Studies of the Constituents of *Astragalus membranaceus* BUNGE. III.[1] Structures of Triterpenoidal Glycosides, Huangqiyenins A and B, from the Leaves

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Two new cycloartane-type triterpenoidal saponins, huangqiyenins A and B, were isolated from the leaves of *Astragalus membranaceus* BUNGE (Leguminosae) collected in Heilongjiang Province, China, and established as 3-*O*-*β*-D-glucopyranosyl-(20*R*,24*S*)-16*β*,25-dihydroxy-20,24-epoxy-9,19-cyclolanost-6-one and 3-*O*-*β*-D-glucopyranosyl-(24*S*)-16*β*,24,25-trihydroxy-cyclolanost-6-one, respectively, on the basis of chemical and spectral evidence.

Key words *Astragalus membranaceus*; cycloartane; huangqiyenin A; huangqiyenin B; triterpenoidal saponin

The roots of *Astragalus membranaceus* BUNGE and *A. mongholicus* BUNGE (Chinese name: Huang Qi, Japanese name: Ougi) (Leguminosae) have been used in China as an antiperspirant, diuretic, or tonic.[2] A number of cycloartane-type triterpenoidal glycosides named astragalosides have been isolated from the root and aerial part of *A. membranaceus*[3] and the root of *A. mongholicus*.[4] The isolation and structural determination of two glycosides, named mongholicosides I and II, from the aerial part of *A. mongholicus* have already been reported.[5]

In our continuing studies of the chemical constituents of *Astragalus* spp. plants, we have investigated the cycloartane-type triterpenoidal glycosides, huangqiyenin D,[1] from the leaves of *A. membranaceus* BUNGE collected in the Heilongjiang district, North-East China. This paper deals with the isolation and structural elucidation of two new cycloartane-type triterpenoidal glycosides.

A suspension of the ethanol extract of the leaves in water was filtered, and then the filtrate was extracted with ethyl acetate. The ethyl acetate extract was repeatedly chromatographed on silica gel to give two new glycosides named huangqiyenin A (**1**) and huangqiyenin B (**2**), in yields of 0.004 and 0.003%, respectively.

Compound I (**1**), white powder (MeOH), mp 265—268 °C, showed a strong hydroxyl absorption band at 3400 cm^{-1} and a carbonyl absorption band at 1690 cm^{-1} in the IR spectrum, which is characteristic of a six-membered cyclic ketone. The molecular formula of **1** was found to be $C_{36}H_{58}O_{10}$ by negative high resolution (HR)-FAB-MS displaying a peak due to $[M-H]^-$ at *m/z* 649.3962 (100%), and the field desorption mass spectrum (FD-MS) exhibited a peak corresponding to $[M^+]$ at *m/z* 650. In the ^{1}H-NMR spectrum (in C_5D_5N), there were signals due to cyclopropane methylene protons at δ 0.11 and 0.67 ppm (each 1H, d, $J=5.1$ Hz) and seven tertiary methyls at δ 0.99, 1.31, 1.33 (6H), 1.38, 1.58 and 1.84 ppm. There was an anomeric proton signal at δ 4.99 ppm (1H, d, $J=7.5$ Hz). Thus, **1** was considered to be a cycloartane-type triterpenoidal glycoside. This observation was supported by the ^{13}C-NMR spectrum (in C_5D_5N, see Table 1). In addition, the presence of a keto function in **1** was also shown by the ^{13}C-NMR signal at δ 211.2 ppm.

Table 1. ^{13}C-NMR Spectral Data for Compounds **1**, **2**, **3**, **4** and Related Compounds **5** and **6**

