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Saponin and Sapogenol. XXXVI.[1] Chemical Constituents of Astragali Radix, the Root of *Astragalus membranaceus* BUNGE. (3). Astragalosides III, V, and VI

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Based on chemical and physicochemical investigations including carbon-13 nuclear magnetic resonance (^{13}C-NMR) examinations and methylation analyses, the structures of three astragalosides, which were isolated from Astragali Radix, the root of Korean *Astragalus membranaceus* BUNGE (Leguminosae), were elucidated: astragaloside III is 3-*O*-[*β*-D-glucopyranosyl (1→2)-*β*-D-xylopyranosyl]cycloastragenol (**3**), astragaloside V is 3-*O*-[*β*-D-glucopyranosyl (1→2)-*β*-D-xylopyranosyl]-25-*O*-*β*-D-glucopyranosyl-cycloastragenol (**5**) and astragaloside VI is 3-*O*-[*β*-D-glucopyranosyl (1→2)-*β*-D-xylopyranosyl]-6-*O*-*β*-D-glucopyranosyl-cycloastragenol (**6**).

Keywords——*Astragalus membranaceus*; 9,19-cyclolanostane-oligoglycosde; cycloastragenol; astragaloside; crude hesperidinase; ^{13}C-NMR

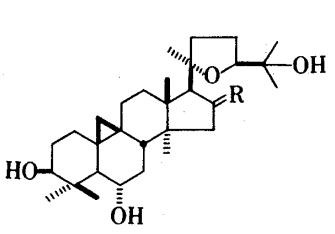
In one of two previous papers on the chemical constituents of Astragali Radix, the root of Korean *Astragalus membranaceus* BUNGE (Leguminosae), we reported the isolation of the oligoglycosidic constituents and the structural elucidation of two genuine aglycones: cycloastragenol (**1**) and soyasapogenol B.[2] Cycloastragenol (**1**) was a new 9,19-cyclolanostane triterpene, and was shown to be converted to the artifact aglycone astragenol, a lanost-9(11)-ene counterpart. In the following paper,[1] we reported the isolation of twelve triterpene-oligoglycosides: acetylastragaloside I, isoastragalosides I and II, astragalosides, I, II, III (**3**), IV (**4**), V (**5**), VI (**6**), VII and VIII and soyasaponin I, and elucidated the structures of seven of them, including acetylastragaloside I, isoastragalosides I and II, astragalosides I, II and IV (**4**). This paper describes the structural elucidation of three of the remaining five oligoglycosides: astragalosides III (**3**), V (**5**) and VI (**6**) on the basis of various evidence including carbon-13 nuclear magnetic resonance (^{13}C-NMR) and methylation analyses and the results of enzymatic degradation.[3]

Astragaloside III (3)

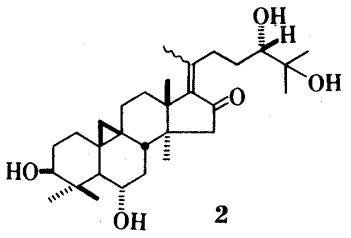
The infrared (IR) spectrum of astragaloside III (**3**) exhibited strong hydroxyl absorption bands characteristic of the glycosidic structure, whereas the field-desorption mass spectrum (FD-MS) gave the highest ion peak at m/z 807, which was ascribable to $(M+Na)^+$. Methanolysis of **3** with 9% hydrogen chloride in dry methanol furnished one mole each of methyl glucoside and methyl xyloside. Enzymatic hydrolysis of **3** with crude hesperidinase provided the genuine aglycone cycloastragenol (**1**) and its 3-*O*-xyloside (**7**).[1]

The ^{13}C-NMR spectrum of astragaloside III (**3**) exhibited signals assignable to two *β*-anomeric carbons of the glucopyranoside and the xylopyranoside residues at δc 105.4 (d) and 105.8 (d). In addition, significant glycosidation shifts[4] were observed of the signals due to 3-C and 2′-C of **3** (Table I). Therefore, astragaloside III (**3**) was shown to possess a 2′-*O*-*β*-D-glucopyranosyl-xylopyranosyl moiety attached to the 3*β*-OH of cycloastragenol (**1**).

