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# Constituents of *Crataegus pinnatifida* var. *psilosa* Leaves (II) —Flavonoids from BuOH Fraction—

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The studies were carried out to evaluate the constituents in the leaves of *Crataegus pinnatifida* var. *psilosa* (Rosaceae) continuously. From the BuOH fraction of the MeOH extract, four flavonoid compounds, hyperoside (**1**), vitexin (**2**), 2″-O-rhamnosylvitexin (**3**) and quercetin-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (**4**) were isolated and identified on the basis of their physico-chemical properties and spectroscopic evidences by comparison with authentic samples.

**Key words:** *Crataegus pinnatifida* var. *psilosa*(Rosaceae), Leaves, Hyperoside, Vitexin, 2″-O-Rhamnosylvitexin, Quercetin-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside

## INTRODUCTION

In the previous paper (Oh *et al*,1993), the isolation of ursolic acid, a mixture of phytosterol, psilosin and (−)epicatechin from the leaves of *Crataegus pinnatifida* var. *psilosa* (Rosaceae) were reported. In the course of continuous work on the same plant, four additional compounds were isolated from the BuOH fraction of *Crataegus pinnatifida* var. *psilosa* leaves.

## MATERIALS AND METHODS

### Instruments

Melting point was obtained on Electrothermal IA 8100. IR and UV spectra were measured on a Shimadzu IR-435 and Varian Cary-3 spectrophotometer respectively. $^1$H-(200 MHz) and $^{13}$C-(50 MHz) NMR were recorded on a Bruker AM-200 NMR spectrometer. EI-MS was taken on a GC-MS/MS-DS, TSQ-700 mass spectrometer and SI-MS was taken on a HITACHI M-2000 by the direct inlet method. GC was carried out to identify sugars by the usual manner with a Shimadzu GC-14A.

### Plant Material

The leaves of *Crataegus pinnatifida* var. *psilosa* were collected in July (1992) at Mt. Kwang Duk of Kang Won Do. After depositing the voucher specimen at the Department of Pharmaceutical Botany, College of Pharmacy, Chung-Ang University. We used for experiment after crushed and air-dried.

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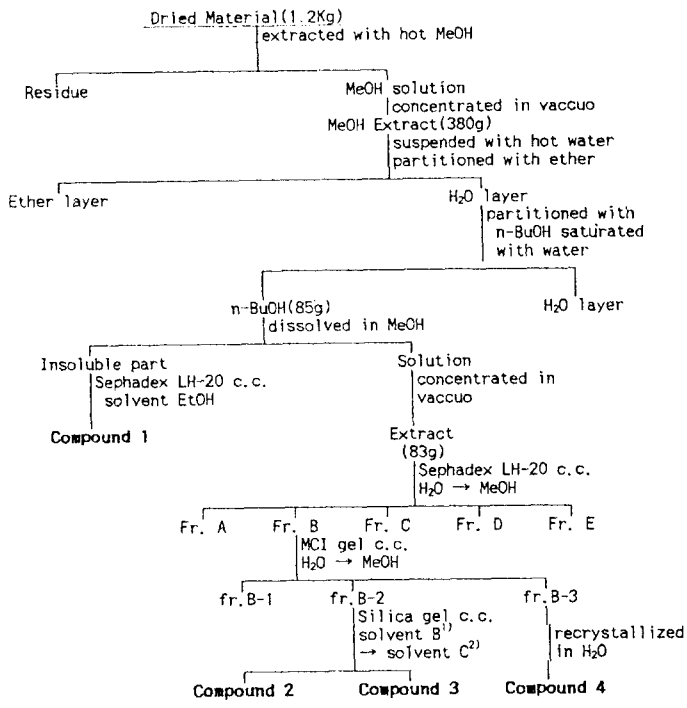
### Extraction and Isolation

The dried material (1.2 kg) was extracted with hot MeOH. The MeOH extracts were suspended in $H_2O$ and partitioned with Ether and n-BuOH, successively. After 85 g of the n-BuOH extracts was dissolved in MeOH, the insoluble fraction was subjected to Sephadex LH-20 column chromatography with EtOH to afford compound **1** and the soluble fraction was chromatographed on Sephadex LH-20 column with $H_2O$ →MeOH (gradient 0 to 100%) to obtain fraction A→ fraction E. Compound **2**, compound **3** and compound **4** were isolated by MCI gel and silica gel column chromatography from fraction B as show in Scheme 1.

