Two New Cycloartane Saponins from the Roots of *Astragalus membranaceus*

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Received September 4, 2007; accepted October 4, 2007

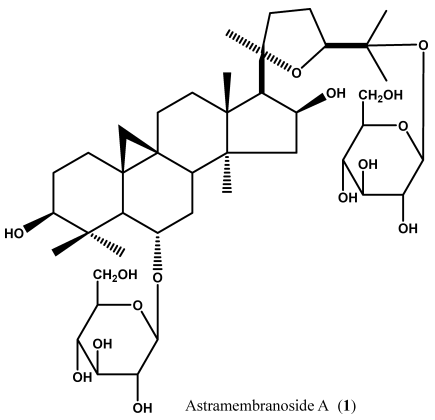
Two new cycloartane-type triterpenoid saponins were isolated from the roots of *Astragalus membranaceus* (FISCH.) BGE. (Leguminosae) cultivated in Kangwon province, Korea. These saponins were named astramembranosides A and B and were established to be cycloastragenol 6,25-di-*O*-*β*-D-glucopyranoside (astramembranoside A) and cyclocanthogenin 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-xylopyranoside (astramembranoside B) on the basis of chemical and spectral evidence. In addition, 12 known saponins were also isolated from the same materials. Although cycloastragenol 3-*O*-xyloside and agroastragalosides I and II have already been isolated from *A. membranaceus* adventitious roots, these three saponins together with brachyoside B and azukisaponin V methyl ester were isolated for the first time from this plant.

Key words *Astragalus membranaceus*; Leguminosae; astramembranoside A; astramembranoside B

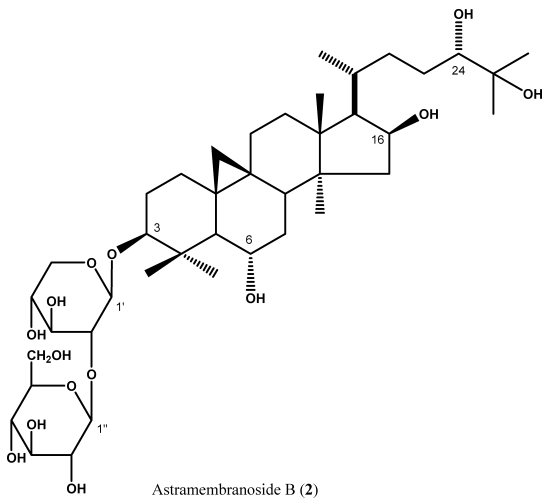
The genus *Astragalus* is one of the largest and most widely distributed genera, comprising 2000 species distributed mainly in northern temperate regions and tropical African mountains.[1] Five species of this genera have been identified in Korea.[2] Astragali Radix, the dry root of *Astragalus membranaceus* (FISCH.) BGE. (Leguminosae) has long been one of the most important tonic herbs used in traditional Chinese medicine. Studies of its pharmacology and clinical use have demonstrated that Astragali Radix has many biological functions, including hepatoprotection,[3,4] neuroprotection against ischemic brain injury,[5,6] immunologic properties,[7—9] cardiotonic[10,11] and antiaging activities,[12] gastroprotection,[13] adjuvant[14] and antitumor effects,[15,16] antiinflammatory effects,[17,18] and stimulation of the growth of new tissues.[19,20] The major bioactive components of *A. membranaceus* are triterpene saponins and isoflavonoids. As part of our efforts to isolate the chemical constituents of Astragali Radix to evaluate *A. membranaceus* qualitatively, we isolated a number of major and minor constituents from the roots of *A. membranaceus* cultivated in Korea. In the present investigation, we report the isolation and elucidation of the structure of two new minor compounds, astramembranosides A (**1**) and B (**2**), from the roots of *A. membranaceus*, together with the isolation of 12 known saponins.

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 EtOAc and BuOH extracts were subjected to sequential column chromatography over silica gel, MCI gel, and RP-18 gel to yield two new minor saponins, astramembranosides A (**1**) and B (**2**), together with 12 known saponins. The known saponins were identified as astragaloside I, astragaloside II, isoastragaloside II, agroastragaloside I, cycloastragenol 3-*O*-xyloside, cycloaraloside A (=cycloastragenol 3-*O*-glucoside), brachyoside B (=cycloastragenol 6-*O*-glucoside), agroastragaloside II, astragaloside III, azukisaponin V methyl ester, astragaloside IV, and cyclocanthoside E based on detailed NMR and MS analyses and comparison with the literature data.

