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A Triterpene Glucosyl Ester from the Roots of *Rubus crataegifolius*

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Along with five known triterpene glycosides, a new triterpene glucosyl ester, named crataegioside, was isolated from the roots of *Rubus crataegifolius* Bunge. The structure was established as ilexosapogenin A 28-*O*-β-D-glucopyranosyl ester by chemical and spectroscopic methods.

Key words: *Rubus crataegifolius*, Rosaceae, Triterpenoids, Oleananes, 28β-Glucosyl Ester of Ilexosapogenin A

INTRODUCTION

Many species of the genus *Rubus* are employed as a folk medicine for the treatment of various diseases including diabetes (Alonso *et al.*, 1980; Niero *et al.*, 1999). Chemical and pharmacological studies have confirmed that some of these plants produce active principles that exert hypoglycemic activity, antibacterial effects against Gram-positive bacteria, and anti-allergic activity against allergic rhinitis, atopic dermatitis, and asthma (Swanston-Flatt *et al.*, 1990; Richards *et al.*, 1994; Nakahara 1996). The plant *Rubus crataegifolius* Bunge (Rosaceae) is ubiquitous in the mountain area of Korea and its fruits have been used as a traditional medicine in Korea and China (Korean name "Bokbun-ja", Chinese name "Fu-pen-zi"). In this paper, we report the isolation and structure elucidation of a new triterpene glucosyl ester, crataegioside, from the roots of the plant.

MATERIALS AND METHODS

General

Mp: uncorr. IR and optical rotations were determined on a JASCO FTIR-8100 and a JASCO DIP-370 instrument, respectively. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Varian AM-500 NMR spectrometer in MeOH-d_4 or $CDCl_3$ with TMS as an internal standard. FABMS data were obtained on a JEOL JMS-D-300 spectrometer.

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Plant material

Roots of *R. crataegifolius* were collected at the Mt. Kumjung, Pusan, Korea in July, 1998. The plant was identified by Prof. Jong Hee Park (Dept. of Pharmacy, Pusan National University, Korea) and the voucher specimen was deposited in the authors laboratory.

Extraction and isolation

The dried roots of the plant (3.6 kg) were extracted with hot MeOH to give an extract (98 g), which was suspended in H_2O and extracted with Et_2O, EtOAc, and *n*-BuOH, successively. A portion (5 g) of the EtOAc extract (19.5 g) was chromatographed on silica gel ($CHCl_3$-MeOH, 5:1) to give four frs. I-IV in order of elution. Fr. III was rechromatographed on silica gel ($CHCl_3$-MeOH-H_2O, 7:3:1, lower phase) to give two mixtures, **1** and **2** (94 mg), and **3** and **4** (79.5 mg). Fr. IV gave a mixture of **5** and **6** (102.5 mg) by silica gel CC ($CHCl_3$-MeOH-H_2O, 65:35:10, lower phase). Each mixture was further puri-fied by HPLC (YMC, ODS-80H, 30 cm × 10 mm i.d., MeOH-H_2O, 7:3) and acetylated with Ac_2O and pyridine to give corresponding acetate mixtures (**1a** and **2a, 3a** and **4a** , **5a** and **6a**). Each of acetate mixtures was subjected to silica gel HPLC separation (m-Porasil, 25 cm × 7.8 mm i.d., *n*-hexane-EtOAc, 20:1) to give respective pure acetate.

1a: Colorless needles, mp 170-171°C, $[\alpha]_D^{24}$ +22.3° ($CHCl_3$, c 1.26). IR $\nu_{Max}^{CHCl_3}$ cm^{-1} : 3440 (w, OH), 1738, 1225 (OAc and ester), ^{1}H NMR ($CDCl_3$): δ 0.98, 1.01, 1.05, 1.07, 1.23, 1.36 (each 3H, *s*, 6 × *tert*-CH_3), 1.98 (3H, *s*), 2.01 (6H, *s*), 2.03 , 2.06, 2.08 (each 3H, *s*) (OAc), 2.52 (1H,

s, H-18b), 3.33 (1H, *m*, H-19b), 3.54, 3.98 (2H, *q*, *J*=12 Hz, H_2-23), 5.38 (1H, *m*, H-12), 5.50 (1H, *d*, *J*=7.8 Hz, H-1')

Deacetylation of 1a-6a

Each of **1a-6a** was mixed with 2M-K_2CO_3 in 80% aq. MeOH (20 ml) and kept stirring at room temp. for 3 h. The reaction mixture was diluted with H_2O (20 ml) and extracted with EtOAc (40 ml × 2) and the total EtOAc extract was washed with H_2O, dried over anhydrous $MgSO_4$, and evaporated *in vacuo* to give natural glycosides **1-6** [**1** (44 mg), **2** (32 mg), **3** (31 mg), **4** (28 mg), **5** (38 mg), and **6** (54 mg)].

