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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: 27 July 2010

To cite this Article Xu, Tun-Hai , Xu, Ya-Juan , Xie, Sheng-Xiu , Zhao, Hong-Feng , Han, Dong , Li, Yu , Niu, Jian-zhao and Xu, Dong-Ming(2008) 'Two new furostanol saponins from *Tribulus terrestris* L.', Journal of Asian Natural Products Research, 10: 5, 419 — 423

To link to this Article: DOI: 10.1080/10286020801966575
URL: http://dx.doi.org/10.1080/10286020801966575

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Journal of Asian Natural Products Research
Vol. 10, No. 5, May 2008, 419–423

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Two new furostanol saponins from *Tribulus terrestris* L.

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(*Received 30 December 2006; final version received 21 July 2007*)

Two new furostanol saponins, tribufurosides B (**1**) and C (**2**), were isolated from the fruits of *Tribulus terrestris* L. With the help of chemical and spectral analyses (IR, MS, 1D NMR and 2D NMR), the structures of two new furostanol saponins were established as 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furost-20(22)-en-2α,3β,26-triol-3-*O*-β-D-galactopyranosyl(1 → 2)-β-D-glucopyranosyl(1 → 2)-β-D-galactopyranoside (**1**) and (25*S*)-5α-furost-20(22)-en-12-one-3β, 26-diol-26-*O*-β-D-glucopyranoside (**2**).

Keywords: tribufurosides B and C; *Tribulus terrestris* L; zygophyllaceae; furostanol saponin

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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 were isolated.[2–4] In this paper, we report the structure elucidation of two new furostanol saponins, tribufurosides B (**1**) and C (**2**), by using 1D, 2D NMR techniques, ESI-MS analysis as well as chemical methods.

2. Results and discussion

Tribufuroside B (**1**), obtained as a white powder, showed a red colouration with Ehrlich reagent. The IR spectrum showed absorptions for hydroxyl groups ($3400\,cm^{-1}$) and double bond ($1642\,cm^{-1}$). An acidic hydrolysis of **1** with mineral acid afforded galactose and glucose as the sugar components identified by a comparison with authentic samples on TLC. Compound **1** exhibited the molecular formula $C_{51}H_{84}O_{24}$ by its HRMS analysis. The ESI-MS of **1** showed a positive ion peak at *m/z* 1103 $[M + Na]^+$, indicating a molecular weight of 1080 and significant ion peaks at *m/z* 941 $[M + Na\text{-}162]^+$, 779 $[M + Na\text{-}162\text{-}162]^+$ and 617 $[M + Na\text{-}162\text{-}162\text{-}162]^+$, corresponding to the loss of a hexosyl moiety continuously, showing the presence of a linear sugar chain of galactose–glucose–galactose. The ^{1}H NMR and ^{13}C NMR spectral data (Table 1) of **1** are assigned unequivocally according to ^1H–^1H COSY, HMQC and HMBC analyses. The ^{1}H NMR spectrum of **1** showed diagnostic signals of four methyl groups at δ 0.56 (3H, s, CH_3-18), 0.59 (3H, s, CH_3-19), 1.49 (3H, s, CH_3-21), and 0.90 (3H, d, $J = 7.3\,Hz$, CH_3-27), and three

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ISSN 1028-6020 print/ISSN 1477-2213 online
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DOI: 10.1080/10286020801966575
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Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (δ_C, 125 MHz, C_5D_5N).

1				**2**			
1	45.8	C-3 Gal-1	105.2	1	37.1	C-26 Glu-1	105.1
2	70.7	2	81.1	2	32.3	2	74.1
3	86.1	3	76.8	3	70.4	3	78.2
4	34.5	4	71.9	4	38.4	4	70.9
5	44.8	5	75.7	5	45.1	5	78.9
6	28.3	6	61.9	6	28.9	6	62.7
7	32.5	Glc-1	107.0	7	31.6		
8	34.3	2	84.8	8	33.6		
9	54.5	3	77.9	9	55.8		
10	37.0	4	72.9	10	36.5		
11	21.7	5	78.1	11	39.2		
12	39.9	6	63.3	12	213.1		
13	43.9	Gal′-1′	103.8	13	57.7		
14	54.8	2′	75.3	14	54.5		
15	34.5	3′	79.1	15	33.9		
16	84.6	4′	70.6	16	83.1		
17	64.7	5′	78.8	17	56.4		
18	14.6	6′	61.9	18	14.3		
19	13.9	C-26		19	12.1		
20	103.6	Glc′-1′	105.0	20	103.3		
21	11.9	2′	75.1	21	11.8		
22	152.5	3′	78.6	22	153.3		
23	23.8	4′	71.9	23	23.8		
24	31.6	5′	78.4	24	32.1		
25	33.6	6′	63.0	25	34.3		
26	75.1			26	75.0		
27	17.5			27	17.4		

