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STEROIDAL SAPONINS FROM FRUITS OF *TRIBULUS TERRESTRIS*

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

Abstract—Further studies on the constituents of the fruits of *Tribulus terrestris* led to the isolation of five new steroidal saponins (terrestrosin A–E), (25*R*,*S*)-5α-spirostan-3β-ol-3-*O*-β-D-galactopyranosyl(1-2)-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside, (25*R*,*S*)-5α-spirostan-3β-ol-3-*O*-β-D-glucopyranosyl(1-4)-[α-L-rhamnopyranosyl(1-2)]-β-D-galactopyranoside, (25*R*,*S*)-5α-spirostan-12-on-3β-ol-3-*O*-β-D-galactopyranosyl(1-2)-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside, hecogenin 3-*O*-β-D-galactopyranosyl(1-2)-[β-D-xylopyranosyl(1-3)]-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside and (25*R*,*S*)-5α-spirostane-2α,3β-diol-3-*O*-β-D-galactopyranosyl(1-2)-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside, together with five known steroidal saponins, desgalactotigonin, F-gitonin, desglucolanatigonin, gitonin and tigogenin 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. The structures of the new saponins were elucidated on the basis of spectroscopic analyses, including two-dimensional NMR techniques, and chemical reactions.

INTRODUCTION

The genus *Tribulus* (Zygophyllaceae) comprises *ca* 20 species which grow as shrubs in subtropical areas around the world. There are only two species distributed in China, *T. terrestris* and *T. cistoides*. In traditional Chinese medicine, the fruit of *T. terrestris*, which is known as Ci Ji Li, has been used against various diseases for a long time. Recently, the crude saponin fraction of the whole plant has been used as a cordial drug. Some chemical constituents of this plant have been reported [1]. Although no detailed chemical investigation has been performed on *T. terrestris* growing in China, four pairs of sapogenins, tigogenin and neotigogenin, gitogenin and neogitogenin, hecogenin and neohecogenin, and manogenin and neomanogenin, have been isolated by hydrolysis of the crude saponins obtained from the plant [2]. This paper reports the isolation and characterization of the spirostanol saponins from the fruits of this plant.

RESULTS AND DISCUSSION

The crude saponin fraction of *T. terrestris* was fractionated by a combination of silica gel chromatography and HPLC on silica gel RP-18 and on polyamine, to afford compounds **1**–**10**. Compounds **1**–**5** were identified as the known desgalactotigonin, F-gitonin [3], desglucolanatigonin II, gitonin [4] and tigogenin 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 [5], respectively, based on their NMR spectral data and by a comparison of the their physical properties with those reported in the literature for these saponins.

Compound **6** was obtained as needles from methanol. Its molecular formula, $C_{45}H_{74}O_{18}$, was established by high resolution FAB mass spectrometry. Upon acid hydrolysis, **6** yielded D-glucose and D-galactose as sugar components. The ^{1}H NMR spectrum of **6** displayed three doublet signals of anomeric protons at δ 4.86, 5.06 and 5.09 with coupling constants of 7.5, 7.6 and 7.6 Hz, respectively, diagnostic of a β-configuration for all the three sugars.

The sequential assignments of the ^{1}H resonances for each monosaccharide were established by a detailed inspection of the ^{1}H–^{1}H COSY, TOCSY and difference spectra of homonuclear Hartmann–Harn experiment spectra. Comparison of the ^{13}C shifts thus assigned with those of the reference methyl glycoside [6] and taking into account the known effect of the *O*-glycosylation and the result of acid hydrolysis indicated that **6** has a terminal β-D-galactopyranosyl unit, a 2-substituted β-D-glucopyranosyl unit and a 4-substituted β-D-galactopyranosyl unit.

