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# Flavonoids from the Leaves of *Betula platyphylla* var. *latifolia*

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**Abstract**—Chemical examination of the leaves of *Betula platyphylla* var. *latifolia* has led to the isolation and characterization of five flavonoid glycosides including two C-glucosyl flavonoids. The structures of these compounds were elucidated as myricetin 3-O-α-L-rhamnoside (myricitrin), quercetin-3-O-β-D-glucopyranoside (isoquercitrin), quercetin-3-O-β-D-galactopyranoside (hyperoside), nalingenin-6-C-β-D-glucopyranoside (hemiphloin) and aromadendrin-6-C-β-D-glucopyranoside (6-C-glucosyldihydrokaempferol) on the basis of physico-chemical and spectroscopic evidences.

**Keywords**—*Betula platyphylla* var. *latifolia* · Betulaceae · flavonoid

In previous paper,[1] the author reported a new diarylheptanoid named betulatetraol together with known phenylpropanoid, flavan-3-ol and its glycosides and proanthocyanidins. Continuous work on phenolic compounds of this plant has led to the isolation and structural elucidation of three flavonol glycosides myricitrin(**1**),[2] quercetin-3-O-β-D-glucopyranoside(**2**),[3] quercetin-3-O-β-D-galactopyranoside(**3**),[4] and two C-glucosyl flavonoids, nalingenin-6-C-β-D-glucopyranoside(**4**)[5] and aromadendrin 6-C-β-D-glucopyranoside(**5**)[6] from the leaves.

## Results and Discussion

Fresh leaves of *B. platyphylla* var. *latifolia* were extracted with aqueous acetone and the extract was subjected to a combination of chromatographies over Sephadex LH-20, MCI-gel CHP 20P and Cosmosil 140 $C_{18}$-OPN to afford five flavonoids.

Compound **1** showed dark green coloration with ferric chloride and UV spectra exhibited characteristic absorptions for flavonoid. The $^1$H-NMR spectrum of **1** exhibited secondary methyl signals at δ0.93 for rhamnosyl methyl together with anomeric proton doublet at δ5.48(1H, d, $J$=1.3Hz, Rha-1) as a α-configuration. It showed two *meta*-coupled doublets at δ6.26(1H, d, $J$=2Hz, H-6) and δ6.46(1H, d, $J$=2Hz, H-8) which means the common 5,7-dihydroxylation pattern in A-ring and the B-ring signals were observed as a two-proton singlet at δ7.10 having 3′,4′,5′-oxygenation pattern.

The MS spectrum showed $[M]^+$ ion peak at $m/z$ 464 and fragment ion peak at $m/z$ 318 indicating an aglycone(myricetin) and a rhamnose lost($[M-146]^+$).

Acid hydrolysis of **1** yielded myricetin as its genin and rhamnose as the sugar.

From these results compound **1** was identified as myricitrin(myricetin 3-O-α-L-rhamnoside) and direct comparison with an authentic standard supported this conclusion(co-TLC).

Compound **2** was well known flavonoid, quercetin 3-O-β-D-glucopyranoside, showing typical

ABX pattern in B-ring region [δ6.96(1H, d, $J$=8.5Hz, H-5′), 7.69(1H, dd, $J$=2.2, 8.5Hz, H-6′), 7.82(1H, d, $J$=2.2Hz, H-2′)], 5,7-dihydroxylation pattern in A-ring region [δ6.29 (1H, d, $J$=2.2Hz, H-6], 6.66(1H, d, $J$=2.2Hz, H-8)] and a glucose anomeric proton at δ5.55 as a β-configuration($J$=7.8Hz) in the $^1$H-NMR spectrum.

The identification was established by direct comparison(co-TLC) with an authentic sample.

The MS spectrum of 3 exhibited $[M]^+$ ion peak at $m/z$ 464 and a fragment ion peak at $m/z$ 302 arising from an aglycone unit and a hexose sugar lost ($[M-162]^+$). In $^1$H-NMR spectrum, the chemical shifts and coupling patterns of 3 were very similar to those of compound 2 but the $^{13}$C-NMR spectrum of 3 showed galactose moiety at sugar region [δ101.7 (Gal-1), 75.7(Gal-5), 73.1(Gal-3), 71.1(Gal-2), 67.9(Gal-4), 60.1(Gal-6)][7].

Acid hydrolysis of 3 afforded quercetin and galactose.

