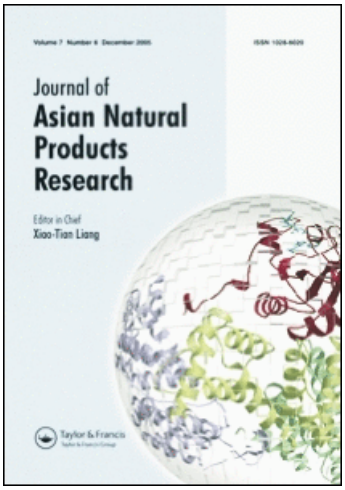

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ORIGINAL ARTICLE

Two new furostanol saponins from *Tribulus terrestris*

Ya-Juan Xu[ab], Tun-Hai Xu[c]*, Hai-Ou Zhou[a], Bo Li[d], Sheng-Xu Xie[a], Yun-Shan Si[a], Yue Liu[a], Tong-Hua Liu[c] and Dong-Ming Xu[a]

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Two new furostanol saponins were isolated from the fruits of *Tribulus terrestris* L. Their structures were established as 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furost-20(22)-en-3β,26-diol-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-β-D-galactopyranoside (**1**) and 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furost-20(22)-en-12-one-3β,26-diol-3-*O*-β-D-galactopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**2**) on the basis of spectroscopic data as well as chemical evidence.

Keywords: *Tribulus terrestris* L.; *Zygophyllaceae*; furostanol saponin

1. Introduction

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 the treatment of eye trouble, edema, abdominal distention, high blood pressure, and cardiovascular diseases. In India, it has long been used as a medicine against impotency and cardiovascular diseases [1]. Recently, a new drug named 'Xin-nao-shu-tong' has been made of the crude saponin fraction of this plant, which showed significant effects for the treatment of various cardiac diseases including coronary heart disease, myocardial infarction, cerebral arteriosclerosis, and the sequelae of cerebral thrombosis [2,3]. Some chemical constituents of this plant have been reported [4–8]. In the preceding paper, we had reported the isolation and structural elucidation of three steroidal glycosides obtained from the fruits of this plant [9,10]. As a continuation to this study, we now describe the isolation and structural elucidation of two new furostanol saponins obtained from the fruits of *T. terrestris*.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. The IR spectrum showed absorptions for hydroxyl groups (3415 cm^{-1}) and double bond (1641 cm^{-1}). Its molecular formula was assigned as $C_{51}H_{84}O_{22}$ on the negative ion HR-ESI-MS at *m/z* 1047.5303 $[M - H]^-$. The negative ion ESI-MS also showed fragment ion peaks at *m/z* 1047 $[M - H]^-$, 885 $[M - H - 162]^-$,