Complete methylation of **3** with methyl iodide and dimsyl carbanion[5] provided the nona-*O*-methyl derivative (**3a**), which gave two *β*-anomeric proton signals in its proton nuclear magnetic resonance (^{1}H-NMR) spectrum. Methanolysis of **3a** liberated methyl 2,3,4,6-tetra-



1 : R=β-OH, α-H
(cycloastragenol)
1a : R=O



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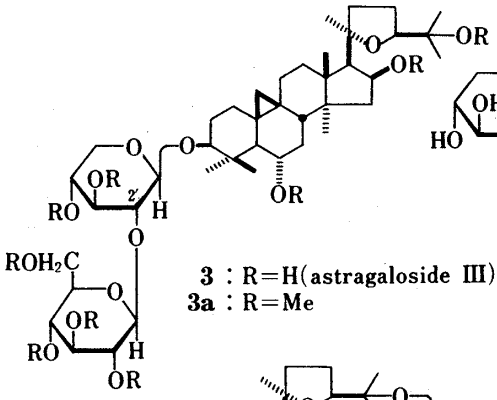
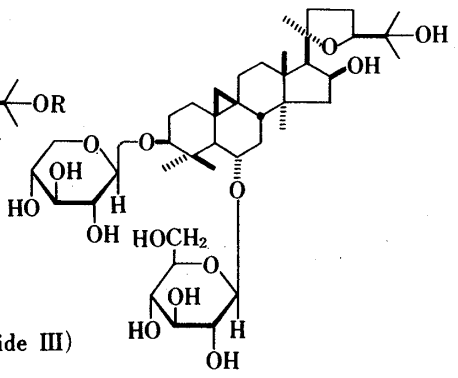
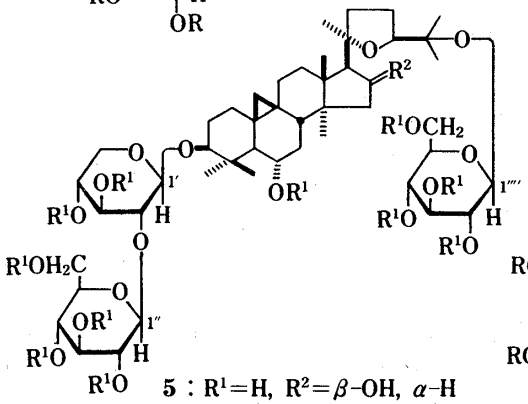


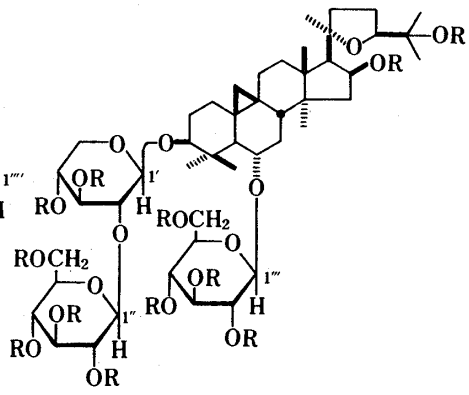
3 : R=H(astragaloside III)
3a : R=Me



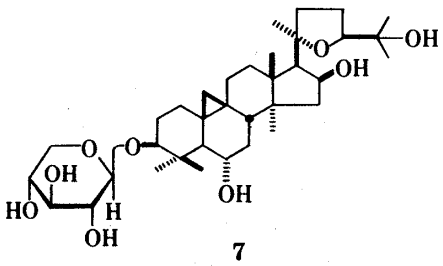
4 : astragaloside IV



5 : R^1=H, R^2=β-OH, α-H
(astragaloside V)
5a : R^1=Me, R^2=β-OMe, α-H
5b : R^1=Ac, R^2=β-OH, α-H
5c : R^1=Ac, R^2=O



