Filiasparosides A−D, Cytotoxic Steroidal Saponins from the Roots of *Asparagus filicinus*

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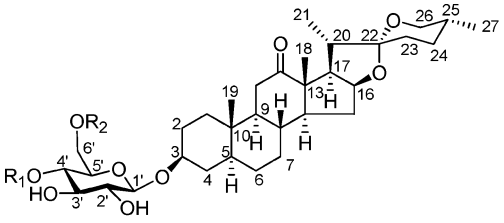
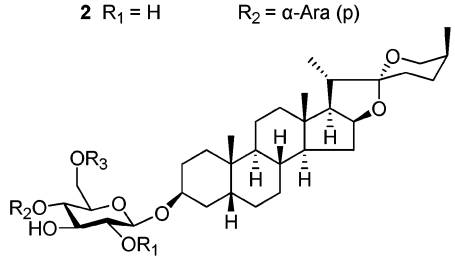
Four new steroidal saponins, filiasparosides A−D (**1**−**4**), together with known aspafiliosides A (**5**) and B (**6**) were isolated from the roots of *Asparagus filicinus.* The structures of these new compounds were elucidated by detailed spectroscopic study and chemical analysis. Compounds **1**−**6** were cytotoxic against human lung carcinoma (A549) and breast adenocarcinoma (MCF-7) tumor cell lines with EC_{50} values of 2.3−16.8 μg/mL. Compound **3** showed the most potent cytotoxicity, with EC_{50} values of 2.3 and 3.0 μg/mL toward A549 and MCF-7 cell lines, respectively.

The dried root of *Asparagus filicinus* Buch.−Ham (Liliaceae) is used under the name "Xiao-Bai-Bu" as a Chinese herb for the treatment of cough, tracheitis, and pneumonia.[1] Some steroidal saponins were isolated from this plant previously,[2−6] but the bioactivity of these compounds was not reported. In our study of bioactive compounds from *Asparagus* medicinal plants, we found that the ethanol extract of the roots of *A. filicinus* exhibited cytotoxic activity. Further fractionation of this extract resulted in the isolation of four new steroidal saponins, filiasparosides A−D (**1**−**4**), and two known compounds, aspafiliosides A (**5**) and B (**6**).[2] In this paper, we present the isolation and structure elucidation of the new compounds, as well as *in vitro* cytotoxicity evaluation of all isolates against human lung carcinoma (A-549) and human breast adenocarcinoma (MCF-7) cell lines.



1 R_1 = β-Xyl (p) R_2 = α-Ara (p)
2 R_1 = H R_2 = α-Ara (p)



3	R_1 = β-Xyl (p)	R_2 = α-Rha (p)	R_3 = H
4	R_1 = H	R_2 = H	R_3 = α-Ara (p)
5	R_1 = H	R_2 = β-Xyl (p)	R_3 = H
6	R_1 = H	R_2 = β-Xyl (p)	R_3 = α-Ara (p)

Results and Discussion

Filiasparoside A (**1**), obtained as a white, amorphous solid, was assigned the molecular formula $C_{43}H_{68}O_{17}$ on the basis of HRESIMS (*m*/*z* 879.4360, $[M + Na]^+$). The IR spectrum exhibited absorptions for hydroxyl groups (3449, 1039 cm^{-1}) and strong absorption bands at 981, 919, 898, and 857 cm^{-1} characteristic of spirostane-type steroidal sapogenins. The weaker intensity of the band at 919 compared with that at 898 cm^{-1} showed that **1** belonged to the 25*R* series of spirostanes.[7] The ^1H NMR spectrum of **1** showed four methyl proton signals at δ_H 1.09 (s, Me-18), 0.71 (s, Me-19), 1.38 (d, J = 6.6 Hz, Me-21), and 0.69 (s, Me-27). A signal at δ_C 212.7 in the ^{13}C NMR spectrum implied the existence of a carbonyl carbon. The HMBC correlations of the proton signals at H_2-11 (δ_H 2.29, 2.40) and CH_3-18 (δ_H 1.09) with the carbon signal at δ_C 212.7 indicated that the carbonyl was present at C-12 (see Figure 1). Acid hydrolysis of **1** yielded an aglycone (**1a**) that was identified as hecogenin by comparing its ^{13}C NMR data with literature values.[8]

