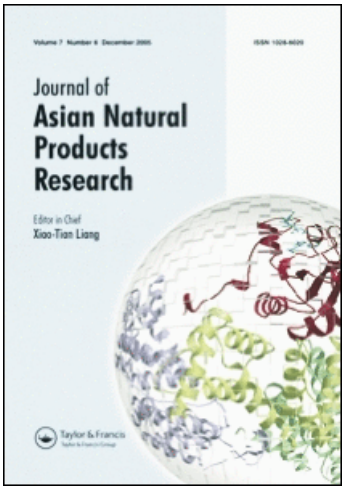

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Two new furostanol saponins, tribufurosides D (**1**) and E (**2**), were isolated from the fruits of *Tribulus terrestris* L. With the help of chemical and spectral analyses (IR, MS, 1D, and 2D NMR), the structures of the two new furostanol saponins were established as 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furost-12-one-2α,3β,22α,26-tetraol-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**1**) and 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furost-12-one-2α,3β,22α,26-tetraol-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (**2**).

Keywords: *Tribulus terrestris* L.; Zygophyllaceae; furostanol saponins; tribufurosides D and E

1. Introduction

Tribulus terrestris L. is an annual creeping herb growing on roadsides and hills in China. The fruits of *T. terrestris*, a Chinese traditional medicine named "Ji Li," are used for treating eye trouble, edema, skin itch, high blood pressure, and cardiovascular diseases [1]. In previous studies on the constituents of the fruits of *T. terrestris*, several steroidal glycosides [2–5] were isolated. In this paper, we report the isolation and structural elucidation of two new furostanol saponins, tribufurosides D (**1**) and E (**2**), using 1D and 2D NMR techniques, ESI-MS analysis as well as chemical methods.

2. Results and discussion

Tribufuroside D (**1**), obtained as a white powder, showed a red coloration with Ehrlich reagent. The IR spectrum showed absorptions for hydroxyl groups ($3420\,cm^{-1}$) and carbonyl group ($1702\,cm^{-1}$). Compound **1** exhibited the molecular formula $C_{45}H_{74}O_{21}$ by its HR-ESI-MS analysis. The ESI-MS of **1** showed a quasi-molecular ion peak at *m/z* 949 $[M-H]^-$, indicating a molecular weight of 950 and significant ion peaks at *m/z* 787 $[M - H - 162]^-$, 607 $[M - H - H_2O - 162 - 162]^-$, 445 $[M - H - H_2O - 162 - 162 - 162]^-$, corresponding to the loss of a hexosyl moiety continuously, showing the presence of a linear sugar chain of galactose–glucose. The ^1H and ^{13}C NMR spectral data (Table 1) 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 0.98

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Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (δ_C, 125 MHz, C_5D_5N).

No.	**1**	**2**	No.	**1**	**2**
1	45.3	45.3	C-3 Gal 1	103.6	103.6
2	70.4	70.4	2	73.2	73.2
3	84.6	84.6	3	75.5	75.4
4	34.4	34.4	4	80.3	80.3
5	44.6	44.6	5	75.9	75.9
6	28.5	28.5	6	61.1	61.1
7	31.8	31.8	Glc 1	107.3	107.3
8	34.0	34.0	2	75.5	75.4
9	55.8	55.8	3	78.9	78.9
10	37.2	37.2	4	72.4	72.4
11	38.3	38.3	5	78.8	78.8
12	212.9	212.9	6	63.3	63.3
13	55.6	55.6	C-26 Glc′1′	105.3	105.1
14	55.9	55.9	2′	75.4	75.3
15	31.8	31.8	3′	78.6	78.6
16	79.8	79.8	4′	71.9	71.9
17	55.2	55.2	5′	78.6	78.6
18	16.4	16.4	6′	62.9	62.9
19	13.0	13.0			
20	41.4	41.4			
21	15.4	15.4			
22	110.9	110.9			
23	37.4	37.4			
24	27.9	27.9			
25	33.9	33.8			
26	75.4	75.3			
27	17.6	17.6			

