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A New Iridoid Glycoside from the Rhizomes of *Cyperus rotundus*

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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, antipyretic, analgesic, and antiemetic remedy for dysentery and women's diseases.[1,2] Previous phytochemical studies on this plant have led to the isolation and identification of iridoid glycosides, flavonoids, glycosides and furochromones, and many new sesquiterpenoids.[3-7] Our studies indicated that the 95% aqueous ethanol extract of rhizomes of *Cyperus rotundus* showed considerable inhibition activity against macrophages respiratory burst (MRB).[2] To further investigate the constituents and screen the bioactive constituents from its rhizomes, a phytochemical study was performed that resulted in the isolation of a new iridoid glycoside and two known phenolic glycosides . Two known compounds, including Pungenin (**2**), Salidroside (**3**) were isolated for the first time from *Cyperus rotundus*.[8,9] In the present article, we describe the structural elucidation of a new compound named rotunduside C, together with the MRB inhibitory activity test performed for all isolated compounds (Figrue 1).

Rotunduside C (**1**) was isolated as a white amorphous powder and gave a molecular formula of $C_{34}H_{44}O_{19}$, as determined by HRESIMS (*m*/*z* 779.2373 $[M+Na]^+$), requiring an index of hydrogen deficiency of 13. Positive result of Wieffering field test indicated that 1 could be a iridoid. The IR spectrum showed absorptions at 3212-3449, 1603, and 1699 cm^{-1} consistent with the presence of hydroxyl, phenyl, and α,β-unsaturated carboxyl groups respectively. Analysis of the ^1H and ^{13}C NMR spectroscopic data (Table 1) led to the identification of the following representative signals: δ 7.63 (1H, s), 7.55 (1H, d, J = 16 Hz), 6.79 (2H, d, J = 1.8 Hz), 6.14 (1H, d, J = 16 Hz), 5.26 (1H, d, J = 7.5 Hz), 4.24, 4.19 (each 1H, d, J = 14 Hz), 3.58 (3H, s), 3.86 (6H, s) as well as two anomeric protons δ 4.79 (1H, d, J = 7.8 Hz), 4.48 (1H, d, J = 7.6 Hz). The ^{13}C NMR spectra displayed 34 signals, of which 13 resonated in the region 60-90 ppm, 6 carbon resonances for a phenyl group, 3 carbon signals for a α,β-unsaturated carboxyl group, and 2 methine signals at δ 100.1 and 103.6 suggested the presence of two anomeric carbons of two hexose residues. All the information mentioned above supports 1 be an iridoid diglycoside.

The ^1H and ^{13}C NMR spectroscopic data of **1** were similar to those of rotunduside B,[7] with the exception of two hydroxyl groups at C-3''' and C-5''' in rotunduside B, instead of two methoxyl groups (δ3.86; δ56.9) in **1**. The suggestion was in accord with the observation of the downfield shift of C-3''' and C-5''' signals from δ 148.6 in rotunduside B to δ 149.4 in **1**. This was further established by the HMBC correlations from the methoxyl protons (δ3.86) to C-3''' and C-5''' (Figrue 2). Therefore, the structure of **1**, which was established as shown in **1**, is a new natural compound, which we named rotunduside C.

All the isolated compounds were tested for their inhibitory activity against MRB with chemiluminescence detection. Rutin (IC_{50} 15.07 ± 2.51 μmol/L) and dexamethasone (IC_{50} 355.14 ± 45.76 μmol/L) were used as control test reagents. The IC_{50} values for **1-3** were 47.13 ± 0.24, 174.11 ± 5.03 and 168.91 ± 1.57 μmol/L, respectively. Compound **1** showed moderate activity with IC_{50} value of approximately 47 μM. The weak MRB inhibitory activity of the initial extract (compounds **1**–**3**) is thus presumably due to other as yet unidentified compounds. One possible reason for this is that compounds with highly MRB inhibitory activity are low in the BuOH-soluble fraction of the 95% aqueous ethanol extract from the rhizomes of *Cyperus rotundus*. Another

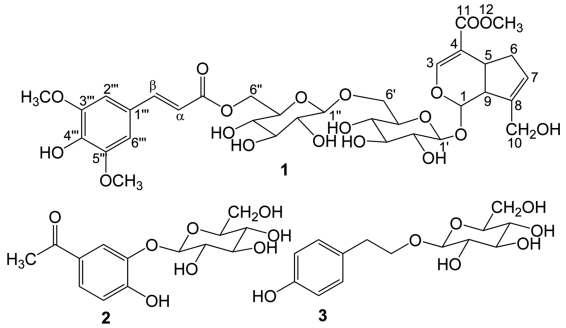

Figure 1. Chemical structures of compounds **1-3** isolated from the rhizomes of *Cyperus rotundus.*