Fragment ions at *m*/*z* 725 $[M + H - 132]^+$, 593 $[M + H - 132 - 132]^+$, and 431 $[M + H - 132 - 132 - 162]^+$ in the ESIMS spectrum showed that there were three sugar units in **1**. Anomeric proton signals of three sugar units were observed in the ^1H NMR spectrum at δ_H 4.94 (d, J = 7.4 Hz), 5.10 (d, J = 7.4 Hz), and 5.56 (d, J = 7.8 Hz), with three corresponding anomeric carbons at δ_C 102.1, 105.7, and 105.1 in the ^{13}C NMR spectrum. Acid hydrolysis of **1** yielded glucose, xylose, and arabinose, identified by TLC and GC analysis. The ^1H NMR coupling constants ($^3J_{1,2}$ > 7 Hz) were consistent with a β-configuration for the glucose and xylose and an α-configuration for the arabinose. The sequences of the sugar chains were determined by analysis of 2D NMR spectroscopic data. Starting from the anomeric proton signal at δ_H 4.94, the proton resonances at δ_H 3.89 (1 H, m), 4.26 (1 H, m), 4.51 (1 H, m), 4.02 (1 H, m), 4.76 (1 H, dd, J = 3.1, 10.8 Hz), and 4.86 (1 H, d, J = 10.8 Hz) were assigned to the glucose H-2, H-3, H-4, H-5, and H_2-6, respectively, on the basis of the $^1H-^1H$ COSY analysis. The carbon signals at δ_C 102.1, 74.8, 76.3, 79.8, 74.8, and 68.0 were assigned to the glucose C-1, C-2, C-3, C-4, C-5, and C-6, respectively, from the HSQC analysis. The proton and carbon signals of the arabinose and xylose moieties were fully assigned by the same method.

Long-range correlations between the following proton and carbon signals, H-1 (δ_H 4.94) of glucose and C-3 (δ_C 77.3) of the aglycone, H-1 (δ_H 5.56) of xylose and C-4 (δ_C 79.8) of glucose, H-1 (δ_H 5.10) of arabinose and C-6 (δ_C 68.0) of glucose, in the HMBC spectrum indicated that the trisaccharide moiety was attached to C-3 of the aglycone and that the xylose and arabinose were linked at C-4 and C-6 of the inner glucose, respectively (see Figure 1). In conclusion, the structure of **1** was determined as (25*R*)-3β-hydroxy-5α-spirostan-12-one-3-*O*-β-xylopyranosyl(1→4)-[α-arabinopyranosyl(1→6)]-β-glucopyranoside.

Filiasparoside B (**2**), obtained as a white, amorphous solid, was determined to have the molecular formula $C_{38}H_{60}O_{13}$ on the basis of HRESIMS (*m*/*z* 723.3961, $[M - H]^-$). Comparison of the ^1H and ^{13}C NMR spectroscopic data for the aglycone moieties of **2** and **1** suggested that they contain the same aglycone. The ^1H NMR

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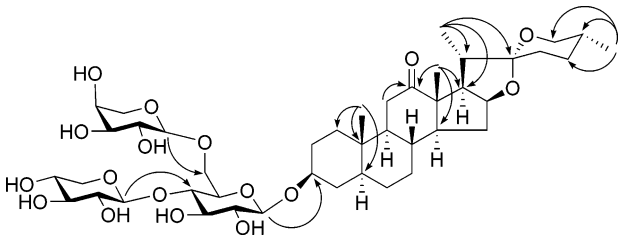

Figure 1. Key HMBC correlations of **1**.

and ^{13}C NMR spectra of **2** showed two anomeric protons at δ_H 4.95 (d, $J = 7.8$ Hz) and 4.96 (d, $J = 7.0$ Hz) and two anomeric carbons at δ_C 102.1 and 105.4, suggesting that there were only two sugar units in **2**. The differences in the ^1H and ^{13}C NMR spectra of **1** and **2** showed that **2** lacked the xylose unit found in **1**. ^3J-correlations were observed between the arabinose H-1 (δ_H 4.96) and the glucose C-6 (δ_C 69.5) as well as the glucose H-1 (δ_H 4.95) and the aglycone C-3 (δ_C 76.8) in the HMBC spectrum. These findings indicated that the terminal arabinose was linked at C-6 of the inner glucose and the sugar chain was attached to C-3 of the aglycone. The carbon and proton signals of **2** were fully assigned by $^1H-^1H$ COSY, TOCSY, HSQC, and HMBC experiments. In conclusion, the structure of **2** was elucidated as (25*R*)-3β-hydroxy-5α-spirostan-12-one-3-*O*-α-arabinopyranosyl(1→6)-β-glucopyranoside.