Astramembranoside A (**1**) was obtained as an amorphous white powder. High-resolution (HR) FAB-MS exhibited an ion peak for $[M-H]^-$ at *m/z* 813.4626, which is compatible with the molecular formula $C_{42}H_{70}O_{15}$. The ^{1}H-NMR spectrum of **1** (Table 1) revealed the presence of a cyclopropane methylene group with signals at δ 0.27 (1H, d, J=3.7 Hz) and 0.64 (1H, d, J=3.7 Hz) and also contained signals for seven tertiary methyl groups at δ 0.92—1.98 and for oxygenated methine and methylene protons ascribable to sugar



Astramembranoside A (**1**)



Astramembranoside B (**2**)

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Table 1. ^{1}H- and ^{13}C-NMR Data of Astramembranosides A (**1**) and B (**2**) in Pyridine-d_5

Position	**1**[a]		**2**[b]	
	δ_H	δ_C (DEPT)	δ_H	δ_C (DEPT)
1	1.26[c]; 1.59[c]	32.6 (CH_2)	1.28[c]; 1.65[c]	32.5 (CH_2)
2	1.97[c]; 2.03[c]	31.3 (CH_2)	2.02[c]; 2.38[c]	30.4 (CH_2)
3	3.63 (dd, 4.6, 10.8)	78.3 (CH)	3.58 (dd, 4.5, 12.0)	88.6 (CH)
4	—	42.5 (C)	—	42.8 (C)
5	1.92 (d, 9.2)	52.5 (CH)	1.75 (d, 9.0)	54.0 (CH)
6	3.91[c]	79.6 (CH)	3.77 (ddd, 4.5, 9.0, 9.0)	67.7 (CH)
7	1.84[a]; 2.29[c]	34.5 (CH_2)	1.65[c]; 1.82[c]	38.3 (CH_2)
8	1.93[c]	46.0 (CH)	1.95[c]	46.7 (CH)
9	—	21.1 (C)	—	21.4 (C)
10	—	29.5 (C)	—	29.1 (C)
11	1.27[c]; 1.86[c]	26.3 (CH_2)	1.16[c]; 1.88[c]	26.3 (CH_2)
12	1.60[c]	33.5 (CH_2)	1.66[c]; 2.30[c]	33.2 (CH_2)
13	—	45.3 (C)	—	45.7 (C)
14	—	46.2 (C)	—	46.8 (C)
15	1.73 (dd, 6.5, 12.4) 2.27 (dd, 7.2, 12.4)	45.8 (CH_2)	1.77 (dd, 4.5, 12.5) 2.18 (dd, 7.5, 12.5)	48.3 (CH_2)
16	4.89 (m)	73.6 (CH)	4.74 (m)	72.0 (CH)
17	2.44 (d, 7.8)	58.1 (CH)	1.81 (dd, 3.5, 7.5)	57.3 (CH)
18	1.38 (s)	21.3 (CH_3)	1.41 (s)	18.8 (CH_3)
19	0.27 (d, 3.7) 0.64 (d, 3.7)	29.4 (CH_2)	0.28 (d, 4.0) 0.58 (d, 3.5)	29.6 (CH_2)
20	—	87.2 (C)	2.39[c]	28.6 (CH)
21	1.29 (s)	27.8 (CH_3)	1.10 (d, 6.5)	18.3 (CH_3)
22	2.82 (dd, 11.4, 20.2) 1.60[c]	35.1 (CH_2)	1.46[c]; 2.30[c]	33.0 (CH_2)
23	1.95[c]; 2.35[c]	26.1 (CH_2)	1.72[c]; 2.00[c]	27.9 (CH_2)
24	3.90[c]	82.1 (CH)	3.98 (dt, 3.5, 9.5)	77.2 (CH)
25	—	78.7 (C)	—	72.5 (C)
26	1.43 (s)	22.9 (CH_3)	1.47 (s)	25.8 (CH_3)
27	1.67 (s)	25.7 (CH_3)	1.49 (s)	26.5 (CH_3)
28	1.98 (s)	29.1 (CH_3)	1.95 (s)	28.8 (CH_3)
29	1.43 (s)	16.1 (CH_3)	1.43 (s)	16.6 (CH_3)
30	0.92 (s)	19.9 (CH_3)	1.05 (s)	20.1 (CH_3)
HO-3	5.80 (d, 4.4)			
HO-16	4.85 (s)			
HO-24			5.77 (br d, 2.5)	
1′	4.96 (d, 7.9)	105.0 (CH)	4.93 (d, 6.5)	105.7 (CH)
2′	4.06[c]	75.6 (CH)	4.27 (t, 9.0)	83.4 (CH)
3′	4.26[c]	79.4 (CH)	4.22[c]	77.9 (CH)
4′	4.24[c]	71.9 (CH)	4.14[c]	71.0 (CH)
5′	3.96 (m)	78.2 (CH)	3.65 (dd, 9.6, 10.8) 4.29[c]	66.7 (CH_2)
6′	4.33 (dd, 3.5, 11.6) 4.49 (br d, 11.6)	63.0 (CH_2)	—	
1″	5.09 (d, 7.6)	99.0 (CH)	5.42 (d, 8.0)	106.2 (CH)
2″	4.06[c]	75.2 (CH)	4.14 (t, 9.0)	77.1 (CH)
3″	4.21[c]	78.6 (CH)	3.96 (t, 9.3)	78.3 (CH)
4″	4.18 (t, 9.5)	71.4 (CH)	4.36 (t, 9.5)	71.7 (CH)
5″	3.90[c]	78.1 (CH)	4.20 (m)	78.0 (CH)
6″	4.44 (br d, 12.4) 4.32[c]	62.8 (CH_2)	4.48 (br d, 9.5) 4.52 (br d, 9.5)	62.8 (CH_2)

