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Quercetin Glycosides from Bark of Maple (*Acer komarovii* Pojark.)*1

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ABSTRACT

The chemical constituents of *Acer komarovii* Pojark. which belongs to Aceraceae has never been reported. The bark of *A. komarovii* was extracted with 70% aqueous acetone, and the concentrated extract was successively partitioned with *n*-hexane, dichloromethane, ethyl acetate and H_2O. From the ethyl acetate soluble fraction, four compounds were isolated by the repeated Sephadex LH-20 and RP C-18 column chromatography. From the results of spectroscopic methods including FAB-MS, 1D and 2D NMR, the structures of the compounds were determined as quercetin **(1)**, guaijaverin **(2)**, hirsutrin **(3)** and hyperin **(4)**. These compounds **(1-4)** have not been reported in this tree yet.

Keywords : *Acer komarovii*, quercetin, guaijaverin, hirsutrin, hyperin

1. INTRODUCTION

Acer komarovii (Acer tschonoskii var. *rubripes)*, one of the rare maple species growing in Korea, has a narrow distribution in Korean peninsula to northeast China and Russia. This species grows on higher mountain slopes, dry bluffs or foothills, but reaches up to high elevation (ca. 1500 m) in Korea. It is not much used in the wood industry, because it is shrubby tree species[1,2].

Up to now, there is no any previous phytochemical study that has been carried out on *A. komarovii*. Previous chemical reports on the genus *Acer* include the isolation of diarylheptanoids[3,4], flavonoids[5-7], phenylethyl glycosides[5], coumarinolignans[8], neolignan glycoside[9], stilbene glycosides[10], hydrolysable tannins[11-14]. The aim of this study was to investigate the chemical constituents of *A. komarovii* bark, which were separated by column chromatographic method. Their chemical structures were identified by spectroscopic methods including ^{1}H NMR, ^{13}C NMR, HMBC and FAB-MS.

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2. MATERIALS and METHODS

2.1. General Experimental

^{1}H (400 and 600 MHz) and ^{13}C NMR (100 and 125 MHz) spectra were recorded on a Bruker Avance DPX 400 and 600 spectrometers using tetramethylsilane (TMS) as an internal standard, and chemical shift are given in δ (ppm). Positive FAB-MS were performed with a Micromass Autospec M363 spectrometer using *m*-nitro benzyl alcohol (NBA) as a matrix. Column chromatography was conducted with lipophilic Sephadex LH-20 (25～100 μm, Sigma). Medium pressure liquid chromatography (MPLC) was carried out by a Combiflash Retrieve (ISCO) apparatus with the columns containing RP C-18 derivatized silica (230～400 mesh). Thin layer chromatography (TLC) was carried out on DC-Plastikfolien Cellulose F (Merck) plates and developed with TBAW (*t*-BuOH-HOAc-H_2O (3 : 1 : 1, v/v/v)) and 6% aqueous HOAc. Spots were detected by spraying vanillin (vanillin-EtOH-H_2SO_4 (15 : 250 : 2.5, w/v/v) and 1% methanolic $FeCl_3$ on a plate, and then followed by heating.

2.2. Plant Material

The bark of *Acer komarovii* was collected at Jeongseon, Gangwon province in September 2008 and identified by Professor Wan-Geun Park of the Department of Forestry. A voucher specimens were deposited in the laboratory at the Department of Wood Science and Engineering, Kangwon National University.

2.3. Extraction and Isolation

The bark of *A. komarovii* (3.96 kg) was extracted with 12 ℓ of 70% aqueous acetone at room temperature for 5 days. After filtration (Advantec No. 2), the residue was repeated the above procedure two times. The filtrates were combined and evaporated with a rotary evaporator under reduced pressure at 40°C and the residues (500 g) was suspended in water (1 ℓ) and successively extracted with 2 ℓ of *n*-hexane, 3 ℓ of dichloromethane (DCM) and 5 ℓ of ethyl acetate (EtOAc). The extract was evaporated under reduced pressure to obtain the *n*-hexane fraction (32.4 g), DCM fraction (56.6 g), EtOAc fraction (123.2 g) and H_2O fraction (199.4 g).

