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Antineoplastic Agents. 534. Isolation and Structure of Sansevistatins 1 and 2 from the African *Sansevieria ehrenbergii*[†,1]

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Using bioactivity-directed isolation procedures, three new spirostanol saponins designated sansevierin A (**1**), sansevistatin 1 (**2**), and sansevistatin 2 (**3**) were isolated (10^{-5} % yield) from the $CH_3OH-CH_2Cl_2$ extract of *Sansevieria ehrenbergii*, accompanied by three known steroidal saponins (**4**−**6**). The structures were determined on the basis of chemical methods and spectroscopic analysis, especially 1D and 2D NMR experiments. Each of the saponins was evaluated against the P388 lymphocytic leukemia cell line and a panel of human cancer cell lines. Except for **1**, all were found to cause inhibition of cancer cell growth. In addition, most of the saponins exhibited antimicrobial activity, particularly against the pathogenic fungi *Candida albicans* and *Cryptococcus neoformans*.

The genus *Sansevieria* (Agavaceae) includes some 60 species indigenous to Africa, Saudi Arabia, and Madagascar, with some now more widespread as invasive ornamentals (e.g., Australia)[2]. Prior chemical studies of this genus appear confined to *S. hyacinthoides*[3] (where 25*S*-ruscogenin as well as a new pregnane were isolated) and *S. trifasciata*,[4] which led to sansevierigenin,[3a] four new pregnane glycosides,[4a] 10 new steroidal saponins,[4b] and 1-phosphotransferase.[5] Interestingly, a potted specimen of the latter species provided a *Streptomyces griseus* strain found to biosynthesize a potent polyene-type antifungal.[5] *S. hyacinthoides* is known as a medicinal plant in the Yucatan Peninsula of Mexico.[3b]

Specimens of *S. ehrenbergii* collected (1966) in Kenya were found in 1978 to give EtOH extracts that had preliminary in vivo activity in the U.S. National Cancer Institute (NCI) murine colon 8 tumor (T/C 133 at 28 mg/kg and toxic at 58 mg/kg) and inhibited growth (ED_{50} 1.2−5.6 μg/mL) of the P388 lymphocytic leukemia (PS) and KB cell lines. In 1979, a scale-up re-collection of the aerial portion and base of this plant was completed as part of the NCI worldwide evaluations of terrestrial plants as potential sources of new anticancer drugs. The stems, leaves, and base were extracted with $CH_3OH-CH_2Cl_2$ (1:1) by a modified Bligh and Dyer sequence.[6] After phase separation by addition of water, the CH_2Cl_2 and CH_3OH-H_2O fractions (PS ED_{50} 2.4 and 2.5 μg/mL, respectively) were evaluated against P388 leukemia, the only cancer line available to us in 1980. The PS-active CH_2Cl_2 fraction proved to be toxic[3c] at all doses tried with that cell line in vivo. Therefore, this plant was assigned a low priority until it was reactivated for study in 1998, when a minipanel of human solid tumor cell lines became available to support our isolation procedures.

Results and Discussion

A portion of this material was extracted as before, and the CH_3OH-H_2O fraction partitioned. Active CH_2Cl_2 and ethyl acetate fractions were subjected to a series of chromatographic separations on Sephadex LH-20 followed by HPLC to afford three new steroidal saponins designated sansevierin A (**1**), sansevistatin 1 (**2**), and sansevistatin 2 (**3**) and three known steroidal saponins (**4**−**6**). The absolute configurations of the sugar residues were assumed to be D for xylose and glucose and L for rhamnose and arabinose, consistent with the usual stereochemistry of naturally occurring monosaccharides. The configurations of the anomeric carbons of D-glucopyranosyl, L-rhamnopyranosyl, L-arabinopyranosyl, and D-xylopyranosyl residues were determined as β, α, α, β, respectively, according to the corresponding NMR coupling constants of anomeric protons along with the chemical shifts of the anomeric carbons. Steroidal glycosides **4** and **5** were readily identified as 3β-*O*-[α-L-rhamnopyranosyl-(1→2)][α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl-(25*R*)-spirost-5-ene (dioscin) and 3β-*O*-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl-(25*R*)-spirost-5-ene by comparison of their spectroscopic data with those reported.[7] Saponin **6**[8,9] was found to be 3β-*O*-[α-L-rhamnopyranosyl-(1→2)][β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl-(25*R*)-spirost-5-ene by mass measurements and the results of 1D and 2D NMR experiments.

