Depression which is associated with substantial disability is a major public health issue world-wide, with a high lifetime prevalence ranging from 2 to 15%.[1] Recently, more herbal medicine has been used as alternative therapy for depression.2–4) Due to its natural constituent and availability, natural herbs which obtained from natural sources are believed to provide less untoward effect profiles and provide greater effectiveness as compared to synthetic drug available over the market.[3] The rhizome of *Cyperus rotundus* is a kind of traditional Chinese medicine named “Xiangfuzi,” which is widely used in folk medicine as an anti-inflammatory, antidepressant, analgesic, and antiemetic remedy for dysentery and women’s diseases.[6,7] In previous research, several phenolic glycosides with antidepressant-like effect from the rhizomes of *Cyperus rotundus* have been proven to possess antidepressant activity, two new iridoid glycosides, rotunduside G (1) and rotunduside B (2), were isolated from the rhizomes of *Cyperus rotundus*, together with four known ones, negundoside (3), nishindaside (4), isoorleuropein (5) and neonuezhenide (6). Their structures were elucidated on the basis of spectroscopic methods and from literature values. In mice models of despair, 1 and 2 showed significant antidepressant activity.

**Note**

New Iridoid Glycosides with Antidepressant Activity Isolated from *Cyperus rotundus*

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Received September 6, 2015; accepted October 5, 2015

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**Key words** *Cyperus rotundus*; rotunduside G; rotunduside H; antidepressant

**Results and Discussion**

The phytochemical study of 95% aqueous ethanol extract obtained from the rhizomes of *Cyperus rotundus* afforded six compounds. The structures of new compounds, rotunduside G (1) and rotunduside H (2), were determined by the one dimensional (1D)- and 2D-NMR elucidations, and mass spectral analysis. Rotunduside G (1) was obtained as a white amorphous powder. Its molecular formula was assigned as C_{43}H_{56}O_{25} on the basis of positive-ion high resolution electrospray ionization mass spectrometry (HR-ESI-MS) (m/z 995.3009 [M+Na^+] and ^{13}C-NMR data. Infrared (IR) spectrum showed the absorption bands for hydroxyl (3200–3445 cm\(^{-1}\)), phenyl (1612, 1590 cm\(^{-1}\)) and α,β-unsaturated carboxyl (1699 cm\(^{-1}\)) groups. Positive result of Wieffering field test indicated that 1 could be an iridoid.[15] The following key proton signals were obviously observed in the 1H-NMR spectra of 1: five olefinic protons at δ 7.55 (1H, d, J=161Hz), 7.63 (1H, s), 6.29 (1H, d, J=161Hz), 5.77 (1H, dq, J=2.1, 2.0, 2.0, 2.0Hz), and 5.37 (1H, t, J=6.81Hz), four oxygenated methylene groups at δ 4.24, 4.19 (each 1H, d, J=14Hz), 4.17 (1H, dd, J=12.0, 2.0Hz), 3.86 (1H, m), 4.53 (1H, dd, J=12.1, 2.0Hz), 4.26 (1H, dd, J=12.1, 6.1Hz), and 4.56 (2H, d, J=6.8Hz), a methoxyl proton at δ 3.58 (3H, s), two aromatic protons at δ 6.69 (2H, s), and three anomic protons at δ 4.79 (1H, d, J=7.8Hz), 4.48 (1H, d, J=7.6Hz) and 4.88 (1H, d, J=7.6Hz). The 13C-NMR spectra of 1 also displayed a pair of signals due to the α,β-unsaturated ester groups at δ 166.8, 167.6, 150.3, 147.8, 115.3 and 111.6, and three anomic carbons at δ 100.5, 103.9 and 102.8. Acid hydrolysis of 1 yielded D-glucose and D-glucuronic acid, which were identified by comparison with the respective authentic samples by gas chromatography (GC) analysis.