The EtOAc-soluble fraction (70 g) was chromatographed on a Sephadex LH-20 column (5 × 60 cm) eluting with MeOH-H_2O (3 : 1, 4 ℓ) to obtain 5 fractions (Fr. 1～Fr. 5). Fr. 1 was rechromatographed on a Sephadex LH-20 and MPLC RP C-18 (3 × 15 cm) column with EtOH-hexane (3 : 1, 1 ℓ) and MeOH-H_2O (1 : 5, 1 : 3 and 1 : 2, 1 ℓ) to give compound **4** (220 mg). Fr. 2 and Fr. 3 were subjected to a Sephadex LH-20 column chromatography eluting with MeOH-H_2O (1 : 1, 2 ℓ) and EtOH-hexane (3 : 1, 1 ℓ), respectively. Fr. 2-4 was purified by Sephadex LH-20 column chromatography eluting MeOH-H_2O (1 : 5, 2 ℓ) to afford compounds **2** (20 mg), **3** (152 mg) and **4** (435 mg). Fr. 3-2 was rechromatographed on a Sephadex LH-20 column with EtOH-hexane (3 : 1, 1 ℓ) to give compound **1** (205 mg).

2.3.1. Quercetin (1)

R_f : 0.58 (TBAW) and 0.00 (6% AcOH). FAB-MS : Calculated for $C_{15}H_{10}O_7$ 302, Found *m/z* 303 $[M+H]^+$. ^{1}H-NMR (400 MHz, δ, $(CD_3)_2CO$) : 6.27 (1H, *d*, *J* = 2.1 Hz, H-6), 6.52 (1H, *d*, *J* = 2.1 Hz, H-8), 7.00 (1H, *d*, *J* = 8.5 Hz, H-5′), 7.70 (1H, *dd*, *J* = 2.1, 8.5 Hz, H-6′), 7.82 (1H, *d*, *J* = 2.1 Hz, H-2′), 12.19 (1H, *s*, 5-OH). ^{13}C-NMR (100 MHz, δ, $(CD_3)_2CO$) : 94.45 (C-8), 99.17 (C-6), 104.08 (C-10), 115.71 (C-2′), 116.18 (C-5′), 121.44 (C-6′), 123.71 (C-1′), 136.77 (C-3), 145.93

(C-3′), 147.02 (C-2), 146.42 (C-4′), 157.75 (C-9), 162.28 (C-5), 165.13 (C-7), 176.60 (C-4).

2.3.2. Guaijaverin (quercetin-3-O-α-L-arabinopyranose) (2)

R_f : 0.58 (TBAW) and 0.09 (6% AcOH). FAB-MS : Calculated for $C_{20}H_{18}O_{11}$ 434, Found *m/z* 435 $[M+H]^+$, 457 $[M+Na]^+$. ^{1}H-NMR (400 MHz, δ, CD_3OD) : 3.45 (1H, *dd*, *J* = 3.0, 13.5 Hz, H-5″a), 3.65 (1H, *dd*, *J* = 3.1, 8.4 Hz, H-3″), 3.82 (1H, *m*, H-4″), 3.84 (1H, *m*, H-5″b), 3.90 (1H, *dd*, *J* = 6.6, 8.4 Hz, H-2″), 5.16 (1H, *d*, *J* = 6.6 Hz, H-1″), 6.19 (1H, *d*, *J* = 2.1 Hz, H-6), 6.38 (1H, *d*, *J* = 2.1 Hz, H-8), 6.87 (1H, *d*, *J* = 8.5 Hz, H-5′), 7.57 (1H, *dd*, *J* = 2.1, 8.5 Hz, H-6′), 7.74 (1H, *d*, *J* = 2.1 Hz, H-2′). ^{13}C-NMR (100 MHz, δ, CD_3OD) : 67.01 (C-5″), 69.17 (C-4″), 72.94 (C-2″), 74.19 (C-3″), 94.74 (C-8), 99.92 (C-6), 104.72 (C-1″), 105.68 (C-10), 116.21 (C-2′), 117.51 (C-5′), 122.92 (C-1′), 123.08 (C-6′), 135.70 (C-3), 146.00 (C-3′), 149.99 (C-4′), 158.45 (C-9), 158.74 (C-2), 163.07 (C-5), 166.06 (C-7), 179.51 (C-4).

