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A New Stilbene Diglycoside from *Rheum undulatum*

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A new stilbene diglycoside, piceatannol-3, 4'-O-β-D-diglucopyranoside (I), together with desoxyrhaponticin (II), emodin-1-O-β-D-glucopyranoside (III), and physcion-8-O-b-D-glucopyranoside (IV), were isolated from the rhizomes of cultivated Korean rhubarb rhizomes (*Rheum undulatum*), Jong DaeWhang, and the structures of I-IV were identified on the basis of chemical and spectral evidences.

Key words: Stilbene diglycoside, Piceatannol-3, 4'-O-β-D-diglucopyranoside, Desoxyrhponticin, Emodin-1-O-β-D-glucopyranoside, Physcion-8-O-β-D-glucopyranoside, Cultivated Korean rhubarb rhizomes, *Rheum undulatum*

INTRODUCTION

Cultivated Korean rhubarb rhizomes (*Rheum undulatum* Linne, Polygonaceae) have been used as Chinese herbal medicine as stomachic bitter, laxative and purgative with secondary astringent action in indigestion, analgesic, and anti-bloodstagnancy, in Korea. (Yook, 1992). In the previous paper (Ko *et al.*, 1995, 1998), the isolation of five stilbene derivatives (desoxyrhapontigenin, rhapontigenin, piceatannol, rhaponticin, piceatannol-3'-O-β-D-glucopyranoside) and six anthraquinone derivatives (chrysophanic acid, emodin, physcion, chrysophanol-8-O-β-D-glucopyranoside, emodin-8-O-β-D-glucopyranoside, aloe-emodin-8-O-β-D-glucopyranoside) from cultivated Korean rhubarb rhizomes (*Rheum undulatum*) have been reported. Regarding phytochemical studies of Rhubarb, anthraquinone derivatives (Tsukida *et al.*, 1954, Uchibayashi *et al.*, 1961, Okabe *et al.*, 1973, Oshio *et al.*, 1974, 1978, Holzschuh *et al.*, 1982, Khetwal *et al.*, 1988, Rawat *et al.*, 1989, Miyamoto *et al.*, 1967, 1972, and Yamagishi *et al.*, 1987), and stilbene derivatives (Yaki *et al.*, 1971 and Kashiwada *et al.*, 1984a, 1984b, 1988) have been reported.

For the continuous study of phytochemical components, cultivated Korean Rhubarb Rhizomes (rhizomes of *Rheum undulatum*, JongDaeWhang) were investigated. Two stilbene derivatives, piceatannol-3, 4'-O-β-D-diglucopyranoside (3', 5-dihydroxystilbene-3, 4'-O-β-D-diglucopyranoside), desoxyrhaponticin and two anthraquinone derivatives, emodin-1-O-β-D-glucopyranoside, physcion-8-O-β-D-glucopyranoside were isolated and characterized on the basis of physico-chemical and spectro-scopic evidences. Stilbene diglycoside, piceatannol-3, 4'-O-β-D-diglucopyranoside represents the first report.

MATERIALS AND METHODS

Instruments

Melting points were determined on Electrothermal IA 8100 apparatus and are uncorrected. IR and UV spectra were obtained with a Bruker IFS48 FT-IR and a Varian Cary-3 spectrophotometer, respectively. PMR and CMR spectra were measured with either a Varian (Gemini 2000) (300 MHz) or a Bruker AMX-500 (500 MHz) spectrometer with tetramethylsilanes as an internal standard. Mass spectrum was taken on a GC-MS/MS-DS, TSQ 700, and Autospec (Micromass) mass spectrometer.

Plant material

Cultivated Korean rhubarb rhizomes (*Rheum undulatum*) were collected in May (1991) at Chong Ju of Chung Chong Buk Do. The voucher specimen was deposited at the Department of Pharmacal Resources Botany, College of Pharmacy, Chung-Ang University.

