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Phytochemical Studies on *Astragalus* Root (1) - Saponins

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Abstract – From the 70% EtOH extract of the roots of *Astragalus membranaceus* (Leguminosae), fifteen saponins were isolated and identified as astragaloside I (**1**), isoastragaloside II (**2**), astragaloside II (**3**), agroastragaloside I (**4**), cyclogaleginoside B (**5**), cycloaraloside A (**6**), brachyoside B (**7**), agroastragaloside II (**8**), astragaloside III (**9**), astragaloside IV (**10**), astramembranoside A (**11**), astramembranoside B (**12**), cylocanthoside E (**13**), cyclounifolioside B (**14**) and azukisaponin V methyl ester (**15**) by spectroscopic methods. Ten compounds **1** - **3**, **5** - **7**, **9** - **11** and **14** have cycloastragenol as an aglycon, and four compounds **4**, **8**, **12**, and **13** have cyclocanthogenin as an aglycon. The hairy roots of *A. membranaceus* were shown to produce previously unreported cycloartane-type saponins such as agroastragalosides I (**4**) and II (**8**) and cycloastragenol 3-*O*-β-D-xyloside (**5**), together with the known saponins. This is the first report of these saponins (**4**, **5**, and **8**) from the intact plant. Although the occurrence of the oleanane-type triterpene saponin, azukisaponin V methyl ester (**15**), in *Astragalus* plants has been demonstrated by others, this is the first report of the azukisaponin V methyl ester (**15**) from the *Astragalus* plants.

Key words – *Astragalus membranaceus*, Leguminosae, saponin

Introduction

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 (Evans, 2002; Mamedova and Isaev, 2004). Five species of this genera have been identified in Korea (Lee, 1989). 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 (Tang and Eisenbrand, 1992; Pistelli, 2002). The major active components of *A. membranaceus* are triterpene saponins, isoflavonoids and polysaccharides (Verotta and El-Sebakhy, 2001). As part of our efforts to isolate the chemical constituents of Astragali Radix to evaluate *A. membranaceus* quantitatively, we isolated a number of major and minor constituents from the roots of *A. membranaceus* cultivated in Jungsun, Kangwon Province, Korea. In the present investigation, we reported the isolation and identification of the structure of fifteen saponins.

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Experimental

General – The optical rotations were determined on a Jasco P-1020 polarimeter. The FAB mass spectrum was obtained on a VG-VSEQ spectrometer. The NMR spectra were measured in pyridine-d_5 on a Bruker Avance-600 (600 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) 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 EtOAc fraction (143.8 g) was fractionated by column chromatography over silica gel with CH_2Cl_2/MeOH (gradient) to yield 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 column chromatography (CH_2Cl_2/MeOH/H_2O = 14 : 1 : 1) afforded **1** (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 afford **2** (3 mg) and **3** (350 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 column chromatography with 80% MeOH to yield **4** (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 afford **5** (5 mg) and **6** (3 mg) from E-47-12-38-28. Subfraction 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 **7** (5 mg) from subfraction E-47-12-73-45. The BuOH soluble fraction was fractionated by silica gel column chromatography on silica gel (CH_2Cl_2/MeOH/H_2O = 7 : 1 : 0.5 → 7 : 2 : 05 → 7 : 3 : 1) to yield 39 fractions (Fr. B-01 - Fr. B-39). Fr. B-18 (2.87 g) was purified on silica gel column with EtOAc and then EtOAc saturated with H_2O/MeOH (gradient) to yield **3** (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 **8** (10 mg) from subfraction B-19-14 and **9** (35 mg) from subfraction B-19-21. Subfraction B-19-40 was purified on silica gel column with (CH_2Cl_2/MeOH/H_2O = 7 : 1 : 0.5) to afford **15** (6 mg). Subfraction B-20 (1.5 g) was rechromatographed on an RP-18 column with 80% MeOH to yield **10** (250 mg) from subfraction B-20-23. Subfraction B-21-5 (80 mg) was purified on silica gel column with EtOAc saturated with H_2O/MeOH (gradient) to yield **11** (4 mg), **12** (15 mg), and **13** (10 mg). Subfraction B-24 (7 g) was purified on silica gel column with EtOAc saturated with H_2O/MeOH (gradient) to yield subfraction B-24-58 (685 mg), which was further purified on silica gel column with (CH_2Cl_2/MeOH/H_2O = 7 : 1 : 0.5) to afford B-24-58-53 (13 mg). Subfraction B-24-58-53 (13 mg) was rechromatographed on an RP-18 column with 80% MeOH to yield **14** (2 mg) and **13** (8 mg).

Astragaloside I (1) – Amorphous white powder. $[\alpha]_D^{25}$ + 15.3° (c, 0.11 in MeOH). ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 1.40 (3H, s, 18-CH_3), 1.28 (6H, s, 21, 27-CH_3), 1.57 (3H, s, 26-CH_3), 1.77 (3H, s, 28-CH_3), 1.24 (3H, s, 29-CH_3), 0.91 (3H, s, 30-CH_3), 0.18 (1H, d, J = 4.5 Hz, H-19a), 0.54 (1H, d, J = 3.9 Hz, H-19b), 1.86 (1H, d, J = 9.0 Hz, H-5α), 3.37 (1H, dd, J = 4.2, 11.1 Hz, H-3α), 3.76 (1H, ddd, J = 3.6, 8.4, 8.4 Hz, H-6β), 5.00 (1H, overlap, H-16α), 2.50 (1H, d, J = 8.1 Hz, H-17α), 3.12 (1H, dd, J = 10.5, 20.4 Hz, H-22a), 3.86 (1H, dd, J = 5.4, 8.7 Hz, H-24), 4.80 (1H, d, J = 7.8 Hz, H-1'), 4.91 (1H, d, J = 7.8 Hz, H-1"), 1.94, 2.02 (3H each, s, OAc); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 1; FAB-MS *m/z* 867 $[M-H]^-$, 825 $[(M-H)-42]^-$.

