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An Ellagic Acid Rhamnoside from the Roots of *Potentilla discolor* with Protein Glycation and Rat Lens Aldose Reductase Inhibitory Activity

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Abstract – Four glycosides, rosamultin (**1**), tetracentronside B (**2**), 4-*O*-methylellagic acid 3-*O*-α-$_L$-rhamnopyranoside (**3**), and vanillic acid 4-*O*-β-$_D$-glucopyranoside (**4**), isolated from the roots extract of *Potentilla discolor*, were subjected to *in vitro* bioassays to evaluate the inhibitory activity on advanced glycation end products (AGEs) formation and rat lens aldose reductase (RLAR). Compound **3** exhibited a significant inhibitory activity against both AGEs formation and RLAR with IC_{50} values of 79.5 and 8.03 μM, respectively. All the compounds (**1 - 4**) were isolated for the first time from this plant.
Keywords – *Potentilla discolor*, Rosaceae, diabetic complications, AGEs, aldose reductase.

Introduction

Potentilla discolor Bunge (Rosaceae) is a perennial herb native in Korea, China, and Japan which can be distinguished by the densely pubscent on the both sides of leaflets and the divided thick spindle-shape roots from the other related species such as *P. fragarioides* var. *major* Maxim. and *P. yokusaiana* Makino (Lee *et al.*, 1996). Its dried roots are used as a Traditional Chinese Medicine for the treatment of diarrhea and hemorrhage (Feng *et al.*, 1996). *P. discolor* is one of the botanical origins of Korean folk medicine "Jin Hae Cho Ip" which has been used as a remedy for neuralgia and as an invigorating drug after a childbirth (Park *et al.*, 2004). However, to the best of our knowledge, there are just few prior reports on secondary metabolites of *P. discolor*; on the isolations of phenolic acids and flavonoids from the whole plants (Liu *et al.*, 1984), triterpenoids from the aerial parts (Jang *et al.*, 2006a), and hydrolysable tannins from the roots (Feng *et al.*, 1996). In our ongoing project directed toward the discovery of preventive agents for diabetic complications from the herbal medicines (Jang *et al.*, 2006b), the roots of *Potentilla discolor* was chosen for more detailed investigation, since the 80% EtOH extract showed a significant *in vitro* inhibitory effect on advanced glycation end products (AGEs) and aldose reductase (AR). Direct evidence indicating the contribution of AGEs in the progression of diabetic complications in different lesions of the kidneys, the rat lens, and in atherosclerosis has been recently reported (Bucala and Vlassara, 1995; Kalousova *et al.*, 2004). AR, the key enzyme in the polyol pathway, also has been demonstrated to play important roles in the pathogenesis of diabetic complications and cataract formation (Beyer-Mears and Cruz, 1985). Thus, the design and discovery of inhibitors of AGEs formation or AR can offer a promising therapeutic approach for the prevention of diabetic or other pathogenic complications (Forbes *et al.*, 2003; Yabe-Nishimura, 1998).

Further fractionation of the EtOAc- and BuOH-soluble fractions of the 80% EtOH extract of the roots of *P. discolor* led to the isolation of four glycosides (**1 - 4**). The isolation and biological evaluation utilizing AGEs and RLAR inhibitory assays of the isolates are described herein.

Experimental

Instruments and reagents – Melting points were measured on an IA9100 melting point apparatus (Barnstead International, USA) and were quoted uncorrected. Optical rotations were obtained using a digital polarimeter (Jasco, Japan) at 25 °C. LRESI were recorded on a Mariner mass spectrometer (Perspective Biosystem, USA). NMR experiments were conducted on a DRX-300 FT-NMR (Bruker, Germany), and the chemical shifts were referenced to the residual solvent signals. TLC analysis was performed on Kieselgel 60 F_{254}

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(Merck) plates (silica gel, 0.25 mm layer thickness); compounds were visualized by dipping plates into 10% (v/v) H_2SO_4 reagent (Aldrich) and then heated at 110 °C for 5 - 10 min. Silica gel (Merck 60A, 70 - 230 or 230 - 400 mesh ASTM), reversed-phase silica gel (YMC Co., ODS-A 12 nm S-150 μm), and sephadex LH-20 (Amersham Pharmacia Biotech) were used for column chromatography. All solvents used for the chromatographic separations were distilled before use.