6 : R=H(astragaloside VI)
6a : R=Me



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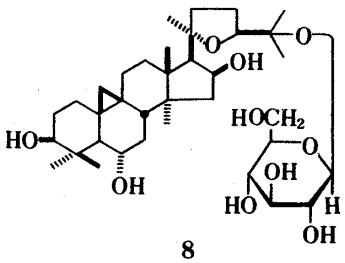

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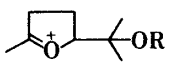

i : R=H
iii : R=Me

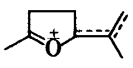

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Chart 1

TABLE I. ^{13}C-NMR Data for Cycloastragenol (**1**) and Astragalosides (in d_5-pyridine, δc)[a]

		1	3	4	5	6	7	8
Aglycone moiety	C-3	78.4	88.8	88.7	88.6	88.5	88.7	78.3(d)[b]
	C-5	54.0	54.2	54.2	54.0	52.3	54.2	53.9(d)
	C-6	68.5	68.2	79.2	67.9	79.1	68.2	68.3(d)
	C-16	73.6	73.7	73.5	73.5	73.4	73.4	73.5(d)
	C-17	58.5	58.7	58.7	58.2	58.2	58.6	58.2(d)
	C-20	87.4	87.4	87.3	87.2	87.2	87.2	87.2(s)
	C-24	81.8	82.2	82.0	82.2	82.2	82.1	82.1(d)
	C-25	71.4	71.2	71.5	78.6	71.3	71.1	78.6(s)
3-*O*-β-D-Xylopyranosyl moiety	C-1′		105.8[b]	107.1	105.7[b]	105.9[b]	107.1(d)	
	C-2′		83.1	75.2	83.0	83.5	75.1(d)	
	C-3′		77.5	77.7	77.6	77.8	77.8(d)	
	C-4′		71.5	71.3	71.4	70.8	71.1(d)	
	C-5′		66.4	66.6	66.4	66.3	66.5(t)	
2′-*O*-β-D-Glucopyranosyl moiety	C-1″		105.4[b]		105.3[b]	105.2(d)[b]		
	C-2″		76.7		76.6	76.6(d)		
	C-3″		78.1		78.3	78.7(d)		
	C-4″		72.2		71.9	71.9(d)		
	C-5″		77.9		77.9	77.8(d)		
	C-6″		63.2		62.9[c]	63.2(t)[c]		
6-*O*-β-D-Glucopyranosyl moiety	C-1‴			105.0		104.9(d)[b]		
	C-2‴			75.6		75.5(d)		
	C-3‴			79.0		78.7(d)		
	C-4‴			72.9		71.9(d)		
	C-5‴			77.9		77.8(d)		
	C-6‴			63.4		63.0(t)[c]		
25-*O*-β-D-Glucopyranosyl moiety	C-1⁗				98.7			98.8(d)
	C-2⁗				75.0			75.1(d)
	C-3⁗				78.6			78.4(d)[b]
	C-4⁗				71.4			71.4(d)
	C-5⁗				77.9			77.8(d)
	C-6⁗				62.8[c]			62.8(t)

a) The carbon signals affected by glycosidation shifts are underlined. The off-resonance patterns of the signals are given in parentheses with abbreviations: d=doublet, s=singlet and t=triplet.
b, c) Assignments may be interchangeable within the same column.

O-methylglucopyranoside (**a**) and methyl 3,4-di-*O*-methylxylopyranoside (**b**).

Based on the above-mentioned evidence, the structure of astragaloside III was determined to be 3-*O*-[β-D-glucopyranosyl (1→2)-β-D-xylopyranosyl]cycloastragenol (**3**).

Astragaloside V (5)

Enzymatic hydrolysis of astragaloside V (**5**) with crude hesperidinase yielded cycloastragenol (**1**), 3-*O*-xylopyranosyl-cycloastragenol (**7**), astragaloside III (**3**) and a new glucoside (**8**).

