SHORT REPORT

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A New Eremophilanolide from the Fresh Roots of *Rehmannia glutinosa*

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Abstract: A new eremophilanolide, serratifolide F (**1**), and six known compounds were isolated from the fresh roots of *Rehmannia glutinosa*. Their structures were characterized by analysis of NMR, CD, HRESIMS, and comparison of the data in previous literatures. The protective effects against lipopolysaccharide (LPS)-stimulated damage on normal rat kidney tubule epithelioid (NRK-52e) cells of the compounds were evaluated using MTT assay and real time cellular analysis (RTCA). The results showed that compound **3** exhibited renoprotective activity with EC_{50} value of 45.1 μM.

Keywords: Scrophulariaceae; *Rehmannia glutinosa*; eremophilanolide; glycosides; renoprotective activity.

1. Plant Source

The fresh roots of *Rehmannia glutinosa* Libosch were collected from Jiaozuo, Henan Province in China, in November 2018, which was identified by Prof. Cheng-Ming Dong. The voucher specimen (No.20181101A) was deposited at the Department of Natural Medicinal Chemistry, Henan University of Chinese Medicine, Zhengzhou, China.

2. Previous Studies

R. Glutinosa, belonging to the Scrophulariaceae family, is mainly distributed in the provinces of Henan, Shandong and Hubei in China. As a traditional Chinese herbal medicine, it is believed that *R. glutinosa* possessed the effects of reducing *fever* and activating blood circulation, nourishing *Yin* and tonifying the kidney [1]. There have been many reports of the pharmacological functions and activities, such as anti-osteoporosis [2], neuroprotective activities [3], and anti-hypertensive activities [4].

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Chemical studies of *R. glutinosa* show that many bioactive compounds have been isolated, such as iridoid glycosides and phenethyl alcohol glycosides [5-7], which established a foundation of pharmacological research.

3. Present Study

In the continuing research on the bioactive constituents of *R. glutinosa* [8-9], one new eremophilanolide, serratifolide F (**1**), and six known compounds (**2**–**7**) (Figure 1) were isolated from the fresh roots of this plant. Herein, the isolation, structure elucidation, and bioactivity of compounds were elucidated.

The fresh roots (80 kg) were cut into small pieces and extracted with 70 % aqueous acetone (2 × 150 L, smashed tissue extraction). The extract (15 kg) was suspended in water and sequentially partitioned with petroleum ether, CH_2Cl_2, EtOAc, and *n*-BuOH for fifteen times. The EtOAc fraction (80.0 g) was passed through silica gel CC (12 × 150 cm) with a CH_2Cl_2-MeOH (100:0→0:100) gradient to obtain E1–E5. Fraction E5 (16.5 g) was rechromatographed with MCI gel CHP-20 CC eluted with MeOH/H_2O (v/v) from 0% to 100% to give E5-1–E5-5. Subfraction E5-2 (8.3 g) was separated by ODS gel CC with MeOH/H_2O (v/v) from 10% to 100% to yield E5-2-1–E5-2-8. Subfraction E5-2-3 (1.1 g) was purified by Sephadex LH-20 CC (MeOH-H_2O 30:70→100:0) and fractionated by semipreparative HPLC (MeOH-H_2O 25:75) to produce compounds **2** (6.4 mg) and **7** (3.6 mg). Subfraction E5-2-5 (1.3 g) was separated by Toyopearl HW-40C (MeOH-H_2O 50:50) and fractionated by semi-preparative HPLC (MeOH-H_2O 45:55) to produce compound **1** (3.2 mg). The *n*-BuOH fraction (380.0 g) was separated by Diaion HP-20 CC(18 × 160 cm) eluted with a MeOH-H_2O (100:0→0:100) gradient and yielded F1–F8. Fraction F4 (10 g) was subjected to silica gel CC (8 × 140 cm) with a CH_2Cl_2-MeOH (170:1→6:1) gradient to produce F4-1–F4-7. Subfraction F4-2 (1.2 g) was rechromatographed with Toyopearl HW-40C column (MeOH-H_2O 50:50) to obtain F4-2-1–F4-2-6. Subfraction F4-2-5 (445.1 mg) was purified by semipreparative HPLC (MeOH-H_2O 34:66) to produce compounds **6** (2.7 mg) and **4** (8.2 mg). Subfraction F4-2-6 (1.1 g) was separated by Sephadex LH-20 CC (MeOH-H_2O 70:30), followed by purification by semipreparative HPLC eluting with MeOH-H_2O (21:79) to give compounds **5** (5.1 mg) and **3** (4.3 mg).