In the rotating frame nuclear Overhauser effect difference spectra (ROEDS) spectrum, the anomeric proton signals at δ 5.09 (galactose), 5.06 (2-substituted glucose) and 4.86 (5-substituted galactose) showed

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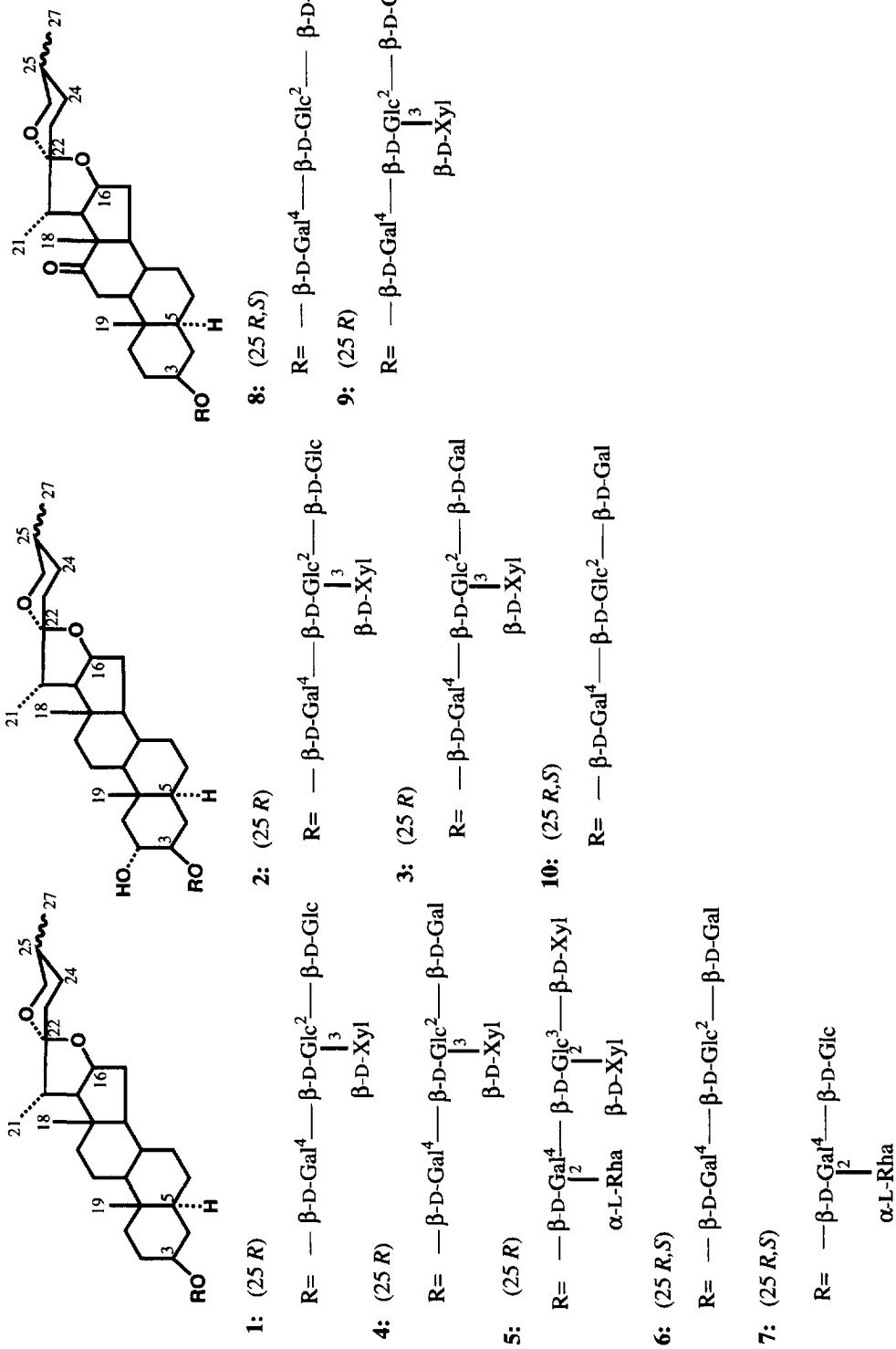

correlations with the proton signals at δ 4.13 (H-2 of 2-substituted glucose), 4.52 (H-4 of 4-substituted galactose) and 3.93 (H-3 of aglycone), respectively (Fig. 1).

