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STEROIDAL SAPONINS FROM FRUITS OF *TRIBULUS TERRESTRIS*

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Key Word Index—*Tribulus terrestris*; Zygophyllaceae; fruits; furostanol saponins.

Abstract—Further studies on the constituents of the fruits of *Tribulus terrestris* led to the isolation of six new furostanol saponins, 26-*O*-β-D-glucopyranosyl (25*R*)-furostane-2α,3β,22α,26-tetrol-3-*O*-β-D-glucopyranosyl (1-4)-β-D-galactopyranoside, 26-*O*-β-D-glucopyranosyl (25*R,S*)-5α-furostane-2α,3β,22α,26-tetrol-3-*O*-β-D-galactopyranosyl(1-2)-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside, 26-*O*-β-D-glucopyranosyl (25*R,S*)-5α-furostane-3β,22α,26-triol-3-*O*-β-D-galactopyranosyl(1-2)-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside, 26-*O*-β-D-glucopyranosyl (25*R,S*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-β-D-galactopyranosyl(1-2)-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside, 26-*O*-β-D-glucopyranosyl (25*R,S*)-furost-5-ene-3β,22α,26-triol-3-*O*-β-D-galactopyranosyl(1-2)-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside, 26-*O*-β-D-glucopyranosyl (25*R*)-5α-furost-20(22)-en-12-one-3β,26-diol-3-*O*-β-D-galactopyranosyl(1-2)-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside, named terrestrosin F–K, respectively. The structures were elucidated on the basis of spectroscopic studies of the isolated compounds and their hydrolysed products.

INTRODUCTION

Tribulus terrestris L. is an annual creeping herb growing on roadsides and hills in China. It is also distributed in Japan, Korea, western part of Asia, southern part of Europe and Africa. In traditional Chinese medicine, the fruit of *T. terrestris*, which is known as 'Ci Ji Li', have long been used for the treatment of eye trouble, edema and abdominal distention, emission and morbid leucorrhea as well as vitiligo [1].

We previously reported the isolation of ten spirostanol-type saponins from the fruits of *T. terrestris* growing in China, among them five compounds (terrestrosin A–E) were new saponins [2]. In a continuation of our study on the same plant, we now report the isolation and structural elucidation of six furostanol saponins.

RESULTS AND DISCUSSION

The crude saponin fraction of *T. terrestris* was subjected to repeated silica gel, reversed phase RP-18 column chromatography and preparative HPLC to afford compounds **1–6**. All the compounds **1–6** were easily deduced to be furostanol saponins on the basis of the colour reaction with Ehrlich's spray reagent on TLC [3] and their C-22α-configurations were confirmed based on 2D ROE spectrum of compound **3**. On acid hydrolysis, they yielded glucose and galactose as sugar residues.

Compound **3** gave a red colour with Ehrlich's reagent. Its molecular formula was determined as $C_{51}H_{86}O_{24}$ from the high-resolution negative FAB-mass spectrometry. The ^{1}H NMR spectrum of **3** displayed four doublet signals of anomeric protons at δ 4.75, 4.85, 5.05 and 5.08 with 7.6, 7.0, 7.6 and 7.5 Hz coupling constants, respectively, diagnostic of the β-D-configuration for all four sugars. All the ^{13}C NMR signals of the sugar moieties of **3** were identical with those of terrestrosin A [2], except for a set of additional signals corresponding to a β-D-glucopyranosyl unit. On enzymatic hydrolysis with β-glucosidase, **3** afforded terrestrosin A (**3S**) and D-glucose indicating **3** is the 26-*O*-(β-D-glucopyranoside) of spirostanol form saponin (**3S**). In the ^{13}C NMR spectrum, the signals due to the aglycone moiety were indicative of a 3,26-di-*O*-glycosylated 5α-furostane-3β,22,26-triol structure [4]. The PROESY (phasesensitive rotating frame nuclear Overhause effect spectra) spectrum of **3** confirmed the C-22 configuration to be α in which the cross peak was observed between the H-20 proton (δ 2.19 *m*) and the H-23 protons (δ 1.97 *m*). The C-25 configuration of **3** was deduced to be a *R,S*-mixture based on the ^{1}H NMR signals at δ3.47 (1H, *dd*, J = 7.8, 8.5 Hz, Ha-26, 25*S*) and 3.60 (1H, *dd*, J = 6.4,