1: Amorphous solid, mp 251-253°C, $[\alpha]_D^{24}$ +19.2° (MeOH, c0.95), IR$\nu_{Max}^{CHCl_3}$ cm^{-1}: 3420 (OH), 1720 (ester). ^{1}H NMR (500 MHz, MeOH-d_4): δ 0.94, 1.04, 1.08, 1.20, 1.24, 1.36 (each 3H, *s*, 6 × *tert*-CH_3), 3.28 (1H, *br s*, H-18β), 3.32 (1H, *m*, H-19β) 5.55 (1H, *t*, H-12), 6.32 (1H, *d*, *J*=7 Hz, H-1'). ^{13}C NMR: Table I. FABMS: *m/z* 673 $(M+Na)^+$, HR-FABMS (positive) *m/z*: 673.8380 $(M+Na)^+$ ($C_{36}H_{58}O_{10}Na$ requires 673.8392).

Alkaline hydrolysis of compounds 2-6

A solution of **2-6** (10 mg each) in 5% KOH-EtOH (10 ml) was refluxed for 1 h. The whole mixture was neutralized with Dowex 50 WX8 (H^+ form) and the resin was filtered. After removal of the solvent *in vacuo* from the filterate, the residue was purified by reversed-phase HPLC (YMC, ODS-80H, H_2O-MeOH, 1:9) to afford each aglycone. Aglycones were identified as rotundic acid for **2**, arjungenin for **3**, 23-hydroxy-tormentic acid for **4**, barrinic acid for **5**, and 2α,3β,19α-trihydroxyurs-12-ene-23,28-dioic acid for **6**, respectively. Identification was made based on mp, $[\alpha]_D$, IR, and ^{1}H NMR data for **4**, while those of other compounds were made based on IR, ^{1}H NMR and ^{13}C NMR data.

Acid hydrolysis of 1

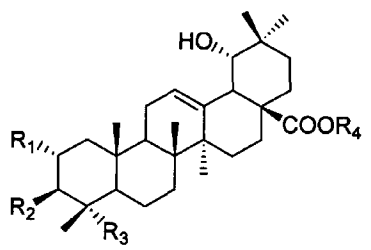
A solution of **1** (3 mg) in 5% aq. H_2SO_4-1,4-dioxane (1:1, v/v, 2 ml) was heated under reflux for 1 h. The reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was filtered off. The residue obtained after removal of the solvent was passed through the ODS (Cosmosil 75C_{18}-OPN, Nakalai, Japan) column with H_2O. The H_2O eluate was concentrated. And the residue was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.5 ml) in pyridine for 1 h. The supernatant was subjected to GLC analysis. GLC: FID detector, carrier gas: N_2 at 40 ml min^{-1}, OV-17 on Uniport HP (80-100 mesh), 2 m × 3 mm i.d. column, column temp.: 150°C, injection temp.: 180°C, t_R: 6.2.

Enzymatic hydrolysis of 1

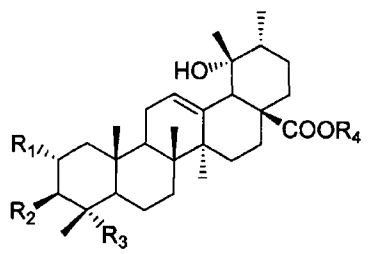
To a solution of **1** (8 mg) in 0.1 M acetate buffer (pH

Table I. ^{13}C NMR data for **1** (MeOH-d_4, δ)

Carbon	δ	Carbon	δ
1	38.6	21	29.1
2	27.7	22	33.6
3	72.7	23	66.3
4	42.9	24	13.0
5	48.6	25	15.8
6	18.7	26	17.5
7	33.0	27	24.6
8	40.0	28	177.3
9	48.4	29	28.9
10	37.4	30	24.8
11	24.2		
12	123.6	glc	
13	144.4	1'	95.9
14	42.1	2'	74.1
15	28.4	3'	79.3
16	29.2	4'	71.3
17	46.1	5'	79.0
18	44.8	6'	62.3
19	79.0		
20	35.7		



