Natural Product Sciences
14(2) : 131-137 (2008)

Phytochemical Studies on *Astragalus* Root (2) – Flavonoids and a Lignan

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Abstract – From the 70% EtOH extract of the roots of *Astragalus membranaceus* (Leguminosae), eleven flavonoid derivatives and a lignan, were isolated and identified as liquiritigenin (**1**), daidzein (**2**), formononetin (**3**), sophorophenolone (**4**), calycosin (**5**), methylnissolin (**6**), isomucronulatol (**7**), isomucronulatol 7-*O*-glucoside (**8**), methylnissolin 3-*O*-glucoside (**9**), calycosin 7-*O*-glucoside (**10**), (+)-syringaresinol *O*-β-D-glucoside (**11**), and isomucronulatol 7,2'-di-*O*-glucoside (**12**), by spectroscopic methods. This is the second report of the isoflavonoid derivatives sophorophenolone (**4**) and isomucronulatol 7,2'-di-*O*-glucoside (**12**) from a natural source, as well as the first report of compounds liquiritigenin (**1**), daidzein (**2**) and (+)-syringaresinol *O*-β-D-glucoside (**11**) from the species *A. membranaceus*.

Keywords – *Astragalus membranaceus*, Leguminosae, flavonoid, lignan

Introduction

In previous papers, we reported the isolation of fourteen cycloartane saponins and an oleanane-type triterpene saponin, azukisaponin V methyl ester from Astragali Radix, the dry root of Korean *Astragalus membranaceus* (FISCH.) BGE. (Leguminosae), and elucidated the structures of the saponins as astragalosides I, II, III, IV and isoastragaloside II as the major components as well as the minor saponins, cyclogaleginoside B, cycloaraloside A, brachyoside B, agroastragalosides I and II, cyclocanthoside E, cyclounifolioside B and astramembranosides A and B, among which astramembranosides are new saponins (Kim, *et al*., 2008a,b). This paper describes the structural elucidation of eleven flavonoid derivatives and a lignan, (+)-syringaresinol *O*-β-D-glucoside (**11**) from the same roots of *A. membranaceus* on the basis of various spectroscopic data.

Experimental

General – The optical rotations were determined on a Jasco P-1020 polarimeter. The UV spectra were determined on a Hitachi JP/U-3010 spectrophotometer. The IR spectra were recorded on a Jasco FT/IR-5300 spectrometer. The EIMS was performed on a Hewlett Packard 5989B or a Jeol JMS-700 mass spectrometer. The FAB mass spectrum was obtained on a VG-VSEQ 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. TLC was performed on silica gel 60 F_{254} (Merck, art. no. 5715), PR-18 (Merck, art. no. 5685) and cellulose plates (Merck, art. no. 5716).

Plant Material – The roots of *A. membranaceus* were cultivated in Jungsun, Kangwon province, Korea, for three years, harvested in September 2004, and authenticated by Prof. Lee J.-H. (College of Oriental Medicine, Dongguk University). A voucher specimen (LJH2005-12) was deposited in the herbarium of the College of Oriental Medicine, Dongguk University.

Extraction and Isolation – The roots of *A. membranaceus* (17.8 kg) were chopped into small pieces and refluxed with 70% EtOH for 3 h at 70 - 80 °C (3 L × 7). The 70% EtOH extract was evaporated to dryness under reduced pressure and then partitioned successively between H_2O and hexane (137 g), EtOAc (145 g), and then BuOH (340 g). The hexane fraction (137 g) was fractionated by column chromatography over silica gel with hexane/EtOAc (gradient) to yield 12 subfractions (Fr. H-01-Fr. H-12). Fraction H-9 (419 mg) was chromatographed on a silica gel column with CH_2Cl_2/MeOH (10 : 0.1 → 10 : 0.3) to afford H-9-10 (86 mg) and repeated silica gel column chromatography (hexane/EtOAc = 5 : 1 → 5 : 2) followed by an RP-18 column with 100% MeOH to afford **1** (2 mg) from H-9-10-5. The

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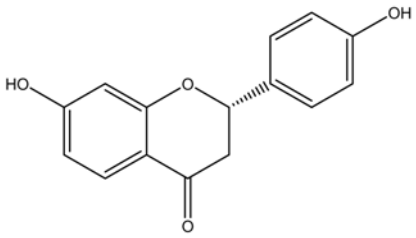
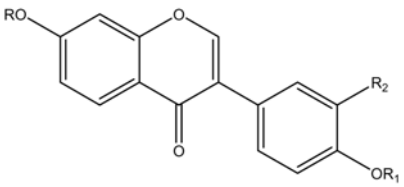
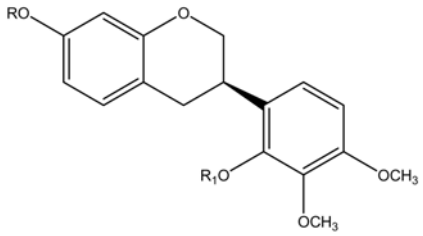


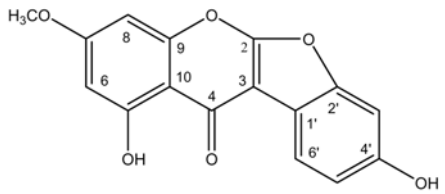
Liquiritigenin (**1**)