Methanolysis of the new glucoside (**8**) provided methyl glucoside. In the ^{13}C-NMR spectrum of **8**,the 25-C signal exhibited a glycosidation shift and there was a signal at δc 98.8 assignable to the anomeric carbon of the glucopyranosyl moiety (Table I). Since the anomeric carbon signal of a glycosyl residue attached to a tertiary hydroxyl group is known to resonate at higher field as compared with an ordinary anomeric carbon,[6] the signal observed at δc 98.8 was assignable to the β-anomeric carbon of the glucopyranosyl residue attached to 25-OH of the aglycone. Consequently, it became clear that **8** was a 25-*O*-β-D-glucopyranosyl-cycloastragenol.

Similarly, the ^{13}C-NMR spectrum of astragaloside V (**5**) showed a signal at δc 98.7, which was ascribable to the anomeric carbon signal of a β-glycosyl residue attached to a tertiary hydroxyl group, together with two ordinary β-anomeric carbon signals at δc 105.3 and 105.7. In addition, significant glycosidation shifts were observed for the 3-C and 25-C signals of the aglycone and the 2′-C signal of the xylopyranoside moiety (Table I). Therefore, astragaloside V (**5**) was demonstrated to be a 25-*O*-β-D-glucopyranosyl derivative of astragaloside III (**3**).

Complete methylation of astragaloside V (**5**) with methyl iodide and dimsyl carbanion furnished the dodeca-*O*-methyl derivative (**5a**), whose ^{1}H-NMR spectrum showed three β-anomeric proton signals. Methanolysis of **5a** provided methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**) and methyl 3,4-di-*O*-methylxylopyranoside (**b**) in 2:1 ratio. These findings further confirmed the structure of carbohydrate moieties of **5**.

Ordinary acetylation of astragaloside V (**5**) provided the undeca-*O*-acetate (**5b**), which, on subsequent pyridinium chlorochromate (PCC) oxidation,[7] was converted to the 16-oxo-undeca-*O*-acetate (**5c**). The circular dichroism (CD) spectrum of **5c** showed a negative CD maximum characteristic of a 16-oxo-9,19-cyclolanostane derivative.[1,2,8] Alkaline treatment[1,2] followed by enzymatic hydrolysis with crude hesperidinase of **5c** provided 16-oxo-cycloastragenol (**1a**)[2] and the enone (**2**).[2] Consequently, the presence of a free 16-OH function in astragaloside V (**5**) was chemically substantiated.

Based on the accumulated evidence mentioned above, the structure of astragaloside V was determined to be 3-*O*-[β-D-glucopyranosyl (1→2)-β-D-xylopyranosyl]-25-*O*-β-D-glucopyranosyl-cycloastragenol (**5**). Astragaloside V (**5**) seems to be a rare example of an oligoglycoside having a glycosyl residue attached to a tertiary hydroxyl group at 25-C of a cyclolanostane-triterpene.

Astragaloside VI (6)

Enzymatic hydrolysis of astragaloside VI (**6**) with crude hesperidinase provided astragaloside IV (**4**) in good yield. The ^{13}C-NMR spectrum of **6** showed three β-anomeric carbon signals and glycosidation shifts of the 3-C, 6-C and 2′-C signals were seen (Table I). Consequently, astragaloside VI (**6**) was suggested to possess a β-glucopyranosyl residue attached to the 2′-OH function in the xylopyranoside moiety of astragaloside IV (**4**).

Complete methylation of astragaloside VI (**6**) furnished the dodeca-*O*-methyl derivative (**6a**). The ^{1}H-NMR spectrum of the methyl ether (**6a**) exhibited three β-anomeric proton signals, while methanolysis of **6a** yielded methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**) and methyl 3,4-di-*O*-methylxylopyranoside (**b**) in 2:1 ratio. Consequently, the carbohydrate sequence in **6** was confirmed and the full structure of astragaloside VI was elucidated to be 3-*O*-[β-D-glucopyranosyl (1→2)-β-D-xylopyranosyl]-6-*O*-β-D-glucopyranosyl-cycloastragenol (**6**).

