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Chemical Constituents of *Lathyrus davidii*

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Abstract – From the MeOH extract of the whole plants of *Lathyrus davidii* (Fabaceae), thirteen constituents were isolated and identified as the flavonoids astragalin, isoquercitrin, nicotiflorin, and rutin, as the saponins soyasapogenol B 3-*O*-β-D-glucuronopyranoside, azukisaponins II and V, soyasaponins II and V and as 4-*O*-β-D-glucopyranosyl syringic acid, uracil and *n*-hexacosanol. Five saponins and 4-*O*-β-D-glucopyranosyl syringic acid were isolated from the BuOH fraction as their methyl esters. Ombuoside (rutin 7,4'-di-*O*-methyl ether) was also isolated from the methylated BuOH-soluble fraction. However, no ombuoside was detected in the HPLC analysis of the nonmethylated BuOH fraction. Therefore, ombuoside is an artifact derived from methylation of rutin. All of these compounds were isolated for the first time from this plant.
Key words – *Lathyrus davidii*, Fabaceae, saponin, flavonoid, phenylpropanoid

Introduction

Lathyrus is one of the largest genus in tribe Fabaceae. *L. davidii* Hance is a relatively unknown leguminous plant that grows along the deep mountains in Korea and has been used as a diuretic and a tonic in folk medicine (Lee, 1989). Although the seed oil composition and β-sitosterol have been identified from *L. davidii*, no further phytochemical work has been reported on this plant (Endo, 1967). In our ongoing research to isolate and identify components of the whole plants of *L. davidii*, thirteen constituents were isolated and identified based on the spectral data.

Experimental

General – The optical rotations were determined on a Jasco P-1020 polarimeter. The UV spectra were obtained on a Hitachi U-3010 spectrophotometer and the IR spectra were recorded on a Jasco FT/IR 4200 spectrometer. The EI-MS was performed on a Hewlett Packard 5989B mass spectrometer. The FAB mass spectrum was obtained on a Jeol JMS-700 spectrometer. The NMR spectra were measured on a Bruker Avance-500 (500 MHz) or a Varian Gemini-2000 (300 MHz), and the chemical shifts were referenced to TMS. The HPLC analysis was carried out on Agilent 1200 HPLC system composed of a binary pump with a degasser, a column oven, a DAD detector and an autosampler. Sample analysis was performed on a Shieseido Capcell Pak C_{18} UG120 column (5 μm, 4.6 mm × 150 mm) at room temperature. The detection wavelength was set at 245 nm. The mobile phases consisted of 100% MeOH (A) and 100% H_2O (B) using a gradient elution of 30% A at 0 - 3 min, 95% A at 3 - 10 min, and 95% A at 10 - 25 min. The flow rate was 1 mL/min. Gas chromatographic analysis was performed with a Hewlett Packard 5890 Series II Plus gas chromatograph equipped with an H_2 flame ionization detector. The column was an HP-5 capillary column (30 m × 0.25 mm, 0.25 μm): column temperature, 50 → 250 °C; injection and detection temperature, 280 °C; and He flow rate, 1 mL/min. TLC was performed on silica gel 60 F_{254} (Merck) and RP-18_{254s} (Merck).

Plant Material – The whole plants of *L. davidii* were collected from Mt. Baiktou, Korea in June 2007, and authenticated by one of the authors (KiHwan Bae). A voucher specimen (CNU 1144) has been deposited at the College of Pharmacy, Chungnam National University.

Extraction and Isolation – The dried whole plant of *L. davidii* (1.4 kg) was refluxed with MeOH for 4 h at 70 - 80 °C (6 L × 4). The MeOH extract was evaporated to dryness under reduced pressure and then partitioned successively between H_2O and hexane (27.4 g), CH_2Cl_2 (5.1 g), EtOAc (6.3 g) and BuOH (35.4 g). The EtOAc fraction (6.3 g) was fractionated by column chromatography over silica gel with hexane/EtOAc (stepwise) to

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yield subfractions (Fr. E-1 - Fr. E-547). Fr. E-7 was crystallized from CH_2Cl_2 to afford **1** (30 mg). Fr. E-165 (271 mg) was further purified on a silica gel column (CH_2Cl_2 / MeOH / H_2O = 7 : 0.5 : 0.5 → 7 : 1 : 0.5). Subfraction E-165-51 (28 mg) was chromatographed on an RP-18 column with 50% MeOH to afford **2** (11 mg). Fr. E-181 (483 mg) was further purified on a silica gel column (CH_2Cl_2 / MeOH / H_2O = 7 : 0.5 : 0.5 → 7 : 3 : 1) to afford **3** (60 mg). Fr. E-200 (199 mg) was further purified on a silica gel column (CH_2Cl_2/MeOH/H_2O = 7 : 1 : 0.5 → 7 : 3 : 1). Subfraction E-200-37 was crystallized in MeOH to afford **4** (10 mg). Fr. E-213 (804 mg) was further purified on a silica gel column (CH_2Cl_2 / MeOH / H_2O = 7 : 1 : 0.5 → 7 : 2 : 1). Subfraction E-213 - 103 (38 mg) was chromatographed on an RP-18 column with 50% MeOH to afford **5** (30 mg). A portion of the BuOH fraction (35.4 g) was desalted with 0.02 N H_2SO_4 in 60% dioxane solution, and then methylated with CH_2N_2 (Rao, *et al*., 1985). A portion of the methylated BuOH fraction (26.7 g) was subjected to SiO_2 column chromatography. Elution with CH_2Cl_2/MeOH/H_2O (7 : 2 : 1) gave subfractions (Fr. B-1 - Fr. B-291). Fr. B-10 (300 mg) was further purified on a silica gel column (hexane / EtOAc = 5 : 8). Subfraction B-10-21 was crystallized in MeOH to afford **6** (2 mg). Fr. B-14 (150 mg) was further purified on a silica gel column (hexane/ EtOAc=5:8). Subfraction B-14-16 and B-14-25 were crystallized in MeOH to afford **7** (2 mg) and **8** (5 mg), respectively. Fr. B-22 was crystallized in MeOH to afford **9** (20 mg). Fr. B-33 (40 mg) was chromatographed on an RP-18 column with 90% MeOH to afford **10** (5 mg). Fr. B-53 was crystallized in MeOH to afford **11** (10 mg). Fr. B-61 (495 mg) was chromatographed on an RP-18 column with 90% MeOH to afford **12** (370 mg). Fr. B-117 was crystallized in MeOH to afford **13** (30 mg).