*Serratifolide F **(1)**:* Colorless amorphous solid; $[\alpha]_D^{20}$ = – 1.5 (*c* = 0.05, MeOH); IR (iTR) ν_{max} = 3340, 2925, 1737, 1674, 1446, 1020, 799 cm^{-1}; UV(MeOH) λ_{max} = 218 nm; HRESIMS *m/z* 465.1749 $[M+Na]^+$ (calcd for $C_{21}H_{30}O_{10}Na$, 465.1732); ^1H NMR (500 MHz, CD_3OD): δ (ppm) = 7.04 (1H, t, *J* = 3.9 Hz, H-1), 4.91 (2H, d, *J* = 8.6 Hz, H-12), 4.78 (1H, d, *J* = 14.6 Hz, H-13a), 4.67 (1H, d, *J* = 14.6 Hz, H-13b), 4.27 (1H, d, *J* = 7.8 Hz, H-1'), 3.86 (1H, d, *J* = 11.8 Hz, H-6'a), 3.67 (1H, m, H-6'b), 3.28 (1H, *overlap*, H-5'), 3.33 (1H, overlap, H-3'), 3.27 (1H, overlap, H-4'), 3.18 (1H, overlap, H-2'), 2.85 (1H, d, *J* = 13.9 Hz, H-6a), 2.75 (1H, d, *J* = 13.9 Hz, H-6b), 2.15 (2H, t, *J* = 4.9 Hz, H-2), 1.80 (1H, overlap, H-3a), 1.76 (1H, overlap, H-4), 1.40 (1H, m, H-3b), 1.18 (3H, s, H-15), 0.93 (3H, d, *J* = 6.8 Hz, H-14); ^{13}C NMR (125 MHz, CD_3OD): δ (ppm) = 177.6 (C, C-8), 170.8 (C, C-9), 162.4 (C, C-11), 143.3 (CH, C-1), 137.1 (C, C-10), 126.8 (C, C-7), 104.2 (CH, C-1'), 78.1 (CH, C-5'), 77.9 (CH, C-3'), 74.8 (CH, C-2'), 72.3 (CH_2, C-12), 71.5 (CH, C-4'), 65.2 (C, C-13), 62.7 (CH_2, C-6'), 41.9 (C, C-5), 36.6 (CH, C-4), 33.1 (CH_2, C-6), 26.7 (CH_2, C-3), 25.1 (CH_2, C-2), 21.9 (CH_3, C-15), 16.2 (CH_3, C-14).

*Acid Hydrolysis of Compound **1***: The sugar was determined by the method of acid hydrolysis as previous literature [10]. By comparison with the retention time of standard substance, the sugar was identified as D-glucose (t_R = 18.3 min).

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Activity Assays: MTT assay: The testing method of cell viability was applied as previous report [9]. RTCA assay: Briefly, the NRK 52e cells were distributed into 16-well plates at 1 × 10^4 cells/well in 150 μL of medium. After 24 h, the cells were treated with LPS (1 μg/mL) and the test compounds at

five concentrations (0.1, 1, 10, 50, 100 μM). Then, the signal was measured every 5 min for the following time [10].