Isoastragaloside II (2) – Amorphous white powder. ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 1.41 (3H, s, 18-CH_3), 1.28 (6H, s, 21, 27-CH_3), 1.57 (3H, s, 26-CH_3), 1.97 (6H, s, 28-CH_3, OAc), 1.31 (3H, s, 29-CH_3), 0.93 (3H, s, 30-CH_3), 0.20 (1H, d, J = 4.5 Hz, H-19a), 0.58 (1H, d, J = 3.9 Hz, H-19b), 1.88 (1H, d, J = 9.0 Hz, H-5), 3.48 (1H, dd, J = 3.6, 11.4 Hz, H-3), 3.77 (1H, ddd, J = 4.2, 8.2, 8.2 Hz, H-6), 4.94 (1H, overlap, H-16), 2.52 (1H, d, J = 7.8 Hz, H-17), 3.13 (1H, dd, J = 10.8, 21.0 Hz, H-22a), 3.87 (1H, dd, J = 5.4, 8.7 Hz, H-24), 4.83 (1H, d, J = 7.5 Hz, H-1'), 4.89 (1H, d, J = 7.8 Hz, H-1"); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 1.

Astragaloside II (3) – Amorphous white powder. $[\alpha]_D^{25}$ + 32.5° (c, 0.13 in MeOH). ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 1.40 (3H, s, 18-CH_3), 1.28 (9H, s, 21, 27, 29-CH_3), 1.57 (3H, s, 26-CH_3), 1.82 (3H, s, 28-CH_3), 0.92 (3H, s, 30-CH_3), 0.17 (1H, d, J = 4.2 Hz, H-19a), 0.54 (1H, d, J = 3.6 Hz, H-19b), 1.88 (1H, d, J = 9.0 Hz, H-5α), 3.40 (1H, dd, J = 4.2, 11.4 Hz, H-3α), 3.77 (1H, ddd, J = 3.9, 8.7, 8.7 Hz, H-6β), 4.96 (1H, overlap, H-16α), 2.51 (1H, d, J = 8.1 Hz, H-17α), 3.12 (1H, dd, J = 10.8, 20.4 Hz, H-22a), 3.87 (1H, dd, J = 5.4, 9.0 Hz, H-24), 4.79 (1H, d, J = 7.8 Hz, H-1'), 4.92 (1H, d, J = 7.5 Hz, H-1"), 2.02 (3H, s, OAc); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 1; FAB-MS *m/z* 825 $[M-H]^-$, 783 $[(M-H)-42]^-$, 489 $[(M-H)-42-132-162]^-$.

Agroastragaloside I (4) – Amorphous white powder. $[\alpha]_D^{23}$ + 17.6° (c, 0.11 in MeOH). ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 1.38 (3H, s, 18-CH_3), 1.45 (3H, s, 27-CH_3), 1.47 (3H, s, 26-CH_3), 1.76 (3H, s, 28-CH_3), 1.24 (3H, s, 29-CH_3), 0.95 (3H, s, 30-CH_3), 1.07 (3H, d, J = 6.6 Hz, 21-CH_3), 0.17 (1H, d, J = 4.5 Hz, H-19a), 0.52 (1H, d, J = 3.9 Hz, H-19b), 1.94, 2.02 (3H each, s, OAc), 3.38 (1H, dd, J = 4.5, 11.7 Hz, H-3), 1.89 (1H, d, J = 9.0 Hz, H-5), 3.77 (1H, ddd, J = 3.6, 7.8, 7.8 Hz, H-6), 4.69 (1H, dd, J = 6.9, 11.7 Hz, H-16), 3.94 (1H, dd, J = 1.2, 10.8 Hz, H-24), 4.79 (1H, d, J = 7.5 Hz, H-1'), 4.93 (1H, d, J = 7.8 Hz, H-1"); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 4; FAB-MS *m/z* 1023 $[M-NBA]^-$, 869 $[M-H]^-$, 809 $[(M-H)-HOAc]^-$; *m/z* 961 $[(M-H)-Glycerol]^-$, 869 $[M-H]^-$, 827 $[(M-H)-42]^-$, 809 $[(M-H)-HOAc]^-$.

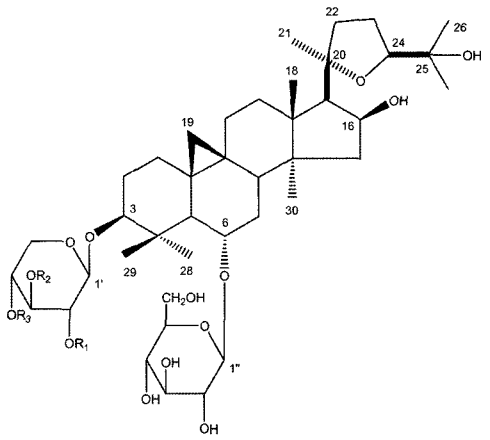
Table 1. ^{13}C-NMR spectral data of **1**, **2**, **3**, and **10**