Daidzein (**2**) R= R_1= R_2= H
Formononetin (**3**) R= R_2= H R_1= CH_3
Calycosin (**5**) R= H R_1= CH_3 R_2= OH
Calycosin 7-*O*-β-glucoside (**10**) R= Glucose R_1= CH_3 R_2= OH

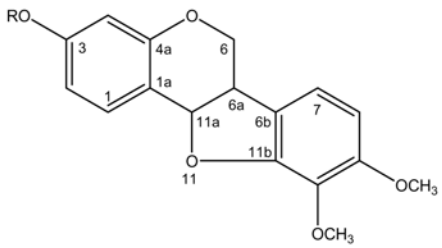


Isomucronulatol (**7**) R= R_1= H
Isomucronulatol 7-*O*-β-glucoside (**8**) R= Glucose R_1 = H
Isomucronulatol 7,2′-di-*O*-glucoside (**12**) R= R_1 = Glucose

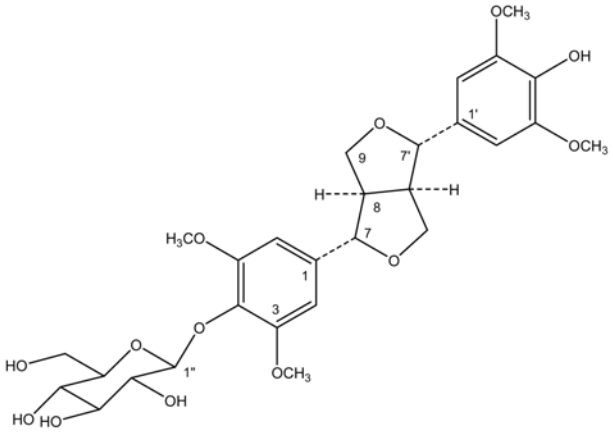


Sophorophenolone (**4**)

EtOAc fraction (143.8 g) was fractionated by column chromatography over silica gel with CH_2Cl_2/MeOH (gradient) to yield 51 subfractions (Fr. E-01-Fr. E-51). Fr.



Methylnissolin (**6**) R= H
Methylnissolin 3-*O*-β-glucoside (**9**) R= Glucose



(+)-Syringaresinol β-D-glucoside (**11**)

E-21 (1.3 g) was chromatographed on a silica gel column with hexane/EtOAc (10 : 0.5 → 10 : 2) to yield **7** (15 mg). Subfraction E-21-24 (1.0 g) was rechromatographed on an RP-18 column with 90% MeOH to give **4** (3 mg). Fr. E-23 (1.0 g) was further purified on a silica gel column with hexane/EtOAc (gradient) to yield **2** (1 mg), **3** (150 mg), and **6** (1 mg). Subfraction E-26 (1.0 g) was further purified on a silica gel column with CH_2Cl_2/MeOH/H_2O (7 : 0.5 : 0.5) to afford **5** (110 mg). Fr. E-40 (17.6 g) was purified on a silica gel column with hexane/EtOAc (gradient) to yield **8** (1,457 mg) and **9** (344 mg). Fraction E-45 (20 g) was purified on a silica gel column with EtOAc/MeOH/H_2O (100 : 1 : 0.5 → 100 : 2 : 1 → 100 : 8 : 4) to afford **10** (1,410 mg). The BuOH soluble fraction (170 g) was fractionated by silica gel column chromatography with CH_2Cl_2/MeOH/H_2O (7 : 1 : 0.5 → 7 : 2 : 0.5 → 7 : 3 : 1) to yield 39 fractions (Fr. B-01-Fr. B-39). Fr. B-12 (0.8 g) was purified on silica gel column with EtOAc and then EtOAc saturated with H_2O/MeOH (gradient) to yield subfraction B-12-65 (105 mg), which was further purified on an RP-18 column with 40% MeOH to yield **11** (63 mg). Subfraction B-20 (1.5 g) was purified on an RP-18 column with 80% MeOH to yield

subfraction B-20-85 (24 mg), which was rechromatographed on a silica gel column with CH_2Cl_2/MeOH/H_2O (7 : 1 : 0.5 → 7 : 2 : 0.5 → 7 : 3 : 1) to afford **12** (10 mg).