Comparison of 1H- and 13C-NMR spectra of 1 with those of rotunduside B[9] indicated that 1 had one more glucuronic acid [δH 4.88 (1H, d, J=7.6Hz); δC 102.8, 72.9, 75.3, 71.6, 74.8, 171.7][16] attached at C-4” (δC 141.3). The suggestion was in accord with the observation of the downfield shift of C-4” signal from δ 140.1 in rotunduside B to δ 141.3 in 1. This was further established by the heteronuclear multiple bond correlation (HMBC) correlation from H-1” [δH 4.88 (1H, d, J=7.6Hz)] to C-4” (δC 141.3). Meantime, the occurrence of a prenyl group in the molecule could be easily deduced from the 1H- and 13C-NMR spectra [δH 4.56 (2H, d, J=6.81Hz), 5.37 (1H, t, J=6.8Hz), 1.68 (3H, s), 1.77 (3H, s); δC 60.8, 121.7, 138.4, 25.9, and 18.1][17]. The detailed 2D-NMR analysis of 1H–1H correlated spectroscopy (1H–1H-COSY) and HMBC correlations also implied that 1 had a prenyl group (Fig. 2). Moreover, the prenyl group was attached to the C-6” (δC 171.7) (Fig. 2). Therefore, the structure of 1, which was
Fig. 1. Chemical Structures of Compounds 1–6 Isolated from the Rhizomes of *Cyperus rotundus*

Fig. 2. Key HMBC and $^1$H–$^1$H-COSY Correlations of Compounds 1 and 2
was performed in the same manner as that of antidepressant activity. 


Table 1. Effect of Compounds 1-6 on the Forced Swimming Test (FST) and Tail Suspension Test (TST) in Mice (Mean±S.E.M.)

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>FST immobility duration (s)</th>
<th>Reduction (%)</th>
<th>TST immobility duration (s)</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>90.3±36.1</td>
<td></td>
<td>114.6±19.9</td>
<td></td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>8</td>
<td>33.5±12.9**</td>
<td>62.9</td>
<td>44.9±17.8**</td>
<td>60.8</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>34.1±14.3**</td>
<td>62.2</td>
<td>49.6±23.1**</td>
<td>56.7</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>36.7±11.8**</td>
<td>59.4</td>
<td>54.8±20.9**</td>
<td>52.2</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>66.4±21.4</td>
<td>26.5</td>
<td>99.1±25.3</td>
<td>13.5</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>89.1±16.6</td>
<td>1.3</td>
<td>100.4±27.5</td>
<td>12.4</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>71.3±18.5</td>
<td>21.0</td>
<td>97.3±24.4</td>
<td>15.1</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>83.8±14.7</td>
<td>7.2</td>
<td>89.6±29.1</td>
<td>21.8</td>
</tr>
</tbody>
</table>

**p<0.01, significant as compared to the control group.

established as shown in I, is a new natural compound, which we named rotunduside G.

Rotunduside H (2) was isolated as a white amorphous powder. Its HR-ESI-MS exhibited a quasi-molecular ion peak at m/z 1011.2954 (calculated value 1011.2958) [M+Na]+, which indicated a molecular formula of C_{46}H_{68}O_{26}. The 1H-NMR spectrum of 2 exhibited signals due to four olefonic protons at δ 7.63 (1H, s), 7.54 (1H, d, J=16Hz), 6.29 (1H, d, J=16Hz), 5.79 (1H, dq, J=2.1, 2.0, 2.0, 2.0Hz), along with four oxygenated methylene groups at δ 4.23, 4.17 (each 1H, J=12.4, 2.2 Hz), 3.87 (1H, dd, J=12.4, 6.6Hz), 5.66 (2H, s). The signals of three anomeric protons at δ 4.81 (1H, d, J=7.5Hz), 4.49 (1H, d, J=7.8Hz), 4.89 (1H, d, J=7.4Hz) were also found. Acid hydrolysis of 2 was performed in the same manner as that of I and β-glucose and β-glucuronic acid as sugar residue were identified by GC analysis. 