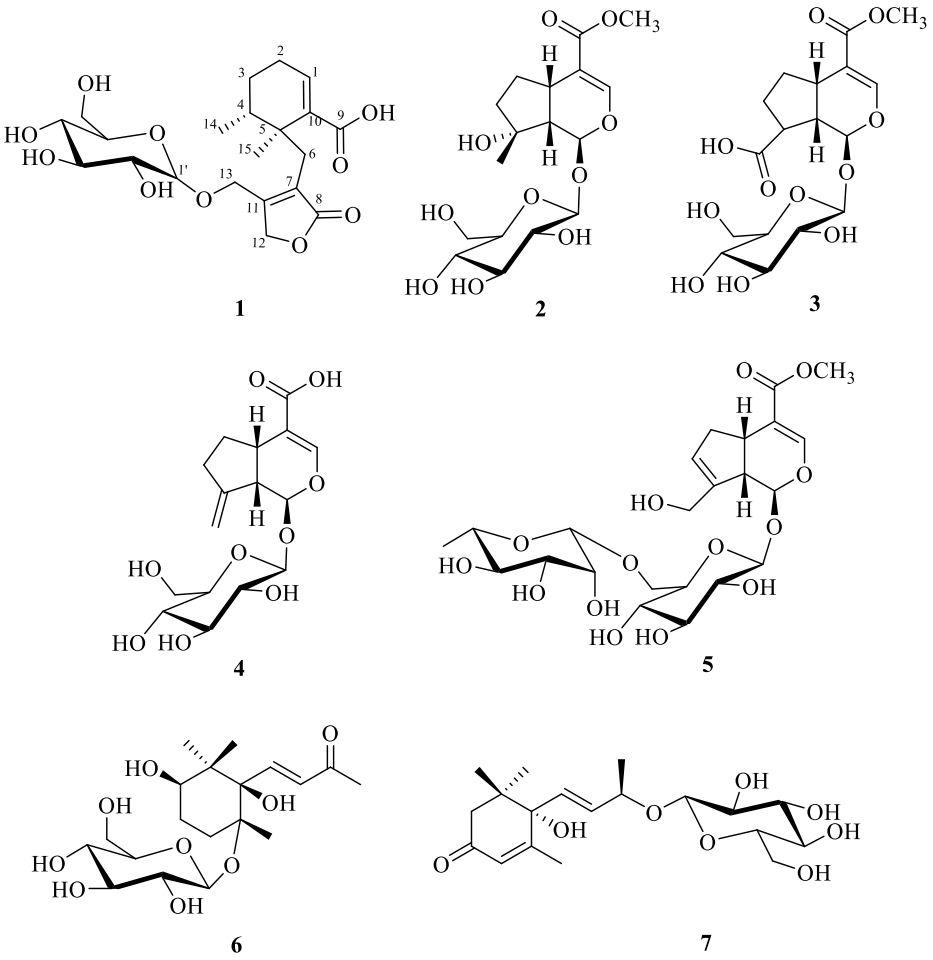

Figure 1. The structures of compounds **1-7**

Compound **1** was isolated as a colorless, amorphous solid. The IR absorption bands indicated the presence of hydroxyl (3340 cm^{-1}), carbonyl (1737, 1674 cm^{-1}) groups. The molecular formula $C_{21}H_{30}O_{10}$ was determined by HRESIMS data (*m/z* 465.1749 $[M+Na]^+$).The ^1H NMR spectral data of **1** displayed resonances for an olefinic proton [δ_H 7.04 (1H, t, *J* = 3.9 Hz, H-1)], two oxygenated methylenes [δ_H 4.91 (2H, d, *J* = 8.6 Hz, H-12), 4.78 (1H, d, *J* = 14.6 Hz, H-13a), 4.67 (1H, d, *J* = 14.6 Hz, H-13b)], three methylenes [δ_H 2.85 (1H, d, *J* = 13.9 Hz, H-6a), 2.75 (1H, d, *J* = 13.9 Hz, H-6b), 2.15 (2H, t, *J* = 4.9 Hz, H-2), 1.80 (1H, overlap, H-3a), 1.40 (1H, m, H-3b)], and two methyl groups [δ_H 1.18 (3H, s, H-15), 0.93 (3H, d, *J* = 6.8 Hz, H-14)]. Its ^{13}C NMR data revealed the presence of an ester carbonyl carbon [δ_C 177.6 (C-8)], a carboxylic carbon [δ_C 170.8 (C-9)], four olefinic carbons [δ_C 162.4 (C-11), 143.3 (C-1), 137.1 (C-10), 126.8 (C-7)], two oxygenated methylenes [δ_C 72.3 (C-12), 65.2 (C-13)], three methylenes [δ_C 33.1 (C-6), 26.7 (C-3), 25.1 (C-2)], and two methyl carbons [δ_C 21.9 (C-15), 16.2 (C-14)]. These spectroscopic features suggested that the structure of **1** was very similar to that of 4,5,11-trimethyl-8,9-*seco*-1(10),7(11)-eremophiladien-8,12-olid-9-oic acid [11], except for the presence of a hexose moiety and an oxygenated methylene at C-13. The hexose moiety was determined to be D-glucose by chiral-phase HPLC analysis. The anomeric proton (δ_H 4.27) had a large coupling constant (*J* = 7.8 Hz), indicating a *β*-configuration. Furthermore, the HMBC crosspeaks from H-1' (δ_H 4.27) to C-13 (δ_C 62.5) established the D-glucose is attached to the C-13 (Figure 2). The NOESY correlations between H_3-15 (δ_H 1.18) and H_3-14 (δ_H 0.93) indicated that the relative configuration of compound **1** (see supporting information S12). To determine the absolute configuration, its experimental circular dichroism spectra (CD) were compared with calculated