2.3.3. Hirsutrin (quercetin-3-O-β-D-glucopyranose) (3)

R_f : 0.64 (TBAW) and 0.07 (6% AcOH). FAB-MS : Calculated for $C_{21}H_{20}O_{12}$ 464, Found *m/z* 465 $[M+H]^+$. ^{1}H-NMR (400 MHz, δ, CD_3OD) : 3.22 (1H, *m*, H-5″), 3.35 (1H, *t*, *J* = 8.6, 9.5 Hz, H-4″), 3.43 (1H, *t*, *J* = 8.5, 9.1 Hz, H-3″), 3.48 (1H, *t*, *J* = 7.5, 9.0 Hz, H-2″), 3.57 (1H, *dd*, *J* = 5.3, 11.9 Hz, H-6″a), 3.71 (1H, *dd*, *J* = 2.3, 11.9 Hz, H-6″b), 5.25 (1H, *d*, *J* = 7.4 Hz, H-1″), 6.19 (1H, *d*, *J* = 2.1 Hz, H-6), 6.38 (1H, *d*, *J* = 2.1 Hz, H-8), 6.87 (1H, *d*, *J* = 8.5 Hz, H-5′), 7.58 (1H, *dd*, *J* = 2.2, 8.5 Hz, H-6′), 7.71 (1H, *d*, *J* = 2.2 Hz, H-2′). ^{13}C-NMR (100 MHz, δ, CD_3OD) : 62.58 (C-6″), 71.24 (C-4″), 75.76 (C-2″), 78.14 (C-5″), 78.41 (C-3″), 94.75 (C-8), 99.91 (C-6), 104.35 (C-1″), 105.75 (C-10), 116.03 (C-2′), 117.60 (C-5′), 123.10 (C-1′), 123.24 (C-6′), 135.66 (C-3), 145.93 (C-3′), 149.88 (C-4′), 158.48 (C-9), 159.04 (C-2), 163.06 (C-5), 166.02 (C-7), 179.52 (C-4).

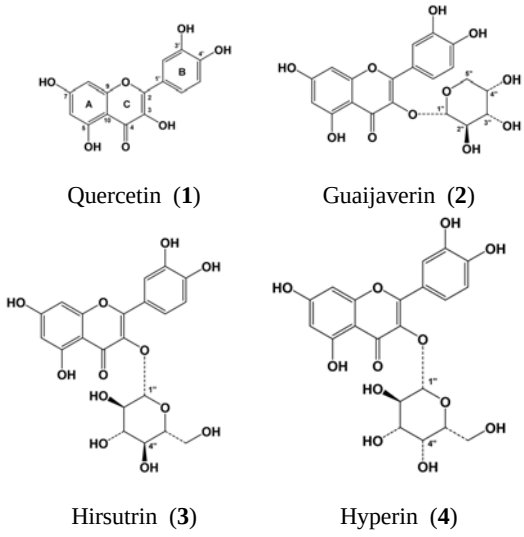

Fig. 1. Chemical structures of compounds **1-4** isolated from *A. komarovii* bark.

2.3.4. Hyperin (quercetin-3-O-β-D-galctopyranose) (4)

R_f : 0.43 (TBAW) and 0.11 (6% AcOH). FAB-MS : Calculated for $C_{21}H_{20}O_{12}$ 464, Found *m/z* 465 $[M+H]^+$. ^{1}H-NMR (600 MHz, δ, $(CD_3)_2SO$) : 3.30 (1H, *t*, *J* = 5.9, 5.9 Hz, H-5″), 3.34 (1H, *dd*, *J* = 3.1, 9.3 Hz, H-3″) 3.38 (1H, *dd*, *J* = 6.0, 10.3 Hz, H-6″b), 3.46 (1H, *dd*, *J* = 5.8, 10.3 Hz, H-6″a), 3.57 (1H, *t*, *J* = 8.3, 8.9 Hz, H-2″), 3.66 (1H, *br d*, *J* = 2.8 Hz, H-4″), 5.38 (1H, *d*, *J* = 7.7 Hz, H-1″), 6.20 (1H, *d*, *J* = 1.2 Hz, H-6), 6.40 (1H, *d*, *J* = 1.2 Hz, H-8), 6.82 (1H, *d*, *J* = 8.5 Hz, H-5′), 7.53 (1H, *d*, *J* = 1.9 Hz, H-2′), 7.67 (1H, *dd*, *J* = 1.9, 8.5 Hz, H-6′), 12.63 (1H, *s*, 5-OH). ^{13}C-NMR (125

MHz, δ, $(CD_3)_2SO$) : 60.02 (C-6″), 67.80 (C-4″), 71.09 (C-2″), 73.07 (C-3″), 75.72 (C-5″), 93.39 (C-8), 98.56 (C-6), 101.68 (C-1″), 103.77 (C-10), 115.06 (C-2′), 115.82 (C-5′), 120.98 (C-1′), 121.89 (C-6′), 133.36 (C-3), 144.71 (C-3′), 148.35 (C-4′), 156.11 (C-9), 156.19 (C-2), 161.11 (C-5), 164.09 (C-7), 177.36 (C-4).

3. RESULTS and DISCUSSION

Quercetin (**1**) and its glycosides (**2-4**) (Fig. 1) were obtained from the EtOAc-soluble fraction of *A. komarovii* bark by column chromatography using Sephadex LH-20 and RP C-18 MPLC. Their structures were determined by analysis of spectroscopic data using NMR and FAB-MS.