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oxymethines at δ 3.62 (1H, m, H-3), 3.79 (1H, m, H-2), and 4.53 (1H, m, H-16), one oxymethylene at δ 3.49 (1H, dd, J = 7.0, 9.5 Hz, H-26), 4.08 (1H, m, H-26), and four anomeric proton doublets at δ 4.71 (1H, d, $J = 7.5$ Hz, glc′-H-1′), 4.84 (1H, d, $J = 7.6$ Hz, gal′-H-1′), 5.04 (1H, d, $J = 7.5$ Hz, glc-H-1), and 5.15 (1H, d, $J = 7.5$ Hz, gal-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 (δ 11.9, 13.9, 14.7, 17.5), four carbons bearing a hydroxyl group (δ 70.7, 75.1, 86.1, 84.6), and four anomeric carbons (δ 103.8, 105.0, 105.2, 107.0). In addition, resonances for the quaternary C-20, C-22 at δ 103.5, 152.5 suggested that **1** possessed a double bond between C-20 and C-22.[5] In the HMBC spectrum, the methyl protons at δ 1.49 (CH_3-21) showed long-range correlations with C-17, C-20 and C-22; CH_3-19 with C-10, C-1, C-5 and C-9; CH_3-18 with C-13, C-14, C-12 and C-17; and CH_3-27 with C-24, C-25, and C-26. Additionally, the ^{1}H NMR and ^{13}C NMR aglycone signals of **1** were made by comparison with those of trigoneoside VIII,[6] and were confirmed by ^{1}H–^{1}H COSY, DEPT, HMQC and HMBC spectral analyses (Table 1). Thus, the aglycone moiety of **1** was deduced to be a 5α-furost-20(22)-en-2α,3β, 26-triol. The 25R configuration of **1** was confirmed by the comparison of its 26-methylene signals of **1** with those of trigoneoside Ia,[7] and trigoneoside Xa,[8] in the ^{1}H NMR spectrum.

As described above, the sugar moiety of **1** consisted of glucose and galactose. The coupling constants of the anomeric protons revealed the β configurations for glucoses and galactoses.[9,10]

The positions of the sugar residues in **1** were defined unambiguously by the HMBC

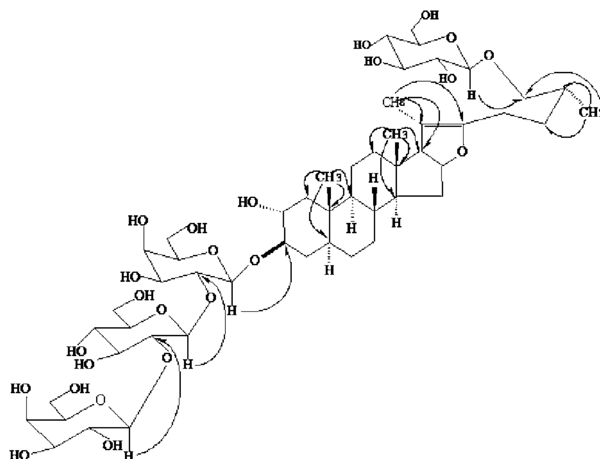

Figure 1. Key HMBC correlations for **1**.

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experiment (Figure 1). HMBC correlations between H-1 of the inner galactose and C-3 (δ 86.1) of the aglycone, H-1 (δ 5.04) of glucose and C-2 (δ 81.1) of the inner galactose, and H-1′ (δ 4.84) of the terminal galactose and C-2 (δ 84.8) of glucose indicated that a trisaccharide moiety 3-*O*-β-D-galactopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside was linked to C-3 of the aglycone. Additionally, an HMBC correlation between H-1′ (δ 4.71) of glucose′ and C-26 (δ 75.1) of the aglycone indicated that the glucose′ was linked to C-26 of the aglycone. On the basis of the above evidence, the structure of **1** was elucidated as 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furost-20(22)-en-2α,3β,26-triol-3-*O*-β-D-galactopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranoside.

