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 pubescent 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$_{254}$
(Merck) plates (silica gel, 0.25 mm layer thickness); compounds were visualized by dipping plates into 10% (v/v) H2SO4 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 Youngchoon, Kyungbuk, Korea in May, 2005 and were identified by Prof. Jo-Hwan Kim, Daejeon University. A voucher specimen (no. KIOM-P031) has been deposited at the Herbarium of the 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 H2O (1.5 L) and then partitioned successively with n-hexane (3 × 2 L), EtOAc (3 × 2 L), and BuOH (3 × 2 L) to afford 6-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 (CHCl3 - MeOH - H2O - Na2SO4) (1.5 L) and then partitioned successively with CHCl3, MeOH–H2O (26.0 g), EtOAc (20.9 g), and BuOH (11.5 g) to afford 9 pooled fractions (fractions B01 - B09).

Fractions B04 (650 mg) and B07 (1.41 g) were subjected to sephadex LH-20 column chromatography (3.8 × 47 cm, 80% MeOH) to give compounds 3 (25 mg) and 4 (24 mg), respectively.

Rosaumalin (1) — White powder; mp 204 - 205 °C (lit. 206 - 210 °C) (Young et al., 1987); 1H-NMR (pyridine-d5, 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-5), 2.93 (1H, s, H-18), 1.67, 1.40, 1.26, 1.22, 1.10, 1.07 (each 3H, s, 6×-CH3), 1.08 (3H, overlapped, CH3-20); 13C-NMR (pyridine-d5, 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), 56.5 (C-5), 54.8 (C-18), 49.0 (C-17), 48.4 (C-1), 48.2 (C-9), 42.5 (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); LR-ESI-MS m/z: 763 ([M + Na]+).

**Tetracentronside B (2)** — White powder; mp 155 - 156 °C (lit. 156 - 157 °C) (Yi et al., 2000); [α]D25 -13.5° (c 0.2, MeOH) [lit. [α]D25 -12.1° (c 0.28, MeOH)] (Yi et al., 2000); 1H-NMR (CD3OD, 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'), 5.87 (4H, s, OCH2O-2'), 4.19 (4H, d, J = 7.5 Hz, H-1'), 3.88 (2H, m, H-18), 1.90 (2H, m, H-9α/H-9β). 35.0 - 3.69 (2H, m, H-9β/H-19β), 2.53 - 2.71 (4H, m, H-7/H-7'). 203 (1H, m, H-8), 190 (1H, m, H-8'); 13C-NMR (CD3OD, 75 MHz) δ 1490 (C-3/C-3'), 1481 (C-4/C-4'), 1388 (C-1), 138.2 (C-1'), 123.3 (C-6), 122.3 (C-6'), 110.5 (C-5), 110.4 (C-5'), 108.8 (C-2/C-2'), 104.7 (C-1''), 102.0 (OCH2O-2'), 782 (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'); LR-ESI-MS m/z: 543 ([M + Na]+).

4-O-Methyljellyacid 3'-O-α-L-rhamnopyranoside (3) — Yellowish powder; mp 357 - 359 °C (lit. >360 °C) (Yazaki and Hillis, 1976); 1H-NMR (DMSO-d6, 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, OC6H3), 3.93 (1H, t-like, H-2'), 3.72 (1H, m, H-3'), 3.68 (1H, m, H-3'), 3.26 (1H, t, J = 9.5 Hz, H-4'), 1.11 (3H, d, J = 6.3 Hz, H-6'); 13C-NMR...