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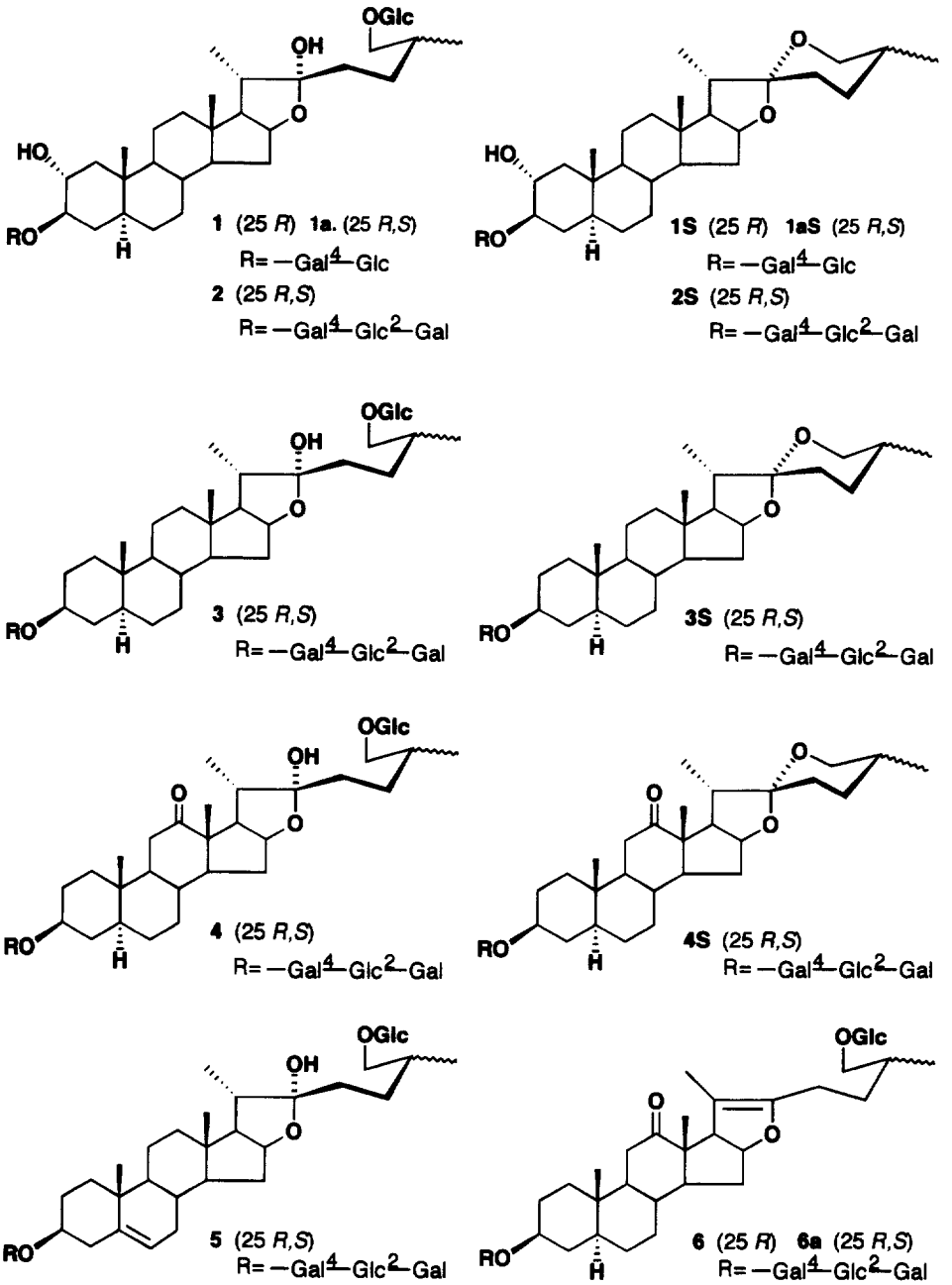

Scheme. 1. Chemical formulae of compounds **1**–**6** and prosapogenins **1S**, **2S**, **3S** and **4S**.

9.3 Hz, Ha-26, 25*R*), which was also determined by analysis of the ^{13}C NMR spectrum of prosapogenin **3S**. Therefore, the structure of **3** was established to be 26-*O*-*β*-D-glucopyranosyl (25*R*,*S*)-5*α*-furostane-3*β*, 22*α*,26-triol-3-*O*-*β*-D-galactopyranosyl(1-2)-*β*-D-glucopyranosyl(1-4)-*β*-D-galactopyranoside, and was named terrestrosin H.

