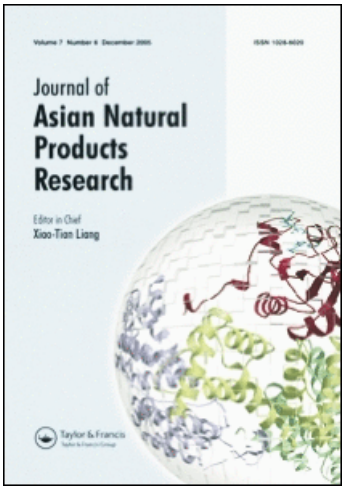

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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Online publication date: 26 March 2010

To cite this Article Liu, Tao , Chen, Gang , Yi, Guo-Qing , Xu, Jian-Kun , Zhang, Tian-Long , Hua, Hui-Ming and Pei, Yue-Hu(2010) 'New pregnane and steroidal glycosides from *Tribulus terrestris* L.', Journal of Asian Natural Products Research, 12: 3, 209 — 214
To link to this Article: DOI: 10.1080/10286020903535419
URL: http://dx.doi.org/10.1080/10286020903535419

Journal of Asian Natural Products Research
Vol. 12, No. 3, March 2010, 209–214

Taylor & Francis
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ORIGINAL ARTICLE

New pregnane and steroidal glycosides from *Tribulus terrestris* L.

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(*Received 31 October 2009; final version received 7 December 2009*)

Three new steroidal saponins were isolated from the fruits of *Tribulus terrestris* L. Their structures were elucidated by spectroscopic and chemical analysis as 16β-(4′-methyl-5′-*O*-β-D-glucopyranosyl-pentanoxy)-5α-pregn-3β-ol-12,20-dione-3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**1**), 2α,3β-dihydroxy-5α-pregn-16-en-20-one 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**2**) and 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostan-20(22)-en-2α,3β,26-triol-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**3**).

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

1. Introduction

Tribulus terrestris (Zygophyllaceae) is an annual creeping herb growing on roadsides and hills in China. The fruit of *T. terrestris*, known as 'jili' in traditional Chinese medicine, has been used against various diseases for a long time. Pharmacological studies have shown that saponins of this plant are the main active components. This paper reports the isolation and structural elucidation of two new pregnane glycosides and one new furostanol saponin based on extensive spectroscopic analysis [1,2].

2. Results and discussion

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D^{20} + 2.3$ ($c = 1.55$, MeOH). Its molecular formula was determined to be $C_{51}H_{82}O_{26}$ by HR-ESI-MS at *m/z* 1133.4990 $[M+Na]^+$. The ^1H and ^{13}C NMR spectra of **1** (Tables 1 and 2) revealed the presence of four methyl groups on the aglycone and were essentially analogous to the aglycone of previously reported compound **4** (5α-furostan-20(22)-en-12-one-3β,26-diol) [3]. However, the aglycone of **1** was different from **4** in the lack of the signals assignable to the olefinic group forming the bond between C-20 and C-22, and the presence of a ketone carbonyl carbon signal at δ 204.4 and an ester carbonyl carbon signal at δ 173.3. The HMBC spectrum (Figure 1) exhibited correlations of the ketone carbonyl carbon with H-17 at δ 3.26 (1H, d, $J = 7.7$ Hz) and Me-21 at δ 2.25 (3H, s) and correlations of the ester carbonyl carbon with H-16 at δ 5.61 (1H, m) and H_2-23 at δ 2.44 and 2.36, indicating that C-20 and C-22 of **1** were the carbonyl groups, instead of the olefinic group in **4**. The *J* value of 7.7 Hz between H-16 and H-17 suggested the β-configuration of the side chain attached to C-16. This information was supported by the NOESY spectra (Figure 1). Acid hydrolysis

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020903535419
http://www.informaworld.com

Table 1. ^{1}H and ^{13}C NMR (pyridine-d_5) spectral data of the aglycone moieties of compounds **1**–**3**.

