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# Antidiabetic Stilbene and Anthraquinone Derivatives from *Rheum undulatum*

**Sang Zin Choi, Sung Ok Lee, Ki Uk Jang, Sung Hyun Chung[1], Sang Hyun Park[1], Hee Chol Kang[2], Eun Young Yang[2], Hi Jae Cho[2], and Kang Ro Lee**

*Natural Products Laboratory, College of Pharmacy, SungKyunKwan University, Suwon 440-746, Korea, [1]Pharmacology Lab, College of Pharmacy, Kyung Hee university, Seoul 130-701, Korea, and [3]Natural products Project, R&D Center of Pharmaceuticals of Science & Technology, Ichon 467-812, Korea*

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The antidiabetic-activity-guided fractionation and isolation of the 80% EtOH extracts obtained from cultivated Korean Rhubarb rhizomes (*Rheum undulatum*, Polygonaceae) led to the isolation and characterization of one stilbene, desoxyrhapontigenin (**1**) and two anthraquinones, emodin (**2**) and chrysophanol (**3**). Their structures were established by chemical and spectroscopic methods. Compounds **1**, **2**, and **3** inhibited postprandial hyperglycemia by 35.8, 29.5, 42.3%, respectively.

**Key words:** *Rheum undulatum*, Polygonaceae, Stilbene, Anthraquinone, Antidiabetic

## INTRODUCTION

*Rheum undulatum* (Polygonaceae, RU), a perennial herb, is mainly distributed and cultivated in South Korea. Cultivated Korean Rhubarb rhizomes (*Rheum undulatum*, Polygonaceae) have been used in Chinese herbal medicine as a laxative and anti-bloodstagnancy drug (Bae, 2001). In our pervious study, RU showed significant antidiabetic activity and, consequently we tried to isolate the active ingredients from an 80% ethanol extract of RU using the bioassay-guided isolation of the antidiabetic agent(s). In a previous paper (Matsuda *et al.*, 2001), stilbene derivatives and anthraquinones were reported from Rhubarb rhizomes. Repeated column chromatographic separation guided by a diabetic activity test based on oral glucose tolerance test led to the isolation of one stilbene, desoxyrhapontigenin (**1**) and two anthraquinones, emodin (**2**) and chrysophanol (**3**). This paper describes the isolation, structural characterization and antidiabetic activities of these compounds.

Correspondence to: Kang Ro Lee, Natural Products Laboratory, College of Pharmacy, SungKyunKwan University, 300 Chonchon-dong, Jangan-ku, Suwon 440-746, Korea
Tel: 82-331-290-7710, Fax: 82-331-292-8800
E-mail: krlee@skku.ac.kr

## MATERIALS AND METHODS

### General experimental procedure

Mps: uncorr. Optical rotations: Jasco P-1020 Polarimeter. NMR: Bruker AMX 500 and Varian UNITY INOVA 500. IR: in CCl4, Nicolet model 205 FT-IR spectrophotometer. MS: VG70-VSEQ mass spectrometer. Column chromatography: Silica gel 60 (Merck, 70~230 mesh and 230~400 mesh), Lichroprep. RP-18 (Merck) and Sephadex LH-20. TLC: Merck precoated Si gel $F_{254}$ plates and RP-18 $F_{254s}$ plates. LPLC: Merck Lichroprep Lobar®-A Si 60 (240 × 10 mm).

### Plant materials

The cultivated Korean rhubarb rhizomes (*Rheum undulatum*, Polygonaceae) were purchased at a market in Seoul, Korea in July 2003. A voucher specimen (SKK-03-010) was deposited at the College of Pharmacy in SungKyunKwan University.

### Extraction, separation and purification of the compounds

The dried material (4 kg) was extracted with 80% aqueous EtOH (10 L × 3) at room temperature for one week and then at 60-70°C for two days. The resultant 80% EtOH extract (RE, 1400 g) was subjected to successive solvent partitioning to give $CHCl_3$ (RE-HC, 36 g), n-BuOH (RE-B, 950 g) and $H_2O$ (RE-W, 340 g) soluble fractions. The n-BuOH fraction significantly prevented postprandial

