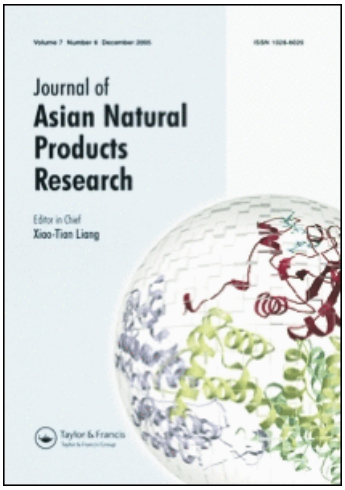

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Su, Lan , Feng, Sheng-Guang , Qiao, Li , Zhou, Yu-Zhi , Yang, Rui-Ping and Pei, Yue-Hu(2009) 'Two new steroidal saponins from *Tribulus terrestris*', Journal of Asian Natural Products Research, 11: 1, 38 — 43
To link to this Article: DOI: 10.1080/10286020802413130
URL: http://dx.doi.org/10.1080/10286020802413130

Journal of Asian Natural Products Research
Vol. 11, No. 1, January 2009, 38–43

Two new steroidal saponins from *Tribulus terrestris*

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(*Received 9 April 2008; final version received 26 June 2008*)

Two new steroidal saponins and two known flavonoid glycosides were isolated from the fruits of *Tribulus terrestris*. Their structures were assigned by spectroscopic analysis and chemical reaction as 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-β-D-glucopyranosyl (1 → 2)-β-D-glucopyranosyl(1 → 4)-β-D-galactopyranoside **(1)**, 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furostan-22-methoxy-2α,3β,26-triol-3-*O*-β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl(1 → 4)-β-D-galactopyranoside **(2)**, kaempferol-3-gentiobioside **(3)**, and isorhamnetin-3-gentiobioside **(4)**.

Keywords: *Tribulus terrestris*; steroidal saponins; flavonoid glycosides

1. Introduction

Tribulus terrestris L. is found growing in subtropical areas around the world. The fruits of *T. terrestris* L. have been used in traditional Chinese medicine for the treatment of eye trouble, edema, abdominal distention, emission, morbid leucorrhea, sexual dysfunction, and veiling. It has also been used as medicine in India, South Africa, and Japan. Some steroidal saponins have previously been isolated from this plant as the active components [1–8]. The quantity of main flavonoids was about 1.5 times that of main saponins. This indicated that the flavonoids contained in *T. terrestris* should be studied, developed, and further used [9]. This paper reports the structural assignment of two new furostanol saponins and two known flavonoid glycosides, based on extensive spectroscopic analysis, including 2D-NMR spectral data, and chemical evidence. Their structures were assigned as 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-β-D-glucopyranosyl (1 → 2)-β-D-glucopyranosyl (1 → 4)-β-D-galactopyranoside **(1)**, 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furostan-22-methoxy-2α, 3β,26-triol-3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl(1 → 4)-β-D-galactopyranoside **(2)**, kaempferol-3-gentiobioside **(3)**, and isorhamnetin-3-gentiobioside **(4)** (Figure 1). Compounds **1** and **2** are new steroidal saponins.

2. Results and discussion

Compound **1** was a furostanol saponin as indicated by the positive Ehrlich reagent. The molecular formula was assigned as $C_{51}H_{84}O_{25}$ on the basis of the HR-ESI-MS at *m/z* 1119.5205 $[M + Na]^+$. The ^1H NMR spectrum showed signals for four steroid methyls at δ 1.11 (3H, s, H-18), 0.67 (3H, s, H-19), 1.53 (1H, d, $J = 7.2$ Hz, H-21), and 0.98 (1H, d, $J = 6.6$ Hz, H-27). The C-25 configuration was deduced to be *R* on the basis of the difference in chemical shifts ($\Delta_{ab} = \delta_a - \delta_b$) of the geminal protons at

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802413130
http://www.informaworld.com

