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Steroids from the Roots of *Asparagus officinalis* and Their Cytotoxic Activity

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Abstract

One new (Sarsasapogenin O) and seven known steroids were isolated from the roots of *Asparagus officinalis* L. Their structures were elucidated on the basis of spectroscopic analysis, including various 2D-NMR techniques, hydrolysis, and by comparison of spectral data of known compounds. These compounds together with nine steroids which were previously isolated from this plant, were tested for cytotoxic activity. Among them, eight compounds displayed significant cytotoxicities against human A2780, HO-8910, Eca-109, MGC-803, CNE, LTEP-a-2, KB and mouse L1210 tumor cells.

Key words: *Asparagus officinalis*; cytotoxic activity; Sarsasapogenin O; steroid.

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Asparagus officinalis L. is a kind of liliaceous perennial plant, the young shoots of which are a popular vegetable used in salads, vegetable dishes, and soups in the world. It has also been used as a traditional herbal medicine in both European and Asian countries; its dried roots are used as an approved medicinal product in the treatment of inflammatory diseases of the urinary tract for prevention of kidney stones in Europe, and as a tonic, antifebrile, anticancer, and antitussive drug in China for a long time (Zhong Hua Ben Cao Editorial Board 1999).

Pharmacological studies on this plant have demonstrated anti-inflammatory (Jang et al. 2004), cytotoxic (Shao et al. 1997), antimutagenic (Tang and Gao 2001) and antifungal activities (Shimoyamada et al. 1990). A considerable number of chemical constituents, including steroidal saponins (Lazur′evskii and Goryano 1976; Pant et al. 1988; Shimoyamada et al. 1990; Shao et al. 1997; Yamamori et al. 2002), flavonoids (Kartnig et al. 1985), oligosaccharides (Fukushi et al. 2000), and amino acid derivatives (Kasai and Sakamura 1981) have been isolated from its roots and shoots. Steroidal saponins were shown to be the major components and suggested to be responsible for the biological activities of this plant (Shimoyamada et al. 1990; Shao et al. 1997).

The present study undertook the isolation and structural elucidation of one new steroidal saponin, namely Sarsasapogenin O (**1,** Figure 1), along with seven known steroids, asparagoside A (**2**) (Tori et al. 1981), (25*R*)-5β-spirostan-3β-ol 3-*O*-β-D-glucopyranoside (**3**) (Seo et al. 1978), sarsasapogenin (**4**) (Tori et al. 1981), sarsasapogenone (**5**) (Loader et al. 2003), (25*S*)-neospirost-4-en-3-one (**6**) (Achenbach et al. 1996), 25*S*-spirosta-1, 4-dien-3-one (**7**) (Kimura et al. 1967), and stigmasterol (**8**) from the roots of *A. officinalis*. The structures of steroids were elucidated on the basis of spectral and chemical methods. Furthermore, these isolated compounds and nine steroidal saponins (**9–17**), which were also isolated from this plant in a previous study (Huang and Kong 2006) have been evaluated for their cytotoxic activity.

Results and Discussion

Compound **1** was obtained as a white amorphous that showed positive reaction in the Liebermann-Burchard and Molish test. Its IR spectrum showed strong absorptions for hydroxy groups (3 354 and 1 070 cm^{-1}). The molecular formula of **1** was determined as $C_{41}H_{66}O_{14}$ on the high-resolution ESI-MS (negative-ion mode), which gave the quasi-molecular ion peak at *m/z* 781.437 0 $[M-H]^-$ (calcd. 781.437 9 $C_{41}H_{65}O_{14}$).