hyperglycemia (Fig. 1). Thus, the n-BuOH extract (150 g) was chromatographed on a silica gel column using a gradient solvent system of $CHCl_3$:MeOH:$H_2O$ (9:3:0.1~0:1:0) to give four fractions (R1: 1.3L, R2: 1.2L, R3: 5.3L and R4: 3.5L). Fraction R2 (7.5 g) exhibited the most potent hypoglycemic activity (Fig. 2) and was therefore chromatographed over a silica gel column eluted with $CHCl_3$:MeOH:$H_2O$ (9:2:0.1) to give three subfractions (R2-1~R2-3). Subfraction R2-1 (400 mg) exhibited the most potent hypoglycemic activity (Fig. 3). This subfraction (R2-1, 400 mg) was chromatographed on an RP Lobar®-A column (85% MeOH), and purified with a Sephadex LH-20 (MeOH), Lobar,-A column ($CHCl_3$:EtOAc:MeOH=3:1:0.5) and HPLC (RP, 60°¦90%MeOH) to yield compounds **1** (4 mg), **2** (15 mg) and **3** (4 mg).

### Desoxyrhapontigenin (1)

Yellow powder, m.p. 180°C; EI-MS *m/z* (rel. int.) : 242 ($M^+$, 100), 181 (16), 152 (7), 149 (5), 57 (9); $^1$H-NMR (500 MHz, Acetone-$d_6$) : $\delta$ 7.53 (2H, dd, *J*=2.0, 8.5 Hz, H-2',6'), 7.06 (1H, d, *J*=16.5 Hz, olefinic H), 6.96 (2H, dd, *J*=2.0, 8.5 Hz, H-3',5'), 6.96 (1H, d, *J*=16.5 Hz, olefinic H), 6.57 (2H, d, *J*=2.0 Hz, H-2,6), 6.30 (1H, d, *J*=2.0 Hz, H-4), 3.83 (3H, s, $OCH_3$); $^{13}$C-NMR (125 MHz, Acetone-$d_6$) : $\delta$ 159.8 (C-4'), 159.0 (C-3,5), 140.1 (C-1), 130.4 (C-1'), 128.1 (C-β), 127.9 (C-2',6'), 126.9 (C-*β*), 114.3 (C-3',5'), 105.1 (C-2,6), 102.2 (C-4), 54.9 ($OCH_3$).

### Emodin (2)

Yellow powder, m.p. 267°C; FAB-MS *m/z* : 271 ($[M+H]^+$); $^1$H-NMR (500 MHz, Acetone-$d_6$) : $\delta$ 7.55 (1H, br.s, H-4), 7.23 (1H, d, *J*=2.0 Hz, H-2), 7.12 (1H, br.s, H-5), 6.64 (1H, d, *J*=2.0 Hz, H-7), 2.47 (3H, s, $CH_3$); $^{13}$C-NMR (125 MHz, Acetone-$d_6$) : $\delta$ 190.9 (C-9), 181.5 (C-10), 166.0 (C-6), 165.4 (C-8), 162.4 (C-1), 148.8 (C-3), 135.9 (C-10a), 133.6 (C-4a), 124.2 (C-2), 120.8 (C-4), 113.8 (C-9a), 109.6 (8a), 109.1 (C-5), 108.1 (C-7), 21.3 ($CH_3$).

### Chrysophanol (3)

Yellow powder, m.p. 196°C; EI-MS *m/z* (rel. int.) : 254 ($M^+$, 100), 237 (28), 226 (15), 197 (10), 152 (10); $^1$H-NMR (500 MHz, Acetone-$d_6$) : $\delta$ 7.84 (1H, dd, *J*=8.0, 1.5 Hz, H-5), 7.83 (1H, t, *J*=8.0 Hz, H-6), 7.65 (1H, br.s, H-4), 7.37 (1H, dd, *J*=1.5, 8.0 Hz, H-7), 7.21 (1H, br.s, H-2), 2.11 (3H, s, $CH_3$); $^{13}$C-NMR (125 MHz, Acetone-$d_6$) : $\delta$ 192.9 (C-9), 181.7 (C-10), 162.7 (C-1), 162.7 (C-8), 149.8 (C-3), 137.5 (C-6), 134.1 (C-4a), 133.8 (C-10a), 124.6 (C-7), 124.3 (C-2), 120.9 (C-4), 119.6 (C-5), 116.2 (C-8a), 114.2 (C-9a), 21.4 ($CH_3$).