Comparison of the ^{1}H and ^{13}C NMR signals due to the aglycone moiety with signals of sapogenins previously isolated from this plant [2] showed the aglycone of **6** is a mixture of tigogenin (25*R*) and neotigogenin (25*S*). Thus, the full structure of **6** was deduced as (25*R*,*S*) - 5α - spirostan - 3β - ol - 3 - *O* - β - D - galactopyranosyl(1-2)-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside and the compound was given the name terrestrosin A.

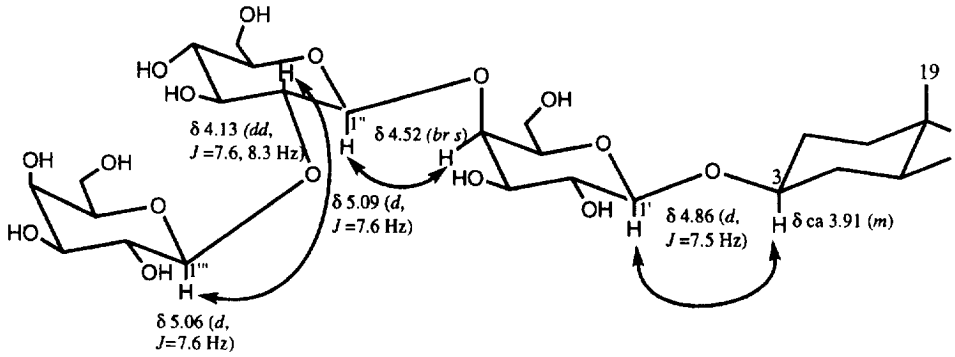

Fig. 1. ROE correlations of the saccharide moieties of compound **6** in pyridine-d_5.

Compound **7** ($C_{45}H_{74}O_{17}$) was obtained as microneedles from methanol–chloroform. The FAB mass spectrum showed that **7** was a triglycoside. This was confirmed by acid hydrolysis, where D-galactose, D-glucose and L-rhamnose were obtained. The fragment ion peak at m/z 739 [885 − 146 (rhamnose)] and 723 [885 − 162 (hexose)] indicated the presence of a terminal rhamnose unit and a terminal hexose unit. On comparison of the ^{1}H and ^{13}C NMR spectra of **7** with those of the prosapogenin neohecogenin 3-*O*-*β*-D-glucopyranosyl(1-4)-[*α*-L-rhamnopyranosyl(1-2)]-*β*-D-galactopyranoside [7], the signals due to sugar moieties were fully superimposable, indicating that they have the same sugar sequence. The spectra showed that the aglycone of **7** was a mixture of tigogenin and neotigogenin [2]. Thus, the structure of **7** was identified to be (25*R*,*S*)-5*α*-spirostan-3*β*-ol-3-*O*-*β*-D-glucopyranosyl(1-4)-[*α*-L-rhamnopyranosyl(1-2)]-*β*-D-galactopyranoside, and it was given the name terrestrosin B.

Compound **8** ($C_{45}H_{72}O_{19}$) was obtained as microneedles from methanol. Its ^{1}H and ^{13}C NMR spectra indicated the presence of three anomeric protons and carbons, and all the ^{13}C NMR signals of sugar moieties were almost superimposable with those of **6**. Comparison of the ^{1}H and ^{13}C NMR signals due to the aglycone moiety with that of the sapogenin previously obtained from the same extract [2] suggested the aglycone of **8** was a mixture of hecogenin and neohecogenin. Acid hydrolysis of **8** gave D-glucose and D-galactose. Thus, the structure of **8** was assigned as (25*R*,*S*)-5*α*-spirostan-12-on-3*β*-ol-3-*O*-*β*-D-galactopyranosyl(1-2)-*β*-D-glucopyranosyl(1-4)-*β*-D-galactopyranoside, and it was given the name terrestrosin C.