**Compound 1:** Compound **1** was obtained by crystallization from MeOH to yield a yellow powder. mp 229-239°; $[\alpha]_D = -74.0°$ (c, 0.5, pyridine); IR $\nu_{max}$ ($cm^{-1}$): 3300 (OH), 1655 (C=O); UV $\lambda_{max}$(nm): 258, 362; $^1$H-NMR (200 MHz, DMSO-$d_6$) δ: 12.65 (1H, s, 5-OH), 7.67 (1H, dd, J=2.2, 8.5 Hz, H-6′), 7.51 (1H, d, J=2.2 Hz, H-2′), 6.82 (1H, d, J=8.5 Hz, H-5′), 6.40 (1H, d, J=2.0 Hz, H-8), 6.21 (1H, d, J=2.0 Hz, H-6), 5.39 (1H, d, J=7.6 Hz, H-1″); $^{13}$C-NMR: see Table I; SI-MS (m/z): 465 $[M+H]^+$, 303 $[M+H\text{-Hexose}]^+$.




**Scheme 1.** Isolation of Compound **1**, **2**, **3** and **4** from n-BuOH extract.
1) Solvent B=$CHCl_3$ : MeOH : $H_2O$ (80 : 25 : 2.5)
2) Solvent C=$CHCl_3$ : MeOH : $H_2O$ (60 : 35 : 8)

**Compound 2:** Compound **2** was obtained by crystallization from MeOH to yield a yellow powder. mp 227-228°; $[\alpha]_D=-38.0°$ (c, 0.5, pyridine); IR $\nu_{max}$ ($cm^{-1}$): 3400 (OH), 1645 (C=O); UV $\lambda_{max}$ (nm): 269, 325; $^1$H-NMR (200 MHz, DMSO-$d_6$) δ: 13.13 (1H, s, 5-OH), 8.00 (2H, d, J=8.4 Hz, H-2', 6'), 6.92 (2H, d, J=8.4 Hz, H-3', 5'), 6.71 (1H, s, H-3), 6.29 (1H, s, H-6), 4.72 (1H, d, J=9.8 Hz, H-1''); $^{13}$C-NMR: see Table I; SI-MS (m/z): 433$[M+H]^+$, 283.

**Compound 3:** Compound **3** was obtained by crystallization from MeOH to yield a yellow powder. mp 224-226°; $[\alpha]_D=-60.0°$ (c, 0.5, pyridine); IR $\nu_{max}$ ($cm^{-1}$): 3300 (OH), 1654 (C=O); UV $\lambda_{max}$ (nm): 269, 331; $^1$H-NMR (200 MHz, DMSO-$d_6$) δ: 13.17 (1H, s, 5-OH), 8.10 (2H, d, J=8.7 Hz, H-2', 6'), 6.95 (2H, d, J=8.7 Hz, H-3', 5'), 6.81 (1H, s, H-3), 6.30 (1H, s, H-6), 5.00 (1H, s, H-1'''), 4.80 (1H, d, J=10.0 Hz, H-1''), 0.50 (3H, d, J=6.1 Hz, rhamnosyl $CH_3$); $^{13}$C-NMR: see Table I; EI-MS (m/z): 578 $[M]^+$, 433, 283.