The 13C-NMR spectrum revealed 43 carbon signals, including one carbonyl at δC 100.7, 104.1, 102.9. A pair of α,β-unsaturated ester signals were found at δ 168.8, 167.8, 150.3, 147.9, 115.5, 111.4. The 1H- and 13C-NMR spectroscopic data of 2 were similar to those of I, with the exception of a 3′′,4′′-methyl-2′′-oxobutyl group [δH 5.66 (2H, s), 2.87 (1H, m), 1.23 (6H, d, J=8.0Hz); δC 68.4, 205.8, 36.7, 17.4, 17.4] at C-6′′ in 2, instead of a prenyl group in I. This was revealed by the 1H-1H correlations of the spin system H-3 with 95% aqueous EtOH (150 L × 2 h). After removing the solvent under reduced pressure, the residue was suspended in water and then sequentially extracted with petroleum ether, CH_{2}Cl_{2}, EtOAc and n-BuOH. The n-BuOH extract (152 g) was submitted through a column chromatography (CC) of high porous absorption resin (Diaion HP-20), eluting with H_{2}O and CH_{3}OH. The methanol fraction (98 g) was extracted three times under reflux with 95% aqueous EtOH (150L×2h). After removing the solvent under reduced pressure, the residue was suspended in water and then sequentially extracted with petroleum ether, CH_{2}Cl_{2}, EtOAc and n-BuOH. The n-BuOH extract (152 g) was submitted through a column chromatography (CC) of high porous absorption resin (Diaion HP-20), eluting with H_{2}O and CH_{3}OH. The methanol fraction (98 g) was repeatedly CC over normal and reverse phase silica gel to afford four fractions (Fr. 1–4). Fraction 1 was subjected to ODS CC eluting with CH_{3}OH–H_{2}O (0 : 1–1 : 0) and silica gel with CHCl_{3}–MeOH–H_{2}O (8 : 2 : 0.3) to give compound 3 (26mg). Fraction 2 was subjected to ODS CC eluting with CH_{3}OH–H_{2}O (0 : 1–1 : 0) and silica gel with CHCl_{3}–MeOH–H_{2}O (8.5 : 15 : 0.15 : 7.3 : 0.3) to give compounds 4 (28mg), 5 (19mg), and 6 (25mg). Fraction 3 was subjected to ODS CC eluting with CH_{3}OH–H_{2}O (7 : 3) and silica gel with CHCl_{3}–MeOH–H_{2}O (8 : 2 : 0.2 : 7.3 : 0.3) to give compound 1 (24mg) and 2 (19mg).

Rotunduside G (I) 

White amorphous powder; [α]D^{25} = 69.6 (c=1.0, MeOH); IR ν_{max} (KBr): 3200–3445, 1601, and 1697 cm^{-1}. UV (MeOH) λ_{max}
1.23 (6H, d, J = 6.7 Hz, H-1), 1.68 (3H, s, H-4), 4.88 (1H, d, J = 7.1 Hz, H-1′), 4.56 (2H, d, J = 6.8 Hz, H-1′′), 1.68 (3H, s, H-4′′), 1.77 (3H, s, H-5′′).

Rotunduside H (2)