Compound **9** ($C_{50}H_{80}O_{23}$) was obtained as microneedles from methanol. Its ^{1}H and ^{13}C NMR spectra indicated the presence of four anomeric protons and carbons. Further inspection of the ^{13}C NMR signals due to sugar moieties led to the conclusion that **9** possessed the same sugar sequence as those of **4**, which was also elucidated by using 2D NMR techniques. The ^{1}H and ^{13}C NMR signals due to the aglycone indicated that **9** was the 3-*O*-glycoside of hecogenin [2]. Acid hydrolysis of **9** gave D-glucose, D-galactose and D-xylose. Accordingly, **9** was established to be hecogenin 3-*O*-*β*-D-galactopyranosyl(1-2)-[*β*-D-xylopyranosyl(1-3)]-*β*-D-glucopyranosyl(1-4)-*β*-D-galactopyranoside, and it was given the name terrestrosin D.

Compound **10** ($C_{45}H_{74}O_{19}$) was obtained as needles from methanol. Acid hydrolysis of **10** gave D-glucose and D-galactose. The comparison of the ^{13}C NMR spectrum of **10** with that of **6** showed they have the same sugar sequence. In addition, the ^{1}H and ^{13}C NMR spectra indicated that the aglycone of **10** was a mixture of gitogenin and neogitogenin [2]. Therefore, **10** was deduced to be (25*R*,*S*)-5*α*-spirostane-2*α*,3*β*-diol-3-*O*-*β*-D-galactopyranosyl(1-2)-*β*-D-glucopyranosyl(1-4)-*β*-D-galactopyranoside, and it was given the name terrestrosin E.

Based on the isolated compounds, when the number of sugar moieties is three (**6**, **7**, **8** and **10**), the aglycone is a mixture of 25*R* and 25*S*. However, if the number of sugar moeities is four or five, the configuration of the aglycone is 25*R* (>90%). In contrast, the aglycones of the reported pure compounds isolated from *T. terrestris* grown in India are all 25*S* regardless of the number of sugar moieties [8]. The saponins having a terminal galactopyranosyl unit were isolated from the genus for the first time. The combined use of the 2D NMR techniques allowed the structural assignments of the sugar moieties without such chemical degradation studies as permethylation followed by hydrolysis or partial hydrolysis, which often consume relatively large amount of materials.

EXPERIMENTAL

Mps: Yanaco micro hot-stage; uncorr. Optical rotation: Union PM-101. NMR (ppm, *J* Hz): JEOL JNM-GX 400, 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 a 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; spots were visualized by spraying with 10% H_2SO_4 followed by heating. Acid hydrolysis of glycosides and identification of resulting monosaccharides: see ref. [9].

Plant material. The fruits of *T. terrestris* L. were bought in 1987 from a company in Lai-yuan county, Hebei province, China, that sells medicinal plants (it was collected in Henan 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 materials were extracted with 80% EtOH. The extract obtained was subjected to CC on silica gel using $CHCl_3$, Me_2CO and MeOH, sequentially. Crude saponin and non-saponin frs were sepd from MeOH by Me_2CO pptn.

Part of the saponin obtained (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 alone. Fr. 3 (2.46 g) was further chromatographed over LiChroprep RP-18 CC using gradient elution with 25–50% aq. MeCN to give 11 frs. Fr. 3-6 (143 mg) was repeatedly subjected to prep. ODS-HPLC with MeOH–H_2O (4:1) and prep. polyamine-HPLC with CH_3CN–H_2O (87:13) to give **8** (16 mg) and **9** (23 mg). Fr. 3-8 (105 mg) was subjected to prep. ODS-HPLC with MeOH–H_2O (83:17) and prep. polyamine-HPLC with MeCN–H_2O (17:3; 43:7) to afford **2** (3.5 mg), **3** (19 mg) and **10** (20 mg). Fr. 3-10 (190 mg) was subjected to prep. ODS-HPLC with MeOH–H_2O (43:7) to yield **5** with a few impurities and Fr. 3-10-2. Compound **5** was purified by prep. polyamine-HPLC with MeCN–H_2O (17:3); **5** (30 mg). Fr. 3-10-2 (71 mg) afforded **1** (8 mg), **4** (28 mg), **6** (24 mg) and **7** (3.5 mg) after subjecting it to prep. polyamine-HPLC with CH_3CN–H_2O (17:3; 87:13).

