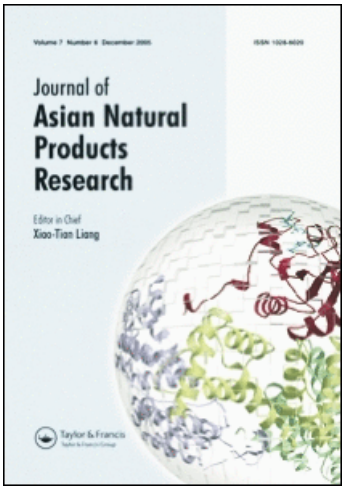

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ORIGINAL ARTICLE

Two new steroidal saponins from *Tribulus terrestris* L.

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Two new steroidal saponins were isolated from the fruits of *Tribulus terrestris* L. Their structures were elucidated by spectroscopic and chemical analysis as (23*S*,24*R*,25*R*)-5α-spirostane-3β,23,24-triol-3-*O*-{α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-β-D-galactopyranoside} (**1**) and (23*S*,24*R*,25*S*)-5α-spirostane-3β,23,24-triol-3-*O*-{α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-β-D-galactopyranoside} (**2**).

Keywords: *Tribulus terrestris* L.; steroidal glycosides; spirostanol saponins

1. Introduction

Tribulus terrestris L. is an annual plant, widely distributed in subtropical areas around the world. Its fruits, a traditional Chinese medicine named 'jili', are used for the treatment of eye problems, edema, emission, sexual dysfunction, high blood pressure, and cardiovascular diseases. In the literature, more than 90 different glycosidic saponins have been reported in this plant [1,2]. Many pharmaceutical preparations and food supplements with these saponins as the active components are commercially available. In this paper, we report the isolation and structural elucidation of two new compounds using 1D and 2D NMR techniques.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ −31.2 ($c = 0.60$, MeOH). Its molecular formula was determined to be $C_{45}H_{74}O_{19}$ by HR-ESI-MS at *m/z* 941.7722 $[M+Na]^+$. The ^{1}H NMR spectrum of **1** showed signals for four steroidal methyl groups at δ 0.79 (3H, s, Me-19), 1.00 (3H, s, Me-18), 1.19 (3H, d, $J = 7.0$ Hz, Me-21), and 1.37 (3H, d, $J = 7.0$ Hz, Me-27), four oxygenated methine protons at δ 3.92 (1H, m, H-3), 4.59 (1H, m, H-16), 4.03 (1H, d, $J = 9.6$ Hz, H-23), and 4.47 (1H, dd, $J = 9.6$, 5.4 Hz, H-24), two oxygenated methylene protons at δ 4.11 (1H, m, H-26a) and 3.49 (1H, t, $J = 10.2$ Hz, H-26b), as well as signals for three anomeric protons at δ 4.91 (1H, d, $J = 7.6$ Hz, H-1′), 6.22 (1H, br s, H-1″), and 5.18 (1H, d, $J = 7.8$ Hz, H-1‴). The signal at δ 1.68 was due to the methyl group of 6-deoxyhexopyranose. The above information was supported by the ^{13}C NMR spectrum. The ^{13}C NMR spectrum of **1** showed signals for five methyl groups at δ 11.3, 12.4, 14.5, 16.9, 18.6, four oxygenated carbons at δ 69.5, 72.0, 76.9, 82.1, and

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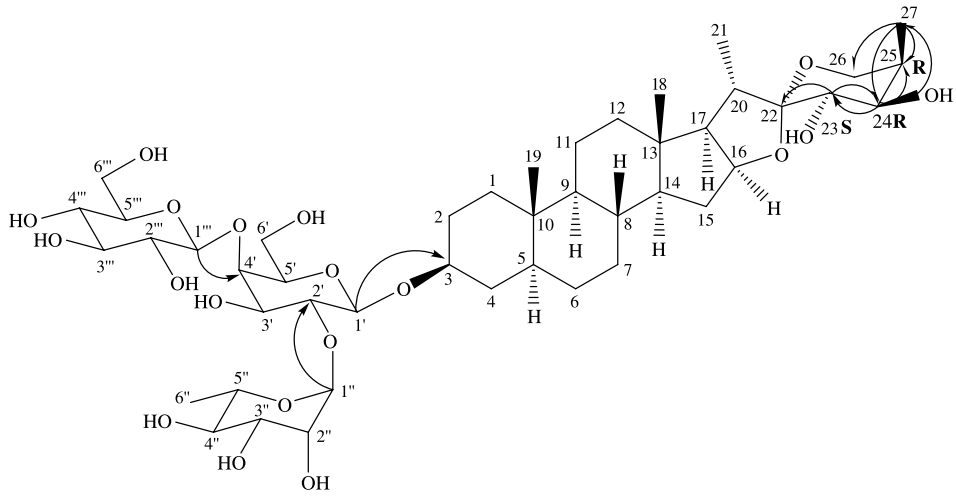

