Five New Steroidal Saponins from *Solanum chrysotrichum* Leaves and Their Antimycotic Activity

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Using bioactivity-directed isolation procedures, five new spirostan saponins and two sterol glycosides have been isolated from *Solanum chrysotrichum* leaves. The structure of these compounds was established based upon spectroscopic measurements, especially 1D and 2D NMR data of their peracetate derivatives. These compounds showed antimycotic activity. The most active compound is 6α-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-quinovopyranosyl-(25*R*)-5α-spirostan-3*β*,23α-ol (**2**) (MIC =12.5, 12.5, 100, and 200 *μ*g/mL against *Trichophyton mentagrophytes, T. rubrum, Aspergillis niger,* and *Candida albicans*, respectively).

Solanum chrysotrichum Schldh. (Solanaceae), commonly known as "sosa", is common in a limited area of the highlands of Chiapas.[1,2] A previous field ethnobotanical study carried out in 200 small rural communities determined that dried or fresh leaves of *S. chrysotrichum* are used for the treatment of resistant skin mycosis, being particularly recommended to cure *Tinea pedis*, and in the treatment of abscesses, sores, wounds, burns, and other similar lesions.[3]

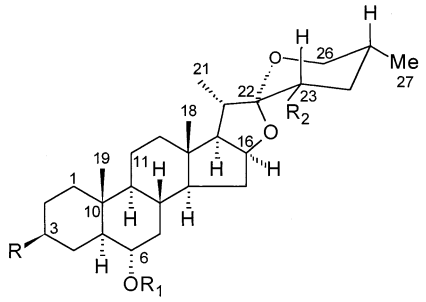
Studies performed with the methanol extract of the leaves demonstrated its in vitro antifungal properties against *Trichophyton mentagrophytes, Trichophyton rubrum*, and *Microsporum gypseum*, and clinical trials demonstrated its efficiency against *Tinea pedis*.[3] A previous phytochemical study on this plant allowed the isolation of a saponin-rich fraction with antimycotic activity against *T. mentagrophytes*, from which the major antimycotic spirostan saponin named SC-1 (**1**) was isolated.[4] This paper reports the isolation and structural elucidation of five additional saponins (SC-2 to SC-6) from the antimycotic saponin-rich fraction of this plant.

Results and Discussion

The MeOH extract of the leaves of *S. chrysotrichum* was partitioned between chloroform and water, to yield an antimycotic chloroform-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−$CHCl_3$−MeOH. The most active fraction was purified by column chromatography and RP-HPLC to give five new spirostan saponins (**2**−**6**), which were characterized as their peracetate derivatives (**2a**−**6a**). Two known compounds were also isolated and identified as 3-*O*-*β*-D-sitosterol glycoside and 3-*O*-*β*-D-stigmasterol glycoside by comparison of their spectroscopic data with those previously described.[5,6]

Their ^{1}H and ^{13}C NMR spectra indicated that saponins **2**−**5** consist of identical aglycone portions but with different saccharide chains. Acid hydrolysis of **2**−**5** afforded chlorogenin (**7**, (25*R*)-5α-spirostan-3*β*,6α-diol), which was identified by comparing its ^{1}H, ^{13}C NMR and mass spectra with those in the literature.[7−9] The hydrolysis product **7** showed a molecular ion $[M]^+$ at *m*/*z* 432 ($C_{27}H_{44}O_4$) and a characteristic fragment ion at *m*/*z* 139 ($C_9H_{15}O$) arising from the spiroketal moiety in the EI-mass spectrum.[10] The ^{1}H NMR spectrum of **7** showed C-18, C-19, C-21, and C-27 methyl groups at *δ* 0.74 (s), 0.83 (s) 1.31 (d), and 1.31 (d), respectively, and 27 carbon signals comprised of those ascribable to the signals of C-3 (70.90), C-6 (68.52), C-16 (81.01), C-17 (62.83), C-22 (109.18), and C-26 (66.76) observed in the ^{13}C NMR spectrum.[8] Most of the signals of the peracetate aglycone of **2a**−**5a** could be assigned through ^2J and ^3J connectivities from the two angular and the two secondary methyl proton resonances in the HMBC spectrum (Tables 1 and 2).

Compound **2a** was obtained as a white amorphous powder after acetylation of the natural product SC-2 (**2**).



