Secocycloartane Triterpenoidal Saponins from the Leaves of *Astragalus membranaceus* BUNGE

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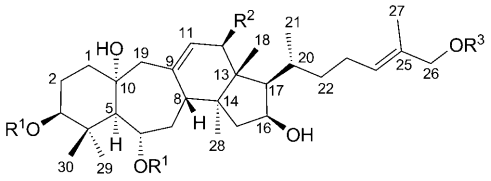
Two new 9,10-secocycloartane triterpenoidal saponins, named huangqiyenins E (**1**) and F (**2**) were isolated from the leaves of *Astragalus membranaceus* BUNGE. Enzymatic hydrolysis of **1** and **2** afforded the aglycones huangqiyegenins III (**3**) and IV (**4**), resp. Further alkaline hydrolysis of **3** provided trideacetylhuangqiyegenin III (**5**). Their structures were established by detailed spectroscopic analysis as (3*S*,5*R*,6*S*,10*R*,12*β*,16*β*,24*E*)-26-(*β*-D-glucopyranosyloxy)-10,16-dihydroxy-4,4,14-trimethyl-9,19-cyclo-9,10-secocholesta-9(11),24-diene-3,6,12-triyl triacetate (**1**), (3*S*,5*R*,6*S*,10*R*, 16*β*,24*E*)-26-(*β*-D-glucopyranosyloxy)-10,16-dihydroxy-4,4,14-trimethyl-9,19-cyclo-9,10-secocholesta-9(11), 24-diene-3,6-diyl diacetate (**2**), (3*S*,5*R*,6*S*,10*R*,12*β*,16*β*,24*E*)-10,16,26-trihydroxy-4,4,14-trimethyl-9,19-cyclo-9,10-secocholesta-9(11),24-diene-3,6,12-triyl triacetate (**3**), (3*S*,5*R*,6*S*,10*R*,16*β*,24*E*)-10,16,26-trihydroxy-4,4,14-trimethyl-9,19-cyclo-9,10-secocholesta-9(11),24-diene-3,6-diyl diacetate (**4**), and (3*S*,5*R*, 6*S*,10*R*,12*β*,16*β*,24*E*)-4,4,14-trimethyl-9,19-cyclo-9,10-secocholesta-9(11),24-diene-3,6,10,12,16,26-hexol (**5**), resp.

Introduction. – *Astragalus membranaceus* BUNGE is a perennial herb, which belongs to the family Leguminosae and is widely distributed in China's Inner Mongolia, Shanxi, Gansu, and Heilongjiang, *etc*. The genus *Astragalus* contains about more than 1600 species in the world, mainly distributed in the sub-tropical and temperate regions of the earth except Oceania. There are *ca.* 130 species in China, ten of which are medicinally used [1]. As a renowned traditional Chinese medicine, *Radix Astragali* (named Huangqi in China), roots of *A. membranaceus* BUNGE or *A. membranaceus* var. *mongholicus*, has been widely used for the treatment of carbuneulosis, swelling, and myospasmia. Radix Astragali is one of the most renowned 'tonifying *qi* drug' in the China's ancient book '*Shen Nong's Herb*'.

Radix Astragali is a relatively precious medicine and has played an quite important role in clinic. Up to now, a large number of chemical constituents such as triterpenes [2–11], flavonoids [12–14], and polysaccharides [15–18] have been isolated from the roots. Saponins, mainly cycloartane-type triterpene glycosides, are characteristic constituents of the genus *Astragalus*. Consideration of sustainability of the plant as source of the drug prompted us to investigate the leaves of *A. membranaceus* BUNGE. In the previous work of this series, we described four cycloartane-type triterpenoidal saponins named huangqiyenins A–D [19–21], isolated from the leaves of this plant collected in the Heilongjiang District, Northeast of China. Herein, we report the isolation and structural determination of two further unusual secocycloartane triterpenoidal saponins named huangqiyenins E (**1**) and F (**2**). It is worth mentioning

that several 9,10-secocycloartane type triterpene saponins and triterpene alkaloids had been discovered from the families Ranunculaceae and Buxaceae [22–24], respectively, but not yet from the Leguminosae family.



	R^1	R^2	R^3
1	Ac	AcO	Glc-p
2	Ac	H	Glc-p
3	Ac	AcO	H
4	Ac	H	H
5	H	OH	H

Results and Discussion. – The BuOH soluble part, obtained from the partition of the MeOH extract from the leaves of *A. membranaceus* BUNGE was chromatographed on SiO_2 column, to give ten fractions (*Frs. I–X*). *Fr. III* was repeatedly chromatographed on SiO_2 and HPLC (*ODS* column) to give two saponins named huangqiyenins E (**1**) and F (**2**). On enzymatic hydrolysis of **1** and **2**, the secocycloartane triterpenoidal aglycones **3** and **4** were obtained, respectively, and, on alkaline hydrolysis of **3**, the trideacetyl sapogenin **5** was obtained.