Figure 1. The structure and key HMBC correlations of compound **1**.

three anomeric carbons at δ 99.9, 102.4, 107.2. Comparison of the proton and carbon chemical shifts of **1** with those of tigogenin-3-*O*-{α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-β-D-galactopyranoside} [3] revealed that the structure of the A–E ring parts was identical to that of the reference compound, including the orientations of C-3, C-5, and C-20 (3β, 5α, 20α). However, significant differences were recognized in the signals from the F ring (C-22–C-27). In the HMBC spectrum (Figure 1), the methyl protons at δ 1.37 (Me-27) showed correlations with the carbons at δ 37.1 (C-25), 63.9 (C-26), and 72.0 (C-24), the proton at δ 4.47 (H-24) with the carbons at δ 11.3 (C-27), 37.1 (C-25), and 69.5 (C-23), and the proton at δ 4.03 (H-23) with the carbons at δ 72.0 (C-24) and 113.3 (C-22), indicating the attachments of two hydroxyl groups at C-23 and C-24. The *J* values of the protons at δ 4.03 (1H, d, $J = 9.6$ Hz, H-23) and 4.47 (1H, dd, $J = 9.6$, 5.4 Hz, H-24) provided evidences for the 23*S*, 24*R*, and 25*R* configurations (Figure 2) [4]. Thus, its aglycone moiety was deduced to be (23*S*,24*R*,25*R*)-5α-spirostane-3β,23,24-triol, a new steroidal sapogenin. Acid hydrolysis of **1** gave L-rhamnose, D-glucose, and D-galactose in a ratio of 1:1:1 on the basis of GC analysis. The α-anomeric configuration for the rhamnosyl group was determined by its C-5 data (δ 69.4). The β-anomeric configurations for both the glucosyl and galactosyl groups were judged from their coupling constants ($J_{1,2} > 7.0$ Hz) [2]. The positions of the sugar residues in **1** were defined by the HMBC experiment (Figure 1). A cross-peak between the ^{1}H NMR signal at δ 4.91 (H-1′, galactosyl group) and the carbon signal at δ 76.9 (C-3, aglycone) indicated glycosylation of the aglycone at C-3. Similarly, anomeric protons at δ 6.22 (H-1″, rhamnosyl group) and δ 5.18 (H-1‴, glucosyl group) showed cross-peaks with the carbon signals at δ 77.0 (C-2′, galactosyl group) and 81.3 (C-4′, galactosyl group), respectively. In conclusion, the structure of **1** was elucidated

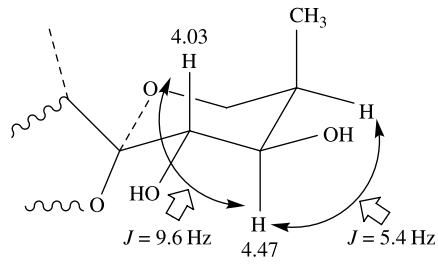

Figure 2. The ^{1}H NMR chemical shifts and the *J* values of the F ring of compound **1**.

as (23*S*,24*R*,25*R*)-5α-spirostane-3β,23,24-triol-3-*O*-{α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-β-D-galactopyranoside}.