Filiasparoside C (**3**) was obtained as a white, amorphous solid. Its molecular formula was determined as $C_{44}H_{72}O_{16}$ on the basis of HRESIMS data (*m*/*z* 879.4722, $[M + Na]^+$). The IR spectrum of **3** exhibited absorptions for hydroxyl groups (3442, 1449 cm^{-1}) and strong absorption bands at 984, 914, 890, and 865 cm^{-1} characteristic of a spirostane-type steroidal sapogenin. However, unlike **1**, the weaker intensity of the band at 890 than 914 cm^{-1} showed that **3** belonged to the 25*S* series of spirostanes.[7] The ^1H NMR spectrum of **3** showed four methyl proton signals at δ_H 0.78 (s, Me-18), 1.06 (s, Me-19), 1.04 (d, $J = 7.0$ Hz, Me-27), and 1.12 (d, $J = 6.6$ Hz, Me-21) and two typical methylene proton signals at δ_H 3.34 (d, $J = 11.3$ Hz, 26-Ha) and 4.04 (26-Hb) in the spirostanol. Acid hydrolysis of **3** yielded the aglycone (**3a**) that was identified as sarsasapogenin by comparison of its ^{13}C NMR data with those in the literature.[8]

Fragment ions at *m*/*z* 725 $[M + H - 132]^+$, 579 $[M + H - 132 - 146]^+$, and 417 $[M + H - 132 - 146 - 162]^+$ in the ESIMS spectrum suggested that there were three sugar units in **3**. The ^1H NMR spectrum displayed three anomeric protons at δ_H 4.82 (d, $J = 7.2$ Hz), 5.02 (d, $J = 7.5$ Hz), and 6.38 (s), with corresponding carbon signals at δ_C 101.8, 105.6, and 101.5, respectively, confirming that the sugar moiety of **3** consists of three units. Complete acid hydrolysis of **3** yielded glucose, xylose, and rhamnose, identified by TLC and GC analysis. ^1H NMR coupling constants ($^3J_{1,2} > 7$ Hz) for anomeric protons indicated that the anomeric carbon configurations were β for the xylose and glucose moieties. An α-configuration for rhamnose was deduced from the C-5 signal of rhamnose at δ_C 69.4.[9] The combined use of $^1H-^1H$ COSY, TOCSY, HSQC, and HMBC experiments allowed the sequential assignments of all resonances for each monosaccharide. Starting from the anomeric proton signal at δ_H 4.82, the proton resonances at δ_H 4.20 (1 H, m), 4.22 (1 H, m), 4.25 (1 H, m), 3.78 (1 H, m), 4.40 (1 H, dd, $J = 3.1$, 12.4 Hz), and 4.50 (1 H, dd, $J = 3.7$, 12.4 Hz) were assigned to the glucose H-2, H-3, H-4, H-5, and H_2-6, respectively, on the basis of $^1H-^1H$ COSY analysis. The carbon signals at δ_C 101.8, 81.5, 77.5, 76.7, 76.1, and 61.6 were assigned to the glucose C-1, C-2, C-3, C-4, C-5, and C-6, respectively, from the HSQC analysis. The proton and carbon signals of xylose and rhamnose were fully assigned by the same method.

The glucose C-2 and C-4 resonances were downfield shifted to δ_C 81.5 and 76.7 in **3** from δ_C 74.2 and 70.8 in 1-OCH_3-glucose,[10]

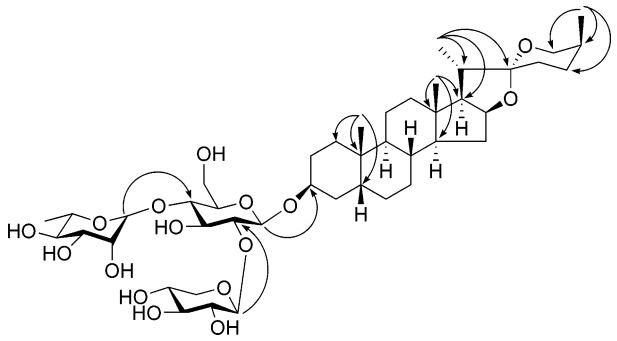

Figure 2. Key HMBC correlations of **3**.