Compound **1**, named huangqiyenin E, was obtained as white amorphous powder. The molecular formula was determined by HR-FAB-MS (positive-ion mode) as $C_{42}H_{66}O_{14}$ from the $[M+Na]^+$ signal at *m/z* 817.4343 (calc. for $C_{42}H_{66}NaO_{14}^+$: 817.4350), indicating ten degrees of unsaturation. Enzymatic hydrolysis of **1** with cellulase and chromatography of the hydrolysate on SiO_2 afforded a new triterpenoidal sapogenin, named huangqiyegenin III (**3**) as aglycone, and gave D-glucose as the sugar component. The ^{1}H-NMR and ^{13}C-NMR spectra (C_5D_5N) of **1** revealed signals due to an anomeric H-atom of a glucopyranosyl moiety at δ(H) 4.91 (*d*, $J = 7.6$), and the signal due to an anomeric C-atom at δ(C) 103.5, respectively. From the coupling constant of the anomeric H-atom, the glucose was determined to be β-configurated.

Structure elucidation of the triterpene moiety was performed on the aglycone huangqiyegenin III (**3**), obtained as white amorphous powder. The molecular formula of **3** was found to be $C_{36}H_{56}O_9$ by HR-FAB-MS (positive-ion mode) and HR-EI-MS. Alkaline hydrolysis of **3** in 0.25N NaOH ($EtOH/H_2O$) solution, gave a triterpenoid named trideacetylhuangqiyegenin III (**5**). Trideacetylhuangqiyegenin III (**5**) was obtained as white amorphous powder. The molecular weight and molecular formula of **5** were determined to be 506 and $C_{30}H_{50}O_6$, respectively, by HR-FAB-MS (positive-ion mode) displaying a peak due to $[M+Na]^+$ at *m/z* 529.3495 (calc. 529.3505), and HR-EI-MS exhibiting a peak corresponding to $[M-H_2O]^+$ at *m/z* 488.3492 (calc. 488.3502), indicating six degrees of unsaturation. The ^{1}H-NMR spectrum of **5** showed signals due to five tertiary Me groups at δ(H) 1.10, 1.38, 1.43, 1.70, and 1.83 (Me(28), Me(30), Me(18), Me(29), and Me(27), resp.), a secondary Me group at δ(H) 1.64 (*d*,

$J = 6.7$; Me(21)), and a HO–CH_2 group at δ(H) 4.25 (CH_2(26)), along with signals due to four O-bearing CH groups and two olefinic H-atoms (*Table 1*). No signals due to H-atoms of a cyclopropane ring were observed. The ^{13}C-NMR spectrum of **5** showed the presence of six Me groups, a HO–CH_2 group, seven CH_2 groups, eight CH groups, thereof four O-bearing, four quaternary C-atoms, thereof one O-bearing, and two pairs of olefinic C-atoms assigned to trisubstituted C=C bonds (*Table 2*). These data suggested that trideacetylhuangqiyegenin III (**5**) was a tetracyclic triterpenoid. The ^{1}H- and ^{13}C-NMR signals were assigned by the use of DQF-COSY, HMQC, and HSQC-TOCSY, which indicated the presence of the partial structures shown in the *Figure*. The full connectivity was deduced from the HMBC correlations (*Figure*). In particular, key correlations from H_α–C(19) to C(5), C(8), C(9), C(10), and C(11), and H_β–C(19) to C(8), C(9), C(10), and C(11) showed that the CH_2(19) group must be located between C(9) and C(10). Furthermore, these data led us to suppose that **5** is a novel cycloartane type triterpenoid possessing a cycloheptane *B* ring arising from the cleavage of the 9,10-bond of cycloartane. This proposal was supported by other evidences. The coupling constants ($J = 15.5$, 1.2) of H_α–C(19) and H_β–C(19) indicated that C(19) was connected to two quaternary C-atoms (C(10) and C(9)), and the CH_2 H-atoms at C(19) showed allylic coupling with the H–C(11) H-atom. In the DQF-COSY spectrum, the olefinic H-atom signal due to H–C(11) at δ(H) 5.61 showed long-range coupling with the signals for H_α–C(19) and H_β–C(19) at δ(H) 2.66 and 3.16, respectively. The relative configuration of **5** was determined on the basis of H-atom coupling constants, nuclear *Overhauser* effect (NOE) experiments, and comparison of ^{13}C-NMR data of **5** with those of mongholicoside I, a compound previously isolated from *A. mongholicus* which possessed a (24*E*) side chain moiety [25]. In particular, the ^{13}C-NMR data of the side chain were almost identical in both compounds, which enabled the assignment of the C(24) = C(25) bond as (*E*) in **5**. The configuration at the C(3) position of **5** could