Experimental[9]

Astragaloside III (3)———Astragaloside III (**3**, 0.1 g) was isolated from Korean Astragali Radix (8 kg) as described in the previous paper.[1] **3**, mp 245—247°C (colorless needles from MeOH), $[\alpha]_D^{18}$ +21.4° (c=0.83, MeOH). *Anal.* Calcd for $C_{41}H_{68}O_{14}\cdot H_2O$: C, 61.32; H, 8.79. Found: C, 61.32; H, 8.54. IR ν_{max}^{KBr} cm^{-1}: 3370, 1070. ^{13}C-NMR: as given in Table I. MS m/z (%): 143 (**i**, 100), 125 (**ii**, 17). FD-MS m/z: 807 $(M+Na)^+$.

Methanolysis of 3———A solution of **3** (2 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 h. After neutralization with Ag_2CO_3 powder, the reaction mixture was filtered to remove the inorganic material. The residue, obtained by evaporation of the solvent under reduced pressure, was dried and dissolved in pyridine (0.1 ml), and the solution was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.2 ml) for 10 min. The reaction mixture was then subjected to gas liquid chromatography (GLC) analysis to identify the TMS derivatives of methyl glucoside and methyl xyloside. The composition (1:1) was determined from the relative peak areas on GLC, *etc.* GLC: 1) 5% silicone SE-52 on Chromosorb WAW DMCS (80—100 mesh); 2 m×3 mm glass column; column temp., 190°C; carrier gas, N_2; flow rate,33 ml/min. t_R: TMS-methyl glucoside 7′30″ (major), 7′59″; TMS-methyl xyloside 3′21″ (major), 3′33″.

Enzymatic Hydrolysis of 3 with Crude Hesperidinase——A solution of **3** (85 mg) in a water–acetone (9:1) mixture (10 ml) was treated with crude hesperidinase[1,2] (1 g) and the whole mixture was incubated with stirring at 37°C for 24 h. The reaction mixture was extracted with *n*-BuOH and the *n*-BuOH extract was passed through a Celite 535 (5 g) column to remove the insoluble material. The residue, which was obtained by evaporation of the solvent from the eluate, was subjected to column chromatography (SiO_2 5 g, $CHCl_3$–MeOH=10:1) to furnish cycloastragenol (**1**, 8 mg) and the 3-*O*-xyloside (**7**, 34 mg), which were identified by mixed mp determination and TLC (for **1**: $CHCl_3$–MeOH=20:1, *n*-hexane–AcOEt=1:5; for **7**: a lower phase of $CHCl_3$–MeOH–H_2O=10:3:1, *n*-BuOH–AcOEt=4:1, $CHCl_3$–MeOH–AcOEt–H_2O=15:9:23:3) and IR (KBr) comparisons with the respective authentic samples.[1,2]

Complete Methylation of 3——A solution of **3** (65 mg) in dimethyl sulfoxide (DMSO) (3 ml) was treated with dimsyl carbanion (10 ml)[5] and the whole solution was stirred under an N_2 atmosphere for 2 h. The reaction mixture was then treated with CH_3I (4 ml) and the whole mixture was stirred in the dark for a further 2 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed successively with aq. $Na_2S_2O_3$ and water, then dried, and the solvent was evaporated off under reduced pressure. The residue was purified by column chromatography (SiO_2 4 g, benzene–acetone=10:1) to furnish the nona-*O*-methyl derivative (**3a**, 50 mg). **3a**, white powder,[10] $[\alpha]_D^{17}$ +25.4° (*c*=1.3, $CHCl_3$). *Anal.* Calcd for $C_{50}H_{86}O_{14}$: C, 65.90; H, 9.51. Found: C, 65.75; H, 9.78. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1095. ^{1}H-NMR ($CDCl_3$, δ): 0.22, 0.51 (1H each, both br d, 19-H_2), 0.95 (6H), 1.14 (3H), 1.22, 1.24 (6H each) (all s, *tert*-CH_3×7), 2.38 (1H, d, *J*=8 Hz, 17-H), 3.11 (3H), 3.24 (6H), 3.38, 3.44, 3.51 (3H each), 3.58 (6H), 3.62 (3H) (all s, OCH_3×9), 4.36, 4.66 (1H each, both d, *J*=7 Hz, anomeric H×2). MS *m/z* (%): 347 (100), 219 (9), 187 (65), 157 (**iii**, 24).