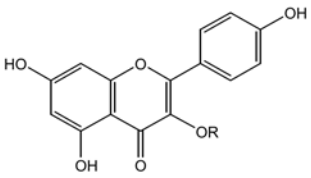
***n*-Hexacosanol (1)** – Amorphous white powder. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t-like, J = 7.2 Hz, CH_3), 1.25 [s, $(CH_2)_n$], 1.56 (2H, m, C$\underline{H_2}$CH$_2$OH), 3.64 (2H, t, J = 6.6 Hz, C$\underline{H_2}$OH); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ: 63.1 (C$\underline{H_2}$OH), 32.8 (C-2), 31.9 (C-24), 29.7 (C-4 ~ 22), 29.4 (C-23), 25.7 (C-3), 22.7 (C-25), 14.1 (CH_3); Peak 1 (t_R 31.611 min, *n*-hexacosanol, 57.2 %), GC/MS (rel. int., %) *m/z* 364 $[M - H_2O]^+$ (1), 336 (1), 209 (2), 195 (2), 181 (3), 167 (5), 153 (8), 139 (12), 125 (24), 111 (46), 97 (81), 83 (83), 57 (100).

Astragalin (2) – Yellow needles. $[\alpha]_D^{23}$ –9.9° (*c* 0.13 in MeOH). IR ν_{max} 3364, 1656, 1607, 1506, 1361, 1284, 1209, 1179, 1070, 1015, 840 cm^{-1}; UV λ_{max} (MeOH) 265 (log ε 4.57), 295 (sh, 4.32), 350 (4.50) nm; λ_{max} (NaOMe) 274 (4.63), 326 (4.37), 402 (4.68) nm; λ_{max} ($AlCl_3$) 234 (sh, 4.47), 274 (4.56), 303 (4.30), 351 (4.43), 401 (4.44) nm; λ_{max} ($AlCl_3$/HCl) 234 (sh, 4.47), 274 (4.56), 302 (4.30), 346 (4.42), 401 (4.38) nm; λ_{max} (NaOAc) 273 (4.66), 304 (4.38), 371 (4.44) nm; λ_{max} (NaOAc/H_3BO_3) 266 (4.57), 299 (sh, 4.33), 352 (4.49) nm; ^{1}H-NMR (300 MHz, CD_3OD) δ: 3.53 (1H, dd, J = 5.4, 12.0 Hz, H-6"a), 3.69 (1H, dd, J = 2.1, 12.0 Hz, H-6"b), 5.23 (1H, d, J = 7.5 Hz, H-1"), 6.16 (1H, brs, H-6), 6.35 (1H, brs, H-8), 6.87 (2H, d, J = 8.7 Hz, H-3', 5'), 8.03 (2H, d, J = 8.7 Hz, H-2', 6'); ^{13}C-NMR (75.5 MHz, CD_3OD) δ: see Table 1; FAB-MS *m/z* 471 $[M + Na]^+$, 449 $[M + H]^+$, 287 $[(M + H) - 162]^+$.

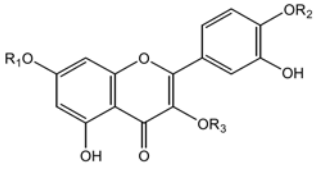
Table 1. ^{13}C-NMR spectral data of **2**, **3**, **4**, **5**, and **11**

Position	**2***	**3***	**4***	**5**#	**11**#	Position	**2***	**3***	**4***	**5**#	**11**#
2	158.5	158.4	158.5	156.6	157.0	1"	104.1	104.3	104.6	101.4	101.4
3	135.4	135.6	135.5	133.5	134.1	2"	75.7	75.7	75.7	74.3	74.3
4	179.4	179.5	179.4	177.5	177.8	3"	78.0	78.1	78.1	76.6	76.6
5	163.0	163.0	163.0	161.4	161.1	4"	71.3	71.2	71.4	70.2	70.1
6	100.1	99.9	100.0	98.9	98.2	5"	78.4	78.4	77.2	76.1	76.1
7	166.5	166.0	166.1	164.5	165.4	6"	62.6	62.5	68.5	67.2	67.1
8	94.9	94.7	94.9	93.8	92.5	1"'			102.4	100.9	101.0
9	159.0	159.0	159.4	156.8	156.7	2"'			72.1	70.7	70.6
10	105.5	105.7	105.6	104.1	105.3	3"'			72.3	70.6	70.8
1'	122.7	123.0	122.7	121.8	122.7	4"'			73.9	72.0	72.1
2'	132.3	116.0	132.4	115.4	116.0	5"'			69.7	68.4	68.5
3'	116.0	145.9	116.1	145.0	146.1	6"'			17.9	17.9	17.9
4'	161.5	149.8	161.5	148.6	150.4	7-OCH_3					55.9
5'	116.0	117.6	116.1	116.4	111.6	4'-OCH_3					56.3
6'	132.3	123.2	132.4	121.3	121.8						

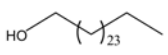
*in CD_3OD; #in DMSO-d_6



2 R = Glc
4 R = Rha-(1→6)-Glc



3 $R_1 = R_2 = H$ R_3 = Glc
5 $R_1 = R_2 = H$ R_3 = Rha-(1→6)-Glc
11 $R_1 = R_2 = CH_3$ R_3 = Rha-(1→6)-Glc



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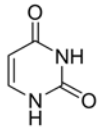

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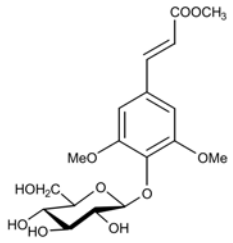

