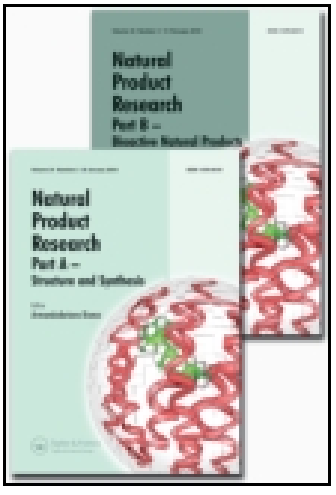

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Furostanol saponins from the fruits of Tribulus terrestris

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Furostanol saponins from the fruits of *Tribulus terrestris*

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Two new steroidal saponins were isolated from the fruits of *Tribulus terrestris*. Their structures were assigned by spectroscopic analysis and colour reaction as 26-*O*-*β*-D-glucopyranosyl-(25R)-5*α*-furostane-12-one-3*β*,22*α*,26-triol-3-*O*-*β*-D-glucopyranosyl(1 → 4)-*β*-D-galactopyranoside (**1**); 26-*O*-*β*-D-glucopyranosyl-25(R)-5*α*-furostan-12-one-3*β*,22*α*,26-triol-3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*O*-[*β*-D-glucopyranosyl-(1 → 4)]-*β*-D-galactopyranoside (**2**).

Keywords: *Tribulus terrestris*; steroidal saponins; furostanols

1. Introduction

Tribulus terrestris L., growing in subtropical areas around the world, the fruits of which were used as the treatment of eye trouble, oedema, abdominal distention, emission, morbid leucorrhea, sexual dysfunction and veiling in traditional Chinese medicine. In addition, it was also used as medicine in India, South Africa and Japan. Many pharmaceutical preparations and food supplements were for sale based on the saponin fraction of this plant. Such as 'Tribestane' and 'Vitanone' were used for the treatment of impotency, 'tribusaponins' and 'Xin-nao-shu-tong' were used for the treatment of cardiovascular disease (Bedir, Khan, & Walker, 2002; Dinchev et al., 2008; Kostova & Dinchev, 2005; Li, Li, Li, & Yang, 2006; Li & Yang, 2006; Neychev, Nikolova, Zhelev, & Mitev, 2007; Yang, Qu, & Sun, 2005; Zhang, Qu, & Zhou, 2006). This article reports the structural assignment of the new saponins based on extensive spectroscopic analysis and chemical evidence.

2. Results and discussion

Two new compounds were isolated from the chloroformic fraction of the alcoholic extract of *T. terrestris* L. by HPLC. The structures of the isolated compounds were established based on 1D and 2D NMR spectral data.

Compound **1** was obtained as an amorphous solid with a molecular formula of $C_{45}H_{74}O_{20}$, as determined by data of the negative-ion HRESI–MS (*m*/*z* 933.4684 $[M-H]^-$). The ^{1}H-NMR spectrum of **1** showed proton signals for four steroidal methyl groups at δ1.12 (3H, s, H-18), 0.67 (3H, s, H-19), 1.54 (3H, d, $J = 7.0$ Hz, H-21) and