White amorphous powder; [α]D 25 −71.3 (c = 1.0, MeOH); IR νmax (KBr): 3200–3445, 1600, 1698 cm−1; UV (MeOH) λmax (log ε): 325 (4.63, 366), and 236 (4.49) nm; HR-ESI-MS m/z: 1011.2954 [M+Na]+ (Calcd for C43H54O25Na, 1011.2958). 1H-NMR (400 MHz, CD3OD) δ: 5.28 (1H, d, J = 7.5 Hz, H-1), 7.63 (1H, s, H-3′), 3.15 (1H, dt, J = 8.0, 8.0, 2.4 Hz, H-5′), 2.40 (1H, ddd, J = 18.4, 2.4, 2.1 Hz, H-6a′), 2.82 (1H, ddd, J = 18.4, 8.0, 2.0 Hz, H-6b′), 5.79 (1H, dq, J = 2.1, 2.0, 2.0, 2.0 Hz, H-7′), 3.06 (1H, t, J = 7.8 Hz, H-9′), 4.23, 4.17 (each 1H, d, J = 13.8 Hz, H-10′), 3.60 (3H, s, H-12′), 4.81 (1H, d, J = 7.5 Hz, H-1′′), 3.13–3.69 (4H, m, H-2′,3′,4′,5′), 4.15 (1H, dd, J = 12.4, 2.2 Hz, H-6′a), 3.87 (1H, dd, J = 12.4, 6.6 Hz, H-6′b), 4.49 (1H, dd, J = 7.8 Hz, H-1′), 3.31–3.58 (4H, m, H-2′,3′,4′,5′), 4.52 (1H, dd, J = 12.0, 2.0 Hz, H-7′), 4.27 (1H, d, J = 6.1 Hz, H-6′b), 6.70 (2H, s, H-2′, H-6′), 7.54 (1H, d, J = 16.1 Hz, H-6′a), 6.29 (1H, d, J = 16.1 Hz, H-β), 4.89 (1H, d, J = 7.4 Hz, H-1′′), 4.31 (1H, d, J = 8.0 Hz, H-5′′), 5.66 (2H, s, H-1′′′), 2.87 (1H, m, H-3′′′), 1.23 (6H, d, J = 8.0 Hz, H-4′′′, H-5′′′). 13C-NMR (100 MHz, CD3OD) δ: 98.1 (C-1), 150.3 (C-3), 111.4 (C-4), 35.3 (C-5), 39.1 (C-6), 127.3 (C-7), 147.1 (C-8), 46.6 (C-9), 60.9 (C-10), 167.7 (C-11), 51.9 (C-12), 100.5 (C-1′), 75.3 (C-2′), 78.4 (C-3′), 71.8 (C-4′), 78.0 (C-5′), 70.1 (C-1′′), 75.3 (C-2′′), 77.9 (C-3′′), 71.2 (C-4′′), 74.6 (C-5′′), 64.4 (C-6′′), 128.6 (C-1′′′), 107.1 (C-2′′′), 143.1 (C-3′′′), 144.4 (C-4′′′), 168.6 (C=O), 115.3 (C-α′′′), 147.8 (C-β′′′), 102.8 (C-1′′′′), 72.9 (C-2′′′′), 75.3 (C-3′′′′), 71.6 (C-4′′′′), 74.8 (C-5′′′′), 171.7 (C-6′′′′), 60.8 (C-1′′′′′), 121.7 (C-2′′′′′), 138.4 (C-3′′′′′), 25.9 (C-4′′′′′), 18.1 (C-5′′′′′).

Acknowledgments

This study was supported by the National Natural Science Foundation of China (31400295).

Acid Hydrolysis of Compounds 1 and 2

Each compound (1.0 mg) was heated at 95°C with dioxane (0.5 mL) and 5% H2SO4 (0.5 mL) for 1 h. After neutralization with Amberlite IRA-400 (OH− form), each reaction mixture was concentrated and the residue was passed through a Sep-Pak C18 cartridge with H2O. The eluate was concentrated and the residue was treated with i-cysteine methyl ester hydrochloride (1 mg) in pyridine (0.125 mL) at 60°C for 1 h. The solution was then treated with N,O-bis(trimethylsilyl)trifluoroacetamide (0.05 mL) at 60°C for 1 h. The supernatant was applied to GC; GC conditions: column, Supelco SPB-5, 30 m×0.25 mm; column temperature, 230°C; N2 flow rate, 0.8 mL/min; tR, 22.80 min (β-glucose), 22.01 min (α-glucose), 22.87 min (d-glucuronic acid), 22.09 min (l-glucuronic acid), 20.21 min (d-glucose) and l-glucuronic acid were all detected in compounds 1 and 2.

Statistical Analysis

Data analysis was performed by one-way analysis of variance with the Dunnett’s post-hoc test for multiple comparisons by SPSS 10.0 software. Data were expressed as the mean±standard error of the mean (S.E.M.). The level of statistical significance was set at p<0.05.
the China Spark Program (2014GA780014), Science and Technology Planning Project of Guangdong Province (2014A020221057).

**Conflict of Interest** The authors declare no conflict of interest.

**References**


