Constituents of the Stems of *Rumex japonicus* with Advanced Glycation End Products (AGEs) and Rat Lens Aldose Reductase (RLAR) Inhibitory Activity

Jong Min Kim, Yun Mi Lee, Dae Sik Jang and Jin Sook Kim*

Department of Herbal Pharmaceutical Development, Korea Institute of Oriental Medicine, Daejeon 305-811, Korea

Received January 3, 2006; Accepted March 8, 2006

Four ursane-type triterpenoids, 2α,3α,19α-trihydroxy-24-norurs-4(23),12-dien-28-oic acid (1), 4(*R*),23-epoxy-2α,3α,19α-trihydroxy-24-norurs-12-en-28-oic acid (2), myrianthic acid (3), and tormentic acid (4), and a phenolic compound, ethyl gallate (5), from an EtOAc-soluble extract of the stems of *Rumex japonicus*, were subjected to *in vitro* bioassays to evaluate advanced glycation end products (AGEs) and rat lens aldose reductase (RLAR) inhibitory activity. Compounds 1 and 5 exhibited a significant inhibitory activity on AGEs formation with IC_{50} value of 87 μM and on RLAR with IC_{50} value of 14.3 μM, respectively. Ethyl gallate (5) was isolated for the first time from this plant.

Key words: *Rumex japonicus,* Polygonaceae, 24-nor-Triterpenoid, Ethyl gallate, AGEs, Aldose reductase.

The Diabetes Control and Complication Trial (DCCT) has identified hyperglycemia as the main risk-factor for the development of complications.[1] Persistent hyperglycemia induces abnormal changes such as the formation of advanced glycation end products (AGEs),[2] the increase of sorbitol through the polyol pathway,[3] the overactivation of protein kinase C isoforms due to the synthesis of DAG.[4] 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.[5,6] Aldose reductase (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.[7] 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.[8,9]

In our ongoing project directed toward the discovery of preventive agents for diabetic complications from the herbal medicines and prescriptions,[10] the stems of *Rumex japonicus* was chosen for more detailed investigation, since the EtOAc-soluble fraction of an 80% EtOH extract showed a significant *in vitro* inhibitory effect on advanced glycation end products (AGEs). *Rumex japonicus* Houtt. (Polygonaceae) is a perennial plant and is widely distributed in Korea. The roots of this plant have been used as a Chinese drug (Rumecis Radix) for the treatment of heat phlegm, jaundice, constipation, scabies, and uterine hemorrhage.[11] *R. japonicus* have shown to possess various biological and pharmacological activities including antioxidation,[12] cytotoxicity,[13] and antimicrobial.[14] Previous phytochemical investigation on the roots and leaves of *R. japonicus* have resulted in the isolation of several flavonoids, anthraquinone derivatives, and naphthalene derivatives.[13-17] However, no pharmacological studies regarding diabetic complications have been carried out on the stems of this plant to date. The biological evaluation utilizing AGEs and RLAR inhibitory assays of the four ursane-type triterpenoids **1-4**, isolated from an EtOAc-soluble extract of the stems of *R. japonicus* in our previous work[18] and ethyl gallate (**5**), isolated from this plant for the first time in this study, are described herein.

Materials and Methods

Plant materials. The stems of *Rumex japonicus* Houtt. (Polygonaceae) were collected in Jeonmin-dong, Yuseong-gu, Daejeon, Korea, in July, 2004 and were identified by a plant taxonomist, Prof. J.-H. Kim., Daejeon University. A voucher specimen (no. KIOM-P005) has been deposited at the Herbarium of Department of Herbal Pharmaceutical Development, Korea Institute of Oriental Medicine, Korea.

General experimental procedures. Melting points were measured on an IA9100 melting point apparatus (Barnstead International, USA) and were quoted uncorrected. LREI was recorded on an Autospec (Micromass, UK). 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) H_2SO_4 reagent (Aldrich) and then heat treated at 110°C for 5-10 min. Silica gel (Merck 60A, 70-230 or 230-400 mesh ASTM) and Sephadex LH-20 (Amersham Pharmacia Biotech) were used for column chromatography. All solvents used for the chromatographic separations were distilled before use.