Methanolysis of 3a——A solution of **3a** (2 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 h. After neutralization with Ag_2CO_3, the reaction mixture was filtered to remove the inorganic material. From the filtrate, methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**) and methyl 3,4-di-*O*-methylxylopyranoside (**b**) were identified by thin-layer chromatography (TLC) (benzene–acetone=2:1; *n*-hexane–AcOEt=1:3) and GLC (in 1:1 ratio) comparisons. GLC: 2) 15% polyneopentylglycol succinate on Chromosorb WAW (80—100 mesh); 2m×3 mm glass column; column temp., 180°C; N_2 flow rate, 35 ml/min. t_R: **a** 6′25″, 8′35″ (major); **b** 7′23″ (major), 8′35″. 3) 15% ethylene glycol succinate polyester on Uniport B (80—100 mesh); 1 m×3 mm glass column; column temp.,180°C; N_2 flow rate, 35 ml/min. t_R: **a** 4′18″, 6′01″ (major); **b** 6′30″ (major), 8′01″.

Astragaloside V (5)——Astragaloside V (**5**, 0.1 g) was obtained from Korean Astragali Radix (8 kg) as reported previously.[1] **5**, mp 202—204°C (colorless fine crystals from MeOH), $[\alpha]_D^{14}$ +7.2° (*c*=1.0, MeOH). *Anal.* Calcd for $C_{47}H_{78}O_{19}\cdot 3H_2O$: C, 56.39; H, 8.46. Found: C, 56.48; H, 8.43. IR ν_{max}^{KBr} cm^{-1}: 3400, 1075, 1035. ^{13}C-NMR: as given in Table I.

Enzymatic Hydrolysis of 5 with Crude Hesperidinase——A solution of **5** (180 mg) in water (10 ml) was treated with crude hesperidinase (800 mg) and the whole mixture was incubated with stirring at 35°C for 16 h. The reaction mixture was extracted with $CHCl_3$–MeOH (5:1) and the organic extract was washed with water and dried over $MgSO_4$ powder. Evaporation of the solvent from the filtrate under reduced pressure gave the product,which was purified by preparative TLC ($CHCl_3$–MeOH–H_2O=65:35:10, lower phase) to furnish cycloastragenol (**1**, 2 mg), the 3-*O*-xyloside (**7**, 25 mg), astragaloside III (**3**, 35 mg) and 25-*O*-glucopyranosyl-cycloastragenol (**8**, 20 mg). **1** was identified by comparison with an authentic sample as described above,and **3** and **7** were identified by mixed mp determination and TLC (as described above) and IR (KBr) comparisons. **8**, mp 255—256°C (colorless fine crystals from MeOH), $[\alpha]_D^{17}$ +30.8° (*c*=1.0, MeOH). *Anal.* Calcd for $C_{36}H_{60}O_{10}\cdot H_2O$: C, 64.45; H, 9.32. Found: C, 64.49; H, 9.67. IR ν_{max}^{KBr} cm^{-1}: 3420, 1070. ^{13}C-NMR: as given in Table I.

Methanolysis of 8——A solution of **8** (2 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 h. The reaction mixture was worked up as described for the methanolysis of **3** and the product was trimethylsilylated and shown to be identical with the TMS derivative of methyl glucoside by GLC as described above.