	1	**3**	**5**[6]	**2**	**4**	**6**[7]
C-1	29.0	30.8	32.8	29.1	30.7	32.5
C-2	30.3	30.3	31.4	30.4	30.4	31.3
C-3	87.8	77.7	78.3	87.9	77.6	78.0
C-4	41.4	41.1	42.4	41.3	41.4	41.1
C-5	57.8	58.1	53.9	57.9	57.9	47.6[a]
C-6	211.2	211.5	68.3	211.4	211.8	21.5
C-7	41.5	41.7	38.8	41.6	41.6	26.5
C-8	42.8	43.5	47.2	42.7	43.0	48.4[a]
C-9	21.5	21.7	20.9	21.8	21.8	20.0
C-10	30.2	31.0	29.9	30.1	30.4	26.8
C-11	26.4	26.7	26.3	26.7	26.7	26.5
C-12	33.2	33.4	33.4	33.2	33.2	33.3[b]
C-13	45.3	45.6	45.0	45.9	45.8	45.8
C-14	47.2	47.3	46.2	47.8	47.8	47.1
C-15	44.2	44.6	46.7	46.0	45.9	48.8
C-16	72.9	73.0	73.4	71.4	71.5	72.0
C-17	57.7	57.9	58.4	56.6	56.6	57.5
C-18	18.5	18.7	21.6	18.4	18.5	18.3
C-19	22.1	22.9	31.0	22.7	21.9	30.3
C-20	87.1	87.2	87.2	28.8	28.8	28.7
C-21	28.6	28.6	28.6	15.7	15.7	19.4
C-22	35.0	35.2	34.9	32.9	32.9	33.1[b]
C-23	26.8	26.5	26.4	28.0	28.0	27.9
C-24	81.7	81.9	81.7	77.3	77.2	77.2
C-25	71.3	71.4	71.2	72.5	72.6	72.5
C-26	27.1	27.1	27.2	25.7	25.7	25.6
C-27	28.1	28.1	28.2	26.4	26.5	26.2
C-28	19.1	19.2	20.2	19.1	19.1	20.3
C-29	26.8	27.3	29.4	27.0	27.5	26.5
C-30	15.3	14.7	16.1	15.4	14.9	14.8
Glc-1	106.8			106.9		
Glc-2	75.8			75.8		
Glc-3	78.7			78.7		
Glc-4	71.8			71.8		
Glc-5	78.3			78.3		
Glc-6	63.1			63.1		

a, b) Ambiguous assignments are shown by identical letters within the column.

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Table 2. ^{1}H–^{13}C Long Range Correlations of **3**

H		C				
H-5	2.47	41.1 (C-4)	211.5 (C-6)	31.0 (C-10)	22.9 (C-19)	14.7 (C-30)
H-7	2.27	211.5 (C-6)	43.5 (C-8)	21.7 (C-9)	47.3 (C-14)	
H-8	2.69	41.7 (C-7)	21.7 (C-9)	19.2 (C-28)		
H-17	2.52	45.6 (C-13)	18.7 (C-18)	87.2 (C-20)	28.6 (C-21)	
H-19	0.21	30.8 (C-1)	26.7 (C-11)			
H-19	0.75	58.1 (C-5)	43.5 (C-8)			
H-21	1.32	87.2 (C-20)	35.2 (C-22)			
H-26	1.28	81.9 (C-24)	71.4 (C-25)	28.1 (C-27)		
H-27	1.52	81.9 (C-24)	71.4 (C-25)	27.1 (C-26)		
H-28	0.99	43.5 (C-8)	45.6 (C-13)	47.3 (C-14)	44.6 (C-15)	

On enzymatic hydrolysis with crude hesperidinase, **1** gave an aglycone named huangqiyegenin I (**3**) and glucose.

Compound **3**, white powder (MeOH), exhibited an ion peak corresponding to $[M^+]$ at *m/z* 488 and a characteristic ion fragment[6] at *m/z* 143 on a side chain in the electron impact mass spectrum (EI-MS).

By comparison of the ^{1}H- and ^{13}C-NMR spectra of **3** with those of 6α-hydroxyl cyclolanostane-type compounds such as cycloastragenol (**5**),[7] it was found that the signals due to the C, D ring and side chain of **3** were in good agreement with those of these compounds except for the upfield shift of the C-18 at 21.6 ppm of **5** to 18.7 ppm for **3**, as shown in Table 1. At the same time, it appeared that a keto function was located in the A or B ring. All the carbon signals of **3** were assigned on the basis of the ^{1}H–^{1}H correlation spectrum (^{1}H–^{1}H COSY) and ^{1}H–^{13}C COSY coupled with the long range ^{1}H–^{13}C COSY. In the ^{1}H-NMR spectrum of **3**, the signal due to the H-5 proton was displaced downfield by 0.75 ppm. In addition, the downfield shift signal of H-7 was also similar to the H-5, while the signal due to the H-6 is absent compared with those of **5**. In the long range ^{1}H–^{13}C COSY of **3** (see Table 2), the singlet signal due to the H-5 at 2.47 ppm and the doublet signal due to the H-7 at 2.27 ppm showed long range correlations with the keto functional carbon at 211.5 ppm. Consequently, the position of the keto function of **3** must be located at C-6. The carbon signal at 18.7 ppm can be assigned as C-18 by long range correlation from H-17 and other evidence. Thus **3** was characterized as (20*R*,24*S*)-3β,16β,25-trihydroxy-20,24-epoxy-9,19-cyclolanost-6-one. On comparision with **3**, the glycosylation site of **1** was suggested from the ^{13}C-NMR spectrum. As shown in Table 1, the signal of the oxygenated carbon C-3 appeared at lower field, by 10.1 ppm, by the glycosylation shift[8] than the corresponding signal of the aglycone (**3**), while the oxygenated carbons C-16 and C-25 exhibited almost the same chemical shifts. Therefore, the glucose must be located at the C-3 position. On the basis of the coupling constant (J=7.5 Hz) of the anomeric proton of **1**, the glucose has the β-configuration. Based on these results, the structure of **1** was established as 3-*O*-β-D-glucopyranosyl-(20*R*,24*S*)-16β,25-dihydroxy-20,24-epoxy-9,19-cyclolanost-6-one as shown in Chart 1.