a) 600 MHz; *b*) 500 MHz. *c*) Signal patterns are unclear due to overlapping.

units. Additionally, two anomeric protons at δ 4.96 (1H, d, J=7.9 Hz, H-1′) and 5.09 (1H, d, J=7.6 Hz, H-1″) were observed, indicative of the presence of two β-linked sugar units. The sugar was identified as glucose by acid hydrolysis and TLC comparison with an authentic sample. The absolute configuration of glucopyranose was determined to be the D-form based on GLC analysis of the thiazolidine derivative.[21] A comparison of spectroscopic data with those of cycloastragenol 6-*O*-β-D-glucopyranoside (brachyoside B)[22] isolated from this experiment indicated that the two compounds are very similar except for the presence of an additional glucopyranose moiety and the attachment of the glucopyranose moiety at C-25. This was obvious from the anomeric carbon resonance attributed to the second glucose unit at C-25 (Table 1) which was found to be shifted upfield at δ 99.0. It has been reported that the anomeric carbon signal of tertiary alcoholic β-glucosides appears at a significantly higher field (δ *ca.* 99) than those of primary (δ *ca.* 104) and secondary (δ *ca.* 102) alcoholic β-glucosides.[23,24] The position of each sugar residue was unambiguously determined in a heteronuclear multiple-bond correlation (HMBC) experiment, which showed long-range correlations between H-1′ (δ 4.96) of the