Compound **1** gave a red colour with Ehrlich's reagent. Its molecular formula, $C_{45}H_{76}O_{20}$, was established by high-resolution negative FAB-mass spectrometry. The ^1H NMR spectrum of **1** displayed three doublet signals of anomeric protons at δ 4.78, 4.90 and 5.24 with 7.9, 7.6 and 7.9 Hz coupling constants, respectively, diagnostic of a *β*-D-configuration for all three sugars (Table 1). In the ^{13}C NMR spectrum, the signals due to the aglycone moiety were indicative of a 3,26-di-*O*-glycosylated 5*α*-furostane-2*α*,3*β*,22*α*,26-tetrol structure [4]. Comparison of the ^{13}C NMR chemical shifts thus assigned with those of the reference methyl glycoside [5] and taking into account the known effect of the *O*-glycosylation and the result of acid hydrolysis indicated that **1** has two terminal *β*-D-glucopyranosyl units and a 4-substituted *β*-D-galactopyranosyl unit.

On enzymatic hydrolysis with *β*-glucosidase, **1** yielded prosapogenin **1S** and D-glucose. Compound **1S** was identical with the reported prosapogenin, gitogenin 3-*O*-*β*-D-glucopyranosyl(1-4)-*β*-D-galactopyr-

Table 1. ^{1}H NMR spectral data for compounds **1–6** in pyridine d_5 (δ values; 500 MHz)

		1	**1a**		**2**		**3**		**4**		**5**		**6**	**6a**	
Aglycone	moiety	25 *R*	25 *R*	25 *S*	25 *R*	25 *S*	25 *R*	25 *S*	25 *R*	25 *S*	25 *R*	25 *S*	25 *R*	25 *R*	25 *S*
	H-18 *s*	0.86	0.85	0.85	0.84	0.84	0.85	0.85	1.09	1.09	0.90	0.90	0.92	0.90	0.88
	H-19 *s*	0.73	0.73	0.73	0.71	0.71	0.65	0.65	0.71	0.71	0.88	0.88	0.68	0.65	0.66
	H-21 *d*	1.30	1.30	1.28	1.29	1.28	1.29	1.28	1.52	1.50	1.31	1.29	1.72	1.72	1.71
		(7.0)	(6.4)	(6.4)	(7.0)	(7.0)	(6.9)	(6.9)	(7.0)	(7.0)	(7.0)	(7.0)	(*s*)	(*s*)	(*s*)
	H-27 *d*	1.02	1.02	0.98	1.01	0.97	1.00	0.96	1.00	0.96	1.01	0.98	1.01	1.01	1.00
		(6.7)	(6.6)	(6.6)	(6.7)	(6.7)	(6.6)	(6.8)	(6.5)	(6.5)	(6.6)	(6.7)	(6.6)	(6.6)	(6.6)
Sugar	moiety														
3-*O*-Gal	H-1 *d*	4.90	4.90		4.90		4.85		4.86		4.87		4.85	4.87	
		(7.6)	(7.6)		(7.8)		(7.0)		(7.8)		(7.6)		(7.3)	(7.4)	
Glc	H-1 *d*	5.24	5.23		5.14		5.08		5.11		5.10		5.09	5.11	
		(7.9)	(7.8)		(7.6)		(7.5)		(7.6)		(7.6)		(7.6)	(4.7)	
Gal	H-1 *d*				5.11		5.05		5.08		5.08		5.07	*O*	
					(7.5)		(7.6)		(7.6)		(7.5)		(7.6)		
26-*O*-Glc	H-1 *d*	4.78	4.78		4.77		4.75		4.78		4.77		4.79	4.81	
		(7.9)	(7.6)		(7.8)		(7.6)		(7.8)		(7.8)		(7.6)	(7.6)	

J values (in parentheses) are reported in Hz.

anoside, based on the analysis of their NMR spectral data and by a comparison of their physical properties [4]. Thus, the structure of **1** was established to be 26-*O*-β-D-glucopyranosyl (25*R*)-5α-furostane-2α,3β,22α,26-tetrol-3-*O*-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside, and was named terrestrosin F.

In addition to **1**, the furostanol saponin **1a** was also obtained. Compound **1a** was determined as a 25*R*,*S*-mixture of **1**, based on the NMR data and the analysis of the ^{1}H NMR spectra signals of the prosapogenin **1aS** with those of the prosapogenin **1S**.