	R_1	R_2	R_3	R_4
1	H	OH	CH_2OH	Glc
1a	H	OAc	CH_2OAc	Glc-$(OAc)_4$
2	OH	OH	CH_2OH	Glc
2a	OAc	OAc	CH_2OAc	Glc-$(OAc)_4$
3	OH	OH	COOH	Glc
3a	OAc	OAc	COOH	Glc-$(OAc)_4$



	R_1	R_2	R_3	R_4
4	H	OH	CH_2OH	Glc
4a	H	OAc	CH_2OAc	Glc-$(OAc)_4$
5	OH	OH	CH_2OH	Glc
5a	OAc	OAc	CH_2OAc	Glc-$(OAc)_4$
6	OH	OH	COOH	Glc
6a	OAc	OAc	COOH	Glc-$(OAc)_4$

4.5, 6 ml) was added naringinase (Sigma, 10 mg) and the mixture was stirred at 40°C for 24 h, poured into EtOH and the whole mixture was evaporated to dryness. The residue was subjected to HPLC (YMC, ODS-80H, 30 cm × 10 mm i.d, H_2O-MeOH, 1:9) to give an aglycone, which was identified to be ilexosapogenin A by comparison of physical data (IR, ^1H NMR, and ^{13}C NMR) with reported values.

Alkaline hydrolysis of 1

A solution of **1** (10 mg) was treated as above to give the same aglycone which was identified by TLC.

RESULTS AND DISCUSSION

The methanolic extract of the roots of *R. crataegifolius* was suspended in H_2O and extracted with Et_2O, EtOAc, and *n*-BuOH, successively. The EtOAc extract was subjected to Sephadex LH-20 and silica gel column chromatography to afford three fractions containing glycosides. Each fraction was acetylated after purification by reversed-phase HPLC, and resulting acetate mixtures were subjected to normal-phase HPLC separation to give each acetate, **1a-6a**. The acetates were deacetylated by treating with 2M-K_2CO_3 in 80% aq. MeOH to give parent glycosides **1-6**.

Compounds **2-6** were assumed to be β-D-gluco-pyranosyl esters of triterpenes as indicated by anomeric proton signals at δ 6.29-6.32 as doublets (J=7-8 Hz) in ^1H NMR and an anomeric carbon signals at d 95.8-95.9 in ^{13}C NMR. D-Glucose was liberated from compounds **2-6** as common sugar constituent on acid hydrolysis, and corresponding aglycones were obtained on alkaline hydrolysis. Each aglycone of the respective glycoside was identified by comparing the IR, ^1H NMR and ^{13}C NMR data with those of reported values, i.e. arjungenin (Honda *et al.*, 1976) for **2**; barrinic acid (Barua *et al.*, 1972) for **3**; rotundic acid (Oyama *et al.*, 1968) for **4**; 23-hydroxytormentic acid (Seto *et al.*, 1984) for **5**; and 2α,3β,19α-trihydroxyurs-12-ene-23,28-dioic acid (Gao *et al.*, 1985) for **6**, respectively. Thus, compounds **2-6** were identified as arjunglycoside I (Honda *et al.*, 1976), 28-β-D-glucosyl ester of barrinic acid (Pal *et al.*, 1991), pedunculoside (Athayde *et al.*, 1999), dotorioside II (Im *et al.*, 1994), and suavissimoside R1 (Gao *et al.*, 1985), respectively.

A new compound, crataegioside (**1**), $[\alpha]_D^{25}$ +19.2°, was obtained as an amorphous solid. The IR spectrum of **1** showed absorption bands at 3420 (OH) and 1720 cm^{-1} (ester). In the FABMS of **1**, quasimolecular ion peak was observed at *m/z* 673 $(M+Na)^+$, and HR-FABMS analysis revealed the molecular formula of **1** to be $C_{36}H_{58}O_{10}$. Acid hydrolysis of **1** afforded D-glucose and the aglycone with traces of artifacts. Alkaline hydrolysis and enzymatic hydrolysis with naringinase afforded a genuine aglycone, which showed identical IR, ^1H, and ^{13}C NMR data with those of ilexosapogenin A, an aglycone constituent of ilexoside XXXII (Amimoto *et al.*, 1992). Compound **1** showed an anomeric proton signal at d 6.32 (*d*, J = 7 Hz) and anomeric carbon signal at δ 95.9, respectively, in its ^1H and ^{13}C NMR spectrum. In the HMBC experiment on **1**, the ester carbon signal at δ 177.3 (C-28) gave a cross peak with the anomeric proton signal at δ 6.32, indicating that the glucopyranosyl group is linked to C-28 of the aglycone. Accordingly, **1** was postulated as ilexosapogenin A 28-*O*-β-D-glucopyranosyl ester.

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