Desgalactotigonin (**1**). Microneedles from MeOH, mp 233–236° [dec.]; $[\alpha]_D^{28}$ −44.7° (pyridine; *c* 0.38); FAB-MS (neg.) *m/z*: 1033 $[M-H]^-$. ^1H NMR: Table 1. ^{13}C NMR: Table 2.

F-gitonin (**2**). Microneedles from MeOH, mp 260–263°; $[\alpha]_D^{24}$ −51.6° (pyridine; *c* 0.21); FAB-MS (neg.) *m/z*: 1049 $[M-H]^-$. ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Gitonin (**3**). Needles from MeOH, mp 250–253°; $[\alpha]_D^{19}$ −40° (pyridine; *c* 1.0); FAB-MS (neg.) was same as **2**. ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Desglucolanatigonin (**4**). Microneedles from MeOH, mp 240–243° [dec.]; $[\alpha]_D^{24}$ −37.5° (pyridine; *c* 1.37); FAB-MS (neg.) was same as **1**. ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Tigogenin 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* (**5**). Needles from MeOH, mp: >300°; $[\alpha]_D^{24}$ −65.1° (pyridine; *c* 0.56); HR-EAB-MS (neg) *m/z*: 1149.5690 $[C_{55}H_{90}O_{25}-H]^-$, require 1149.5696. FAB-MS (neg.) *m/z*: 1149 $[M-H]^-$, 1017 $[M-Xyl]^-$, 885 $[M-2Xyl]^-$, 871 $[M-Xyl-Rha]^-$, 723 $[M-2Xyl-Glc]^-$, 577 $[723-Rha]^-$. ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Terrestrosin A (**6**). Needles from MeOH, mp 227–230°; $[\alpha]_D^{24}$ −56.5° (pyridine; *c* 0.65); HR-FAB-MS (neg) *m/z*: 901.4763 $[C_{45}H_{74}O_{18}-H]^-$ requires 901.4797. FAB-MS (neg.) *m/z*: 901 $[M-H]^-$, 739 $[M-Gal]^-$, 577 $[M-Gal-Glc]^-$. ^1H NMR: Table 1; ^{13}C NMR: Table 2; 2D NMR: Table 3.

Terrestrosin B (**7**). Needles from MeOH–$CHCl_3$, mp

Table 1. ^1H NMR spectral data for compounds **1**–**10** in pyridine-d_5 (*δ* values; 400 MHz)

