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Article

Constituents of the Fruits of *Rumex japonicus* with Inhibitory Activity on Aldose Reductase

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Five anthraquinones, emodin (1), ω-hydroxyemodin (2), chrysophanol-8-*O*-β-D-glucoside (3), emodin-8-*O*-β-D-glucoside (4), and physcion-8-*O*-β-D-glucoside (5), and five flavonoids, kaempferol-3-*O*-β-D-glucoside (6), quercetin (7), quercitrin (8), isoquercitrin (9), and (+)-catechin (10), were isolated from the EtOAc-soluble extract of the fruits of *Rumex japonicus*. The structures of 1-10 were identified by spectroscopic methods including NMR studies. This is the first report on the isolation of compounds 3-5 from this plant. The isolates were subjected to *in vitro* bioassays to evaluate their inhibitory activities on the rat lens aldose reductase (RLAR), among which two anthraquinones (1 and 4), and five flavonols (5-9) showed significant activities on RLAR.

Key words : *aldose reductase, anthraquinone, flavonoid, Polygonaceae,* Rumex japonicus

Rumex japonicus Houtt. (Polygonaceae) is a perennial plant widely distributed in Korea. The roots of this plant have been used as a Chinese drug (Rumecis Radix) for the treatments of heat phlegm, jaundice, constipation, scabies, and uterine hemorrhage [Bae, 2000]. Through numerous studies, *R. japonicus* has been revealed to possess various biological and pharmacological activities including antioxidation [Li *et al*., 2000], cytotoxicity [Kim *et al*., 1998], and antimicrobial [Aritomi *et al*., 1965; Nishina *et al*., 1993] activities. Previous phytochemical investigations of *R. japonicus* have resulted in the isolation of several flavonoids [Aritomi *et al*., 1965], anthraquinone derivatives [Zee *et al*., 1998; Li *et al*., 2000], naphthalene derivatives [Aritomi *et al*., 1965; Nishina *et al*., 1993; Li *et al*., 2000; Hwang *et al*., 2004], and triterpenoids [Jang *et al*., 2005].

In our ongoing project directed toward the discovery of preventive agents from the herbal medicines for the treatment of diabetic complications [Jang *et al*., 2006], the fruits of *R. japonicus* were chosen for a more detailed investigation, because the EtOAc-soluble fraction of the MeOH extract showed a significant inhibitory activity on the AR *in vitro*. 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 AR can offer a promising therapeutic approach for the prevention of diabetic and other pathogenic complications [Yabe-Nishimura, 1998].

In the present study, five anthraquinones (**1-5**) and five flavonoids (**6-10**) were isolated from the EtOAc-soluble extract of the fruits of *R. japonicus*. The isolates were subjected to *in vitro* bioassays to evaluate their inhibitory activity on the RLAR. The biological evaluation of the isolates are described herein.

Materials and Methods

Plant materials. The fruits of *R. japonicus* Houtt. (Polygonaceae) were collected from Daejeon City, Korea, in June, 2006 and were identified by Prof. Joo-Hwan Kim of the Division of Life Science, Daejeon University, Korea. A voucher specimen (no. KIOM-RuJa01) 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, Dubuque, Iowa) and were quoted (uncorrected). LRMS was recorded on an Autospec

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Abbreviations: AR, aldose reductase; CC, column chromatography; EtOAc, ethyl acetate; FT-NMR, Fourier transform nuclear magnetic resonance; LRMS, low resolution mass spectroscopy; RLAR, rat lens aldose reductase; TLC, thin layer chromatography

(Micromass, Manchester, UK). NMR experiments were conducted on a DRX-300 FT-NMR (Bruker, Karlsruhe, Germany), and the chemical shifts were referenced to the residual solvent signals. The TLC analysis was performed on the Kieselgel 60 F_{254} (Merck, Darmstadt, Germany) plates (silica gel, 0.25 mm layer thickness); the compounds were visualized by dipping the plates into 10%(v/v) H_2SO_4 reagent (Aldrich), followed by heating 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 the column chromatography. All solvents used for the chromatographic separations were distilled before use.