Compound **2** gave a red colour with Ehrlich's reagent. The high-resolution negative FAB-mass spectrometry determined its molecular formula as $C_{51}H_{86}O_{25}$. The ^{1}H NMR spectrum of **2** displayed four doublet signals of anomeric protons at δ 4.77, 4.90, 5.11 and 5.14 with 7.8, 7.8, 7.5 and 7.6 Hz coupling constants, respectively, diagnostic of a β-D-configuration for all four sugars (Table 1). In the ^{13}C NMR spectrum (Table 2), the signals due to the aglycone moiety were almost superimposable on those of **1a**, while the signals due to the sugar moiety were identical with those of terrestrosin E [2], except for a set of additional signals corresponding to a β-D-glucopyranosyl unit. On enzymatic hydrolysis with β-glucosidase, **2** afforded prosapogenin **2S** and D-glucose. Compound **2S** was identical with terrestrosin E based on the analysis of their NMR spectral data and by a comparison of their physical properties. Thus, the structure of **2** was established to be 26-*O*-β-D-glucopyranosyl (25*R*,*S*)-5α-furostane-2α,3β,22α,26-tetrol-3-*O*-β-D-galactopyranosyl(1-2)-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside, and was named terrestrosin G.

Compound **4** gave a red colour with Ehrlich's reagent. The high-resolution negative FAB-mass spectrometry determined its molecular formula as $C_{51}H_{84}O_{25}$. In the ^{13}C NMR spectrum of **4**, the signals due to the aglycone moiety were indicative of a 3,26-di-*O*-glycosylated 5α-furostan-12-one-3β,22α,26-triol structure [6]. Its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) indicated the presence of four anomeric protons and carbons, and all the ^{13}C NMR signals due to sugar moieties were almost superimposable on those of **3**. On enzymatic hydrolysis with β-glucosidase, compound **4** afforded terrestrosin C (**4S**) [2] and D-glucose. The C-25 configuration of **4** was deduced to be an *R*,*S*-mixture based on its ^{1}H NMR spectrum, which was also determined by analysis of ^{13}C NMR spectra of prosapogenin **4S**. Therefore, the structure of **4** was established to be 26-*O*-β-D-glucopyranosyl (25*R*,*S*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-β-D-galactopyranosyl(1-2)-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside, and was named terrestrosin I.

Compound **5** gave a red colour with Ehrlich's reagent. From the high-resolution negative FAB-mass spectrometry, its molecular formula was determined as $C_{15}H_{84}O_{24}$. It showed a quasi-molecular ion peak, $[M-H]^-$, at *m*/*z* 1079 in the negative FAB-mass spectrum, indicating that **5** had one additional double bond compared with **3**, this was supported by the ^{1}H NMR signal at δ 5.30 (olefinic 1H, *br*, *s*, H-6) and the ^{13}C NMR signals at δ 121.6 (C-6) and 141.1 (C-5). On comparison of the ^{1}H and ^{13}C NMR spectra of **5** (Table 2) with those of the 25*R*-epimer of the reported compound PO-8 [7], the signals due to the aglycone moiety were indicative of a 3,26-di-*O*-glycosylated furost-5-ene-3β,22α,26-triol structure. In the ^{1}H NMR spectrum, the four signals at δ 1.31, 1.29, 0.98, 1.01 and the signals at δ 3.48 (1H, *dd*, J = 6.9, 9.5 Hz, Ha-26, 25*S*) and 3.61(1H, *dd*, J = 6.9, 9.5 Hz, Ha-26, 25*R*) indicated the C-25 configuration of **5** to be an *R*,*S*-mixture. The ^{1}H and ^{13}C NMR spectra of **5** indicated four anomeric protons and carbons, and all the ^{13}C NMR signals were fully superimposable on those of **3**, indicating that they have the same sugar sequence.

