Antimycotic Spirostanol Saponins from *Solanum hispidum* Leaves and Their Structure−Activity Relationships

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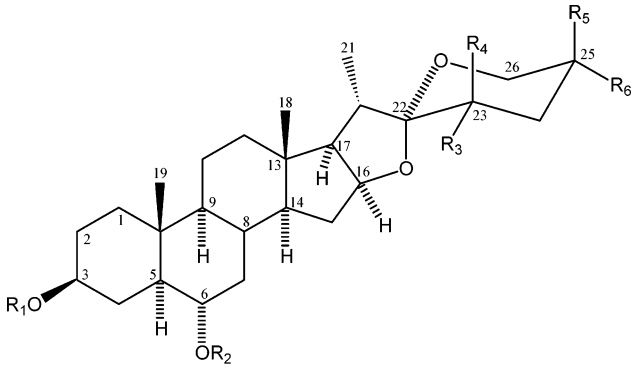
A new spirostanol saponin, together with three known saponins, were isolated from the leaves of *Solanum hispidum*. The structure of the new saponin was elucidated as 6α-*O*-*β*-D-quinovopyranosyl-(25*S*)-5α-spirostan-3*β*-ol (**1**) on the basis of spectroscopic analysis (^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, HMQC, HMBC, and HRFABMS). All of the isolated compounds showed antimycotic activity. The most active compound was 6α-*O*-[*β*-D-xylopyranosyl-(1→3)-*β*-D-quinovopyranosyl]-(25*S*)-5α-spirostan-3*β*-ol (**2**) (MIC = 25 *μ*g/mL against both *Trichophyton mentagrophytes* and *T. rubrum*). The structure−activity relationships of the isolated compounds and those isolated from *S. chrysotrichum* are discussed.

A number of *Solanum* species known as "sosa" including *S. chrysotrichum*, *S. lanceolatum*, and *S. hispidum* are employed in treating several skin mycosis conditions, principally athlete's foot,[1,2] and chemical and pharmacological investigations on *S. chrysotrichum* have demonstrated that the steroidal saponins are the bioactive components.[3,4] *S. hispidum* Pers. (Solanaceae) is a small tree growing in the highlands of Chiapas, México, where the leaves of this species have been well documented as folk medicine for curing symptoms associated with skin mycosis.[5] This species also has been used as a folk remedy for curing ulcers, wounds, and burns in Guatemala and India.[6,7] With regard to the constituents of *S. hispidum*, some steroidal sapogenins, e.g., hispigenin, neochlorogenin, and paniculogenin, have been isolated.[8−12] However, until now, no chemical study has been reported on the saponin fraction. As a part of a study on the efficacy of topical herbal remedies combined with a search for novel structures with antimycotic activity, we have initiated the chemical and pharmacological investigation on this plant.

Using bioactivity-directed isolation procedures, four spirostanol saponins including the new spirostanol saponin 6α-*O*-*β*-D-quinovopyranosyl-(25*S*)-5α-spirostan-3*β*-ol (**1**) and the known 6α-*O*-[*β*-D-xylopyranosyl-(1→3)-*β*-D-quinovopyranosyl]-(25*S*)-5α-spirostan-3*β*-ol (**2**), 6α-*O*-[*β*-D-xylopyranosyl-(1→3)-*β*-D-quinovopyranosyl]-(25*S*)-5α-spirostan-3*β*,23*β*-ol (**3**), and 6α-*O*-[*β*-D-xylopyranosyl-(1→3)-*β*-D-quinovopyranosyl]-(25*S*)-5α-spirostan-3*β*,23α-ol (**4**), were isolated from *S. hispidum* leaves. Compounds **2** and **3** were previously isolated from *S. torvum*,[13] and compound **4** from *S. paniculatum*.[14] In this paper we provide an account of the isolation and structural elucidation of the new saponin, as well as the NMR data of saponin **4** that have not been described. Also we describe the antimycotic activity of all four saponins against *Trichophyton mentagrophytes*, *T. rubrum*, *Microsporum gypseum*, and *Candida albicans*, as well as their structure−activity relationships in comparison with those isolated from *S. chrysotrichum*, whose standardized phytodrug was proved to be effective to treat *Tinea pedis*.[15]