	1		**2**		**3**	
No.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	36.6		45.5		45.8	
2	29.7		70.4	3.93 (m)	70.5	3.95 (m)
3	76.8	3.87 (m)	84.6	3.83 (m)	84.7	3.85 (m)
4	34.6		34.1		34.0	
5	44.4		44.9	0.98 (m)	44.7	
6	28.5		28.1		28.1	
7	31.3		31.9		32.4	
8	33.7		33.2		34.4	
9	56.3		54.9		54.4	
10	36.4		37.0		36.9	
11	37.5		21.5		21.6	
12	212.0		35.2		39.8	
13	57.1		46.5		43.8	
14	53.9		56.3		54.7	
15	34.5		32.2		34.4	
16	73.4	5.61 (m)	144.7	6.58 (m)	84.5	4.60 (m)
17	59.1	3.26 (d, $J = 7.7$ Hz)	155.4		64.6	
18	14.1	1.50 (s)	16.2	0.87 (s)	14.5	0.68 (s)
19	11.7	0.62 (s)	13.3	0.70 (s)	13.4	0.71 (s)
20	204.4		196.3		103.7	
21	30.7	2.25 (s)	27.1	2.22 (s)	11.8	1.61 (s)
22	173.3				152.4	
23	32.2	2.36, 2.44 (m)			23.7	
24	29.0				31.5	
25	33.4				33.5	
26	74.7	3.69, 4.02 (m)			75.0	3.63, 3.95 (m)
27	17.0	0.90 (d, $J = 6.5$ Hz)			17.4	1.01 (d, $J = 6.5$ Hz)

of **1** gave D-glucose and D-galactose in a ratio of 3:1 on the basis of GC analysis. The β-anomeric configurations for both glucose and galactose were judged from their coupling constants ($J_{1,2} > 7.0$ Hz) [1]. The HMBC experiment showed correlations between the proton signal at δ 4.88 (H-1′, galactosyl group) and the carbon signal at δ 76.8 (C-3, aglycone), between H-1″ at δ 5.14 and C-4′ at δ 81.1, between H-1‴ at δ 5.22 and C-2″ at δ 86.2, and between H-1⁗ at δ 4.78 and C-26 at δ 74.7. Accordingly, the structure of **1** was determined to be 16β-(4′-methyl-5′-*O*-β-D-glucopyranosyl-pentanoxy)-5α-pregn-3β-ol-12,20-dione-3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside.

Compound **2** was obtained as a white amorphous powder, $[\alpha]_D^{20} + 3.9$ ($c = 0.89$, MeOH). Its molecular formula was determined to be $C_{33}H_{52}O_{13}$ by HR-ESI-MS at *m/z* 679.3305 $[M+Na]^+$. The ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2) revealed the presence of three methyl groups on the aglycone and were essentially analogous to the aglycone of previously reported compound **5** (2α,3β-dihydroxypregna-5,16-dien-20-one) [4]. However, the aglycone of **2** was different from **5** in the lack of the signals assignable to the olefinic group forming the bond between C-5 and C-6, and a methine carbon signal at δ 44.9 and a methylene carbon signal at δ 28.1 appeared in the ^{13}C NMR spectrum. The HMBC spectrum (Figure 2) showed correlation of the methine carbon with Me-19 at δ 0.70 (3H, s) and correlation of the methylene carbon with H-5 at δ 0.98 (1H, m). All

Table 2. ^{1}H and ^{13}C NMR (pyridine-d_5) spectral data of the sugar moieties of compounds **1**–**3**.

	1		**2**		**3**	
No.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
(3-*O*)-β-D-Gal	102.3	4.88 (d, $J = 7.6$ Hz)	103.5	4.92 (d, $J = 7.7$ Hz)	103.5	4.93 (d, $J = 7.6$ Hz)
2	73.4		73.1		73.1	
3	75.6		75.8		75.8	
4	81.1		80.2		80.2	
5	75.3		76.0		76.0	
6	60.5		61.0		61.0	
(Gal^4)-β-D-Glc	105.3	5.14 (d, $J = 7.6$ Hz)	107.2	5.27 (d, $J = 7.7$ Hz)	107.2	5.27 (d, $J = 7.7$ Hz)
2	86.2		75.2		75.2	
3	78.2		78.8		78.8	
4	70.3		72.3		72.3	
5	77.7		78.6		78.6	
6	61.6		63.1		63.1	
(Glc^2)-β-D-Glc	107.0	5.22 (d, $J = 7.0$ Hz)				
2	77.0					
3	79.0					
4	71.7					
5	78.5					
6	63.2					
(26-*O*)-β-D-Glc	104.9	4.78 (d, $J = 7.8$ Hz)			104.9	4.83 (d, $J = 7.7$ Hz)
2	75.2				75.2	
3	78.6				78.7	
4	71.8				71.8	
5	78.5				78.6	
6	62.9				62.9	