Extraction and isolation. The fresh and cut plant material

*Corresponding author
Phone: +82-42-868-9465; Fax: +82-42-863-9466
E-mail: jskim@kiom.re.kr

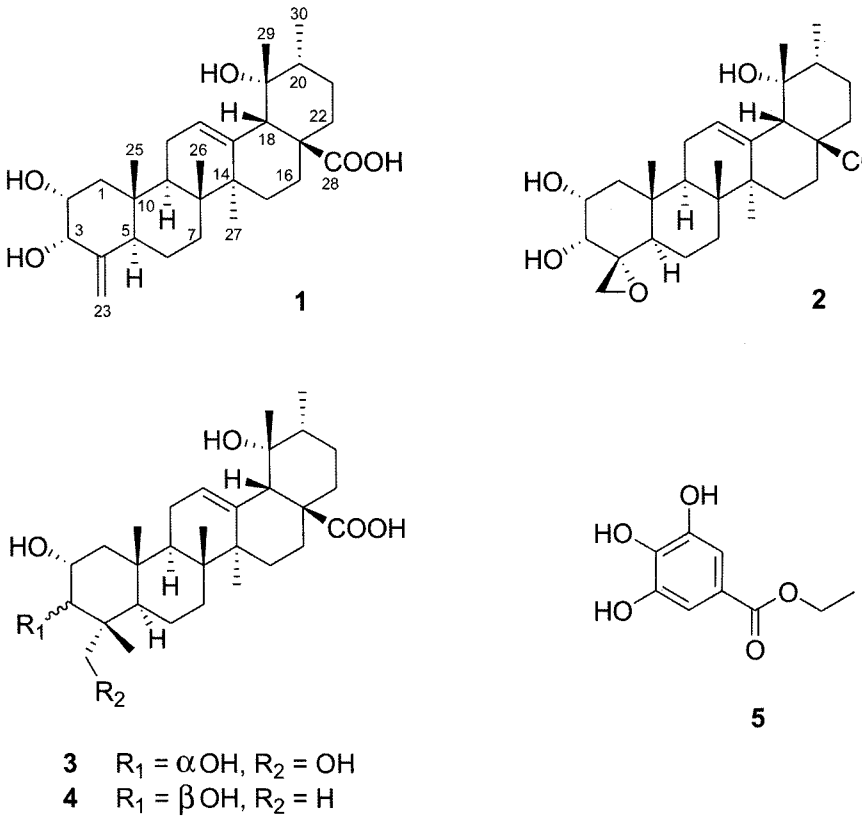

Fig. 1. Structures of compounds 1-5 from the stems of *Rumex japonicus*.

(7.7 kg) was extracted with 80% EtOH (3 × 20 *l*) by maceration. The extracts were combined and concentrated *in vacuo* at 40°C. The concentrated extract (185 g) was suspended in H_2O (1.5 *l*) and then partitioned with *n*-hexane (3 × 1.5 *l*) to afford a *n*-hexane-soluble fraction (7.6 g) on drying. Next, the aqueous partition was partitioned again with EtOAc (3 × 1.5 *l*) to give an EtOAc-soluble fraction (24.0 g) and an aqueous residue. From the EtOAc-soluble fraction (21.0 g), four ursane-type triterpenoids **1-4** were isolated by chromatographic methods and identified by spectroscopic methods as our previous study (Jang *et al.*, 2005). Fraction F05 [eluted with $CHCl_3$-MeOH (19 : 1 v/v); 1.98 g] from the EtOAC-soluble fraction was chromatographed through silica gel (4.6 × 36 cm, 230-400 mesh; *n*-hexane-EtOAc-MeOH gradient from 6 : 3.5 : 0.5 to 2 : 2 : 1 v/v) to produce 10 subfractions (fractions 0501-0510). Ethyl gallate (**5**, 14.3 mg) was purified from the fraction 0503 by Sephadex LH-20 column chromatography (3.6 × 32 cm, 12 nm S-150 μm; 100% MeOH).