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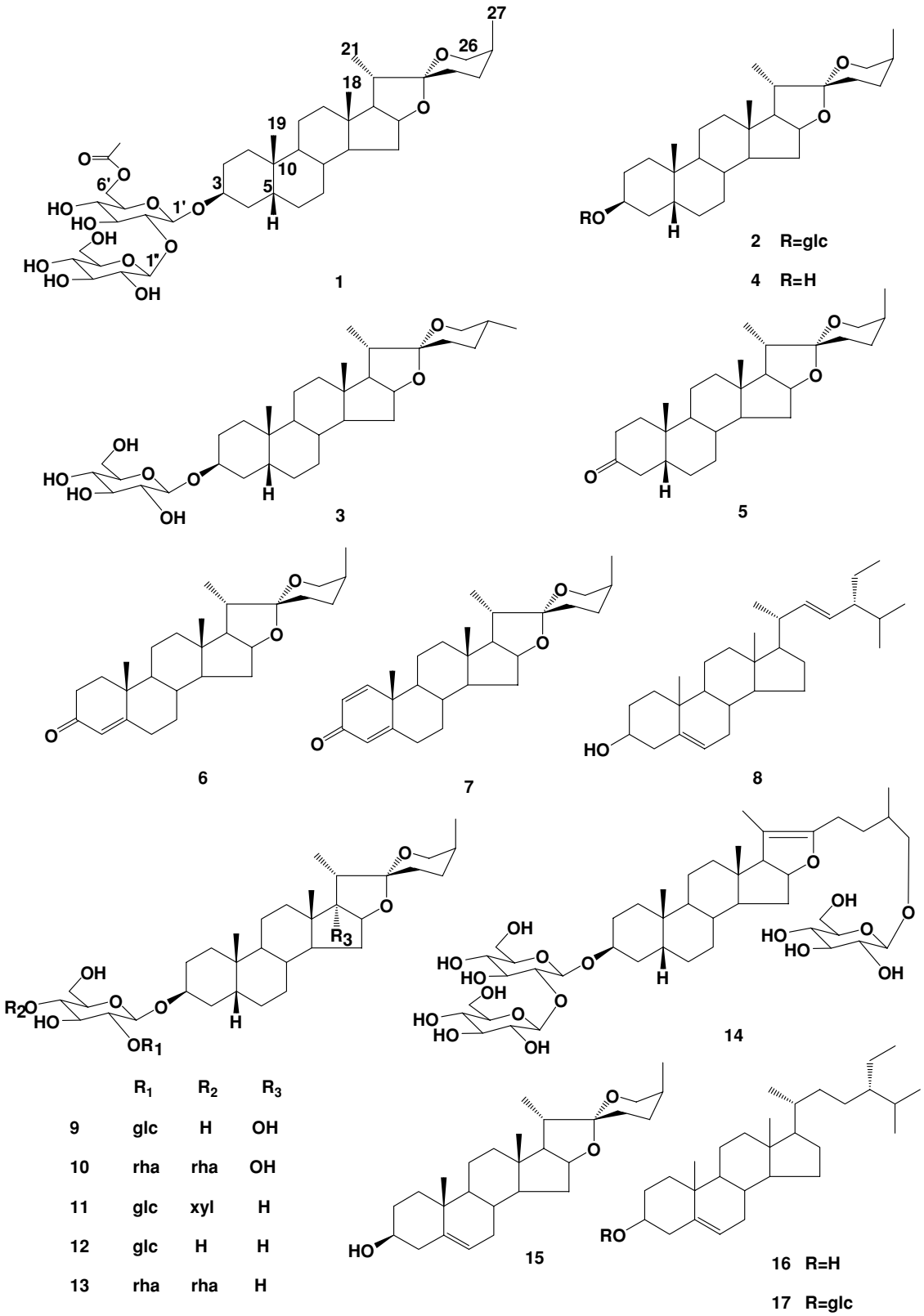

Figure 1. Structures of compounds **1–17.**

In the ^{1}H NMR spectrum of **1**, two tertiary methyl groups at δ 0.78 (s) and 1.00 (s) assignable to the C-18 and C-19 methyl groups, two secondary methyl groups at δ 0.98 (d, $J = 7.0$ Hz) and 1.06 (d, $J = 7.1$ Hz) ascribable to the C-27 and C-21 methyl groups, and two typical proton signals of H-26 [δ 3.26 (d, $J = 11.0$ Hz), 3.92 (dd, $J = 11.0$, 2.5 Hz)] in the spirostanol were observed, which might indicate that the absolute configuration of C-25 was *S* type (Boll and Philipsborn 1965). Comparisons of ^{13}C NMR spectrum with that of asparanin A (Zhang et al. 2004) indicate that they share the same aglycone. The 5 β configuration was further confirmed by the chemical shift of C-19 (δ 24.96) (Agrawal et al. 1985).

