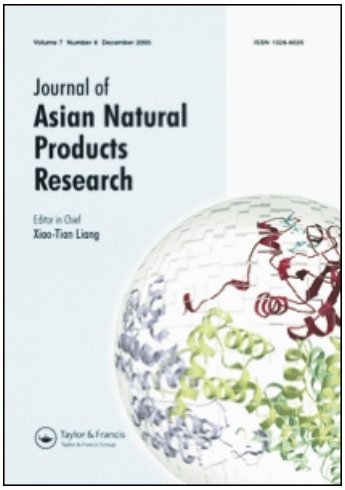


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Terrestrinins A and B, two new steroid saponins from *Tribulus terrestris*

Jin-Wen Huang; Chang-Heng Tan; Shan-Hao Jiang; Da-Yuan Zhu

Online publication date: 12 May 2010

To cite this Article Huang, Jin-Wen , Tan, Chang-Heng , Jiang, Shan-Hao and Zhu, Da-Yuan(2003) 'Terrestrinins A and B, two new steroid saponins from *Tribulus terrestris*', Journal of Asian Natural Products Research, 5: 4, 285 — 290
To link to this Article: DOI: 10.1080/1028602031000111996
URL: http://dx.doi.org/10.1080/1028602031000111996

Journal of Asian Natural Products Research, 2003 VOL. 5 (4), pp. 285–290

TERRESTRININS A AND B, TWO NEW STEROID SAPONINS FROM *TRIBULUS TERRESTRIS*

JIN-WEN HUANG, CHANG-HENG TAN, SHAN-HAO JIANG and DA-YUAN ZHU*

State Key Laboratory of Drug Research, Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 294 Taiyuan Road, Shanghai 200031, People's Republic of China

(Received 23 December 2002; Revised 18 February 2003; In final form 28 February 2003)

Two new steroid saponins, named terrestrinins A (**1**) and B (**2**), along with six known compounds were isolated from the Chinese medicine herb *Tribulus terrestris*, and their chemical structures were elucidated as 26-*O*-β-D-glucopyranosyl-(25*S*)-furostan-4(5),20(22)-diene-3,12-dione (**1**) and 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furostane-3β,22α,26-triol-3-*O*-β-D-xylopyranosyl(1 → 3)-[(β-D-xylopyranosyl(1 → 2)]-β-D-glucopyranosyl(1 → 4)-[α-L-rhamnopyranosyl(1 → 2)]-β-D-galactopyranoside (**2**) on the basis of spectroscopic techniques.

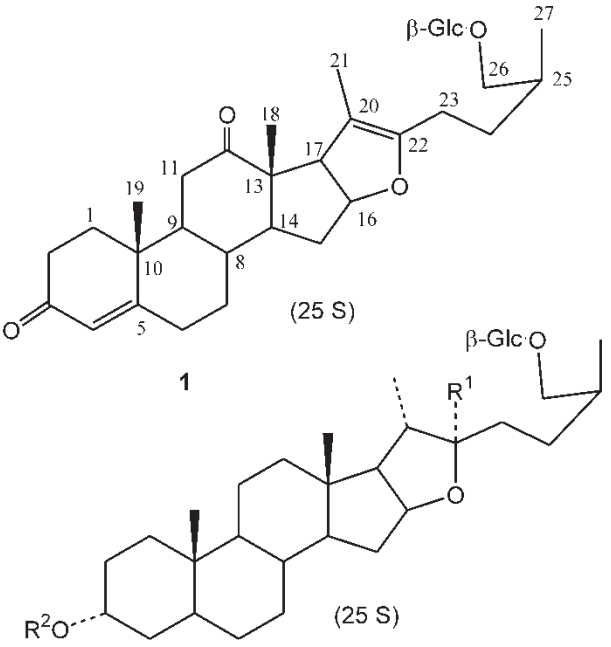
Keywords: *Tribulus terrestris*; Steroid saponins; Terrestrinin A; Terrestrinin B