	R_1	R_2	R_3	R_4	R_5	R_6
1	H	Qui	H	H	Me	H
1a	Ac	Qui $(OAc)_3$	H	H	Me	H
2	H	Xyl(1→3)-Qui	H	H	Me	H
2a	Ac	Xyl(1→3)-Qui $(OAc)_5$	H	H	Me	H
3	H	Xyl(1→3)-Qui	H	OH	Me	H
3a	Ac	Xyl(1→3)-Qui $(OAc)_5$	H	OAc	Me	H
4	H	Xyl(1→3)-Qui	OH	H	Me	H
4a	Ac	Xyl(1→3)-Qui $(OAc)_5$	OAc	H	Me	H
5	H	Xyl(1→3)-Qui	H	H	H	Me
6	H	Xyl	H	H	H	Me
7	H	Qui	H	H	H	Me
8	H	Rha(1→3)-Qui	H	H	H	Me
9	H	Rha(1→3)-Qui	OH	H	H	Me

Results and Discussion

The MeOH extract of the leaves of *S. hispidum* was partitioned between CH_2Cl_2 and H_2O, to yield an antimycotic CH_2Cl_2-soluble fraction and an inactive aqueous fraction. The active extract, mainly composed of steroidal saponins, was subjected to vacuum liquid chromatography on silica gel eluted with *n*-hexane−CH_2Cl_2−MeOH. The most active fraction was purified by column chromatography and RP-HPLC to give four spirostan saponins (**1**−**4**), which were characterized by means of spectroscopic data interpretation of the natural products and their peracetate derivatives (**1a**−**4a**).

Compound **1** was obtained as an amorphous solid, $[\alpha]^{25}_D$ −18° (*c* 0.002, Py). In the positive-ion FABMS of **1**, quasimolecular ion peaks at *m/z* 601 $[M + Na]^+$ and 579 $[M + H]^+$ were observed, and HRFABMS analysis revealed the molecular formula to be $C_{33}H_{54}O_8$ (*m/z* = 579.7982 $[M + H]^+$). Acid hydrolysis of **1** yielded quinovose (identified

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Table 1. ^{1}H NMR Spectral Data of Compounds **1**, **4** (300 MHz, Py-d_5), and **1a**−**4a** (500 MHz, $CDCl_3$)

