Four New Oleanene Derivatives from the Seeds of *Astragalus complanatus*[1)]

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Four new methyl ester derivatives of oleanene glycosides (3—6) were isolated from the seeds of *Astragalus complanatus* R. BR. together with two known triterpene glycosides, astragaloside VIII methyl ester (1) and soyasaponin I methyl ester (2) after treatment with diazomethane during separation procedure. The structures of 3—6 were elucidated as 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl(1→2)-6-*O*-methy-β-D-glucuronopyranosyl-soyasapogenol B 22-*O*-β-D-glucopyranoside, 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-6-*O*-methyl-β-D-glucuronopyranosyl-soyasapogenol B 22-*O*-β-D-glucopyranoside, 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl(1→2)-6-*O*-methyl-β-D-glucuronopyranosyl 3β,22β,24-trihydroxy-11-oxo-olean-12-ene and 3-*O*-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl(1→2)-6-*O*-methyl-β-D-glucuronopyranosyl 3β,22β,24-trihydroxy-11-oxo-olean-12-ene, respectively.

Keywords *Astragalus complanatus*; Leguminosae; soyasapogenol B; 3β,22β,24-trihydroxy-11-oxo-olean-12-ene; astragaloside VIII; soyasaponin I; oleanene glycoside; glucuronide

Astragalus complanatus (Leguminosae) occurs very commonly in the north of China. Its seeds, Astragali Semen (沙苑子), have been used in traditional Chinese medicine as a tonic for liver and kidney. We previously reported the isolation of flavonoids.[2)] Our further investigation on the chemical constituents has revealed the occurrence of triterpene glycosides. This paper describes the isolation and structure elucidation of four new oleanene glycosides.

The methanol extract of Astragali Semen was partitioned between *n*-hexane and 80% MeOH, and then the MeOH extract was further partitioned with 1-BuOH and water. Removal of the solvent of the organic layer gave a residue, which was methylated with CH_2N_2 and separated by normal and reversed phase column chromatographies to yield six oleanene glycosides (**1**—**6**).

Compounds **1** and **2** were identified with the methyl esters of astragaloside VIII[3)] and soyasaponin I,[4)] respectively, by positive fast atom bombardment mass spectrum (FAB-MS) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum as listed in Table I.

Compound **3**, a whith powder, $[\alpha]_D$ −68.3° (MeOH), on acid hydrolysis, provided a sapogenol which was identical with soyasapogenol B with respect to mp, $[\alpha]_D$ and thin layer chromatography (TLC). The high resolution fast atom bombardment mass spectrum (HR-FAB-MS) of **3** indicated a quasi-molecular ion at *m/z* 1111.5641 due to $[M+Na]^+$ ($C_{54}H_{88}O_{22}Na$). A comparative study of the ^{13}C-NMR spectrum of **3** with that of astragaloside VIII methyl ester (**1**) led to the identification at the sugar residue as listed in Table I, which suggests the occurrence of a terminal β-D-glucopyranosyl moiety and an α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl (1→2)-6-*O*-methyl-β-D-glucuronopyranosyl group. Among the carbon signals resulting from the sapogenol, the one due to C-22 was shifted to 82.5 ppm, about 7 ppm lower than that of the free C-22-OH when the ^{13}C-NMR spectrum was compared with that of astragaloside VIII methyl ester (**1**), therefore the glucopyranosyl moiety was attached to the C-22-OH in **3**. Consequently, the structure of **3** was characterized as 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl (1→2)-6-*O*-methyl-β-D-glucuronopyranosyl-soyasapogenol B 22-*O*-β-D-glucopyranoside.

Compound **4**, a white powder, $[\alpha]_D$ −37.0° (MeOH), showed a peak due to $[M+Na]^+$ ($C_{55}H_{90}O_{23}Na$) at *m/z* 1141.5759 which was higher by 30 mass units than that of **3** in the HR-FAB-MS spectrum. Compound **4**, on acid hydrolysis, afforded soyasapogenol B as a sapogenol. Signals in the ^{13}C-NMR spectrum suggested the appearance of a terminal β-D-glucopyranosyl moiety and an α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-6-*O*-methyl-β-D-glucuronopyranosyl group, the latter of which was identical with that of soyasaponin I methyl ester (**2**), as listed in Table I. On the other hand, the signal due to the aglycone moiety (Table I) displayed a downfield shift