Tribufuroside C (**2**), obtained as a white powder, showed a red colouration with Ehrlich reagent. The IR spectrum showed absorption bands for hydroxyl groups (3423 cm^{-1}), carbonyl group (1703 cm^{-1}) and double bond (1630 cm^{-1}). An acidic hydrolysis of **2** with mineral acid afforded glucose as the sugar component identified on TLC by comparison with authentic sample. ESI-MS spectrum of **2** showed a quasi-molecular ion peak at *m*/*z* 591 [M − H], indicating a molecular weight of 592. The molecular formula of **2** was determined to be as $C_{33}H_{52}O_9$ by HRMS (*m*/*z* 615.3630 $[M + Na]^+$). A significant ion peak at *m*/*z* 429 [M-162-H], corresponding to the loss of glucose, was in agreement with the presence of glucose. The ^{1}H NMR spectrum of **2** showed diagnostic signals of four methyl groups at δ 0.84 (3H, s, CH_3-18), 0.71 (3H, s, CH_3-19), 1.64 (3H, s, CH_3-21), and 0.93 (3H, d, $J = 6.8$ Hz, CH_3-27), and signals of two oxymethines at δ 3.85 (1H m, H-3), 4.63 (1H m, H-16), and one oxymethylene at δ 3.48 (1H, dd, $J = 7.0$, 9.5 Hz, H_a-26), 4.07 (1H, m, H_b-26). In addition, an anomeric proton at δ 4.72 (1H, d, $J = 7.5$ Hz, glc-H-1) was observed, consistent with the presence of one monosaccharide. The ^{13}C NMR spectrum of **2** showed signals of four angular methyl groups (δ 11.8, 12.1, 14.3, 17.4), one ketone carbonyl group (δ 213.1) and an anomeric carbon (δ 105.1). In addition, resonances for the quaternary C-20, C-22 at δ 103.3, 153.3 suggested that **2** possesses a double bond between C-20 and C-22.[5] Additionally, the ^{13}C NMR spectral data of the aglycone of **2** (Table 1) were almost consistent with those of the aglycone of terrestosin K,[11] except that the signal of C-3 δ 70.4 was shielded by $\Delta\delta$ 7.84, and signals of C-2 (δ 32.3) and C-4 (δ 38.4) were deshielded by $\Delta\delta$ 2.52 and 3.61, respectively, indicating that the 3-β-hydroxy

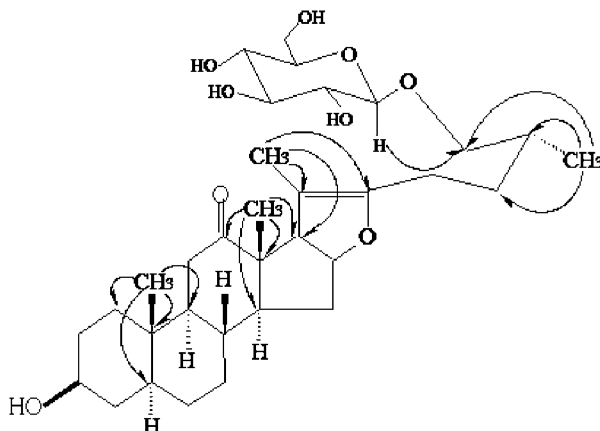

Figure 2. Key HMBC correlations for **2**.

group of the aglycone of **2** was not glycosidated. Thus, the aglycone moiety of **2** was deduced to be a 5α-furost-20(22)-en-12-one-3β, 26-diol. The 25R configuration of **2** was confirmed by the comparison of its 26-methylene signals with those of trigoneosides Ia,[7] and Xa,[8] in the ^{1}H NMR spectrum.

The coupling constant of the anomeric proton revealed the β configuration for glucose.[9,10]

The position of the sugar in **2** was defined unambiguously by the HMBC experiment (Figure 2). An HMBC correlation between H-1 of glucose and C-26 of the aglycone indicated that the glucose was linked to C-26 of the aglycone. On the basis of all these evidence, **2** was identified as (25*R*)-5α-furost-20(22)-en-12-one-3β, 26-diol-26-*O*-β-D-glucopyranoside.