electronic circular dichroism (ECD) spectra [12-13], the (4*S*, 5*R*) of **1** was determined (Figure. 3). Accordingly, the structure of **1** was elucidated and named as serratifolide F.

Six known compounds were identified as mussaenoside (**2**) [14], 11-methylforsythide (**3**) [15], 7-deoxygardoside (**4**) [16], 1-*O*-*α*-L-rhamnopyranosyl(1-6)-*β*-D-glucopyranoside (**5**) [17], melasmoside (**6**) [18], (6*S*, 9*R*)-roseoside (**7**) [19] by comparison of the NMR data with previously reported.

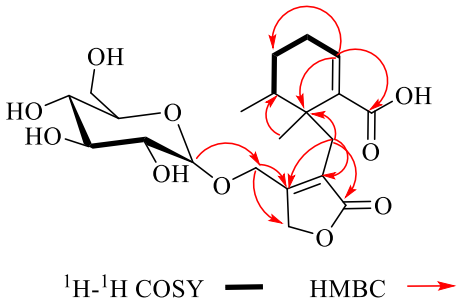

Figure 2. The key ^{1}H-^{1}H COSY and HMBC correlations of compound **1**

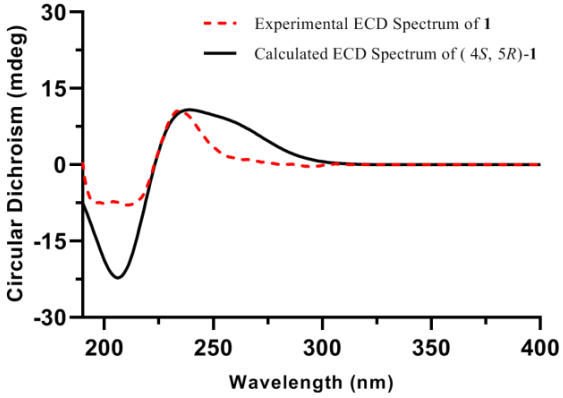

Figure 3. Experimental and calculated ECD spectra of compound **1**

In preliminary bioassays, the cytoprotective effects of the compounds (**1**–**7**) were evaluated against LPS-induced NRK 52e cells using the MTT and RTCA assay. The results (Table 2) demonstrated that compound **3** exhibited protective activity against NRK-52e cells injury induced by LPS with EC_{50} value of 45.1 μM.

Table 1. The effect of compounds **1**-**7** on NRK-52e cell injury induced by LPS.

Group	Dose (μM)	Cell viability
CON	-	0.777 ± 0.020***
LPS	-	0.647 ± 0.054
1	10	0.644 ± 0.058
2	10	0.589 ± 0.055
3	10	0.772 ± 0.051***
4	10	0.649 ± 0.047
5	10	0.642 ± 0.037
6	10	0.632 ± 0.044
7	10	0.603 ± 0.043

*** P<0.001 compared with the LPS group. These data represent the average values of three repeated experiments (mean ± SD)

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Supporting Information

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