### Oral glucose tolerance test (OGTT)

ICR mice were fasted for 10 h followed by the oral administration of glucose (1.5 g/kg). The blood samples were collected at 0 (prior to glucose administration), 30, 60, and 120 min after the glucose administration. Blood was withdrawn from the orbital venous plexus using a heparinized capillary tube. The blood samples were placed on ice and centrifuged at 5000 rpm at 4°C for 15 min and the plasma was stored at -20°C until the assay. The plasma glucose concentration was determined by the glucose oxidase method (Trinder, 1969).

### Statistical analysis

All data were expressed as means ± S.E.M. For multiple comparisons, an analysis of variance (ANOVA) was carried out, followed by Fishers protected least significant difference test as a post hoc test (Statview, SAS Institute, Carry, U.S.A.). A value of $P < 0.05$ was considered significant.

## RESULTS AND DISCUSSION

The 80% ethanol extract of RU was subject to solvent fractionation, and the n-butanol fraction (RE-B) showed the most potent antidiabetic activity when each fraction was administered at a dose level corresponding to a dose of 1000 mg/kg of the RU ethanol extract. The n-butanol fraction administered at a dose level of 486 mg/kg inhibited postprandial hyperglycemia by 42% (Fig. 1). The antidiabetic activity of the R2 fraction was then effectively enriched by silica gel chromatography, as shown in Fig. 2. The R2 fraction was further purified using a silica gel column to give the R2-1, R2-2 and R2-3 subfractions. As shown in Fig. 3, The R2-1 subfraction significantly inhibited postprandial hyperglycemia by 35%. The R2-1 subfraction

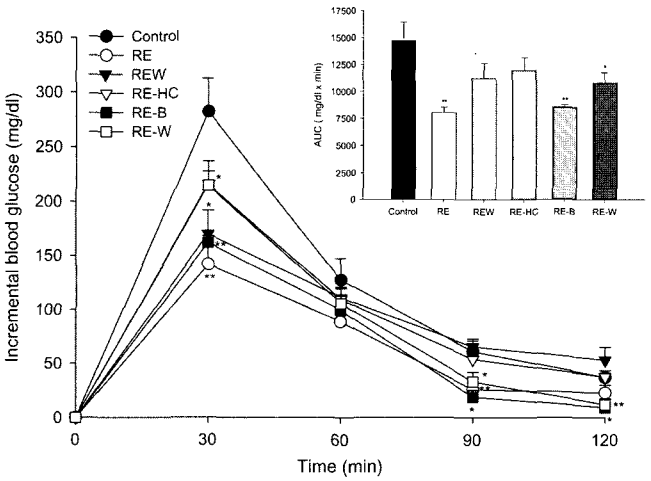


**Fig. 1.** Effect of solvent fractions of the 80% EtOH extract of *Rheum undulatum* on oral glucose tolerance test in ICR mice. The doses administered for RE, REW, RE-HC, RE-B and RE-W were 1000, 100, 70, 486, and 290 mg/kg, respectively. **P<0.01, *P<0.05 compared to control.

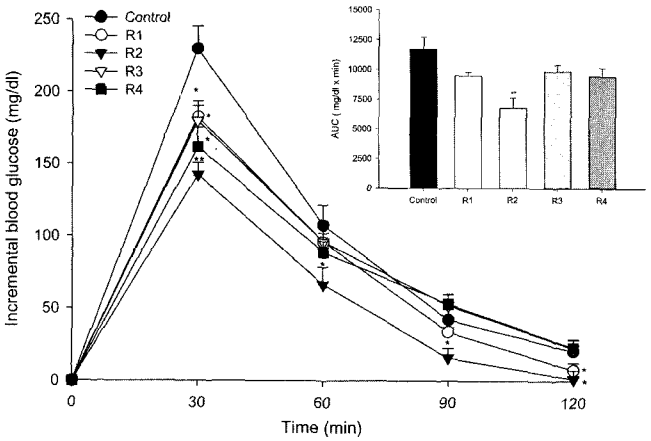


**Fig. 2.** Effect of R1~R4 subfractions of the n-BuOH fraction on oral glucose tolerance test in ICR mice. The doses administered for R1, R2, R3, and R4 were 13.5, 31.5, 153:5, 306.5 mg/kg, respectively. **P<0.01, *P<0.05 compared to control.