Compound **2**, white powder (MeOH), mp 272—274 °C, also showed a strong hydroxy absorption band at 3400

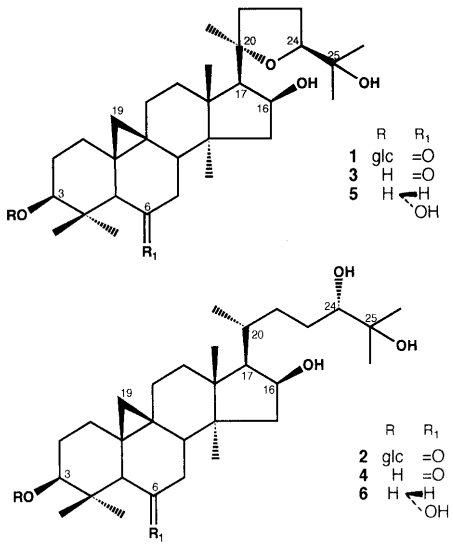

Chart 1. Structures of Compounds **1**, **2**, **3**, **4**, **5** and **6**

cm^{-1} and a carbonyl absorption band at 1690 cm^{-1} in the IR spectrum. The molecular formula of **2** was found to be $C_{36}H_{60}O_{10}$, by negative HR-FAB-MS, exhibiting an ion peak corresponding to $[M-H]^-$ at *m/z* 651.4175 (100%) and the FD-MS spectrum showed a molecular ion peak at *m/z* 652. The ^{1}H- and ^{13}C-NMR spectra of **2** were generally similar to that of **1**, except for the signals due to the side chain, so that compound **2** is also a cycloartane-type triterpenoidal glycoside. Compound **2** gave an aglycone named huangqiyegenin II (**4**) and glucose as the sugar moiety following enzymatic hydrolysis with crude hesperidinase.

The EI-MS spectrum of **4**, white powder (MeOH), exhibited a molecular ion peak at *m/z* 490 $[M^+]$. A molecular weight of **4** is 2 mass units higher than that of compound **3** and no characteristic fragment at *m/z* 143. It is possible that **4** has a chain structure, but is not cyclic. The structure of the side chain of **4** was determined by comparing the ^{13}C-NMR spectrum of **4** with a related compound. In the ^{13}C-NMR spectrum of cyclofoetigenin A (**6**) isolated from *Thalictrum foetidum* by Ganenko *et al.*,[9] which has a (24*S*),25-diol group side chain, the C-24 and C-25 carbons resonate at 77.2 and 72.5 ppm, respectively. The signals of the same carbon in the spectrum of **4** were observed at 77.2 and 72.6 ppm, while the *R* configuration at C-24 resonates at *ca.* 80 ppm.[10] The good agreement in the chemical shifts of the carbon under

consideration showed that the side chain of **4** also had the 24*S*,25-diol group. Thus, **4** was established as (24*S*)-3β,16β,24,25-tetrahydroxy-9,19 cyclolanost-6-one.

By comparison of the ^{13}C-NMR spectral data of **2** (see Table 1) with huangqiyegenin II (**4**), it was found that the signal due to the C-3 of **2** shifted to a lower field by approximately 10.3 ppm, while the signals of C-16, C-24, C-25 showed almost identical chemical shifts. This indicates that the glucose unit of **2** is located at the C-3 position of huangqiyegenin II (**4**). In the ^{1}H-NMR spectrum of **2**, the coupling constants of the signal due to the anomeric proton of glucose was 8.1 Hz, so that the glucose has the β-configuration.

From the above evidence, the structure of huangqiyenin B (**2**) was established as 3-*O*-β-D-glucopyranosyl-(24*S*)-16β,24,25-trihydroxy-cyclolanost-6-one (Chart 1).

The structures of the other saponins isolated from the leaves of this plant are now under investigation.

Experimental

General Procedure All melting points were determined on a Yanagimoto micromelting point apparatus and were uncorrected. Optical rotations were measured with a Union PM-101 automatic digital polarimeter. IR spectra were recorded on a Beckman-1300 spectrometer. Mass spectra were obtained with a JEOL JMS SX-102 mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL GX-400 spectrometer in C_5D_5N using tetramethylsilane as an internal standard.