INTRODUCTION

Tribulus terrestris Linn. (Zygophyllaceae) is known as "*Baijili*" in Chinese folk medicine and has been used for treatment of eye trouble, edema, abdominal distention and promoting blood circulation to remove blood stasis [1]. Up to now, more than twenty steroid saponins have been reported from this plant [2–7]. Pharmacological studies have shown that the saponins of this plant are the main active components in treatment of cardiac diseases [8,9]. A chemical investigation on *T. terrestris* resulted in the isolation of terrestrinins A (**1**) and B (**2**), two new steroid saponins whose structures have been determined as 26-*O*-β-D-glucopyranosyl-(25*S*)-furostan-4(5),20(22)-diene-3,12-dione (**1**) and 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furostane-3β,22α,26-triol-3-*O*-β-D-xylopyranosyl(1 → 3)-[β-D-xylopyranosyl(1 → 2)]-β-D-glucopyranosyl (1 → 4)-[α-L-rhamnopyranosyl (1 → 2)]-β-D-galactopyranoside (**2**), along with six known compounds, 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furostane-22α-methoxy-3β, 26-diol-3-*O*-β-D-xylopyranosyl(1 → 3)-[β-D-xylopyranosyl(1 → 2)]-β-D-glucopyranosyl(1 → 4)-[α-L-rhamnopyranosyl (1 → 2)]-β-D-galactopyranoside (**3**) [3], agovoside A (**4**) [4], terrestrosin D (**5**) [5], hecogenin-3-*O*-β-D-glucopyranosyl (1 → 2)-[β-D-xylopyranosyl (1 → 3)]-β-D-glucopyranosyl(1 → 4)-β-D-galactopyranoside (**6**) [6], hecogenin-3-*O*-β-D-xylopyranosyl(1 → 2)-[β-D-xylopyranosyl(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl(1 → 2)]-β-D-galactopyranoside (**7**) [7], and tigogenin

*Corresponding author. Tel.: +86-21-64311833. Ext. 318. Fax: +86-21-64370269.
E-mail: dyzhu@mail.shcnc.ac.cn

ISSN 1028-6020 print/ISSN 1477-2213 online © 2003 Taylor & Francis Ltd
DOI: 10.1080/1028602031000111996



2 R^1=OH, R^2=β–Gal_2^4-β-Glc_2^3-β-Xyl
α–Rha^4 β-Xyl^4

3 R^1=OMe, R^2=β–Gal_2^4-β-Glc_2^3-β-Xyl
α–Rha^4 β-Xyl^4

FIGURE 1 Structures of **1**–**3**.

3-*O*-β-D-xylopyranosyl(1 → 2)-[β-D-xylopyranosyl (1 → 3)]-β-D-glucopyranosyl (1 → 4)-[α-L-rhamnopyranosyl(1 → 2)]-β-D-galactopyranoside (**8**) [5]. Here, we report the isolation and structure elucidation of the two new steroid saponins (Fig. 1).

RESULTS AND DISCUSSION

Terrestrinin A (**1**) is red under ultraviolet light, its molecular formula $C_{33}H_{48}O_9$ was established by HR-EIMS (found M^+ 588.3293, $C_{33}H_{48}O_9$ requires 588.3298). Upon acid hydrolysis, **1** yielded D-glucose as the sugar moiety. The ^{1}H NMR spectrum of **1** showed an anomeric proton at δ 4.28 (d, $J = 7.6$ Hz), demonstrating a β-configuration of the sugar.

The ^{13}C NMR spectrum showed that the aglycone moiety possesses one saturated (δ 212.2) and one conjugated (δ 198.8) carbonyl group, and two pairs of double bonds (δ 168.4, 152.3, 124.8, and 103.4), which, combined with the molecular formula, means that **1** should be a pentcyclic furostan saponin with cleavage of F ring. The same A–D rings as those of (25*S*)-spirostan-4-ene-3,12-dione (**9**) [9] were elucidated by ^{13}C NMR data comparison of **1** and **9**. The remaining double bond was assigned between C-20 and C-22 on the basis of consideration of the biogenetic pathway from furostan saponin, and the methyl NMR signals (δ_H: 1.58, s, and δ_C: 11.2, 21-Me). In the HMBC spectrum, the anomeric proton of glucose showed long-range correlation with the oxy-bearing methine carbon (δ_c 75.1, C-26),