**Compound 4:** Compound **4** was obtained by crystallization from $H_2O$ to yield yellow needles. mp 186-188°; $[\alpha]_D=-81.7°$ (c, 0.6, pyridine); IR $\nu_{max}$ ($cm^{-1}$): 3390 (OH), 1655 (C=O); UV $\lambda_{max}$ (nm): 258, 360; $^1$H-NMR (200 MHz, DMSO-$d_6$) δ: 7.63 (1H, dd, J=2.1, 8.5 Hz, H-6'), 7.50 (1H, d, J=2.1 Hz, H-2'), 6.79 (1H, d, J=8.5 Hz, H-5'), 6.37 (1H, d, J=2.0 Hz, H-8), 6.16 (1H, d, J=2.0 Hz, H-6), 5.29 (1H, d, J=7.5 Hz, H-1''), 4.40 (1H, s, H-1'''), 1.07 (3H, d, J=6.0 Hz, rhamnosyl $CH_3$); $^{13}$C-NMR: see Table I; EI-MS (m/z): 610 $[M]^+$, 302, 153.

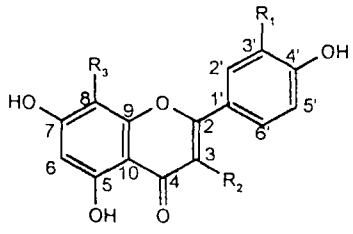
**Table I.** $^{13}$C-NMR spectral data of compound **1**, **2**, **3** and **4** (pyridin-$d_5$, 50 MHz)

| Carbon No. | Compound 1 | Compound 2 | Compound 3 | Compound 4 |
|---|---|---|---|---|
| 2 | 156.4 | 164.1 | 164.6 | 156.3 |
| 3 | 133.7 | 102.6 | 103.1 | 133.4 |
| 4 | 177.7 | 182.3 | 182.6 | 177.2 |
| 5 | 161.4 | 160.6 | 161.6 | 161.1 |
| 6 | 98.9 | 98.3 | 99.2 | 98.6 |
| 7 | 164.3 | 162.7 | 162.9 | 164.3 |
| 8 | 93.7 | 104.2 | 104.7 | 93.5 |
| 9 | 156.4 | 156.3 | 156.4 | 156.3 |
| 10 | 104.1 | 104.8 | 105.0 | 103.7 |
| 1' | 121.4 | 121.8 | 122.2 | 121.8 |
| 2' | 115.4 | 129.1 | 129.5 | 115.1 |
| 3' | 145.0 | 116.0 | 116.5 | 144.7 |
| 4' | 148.6 | 161.3 | 161.1 | 148.4 |
| 5' | 116.1 | 116.0 | 116.5 | 115.8 |
| 6' | 122.2 | 129.1 | 129.5 | 120.9 |
| Galactose | | | | |
| 1'' | 102.0 | | | 101.9 |
| 2'' | 71.4 | | | 71.0 |
| 3'' | 73.4 | | | 72.9 |
| 4'' | 68.1 | | | 67.9 |
| 5'' | 76.0 | | | 73.4 |
| 6'' | 60.3 | | | 65.0 |
| Glucose | | | | |
| 1'' | | 73.8 | 72.5 | |
| 2'' | | 70.9 | 75.7 | |
| 3'' | | 78.6 | 80.6 | |
| 4'' | | 70.7 | 70.6 | |
| 5'' | | 82.0 | 82.1 | |
| 6'' | | 61.5 | 61.7 | |
| Rhamnose | | | | |
| 1''' | | | 101.1 | 99.9 |
| 2''' | | | 70.6 | 70.5 |
| 3''' | | | 70.8 | 70.3 |
| 4''' | | | 72.1 | 71.8 |
| 5''' | | | 68.8 | 68.2 |
| 6''' | | | 18.2 | 17.8 |

**Hydrolysis of Compound 1, 3 and 4:** Compound **1**, **3** and **4** (each 20 mg) were hydrolyzed by using a known procedure. The hydrolysate was diluted with $H_2O$ and extracted with $CHCl_3$. The residue from the $CHCl_3$ extract was chromatographed on a Sephadex LH-20 column using an eluting solvent system of EtOH. The elutes were concentrated, and the residues were recrystallized from MeOH. The compounds obtained were identified by direct comparison with authentic samples. After being neutralized with Amberlite MB-3, the filtrate was concentrated to a small volume, and examined by TLC and G.C., to identify the type of sugar moiety from compound **1**, **3** and **4**.