Table 2. ^{13}C NMR spectral data for **1-6** in pyridine-d_5 (δ values; 125 MHz)

	1 (1a)	**2**	**3**	**4**	**5**	**6 (6a)**
1	45.6	45.5	37.1	36.6	37.5	36.7
2	70.5	70.4	30.0	29.7	30.1	29.8
3	84.9	85.0	77.9	77.9	78.0	77.9
4	34.3	34.2	34.8	34.7	39.4	34.8
5	44.7	44.7	44.6	44.5	141.1	44.6
6	28.2	28.1	28.9	28.5	121.6	28.6
7	32.3	32.3	32.4	31.6	32.3	31.1
8	34.6	34.5	35.2	34.3	31.7	34.8
9	54.5	54.4	54.4	55.7	50.4	55.6
10	36.9	36.8	35.7	36.2	37.1	36.3
11	21.5	21.4	21.2	37.9	21.1	38.2
12	40.2	40.1	40.1	213.0	40.0	212.9
13	41.1	41.0	41.0	55.5	40.8	57.6
14	56.3	56.2	56.3	55.8	56.6	54.2
15	32.4	32.3	32.3	31.7	32.4	33.8
16	81.1	81.1	81.1	79.6	81.1	83.0
17	63.9	63.9	63.9	55.0	63.8	56.2
18	16.7	16.7	16.7	16.2	16.4	14.1
19	13.4	13.4	12.3	11.7	19.4	11.8
20	40.7	40.6	40.6	41.2	40.8	103.2
21	16.4	16.4	16.4	15.2	16.5	11.5
22	110.7	110.6	110.6	110.7	110.7	153.2
23	37.1	37.1	37.0	37.0	37.1	23.7
24	28.4 (28.3)	28.32 (28.26)	28.34 (28.30)	28.33 (28.26)	28.32 (28.26)	31.4 (31.3)
25	34.1 (34.4)	34.2 (34.4)	34.2 (34.4)	34.2 (34.3)	34.36 (34.42)	33.5 (33.7)
26	75.2 (75.3)	75.1 (75.2)	75.1 (75.2)	75.16 (75.22)	75.2 (75.3)	74.9 (75.1)
27	17.4	17.4	17.4	17.41 (17.37)	17.4	17.3 (17.1)
3-*O*-Gal 1	103.6	103.3	102.3	102.4	102.8	102.4
2	73.1	72.6	73.1	73.1	73.2	73.1
3	75.8	75.6	75.7	75.7	75.8	75.7
4	80.1	80.2	80.4	80.3	80.3	80.3
5	75.9	75.6	75.2	75.3	75.2	75.2
6	60.9	60.3	60.4	60.4	60.5	60.5
Glc 1	107.2	105.1	105.2	105.2	105.2	105.2
2	75.4	84.9	84.9	85.0	85.1	85.0
3	78.8	77.9	77.4	77.2	77.4	77.4
4	72.3	72.1	72.2	72.2	72.2	72.1
5	78.5	77.9	77.9	77.9	78.0	78.0
6	63.2	63.2	63.2	63.2	63.3	63.2
Gal 1		107.2	107.2	107.2	107.3	107.2
2		74.3	74.4	74.4	74.4	74.3
3		74.3	74.1	74.1	74.2	74.2
4		70.8	70.8	70.8	70.9	70.8
5		77.5	77.4	77.4	77.4	77.4
6		63.0	62.9	62.8	63.1	63.0
26-*O*-Glc 1	104.9 (105.1)	104.8 (105.1)	104.9 (105.1)	104.9 (105.0)	104.9 (105.1)	104.8 (105.0)
2	75.2	75.2	75.1	75.2	75.2	75.1
3	78.4	78.5	78.5	78.5	78.6	78.6
4	71.8	71.6	71.6	71.7	71.8	71.8
5	78.6	78.4	78.4	78.4	78.4	78.4
6	62.9	62.8	62.7	62.9	62.9	62.9

* Shifts for the C-25 isomers of **1a**, **2–5** and **6a** were shown in the parentheses.

Therefore, the structure of **5** was established to be 26-*O*-β-D-glucopyranosyl (25*R*,*S*)-furost-5-ene-3β,22α,26-triol-3-*O*-β-D-galactopyranosyl(1-2)-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside, and was named terrestrosin J.

Compound **6** gave a red colour with Ehrlich's reagent. The molecular formula of **6** was determined to be $C_{51}H_{82}O_{24}$ by the high resolution negative FAB-mass spectrometry. Comparison of the FAB-mass spectrometry (neg.) of **6** with that of **4** showed that all the peaks in the spectrum of **6** were 18 mass units lower than the corresponding ion peaks of **4**.