Sansevierin A (**1**) was obtained as an amorphous solid. The molecular formula of saponin **1** was deduced as $C_{39}H_{62}O_{13}$ from the HRFABMS (*m/z* 745.4319 $[M + Li]^+$, calcd 745.4350). The ^1H NMR spectrum displayed two anomeric proton signals at δ 6.38 (br s) and 4.97 (d, J = 6.8 Hz) in addition to signals of the aglycon corresponding to two tertiary methyl proton signals (at δ 0.90 and 1.06), two secondary methyl proton signals at δ 0.66 (d, J = 6.0 Hz) and 1.13 (d, J = 6.8 Hz), and an olefinic proton signal at δ 5.77 (d, J = 4.8 Hz), and acid hydrolysis of sansevierin A (**1**) gave D-glucose and L-rhamnose. The molecular weight of the aglycon moiety was calculated to be 430 amu, greater than that of diosgenin (**7**) by 16 amu. By comparison of the NMR signals of the sterol unit of **1** with those of saponins **4**−**6** and **7**, one more tertiary carbon

[†] Dedicated to Professor Carl Djerassi, a great pioneer in advancing natural products chemistry, on the occasion of his 80th birthday.

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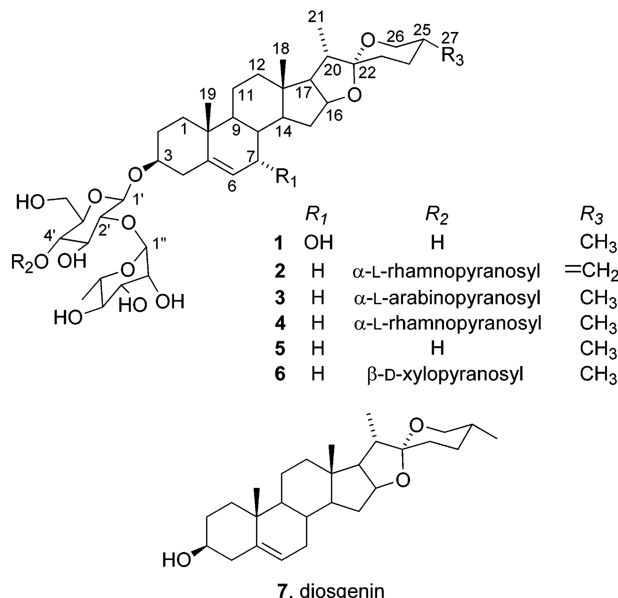

7, diosgenin

(δ 64.61) and one less secondary carbon were found (Table 1). In the ^{1}H−^{1}H COSY spectrum, the olefinic proton signal at δ 5.77 showed a correlation with a proton linked to an oxygenated carbon at δ 4.00. Thus, the hydroxyl group was presumed to be at C-7, which was confirmed by HMBC correlations of C-7/H-6, C-5/H-7, and C-6/H-7. The orientation of the 7-hydroxyl group was assigned as α because of the resonance of C-7 (δ 64.61), whereas with 7β-hydroxylation, the signal for C-7 would be at δ 72.6.[10,11] When a 7α-hydroxyl group is present, γ-gauche effects would be expected at C-9 and C-14, which were found in sansevierin A (**1**) to be moved upfield (−7.73 and −7.80 ppm) relative to their respective signals in saponin **5**. The effects of the 7β-isomer on C-9 and C-14 would be less, as a γ-gauche orientation is not possible. The HMBC spectra also showed the correlation of H-1″ (δ 6.38) of rhamnose to C-2′ (δ 79.67) of glucose as well as the anomeric carbon of glucose at δ 100.38 with the H-3 (δ 3.84) of the sterol. The sansevierin A (**1**) structure was, therefore, determined as 3β-*O*-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl-7α-hydroxyl-(25*R*)-spirost-5-ene.