## RESULTS AND DISCUSSION

The n-butanol soluble fraction of the methanol ext-



| | R1 | R2 | R3 |
|---|---|---|---|
| Compound 1 | OH | O--Gal | H |
| Compound 2 | H | H | Glc |
| Compound 3 | H | H | Glc 2→Rha |
| Compound 4 | OH | O-- Gal 6→Rha | H |

ract of the leaves of *Crataegus pinnatifida* var. *psilosa* was successively chromatographed on silica gel, MCI gel and Sephadex LH-20 gel.

Two compounds (compound **1**, **4**) were identified as flavonol O-glycosides and the remaining two compounds (compound **2**, **3**) were identified as flavone C-glycosides.

Compound **1**, a yellow powder, mp 229-239°, was insoluble in $CHCl_3$, ether and acetone but soluble in MeOH. Compound **1** was detected by $FeCl_3$ and Mg-HCl as a positive reaction, suggesting it to be a flavonoid. The broad band hydroxyl absorption at 3300 $cm^{-1}$, the conjugated carbonyl absorption of the γ-pyron functionality at 1655 $cm^{-1}$, a strong absorption of the aromatic ring at 1610, 1550 and 1505 $cm^{-1}$ were observed. The UV $\lambda_{max}$ absorbances of compound **1**, 258 and 362 nm (in MeOH), were appeared in a typical pattern of the flavonol. Upon treatment with various shift reagents, compound **1** showed the typical pattern of the quercetin type flavonoids (Markham, 1982; Mabry *et al.*, 1970). The SIMS spectrum showed a molecular ion at m/z 465 $(M+H)^+$ and other fragment ions at m/z 311 (RDA, B ring), 153 (RDA, A ring) and 303 (M+H-Hexose).

The $^1$H-NMR spectrum (DMSO-$d_6$) showed a double doublet signal at δ 7.67 (1H, J=2.2, 8.5 Hz, H-6'), a doublet signal at δ 7.51 (1H, J=2.2 Hz, H-2') and a doublet signal at δ 6.82 (1H, J=8.5 Hz, H-5') on the B ring. The resonances at δ 6.40 and δ 6.21 were assigned to H-8 (J=2.0 Hz) and H-6 (J=2.0 Hz), respectively. The anomeric proton signal δ 5.39 at (J=7.6 Hz) supported our assigrment for the β-configuration. $^{13}$C-NMR spectrum (DMSO-$d_6$) showed a carbonyl signal at δ 177.7 (C-4), and a disubstituted methylene signal at δ 133.7 (C-3). Also the C-3' and C-4' of the B ring appeared at δ 145.0 and δ 148.6, respectively indicating substitution (Harbone *et al.*, 1982). Carbon signals of the sugar moiety were found at δ 102.0, δ 71.4, δ 73.4, δ 68.1, δ 76.0 and δ 60.3 (Agrawal, 1989). On hydrolysis, compound **1** gave quercetin and galactose.

These data suggest that compound **1** is quercetin-3-O-β-D-galactopyranoside (hyperoside), and melting point and other physical data were identical with those of an authentic sample.