Compound **2** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ −15.8 (c = 1.20, MeOH). Its molecular formula was determined to be $C_{45}H_{74}O_{19}$ by HR-ESI-MS at *m/z* 941.7721 $[M+Na]^+$. The ^1H NMR spectrum of **2** showed five methyl proton signals at δ 0.80 (3H, s, Me-19), 1.02 (3H, s, Me-18), 1.19 (3H, d, J = 6.9 Hz, Me-21), 1.09 (3H, d, J = 6.5 Hz, Me-27), and 1.68 (3H, d, J = 6.2 Hz, Rha-6) and three anomeric protons at δ 4.91 (1H, d, J = 7.6 Hz, H-1′), 6.22 (1H, br s, H-1″), and 5.18 (1H, d, J = 7.8 Hz, H-1‴). The ^1H and ^{13}C NMR spectra of **2** (Table 1) were closely related to those of **1**, except for the F ring. In the HMBC spectrum (Figure 3), the methyl protons at δ 1.09 (Me-27) showed correlations with the carbons at δ 39.5 (C-25), 64.5 (C-26), and 76.1 (C-24). The proton at δ 3.96 (H-24) showed correlations with the carbons at δ 13.7 (C-27), 39.5 (C-25), and 73.8 (C-23), and the proton at δ 3.86 (H-23) showed correlations with the carbons at δ 76.1 (C-24) and 113.3 (C-22), indicating the attachments of two hydroxyl groups at C-23 and C-24 (Figure 3). The configurations of C-23, C-24, and C-25 were 23*S*, 24*R*, and 25*S* because of the large J values of the protons at δ 3.86 (H-23) (1H, d, J = 9.1 Hz) and 3.96 (H-24) (1H, dd, J = 9.1, 9.5 Hz) (Figure 4). Thus, the aglycone moiety of **2** was deduced to be (23*S*,24*R*,25*S*)-5α-spirostane-3β,23,24-triol, a new steroidal sapogenin. On comparison of ^1H and ^{13}C NMR spectral data of the sugar moiety of **2** with those of **1** indicated that they have the same sugar chain. On the basis of all these evidences, **2** was elucidated as (23*S*,24*R*,25*S*)-5α-spirostane-3β,23,24-triol-3-*O*-{α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-β-D-galactopyranoside}.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were taken on a Bruker IFS-55 infrared spectrophotometer. The NMR spectral data were recorded on Bruker AV-600 (600 MHz for ^1H and 150 MHz for ^{13}C) in C_5D_5N with TMS as the internal standard. The HR-ESI-MS data were obtained on the Micross Mass Autospec-UltimaE TOF mass spectrophotometer. Chromatography was performed on silica gel (200–300 mesh; Qingdao Haiyang Chemical Factory, Qingdao, China), and purified by HPLC (Shimadzu LC-8A, RID-10A, Kyoto, Japan). GC analysis was performed on a Shimadzu GC-2010 gas chromatograph equipped with an H_2 flame ionization detector and a DB-5 quartz capillary column (30 m × 0.25 mm × 0.25 μm).

3.2 Plant material

The fruits of *T. terrestris* were bought from Henan Province, China, and identified by Prof. Qishi Sun of Shenyang Pharmaceutical University. A voucher specimen (No. 0093) is deposited in the School of Traditional Chinese Materia Medica of Shenyang Pharmaceutical University.

3.3 Extraction and isolation

The fruits of *T. terrestris* (5 kg) were extracted with 75% EtOH for three times, 2 h each. The extract (200 g) was successively partitioned with $CHCl_3$, EtOAc, and *n*-BuOH. The *n*-BuOH-soluble fraction (65 g) was subjected to a silica gel column, eluted with $CHCl_3$–CH_3OH (from 100:1 to 0:1), yielding eight fractions. Fraction 2 (4 g) was subjected to HPLC and eluted with MeOH (68%) by a flow rate of 3 ml/min, which afforded compounds **1** (15 mg) at 28.9 min and **2** (22 mg) at 25.8 min.

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** (in pyridine-d_5).

	1		**2**			**1**		**2**	
No.	δ_C	δ_H	δ_C	δ_H	Sugar unit	δ_C	δ_H	δ_C	δ_H
1	37.2		37.2		β-D-Gal 1′	99.9	4.91 (d, $J = 7.6$ Hz)	99.9	4.91 (d, $J = 7.6$ Hz)
2	29.9		29.9		2	77.0		77.0	
3	76.9	3.92 (m)	76.9	3.92 (m)	3	76.4		76.4	
4	34.4		34.4		4	81.3		81.3	
5	44.6		44.6		5	75.2		75.2	
6	29.0		29.0		6	61.1		61.0	
7	32.4		32.4		α-L-Rha 1″	102.4	6.22 (br s)	102.4	6.22 (br s)
8	35.2		35.1		2	72.5		72.4	
9	54.4		54.4		3	72.8		72.8	
10	35.9		35.9		4	74.1		74.1	
11	21.3		21.3		5	69.5		69.4	
12	40.5		40.5		6	18.7	1.68 (d, $J = 6.2$ Hz)	18.6	1.68 (d, $J = 6.2$ Hz)
13	41.3		41.3		β-D-Glc 1‴	107.2	5.18 (d, $J = 7.8$ Hz)	107.2	5.18 (d, $J = 7.8$ Hz)
14	56.5		56.4		2	75.6		75.6	
15	32.0		32.0		3	78.9		78.9	
16	82.1	4.59 (m)	82.1	4.66 (m)	4	72.2		72.2	
17	62.0		62.1		5	78.6		78.5	
18	16.9	1.00 (s)	16.9	1.02 (s)	6	63.1		63.1	
19	12.4	0.79 (s)	12.4	0.80 (s)					
20	36.7		36.5						
21	14.5	1.19 (d, $J = 7.0$ Hz)	14.6	1.19 (d, $J = 6.9$ Hz)					
22	113.3		113.3						
23	69.5	4.03 (d, $J = 9.6$ Hz)	73.8	3.86 (d, $J = 9.1$ Hz)					
24	72.0	4.47 (dd, $J = 9.6$, 5.4 Hz)	76.1	3.96 (dd, $J = 9.1$, 9.5 Hz)					
25	37.1		39.5						
26	63.9	4.11 (m) 3.49 (t, $J = 10.2$ Hz)	64.5	3.65 (2H, m)					
27	11.3	1.37 (d, $J = 7.0$ Hz)	13.7	1.09 (d, $J = 6.5$ Hz)					