Carbon No.	**1**	**2**	**3**	**10**	Carbon No.	**1**	**2**	**3**	**10**
1	32.0	32.1	32.0	32.2	1'	104.0	107.3	104.7	107.7
2	29.8	30.1	29.9	30.2	2'	73.1	73.1	75.6	75.6
3	89.2	88.7	88.9	88.5	3'	76.8	79.2	76.3	78.1
4	42.2	42.6	42.3	42.6	4'	68.8	69.2	71.3	71.8
5	52.4	52.4	52.5	52.5	5'	66.7	66.7	67.1	67.0
6	79.3	79.3	79.3	79.3	1"	105.2	105.3	105.2	105.2
7	34.9	34.6	34.9	34.6	2"	75.6	75.6	75.6	75.6
8	45.9	46.2	46.1	45.7	3"	79.2	79.3	79.3	79.2
9	21.2	21.1	21.2	21.1	4"	71.8	71.8	71.9	71.3
10	28.9	28.9	28.9	29.0	5"	78.2	78.2	78.2	78.5
11	26.1	26.1	26.1	26.2	6"	63.1	63.1	63.1	63.1
12	33.3	33.3	33.5	33.4	$CO\underline{C}H_3$	20.8	21.2	21.2	
13	45.0	45.0	45.8	45.0		20.9			
14	46.2	46.2	46.2	46.2	$\underline{C}OCH_3$	169.9	170.8	170.1	
15	46.3	45.7	45.8	46.2		170.5			
16	73.4	73.3	73.4	73.4					
17	58.2	58.2	58.2	58.2					
18	21.2	21.2	21.2	21.1					
19	29.1	28.8	29.2	28.8					
20	87.2	87.2	87.2	87.2					
21	27.1	27.1	27.1	27.1					
22	34.9	34.9	35.0	34.9					
23	26.5	26.5	26.5	26.4					
24	81.7	81.6	81.7	81.7					
25	71.2	71.2	71.3	71.3					
26	28.2	28.2	28.2	28.2					
27	28.3	28.6	28.3	28.6					
28	28.6	28.6	28.6	28.6					
29	16.5	16.6	16.5	16.6					
30	19.9	19.8	19.9	19.8					

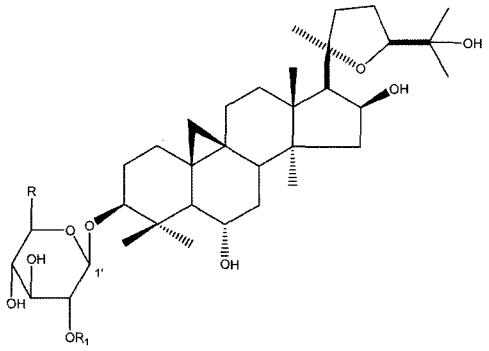
Cyclogaleginoside B (cycloastragenol 3-*O*-β-D-xyloside, 5) – Amorphous white powder. ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 1.41 (3H, s, 18-CH_3), 1.28 (3H, s, 21-CH_3), 1.57 (3H, s, 26-CH_3), 1.30 (3H, s, 27-CH_3), 2.00 (3H, s, 28-CH_3), 1.33 (3H, s, 29-CH_3), 0.99 (3H, s, 30-CH_3), 0.27 (1H, d, J = 3.9 Hz, H-19a), 0.56 (1H, d, J = 3.9 Hz, H-19b), 3.63 (1H, dd, J = 4.2, 11.7 Hz, H-3), 3.73 (1H, overlap, H-6), 5.01 (1H, overlap, H-16), 2.53 (1H, d, J = 7.8 Hz, H-17), 3.10 (1H, dd, J = 10.2, 20.1 Hz, H-22a), 3.87 (1H, dd, J = 5.4, 8.7 Hz, H-24), 4.92 (1H, d, J = 7.5 Hz, H-1'); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 2.

Cycloaraloside A (astraverrucin I, huangqiyie saponin C, cycloastragenol 3-*O*-β-D-glucoside, 6) – Amorphous white powder. $[\alpha]_D^{25}$ + 22.7° (c, 0.13 in MeOH). ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 1.41 (3H, s, 18-CH_3), 1.28 (3H, s, 21-CH_3), 1.57 (3H, s, 26-CH_3), 1.30 (3H, s, 27-CH_3), 2.01 (3H, s, 28-CH_3), 1.34 (3H, s, 29-CH_3), 0.99 (3H, s, 30-CH_3), 0.21 (1H, d, J = 3.9 Hz, H-19a), 0.54 (1H, d, J = 3.9 Hz, H-19b), 3.65 (1H, dd, J = 4.8, 12.3 Hz, H-3), 3.74 (1H, ddd, J = 4.8, 9.0, 9.0 Hz, H-6), 4.95 (1H, overlap, H-16), 2.53 (1H, d, J = 7.8 Hz, H-17), 3.10 (1H, dd, J = 11.4, 20.4 Hz, H-22a), 3.87 (1H, dd, J = 5.4, 9.0 Hz, H-24), 5.01 (1H, d, J = 8.1 Hz, H-1'); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 2; FAB-MS *m/z* 651 $[M-H]^-$, 489 $[(M-H)-162]^-$.

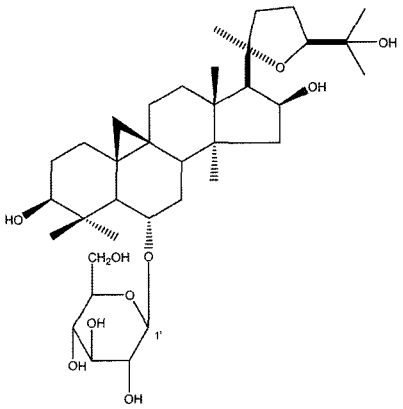
Brachyoside B (cycloastragenol 6-*O*-β-D-glucopyranoside, 7) – Amorphous white powder. $[\alpha]_D^{23}$ + 35.8° (c, 0.10 in MeOH). ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 1.41 (3H, s, 18-CH_3), 1.29 (3H, s, 21-CH_3), 1.57 (3H, s, 26-CH_3), 1.43 (3H, s, 27-CH_3), 1.96 (3H, s, 28-CH_3), 1.30 (3H, s, 29-CH_3), 0.92 (3H, s, 30-CH_3), 0.27 (1H, d,



