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CONSTITUENTS OF *ASTRAGALUS MEMBRANACEUS*

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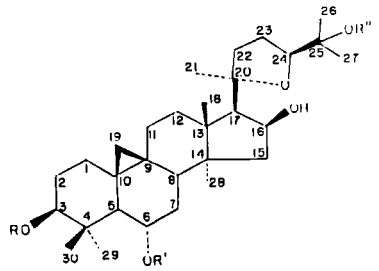
ABSTRACT.—Two glycosides have been isolated from *Astragalus membranaceus* var. *mongholicus*, and their structures have been determined to be 3-*O*-β-D-xylopyranosyl-25-*O*-β-D-glucopyranosyl-cycloastragenol and (3*R*)-(−)-7,2′-dihydroxy-3′,4′-dimethyl isoflavan-7-*O*-β-D-glucopyranoside by spectroscopic means and chemical transformations.

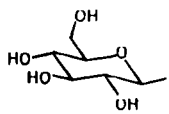
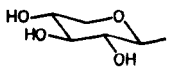
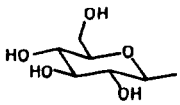
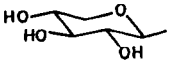
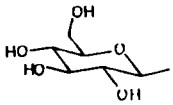
Astragalus membranaceus Bge. var. *mongholicus* (Bge.) Hsiao (Leguminosae) is a medicinal plant frequently used as a traditional medicine in China, Japan, Korea, and Southeast Asia. It has been reported (1) to strengthen superficial resistance and to promote the discharge of pus and the growth of new tissue. The crude extract has shown significant antiviral and immunostimulant activities and inhibits the activities of murine retroviral reverse transcriptase and human DNA polymerases (2).

The previous studies of chemical constituents from this species have resulted in the isolation of saponins, flavonoids, polysaccharides, free amino acids (3,4), and an isoflavan glycoside (5).

During our study of the polar constituents, we have isolated a new saponin, isoastragaloside IV [**1**], which is an isomer of the previously reported astragaloside IV [**2**] (6) together with an isoflavan glycoside **5** from the *n*-BuOH-soluble portion of the EtOH extract. In this paper, we report the isolation and structure elucidation by spectroscopic means and chemical transformation of these two compounds.

Isoastragaloside IV [**1**] shows almost the same polarity as astragaloside IV [**2**] by co-tlc comparison. Its molecular formula, $C_{41}H_{68}O_{14}$, was determined by fabms which displayed prominent pseudomolecular ions at *m/z* 807 $[M + Na]^+$ and 785 $[M + H]^+$.



	R	R′	R″
1		H	
2			H
3		H	H
4	H	H	

The ^{1}H-nmr spectrum of **1** showed two anomeric doublets at δ 4.90 ppm ($J = 7.0$ Hz) and δ 5.06 ppm ($J = 7.5$ Hz) in the downfield region, indicative of the presence of two β-linked sugars (6,8). The upfield region was generally similar to that of astragaloside IV [**2**]. Two doublets at δ 0.28 ppm and δ 0.55 ppm for cyclopropane-

TABLE 1. ^{13}C-nmr Data for Compounds **1–4** in Pyridine-d_5.[a]

Carbon	Compound			
	1	**2**[b]	**3**[c]	**4**[d]
C-1	32.4	32.6	32.5	
C-2	30.3	30.5	30.4	
C-3	88.7	88.7	88.7	78.3
C-4	42.6	42.8	42.7	
C-5	53.7	53.7	54.1	53.9
C-6	68.1	79.4	68.0	68.3
C-7	38.4	39.1	38.7	
C-8	47.1	47.1	47.1	
C-9	20.8	21.3	21.0	
C-10	29.9	30.5	29.9	
C-11	26.3	26.4	26.3	
C-12	33.3	33.6	33.4	
C-13	45.1	45.2	45.0	
C-14	46.0	45.9	46.1	
C-15	46.8	46.4	46.7	
C-16	73.4	73.6	73.4	73.5
C-17	58.0	58.4	58.4	58.2
C-18	21.6	21.4	21.5	
C-19	30.8	31.6	30.6	
C-20	87.3	87.4	87.3	87.2
C-21	27.9	27.3	28.6	
C-22	35.0	35.1	34.9	
C-23	25.9	26.7	26.5	
C-24	82.1	81.8	81.7	82.1
C-25	78.2	71.4	71.3	78.6
C-26	23.0	28.0	27.2	
C-27	25.7	28.8	28.2	
C-28	19.9	20.1	20.2	
C-29	28.8	29.2	29.0	
C-30	16.6	16.9	16.7	
xylose				
C-1′	107.4	107.7	107.7	
C-2′	75.3	75.7	75.7	
C-3′	78.1	78.2	78.6	
C-4′	71.0	71.4	71.3	
C-5′	66.8	67.1	67.1	
glucose				
C-1″	98.6	105.2		98.8
C-2″	74.7	75.7		75.1
C-3″	78.8	79.2		78.4
C-4″	71.1	71.9		71.4
C-5″	77.7	78.6		77.8
C-6″	62.4	63.2		62.8