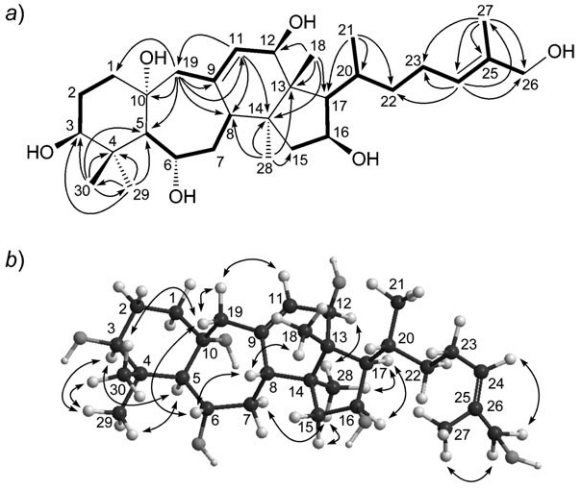


Figure. a) *^{1}H,^{1}H-COSY* (bold lines) *and key HMBC* (arrows) *correlations of* **5**. b) *Key NOE correlations of* **5**.

Table 1. *^{1}H-NMR Data* (in C_5D_5N) *of* **1**–**5**. At 500 MHz; δ in ppm, J in Hz.