		1	**2**	**3**	**4**	**5**	**6**		**7**		**8**		**9**	**10**	
		25*R*	25*R*	25*R*	25*R*	25*R*	25*R*	25*S*	25*R*	25*S*	25*R*	25*S*	25*R*	25*R*	25*S*
Aglycone moiety															
	H-18 *s*	0.80	0.79	0.79	0.80	0.79	0.80	0.79	0.80	0.79	1.06	1.06	1.05	0.81	0.80
	H-19 *s*	0.63	0.68	0.69	0.63	0.84	0.65	0.65	0.85	0.85	0.64	0.64	0.62	0.70	0.70
	H-21 *d*	1.11	1.11	1.11	1.11	1.12	1.11	1.12	1.12	1.12	1.34	1.34	1.33	1.10	1.11
		(7.0)	(6.8)	(7.0)	(7.1)	(6.6)	(7.1)	(7.1)	(7.0)	(7.0)	(7.0)	(7.0)	(7.0)	(6.8)	(6.6)
	H-27 *d*	0.68	0.67	0.70	0.68	0.67	0.68	1.06	0.67	1.05	0.67	1.04	0.67	0.70	1.05
		(5.8)	(5.5)	(6.3)	(5.9)	(4.9)	(5.9)	(7.1)	(6.2)	(7.0)	(5.7)	(4.8)	(5.7)	(7.1)	(7.1)
Sugar moiety															
3-*O*-Gal	H-1 *d*	4.83	4.91	4.91	4.86	4.83	4.86		4.91		4.88		4.87	4.93	
		(7.6)	(7.7)	(7.8)	(7.6)	(7.8)	(7.5)		(7.7)		(7.7)		(7.5)	(7.7)	
Glc	H-1 *d*	5.12	5.21	5.15*	5.12	4.97*	5.09		5.18		5.13*		5.16*	5.16*	
		(8.3)	(8.1)	(8.0)	(8.1)	(7.8)	(7.6)		(7.9)		(7.3)		(7.9)	(7.7)	
Gal or Glc	H-1 *d*	5.52	5.59	5.48	5.42		5.06				5.10*		5.45	5.14*	
		(7.8)	(7.7)	(7.8)	(7.8)		(7.5)				(7.7)		(7.6)	(7.3)	
Xyl	H-1 *d*	5.17	5.25	5.09*	5.04	5.23*							5.05*		
		(7.8)	(7.9)	(7.6)	(7.6)	(7.8)							(7.1)		
Xyl	H-1 *d*					5.41*									
						(7.8)									
Rha	H-1 *s*					6.17			6.23						
Rha	H-6 *d*					1.70			1.69						
						(5.9)			(6.2)						

J values (in parentheses) are reported in Hz.

*Signals may be interchangeable within each column.

Table 2. ^{13}C NMR spectral data for compounds **1–10** in pyridine-d_5 (δ values; 100 MHz)