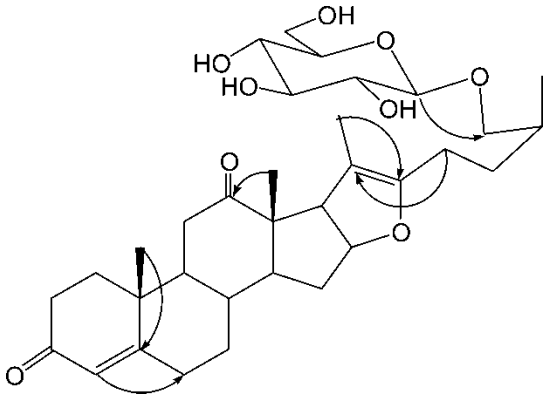


FIGURE 2 Significant HMBC correlations of **1** (H to C).

suggesting that the sugar moiety was connected to C-26 (Fig. 2). C-25 was determined to have an *S*-configuration by means of inspection of the ^{13}C NMR data of C-27 (δ 16.8) and C-26 (δ 75.1) [10]. Thus, the structure of **1** was established to be 26-*O*-β-D-glucopyranosyl-(25*S*)-furostan-4(5),20(22)-diene-3,12-dione.

Terrestrinin B (**2**) yielded a red color with Ehrlich's reagent, indicating a furostan saponin. Its ^{13}C and DEPT NMR spectra displayed 61 carbon atoms ($CH_3 \times 5$, $CH_2 \times 16$, CH × 37, C × 3) and 84 carbon-bonded protons. Combined with the positive quasi molecular ion at *m/z* 1313 ($[M + H - H_2O]^+$) in the ESI-MS, this suggests that the molecular formula of **2** is $C_{61}H_{102}O_{31}$, with 18 hydroxyl groups.

Acid hydrolysis of **2** yielded D-galactose, L-rhamnose, D-xylose, and D-glucose. The ^{13}C NMR spectrum of **2** was highly superimposable with that of **3**, except for the lack of a methoxyl signal (δ_H 3.25 and δ_C 47.2), as well as two carbon signals at δ 112.5 (s) and 30.8 (t) instead of δ 110.6 and 37.2 (C-22 and C-23 of **3**), respectively. This showed both have the same sugar moieties and steroid skeleton with the exception of a hydroxyl rather than a methoxyl group located at C-22 in **2**. Aside from those signals of the aglycone, five doublet anomeric protons at δ 4.85, 4.99, 5.25, 5.42 and 4.81 with $J = 8.0$, 8.0, 7.6, 7.7 and 7.8 Hz, respectively, were observed in the ^1H NMR spectrum, affirming the β-configurations for five sugars; there was also a proton single peak at δ 6.20, along with a ^{13}C NMR peak at δ 18.5, disclosing the presence of an α-rhamnose unit. The positions of those sugars were further confirmed to have the same sequence with that of **3** by the HMBC spectrum (Fig. 3). Therefore, terrestrinin B was established as 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furostane-3β,22α,26-triol-3-*O*-β-D-xylopyranosyl(1 → 3)-[β-D-xylopyranosyl(1 → 2)]-β-D-glucopyranosyl(1 → 4)-[α-L-rhamnopyranosyl(1 → 2)]-β-D-galactopyranoside.