	1	1a	2a	3a	4	4a
H-3	3.75, m	4.62, m	4.62, dddd (11.0, 11.0, 6.5)	4.62, m	3.78, m	4.62, dddd (11.0, 11.0, 6.0, 5.5)
H-6	3.70−3.74, m	3.25, m	3.21, ddd (10.5, 10.5, 4.5)	3.22, m	3.71−3.75, m	3.21, ddd (10.5, 10.5, 4.5)
H-16	4.46 dd (7.6, 7.4)	4.40, ddd (7.2, 7.2, 6.8)	4.40, ddd (7.5, 7.5, 7.5)	4.48, ddd (7.5, 7.0, 7.0)	4.57, dd (6.9, 6.6)	4.46, ddd (7.5, 7.5, 7.5)
H-18	0.82, s	0.75, s	0.74, s	0.78, s	0.81, s	0.78, s
H-19	0.86, s	0.85, s	0.84, s	0.84, s	0.98, s	0.84, s
H-21	1.13, d (6.7)	0.98, d (6.4)	0.98, d (6.5)	0.97, d (7.0)	1.12, d (7.2)	0.97, d (7.0)
H-23				4.77, t (3.0)	3.96−4.06, m	5.05, dd (12.0, 5.0)
H-26α	3.91, m	3.94, dd (11.2, 2.8)	3.93, dd (11.0, 3.0)	3.91, dd (11.0, 2.5)	4.08−4.18, m	3.91, dd (11.0, 3.0)
H-26*β*	3.32, d (10.5)	3.30, d (11.2)	3.29, d (11.0)	3.25, d (11.0)	3.29, d (11.1)	3.25, d (11.0)
H-27	1.06, d (7.0)	1.07, d (7.2)	1.07, d (7.0)	1.11, d (6.8)	1.18 d (6.9)	1.11, d (7.0)
Qui-1′	4.81, d (7.4)	4.47, d (8)	4.32, d (8.5)	4.32, d (8.5)	4.77, d (7.2)	4.32, d (8.0)
2′	4.05, m	4.93, dd (10.0)	4.93, dd (9.5, 8.5)	4.93, dd (9.5, 8.5)	3.96−4.06, m	4.93, dd (10.0, 8.0)
3′	4.05, m	5.12, t (9.6)	3.72, t (9.5)	3.72, t (9.5)	3.96−4.06, m	3.73, t (10.0)
4′	3.58, m	4.81, t (9.6)	4.70, t (9.5)	4.70, t (9.5)	3.61, m	4.70, t (10.0)
5′	3.70, m	3.52, m	3.45, m	3.45, m	3.71−3.75 m	3.44, m
6′	1.57, d (6.4)	1.20, d (6)	1.17, d (6.0)	1.18, d (6)	1.58, d (6)	1.18, d (6.0)
Xyl-1″			4.55, d (6.0)	4.55, d (6.0)	5.18, d (7.8)	4.55, d (6.0)
2″			4.74, dd (7.5, 6.0)	4.74, dd (7.0, 6.0)	3.96−4.06, m	4.74, dd (7.5, 6.0)
3″			5.01, t (7.5)	5.01, t (7)	4.08−4.18, m	5.02, t (7.5)
4″			4.83, ddd (7.5, 7.0, 4.5)	4.83, ddd (7.0, 7.0, 4.5)	4.08−4.18, m	4.83, ddd (7.5, 7.5, 4.5)
5α″			3.35, dd (12.0, 7.5)	3.35, dd (12.0, 7.0)	3.71−3.75 m	3.35, dd (11.5, 7.5)
5*β*″			4.09, dd (12.0, 4.5)	4.09, dd (12.0, 4.5)	4.29, dd (11.1, 4.5)	4.09, dd (11.5, 4.5)

by co-TLC with an authentic sample) and an aglycone which was identified as neochlorogenin [(25*S*)-5α-spirostan-3*β*,6α-diol][10,16] on the basis of the DEPT, NOESY, HMQC, and HMBC spectra of its tetraacetate derivative (**1a**). The ^{1}H NMR spectrum of **1a** (Table 1) displayed the following representative signals: four steroid methyl protons at δ 0.75 (s), 0.85 (s), 0.98 (d, J = 6.4 Hz), and 1.07 (d, J = 7.2 Hz), one anomeric proton at δ 4.47 (d, J = 8.0 Hz), and the methyl protons of a 6-deoxyhexopyranose at δ 1.20 (d, J = 6.0 Hz). The ^{13}C NMR spectrum of **1a** (Table 2) showed a total of 29 carbons for the acetylated aglycone including four methyl, 10 methylene, and 10 methine groups. Twelve additional carbons belonged to a sugar unit. The signals of C-22, C-25, and C-26 (δ 109.91, 27.24, and 65.34) and also of C-5, C-6, and C-7 (δ 49.79, 80.85, and 39.87) allowed the identification of neochlorogenin as the aglycone of **1a**.[10,16] The 25*S* configuration of **1a** was confirmed by the ^{1}H NMR parameters of the H-26 methylene protons at δ 3.94 (dd, $J_{26ax,26eq}$ = 11.2 Hz, $J_{26ax,25eq}$ = 2.8 Hz) and 3.30 (d, $J_{26eq,26ax}$ = 11.2 Hz). Full assignments of the proton and carbon resonances of the aglycone were secured from the COSY, NOESY, HMQC, and HMBC spectral data (Tables 1 and 2). Regarding the sugar portion, evaluation of spin−spin coupling and chemical shifts allowed the identification of *β*-D-quinovopyranose as the sugar residue. The position of the sugar residue in **1a** was defined unambiguously to be at C-6 due to the long-range correlation between C-6 (δ 80.85) of the aglycone and H-1 (δ 4.47) of the quinovopyranosyl unit, which was confirmed from the interaction between qui H-1 (δ 4.47)/aglycon H-6 (δ 3.26) observed in the NOESY spectrum. Moreover, the α-configuration of the C-6-*O*-quinovopyranose moiety was evident from the NOESY interaction observed between H-6 (δ 3.25) and H-19 (δ 0.85). On the basis of this information, the natural product was identified as 6α-*O*-*β*-D-quinovopyranosyl-(25*S*)-5α-spirostan-3*β*-ol (**1**), a new spirostan saponin.