Position	**1**	**2**	**3**	**4**	**5**
1α	1.86–1.94 (*m*)	1.92–1.99 (*m*)	1.83–1.90 (*m*)	1.93–1.99 (*m*)	1.92–1.98 (*m*)
1β	1.86–1.94 (*m*)	1.92–1.99 (*m*)	1.83–1.90 (*m*)	1.93–1.99 (*m*)	1.92–1.98 (*m*)
2α	1.71–1.79 (*m*)	1.70–1.77 (*m*)	1.64–1.72 (*m*)	1.71–1.79 (*m*)	1.80–1.87 (*m*)
2β	1.94–1.99 (*m*)	1.87–1.92 (*m*)	1.96–2.00 (*m*)	1.86–1.93 (*m*)	1.96–2.01 (*m*)
3	4.93 (*dd*, *J* = 7.0, 3.0)	4.95 (*dd*, *J* = 7.4, 2.5)	4.94 (*dd*, *J* = 10.0, 3.1)	4.95 (*dd*, *J* = 6.7, 2.5)	3.70 (*dd*, *J* = 10.3, 4.0)
5	2.29 (*d*, *J* = 6.1)	2.31 (*d*, *J* = 8.0)	2.30 (*d*, *J* = 5.6)	2.31 (*d*, *J* = 8.2)	2.23 (*d*, *J* = 4.6)
6	5.53 (*ddd*, *J* = 7.3, 6.1, 3.3)	5.54–5.62 (*m*)	5.52–5.60 (*m*)	5.54–5.62 (*m*)	4.41–4.49 (*m*)
7α	1.84–1.91 (*m*)	2.00–2.09 (*m*)	1.83–1.90 (*m*)	2.00–2.10 (*m*)	3.28–3.36 (*m*)
7β	1.84–1.91 (*m*)	2.00–2.09 (*m*)	1.83–1.90 (*m*)	2.00–2.10 (*m*)	1.92–1.98 (*m*)
8	2.55 (br. *d*, *J* = 9.0)	2.52 (br. *d*, *J* = 10.7)	2.56 (br. *d*, *J* = 10.2)	2.53 (br. *d*, *J* = 10.2)	2.56 (br. *d*, *J* = 8.7)
11	5.40 (br. *s*)	5.43 (br. *s*)	5.40 (br. *s*)	5.43 (br. *s*)	5.61 (br. *s*)
12α	5.61 (br. *s*)	2.00–2.09 (*m*)	5.64 (br. *s*)	2.00–2.10 (*m*)	4.41 (br. *s*)
12β	–	2.00–2.09 (*m*)	–	2.00–2.10 (*m*)	–
15α	2.20–2.26 (*m*)	2.14–2.24 (*m*)	2.26–2.36 (*m*)	2.16–2.28 (*m*)	2.18–2.26 (*m*)
15β	2.01–2.07 (*m*)	2.00–2.09 (*m*)	2.16–2.23 (*m*)	2.00–2.10 (*m*)	1.99–2.09 (*m*)
16	4.62–4.68 (*m*)	4.58–4.67 (*m*)	4.64–4.72 (*m*)	4.66 (*ddd*, *J* = 11.3, 7.6, 4.2)	4.68–4.76 (*m*)
17	2.07–2.11 (*m*)	1.77–1.84 (*m*)	2.06–2.11 (*m*)	1.78–1.86 (*m*)	2.18–2.26 (*m*)
18	1.38 (*s*)	1.27 (*s*)	1.39 (*s*)	1.28 (*s*)	1.43 (*s*)
19α	2.57 (*dd*, *J* = 15.8, 1.5)	2.56 (*dd*, *J* = 15.0, 1.5)	2.59 (*dd*, *J* = 15.0, 1.2)	2.55 (*dd*, *J* = 15.0, 1.5)	2.66 (*dd*, *J* = 15.5, 1.2)
19β	2.97 (*dd*, *J* = 15.8, 1.5)	2.92–2.98 (*m*)	2.98 (*dd*, *J* = 15.0, 1.2)	2.92–2.99 (*m*)	3.16 (*dd*, *J* = 15.5, 1.2)
20	2.26–2.33 (*m*)	2.28–2.36 (*m*)	2.26–2.36 (*m*)	2.32–2.39 (*m*)	2.33–2.37 (*m*)
21	1.16 (*d*, *J* = 6.5)	1.06 (*d*, *J* = 6.5)	1.17 (*d*, *J* = 5.3)	1.10 (*d*, *J* = 6.6)	1.64 (*d*, *J* = 6.7)
22	2.05–2.08 (*m*), 1.27–1.32 (*m*)	2.09–2.14 (*m*), 1.33–1.37 (*m*)	2.11–2.16 (*m*), 1.29–1.35 (*m*)	2.10–2.16 (*m*), 1.34–1.39 (*m*)	2.26–2.33 (*m*), 1.48–1.54 (*m*)
23	2.26–2.33 (*m*), 2.20–2.26 (*m*)	2.28–2.36 (*m*), 2.14–2.24 (*m*)	2.26–2.36 (*m*), 2.16–2.23 (*m*)	2.29–2.34 (*m*), 2.16–2.28 (*m*)	2.37–2.43 (*m*), 2.26–2.33 (*m*)
24	5.73 (*t*, *J* = 6.6)	5.73 (*t*, *J* = 6.6)	5.82 (*t*, *J* = 6.0)	5.81 (*t*, *J* = 6.2)	5.82 (*dt*, *J* = 7.0, 1.2)
26	4.50 (*d*, *J* = 11.5), 4.24 (*d*, *J* = 11.5)	4.50 (*d*, *J* = 11.5), 4.23 (*d*, *J* = 11.5)	4.30 (br. *s*), 4.30 (br. *s*)	4.29 (br. *s*), 4.29 (br. *s*)	4.25 (br. *s*), 4.25 (br. *s*)
27	1.81 (*s*)	1.80 (*s*)	1.85 (*s*)	1.83 (*s*)	1.83 (*s*)
28	0.99 (*s*)	0.89 (*s*)	1.01 (*s*)	0.90 (br. *s*)	1.10 (*s*)
29	1.14 (*s*)	1.18 (*s*)	1.15 (*s*)	1.18 (*s*)	1.70 (*s*)
30	1.17 (*s*)	1.18 (*s*)	1.18 (*s*)	1.18 (*s*)	1.38 (*s*)
3-AcO	2.10 (*s*)	2.06 (*s*)	2.06 (*s*)	2.06 (*s*)	–
6-AcO	2.09 (*s*)	2.05 (*s*)	2.06 (*s*)	2.05 (*s*)	–
12-AcO	2.19 (*s*)	–	2.19 (*s*)	–	–
β-D-Glucopyranoside:					
1	4.91 (*d*, *J* = 7.6)	4.91 (*d*, *J* = 7.8)	–	–	–
2	4.09 (*t*, *J* = 7.6)	4.08 (*t*, *J* = 7.8)	–	–	–
3	4.25–4.32 (*m*)	4.26–4.33 (*m*)	–	–	–
4	4.25–4.32 (*m*)	4.26–4.33 (*m*)	–	–	–
5	3.92–4.00 (*m*)	3.94–4.01 (*m*)	–	–	–
6	4.57 (*dd*, *J* = 11.0, 2.0), 4.41 (*dd*, *J* = 11.0, 4.6)	4.57 (*dd*, *J* = 11.5, 2.0), 4.40 (*dd*, *J* = 11.5, 5.0)	–	–	–

Table 2. *^{13}C-NMR Data* (in C_5D_5N) *of* **1**–**5**. At 125 MHz; δ(C) (multiplicity).