[a]Carbon signals affected by glycosylation are underlined.
[b]For partial assignment see Kitagawa *et al.* (6).
[c]For partial assignment see Kitagawa *et al.* (6,7).
[d]Data from Kitagawa *et al.* (6).

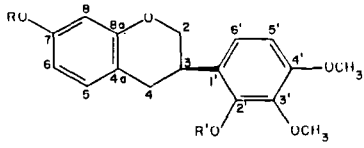
methylene protons, and seven methyl singlets at δ 0.93 ppm, δ 1.27 ppm, δ 1.30 ppm, δ 1.33 ppm, δ 1.42 ppm, δ 1.66 ppm, and δ 1.99 ppm were consistent with a cycloastragenol aglycone moiety (8).

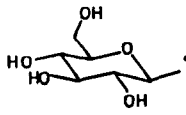
The ^{13}C-nmr spectrum of **1** displayed a total of 41 carbon signals. Based on a DEPT experiment and comparison with ^{13}C-nmr data of related compounds **2** (6,7), **3** (6), and **4** (7), all signals could be assigned as in Table 1. The xylose unit and glucose unit of **1** are attached to C-3 and C-25, respectively, in view of coincident ^{13}C chemical shifts for C-3 (of **3**) and C-25 (of **4**). The anomeric carbon signal at δ 98.6 ppm also indicated the glucose unit must be attached to C-25 by comparison with compounds **2** and **4**. The glycosylation also affected signals C-26 and C-27 of **1** as observed in asernesteroside C (8). Thus, isoastragaloside IV is formulated as 3-*O*-β-D-xylopyranosyl-25-*O*-β-D-glucopyranosyl-cycloastragenol [**1**].

The high resolution ei mass spectrum of compound **5** displayed a weak molecular ion at *m/z* 464.1698 (calcd 464.1682) corresponding to $C_{23}H_{28}O_{10}$ and a base peak at *m/z* 302.1159 consistent with loss of a sugar moiety ($C_6H_{10}O_5$). Prominent ions at *m/z* 180.0787 ($C_{10}H_{12}O_3$) and 167.0707 ($C_9H_{11}O_3$) were indicative of an isoflavan B ring substituted with one OH and two OMe groups (9–12).

The ^{1}H-nmr spectrum of **5** in DMSO-d_6 (Table 2) revealed the presence of two methoxyls and five aromatic protons, two of which corresponded to an AB system with ortho couplings in a tetrasubstituted benzene ring, while the remainder constituted an ABX system for a 1,2,4-trisubstituted benzene ring. Other signals were consistent with protons at C-2, C-3, and C-4 of an isoflavan system (12–14) and a sugar moiety (15). In nOe experiments a 1% enhancement of the δ 3.68 (3′-OMe) signal was observed upon irradiation at δ 8.96 (2′-OH), while a 3% enhancement of the δ 3.75 (4′-OMe) signal was noted when δ 6.46 (H-5′) was irradiated. Thus, the positions of substituents in the tetrasubstituted aromatic ring were established.

To determine the structure of the aglycone, **5** was hydrolyzed by 2 N HCl to yield D-glucose and (3*R*)-(−)-7,2′-dihydroxy-3,4-dimethoxy isoflavan [**6**], whose ^{1}H-nmr

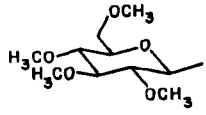


5 R=  , R′=H

6 R=R′=H

7 R=R′=Ac

8 R=H, R′=Ac

9 R=  , R′=Me

10 R=H, R′=Me

TABLE 2. ^{1}H-nmr Data for Compounds **5–10** (δ values in ppm).