Liquiritigenin (1) – Amorphous white powder. $[\alpha]_D^{26}$ –1.8° (*c* 0.1 in pyridine). IR ν_{max} (KBr) 3406 (OH), 1608 (α,β-unsat. CO), 1512, 1458 (aromatic C = C) cm^{-1}; UV λ_{max} (MeOH) 274 (log ε 4.18), 309 (3.95) nm; (CH_3ONa) 246 (4.35), 298 (3.98), 327 (4.29), 335 (4.34) nm; ($AlCl_3$) 273 (4.18), 309 (3.95) nm; ($AlCl_3$ + HCl) 273 (4.17), 310 (3.94) nm; (NaOAc) 250 (4.25), 282 (4.10), 327 (4.31), 335 (4.34) nm; (NaOAc + H_3BO_3) 274 (4.18), 310 (3.98) nm; ^{1}H-NMR (300 MHz, CD_3OD) δ: 2.69 (1H, dd, *J* = 3.0, 16.8 Hz, H-3a), 3.05 (1H, dd, *J* = 12.9, 16.8 Hz, H-3b), 5.38 (1H, dd, *J* = 3.0, 12.9 Hz, H-2), 6.34 (1H, dd, *J* = 2.1 Hz, H-8), 6.49 (1H, dd, *J* = 2.1, 8.8 Hz, H-6), 6.81 (2H, d, *J* = 8.7 Hz, H-3', 5'), 7.32 (2H, d, *J* = 8.4 Hz, H-2', 6'), 7.72 (1H, d, *J* = 8.8 Hz, H-5); ^{13}C-NMR (75.5 MHz, CD_3OD) δ: 44.9 (C-3), 81.1 (C-2), 103.8 (C-8), 111.7 (C-6), 115.0 (C-10), 116.3 (C-3', 5'), 129.0 (C-2', 6'), 129.8 (C-5), 131.4 (C-1'), 159.0 (C-4'), 165.3 (C-9), 166.8 (C-7), 193.5 (C-4); EIMS (rel. int., %) *m/z* 256 $[M]^+$ (29), 228 $[M - CO]^+$ (2), 163 [M – ring B]$^+$ (18), 137 $[A_1 + H]^+$ (100), 120 $[B_3]^+$ (67), 107 $[HOC_6H_4CH_2]^+$ (25).

Daidzein (2) – Amorphous white powder. ^{1}H-NMR (300 MHz, CD_3OD) δ: 6.84 (1H, d, *J* = 2.1 Hz, H-8), 6.93 (1H, dd, *J* = 2.1, 8.1 Hz, H-6), 6.98 (2H, d, *J* = 9.0 Hz, H-3', 5'), 7.46 (2H, d, *J* = 9.0 Hz, H-2', 6'), 8.05 (1H, d, *J* = 8.1 Hz, H-5), 8.15 (1H, s, H-2); ^{13}C-NMR (75.5 MHz, CD_3OD) δ: see Table 1; EIMS (rel. int., %) *m/z* 254 $[M]^+$ (100), 253 $[M - H]^+$ (45), 225 $[M - HCO]^+$ (8), 197 $[M - HCO - CO]^+$ (8), 137 $[A_1 + H]^+$ (41), 136 $[A_1]^+$ (10), 118 $[B_1]^+$ (50), 108 $[A_1 - CO]^+$ (24), 89 (23).

Formononetin (3) – Amorphous white powder. IR ν_{max} (KBr) 3130 (OH), 1647 (α,β-unsat. CO), 1597, 1514, 1454 (aromatic C = C) cm^{-1}; UV λ_{max} (MeOH) 247 (log ε 4.51), 300 (4.17) nm; (CH_3ONa) 255 (4.62), 335 (4.21) nm; ($AlCl_3$) 247 (4.55), 300 (4.17) nm; ($AlCl_3$ + HCl) 247 (4.54), 300 (4.16) nm; (NaOAc) 254 (4.60), 335 (4.15) nm; (NaOAc + H_3BO_3) 247 (4.57), 302 (4.18) nm; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.78 (3H, s, OCH_3), 6.87 (1H, d, *J* = 2.1 Hz, H-8), 6.93 (1H, dd, *J* = 2.1, 9.0 Hz, H-6), 6.98 (2H, d, *J* = 8.7 Hz, H-3', 5'), 7.50 (2H, d, *J* = 8.7 Hz, H-2', 6'), 7.96 (1H, d, *J* = 9.0 Hz, H-5), 8.32 (1H, s, H-2), 10.77 (1H, br s, OH); ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 3.68 (3H, s, OCH_3), 7.07 (2H, d, *J* = 8.7 Hz, H-3', 5'), 7.10 (1H, d, *J* = 2.1 Hz, H-8), 7.21 (1H, dd, *J* = 2.1, 8.4 Hz, H-6), 7.77 (2H, d, *J* = 8.7 Hz, H-2', 6'), 8.14 (1H, s, H-2), 8.45 (1H, d, *J* = 8.4 Hz, H-5); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 1; EIMS (rel. int., %) *m/z* 268 $[M]^+$ (100), 253 $[M - CH_3]^+$ (17), 225 $[M - CH_3 - CO]^+$ (6), 136 $[A_1]^+$ (3), 132 $[B_1]^+$ (60), 117 $[B_1 - CH_3]^+$ (11), 108 $[A_1 - CO]^+$ (3), 89 $[B_1 - CH_3 - CO]^+$ (13).