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0.98 (3H, d, $J = 6.5$ Hz, H-27), two methine proton signals at δ 3.87 (1H, m, H-3) and 4.86 (1H, m, H-16) which was indicative of secondary alcoholic functions, two methylene proton signals at δ 4.08 (1H, m, H-26 a) and 3.61 (1H, dd, $J = 6.0$, 9.2, H-26 b) ascribable to a primary alcoholic function, and three anomeric protons at δ 4.86 (1H, d, $J = 7.6$ Hz, H-1′), 5.28 (1H, d, $J = 7.7$ Hz, H-1″), and 4.81 (1H, $J = 7.8$ Hz, H-1‴). The ^{13}C-NMR spectrum displayed signals ascribable to a carbonyl function at δ 213.0 (C-12), a hemiacetal function at δ 110.8 (C-22), two secondary alcoholic functions at δ 76.9 (C-3) and 79.7 (C-16), and one primary alcoholic function at δ 75.25 (C-26), suggesting the occurrence of a 3,26-bisdesmosidic furostanol saponin. In the HMBC spectrum, the methyl protons at δ 1.12 (18-CH_3) showed long-range correlation with the carbons at δ 213.0 (C-12), 55.6 (C-13), 55.8 (C-14) and 55.1 (C-17), indicating the attachment of a carbonyl group at C-12. Comparison of the signals from the sterol part of **1** in the ^{13}C-NMR spectra with those from the sterol part of 26-*O*-β-D-glucopyranosyl-(25R)-5α-furostane-12-one-3β,22α,26-triol-3-*O*-β-D-glucopyranosyl (1 → 2)-β-D-galactopyranoside (Cai et al., 1999) showed that the sterol part of **1** was the same as that of the compound in the literature, including the orientations of the C-3 oxygen atom, C-5 hydrogen atom and C-22 hydroxyl group (3β, 5α, 22α). Acid hydrolysis of **1** with 2 M HCl in CH_3OH–H_2O (4 : 1) gave glucose and galactose in a ratio of 2 : 1. The β-anomeric configurations for both the glucose and galactose were judged from its coupling constants ($J_{1,2} > 7.0$ Hz). The absolute configurations of the sugar units were determined to be D-glucose and D-galactose on the basis of GC analysis. In the HMBC spectrum, a cross-peak from proton signal at δ 4.86 (H-1′, galactose) to the carbon signal at δ 76.9 (C-3, aglycone), from δ 4.81(H-1‴, glucose) to δ 75.25 (C-26) were observed, indicating the glycosylation of the aglycone at C-3 and C-26. Similarly, anomeric proton signal at δ 5.28 (H-1″, terminal glucose) showed cross-peak with the carbon signal at δ 80.1 (C-4′ of the galactose). Thus, the structure of **1** was established to be 26-*O*-β-D-glucopyranosyl-(25R)-5α-furostane-12-one-3β,22α,26-triol-3-*O*-β-D-glucopyranosyl (1 → 4)-β-D-galactopyranoside, named Tribulusaponin A.

Compound **2** was obtained as an amorphous solid with a molecular formula of $C_{51}H_{84}O_{24}$, as determined by data of the negative-ion HRESI–MS (m/z 1079.5216 $[M - H]^-$). The ^{1}H-NMR spectrum showed signals for steroid methyl protons at δ 1.07 (3H, s, H-18), 0.87 (3H, s, H-19), 1.53 (3H, d, $J = 7.2$, H-21), 0.96 (3H, d, $J = 6.6$ Hz, H-27), along with signals for four anomeric protons at δ 4.87 (1H, d, $J = 7.2$ Hz, H-1′), 6.19 (1H, br s, H-1″), 5.16 (1H, d, $J = 7.8$ Hz, H-1‴), 4.79 (1H, d, $J = 7.8$ Hz, H-1⁗). Comparison of the ^{13}C-NMR data for the aglycon moiety of **2** with those of **1** revealed that the aglycon moiety was identical to that of **1**. Sugars obtained on acid hydrolysis of **2** were identified as D-galactose, D-glucose and L-rhamnose in a ratio of 1 : 2 : 1 on the basis of thin layer chromatography (TLC) and GC analysis. An α-anomeric configuration for the rhamnose unit was concluded from its C-5 chemical shift (δ 69.5). The β-anomeric configurations for both the glucose and galactose were judged from their coupling constants ($J_{1,2} > 7.0$ Hz). The HMBC correlations (Figure 1) from δ 4.87 (H-1′ of galactose) to δ 76.8 (C-3 of aglycone), from δ 6.19 (H-1″ of rhamnose) to δ 77.0 (C-2′ of glucose), from δ 5.16 (H-1‴ of glucose) to δ 81.3 (C-4′ of galactose) and from δ 4.79 (1‴ of glucose) to δ 75.3 (C-26 of aglycone) indicated that the sugar chain was attached to C-3 of the aglycone and the rhamnose and glucose were linked at C-2′ and C-4′ of the inner galactose, respectively, the other glucose was linked at C-26 of aglycone. Accordingly, the structure of compound **2** was determined to be 26-*O*-β-D-glucopyranosyl-(25R)-5α-furostane-12-one-3β,22α,26-triol-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 4)]-β-D-galactopyranoside, named Tribulusaponin B.