Compound **4** was obtained as a white amorphous powder which showed the molecular ion $[M]^+$ at *m*/*z* 726.3 in the EIMS. From this, together with its ^{13}C NMR data (Table 2), the molecular formula was determined as $C_{38}H_{62}O_{13}$. The ^{13}C NMR spectrum of **4** showed a total of 27 carbons for the aglycone including four methyl, nine methylene, and 11 methine groups. Eleven additional carbons belonged to sugar units. These sugar moieties were identified as quinovose and xylose by direct comparison (co-chromatography) of the hydrolysis product of **4** and comparison of the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2). Moreover, the presence of these sugar units in the peracetate derivative (**4a**) was deduced from the ^{1}H NMR anomeric proton signals at δ 4.32 (d, J = 8 Hz) and 4.55 (d, J = 6 Hz), which were compatible with the ^{13}C NMR signals for anomeric ether-linked carbons at δ 102.12 and 100.92, respectively. Comparison of the ^{13}C signals of the aglycone of **4a** (Table 2) showed that most of the signals were in good agreement with literature data for paniculogenin.[10,15] The interglycosidic linkage of the two sugar units was derived from the HMBC correlations and NOESY interactions. A cross-peak due to long-range correlations between the anomeric proton of xylose at δ 4.55 (d, J = 6.0 Hz) and C-3 of the quinovopyranosyl unit at δ 80.65 indicated that xylopyranose was the terminal saccharide unit. The position of the sugar residue in **4a** was defined unambiguously to be at C-6 due to the long-range correlation observed between C-6 (δ 80.43) of the aglycone and H-1 (δ 4.32) of the quinovopyranosyl unit. On the basis of all this evidence, this compound was identified as 6α-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-quinovopyranosyl-(25*S*)-5α-spirostan-3*β*,23α-ol (**4**). This compound was previously isolated from *S. paniculatum*,[14] and the NMR data of the natural product (**4**) as well as of its peracetate derivative (**4a**) are described for the first time.

All the saponins isolated from *S. hispidum* showed toxicity in different degrees against the dermatophytes *T. mentagrophytes* and *T. rubrum*, but not against *A. niger* and *C. albicans* (Table 3). Several preliminary conclusions can be made about the importance of certain functional

Table 2. ^{13}C NMR Spectral Data of Compounds **1** and **4** (75 MHz, Py-d_5) and **1a**−**4a** (125 MHz, $CDCl_3$)