Extraction and isolation

The dried material (120 g) was extracted with hot water for 4 h and followed by concentration in vacuo. The water extract (50 g, yield 41.7%) was subjected to column chromatography over Sephadex LH-20 (200 g)

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eluting sequentially with H_2O (3.5 L), 20% MeOH (2.5 L), 40% MeOH (2.5 L), 60% MeOH (2.0 L), and 80% MeOH (2.0 L). The eluates were grouped based on TLC pattern to yield fractions designated as F1-F5 : void volumn (0.7 L), F1 (3.3 L), F2 (2.3 L), F3 (2.4 L), F4 (1.9 L), and F5 (1.9 L).

The F3 fraction (3.2 g) was chromatographed on silica gel column (300 g) with eluting solvents of $CHCl_3$-MeOH-H_2O (80:25:2.5) to give six subfractions (F31-F36).

The F36 fraction (400 mg) was further subjected to ODS (C-18) column chromatography (200 g, 30% MeOH) to afford compound **I** (50 mg). The F33 fraction (500 mg) was rechromatographed over ODS (C-18) column chromatography (200 g, 70% MeOH) to afford compound **II** (50 mg) and **III** (30 mg).

The F2 fraction (10.8 g) was rechromatographed on a Sephadex LH-20 column (200 g) with eluting solvent of 50% MeOH to give three subfractions (F21-F23).

The F23 fraction (300 mg) was further subjected to ODS (C-18) column chromatography (200 g, 70% MeOH) to afford compound **IV** (30 mg).

Acid hydrolysis of compound I, II, III, and IV

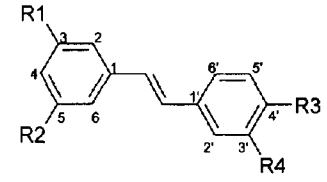
Compound **I**, **II**, **III**, and **IV**(each 10 mg) were hydrolyzed by acid using the method described in general procedure. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. Then, $CHCl_3$ part thus obtained was washed with H_2O and concentrated to give aglycone. The water layer was neutralized with mixed bed resin TMD-8 column and concentrated to give sugars. This aglycones and sugars were identified by direct comparison with authentic samples.

Compound I : Colorless needles (dil. acetone), mp: 282°C ; IR ν_{max}^{KBr} cm^{-1} : 3502 (OH), 1647, 1507 (C=C) ; UV λ_{max} MeOH : 220, 300, 320; FABMS (negative) *m/z*: 567 $[M-H]^-$, 405$[M-Glu]^-$, 243$[M-2Glu]^-$; ^{1}H-NMR (Acetone-d_6+D_2O, 300 MHz): δ 4.93, 4.99 (each 1H, d, *J*=7.5 Hz, anomeric H), 6.55 (1H, s, H-4), 6.74 (1H, s, H-6), 6.84 (1H, s, H-2), 6.89 (1H, d, *J*=8.4 Hz, H-5'), 6.98 (1H, d, *J*=16.2 Hz, olefinic H), 7.11 (1H, d, *J*=16.5 Hz, olefinic H), 7.16 (1H, dd, *J*=1.8, 8.4 Hz, H-6'), 7.54 (1H, d, *J*=1.8 Hz, H-2'); ^{13}C-NMR (Acetone-d_6 +D_2O, 75.5 MHz): 140.7 (C-1), 106.8 (C-2), 160.1 (C-3), 104.1 (C-4), 159.4 (C-5), 108.1 (C-6), 130.5 (C-1'), 116.2 (C-2'), 148.1 (C-3'), 146.7 (C-4'), 117.0 (C-5'), 123.3 (C-6'), 127.2 (α), 129.5 (β), 101.9 (3G-1), 74.3 (3G-2), 77.6 (3G-3), 71.0 (3G-4), 77.0 (3G-5), 62.2 (3G-6), 103.7 (4'G-1), 74.4 (4'G-2), 78.0 (4'G-3), 71.0 (4'G-4), 77.5 (4'G-5), 62.3 (4'G-6)