Sansevistatin 1 (**2**) was also obtained as an amorphous powder. The molecular formula of **2** was deduced to be $C_{45}H_{70}O_{16}$ from the HRAPCIMS (*m/z* 867.4761 [M + H]$^+$, calcd 867.4742). The ^{1}H NMR spectrum showed two tertiary methyl proton signals at δ 0.81 and 1.04, a secondary methyl proton signal at δ 1.08 (d, J = 6.8 Hz), exomethylene proton signals at δ 4.78 and 4.81 (each 1H, br s), an olefinic proton signal at δ 5.31 (br d, J = 4.4 Hz), and three anomeric proton signals at δ 6.40 (br s), 5.86 (br s), and 4.95. Acid hydrolysis of **2** gave D-glucose and L-rhamnose. The above data, and the observation of a distinctive quaternary carbon signal at δ 109.51 and three anomeric carbon signals at δ 100.39, 102.15, and 103.01, led to the hypothesis that sansevistatin 1 (**2**) is a spirostane saponin with three sugar units (Table 1). Comparison of the ^{1}H and ^{13}C NMR spectra with those of **4** revealed that the signals were similar except for the appearance of signals for an exocyclic olefin (δ_H 4.78 and 4.81, δ_C 144.46 and 108.79) and the disappearance of the signals of a secondary methyl (CH_3-27) and a methane (CH-25). Thus, **2** was proposed to be a 25,27-dehydro derivative of sterol **4**, and this was confirmed by the HMBC correlations of the exomethylene proton signals given above with C-24 and C-26. The HMBC spectral correlations of C-2′ of D-glucose with the anomeric proton of one L-rhamnose, of C-4′ of D-glucose with an anomeric proton of another L-rhamnose, and C-1′ of D-glucose with H-3 of the aglycon were also observed. Thus, sansevistatin 1 (**2**) was determined to be 3β-*O*-[α-L-rhamnopyranosyl-(1→2)][α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosylspirosta-5,25(27)-diene.

Sansevistatin 2 (**3**, amorphous powder) showed a [M + Li]$^+$ signal at *m/z* 861.4864 by HRFABMS, corresponding to the molecular formula $C_{44}H_{70}O_{16}$. Upon acid hydrolysis, L-arabinose, D-glucose, L-rhamnose, and diosgenin were detected. Comparison of the NMR carbon signals corresponding to saponin **3** with those of **5** showed that **3** contains the aglycon and glycoside units of **5** and also has an α-L-arabinopyranosyl unit, which must be linked to C-4′ of the glucose unit, as a glycosidation shift of the C-4′ signal (+9.61 ppm) was observed in the spectrum of **3**, relative to that of **5**. HMBC correlations of C-2′ of D-glucose with the anomeric proton of L-rhamnose, of C-4′ of D-glucose with the anomeric proton of L-arabinose, and of C-1′ of D-glucose with H-3 of the aglycon confirmed the linkage sites. Thus, **3** was deduced as 3β-*O*-[α-L-arabinopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl-(25*R*)-spirost-5-ene.

The cytotoxic activities of steroidal saponins have been shown to be sensitive to the monosaccharides constituting the sugar moieties and their sequences as well as to the structures of the aglycons.[12] The cytotoxic effects of some steroidal saponins may result from their nonspecific detergent effects[13] on membrane architecture.[14] After saponin treatment, tumor cells aggregate at early stages. In the present study, the steroidal sapogenin diosgenin (**7**) showed negligible activity, while **2**−**6** were significantly active cancer cell growth inhibitors (Table 2). Clearly, the glycoside unit is essential for cancer cell line growth inhibition in the diosgenin series, and introduction of a 7α-hydroxyl group eliminates such biological activity (cf. **1**, Table 2).

Compounds **2**−**6** were very active against the pathogenic yeasts *Cryptococcus neoformans* and *Candida albicans* (Table 3), with most minimum inhibitory concentrations (MICs) in the 1−2 μg/mL range. In addition to antifungal activity, saponins **3**−**5** had anti-Gram-positive antibacterial activity. Compound **6** was available in sufficient quantity to determine whether its mode of action against *C. albicans* was fungistatic or fungicidal. The minimum fungicidal activity (MFC)/MIC ratio was ≤2, indicating fungicidal activity.

Experimental Section

General Experimental Procedures. All solvents used in the extraction and isolation processes were redistilled prior to use. Column chromatography was performed with Sephadex LH-20 from Pharmacia. The HPLC separations were achieved using a Zorbax SB C_{18} column with aqueous CH_3OH (80%) as mobile phase. The chromatograms were recorded with both a diode array detector (HP1100 series) and an evaporative light scattering detector (SEDEX model 55). The uncorrected melting points were observed using a Fisher-Johns apparatus. The optical rotation measurements were recorded with a Perkin-Elmer 241 polarimeter. The IR spectral data were obtained with a Thermo Nicolet Avatar 360 E.S.P. spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded with a Varian Inova 500 MHz instrument. High-resolution APCI mass spectra were obtained from a JEOL-LC Mate LCMS system and HRFABMS [3NBA-Li inactive] at Washington University Mass Spectrometry Center, St. Louis, Missouri.