	1	1a	2a	3a	4	4a
C-1	37.70	36.92	36.74	36.69	37.69	36.69
C-2	32.08	27.31	27.15	27.08	32.08	27.06
C-3	70.58	73.43	73.32	73.28	70.62	73.27
C-4	33.10	28.41	28.27	28.20	33.19	28.18
C-5	51.25	49.79	49.57	49.54	51.29	49.52
C-6	78.12	80.85	80.70	80.48	79.55	80.43
C-7	41.37	39.87	39.71	39.20	41.47	39.55
C-8	34.15	34.04	33.87	33.94	34.14	33.87
C-9	53.86	53.40	53.25	53.17	53.97	53.13
C-10	36.75	36.63	36.43	36.36	36.23	36.39
C-11	21.22	20.90	20.90	20.67	21.34	20.75
C-12	40.38	40.23	40.01	39.91	40.47	39.96
C-13	40.04	40.73	40.54	40.89	41.38	40.96
C-14	56.36	56.01	55.85	55.96	56.40	55.81
C-15	32.05	31.35	31.67	29.67	32.17	29.67
C-16	81.11	80.94	80.74	81.48	81.72	81.18
C-17	62.80	62.09	61.98	63.72	62.45	61.30
C-18	16.58	16.65	16.45	16.13	16.87	16.05
C-19	13.55	13.55	13.37	13.32	13.61	13.31
C-20	42.47	42.38	42.22	40.50	37.81	36.39
C-21	14.79	14.51	14.30	15.90	14.54	13.89
C-22	109.63	109.91	109.68	107.62	112.37	109.21
C-23	26.34	26.14	25.99	71.84	63.37	65.47
C-24	26.16	25.98	25.82	30.03	36.11	31.56
C-25	27.50	27.24	27.07	25.77	30.00	29.52
C-26	67.07	65.34	65.14	64.50	64.29	64.13
C-27	16.25	16.25	16.06	19.30	17.57	17.13
Qui-1′	105.12	102.15	102.11	102.13	105.26	102.12
2′	75.80	73.49	73.24	73.16	74.75	73.18
3′	78.20	73.22	80.58	80.67	87.74	80.65
4′	76.67	72.34	73.82	73.73	75.30	73.75
5′	72.25	69.96	69.79	69.70	72.31	69.70
6′	18.54	17.62	17.51	17.50	18.59	17.46
Xyl-1″			100.93	100.93	106.49	100.92
2″			69.86	69.81	74.82	69.81
3″			70.29	70.26	78.22	70.25
4″			68.55	68.50	70.91	68.50
5″			61.43	61.41	67.43	61.41

Table 3. Antimycotic Activity of Extracts and Compounds Isolated from *Solanum hispidum* and *S. chrysotrichum* (MIC values; μg/mL)

	T. m.[a]	*T. r.*[b]	*A. n.*[c]	*C. a.*[d]
MeOH extract	10000	nt[f]	nt	nt
CH_2Cl_2 fraction	8000	nt	nt	nt
fraction 1	>10000	nt	nt	nt
fraction 2	>10000	nt	nt	nt
fraction 3	>10000	nt	nt	nt
fraction 4	400	400	800	800
1	25	50	>400	>400
2	25	25	>400	>400
3	100	100	>400	>400
4	25	25	>400	>400
5	12.5	12.5	100	200
6[e]	100	100	>400	200
7	25	50	>400	200
8[e]	200	50	400	200
9[e]	200	200	200	200
Nystatin	nt[f]	nt[f]	nt[f]	8
Miconazole	4	4	8	nt[f]

[a] *T. m.* = *Trichophyton mentagrophytes*. [b] *T. r.* = *Trichophyton rubrum*. [c] *A. n.* = *Aspergillus niger*. [d] *C. a.* = *Candida albicans*. [e] Taken from ref 4. [f] nt = not tested.

groups in the studied compounds for their antimycotic activity. A comparison of the activity of **1** and **2** shows that the first lacks the xylopyranose unit attached at C-3 of the quinovopyranose and is slightly less toxic against *T. rubrum*, but has the same activity against *T. mentagrophytes* than **2**. Comparison of the activities of **2**, **3**, and **4**, which have identical structure of the disaccharide chain but different aglycone, shows that the presence of an additional 23α-hydroxy in **4** has no effect on the activity against the dermatophytes *T. mentagrophytes* and *T. rubrum*, while the presence of the 23*β*-hydroxy in **3** remarkably diminishes the activity against these microorganisms.