showing that the terminal xylose and rhamnose were linked at the C-2 and C-4 positions of the inner glucose. ^3J-correlations between H-1 (δ_H 5.02) of xylose and C-2 (δ_C 81.5) of glucose and between H-1 (δ_H 6.38) of rhamnose and C-4 (δ_C 76.7) of glucose in the HMBC spectrum also supported the above observations. In the HMBC spectrum, long-range correlation between the glucose H-1 (δ_H 4.82) and the aglycone C-3 (δ_C 75.8) indicated that the sugar chain was attached to C-3 of the aglycone (see Figure 2). On the basis of the observed data, the structure of **3** was established as (25*S*)-5β-spirostan-3β-hydroxy-3-*O*-β-xylopyranosyl(1→2)-[α-rhamnopyranosyl(1→4)]-β-glucopyranoside.

Filiasparoside D (**4**), obtained as a white, amorphous solid, has the molecular formula $C_{38}H_{62}O_{12}$ from HRESIMS measurement (*m*/*z* 709.4167, $[M - H]^-$). Comparison of the ^1H NMR and ^{13}C NMR spectroscopic data showed that **4** and **3** possess the same aglycone. Two anomeric proton signals at δ_H 4.86 (d, $J = 7.8$ Hz) and 4.98 (d, $J = 7.4$ Hz) and two anomeric carbon signals at δ_C 102.9 and 105.3 were observed in the ^1H NMR and ^{13}C NMR spectra, respectively, suggesting that the sugar moiety of **4** consists of two units. The ^{13}C NMR data for the sugar moieties of **4** agreed well with those of **2**, so these two compounds should contain the same sugar moieties. As for **2**, ^3J-correlations [arabinose H-1 (δ_H 4.98) with glucose C-6 (δ_C 69.6); glucose H-1 (δ_H 4.86) with aglycone C-3 (δ_C 74.3)] in the HMBC spectrum of **4** indicated that the terminal arabinose was linked at C-6 of the inner glucose and the sugar chain was attached to C-3 of the aglycone. All carbon and proton signals of **4** were fully assigned by $^1H-^1H$ COSY, TOCSY, HSQC, and HMBC experiments. From the above data, the structure of **4** was elucidated as (25*S*)-5β-spirostan-3β-hydroxy-3-*O*-α-arabinopyranosyl(1→6)-β-glucopyranoside.

Compounds **5** and **6** were identified as aspafiliosides A and B by comparing their ^1H NMR and ^{13}C NMR data with reported data.[2] All isolates were evaluated for *in vitro* cytotoxicity against human lung carcinoma (A549) and breast adenocarcinoma (MCF-7) cell lines. The results are listed in Table 5. Compounds **1**−**6** showed varying degrees of cytotoxic activity against the two cell lines, except that **5** had no effect on the MCF-7 cell line ($EC_{50} > 20$ μg/mL). The most potent compound, **3**, exhibited significant cytotoxicity against A549 and MCF-7 cell lines with EC_{50} values of 2.3 and 3.0 μg/mL, respectively. Compound **4** was as potent against the A549 cell line (EC_{50} 2.4 μg/mL) but less potent against the MCF-7 cell line (EC_{50} 10.3 μg/mL).

Because compounds **3**−**6** have the same aglycone, differences in their cytotoxic activities likely are related to the numbers, identities, and linkages of the sugar moieties. Compound **3** is the only compound with an attached rhamnose unit or with a sugar at C-2 of the inner glucose. The aglycone unit also affects activity, as compounds **2** and **4** have the same sugar units and linkage positions, but the cytotoxic activity of **2** is weaker than that of **4**. The aglycones differ in the stereochemistries at C-5 and C-25 and in the presence of a carbonyl at C-12. This latter difference has been associated previously with decreased cytotoxicity, as discussed in the literature.[11]

Table 1. ^{1}H NMR (400 MHz) Data of the Aglycone Moieties of **1**−**4** (pyridine-d_5, δ in ppm, J in Hz)