1 R_1= R_2= Ac R_3= H
2 R_1= R_3= H R_2= Ac
3 R_1= Ac R_2= R_3= H
10 R_1= R_2= R_3= H



5 R= R_1= H
6 R= CH_2OH R_1= H
9 R_1= Glc R= H
14 R= CH_2OH R_1= Glc



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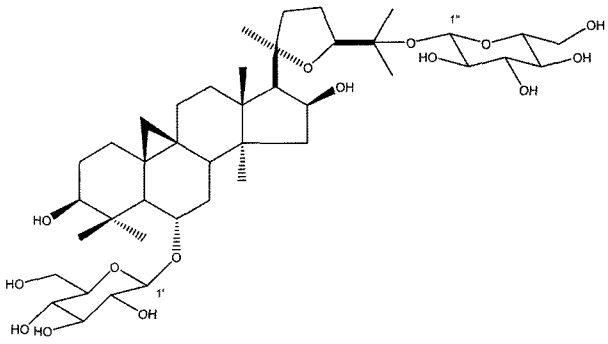

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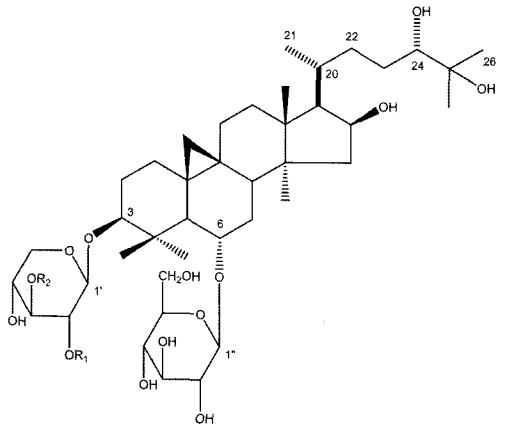
J=4.2 Hz, H-19a), 0.63 (1H, d, J=3.9 Hz, H-19b), 1.88 (1H, d, J=9.0 Hz, H-5), 3.60 (1H, dd, J=4.5, 11.1 Hz, H-3), 3.84 (1H, ddd, J=3.0, 8.7, 8.7 Hz, H-6), 5.00 (1H, overlap, H-16), 2.51 (1H, d, J=8.1 Hz, H-17), 3.13 (1H, dd, J=11.4, 20.7 Hz, H-22a), 3.87 (1H, dd, J=5.4, 9.3 Hz, H-24), 4.94 (1H, d, J=7.8 Hz, H-1'); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 3; FAB-MS *m/z* 805 [M – NBA]$^-$, 651 [M – H]$^-$, 489 [(M – H) – 162]$^-$.

Agroastragaloside II (8) – Amorphous white powder. $[\alpha]_D^{25}$ + 40.4° (c, 0.15 in MeOH). ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 1.36 (3H, s, 18-CH_3), 1.44 (3H, s, 27-CH_3), 1.46 (3H, s, 26-CH_3), 1.78 (3H, s, 28-CH_3), 1.25 (3H, s, 29-CH_3), 0.95 (3H, s, 30-CH_3), 1.05 (3H, d, J=6.3 Hz, 21-CH_3), 0.15 (1H, d, J=4.2 Hz, H-19a), 0.50 (1H, d, J=3.9 Hz, H-19b), 2.02 (3H, s, OAc), 3.38 (1H, dd, J=4.2, 11.4 Hz, H-3), 1.88 (1H, d, J=8.7 Hz, H-5), 3.75 (1H, ddd, J=3.6, 7.8, 7.8 Hz, H-6), 4.67 (1H, dd, J=6.9, 11.7 Hz, H-16), 3.92 (1H, brd, J=9.9 Hz, H-24), 4.75 (1H, d, J=7.8 Hz, H-1'), 4.90 (1H, d, J=8.1 Hz, H-1"); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 4; FAB-MS *m/z* 827 [M – H]$^-$, 785 [(M – H) – 42]$^-$, 767 [(M – H) – HOAc]$^-$, 605 [(M – H) – HOAc – 162]$^-$.

Astragaloside III (9) – Amorphous white powder. $[\alpha]_D^{25}$ + 23.7° (c, 0.13 in MeOH). ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 1.41 (6H, s, 18, 27-CH_3), 1.29 (3H, s, 21-CH_3), 1.28 (3H, s, 26-CH_3), 1.91 (3H, s, 28-CH_3), 1.55 (3H, s, 29-CH_3), 0.88 (3H, s, 30-CH_3), 0.26 (1H, d, J=4.2 Hz, H-19a), 0.54 (1H, d, J=3.6 Hz, H-19b), 3.52 (1H, dd, J=4.2, 11.7 Hz, H-3α), 3.70 (1H, ddd, J=3.6, 8.4, 8.4 Hz, H-6β), 5.06 (1H, overlap, H-16α), 2.51 (1H, d, J=7.8 Hz, H-17α), 3.07 (1H, dd, J=10.8, 20.1 Hz, H-22a), 3.86 (1H, dd, J=5.4, 8.4 Hz, H-24), 4.88 (1H, d, J=6.9 Hz, H-1'), 5.38 (1H, d, J=7.8 Hz, H-1"); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 2; FAB-MS *m/z* 783 [M – H]$^-$, 621 [(M – H) – 162]$^-$, 489 [(M – H) – 162 – 132]$^-$.