Recently we reported the isolation and characterization of five antimycotic saponins from *S. chrysotrichum* (**5**−**9**);[4] these saponins differ from those of *S. hispidum* in the configuration at C-25. *S. hispidum* contains 25*S* spirostane aglycones, while those from *S. chrysotrichum* are 25*R*-spirostanes; therefore, the activities of these compounds against the dermatophytes should be compared. To obtain a meaningful comparison between those two sets of compounds, saponins **5** and **7**, isolated from *S. chrysotrichum*,[4] were included in the same antimycotic assay (vide infra). The MIC values obtained for compounds **5** and **7** were almost identical with those obtained in ref 4; so this allowed us to compare the MIC values of all compounds. The antimycotic activities of the saponins of *S. chrysotrichum* were included in Table 3. Despite the poor activity of the saponins against *C. albicans* and *A. niger*, in general, all the saponins from *S. chrysotrichum* are most active against all the studied microorganisms. The most active compound is **5**, and comparison with **2** shows that change in the orientation of the methyl group at C-25 from equatorial in **5** to axial in **2** diminishes the activity. However, **1** and **7** have similar activities. These results indicate that both the 25*R* configuration and the disaccharide moiety (Xyl-(1→3)-Qui) at C-6 of the aglycone must be present for the activity. This is evident comparing the activities of **8** and **9**, which have different disaccharide chains than **5** and show much less activity. Finally, comparison of the activities of **4** and **9** shows that **4** is more active than **9** due to the presence of the disaccharide unit (Xyl-(1→3)-Qui) in **4**, which indicates that the presence of this disaccharide is more important than the configuration at C-25. Comparison of **3** and **9** shows that **3** is more active due to the presence of the (Xyl-(1→3)-Qui) moiety, but is less active than **4** due to the orientation of the hydroxyl group at C-23.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 digital polarimeter at 25 °C. IR spectra were recorded on a Bruker Vector 22 FTIR. All NMR spectra were recorded on Varian VXR-300 and Varian Unity Plus-500 at 300 and 500 MHz, respectively, for ^1H NMR, $^1H-^1H$ COSY, HMQC, and HMBC, and 75 and 125 MHz for ^{13}C NMR and ^{13}C DEPT, using pyridine-d_5 and $CDCl_3$ as solvents. Chemical shifts are reported in ppm relative to TMS. EIMS, FABMS, and HRFABMS were performed using Thermofinnigan Polaris Q, Hewlett-Packard 5985-B, and JEOL-AX 505 HA mass spectrometers, respectively. HPLC (Waters) was performed using a Merck Lichrospher C18 124 × 4 mm, 5 μm column.

Plant Material. Leaves of *S. hispidum* (900 g) were collected in the highlands of Chiapas, Mexico, on September 26, 1999, and identified by Biol. Abigaíl Aguilar from the IMSS-Mexico Herbarium (IMSSM), where voucher specimens (code number: 10122) have been deposited.

Extraction and Isolation. Leaves were extracted by maceration at room temperature three times with MeOH (5 L) and concentrated to dryness under reduced pressure. The MeOH extract (122.17 g) was suspended in H_2O and partitioned with CH_2Cl_2 to yield an active CH_2Cl_2-soluble fraction and an inactive aqueous-soluble fraction. The CH_2Cl_2 extract (14 g, 1.55%) was fractionated on a vacuum liquid chromatography column (VLC: 70 g silica gel) eluting with *n*-hexane−CH_2Cl_2−MeOH mixtures of increasing polarity to yield four fractions: fraction 1, 4.0 g (100:0:0); fraction 2, 2.85 g

(95:5:0); fraction 3, 1.32 g (9:1:0); fraction 4, 1.0 g (0:0:100). The most active fraction (fraction 4) was chromatographed on silica gel (30 g) with a gradient mixture of CH_2Cl_2−MeOH to give two fractions. Fraction 1 (28 mg), eluted with CH_2Cl_2−MeOH (95:5), was subjected to reversed-phase HPLC on a Lichrospher C18 column (5 μm, 124 × 4 mm) with CH_3CN−H_2O (55:45) as the isocratic eluent system and a flow rate of 1 mL/min, to afford 7 mg of compound **1** (t_R 5.69 min). Fraction 2 (0.384 g), eluted with CH_2Cl_2−MeOH (9:1), was further chromatographed on a C18 Sep-pack cartridge (Waters, 35 cm^3, 10 g) with CH_3CN−H_2O (3:2) as the isocratic eluent system to give 16.1 mg of **2**[13] and 122 mg of a mixture of two compounds, which were separated by preparative C18 TLC, using CH_3CN−H_2O (7:3) as eluent to yield 21.2 mg of **3**[13] (R_f = 0.32) and 27.4 mg of **4**[14] (R_f = 0.28).