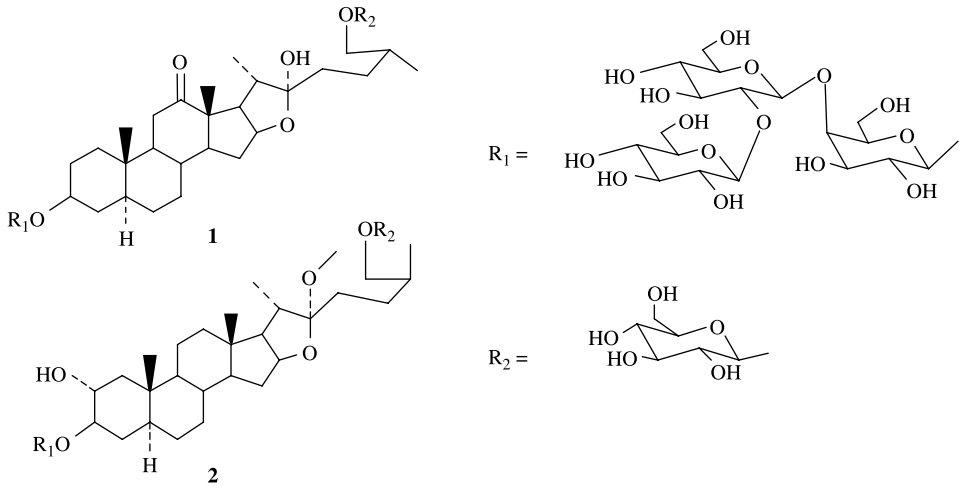

Figure 1. Structures of compounds **1**–**2**.

H_2-26 ($\Delta_{ab} = 0.47$ ppm). It has been described that Δ_{ab} is usually >0.57 ppm in 25*S* compounds and <0.48 in 25*R* compounds [10,11]. The ^{13}C NMR spectrum of **1** exhibited the signal of a 5α-steroidal saponin (C-5: 44.5, C-9: 55.8, C-19: 11.8), because for the 5α compounds the chemical shifts of C-5, C-9, and C-19 appear at ~43–46, ~54–56, and ~11–14 ppm, respectively, and for the 5β compounds the chemical shifts of these carbons are observed at ~35–36.5, ~40, and ~24 ppm, respectively [12]. A carbonyl signal at δ 213.0 could be assigned to C-12. These assignments can be confirmed through long-range couplings in the HMBC spectrum. Comparison of the ^{1}H and ^{13}C NMR assignments of the aglycone moiety of **1**, which were established by the analysis of the HMQC and HMBC spectra, with those of 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostane-12-one-3β,22α,26-triol-3-*O*-β-D-glucopyranosyl(1 → 2)-β-D-glucopyranoside, isolated from *T. terrestris* [13], revealed that the structure of the aglycone moiety of **1** was identical to that of the reference compound. Thus, the aglycone moiety was identified as (25*R*)-5α-furostane-12-one-3β,22α,26-triol.

The ^{13}C NMR spectrum of **1** showed, in addition to the aglycone signals, 24 signals ascribable to a sugar portion made up of one galactose unit and three glucose units (Table 1). The ^{1}H and ^{13}C NMR spectra of **1** showed signals for four anomeric protons at δ 4.86 (1H, d, $J = 7.2$ Hz, H-1′), 5.12 (1H, d, $J = 7.8$ Hz, H-1″), 5.20 (1H, d, $J = 7.2$ Hz, H-1‴), and 4.79 (1H, d, $J = 7.2$ Hz, H-1⁗) with their corresponding anomeric carbons at δ 102.4, 105.2, 107.0, and 105.0, respectively (Table 1). The ^{1}H NMR coupling constants ($J_{1,2} > 7.0$ Hz) for anomeric protons revealed that both the galactose and glucose have a β-configuration. The HMBC correlations from H-1′ of galactose to C-3 of aglycone, H-1″ of glucose to C-4′ of galactose, H-1″ to C-2″ of glucose, and H-1″ to C-26 of the aglycone indicated that the sugar chain was attached to C-3 of the aglycone, and that the glucose was linked at C-4′ of the inner galactose, the terminal glucose was linked at C-2″ of the glucose, and another glucose was linked at C-26 of aglycone. The configurations of the sugar units were assigned after hydrolysis of **1** with 2 M HCl. The hydrolysate was acetylated, and GC retention times of each sugar were compared with those of the authentic samples prepared in the same manner. In this way, the sugar units of **1** were determined to be D-glucose and D-galactose in the ratio 3:1. In conclusion, the structure of compound **1** was elucidated as 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-β-D-glucopyranosyl(1 → 2)-β-D-