Plant Material. The stems, leaves, and base of *Sansevieria ehrenbergii* Schweinf. ex Baker. were initially collected in Southern Province, Kenya, in November 1966 under the direction of Dr. Robert Perdue, United States Department of Agriculture (USDA), and Dr. Jonathan L. Hartwell, National

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Assignments for Saponins **1**−**3** (in pyridine-d_5)

	sansevierin A (**1**)		sansevistatin 1 (**2**)		sansevistatin 2 (**3**)	
position	^{1}H δ (J Hz)	^{13}C δ	^{1}H δ (J Hz)	^{13}C δ	^{1}H δ (J Hz)	^{13}C δ
1	0.91	37.20	0.96	37.69	0.95	37.50
	1.69		1.75		1.72	
2	1.87	30.03	2.06	30.37	1.88	30.17
	2.04		1.84		2.10	
3	3.84	77.61	3.88	78.20	3.87	78.16
4	2.83 (2H)	38.94	2.73 (2H)	39.17	2.75 (2H)	38.97
5		143.91		140.84		140.77
6	5.77 d (4.8)	125.96	5.31 br d (4.4)	121.85	5.29 d (6.0)	121.83
7	4.00	64.61	1.55	32.50	1.43	32.32
			1.86		1.85	
8	1.64	37.95	1.51	31.88	1.44	31.85
9	1.59	42.56	0.89	50.45	0.89	50.30
10		37.95		37.32		37.15
11	1.48 (2H)	20.95	1.41 (2H)	21.31	1.46 (2H)	21.12
12	1.21	39.77	1.10	39.97	1.08	39.87
	1.73		1.67		1.69	
13		40.31		40.66		40.47
14	2.06	49.85	1.03	56.78	1.00	56.64
15	1.61	32.39	1.46	32.36	1.45	32.24
	2.56		2.00		2.03	
16	4.63	81.41	4.53	81.54	4.54	81.12
17	1.89	63.07	1.78	63.04	1.80	62.92
18	0.90 s	16.39	0.81 s	16.53	0.82 s	16.35
19	1.06 s	18.32	1.04 s	19.62	1.04 s	19.41
20	1.99	42.09	1.93	42.03	1.95	41.99
21	1.13 d (6.8)	15.13	1.08 d (6.8)	15.22	1.13 d (7.0)	15.06
22		109.29		109.51		109.27
23	1.68 (2H)	31.90	1.80 (2H)	33.40	1.67 (2H)	31.70
24	1.56 (2H)	29.31	2.24 d (13.6)		29.17	29.28
			2.73 d (13.6)			
25	1.56	30.64		144.46	1.58	30.62
26	3.46	66.83	4.02 d (12.0)	65.15	3.49	66.88
	3.53		4.45 d (12.0)		3.58	
27	0.66 d (6.0)	17.35	4.78 br s	108.79	0.69 d (5.5)	17.34
			4.81 br s			
Glc						
1′	4.97 d (6.8)	100.38	4.95	100.39	4.94 d (7.5)	100.03
2′	4.26	79.67	4.18	78.11	4.20	77.60
3′	4.26	77.69	4.20	77.91	4.20	77.36
4′	4.14	71.88	4.38	78.71	4.19	81.44
5′	3.88	78.39	3.63	77.08	3.85	76.28
6′	4.34	62.75	4.22	61.44	4.44	61.67
	4.52		4.08		4.54	
Rha						
1″	6.38 br s	102.07	6.40 br s	102.15	6.25 br s	102.01
2″	4.79	72.62	4.83	72.68	4.80	72.50
3″	4.61	72.86	4.62	72.99	4.60	72.83
4″	4.36	74.19	4.36	74.28	4.36	74.18
5″	4.98	69.53	4.98	69.66	4.93	69.61
6″	1.88 d (6.8)	18.69	1.76 d (6.4)	18.88	1.77 d (6.0)	18.70
			Rha		Ara	
1‴			5.86 br s	103.01	5.03 d (8.0)	105.92
2‴			4.67	72.68	3.98	72.54
3‴			4.53	72.90	4.13	74.59
4‴			4.33	74.08	4.18	69.61
5‴			4.93	70.57	3.68	67.90
					4.26	
6‴			1.63	18.72		

Cancer Institute (NCI). Re-collections in 1977−1979 were directed by Drs. James A. Duke (USDA), John D. Douros (NCI), and Matthew Suffness (NCI). A voucher specimen (8083) of the initial collection of this plant has been retained by the United States National Arboretum, Washington, DC 20002-1958.