position	1	2	3	4
1	0.82, 1.36, m	1.32, 0.78, m	1.46, 1.82, m	1.68, 1.76, m
2	1.65, 2.10, m	1.58, 2.07, m	1.49, 1.92, m	1.70, 1.98, m
3	3.92, m	3.95, m	4.22, m	4.38, m
4	1.43, 1.85, m	1.32, 1.79, m	1.50, 1.78, m	1.48, 1.70, m
5	0.90, m	0.82, m	2.14, m	1.98, m
6	1.13, m	1.10, m	1.28, 1.34, m	1.02, 1.05, m
7	1.67, 1.72, m	1.60, 1.68, m	0.97, 1.31, m	0.93, 1.22, m
8	1.76, m	1.71, m	1.53, m	1.45, m
9	0.92, m	0.84, m	1.02, m	1.02, m
10				
11	2.29, dd (5.2, 14.0)	2.20, dd (4.6, 14.1)	1.32, m	1.30, 1.26, m
	2.40, t (14.0)	2.34, t (14.1)		
12			1.29, m	1.24,1.64, m
13				
14	1.36, m	1.30, m	1.08, m	1.03, m
15	1.58, 2.13, m	1.52, 2.07, m	1.38, 2.01, m	1.38, 2.00, m
16	4.50, m	4.48, m	4.55, m	4.57, m
17	2.79, dd (1.6, 6.8)	2.74, dd (1.9, 7.0)	1.80, m	1.82, m
18	1.09, s	1.05, s	0.78, s	0.78, s
19	0.71, s	0.66, s	1.06, s	0.80, s
20	1.94, m	1.90, m	1.88, m	1.99, m
21	1.38, d (6.6)	1.30, d (7.0)	1.12, d (6.6)	1.14, d (7.0)
22				
23	0.78, 1.58, m	1.49, 1.58, m	1.38, 1.88, m	1.42, 1.88, m
24	1.58, m	1.54, m	1.43, 2.13, m	1.33, 2.12, m
25	1.59, m	1.55, m	1.57, m	1.56, m
26	3.50, t (10.4)	3.48, m	3.34, d (11.3)	3.36, d (9.3)
	3.60, dd (3.3, 10.4)	3.56, dd (2.6, 9.8)	4.04, m	4.04, m
27	0.69, s	0.65, s	1.04, d (7.0)	1.04, d (7.0)

Table 2. ^{13}C NMR (100 MHz) Data of the Aglycone Moieties of **1**−**4** (pyridine-d_5, δ in ppm)

position	1	2	3	4
1	36.5 t	36.4 t	30.7 t	30.4 t
2	29.7 t	29.7 t	27.4 t	26.8 t
3	77.3 d	76.8 d	75.8 d	74.3 d
4	34.6 t	34.5 t	30.8 t	30.9 t
5	44.3 d	44.1 d	37.0 d	36.8 d
6	28.5 t	28.4 t	26.7 t	26.8 t
7	31.7 t	31.6 t	26.6 t	26.6 t
8	34.3 d	34.1 d	35.4 d	35.4 d
9	55.3 d	55.2 d	40.2 d	40.1 d
10	36.2 s	36.1 s	35.2 s	35.1 s
11	37.9 t	37.8 t	21.0 t	21.0 t
12	212.7 s	212.6 s	40.2 t	40.1 t
13	55.4 s	55.2 s	40.7 s	40.7 s
14	55.8 d	55.7 d	56.4 d	56.3 d
15	31.4 t	31.3 t	32.0 t	32.0 t
16	79.6 d	79.5 d	81.2 d	81.2 d
17	54.2 d	54.1 d	62.8 d	62.8 d
18	16.1 q	15.9 q	16.5 q	16.5 q
19	11.7 q	11.5 q	23.7 q	23.7 q
20	42.6 d	42.5 d	42.3 d	42.3 d
21	13.9 q	13.8 q	14.8 q	14.8 q
22	109.3 s	109.2 s	109.5 s	109.6 s
23	31.6 t	31.5 t	26.2 t	26.2 t
24	29.2 t	29.1 t	26.0 t	26.1 t
25	30.5 d	30.4 d	27.5 d	27.4 d
26	66.9 t	66.8 t	64.9 t	64.9 t
27	17.3 q	17.2 q	16.1 q	16.1 q

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a JASCO P-1020 polarimeter at room temperature. IR spectra were recorded as KBr pellets on a 360 FT-IR Nicolet spectrophotometer. 1D and 2D NMR spectra were taken on a Bruker DRX-400 spectrometer in pyridine-d_5. Mass spectra were determined on a HP 5989A mass spectrometer for ESIMS and Q-T of a micro mass spectrometer for HRESIMS. TLC was carried out on plates precoated with RP_{18} (Merck) and silica gel HF_{254} (Qingdao Marine Chemistry Ltd.). Spots on the plates were visualized by spraying with 10% H_2SO_4, followed by heating. Column chromatography (CC) was performed on silica gel (200−300 and 300−400 mesh, Qingdao Marine Chemical Factory), Lichroprep RP_{18} gel (40−60 μm, Merck, Darmstadt, Germany), and MCI (75−150 μm, Mitsubishi Chemical). GC was performed on an Agilent Technologies HP6890 gas chromatograph equipped with an H_2 flame ionization detector. Standards glucose, rhamnose, arabinose, and xylose were purchased from National Institute for the Control of Pharmaceutical and Biological Products, China.