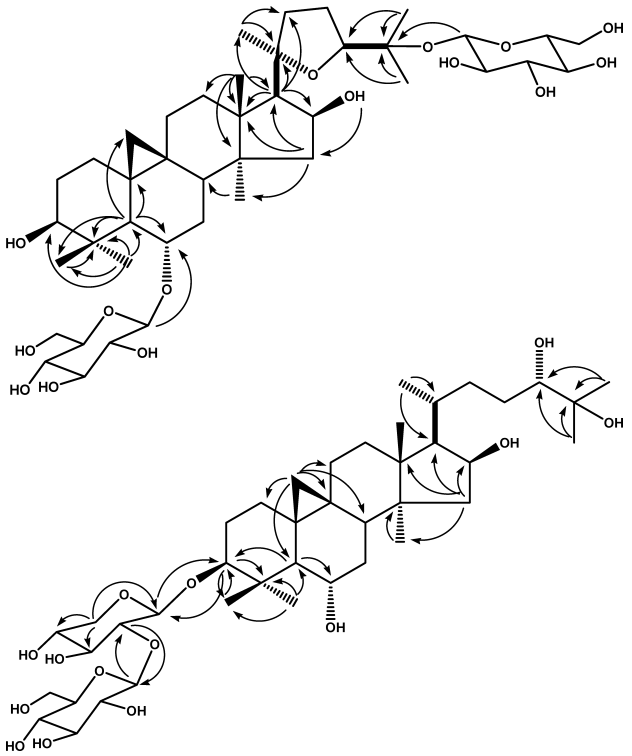

Fig. 1. Key HMBC Correlations for **1** (Upper Part) and **2** (Lower Part)

glucose and C-6 of cycloastragenol (δ 79.6), and between H-1″ of the second glucose at δ 5.09 with C-25 (δ 78.7), as indicated in Fig. 1. The structure of astramembranoside A (**1**) was thus determined to be (20*R*,24*S*)-6-*O*-β-D-glucopyranosyl-25-*O*-β-D-glucopyranosyl-3β,6α,16β,25-tetrahydroxy-20,24-epoxycycloartane (=cycloastragenol 6,25-di-*O*-β-D-glucopyranoside).

Astramembranoside B (**2**) was also obtained as an amorphous white powder. The HR-FAB-MS displayed a pseudomolecular ion peak $[M-H]^-$ at *m*/*z* 785.4666, indicating the molecular formula $C_{41}H_{70}O_{14}$. The NMR data of **2** were consistent with the presence of a cycloartane-type triterpene diglycosidic structure: characteristic signals due to cyclopropane methylene protons (δ 0.28, 1H, d, J=4.0 Hz; 0.58, 1H, d, J=3.5 Hz); six tertiary methyl groups (δ 1.05—1.95); a secondary methyl group (δ 1.10, 3H, d, J=6.5 Hz); and two anomeric protons (δ 4.93, d, J=6.5 Hz; 5.42, d, J=8.0 Hz) for two β-linked sugar units. Acid hydrolysis of **2** gave sugars identified as D-xylose and D-glucose as described for **1**.[21] The FAB-MS fragments at *m*/*z* 623 $[(M-H)-162]^-$ and 491 $[(M-H)-162-132]^-$ showed the presence of a diglycosidic sugar sequence that appeared to be that of glucosyl-xyloside.[25] The ^{13}C-NMR resonances arising from the rings of the sapogenol and sugar moieties were very close to those of astragaloside III,[26,27] except for the signals assigned to the side-chain moiety. These results indicate that **2** has the same sequence for the sugar linkage as astragaloside III and the side-chain structure of **2** appeared to have an acyclic side chain. This was supported by the ^{1}H-NMR spectrum, which showed a secondary methyl signal for the 21-CH_3 group (δ 1.10, d, J=6.5 Hz). Starting from the secondary methyl group, the partial structure of the acyclic side chain, CH_3–CH(C)–CH_2–CH_2–CH(C)–OH, could be deduced from ^{1}H–^{1}H correlation spectroscopy (COSY) and heteronuclear multiple-quantum correlation (HMQC) spectra. Therefore, instead of the epoxide ring seen in **1**, there was a hydroxyl group at C-24. The ^{13}C-NMR data for C-24 are comparable to those reported for analogous compounds having a 24*S* configuration.[27—30] The ^{13}C-NMR chemical shift for C-24 can be regarded as a characteristic parameter in the determination of the absolute configurations of C-24. In the case of the 24*R* configuration, the chemical shift for C-24 gives resonance at 80.0—80.5 ppm, while for the 24*S* configuration the chemical shift for C-24 gives resonance at 77.0—77.2 ppm.[28] All these observations support the presence of cyclocanthogenin[31] as the aglycon moiety. The sequence of the sugars and binding site at the aglycon of **2** were unambiguously determined in the HMBC experiment, which showed long-range correlations between the H-1′ of xylose and C-3 of cyclocanthogenin and between the H-1″ of the terminal glucose and C-2′ of xylose, as indicated in Fig. 1. Consequently, the structure of astramembranoside B (**2**) was established as 3-*O*-β-D-glucopyranosyl(1→2)-β-D-xylopyranosyl-3β,6α,16β,24*S*,25-pentahydroxy cycloartane (=cyclocanthogenin 3-*O*-β-D-glucopyranosyl(1→2)-β-D-xylopyranoside).