Plant material – The roots of *Potentilla discolor* Bunge (Rosaceae) were collected in Youngcheon, Kyungbuk, Korea in May, 2005 and were identified by Prof. Joo-Hwan Kim, Daejeon University. A voucher specimen (no. KIOM-P031) has been deposited at the Herbarium of Department of Herbal Pharmaceutical Development, Korea Institute of Oriental Medicine, Daejeon 305-811, Korea.

Extraction and isolation – The dried and cut plant material (865 g) was extracted with 80% EtOH (3 × 10 L) by maceration at room temperature for 3 days. The extracts were combined and concentrated *in vacuo* at 40 °C. The concentrated extract (110 g) was suspended in H_2O (1.5 L) and then partitioned successively with *n*-hexane (3 × 2 L), EtOAc (3 × 2 L), and BuOH (3 × 2 L) to afford the *n*-hexane-soluble (11.5 g), EtOAc-soluble (26.0 g), BuOH-soluble (20.9 g), and aqueous fractions (55.0 g), respectively. The EtOAc-soluble fraction was chromatographed through silica gel (6.2 × 45 cm, 70 - 230 mesh) as stationary phase using a solvent system ($CHCl_3$-MeOH gradient from 60 : 1 to 0 : 1 v/v) to afford 8 pooled fractions (fractions E01 - E08). Compounds **1** (16 mg) and **2** (19 mg) were purified from fractions E06 (400 mg) and E04 (390 mg) by reversed-phase column chromatography (2.8 × 38 cm, MeOH–H_2O gradient from 1 : 1 to 7 : 3 v/v), respectively. The BuOH-soluble fraction was chromatographed through silica gel (6.2 × 45 cm, 70 - 230 mesh) as stationary phase using a solvent system ($CHCl_3$-MeOH-H_2O gradient from 7 : 4 : 1 to 0 : 0 : 1 v/v) to afford 9 pooled fractions (fractions B01–B09). Fractions B04 (650 mg) and B07 (1.41 g) were subjected sephadex LH-20 column chromatography (3.8 × 47 cm, 80% MeOH) to give compounds **3** (25 mg) and **4** (24 mg), respectively.

Rosamultin (1) – White power; mp 204 - 205 °C (lit. 206 - 210 °C) (Young *et al.*, 1987); ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 6.30 (1H, d, J = 7.8 Hz, H-1'), 5.54 (1H, t-like, H-12), 4.51 - 4.10 (m, glucosyl-H), 4.07 (1H, m, H-2), 3.38 (1H, d, J = 9.3 Hz, H-3), 2.93 (1H, s, H-18), 1.67, 1.40, 1.26, 1.22, 1.10, 1.07 (each 3H, s, 6×-CH_3), 1.08 (3H, overlapped, CH_3-20); ^{13}C-NMR (pyridine-d_5, 75 MHz) δ 177.3 (C-28), 139.6 (C-13), 128.7 (C-12), 96.2 (C-1'), 84.2 (C-3), 79.6 (C-5'), 79.3 (C-3'), 74.4 (C-2'), 73.0 (C-19), 71.6 (C-4'), 69.0 (C-2), 62.7 (C-6'), 56.3 (C-5), 54.8 (C-18), 49.0 (C-17), 48.4 (C-1), 48.2 (C-9), 42.54 (C-20), 42.50 (C-14), 40.7 (C-8), 40.2 (C-10), 38.8 (C-4), 38.0 (C-22), 33.8 (C-7), 29.7 (C-23), 29.5 (C-15), 27.3 (C-29), 27.0 (C-21), 26.5 (C-16), 24.5 (C-27), 23.3 (C-11), 19.4 (C-6), 18.0 (C-24), 17.8 (C-26), 17.3 (C-30), 17.0 (C-25); LRESIMS *m/z*: 673 ([M + Na]$^+$).

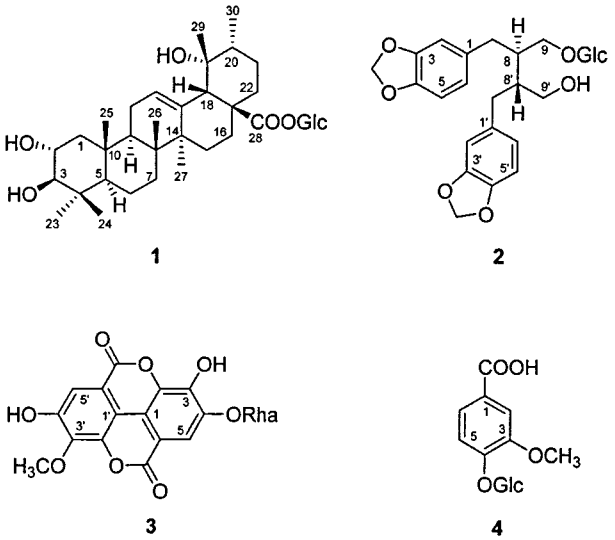

Fig. 1. Structures of compounds **1** - **4** from the roots of *Potentilla discolor*.