The comparison of the $^{13}$C-NMR spectrum of 3 with quercetin as its aglycone revealed glycosylation shift at C-2(+9.3 ppm), C-3(−2.2 ppm), and C-4(+1.7 ppm), suggesting a sugar unit was attached at C-3 of quercetin.[10]

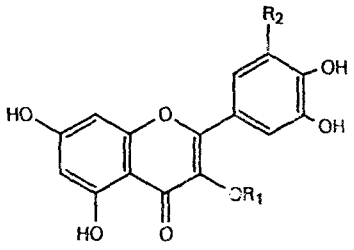
Thus compound 3 was identified as quercetin 3-O-β-D-galactopyranoside and direct comparison (co-TLC) with an authentic standard supported this conclusion.

Compound 4 was obtained as white amorphous powder and gave a greenish brown color in the ferric chloride. The UV spectrum of 4, λmax (MeOH) at 295, 340(sh)nm and $^1$H-NMR signals at δ2.72(1H, dd, $J$=3.17Hz, H-3$eq$), δ3.27 (1H, dd, $J$=12, 17Hz, H-3$ax$) and δ5.41(1H, dd, $J$=3, 12Hz, H-2) showing the couplings of the C-2 proton with the two C-3 protons indicated to be a flavanone.[11]

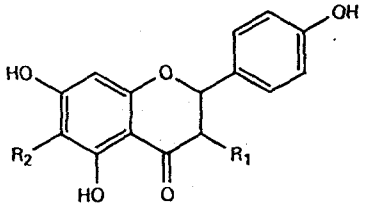
The $^1$H NMR spectrum of 4 also showed a sugar moiety at δ4.49(d, $J$=9.7Hz) and one proton singlet at δ5.95 suggesting C-glycosyl type. And typical four-protons of two doublets (each $J$=8.5Hz) at δ6.78 and 7.31 showed $A_2B_2$ pattern for the B-ring. The $^{13}$C-NMR spectrum of 4 exhibited a C-glucosyl moiety at δ73.0(Glc-1), 70.3(Glc-2), 79.0(Glc-3), 70.7 (Glc-4), 81.5(Glc-5), 61.5(Glc-6).[8]

The MS spectrum of 4 also exhibited $[M]^+$ ion peak at $m/z$ 434 and a fragment ion peak at $m/z$ 285 which consists of aglycone fragment containing a $CH_2$ remnant of C-linked sugar[12]. From these results, the aglycone type was defined as nalingenin and the sugar was glucose.

The $^{13}$C-NMR spectrum of 4 provided information for its assignment of 6-C glucosyl position from the signals at δ94.8(C-8) and 105.8(C-6) compared with the signals of its aglycone, nalingenin at δ95.9(C-6) and 95.0(C-8).




| | $R_1$ | $R_2$ |
|---|---|---|
| 1 | α-L-rhamnose | OH |
| 2 | β-D-glucose | H |
| 3 | β-D-galactose | H |
| 3a | H | H |




| | $R_1$ | $R_2$ |
|---|---|---|
| 4 | H | β-D-glucose |
| 4a | H | H |
| 5 | OH | β-D-glucose |
| 5a | OH | H |

Futhermore, the upfield shift in the signals of adjacent carbons at δ162.8(−0.8 ppm, C-5) and 165.8(−0.9 ppm, C-7) supported the attachment of glucosyl moiety at C-6[13].

Thus the structure of compound **4** was confirmed to be 6-C-glucosyl nalingenin(hemiphloin)[9].

Compound **5** suggested to be dihydroflavonol from UV maxima at 293, 340(sh)nm and a pair of doublets(each for one proton) at δ4.62 and 5.02 with $J$=11.4Hz typical for 1,2-diaxial protons in C-ring region[11].

The $^1$H-NMR spectrum of **5** clearly showed two doublets of four protons at δ6.79 and 7.33 with $J$=8.5Hz, typical of the $A_2B_2$ pattern in B-ring and a singlet at δ5.91 (1H) in A-ring region and a doublet for one proton at δ4.85 with $J$=9.8Hz as an anomeric proton indicated 6-C or 8-C glycosyl dihydrokaempferol.

The $^{13}$C-NMR spectrum showed a C-glucosyl moiety at δ75.0(Glc-1), 72.6(Glc-2), 80.1 (Glc-3), 71.8(Glc-4), 82.4(Glc-5), 62.9(Glc-6)[8], and the signals arising from the $^{13}$C-NMR spectrum of **5** except glucose moiety similar to its postulated aglycone, aromadendrin[14].

The position of the glucosyl group was determined by comparing the $^{13}$C-NMR spectra of **5** with aromadendrin. The signals of C-6 and C-8 in aromadendrin was δ97.5 and 96.4 respectively in the literature[14] but **5** showed its C-6, C-8 signals at δ106.2, 96.5 respectively.