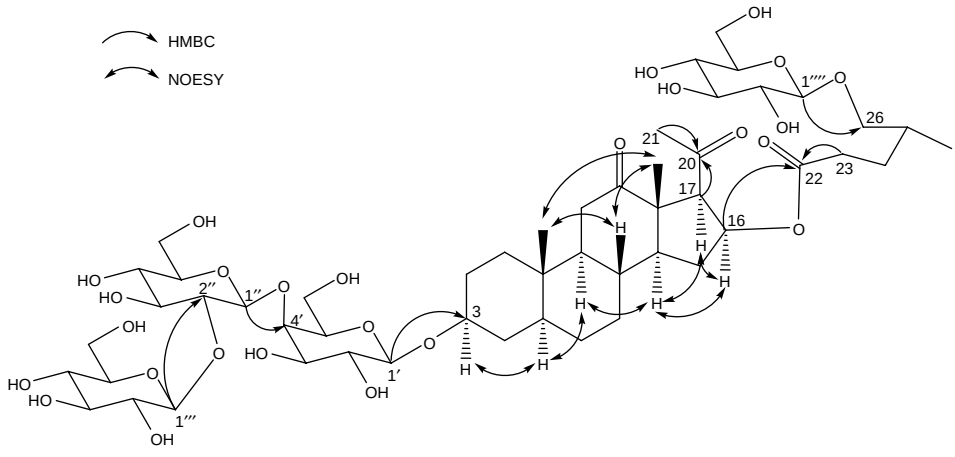

Figure 1. The structure and key HMBC and NOESY correlations of compound **1**.

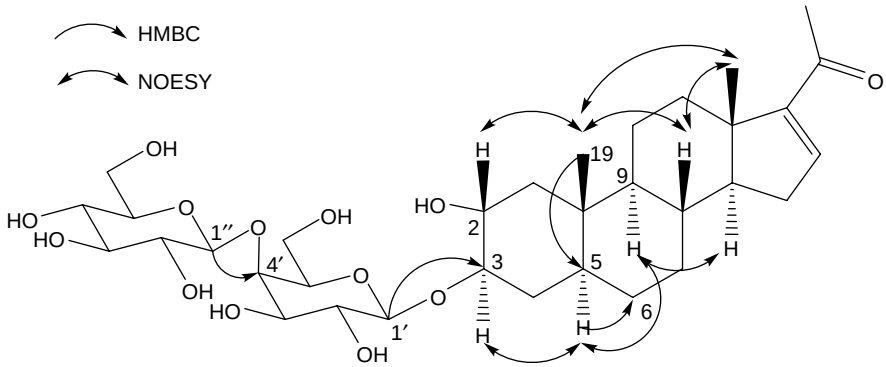

Figure 2. The structure and key HMBC and NOESY correlations of compound **2**.

these evidences indicated that the double bond between C-5 and C-6 was saturated. The orientation of C-5 is α because of the chemical shifts of C-5 (δ 44.9), C-9 (δ 54.9), and C-19 (δ 13.3). For 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 5β compounds, the chemical shifts of these carbons are observed at ~35–36.5, ~40, and ~24 ppm, respectively [1]. Thus, the aglycone moiety of **2** was deduced to be 2α,3β-dihydroxy-5α-pregn-16-en-20-one. The above conclusion was also confirmed by the NOESY spectra (Figure 2). Acid hydrolysis of **2** afforded D-glucose and D-galactose in a ratio of 1:1 on the basis of GC analysis. The β-anomeric configurations for both glucose and galactose were judged from their coupling constants. The HMBC experiment showed correlations between the proton signal at δ 4.92 (H-1′, galactosyl group) and the carbon signal at δ 84.6 (C-3, aglycone), between H-1″ at δ 5.27 and C-4′ at δ 80.2. In conclusion, the structure of **2** was elucidated as 2α,3β-dihydroxy-5α-pregn-16-en-20-one 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside.

Compound **3** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ + 0.16 (c = 1.15, MeOH). Its molecular formula was determined to be $C_{45}H_{74}O_{19}$ by HR-ESI-MS at m/z 941.7723 $[M+Na]^+$. Comparing the ^1H and ^{13}C NMR spectra of **3** (Tables 1 and 2) with those of reported steroidal sapogenin [5], we confirm the aglycone moiety as (25*R*)-5α-furostane-20(22)-en-2α,3β,26-triol. The above conclusion was also confirmed by the NOESY spectra (Figure 3). Acid hydrolysis of **3** afforded D-glucose and D-galactose in a ratio of 2:1 on the basis of GC analysis. The β-anomeric configurations for both glucose and galactose were judged from their coupling constants. The positions of the sugar residues in **3** were defined by the HMBC experiment (Figure 3). The cross-peaks between H-1′ at δ 4.93 and C-3 at δ 84.7, between H-1″ at δ 5.27 and C-4′ at δ 80.2 indicated that a sugar moiety, 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside, was linked to the aglycone at C-3. Additionally, a cross-peak between H-1‴ at δ 4.83 and C-26 at δ 75.0 definitively proved that the other glucose was linked to C-26 of the aglycone.