In addition, the known saponins were identified as astragaloside I,[27,32] astragaloside II,[29,32] isoastragaloside II,[32] agroastragaloside I,[27] cycloastragenol 3-*O*-xyloside,[29] cycloaraloside A (=cycloastragenol 3-*O*-glucoside),[33] brachyoside B (=cycloastragenol 6-*O*-glucoside),[22] agroastragaloside II,[29] astragaloside III,[26,27] azukisaponin V methyl ester,[34] astragaloside IV,[27,32] and cyclocanthoside E[35] by comparison of their physical and spectral data with those previously reported. The hairy roots of *A. membranaceus* were shown to produce previously unreported cycloartane-type saponins such as agroastragaloside I,[27] cycloastragenol 3-*O*-xyloside,[29] and agroastragaloside II,[29] together with the known saponins. To the best of our knowledge, this is the first report of these saponins from the intact plant. Although the occurrence of the oleanane-type triterpene saponin, azukisaponin V, in *Astragalus* plants has been demonstrated by others,[36—38] this is the first report of the azukisaponin V methyl ester from the *Astragalus* species.

Experimental

General The optical rotations were determined on a Jasco P-1020 polarimeter. The IR spectra were recorded on a Jasco FT/IR-5300 spectrometer. The EI-MS was performed on a Hewlett Packard 5989B mass spectrometer. The FAB mass spectrum was obtained in a glycerol matrix in negative-ion mode on a VG-VSEQ spectrometer. The NMR spectra were measured in pyridine-d_5 on a Varian Inova 500 instrument (500 MHz) or a Bruker Avance-600 instrument (600 MHz), and the chemical shifts were referenced to TMS. Gas chromatographic analysis was performed with a Hewlett Packard 5890 Series II gas chromatograph equipped with an H_2 flame ionization detector. The column was an HP-5 capillary column (30 m×0.32 mm×0.25 mm): column temperature, 200 °C; injector and detector temperature, 290 °C; and He flow rate, 30 ml/min. TLC was performed on silica gel 60 F_{254} (Merck) and cellulose plates (art. no. 5716, Merck).