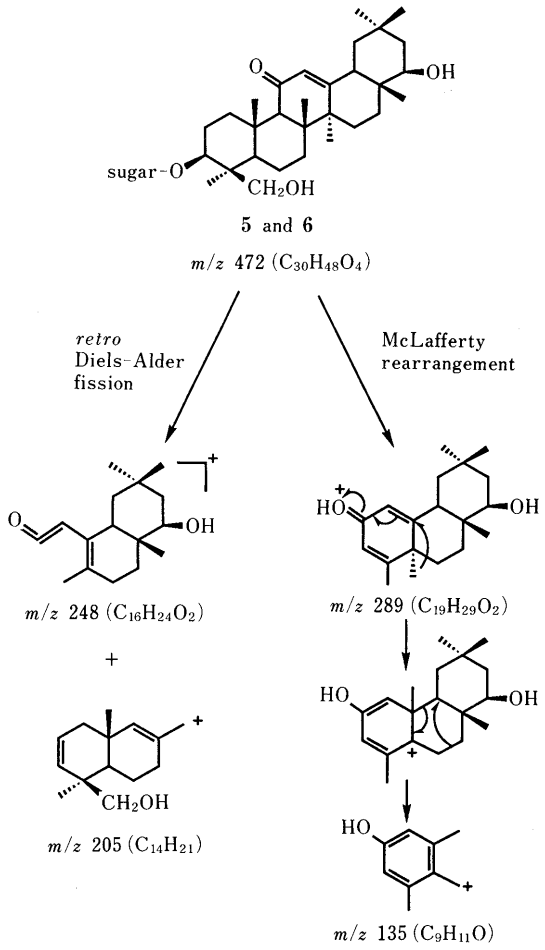


Chart 1. EI-MS of **5** and **6**

of about 7 ppm at C-22 where the glucopyranosyl moiety was attached. On the basis of the above data, the structure of **4** could be represented as 3-*O*-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl(1→2)-6-*O*-methyl-β-D-glucuronopyranosyl-soyasapogenol B 22-*O*-β-D-glucopyranoside.

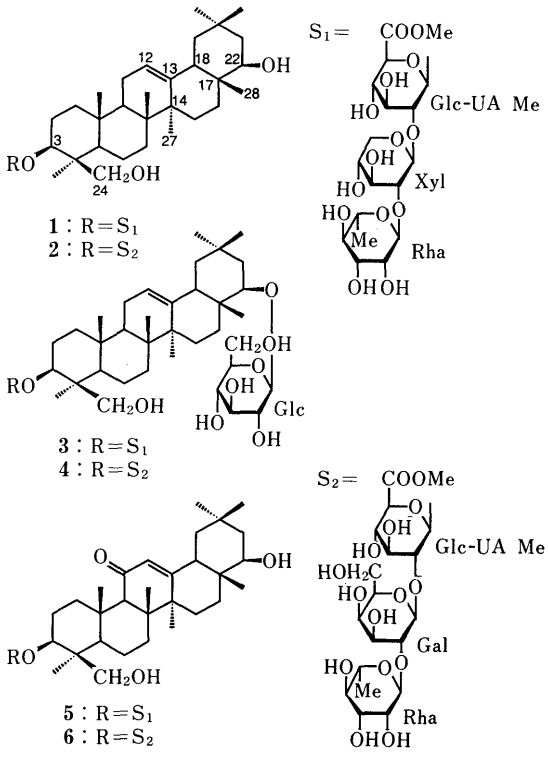
TABLE I. ^{13}C-NMR Spectral Data for Compounds **1**—**6** (Pyridine-d_5)

