic resonance (NMR), carbon nuclear magnetic resonance and electrospray mass spectrometry; acetylcholineterase inhibitory activity of the compounds was detected using improved Ellman method. **Result**: Four cyclopeptides were isolated and identified as discarine-M (**1**), fumitremorgin C (**2**), fructigenine B (**3**), spirotryprostatin A (**4**). Compound **1** showed moderate anti-acetylcholineterase activity with IC_{50} value of 56 μmol·L^{-1}. **Conclusion**: All of the four cyclopeptides were isolated and obtained from *Talaromyces* genus for the first time.

［**Key words**］ cyclopeptides; *Talaromyces*; *Tripterygium wilfordii*

Endophytic fungi are microorganisms that live in the intercellular spaces of stems, petioles, roots and

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leaves of plants, without causing visible disease symptoms[1]. Recently, many novel bioactive compounds were isolated from fungal endophytes[2]. *Talaromyces* are a kind of interesting endophytic fungi genus in plant, which have produced many novel natural products such as alkaloids[3-4], anthracenones[5], oxaphenalenones[6], polyesters[7], etc. During our research on chemical components of endophytic fungi of plant, we obtained an endophytic fungus *Talaromyces* sp. GT-5 (GenBank Accession No. KF934202) which was isolated from *Tripterygiun wilfordii*. Through further chemical investigation, four cyclopeptides (**1-4**) were isolated from this strain. Herein, we report the isolation and identification of compounds (**1-4**) and their acetylcholineterase inhibitory activity. Compounds **1-4** were obtained from *Talaromyces* genus for the first time.

1 Material

ESIMS were measured on Bruker APEXII NMR spectra were recorded with Bruker Avance 400 NMR spectrometer. Optical rotations were measured using Jasco P-1030 polarimeter. Silica gel 200-300 mesh for column chromatography and silica GF_{254} for TLC were supplied by Qingdao Marine Chemical Inc., China. HPD-100 macroporous resin and MCI-CHP 20P gel (75-150 μm) were purchased from Hebei Cangzhou Chemical Inc. and Mitsubishi Chemical Holdings Corp. respectively.

2 Fungus isolation and identification

Talaromyces sp. GT-5 was isolated from the rhizomes of *Tripterygium wilfordii* using an established surface sterilization protocol. Briefly, plant tissues were washed with tap water, cut under sterile conditions into small pieces (2-3 cm) and immersed in 0.1% aqueous $HgCl_2$ solution for 6 min. Traces of aqueous $HgCl_2$ solution were removed by washing the plant tissues in sterile distilled water, followed by immersion in 70% EtOH for 3 min. The sterilized rhizomes were then rinsed with distilled water and dried with sterile absorbent paper. The outer tissues were then removed, and the internal tissues were cut into 0.5-1 cm sections and deposited on a Petri dish (3-5 pieces by plate) containing PDA (potato-dextrose agar) and streptomycin sulfate ($0.1\ g \cdot L^{-1}$). Distilled water used for surface sterilization was also incubated to ensure sterile conditions. The plates were incubated at 28 ℃ for five days. Fungal hyphal tips that emerged out of the plant tissues were picked and grown on potato dextrose agar in pure culture. The strain sequence was submitted to GenBank and given an Accession number KF934202. Based on the similarity and morphological characteristics in GenBank search for DNA sequence, the fungus was taxonomically identified as *Talaromyces* genus.

3 Fermentation, extraction and isolation

The endophytic fungus Gt-5 was cultivated in the PDA medium (50 L) for 5 days at 28 ℃ in a fermentor. The culture was filtered and the broth was extracted with EtOAc (50 L × 2) to afford the crude extract (10 g), which was dissolved into 50 mL ethanol and subjected to column chromatography over a HPD-100 macroporous resin with a gradient solvent system EtOH-H_2O (30%, 50%, 70%, 90%) to afford four fractions (Fr. 1 to Fr. 4). Fr. 2 was subjected to MCI-CHP20P gel column chromatography with a gradient solvent system EtOH-H_2O (40%, 50%, 60%, 70%, 80%) to afford five fractions (Fr. 2.1 to Fr. 2.5). Fr. 2.2 was further purified by silica gel column chromatography, and eluted with CH_3Cl-CH_3OH (5∶1) to afford compound **1** (4 mg) and compound **3** (2 mg). Fr. 2.3 was further purified by silica gel column chromatography, and eluted with CH_3Cl-CH_3OH (8∶1) to afford compound **2** (8 mg) and compound **4** (3 mg).