Plant Extraction and Initial Separation. The dried and chipped plant (22 kg) was extracted with CH_2Cl_2−CH_3OH (1:1, 300 L, divided among two polyethylene containers) at ambient temperature. The extract was separated into CH_2Cl_2 and CH_3OH−H_2O phases by addition (30 vol %) of H_2O. Removal of solvent from the CH_2Cl_2 fraction gave 545.7 g (P388 ED_{50} 33.7 μg/mL) and from the CH_3OH−H_2O fraction gave 533.5 g (P388 ED_{50} 3.4 μg/mL). The ambient extraction with CH_2Cl_2−CH_3OH (1:1) was repeated a second time to yield a 170.8 g CH_2Cl_2 fraction (P388 ED_{50} 19.6 μg/mL) and a 318.3 g CH_3OH−H_2O fraction (P388 ED_{50} 2.2 μg/mL). A 200 g portion was taken from each CH_3OH−H_2O extract, and the combined material was partitioned between CH_3OH−H_2O (9:1) and hexane. The hexane phase provided 10.8 g (P388 ED_{50} 3.2 μg/mL). The methanolic phase was diluted to CH_3OH−H_2O (3:2), and partitioning with CH_2Cl_2 provided 45.5 g (P388 ED_{50} 1.4 μg/mL) of a CH_2Cl_2 extract. Methanol was removed from the remaining CH_3OH−H_2O fraction, and the aqueous phase was then partitioned with EtOAc (136 g; P388 ED_{50} 1.9 μg/mL) and n-BuOH (42.9 g; P388 ED_{50} 22.8 μg/mL). The remaining H_2O fraction amounted to 86 g (P388 ED_{50} > 100 μg/mL).

Table 2. Murine P388 Lymphocytic Leukemia Cell Line (ED_{50}) and Human Cancer Cell Line Inhibition Values (GI_{50}) Expressed in μg/mL for Saponins **1**−**6** and Diosgenin (**7**)

cancer cell line[a]	1	2	3	4	5	6	7
P388	>10	1.6	1.7	1.5	1.5	2.6	4.3
BXPC-3	>10	1.1	0.93	1.1	1.8	1.7	>10
MCF-7	>10	1.1	0.62	1.6	2.0	1.4	>10
SF-268	>10	1.3	0.68	1.2	1.8	1.3	>10
NCI-H460	>10	0.43	0.26	1.6	1.8	1.2	>10
KM20L2	>10	0.47	0.22	1.6	1.7	0.5	>10
DU-145	>10	1.0	0.42	1.6	1.6	1.1	8.5

[a] Cancer type: P388 (lymphocytic leukemia); BXPC-3 (pancreas adenocarcinoma); MCF-7 (breast adenocarcinoma); SF268 (CNS glioblastoma); NCI-H460 (lung large cell); KM20L2 (colon adenocarcinoma); DU-145 (prostate carcinoma).

Isolation Procedures. A CH_3OH solution of the PS-active CH_2Cl_2 fraction (45.4 g) was passed through a Sephadex LH-20 column and eluted with the same solvent. Both P388 and human cancer cell line active fractions (a, b, and c) were obtained. Fraction b was rechromatographed on a Sephadex LH-20 column, using $CH_3OH-CH_2Cl_2$ (3:2) as eluent, to provide active fraction d, which was again chromatographed using a Sephadex LH-20 column, with hexane−CH_2Cl_2−CH_3OH−2-propanol (4:1:1:1) as eluent, to provide active fraction e. Fraction e was chromatographed on a silica gel column, and fractions were eluted with $CH_2Cl_2-CH_3OH$ (9:1 → 7:3) to afford a pure sample of **4** (620 mg, 1.5×10^{-3} % yield). Fraction c was rechromatographed on Sephadex LH-20 columns with $CH_3OH-CH_2Cl_2$ (3:2) and hexane−CH_2Cl_2−CH_3OH−2-propanol (4:1:1:1 and 8:1:2:2), successively, and provided **4** (100 mg, 2.4×10^{-4} % yield) and **5** (140 mg, 3.4×10^{-4} % yield).