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723 $[M - H - 162 - 162]^-$, 577 $[M - H - 162 - 162 - 146]^-$, and 415 $[M - H - 162 - 162 - 146 - 162]^-$. The ^1H and ^{13}C NMR spectral data of **1** are assigned unequivocally according to ^1H–^1H COSY, HMQC, and HMBC analysis. The ^1H NMR spectrum of **1** showed diagnostic signals of four methyl groups at δ 0.58 (3H, s, CH_3-18), 0.75 (3H, s, CH_3-19), 1.51 (3H, s, CH_3-21), 0.90 (3H, d, $J = 7.0$ Hz, CH_3-27), two oxymethines at δ 3.87 (1H, m, H-3), 4.74 (1H, m, H-16), one oxymethylene at δ 3.49 (1H, dd, $J = 7.0$, 8.5 Hz, H-26), 4.07 (1H, m, H-26), and three anomeric doublets and one singlet at δ 4.80 (1H, d, $J = 7.0$ Hz, Gal-H-1), 5.06 (1H, d, $J = 7.5$ Hz, Glc-H-1), 6.11 (1H, br s, Rha-H-1), 4.71 (1H, d, $J = 7.5$ Hz, Glc′-H-1′). This information was supported by the ^{13}C NMR spectral data of **1**. The ^{13}C NMR spectrum of **1** showed signals of four angular methyl groups at δ 14.5, 12.6, 11.9, 17.5, three carbons bearing oxygen at δ 76.7, 84.6, 75.4, and four anomeric carbons at δ 100.1, 107.4, 102.5, 105.0. Comparison of ^1H and ^{13}C NMR assignment of the aglycone moiety of **1** with that of compound **8** {26-*O*-β-D-glucopyranosyl-22-methoxyl-(5α,25*R*)-furostan-3β,26-diol-3-*O*-β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside} [11] revealed that the structure of the A–D ring parts (C-1—C-19) of **1** was identical to that of compound **8**, including the orientation of the C-3 oxygen atom and H-5 hydrogen atom, but with significant differences in the signals from the E ring (C-20—C-27). The ^1H NMR spectrum of compound **8** showed the presence of two singlet methyl signals and two doublet methyl signals, while the ^1H NMR spectrum of **1** showed the presence of three singlet methyl signals and only one doublet methyl signal. The difference between the two compounds is that **1** possesses a double bond between C-20 and C-22, which was suggested by the ^1H NMR signals at δ 1.51 (3H, s, H_3-21) and 2.31 (1H, d, $J = 10.3$ Hz, H-17), and two quaternary carbon signals at δ 103.8 (C-20) and 152.4 (C-22) in the ^{13}C NMR spectrum [12,13]. In the HMBC spectrum, the methyl protons at δ 0.58 (CH_3-18) showed long-range correlations with the carbons at δ 43.9 (C-13), 54.6 (C-14), 40.0 (C-12), and 64.8 (C-17). The methyl protons at δ 0.75 (CH_3-19) showed long-range correlations with the carbons at δ 35.2 (C-10), 36.1 (C-1), 44.8 (C-5), and 54.9 (C-9). The methyl protons at δ 1.51 (CH_3-21) showed long-range correlations with the carbons at δ 64.8 (C-17), 103.8 (C-20), and 152.4 (C-22). The methyl protons at δ 0.90 (CH_3-27) showed long-range correlations with the carbons at δ 31.6 (C-24), 32.7 (C-25), and 75.4 (C-26). Thus, the aglycone moiety of **1** was deduced to be a 5α-furost-20(22)-en-3β,26-diol structure. The 25*S* configuration of **1** was confirmed by comparison of the 26-methylene signals for **1** with those of trigoneosides Ia and Xa in the ^1H NMR spectrum [14,15]. The proton signals assignable to the 26-methylene group [δ 3.49 (1H, dd, $J = 7.0$, 8.5 Hz, H-26), 4.07 (1H, m, H-26)] in the ^1H NMR spectrum of **1** were very similar to those of trigoneosides Ia and Xa.

Acid hydrolysis of **1** with mineral acid afforded galactose, rhamnose, and glucose as the sugar components identified on TLC by comparison with authentic samples. The coupling constants of the anomeric signals revealed the configuration for galactose, rhamnose, and glucose. The ^{13}C NMR signals due to sugar moieties were almost superimposable on those of 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furostan-12-one-22-methoxy-3β,26-diol-3-*O*-{α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 4)]-β-D-galactopyranoside} [16]. The 3,26-bisdesmoside structure of **1** was characterized by a HMBC experiment (Figure 1). Namely, long-range correlations were observed between H-1 of Gal