Extraction and isolation. The dried and ground plant materials (1.5 kg) were extracted with MeOH (3×8 L) by maceration for 2 d at room temperature. The solvent was evaporated *in vacuo* at 40°C to afford the MeOH extract (250 g), which was then suspended in water (1 L), and sequentially partitioned with *n*-hexane (3×1 L), CH_2Cl_2 (3×1 L), EtOAc (3×1 L), and *n*-BuOH (3×1 L). The EtOAc-soluble fraction (64 g) was chromatographed over silica gel (12×40 cm, 70-230 mesh) using a $CHCl_3$/MeOH/H_2O gradient (from 7 : 1 : 0.1→2 : 1 : 0.1 v/v, finally 100% MeOH) to yield 20 pooled fractions (F01-F20). Emodin (**1**, 30 mg) was purified from F02 [eluted with $CHCl_3$/MeOH/H_2O (7 : 1 : 0.1 v/v); 1.4 g] by recrystallization in MeOH. F04 [eluted with $CHCl_3$/MeOH/H_2O (7 : 1 : 0.1 v/v); 0.3 g] was chromatographed through silica gel (4×36 cm, 230-400 mesh; *n*-hexane-EtOAc=1 : 1 v/v) to produce ω-hydroxyemodin (**2**, 2 mg). Subsequently, F07 [eluted with $CHCl_3$/MeOH/H_2O (6 : 1 : 0.1 v/v); 0.1 g] was further fractionated through a reversed phase silica gel CC [3×37 cm, MeOH-H_2O (1 : 1 v/v)] to afford chrysophanol-8-*O*-β-D-glucoside (**3**, 12 mg) and physcion-8-*O*-β-D-glucoside (**5**, 40 mg). Emodin-8-*O*-β-D-glucoside (**4**, 45 mg) and quercetin (**7**, 30 mg) were obtained from F011 [eluted with $CHCl_3$/MeOH/H_2O (6 : 1 : 0.1 v/v); 0.5 g] and F012 [eluted with $CHCl_3$/MeOH/H_2O (4 : 1 : 0.1 v/v); 0.32 g], respectively, by repeated CC. F015 [eluted with $CHCl_3$/MeOH/H_2O (4 : 1 : 0.1 v/v); 0.7 g] was subjected to the silica gel CC (4×35 cm, 230-400 mesh; EtOAc/MeOH/H_2O=19 : 1 : 0.5 v/v) to give kaempferol-3-*O*-β-D-glucoside (**6**, 100 mg) and (+)-catechin (**10**, 10 mg). Quercitrin (**8**, 260 mg) and isoquercitrin (**9**, 60 mg) were purified from F017 [eluted with $CHCl_3$/MeOH/H_2O (2 : 1 : 0.1 v/v); 1.0 g] and F019 [eluted with $CHCl_3$/MeOH/H_2O (2 : 1 : 0.1 v/v); 8.0 g], respectively, by repeated CC.

Measurement of RLAR activity. The rat lens were removed from the eyes of 8 weeks old Sprague-Dawley rats (Dae-Han Bio Link Co., Umsung, Korea), each 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 RLAR. The RLAR activity was assayed according to the methods described previously [Kim and Oh, 1999; Matsuda *et al.*, 2002] with slight modifications. The incubation mixture (total 1.0 mL) 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 the enzyme fraction (50 μL), with or without the sample solution (25 μL). The reaction was initiated by adding NADPH at 37°C and stopped by adding 0.5 M HCl (0.3 mL), followed by the addition of 6 M NaOH containing 10 mM imidazole (1 mL). The solution was heated at 60°C for 10 min to convert NADP into a fluorescent product. The fluorescence was measured using a spectrofluorometric detector (Shimadzu RF-5301PC, Kyoto, Japan, Ex: 360, Em: 460 nm). The RLAR assay was 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.