Plant Material. The crude drug was purchased from Qujing Company of Chinese Materia Medica, Yunnan, People's Republic of China, in July of 2004 and was identified as the roots of *Asparagus filicinus* Buch.−Ham (Liliaceae) by one of the authors (D.-F.C.). A voucher specimen (DFC-XBB20040701) is deposited in the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, People's Republic of China.

Extraction and Isolation. The roots of *A. filicinus* (6 kg) were crushed to a coarse powder and then extracted with EtOH (20 L × 3) at room temperature. The EtOH extract was used to prepare the crude saponins (170 g), a portion (150 g) of which was chromatographed on silica gel (2 kg) with a $CHCl_3$−MeOH gradient (90:10 to 0:100) to give six fractions. Fraction 2 (10 g), eluted with $CHCl_3$−MeOH (80:20), was chromatographed on a silica gel column with $CHCl_3$−MeOH−H_2O (6:1:0.1) and then subjected to repeated MCI column chromatography with a gradient solvent system (MeOH−H_2O, 7:3 to 9:1) to yield **2** (5 mg) and **4** (4 mg). Fraction 3 (15 g) was subjected to silica gel column chromatography with $CHCl_3$−MeOH−H_2O (4:1:0.2) to give four fractions. Fraction 3-2 was subjected to repeated RP_{18} column chromatography with MeOH−H_2O (6:4 to 9: 1) to yield **1** (100 mg) and **3** (20 mg). Fraction 4 (20 g) was subjected to a silica gel column with $CHCl_3$−MeOH−H_2O (7:3:0.5) to give six fractions. Fraction 4-2 was subjected to repeated column chromatography with $CHCl_3$−MeOH−H_2O (7:3:0.5) to yield **5** (200 mg) and **6** (130 mg).

Filiasparoside A (1): white, amorphous solid; $[\alpha]_D^{22}$ +39.2 (*c* 0.02, MeOH−$CHCl_3$); IR (KBr) ν_{max} 3449 (OH), 2927 (CH), 1707 (C=O), 1039 (OH), 981, 919, 898, 857 cm^{-1}; ESIMS: *m/z* 725 [M + H − 132]$^+$, 593 [M + H − 132 − 132]$^+$, 431 [M + H − 132 − 132 − 162]$^+$; ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data, see Tables 1−4; HRESIMS *m/z* 879.4360 (calcd for $C_{44}H_{68}O_{17}Na$ [M + Na]$^+$, 879.4356).

Filiasparoside B (2): white, amorphous solid; $[\alpha]_D^{22}$ +20.0 (*c* 0.02, MeOH−$CHCl_3$); IR (KBr) ν_{max} 3443 (OH), 2935 (CH), 1705 (C=O), 1455 (OH), 1058 (OH), 972, 918, 901, 861 cm^{-1}; ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data, see Tables 1−4; HRESIMS *m/z* 723.3961 (calcd for $C_{38}H_{60}O_{13}$ [M − H]$^-$, 723.3956).

Table 3. ^{1}H NMR (400 MHz) Data of the Sugar Moieties of **1**−**4** (pyridine-d_5, δ in ppm, J in Hz)

position	**1**	**2**	**3**	**4**
3-O-Glu				
1	4.94, d (7.4)	4.95, d (7.8)	4.82, d (7.2)	4.86, d (7.8)
2	3.89, m	3.98, m	4.20, m	4.00, m
3	4.26, m	4.24, m	4.22, m	4.20, m
4	4.51, m	4.14, m	4.25, m	4.14, m
5	4.02, m	4.14, m	3.78, m	4.08, m
6	4.76, dd (3.1, 10.8)	4.32, m	4.40, dd (3.1, 12.4)	4.32, m
	4.86, d (10.8)	4.86, d (10.1)	4.50, dd (3.7, 12.4)	4.84, dd (2.0, 11.6)
Xyl				
1	5.56, d (7.8)		5.02, d (7.5)	
2	4.07, m		3.96, dd (8.4, 7.5)	
3	4.35, m		4.08, m	
4	4.23, m		4.14, m	
5	3.97, 4.26, m		3.66, t (10.4)	
			4.24, m	
Ara				
1	5.10, d (7.4)	4.96, d (7.0)		4.98, d (7.4)
2	4.49, m	4.46, m		4.48, m
3	4.05, m	4.14, m		4.18, m
4	4.24, m	4.30, m		4.32, m
5	3.71, d (11.3)	3.73, d (10.1)		3.76, dd (2.9,10.5)
	4.26, m	4.28, m		4.28, m
Rha				
1			6.38, s	
2			4.77, m	
3			4.55, m	
4			4.32, t (9.2)	
5			4.78, m	
6			1.74, d (6.3)	