(3H, s, CH_3-18), 0.62 (3H, s, CH_3-19), 1.42 (3H, s, CH_3-21), 0.90 (3H, d, $J = 7.3$ Hz, CH_3-27), and three oxymethines at δ 3.69 (1H, m, H-3), 3.81 (1H, m, H-2), 4.74 (1H, m, H-16), one oxymethylene at δ 3.49 (1H, dd, $J = 7.0$, 9.5 Hz, H-26), 4.07 (1H, m, H-26), and three anomeric proton doublets at δ 4.68 (1H, d, $J = 7.6$ Hz, gal-H-1), 5.14 (1H, d, $J = 7.5$ Hz, glc-H-1), 4.79 (1H, d, $J = 7.5$ Hz, glc′-H-1′). This information was supported by ^{13}C NMR spectral data of **1**. The ^{13}C NMR spectrum of **1** showed signals of four angular methyl groups at δ 16.4, 13.0, 15.4, 17.6, three carbons bearing oxygen at δ 84.6, 75.4, 79.8, and three anomeric carbons at δ 103.6, 107.3, 105.3. Comparison of ^1H and ^{13}C NMR assignment of the aglycone moiety of **1** with those of 5α-(25*R*)-spirostan-2α,3β-diol-12-one (manogenin) [6] revealed that the structure of the A–D ring parts (C-1–C-19) of **1** was identical to that of the reference compound, including the orientation of the C-2 and C-3 oxygen atoms, but with significant differences in the signals from the E-ring (C-20–C-27). Calculated from the ESI-MS, the molecular weight of the aglycone moiety was 464, 14 more than that of terrestrosin F [7]. In comparison to terrestrosin F, there was one quaternary carbon more and one secondary carbon less in the ^{13}C NMR spectrum, and the IR spectrum showed absorptions for carbonyl group. The ^{13}C NMR spectral data of the aglycone of **1** (Table 1) were almost consistent with those of the aglycone of terrestrosin F, except for the signals of C-12 at δ 212.9, C-11 at δ 38.3, and C-13 at δ 55.6 of ring C. The downfield shift observed in the ^{13}C NMR spectrum at δ 212.9 (C-12), 38.3

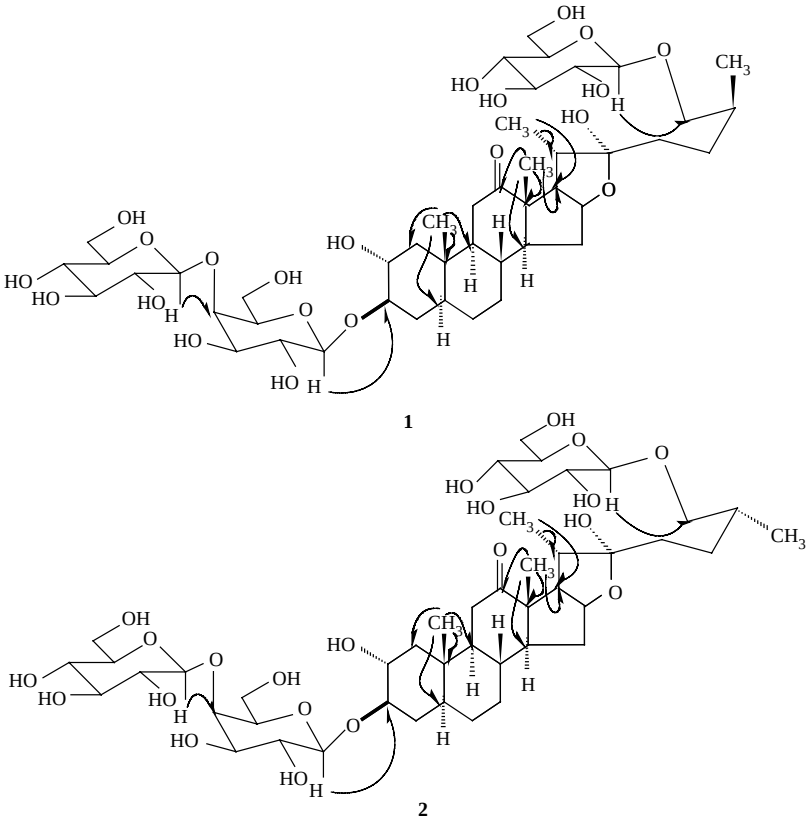

Figure 1. Key HMBC correlations for **1** and **2**.