Complete methylation of 5——A solution of **5** (130 mg) in DMSO (5 ml) was treated with dimsyl carbanion (10 ml) and the whole mixture was stirred under an N_2 atmosphere for 2.5 h. After addition of CH_3I (10 ml), the whole mixture was stirred in the dark for 1 h. Work-up of the reaction mixture as described for the methylation of **3** gave the product,which was purified by column chromatography (SiO_2 15 g, benzene–acetone=10:1) to furnish the dodeca-*O*-methyl derivative (**5a**, 70 mg). **5a**, white powder, $[\alpha]_D^{14}$ +17.1° (*c*=1.0, $CHCl_3$). *Anal.* Calcd for $C_{59}H_{102}O_{19}$: C, 63.53; H, 9.22. Found: C, 63.61; H, 9.20. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1100. ^{1}H-NMR ($CDCl_3$, δ): 0.20, 0.47 (1H each, both d, *J*=4 Hz, 19-H_2), 0.94, 1.16 (3H each), 1.25 (15H) (all s, *tert*-CH_3×7), 2.39 (1H, d, *J*=8 Hz, 17-H), 3.13, 3.23 (3H each), 3.38 (6H), 3.44 (3H), 3.50 (9H), 3.54 (3H), 3.60 (9H) (all s, OCH_3×12), 4.34, 4.51, 4.64 (1H each, all d, *J*=7 Hz, anomeric H×3). MS *m/z* (%): 347 (50), 187 (100), 157 (**iii**, 65).

Methanolysis of 5a——A solution of **5a** (7 mg) in 9% HCl–dry MeOH (0.5 ml) was heated under reflux for 1.5 h. Work-up of the reaction mixture as described for the methanolysis of **3a** provided the product, from which methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**) and methyl 3,4-di-*O*-methylxylopyranoside (**b**) were

identified by TLC and GLC comparisons (as described in the case of **3a**).

Acetylation of 5——A solution of **5** (140 mg) in Ac_2O–pyridine (1:1, 2 ml) was allowed to stand at 17°C for 3 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed successively with aq. dil. HCl, aq. sat. $NaHCO_3$ and water, then dried. Evaporation of the solvent under reduced pressure provided the undeca-*O*-acetate (**5b**, 150 mg). **5b**, white powder, $[\alpha]_D^{20}$ −8.4° (c=1.0, $CHCl_3$). *Anal.* Calcd for $C_{69}H_{100}O_{30}$: C, 58.80; H, 7.15. Found: C, 58.82; H, 7.20. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3540, 1755, 1215, 1050.

PCC Oxidation of 5b——A solution of **5b** (89 mg) in CH_2Cl_2 (6 ml) was treated with PCC (180 mg) and the whole mixture was stirred at 18°C for 10 h. After dilution with ether, the reaction mixture was passed through a Florisil (100—200 mesh, 10 g) column. Evaporation of the solvent from the eluate furnished the 16-oxo-undeca-*O*-acetate (**5c**, 87 mg). **5c**, white powder, $[\alpha]_D^{17}$ −30.8° (c=1.0, $CHCl_3$). *Anal.* Calcd for $C_{69}H_{98}O_{30}$: C, 58.88; H, 7.02. Found: C, 58.56; H, 7.33. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1750, 1220, 1045. CD (c=1.165×10^{-1}, MeOH): $[\theta]_{340}$ 0, $[\theta]_{310}$ −17900 (neg. max.), $[\theta]_{260}$ 0.

Alkaline Treatment followed by Enzymatic Hydrolysis of 5c——A solution of **5c** (70 mg) in MeOH (3 ml) was treated with 10% NaOMe–MeOH (3 ml), and the whole solution was stirred at 19°C for 30 min. After neutralization with Dowex 50w×8 (H^+ form), the reaction mixture was filtered to remove the resin. Removal of the solvent from the filtrate under reduced pressure furnished the residue, which was dissolved in H_2O–MeOH (10:1, 5.5 ml). The solution was then incubated added with crude hesperidinase (500 mg) with stirring at 35°C for 24 h. The reaction mixture was extracted with AcOEt. The AcOEt extract was washed with water and dried, and the solvent was evaporated off under reduced pressure. The resulting product was purified by preparative TLC ($CHCl_3$–MeOH=8:1) to furnish 16-oxo-cycloastragenol (**1a**, 7 mg) and the enone (**2**, 12 mg). **1a** was shown to be identical with an authentic sample[2] by mixed mp determination and TLC (as described for **1**) and IR (KBr) comparisons. **2** was shown to be identical with an authentic sample[2] by TLC (as described for **1**) and ^{1}H-NMR ($CDCl_3$) comparisons.[11]