Position	**1**	**2**	**3**	**4**	**5**
1	43.0 (*t*)	44.0 (*t*)	44.3 (*t*)	44.1 (*t*)	44.1 (*t*)
2	24.9 (*t*)	24.8 (*t*)	25.2 (*t*)	24.8 (*t*)	29.0 (*t*)
3	79.7 (*d*)	79.7 (*d*)	79.7 (*d*)	79.7 (*d*)	77.9 (*d*)
4	39.4 (*s*)	39.3 (*s*)	39.4 (*s*)	39.3 (*s*)	40.8 (*s*)
5	57.8 (*d*)	57.9 (*d*)	57.7 (*d*)	57.8 (*d*)	62.1 (*d*)
6	76.6 (*d*)	77.3 (*d*)	76.6 (*d*)	77.2 (*d*)	73.1 (*d*)
7	32.7 (*t*)	32.9 (*t*)	32.7 (*t*)	32.9 (*t*)	37.7 (*t*)
8	43.3 (*d*)	43.6 (*d*)	44.0 (*d*)	43.8 (*d*)	43.9 (*d*)
9	140.0 (*s*)	136.8 (*s*)	140.0 (*s*)	136.8 (*s*)	138.1 (*s*)
10	72.2 (*s*)	72.6 (*s*)	72.2 (*s*)	72.6 (*s*)	72.9 (*s*)
11	125.8 (*d*)	124.2 (*d*)	125.7 (*d*)	123.8 (*d*)	130.7 (*d*)
12	77.2 (*d*)	38.3 (*t*)	77.2 (*d*)	38.3 (*t*)	72.8 (*d*)
13	48.8 (*s*)	44.9 (*s*)	48.8 (*s*)	44.9 (*s*)	49.2 (*s*)
14	50.0 (*s*)	46.9 (*s*)	50.0 (*s*)	46.9 (*s*)	50.2 (*s*)
15	47.0 (*t*)	47.2 (*t*)	47.0 (*t*)	47.6 (*t*)	47.5 (*t*)
16	70.7 (*d*)	70.8 (*d*)	70.7 (*d*)	70.7 (*d*)	71.3 (*d*)
17	56.7 (*d*)	55.8 (*d*)	56.7 (*d*)	55.8 (*d*)	56.9 (*d*)
18	12.0 (*q*)	16.5 (*q*)	12.0 (*q*)	16.5 (*q*)	11.1 (*q*)
19	47.7 (*t*)	48.0 (*t*)	47.1 (*t*)	48.3 (*t*)	47.8 (*t*)
20	30.3 (*d*)	30.8 (*d*)	30.3 (*d*)	30.8 (*d*)	30.3 (*d*)
21	18.6 (*q*)	18.2 (*q*)	18.6 (*q*)	18.2 (*q*)	19.5 (*q*)
22	37.2 (*t*)	36.5 (*t*)	37.2 (*t*)	36.4 (*t*)	37.1 (*t*)
23	25.9 (*t*)	25.6 (*t*)	25.8 (*t*)	25.5 (*t*)	25.6 (*t*)
24	129.4 (*d*)	129.4 (*d*)	125.9 (*d*)	125.7 (*d*)	125.9 (*d*)
25	131.9 (*s*)	131.9 (*s*)	135.9 (*s*)	135.9 (*s*)	135.3 (*s*)
26	75.2 (*t*)	75.2 (*t*)	68.2 (*t*)	68.2 (*t*)	68.0 (*t*)
27	14.2 (*q*)	14.2 (*q*)	14.0 (*q*)	14.0 (*q*)	13.5 (*q*)
28	18.6 (*q*)	18.2 (*q*)	18.5 (*q*)	18.2 (*q*)	18.5 (*q*)
29	28.4 (*q*)	29.0 (*q*)	28.3 (*q*)	28.8 (*q*)	30.0 (*q*)
30	18.6 (*q*)	18.2 (*q*)	18.6 (*q*)	18.4 (*q*)	18.4 (*q*)
3-AcO	21.8 (*q*), 170.3 (*s*)	21.8 (*q*), 170.3 (*s*)	21.7 (*q*), 170.3 (*s*)	21.8 (*q*), 170.3 (*s*)	
6-AcO	21.0 (*q*), 170.3 (*s*)	21.0 (*q*), 170.3 (*s*)	21.0 (*q*), 170.3 (*s*)	21.0 (*q*), 170.3 (*s*)	
12-AcO	21.7 (*q*), 170.7 (*s*)		21.7 (*q*), 170.8 (*s*)		
β-D-Glucopyranoside:					
1	103.5 (*d*)	103.6 (*d*)			
2	75.3 (*d*)	75.2 (*d*)			
3	78.7 (*d*)	78.6 (*d*)			
4	71.8 (*d*)	71.8 (*d*)			
5	78.5 (*d*)	78.4 (*d*)			
6	62.0 (*t*)	62.9 (*t*)			

be assigned as β, according to the coupling constant of H–C(3) at δ(H) 3.70 (*dd*, J = 10.3, 4.0). In the NOE difference spectrum of **5**, irradiation at Me(29) and Me(18) enhanced the signal intensities of H–C(3) and H–C(5), and of H–C(8), respectively. Irradiation at Me(28) and Me(30) enhanced the signal intensities of H–C(12), H–C(16), and H–C(17), and of H–C(6) and H_β–C(19), respectively. Irradiation at H–C(8) enhanced the signal intensities of H–C(6) and Me(18), and irradiation at