4 Acetylcholineterase (AChE) assays

The procedure of testing AChE inhibitory activity was same with that reported in previous literature[8]. Briefly, 140 μL of $0.1\ mol \cdot L^{-1}$ sodium phosphate buffer (pH 8.0), 20 μL sample solution and 15 μL enzyme solution were mixed and incubated at 4 ℃ for 20 min. 10 μL of $0.01\ mol \cdot L^{-1}$ 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) was added and the reaction was then started by adding 10 μL of $0.075\ mol \cdot L^{-1}$ ATCI. After the reaction solution was incubated at 37 ℃ for 20 min, the optical densities were measured in a 96-well plate reader at 405 nm immediately. Blank positive controls were set up by adding 20 μL huperzine A ($100\ mg \cdot L^{-1}$ in phosphate

buffered saline) instead of 20 μL sample solution. Blanks were set up by adding 20 μL buffer solution instead of 20 μL sample solution. Experiment controls were set up by adding 15 μL buffer solution instead of 15 μL enzyme solution in order to deduct sample background. All reactions were carried out thrice. The inhibition rate (%) was calculated by the following equation:

Inhibition rate = (Blank-Blankpositivecontrol)-(Experiment-Experimentcontrol)/(Blank-Blankpositivecontrol) ×100%

The IC_{50} values (the concentration of test compounds that inhibit the hydrolysis of substrates by 50%) were determined by spectrophotometric measurement of the effect of increasing concentrations of test compounds on enzyme activity. The IC_{50} values were calculated using EZ-Fit Enzyme Kinetics Program (Perrella Scientific Inc., Amherst, USA).

5 Structural elucidation

Discarine-M 1 Amorphous powder, $[\alpha]_D^{20}$ −178.8 (*c* 0.1, MeOH-$CHCl_3$, 1∶1), ESI-MS *m/z* 457.8 $[M+H]^+$, ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 8.46 (1H, d, *J* = 10.4 Hz, NH-20), 7.60 (1H, d, *J* = 10.0 Hz, NH-9), 7.15 (1H, d, *J* = 8.0 Hz, H-14), 7.10 (1H, d, *J* = 8.0 Hz, H-15), 7.01 (1H, d, *J* = 8.0 Hz, H-16), 6.90 (1H, d, *J* = 8.0 Hz, H-13), 6.74 (1H, d, *J* = 8.0 Hz, NH-6), 6.64 (1H, m, H-23), 6.50 (1H, m, H-10), 6.40 (1H, d, *J* = 8.0 Hz, H-11), 5.86 (H, d, *J* = 14.0 Hz, H-22), 4.90 (1H, m, H-3), 4.57 (1H, m, H-4), 3.80 (1H, m, H-7), 2.33 (1H, m, H-24), 2.10 (1H, m, H-17), 1.41 (1H, m, H-27), 1.34 (1H, m, H-27′), 1.28 (1H, m, H-28), 1.10 (3H, d, *J* = 6.4 Hz, Me-19), 0.98 (3H, d, *J* = 6.4 Hz, Me-26), 0.95 (3H, d, *J* = 6.4 Hz, Me-25), 0.82 (3H, d, *J* = 6.4 Hz, Me-18), 0.71 (3H, d, *J* = 6.4 Hz, Me-29), 0.48 (3H, d, *J* = 6.4 Hz, Me-30); ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 171.4 (C-5), 168.0 (C-8), 164.1 (C-21), 155.8 (C-1), 149.7 (C-23), 131.1 (C-128), 130.9 (C-13), 130.3 (C-16), 125.7 (C-10), 122.8 (C-15), 121.4 (C-14), 121.0 (C-22), 116.8 (C-11), 82.2 (C-3), 54.9 (C-4), 52.1 (C-7), 38.6 (C-27), 30.0 (C-24), 28.2 (C-17), 23.4 (C-28), 23.1 (C-29), 21.5 (C-26), 21.4 (C-25), 21.3 (C-30), 20.4 (C-19), 14.4 (C-18)[9].