		1	**2**	**3**	**4**	**5**	**6**		**7**		**8**		**9**	**10**	
	C	*25R*	*25R*	*25R*	*25R*	*25R*	*25R*	*25S*	*25R*	*25S*	*25R*	*25S*	*25R*	*25R*	*25S*
	1	37.2	45.5	45.7	37.2	37.1	37.2		37.2		36.6		36.5	45.5	
	2	29.9	70.4	70.5	30.0	29.9	30.1		29.9		29.7		29.7	70.4	
	3	78.5	84.2	84.6	78.4	78.7	78.0		76.9		77.9		78.4	84.7	
	4	34.8	34.1	34.2	34.9	34.4	35.0		34.4		34.3		34.2	34.2	
	5	44.7	44.6	44.8	44.8	44.7	44.8		44.6		44.1		44.4	44.6	
	6	28.9	28.1	28.1	28.9	29.0	28.9		28.9		28.5		28.5	28.0	
	7	32.4	32.2	32.3	32.4	32.4	32.4		32.4		31.7		31.7	32.2	
	8	35.3	34.6	34.6	35.3	35.3	35.3		35.2		34.7		34.6	34.5	
	9	54.4	54.3	54.5	54.5	54.5	54.4		54.6		55.3		55.3	54.3	
	10	35.8	36.8	36.9	35.8	35.9	35.8		35.9		36.2		36.2	36.8	
	11	21.3	21.4	21.5	21.3	21.3	21.3		21.2		37.9		37.9	21.4	
	12	40.1	40.0	40.1	40.2	40.2	40.2		40.1		212.7		212.7	40.0	
	13	40.8	40.7	40.8	40.8	40.8	40.8		40.8		55.5		55.4	40.7	
	14	56.4	56.3	56.4	56.5	56.5	56.4		56.4		55.9		55.8	56.2	
	15	32.1	32.1	32.1	32.1	32.1	32.1		32.1		31.4		31.3	32.0	
	16	81.1	81.1	81.2	81.1	81.1	81.1	81.1	81.1	81.2	79.7	79.7	79.6	81.1	81.1
	17	63.0	63.1	63.1	63.1	63.1	63.1	63.0	63.1	63.0	54.2	54.1	54.2	63.1	63.0
	18	16.5	16.6	16.6	16.6	16.5	16.6		16.6		16.0		16.0	16.5	
	19	12.3	13.4	13.5	12.3	12.4	12.3		12.4		11.7		11.6	13.4	
	20	42.0	42.0	42.0	42.0	42.0	42.0	42.4	42.0	42.5	42.6	43.0	42.5	41.9	42.4
	21	14.9	15.0	15.0	15.0	15.0	15.0	14.8	15.0	14.8	13.9	13.7	13.8	15.0	14.8
	22	109.2	109.2	109.2	109.2	109.2	109.2	109.7	109.2	109.7	109.3	109.7	109.2	109.2	109.6
	23	31.8	31.8	31.9	31.8	31.9	31.8	26.3	31.8	26.3	31.6	26.3	31.6	31.7	26.3
	24	29.2	29.2	29.3	29.3	29.3	29.3	26.1	29.2	26.1	29.2	26.1	29.1	29.2	26.1
	25	30.6	30.6	30.6	30.6	30.6	30.6	27.5	30.4	27.5	30.4	27.5	30.5	30.5	27.5
	26	66.9	66.8	66.9	66.9	66.9	66.9	65.1	66.8	65.1	66.9	65.1	66.9	66.8	65.0
	27	17.2	17.3	17.3	17.3	17.3	17.3	16.2	17.3	16.3	17.2	16.2	17.2	17.2	16.2
3-*O*-Gal	1	102.5	103.3	103.3	102.4	100.2	102.5		99.9		102.3		102.2	103.3	
	2	73.1	72.6	72.5	73.1	81.3	73.2		77.0		73.1		73.0	72.6	
	3	75.5	75.7	75.8	75.7	75.8	75.8		76.4		75.8		75.7	75.5	
	4	79.8	79.4	79.4	79.6	78.9	80.3		81.3		80.4		79.5	80.2	
	5	75.3	75.5	75.7	75.4	77.7	75.2		75.6		75.2		75.2	75.6	
	6	60.6	60.6	60.5	60.6	60.4	60.5		61.0		60.4		60.5	60.4	
Glc	1	104.9	104.8	105.2	105.4	105.0*	105.2		107.2		105.3		105.5	105.1	
	2	81.2	81.2	81.2	81.0	81.4	85.0		75.2		85.0		81.0	84.9	
	3	87.0	87.0	86.1	85.9	87.7	77.6		78.9		77.5		85.5	78.0	
	4	70.4	70.4	70.5	70.5	70.4	72.2		72.4		72.2		70.5	72.1	
	5	77.6	77.6	77.6	77.5	77.1	77.9		78.5		77.9		77.3	78.0	
	6	63.0	63.0	63.0	63.0	63.0	63.3		62.8		63.2		63.0	63.2	
Gal or Glc	1	104.7	104.7	105.3	105.2		107.2				107.3		105.3	107.2	
	2	76.1	76.1	73.7	73.3		74.4				74.4		73.7	74.3	
	3	77.5	78.1*	74.4	74.1		74.2				74.1		73.9	74.3	
	4	71.1	71.1	70.5	70.3		70.8				70.9		70.3	70.8	
	5	77.8	78.7*	77.2	77.2		77.4				77.1		77.3	77.5	
	6	62.5	62.7	62.6	62.4		62.9				63.0		62.4	62.9	
Xyl	1	104.9	105.0	104.9	104.8	105.6*							104.7		
	2	75.0	75.1	75.1	75.0	75.0†							74.9		
	3	78.5	78.5*	78.5	77.7	76.6‡							77.5		
	4	70.7	70.8	70.8	70.7	70.7§							70.7		
	5	67.2	67.3	67.3	67.2	67.3‖							67.2		
Xyl	1					105.3*									
	2					75.1†									
	3					76.7‡									
	4					70.8§									
	5					67.5‖									
Rha	1					101.9			102.3						
	2					72.4			72.2						
	3					72.7			72.7						
	4					74.0			74.1						
	5					69.3			69.4						
	6					18.4			18.6						

*,†,‡,§,‖Signals may be interchangeable within each column.