A CH_3OH solution of the active EtOAc fraction (136 g) was applied to a Sephadex LH-20 column and eluted with CH_3OH. Both P388 and human cancer cell line active fractions (f, g, and h) were obtained. Fraction f was rechromatographed on Sephadex LH-20 columns with $CH_3OH-CH_2Cl_2$ (3:2), hexane−CH_2Cl_2−CH_3OH−2-propanol (4:1:1:2), hexane−toluene−CH_2Cl_2−EtOH (1:1:1:1), and hexane−CH_2Cl_2−CH_3OH−2-propanol (8:1:2:2), successively, to afford **6** (38 mg, 9.0×10^{-5} % yield). Fraction g was rechromatographed on a Sephadex LH-20 column, with $CH_3OH-CH_2Cl_2$ (3:2) as eluent, to provide active fraction i. Fraction h was rechromatographed on a Sephadex LH-20 column, with $CH_3OH-CH_2Cl_2$ (3:2) as eluent, to provide active fraction j. Fractions i and j were combined and chromatographed on a Sephadex LH-20 column, with hexane−toluene−CH_2Cl_2−EtOH (1:1:1:1) as eluent, to provide active fraction k. Fraction k was passed through a silica gel column, eluted with $CH_2Cl_2-CH_3OH$ (9:1 → 7:3), and active fraction 1 was obtained. Fraction 1 was rechromatographed on a Sephadex LH-20 column, with hexane−CH_2Cl_2−CH_3OH−2-propanol (8:1:2:2) as eluent, and active fraction m was obtained. Fraction m was rechromatographed on a Sephadex LH-20 column, with hexane−EtOAc−CH_3OH (4:5:1) as eluent, and active fraction n was obtained. Fraction n was further separated by HPLC, by use of a Zorbax SB C_{18} column (25 cm × 4.6 mm, 5 μm) and an isocratic mobile phase (75% CH_3OH in H_2O for 20 min). Peaks were monitored at 210 nm; flow rate was 1.5 mL/min. Pure **1** (8 mg, 1.9×10^{-5} % yield), **2** (12 mg, 2.9×10^{-5} % yield), **3** (20 mg, 4.8×10^{-5} % yield), and **4** (10 mg, 2.4×10^{-5} % yield) were obtained.

Sansevierin A (1): amorphous solid from CH_3OH; mp 226−230 °C; $[\alpha]_D^{24}$ −104.0° (*c* 0.62, CH_3OH,); IR ν_{max} 3379, 2928, 1454, 1377, 1038 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) data, see Table 1; HRFABMS *m/z* 745.4319 $[M + Li]^+$ (calcd for $C_{39}H_{62}O_{13}$ Li, 745.4350).

Sansevistatin 1 (2): amorphous powder from CH_3OH; mp 267−269 °C; $[\alpha]_D^{24}$ −102.0° (*c* 0.40, CH_3OH); IR ν_{max} 3381, 2937, 1451, 1376, 1135, 1036 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) data, see Table 1; HRAPCIMS *m/z* 867.4761 $[M + H]^+$ (calcd for $C_{45}H_{71}O_{16}$, 867.4742).

Sansevistatin 2 (3): amorphous powder from CH_3OH; mp 280−282 °C; $[\alpha]_D^{24}$ −87.1° (*c* 0.68, C_5H_5N); IR ν_{max} 3390, 2944, 1456, 1377, 1142, 1051 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) data, see Table 1; HRFABMS *m/z* 861.4864 $[M + Li]^+$ (calcd for $C_{44}H_{70}O_{16}$ Li, 861.4824).

Saponin 4: colorless needles from CH_3OH; mp 287−288 °C; lit.[7] mp 275−277 °C; $[\alpha]_D^{25}$ −102.2° (*c* 1.00, CH_3OH).

Saponin 5: amorphous powder from CH_3OH; mp 235−237 °C; lit.[7] mp 238−240 °C; $[\alpha]_D^{25}$ −99.4° (*c* 0.50, CH_3OH).