From these results the downfield shift at δ106.2 was assigned to C-6 and similar results were reported for 6-C-methylaromadendrin[14].

Futhermore, the upfield shift in the signals of adjacent carbons (C-5 and C-7) at δ164.0 (−1.3 ppm) and 167.5(−1.3 ppm) respectively supported this conclusion[13].

Thus the structure of compound **5** was elucidated as aromadendrin-6-C-β-D-glucopyranoside (6-C-glucosyldihydrokaempferol)[9].

## Experimental

**General**—NMR spectra were recorded at 100 and 300 MHz($^1$H-NMR), 25.05 and 75.47 MHz ($^{13}$C-NMR). Chemical shifts are given in δ (ppm) scale with TMS as int. std. MS were measured at 70eV. CC was carried out on Sephadex LH-20(25∼10 μm, Pharmacia), MCI-gel CHP 20P(75∼150 μm; Mitsubishi) and cosmosil 140 $C_{18}$-OPN(Nacalai). TLC was conducted on precoated silica gel 60 $F_{254}$(Merck) and precoated cellulose $F_{254}$ plates(Merck). Spots were detected under UV and by spraying with $FeCl_3$ and dil. $H_2SO_4$, followed by heating.

**Plant material**—Leaves of *B. platyphylla* var. *latifolia* were collected Mt. Kuanak near Seoul city, Korea.

**Extraction and isolation**—Fresh leaves(5.5 kg) were extracted with 80% aq. $Me_2CO$ at room temp. After removal of $Me_2CO$ *in vacuo*, the aq. solution was filtered. The filtrate was concentrated and then applied to Sephadex LH-20 column.

Elution with $H_2O$ containing increasing proportion of MeOH afforded 3 frs, I(150 g), II(225 g) and III(320 g). Repeated CC of fr. I on MCI-gel CHP 20P with an $H_2O$-MeOH gradient system and Sephadex LH-20 with EtOH gave 6-C-glucosyl aromadendrin(**5**, 150 mg).

CC of fr. II over MCI-gel, cosmosil 140 $C_{18}$-OPN with an $H_2O$-MeOH gradient system furnished 6-C-glucosyl nalingenin(**4**, 100 mg).

CC of fr. III over MCI-gel with an $H_2O$-MeOH gradient system and Sephadex LH-20 with EtOH afforded myricitrin(**1**, 50 mg), quercetin-3-O-β-D-glucopyranoside(**2**, 1 g) and quercetin-3-O-β-D-galactopyranoside(**3**, 2 g).

**Myricitrin**(**1**)—Yellow amorphous powder, $FeCl_3$: dark green, $[\alpha]_D^{26}$ −126.0° (MeOH: c 0.3); MS $m/z$ 464 $[M]^+$, 318 $[M-Rha]^+$; $^1$H-NMR(100 MHz, $Me_2CO$-$d_6$+$D_2O$) δ: 0.93

(3H, d, $J$=5.9Hz, Rha-$CH_3$), 3.76(1H, dd, $J$=3.6, 9Hz, Rha-3), 5.48(1H, d, $J$=1.2 Hz, anomeric H), 6.26(1H, d, $J$=2Hz, H-6), 6.46(1H, d, $J$=2Hz, H-8), 7.10(2H, s, H-2′ and H-6′); UV, $\lambda_{max}^{MeOH}$ nm(log $\varepsilon$) 256, 297, 308, 353; $\lambda_{max}^{MeOH+NaOH}$ nm 261 sh, 293, 369(dec); $\lambda_{max}^{NaOAc}$ 270, 308, 369; $\lambda_{max}^{NaOAc+H_3BO_3}$ nm 259, 294, 374; $\lambda_{max}^{AlCl_3}$ nm 380.

**Acid hydrolysis of 1**—A solution of **1** (20 mg) in 5% HCl was refluxed for 3hr, and the reaction mixture was extracted by EtOAc. The organic part was concentrated and then applied to a column of Sephadex LH-20 with a $H_2O$-MeOH in gradient to give yellow powder, which was identified as myricetin by direct comparision with an authentic sample(co-TLC).

The remaining aqueous solution was neutralized by Amberlite MB-3 and concd. L-Rhamnose was detected by TLC.