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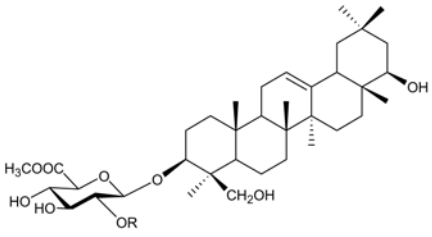

7 R = H
9 R = Ara
10 R = Glc
12 R = Rha-(1→2)-Ara-(1→2)
13 R = Rha-(1→2)-Glc-(1→2)

Isoquercitrin (3) – Yellow needles. $[\alpha]_D^{27}$ –3.2° (*c* 0.18 in MeOH). IR ν_{max} 3388, 1655, 1604, 1504, 1359, 1301, 1197, 1061, 1011, 934, 798 cm^{-1} ; UV λ_{max} (MeOH) 256 (log ε 4.41), 263 (sh, 4.41), 296 (sh, 4.03), 357 (4.35) nm; λ_{max} (NaOMe) 271 (4.46), 330 (4.06), 408 (4.42) nm; λ_{max} ($AlCl_3$) 274 (4.47), 306 (sh, 3.93), 334 (3.80), 435 (4.45) nm; λ_{max} ($AlCl_3$/HCl) 269 (4.40), 299 (sh, 4.00), 361 (sh, 4.17), 393 (sh, 4.24), 403 (4.27) nm; λ_{max} (NaOAc) 271 (4.43), 325 (sh, 4.11), 381 (4.27), 402 (sh, 4.20) nm; λ_{max} (NaOAc/H_3BO_3) 261 (4.47), 295 (sh, 3.95), 378 (4.35) nm; ^{1}H-NMR (300 MHz, CD_3OD) δ: 3.21 (1H, m, H-5"), 3.56 (1H, dd, *J* = 5.4, 11.7 Hz, H-6"a), 3.71 (1H, dd, *J* = 2.4, 11.7 Hz, H-6"b), 5.25 (1H, d, *J* = 7.5 Hz, H-1"), 6.20 (1H, d, *J* = 1.8 Hz, H-6), 6.39 (1H, d, *J* = 1.8 Hz, H-8), 6.86 (1H, d, *J* = 8.7 Hz, H-5'), 7.58 (1H, dd, *J* = 2.1, 8.7 Hz, H-6'), 7.70 (1H, d, *J* = 2.1 Hz, H-2'); ^{13}C-NMR (75.5 MHz, CD_3OD) δ: see Table 1; FAB-MS *m/z* 487 $[M + Na]^+$, 465 $[M + H]^+$, 303 $[(M + H) - 162]^+$.

Nicotiflorin (4) – Yellow needles. $[\alpha]_D^{27}$ –11.4° (*c* 0.45 in MeOH). IR ν_{max} 3376, 2926, 1656, 1607, 1507, 1453, 1362, 1281, 1211, 1181, 1065, 886, 839, 810 cm^{-1}; UV λ_{max} (MeOH) 265 (log ε 4.08), 298 (sh, 3.85), 349 (3.96) nm; λ_{max} (NaOMe) 274 (4.15), 325 (3.93), 400 (4.15) nm; λ_{max} ($AlCl_3$) 273 (4.07), 304 (3.85), 352 (3.92), 396 (3.94) nm; λ_{max} ($AlCl_3$/HCl) 273 (4.06), 305 (sh, 3.84), 347 (3.92), 395 (3.89) nm; λ_{max} (NaOAc) 273 (4.17), 306 (3.92), 381 (3.98) nm; λ_{max} (NaOAc/H_3BO_3) 265 (4.13),

298 (sh, 3.91), 352 (4.00) nm; ^{1}H-NMR (300 MHz, CD_3OD) δ: 1.11 (3H, d, *J* = 6.3 Hz, H-6'"), 3.51 (1H, dd, *J* = 3.3, 9.6 Hz, H-3'"), 3.62 (1H, dd, *J* = 1.5, 3.3 Hz, H-2'"), 4.51 (1H, d, *J* = 1.5 Hz, H-1'"), 5.13 (1H, d, *J* = 7.8 Hz, H-1"), 6.21 (1H, d, *J* = 2.1 Hz, H-6), 6.41 (1H, d, *J* = 2.1 Hz, H-8), 6.89 (2H, d, *J* = 9.0 Hz, H-3', 5'), 8.06 (2H, d, *J* = 9.0 Hz, H-2', 6'); ^{13}C-NMR (75.5 MHz, CD_3OD) δ: see Table 1; FAB-MS *m/z* 617 $[M + Na]^+$, 595 $[M + H]^+$, 449 $[(M + H) - 146]^+$, 287 $[(M + H) - 146 - 162]^+$.

Rutin (5) – Yellow needles $[\alpha]_D^{27}$ +2.6° (*c* 0.16 in MeOH). IR ν_{max} 3364, 1655, 1602, 1505, 1456, 1362, 1296, 1204, 1065, 1015, 808 cm^{-1}; UV λ_{max} (MeOH) 256 (log ε 4.33), 267 (sh, 4.26), 297 (sh, 3.96), 358 (4.23) nm; λ_{max} (NaOMe) 271 (4.39), 329 (3.99), 395 (sh, 4.31), 410 (4.37) nm; λ_{max} ($AlCl_3$) 274 (4.39), 307 (sh, 3.85), 335 (sh, 3.74), 403 (sh, 4.02), 435 (4.38) nm; λ_{max} ($AlCl_3$/HCl) 269 (4.33), 300 (sh, 3.94), 404 (4.23) nm; λ_{max} (NaOAc) 269 (4.36), 324 (sh, 4.03), 385 (4.22), 402 (sh, 4.19) nm; λ_{max} (NaOAc/H_3BO_3) 261 (4.40), 297 (sh, 3.92), 379 (4.27), 402 (sh, 4.15) nm; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 0.98 (3H, d, *J* = 6.0 Hz, H-6'"), 3.38 (1H, dd, *J* = 0.9, 2.4 Hz, H-2'"), 4.37 (1H, d, *J* = 0.9 Hz, H-1'"), 5.33 (1H, d, *J* = 7.5 Hz, H-1"), 6.17 (1H, d, *J* = 1.8 Hz, H-6), 6.36 (1H, d, *J* = 1.8 Hz, H-8), 6.83 (1H, d, *J* = 8.7 Hz, H-5'), 7.52 (1H, brs, H-2'), 7.55 (1H, dd, *J* = 2.1, 8.7 Hz, H-6'), 12.57 (1H, brs, 5-O<u>H</u>); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: see Table 1; FAB-MS *m/z* 633 $[M + Na]^+$, 611 $[M + H]^+$, 465 $[(M + H) - 146]^+$, 303 $[(M + H) - 146 - 162]^+$.