Plant Material The roots of *A. membranaceus* were cultivated in Jungsun, Kangwon province, Korea, for three years, harvested in September 2004, and authenticated by Dr. J.-H. Lee (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 EtOAc fraction (143.8 g) was fractionated by column chromatography (CC) over silica gel with CH_2Cl_2/MeOH (gradient) to yield 51 subfractions (Fr. E-01—Fr. E-51). Fr. E-45 (20 g) was further purified on a silica gel column (EtOAc/MeOH/H_2O; 100 : 1 : 0.5→100 : 2 : 1) to yield 70 subfractions (Fr. E-45-01—Fr. E-45-70). Subfraction E-45-50 (4.5 g) was chromatographed on an RP-18 column with 80% MeOH to afford E-45-50-8 (3.2 g) and repeated silica gel CC ($CHCl_3$/MeOH/H_2O; 7 : 0.5 : 0.5) afforded astragaloside I (1.61 g) from E-45-50-8-25. Subfraction E-45-68 (1.5 g) was further purified on an RP-18 column with 80% MeOH to yield astragaloside II (350 mg) and isoastragaloside II (3 mg) from E-45-68-64. Fr. E-47 (3.8 g) was purified on an MCI gel column (MeOH) to yield subfraction E-47-12 (400 mg) and repeated RP-18 CC with 80% MeOH afforded agroastragaloside I (8 mg) from E-47-12-54. Subfraction E-47-12-38 (20 mg) was further purified on an RP-18 column with 70% MeOH to yield cycloastragenol 3-*O*-xyloside (5 mg) and cycloastragenol 3-*O*-glucoside (3 mg) from E-47-12-38-28. Fr. E-47-12-73 (30 mg) was further purified on a silica gel column with EtOAc and then EtOAc saturated with H_2O/MeOH (gradient) to yield cycloastragenol 6-*O*-glucoside (5 mg) from subfraction E-47-12-73-45. The BuOH-soluble fraction was fractionated by CC on silica gel (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-18 (2.87 g) was further purified on a silica gel column with EtOAc and then EtOAc saturated with H_2O/MeOH (gradient) to yield astragaloside II (21 mg) from subfraction B-18-69. Subfraction B-19 (1.5 g) was further purified on an RP-18 column with 80% MeOH to yield agroastragaloside II (10 mg) from subfraction B-19-14 and astragaloside III (35 mg) from subfraction B-19-21. Subfraction B-19-40 was further purified on a silica gel column with CH_2Cl_2/MeOH/H_2O (7 : 1 : 0.5) to afford azukisaponin V methyl ester (6 mg). Fr. B-20 (1.5 g) was rechromatographed on an RP-18 column with 80% MeOH to afford astragaloside IV (250 mg) from subfraction B-20-23. Fr. B-21-5 (80 mg) was purified on a silica gel column with EtOAc saturated with H_2O/MeOH (gradient) to yield cycloastragenol 6,25-di-*O*-glucoside (**1**, 4 mg), cyclocanthogenin 3-*O*-glucosyl(1→2)-xyloside (**2**, 15 mg), and cyclocanthoside E (10 mg). The known compounds were identified as astragaloside I, astragaloside II, isoastragaloside II, agroastragaloside I, cycloastragenol 3-*O*-xyloside, cycloaraloside A (=cycloastragenol 3-*O*-glucoside), brachyoside B (=cycloastragenol 6-*O*-glucoside), agroastragaloside II, astragaloside III, azukisaponin V methyl ester, astragaloside IV, and cyclocanthoside E after detailed NMR and MS analyses and comparison with the literature data.

Astramembranoside A (=Cycloastragenol 6,25-Di-*O*-*β*-D-Glucopyranoside, **1**): Amorphous white powder. $[\alpha]_D^{18}$ +23.5° (*c*=0.11, MeOH). (−)-HR-FAB-MS *m/z*: 813.4626. Calcd for $C_{42}H_{69}O_{15}$: 813.4636. FAB-MS *m/z*: 813 $[M-H]^-$. ^{1}H- and ^{13}C-NMR data (pyridine-d_5): Table 1.

Astramembranoside B (=Cyclocanthogenin 3-*O*-*β*-D-Glucopyranosyl(1→2)-*β*-D-xylopyranoside, **2**): Amorphous white powder. $[\alpha]_D^{25}$ +32.1° (*c*=0.15, MeOH). (−)-HR-FAB-MS *m/z*: 785.4666. Calcd for $C_{41}H_{69}O_{14}$: 785.4687. FAB-MS *m/z*: 785 $[M-H]^-$, 623 $[(M-H)-162]^-$, 491 $[(M-H)-162-132]^-$. ^{1}H- and ^{13}C-NMR data (pyridine-d_5): Table 1.

Acid Hydrolysis of 1 and 2 and Determination of the Absolute Configuration of Sugars[21,22] Saponins **1** and **2** (2 mg each) were refluxed separately with 5% HCl in 60% aqueous dioxane (10 ml) for 2 h. The reaction mixture was neutralized with Ag_2CO_3, filtered, and then concentrated to dryness *in vacuo* to give a residue. The residue was treated with L-cysteine methyl ester hydrochloride (1 mg) in pyridine (0.2 ml) at 60 °C for 1 h. The solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.05 ml) at 60 °C for 1 h. After the addition of *n*-hexane and water, the *n*-hexane layer was removed and checked using gas chromatography. D-Glucose (t_R 37.87 min) was identified from **1** and D-xylose (t_R 18.38 min) and D-glucose from **2**. The retention times (t_R) of the L-glucose and L-xylose were 39.90 and 40.65 min and 20.90 min, respectively.

Acknowledgments 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 Oriental Herbal Medicines (Project 8: Paeoniae Radix and Astragali Radix) by the Korea Food & Drug Administration. The authors are grateful to the staff members of the Korea Basic Science Institute for recording NMR and MS spectra.

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