Isolation of Compounds 5−9. Saponins **5**−**9** were obtained from the MeOH extract of *S. chrysotrichum* as described in ref 4.

Acid Hydrolysis of Saponins 1 and 4. Saponins **1** (2 mg) and **4** (2.5 mg) in 0.5 M HCl (dioxane−H_2O, 1:1, 5 mL) were refluxed separately on a water bath at 100 °C for 2 h. After cooling, the nonpolar reaction product was separated by precipitation with ice (5 g) and filtration. The aqueous layer was neutralized with NH_4OH and reduced to dryness by lyophilization. The sugars were analyzed by silica gel TLC [EtOAc−MeOH−H_2O−AcOH (11:2:2:2)] by comparison with standard sugars.

Acetylation of Compounds 1−4. Saponins **1** (5 mg), **2** (10 mg), **3** (12 mg), and **4** (13 mg) were each treated with Ac_2O (1 mL) and pyridine (0.5 mL) for 3 h. Extraction with $CHCl_3$, after addition of H_2O (2 mL) into the reaction mixture and crystallization from *n*-hexane−EtOAc, afforded the peracetate derivatives **1a** (6 mg), **2a** (12 mg), **3a** (15 mg), and **4a** (17 mg).

Saponin 1: white amorphous powder; mp 190−194 °C; $[\alpha]^{25}_D$ −18° (*c* 0.002, Py); IR (KBr) ν_{max} 3500−3300 (OH), 2925 (CH) cm^{-1}; ^1H NMR (Py-d_5, 300 MHz), see Table 1; ^{13}C NMR (Py-d_5, 300 MHz), see Table 2; FABMS *m/z* 601 $[M + Na]^+$, 579 $[M + H]^+$, 431 $[M - qui]^+$; HRFABMS *m/z* 579.7982 (calcd for $C_{33}H_{55}O_8$, 579.7933).

Compound 1a: colorless powder; mp 52−55 °C; $[\alpha]^{25}_D$ −11° (*c* 0.2, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2922, 1757 (ester), 1452, 1376, 1050 (C−O−C), 757 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$), see Table 2.

Saponin 2: colorless powder; mp 264−265 °C; $[\alpha]^{25}_D$ −32° (*c* 0.2, Py) [lit.[13] $[\alpha]^{27}_D$ −18.9° (*c* 2.0, MeOH)]; IR (KBr) ν_{max} 3423 (OH), 2929 (CH), 1077−1047 (C−O−C) cm^{-1}; ^1H NMR (Py-d_5, 300 MHz) δ 0.82 (3H, s, H-18), 0.87 (3H, s, H-19), 1.07 (3H, d, *J* = 6.9, H-27), 1.13 (3H, d, *J* = 6.6 Hz, H-21), 1.55 (3H, d, *J* = 6 Hz, qui H-6), 2.51 (1H, brd, *J* = 12.3 Hz, H-7), 3.17 (1H, brd, *J* = 11.7 Hz, H-4), 3.34 (1H, d, *J* = 10.5 Hz, H-26), 4.06 (2H, m, qui H-2, qui H-3), 4.48 (1H, dd, *J* = 7.5, 7.2 Hz, H-16), 4.80 (1H, d, *J* = 7.2 Hz, qui H-1), 5.20 (1H, d, *J* = 7.5 Hz, xyl H-1).

Compound 2a: colorless powder; mp 82−85 °C; $[\alpha]^{25}_D$ −12.7° (*c* 0.72, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2918, 1749 (ester), 1455, 1370, 1055 (C−O−C), 758 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2.