H_β–C(19) and H_α–C(19) enhanced the signal intensities of H_α–C(19) and Me(30), and of H_β–C(19) and H–C(11), respectively. From these data, H–C(3), H–C(5), H–C(12), H–C(16), H–C(17), and H_α–C(19) could be assigned as α-configurated, and H–C(6), H–C(8), and H_β–C(19) could be assigned as in β-configuration, respectively. In addition, the interaction of H_β–C(19) and Me(30) in the NOE difference spectrum indicated that the CH_2(19) group was β-oriented. Therefore, the OH group at C(10) could be assigned as α-configurated. Based on these results, the structure of **5** was established as (3*S*,5*R*,6*S*,10*R*,12β,16β,24*E*)-4,4,14-trimethyl-9,19-cyclo-9,10-secocholesta-9(11),24-diene-3,6,10,12,16,26-hexol.

To clarify the linking position of three AcO groups in huangqiyegenin III (**3**), HMBC and HSQC-TOCSY spectra were recorded. In the HMBC spectrum of **3**, the H-atoms due to H–C(3) (δ(H) 4.94) and H–C(12) (δ(H) 5.64) showed connectivity with the signals of the CO C-atoms of the AcO group at δ(C) 170.3 and the AcO group at δ(C) 170.8, respectively. By comparing the ^{1}H- and ^{13}C-NMR data of **3** with those of **5**, it was found that the chemical shifts of the signals due to H–C(16) and CH_2(26) of **3** were almost unchanged, while signals due to H–C(3), H–C(6), and H–C(12) were shifted downfield by 1.24, 1.11, and 1.23 ppm, respectively. At the same time, the chemical shifts due to C(10), C(16), and C(26) of **3** were almost unchanged, but the signals due to C(3), C(6), and C(12) of **3** were shifted downfield by 1.8, 3.5, and 4.4, respectively. Therefore, the three AcO groups of **3** should be linked to the 3-, 6-, and 12-position, respectively. Based on these results, the structure of **3** was deduced to be (3*S*,5*R*,6*S*,10*R*,12β,16β,24*E*)-10,16,26-trihydroxy-4,4,14-trimethyl-9,19-cyclo-9,10-secocholesta-9(11),24-diene-3,6,12-triyl triacetate.

All the signals of the ^{1}H- and ^{13}C-NMR spectra of huangqiyenin E (**1**) could be assigned by using DQF-COSY, HMQC, and HSQC-TOCSY (*Tables 1* and *2*). By comparison of the ^{13}C-NMR data of **1** with huangqiyegenin III (**3**), it was found that the signals of C(26) and C(24) were shifted downfield by 7.0 and 3.5 ppm, respectively, while the signal for C(25) was shifted highfield by 4.0 ppm, and other signals remained almost unchanged. This indicated that the glucose residue of **1** should be attached to C(26). Thus the structure of huangqiyenin E (**1**) was established as (3*S*,5*R*,6*S*,10*R*,12β,16β,24*E*)-26-(β-D-glucopyranosyloxy)-10,16-dihydroxy-4,4,14-trimethyl-9,19-cyclo-9,10-secocholesta-9(11),24-diene-3,6,12-triyl triacetate.

Compound **2**, named huangqiyenin F, was obtained as a white amorphous powder. The molecular weight and molecular formula of **2** were determined to be 736 and $C_{40}H_{64}O_{12}$, respectively, by HR-FAB-MS (positive-ion mode), indicating nine degrees of unsaturation. Enzymatic hydrolysis of **2** with the same method as described for **1** gave a new triterpenoidal sapogenin named huangqiyegenin IV (**4**) and D-glucose. The ^{1}H- and ^{13}C-NMR data of **2** revealed the presence of an anomeric H-atom at δ(H) 4.91 (*d*, $J = 7.8$) and an anomeric C-atom at δ(C) 103.6 ppm, respectively. The coupling constant revealed the β-configuration of the glucosyl residue.