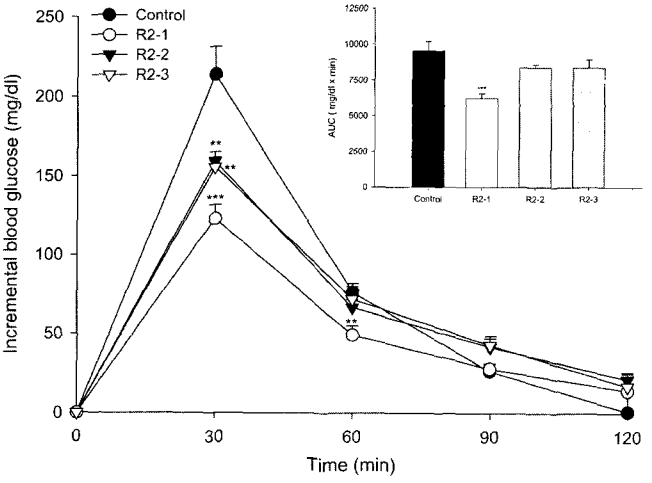


**Fig. 3.** Effect of R2-1~R2-3 of the R2 subfraction on oral glucose tolerance test in ICR mice. The doses administered for R2-1, R2-2, and R2-3 were 7.8, 45.1and 41.2mg/kg, respectively. ***P<0.001, **P<0.01 compared to control.

was further purified to give compounds **1**, **2**, and **3**.

Compound **1** was obtained as a yellow powder. The EI-MS spectrum of **1** showed a molecular ion peak at *m/z* 242. The $^1$H-NMR spectrum showed seven aromatic proton peaks at δ 6.30~7.53, two doublet signals at δ 6.96 (1H, d, *J*=16.5 Hz) and 7.06 (1H, d, *J*=16.5 Hz) and a methoxyl proton signal at δ 3.83 (3H, s). Also the aromatic proton signals at δ 6.57 (2H, d, *J*=2.0 Hz, H-2,6), 6.30 (1H, d, *J*=2.0 Hz, H-4) showed meta coupling. The $^{13}$C-NMR spectrum exhibited the presence of 15 carbon signals, consisting of fourteen olefinic carbon signals at δ 102.2~159.8 and one methoxyl carbon signal at δ 54.9. These spectral data suggested that **1** was a stilbene with a methoxyl group. Thus, the structure of **1** was determined to be 3,5-dihydroxy-4'-methoxyl stilbene (desoxyrhapontigenin). The NMR spectral and physical data of the compound **1** were in good agreement with those reported in a previous paper (Ko *et al.*, 1998).

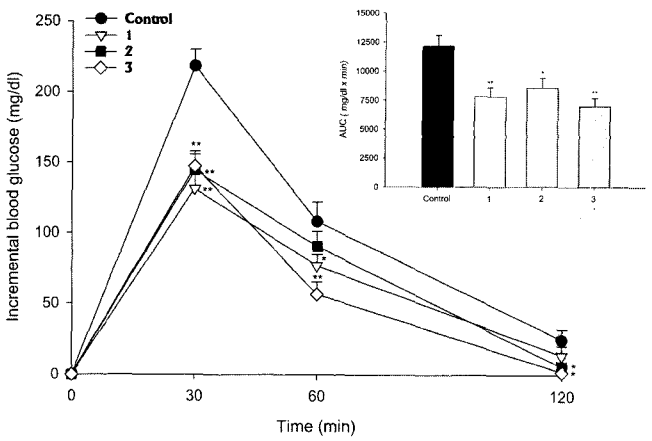


**Fig. 4.** Effect of compounds **1~3** on oral glucose tolerance test in ICR mice. The doses administered for compound 1, 2, and 3 were 0.21, 0.45, and 0.18 mg/kg, respectively. **P<0.01, *P<0.05 compared to control.