Fumitremorgin C 2 Colorless amorphous powder, $[\alpha]_D^{20}$ −14.3 (*c* 0.1, MeOH), ESI-MS *m/z* 380.6 $[M+H]^+$, ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H: 7.71 (1H, s, H-1), 7.43 (1H, d, *J* = 8.0 Hz, H-16), 6.86 (1H, s, H-19), 6.80 (1H, d, *J* = 8.0 Hz, H-17), 5.98 (1H, d, *J* = 9.2 Hz, H-3), 4.90 (1H, d, *J* = 9.2 Hz, H-21), 4.18 (1H, dd, *J* = 11.6 Hz, 4.8 Hz, H-12), 4.11 (1H, t, *J* = 8.0 Hz, H-6), 3.83 (3H, s, OMe), 3.64 (2H, m, H-9), 3.50 (1H, dd, *J* = 16.0 Hz, 4.8, H-13a), 3.09 (1H, dd, *J* = 16.0 Hz, 11.6 Hz, H-13b), 2.38 (1H, m, H-7a), 2.25 (1H, m, H-7b), 2.06 (1H, m, H-8a), 1.99 (3H, s, H-24), 1.89 (1H, m, H-8b), 1.68 (3H, s, H-23); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C: 169.5 (C-5), 165.7 (C-11), 156.6 (C-18), 137.0 (C-20), 133.9 (C-22), 132.2 (C-2), 124.2 (C-21), 120.7 (C-15), 118.8 (C-16), 109.5 (C-17), 106.3 (C-14), 95.3 (C-19), 59.2 (C-6), 56.8 (C-12), 55.7 (C-25), 51.0 (C-3), 45.4 (C-9), 28.6 (C-7), 25.7 (C-23), 23.0 (C-8), 21.9 (C-13), 18.1 (C-24)[10].

Fructigenine B 3 Colorless crystal, $[\alpha]_D^{20}$ −160.8 (*c* 0.1, $CHCl_3$), ESI-MS *m/z* 410 $[M+H]^+$. ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H: 7.98 (1H, d, *J* = 8.0 Hz, H-7), 7.32 (1H, t, *J* = 8.0 Hz, H-8), 7.26 (1H, d, *J* = 8.0 Hz, H-10), 7.14 (1H, dd, *J* = 7.8 Hz, 7.2 Hz, H-9), 5.99 (1H, s, H-2), 6.06 (1H, s, H-5a), 5.80 (1H, dd, *J* = 10.4 Hz, 17.2 Hz, H-17), 5.10 (2H, dd, *J* = 11.2 Hz, 5.0 Hz, H-18), 4.00 (1H, d, *J* = 8.0 Hz, H-3), 3.86 (1H, dd, *J* = 5.6 Hz, 10.8 Hz, H-11a), 2.64 (3H, s, H-20), 2.61 (1H, t, *J* = 12.1 Hz, H-12a), 2.40 (1H, t, *J* = 12.0 Hz, H-12b), 1.99 (1H, m, H-13), 1.68 (1H, m, H-11a), 1.56 (1H, m, H-11b), 1.15 (3H, s, H-16a), 0.99 (3H, s, H-16b), 0.98 (3H, d, *J* = 6.6 Hz, H-14), 0.91 (3H, d, *J* = 6.6 Hz, H-15); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C: 172.4 (C-19), 171.1 (C-4), 168.5 (C-1), 144.7 (C-7a), 144.3 (C-17), 134.1 (C-10a), 129.8 (C-8.10), 126.1 (C-9), 120.1 (C-7), 114.8 (C-18), 80.8 (C-5a), 62.2 (C-10b), 60.4 (C-11a), 54.4 (C-3), 41.4 (C-16), 39.8 (C-

11), 36.3 (C-12), 24.0 (C-20), 23.7 (C-13), 23.4 (C-15), 22.7 (C-14), 22.2 (C-16a, 16b)[11].

Spirotryprostatin A 4 Colorless acicular crystals. $[\alpha]_D^{20}$ −31.2 (*c* 0.1, $CHCl_3$), ESI-MS *m/z* 396.0 $[M+H]^+$. ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H: 7.51 (1H, s, H-1), 6.93 (1H, d, *J* = 8.4 Hz, H-4), 6.50 (1H, d, *J* = 8.4 Hz, H-5), 6.43 (1H, s, H-7), 5.00 (2H, m, H-18, 9), 4.77 (1H, *J* = 9.0 Hz, H-19), 4.29 (1H, t, *J* = 8.4 Hz, H-12), 3.80 (3H, s,-OMe), 3.68 (2H, m, H-15), 2.60 (1H, dd, *J* = 10.8 Hz, 13.2, H-13b), 1.95-2.41 (7H, m, H-13a, 14, 15, 8), 1.59 (3H, s, H-21), 1.25 (3H, s, H-22)[12].

The acetylcholineterase (AChE) inhibitory activity of compounds **1-4** was evaluated according to the method in previous literature[8]. The results showed that compound **1** had moderate anti-acetylcholineterase activity with IC_{50} value of 56 μmol·L^{-1}. However, other compounds showed no such activity.

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