Structure elucidation of the triterpene moiety was performed on the aglycone huangqiyegenin IV (**4**), obtained as a white amorphous powder after enzymatic hydrosis. The molecular weight and molecular formula of **4** were found to be 574 and $C_{34}H_{54}O_7$ by HR-FAB-MS (positive-ion mode) and HR-EI-MS, indicating eight degrees of unsaturation. The ^{1}H- and ^{13}C-NMR data of **4** were basically similar to those of **3**, except for the signals due to the *C* ring. However, the number of CH_2 groups of **4**

is one more than that of **3**, and the number of O-bearing CH and AcO groups of **4** is one less than that of **3**. In addition, the molecular weight of **4** is 58 mass units less than that of **3**, as shown in the HR-FAB-MS (positive-ion mode) spectrum. Besides, it was found that the signals due to C(9), C(11), C(12), C(13), and C(14) in the *C* ring of **4** were shifted upfield by 3.2, 1.9, 38.9, 3.9, and 3.1 ppm compared to **3**, respectively, while the other C-atoms of **4** showed almost identical chemical shifts. In the HMBC spectrum of **4**, the olefinic H-atom signal due to H–C(11) at δ(H) 5.43 showed correlations with the C-atom signals at δ(C) 38.3 (*t*, C(12)), and 44.9 (*s*, C(13)), respectively. Those data indicated that C(12) of **4** is a CH_2 group. From above evidence, the structure of huangqiyegenin IV (**4**) was established as (3*S*,5*R*,6*S*,10*R*,16β,24*E*)-10,16,26-trihydroxy-4,4,14-trimethyl-9,19-cyclo-9,10-secocholesta-9(11),24-diene-3,6-diyl diacetate.

By comparison of the ^{13}C-NMR data of **2** with huangqiyegenin IV (**4**), it was found that the signals of C(26) and C(24) were shifted downfield by 7.0 and 3.7 ppm, respectively, while the signal for C(25) was shifted upfield by 4.0 ppm, and the other signals remained almost unchanged. This indicated that the β-D-glucose of **2** should be attached to C(26). Thus **2** was established as (3*S*,5*R*,6*S*,10*R*,16β,24*E*)-26-(β-D-glucopyranosyloxy)-10,16-dihydroxy-4,4,14-trimethyl-9,19-cyclo-9,10-secocholesta-9(11),24-diene-3,6-diyl diacetate.

Experimental Part

General. Anal. TLC (Kieselgel *60 F_{254}*, *Merck*, Germany). Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, *Kieselgel 60*, *Merck*, Germany). Prep. HPLC: *Waters Delta-600 pump* (*Waters 2414* refractive index detector); *TSK-gel ODS-120T* (10 µm, 40 × 300 mm, *Tosoh*, Japan). Optical rotations: *Union PM-101*. IR Spectra: *Shimadzu FTIR-8400S*. NMR Spectra: *JEOL LA-500 NMR* spectrometer; at 500 MHz for (^{1}H) and 125 MHz (^{13}C); chemical shifts δ in ppm rel. to Me_4Si as internal standard, coupling constant *J* in Hz. FAB-MS, HR-FAB-MS, and HR-EI-MS: *Finnigan MAT8430* (Matrix: glycerol), *VG Autospec-3000 spectrometer* (Matrix: glycerol) and *JEOL JMS DX-302* mass spectrometer, resp.; in *m*/*z*.

Plant Material. The leaves of *A. membranaceus* BUNGE were collected in Daxing'anling, Heilongjiang Province, P. R. China, in August 1995, and identified by Prof. *Zhenyue Wang*. A voucher specimen (No. 19950026) is deposited with the Herbarium of Heilongjiang University of Chinese Medicine, P. R. China.

Extraction and Isolation. The air dried leaves of *A. membranaceus* BUNGE (1.2 kg) were defatted with petroleum ether (PE; 5 l, 2 ×) for 2 h each time (80°). Then the residue was extracted with MeOH under reflux (5 l, 3 ×) for 3 h each time and the extract was concentrated *in vacuo* to a syrup. A suspension of the MeOH extract (310 g) in H_2O (5 l) was extracted in BuOH (5 l, 3 ×) sat. with H_2O. The BuOH layer was concentrated to dryness *in vacuo*, to give the BuOH extract (119 g). A portion of the BuOH extract (60 g) was chromatographed over a SiO_2 column with $CHCl_3$/MeOH/H_2O 6:1:0.1 and then $CHCl_3$/MeOH/H_2O (3:1:0.1) to give ten fractions, *Frs. I–X*, in order of elution. *Fr. III* (10.5 g) was rechromatographed over SiO_2 with $CHCl_3$/MeOH/H_2O 5:1:0.1 and separated further by prep. HPLC using a *TSK*-gel *ODS-120T* column (10 µm, 40 × 300 mm, flow rate 8 ml/min) with MeOH/H_2O 3:7 to give **1** (205 mg; t_R 15.5 min) and **2** (140 mg; t_R 22.3 min), resp.