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_H	δ_C	δ_H	δ_C	Sugar unit	δ_H	δ_C	δ_H	δ_C
1	0.69 m	36.7	1.16 m	45.7	Galactose				
	1.31 m		2.18 m						
2	1.58 m	29.8	4.17 m	70.5	1′	4.86 d (7.2)	102.4	4.93 d (7.3)	103.4
	2.00 m								
3	3.87 m	76.8	3.85 m	84.7	2′	4.45 m	73.3	4.56 m	72.7
4	1.28 m	34.7	1.48 m	34.4	3′	4.05 m	75.6	4.07 m	75.5
	1.80 m		1.89 m						
5	0.86 m	44.5	0.99 m	44.7	4′	4.55 m	81.1	4.55 m	81.0
6	1.12 m	28.6	1.02 m	28.1	5′	4.06 m	75.3	4.06 m	75.5
			1.12 m						
7	0.70 m	31.8	0.77 m	30.0	6′	4.22 m	60.6	4.21 m	60.4
	1.52 m		1.47 m			4.75 m		4.72 m	
8	1.72 m	34.3	1.36 m	34.5	Glucose				
9	0.89 m	55.8	0.57 m	54.4	1‴	5.12 d (7.8)	105.2	5.15 d (7.8)	105.0
10		36.3		36.9	2‴	4.12 m	86.1	4.12 m	86.1
11	2.22 m	38.0	1.21 m	21.4	3‴	3.92 m	78.2	3.86 m	78.3
	2.39 m		1.44 m						
12		213.0	1.02 m	40.1	4‴	3.92 m	70.5	3.92 m	70.5
			1.65 m						
13		55.7		41.1	5‴	3.86 m	77.7	3.92 m	77.8
14	1.34 m	55.9	1.00 m	56.3	6‴	4.33 m, 4.57 m	61.6	4.35 m, 4.58 m	61.8
15	1.60 m	31.8	1.42 m	32.1	Glucose				
	2.10 m		2.00 m						
16	4.86 m	79.7	4.55 m	81.3	1⁗	5.20 d (7.2)	107.0	5.28 d (7.2)	106.9
17	2.89 m	55.1	1.89 m	64.3	2⁗	4.04 m	77.1	4.07 m	76.7
18	1.11 s	16.3	0.85 s	16.4	3⁗	4.22 m	79.0	4.22 m	79.0
19	0.67 s	11.8	0.76 s	13.4	4⁗	3.96 m	71.8	3.96 m	71.8
20	2.18 m	41.3	2.20 m	40.5	5⁗	3.93 m	78.5	3.93 m	78.5
21	1.53 d (7.2)	15.3	1.14 d (7.2)	16.3	6⁗	4.07 m, 4.61 m	63.2	4.08 m, 4.58 m	63.2
22		110.8		112.8	26-*O*-Glucose				
23	2.03 m	37.1	1.95 m, 2.04 m	31.0	1⁗	4.79 d (7.2)	105.0	4.83 d (7.7)	105.2
24	1.65 m	28.4	1.69 m	28.2	2⁗	4.04 m	75.2	4.06 m	75.2
	2.04 m		2.04						