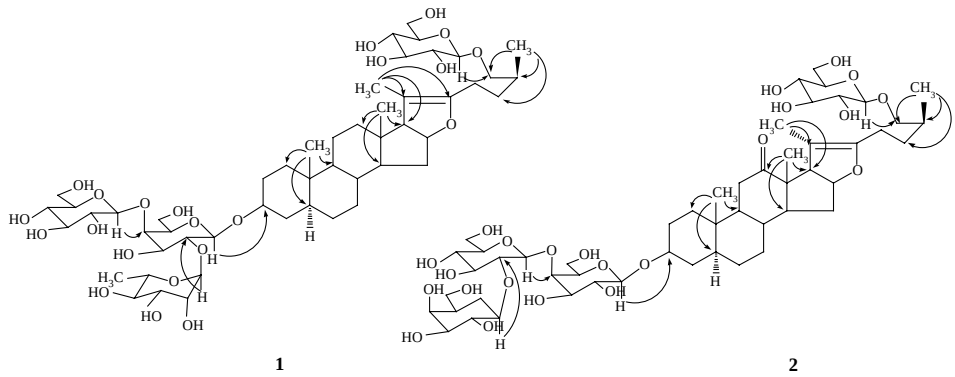

Figure 1. Key HMBC correlations for **1** and **2**.

at δ 4.80 and C-3 of the aglycone at δ 76.7, between H-1 of Glc at δ 5.06 and C-4 of Gal at δ 81.6, between H-1 of Rha at δ 6.11 and C-2 of Gal at δ 77.1, and between H-1′ of Glu′ at δ 4.71 and C-26 of the aglycone at δ 75.4. Consequently, the structure of **1** was elucidated to be 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furost-20(22)-en-3β,26-diol-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-β-D-galactopyranoside.

Compound **2**, isolated as a white amorphous powder, was also deduced to possess a furostanol structure by the Ehrlich test. The IR spectrum showed absorption bands for hydroxyl groups (3420 cm^{-1}), carbonyl group (1701 cm^{-1}), and double bond (1625 cm^{-1}). Its molecular formula was determined as $C_{51}H_{84}O_{24}$ on the basis of the ^{13}C NMR spectral data and negative ion HR-ESI-MS at *m/z* 1077.5109 $[M - H]^-$. The negative ion ESI-MS of **2** showed a quasi-molecular ion peak at *m/z* 1077 $[M - H]^-$ and fragment ion peaks at *m/z* 915 $[M - H - 162]^-$, 753 $[M - H - 162 - 162]^-$, and 591 $[M - H - 162 - 162 - 162]^-$. Acid hydrolyzation of **2** 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 configuration for galactose and glucose. The NMR signals of **2** were assigned in detail with the aid of $^1H-^1H$ COSY, HMQC, and HMBC spectra, as shown in Table 1. In a comparison of the ^{13}C NMR signals for aglycone of **2** with those of the known saponin of tribufuroside C [9], all signals due to the aglycone of **2** were almost superimposable with those of tribufuroside C, indicating that the aglycone of **2** was the same as that of tribufuroside C. The 25*S* configuration of **2** was confirmed by comparison of 26-methylene signals of **2** with those of trigoneosides Ia and Xa [11,14] in the ^1H NMR spectrum. The chemical shifts of the 26-methylene at δ 4.06 (1H, m, H-26) and 3.47 (1H, dd, $J = 7.5$, 8.5 Hz, H-26) of **2** were very similar to those of trigoneosides Ia and Xa [14,15]. Thus, the aglycone moiety of **2** was deduced to be a (25*S*)-5α-furost-20(22)-en-12-one-3β,26-triol. The 3,26-bisdesmoside structure of **2** was characterized by a HMBC experiment. The long-range correlations were observed between H-1 of Gal at δ 4.62 and C-3 of the aglycone at δ 78.1, H-1 of Glc at δ 5.02 and C-4 of Gal at δ 81.2, H-1′ of the terminal Gal′ at δ 5.10 and C-2 of Glc at δ 86.2, and H-1′ of Glc′ at δ 4.78 and C-26 of the aglycone at δ 75.2. On the basis of the above evidence, the structure of **2** was elucidated as 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furost-20(22)-en-12-one-3β,26-diol-3-*O*-β-D-galactopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside.

Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (δ_C, 125 MHz, pyridine-d_5).