Compound **2** was obtained as a yellow powder. The FAB-MS spectrum of **2** showed a molecular ion peak at *m/z* 271 ([M+H]$^+$). The $^1$H-NMR spectrum showed pairs of meta coupling protons at δ 7.55 (1H, br.s, H-4) and 7.23 (1H, d, *J*=2.0 Hz, H-2), and at δ 7.12 (1H, br.s, H-5) and 6.64 (1H, d, *J*=2.0 Hz, H-7), and a methyl proton at δ 2.47 (3H, s). The $^{13}$C-NMR spectrum showed two carbonyl carbons at δ 181.5 and 190.9, two oxygenated quaternary carbons at δ 162.4 and 165.4, and a methyl carbon at δ 21.3. These spectral data suggested that **2** was an anthraquinone derivative. Thus, the structure of **2** was determined to be 1,6,8-trihydroxy-3-methyl anthraquinone (emodin). The NMR spectral and physical data of compound **2** were in good agreement with those reported in a previous paper (Kim *et al.*, 1998).

Compound **3** was obtained as a yellow powder. The EI-MS spectrum of **3** showed a molecular ion peak at *m/z* 254. The $^1$H-NMR spectrum showed ABC coupling protons at δ 7.84 (1H, dd, *J*=8.0, 1.5 Hz, H-5), 7.83 (1H, t, *J*=8.0 Hz, H-6), and 7.37 (1H, dd, *J*=1.5, 8.0 Hz, H-7), a pair of singlet signals at δ 7.65 (1H, br. s, H-4) and 7.21 (1H, br. s, H-2) and a methyl proton at δ 2.11 (3H, s). The $^{13}$C-NMR spectrum showed two carbonyl carbons at δ 181.7 and 192.9, two oxygenated quaternary carbons at δ 162.7 and 162.7 and a methyl carbon at δ 21.4. These spectral data suggested that **3** was an anthraquinone derivative. Thus, the structure of **3** was determined to be 1,8-dihydroxy-3-methyl anthraquinone (chrysophanol). The NMR spectral and physical data of compound **3** were in good agreement with those reported in a previous paper (Kim *et al.*, 1998). Compounds **1**, **2**, and **3** inhibited

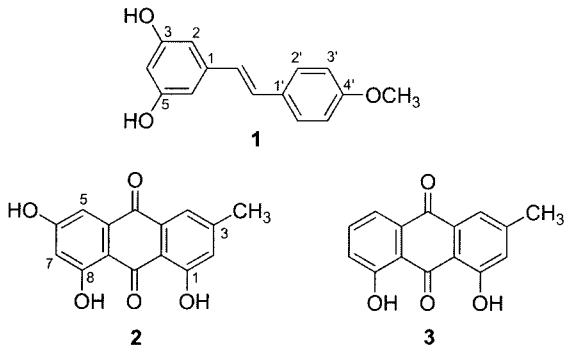


**Fig. 5.** Structures of compounds **1~3**

postprandial hyperglycemia by 35.8, 29.5, and 42.3%, respectively.

Although it is widely known that RU has been utilized to treat senile constipation, dyslipidemia and liver dysfunction, in our previous study using db/db mice, RU was also found to reduce the body weight and plasma glucose levels. The overexpression of glucose transporter-4 (GLUT-4) in skeletal muscle and the alteration of the hepatic enzyme activities were previously found to be responsible for the plasma glucose lowering activity of RU. To the best of our knowledge, however, there are no known active ingredients in RU which have been reported to show antidiabetic activity. Ko *et al.* reported that among the three stilbenes (desoxyrhapontigenin, rhapontigenin, and piceatannol) contained in cultivated Korean rhubarb rhizomes (*Rheum undulatum*) rhapontigenin and desoxyrhapontigenin strongly inhibitied the aggregation induced by arachidonic acid and collagen. These inhibitory effects may partially contribute to the anti-blood stagnancy activity of rhubarb (Ko *et al*, 1999). Chen *et al.* recently reported that among the three structurally related anthraquinones, including emodin, physcion, and chrysophanol, emodin showed the most potent cytotoxic effects on HL-60 cells, with these effects being accompanied by the dose- and time-dependent appearance of the characteristics of apoptosis including an increase in the DNA ladder intensity, morphological changes, the appearance of apoptotic bodies, and an increase in the number of hypodiploid cells. (Chen *et al*, 2002).

We are currently attempting to determine the hypoglycemic mechanism of the isolated three compounds which were isolated in this study, using several types of *in vitro* experiments, such as the hepatic glucose production assay using the H4IIE hepatoma cell line, the insulin secretion assay using primary pancreatic beta cells, and the insulin resistance related assay using differentiated adipocytes.

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