Saponin 3: white amorphous powder; mp 205−210 °C; $[\alpha]^{25}_D$ −43.61° (*c* 0.02, Py) [lit.[13] $[\alpha]^{27}_D$ −30.7° (*c* 2.70, MeOH)]; IR (KBr) 3436 (OH), 2929 (CH), 1077−1049 (C−O−C) cm^{-1}; ^1H NMR (Py-d_5, 300 MHz) δ 0.82 (3H, s, H-18), 0.86 (3H, s, H-19), 1.54 (3H, d, *J* = 6.5 Hz, H-27), 1.57 (3H, d, *J* = 6 Hz, qui H-6), 3.54 (1H, d, *J* = 10.5 Hz, H-26), 3.61 (1H, m, qui H-4), 3.70 (3H, m, H-6, qui H-5, xyl H-5), 3.75 (1H, m, H-3), 4.03 (4H, m, H-23, qui H-2, qui H-3, xyl H-2), 4.16 (3H, m, H-26, xyl H-3, xyl H-4), 4.30 (1H, dd, *J* = 10.8, 4.8 Hz, xyl H-5), 4.63 (1H, dd, *J* = 7.8, 7.5 Hz, H-16), 4.80 (1H, d, *J* = 6.9 Hz, qui H-1), 5.21 (1H, d, *J* = 7.5 Hz, xyl H-1).

Compound 3a: white amorphous powder; mp 142−145 °C; $[\alpha]^{25}_D$ −45.3° (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2923, 1756 (ester), 1452, 1376, 1050 (C−O−C), 757 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2.

Saponin 4: white amorphous powder; mp 264−265 °C (lit.[14] 262−264 °C); $[\alpha]^{25}_D$ −60° (c 0.2, Py) [lit.[14] $[\alpha]^{27}_D$ −61.2 (*c* 1.1, Py)]; IR (KBr) ν_{max} 3422 (OH), 2928 (CH), 1075−1047 (C−O−C) cm^{-1}; ^1H NMR (Py-d_5, 300 MHz), see Table 1; ^{13}C NMR (Py-d_5, 300 MHz), see Table 2; IEMS *m/z* 726.3 $[M]^+$ (1.2), 593.2 $[M - Xyl]^+$ (0.5), 429 $[M - Xyl - Qui - H_2O]^+$ (4), 396.3 (14), 283.2 (40), 161.1 (51), 133.1 (100), 107.1 (63).

Compound 4a: white amorphous powder; mp 129−132 °C; $[\alpha]^{25}_D$ −19° (*c* 0.25, $CHCl_3$); IR (KBr) ν_{max} 2922, 1750 (ester), 1451, 1370, 1050 (C−O−C), 756 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2.

Antifungal Activity. Antimycotic evaluation of *S. hispidum* extracts and isolated compounds was performed following the conventional agar dilution assay procedure[17] for activity against *T. mentagrophytes* (ATCC 28185), *T. rubrum* (ATCC 28188), *A. niger* (ATCC 10335), and *Candida albicans* (ATCC 10231). The filamentous fungi were maintained on potato dextrose agar (PDA) at 27 °C. Sabouraud glucose agar (SGA) was used to maintain *C. albicans* and as assay medium. The stock solution of the extracts, pure compounds (**1**−**4** from *S. hispidum*, and **5** and **7** from *S. chrysotrichum*), and reference compounds (Nystatin and Miconazole) in DMSO were diluted to give serial 2-fold dilutions in the range 10−1.25 mg/mL, 400−3.125 μg/mL, and 128−1 μg/mL, respectively. Those were added to the medium. The final concentration of DMSO did not exceed 2%. A final inoculum of 10^5 cell/mL for *C. albicans* and 10^6 spore/mL for the filamentous fungi counted in a hematocytometer was applied to the top of solidified agar with a loop calibrated to deliver 0.010 mL. Experiments were done in triplicate. Plates were incubated at 29 °C for 72, 24, and 48 h for filamentous fungi, *C. albicans*, and *A. niger*, respectively. Minimum inhibitory concentrations (MICs) were defined as the lowest concentration that inhibited visible growth on agar.

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