Uracil (6) – Needles. IR ν_{max} (KBr) 3429, 3111, 2934, 1713, 1643, 1452, 1417, 1390, 1234, 993, 858, 759 cm^{-1}; UV λ_{max} (MeOH) 257 (log ε 3.68) nm; ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 5.79 (1H, d, *J* = 7.8 Hz, H-5), 7.50 (1H, d, *J* = 7.8 Hz, H-6); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: 101.2 (C-5), 142.1 (C-6), 153.2 (C-2), 165.7 (C-4); EI-MS (rel. int., %) *m/z* 112 $[M]^+$ (71), 69 $[M - HNCO]^+$ (100).

3-*O*-β-D-Glucuronopyranosyl soyasapogenol B methyl ester (7) – Amorphous white powder. $[\alpha]_D^{27}$ –2.7° (*c* 0.15 in MeOH). IR ν_{max} (KBr) 3389, 2925, 1736, 1518, 1442, 1377, 1249, 1165, 1082, 1043, 913 cm^{-1}; ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 0.83 (3H, s, 25-CH_3), 0.98 (3H, s, 26-CH_3), 0.99 (3H, s, 29-CH_3), 1.22 (3H, s, 28-CH_3), 1.27 (3H, s, 27-CH_3), 1.29 (3H, s, 30-CH_3), 1.56 (3H, s, 23-CH_3), 2.39 (1H, brd, *J* = 12.0 Hz, H-18), 3.57 (1H, dd, *J* = 4.5, 10.5 Hz, H-3), 3.63 (1H, m, H-22), 3.62 (1H, d, *J* = 10.8 Hz, H-24a), 3.72 (3H, s, CO_2CH_3), 4.07 (1H, t, *J* = 9.0 Hz, H-2'), 4.28 (1H, t, *J* = 9.0 Hz, H-3'), 4.38 (1H, d, *J* = 10.8 Hz, H-24b), 4.48 (1H, t, *J* = 9.0 Hz, H-4'), 4.64 (1H, d, *J* = 9.0 Hz, H-5'), 5.12 (1H, d, *J* = 7.8 Hz, H-1'), 5.30 (1H, brs, H-12); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 2; FAB-MS *m/z* 671 $[M + Na]^+$, 649 $[M + H]^+$.

4-*O*-β-D-Glucopyranosyl sinapic acid methyl ester (8) – Needles. $[\alpha]_D^{27}$ –17.4° (*c* 0.10 in MeOH). IR ν_{max} 3348, 2925, 1709, 1637, 1587, 1504, 1457, 1318, 1281, 1128, 1061, 980, 822 cm^{-1}; UV λ_{max} (MeOH) 300 (log ε 3.81) nm; ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 3.74 (3H, s, CO_2CH_3), 3.77 (6H, s, 3, 5-OCH_3), 5.94 (1H, d, *J* = 7.2 Hz, H-1'), 6.67 (1H, d, *J* = 15.9 Hz, H-8), 6.98 (2H, s, H-2, 6), 7.84 (1H, d, *J* = 15.9 Hz, H-7); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: 51.4 (COO<u>C</u>H_3), 56.6 (OCH_3), 62.5 (C-6'), 71.6 (C-4'), 76.0 (C-2'), 78.4 (C-3'), 78.9 (C-5'), 104.3 (C-1'), 107.0 (C-2, 6), 117.6 (C-8), 130.4 (C-1), 138.0 (C-4), 145.2 (C-7), 153.8 (C-3, 5), 167.4 (C-9); EI-MS (rel. int., %) *m/z* 238 [genin, $M - 162]^+$ (100), 223 $[genin - CH_3]^+$ (4), 207 $[genin - OCH_3]^+$ (21), 180 $[(CH_3O)_2 HOC_6H_2$-$CH{=}CH_2]^+$ (6), 175 $[genin - OCH_3 - CH_3OH]^+$ (14), 147 $[175 - CO]^+$ (7); FAB-MS *m/z* 423 $[M + Na]^+$, 391 $[(M + Na) - CH_3OH]^+$, 261 $[(M + Na) - 162]^+$, 239 $[(M + H) - 162]^+$.

Soyasaponin IV methyl ester (9) – Amorphous white powder. $[\alpha]_D^{27}$ +22.2° (*c* 0.50 in MeOH). IR ν_{max} (KBr) 3434, 2951, 1735, 1633, 1384, 1174, 1139, 1056, 785 cm^{-1}; ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 0.73 (3H, s, 25-CH_3), 0.94 (3H, s, 26-CH_3), 0.97 (3H, s, 29-CH_3), 1.20 (3H, s, 28-CH_3), 1.23 (3H, s, 27-CH_3), 1.27 (3H, s, 30-CH_3), 1.28 (3H, s, 23-CH_3), 2.37 (1H, *J* = 13.5 Hz, H-18), 3.37 (1H, dd, *J* = 4.2, 11.1 Hz, H-3), 3.65 (1H, d, *J* = 12.6 Hz, H-5"), 3.72 (3H, s, CO_2CH_3), 4.02 (1H, dd, *J* = 3.6, 9.3 Hz, H-3"), 4.49 (1H, d, *J* = 9.3 Hz, H-5'), 4.88 (1H, d, *J* = 7.5 Hz, H-1'), 5.28 (1H, brs, H-12), 5.43 (1H, d, *J* = 7.2 Hz, H-1"); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 2; FAB-MS *m/z* 779 $[M - H]^-$.