Enzymatic Hydrolysis of **1** *and* **2**. Huangqiyenins E (**1**) (58 mg) and F (**2**) (65 mg) in H_2O (10 ml) were incubated at 40° for 36 h with cellulase (100 mg, *Onozuka R-10*, 2900u/g, No. L0012, Yakult, Japan). The mixtures were extracted with AcOEt (5 ml, 3 ×) and then the AcOEt layers were evaporated to dryness. The products were chromatographed over a SiO_2 column with AcOEt/MeOH 15:1: **1** gave huangqiyegenin III (**3**) (21 mg), and **2** gave huangqiyegenin IV (**4**) (25 mg). The sugar was confirmed as D-glucose in the H_2O layers by comparison with an authentic sample on TLC (BuOH/AcOH/H_2O 4:1:5;

upper layer, R_f 0.14, spray with naphthalen-1-ol/H_2SO_4 acid) and by measuring its optical rotation ($[\alpha]_D^{20} = +45.6$ (**1**), $+43.2$ (**2**)).

Alkaline Hydrolysis of **3**. Huangqiyegenin III (**3**) (15 mg) in 0.25N NaOH (EtOH/H_2O 1:1; 3 ml) was refluxed at 80° for 3 h. The mixture was extracted with AcOEt, and then the AcOEt layer was evaporated to dryness. The product was chromatographed on prep. HPLC using an *Aquasil SS-652N* column with AcOEt/MeOH (15:1), to give **5** (5 mg), which was named trideacetylhuangqiyegenin III.

Huangqiyenin E (=*(3S,5R,6S,10R,12β,16β,24E)-26-(β-*D*-Glucopyranosyloxy)-10,16-dihydroxy-4,4,14-trimethyl-9,19-cyclo-9,10-secocholesta-9(11),24-diene-3,6,12-triyl Triacetate*; **1**). White amorphous powder. $[\alpha]_D^{20} = +62.3$ ($c = 0.5$, MeOH). IR (KBr): 3435, 2949, 2878, 1728, 1713, 1375, 1248, 1188, 1163, 1078, 1022. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (pos.): 817 ($[M+Na]^+$), 833 ($[M+K]^+$). HR-FAB-MS (pos.): 817.4343 ($[M+Na]^+$, $C_{42}H_{66}NaO_{14}^+$; calc. 817.4350).

Huangqiyenin F (=*(3S,5R,6S,10R,16β,24E)-26-(β-*D*-Glucopyranosyloxy)-10,16-dihydroxy-4,4,14-trimethyl-9,19-cyclo-9,10-secocholesta-9(11),24-diene-3,6-diyl Diacetate*; **2**). White amorphous powder. $[\alpha]_D^{20} = +66.3$ ($c = 0.4$, MeOH). IR (KBr): 3420, 2937, 2887, 1717, 1701, 1373, 1256, 1190, 1161, 1078, 1024. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (pos.): 775 ($[M+K]^+$), 759 ($[M+Na]^+$). HR-FAB-MS (pos.): 759.4276 ($[M+Na]^+$, $C_{40}H_{64}NaO_{12}^+$; calc. 759.4296).

Huangqiyegenin III (=*(3S,5R,6S,10R,12β,16β,24E)-10,16,26-Trihydroxy-4,4,14-trimethyl-9,19-cyclo-9,10-secocholesta-9(11),24-diene-3,6,12-triyl Triacetate*; **3**). White amorphous powder. $[\alpha]_D^{20} = +12.0$ ($c =$ 0.13, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-FAB-MS (pos.): 655.3804 ($[M+Na]^+$, $C_{36}H_{56}NaO_9^+$; calc. 655.3822). HR-EI-MS (pos.): 614.3815 ($[M-H_2O]^+$, $C_{36}H_{54}O_8^+$; calc. 614.38187).

Huangqiyegenin IV (=*(3S,5R,6S,10R,16β,24E)-10,16,26-Trihydroxy-4,4,14-trimethyl-9,19-cyclo-9,10-secocholesta-9(11),24-diene-3,6-diyl Diacetate*; **4**). White amorphous powder. $[\alpha]_D^{20} = +8.1$ ($c =$ 0.09, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-FAB-MS (pos.): 597.3765 ($[M+Na]^+$, $C_{34}H_{54}NaO_7^+$; calc. 597.3767). HR-EI-MS (pos.): 556.3770 ($[M-H_2O]^+$, $C_{34}H_{52}O_6^+$; calc. 556.3764).

Trideacetylhuangqiyegenin III (=*(3S,5R,6S,10R,12β,16β,24E)-4,4,14-Trimethyl-9,19-cyclo-9,10-secocholesta-9(11),24-diene-3,6,10,12,16,26-hexol*; **5**). White amorphous powder. $[\alpha]_D^{20} = +8.9$ ($c = 0.10$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-FAB-MS (pos.): 529.3495 ($[M+Na]^+$, $C_{30}H_{50}NaO_6^+$; calc. 529.3505). HR-EI-MS (pos.): 488.3492 ($[M-H_2O]^+$, $C_{30}H_{48}O_5^+$; calc. 488.3502).

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