EXPERIMENTAL

General Experimental Procedures

All melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. The optical rotations were measured using a Perkin–Elmer 241 MC polarimeter in $CHCl_3$ or C_5H_5N. The IR spectra were recorded on a Nicolet Magna 750 FTIR (KBr) spectrophotometer. All MS data were obtained with a MAT-95 mass spectrometer. NMR spectra were recorded on a Bruker AM × 400 instrument in $CDCl_3$ or C_5D_5N with TMS as internal standard. Silica gel (200–300, 400 mesh) and precoated plates of silica gel

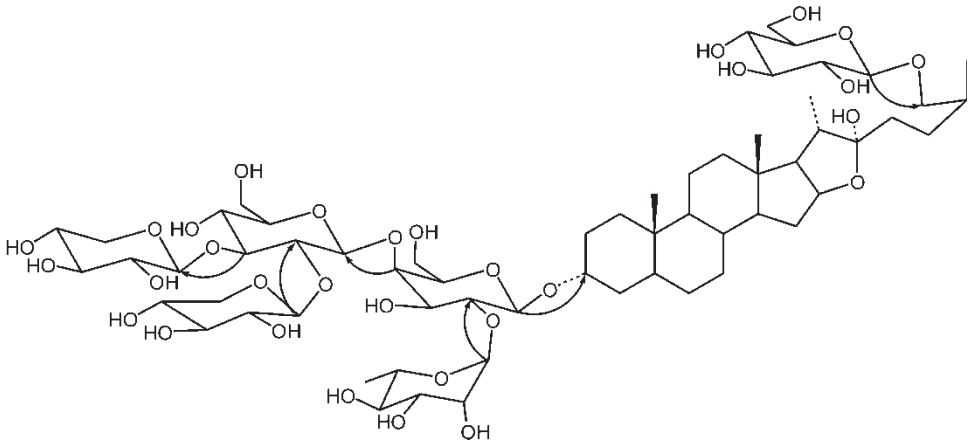


FIGURE 3 Important HMBC correlations of **2** (H to C).

($HSGF_{254}$) (Qingdao Haiyang Chemical Group Co. Ltd, Qingdao, China) were used for column chromatography (CC) and TLC, respectively.

Plant Material

Fresh fruit of *Tribulus terrestris* Linn. (Zygophyllaceae) was collected in Shandong Province, China, and was identified by Professor S.H. Jiang. The voucher specimen is deposited at the Herbarium of the Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

Extraction and Isolation

The air-dried fruit of *T. terrestris* (10 kg) was extracted with 80% ethanol (10 l × 3). The extract was concentrated to 3 l, then extracted successively with $CHCl_3$, EtOAc and n-BuOH (each 3 l × 3). The n-BuOH layers were then dried to give crude saponins (60 g). Part of the crude saponins (30 g) was separated into six fractions over silica gel CC with $CHCl_3$–MeOH gradients (10:1, 8:2, 7:3, 6:4, 1:1, 0:1, each 2.5 l). Fr.1 was further chromatographed over a silica gel column with $CHCl_3$–MeOH (10:1, 2 l) as eluent to give **1** (200 mg) and **4** (300 mg); Fr.3 was subjected to silica gel CC and eluted with $CHCl_3$–MeOH–H_2O (65:35:10, organic layer, 2 l) to give **5** (55 mg), **6** (60 mg), **7** (45 mg) and **8** (50 mg), successively. Chromatography of Fr.4 on an ODS silica gel column eluted with MeOH–H_2O (80:20, 1 l) and a silica gel column, with $CHCl_3$–EtOH–H_2O (10: 10: 2.5, 1 l) as eluent, yielded **2** (55 mg) and **3** (60 mg), respectively.

Acid Hydrolysis of Saponins

Saponins (10 mg of **1** and **2**, respectively) were hydrolyzed with 2 M HCl-1,4-dioxane (1:1, 5 ml), refluxed for 1 h, the 1,4-dioxane removed under reduced pressure, and extracted with CH_2Cl_2 (3 × 3 ml). D-Glucose was detected in the remaining H_2O layer of **1**, and D-glucose, L-rhamnose, D-galactose, and D-xylose were observed in that of **2**, respectively, by means of TLC comparison with authentic samples (solvent system: n-BuOH–pyridine–H_2O, 6:4:3; R_f: D-glucose 0.24; D-xylose, 0.43; D-galactose, 0.19; L-rhamnose, 0.38).

