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TWO SAPOGENINS FROM *TRIBULUS TERRESTRIS*

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Key Word Index—*Tribulus terrestris* L.; Zygophyllaceae; steroidal sapogenins.

Abstract—Studies on the constituents of *Tribulus terrestris* L. led to the isolation of two new steroidal sapogenins, (5α, 25*R*)-spirostan-3,6,12-trione and 25*R*-spirostan-4-ene-3,6,12-trione, together with five known steroidal sapogenins, tigogenin, hecogenin, gitogenin, hecogenone, and 25*R*-spirostan-4-ene-3,12-dione. The structures of the new sapogenins were established on the basis of chemical and spectroscopic evidence, especially 2D NMR spectroscopic techniques. © 1998 Elsevier Science Ltd. All rights reserved

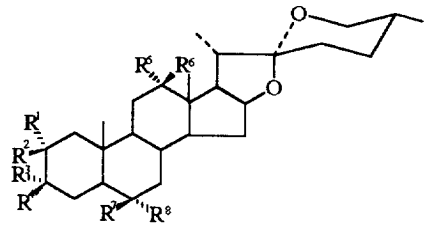
INTRODUCTION

Tribulus terrestris L. is a traditional Chinese medicine which 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 [1–4]. In this paper, we report the isolation and structural elucidation of five known and two new steroidal sapogenins.

RESULTS AND DISCUSSION

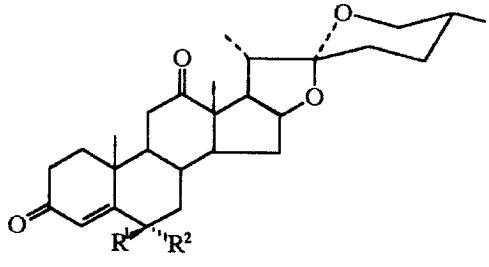
The material obtained after ethanolic extraction of *T. terrestris* L. was partitioned between water and chloroform. The chloroform fraction was further fractionated by silica gel chromatography to afford compounds **1**–**7**. Compounds **1**–**4** were identified as tigogenin, hecogenin, gitogenin and hecogenone.

Compound **5** was obtained as needles from chloroform. This constituent was considered to have both a saturated and an α,β-unsaturated ketone based on the ultraviolet (λ_{max}^{MeOH} 238.2 nm) and infrared spectra (C=O, 1716, 1682 cm^{-1}; C=C, 1621 cm^{-1}). Comparing with hecogenone, ^1H NMR, ^{13}C NMR and DEPT spectroscopic data indicated that the structure of **5** was 25*R*-spirostan-4-ene-3,12-dione (Tables 1 and 2). This structure was confirmed by comparison of the ^1H NMR and ^{13}C NMR spectra with the known steroidal sapogenin, (5α, 25*S*)-spirostan-4-ene-3,12-dione [5]. In the ^1H NMR spectrum of **5** in $CDCl_3$ the absorption of H-26 "eq" and H-26 "ax" (δ 3.49 and 3.34) was observed as a narrow doublet, characteristic of the 25*R*-configuration; the 25*S*-configuration produces signals at δ 3.32 and 3.93 [5]. The ^{13}C NMR spectrum showed the chemical shifts of the F-ring



R^n=H unless otherwise stated

1 R^4 = OH
2 R^4 = OH, R^5R^6 = O
3 R^1 = R^4 = OH
4 R^3R^4 = R^5R^6 = O
6 R^3R^4 = R^5R^6 = R^7R^8 = O



5 R^1 = R^2 = H
7 R^1R^2 = O

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Table 1. ^{1}H NMR spectral data of compounds **5**–**7** in $CDCl_3$

Compound	Me-18	Me-19	Me-21	Me-27	H-16	H-26		Others
5	1.11 *s*	1.28 *s*	1.07 *d* (7.0)	0.80 *d* (6.3)	4.36 *m*	3.34 *t*	3.49 *dd*	5.78 {*s*, H-4}
6	1.09 *s*	1.06 *s*	1.08 *d* (6.3)	0.80 *d* (6.3)	4.38 *m*	3.34 *t*	3.49 *dd*	2.09; 2.51 {*m*; H-7} 2.58 {H-4} 2.39 {H-2} 2.61 {H-5} 1.83 {H-9} 1.71 {H-14} 2.55 {H-17}
7	1.13 *s*	1.28 *s*	1.08 *d* (7.0)	0.80 *d* (6.3)	4.38 *m*	3.34 *t*	3.49 *dd*	6.26 {*s*; H-4} 2.12 *m*; 2.82 *dd* {H-7} 2.54 {*m*; H-2} 1.87 {H-9} 1.64 {*m*; H-14} 2.58 {*m*; H-17}

Table 2. ^{13}C NMR chemical shift (δ) of compounds **4**–**7** and 25*S*-spirostan-4-ene-3,12-dione in $CDCl_3$