Azukisaponin II methyl ester (10) – Amorphous white powder. $[\alpha]_D^{27}$ +9.2° (*c* 0.10 in MeOH). IR ν_{max} 3303, 2942, 1727, 1651, 1457, 1381, 1253, 1170, 1076, 1048 cm^{-1}; ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.71 (3H, s, 25-CH_3), 0.93 (3H, s, 26-CH_3), 0.97 (3H, s, 29-CH_3), 1.21 (3H, s, 28-CH_3), 1.22 (3H, s, 27-CH_3), 1.27 (3H, s, 30-CH_3), 1.36 (3H, s, 23-CH_3), 3.36 (1H, d, *J* = 11.3 Hz, H-24a), 3.42 (1H, dd, *J* = 4.5, 11.6 Hz, H-3), 3.77 (3H, s, CO_2CH_3), 4.52 (1H, d, *J* = 9.7 Hz, H-5'), 4.92 (1H, d, *J* = 7.9 Hz, H-1'), 5.29 (1H, brs, H-12), 5.60 (1H, d, *J* = 7.8 Hz, H-1"); ^{13}C-NMR (125.8 MHz, pyridine-d_5) δ: see Table 2; FAB-MS *m/z* 809 $[M - H]^-$.

Ombuoside (11) – Yellow needles. $[\alpha]_D^{27}$ –22.1° (*c* 0.10 in MeOH). IR ν_{max} 3377, 1649, 1580, 1495, 1439, 1353, 1208, 1062, 1005, 829, 803 cm^{-1}; UV λ_{max} (MeOH)

Table 2. ^{13}C-NMR spectral data of **7**, **9**, **10**, **12**, and **13** in pyridine-d_5

Position	**7**	**9**	**10**	**12**	**13**	Position	**7**	**9**	**10**	**12**	**13**
1	38.7	38.7	38.6	38.7	38.6	1'	106.5	105.2	104.9	105.4	105.4
2	26.9	26.7	26.6	26.7	26.7	2'	75.3	78.7	81.3	76.4	78.3
3	89.1	90.6	90.9	91.1	91.8	3'	77.9	77.0	78.0	78.0	78.5
4	44.4	44.0	43.8	43.9	43.8	4'	73.2	73.5	72.6	73.7	73.6
5	56.1	56.1	56.2	56.1	56.3	5'	77.4	77.7	77.0	76.9	76.8
6	18.9	18.6	18.6	18.5	18.6	6'	170.7	170.3	170.5	170.3	170.5
7	33.3	33.3	33.2	33.3	33.3	OCH_3	52.1	52.1	52.2	52.1	52.3
8	40.0	39.9	39.9	39.9	40.0	1"		104.8	104.8	101.8	102.1
9	47.8	47.8	47.7	47.8	47.9	2"		73.0	75.7	77.6	77.9
10	36.6	36.5	36.4	36.5	36.5	3"		74.9	78.4	75.8	79.2
11	24.1	24.0	24.0	24.0	24.1	4"		70.2	69.9	70.5	69.8
12	122.5	122.4	122.4	122.3	122.4	5"		67.5	78.2	66.9	78.2
13	144.8	144.8	144.8	144.8	145.0	6"			61.6		61.5
14	42.4	42.3	42.4	42.3	42.5	1"'				102.4	102.2
15	26.4	26.4	26.4	26.4	26.5	2"'				72.3	72.4
16	28.8	28.6	28.6	28.6	28.8	3"'				72.7	72.8
17	38.0	38.0	38.0	38.0	38.1	4"'				74.3	74.4
18	45.3	45.3	45.3	45.3	45.4	5"'				69.4	69.5
19	46.8	46.8	46.7	46.7	46.9	6"'				18.9	19.1
20	30.9	30.9	30.9	30.9	31.0						
21	42.3	42.3	42.2	42.3	42.4						
22	75.6	75.5	75.6	75.5	75.7						
23	23.3	22.5	22.6	22.9	22.9						
24	63.2	63.3	63.3	63.4	63.5						
25	15.5	15.7	15.6	15.8	15.8						
26	17.0	16.9	17.0	16.9	17.1						
27	25.7	25.7	25.7	25.7	25.8						
28	21.2	21.1	21.1	21.1	21.3						
29	33.4	33.3	33.2	33.3	33.4						
30	28.7	28.6	28.7	28.6	28.8						

255 (log ε 4.40), 267 (sh, 4.33), 353 (4.28) nm; λ_{max} (NaOMe) 240 (sh, 4.41), 271 (4.51), 381 (4.23) nm; λ_{max} ($AlCl_3$) 234 (4.12), 269 (4.40), 299 (3.99), 362 (sh, 4.15), 402 (4.26) nm; λ_{max} ($AlCl_3$/HCl) 236 (4.11), 268 (4.38), 299 (4.00), 362 (sh, 4.15), 399 (4.21) nm; λ_{max} (NaOAc) 256 (4.39), 267 (sh, 4.35), 296 (sh, 4.05), 354 (4.26) nm; λ_{max} (NaOAc/H_3BO_3) 256 (4.39), 267 (sh, 4.35), 296 (sh, 4.05), 354 (4.26) nm; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 0.96 (3H, d, J = 6.3 Hz, H-6'"), 3.85 (6H, s, 2 × OCH_3), 4.38 (1H, brs, H-1'"), 5.38 (1H, d, J = 7.8 Hz, H-1"), 6.36 (1H, d, J = 2.1 Hz, H-6), 6.68 (1H, d, J = 2.1 Hz, H-8), 7.03 (1H, d, J = 8.4 Hz, H-5'), 7.53 (1H, d, J = 2.1 Hz, H-2'), 7.72 (1H, dd, J = 2.1, 8.4 Hz, H-6'), 12.52 (1H, brs, 5-O<u>H</u>); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: see Table 1; EI-MS (rel. int., %) *m/z* 330 [aglycon]$^+$ (100), 315 [aglycon – CH_3]$^+$ (48), 301 [aglycon – CHO]$^+$ (7), 287 [aglycon – CH_3CO]$^+$ (19), 259 [aglycon – CH_3CO – CO]$^+$ (23), 231 [aglycon – CH_3CO – 2CO]$^+$ (13), 167 [A_1 + H]$^+$ (6), 151 [B_2]$^+$ (8), 123 [B_2 – CO]$^+$ (14); FAB-MS *m/z* 661 [M + Na]$^+$, 639 [M + H]$^+$, 493 [(M + H) – 146]$^+$, 331 [(M + H) – 146 – 162]$^+$.