	1	2	3	4	5	6
C-1	38.8	38.6	38.8	38.6	39.5	39.3
C-2	26.7[a]	26.7[a]	25.9[a]	26.0[a]	26.7	26.7[a]
C-3	91.1	91.3	91.1	91.1	91.0	90.9
C-4	44.3	43.9	44.3	43.9	44.7	44.5
C-5	56.4	56.1	56.3	56.1	56.1	55.9
C-6	18.6	18.5	18.6	18.5	17.9	17.7
C-7	33.3	33.2	33.3	33.3	33.2	33.0
C-8	39.9	39.9	39.7	39.8	44.0	43.8
C-9	47.7	47.8	47.7	47.8	61.8	61.6
C-10	36.5	36.5	36.5	36.4	37.0	36.6
C-11	24.0	24.1	23.9	24.0	199.4	199.3
C-12	122.4	122.3	122.5	122.5	128.4	128.2
C-13	144.8	144.9	144.3	144.4	169.3	169.7
C-14	42.4	43.9	42.2	42.3	45.4	45.0
C-15	26.4[a]	26.4[a]	26.6[a]	26.7[a]	26.7	26.5[a]
C-16	28.6	28.6	28.6	28.6	27.9	27.7
C-17	38.0	38.0	37.4	37.4	37.8	36.8
C-18	45.3	45.3	45.8	45.8	45.5	45.2
C-19	46.7	46.8	46.4	46.4	45.2	45.0
C-20	30.9	30.9	30.5	30.6	30.9	30.7
C-21	42.4	42.3	37.4	37.5	42.0	41.8
C-22	75.5	75.6	82.5	82.5	74.9	74.7
C-23	23.0	23.0	22.9	23.0	23.0[a]	22.7[b]
C-24	62.8	63.6	63.0	63.6	62.8	63.3
C-25	15.6	15.8	15.6	15.8	16.5	16.7
C-26	17.0	17.0	16.9	16.9	18.7	18.5
C-27	25.6	25.7	25.2	25.3	23.1[a]	22.8[b]
C-28	28.6	28.7	28.6	28.7	28.3	28.1
C-29	33.3	33.2	32.3	32.5	33.2	32.9
C-30	21.1	21.2	21.0	21.1	21.7	21.5
Glc-UA						
1	105.4	105.5	105.4	105.5	105.5	105.3
2	78.6[b]	78.6	78.8[b]	78.9	78.6[b]	78.4
3	76.8	76.5[b]	76.8	76.4[b]	76.8	76.3[c]
4	73.6	73.6	73.6	73.6	73.6	73.4
5	77.5	77.7	77.5	77.7	77.5	77.5
6	170.4	170.4	170.4	170.4	170.3	170.1
COOMe	52.1	52.2	52.0	52.1	52.1	52.0
Xyl						
1	102.5		102.5		102.6	
2	79.5		79.4		79.5	
3	78.2[b]		78.1[b]		78.2[b]	
4	70.8		70.8		70.9	
5	66.8		66.8		66.9	
Gal						
1		101.8		101.7		101.5
2		76.8[b]		76.6[b]		76.3[c]
3		76.6[b]		76.5[b]		76.2[c]
4		71.2		71.2		71.0
5		76.9[b]		76.9[b]		76.7[c]
6		61.6		61.6		61.6
Rha						
1	102.3	102.5	102.3	102.4	102.4	102.2
2	72.4[c]	72.4[c]	72.4[c]	72.4[c]	72.4[c]	72.2[d]
3	72.8[c]	72.8[c]	72.7[c]	72.8[c]	72.8[c]	72.5[d]
4	74.3	74.3	74.3	74.3	74.3	74.1
5	69.4	69.4	69.4	69.4	69.5	69.2
6	18.9	19.0	18.9	19.0	19.0	18.7
Glc						
1			102.6	102.6		
2			75.2	75.3		
3			78.2[b]	78.2[d]		
4			71.9[c]	72.0[c]		
5			78.5[b]	78.3[d]		
6			62.8	63.1		

a—*d*) In each vertical column may be interchanged.

Compound **5**, a white powder, $[\alpha]_D$ +13.3° (MeOH), exhibited absorption bands due to α,β-unsaturated carbonyl (1660 cm^{-1}) and carboxyl ester (1746 cm^{-1}) groups together with hydroxyl groups (3432 cm^{-1}) in the infrared (IR) spectrum. The HR-FAB-MS spectrum of **5** showed a peak due to $[M+Na]^+$ ($C_{48}H_{76}O_{18}Na$) at *m/z* 963.4908. Moreover, its electron impacting mass spectrum (EI-MS) disclosed characteristic peaks at *m/z* 289, 135 and 248, 205 due to McLafferty rearrangement[5] and *retro* Diels–Alder fission[6] as shown in Chart 1, which suggested that the sapogenol in **5** possessed the partial structure of the 11-oxo-olean-12-ene system. The ^{1}H-NMR spectrum of **5** showed seven tertiary methyl groups and an olefinic proton at δ 5.81 (1H, s) assignable to the H-C-12. The ^{13}C-NMR spectrum (Table I) showed signals due to C-11, 12 and 13 at δ 199.4, 128.4 and 169.3, respectively, and signals due to C-8, 9, 14, 26 and 27 exhibited the C-11 ketonization shifts[7] (+4.1, +14.1, +3.0, +1.7 and −2.5 ppm, respectively), in comparison with those of **1**, which also supported the above partial structure. Moreover, the ^{13}C-NMR spectrum suggested the presence of trihydroxy groups attached to C-3β, 22β and 24, and the sugar moiety was connected with C-3-OH, so that the structure of a new sapogenol of **5** was constructed as 3β,22β,24-trihydroxy-11-oxo-olean-12-ene. Signals due to the sugar moiety were superimposable on those of astragaloside VIII methyl ester (**1**). The structure of compound **5** was therefore characterized as 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl(1→2)-6-*O*-methyl-β-D-glucuronopyranosyl 3β,22β,24-trihydroxy-11-oxo-olean-12-ene.

Compound **6**, a white powder, $[\alpha]_D$ +8.0° (MeOH), showed a peak at *m/z* 993.5067 due to $[M+Na]^+$



($C_{49}H_{78}O_{19}Na$), which was higher by 30 mass units than that of **5** in the HR-FAB-MS. According to the EI-MS, ^1H- and ^{13}C-NMR (Table I) spectra, the sapogenol was shown to be the same as that of **5**, and the sugar moiety was identical with that of soyasaponin I methyl ester (**2**). The structure of **6** was thus characterized as 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-6-*O*-methyl-β-D-glucuronopyranosyl 3β,22β,24-trihydroxy-11-oxo-olean-12-ene.