Ethyl gallate (5); White powder; mp 197-198°C; EIMS *m/z* (rel. int.): 198 $[M]^+$ (38), 170 (20), 153 (100), 125 (23), 107 (9), 79 (22); ^{1}H-NMR (CD_3OD, 300 MHz) δ 7.06 (2H, s, galloyl-H), 4.28 (2H, q, *J* = 7.2, OCH_2), 1.36 (3H, t, *J* = 7.2, CH_3); ^{13}C-NMR (CD_3OD, 75 MHz) δ 167.5 (COO), 145.5 (C-3/C-5), 138.7 (C-4), 120.8 (C-1), 109.0 (C-2/C-6), 60.7 (OCH_2), 13.6 (CH_3).

Determination of AGEs formation. According to the method of Vinson and Howard,[19] the reaction mixture, 10 mg/ml of bovine serum albumin (Sigma, St Louis, MO, USA) in 50 mM phosphate buffer (pH 7.4) with 0.02% sodium azide to prevent bacterial growth was added to 0.2 M fructose and glucose. The reaction mixture was then mixed with compounds or aminiguanidine (Sigma, St Louis, MO, USA). After incubating at 37°C for 7 days, the fluorescent reaction products were 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 previously[20,21] 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 ursane-type triterpenoids, 2α,3α,19α-trihydroxy-24-norurs-4(23),12-dien-28-oic acid (**1**), 4(*R*),23-epoxy-2α,3α,19α-trihydroxy-24-norurs-12-en-28-oic acid (**2**), myrianthic acid (**3**), and tormentic acid (**4**), were isolated from an EtOAc-soluble extract of the stems of *R. japonicus* and identified by spectroscopic methods as our previous study.[18] In this study, a phenolic compound, ethyl gallate (**5**), was isolated for the first time from this plant, and was identified as by physical and spectroscopic data (mp, ^{1}H-NMR, ^{13}C-NMR, and MS) measurement and by comparison with published values.[22]

All the isolates **1-5** were subjected to *in vitro* bioassays to evaluate AGEs and RLAR inhibitory activities. In the RLAR inhibitory assay, only ethyl gallate (**5**) showed a good activity with observed IC_{50} value of 14.3 μM (IC_{50} value of a positive control tetramethyleneglutaric acid: 24.1 μM). While compound **1**, a rare 24-nor-ursane triterpenoid with exocyclic methylene at the position C-4, exhibited more inhibitory activity against AGEs formation with IC_{50} value of 87 μM than a well known positive control aminoguanidine (IC_{50} value of 473 μM) while others was found to be inactive. It seems that the inhibitory potency of compound **1** is due to its exomethylene group at position C-4 when it is compared with other inactive analogues **2-4** isolated from *R. japonicus* (Fig. 1). Although a number of natural inhibitors of AGEs formation have been reported up to date, many of them are flavonoids with IC_{50} values ranging from 90 to 200 μM.[23] Thus, 2α,3α,19α-trihydroxy-24-norurs-4(23),12-dien-28-oic acid (**1**) seems worthy of additional biological testing to more fully evaluate its potential as a new drug for diabetic complications.

Acknowledgments

We thank Prof. Joo-Hwan Kim, Division of Life Science, Daejeon University, Daejeon, Korea, for identifying the plant material. We also thank Korea Basic Science Institute (KBSI) for running NMR and MS experiments. This research was supported by a grant [M 10413010001] from the Ministry of Science and Technology, the Korean Government.