Soyasaponin II methyl ester (12) – Amorphous white powder. $[\alpha]_D^{22}$ –3.1° (*c* 0.26 in MeOH). IR ν_{max} 3388, 2942, 1740, 1650, 1455, 1378, 1221, 1136, 1050, 910, 779 cm^{-1}; ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 0.71 (3H, s, 25-CH_3), 0.94 (3H, s, 26-CH_3), 0.97 (3H, s, 29-CH_3), 1.20 (3H, s, 28-CH_3), 1.26 (3H, s, 27-CH_3), 1.27 (3H, s, 30-CH_3), 1.40 (3H, s, 23-CH_3), 1.73 (1H, d, J = 6.3 Hz, H-6'"), 2.37 (1H, brd, J = 12.6 Hz, H-18), 3.25 (1H, d, J = 9.9 Hz, H-24a), 3.36 (1H, dd, J = 4.2, 11.7 Hz, H-3), 3.54 (1H, d, J = 12.0 Hz, H-5"a), 3.72 (3H, s, CO_2CH_3), 4.11 (1H, brd, J = 12.0 Hz, H-5"b), 4.25 (1H, d, J = 9.9

Hz, H-24b), 4.51 (1H, d, J = 9.6 Hz, H-5'), 4.61 (1H, dd, J = 3.6, 9.3 Hz, H-3'"), 4.75 (1H, d, J = 3.6 Hz, H-2'"), 4.92 (1H, d, J = 7.2 Hz, H-1'), 5.28 (1H, brs, H-12), 5.54 (1H, d, J = 7.2 Hz, H-1"), 6.20 (1H, brs, H-1'"); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 2; FAB-MS *m/z* 925 $[M-H]^-$, 779 $[(M-H)-146]^-$.

Azukisaponin V methyl ester (13) – Amorphous white powder. $[\alpha]_D^{23}$ −3.9° (*c* 0.33 in MeOH). IR ν_{max} 3388, 2945, 1743, 1650, 1456, 1381, 1046, 912 cm^{-1}; ^{1}H-NMR (75.5 MHz, pyridine-d_5) δ: 0.67 (3H, s, 25-CH_3), 0.92 (3H, s, 26-CH_3), 0.96 (3H, s, 29-CH_3), 1.19 (3H, s, 28-CH_3), 1.23 (3H, s, 27-CH_3), 1.25 (3H, s, 30-CH_3), 1.43 (3H, s, 23-CH_3), 1.75 (3H, d, J = 6.0 Hz, H-6'"), 2.35 (1H, brd, J = 12.0 Hz, H-18), 3.28 (1H, d, J = 11.7 Hz, H-24a), 3.36 (1H, dd, J = 3.9, 11.7 Hz, H-3), 3.74 (3H, s, CO_2CH_3), 4.94 (1H, d, J = 8.1 Hz, H-1'), 5.27 (1H, brs, H-12), 5.81 (1H, d, J = 7.5 Hz, H-1"), 6.35 (1H, brs, H-1'"); ^{13}C-NMR (300 MHz, pyridine-d_5) δ: see Table 2; FAB-MS *m/z* 955 $[M-H]^-$, 809 $[(M-H)-146]^-$.

Results and Discussion

The dried whole plants of *L. davidii* were crushed, extracted with MeOH, and successively partitioned with H_2O and hexane, CH_2Cl_2, EtOAc, and then BuOH. The BuOH-soluble fraction was methylated with CH_2N_2 to facilitate separation and purification (Kim, *et al*., 2002b; Rao, *et al*., 1985). The EtOAc and methylated BuOH-soluble extracts were subjected to a series of chromatographic separations, which led to the isolation of five (**1** - **5**) and eight (**6** - **13**) compounds, respectively. Compound **1** was identified as *n*-alkanol that contained *n*-hexacosanol as a major component according to NMR and GC/MS data (Kim, *et al*., 2002a). The well-known flavonol glycosides from Fabaceae such as astragalin (**2**), isoquercitrin (**3**), nicotiflorin (**4**), and rutin (**5**) were identified based on detailed UV, NMR, and MS analyses (Markham, 1982; Kang, *et al*., 1985; Kang, *et al*., 1998). Compounds **6** and **8** were identified as uracil (Lee, *et al*., 2002; Ryu, *et al*., 2002) and 4-*O*-β-D-glucopyranosyl sinapic acid methyl ester, respectively (Hashimoto, *et al*., 1992). Compounds **7**, **9**, **10**, **12** and **13** were readily classified with the aid of the characteristic NMR spectra as olean-12-ene triterpenoid saponins that existed as the aglycon soyasapogenol B (Kang, *et al*., 1988). However, these compounds differ from each other due to the presence of different sugar moieties. Acid hydrolysis of **7** on a TLC plate allowed identification of glucuronic acid as the sugar moiety (Kang, *et al*., 1997; Amoros and Girre, 1987). The positive ion mode FAB mass spectrum showed quasimolecular ions at *m/z* 671 $[M+Na]^+$ and 649 $[M+H]^+$. Diagnostic features in the ^{1}H-NMR spectrum of **7** were the presence of seven angular methyl singlet signals at δ 0.83, 0.98, 0.99, 1.22, 1.27, 1.29, and 1.56, an olefinic proton at δ 5.30 and an anomeric proton signal at δ 5.12 (d, J = 7.8 Hz), reminiscent of the well-known leguminous soyasaponins. Therefore, compound **7** was established as 3-*O*-β-D-glucuronopyranosyl soyasapogenol B methyl ester (Kang, *et al*., 1988; Sakamoto, *et al*., 1992). An inspection of the ^{1}H- and ^{13}C-NMR spectra of **9** readily indicated the presence of two monosaccharide units through easily identifiable signals for anomeric protons and carbons. Acid hydrolysis of **9** on TLC plate afforded glucuronic acid and arabinose as the sugar components. (−)-FAB-MS showed a pseudomolecular ion peak at *m/z* 779 $[M-H]^-$, which is consistent with a disaccharide glycoside carrying one mole each of arabinose and glucuronic acid methyl ester, and an aglycon, soyasapogenol B. Comparison of the ^{13}C-NMR data of **9** with **7** showed that the signals for C-2' of **9** were significantly shifted downfield by +3.4 ppm due to a glycosidation shift (Byun, *et al*., 2004; Kim, *et al*., 2008). The ^{13}C-NMR spectrum of **9** was superimposable on that of soyasaponin IV methyl ester (Cui, *et al*., 1992). The negative FAB mass spectrum of **10** showed a pseudomolecular ion peak at *m/z* 809 $[M-H]^-$, suggesting the presence of one hexose (glucose) unit rather than pentose. Acidic hydrolysis of **10** gave sugars identified as glucose and glucuronic acid as described in **7**. The ^{13}C-NMR resonances arising from the aglycon and sugar moieties were superimposable on those of azukisaponin II methyl ester (Kang, *et al*., 1988; Kang, *et al*., 1998; El-Sebakhy, *et al*., 2000), which was further confirmed by direct comparison with an authentic sample. Acidic hydrolysis of **12** and **13** gave sugars identifed as arabinose, rhamnose and glucuronic acid in **12** and glucose, rhamnose and glucuronic acid in **13** as described in **10**. The negative ion mode FAB-MS of **12** and **13** showed fragment ions at *m/z* 925 $[M-H]^-$, 779 $[(M-H)-146]^-$ and *m/z* 955 $[M-H]^-$ and 809 $[(M-H)-146]^-$, respectively, which were consistent with the presence of glucuronic acid methyl ester, arabinose (glucose) and the terminal sugar, rhamnose. The ^{13}C-NMR resonances arising from the sugar moieties of **12** and **13** were very close to those of **9** and **10**, respectively, except for the signals assigned to the terminal sugar, rhamnose. Comparison of the ^{13}C-NMR data of **13** with **10** as well as **12** with **9** showed that the signals for C-2" of both were significantly shifted downfield by +2.2 and +4.6 ppm, respectively, due to a glycosidation shift. The large