Extraction and Separation The dried leaves of *Astragalus membranaceus* BUNGE (4.5 kg), collected in Heilongjiang, China, were extracted with EtOH at 80 °C for 8 h and the solvent was then evaporated under reduced pressure. The EtOH extract (600 g) was suspended in H_2O, filtered and then extracted with ethyl acetate; the solvent was evaporated under reduced pressure. The ethyl acetate extract (30 g) was chromatographed on a column of silica-gel with $CHCl_3$–MeOH (10 : 1, 5 : 1) to give **1** (170 mg) and **2** (135 mg).

Huangqiyenin A (1) A white powder (MeOH), mp 265—268 °C, $[\alpha]_D$ +32.1° (c=0.56, MeOH). IR cm^{-1}: 3400 (OH), 1690 (C=O). FD-MS m/z: 650 (M^+). HR-FAB-MS (neg.) m/z: 649.3962 $(M-H)^-$, $C_{36}H_{58}O_{10}$. ^{1}H-NMR (C_5D_5N) δ: 4.99 (1H, d, J=7.5 Hz), 1.84 (3H, s), 1.58 (3H, s), 1.38 (3H, s), 1.33 (6H, s), 1.31 (3H, s), 0.99 (3H, s), 0.67 (1H, d, J=5.1 Hz), 0.11 (1H, d, J=5.1 Hz). ^{13}C-NMR spectrum: see Table 1.

Huangqiyenin B (2) A white powder (MeOH), mp 272—274 °C, $[\alpha]_D$ +54.4° (c=0.60, C_5H_5N). IR cm^{-1}: 3400 (OH), 1690 (C=O). FD-MS m/z: 652 (M^+). HR-FAB-MS (neg.) m/z: 651.4175 $(M-H)^-$. $C_{36}H_{60}O_{10}$. ^{1}H-NMR (C_5D_5N) δ: 4.95 (1H, d, J=8.1 Hz), 1.87 (3H, s), 1.51 (3H, s), 1.48 (3H, s), 1.37 (3H, s), 1.29 (3H, s), 1.09 (3H, d, J=6.6 Hz), 0.98 (3H, s), 0.70 (1H, d, J=5.1 Hz), 0.10 (1H, d, J=5.1 Hz). ^{13}C-NMR spectrum: see Table 1.

Huangqiyegenin I (3) A white powder (MeOH), $[\alpha]_D$ +74.4° (c=0.59, $CHCl_3$). EI-MS m/z: 488 (M^+), 143. $C_{30}H_{48}O_5$. ^{1}H-NMR (C_5D_5N) δ: 4.98 (1H, ddd, J=6.2, 7.7, 7.9 Hz, H-16), 3.88 (1H, dd, J=5.3, 9.0 Hz, H-24), 3.46 (1H, dd, J=4.4, 11.3 Hz, H-3), 1.64 (3H, s, 29-CH_3), 1.52 (3H, s, 27-CH_3), 1.35 (3H, s, 18-CH_3), 1.34 (3H, s, 30-CH_3), 1.32 (3H, s, 21-CH_3), 1.28 (3H, s, 26-CH_3), 0.99 (3H, s, 28-CH_3), 0.75 (1H, d, J=5.3 Hz), 0.21 (1H, d, J=5.3 Hz). ^{13}C-NMR spectrum: see Table 1.

Huangqiyegenin II (4) A white powder (MeOH), $[\alpha]_D$ +112.5° (c=0.40, $CHCl_3$). EI-MS m/z: 490 (M^+). $C_{30}H_{50}O_5$. ^{1}H-NMR (C_5D_5N) δ: 1.76 (3H, s), 1.51 (3H, s), 1.48 (3H, s), 1.40 (3H, s), 1.30 (3H, s), 1.08 (3H, d, J=6.4 Hz), 0.97 (3H, s), 0.81 (1H, d, J=5.1 Hz), 0.20 (1H, d, J=5.1 Hz). ^{13}C-NMR spectrum: see Table 1.

Enzymatic Hydrolysis of 1 and 2 A solution of **1** (35 mg) and **2** (30 mg) in water (5 ml) were incubated at 40 °C for 36 h with crude hesperidinase (70 mg). The reaction mixtures were extracted with $CHCl_3$ and then the $CHCl_3$ layer were evaporated to dryness. The products were chromatographed on a silica gel column [solvent: $CHCl_3$–MeOH (15 : 1)], **1** gave huangqiyegenin I (**3**) (15 mg) and **2** gave huangqiyegenin II (**4**) (11 mg). The H_2O layers were examined to identify glucose by direct comparison on silica-gel TLC with an authentic sample.

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