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FUROSTANOL GLYCOSIDES FROM LEAVES OF THE CHINESE PLANT *Tribulus terrestris*

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*The structures of five furostanol glycosides (**1–5**), of which the 26-*O*-β-*D*-glucopyranosyl-(25S),5α-furost-20(22)-en-12-one-2α,3β,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside (**1**) was new, from the leaves of* Tribulus terrestris *L. were established using chemical and NMR spectroscopic methods.*

Keywords: *Tribulus terrestris* L., Zygophyllaceae, furostanol glycosides.

Tribulus terrestris L. is an annual creeping herb widespread in China. It is also distributed in Japan, Korea, the western part of Asia, the southern part of Europe, and Africa. In traditional Chinese medicine, the fruit of *T. terrestris* is used for treating eye problems, edema, abdominal distention, high blood pressure, and cardiovascular diseases [1]. In the previous studies, several steroidal saponins and flavonoid constituents were isolated from the fruit and leaf of this plant [2–5]. Later, much more attention was paid to studies of the chemical components of *T. terrestris*, and more than 30 steroidal saponins have been isolated from this plant [6–9]. Recently, the crude saponin fraction of the leaves of this plant has been used as a cordial drug. In this paper, we report the structure of the five furostanol glycosides from the dried leaves of this plant.

Compounds **2–5** were identified as 26-*O*-*β*-D-glucopyranosyl-(25*R*),5*α*-furost-20(22)-en-12-one-3*β*,26-diol-3-*O*-*β*-D-galactopyranosyl(1→2)-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**2**) [6], 26-*O*-*β*-D-glucopyranosyl-(25*R*,*S*),5*α*-furost-20(22)-en-12-one-3*β*,26-diol-3-*O*-*β*-D-glucopyranosyl(1→4)-*β*-D-galactopyranoside (**3**) [7], 26-*O*-*β*-D-glucopyranosyl-(25*R*),5*α*-furost-12-one-3*β*,22*α*,26-triol-3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-galactopyranoside (**4**) [7], and 26-*O*-*β*-D-glucopyranosyl-(25*R*),5*α*-furost-22-methoxy-3*β*,26-diol-3-*O*-[*β*-xylopyranosyl(1→2)-[*β*-xylopyranosyl(1→3)]-*β*-D-glucopyranosyl-(1→4)-[*α*-rhamnopyranosyl(1→2)]-*β*-D-galactopyranoside (**5**) [8], respectively, which were isolated from the fruits of *Tribulus terrestris* L. Compounds **2–5** were identified by comparison of the ESI-MS, ^{13}C NMR, melting point (mp), and optical rotation data ($[\alpha]_D$) with reference data. They were all isolated for the first time from the leaves of *Tribulus terrestris* L.

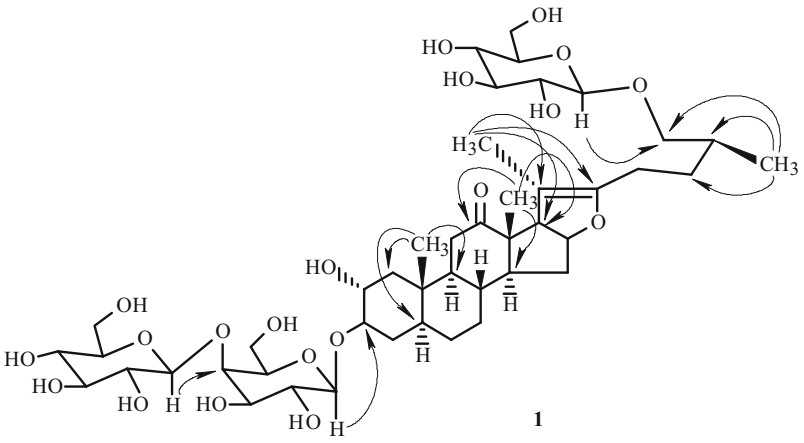

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Fig. 1. Key HMBC correlations (H→C) Compound **1**.

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TABLE 1. Chemical Shifts for ^{13}C Atoms of Aglycons of Compounds **1–5** and Protons of the Aglycon of Compound **1** (C_5D_5N, 0 = TMS, δ, ppm, J/Hz)

C atom	1		2	3	4	5
	δ_C	δ_H	δ_C			
1	45.34		36.84	38.14	36.65	37.30
2	70.32	3.80	29.91	29.99	29.68	29.82
3	84.50	3.69	78.17	78.17	78.53	77.10
4	33.94		34.80	36.08	34.37	34.13
5	44.60		44.63	46.63	44.59	44.56
6	27.96		28.74	29.74	27.94	28.87
7	31.47		31.99	32.29	31.80	32.23
8	33.99		34.23	35.23	34.37	34.96
9	55.58		59.69	57.69	55.78	54.93
10	37.42		36.46	37.46	36.91	36.11
11	38.44		38.33	39.33	37.60	21.12
12	212.75		213.02	214.32	213.02	39.91
13	57.71		57.70	58.70	55.60	41.14
14	54.16		54.31	56.11	55.78	56.23
15	33.85		33.85	34.85	32.03	32.01
16	83.13	4.62	83.32	84.32	80.20	81.30
17	56.38	3.29	56.37	56.37	55.09	64.27
18	14.32	0.80	14.31	14.81	16.39	16.35
19	13.00	0.63	11.88	12.28	11.64	12.38
20	103.57		103.28	104.28	41.06	40.46
21	11.77	1.61	11.77	11.77	15.36	16.10
22	153.30		153.30	154.30	110.88	112.50
23	23.85		23.83	24.23	37.23	30.71
24	31.56		31.45	33.33	28.50	28.09
25	33.63		33.93	34.29	34.37	34.15
26	75.09	3.48, 4.06	75.26	75.95	75.19	75.06
27	17.45	0.90 (J = 6.2)	17.25	17.80	17.45	17.08