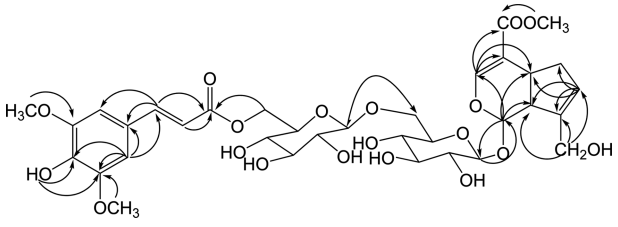

Figure 2. Key HMBC (→) correlations of **1**.

possible reason is compounds with increased activity may be found in other extraction fractions (ethyl acetate extract, chloroform extract and petroleum ether extract) of the 95% aqueous ethanol extract from the rhizomes of this medicine plant. Therefore, more chemical work needs to be done in the future.

Experimental Section

Reagents and Instruments. UV spectra were recorded on a Hewlett-Packard HP-845 UV-VIS spectrophotometer. Specific rotation measurements were recorded on a Perkin-Elmer 242 MC polarimeter. IR spectra were recorded on a Nicolet 470 spectrometer and MS on a Varian MAT-212 mass spectrometer and a Shimadzu GC-MS model QP2010 Plus spectrometer, respectively. NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^{1}H NMR) using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as internal standard. The chemiluminescence value was recorded by BPCL-1-G-C Ultraweak Luminescence Analyzer (Beijing Institutes for Biophysics, Chinese Academy of Science). RPMI-1640, Phorbol 12-myristate 13-acetate (PMA) and fetal calf serum (FCS) were obtained from GIBCO (USA), respectively. Column chromatography separations were carried out onsilica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd, Qingdao, P.R. China), ODS (50 mesh, AA12S50, YMC), and Diaion HP-20 (Pharmacia, Peapack, New Jersey, USA). All other chemicals used were of biochemical reagent grade.

Plant Material. The rhizomes of *Cyperus rotundus* were collected in Zhanjiang, Guangdong Province of China in September 2009, and were identified by Wen-qing Yin (School of Chemistry & Chemical Engineering of Guangxi Normal University, Ministry of Education Key Laboratory of Chemistry and Molecular Engineering of Medicinal Resource, Guilin). A voucher specimen (No. 20090903) has been deposited in the authors' laboratory.

Extraction and Isolation. The dry rhizomes of *Cyperus rotundus* (10 kg) were extracted three times under reflux with 95% aqueous ethanol (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_2Cl_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_2O and CH_3OH. The methanol fraction (98 g) was repeatedly CC over normal and reverse phase silica gel to afford four fractions (Frs.1-4). Fr.3 was subjected to ODS CC eluting with CH_3OH-H_2O (0:1-1:0 and silica gel with $CHCl_3$-MeOH-H_2O (8:2:0.2-7:3:0.3) to afford compounds **1** (31 mg). Fr.4 was subjected to ODS CC eluting with CH_3OH-H_2O (6:4) and silica gel with $CHCl_3$-MeOH-H_2O (8:2:0.2-7:3:0.3) to give compounds **2** (25 mg) and **3** (19 mg).

Macrophages Respiratory Burst Inhibitory Activity. The murine macrophage-like cell line RAW 264.7 was routinely cultivated at 37 °C, 5% CO_2 in RPMI-1640 supplemented with 10% FCS (Hyclone, America), 100 μg/mL streptomycin, 118 μg/mL ampicillin, and 2 mg/mL sodium bicarbonate. After 72 h, the macrophage cellsformed a confluent monolayer. The monolayer cells were digested with trypsin. After washed with PBS without Ca^{2+} and Mg^{2+}, the deposited cells were suspended with RPMI-1640 without FCS (approximately 2×10^6 cell/mL) in the vitreous culture flask.[10,11]

The details of chemiluminescence assay procedure were according to the method described in the literature.[12] Tested

Table 1. ^{13}C and ^{1}H NMR spectroscopic data of **1** (in C_5D_5N)