Proton	Compound						
	5[a]	**6**		**7**	**8**	**9**	**10**
	DMSO-d_6	$CDCl_3$	$(CD_3)_2CO$	$CDCl_3$		$CDCl_3$	$CDCl_3$
H-2	3.96 t (10)	4.11	3.99	3.96	3.94	3.99	3.98
	4.20 dq (10,2)	4.33	4.25	4.28	4.25	4.28	4.29
H-3	3.48 m	3.52	3.49	3.26	3.25	3.50	3.50
H-4	2.89 m	2.95	2.90	2.93	2.88	2.90	2.90
H-5	6.99 d (8)	6.93	6.90	7.06	6.92	6.97	6.93
H-6	6.55 dd (8,2)	6.40	6.36	6.63	6.40	6.59	6.42
H-8	6.47 d (2)	6.38	6.28	6.60	6.37	6.58	6.39
H-5′	6.46 d (9)	6.44	6.51	6.83	6.82	6.65	6.67
H-6′	6.79 d (9)	6.78	6.84	6.89	6.88	6.79	6.81
H-1″	4.76 d (7)					4.79	
OMe	3.75 s	3.92	3.83	3.84	3.84	3.90, 3.88	3.90
	3.68 s	3.85	3.79	3.86	3.86	3.85, 3.65 (×2)	3.88
						3.55, 3.39	3.55
OAc				2.29	2.35		
				2.35			
OH	8.96 s, 5.31 d (5), 5.05 d (4), 5.12 d (5), 4.58 t (5)						

[a]*J* values in Hz in parentheses.

spectra and ei mass spectra were identical with reported data (12,13). Compound **6** was treated with pyridine and Ac_2O and readily converted to the diacetate **7** and the monoacetate **8**. The diacetate **7** was identical with an authentic sample of (3*R*)-(−)-isomucronulatol diacetate in ^{1}H nmr, ei mass spectral, and tlc behavior. The aglycone **6**, first isolated from *Glycyrrhizia glabra* as a phytoalexin (9), has been synthesized in racemic form (16).

The location of the β-D-glucopyranosyl unit at C-7 in **5** was confirmed by permethylation of **5** to give permethylated product **9**, and the hydrolysis of **9** gave 2,3,4,6-tetramethyl glucose and a new product 7-hydroxy-2′,3′,4′-trimethyl isoflavan [**10**].

The ^{13}C-nmr chemical shift assignments were facilitated by comparison with those of related isoflavans (17, 18). To date no ^{13}C-nmr data for isomucronulatol or its structural isomers, mucronulatol and laxifloran, have been reported.

Thus, compound **5** is formulated as (3*R*)-(−)-7,2′-dihydroxy-3′,4′-dimethylisoflavan-7-*O*-β-D-glucopyranoside. This structure has been proposed for an isoflavan glycoside isolated from *Astragalus mongholicus* by G. Lu and co-workers (5); however, the data presented do not permit an unambiguous structural assignment in our view, and the mp (158–163°) differs significantly from ours (145–147°). It is possible that the compound isolated by Lu *et al.* is the racemate of **5** because no optical rotation was reported.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined using a Kofler hot stage instrument and are uncorrected. The uv spectra were measured on a Perkin-Elmer Lambda 5 spectrometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Nmr spectra were recorded on a Varian XL-200 instrument using TMS as internal standard. Mass spectra were obtained with MAT 711 or Kratos MS-50 instruments.

TABLE 3. ^{13}C-nmr Data for Compounds **5**, **6**, and **10**.

Carbon	Compound		
	5	**6**	**10**
	DMSO-d_6	$CDCl_3$	$CDCl_3$
C-2	69.12	69.68	70.44
C-3	31.19	32.07	31.76
C-4	29.60	30.11	31.29
C-4a	115.67	114.62	114.59
C-5	129.97	130.38	130.37
C-6	108.68	107.84	107.92
C-7	156.61	155.17	155.12
C-8	103.06	103.18	103.21
C-8a	154.36	154.78	154.96
C-1′	120.70	120.20	127.27
C-2′	148.02	147.41	152.63
C-3′	135.98	135.32	142.31
C-4′	151.55	151.07	151.94
C-5′	103.72	103.59	107.40
C-6′	121.38	121.80	121.39
OMe	60.14	60.95	61.32
	55.50	55.77	60.75
			55.98
C-1″	100.55		
C-2″	73.12		
C-3″	76.89		
C-4″	69.61		
C-5″	76.75		
C-6″	60.61		

PLANT MATERIAL.—The plant samples of *A. membranaceus* var. *mongholicus* used in this study were collected in Inner Mongolia, China. A voucher specimen is kept at the Herbarium of NICPBP, Beijing.

EXTRACTION AND ISOLATION.—The air-dried roots (6 kg) were cut into small pieces and extracted with 95% EtOH under reflux. The aqueous solution was extracted with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH successively to yield the extracts of *n*-hexane (40 g), $CHCl_3$ (20 g), EtOAc (20 g), and *n*-BuOH (50 g).