(C-11), and 55.6 (C-13) suggested that the aglycone has a carbonyl group at C-12. The ^{13}C NMR aglycone signals of **1** were made by comparison with those of terrestrosin F [7], and were confirmed by ^{1}H–^{1}H COSY, DEPT, HMQC, and HMBC spectral analysis (Table 1). In the HMBC spectrum, the methyl protons at δ 0.98 (CH_3-18) showed long-range correlations with carbons at δ 55.6 (C-13), 55.9 (C-14), 212.9 (C-12), and 55.2 (C-17), as shown in Figure 1, indicating the attachment of the keto group at C-12. Thus, aglycone moiety of **1** was deduced to be a 5α-furost-12-one-2α,3β,22α,26-tetraol. The 25*S* configuration of **1** was confirmed by comparison of 26-methylene signals of **1** with those of trigoneoside Ia [8] and trigoneoside Xa [9] in the ^{1}H NMR spectrum. The proton signals assignable to the 26-methylene group [δ 3.48 (1H, dd, $J = 7.0$, 9.5 Hz, H_a-26), 4.07 (1H, m, H_b-26)] in the ^{1}H NMR spectrum of **1** were very similar to those of trigoneoside Ia and trigoneoside Xa [8,9].

An acidic hydrolysis of **1** 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 β-configuration for glucose and galactose [10,11]. The positions of the sugar residues in **1** were defined unambiguously by the HMBC experiment (Figure 1). Cross-peaks due to long-range correlations between gal-H-1 (δ 4.68) of galactose and C-3 (δ 84.6) of the aglycone; between glc-H-1 (δ 5.14) of glucose and

C-4 (δ 80.3) of galactose indicated that a disaccharide 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′ (δ 4.79) of glucose′ and C-26 (δ 75.4) of the aglycone definitively proved that the glucose′ was linked to C-26 of the aglycone. On the basis of all of these evidences, **1** was identified as 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furost-12-one-2α,3β,22α,26-tetraol-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside.

Tribufuroside E (**2**), obtained as a white powder, was also deduced to possess a furostanol structure based on the positive Ehrlich test. The IR spectrum of **2** was similar to that of **1**. The ESI-MS of **2** showed quasi-molecular ion peak at *m/z* 949 $[M-H]^-$, and fragment ion peaks at *m/z* 787 $[M-H-162]^-$, 607 $[M-H-H_2O-162-162]^-$, 445$[M-H-H_2O-162-162-162]^-$, were observed in the ESI-MS. The HR-MS analysis revealed the molecular formula of **2** to be $C_{45}H_{74}O_{21}$, which was the same as that of **1**. The ^1H and ^{13}C NMR spectral data (Table 1) of **2** were assigned unequivocally according to $^1H-^1H$ COSY, HMQC, and HMBC analysis. The ^1H and ^{13}C NMR (Table 1) spectra of **2** were shown to be superimposable to those of **1**, except for the 26 methylene signals [δ 3.61 (1H, dd, $J = 7.0$, 9.5 Hz, H_a-26), 3.97 (1H, m, H_b-26)], which showed the 25*R*-configuration [8,9]. Thus, the aglycone moiety of **2** was deduced to be a (25*R*)-5α-furost-12-one-2α,3β,22α,26-tetraol.

An acidic hydrolysis 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 glucose and galactose [10,11]. The 3,26-bisdesmoside structure of **2** was identified by a HMBC experiment (Figure 1). Cross-peaks due to long-range correlations between gal-H-1 (δ 4.68) of galactose and C-3 (δ 84.6) of the aglycone; between glc-H-1 (δ 5.14) of glucose and C-4 (δ 80.3) of galactose indicated that a disaccharide 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′ (δ 4.79) of glucose′ and C-26 (δ 75.4) of the aglycone definitily proved that the glucose′ was linked to C-26 of the aglycone. Finally, by comparison of the NMR spectral data for **2** with those for **1**, the structure of tribufuroside E was determined to be 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furost-12-one-2α,3β,22α, 26-tetraol-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Kofler microscope apparatus and are uncorrected. The optical rotations were determined on WZZ-15 autopolarimeter. The IR spectra were measured on a Y-Zoom scroll Fourier transform infrared spectrometer with a KBr disc. The HR-ESI-MS were recorded on IonSpec HRESI FT-ICR (Lake Forest, Irvine, CA, USA), 7.0 T (Cryomagnetics, Oak Ridge, TN, USA) and the ESI-MS were recorded on a LCQ-1700 ESI-MS instrument. The NMR spectra were obtained on a Bruker AM-500 instrument, using TMS as an internal standard. HPLC was performed using an ODS column (Shim-park 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 in Tianjin Gel Co. (Tianjin, China). Spots were detected after spraying with 10% H_2SO_4.