Compound **1** showed a quasi-molecular ion peak due to $[M-H]^-$ at *m/z* 781 and two fragment ion peaks due to $[M-CH_3CO]^-$ and $[M-CH_3CO\text{-glc}]^-$ at *m/z* 739 and 577 in the negative ESI-MS. Furthermore, signals for two anomeric protons at δ 4.64 ($J = 7.5$ Hz) and 4.43 ($J = 7.8$ Hz) could be readily assigned. Acid hydrolysis of **1** with 1M hydrochloric acid in dioxane-H_2O (1:1) led to the production of aglycone sarsasapogenin, which was identified by TLC with authentic samples and comparison of the ^{13}C NMR chemical shift from published reports (Tori et al. 1981). The monosaccharides obtained after hydrolysis of **1** were conformed only as D-glucose, the identity of which was confirmed by optical rotations and TLC comparison with authentic samples. The ^{1}H NMR and ^{13}C NMR data (Table 1) of **1** exhibited that **1** contained two glucose residue. The configurations of the anomeric positions of two glucoses were assigned as β by judging from their large coupling constants between H-1 and H-2 of the sugar ring protons ($J_{1',2'} = 7.5$ Hz, $J_{1'',2''} = 7.8$ Hz, respectively). The linkage of the sugars was deduced from the ^3J-correlations between the protons and respective related carbons, H-1′ (δ 4.43) with C-3 (δ 76.73), H-1′′ (δ 4.64) with C-2′ (δ 81.50), and H-6′ (δ 4.32, 4.18) with C=O (δ 173.25) in the HMBC spectrum. All of the proton and carbon signals were fully assigned by TOCSY, HSQC, and HMBC experiments (Table 1). In conclusion, the structure of **1** was elucidated as (25*S*)- 5 β-spirostan-3β-ol 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-6-*O*-acetylglucopyranoside, named Sarsasapogenin O.

All of the compounds were evaluated for their *in vitro* cytotoxic activity against a variety of types of cancer cell lines. Some of them showed moderate to significant cytotoxic activity. As shown in Table 2, compounds **1**, **11,** and **12** showed significant inhibitory effects on the proliferation of tumor cells. Compound **1** inhibited the proliferation of the CNE cell line with an IC_{50} value of 5.50 μM. When a mouse L1210 cell was treated with compound **11**, the IC_{50} value was 1.46 μM. Compound **12** showed great inhibitory effects on Eca-109, MGC-803, L1210, LTEP-a-2 and the KB cell line with IC_{50} values of 4.03, 3.72, 4.32, 3.16 and 1.38 μM, respectively. The results also suggested that compounds **2**, **3**, **4**, **6**, and **13** showed moderate cytotoxic activity on tumor cells.

Table 1. NMR data of compound **1** (in CD_3OD, *J* in Hz) [a]