Astragaloside VI (6)——Astragaloside VI (**6**, 0.3 g) was obtained from Korean Astragali Radix (8 kg) as described previously.[1] **6**, mp 290—291°C (colorless fine crystals from MeOH). $[\alpha]_D^{14}$ +17.3° (c=1.0, MeOH). *Anal.* Calcd for $C_{47}H_{78}O_{19}\cdot H_2O$: C, 58.49; H, 8.36. Found: C, 58.14; H, 8.44. IR ν_{max}^{KBr} cm^{-1}: 3400, 1075, 1033. ^{13}C-NMR: as given in Table I. MS m/z (%): 143 (**i**, 100), 125 (**ii**, 34).

Enzymatic Hydrolysis of 6 with Crude Hesperidinase——A solution of **6** (100 mg) in water (10 ml) was incubated with crude hesperidinase (1 g) with stirring at 35°C for 36 h. The reaction mixture was extracted with $CHCl_3$–MeOH (5:1). The organic extract was washed with water and dried over $MgSO_4$ powder. Removal of the solvent under reduced pressure gave the product, which was purified by preparative TLC ($CHCl_3$–MeOH–H_2O=65:35:10, lower phase) to furnish astragaloside IV (**4**, 35 mg). **4** was shown to be identical with an authentic sample[1] by mixed mp determination and TLC ($CHCl_3$–MeOH–H_2O=65:35:10, lower phase; *n*-BuOH–AcOEt–H_2O=4:1:5, upper phase) and IR (KBr) comparisons.

Complete Methylation of 6——A solution of **6** (100 mg) in DMSO (5 ml) was treated with dimsyl carbanion (10 ml), and the reaction mixture was stirred under an N_2 atmosphere for 2.5 h. After addition of CH_3I (10 ml), the whole mixture was stirred in the dark for 1 h. The reaction mixture was worked-up as in the case of **3a**, and the product was purified by column chromatography (SiO_2 5 g, benzene–acetone=5:1) to furnish the dodeca-O-methyl derivative (**6a**, 60 mg). **6a**, white powder, $[\alpha]_D^{14}$ +21.6° (c=0.5, $CHCl_3$). *Anal.* Calcd for $C_{59}H_{102}O_{19}$: C, 63.53; H, 9.22. Found: C, 63.42; H, 9.23. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1095. ^{1}H-NMR ($CDCl_3$, δ): 0.22, 0.51 (1H each, both d, J=5 Hz, 19-H_2), 0.99 (6H), 1.08, 1.11 (3H each), 1.15 (9H) (all s, *tert*-CH_3×7), 2.38 (1H, d, J=8 Hz, 17-H), 3.09, 3.24 (3H each), 3.36 (6H), 3.43 (3H), 3.50 (9H), 3.56 (6H), 3.59, 3.61 (3H each) (all s, OCH_3×12), 4.26, 4.37, 4.63 (1H each, all d, J=7 Hz, anomeric H×3). MS m/z (%): 347 (59), 219 (9), 187 (100), 157 (**iii**, 11).

Methanolysis of 6a——A solution of **6a** (5 mg) in 9% HCl–dry MeOH (0.5 ml) was heated under reflux for 1 h. Work-up of the reaction mixture as in the case of **3a** furnished the product, from which methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**) and methyl 3,4-di-*O*-methylxylopyranoside (**b**) were identified by TLC and GLC (in 2:1 ratio) comparisons as described above.

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9) The instruments used to obtain the physical data and the experimental conditions for chromatography were the same as described in our previous paper.[2]
10) All attempts at crystallization were unsuccessful. These compounds are therefore described as they appeared in noncrystalline form.
11) The composition of the 17(20)-ene geometric isomers in the present enone (**2**) was shown by ^{1}H-NMR analysis to be almost identical with that of the enone obtained previously.[2]