Terrestrinin A (**1**): Amorphous powder, mp 134–136°C, $[\alpha]_D^{20}$: +56.5 (*c* 0.56, $CHCl_3$). IR (ν_{max}^{KBr} cm^{-1}): 3412, 2918, 1707, 1670, 1450, 1379, 1269, 1238, 1076, 1040, 752. EIMS: *m/z* = 588 $[M]^+$, 480, 426, 181, 109, 69. HR-EIMS (*m/z*): $C_{33}H_{48}O_9$, found: 588.3293 (M^+), calcd: 588.3298. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 5.78 (1H, s, H-4), 4.71 (1H, m, H-16), 4.28 (1H, d, *J* = 7.6 Hz, H-1′ of Glc), 1.58 (3H, s, H_3-21), 1.28 (3H, s, H_3-19), 0.98 (3H, s, H_3-18), 0.93 (3H, d, *J* = 6.6 Hz, H_3-27). ^{13}C NMR ($CDCl_3$, 100 MHz) data: see Table I.

Terrestrinin B (**2**): $C_{61}H_{102}O_{31}$, amorphous powder, mp 268–270°C, $[\alpha]_D^{20}$ −17.1 (*c* 0.51, C_5H_5N). IR (ν_{max}^{KBr} cm^{-1}): 3406, 1641, 1452, 1382, 1161, 1074, 1043, 980. ESI-MS: *m/z* = 1313 $[M + H - H_2O]^+$. ^{1}H NMR (C_5D_5N, 400 MHz) δ (ppm): 6.20 (1H, s, Rha-H-1), 5.42 (1H, d, *J* = 7.7 Hz), 5.25 (1H, d, *J* = 7.6 Hz), 5.01 (1H, br.s, H-16), 4.99 (1H, d, *J* = 8.0 Hz), 4.85 (1H, d, *J* = 8.0 Hz), 4.81 (1H, d, *J* = 7.8 Hz), 3.98 (1H, m, H-3), 1.78 (3H, d, *J* = 5.8 Hz, Rha-Me), 1.40 (3H, d, *J* = 6.5, H_3-21), 1.05 (3H, d, *J* = 6.5, H_3-27), 0.96 (3H, s, H_3-18), 0.94 (3H, s, H_3-19). ^{13}C NMR (C_5D_5N, 100 MHz) data see Table I.

Compound **3**: Amorphous powder, $C_{62}H_{104}O_{31}$. IR (ν_{max}^{KBr} cm^{-1}): 3423, 1637, 1373, 1161, 1045, 980, 895. ESI-MS: *m/z* = 1344 $[M]^+$, 1367 $[M + Na]^+$, 695 $[M/2 + Na]^+$. ^{1}H NMR (C_5D_5N, 400 MHz) δ (ppm): 6.19 (1H, s), 5.42 (1H, d, *J* = 7.7 Hz), 5.23 (1H, d, *J* = 7.8 Hz), 4.99 (1H, d, *J* = 7.9 Hz), 4.84 (1H, d, *J* = 7.8 Hz), 4.84 (1H, d, *J* = 7.8 Hz), 3.25 (s, 22-OMe), 1.70 (d, *J* = 6.2 Hz, Me of Rha), 1.16 (3H, d, *J* = 6.94, H_3-21), 0.98

TABLE I ^{13}C NMR data of **1** (in $CDCl_3$), **2** and **3** (in C_5D_5N)