References

1. The Diabetes Control and Complication Trial Research Group (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N. Engl. J. Med.* **329**, 977-986.
2. Makita, Z., Radoff, S., Rayfield, E. J., Yang, Z. H., Skolnik, E., Delaney, V., Friedman, E. A., Cerami, A. and Vlassara, H. N. (1993) Advanced glycosylation end products in patients with diabetic nephropathy. *N. Eng. J. Med.* **325**, 836-842.
3. Shinohara, R., Mano, T., Nagasaka, A., Sawai, Y., Uchimura, K., Hayashi, R., Hayakawa, N., Nagata, M., Makino, M., Kakizawa, H., Itoh, Y., Nakai, A. and Itoh, M. (1998) Effects of thyroid hormone on the sorbitol pathway in streptozotocin-induced diabetic rats. *Biochim. Biophys. Acta* **1425**, 577-586.
4. Larkins, R. G. and Dunlop, M. E. (1992) The link between hyperglycaemia and diabetic nephropathy. *Diabetologia* **35**, 499-504.
5. Bucala, R. and Vlassara, H. (1995) Advanced glycosylation end products in diabetic renal and vascular disease. *Am. J. Kidney Dis.* **26**, 875-888.
6. Kalousova, M., Zima, T., Tesar, V., Stipek, S. and Sulkova, S. (2004) Advanced glycation end products in clinical nephrology. *Kidney Blood Press Res.* **27**, 18-28.
7. Beyer-Mears, A. and Cruz, E. (1985) Reversal of diabetic cataract by sorbinil, an aldose reductase inhibitor. *Diabetes* **34**, 15-21.
8. Forbes, J. M., Cooper, M. E., Oldfield, M. D. and Thomas, M. C. (2003) Role of advanced glycation end products in diabetic nephropathy. *J. Am. Soc. Nephrol.* **14**, S254-S258.
9. Yabe-Nishimura, C. (1998) Aldose reductase in glucose toxicity: a potential target for the prevention of diabetic complications. *Pharmacol. Rev.* **50**, 21-33.
10. Kim, J. S., Kim, H. and Ko, J. H. (2002) Studies on the processing of herbal medicines (III)-HPLC analysis of magnolol and inhibitory effects on the formation of advanced glycation endproducts (AGEs) *in vitro* of unprocessed and processed Magnolia Bark. *Kor. J. Pharmacogn.* **33**, 308-311.
11. Bae, K., Medicinal Plants of Korea, Kyo-Hak Publishing Co., Seoul, 2000, p 92.
12. Li, Y. P., Takamiyagi, A., Ramzi, S. T. and Nonaka, S. (2000) Inhibitory effect of *Rumex japonicus* Houtt on the porphyrin photooxidative reaction. *J. Dermatology*, **27**, 761-768.
13. Kim, D. K., Choi, S. U., Ryu, S. Y., Lee, K. R. and Zee, O. P. (1998) Cytotoxic constituents of *Rumex japonicus*. *Yakhak Hoeji* **42**, 233-237.
14. Nishina, A., Kubota, K. and Osawa, T. (1993) Antimicrobial components, trachrysone and 2-methoxystypandrone, in *Rumex japonicus* Houtt. *J. Agric. Food Chem.*, **41**, 1772-1775.
15. Aritomi, M., Kiyota, I. and Mazaki, T. (1965) Flavonoid constituents in leaves of *Rumex acetosa* and *R. japonicus*, *Chem. Pharm. Bull.* **13**, 1470-1471.
16. Zee, O. P., Kim, D. K., Kwon, H. C. and Lee, K. R. (1998) A new epoxynaphthoquinol from *Rumex japonicus*, *Arch. Pharm. Res.* **21**, 485-486.

17. Hwang, S. W., Ha, T. J., Lee, J. R., Lee, J., Nam, S. H., Park, K. H. and Yang, M. S. (2004) Isolation of anthraquinone derivatives from the root of *Rumex japonicus* H. *J. Korean Soc. Appl. Biol. Chem.* **47**, 274-278.
18. Jang, D. S., Kim, J. M., Kim, J. H. and Kim, J. S. (2005) 24-nor-ursane type triterpenoids from the stems of *Rumex japonicus*. *Chem. Pharm. Bull.* **53**, 1594-1596.
19. Vinson, J. A. and Howard III, T. B. (1996) Inhibition of protein glycation and advanced glycation end products by ascorbic acid and other vitamins and nutrients. *J. Nutr. Biochem.* **7**, 659-663.
20. Kim, H. Y. and Oh, J. H. (1999) Screening of Korean forest plants for rat lens aldose reductase inhibition. *Biosci. Biotechnol. Biochem.* **63**, 184-188.
21. Matsuda, H., Morikawa, T., Toguchida, I. and Yoshikawa, M. (2002) Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity. *Chem. Pharm. Bull.* **50**, 788-795.
22. Park, W. Y. (1996) Phenolic compounds from *Acer ginnala* Maxim. *Kor. J. Pharmacog.* **27**, 212-218.
23. Matsuda, H., Wang, T., Managi, H. and Yoshikawa, M. (2003) Structural requirements of flavonoids for inhibition of protein glycation and radical scavenging activities. *Bioorg. Med. Chem.* **11**, 5317-5323.