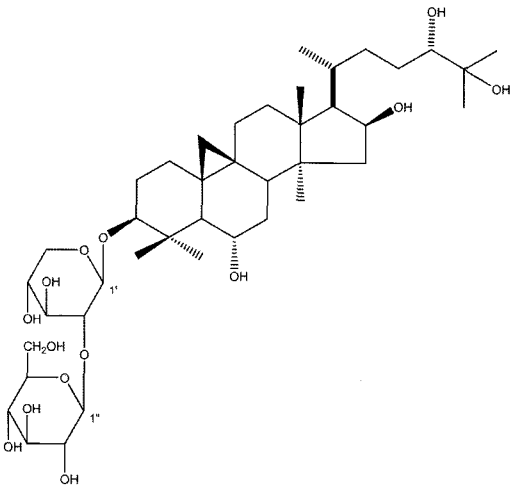
Astragaloside IV (10) – Amorphous white powder. $[\alpha]_D^{25}$ + 22.7° (c, 0.11 in MeOH). ^{1}H-NMR (300 MHz,



4 $R_1 = R_2 = Ac$

8 $R_1 = Ac$ $R_2 = H$

13 $R_1 = R_2 = H$



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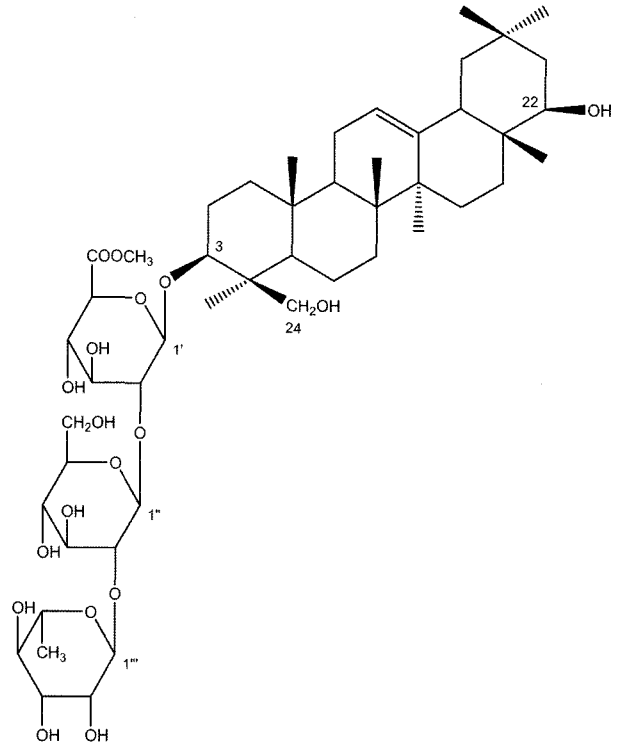
pyridine-d_5) δ: 1.40 (3H, s, 18-CH_3), 1.29 (6H, s, 21, 27-CH_3), 1.57 (3H, s, 26-CH_3), 2.02 (3H, s, 28-CH_3), 1.36 (3H, s, 29-CH_3), 0.93 (3H, s, 30-CH_3), 0.19 (1H, d, J = 4.2 Hz, H-19a), 0.58 (1H, d, J = 3.9 Hz, H-19b), 1.90 (1H, d, J = 9.0 Hz, H-5α), 3.51 (1H, dd, J = 4.2, 11.7 Hz, H-3α), 3.78 (1H, ddd, J = 3.3, 7.8, 7.8 Hz, H-6β), 4.94 (1H, overlap, H-16α), 2.51 (1H, d, J = 7.5 Hz, H-17α), 3.12 (1H, dd, J = 11.0, 20.4 Hz, H-22a), 3.87 (1H, dd, J = 5.1, 8.7 Hz, H-24), 4.84 (1H, d, J = 7.5 Hz, H-1'), 4.90 (1H, d, J = 8.1 Hz, H-1"); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 1; FAB-MS *m/z* 783 $[M - H]^-$, 651 $[(M - H) - 132]^-$, 621 $[(M - H) - 162]^-$.

Astramembranoside A (11) – Amorphous white powder. $[\alpha]_D^{18}$ +23.5° (c, 0.11 in MeOH). ^{1}H-NMR (600 MHz, pyridine-d_5) δ: 1.38 (3H, s, 18-CH_3), 1.29 (3H, s, 21-CH_3), 1.43 (6H, s, 26, 29-CH_3), 1.67 (3H, s, 27-CH_3), 1.98 (3H, s, 28-CH_3), 0.92 (3H, s, 30-CH_3), 0.27 (1H, d, J = 3.7 Hz, H-19a), 0.64 (1H, d, J = 3.7 Hz, H-19b), 1.73



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(1H, dd, J = 6.5, 12.4 Hz, H-15a), 1.92 (1H, d, J = 9.2 Hz, H-5α), 2.27 (1H, dd, J = 7.2, 12.4 Hz, H-15b), 3.63 (1H, dd, J = 4.6, 10.8 Hz, H-3α), 3.90-3.92 (1H, overlap, H-6β), 4.89 (1H, m, H-16α), 2.44 (1H, d, J = 7.8 Hz, H-17α), 2.82 (1H, dd, J = 11.4, 20.2 Hz, H-22a), 4.44 (1H, brd, J = 12.4 Hz, H-6"), 4.49 (1H, brd, J = 11.6 Hz, H-6'), 4.85 (1H, s, 16-OH), 4.96 (1H, d, J = 7.9 Hz, H-1'), 5.09 (1H, d, J = 7.6 Hz, H-1"), 5.80 (1H, d, J = 5.3 Hz, 3-OH); ^{13}C-NMR (125.8 MHz, pyridine-d_5) δ: see Table 3; FAB-MS *m/z* 813 $[M - H]^-$.