No.	δ_H	δ_C	HMBC
1	1.84 (1H, m) 1.47 (1H, m)	31.84	C-2,3,10,19
2	1.91 (1H, m) 1.40 (1H, m)	27.71	C-10
3	3.18 (1H, m)	76.73	C-1,4,1′
4	1.52 (1H, m) 1.47 (1H, m)	31.95	
5	2.17 (1H, m)	38.20	C-10
6	1.90 (1H, m) 1.17 (1H, m)	28.17	C-5
7	1.18 (1H, m) 1.05 (1H, m)	28.24	C-6
8	1.61 (1H, m)	37.20	
9	1.41 (1H, m)	41.92	
10		36.65	
11	1.42 (1H, m) 1.06 (1H, m)	22.56	C-9,12
12	1.74 (1H, m) 1.30 (1H, m)	41.92	C-9,11
13		42.33	
14	1.21 (1H, m)	58.16	C-18
15	1.96 (1H, m) 1.23 (1H, m)	33.19	C-14,16
16	4.38 (1H, m)	82.59	
17		64.21	
18	0.78 (3H, s)	17.44	C-12,13,17
19	1.00 (3H, s)	24.96	C-1,5,9,10
20	1.84 (1H, m)	43.96	C-13,21
21	1.06 (3H, d, *J*=7.1)	15.21	C-17,20
22		111.57	
23	1.92 (1H, m) 1.43 (1H, m)	27.49	C-20
24	2.02 (1H, m) 1.31 (1H, m)	27.26	C-23
25	1.67 (1H, m)	29.02	C-27
26	3.92 (1H, dd, $J = 11$, 2.5)	66.61	C-22
	3.26 (1H, d, $J = 11$)		
27	0.98 (3H, d, $J = 7.0$)	16.90	C-25,26
Glc			
1′	4.43 (1H, d, $J = 7.5$)	101.93	
2′	3.53 (1H, m)	81.51	C-3′
3′	4.02 (1H, m)	77.50	
4′	3.23(1H, m)	72.09	C-6′
5′	3.54 (1H, m)	78.60	C-3′
6′	4.32 (1H, m) 4.18 (1H, m)	65.22	C=O
Glc′			
1′′	4.64 (1H, d, $J = 7.8$)	105.04	C-2′
2′′	3.42 (1H, m)	75.59	
3′′	3.36 (1H, m)	78.37	
4′′	3.27 (1H, m)	72.02	C-3′′
5′′	3.23 (1H, m)	79.04	C-1′′,6′′
6′′	3.82 (1H, m) 3.66 (1H, m)	63.71	
C-CH_3	2.02 (3H, s)	21.20	C=O
C=O		173.25	

[a]Experiments were run at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Assignments are based on COSY, HMQC, HMBC, and NOESY experiments.

Table 2. *In vitro* cytotoxic activity (IC_{50}, in μM) of compounds from roots of *Asparagus officinalis*

Compound	Ovarian[a]		Esophageal[a]	Gastric[a]	Nasopharyngeal[a]	Lung[a]	Nasophyrnx	Leukemia[b]
	A2780	HO-8910	Eca-109	MGC-803	CNE	LTEP-a-2	KB	L1210
1	10.57	–	–	8.39	5.50	–	–	–
2	10.22	5.04	–	–	–	–	–	–
3	–	24.83	–	–	–	–	–	12.33
4	6.09	–	–	–	–	–	–	–
6	18.85	–	–	–	–	–	–	–
11	–	–	2.91	–	–	2.85	2.66	1.46
12	–	–	4.03	3.72	–	3.16	1.38	4.32
13	–	–	10.15	–	12.88	–	–	–
Doxorubicin	0.15	1.45	0.36	0.72	0.58	1.33	0.43	0.26

[a]Human carcinoma; [b]Mouse carcinoma; –, ≥ 50 μM.

Experimental

General experimental procedures

Optical rotations were measured on a JASCO P-1020 polarimeter. IR spectra were obtained on a Shimadzu ftir-8 400 s spectrometer. One- and two-dimensional ^{1}H and ^{13}C NMR spectra were conducted on Bruker DRX-500 MHz spectrometers and tetramethylsilane (TMS) as the internal standard. HR-ESI-MS and ESI-MS were respectively measured on Micro-Q-TOF and Agilent 1100-LC/MSD Trap mass spectrometers. Column chromatography was carried out over silica gel (200–300 mesh, Qingdao Marine Chemistry Ltd., China), RP-C_{18} (40–63 μm, Waters, MA, USA), and Sephadex LH-20 (20–100 μm, Pharmacia, Oslo, Norway). Thin layer chromatography (TLC) was carried out on plates precoated with Merck RP-C_{18} and silica gel HF_{254} (Qingdao Marine Chemistry Ltd.). All solvents used for chromatographic separations were purchased from Jiangsu Hanbom Science & Technology Co. Ltd. and were distilled before use.