No.	**1**	**2**	No.	**1**	**2**
1	36.1	36.8	C-3-O-		
2	29.1	29.9	Gal-1	100.1	102.5
3	76.7	78.1	2	77.1	73.5
4	34.5	34.8	3	76.6	76.2
5	44.8	44.6	4	81.6	81.2
6	29.1	28.7	5	75.8	75.4
7	31.1	32.0	6	61.2	60.6
8	34.5	34.2	Glu-1	107.4	105.2
9	54.9	55.6	2	75.4	86.2
10	35.2	36.4	3	78.7	77.9
11	21.6	38.3	4	72.4	72.0
12	40.0	213.0	5	78.8	78.4
13	43.9	57.7	6	63.2	63.3
14	54.6	54.3	Gal-1′		107.1
15	33.6	33.8	2′		74.7
16	84.6	83.4	3′		74.1
17	64.8	56.3	4′		71.0
18	14.5	14.3	5′		76.6
19	12.6	11.9	6′		63.0
20	103.8	103.3	Rha-1	102.5	
21	11.9	11.7	2	72.6	
22	152.4	153.3	3	72.9	
23	23.8	23.8	4	74.3	
24	31.6	31.4	5	69.6	
25	32.7	33.8	6	18.8	
26	75.4	75.2	C-26-O-		
27	17.5	17.3	Glu′-1′	105.0	105.3
			2′	75.4	75.4
			3′	78.7	79.4
			4′	71.9	71.9
			5′	78.7	78.9
			6′	63.0	61.7

3. Experimental

3.1 General experimental procedures

Melting points were determined by an electrothermal Yanaco MP-S3 micromelting point apparatus and are uncorrected. The optical rotations were determined on a WZZ-15 autopolarimeter. The IR spectra were measured on a Y-Zoom scroll Fourier transform infrared spectrometer with a KBr disk. The NMR spectra were obtained on a Bruker AM-500 instrument, using TMS as the internal standard. The HR-ESI-MS was recorded on IonSpec HiResESI FT-ICR (Lake Forest, Irvine, CA, USA), 7.0 T (Cryomagnetics, Oak Ridge, TN, USA), and the ESI-MS was recorded on a LCQ-1700 ESI-MS instrument. HPLC was performed using an ODS column (Shimpark PREF-ODS, 250 × 4.6 mm). Column chromatography was performed on silica gel (200–300 mesh; Qingdao Oceanic Chemical Industry, Qingdao, China) and reversed silica gel (25 × 2.5 cm; Nacalai Tesque, Kyoto, Japan). Macroporous resin D_{101} was made by Tianjin Gel Co. (Tianjin, China). Spots were detected after spraying with 10% H_2SO_4.

3.2 Plant material

The fruits of *T. terrestris* were collected from Baicheng, Jilin Province of China, in September 2006 and identified by

Prof. Minglu Deng, Changchun College of Traditional Chinese Medicine. A voucher specimen (No. 060925) has been deposited in the Herbarium of the Academy of Traditional Chinese Medicine and Material Medica of Jilin Province.

3.3 Extraction and isolation

The dried and powdered fruits (15 kg) of *T. terrestris* were exhaustively extracted with 60% EtOH. The 60% EtOH solution was heated on a steam bath to remove EtOH. The water solution was chromatographed on a 1.5 kg D_{101} porous resin, eluting with water until the eluate was colorless and then with 70% EtOH (12 liters). The 70% EtOH solution was further subjected to neutral resin to remove most of the color material and then evaporated to dryness to give crude saponins (28 g). Part of crude saponins (25 g) was chromatographed on silica gel (200–300 mesh) with $CHCl_3$–MeOH–H_2O gradients 1:0:0, 50:10:1 to 10:10:1, and finally with MeOH, 500 ml per part, to give fractions 1–7. Fraction 4 (5.0 g) was subjected to repeated column chromatography on silica gel (200 mesh, 520 g), eluted with $CHCl_3$–MeOH–*n*-BuOH (8:2:1, 250 ml per part) to afford fractions 4-1–4-6. Fraction 4-4 (330 mg) was subjected to HPLC eluting with 50%, 45% MeOH in turn to give compound **1** (43 mg). Fraction 5 (340 mg) was subjected to HPLC eluting with 50%, 45% MeOH to give compound **2** (36 mg).