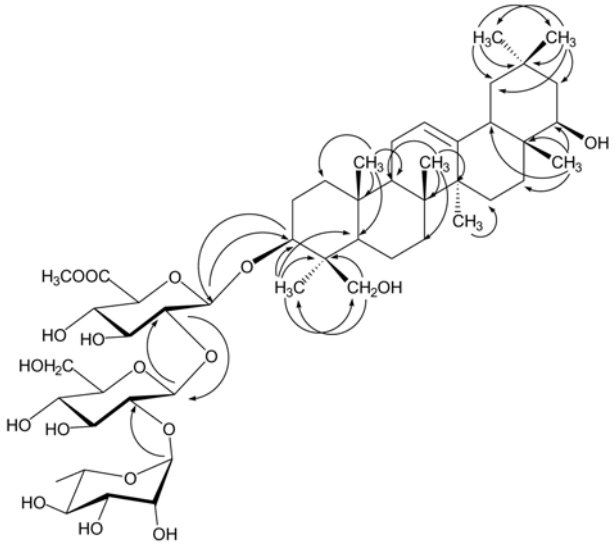

Fig. 1. Key HMBC correlations for **13**.

coupling constant (7.2 Hz) of the arabinose moiety in **9** and **12** suggested that the conformation of arabinopyranosyl moiety had the usual $^4C_1$ conformation rather than the $^1C_4$ conformation (Byun, *et al*., 2004). Thus the structures of **12** and **13** were determined to be soyasapogenol B 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 2)-β-D-glucuronopyranoside methyl ester, soyasaponin II methyl ester (Liu, *et al*., 2006; Wang, *et al*., 2007) and soyasapogenol B 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2)-β-D-glucuronopyranoside methyl ester, azukisaponin V methyl ester (Kang, *et al*., 1988; Kang, *et al*., 1998; Kim, *et al*., 2008), respectively. The sequence of the sugars and binding site at the aglycon of **13** were unambiguously determined in the HMBC experiment as indicated in Fig. 1. The spectroscopic data of **11** showed close similarity to those of rutin (**5**). However, the NMR data showed the presence of two methoxyl groups [δ_H 3.85 (2 × OCH_3); δ_C 55.9, 56.3] in its structure. EI-MS data showed a base peak at *m/z* 330 for an aglycon moiety together with two important fragment ions at *m/z* 167 $[A_1 + H]^+$ and 151 $[B_2]^+$ due to the *retro* Diels-Alder fragmentation, suggesting that the hydroxy-methoxy groups were located at rings A and B. Meanwhile, the UV data were more informative with respect to the location of these methoxyls. A bathochromic shift with decreasing intensity upon addition of NaOMe and no bathochromic effect upon addition of NaOAc indicated the presence of the methoxyl groups at C-7 and C-4' (Markham, 1982). Therefore, the structure of **11** was identified as ombuin 3-*O*-rutinoside (ombuoside) (Inigo, *et al*., 1985; Mitrocotsa, *et al*., 1999). Soyasaponins as well as kaempferol and quercetin glycosides are commonly encountered in leguminous plants (Kang, *et al*., 1998; Okubo and Yoshiki, 1996; Ranabahu and Harborne, 1993). In contrast, ombuoside is described here for the first time in the genus *Lathyrus*. To confirm the presence of this flavonoid in this plant, the nonmethylated BuOH-soluble fraction was analyzed by HPLC. As shown in Fig. 2, the HPLC chromatogram showed no ombuoside. Therefore, ombuoside is an artifact derived from methylation of rutin. All of these compounds were isolated for the first time from this plant.