Sophorophenolone (4) – Amorphous white powder. UV λ_{max} (MeOH) 255 (log ε 4.28), 280 (sh, 3.82), 333 (3.81) nm; (CH_3ONa) 264 (4.21), 314 (3.77), 359 (3.76) nm; ($AlCl_3$) 266 (4.20), 284 (sh, 3.93), 301 (sh, 3.64), 319 (sh, 3.43), 380 (3.80) nm; ($AlCl_3$+HCl) 266 (4.21), 282 (sh, 3.94), 301 (sh, 3.65), 315 (sh, 3.42), 380 (3.82) nm; (NaOAc) 255 (4.26), 280 (sh, 3.82), 300 (sh, 3.64), 333 (3.78) nm; (NaOAc + H_3BO_3) 255 (4.28), 279 (sh, 3.83), 300 (sh, 3.64), 333 (3.80) nm; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.87 (3H, s, OCH_3), 6.49 (1H, d, *J* = 2.1 Hz, H-6), 6.85 (1H, d, *J* = 2.1 Hz, H-8), 6.93 (1H, dd, *J* = 2.1, 8.4 Hz, H-5'), 7.12 (1H, d, *J* = 2.1 Hz, H-3'), 7.72 (1H, d, *J* = 8.4 Hz, H-6'); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: 56.5 (OCH_3), 94.1 (C-8), 97.7 (C-3), 99.0 (C-6), 99.1 (C-3'), 104.1 (C-10), 113.4 (C-1'), 114.1 (C-5'), 121.3 (C-6'), 150.3 (C-2'), 154.7 (C-9), 156.6 (C-4'), 162.1 (C-5), 164.7 (C-7), 164.8 (C-2), 178.4 (C-4); EIMS *m/z* 298 $[M]^+$ (100), 269 $[M - CHO]^+$ (22), 255 (18), 199 (9), 176 (17), 149 (18), 122 (12), 69 (13).

Calycosin (5) – Amorphous white powder. IR ν_{max} (KBr) 3468, 3200 (OH), 1620 (α,β-unsat. CO), 1601, 1579, 1512, 1458 (aromatic C = C) cm^{-1}; UV λ_{max} (MeOH) 248 (log ε 4.53), 290 (4.32) nm; (CH_3ONa) 250 (4.61), 330 (4.33) nm; ($AlCl_3$) 248 (4.51), 291 (4.30) nm; ($AlCl_3$ + HCl) 248 (4.51), 290 (4.29) nm; (NaOAc) 257 (4.63), 333 (4.21) nm; (NaOAc + H_3BO_3) 250 (4.55), 290 (4.32) nm; ^{1}H-NMR (300 MHz, acetone-d_6) δ: 3.87 (3H, s, OCH_3), 6.89 (1H, d, *J* = 2.1 Hz, H-8), 6.97 (1H, d, *J* = 8.4 Hz, H-5'), 6.99 (1H, dd, *J* = 2.1, 8.4 Hz, H-6), 7.06 (1H, dd, *J* = 2.1, 8.4 Hz, H-6'), 7.16 (1H, d, *J* = 2.1 Hz, H-2'), 8.06 (1H, d, *J* = 8.4 Hz, H-5), 8.14 (1H, s, H-2); ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 3.75 (3H, s, OCH_3), 7.03 (1H, d, *J* = 8.1 Hz, H-5'), 7.09 (1H, d, *J* = 2.1 Hz, H-8), 7.19 (1H, dd, *J* = 2.1, 8.5 Hz, H-6), 7.32 (1H, dd, *J* = 2.1, 8.1 Hz, H-6'), 7.79 (1H, d, *J* = 2.1 Hz, H-2'), 8.17 (1H, s, H-2), 8.43 (1H, d, *J* = 8.5 Hz, H-5); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 1; EIMS (rel. int., %) *m/z* 284 $[M]^+$ (100), 269 $[M - CH_3]^+$ (23), 241 $[M - CH_3 - CO]^+$ (30), 213 $[M - CH_3 - 2 \times CO]^+$ (35), 148 $[B_1]^+$ (7), 137 $[A_1 + H]^+$ (24), 133 $[B_1 - CH_3]^+$ (22), 105 $[A_1 - CO - CH_3]^+$ (35).

Methylnissolin (6) – Amorphous white powder. ^{1}H-NMR (300 MHz, CD_3OD) δ: 3.49 – 3.55 (1H, m, H-6a), 3.58 (1H, t, *J* = 10.5 Hz, H-6β), 3.80, 3.82 (3H each, s, 2 × OCH_3), 4.22 (1H, dd, *J* = 3.6, 10.5 Hz, H-6α), 5.51 (1H, d, *J* = 6.3 Hz, H-11a), 6.30 (1H, d, *J* = 2.4 Hz, H-4), 6.49 (1H, dd, *J* = 2.4, 8.7 Hz, H-2), 6.52 (1H, d, *J* = 8.4 Hz, H-8), 6.94 (1H, d, *J* = 8.4 Hz, H-7), 7.32 (1H, d,

J = 8.7 Hz, H-1); EIMS (rel. int., %) *m/z* 300 $[M]^+$ (61), 299 $[M-H]^+$ (10), 285 $[M-CH_3]^+$ (13), 268 $[M-(CH_3O+H)]^+$ (100), 253 $[268-CH_3]^+$ (12), 225 $[253-CO]^+$ (8), 147 $[C_9H_7O_2]^+$ (12).