Table 1 – *continued*

	1		**2**			**1**		**2**	
No.	δ_H	δ_C	δ_H	δ_C	Sugar unit	δ_H	δ_C	δ_H	δ_C
25	1.91 m	34.3	1.90 m	34.2	$3'''$	4.22 m	78.6	4.22 m	78.6
26	3.61 dd (6.0, 9.0), 4.08 m	75.2	3.46 dd (7.1, 8.9), 4.06 m	75.0	$4'''$	4.21 m	71.9	4.22 m	71.7
27	0.98 d (6.6)	17.5	1.03 d (6.8)	17.5	$5'''$	3.79 m	78.5	3.87 m	78.5
OCH_3				47.4	$6'''$	4.35 m, 4.55 m	62.9	4.38 m, 4.55 m	62.9

glucopyranosyl(1 → 4)-β-D-galactopyranoside.

Compound **2** was also a furostanol saponin as indicated by the positive Ehrlich reagent. The molecular formula was assigned as $C_{52}H_{88}O_{25}$ on the basis of the HR-ESI-MS at *m/z* 1135.5516 $[M + Na]^+$. The ^1H NMR spectrum showed signals for four steroid methyl protons at δ 0.85 (3H, s, H-18), 0.76 (3H, s, H-19), 1.14 (3H, d, $J = 7.2$ Hz, H-21), and 1.03 (3H, d, $J = 6.8$ Hz, H-27). The C-25 configuration of **2** was *S*, which was confirmed by the ^1H NMR spectral data at δ 4.06 (H-26a), 3.46 (1H, dd, $J = 7.1$, 8.9 Hz, H-26b) [10,11]. The ^{13}C NMR spectrum of **2** exhibited the signal of a 5α-steroidal saponin (C-5: 44.7, C-9: 54.4, C-19: 13.4) [12]. The appearance of a methoxyl signal around δ 47.4 in the ^{13}C NMR spectrum and the downfield shift of C-22 to 112.8 suggested that the 22-OH was methylated [14]. Comparison of the signals from the sterol part of **2** in the ^1H and ^{13}C NMR spectra, which were established by the analysis of the HMQC and HMBC spectra, with those from the sterol part of capsicosides B, showed that the sterol part of **2** was the same as that of capsicosides B, expect for the configuration of C-25 [15].

The ^1H NMR spectrum showed signals for four anomeric protons at δ 4.93 (1H, d, $J = 7.3$ Hz, H-1′), 5.15 (1H, d, $J = 7.8$ Hz, H-1″), 5.28 (1H, d, $J = 7.2$ Hz, H-1″), and 4.83 (1H, d, $J = 7.7$ Hz, H-1″). On comparison of the ^1H and ^{13}C NMR spectra of **2** with those of **1**, the signals due to the sugar moieties were almost the same, indicating that they have the same sugar chain (Table 1). Thus, the structure of **2** was established to be 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furostan-22-methoxy-2α,3β,26-triol-3-*O*-β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl(1 → 4)-β-D-galactopyranoside.

Compounds **3** and **4** were confirmed as kaempferol-3-gentiobioside and isorhamnetin-3-gentiobioside, respectively, by comparison of their spectral data with those reported in the literature [16–19].