Saponin 6: colorless needles from CH_3OH; mp 262−265 °C; lit.[8] mp 245−250 °C; $[\alpha]_D^{25}$ −91.2° (*c* 0.25, CH_3OH).

Acid Hydrolysis. A solution of saponin **4** (0.10 g) in 1 N HCl (CH_3OH-H_2O, 40 mL) was heated at 55 °C for 24 h. After removal of solvent, the residue was submitted to silica gel chromatography in a gradient mixture of $CH_2Cl_2-CH_3OH$ (95:5 → 70:30) to provide diosgenin (**7**, 10 mg). Separately, 0.5 mg of each (**1**−**6**) saponin was dissolved in CH_3OH (1 mL), followed by addition of H_2O (1 mL) and 2 N HCl (2 mL), and the solution was heated at 80 °C for 24 h. After removal of solvent, the residue was dissolved in 0.5 mL of CH_3OH and examined by TLC. The TLC plates were developed with hexane−ethyl acetate (8:2) for the sapogenins and with n-BuOH−EtOAc−2-propanol−AcOH−H_2O (35:100:60:35:30) and n-BuOH−AcOH−H_2O (4:1:1) for the sugars with authentic samples for comparison. Diosgenin (**7**) was detected in the reactions of compounds **3**−**6**. Both D-glucose and L-rhamnose were detected in each of the reaction products; in addition, L-arabinose was found in the hydrolysis of **3** and D-xylose was noted in the hydrolysis of **6**.

Cancer Cell Line Procedures. Inhibition of human cancer cell growth was assessed using the NCI's standard sulforhodamine B assay as previously described.[15] Briefly, cells in a 5% fetal bovine serum/RPMI1640 medium solution were inoculated in 96-well plates and incubated for 24 h. Serial dilutions of the compounds were then added. After 48 h, the plates were fixed with trichloroacetic acid, stained with sulforhodamine B, and read with an automated microplate reader. A growth inhibition of 50% (GI_{50} or the drug concentration causing a 50% reduction in the net protein increase) was calculated from optical density data with Immunosoft software. Mouse leukemia P388 cells[16] were incubated for 24 h in a 10% horse serum/Fisher medium solution followed by a 48 h incubation with serial dilutions of the compounds. Cell growth inhibition (ED_{50}) was then calculated using a Z1 Beckman/Coulter particle counter.

Antimicrobial Susceptibility Testing. The antibacterial and antifungal activity of the saponins was assessed by the National Committee for Clinical Laboratory Standards (NCCLS) broth microdilution assay.[17,18] Compounds were reconstituted in a small volume of sterile DMSO and diluted

Table 3. Antimicrobial Activities of Saponins **1**−**6** and Diosgenin (**7**)

		range of MIC (μg/mL)						
microorganism[a]	ATCC or (Presque Isle)#	1	2	3	4	5	6	7
Cryptococcus neoformans	90112	64	1−2	1−2	1−2	1	2	*b*
Candida albicans	90028	64	2	*b*	2	2	4−8	*b*
Streptococcus pneumoniae	6303	*b*	*b*	32−64	32−64	16−32	*b*	*b*
Micrococcus luteus	(456)	*b*	*b*	*b*	4−8	*b*	*b*	32

[a] The compounds were inactive (at ≤64 μg/mL) against the following organisms: *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli*, *Enterobacter cloacae*, *Stenotrophomonas maltophilia*, *Neisseria gonorrhoeae*. [b] No inhibition at 64 μg/mL

in the appropriate media immediately prior to susceptibility experiments. The MIC was defined as the lowest drug concentration that inhibited all visible growth of the test organism (optically clear). Assays were repeated on separate days. MFCs were determined by subculturing 100 μL from each well with no visible growth in the MIC broth microdilution series onto drug-free plates. The plates were incubated for 48 h, and the MFC defined as the lowest drug concentration that resulted in $\geq$99.9% reduction in the initial inoculum.