Isomucronulatol (7) – Amorphous white powder. $[\alpha]_D^{22}$ –4.9° (*c* 0.3 in MeOH). UV λ_{max} (MeOH) 228 (sh, log ε 4.48), 280 (3.94), 288 (sh, 3.73) nm; (CH_3ONa) 243 (sh, 4.33), 291 (4.12) nm; ($AlCl_3$) 227 (sh, 4.46), 280 (3.89), 288 (sh, 3.78) nm; ($AlCl_3$ + HCl) 280 (3.91), 289 (sh, 3.78) nm; (NaOAc) 280 (3.91), 288 (sh, 3.80) nm; (NaOAc + H_3BO_3) 280 (3.93), 289 (sh, 3.79) nm; ^{1}H-NMR (300 MHz, CD_3OD) δ: 2.79 (1H, br ddd, J = 1.5, 5.1, 15.6 Hz, H-4β), 2.94 (1H, br dd, J = 10.8, 15.6 Hz, H-4α), 3.44 (1H, m, H-3), 3.78, 3.80 (3H each, s, 2 × OCH_3), 3.95 (1H, t, J = 10.5 Hz, H-2α), 4.22 (1H, ddd, J = 1.8, 3.3, 10.5 Hz, H-2β), 6.21 (1H, d, J = 2.4 Hz, H-8), 6.30 (1H, dd, J = 2.4, 8.1 Hz, H-6), 6.45 (1H, d, J = 8.4 Hz, H-5'), 6.77 (1H, d, J = 8.4 Hz, H-6'), 6.86 (1H, d, J = 8.1 Hz, H-5); ^{13}C-NMR (75.5 MHz, CD_3OD) δ: see Table 1; EIMS (rel. int., %) *m/z* 302 $[M]^+$ (100), 180 $[C_{10}H_{12}O_3$ (ring B)$]^+$ (100), 167 $[C_9H_{11}O_3]^+$ (51), 165 [ring B – CH_3]$^+$ (16), 137 [ring B – CH_3 – CO]$^+$ (13), 133 (22), 123 $[C_7H_7O_2$ (ring A)$]^+$ (12), 94 $[C_7H_6O_2 - CO]^+$ (9).

Isomucronulatol 7-*O*-β-glucoside (8) – Amorphous white powder. $[\alpha]_D^{22}$ –58.2° (*c* 0.22 in pyridine). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.79 (1H, br dd, J = 5.7, 16.5 Hz, H-4β), 2.92 (1H, dd, J = 10.8, 16.5 Hz, H-4α), 3.67, 3.74 (3H each, s, 2 × OCH_3), 3.95 (1H, t, J = 10.2 Hz, H-2α), 4.18 (1H, ddd, J = 1.5, 3.0, 10.2 Hz, H-2β), 4.75 (1H, d, J = 7.2 Hz, H-1"), 6.45 (1H, d, J = 9.0 Hz, H-5'), 6.46 (1H, d, J = 2.7 Hz, H-8), 6.53 (1H, dd, J = 2.7, 8.7 Hz, H-6), 6.77 (1H, d, J = 9.0 Hz, H-6'), 6.99 (1H, d, J = 8.7 Hz, H-5), 8.94 (1H, s, 6'-OH); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: see Table 1; FAB-MS *m/z* 487 [M + Na]$^+$, 465 $[M+H]^+$, 464 $[M]^+$, 303 $[(M+H)-162]^+$, 302 $[M-162]^+$, 180 [ring B]$^+$, 123 [ring A]$^+$; EIMS *m/z* 302 [aglycon]$^+$, 180 [ring B]$^+$, 123 [ring A]$^+$.

Methylnissolin 3-*O*-β-glucoside (9) – Amorphous white powder. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.12 (1H, t, J = 8.7 Hz, H-4'), 3.20 (1H, t, J = 8.7 Hz, H-2'), 3.23 (1H, t, J = 8.7 Hz, H-3'), 3.30 – 3.34 (1H, m, H-5'), 3.42 (1H, dd, J = 5.7, 12.0 Hz, H-6'a), 3.62 – 3.74 (3H, overlap, H-6a, 6β, 6'b), 3.70, 3.73 (3H each, s, 2 × OCH_3), 4.27 (1H, dd, J = 3.6, 10.5 Hz, H-6α), 4.84 (1H, d, J = 7.5 Hz, H-1'), 5.62 (1H, d, J = 6.9 Hz, H-11a), 6.53 (1H, d, J = 8.4 Hz, H-8), 6.55 (1H, d, J = 2.4 Hz, H-4), 6.71 (1H, dd, J = 2.4, 8.7 Hz, H-2), 6.99 (1H, d, J = 8.4 Hz, H-7), 7.41 (1H, d, J = 8.7 Hz, H-1); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: 132.2 (C-1), 110.7 (C-2), 158.7 (C-3), 104.2 (C-4), 156.3 (C-4a), 114.2 (C-1a), 65.9 (C-6), 39.7 (C-6a), 121.8 (C-6b), 118.9 (C-7), 105.3 (C-8), 152.9 (C-9), 133.6 (C-10), 78.4 (C-11a), 151.2 (C-11b), 100.5 (C-1'), 73.4 (C-2'), 76.6 (C-3'), 69.9 (C-4'), 77.2 (C-5'), 60.9 (C-6'), 56.3, 60.1 (2 × OCH_3); EIMS (rel. int., %) *m/z* 300 [aglycon]$^+$ (61), 299 [aglycon – H]$^+$ (10), 285 [aglycon – CH_3]$^+$ (13), 268 [aglycon – (CH_3O + H)]$^+$ (100), 253 [268 – CH_3]$^+$ (12), 225 [253 – CO]$^+$ (8), 147 $[C_9H_7O_2]^+$ (12).