3. Experimental

3.1 General experimental procedures

Optical rotations were obtained on a PerkinElmer 241MC spectropolarimeter at room temperature. IR spectra were measured on a Bruker IFS 55 spectrometer. NMR spectra were taken on a Bruker DRX-300 or DRX-600 spectrometer with TMS as an internal standard. HR-ESI-MS was measured on a TOF micromass spectrometer (Agilent, Milford, MA, USA). Thin layer chromatography (TLC) was carried out on plates precoated with RP-18 gel (Merck, Rahway, NJ, USA) and silica gel F_{254} (Qingdao Marine Chemistry Ltd., Qingdao, China). Spots on the plates were visualized by spraying with Ehrlich reagent, followed by heating. Column chromatography was performed on silica gel (200–300 and 300–400 mesh; Qingdao Marine Chemical Ltd), MPLC (column 3.5 cm × 45 cm, 50 μm; BÜCHI, Postfach, Switzerland), and HPLC (column 10 mm × 250 mm, 5 μm, Shimadzu LC-8; Shimadzu, Kyoto, Japan). GC analysis was performed on a Shimadzu GC-2010 gas chromatograph equipped with an H_2 flame ionization detector, and the column was DB-5 quartz capillary column (30 m × 0.25 mm, 0.25 μm; Agilent).

3.2 Plant material

The fruits of *T. terrestris* L. were bought from Henan Province, China, and identified by Prof. Qi-shi Sun of Shenyang Pharmaceutical University. The voucher specimen (no. sl0093) is deposited at our laboratory.

3.3 Extraction and isolation

The fruits of *T. terrestris* L. (5 kg) were comminuted and extracted with 75% EtOH for three times, and the extract was evaporated under reduced pressure to afford a residue (200 g). The residue was suspended in water, and then extracted with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH successively. The *n*-butanol extract was evaporated *in vacuo* to give a residue (65 g), which was chromatographed over silica gel column eluting with $CHCl_3$–CH_3OH (in gradient, 100:1 to 0:100, v/v) to yield eight major fractions (A–H) on the basis of the TLC analysis. Fraction E (5 g) was further subjected to silica gel column chromatography eluting with $CHCl_3$–CH_3OH (20:1 to 1:1, v/v) to give 10 fractions (E_1–E_{10}). Compound **1** (25 mg) was obtained from fraction E_3 [HPLC (MeOH–H_2O, 50:50)]. Compound **2** (11 mg) was obtained from fraction E_4 [HPLC (MeOH–H_2O, 56:44)]. Compounds **3** (8 mg) and **4** (15 mg) were obtained from fraction E_6 [HPLC (MeOH–H_2O, 38:62)].

3.3.1 Compound *1*

White amorphous power; $[\alpha]_D^{22}$ −22.2 (c = 0.25, pyridine); IR (KBr) ν_{max} 3416 (OH), 2928 (CH), 1705 (C = O), 1384, 1068, 748, 703 cm^{-1}; for ^1H and ^{13}C NMR spectral data (300 MHz, pyridine-d_5), see Table 1; HR-ESI-MS: *m/z* 1119.5205 $[M + Na]^+$ (calcd for $C_{51}H_{84}O_{25}Na$, 1119.5199).

3.3.2 Compound *2*

White amorphous power; $[\alpha]_D^{22}$ −23.1 (c = 0.20, pyridine); IR (KBr) ν_{max} 3420 (OH), 2928 (CH), 1380, 1070, 748, 703 cm^{-1}; for ^1H and ^{13}C NMR spectral data (300 MHz, pyridine-d_5), see Table 1; HR-ESI-MS: *m/z* 1135.5516 $[M + Na]^+$ (calcd for $C_{52}H_{88}O_{25}Na$, 1135.5512).

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 6 h. After cooling, the reaction mixture was diluted to 20 ml with water and extracted with CH_2Cl_2 (3 × 20 ml). The aqueous layer was concentrated to dryness to give a residue and 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 equal volume of acetic anhydride

was added, followed by heating at 90°C for another 2 h. Then, the solution was concentrated to dryness and taken up 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, carrier gas: N_2 (1.5 ml/min), injector and detector temperature: 280°C, injection volume: 1 μl, split ratio: 10:1). The derivatives of D-glucose and D-galactose were detected. R_f (min): 26.09 and 26.59 min, respectively. The standard monosaccharides were subjected to the same reaction and GC analysis under the same condition.

Acknowledgements

The authors are grateful to the members of the Analytical Group in Shenyang Pharmaceutical University for measurements of all spectra.

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