Plant materials

The fresh roots of *Asparagus officinalis* L. were collected from Lishui County, Jiangsu Province, China, in November 2003 and authenticated by Professor M. J. Qin, Department of Medicinal Plants, China Pharmaceutical University. A voucher specimen (KH20031101) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Cell lines

Seven human tumor cell lines, A2780, HO-8910, Eca-109, MGC-803, CNE, LTEP-a-2, KB and a mouse L1210 cell line were used, obtained from the Cell Bank of the Shanghai Institute of Cell Biology. All cells were maintained in Eagle's minimal essential medium (MEM) with 5% of fetal bovine serum (FBS), gentamicin 50 μg/mL. Cultures were held in 75 cm^2 culture flasks at 37 °C, 5% CO_2 and 100% relative humidity, changing media at least twice a week.

Extraction and isolation

The dried and powdered roots of *A. officinalis* (12 kg) were extracted with 80% EtOH (3 × 30 L) for 3 h at 60 °C. The EtOH extract (710 g) was concentrated under reduced pressure, dissolved in water, and successively extracted with petroleum ether (60–90 °C), EtOAc, and *n*-BuOH. Solvent was removed to give the petroleum ether extracts (58 g), EtOAc extracts (67 g), and *n*-BuOH extracts (210 g). The *n*-BuOH fraction was repeatedly column chromatographed on silica gel with gradient elution by $CHCl_3$/MeOH (100:1–0:100) to afford seven fractions 1 (5 g), 2 (7 g), 3 (8 g), 4 (18 g), 5 (35 g), 6 (32 g), and 7 (42 g). Fraction 2 was chromatographed on silica gel eluting with $CHCl_3$/MeOH/H_2O (10:2:0.2) and ODS column with MeOH/H_2O (3:2; 5:2) to yield compounds **1** (16 mg), **2** (42 mg), and **3** (63 mg). The petroleum ether fraction was repeatedly column chromatographed on silica gel with gradient elution by petroleum ether (60–90 °C)/EtOAc (100:1–100:50) to afford five fractions 1 (6 g), 2 (4 g), 3 (5 g), 4 (8 g), and 5 (6 g). Fraction 3 was combined and subjected to silica gel column chromatography elution with petroleum ether/EtOAc (10:2) to afford a number of sub-fractions F_1–F_4. Compounds **4** (53 mg), **5** (12 mg), and **6** (11 mg) were obtained by silica gel column chromatography of sub-fractions F_2 elution with petroleum ether/Me_2CO (10:2) and further purification by Sephadex LH-20 ($CHCl_3$/MeOH = 1:1). Similarly, compounds **7** (10 mg) and **8** (230 mg) were obtained by silica gel column chromatography of sub-fractions F_3 elution with petroleum ether/Me_2CO (10:3) and Sephadex LH-20 ($CHCl_3$/MeOH = 1:1).

Identification

(25*S*)-5β-spirostan-3β-ol 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-6-*O*-acetylglucopyranoside (1)

White amorphous powder, $C_{41}H_{64}O_{14}$, $[\alpha]^{22}_{D}$ −53.48° (*c* 0.11, MeOH). IR (KBr) υ_{max} 3 354, 2 949, 2 930, 1 616, 1 450, 1 382,

1 070, 1 009, 984, 910, 891, and 851 cm^{-1}; ESI-MS *m/z*: 781 $[M-H]^-$, 739 $[M-CH_3CO]^-$, 577 $[M-CH_3CO-C_6H_{10}O_5]^-$; HR-ESI-MS *m/z*: 781.437 0 (calcd for $C_{41}H_{65}O_{14}$ $[M-H]^-$: 781.437 9); For ^1H and ^{13}C NMR, see Table 1.