Experimental

Optical rotations were measured on a JASCO DIP-360 automatic digital polarimeter. The IR spectra were recorded with a Hitachi IR spectrometer, model 270-30. The ^1H- and ^{13}C-NMR spectra were measured with a JEOL JNM-GX 400 NMR spectrometer and chemical shifts are given on a δ(ppm) scale with tetramethylsilane (TMS) as an internal standard. The FAB- and EI-MS were recorded with a JEOL DX-303 HF spectrometer and taken in a glycerol matrix containing NaI, and HR-FAB-MS spectra were measured with a JEOL HX-110 spectrometer. TLC was performed on precoated Kieselgel 60 F_{254} plate (0.2 mm Merck) and detection was achieved by spraying 10% H_2SO_4 followed by heating. Column chromatography was carried out with Sephadex LH-20 (pharmacia), Bondapak C_{18} (Waters Associates) and Kieselgel 60 (70—230 and 230—400 mesh, Merck).

Extraction and Separation The dried seeds (9 kg) of *Astragalus complanatus* collected in a natural habitat in China were extracted with MeOH and the extract (371 g) was partitioned between *n*-hexane and 80% MeOH. The 80% MeOH extract was further partitioned with 1-BuOH and water. The 1-BuOH soluble portion (90 g) was subjected to Sephadex LH-20 column chromatography with water and 10% MeOH → MeOH to afford a number of fractions. The triterpene fr. (7 g) was chromatographed on Bondapak C_{18} column with 50% MeOH→MeOH and three fractions were obtained by TLC monitoring, which were respectively treated with excess diazomethane followed by column chromatography on silica gel using $CHCl_3$–MeOH–H_2O (10 : 1 : 0.1 → 7 : 3 : 0.5) to provide compounds **1** (59 mg), **2** (13 mg), **3** (32 mg), **4** (24 mg), **5** (48 mg) and **6** (16 mg).

Compound 1 A white powder, $[\alpha]_D$ −9.2° (c=0.7, MeOH). Positive FAB-MS (m/z): 949 $[M+Na]^+$, 927 $[M+H]^+$. Identified with astragaloside VIII methyl ester.

Compound 2 A white powder, $[\alpha]_D$ −4.1° (c=1.0, MeOH). Positive FAB-MS (m/z): 979 $[M+Na]^+$, 957 $[M+H]^+$. Identified with soyasaponin I methyl ester.

Compound 3 A white powder, $[\alpha]_D$ −68.3° (c=0.4, MeOH). HR-FAB-MS (m/z): 1111.5641 $[M+Na]^+$ (Calcd for $C_{54}H_{88}O_{22}Na$ 1111.5666). A small amount of **3** was hydrolyzed with 1 N HCl–MeOH to yield a sapogenol, colorless needles, mp 255—257 °C, $[\alpha]_D$ +62.5° (c=0.4, MeOH), identical with soyasapogenol B, methyl α,β-xylopyranoside and methyl α,β-rhamnopyranoside.

Compound 4 A white powder, $[\alpha]_D$ −37.0° (c=0.8, MeOH). HR-FAB-MS (m/z): 1141.5759 $[M+Na]^+$ (Calcd for $C_{55}H_{90}O_{23}Na$ 1141.5772). Acid hydrolysis of **4** gave soyasapogenol B as a sapogenol, methyl α,β-galactopyranoside and methyl α, β-rhamnopyranoside.

Compound 5 A white powder, $[\alpha]_D$ +13.3° (c=0.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3432 (OH), 1746 (COOMe), 1660 (α, β-unsaturated carbonyl). HR-FAB-MS (m/z): 963.4908 $[M+Na]^+$ (Calcd for $C_{48}H_{76}O_{18}Na$ 963.4931). ^1H-NMR (C_5D_5N) δ: 5.81 (1H, s, H-12). EI-MS (m/z): 472 $[M]^+$, 454 $[-H_2O]^+$, 289, 205 [McLafferty rearrangement], 248, 135 [*retro* Diels–Alder fission] as shown in Chart 1.

Compound 6 A white powder, $[\alpha]_D$ +8.0° (c=0.2, MeOH). HR-FAB-MS (m/z): 993.5067 $[M+Na]^+$ (Calcd for $C_{49}H_{78}O_{19}Na$ 993.5036). ^1H-NMR (C_5D_5N) δ: 5.80 (1H, s, H-12). EI-MS (m/z): 472 $[M]^+$, 454 $[M-H_2O]^+$, 289, 205 [McLafferty rearrangement] and 248, 135 [*retro* Diels–Alder fission] as shown in Chart 1.

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