Table 4. ^{13}C NMR (100 MHz) Data of the Sugar Moieties of **1**−**4** (pyridine-d_5, δ in ppm)

position	**1**	**2**	**3**	**4**
3-O-Glu				
1	102.1	102.1	101.8	102.9
2	74.8	75.0	81.5	75.1
3	76.3	78.5	77.5	78.5
4	79.8	71.7	76.7	71.8
5	74.8	77.0	76.1	77.0
6	68.0	69.5	61.6	69.6
Xyl				
1	105.1		105.6	
2	75.0		74.8	
3	78.5		78.2	
4	71.1		70.6	
5	67.4		67.2	
Ara				
1	105.7	105.4		105.3
2	72.5	72.2		72.3
3	74.5	74.3		74.4
4	69.9	69.1		69.1
5	67.5	66.5		66.5
Rha				
1			101.5	
2			72.3	
3			72.6	
4			73.9	
5			69.4	
6			18.7	

Filiasparoside C (3): white, amorphous solid; $[\alpha]_D^{22}$ −60.5 (*c* 0.20, MeOH); IR (KBr) ν_{max} 3442 (OH), 2934 (CH), 1449 (OH), 1048 (OH), 984, 914, 890, 865 cm^{-1}; ESIMS: *m*/*z* 725 $[M + H - 132]^+$, 579 $[M + H - 132 - 146]^+$, 417 $[M + H - 132 - 146 - 162]^+$; ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data, see Tables 1−4; HRESIMS *m*/*z* 879.4722 (calcd for $C_{44}H_{72}O_{16}Na$ $[M + Na]^+$, 879.4718).

Filiasparoside D (4): white, amorphous solid; $[\alpha]_D^{22}$ −25.0 (*c* 0.22, MeOH); IR (KBr) ν_{max} 3417 (OH), 2935 (CH), 1450 (OH), 1068 (OH), 986, 918, 897, 850 cm^{-1}; ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data, see Tables 1−4; HRESIMS *m*/*z* 709.4167 (calcd for $C_{38}H_{62}O_{12}$ $[M - H]^-$, 709.4163).

Acid Hydrolysis of 1. Compound **1** (8 mg) was dissolved in 1 M H_2SO_4−50% EtOH (5 mL). The mixture was refluxed at 100 °C for 6 h. After cooling, the mixture was neutralized with Na_2CO_3 and then extracted with $CHCl_3$ (10 mL × 3). The $CHCl_3$ extract was combined and washed with H_2O (10 mL) and then evaporated to give aglycone **1a** (hecogenin, 2 mg). ^{13}C NMR data (pyridine-d_5, 100 MHz) of **1a**: δ 36.3 (C-1), 29.9 (C-2), 66.3 (C-3), 35.7 (C-4), 44.3 (C-5), 28.6 (C-6), 31.6 (C-7), 33.8 (C-8), 55.4 (C-9), 35.7 (C-10), 37.5 (C-11), 212.3 (C-12), 54.8 (C-13), 55.4 (C-14), 30.8 (C-15), 79.1 (C-16), 53.7 (C-17), 15.5 (C-18), 11.3 (C-19), 42.0 (C-20), 13.3 (C-21), 108.7 (C-22), 31.2 (C-23), 29.4 (C-24), 29.9 (C-25), 69.7 (C-26), 16.7 (C-27). The aqueous layer was concentrated to an appropriate volume (1 mL) and examined by TLC (silica gel) with the solvent system EtOAc−MeOH−HOAc−H_2O (6:2:1:1) for sugar analysis. The remaining aqueous layer was concentrated to dryness to give a residue, which was dissolved in pyridine (2 mL) and trimethylchlorosilane (0.5 mL) and stirred for 6 h at room temperature. The solution was concentrated to dryness and dissolved in water (2 mL), followed by extraction with *n*-hexane (5 mL × 3). The *n*-hexane fraction was analyzed by GC: column HP-5 (30 m × 0.32 mm × 0.25 μm); detector FID, column temp 100/200 °C (programmed increase, 10 °C/min), carrier gas N_2 (2 mL/min); injection and detector temperature 250 °C, injection volume 1 μL, split ratio 1/20. Under these conditions, the retention times of the trimethylsilyl derivatives of arabinose, xylose, and glucose standards were 8.14, 9.14, and 11.21 min, respectively.