^{13}C chemical shift (δ)	Compound				
	4	**25*S****	**5**	**6**	**7**
1	37.6	35.3	35.3	37.4	35.2
2	37.7	32.9	32.3	36.9	33.7
3	210.6	198.6	198.6	209.8	198.4
4	44.4	124.7	124.7	37.1	126.8
5	46.2	168.5	168.4	56.9	158.4
6	28.6	33.7	33.6	107.3	200.1
7	31.4	31.1	31.1	45.8	45.9
8	34.3	34.4	34.4	36.3	32.7
9	54.9	54.6	54.5	54.0	51.7
10	36.2	38.7	38.7	40.6	39.3
11	37.7	37.1	37.1	37.6	36.7
12	212.7	211.9	211.9	211.1	210.6
13	55.1	54.8	54.8	55.1	54.8
14	55.5	54.8	54.8	55.1	55.1
15	31.2	31.1	31.4	31.4	31.4
16	79.1	79.1	78.9	78.8	78.7
17	53.6	53.3	53.5	53.7	53.5
18	16.0	15.9	15.9	16.0	15.9
19	11.1	16.9	16.8	12.3	17.4
20	42.2	42.7	42.2	42.3	42.3
21	13.2	13.0	13.2	13.2	13.2
22	109.3	109.7	109.2	109.3	109.3
23	31.2	25.8	31.2	31.0	31.0
24	28.8	26.0	28.7	28.7	28.8
25	30.2	27.0	30.1	30.2	30.2
26	66.9	65.2	66.9	67.0	67.0
27	17.1	16.0	17.1	17.1	17.1

25*S**: 25*S*-spirostan-4-ene-3,12-dione [5].

characteristic of a 25*R*-configuration (Table 2). Thus compound **5** was identified as 25*R*-spirostan-4-ene-3,12-dione. This is the first record of **5** as a natural product.

Compound **6** was obtained as needles from methanol. Its molecular formula, $C_{27}H_{38}O_5$, was established by HREI-MS. The base peak at *m/z* 139 and a fragment ion at *m/z* 126 were indicative of a lack of substitution in the E- and F-rings [6]. The ^{1}H NMR spectrum of **6** showed signals attributable to the C-18 and C-19 methyl groups at δ 1.09 and 1.06, the C-27 and C-21 methyls at δ 0.80 (3H, *d*, J = 6.3 Hz) and 1.08 (3H, *d*, J = 6.3 Hz). By comparison of the ^{1}H NMR, ^{13}C NMR and DEPT spectra with those of a series of spirostane steroids [7, 8], the structure was assigned as a 5α, 25*R*-sapogenin with three ketones (δ 207.3, 209.8, 211.1). The structure of **6** was determined through the application of 2-dimensional (2D) NMR techniques, including HMQC and HMBC. HMQC and HMBC spectra showed that the signal at δ 211.1 was related to 18-CH_3 (δ 1.09, *s*), which indicated a 12-keto group. The other two ketones (δ 209.8, 207.3) were related to the proton (H-5) at δ 2.61 which was correlated with C-10 (δ 40.6) and C-19 (δ 12.3). The chemical shift of C-5 (δ 56.9) showed a 10.7 ppm upfield shift compared to the corresponding value for hecogenone (δ 46.2, C-5) (Table 2). These data suggest that the two ketones were located close to C-5 and that one of them was in an *ortho*-position to C-5. According to the HMQC and HMBC spectra, the ketone (at δ 207.3) was related to an unequal CH_2 group (δ 2.09; 2.51, *m*), which was correlated with C-8 (δ 36.3), C-9 (δ 54.0) and C-14 (δ 55.1). The other ketone (at δ 209.8) was related to two CH_2 groups (δ 2.39; 2.58) and one of these (δ 2.58) was correlated with the ketone at δ 207.3. Thus, the structure of **6** was established as (5α,25*R*)-spirostan-3,6,12-trione. This structure was confirmed by comparison of the ^{13}C NMR spectrum with those of chlorogenone [9] and hecogenone (Table 2).

Compound **7** was obtained as a white powder. Its molecular formula, $C_{27}H_{36}O_5$, was established by

HREI-MS. It also had the spirostane structure. Its ^1H NMR and ^{13}C NMR spectra indicated the presence of three ketones and an unsaturated double bond. 2D NMR experiments including ^1H-^{13}C COSY and HMBC were used to assign the hydrogen atoms attached to each carbon, to identify adjacent coupled protons and to examine long-range coupled protons. Similarly, the ketone at δ 210.6 was related to 18-CH_3 (δ 1.13, *s*), which showed a 12-keto group. The alkene proton (δ 6.26, *s*) was related to C-10 (δ 39.3), C-5 (δ 158.4), a ketone (δ 200.1) and a CH_2 group (δ 33.7). These data suggested that the unsaturated double bond was either $\Delta^{4,5}$ or $\Delta^{5,6}$. From the ultraviolet spectrum (λ_{max}^{MeOH} 240.4 nm) and the chemical shifts of two ketones (δ 198.4 and δ 200.1), it can be deduced that the two ketones have conjugated with the unsaturated double bond. Thus either a 4-ene-3,6-dione group or a 5-ene-4,7-dione group was possible. From the HMBC measurement, the two ketones (δ 200.1; 198.4) were long-range coupled with two CH_2 groups and the two CH_2 groups had no relationship. This proved the presence of a 4-ene-3,6-dione group. This structure was confirmed from the infrared spectrum (C=O, 1700, 1710 cm^{-1}; C=C, 1620 cm^{-1}) and by comparison of the ^{13}C-spectrum with 25*R*-spirostan-4-ene-3,12-dione. All the above data identified **7** as 25*R*-spirostan-4-ene-3,6,12-trione.