Calycosin 7-*O*-β-glucoside (10) – Amorphous white powder. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.16 (1H, t, J = 9.0 Hz, H-4"), 3.27 – 3.34 (2H, m, H-2", 3"), 3.44 (1H, m, H-5"), 3.48 (1H, dd, J = 5.5, 11.5 Hz, H-6"a), 3.71 (1H, dd, J = 4.5, 11.5 Hz, H-6"b), 3.79 (3H, s, OCH_3), 4.60 (1H, t, J = 6.0 Hz, 6"-OH), 5.08 (1H, t, J = 5.1 Hz, 4"-OH), 5.09 (1H, d, J = 7.8 Hz, H-1"), 5.14 (1H, d, J = 4.5 Hz, 3"-OH), 5.43 (1H, d, J = 4.8 Hz, 2"-OH), 6.96 (2H, br s, H-5', 6'), 7.06 (1H, s, H-2'), 7.13 (1H, dd, J = 2.4, 8.7 Hz, H-6), 7.22 (1H, d, J = 2.4 Hz, H-8), 8.04 (1H, d, J = 8.7 Hz, H-5), 8.39 (1H, s, H-2), 9.02 (1H, s, 3'-OH); ^{13}C-NMR (125.5 MHz, DMSO-d_6) δ: see Table 1; FAB-MS *m/z* 469 $[M+Na]^+$, 447 $[M+H]^+$, 285 $[(M+H)-162]^+$.

(+)-Syringaresinol *O*-β-D-glucoside (11) – Amorphous white powder. $[\alpha]_D^{25}$ –28.5° (*c* 0.5 in MeOH). ^{1}H-NMR (300 MHz, CD_3OD) δ: 3.13 (2H, m, H-8, 8'), 3.18 (1H, m, H-5"), 3.34 – 3.50 (3H, m, H-2", 3", 4"), 3.65 (1H, dd, J = 5.4, 11.7 Hz, H-6"a), 3.77 (1H, dd, J = 2.7, 11.7 Hz, H-6"b), 3.84, 3.85 (6H each, s, 4 × OCH_3), 3.90 (2H, dd, J = 3.6, 9.0 Hz, H-9*ax*, 9'*ax*), 4.24 – 4.31 (2H, m, H-9*eq*, 9'*eq*), 4.71 (1H, d, J = 4.2 Hz, H-7'), 4.76 (1H, d, J = 4.2 Hz, H-7), 4.85 (1H, d, J = 7.5 Hz, H-1"), 6.65 (2H, br s, H-2', 6'), 6.71 (2H, br s, H-2, 6); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: 131.5 (C-1), 133.9 (C-1'), 103.8 (C-2, 6), 104.4 (C-2', 6'), 148.1 (C-3, 5), 152.8 (C-3', 5'), 135.0 (C-4), 137.3 (C-4'), 85.3, 85.5 (C-7, 7'), 53.8, 53.9 (C-8, 8'), 71.3, 71.4 (C-9, 9'), 56.2, 56.6 (OCH_3), 102.8 (C-1"), 74.3 (C-2"), 76.7 (C-3"), 70.1 (C-4"), 77.4 (C-5"), 61.1 (C-6"); EIMS (rel. int., %) *m/z* 418 [aglycon]$^+$ (12), 235 (4), 210 [Ar – CH = CH – $CH_2OH]^+$ (8), 193 [Ar – CH = CH – $CH_2]^+$ (17), 182 [Ar – CHO]$^+$ (45), 181 [Ar – CO]$^+$ (96), 167 [Ar – $CH_2]^+$ (100).

Isomucronulatol 7,2'-di-*O*-glucoside (12) – Amorphous white powder. $[\alpha]_D^{22}$ –24.6° (*c* 0.32 in MeOH). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 2.72 (1H, br dd, J = 4.5, 15.7 Hz, H-4β), 2.86 (1H, dd, J = 11.4, 15.7 Hz, H-4α), 3.63 (1H, m, H-3), 3.74 (3'-OCH_3), 3.77 (4'-OCH_3), 3.85 (1H, t, J = 10.2 Hz, H-2α), 4.33 (1H, br d, J = 8.9 Hz, H-2β), 4.78 (1H, d, J = 7.6 Hz, H-1"), 4.87 (1H, d, J = 7.2 Hz, H-1'"), 6.47 (1H, d, J = 2.3 Hz, H-8), 6.54 (1H, dd, J = 2.3, 8.3 Hz, H-6), 6.80 (1H, d, J = 8.8 Hz, H-5'), 6.91 (1H, d, J = 8.8 Hz, H-6'), 6.98 (1H, d, J = 8.3 Hz, H-5); ^{13}C-NMR

Table 1. ^{13}C-NMR spectral data of isoflavones (**2**, **3**, **5**, **10**) and isoflavans (**7**, **8**, **12**)