The $CHCl_3$ extract (5 g) was subjected to repeated Si gel chromatography to give known compounds glycerol monopalmitate (30 mg), cycloastragenol (5 mg), (3*R*)-(−)-isomucronulatol (20 mg), formononetin (10 mg), and calycosin (20 mg), all identified by comparison of spectral data with literature values.

The *n*-BuOH extract (5 g) was subjected to repeated Si gel chromatography to yield previously reported astragalosides I (40 mg), II (20 mg), and IV (70 mg) [**2**] (6), and compounds **3** (25 mg), **1** (10 mg), and **5** (40 mg).

Isoastragaloside IV [**1**].—Colorless powder, mp 279–283°, $[\alpha]_D$ +17° (c = 0.4, H_2O); ^{1}H nmr (pyridine-d_5) δ ppm 0.28 (1H, d, J = 3.8 Hz, H-19a), 0.55 (1H, d, J = 3.8 Hz, H-19b), 0.93 (3H, s, H-28), 1.27, 1.30, 1.33, 1.42 (12H, 4s, H-21, H-18, H-26, H-30), 1.66 (3H, s, H-27), 1.99 (3H, s, H-29), 4.90 (1H, d, J = 7.0 Hz, H-1′), 4.90 (1H, m, H-16), 5.06 (1H, d, J = 7.5 Hz, H-1″); ^{13}C nmr see Table 1; fabms (glycerol) *m/z* $[M+Na]^+$ 807, $[M+H]^+$ 785.

Compound **5**.—Colorless needles, mp 145–147° (MeOH); uv λ max nm (ε) 276 (4650); $[\alpha]_D$ −14° (c = 0.1, EtOH); hreims *m/z* (%) $[M]^+$ 464.1698 (8), $[M-\text{glucose unit}]^+$ 302.1159 (100), 180.0787 (90), 168.0782 (52), 167.0707 (43), 165.0554 (8), 151.0637 (9), 123.0449 (12); fabms (glycerol) $[M+Na]^+$ 487, $[M+H]^+$ 465; ^{1}H nmr see Table 2; ^{13}C nmr see Table 3.

Hydrolysis of **5**.—Compound **5** (9.4 mg) was dissolved in 20 ml 2 N HCl and heated at 90° for 10 h. The mixture was extracted with $CHCl_3$ (20 ml × 3). The $CHCl_3$ layer was dried with anhydrous Na_2SO_4 and evaporated at 40°. The residue was recrystallized with MeOH to yield an aglycone **6** as colorless crystals (5 mg).

Compound **6**.—Mp 146–148°; $[\alpha]_D$ −16° (c = 0.1, EtOH) (12); ^{1}H nmr see Table 2; ^{13}C nmr see Table 3.

Acetylation of **6**.—Compound **6** (4 mg) was treated with pyridine (2 ml) and Ac_2O (2 ml) overnight. The mixture was chromatographed on a Si gel plate (0.5 mm) using $CHCl_3$-MeOH (19:1) as solvent to yield the monoacetate **8** (1 mg) and the diacetate **7** (3 mg): ^{1}H nmr see Table 2.

Permethylation of **5**.—Compound **5** (17 mg) was added to dimsyl carbanion solution (10 ml), and the mixture was stirred at room temperature for 1 h under N_2. The reaction mixture was treated with MeI (3.5 ml) under ice-cooling, stirred at room temperature in the dark for 1 h, and poured into cold H_2O (20 ml). The whole mixture was extracted with $CHCl_3$ (20 ml × 3). The evaporated $CHCl_3$ layer gave a permethylated product **9** (16 mg) after chromatography on a Si gel column. For ^{1}H-nmr data, see Table 2. Eims *m/z* (%) $[M]^+$ 534 ($C_{28}H_{38}O_{10}$) (22), [M − tetramethyl glucose unit]$^+$ 316 (35), [M − aglycone]$^+$ 218 (100), 194 (29), 187 (17), 181 (19), 179 (14), 147 (9), 116 (24), 111 (16), 101 (19).

Hydrolysis of **9**.—Compound **9** (15 mg) was heated at 90° with 2 N HCl for 10 h. The mixture was evaporated to dryness and chromatographed on a Si gel column to give compound **10**. ^{1}H- and ^{13}C-nmr data for **10** are given in Tables 2 and 3. Eims *m/z* (%) $[M]^+$ 316 (100), 194 (70), 182 (55), 181 (36), 179 (40), 167 (11), 149 (24), 135 (20).

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