Tetracentronside B (2) – White power; mp 155 - 156 °C (lit. 156 - 157 °C) (Yi *et al.*, 2000); $[\alpha]_D^{25}$ –13.5° (c 0.2, MeOH) [lit. $[\alpha]_D^{25}$ –12.1° (c 0.28, MeOH)] (Yi *et al.*, 2000); ^{1}H-NMR (CD_3OD, 300 MHz) δ 6.67 (2H, d, J = 8.4 Hz, H-5/H-5'), 6.61 (2H, d, J = 2.1 Hz, H-2/H-2'), 6.58 (2H, m, H-6/H-6'), 5.87 (4H, s, -OCH_2O-×2), 4.19 (1H, d, J = 7.5 Hz, H-1"), 3.88 (2H, m, H-9α/H-9'α). 3.50-3.69 (2H, m, H-9β/H-9'β), 2.53-2.71 (4H, m, H-7/H-7'), 2.03 (1H, m, H-8), 1.90 (1H, m, H-8'); ^{13}C-NMR (CD_3OD, 75 MHz) δ 149.0 (C-3/C-3'), 148.1 (C-4/C-4'), 138.8 (C-1), 138.2 (C-1'), 123.3 (C-6), 123.2 (C-6'), 110.5 (C-5), 110.4 (C-5'), 108.8 (C-2/C-2'), 104.7 (C-1"), 102.0 (-OCH_2O-×2), 78.2 (C-3"), 78.1 (C-5"), 75.3 (C-4"), 72.5 (C-2"), 70.4 (C-9), 62.9 (C-9'), 62.8 (C-6"), 44.4 (C-8), 41.9 (C-8'), 35.8 (C-7/C-7'); LRESIMS *m/z*: 543 ([M + Na]$^+$).

4-*O*-Methylellagic acid 3'-*O*-α-$_L$-rhamnopyranoside (3) – Yellowish power; mp 357 - 359 °C (lit. > 360 °C) (Yazaki and Hillis, 1976); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 7.45 (1H, s, H-5), 7.38 (1H, s, H-5'), 5.49 (1H, d, J = 1.8 Hz, H-1"), 3.99 (3H, s, OCH_3), 3.93 (1H, t-like, H-2"), 3.72 (1H, m H-3"), 3.68 (1H, m, H-5"), 3.26 (1H, t, J = 9.5 Hz, H-4"), 1.11 (3H, d, J = 6.3 Hz, H-6"); ^{13}C-NMR

(DMSO-d_6, 75 MHz) δ 160.1 (C-7), 159.2 (C-7'), 151.9 (C-4), 148.9 (C-4'), 142.4 (C-3'), 139.5 (C-3), 137.6 (C-2/C-2'), 115.2 (C-6'), 115.0 (C-1'), 113.7 (C-6/C-5'), 112.7 (C-1), 110.1 (C-5), 99.0 (C-1''), 72.2 (C-4''), 70.5 (C-3''), 70.1 (C-2''), 69.1 (C-5''), 60.6 (OCH_3-4), 17.9 (C-6''); LRESIMS *m/z*: 463 ($[M+H]^+$).

Vanillic acid 4-*O*-β-$_D$-glucopyranoside (4) – Yellowish power; mp 134-136 °C (lit. 136 - 138 °C) (Sakushima *et al.*, 1995); ^{1}H-NMR (CD_3OD, 300 MHz) δ 7.64 (1H, overlapped, H-2), 7.62 (1H, s, H-6), 7.21 (1H, d, J = 8.1 Hz, H-5), 5.03 (1H, d, J = 7.2 Hz, H-1'), 3.90 (3H, s, OCH_3), 3.88 - 3.34 (m, glucosyl-H); ^{13}C-NMR (CD_3OD, 75 MHz) δ 169.8 (C-7), 152.0 (C-4), 150.4 (C-3), 126.4 (C-1), 124.8 (C-6), 116.5 (C-5), 114.5 (C-2), 102.1 (C-1'), 78.4 (C-5'), 77.9 (C-3'), 74.9 (C-2'), 71.3 (C-4'), 62.5 (C-6'), 56.8 (OCH_3-3); LRESIMS *m/z*: 353 ($[M+Na]^+$).