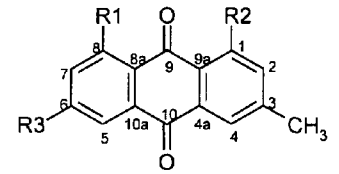
Compound II : Colorless needles (dil. acetone), mp: 227°C ; IR ν_{max}^{KBr} cm^{-1} : 3400(OH), 1590, 1510(C=C) ; UV λ_{max} nm MeOH : 215, 306, 319 ; EIMS (*m/z*): 404$[M]^+$, 242$[M-Glu]^+$; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 3.75 (3H, s, OCH_3), 4.81 (1H, d, *J*=7.2 Hz, anomeric H), 6.36 (1H, s, H-4), 6.59, 6.76 (each 1H, s, H-2, 6), 6.93, 7.08 (each 1H, d, *J*=16.7 Hz, olefinic H), 6.92, 7.05 (each 2H, d, *J*=8.7 Hz, H-3',5' and H-2',6'), 9.50 (1H, OH, disappeared on addition of D_2O) ; ^{13}C-NMR (DMSO-d_6, 75.5 MHz): δ 139.5 (C-1), 105.1 (C-2), 158.7 (C-3), 103.2 (C-4), 158.5 (C-5), 107.6 (C-6), 129.9 (C-1'), 128.1 (C-2'), 113.9 (C-3'), 159.3 (C-4'), 114.4 (C-5'), 128.1 (C-6'), 126.5 (α), 128.5 (β), 55.3 (OCH_3), 100.9 (G-1), 73.5 (G-2), 77.3 (G-3), 70.0 (G-4), 76.9 (G-5), 60.9 (G-6)

Compound III : Orange needles (MeOH), mp : 239°C ;IR ν_{max}^{KBr} cm^{-1} : 1625 (chelated C=O), 1660 (non-chelated C=O) ; UV λ_{max} nm MeOH : 253, 287, 425; EIMS (*m/z*) : 432$[M]^+$, 270$[M-Glu]^+$; ^{1}H-NMR (DMSO-d_6, 300 MHz) : δ 2.37 (3H, s, CH_3), 4.98 (1H, d, *J*=7.5 Hz, anomeric H), 6.29 (1H, d, *J*=2.1 Hz, H-7), 6.85 (1H, d, *J*=2.4 Hz, H-5), 7.45 (1H, s, H-2), 7.60 (1H, s, H-4), 13.40, 13.80 (each 1H, OH, disappeared on addition of D_2O); ^{13}C-NMR: (DMSO-d_6, 75.5 MHz): δ 158.8 (C-1), 122.8 (C-2), 147.3 (C-3), 124.7 (C-4), 109.2 (C-5), 170.2 (C-6), 108.8 (C-7), 165.7 (C-8), 185.6 (C-9), 183.8 (C-10), 134.6 (C-4a), 111.1 (C-8a), 119.8 (C-9a), 135.1 (C-10a), 22.2 (CH_3), 102.1 (G-1), 73.8 (G-2), 76.5 (G-3), 70.2 (G-4), 77.7 (G-5), 61.3 (G-6)