	R	R_1	R_2
1	Qui (1→6)-Glc-((1→6)-Glc-O	H	H
2	OH	Xyl (1→3)-Qui	H
2a	OAc	Xyl (1→3)-Qui $(OAc)_5$	H
3	OH	Xyl	H
3a	OAc	Xyl $(OAc)_3$	H
4	OH	Qui	H
4a	OAc	Qui $(OAc)_3$	H
5	OH	Rha (1→3)-Qui	H
5a	OAc	Rha (1→3)-Qui $(OAc)_5$	H
6	OH	Rha (1→3)-Qui	OH
6a	OAc	Rha (1→3)-Qui $(OAc)_5$	OAc
7	OH	H	H
8	OH	H	OH
8a	OAc	Ac	OAc

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Table 1. ^{1}H NMR Spectral Data of Compounds **2a**−**6a** (400 MHz, $CDCl_3$)

position	**2a**	**3a**	**4a**	**5a**	**6a**
H-1	1.63 0.99	1.65 1.06	1.63 1.06	1.62 0.99	1.05 1.7
H-2	1.82 1.44	1.65 1.21	1.82 1.44	1.65 1.21	1.46 1.92
H-3	4.61 dddd	4.62 dddd	4.62 dddd	4.62 dddd	4.63 dddd
	(11, 10.5, 6, 5.5)	(11, 10, 6, 5.5)	(11, 10.5, 6, 5)	(11, 10.5, 6, 5)	(10.5, 10, 6, 5)
H-4	2.07 1.2	1.61	2.07 1.19	2.05 1.2	1.25 2.09
H-5	1.21	1.75	1.25	1.25	1.22
H-6	3.21 ddd	3.28 ddd	3.2 ddd	3.22 ddd	3.22 ddd
	(11, 10, 4.5)	(10.2, 10, 4.2)	(11, 10, 4.5)	(11, 10, 4.5)	(10, 10, 5)
H-7	2.1 1.05	2.01 1.07	2.1 1.1	2.51 1.27	1.72 2.01
H-8	1.63	1.47	1.63	1.60	1.61
H-9	0.66	0.67	0.68	0.68	0.69
H-11	1.4 1.2	1.45 1.2	1.4 1.2	1.45 1.2	1.2 1.16
H-12	2.08 1.69	1.99 1.61	2.08 1.69	2.01 1.68	1.68 2.08
H-14	1.2 1.15	1.21 1.07	1.19 1.06	1.2 1.14	1.14 1.23
H-15	1.99 1.2	2.01 1.25	1.99 1.20	2.01 1.2	2.05
H-16	4.39 ddd	4.39 ddd	4.39 ddd	4.39 ddd	4.46 ddd
	(7.5,7.4,7)	(7.5, 7.4, 7)	(7.5, 7.4, 7)	(7.5, 7.4, 7)	(7.5, 7.4, 7)
H-17	1.68 dd	1.65 dd	1.77 dd	1.68 dd	0.69 dd
	(8.4, 6.8)	(8.4, 6.8)	(8.4, 6.8)	(8.4, 6.8)	(8.4, 6.4)
H-18	0.74	0.74	0.75	0.75	0.79
H-19	0.84	0.84	0.85	0.84	0.84
H-20	1.85	1.76	1.85	1.80	2.09
H-21	0.95 d (6.4)	0.95 d (6.4)	0.95 d (6.4)	0.95 d (6.4)	0.94 d (6.4)
H-23	1.2 0.7	1.79 1.47	1.68 1.61	1.7 1.2	4.81 m
H-24	1.2 0.68	1.46 1.4	1.15 0.68	1.2 0.65	1.61 1.70
H-25	1.77	1.6	1.25	1.77	1.88
H-26α	3.37 dd	3.28 dd	3.26 dd	3.36 dd	3.36 dd
	(11, 10)	(11, 10)	(11, 10)	(11, 10)	(11, 10)
H-26*β*	3.45 dd	3.46 dd	3.47 dd	3.45 dd	3.46 dd
	(11, 2.4)	(11, 2.4)	(11, 2.4)	(11, 2.4)	(11, 3)
H-27	0.79 d (6.4)	0.78 d (6.4)	0.78 d (6.4)	0.79 d (6.4)	0.83 d (6.4)
Qui-1′	4.33 d (8.0)		4.47 d (8.0)	4.36 d (8.0)	4.37 d (8.0)
2′	4.94 dd		4.93 dd	5.05 dd	5.06 dd
	(9.6, 8)		(9.6, 8)	(9.6, 8)	(9.6, 8)
3′	3.72 t (9.6)		5.12 t (9.6)	3.6 t (9.6)	3.70 t (9.6)
4′	4.70 t (9.6)		4.81 t (9.6)	4.81 t (9.6)	5.00 t (9.6)
5′	3.44 m		3.52 m	3.41 m	3.44 m
6′	1.18 d (6.4)		1.20 d (6.0)	1.17 d (6.4)	1.18 d (6.4)
Xyl-1″	4.54 d (5.6)	4.45 d (7.2)			
2″	4.74 dd	4.89 dd			
	(7.2, 5.6)	(9.2, 7.2)			
3″	5.01 t (7.2)	5.14 t (9.2)			
4″	4.85 ddd	4.45 ddd			
	(7.3, 7.2, 4.4)	(9.5, 9.2)			
5α″	3.35 dd	3.28 dd			
	(7.2, 1.2)	(11, 9.5)			
5*β*″	4.09 dd	4.07 dd			
	(4.4, 1.2)	(11, 5)			
Rha-1‴				4.77 d (1.2)	4.79 d (1.6)
2‴				5.08 m	5.07 m
3‴				5.1 m	5.08 m
4‴				5.13 dd (10, 9.6)	5.02 dd (10, 9.6)
5‴				3.87 dd (10, 6.4)	3.88 dd (10, 6.4)
6‴				1.13 d (6.4)	1.14 d (6.4)