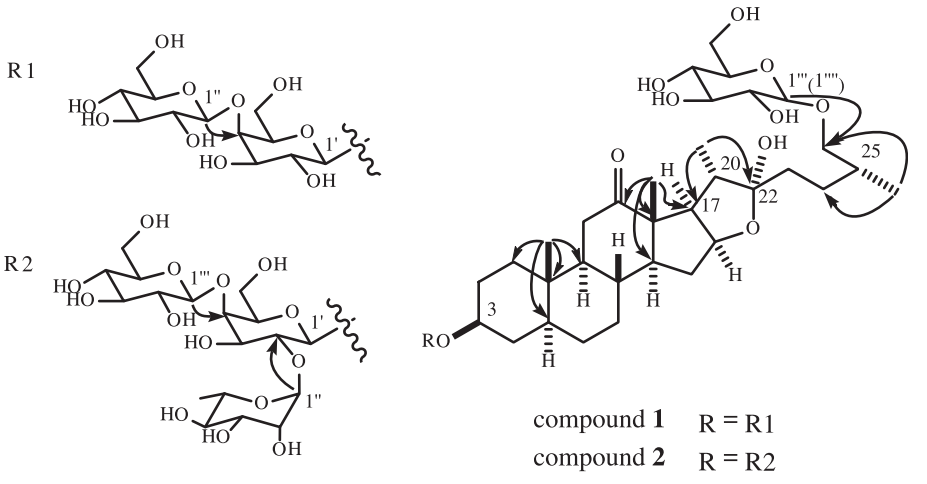

Figure 1. Structures and HMBC correlations of compounds **1** and **2**.

3. Experimental

3.1. *General details*

Optical rotations were obtained using a Perkin Elmer 241MC spectropolarimeter at room temperature. NMR analysis was measured on ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz): Bruker DRX-300 and DRX-600 spectrometer with TMS as internal standard. HRESI–MS was measured on a TOF of micromass spectrometer. TLC was carried out on plates precoated with RP-18 gel (Merck) and silica gel F_{254} (Qingdao Marine Chemistry Ltd.). Spots on the plates were visualised by spraying with Ehrich reagent, followed by heating. Column chromatography (CC) was performed on silica gel (200–300 and 300–400 mesh; Qingdao Marine Chemical Factory), MPLC (BÜCHI, column $3.5 \times 45\,cm^2$, 50 μm) and HPLC (Shimadzu LC-8, column $10 \times 250\,mm^2$, 5 μm). GC analysis was performed on a Shimadzu GC-2010 gas chromatograph equipped with an H_2 flame ionisation detector. The column was DB-5 quartz capillary column ($30\,m \times 0.25\,mm \times 0.25\,\mu m$) with the following conditions: column temperature 160–195°C; programmed increase, $2^{\circ}C\,min^{-1}$; carrier gas, N_2 ($1\,mL\,min^{-1}$); injector and detector temperature, 250°C; injection volume, 1 μL; split ratio, 1/10. Standards D-glucose, D-galactose and L-rhamnose were purchased from National Institute for the Control of Pharmaceutical and Biological Products, China.

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 is deposited at our laboratory (no. 200912a).

3.3. *Extraction and purification of 1 and 2*

The comminuted fruits of *T. terrestris* L. (5 kg) were extracted with 75% EtOH for three times and the extract was evaporated under reduce press to afford a residue (200 g). The residue was suspended in H_2O, and then extracted with petroleum benzin, $CHCl_3$, EtOAc and *n*-BuOH, respectively. The *n*-BuOH layer was then concentrated to dryness giving a crude saponin fr. (65 g). The extract was subjected to CC on silica gel eluted with $CHCl_3$–MeOH (100 : 1–0 : 100), successively. The fraction (CH_3Cl–MeOH = 100 : 20) was subjected to CC on silica gel to gave five fractions. Fraction D (200 mg) was subjected to

HPLC eluted with MeOH/H_2O (55%) to give tribulusaponin A (**1,** 25 mg, 0.0005%) and B (**2,** 22 mg, 0.0005%).