Position	*1*	*2*	*3*	*Position*	*2*	*3*
1	35.3 t	37.2 t	37.2 t	3-O-Gal		
2	32.4 t	29.9 t	29.9 t	1	100.1 d	100.1 d
3	198.8 s	76.9 d	76.9 d	2	76.6 d	76.5 d
4	124.8 d	34.2 t	34.3 t	3	76.6 d	76.7 d
5	168.4 s	44.6 d	44.6 d	4	81.5 d	81.5 d
6	33.7 t	28.9 t	29.0 t	5	75.8 d	75.8 d
7	31.3 t	32.4 t	32.4 t	6	60.4 t	60.4 t
8	34.2 d	35.2 d	35.2 d	Rha		
9	54.6 d	54.4 d	54.4 d	1	102.0 d	102.0 d
10	38.7 s	35.9 s	35.9 s	2	72.5 d	72.5 d
11	37.3 t	21.2 t	21.3 t	3	72.7 d	72.7 d
12	212.2 s	39.9 t	40.2 t	4	74.0 d	74.0 d
13	56.9 s	41.1 s	41.1 s	5	69.4 d	69.4 d
14	53.3 d	56.3 d	56.4 d	6	18.5 q	18.5 q
15	30.7 t	32.0 t	32.4 t	Glc		
16	82.5 d	81.3 d	81.1 d	1	105.0 d	105.0 d
17	55.6 d	64.3 d	64.0 d	2	81.3 d	81.3 d
18	13.9 q	16.4 q	16.7 q	3	87.6 d	87.6 d
19	16.9 q	12.3 q	12.4 q	4	70.4 d	70.4 d
20	103.4 s	40.5 d	40.7 d	5	77.8 d	77.8 d
21	11.2 q	16.3 q	16.5 q	6	62.8 t	62.8 t
22	152.3 s	112.5 s	110.6 s	Xyl		
23	23.2 t	30.8 t	37.2 t	1	105.3 d	105.3 d
24	33.4 t	28.1 t	28.4 t	2	75.1 d	75.1 d
25	32.9 d	34.2 d	34.3 d	3	78.6 d	78.6 d
26	75.1 t	75.1 t	75.3 t	4	70.7 d	70.7 d
27	16.8 q	17.1 q	17.5 q	5	67.3 t	67.6 t
26-O-Glc				Xyl		
1	103.0 d	105.0 d	105.0 d	1	105.9 d	105.9 d
2	73.6 d	75.2 d	75.0 d	2	75.1 d	75.1 d
3	76.3 d	78.5 d	78.5 d	3	79.1 d	79.1 d
4	69.7 d	71.7 d	71.6 d	4	70.9 d	70.9 d
5	75.4 d	78.8 d	78.8 d	5	67.6 t	67.6 t
6	61.7 t	62.9 t	62.9 t	22-OMe		47.2 q

(d, $J = 6.57$, H_3-27), 0.83 (3H, s, H_3-19), 0.77 (3H, s, H_3-18). ^{13}C NMR (C_5D_5N, 100 MHz) data see Table I.

References

[1] Jiangsu New Medical College (1985), *The Dictionary of Traditional Chinese Medicine* (Shanghai Science and Technology Press, Shanghai), p. 1274.
[2] Wu, G., Jiang, S.H., Jiang, F.X., Zhu, D.Y., Wu, H.M. and Jiang, S.K. (1996), *Phytochemistry* **42**, 1677–1681.
[3] Xu, Y.X., Chen, H.S., Liang, H.Q., Gu, Z.B., Liu, W.Y., Leung, W.N. and Li, T.J. (2000), *Planta Med.* **66**, 545–550.
[4] Kintya, P.K. and Bobeiko, V.A. (1979), *Khim. Prir. Soedin.* **15**, 102–103.
[5] Yan, W., Ohtani, K., Kasai, R. and Yamasaki, K. (1996), *Phytochemistry* **42**, 1417–1422.
[6] Mimaki, Y., Kanmoto, T., Sahida, Y., Nishino, A., Satomi, Y. and Nishio, H. (1996), *Phytochemistry* **41**, 1405–1410.
[7] Cai, L.F., Wu, Y.J., Zhang, J.G., Pei, F.K., Xu, Y.J., Xie, S.X. and Xu, D.M. (2001), *Planta Med.* **67**, 196–198.
[8] Tori, K., Seo, S., Terui, Y., Nishikawa, J. and Yasuda, F. (1981), *Tetrahedron Lett.* **22**, 2405–2408.
[9] Xu, Y.X., Chen, H.S., Liu, W.H., Gu, Z.B. and Liang, H.Q. (1998), *Phytochemistry* **49**, 199–201.
[10] Li, X.C., Wang, Y.F., Wang, D.Z. and Yang, C.R. (1990), *Phytochemistry* **29**, 3899–3901.