Acid hydrolysis of Compound 1

Compound **1** (10 mg) was heated in 5% H_2SO_4 in dioxane/H_2O 1:1 (5 mL) at 90 °C for 5 h. After evaporation of the dioxane, the aqueous phase was extracted with CH_2Cl_2 (3×5 mL), the extract washed with H_2O, dried (Na_2SO_4), and evaporated, and the residue purified by column chromatographed (silica gel $CHCl_3$/MeOH = 20:1) to afford aglycon part (3 mg) which is identified using authentic samples by TLC (*n*-hexane/Me_2CO = 5:1; Rf = 0.58) as sarsasapogenin. The acid aqueous layer was neutralized with $BaCO_3$ and freeze-dried. Glucose was identified with authentic samples by TLC (*n*-BuOH/Me_2CO/H_2O = 4:5:1). The sugar was separated by preparative TLC ($CHCl_3$/MeOH/H_2O = 10:5:1): D-glucose (2.5 mg; $[\alpha]_D^{22} + 13.4°$(*c* 0.11, H_2O)).

Asparagoside A (2)

White amorphous powder. IR (KBr) υ_{max}: 3 350, 2 957, 2 930, 2 878, 1 618, 1 448, 1 175, 1 074, 1 010, 914, 889, 850 cm^{-1}; ESI-MS *m/z*: 579 $[M+H]^+$, 435, 255; ^1H NMR (500 MHz, pyridine-d_5) δ: 4.92 (1H, d, J = 7.7 Hz, H-1′), 3.94 (1H, m, H-26a), 3.34 (1H, d, J = 10.8 Hz, H-26b), 1.13 (3H, d, J = 6.9 Hz, H-21), 1.05 (3H, d, J = 7.0 Hz, H-27), 0.82 (3H, s, H-19), 0.80 (3H, s, H-18); ^{13}C NMR (125 MHz, pyridine-d_5) δ: 31.01 (C-1), 27.58 (C-2), 74.35 (C-3), 30.52 (C-4), 37.02 (C-5), 26.79 (C-6), 27.05 (C-7), 35.59 (C-8), 40.30 (C-9), 35.27 (C-10), 21.20 (C-11), 40.35 (C-12), 40.93 (C-13), 56.51 (C-14), 32.18 (C-15), 81.36 (C-16), 63.00 (C-17), 16.62 (C-18), 23.90 (C-19), 42.53 (C-20), 14.91 (C-21), 109.72 (C-22), 26.45 (C-23), 26.24 (C-24), 27.58 (C-25), 65.14 (C-26), 16.31 (C-27), 103.14 (C-1′), 75.43 (C-2′), 78.44 (C-3′), 71.89 (C-4′), 78.78 (C-5′), 62.98 (C-6′).

(25*R*)-5β-spirostan-3β-ol 3-*O*-β-D-glucopyranoside (3)

White amorphous powder. IR (KBr) υ_{max}: 3 414, 2 928, 1 693, 1 645, 1 452, 1 375, 1 076, 1 034, 901, 851 cm^{-1}; ESI-MS *m/z*: 579 $[M+H]^+$, 435, 255; ^1H NMR (500 MHz, CD_3OD) δ: 4.38 (1H, m, H-16), 4.30 (1H, d, J = 7.8 Hz, H-1′), 4.05 (1H, brs, H-3), 1.08 (3H, d, J = 7.1 Hz, H-21), 0.98 (3H, d, J = 6.9 Hz, H-27), 0.97 (3H, s, H-19), 0.78 (3H, s, H-18); ^{13}C NMR (125 MHz, CD_3OD) δ: 29.02 (C-1), 26.80 (C-2), 75.73 (C-3), 33.19 (C-4), 38.37 (C-5), 27.26 (C-6), 27.49 (C-7), 37.27 (C-8), 41.91 (C-9), 36.63 (C-10), 22.53 (C-11), 41.91 (C-12), 42.33 (C-13), 58.16 (C-14), 32.01 (C-15), 82.95 (C-16), 64.23 (C-17), 17.43 (C-18), 24.71 (C-19), 43.95 (C-20), 15.21 (C-21), 111.56 (C-22), 31.54 (C-23), 28.20 (C-24), 28.26 (C-25), 66.60 (C-26), 16.87 (C-27), 103.20 (C-1′), 76.13 (C-2′), 78.77 (C-3′), 72.29 (C-4′), 78.34 (C-5′), 63.36 (C-6′).