Astramembranoside B (12) – Amorphous white powder. $[\alpha]_D^{25}$ +32.1° (c, 0.15 in MeOH). ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 1.05 (3H, s, 30-CH_3), 1.10 (3H, d, J = 6.5 Hz, 21-CH_3), 1.41 (3H, s, 18-CH_3), 1.43 (3H, s, 29-CH_3), 1.47 (3H, s, 26-CH_3), 1.49 (3H, s, 27-CH_3), 1.95 (3H, s, 28-CH_3), 0.28 (1H, d, J = 4.0 Hz, H-19a), 0.58 (1H, d, J = 3.5 Hz, H-19b), 3.58 (1H, dd, J = 4.5, 12.0 Hz, H-3), 1.75 (1H, d, J = 9.0 Hz, H-5), 2.18 (1H, dd, J = 7.5, 12.5 Hz, H-15), 3.77 (1H, ddd, J = 4.5, 9.0, 9.0 Hz, H-6), 4.74 (1H, m, H-16), 3.98 (1H, dt, J = 3.5, 9.5 Hz, H-24), 4.93 (1H, d, J = 6.5 Hz, H-1'), 5.42 (1H, d, J = 8.0 Hz, H-1"); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 4; FAB-MS *m/z* 785 $[M - H]^-$, 623 $[(M - H) - 162]^-$, 491 $[(M - H) - 162 - 132]^-$.

Cyclocanthoside E (13) – Amorphous white powder. $[\alpha]_D^{21}$ + 1.1° (c, 0.5 in pyridine). ^{1}H-NMR (300 MHz,

Table 2. ^{13}C-NMR spectral data of **5**, **6**, **9**, and **14**

Carbon No.	5	6	9	14	Carbon No.	5	6	9	14
1	32.4	32.3	32.4	32.3	1'	107.7	107.0	105.6	105.1
2	30.3	30.5	30.3	30.3	2'	75.6	76.0	83.2	83.6
3	88.7	89.0	88.6	88.9	3'	78.6	78.7	77.8	78.0
4	42.7	42.6	42.7	42.7	4'	71.2	71.8	70.9	71.6
5	54.0	54.0	53.9	54.0	5'	67.1	78.2	66.6	78.1
6	68.0	68.0	67.8	67.8	6'		63.0		62.9
7	38.6	38.6	38.5	38.5	1"			106.0	106.2
8	47.2	47.0	46.8	46.8	2"			76.9	77.1
9	21.0	20.9	21.0	21.0	3"			78.0	78.1
10	29.5	29.5	29.3	29.4	4"			71.7	71.8
11	26.2	26.4	26.2	26.3	5"			78.2	78.2
12	33.3	33.4	33.3	33.4	6"			62.7	62.9
13	45.0	45.0	45.0	45.1					
14	46.1	46.1	46.1	46.2					
15	46.6	46.6	46.5	46.6					
16	73.4	73.4	73.4	73.5					
17	58.3	58.3	58.3	58.4					
18	21.5	21.5	21.4	21.4					
19	30.6	30.2	30.3	30.1					
20	87.2	87.2	87.2	87.3					
21	27.1	27.1	27.1	27.2					
22	34.9	34.9	34.9	34.9					
23	26.4	26.2	26.4	26.5					
24	81.6	81.7	81.6	81.7					
25	71.2	71.2	71.2	71.3					
26	28.2	28.2	28.1	28.2					
27	28.5	28.5	28.5	28.6					
28	28.9	29.0	28.7	28.9					
29	16.6	16.7	16.5	16.6					
30	20.1	20.1	20.1	20.1					

pyridine-d_5) δ: 1.39 (3H, s, 18-CH_3), 1.07 (3H, d, J = 6.6 Hz, 21-CH_3), 1.47 (3H, s, 26-CH_3), 1.45 (3H, s, 27-CH_3), 2.01 (3H, s, 28-CH_3), 1.35 (3H, s, 29-CH_3), 0.97 (3H, s, 30-CH_3), 0.18 (1H, d, J = 4.8 Hz, H-19a), 0.57 (1H, d, J = 3.6 Hz, H-19b), 1.93 (1H, d, J = 8.4 Hz, H-5α), 3.52 (1H, dd, J = 4.2, 11.7 Hz, H-3α), 3.80 (1H, m, H-6β), 4.70 (1H, m, H-16α), 3.90 (1H, overlap, H-24), 4.83 (1H, d, J = 7.5 Hz, H-1'), 4.90 (1H, d, J = 7.2 Hz, H-1"); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 4; FAB-MS *m/z* 940 $[M + NBA + H]^-$, 786 $[M]^-$.

Cyclounifolioside B (14) – Amorphous white powder. $[\alpha]_D^{21}$ + 74.0° (c, 0.1 in pyridine). ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 1.42 (3H, s, 18-CH_3), 1.29 (3H, s, 21-CH_3), 1.57 (3H, s, 26-CH_3), 1.31 (3H, s, 27-CH_3), 1.97 (3H, s, 28-CH_3), 1.31 (3H, s, 29-CH_3), 1.01 (3H, s, 30-CH_3), 0.20 (1H, d, J = 4.2 Hz, H-19a), 0.55 (1H, d, J = 3.9 Hz, H-19b), 2.54 (1H, d, J = 7.8 Hz, H-17α), 3.11 (1H, dd, J = 10.5, 20.1 Hz, H-22a), 3.57 (1H, dd, J = 4.8, 12.0 Hz, H-3α), 3.74 (1H, m, H-6β), 3.88 (1H, dd, J = 5.1, 9.0 Hz, H-24), 4.99 (1H, d, J = 6.9 Hz, H-1'), 5.42 (1H, d, J = 7.8 Hz, H-1"); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 2; FAB-MS *m/z* 968 $[M + NBA + H]^-$, 814 $[M]^-$, 652 $[M - 162]^-$.