Compound **1**, obtained as a white powder, showed a red coloration with Ehrlich reagent. The IR spectrum showed absorptions for hydroxyl groups (3420 cm^{-1}), carbonyl group (1702 cm^{-1}), and double bond (1624 cm^{-1}). Compound **1** exhibited the molecular formula $C_{45}H_{72}O_{20}$ by its HR-MS spectrum. The ESI-MS of **1** showed a negative ion peak at *m/z* 931 $[M - H]^-$, and significant ion peaks at *m/z*: 769 $[M - H - 62]^-$, 607 $[M - H - 162 - 162]^-$, 445 $[M - H - 162 - 162 - 162]^-$, corresponding to the loss of three hexosyl moieties. Calculated from the ESI-MS, the molecular weight of the aglycone moiety was 444, 14 more than that of tribufuroside B [9]. In comparison to tribufuroside B, there was one more quaternary carbon and one less secondary carbon in the ^{13}C NMR spectrum by DEPT measurements, and there were absorptions for carbonyl group in the IR spectrum. The ^{13}C NMR data of the aglycone of **1** (Table 1) were almost consistent with those of the aglycone of tribufuroside B, except that, owing to the carbonyl group in C-12, the signals of C-11 (δ 38.44) and C-13 (δ 57.71) shifted downfield compared with those of tribufuroside B, which showed that the structural difference between the aglycone of **1** and that of tribufuroside B was the carbonyl group at C-12 in the aglycone moiety of **1**. Thus, the aglycone moiety of **1** was deduced to be 5α-furost-20(22)-en-12-one-2α,3β,26-triol. The 25*S* configuration of **1** was confirmed by comparison of the 26-methylene signals for **1** with those for trigoneoside Xa [10] and trigoneoside Ia [11] in the ^1H NMR specrum. The proton signals assignable to the 26-methylene group [δ 3.48 (1H, dd, J = 7.5, 9.5 Hz, H_a-26), 4.06 (1H, m, H_b-26)] in the ^1H NMR specrum of **1** were very similar to those of trigoneoside Ia and trigoneoside Xa [10, 11].

Acid hydrolysis of **1** with mineral acid afforded galactose and glucose as the sugar components, identified on TLC by comparison with authentic samples. The coupling constants of the anomeric signals revealed the β-configuration for glucose and galactose [12, 13].

TABLE 2. Chemical Shifts for ^{13}C Atoms of Carbohydrates of Glycosides **1–5** and Protons of the Carbohydrates of Glycoside **1** (C_5D_5N, 0=TMS, δ, ppm, J/Hz)

C atom	**1**		**2**	**3**	**4**	**5**	
	δ_C	δ_H	δ_C				
			C3-O				
	Gal	Gal	Gal	Gal	Gal	Gal	Xyl
1	103.32	4.80 (d, J = 7.5)	102.54	103.54	103.57	100.07	105.26
2	73.19	4.39	73.56	73.45	73.19	76.55	74.95
3	75.33	4.28	76.02	75.98	75.46	76.59	78.60
4	80.29	4.55	80.85	79.45	80.29	81.20	70.82
5	76.07	4.09	75.45	76.15	76.07	75.61	67.52
6	61.07	4.26, 4.62	60.61	61.61	61.02	60.35	
	Glc	Glc	Glc	Glc	Glc	Glc	Xyl
1	107.28	5.14 (d, J = 7.5)	105.22	106.62	107.30	105.10	105.66
2	75.93	4.10	85.24	75.64	75.91	81.26	74.88
3	78.86	4.32	77.98	78.48	78.86	87.60	78.97
4	72.38	4.22	72.07	72.32	72.37	70.25	70.86
5	78.64	4.01	78.40	78.55	78.62	77.59	67.35
6	63.24	4.64, 4.43	63.30	63.45	63.22	62.67	
			Gal				Rha
1			107.10				101.92
2			74.72				72.21
3			74.19				72.66
4			71.02				73.87
5			77.70				69.31
6			63.00				18.43
			C26-O				
	Glc′-1	Glc′-1	Glc′-1	Glc′-1	Glc′-1	Glc′-1	
1	105.04	4.71 (d, J = 7.5)	105.30	105.10	105.25	104.89	
2	75.33	4.07	75.43	75.63	75.35	75.25	
3	78.75	4.20	79.04	78.74	78.62	78.43	
4	71.89	4.77	71.90	71.78	71.84	71.56	
5	78.64	3.99	78.90	78.60	78.74	78.58	
6	63.01	4.56, 4.35	61.78	63.20	62.95	62.78	