3.3.1 Compound *1*

An amorphous powder, mp 228–230°C, $[\alpha]_D^{25}$ −63.6 (c = 0.25, pyridine), IR (KBr) ν_{max}: 3415, 2929, 1641, 1450, 1380, 1364, 1163, 1076, 1038, 892, 603 cm^{-1}. ^1H NMR (500 MHz, pyridine-d_5) δ: 0.58 (3H, s, CH_3-18), 0.75 (3H, s, CH_3-19), 1.51 (3H, s, CH_3-21), 0.90 (3H, d, J = 7.0 Hz, CH_3-27), 3.87 (1H, m, H-3), 4.74 (1H, m, H-16), 2.31 (1H, d, J = 10.3 Hz, H-17), 3.49 (1H, dd, J = 7.0, 8.5 Hz, H-26), 4.07 (1H, m, H-26), 4.80 (1H, d, J = 7.0 Hz, Gal-H-1), 5.06 (1H, d, J = 7.5 Hz, Glc-H-1), 6.11 (1H, br s, Rha-H-1), 4.71 (1H, d, J = 7.5 Hz, Glc′-H-1′). ^{13}C NMR (125 MHz, pyridine-d_5) spectral data are given in Table 1. HR-ESI-MS *m/z*: 1047.5303 $[M - H]^-$ (calcd for $C_{51}H_{83}O_{22}$, 1047.5376). ESI-MS *m/z*: 1047 $[M - H]^-$, 885 $[M - H - 162]^-$, 723 $[M - H - 162 - 162]^-$, 577 $[M - H - 162 - 162 - 146]^-$, 415 $[M - H - 162 - 162 - 146 - 162]^-$.

3.3.2 Compound *2*

An amorphous powder, mp 214–217°C, $[\alpha]_D^{20}$ −18.9 (c = 0.22, pyridine). IR (KBr) ν_{max}: 3420, 2929, 1701, 1625, 1451, 1380, 1358, 1160, 1070, 1041, 890, 604 cm^{-1}. ^1H NMR (500 MHz, pyridine-d_5) δ: 0.56 (3H, s, CH_3-18), 0.79 (3H, s, CH_3-19), 1.62 (3H, s, CH_3-21), 0.91 (3H, d, J = 6.8 Hz, CH_3-27), 3.82 (1H, m, H-3), 4.65 (1H, m, H-16), 4.06 (1H, m, H-26), 3.47 (1H, dd, J = 7.5, 9.5 Hz, H-26), 4.62 (1H, d, J = 7.3 Hz, Gal-H-1), 5.02 (1H, d, J = 7.6 Hz, Glc-H-1), 5.10 (1H, d, J = 7.5 Hz, Gal′-H-1′), 4.78 (1H, d, J = 7.6 Hz, Glc′-H-1′). ^{13}C NMR (125 MHz, pyridine-d_5) spectral data are given in Table 1. HR-ESI-MS *m/z*: 1077.5109 $[M - H]^-$ (calcd for $C_{51}H_{83}O_{24}$, 1077.5118). ESI-MS *m/z*: 1077 $[M - H]^-$, 915 $[M - H - 162]^-$, 753 $[M - H - 162 - 162]^-$, 591 $[M - H - 162 - 162 - 162]^-$.

3.4 Acid hydrolysis

Compounds **1** and **2** (10 mg each) were dissolved in 1 mol/l HCl in MeOH–H_2O (1:1) and refluxed for 2 h. The reaction mixture was neutralized with $NaHCO_3$. The water phase was chromatographed on the silica gel HPTLC with the system of *n*-BuOH–*i*-PrOH–H_2O (10:5:4, homogeneous), and then the brown colored spots were visualized by spraying with phenylamine-*ortho*-benzene-dicarboxylic acid reagent followed by heating. Glucose,

galactose, and ramnose were detected by comparison with the authentic samples.

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