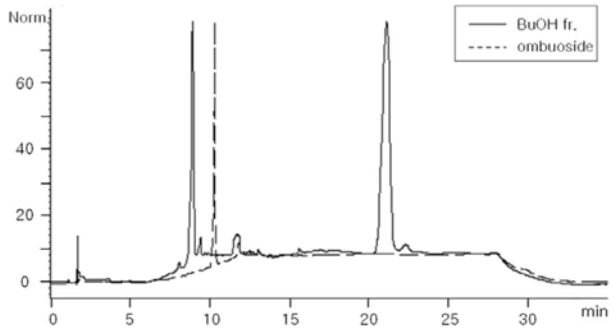

Fig. 2. HPLC chromatogram of the nonmethylated BuOH fraction and ombuoside.

References

Amoros, M. and Girre, R.L., Structure of Two Antiviral Triterpene Saponins from *Anagallis arvensis*. *Phytochemistry* **26**, 787-791 (1987).

Byun, J.H., Kim, J.S., Kang, S.S., Son, K.H., Chang, H.W., Kim, H.P., and Bae, K.H., Triterpenoid Saponins from the Roots of *Sophora koreensis*. *Chem. Pharm. Bull*. **52**, 870-873 (2004).

Cui, B.L., Inoue, J., Takeshita, T, Kinjo, J., and Nohara, T., Triterpene Glycosides from the Seeds of *Astragalus sinicus* L. *Chem. Pharm. Bull*. **40**, 3330-3333 (1992).

Ei-Sebakhy, N.A., Asaad, A.M., Abdallah, R.M., Toaima, S.M., Verotta, L., and Orsini, F., Constituents of Egyptian *Astragalus tribuloides* Del. *Nat. Prod. Sci.* **6**, 11-15 (2000).

Endo, S., Seed oil of itachisasage (*Lathyrus davidii*). *Yukagaku* **16**, 524-526 (1967).

Hashimoto, K., Katsuhara, T., Niitsu, K., Ikeya, Y., Okada, M., and Mitsuhashi, H., Two glycosides from roots of *Asiasarum sieboldi*. *Phytochemistry* **31**, 2477-2480 (1992).

Inigo, R.P.A. and Pomilio, A.B., Flavonoids from *Erythroxylon argentinum*. *Phytochemistry* **24**, 347-349 (1985).

Kang, S.S., Cordell, G.A., Soejarto, D.D., and Fong, H.H.S., Alkaloids and Flavonoids from *Ricinus communis*. *J. Nat. Prod*. **48**, 155-156 (1985).

Kang, S.S., Lee, Y.S., and Lee, E.B., Saponins and Flavonoid Glycosides from Yellow Sweetclover. *Arch. Pharm. Res*. **11**, 197-202 (1988).

Kang, S.S., Kim, J.S., Kim, Y.H., and Choi, J.S., A Triterpenoid Saponin from *Patrinia scabiosaefolia*. *J. Nat. Prod*. **60**, 1060-1062 (1997).

Kang, S.S., Ahn, B.T., Kim, J.S., and Bae, K.-H., Lathyrus saponin, a new trisaccharide glycoside from *Lathyrus japonicus*. *J. Nat. Prod.* **61**, 299-300 (1998).

Kim, J.S., Kang, S.S., Son, K.H., Chang, H.W., Kim, H.P., and Bae, K.H., Constituents from the roots of *Hemerocallis fulva*. *Kor. J. Pharmacogn*., **33**, 105-109 (2002a)

Kim, J.S., Byun, J.H., Kang, S.S., Son, K.H., Chang, H.W., and Kim, H.P., Isolation of flavonoids and a saponin from *Echinosophora koreensis*. *Kor. J. Pharmacogn*. **33**, 110-115 (2002b).

Kim, J.S., Yean, M.H., Lee, E.-J., and Kang, S.S., Phytochemical studies on *Astragalus* Root (1) - Saponins. *Nat. Prod. Sci.* **14**, 37-46 (2008).

Lee, S.H., Kang, S.S., and Shin, K.H., Coumarins and a Pyrimidine from *Angelica gigas* Roots. *Nat. Prod. Sci*. **8**, 58-61 (2002).

Lee, T.B., *Illustrated Flora of Korea*, Hyangmunsa, Seoul, p. 482, 1989.

Liu, Y., Zhang, Q.Y., Chen, H.B., Wang, B., An, D.G., and Zhao, Y.Y., Spectral Assignments and Reference Data. Structural determination of saponins from *Hedysarum polybotrys*. *Magn. Reson. Chem*. **44**, 1128-1130 (2006).

Markham, K.R., *Techniques of flavonoid identification*, Academic Press, London, pp. 36-51, 1982.

Mitrocotsa, D., Skaltsounis, A.-L., Mitaku, S., Harvala, C., and Tillequin, F., Flavonoid and terpene glycosides from European *Ebenus* species. *Biochem. System. Ecol*. **27**, 305-307 (1999).

Okubo, K. and Yoshiki, Y., Oxygen-Radical-Scavenging activity of DDMP-Conjugated Saponins and Physiological Role in Leguminosae Plant. *Adv. Exp. Med. Biol*. **405**, 141-154 (1996).

Ranabahu, P. and Harborne, J.B., The Flavonoids of the Genus *Lathyrus* and a Comparison of Flavonoid Patterns within the Tribe Vicieae. *Biochem. System. Ecol*. **21**, 715-722 (1993).

Rao, G.V., Rao, P.S., Tomimori, T., and Kizu, H., Soyasaponin I from *Rothia trifoliata*. *J. Nat. Prod*. **48**, 135-138 (1985).

Ryu, J.Y., Kim, J.S., and Kang, S.S., Quality evaluation and components of *Euphoria longana*. *Kor. J. Pharmacogn*. **33**, 191-193 (2002).

Sakamoto, S., Kofuji, S., Kuroyanagi, M., Ueno, A., and Sekita, S., Saponins from *Trifolium repens*. *Phytochemistry* **31**, 1773-1777 (1992).

Wang, W., Hai, L.-Q., Zhao, Y.-Y., Chen, H.-B., Wang, B., and Liang, H., Saponins from the roots of *Hedysarum multijugum*. *China J. Chin. Mat. Med*. **32**, 315-317 (2007).

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