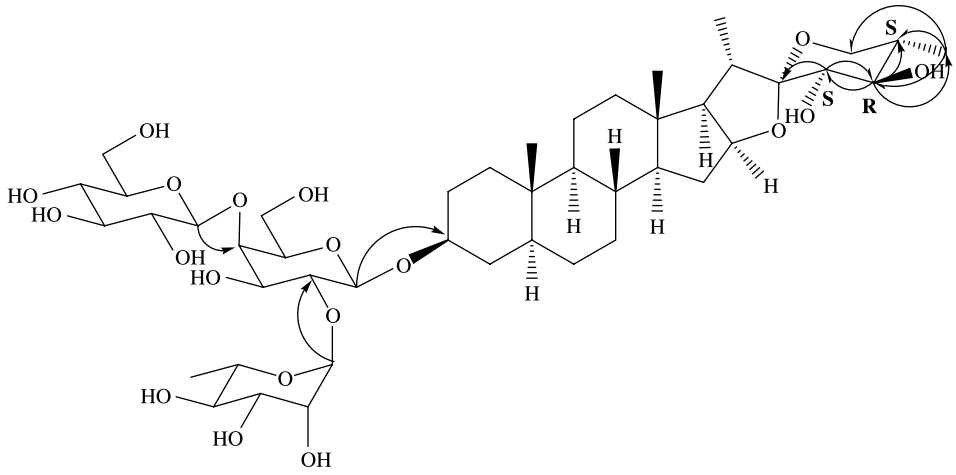

Figure 3. The structure and key HMBC correlations of compound **2**.

*3.3.1 (23S,24R,25R)-5α-Spirostane-3β,23,24-triol-3-O-{α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-β-D-galactopyranoside} (**1**)*

A white amorphous powder; $[\alpha]_D^{20}$ −31.2 (c = 0.60, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3425, 2930, 1637, 1384, 1053, 992, 916, 639; ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS: *m/z* 941.4722 $[M+Na]^+$ (calcd for $C_{45}H_{74}O_{19}Na$, 941.4722).

*3.3.2 (23S,24R,25S)-5α-Spirostane-3β,23,24-triol-3-O-{α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-β-D-galactopyranoside} (**2**)*

A white amorphous powder; $[\alpha]_D^{20}$ −15.8 (c = 1.20, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3419, 2929, 1641, 1384, 1045, 635; ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS: *m/z* 941.4721 $[M+Na]^+$ (calcd for $C_{45}H_{74}O_{19}Na$, 941.4722).

3.4 Acid hydrolysis of 1 and 2

Each solution of compounds **1** and **2** (5 mg) in 2 M HCl–MeOH (4:1, 5 ml) was refluxed at 90°C for 5 h. Then, the reaction mixture was diluted with H_2O and extracted with $CHCl_3$ (3× 20 ml). The water layer was concentrated to dryness to give a residue that was dissolved in pyridine (1 ml), and then L-cysteine methyl ester hydrochloride (2 mg) was added to the solution. The mixture was heated at 60°C for 2 h, and then with the equal volume of acetic anhydride at 90°C for another 2 h. The solution was concentrated to dryness and then dissolved in MeOH (0.5 ml), which was analyzed by GC [column: DB-5 quartz capillary column (30 m × 0.25 mm × 0.25 μm), H_2 flame ionization detector; column temperature: 100–280°C; programmed increase: 10°C min^{-1}; carrier gas: N_2 (1.5 ml/min); injector and detector temperature: 280°C; injection volume: 0.5 μl; split ratio: 10:1]. The derivatives of L-rhamnose, D-glucose,

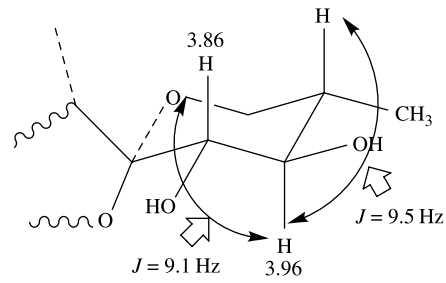

Figure 4. The ^1H NMR chemical shifts and the J values of the F ring of compound **2**.

and D-galactose were detected [R_f (min): 22.72, 26.11, and 26.60, respectively]. The standard monosaccharides were subjected to the same reaction and GC analysis under the same conditions.

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