Azukisaponin V methyl ester (15) – Amorphous white powder. $[\alpha]_D^{21}$ −13.5° (c, 0.4 in pyridine). ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 0.69, 0.94, 0.98, 1.21, 1.24, 1.28, 1.47 (3H each, s, 7 × CH_3), 1.77 (3H, d, J = 6.0 Hz, Rha-CH_3), 2.39 (1H, brd, J = 11.4 Hz, H-18), 3.31 (1H, brd, J = 11.4 Hz, H-24a), 3.38 (1H, dd, J = 4.5, 11.4 Hz, H-3), 3.74 (1H, m, H-22), 3.75 (3H, s, $COOCH_3$), 4.96

Table 3. ^{13}C-NMR spectral data of **7**, **11**, and **15**

Carbon No.	**7**	**11***	**15**	Carbon No.	**7**	**11**	**15**
1	32.6	32.6	38.5	1'	105.2	105.0	105.3
2	29.6	31.3	26.6	2'	75.6	75.6	78.3
3	78.2	78.3	91.7	3'	79.2	79.4	78.5
4	42.4	42.5	43.7	4'	71.8	71.9	73.5
5	52.6	52.5	56.3	5'	78.2	78.2	76.7
6	79.9	79.6	18.6	6'	63.1	63.0	170.4
7	34.9	34.5	33.3	1"		99.0	102.1
8	46.4	46.0	40.0	2"		75.2	77.7
9	21.0	21.1	47.8	3"		78.6	79.2
10	29.5	29.5	36.5	4"		71.4	69.7
11	26.2	26.3	24.1	5"		78.1	78.1
12	33.4	33.5	122.4	6"		62.8	61.4
13	45.0	45.3	144.9	1'''			102.5
14	46.2	46.2	42.4	2'''			72.3
15	46.2	45.8	26.5	3'''			72.8
16	73.4	73.6	28.7	4'''			74.4
17	58.3	58.1	38.0	5'''			69.5
18	21.3	21.3	45.3	6'''			19.0
19	31.3	29.4	46.8	$COOCH_3$			52.1
20	87.2	87.2	30.9				
21	28.6	27.8	42.3				
22	35.0	35.1	75.6				
23	26.5	26.1	22.9				
24	81.7	82.1	63.4				
25	71.3	78.7	15.6				
26	27.1	22.9	17.0				
27	28.2	25.7	25.7				
28	29.1	29.1	28.7				
29	16.2	16.1	33.1				
30	19.9	19.9	21.2				

*125.8 MHz

(1H, d, J = 8.1 Hz, H-1'), 5.29 (1H, brs, H-12), 5.87 (1H, d, J = 7.5 Hz, H-1"), 6.42 (1H, s, H-1'''); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: see Table 3; FAB-MS *m/z* 956 $[M]^-$, 810 $[M - 146]^-$.

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 EtOAc and BuOH soluble extracts were subjected to sequential column chromatography over silica gel, MCI gel, and RP-18 gel to yield fifteen saponins. The well-known cycloartane triterpenoid saponins from *Astragalus* plants such as astragalosides I (**1**), II (**3**), III (**9**), and IV (**10**) as the major components as well as a minor saponin, isoastragaloside II (**2**), were identified based on detailed NMR and MS analyses and direct comparison with the authentic samples (Kitagawa, *et al.*, 1983b,c; Hirotani, *et al.*, 1994a,b; Hirotani, 1999). Comparison of the ^{13}C-NMR data of glycoside moieties for **5** and **6** with the methyl glycosides (Agrawal, 1992) showed the presence of characteristic signals for β-D-xyloside and β-D-glucoside, respectively, as shown in Table 2. Therefore compounds **5** and **6** were readily identified as cyclo-galeginoside B (Hirotani, *et al.*, 1994b) and cycloaraloside A (Isaev, *et al.*, 1989), respectively. The ^{13}C-NMR resonances of **14** arising from the rings of the sapogenol

Table 4. ^{13}C-NMR spectral data of **4**, **8**, **12**, and **13**

Carbon No.	**4**	**8**	**12**	**13**	Carbon No.	**4**	**8**	**12**	**13**
1	32.0	32.0	32.5	32.2	1'	104.0	104.7	105.7	107.7
2	29.8	29.9	30.4	30.2	2'	73.0	75.6	83.4	75.6
3	89.2	88.9	88.6	88.5	3'	76.8	76.2	77.9	78.1
4	42.2	42.2	42.8	42.6	4'	68.8	71.3	71.0	71.8
5	52.4	52.4	54.0	52.5	5'	66.8	67.0	66.7	67.1
6	79.2	79.1	67.7	79.2	1"	105.2	105.1	106.2	105.2
7	34.5	34.4	38.3	34.3	2"	75.6	75.6	77.1	75.6
8	45.8	45.7	46.7	45.5	3"	79.2	79.0	78.3	79.1
9	21.4	21.4	21.4	21.4	4"	71.8	71.8	71.7	71.2
10	28.6	28.6	29.1	28.7	5"	78.2	78.1	78.0	78.5
11	26.2	26.1	26.3	26.2	6"	63.1	63.0	62.8	63.1
12	33.1	33.1	33.2	33.1	$COCH_3$	169.9	170.1		
13	45.7	45.7	45.7	46.2		170.5	21.2		
14	46.8	46.8	46.8	46.9		20.7			
15	47.9	47.8	48.3	47.8		20.8			
16	71.9	71.9	72.0	71.9					
17	57.2	57.1	57.3	57.1					
18	18.6	18.5	18.8	18.4					
19	28.5	28.3	29.6	28.5					
20	28.5	28.6	28.6	28.7					
21	18.3	18.3	18.3	18.3					
22	32.9	32.9	33.0	32.9					
23	27.8	27.8	27.9	27.8					
24	77.0	77.1	77.2	77.0					
25	72.5	72.5	72.5	72.5					
26	25.8	25.8	25.8	25.8					
27	26.4	26.3	26.5	26.4					
28	28.4	28.2	28.8	28.1					
29	16.5	16.5	16.6	16.6					
30	19.8	19.8	20.1	19.8					