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References and Notes

(1) For contribution 533, see: Dang, L. H.; Bettegowda, C.; Agrawal, N.; Cheong, I.; Huso, D.; Frost, P.; Loganzo, F.; Greenberger, L.; Barkoczy, J.; Pettit, G. R.; Smith, A. B.; Gurulingappa, H.; Khan, S.; Parmigiani, G.; Kinzler, K. W.; Zhou, S.; Vogelstein, B. *Cancer Biol. Ther.*, in press.
(2) Batianoff, G. N.; Franks, A. J. *Plant Prot. Q.* **1997**, *12*, 180−186.
(3) (a) González, A. G.; Freire, R.; García-Estrada, M. G.; Salazar, J. A.; Suárez, E. *Tetrahedron* **1972**, *28*, 1289−1297. (b) Gamboa-Angulo, M. M.; Reyes-López, J.; Peña-Rodríguez, L. M. *Phytochemistry* **1996**, *43*, 1079−1081. (c) Woodcock, B. G.; Masinde, E. L. K.; Schnieden, H. *Toxicon* **1982**, *20*, 659−661.
(4) (a) Mimaki, Y.; Inoue, T.; Kuroda, M.; Sashida, Y. *Phytochemistry* **1997**, *44*, 107−111. (b) Mimaki, Y.; Inoue, T.; Kuroda, M.; Sashida, Y. *Phytochemistry* **1996**, *43*, 1325−1331. (c) Kowalczyk, S.; Januszewska, B.; Cymerska, E.; Maslowski, P. *Physiol. Plant.* **1984**, *60*, 31−37.
(5) Staib, F.; Mishra, S. K.; Fromtling, R. A.; Blisse, A.; Kleinkauf, H.; Mahmutoglu, I.; Mueller, K. D.; Salnikow, J.; Hoehne, G.; Schwarz, H. *Arzneim.-Forsch.* **1983**, *33*, 84−87.
(6) Bligh, E. G.; Dyer, W. J. *Can. J. Biochem. Physiol.* **1959**, *37*, 911−917.
(7) Han, X.; Yu, H.; Liu, X.; Bao, X. *Chin. J. Magn. Reson.* **1999**, *16*, 541−546.
(8) Sharma, S. C.; Sharma, R.; Kumar, R. *Phytochemistry* **1983**, *22*, 2259−2262.
(9) Mimaki, Y.; Satou, T.; Ohmura, M.; Sashida, Y. *Nat. Med.* (Tokyo) **1996**, *50*, 308.
(10) Krenn, L.; Kopp, B.; Bamberger, M.; Brustmann, E.; Kubelka, W. *Nat. Prod. Lett*. **1993**, *3*, 139−143.
(11) Blunden, G.; Patel, A. V.; Crabb, T. A. *Phytochemistry* **1990**, *29*, 1771−1780.
(12) (a) Yokosuka, A.; Mimaki, Y.; Sashida, Y. *Phytochemistry* **2002**, *61*, 73−78. (b) Yu, W.; Jin, Z. *J. Am. Chem. Soc.* **2002**, *124*, 6576−6583. (c) Mimaki, Y.; Yokosuka, A.; Kuroda, M.; Sashida, Y. *Biol. Pharm. Bull*. **2001**, *24*, 1286−1289. (d) Dong, M.; Feng, X.-Z.; Wu, L.-J.; Wang, B.-X.; Ikejima, T. *Planta Med.* **2001**, *67*, 853−857. (e) Hufford, C. D.; Liu, S.; Clark, A. M. *J. Nat. Prod.* **1988**, *51*, 94- 98.
(13) (a) Furuya, S.; Takayama, F.; Mimaki, Y.; Sashida, Y.; Satoh, K.; Sakagami, H. *Anticancer Res.* **2000**, *20*, 4189−4194. (b) Furuya, S.; Takayama, F.; Mimaki, Y.; Sashida, Y.; Satoh, K.; Sakagami, H. *Anticancer Res*. **2001**, *21*, 959−964.
(14) Hostettmann, K.; Marston, A. *Saponins*; Cambridge University Press: New York, 1995; pp 287−306.
(15) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A. *J. Natl. Cancer Inst*. **1991**, *83*, 757−766.
(16) Suffness, M.; Douros, J. In *Methods in Cancer Research;* DeVita, V. T., Busch, H., Eds.; Academic Press: New York, 1979; Vol. XVI, Chapter III, pp 73−126.
(17) NCCLS. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard−Second Edition.* NCCLS document M27-A2 (ISBN 1-56238-469-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, 2002.
(18) NCCLS. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard−Fifth Edition.* NCCLS document M7-A5 (ISBN 1-56238-394-9). NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, 2000.

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