Compound **1** was obtained as a yellow amorphous powder. The molecular formula was deduced to be $C_{15}H_{10}O_7$ on the basis of the peak at *m/z* 303 $[M+H]^+$ in the positive FAB-MS. The ^1H NMR spectrum of **1** showed the presence of two *meta*-coupled doublet (J = 2.1 Hz) protons on the A-ring at δ 6.27 and δ 6.52, which were assigned to H-6 and H-8, respectively. Two sets of doublet and one set of double doublet of an ABX spin system at δ 7.82 (*d*, J = 2.1 Hz), δ 7.70 (*dd*, J = 2.1, 8.5 Hz) and δ 7.00 (*d*, J = 8.5 Hz) were observed which were characteristic of H-2′, H-6′ and H-5′, respectively. In the ^{13}C NMR spectrum of **1**, the six oxygenated aromatic carbons appeared at δ 136.77 (C-3), δ 162.28 (C-5), δ 165.13 (C-7), δ 157.75 (C-9), δ 145.93 (C-3′) and δ 146.42 (C-4′), and a carbonyl carbon at δ 176.60 (C-4). Based on the above results and literature[15,16], **1** was identified as quercetin.

Compounds **2**, **3** and **4** were isolated as yellow amorphous powder. In the ^1H and ^{13}C NMR spectra were similar to those of **1**, except for the presence of a sugar moiety. The FAB-MS spectrum of **2** showed the $[M+H]^+$ ion peak at *m/z* 435 and established the molecular formula $C_{20}H_{18}O_{11}$. The anomeric proton signal of **2** appeared at δ 5.16 (*d*, J = 6.6 Hz) and the resonance in the region of δ 3.45～3.90 (5H, m, H-2″, H-3″, F-4″, H-5″) together with the corresponding carbon resonance inferred from the HMQC spectrum suggested the presence of α-L-arabinopyranose. In the HMBC spectrum, a crosspeak between C-3 and H-1″ established the linkage position between quercetin and sugar moieties. The structure of **2** was identifited as guaijaverin (quercetin-3-*O*-α-L-arabinopyranose)[17,18]. The FAB-MS spectra of **3** and **4** showed the $[M+H]^+$ ion peak at *m/z* 465 and established the molecular formula $C_{21}H_{20}O_{12}$. The anomeric proton signals of **3** and **4** were observed at δ 5.25 (*d*, J = 7.4 Hz, H-1″) and δ 5.38 (*d*, J = 7.7 Hz, H-1″). In the ^{13}C NMR spectra, the characteristic signals of sugar moieties indicated the presence of β-D-glucopyranose and β-D-galctopyranose, respectively. In the HMBC spectra of **3** and **4**, a crosspeak between C-3 and H-1″ established the linkage point between quercetin and sugar moieties. Therefore, **3** and **4** were identified as hirsutrin (quercetin-3-*O*-β-D-glucopyranose)[19] and hyperin (quercetin-3-*O*-β-D-galctopyranose) [19,20], respectively.

The present study reported for the first time one flavonol, quercetin (**1**), and three flavonol glycosides, guaijaverin (**2**), hirsutrin (**3**) and hyperin (**4**) in the bark of *A. komarovii*. Quercetin (**1**) and its glycosides (**2-4**) are widespread in higher plants and have been reported from some *Acer* species, such as *A. tegmentosum*[5], *A. mono*[10], *A. negundo*[21] and *A. okamotoanum*[6]. In the genus *Acer* occurring flavonol glycosides, glucosylation or rhamnosylation at C-3 of quercetin and kaempferol are very common, but arbinosylation and galactosylation is rather uncommon. Thus, the presence of guaija-

verin (**2**) and hyperin (**4**) in *A. komarovii* seem to have chemotaxonomic significance.

4. CONCLUSIONS

Quercetin glycosides were isolated by column chromatography using Sephadex LH-20 and RP C-18 from the EtOAc soluble fraction of *Acer komarovii* bark, and elucidated as quercetin (**1**) (205 mg), guaijaverin (quercetin-3-*O*-α-L-arabinopyranose) (**2**) (20 mg), hirsutrin (quercetin-3-*O*-β-D-glucopyranose) (**3**) (152 mg) and hyperin (quercetin-3-*O*-β-D-galctopyranose) (**4**) (655 mg) by a combination of spectroscopic methods (FAB-MS, ^{1}H and ^{13}C NMR, including HMQC and HMBC) and comparison with the literature data. These compounds (**1**-**4**) were isolated for the first time from *Acer komarovii* bark.

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