Position	^{13}C (δ)[a]	^{1}H (δ) (*J* in Hz)	Position	^{13}C (δ)	^{1}H (δ) (*J* in Hz)
1	98.2 (d)	5.26 d (7.5)	1"	103.6 (d)	4.48 d (7.6)
3	150.3 (d)	7.63 s	2"	75.5 (d)	3.30 m
4	111.8 (s)		3"	78.1 (d)	3.44 m
5	36.1 (d)	3.16 ddd (8.0, 8.0, 2.4)	4"	71.6 (d)	3.57 m
6	39.3 (t)	2.39 ddd (18.4, 2.4, 2.1)	5"	74.8 (d)	3.45 m
		2.80 ddd (16.6, 8.0, 2.0)	6"	64.6 (d)	4.53 dd (12.1, 2.0), 4.26 dd (12.0, 6.1)
7	127.3 (d)	5.77 dddd (2.1, 2.0, 2.0, 2.0)	1"'	126.8 (s)	
8	147.1 (s)		2"'	107.6 (d)	6.79 d (1.8)
9	46.9 (d)	3.04 t (7.7)	3"'	149.4 (s)	
10	60.5 (t)	4.24, 4.19 d (14)	4"'	141.3 (s)	
11	167.8 (s)		5"'	149.4 (s)	
12	51.6 (q)	3.58 s	6"'	107.6 (d)	6.79 d (1.8)
1'	100.1 (d)	4.79 d (7.8)	C=O	167.3 (s)	
2'	75.2 (d)	3.11 m	α	114.7 (d)	6.14 d (16.0)
3'	78.1 (d)	3.68 m	β	147.9 (d)	7.55 d (16.0)
4'	72.0 (d)	3.57 m	-OCH_3	56.9 (q)	3.86 s
5'	78.1 (d)	3.54 m, 3.63 m			
6'	70.2 (t)	4.17 dd (12.0, 2.0), 3.86 m			

compounds were prepared as 10 mM top stocks, dissolved in DMSO, and stored at 4 °C. Phorbol 12-myristate 13-acetate (PMA) was applied as triggering agent. Data were collected at a frequency of 6 s/min and chemiluminescence was recorded for up to 30 min. The IC_{50} values were obtained bylinear regression analysis of the dose response curves, which were plots of % inhibition versus concentration.[13]

Rotunduside C (1). White amorphous powder. $[\alpha]_D^{25}$ −77.1° (*c* 1.0, MeOH); IR ν_{max} (KBr): 3212-3449, 2931, 1699 and 1603 cm^{-1}. HRESIMS *m/z* 779.2373 $[M+Na]^+$, Calcd. for $C_{34}H_{44}O_{19}Na$, 779.2349. ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) data see Table 1.

Pungenin (2). White powder, EI-MS *m/z* 314 $[M]^+$. ^1H-NMR (CD_3OD, 500 MHz) δ 2.49 (3H, s, -$COCH_3$), 4.88 (1H, d, *J* = 7.8 Hz, H-1′), 6.95 (1H, d, *J* = 8.4 Hz, H-3), 7.68 (1H, dd, *J* = 8.4 Hz, 2.1 Hz, H-4), 7.86 (1H, d, *J* = 2.1 Hz, H-6); ^{13}C-NMR (CD_3OD, 125 MHz) δ 144.8 (C-1), 155.3 (C-2), 114.6 (C-3), 124.7 (C-4), 131.9 (C-5), 112.8 (C-6), 197.3 (-$COCH_3$), 26.8 (CH_3), 101.4 (C-1′), 74.8 (C-2′), 77.8 (C-3′), 70.9 (C-4′), 78.1 (C-5′), 62.1 (C-6′).

Salidroside (3). White needle, ESI-MS *m/z* 323 $[M+Na]^+$. ^1H-NMR (CD_3OD, 500 MHz) δ 7.07 (2H, d, *J* = 8.4 Hz, H-2, 6), 6.68 (2H, d, *J* = 8.4 Hz, H-3, 5), 2.81 (2H, m, H-7), 3.64 (2H, m, H-8), 4.31 (1H, t, *J* = 6.0 Hz, H-1′), 3.09-4.02 (6H, m, H-2′-6′); ^{13}C-NMR (CD_3OD, 125 MHz) δ 130.6 (C-1), 130.9 (C-2, 6), 116.1 (C-3, 5), 156.9 (C-4), 36.3 (C-7), 72.3 (C-8), 104.3 (C-1′), 75.1 (C-2′), 78.0 (C-3′), 71.4 (C-4′), 78.2 (C-5′), 62.6 (C-6′).

Wieffering Field Test. Field tests involving chemical color tests include heating with dilute hydrochloric acid or acetous copper sulfate. Color reaction in conjunction with thin-layer chromatography (TLC), as developed by wieffering, is currently used.[14] Sample was tested by TLC and heated with acetous copper sulfate. In general, a compound responds positively to Wieffering field test, giving blue colouration, which indicates it as an iridoid.

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