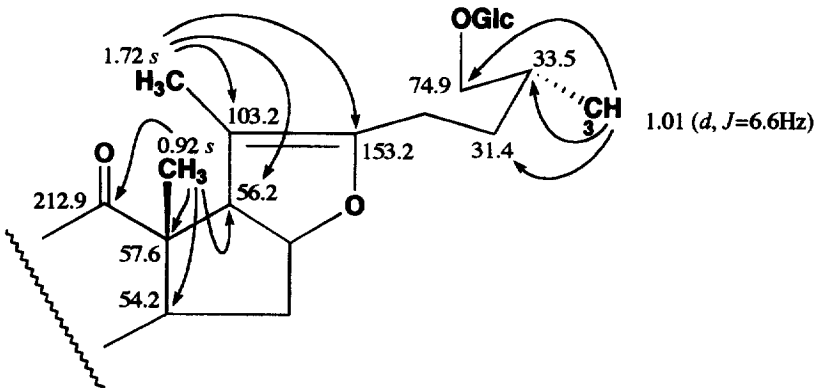

Fig. 1. Long-range ^{1}H- ^{13}C coupling observed in HMBC spectrum of **6**.

Comparison of the ^{1}H and ^{13}C NMR spectra of **6** with those of **4** indicated that they have the same partial structures A, B, C and D-rings. The ^{1}H NMR spectrum of **4** showed the presence of two singlet and two doublet methyl signals while the ^{1}H NMR spectrum of **6** showed the presence of three singlet and only one doublet methyl signals. The difference between the two compounds is that **6** possesses a double bond between C-20 and C-22, which was suggested by the NMR signals at δ_H 1.72 (3H, *s*, 21-CH_3) and 3.39 (1H, *d*, J = 10.3 Hz, 17-H) and two quaternary carbon signals at δ_C 153.2 (C-22) and 103.2 (C-20) [8, 9]. On acid hydrolysis, **6** gave hecogenin which was probably derived from its original aglycone by cyclization of the side chain. The assignments of the aglycone moiety were determined by DEPT, HSQC, HMBC and comparison with the aglycone moiety of **4**. In the HMBC spectrum, the methyl protons at δ 0.92 (18-CH_3) showed long-range correlation with the carbons at δ 57.6 (C-13), 54.2 (C-14), 212.9 (C-12) and 56.2 (C-17), as shown in Fig. 1, indicating the attachment of the keto group at C-12. The methyl protons at δ 0.68 (19-CH_3) showed long-range correlation with the carbons at δ 36.3 (C-10), 36.7 (C-1), 44.6 (C-5) and 55.6 (C-9). The methyl protons at δ 1.72 (21-CH_3) showed long-range correlation with the carbons at δ 56.2 (C-17), 103.2 (C-20) and 153.2 (C-22). The methyl protons at δ 1.01 (27-CH_3) showed long-range correlation with carbons at δ 31.4 (C-24), 33.8 (C-25) and 74.9 (C-26). Thus, its aglycone moiety was deduced to be a 5α-furost-20(22)-en-12-one-3β,26-diol structure. The C-25 configuration of **6** was *R*, which was confirmed by the ^{1}H NMR signals at δ 3.61 (1H, *dd*, J = 5.7, 9.3 Hz, Ha-26, 25*R*). The comparison of ^{1}H and ^{13}C NMR spectra of **6** with those of **4** indicated that they have the same sugar sequence. Thus, the structure of **6** was established to be 26-*O*-β-D-glucopyranosyl (25*R*)-5α-furost-20(22)-en-12-one-3β,26-diol-3-*O*-β-D-galactopyranosyl(1-2)-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside and was named terrestrosin K.

In addition to **6**, the 25*R*,*S*-mixture, **6a**, $[\alpha]_D^{25}$ + 5.3° (pyridine) was also isolated. Compound **6a** was established to be the 25*R*,*S*-mixture based on the ^{1}H NMR signals at δ 3.47 (1H, *dd*, J = 7.0, 8.8 Hz, Ha-26, 25*S*) and 3.60 (1H, *dd*, J = 6.0, 9.0 Hz, Ha-26, 25*R*).

The absolution configurations of the sugars were determined in the course of our studies of the spirostanol saponins from the fruits of *T. terrestris* [2]. We assumed the same configurations for the sugar moieties of the furostanol saponins found in the same extract.

The amount of the isolated saponins indicated that the furostanol saponins were the major constituents of the fruit extract.