Compound **2**, a yellow powder, mp 227-228°, was insoluble in $CHCl_3$ and ether but soluble in MeOH. Compound **2** was detected by $FeCl_3$ and Mg-HCl as a positive reaction, suggesting it to be a flavonoid. The broad band hydroxyl absorption at 3400 $cm^{-1}$, the conjugated carbonyl absorption of the γ-pyron functionality at 1645 $cm^{-1}$ and a strong absorption of the aromatic ring at 1614, 1507 and 1440 $cm^{-1}$. The UV $\lambda_{max}$ absorbances of compound **2**, 269 and 325 nm (in MeOH), were appeared in a typical pattern of the flavone. Upon treatment with various shift reagents, compound **2** showed the typical pattern of apigenin type flavonoids (Markham, 1982; Mabry *et al.*, 1970). The SIMS spectrum showed a molecular ion at m/z 433$(M+H)^+$ and a fragment ion at m/z 283 which is the aglycone fragment containing a $CH_2$ remnant of C-linked sugar. The $^1$H-NMR spectrum (DMSO-$d_6$) (Kim *et al.*, 1993) showed two doublet signal at δ 8.00 (2H, J=8.4 Hz, H-2', 6') and δ 6.92 (2H, J=8.4 Hz, H-3', 5') on the B ring. Singlet signals at δ 6.71 and δ 6.29 were assigned to H-3 and H-6 the on A ring, respectively. The anomeric proton signal of sugar showed β configuration at δ 4.72 (J=9.8 Hz). $^{13}$C-NMR spectrum (DMSO-$d_6$) showed a carbonyl signal at δ 182.3 (C-4), and a non-substituted carbon siganal at δ 102.6 (C-3). The C-4' of the B ring revealed substitution at δ 161.3. Compound **2** was C-glycoside (Kashnikova, 1984), and carbon signals of the sugar moiety were confirmed at δ 73.8, δ 70.9, δ 78.6, δ 70.7, δ 82.0 and δ 61.5. There was no reaction upon attempted acid hydrolysis.

These data suggest that compound **2** is apigenin-8-C-β-D-glucopyranoside (vitexin), and melting point and other physical data were identical with those of an authentic sample.

Compound **3**, a yellow powder, mp 224-226°, was insoluble in $CHCl_3$, ether and acetone but soluble in MeOH and $H_2O$. Compound **3** was detected by $FeCl_3$ and Mg-HCl as a positive reaction, suggesting it to be a flavonoid. The IR and UV spectra were similar to compound **2**. Therefore aglycone of compound **3** was determined to be an apigenin same as compound **2**. The EIMS spectrum showed a molecular ion at m/z 578 $(M^+)$ and a fragment ion at m/z 283 due to the removal of a C-linked sugar and a methyl pentose from the aglycone unit. The $^1$H-NMR (DMSO-$d_6$) and $^{13}$C-NMR (DMSO-$d_6$) (Nikolov *et al.*, 1982; Bykov *et al.*, 1973) spectra were similar to compound **2**, but it appeared that there is additional one mole of rhamnose. In the C-NMR, C-2" of glucose was shifted downfield (4.3 ppm) and adjacent carbon (c-1") was shifted upfield (1.4 ppm) relative to compound **2**. Therefore rhamnose of terminal sugar was attached to C-2" of

glucose (Harborne *et al.*, 1982). On the acid hydrolysis, a vitexin as aglycone part and a rhamnose as sugar part were obtained. Rhamnose was identified by G.C. after prepared derivative with TMS. These data suggest that compound **3** is apigenin-8-C-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (2″-O-rhamnosylvitexin), and melting point and other physical data were identical with those of an authentic sample.

Compound **4**, a yellow needle crystal (in $H_2O$), mp 186-188°, was insoluble in $CHCl_3$ and ether but soluble in MeOH and EtOH. Compound **4** was detected by $FeCl_3$ and Mg-HCl as a positive reaction, suggesting it to be a flavonoid. The IR (Nikolov, 1973) and UV spectra was similar to compound **1**. Therefore aglycone of compound **4** was supposed to be apigenin same as compound **1**. The EIMS spectrum showed a molecular ion at m/z 610 ($M^+$) and a fragment ion at m/z 302 ($M^+$-Hexose-methyl pentose). The $^1$H-NMR (DMSO-$d_6$) (Bykov *et al.*, 1973) and $^{13}$C-NMR (DMSO-$d_6$) spectra were similar to compound **1**, but there appeared additional one mole of rhamnose. In the C-NMR, C-6″ of galactose was shifted downfield (4.2 ppm) and adjacent carbon (c-5″) was shifted upfield (2.4 ppm). Therefore rhamnose of terminal sugar conjugated with the C-6″ of galactose (Harbone *et al.*, 1982). On the acid hydrolysis, a quercetin as aglycone part and a rhamnose and a galactose as sugar part were obtained. Sugars were identified by G.C. after prepared derivative with TMS.

These data suggested that compound **4** was quercetin-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside, and melting point and other physical data were identical with those of an authentic sample.

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