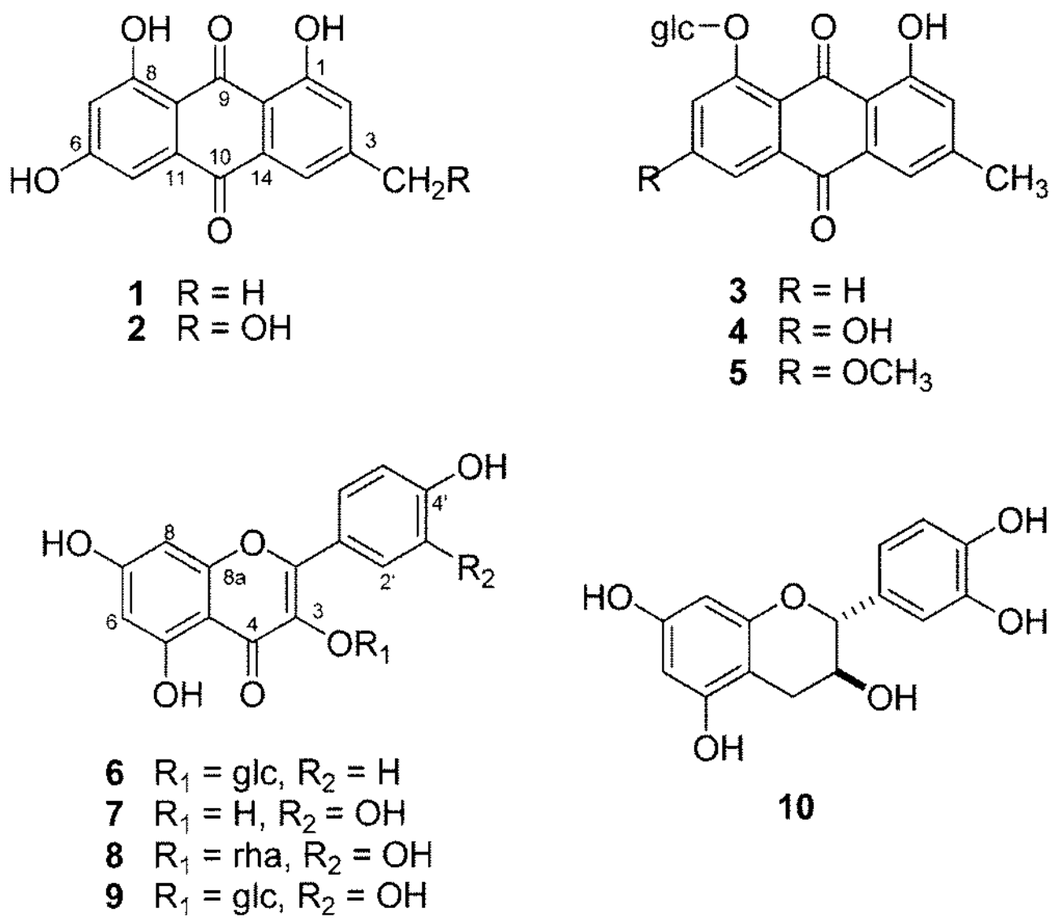

Fig. 1. Structures of compounds 1-10 from the fruits of *Rumex japonicus*.

Results and Discussion

Ten compounds were isolated from the EtOAc-soluble fraction of the fruits of *R. japonicus* and were identified as emodin (**1**) [Lee *et al.*, 2003], ω-hydroxyemodin (**2**) [Lee *et al.*, 2003], chrysophanol-8-*O*-β-D-glucoside (**3**)

Table 1. Inhibitory effects of compounds from the fruits of *R. japonicus* on rat lens aldose reductase (RLAR) *in vitro*[a]

Compound	Inhibitory effects (IC_{50} value; μM)[a]
1	15.8±0.66
2	ND[b]
4	14.4±0.93
6	18.7±2.17
7	7.62±1.67
8	0.17±0.01
9	4.06±1.31
EP[c]	0.067±0.009

[a]Inhibitory effect was expressed as mean±SD of triplicate experiments. IC_{50} values were calculated from the dose inhibition curve. Compounds **3**, **5**, and **10** were not active (IC_{50} value of >100 μM) in this bioassay system.
[b]Not determined because the amount of the available compound was insufficient.
[c]Epalrestat (EP) was used as the positive control.

[Choe *et al.*, 1998], emodin-8-*O*-β-D-glucoside (**4**) [Zhang *et al.*, 2005], physcion-8-*O*-β-D-glucoside (**5**) [Zhang *et al.*, 2005], kaempferol-3-*O*-β-D-glucoside (**6**) [Amani *et al.*, 2006], quercetin (**7**) [Lee *et al.*, 2004], quercitrin (quercetin-3-*O*-α-L-rhamnoside) (**8**) [Lee *et al.*, 2004], isoquercitrin (quercetin-3-*O*-β-D-glucoside) (**9**) [Lee *et al.*, 2004], and (+)-catechin (**10**) [Yokozawa *et al.*, 2002] through the physical and the spectroscopic methods as well as by comparison of the data obtained from those of the published values. To the best of our knowledge, this is the first report on the isolation of compounds **3-5** from this plant, although the presence of compounds **1**, **3**, and **4** was recently confirmed in the roots of *R. japonicas* by HPLC analysis [Koyama *et al.*, 2003].

In this study, we evaluated the isolates obtained from *R. japonicus* for their potential to inhibit the RLAR activity *in vitro* (Table 1). Among the isolates, quercitrin (**8**) exhibited the most potent RLAR inhibitory activity, with observed IC_{50} value of 0.17 μM. Emodin (**1**), emodin-8-*O*-β-D-glucoside (**4**), kaempferol-3-*O*-β-D-glucoside (**6**), quercetin (**7**), and isoquercitrin (**9**) also showed significant inhibitory effects on RLAR, with IC_{50} values ranging from 4.06 to 18.7 μM, whereas chrysophanol-8-*O*-β-D-glucoside (**3**), physcion-8-*O*-β-D-glucoside (**5**), and (+)-catechin (**10**) were not active (IC_{50} value of >100 μM) in this bioassay system. Quercetin (**7**) and quercitrin (**8**) were recently isolated by our group as RLAR inhibitors from the whole plants of *Houttuynia cordata* [Jang *et al.*, 2006]. AR is an enzyme catalyzing the reduction of glucose into sorbitol in the polyol pathway. The polyol pathway in diabetes accelerates the formation of sorbitol in the insulin-insensitive tissues such as nerve, lens, retina and kidney, thereby inducing such diabetic complications as neuropathy, cataract, retinopathy, and nephropathy [Beyer-Mears and Cruz, 1985; Yabe-Nishimura, 1998]. Therefore, the active compounds obtained in this study would be worthy of consideration as potent therapeutic agents for the treatments of diabetic complications and related diseases through additional biological evaluation.

In summary, of the isolates obtained from the fruits of *R. japonicus*, quercitrin (**8**) was the most potent RLAR inhibitor, with compounds **1**, **4**, **6**, **7**, and **9** also possessing the active principles of this plant.

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