Sarsasapogenin (4)

White amorphous powder. IR (KBr) υ_{max}: 3 473, 2 931, 2 864, 1 693, 1 452, 1 383, 1 039, 989, 918, 896, 848 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: 4.41 (1H, q, J = 7.7 Hz, H-17), 4.10 (1H, m, H-3), 3.95 (1H, dd, J = 11.0, 2.7 Hz, H-26), 3.29 (1H, d, J = 11.0 Hz, H-26), 1.08 (3H, d, J = 7.1 Hz, H-21), 0.99 (3H, d, J = 6.9 Hz, H-27), 0.98 (3H, s, H-19), 0.76 (3H, s, H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 30.00 (C-1), 27.88 (C-2), 67.14 (C-3), 33.63 (C-4), 36.59 (C-5), 26.61 (C-6), 26.59 (C-7), 35.34 (C-8), 40.37 (C-9), 35.34 (C-10), 20.95 (C-11), 39.94 (C-12), 40.73 (C-13), 56.54 (C-14), 31.80 (C-15), 81.05 (C-16), 62.21 (C-17), 16.51 (C-18), 23.94 (C-19), 42.18 (C-20), 14.34 (C-21), 109.74 (C-22), 27.14 (C-23), 25.83 (C-24), 26.02 (C-25), 65.18 (C-26), 16.08 (C-27).

Sarsasapogenone (5)

White amorphous powder. ESI-MS *m/z*: 415 $[M+H]^+$, 271, 253; ^1H NMR (500 MHz, $CDCl_3$) δ: 4.42 (1H, q, J = 7.4 Hz, H-16), 3.95 (1H, dd, J = 11.0, 2.7 Hz, H-26a), 3.29 (1H, d, J = 11.0 Hz, H-26b), 2.68 (1H, dd, J = 14.6, 14.0 Hz, H-4α), 2.31 (1H, m, H-2α), 1.08 (3H, d, J = 7.1 Hz, H-21), 1.04 (3H, s, H-19), 1.00 (3H, d, J = 6.7 Hz, H-27), 0.79 (3H, s, H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 37.03 (C-1), 37.19 (C-2), 213.11 (C-3), 42.37 (C-4), 44.26 (C-5), 26.58 (C-6), 26.07 (C-7), 35.24 (C-8), 40.91 (C-9), 35.06 (C-10), 21.05 (C-11), 40.17 (C-12), 40.71 (C-13), 56.31 (C-14), 31.74 (C-15), 80.92 (C-16), 62.14 (C-17), 16.50 (C-18), 22.70 (C-19), 42.19 (C-20), 14.34 (C-21), 109.75 (C-22), 26.00 (C-23), 25.81 (C-24), 27.11 (C-25), 65.19 (C-26), 16.07 (C-27).