EXPERIMENTAL

Optical rotation: Union PM-101. NMR (ppm, *J* Hz): JEOL JNM-GX 500, TMS as int. standard. FAB MS: JEOL JMS-SX 102, direct inlet method. HPLC: a Tosoh HPLC system (pump, HLC-803 D; detector, RI-8000) equipped with D-ODS-5 column (20 mm i.d. × 25 cm YMC) and polyamine-II column (20 mm i.d. × 25 cm YMC) with flow rate of mobile phase 6 ml min^{-1}. CC: Kieselgel 60 (70-230 mesh, Merck) and LiChroprep RP-18 (Merck). TLC: Kieselgel 60 precoated plates, F_{254} (Merck) and HPTLC using RP-18 precoated plates, F_{254} (Merck) or Kieselgel 60 precoated plates (Merck) for acid hydrolysis on TLC and spots were visualized by spraying with Ehrlich's reagent or 10% H_2SO_4 followed by heating.

Plant material. The fruits of *T. terrestris* L. were bought in 1987 from a company in Lai-yuan county, He-bei province, China that sells medicinal plants (it was collected in He-nan province, 1986) and identified by Prof. Jia-shi Li of the Beijing University of Traditional Chinese Medicine. The voucher specimen is deposited at our laboratory.

Extraction and isolation. The fruits were defatted with petrol (bp 60–90°). The defatted material was extracted with 80% EtOH. The extract was subjected to CC on silica gel using $CHCl_3$, Me_2CO and MeOH, successively. Crude saponin was obtained from MeOH with Me_2CO precipitation.

Crude saponin (24 g) was sepd into 7 frs over silica

gel CC with CH_2Cl_2–MeOH–H_2O, (50:10:1; 40:10:1; 30:10:1; 20:10:1; 10:10:1) and finally with MeOH. Fr. 3 (2.46 g) was further chromatographed over LiChroprep RP-18 CC using a gradient elution with 25–50% aq. MeCN to give 11 frs. Fr. 3-1 (770 mg) was repeatedly subjected to prep. ODS-HPLC with 25% aq. MeCN and prep. polyamine-HPLC with 83% aq. MeCN to give **1** (35 mg) and **1a** (20 mg). Fr. 4 (4.20 g) was sepd by CC on reversed-phase silica gel, LiChroprep RP-18 using a gradient elution with 20–30% aq. MeCN and silica gel CC with CH_2Cl_2–MeOH–H_2O (30:10:1) to give **1a** (93 mg) and **3** (199 mg) with a little impurity. Fr. 5 (6.67 g) was subjected to LiChroprep RP-18 CC using a gradient elution with 20–35% aq. MeCN to give 13 frs. Fr. 5-4 (1.25 g) was repeatedly subjected to silica gel CC with CH_2Cl_2–MeOH–H_2O (30:10:1) and ODS-MPLC with 80% aq. MeCN to give Fr. 5-4-4-1 (580 mg) which was almost pure. Part of Fr. 5-4-4-1 (230 mg) was further purified by prep. polyamine-HPLC using 79.5% aq. MeCN to give **4** (40 mg). Fr. 5-6 (1.44 g) was sepd by silica gel CC with CH_2Cl_2–MeOH–H_2O (30:10:1) to give Fr. 5-6-3 (1.17 g) which was almost pure. Part of Fr. 5-6-3 (240 mg) was further purified by prep. polyamine-HPLC using 80% aq. MeCN to give **2** (78 mg). Fr. 5-8 (1.39 g) was repeatedly subjected to ODS-MPLC with 23% and 25% aq. MeCN, silica gel CC with CH_2Cl_2–MeOH–H_2O (30:10:1) and prep. ODS-HPLC with 26.5% and 27% aq. MeCN to give pure **3** (82 mg), **5** (10 mg), **6** (19 mg) and **6a** (32 mg).

After subjecting the frs to silica gel CC, they were refluxed with 30 % Me_2CO to convert the 22-methoxy form to the original 22-hydroxy form then proceeded as described.