Compound IV : Orange needles (MeOH), mp : 231°C



	R1	R2	R3	R4
Compound I	O-Glc	OH	O-Glc	OH
Compound II	OH	O-Glc	OCH_3	H



	R1	R2	R3
Compound III	OH	O-Glc	OH
Compound IV	O-Glc	OH	OCH_3

Fig. 1. Structures of compound **I-IV** from *Rheun undulatum*

;IR ν_{max}^{KBr} cm^{-1} : 3445 (OH), 1634 (chelated C=O), 1594, 1507, 1456 (C=C) ; UV λ_{max} nm MeOH : 220, 280, 420 ; EIMS (*m/z*) : 446[M]$^+$, 284[M-Glu]$^+$; ^{1}H-NMR (DMSO-d_6, 300 MHz) : δ 2.46 (3H, s, CH_3), 4.01 (3H, s, OCH_3), 5.21 (1H, d, *J*=7.8 Hz, anomeric H), 7.22 (1H, s, H-2), 7.23 (1H, d, *J*=1.5 Hz, H-7), 7.42 (1H, d, *J*=2.1 Hz, H-5), 7.54 (1H, s, H-4) ; ^{13}C-NMR : (DMSO-d_6, 75.5 MHz) :δ 161.9 (C-1), 121.6 (C-2), 147.3 (C-3), 124.4 (C-4), 106.6 (C-5), 164.9 (C-6), 107.4 (C-7), 160.9 (C-8), 186.7 (C-9), 182.2 (C-10), 132.2 (C-4a), 119.5 (C-8a), 114.6 (C-9a), 136.5 (C-10a), 21.4 (CH_3), 56.1 (OCH_3), 100.7 (G-1), 73.3 (G-2), 76.6 (G-3), 69.8 (G-4), 77.5 (G-5), 60.8 (G-6)

RESULTS AND DISCUSSION

The water extract from cultivated Korean rhubarb rhizomes (*Rheum undulatum*) was chromatographed on Sephadex LH-20 gel, ODS (C-18) gel, and silica gel successively. Two compounds (compound **I**, **II**) were identified as stilbene derivatives and the remaining two compounds (compound **III**, **IV**) were identified as anthraquinone derivatives.

Compound **I** was obtained as a colorless needles. IR spectrum of compound **I** gave 3502 (OH), 1647, 1507 (C=C) cm^{-1} and UV spectrum 220, 300 and 320 (sh.) nm. On acid hydrolysis, compound I produced piceatannol and D-glucose (2 mole). In the FABMS spectrum of compound I $[M-H]^-$ peak and fragment ions appeared at *m/z* 567, 405, and 243, suggesting it to be a stilbene diglycoside derivative.

The ^{1}H-NMR spectrum (Acetone-d_6+D_2O) showed three singlet signals at δ 6.55, δ 6.74, 6.84 (each 1H, H-4, H-6, H-2), two doublet signals at δ 6.89, δ 7.54 (each 1H, d, *J*=8.4 Hz, *J*=1.8 Hz, H-5', H-2'), and a double doublet signal at δ 7.16 (1H, dd, *J*=1.8, 8.4 Hz, H-6') on aromatic ring, and two doublet signals at δ 6.98 (1H, d, *J*=16.2 Hz) and δ 7.11 (1H, d, *J*=16.5 Hz) showed trans-olefinic protons, and also the anomeric proton signal at δ 4.93 and 4.99 (each 1H, d, *J*=7.5 Hz) supported our assignment for the β-configuration.

The ^{13}C-NMR spectrum (Acetone-d_6+D_2O) showed two anomeric carbon signals at 103.7 and 101.9. The linkage between the anomeric carbons/aromatic carbons and anomeric protons/aromatic protons was determined from HMQC and HMBC spectra. In the HMBC spectrum, C-3 signal (δ 160.1) was correlated to anomeric proton (δ 4.99), H-2 (δ 6.84), and H-4 (δ 6.55), and C-5 signal (δ 159.4) was correlated to H-4 (δ 6.55) and H-6 (δ 6.74), and C-3' signal (d 148.1) was correlated to H-2' (δ 7.54) and H-6' (δ 7.16), and C-4' signal (δ 146.7) was correlated to anomeric proton (δ 4.93), H-5' (δ 6.89), and H-6' (δ 7.16).

On the basis of the above results, the structure of was established as piceatannol-3, 4'-O-β-D-diglucopyranoside (3', 5-dihydroxystilbene-3, 4'-O-β-D-diglucopyranoside).

Compound **II-IV** were identified as desoxyrhaponticin (**II**), emodin-1-O-β-D-glucopyranoside (**III**), physcion-8-O-β-D-glucopyranoside (**IV**) by comparison of physicochemical data and spectral evidences (IR, UV, MS, ^{1}H- and ^{13}C-NMR, and C-H COSY) with those reported in the literatures (Kashiwada *et al*, 1984, Kato *et al*, 1987).

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