3.2 Plant material

The fruits of *T. terrestris* L. were purchased from the company of Chinese Medicinal Materials in Changchun, Jilin Province, China, in September 2004 and identified by Prof. Minglu Deng, Changchun College of Traditional Chinese Medicine. A voucher specimen (No. 040920) has been deposited in the Herbarium of Academy of Traditional Chinese Medicine and Material Medica of Jilin Province.

3.3 Extraction and isolation

The dried and powdered fruits (10 kg) of *T. terrestris* were exhaustively extracted with 60% EtOH, and the extract was concentrated under reduced pressure to obtain a crude residue (310 g), which was chromatographed over a D_{101} macroporous resin column (10 × 80 cm), eluted successively with H_2O, 30% EtOH, and 70% EtOH. The 70% EtOH eluate was concentrated to dryness (29 g saponin mixture) and chromatographed over a silica gel column (200–300 mesh) eluted with $CHCl_3$–MeOH–H_2O (30:10:1–10:10:1) to give fractions 1–5. Fraction 3 (254 mg) was subjected to HPLC (column: 10 × 250 mm, RP-18, 10 μm, flow rate: 3.0 ml/min) with MeOH–H_2O (60:40) as mobile phase to afford **1** (38 mg) and **2** (55 mg).

3.3.1 Tribufuroside D (*1*)

A white powder; mp 204–206°C; $[\alpha]_D^{20}$ –65.5 ($c = 0.30$, MeOH); IR (KBr) (ν_{max}): 3420, 2929, 1702, 1450, 1381, 1367, 1155, 1075, 1038, 893, 604 cm^{-1}. ^1H NMR (500 MHz, pyridine-d_5) δ 0.98 (3H, s, CH_3-18), 0.62 (3H, s, CH_3-19), 1.42 (3H, s, CH_3-21), 0.90 (3H, d, $J = 7.3$ Hz, CH_3-27), 3.69 (1H, m, H-3), 3.81 (1H, m, H-2), 4.74 (1H, m, H-16), 3.49 (1H, dd, $J = 7.0$, 9.5 Hz, H_a-26), 4.07 (1H, m, H_b-26), 4.68 (1H, d, $J = 7.6$ Hz, gal-H-1), 5.14 (1H, d, $J = 7.5$ Hz, glc-H-1), 4.79 (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*: 949.4632 $[M - H]^-$ (calcd for $C_{45}H_{73}O_{21}$, 949.4644). ESI-MS *m/z*: 949 $[M - H]^-$, 787 $[M - H - 162]^-$, 607 $[M - H - H_2O - 162 - 162]^-$, 445 $[M - H - H_2O - 162 - 162 - 162]^-$.

3.3.2 Tribufuroside E (*2*)

A white powder; mp 206–208°C; $[\alpha]_D^{20}$ –141.8 ($c = 0.21$, MeOH); IR (KBr) (ν_{max}): 3420, 2926, 1702, 1450, 1380, 1367, 1153, 1073, 1038, 893, 604 cm^{-1}. ^1H NMR (500 MHz, pyridine-d_5) δ 0.99 (3H, s, CH_3-18), 0.62 (3H, s, CH_3-19), 1.43 (3H, s, CH_3-21), 0.86 (3H, d, $J = 7.3$ Hz, CH_3-27), 3.69 (1H, m, H-3), 3.81 (1H, m, H-2), 4.74 (1H, m, H-16), 3.61 (1H, dd, $J = 7.0$, 9.5 Hz, H_a-26), 3.97 (1H, m, H_b-26), 4.68 (1H, d, $J = 7.6$ Hz, gal-H-1), 5.14 (1H, d, $J = 7.5$ Hz, glc-H-1), 4.79 (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*: 949.4632 $[M-H]^-$ (calcd for $C_{45}H_{73}O_{21}$, 949.4644); ESI-MS *m/z*: 949 $[M-H]^-$, 787 $[M-H-162]^-$, 607 $[M-H-H_2O-162-162]^-$, 445 $[M-H-H_2O-162-162-162]^-$.

3.4 Acid hydrolysis

The saponin (each 10 mg) was heated with 2 M HCl–MeOH (10 ml) under reflux for 3 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The water layer was neutralized with Na_2CO_3, concentrated, and subjected to TLC analysis with authentic samples D-glucose, L-galactose, and developed with CH_2Cl_2–MeOH–H_2O (15:6:1). Detection was carried out with aniline phthalate spray.

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