296–300° [dec]; $[\alpha]_D^{24}$ −90.5 (pyridine; *c* 0.22); HR-FAB-MS (neg.) *m/z*: 885.4839 $[C_{45}H_{74}O_{17}-H]^-$, require 885.4848. FAB-MS (neg.) *m/z*: 885 $[M-H]^-$, 739 $[M-Rha]^-$, 723 $[M-Glc]^-$, 577 $[M-Rha-Glc]^-$. ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Terrestrosin C (**8**). Microneedles from MeOH, mp 213–215°; $[\alpha]_D^{24}$ −16.4° (pyridine; *c* 0.91); HR-FAB-MS (neg.) *m/z*: 915.4636 $[C_{45}H_{72}O_{19}-H]^-$, requires 915.4589. FAB-MS (neg.) *m/z*: 915 $[M-H]^-$, 753 $[M-Gal]^-$, 591 $[M-Gal-Glc]^-$. ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Terrestrosin D (**9**). Microneedles from MeOH, mp 277–279°; $[\alpha]_D^{24}$ −20.4° (pyridine; *c* 1.13); HR-FAB-MS (neg.) *m/z*: 1047.5010 $[C_{50}H_{80}O_{23}-H]^-$, requires 1047.5013. FAB-MS (neg.) *m/z*: 1047 $[M-H]^-$, 915 $[M-Xyl]^-$, 885 $[M-Gal]^-$, 753 $[M-Gal-Glc]^-$, 591 $[M-Xyl-Gal-Glc]^-$. ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Terrestrosin E (**10**). Needles from MeOH, mp 222–225°, $[\alpha]_D^{28}$ −30° (pyridine; *c* 1.14). HR-FAB-MS (neg.) *m/z*: 917.4784 $[C_{45}H_{74}O_{19}-H]^-$, requires 917.4746. FAB-MS (neg.) *m/z*: 917 $[M-H]^-$, 755 $[M-Gal]^-$, 593 $[MGal-Glc]^-$. ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Acid hydrolysis on TLC plate. Saponins were hydrolysed with HCl vapour on the HPTLC precoated plate (80° water bath for 30 min) [10] followed by developing with CH_2Cl_2–MeOH (19:1). This was used for identifying the aglycone by comparison with authentic samples. Saponins **2**, **3** and **10** gave gitogenin, **8** and **9** gave hecogenin, and **1** and **4–7** gave tigogenin.

Table 3. ^1H NMR chemical shift assignments of the saccharide moieties of compound **6** in pyridine-d_5

	^1H
Gal 1′	4.86 *d* (7.5)
2′	4.34 *dd* (7.5, 9.8)
3′	4.04 *dd* (9.8, 3.3)
4′	4.52 (br s)
5′	3.97 (m)
6′	4.19 *dd* (10.7, 5.0)
	4.70 *dd* (10.7, 9.5)
Glc 1″	5.09 *d* (7.6)
2″	4.13 *dd* (7.6, 8.3)
3″	4.19 *dd* (8.5, 8.5)
4″	3.89 *dd* (8.6, 8.6)
5″	3.96 (m)
6″	4.06 (m)
	4.57 *brd* (11.2)
Gal 1‴	5.06 *d* (7.6)
2‴	4.50 *dd* (7.6, 9.8)
3‴	3.97 *dd* (9.8, 3.4)
4‴	4.33 *dd* (3.4, 1.1)
5‴	3.96 (m)
6‴	4.39 *dd* (11.7, 4.7)
	4.56 *dd* (11.7, 5.6)

J values (in parentheses) are reported in Hz.

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