(25*S*)-neospirost-4-en-3-one (6)

White amorphous powder. Positive reaction to the Liebermann-Burchard test. ESI MS *m/z*: 413$[M+H]^+$; ^1H NMR (500 MHz, $CDCl_3$) δ: 5.73 (1H, s, H-4), 4.42 (1H, q, J = 7.5 Hz, H-16), 3.95 (1H, dd, J = 11.0, 2.7 Hz, H-26a), 3.30 (1H, d, J = 11.0 Hz, H-26b), 1.08 (3H, d, J = 7.0 Hz, H-21), 0.99 (3H, d, J = 6.6 Hz, H-27), 1.20 (3H, s, H-19), 0.82 (3H, s, H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 35.73 (C-1), 32.83 (C-2), 199.42 (C-3), 123.92 (C-4), 171.06 (C-5), 33.99 (C-6), 31.71 (C-7), 35.27 (C-8), 53.81 (C-9), 38.69 (C-10), 20.87 (C-11), 39.71 (C-12), 40.38 (C-13), 55.70 (C-14), 29.71 (C-15), 80.73 (C-16), 61.95 (C-17), 16.37 (C-18), 17.33 (C-19), 42.21 (C-20), 14.31 (C-21), 109.75 (C-22), 25.82 (C-23), 25.99 (C-24), 27.10 (C-25), 65.18 (C-26), 16.07 (C-27).

25*S* -spirosta-1, 4-dien-3-one (7)

White amorphous powder. Positive reaction in the Liebermann-Burchard test. ESI MS *m/z*: 411 $[M+H]^+$, 393; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.04 (1H, d, J = 10.2 Hz, H-2), 6.22 (1H, dd, J = 10.2, 2.4 Hz, H-1), 6.08 (1H, s, H-4), 4.41 (1H, q, J = 6.8 Hz, H-16), 3.92 (1H, *dd*, J = 11.0, 2.7 Hz, H-26a), 3.30 (1H, *d*, J = 11.0 Hz, H-26b), 1.08 (3H, d, J = 7.1 Hz, H-21), 1.00 (3H, d, J = 6.9 Hz, H-27), 0.89 (3H, s, H-19), 0.85 (3H, s, H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 155.68 (C-1), 127.59 (C-2), 186.31 (C-3), 123.95 (C-4), 168.94 (C-5), 33.79 (C-6),

32.83 (C-7), 35.25 (C-8), 52.48 (C-9), 39.58 (C-10), 22.67 (C-11), 40.68 (C-12), 42.23 (C-13), 55.30 (C-14), 31.93 (C-15), 80.23 (C-16), 61.95 (C-17), 16.46 (C-18), 18.79 (C-19), 43.61 (C-20), 14.29 (C-21), 109.75 (C-22), 26.00 (C-23), 27.10 (C-24), 29.46 (C-25), 65.20 (C-26), 16.03 (C-27).

Stigmasterol (8)

White amorphous solid, mp 171–172 °C, positive reaction in the Liebermann- Burchard test; TLC: solvent (hexane/EtOAc = 10:1), Rf = 0.52, the same with authentic sample of stigmasterol.

Cytotoxic activity assay

Cytotoxic activities against human and mouse tumor cell lines of these compounds and another nine compounds [sarsasapogenin M (**9**), sarsasapogenin N (**10**), (25*S*)-5β-spirostan-3β-ol 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranoside (**11**), (25*S*)-5β-spirostan-3β-ol 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**12**), (25*S*)-5β-spirostan-3β-ol 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside (**13**), (25*S*)-26-*O*-β-D-glucopyranosyl-5β-furost-20(22)-ene-3β,26-diol 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**14**), yamogenin (**15**), β-sitosterol (**16**), and sitosterol-β-D-glucoside (**17**)], which were isolated from this plant in a previous study (Huang and Kong 2006), were tested using the microtitration colorimetric method of MTT reduction. The basic protocol described by Mossmann was used with some modifications (Mossmann 1983). Compounds were dissolved in dimethylsulphoxide (DMSO) and diluted in culture media prior to the application. Six dilutions of each compound were tested with an incubation period of 48 h. Cytotoxicity is reported as IC_{50} because the recuperation period after exposition was not used (Freshney 2000). Doxorubicin HCl was used as a positive control and the vehicle (DMSO + MEM) as a negative control.

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