The positions of the sugar residues in **1** were defined unambiguously by HMBC experiments (Fig. 1). The cross-peaks due to long-range correlations between Gal H-1 (δ 4.80) of galactose and C-3 (δ 84.50) of the aglycone, and between Glc H-1 (δ 5.14) of glucose and C-4 (δ 80.29) of galactose indicated that the disaccharide moiety, 3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside, was linked to the aglycone at C-3. Additionally, the cross-peaks between H-1 (δ 4.71) of glucose and C-26 (δ 75.09) of the aglycone showed that the glucose was linked to C-26 of the aglycone. On the basis of all of this, **1** was identified as 26-*O*-*β*-D-glucopyranosyl-(25*S*),5*α*-furost-20(22)-en-12-one-2*α*,3*β*,26-triol-3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside.

EXPERIMENTAL

General Methods. ^1H NMR and ^{13}C NMR, HMQC, and HMBC spectra: Bruker AM-500 instrument; ESI-MS: LCQ-1700 ESI-MS instrument; IR spectra: Y-Zoom scroll Fourier transform infrared spectrometer; WZZ-15 optical rotation apparatus.

Plant Material. The fruits of *Tribulus terrestris* L. were purchased from the Chinese Medicinal Materials Company in Changchun, Jilin Province, China, in September 2002, and identified by Professor Minglu Deng, Changchun College of Traditional Chinese Medicine. A voucher specimen (020925) has been deposited in the Herbarium of the Academy of Traditional Chinese Medicine and Materia Medica of Jilin Province.

Extraction and Purification. The dried fruits (5 kg) were exhaustively extracted with 60% EtOH, and the extract was concentrated under reduced pressure to obtain a crude residue (166 g), which was chromatographed over a D_{101} macroporous resin column (10 × 80 cm) and eluted successively with H_2O, 30% EtOH, and 70% EtOH. The 70% EtOH eluate was concentrated to dryness (15 g saponin mixture) and chromatographed over a silica gel column (200–300 mesh) eluted with $CHCl_3$–MeOH–H_2O 30:10:1→10:10:1 to give fractions 1–4. Fraction 2 was subjected to HPLC (column: 10 × 250 mm, RP-18, 10 μm, flow rate: 3.0 mL/min) with MeOH–H_2O (65:35) as mobile phase to afford **1** (45 mg), **3** (29 mg), and **4** (23 mg). Fraction 4 was subjected to HPLC eluting with MeOH–H_2O (60:40) to afford **2** (38 mg), **5** (26 mg).

Acid Hydrolysis of Compound 1. The saponin (10 mg) was heated with 2M HCl–MeOH (10 mL) under reflux for 3 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The water layer was neutralized with Na_2CO_3, concentrated, and subjected to TLC analysis with authentic samples of D-glucose and L-galactose, and developed with CH_2Cl_2–MeOH–H_2O (15:6:1). Detection was carried out with aniline phthalate spray.

Compound 1, $C_{45}H_{72}O_{22}$, white amorphous powder, mp 212°C (dec.), $[\alpha]_D^{25}$ +4.6° (*c* 1.25; pyridine); HR-FAB-MS *m/z*: 931.46276 (calcd for $C_{45}H_{72}O_{22}$, 931.46169); ESI-MS: 931 $[M - H]^-$; IR (KBr, ν_{max}, cm^{-1}): 3420 (OH), 1702 (C=O), 1624 (C=C). ^1H NMR and ^{13}C NMR, as well as HMBC and HMQC; for spectral data, see Tables 1 and 2 and Fig. 1.

Compound 2, $C_{51}H_{82}O_{24}$, white amorphous powder, mp 202°C (dec.), $[\alpha]_D^{25}$ +2.6° (*c* 1.25; pyridine), ESI-MS *m/z*: 1077 $[M - H]^-$; for ^{13}C NMR, see Tables 1 and 2.

Compound 3, $C_{45}H_{72}O_{19}$, white amorphous powder, mp 205°C (dec.), $[\alpha]_D^{20}$ +6.0° (*c* 0.3; pyridine), ESI-MS *m/z*: 915 $[M - H]^-$; for ^{13}C NMR, see Tables 1 and 2.

Compound 4, $C_{45}H_{74}O_{20}$, white amorphous powder, mp 228°C (dec.), $[\alpha]_D^{20}$ –18.4° (*c* 0.50; pyridine), ESI-MS *m/z*: 935 $[M - H]^-$; for ^{13}C NMR, see Tables 1 and 2.

Compound 5, $C_{62}H_{104}O_{31}$, white amorphous powder, mp 216°C (dec.), $[\alpha]_D^{20}$ –69° (*c* 0.50; MeOH), ESI-MS *m/z*: 1369 [M + Na]+; for ^{13}C NMR, see Tables 1 and 2.

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