Carbon No.	**2**[1)]	**3**[2)]	**5**[2)]	**10**[3)]	Carbon No.	**7**[1)]	**8**[3)]	**12**[3)]
2	153.1	152.7	152.8	153.6	2	70.9	69.4	69.7
3	122.8	125.4	126.3	124.4	3	33.5	31.5	30.1
4	175.1	175.6	175.7	174.6	4	31.3	29.9	30.8
5	127.6	128.2	128.2	127.0	5	131.2	130.2	130.0
6	115.5	115.9	115.9	115.6	6	109.0	109.0	108.8
7	162.8	164.1	164.1	161.4	7	157.5	156.9	156.7
8	102.4	103.1	103.1	103.4	8	103.8	103.4	103.9
9	157.7	158.5	158.5	157.0	9	156.3	154.7	154.6
10	116.9	117.9	118.0	118.5	10	114.8	116.0	116.0
1'	123.8	124.6	124.9	123.6	1'	122.4	121.0	128.4
2'	130.4	130.8	117.9	116.4	2'	149.5	148.3	147.6
3'	115.3	114.2	148.0	146.0	3'	137.4	136.3	141.2
4'	157.4	159.9	148.7	147.6	4'	153.1	151.8	152.1
5'	115.3	114.2	112.3	111.9	5'	104.3	104.0	108.6
6'	130.4	130.8	120.4	119.7	6'	122.8	121.7	121.6
OCH_3		55.2	55.9	55.7	4'-OCH_3	56.2	55.8	55.8
1"				100.0	3'-OCH_3	61.0	60.4	60.5
2"				73.1	1"		100.9	100.7
3"				76.5	2"		73.4	73.2
4"				69.6	3"		76.8	76.6
5"				77.2	4"		69.9	69.8
6"				60.6	5"		77.2	77.0
					6"		60.9	60.8
					1'''			103.3
					2'''			74.0
					3'''			76.4
					4'''			70.2
					5'''			77.4
					6'''			61.3

[1)]CD_3OD; [2)]pyridine-d_5; [3)]DMSO-d_6

(125.5 MHz, DMSO-d_6) δ: see Table 1; FAB-MS *m/z* 649 $[M + Na]^+$, 627 $[M + H]^+$, 465 $[(M + H) - 162]^+$, 303 $[(M + H) - 2 \times 162]^+$.

Results and Discussion

The dried roots of *A. membranaceus* were crushed, extracted with 70% EtOH, and partitioned successively with H_2O and hexane, EtOAc, and then BuOH. The hexane, EtOAc and BuOH soluble extracts were subjected to sequential column chromatography over silica gel and RP-18 gel to yield twelve phenolic compounds. The well-known isoflavone derivatives from *Astragalus* plants such as daidzein (**2**), formononetin (**3**), calycosin (**5**), and calycosin 7-*O*-β-glucoside (**10**) were identified based on detailed NMR and MS analyses and direct comparison with the authentic samples (Du, *et al*., 2006; Song, *et al*., 1997a; Kang, *et al*., 2000). Compound **1** showed UV maximum absorptions typical of the flavanone skeleton at 274 and 309 nm (Markham, 1982). In the ^{1}H-NMR spectrum, three typical flavanone skeleton resonances at δ 5.38 (1H, dd, J = 3.0, 12.9 Hz, H-2), 2.69 (1H, dd, J = 3.0, 16.8 Hz, H-3a), and 3.15 (1H, dd, J = 12.9, 16.8 Hz, H-3b) were observed together with *para*-substituted benzene ring [δ 6.81 (2H, d, J = 8.7 Hz, H-3', 5') and 7.32 (2H, d, J = 8.4 Hz, H-2', 6')] and 1,2,4-trisubstituted benzene ring proton signals [δ 6.34 (1H, dd, J = 2.1 Hz, H-8), 6.49 (1H, dd, J = 2.1, 8.8 Hz, H-6) and 7.72 (1H, d, J = 8.8 Hz, H-5)]. These data were superimposable to those for the well-known flavanone, liquiritigenin, which has been obtained from many leguminous plants. Direct comparison with an authentic specimen obtained from our

previous study (Shim, *et al*., 2005) supported for further identification. Compound **4** was obtained an amorphous powder. The UV absorption bands at 255 (4.28), 280 (sh, 3.82), and 333 (3.81) nm indicated similar characteristic absorption bands of isoflavone (Markham, 1982). The addition of $AlCl_3$ or $AlCl_3$ + HCl caused bathochromic shifts in the UV spectrum suggesting the presence of chelated hydroxyl group. Initial recognition of this compound as a coumaronochromone rather than as an isoflavone was based on the absence of a H-2 signal in the ^{1}H-NMR spectrum, the failure to observe either ring A or ring B derived *retro* Diels-Alder fragments in the EIMS, and the unusual UV spectrum (Zhao, *et al*., 2007). Hydroxylation at C-5 and C-4' was evident from UV shift measurements. The ^{1}H-NMR spectrum exhibited an AMX system corresponding to three protons at δ 6.93 (1H, dd, *J* = 2.1, 8.4 Hz, H-5'), 7.12 (1H, d, *J* = 2.1 Hz, H-3'), and 7.72 (1H, d, *J* = 8.4 Hz, H-6'), two *meta*-coupled aromatic protons at δ 6.49 (1H, d, *J* = 2.1 Hz, H-6), and 6.85 (1H, d, *J* = 2.1 Hz, H-8), and a methoxyl singlet signal at δ 3.87 (3H, s, OCH_3). The proposed structure 5,4'-dihydroxy-7-methoxycoumaronochromone, was also supported by EIMS fragmentation and ^{13}C-NMR data. This compound (**4**) was recently isolated from the fruits of *Sophora japonica* under the name of sophorophenolone (Tang, *et al*., 2002). Although the occurrence of the coumarono-chromone derivatives in leguminous plants has been reviewed (Harborne and Boxter, 1999), this is the first report of the sophorophenolone from the *Astragalus* species. The ^{1}H-NMR spectrum of compound **6** suggested a pterocarpan structure due to the splitting pattern of the protons at δ 5.51 (1H, d, *J* = 6.3 Hz, H-11a), 4.22 (1H, dd, *J* = 3.6, 10.5 Hz, H-6α), 3.58 (1H, t, *J* = 10.5 Hz, H-6β), and 3.49 – 3.55 (1H, m, H-6a), and two sets of aromatic protons of rings A and D [δ 7.32 (1H, d, *J* = 8.7 Hz, H-1), 6.49 (1H, dd, *J* = 2.4, 8.7 Hz, H-2), 6.30 (1H, d, *J* = 2.4 Hz, H-4); δ 6.94 (1H, d, *J* = 8.4 Hz, H-7), 6.52 (1H, d, *J* = 8.4 Hz, H-8); δ 3.80, 3.82 (3H each, s, 2 × OCH_3)]. The ^{1}H- and ^{13}C-NMR data were similar to those of (−)-maackiain (Kang, *et al*., 2000; Jung, *et al*., 2005; Kim, *et al*., 2002), supporting the above suggestion. Thus, the structure of **6** was determined as (6a*R*,11a*R*)-3-hydroxy-9,10-dimethoxypterocarpan, methylnissolin, which was isolated from *Lathyrus nissolia* (Robeson and Ingham, 1979; Song, *et al*., 1997b; Ohkawara, *et al*., 2005). Comparison of the spectral data for **6** with those of **9** showed the presence of characteristic signals for β-D-glucoside as indicated in trifolirhizin (Kang, *et al*., 2000; Jung, *et al*., 2005). Therefore compound **9** was identified as methylnissolin 3-*O*-β-glucoside (Song, *et al*., 1997b).