**Quercetin-3-O-β-D-glucopyranoside (2)** — Yellow amorphous powder, $FeCl_3$: green, $[\alpha]_D^{26}$ −11.0°(MeOH; c 0.3). $^1$H-NMR(100 MHz, $Me_2CO$-$d_6$+$D_2O$): δ5.55(1H, d, $J$=7.8 Hz, anomeric H), 6.29(1H, d, $J$=2.2Hz, H-6), 6.65(1H, d, $J$=2.2Hz, H-8), 6.96(1H, d, $J$=8.5Hz, H-5′), 7.69(1H, dd, $J$=2.2, 8.5Hz, H-6′), 7.82(1H, d, $J$=2.2Hz, H-2′).

**Quercetin-3-O-β-D-galactopyranoside (3)** —Yellow amorphous powder, $FeCl_3$: green, $[\alpha]_D^{26}$ −44.0° (MeOH; c 0.3); UV, $\lambda_{max}^{MeOH}$ nm(log $\varepsilon$) 257, 359; $\lambda_{max}^{MeOH+NaOH}$ nm 271, 330, 410; $\lambda_{max}^{NaOAc}$ nm 269, 379; $\lambda_{max}^{NaOAc+H_3BO_3}$ nm 264, 384; $\lambda_{max}^{AlCl_3}$ nm 267, 401; $\lambda_{max}^{AlCl_3+HCl}$ nm 267, 298, 363, 400; MS $m/z$ 464[M]$^+$, 302[M-Gal]$^+$; $^1$H-NMR (100MHz, $Me_2CO$-$d_6$+$D_2O$) δ: 5.22(1H, d, $J$=7.6Hz, anomeric H), 6.28(1H, d, $J$=2Hz, H-6), 6.53(1H, d, $J$=2Hz, H-8), 6.94(1H, d, $J$=8.5 Hz, H-5′), 7.61(1H, dd, $J$=2.2, 8.5Hz, H-6′), 8.07(1H, d, $J$=2.2Hz, H-2′); $^{13}$C-NMR(25.05 MHz, DMSO-$d_6$): see Table I.

**Acid hydrolysis of 3**—A solution of **3** (20 mg) in 5% HCl was refluxed for 3 hr. After cooling, the solution was extracted with EtOAc. TLC examination of EtOAc layer showed the presence of quercetin. The aqueous layer was neutralized with Amberlite MB-3 and concentrated *in vacuo*. D-Galactose was detected by TLC.

**6-C-Glucosylnalingenin (4)**—White amorphous powder, $FeCl_3$: greenish brown, $[\alpha]_D^{26}$+28° (MeOH; c 0.3); UV, $\lambda_{max}^{MeOH}$ nm(log $\varepsilon$) 289, 325sh; $\lambda_{max}^{MeOH+NaOH}$ nm 246, 328; $\lambda_{max}^{NaOAc}$ nm 291, 328; $\lambda_{max}^{NaOAc+H_3BO_3}$ nm 291, 328, MS $m/z$ 434[M]$^+$, 285; $^1$H-NMR(300 MHz, DMSO-$d_6$) δ: 2.72

**Table I.** $^{13}$C-NMR Chemical shifts of compound **3**~**4a** in DMSO-$d_6$ and **5**~**5a** in $CD_3OD$

| Carbon | 3 | 3a[a] | 4 | 4a[a] | 5 | 5a[b] |
|---|---|---|---|---|---|---|
| C-2 | 156.2 | 146.9 | 78.3 | 78.4 | 84.9 | 85.0 |
| C-3 | 133.4 | 135.6 | 21.0 | 42.0 | 73.5 | 73.7 |
| C-4 | 177.4 | 175.7 | 196.6 | 196.2 | 198.8 | 198.4 |
| C-5 | 161.1 | 160.7 | 162.8 | 163.6 | 164.0 | 165.3 |
| C-6 | 98.6 | 98.2 | 105.8 | 95.9 | 106.2 | 97.5 |
| C-7 | 164.0 | 163.9 | 165.8 | 166.7 | 167.5 | 168.8 |
| C-8 | 93.4 | 93.4 | 94.8 | 95.0 | 96.5 | 96.4 |
| C-9 | 156.2 | 156.2 | 161.7 | 162.9 | 163.8 | 164.6 |
| C-10 | 103.8 | 103.0 | 101.5 | 101.8 | 101.7 | 101.9 |
| C-1′ | 121.9 | 122.0 | 128.9 | 128.9 | 129.1 | 129.4 |
| C-2′ | 115.0 | 115.3 | 128.3 | 128.2 | 130.3 | 130.4 |
| C-3′ | 144.7 | 145.0 | 115.2 | 115.2 | 116.2 | 116.3 |
| C-4′ | 148.4 | 147.6 | 157.7 | 157.8 | 159.1 | 159.2 |
| C-5′ | 115.9 | 115.6 | 115.2 | 115.2 | 116.2 | 116.3 |
| C-6′ | 120.9 | 120.0 | 128.3 | 128.2 | 130.3 | 130.4 |
| Galactose | | | Glucose | | Glucose | |
| C-1 | 101.7 | | 73.0 | | 75.0 | |
| C-2 | 71.1 | | 70.3 | | 72.6 | |
| C-3 | 73.1 | | 79.0 | | 80.1 | |
| C-4 | 67.9 | | 70.7 | | 71.8 | |
| C-5 | 75.7 | | 81.5 | | 81.4 | |
| C-6 | 60.1 | | 61.5 | | 62.9 | |