In conclusion, the structure of **3** was elucidated as 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostan-20(22)-en-2α,3β,26-triol-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside.

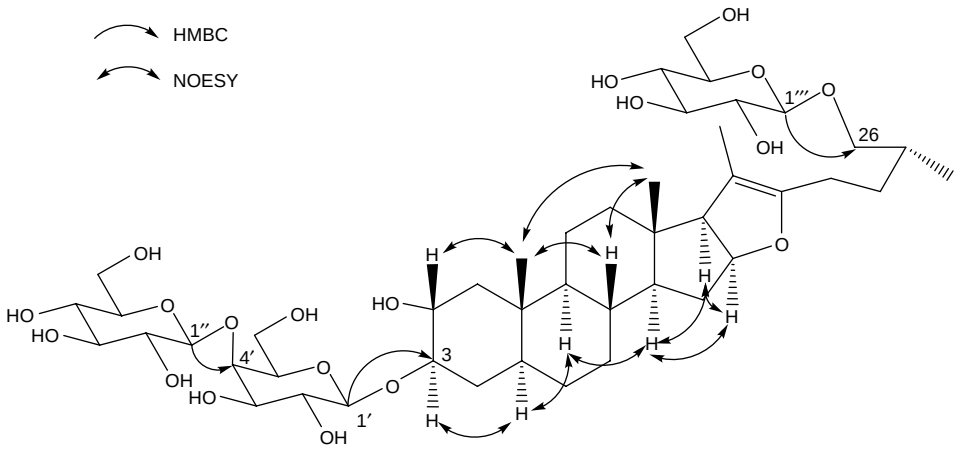

Figure 3. The structure and key HMBC and NOESY correlations of compound **3**.

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 ^{1}H and 150 MHz for ^{13}C) in C_5D_5N with TMS as an 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 three times for 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 the silica gel column, eluted with $CHCl_3$–CH_3OH (100:1–0:1), yielding eight fractions. Fraction 2 (4 g) was subjected to HPLC, eluted with MeOH (50%) by a flow rate of 3 ml/min and afforded compound **2** (25 mg) at 169 min. Fraction 4 (2 g) was subjected to HPLC, eluted with MeOH (67%) by a flow rate of 3 ml/min and afforded compound **3** (15 mg) at 38.7 min. Fraction 5 (3 g) was subjected to HPLC, eluted with MeOH (41%) by a flow rate of 3 ml/min and afforded compound **1** (23 mg) at 87 min.

3.3.1 *16β-(4′-Methyl-5′-O-β-D-glucopyranosyl-pentanoxy)-5α-pregn-3β-ol-12,20-dione-3-O-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**1**)*

White amorphous powder; $[\alpha]_D^{20} + 2.3$ ($c = 1.55$, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3410, 2927, 1706, 1645, 1372, 1070, 893, 633; ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2; HR-ESI-MS:

m/z 1133.4990 $[M+Na]^+$ (calcd for $C_{51}H_{82}O_{26}Na$, 1133.4992).

*3.3.2 2α,3β-Dihydroxy-5α-pregn-16-en-20-one 3-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**2**)*

White amorphous powder; $[\alpha]_D^{20} + 3.9$ ($c = 0.89$, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3407, 2932, 1661, 1586, 1380, 1232, 1164, 1074, 924, 892, 607; ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; HR-ESI-MS: *m/z* 679.3305 $[M+Na]^+$ (calcd for $C_{33}H_{52}O_{13}Na$, 679.3306).

*3.3.3 26-O-β-D-Glucopyranosyl-(25R)-5α-furostan-20(22)-en-2α,3β,26-triol-3-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**3**)*

White amorphous powder; $[\alpha]_D^{20} + 0.16$ ($c = 1.15$, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3421, 2927, 1653, 1380, 1071, 746, 704, 666; ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; HR-ESI-MS: *m/z* 941.4723 $[M+Na]^+$ (calcd for $C_{45}H_{74}O_{19}Na$, 941.4722).

3.4 Acid hydrolysis of compounds 1–3

Each solution of compounds **1**–**3** (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 *in vacuo* to dryness to give a residue which 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, followed by an equal volume of acetic anhydride at 90°C for another 2 h. The solution was concentrated in vacuo dryness and 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; 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 D-glucose and D-galactose were detected [R_f (min): 26.11 and 26.60, respectively]. The standard monosaccharides were subjected to the same reaction and GC analysis under the same conditions.

Acknowledgement

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

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