3.3.1. *Tribulusaponin A (**1**)*

White amorphous power; $[\alpha]_D^{22}-14.2$ (*c* 0.064, pyridine); HRESI–MS *m*/*z* 933.4684 $[M-H]^-$ (Calcd for $C_{45}H_{74}O_{20}$, 933.4695). IR (KBr) υ_{max} 3413 (OH), 2930 (CH), 1709 (C=O), 1388, 1065, 749, 705 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5): δ 0.69, m, 1H, C1-Ha; 1.31, m, 1H, C1-Hb; 1.52, m, 1H, C2-Ha; 1.97, m, 1H, C2-Hb; 3.87, m, 1H, C3-H; 1.28, m, 1H, C4-Ha; 1.80, m, 1H, C4-Hb; 0.86, m, 1H, C5-H; 1.12, m, 2H, C6-H; 0.70, m, 1H, C7-Ha; 1.52, m, 1H, C7-Hb; 1.72, m, 1H, C8-H; 0.89, m, 1H, C9-H; 2.20, m, 1H, C11-Ha; 2.39, m, 1H, C11-Hb; 1.31, m, 1H, C14-H; 1.60, m, 1H, C15-Ha; 2.10, m, 1H, C15-Hb; 4.86, m, 1H, C16-H; 2.89, m, 1H, C17-H; 1.12, s, 3H, C18-H; 0.67, s, 3H, C19-H; 2.18, m, 1H, C20-H; 1.54, d, $J=6.7$ Hz, 3H, C21-H; 1.93, m, 1H, C23-Ha; 2.05, m, 1H, C23-Hb; 1.69, m, 1H, C24-Ha; 2.04, m, 1H, C24-Hb; 1.93, m, 1H, C25-H; 3.61, d, $J=6.0$, 9.2 Hz, 1H, C26-Ha; 4.08, m, 1H, C26-Hb; 0.98, d, $J=6.4$ Hz, 3H, C27-H; 4.86, d, $J=7.6$ Hz, 1H, C1′-H; 4.37, m, 1H, C2′-H; 4.07, m, 1H, C3′-H; 4.69, m, 1H, C4′-H; 4.12, m, 1H, C5′-H; 4.25, m, 1H, C6′-Ha; 4.65, m, 1H, C6′-Hb; 5.28, d, $J=7.7$ Hz, 1H, C1″-H; 4.10, m, 1H, C2″-H; 4.20, m, 1H, C3″-H; 4.06, m, 1H, C4″-H; 3.95, m, 1H, C5″-H; 4.21, m, 1H, C6″-Ha; 4.54, m, 1H, C6″-Hb; 4.81, d, $J=7.8$ Hz, 1H, C1‴-H; 4.04, m, 1H, C1‴-H; 4.22, m, 1H, C3‴-H; 4.18, m, 1H, C4‴-H; 3.94, m, 1H, C5‴-H; 4.38, m, 1H, C6‴-Ha; 4.58, m, 1H, C6‴-Hb. ^{13}C-NMR (150 MHz, pyridine-d_5): δ (C-1) 36.7; (C-2) 29.8; (C-3) 76.9; (C-4) 34.6; (C-5) 44.5; (C-6) 28.6; (C-7) 31.7; (C-8) 34.3; (C-9) 55.8; (C-10) 36.3; (C-11) 38.0; (C-12) 213.0; (C-13) 55.6; (C-14) 55.8; (C-15) 31.7; (C-16) 79.7; (C-17) 55.1; (C-18) 16.3; (C-19) 11.7; (C-20) 41.3; (C-21) 15.3; (C-22) 110.8; (C-23) 37.1; (C-24) 28.4; (C-25) 34.3; (C-26) 75.2; (C-27) 17.5; (C-1′) 102.5; (C-2′) 73.5; (C-3′) 75.5; (C-4′) 80.1; (C-5′) 76.0; (C-6′) 61.1; (C-1″) 107.1; (C-2″) 75.3; (C-3″) 78.7; (C-4″) 72.3; (C-5″) 78.5; (C-6″) 62.9; (C-1‴) 105.0; (C-2‴) 75.3; (C-3‴) 78.6; (C-4‴) 71.7; (C-5‴) 78.5; (C-6‴) 63.1.