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 ESI-MS was recorded on LCQ-1700 ESI-MS instrument. The NMR spectra were obtained on a Bruker AM-500 instrument, using TMS as 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, China) and reversed silica gel (25 × 2.5 cm, Nacalai Tesque, Kyoto, Japan). Macroporous resin D_{101} made in Tianjin gel Co., 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 2002 and identified by Professor Minglu Deng, Changchun College of traditional Chinese medicine. A voucher specimen (No. 020925) 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 (5 kg) of *T. terrestris* were exhaustively extracted with 60% EtOH, and the extract was concentrated under reduced pressure to obtain a crude residue (166 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

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(15 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 1–4.Fraction 3 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** (35 mg). Fraction 2 was subjected to HPLC eluting with MeOH–H_2O (65:35) to afford **2** (78 mg).

3.3.1 Tribufuroside B (1)

White powder, mp 203–206°C, $[\alpha]_D^{18}$ − 17 (*c* 0.31, MeOH), IR (KBr) (ν_{max}): 3400, 2928, 1642, 1451, 1380, 1365, 1165, 1076, 1039, 892, 602 cm^{-1}. ^1H NMR (500 MHz, pyridine-d_5) δ 0.56 (3H, s, CH_3-18), 0.59 (3H, s, CH_3-19), 1.49 (3H, s, CH_3-21), 0.90 (3H, d, $J = 7.3$ Hz, CH_3-27), 3.62 (1H, m, H-3), 3.79 (1H, m, H-2), 3.49 (1H, dd, $J = 7.0$, 9.5 Hz, H_a-26), 4.08 (1H, m, H_b-26), 4.71 (1H, d, $J = 7.5$ Hz, glc′-H-1′), 4.84 (1H, d, $J = 7.6$ Hz, gal′-H-1′), 5.04 (1H, d, $J = 7.5$ Hz, glc-H-1), 5.15 (1H, d, $J = 7.5$ Hz, gal-H-1), and ^{13}C NMR (125 MHz, pyridine-d_5) spectral data are given in Table 1. HRMS *m/z*: 1103.5254 $[M + Na]^+$(calcd for $C_{51}H_{84}O_{24}Na$, 1103.5245). ESI-MS *m/z*: 1103 $[M + Na]^+$, 941 $[M + Na\text{-}162]^+$, 779 $[M + Na\text{-}162\text{-}162]^+$, 617 $[M + Na\text{-}162\text{-}162\text{-}162]^+$.

3.3.2 Tribufuroside C (2)

White powder, mp 211–214°C, $[\alpha]_D^{23}$ − 9.7 (*c* 0.21, MeOH), IR (KBr) (ν_{max}): 3423, 2929, 1703, 1630, 1450, 1381, 1359, 1160, 1072, 1040, 890, 603 cm^{-1}. HRMS *m/z*: 615.3630 $[M + Na]^+$ (calcd for $C_{33}H_{52}O_9Na$, 615.3621). ESI-MS *m/z*: 591 [M − H], 429 [M-162-H]. ^1H NMR (500 MHz, pyridine-d_5) δ 0.84 (3H, s, CH_3-18), 0.71 (3H, s, CH_3-19), 1.64 (3H, s, CH_3-21), 0.93 (3H, d, $J = 6.8$ Hz, CH_3-27), 3.85 (1H, m, H-3), 3.48 (1H, dd, $J = 7.0$, 9.5 Hz, H_a-26), 4.07 (1H, m, H_b-26), 4.72 (1H, d, $J = 7.5$ Hz, glc-H-1), and ^{13}C NMR (125 MHz, pyridine-d_5) spectral data are given in Table 1.

3.4 Acid hydrolysis

The saponin (each 10 mg) was heated with 2M 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, D-xylose and L-rhamnose and developed with CH_2Cl_2–MeOH–H_2O (15:6:1). Detection was carried out with aniline phthalate spray.

Acknowledgements

The authors are grateful to the Program for Changjiang Scholars and Innovative Research Team in University (No. IRT0413) and China International Science and Technology Cooperation program (No. 2006DFA31230).

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