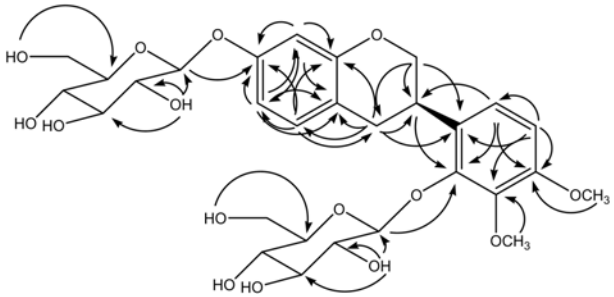


Fig. 1. Key HMBC correlations for isomucronulatol 7,2'-di-*O*-glucoside (**12**).

Compounds **7**, **8** and **12** were readily shown to be an isoflavan-type flavonoid by the characteristic ^{1}H-NMR data. Diagnostic features in the ^{1}H-NMR spectrum of **7** were the presence of proton signals at δ 2.79 (1H, br ddd, *J* = 1.5, 5.1, 15.6 Hz, H-4β), 2.94 (1H, br dd, *J* = 10.8, 15.6 Hz, H-4α), 3.44 (1H, m, H-3), 3.95 (1H, t, *J* = 10.5 Hz, H-2α), and 4.22 (1H, ddd, *J* = 1.8, 3.3, 10.5 Hz, H-2β) together with five aromatic protons, reminiscent of the known phytoalexin, isomucronulatol (Ingham, 1977; Al-Ani and Dewick, 1985; He and Findlay, 1991). Comparison of the ^{13}C-NMR data for **8** and **12** with those of **7** showed the presence of characteristic signals for β-D-glucoside as shown in Table 1. Therefore compound **8** was readily identified as isomucronulatol 7-*O*-β-glucoside (Wang, *et al*., 1990; He and Findlay, 1991; Subarnas, *et al*., 1991). A comparison of spectroscopic data of **12** with those of **8** indicated that the two compounds are very similar except for the presence of an additional glucopyranose moiety at C-2'. The HMBC experiment revealed the correlation between C-2' of isomucronulatol and H-1''' (δ 4.87) of the glucopyranosyl unit as indicated in Fig. 1. Consequently, the structure of **12** was established as isomucronulatol 7,2'-di-*O*-β-D-glucoside, which has been isolated from the same genus, *A. mongholicus* (Subarnas, *et al*., 1991). Compound **11** was obtained as an amorphous powder with negative optical rotation. ^{1}H- and ^{13}C-NMR spectra showed resonances typical of furofuran-type lignan glucoside (Yoo, *et al*., 2002; Kim, *et al*., 2007; Shahat, *et al*., 2004). This was consistent with the structure of (+)-syringaresinol *O*-β-D-glucoside (Yoshizawa, *et al*., 1990), one of the most commonly distributed lignans in terrestrial plants. This is the second report of the isoflavonoid derivatives sophorophenolone (**4**) and isomucronulatol 7,2'-di-*O*-glucoside (**12**) from a natural source, as well as the first report of compounds liquiritigenin (**1**), daidzein (**2**) and (+)-syringaresinol *O*-β-D-glucoside (**11**) from the species *A. membranaceus* (Pistelli, 2002).

Acknowledgment

This work was supported by a grant for the Study of the Isolation of the Bioactive Components and the Identification of the Biomarker Genes from the Oriental Herbal Medicines (Project 8: Paeoniae Radix and Astragali Radix) by the Korea Food & Drug Administration.

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(Accepted June 14, 2008)