Determination of AGEs formation – According to the established method (Vinson and Howard, 1996), 700 μL of the reaction mixture [bovine serum albumin (10 mg/mL, Sigma, St Louis, MO, USA) in 50 mM phosphate buffer (pH 7.4) with 0.02% sodium azide] was added to 100 μL of 0.2 M fructose and glucose. In 1.5 mL screw cap tubes, the reaction mixture was then mixed with 200 μL of serial diluted compounds or aminoguanidine (Sigma, St Louis, MO, USA). After incubating at 37 for 14 days, 200 μL of the fluorescent reaction products were transferred to 96 well plates and assayed on a spectrofluorometric detector (BIO-TEK, Synergy HT, USA; Ex: 350, Em: 450 nm).

Measurement of RLAR activity – Rat lens were removed from the eyes of 8 weeks old Sprague-Dawley rats (Dae-Han Bio Link Co., Umsung, Korea) weighing 100 - 150 g and homogenized in 12 volumes of a 135 mM Na, K-phosphate buffer (pH 7.0) containing 0.5 mM phenylmethylsulfonyl fluoride and 10 mM 2-mercaptoethanol. The homogenate was centrifuged at 100,000 × *g* for 30 min, and the supernatant fluid was used as the crude rat lens aldose reductase (RLAR). RLAR activity was assayed according to the methods described (Kim and Oh, 1999; Matsuda *et al.*, 2002) with slight modification. The incubation mixture contained 135 mM Na, K-phosphate buffer (pH 7.0), 100 mM Lithium sulfate, 0.03 mM NADPH, 1 mM $_{DL}$-glyceraldehyde as a substrate, and 50 μL of enzyme fraction, with or without 25 μL of sample solution, in a total volume of 1.0 mL. The reaction was initiated by the addition of NADPH at 37 °C and stopped by the addition of 0.3 mL of 0.5 M HCl. Then, 1 mL of 6 M NaOH containing 10 mM imidazole was added, and the solution was heated at 60 °C for 10 min to convert NADP to a fluorescent product. Fluorescence was measured using a spectrofluorometric detector (Schimadzu RF-5301PC, Japan, Ex: 360, Em: 460 nm). Both AGEs and RLAR assays were performed in triplicate. The concentration of each test sample giving 50% inhibition of the activities (IC_{50}) was estimated from the least-squares regression line of the logarithmic concentration plotted against the remaining activity.

Results and Discussion

Four glycosides were isolated from the EtOAc- (compounds **1** and **2**) and BuOH-soluble extracts (compounds **3** and **4**) of the roots of *Potentilla discolor*. The isolates, previously known structures, were identified as rosamultin (**1**) (Young *et al.*, 1987), tetracentronside B (**2**) (Yi *et al.*, 2000), 4-*O*-methylellagic acid 3-*O*-α-$_L$-rhamnopyranoside (**3**) (Guo and Yang, 2005), and vanillic acid 4-*O*-β-$_D$-glucopyranoside (**4**) (Sakushima *et al.*, 1995) by physical and spectroscopic data and by comparison with published ones. All the four compounds (**1** - **4**) were isolated for the first time from this plant.

The isolates **1** - **4** were subjected to *in vitro* bioassays to evaluate AGEs and RLAR inhibitory activities. Of these, only 4-*O*-methylellagic acid 3-*O*-α-$_L$-rhamnopyranoside (**3**) showed a significant inhibitory activity with observed IC_{50} values of 79.5 and 8.03 μM against AGEs formation and RLAR (IC_{50} values of the positive controls, aminoguanidine and tetramethyleneglutaric acid: 961 and 24.1 μM, respectively), respectively. Although ellagic acid and its derivatives has been reported to be an AR inhibitor from other plants (Ueda *et al.*, 2004), this is the first report that 4-*O*-methylellagic acid 3-*O*-α-$_L$-rhamnopyranoside inhibits both protein glycation and RLAR. Thus, this compound (**3**) seems worthy of additional biological testing to evaluate more fully its potential as a therapeutic or preventing agent for diabetic complications and related diseases.

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