Table 5. Cytotoxicity (EC_{50} in μg/mL) Data of **1**−**6** against Human Tumor Cell Lines

	cell line	
compound	A549	MCF-7
1	13.3	11.2
2	16.4	16.8
3	2.3	3.0
4	2.4	10.3
5	9.4	>20
6	7.6	10.4
etoposide	0.8	2.8

Acid Hydrolysis of 3. Compound **3** (8 mg) was subjected to acid hydrolysis as described for **1** to give **3a** (sarsasapogenin, 3 mg). The aqueous layer was treated and analyzed as described for **1**, and glucose, rhamnose, and xylose were detected by TLC and GC analysis (retention times 11.21, 8.26, and 9.14 min, respectively). ^{13}C NMR data (pyridine-d_5, 100 MHz) of **3a**: δ 30.6 (C-1), 28.6 (C-2), 66.0 (C-3), 34.4 (C-4), 37.0 (C-5), 27.2 (C-6), 26.9 (C-7), 35.6 (C-8), 40.4 (C-9), 35.6 (C-

10), 21.2 (C-11), 40.1 (C-12), 40.9 (C-13), 56.6 (C-14), 32.2 (C-15), 81.3 (C-16), 63.1 (C-17), 16.6 (C-18), 24.3 (C-19), 42.5 (C-20), 14.9 (C-21), 109.7 (C-22), 26.4 (C-23), 26.2 (C-24), 27.5 (C-25), 65.1 (C-26), 16.3 (C-27).

Growth Inhibition Assay. Drug stock solutions were prepared in DMSO and stored at −70 °C. Upon dilution into culture medium, the final DMSO concentration was ≤1% DMSO (v/v), a concentration without effect on cell replication. The human tumor cell line panel consisted of human lung carcinoma (A549) and breast adenocarcinoma (MCF-7). Cell culture and other procedures have been reported previously.[12] The EC_{50} value is the concentration that inhibited growth by 50% following 2 days of continuous exposure.

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Supporting Information Available: 2D NMR spectra of the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Jiangsu Medical College. *Dictionary of Traditional Chinese Medicines*; Shanghai Science and Technology Press, 1985; p 86.
(2) Ding, Y.; Yang, C. R. *Acta Pharm. Sin.* **1990**, *25*, 509−514.
(3) Cong, X. D.; Ye, W. C.; Che, Ch. T. *Chin. Chem. Lett.* **2000**, *9*, 793−794.
(4) Sharma, S. C.; Thakur, N. K. *Phytochemistry* **1994**, *36*, 469−471.
(5) Sharma, S. C.; Thakur, N. K. *Phytochemistry* **1996**, *41*, 599−603.
(6) Li, Y. F.; Hu, L. H.; Lou, F. Ch.; Hong, J. R.; Li, J.; Shen, Q. *J. Asian Nat. Prod. Res*. **2005**, *7*, 43−47.
(7) Jones, R. N.; Katzenellenbogen, K.; Dobriner, K. *J. Am. Chem. Soc.* **1953**, *75*, 158−166.
(8) Agrawal, P. K.; Jain, D. C.; Gupta, R. K.; Thakur, R. S. *Phytochemistry* **1985**, *24*, 2479−2496.
(9) Gorin, P. A. J. *Carbohydr. Res.* **1975**, *39*, 3−10.
(10) Gorin, P. A. J.; Mazurek, M. *Can. J. Chem*. **1975**, *53*, 1212−1223.
(11) Takechi, M.; Chikari, U.; Tanaka, Y. *Phytochemistry* **1996**, *41*, 121−123.
(12) Cheng, H. H.; Wang, H. K.; Ito, J.; Bastow, K. F.; Tachibana, Y.; Nakanishi, Y.; Xu, Z.; Luo, T. Y.; Lee, K. H. *J. Nat. Prod*. **2001**, *64*, 915−919.

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