3.3.2. *Tribulusaponin B (**2**)*

White amorphous power; $[\alpha]_D^{22}-16.3$(*c* 0.027, pyridine); HRESI–MS *m*/*z* 1079.5216 $[M-H]^-$ (Calcd for $C_{51}H_{83}O_{24}$, 1079.5274). IR (KBr) υ_{max} 3412 (OH), 2933 (CH), 1708 (C=O), 1388, 1065, 749, 700 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5): δ 0.71, m, 1H, C1-Ha; 1.35, m, 1H, C1-Hb; 1.80, m, 1H, C2-Ha; 2.00, m, 1H, C2-Hb; 3.87, m, 1H, C3-H; 1.25, m, 1H, C4-Ha; 1.75, m, 1H, C4-Hb; 0.85, m, 1H, C5-H; 1.15, m, 2H, C6-H; 0.72, m, 1H, C7-Ha; 1.55, m, 1H, C7-Hb; 1.65, m, 1H, C8-H; 0.90, m, 1H, C9-H; 2.25, m, 1H, C11-Ha; 2.41, m, 1H, C11-Hb; 1.37, m, 1H, C14-H; 1.58, m, 1H, C15-Ha; 2.05, m, 1H, C15-Hb; 4.86, m, 1H, C16-H; 2.89, m, 1H, C17-H; 1.07, s, 3H, C18-H; 0.87, s, 3H, C19-H; 2.18, m, 1H, C20-H; 1.53, d, $J=7.2$ Hz, 3H, C21-H; 2.03, m, 2H, C23-H; 1.65, m, 1H, C24-Ha; 2.04, m, 1H, C24-Hb; 1.91, m, 1H, C25-H; 3.59, d, $J=6.6$, 9.6 Hz, 1H, C26-Ha; 3.93, m, 1H, C26-Hb; 0.96, d, $J=6.6$ Hz, 3H, C27-H; 4.87, d, $J=7.2$ Hz, 1H, C1′-H; 4.52, m, 1H, C2′-H; 4.27, m, 1H, C3′-H; 4.59, m, 1H, C4′-H; 4.04, m, 1H, C5′-H; 4.25, m, 1H, C6′-Ha; 4.60, m, 1H, C6′-Hb; 6.19, br. s, 1H, C1″-H; 4.76, m, 1H, C2″-H; 4.56, m, 1H, C3″-H; 4.30, m, 1H, C4″-H; 4.85, m, 1H, C5″-H; 1.68, d, $J=6.1$ Hz, 3H, C6″-H. 5.16, d, $J=7.8$ Hz, 1H, C1‴-H; 4.04, m, 1H, C1‴-H; 4.17, m, 1H, C3‴-H; 4.05, m, 1H, C4‴-H; 3.96, m, 1H, C5‴-H; 4.18, m, 1H, C6‴-Ha; 4.58, m, 1H, C6‴-Hb; 4.79 d, $J=7.8$ Hz, 1H, C1⁗-H; 4.04, m, 1H, C1⁗-H; 4.22, m, 1H, C3⁗-H; 4.21, m, 1H, C4⁗-H; 3.96, m, 1H, C5⁗-H; 4.38, m, 1H, C6⁗-Ha; 4.55, m, 1H, C6⁗-Hb. ^{13}C-NMR (150 MHz, pyridine-d_5): δ (C-1) 36.7; (C-2) 29.8; (C-3) 76.9; (C-4) 34.6; (C-5) 44.5; (C-6) 28.6; (C-7) 31.7; (C-8) 34.3; (C-9) 55.8; (C-10) 36.3; (C-11) 38.0; (C-12) 213.0; (C-13) 55.6; (C-14) 55.9; (C-15) 31.7;

(C-16) 79.7; (C-17) 55.1; (C-18) 16.3; (C-19) 11.7; (C-20) 41.3; (C-21) 15.3; (C-22) 110.8; (C-23) 37.1; (C-24) 28.4; (C-25) 34.3; (C-26) 75.2; (C-27) 17.5; (C-1′) 100.0; (C-2′) 77.0; (C-3′) 76.4; (C-4′) 81.3; (C-5′) 75.3; (C-6′) 61.1; (C-1″) 102.3; (C-2″) 72.5; (C-3″) 72.8; (C-4″) 74.1; (C-5″) 69.5; (C-6″) 18.7; (C-1‴) 107.2; (C-2‴) 75.7; (C-3‴) 78.9; (C-4‴) 72.2; (C-5‴) 78.6; (C-6‴) 63.1; (C-1⁗) 105.0; (C-2⁗ 75.2; (C-3⁗) 78.7; (C-4⁗) 71.8; (C-5⁗) 78.5; (C-6⁗) 62.9.

3.4. *Acid hydrolysis of compounds* 1 *and* 2

A 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 and extracted with CH_2Cl_2 (3 × 20 mL). The aqueous layer was concentrated to appropriate volume (1 mL) and examined by TLC (silica gel) with the solvent system $CHCl_3$/MeOH/H_2O (65 : 35 : 10) for sugar analysis. R_f values of D-glucose, D-galactose and L-rhamnose were 0.25, 0.25 and 0.42, respectively. The remaining 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, 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 analysed by GC (Column: DB-5 quartz capillary column (30 m × 0.25 mm, 0.25 μm)), H_2 flame ionisation detector, column temperature: 160–280°C, programmed increase: 5°C min^{-1}, carrier gas: N_2 (1.5 mL min^{-1}), injector and detector temperature: 280°C, injection volume: 1 μL, split ratio: 10/1. The derivatives of L-rhamnose, D-glucose and D-galactose were detected. Room temperature: 23.87, 28.04 and 28.63 min, respectively. The standard monosaccharides were subjected to the same reaction and GC analysis under the same condition.

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