EXPERIMENTAL

General

Mps: ZMD 83-1 electric hot-stage; uncorr. UV: Shimadzu UV-265 FW. IR: Hitachi 275-50. NMR (ppm, *J* Hz): Bruker AC-300 and Bruker AMX-400, TMS as int. standard. EI-MS: Varian MAT 212, direct inlet method. CC: silica gel H (10–40 μ, made in QuingDao Oceanic Chemical Industry). TLC: silica gel H (10–40 μ), spots were visualized by spraying with 10% H_2SO_4 followed by heating.

Plant material

Tribulus terrestris L. (Zygophyllaceae) was collected in 1993 from Henan province, China, and identified by Prof. H. C. Zheng, Dept. of Pharmacognosy, School of Pharmacy, The Second Military Medical University. A voucher specimen is deposited in the herbarium of this institute.

Extraction and isolation

Air-dried and powdered plants (10.7 kg) were extracted with cool 95% EtOH. After removal of solvent by evapn, the residue was extracted with petrol, $CHCl_3$ and *n*-BuOH, respectively. The $CHCl_3$ layer (66 g) was separated and fractionated by silica gel chromatography with petrol–EtOAc (20:1; 10:1; 5:1) and $CHCl_3$–MeOH (20:1; 10:1) to afford **1** (370 mg), **2** (395 mg), **3** (130 mg) and **5** (57 mg). The medial frs were further separated by prep. TLC with $CHCl_3$–EtOAc (10:1) to yield **4** (14 mg), **6** (11 mg) and **7** (12 mg).

25R-*Spirostan*-4-*ene*-3,12-*dione* (**5**). White needles from CH_3OH. mp 256–257°. Liebermann-Burchard reaction showed positive. UV: λ_{max}^{MeOH} 238.2 nm. IR (ν_{max}^{KBr} cm^{-1}): 1718, 1682 (C=O), 1624 (C=C), 982, 924, 902, 863 (924 < 902). EIMS (probe) 70 eV, *m/z*: 426 $[M]^+$ (42), 398 [M−CO] (13), 367 (16), 354 (49), 312 (100), 283 (8), 269 (25), 139 (85), 126 (48). ^1H NMR: Table 1; ^{13}C NMR: Table 2.

5α,25R-*Spirostan*-3,6,12-*trione* (**6**). White needles from CH_3OH. mp 267–269°. IR (ν_{max}^{KBr} cm^{-1}): 1710, 1714 (C=O), 980, 920, 900, 860 (920 < 900). EIMS (probe) 70 eV, *m/z*: 442.2917 ($[M]^+$, calc. for $C_{27}H_{38}O_5$, 442.2719) (21), 414 [M−CO] (8), 383 $[C_{24}H_{31}O_4^+]$ (12), 370 $[C_{23}H_{30}H_4]$ (28), 355 $[C_{24}H_{31}O_4^+ - CO]$ (15), 328 $[C_{21}H_{28}O_3]$ (27), 299 $[C_{19}H_{23}O_3]$ (6), 285 $[C_{19}H_{25}O_2]$ (33), 139 (100), 126 (39), 115 (77). ^1H NMR: Table 1; ^{13}C NMR: Table 2.

25R-*Spirostan*-4-*ene*-3,6,12-*trione* (**7**). White powder. mp 264–266°. UV: λ_{max}^{MeOH} 240.4 nm. IR (ν_{max}^{KBr} cm^{-1}): 1700, 1710 (C=O), 1620 (C=C), 980, 920, 900, 862 (920 < 900). EIMS (probe) 70 eV, *m/z*: 440.2571 ($[M]^+$, calc. for $C_{27}H_{36}O_5$, 440.2563) (77), 412 [M−CO] (20), 398 [M−42] (4), 381 $[C_{24}H_{29}O_4^+]$ (31), 368 $[C_{23}H_{28}O_4]$ (82), 353 $[C_{24}H_{29}O_4^+ - CO]$ (36), 326 $[C_{21}H_{26}O_3]$ (100), 312 $[C_{24}H_{29}O_4^+ - C_4H_5O^+]$ (34), 297 $[C_{19}H_{21}O_3]$ (15), 283 $[C_{19}H_{23}O_2]$ (41), 139 (63), 126 (11), 115 (47), 69 $[C_4H_5O^+]$ (19). ^1H NMR: Table 1; ^{13}C NMR: Table 2.

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