a) Reference data reported by Markham *et al.* ($^{13}$C NMR Chart).[7]

b) Reference data reported by Agrawal.[15]

(1H, dd, $J$=3, 17Hz, H-3*eq*), 3.27(1H, dd, $J$=12, 17Hz, H-3*ax*), 4.49(1H, d, $J$=9.7Hz, anomeric H), 5.41(1H, dd, $J$=3, 12Hz, H-2), 5.95(1H, s, H-8), 6.78(2H, d, $J$=8.5 Hz, H-3′ and H-5′), 7.31(2H, d, $J$=8.5 Hz, H-2′ and H-6′), 12.72(1H, s, 5-OH); $^{13}$C-NMR (75.47MHz, DMSO-$d_6$): see Table I.

**Aromadendrin 6-C-$\beta$-D-glucopyranoside** (5)—Light brown amorphous powder, $FeCl_3$: brown, $[\alpha]_D^{25}$ +32° (MeOH; c 0.3); UV, $\lambda_{max}^{MeOH}$ nm(log ε) 294, 346sh; $\lambda_{max}^{MeOH+NaOH}$ nm 247, 320; $\lambda_{max}^{NaOAc}$ nm 255, 334; $\lambda_{max}^{NaOAc+H_3BO_3}$ nm 294, 330, $\lambda_{max}^{AlCl_3}$ nm 295, 320sh; $\lambda_{max}^{AlCl_3+HCl}$ nm 312, 389 sh; Positive FAB-MS $m/z$ 451[M+H]$^+$; $^1$H-NMR(300MHz, DMSO-$d_6$) $\delta$: 4.48(1H, $J$=9.8 Hz, anomeric H), 4.56(1H, d, $J$=11.4Hz, H-3), 5.03(1H, d, $J$=11.4Hz, H-2), 5.91(1H, s, H-8), 6.79(2H, d, $J$=8.5Hz, H-3′ and H-5′), 7.33(2H, d, $J$=8.5Hz, H-2′ and H-6′), 12.48 (1H, s, 5-OH).

$^{13}$C-NMR(75.47MHz, DMSO-$d_6$): see Table I.

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## Literature Cited


1. Lee, M.-W., Tanaka, T., Nonaka, G. and Hahn, D.-R.: *Arch. Pharm. Res.* **15**, 211(1992).
2. Mensah, I.A. and Achenbach, H.: *Phytochemistry* **23**, 2043(1984).
3. Park, W.-Y., Lee, S.-C., Ahn, B.-T., Lee, S.-H., Ro, J.-S. and Lee, K.-S.: *Kor. J. Pharmacogn.* **24**, 20(1993).
4. Cho, E.-H. and Kim, I.-H.: *Yakhak Hoeji* **35**, 427(1991).
5. Lorente, F.T., Ferreres, F. and Barberan, F.T.: *Phytochemistry* **21**, 1461(1982).
6. Harborne, J.B.: *The Flavonoids, Advances in Research since 1980*, Chapman and Hall, New York, p. 67 (1980).
7. Harborne, J.B. and Mabry, T.J.: *The Flavonoids, Advances in Research*, Chapman and Hall, London, New York, p. 38 (1982).
8. *ibid.*, p. 46.
9. *ibid.*, p. 459.
10. Tanaka, O.: *Yakugaku Zasshi* **105**, 323(1985).
11. Mabry, T.J., Markham, K.R. and Thomas, M.B.: *The Systematic Identification of Flavonoids*, Springer-Verlag, Berlin, p. 267(1970).
12. Markham, K.R.: *Techniques of Flavonoid Identification*, Academic Press, London, p. 91(1982).
13. *ibid.*, p. 82.
14. Shen, Z. and Theander, O.: *Phytochemistry* **24**, 155(1985).
15. Agrawal, P.K.: *Carbon-13 NMR of Flavonoids*, Elsevier Science Publishing Company Inc., New York, p. 118(1989).