Terrestrosin F (**1**). White powder, $[\alpha]_D^{20} - 20.0°$ (pyridine; *c* 0.60). HR-FAB-MS (neg.) *m/z*: 935.4871 $[C_{45}H_{76}O_{20} - H]^-$, requires 935.4852. FAB-MS (neg.) *m/z*: 935 $[M-H]^-$, 773 $[M-Glc]^-$, 611 $[M-Glc-Gal]^-$. ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Terrestrosin G (**2**). White powder, $[\alpha]_D^{24} - 26.8°$ (pyridine; *c* 0.75). HR-FAB-MS (neg.) *m/z*: 1097.5440 $[C_{51}H_{86}O_{25} - H]^-$, requires 1097.5379. FAB-MS (neg.) *m/z*: 1097 $[M-H]^-$, 935 $[M-Glc]^-$, 773 $[M-Glc-Gal]^-$, 611 $[M-Glc-Gal-Glc]^-$. ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Terrestrosin H (**3**). White powder, $[\alpha]_D^{24} - 20.4°$ (pyridine; *c* 0.54). HR-FAB-MS (neg.) *m/z*: 1081.5400 $[C_{51}H_{86}O_{24}]^-$, requires 1081.5428. FAB-MS (neg.) *m/z*: 1081 $[M-H]^-$, 919 $[M-Glc]^-$, 757 $[M-Glc-Gal]^-$, 595 $[M-Glc-Gal-Glc]^-$. ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Terrestrosin I (**4**). White powder, $[\alpha]_D^{24} - 17.0°$ (pyridine; *c* 0.53). HR-FAB-MS (neg.) *m/z*: 1095.5220 $[C_{51}H_{84}O_{25} - H]^-$, requires 1095.5219. FAB-MS (neg.) *m/z*: 1095 $[M-H]^-$, 933 $[M-Glc]^-$, 771 $[M-Glc-Gal]^-$, 609 $[M-Glc-Gal-Glc]^-$. ^1H NMR: Table 1: ^{13}C NMR: Table 2.

Terrestrosin J (**5**). White powder, $[\alpha]_D^{25} - 42.9°$ (pyridine; *c* 0.79). HR-FAB-MS (neg.) *m/z*: 1079.5280 $[C_{51}H_{84}O_{24} - H]^-$, requires 1079.5273. FAB-MS (neg.) *m/z*: 1079 $[M-H]^-$, 917 $[M-Glc]^-$, 755 $[M-Glc-Gal]^-$, 593 $[M-Glc-Gal-Glc]^-$. ^1H NMR: 5.30 (1H, *br*, *s*, H-6), the other signals see Table 1; ^{13}C NMR: Table 2.

Terrestrosin K (**6**). White powder, $[\alpha]_D^{25} + 3.1°$ (pyridine; *c* 1.31). HR-FAB-MS (neg.) *m/z*: 1077.5140 $[C_{51}H_{82}O_{24} - H]^-$, requires 1077.5125. FAB-MS (neg.) *m/z*: 1077 $[M-H]^-$, 915 $[M-Glc]^-$, 753 $[M-Glc-Gal]^-$, 591 $[M-Glc-Gal-Glc]^-$. ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Acid hydrolysis of **1–6**. A soln of each saponin (about 2 mg) in 2 N HCl–dioxane (1:1, 0.5 ml) was heated at 95° for 2 hr. The reaction mixt. was diluted with H_2O and then extracted with EtOAc. The EtOAc layer and H_2O layer was checked for identification of aglycone and sugar moieties, respectively. Aglycones were identified with TLC by comparison with authentic samples, using CH_2Cl_2–MeOH (50:1) as developing solvent and 10% H_2SO_4 as detection reagent. Saponins **1** and **2** gave gitogenin (R_f 0.09), **3** gave tigogenin (R_f 0.33), **4** and **6** gave hecogenin (R_f 0.20) and **5** gave diosgenin (R_f 0.39). Sugars were checked by TLC using CH_2Cl_2–MeOH–H_2O (15:6:1) as developing solvent and TTC reagent for detection. **1–6** gave glucose (R_f 0.21) and galactose (R_f 0.17), respectively.

Enzymatic hydrolysis of **1–4**. A soln of **1** (20 mg) and β-glucosidase (20 mg) in acetate buffer (5 ml, pH 5.0) was incubated at 37° overnight. The soln was extracted with *n*-BuOH. The *n*-BuOH extract was concd and subjected to prep. polyamine HPLC using 87% aq. MeCN afforded **1S** (5 mg). **1S** was identified as gitogenin 3-*O*-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside based on the physical properties and NMR spectral data [4]. Glucose was detected by TLC as described above.

By the same procedure carried out for **1a** (8 mg), **2** (23 mg), **3** (25 mg) and **4** (32 mg) yielded the corresponding prosapogenins **1aS** (2 mg), **2S** (5 mg), **3S** (4 mg) and **4S** (6 mg), respectively, as well as glucose.

The prosapogenins **2S**, **3S** and **4S** were identical with terrestrosin E, terrestrosin A and terrestrosin C, respectively, isolated earlier from the same plant [2].

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