and sugar moieties were very close to those of astragaloside III (**9**) except for the signals assigned to the inner glycoside moiety. A linkage of the diglycoside of glucosyl(1 → 2)-glucosyl to C-3 of cycloastragenol as for **9** was determined by ^{13}C-NMR data (Agrawal, 1992). The structure of **14** was determined to be cyclounifolioside B (Kucherbaev, *et al.*, 2002). Compound **7** showed a molecular ion peak at *m/z* 651 $[M - H]^-$ in the (–)-FAB mass spectrum. The NMR spectra displayed a general pattern very similar to those of **6**. The major difference was the downfield shift of the oxygenated methine proton signal at C-6 by 0.1 ppm which indicated the existence of one sugar unit at C-6. This result was further supported by the downfield shift of carbon chemical shift of C-6. The structure of **7** was thus determined to be brachyoside B (Bedir, *et al.*, 1998). Compound **11** exhibited a molecular ion peak at *m/z* 813 $[M - H]^-$ in the (–)-FAB mass spectrum. A comparison of spectroscopic data with those of **7** indicated that the two compounds are very similar except for the presence of an additional glucopyranose moiety and the attachment of the glucose moiety at C-25. This was obvious from the anomeric carbon resonance attributed to the second glucose unit at C-25 which was found to be shifted upfield at δ 99.0 (Tori, *et al.*, 1977; Kasai, *et al.*, 1977). Consequently, the structure of **11** was established as cycloastragenol 6,25-di-*O*-β-D-glucoside (**11**), which has been recently isolated from this plant and named as astramembranoside A (Kim, *et al.*, 2008). The ^{13}C-NMR resonances arising from the rings of the sapogenol of the remaining four saponins **4**, **8**, **12**, and **13**

were very close to those of cycloastragenol (Kitagawa, *et al.*, 1983a; Wang, *et al.*, 1989), except for the signals assigned to the side chain moiety. These results indicated that the side chain structure of these saponins appeared to have an acyclic side chain. Therefore, instead of the epoxide ring seen in cycloastragenol, there was a hydroxyl group at C-24. The ^{13}C-NMR chemical shift for C-24 is comparable to those reported for analogous compounds having a 24*S* configuration (Hirotani, *et al.*, 1994a,b; Bedir, *et al.*, 2000). 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 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 (Bedir, *et al.*, 2000). All these observations support the presence of cyclocanthogenin as the aglycon moiety (Fadeev, *et al.*, 1988). The ^{13}C-NMR data of rings A and B as well as sugar moieties of **4**, **8** and **13** were superimposable to those of astragaloside I (**1**), II (**3**) and IV (**10**), respectively, as shown in Tables 1 and 4. Therefore, the structures of compounds **4**, **8** and **13** were identified as agroastragaloside I (Hirotani, *et al.*, 1994a; Hirotani, 1999), agroastragaloside II (Hirotani, *et al.*, 1994b; Hirotani, 1999) and cyclocanthoside E (Isaev, *et al.*, 1992), respectively. In a similar manner, the ^{13}C-NMR data of rings A and B as well as sugar moieties of **12** were identical to those of astragaloside III (**9**), as shown in Tables 2 and 4. Thus, the structure of compound **12** was determined as 3-*O*-β-D-xylopyranosyl(1 → 2)-β-D-glucopyranosyl-(24*S*)-3β,6α,16β,24,25-pentahydroxy-9,19-cyclolanostane, which has been recently isolated from this plant and named as astramembranoside B (Kim, *et al.*, 2008). The hairy roots of *A. membranaceus* were shown to produce previously unreported cycloartane-type saponins such as agroastragalosides I (**4**) and II (**8**) and cycloastragenol 3-*O*-β-D-xyloside (**5**), together with the known saponins (Hirotani, *et al.*, 1994a,b; Hirotani, 1999). This is the first report of these saponins (**4**, **5**, and **8**) from the intact plant. Compound **15** was readily shown to be an olean-12-ene triterpenoid saponin by the characteristic ^{1}H-NMR data. Diagnostic features in the ^{1}H-NMR spectrum of **15** were the presence of 7 angular methyl singlet signals, an olefinic proton at δ 5.29 and three anomeric proton signals at δ 4.96 (d, J = 8.1 Hz), 5.87 (d, J = 7.5 Hz), and 6.42 (s) with rhamnose methyl doublet at δ 1.77 (3H, d, J = 6.0 Hz), reminiscent of the well-known leguminous soyasaponins (Kang, *et al.*, 1988; Kang, *et al.*, 1998; Byun, *et al.*, 2004). The FAB-MS fragment ions at *m/z* 956 $[M]^-$ and 810 $[M - 146]^-$ in negative ion mode suggested that the sugar moiety of **15** was linear chain, glucuronic acid methyl ester-hexose-rhamnose. By comparing the ^{13}C-NMR spectroscopic data of the sugar moiety of **15** with those in the literature (Kang, *et al.*, 1988), it was found that the ^{13}C-NMR data of sugars of **15** was identical to those of the sugar moiety of α-L-rhamnopyranosyl(1 → 2)-β-D-glucopyranosyl(1 → 2)-β-D-6'-methylglucuronopyranoside. Therefore, compound **15** was established as azukisaponin V methyl ester. Although the occurrence of the oleanane-type triterpene saponin, azukisaponin V, in *Astragalus* plants has been demonstrated by others (Pelizzoni, *et al.*, 1996; Gromova, *et al.*, 2001; Avunduk, *et al.*, 2008), this is the first report of the azukisaponin V methyl ester from the *Astragalus* species.

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