Positive-ion HRFABMS of **2a** displayed the molecular ion at *m*/*z* 962.4901 in the HRFABMS, corresponding to the molecular formula $C_{50}H_{74}O_{18}$ (Δ +0.0026 mmu of calcd). The ^{13}C NMR spectrum showed 50 signals, of which 29 were assigned to the acetylated spirostanol moiety and 21 to the saccharide portion. These sugar moieties were identified as quinovose and xylose by direct comparison (cochromatography) of the hydrolysis product of **2** 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 (**2a**) was deduced from the ^{1}H NMR anomeric proton signals at δ 4.33 (d, J = 8 Hz) and 4.54 (d, J = 6 Hz), which were compatible with the ^{13}C NMR signals for anomeric ether linked carbons at δ 102.2 and 101.07, respectively. The interglycosidic linkage of the two sugar units was derived from HMBC correlations and NOESY interactions (Table 1). A cross-peak due to long-range correlations between the anomeric proton of xylose at δ 4.45 (d, J = 8.0 Hz) and C-3 of the quinovopyranosyl unit at δ 80.8 indicated that xylopyranose was the terminal saccharide unit. The position of the sugar residue in **2a** was defined unambiguously to be at C-6 due to the long-range correlation observed between C-6 (δ 80.66) of the aglycone and H-1 (δ 4.36) of the quinovopyranosyl unit. On the basis of all this evidence, the natural product was identified as 6α-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-quinovopyranosyl-(25*R*)-5α-spirostan-3*β*-ol (**2**).

Analysis of the NMR data of compounds **3a** and **4a** and comparison with those of **2a** showed **3a** to differ from **2a** only in the absence of the quinovopyranosyl unit linked at C-6 of the aglycone. Instead, it is attached to a xylopyranosyl unit (Tables 1 and 2), while **4a** differs from **2a** in the absence of the terminal xylopyranosyl unit linked at C-3 of the quinovopyranosyl unit (Tables 1 and 2). Therefore, the structures 6α-*O*-*β*-D-xylopyranosyl-(25*R*)-5α-spirostan-3*β*-ol and 6α-*O*-*β*-D-quinovopyranosyl-(25*R*)-5α-spirostan-

Table 2. ^{13}C NMR Spectral Data of Compounds **2a**−**6a** and Aglycone **8** (100 MHz, $CDCl_3$)

	2a	3a	4a	5a	6a	8
C-1	36.67	36.70	36.69	36.67	36.71	38.40
C-2	27.27	27.1	27.05	27.07	27.09	31.32
C-3	73.43	71.5	73.20	73.25	73.19	70.23
C-4	28.41	28.45	28.14	28.14	28.15	33.3
C-5	49.73	49.96	49.55	49.43	49.5	51.08
C-6	80.69	80.60	80.68	80.78	80.52	66.33
C-7	39.85	39.90	39.98	39.62	39.55	40.86
C-8	34.02	33.80	33.7	33.8	33.8	34.99
C-9	53.4	53.43	53.1	53.12	53.1	53.33
C-10	36.9	36.38	36.30	36.39	36.39	37.99
C-11	20.8	20.72	20.81	21.2	20.78	20.44
C-12	40.15	39.60	39.60	39.91	39.91	38.83
C-13	40.72	40.75	40.50	41.51	41.02	39.26
C-14	56.01	56.06	55.76	55.76	55.78	55.47
C-15	31.56	31.30	31.30	31.66	31.65	30.10
C-16	80.82	80.60	80.62	81.74	81.1	80.92
C-17	61.33	62.20	62.04	61.98	61.42	61.08
C-18	17.22	17.10	17.09	17.12	16.09	15.87
C-19	13.55	13.20	13.32	13.36	13.35	12.84
C-20	41.85	41.81	41.60	41.6	36.03	40.52
C-21	14.59	14.49	14.46	14.49	14.09	13.41
C-22	109.36	109.15	109.20	109.24	108.58	110.27
C-23	30.98	30.20	31.65	31.3	68.6	68.29
C-24	28.99	28.70	28.7	28.76	34.0	36.82
C-25	30.43	31.6	30.22	30.24	30.65	35.85
C-26	65.2	66.8	66.82	66.82	65.68	65.34
C-27	16.55	16.40	16.41	16.44	16.4	15.88
Qui-1′	102.23		101.92	102.1	102.17	
2′	73.43		72.11	72.12	72.16	
3′	80.8		72.97	81.93	81.88	
4′	73.98		73.23	74.41	74.43	
5′	69.9		69.72	69.8	69.87	
6′	17.66		17.39	17.49	17.44	
Xyl-1″	101.07	102.39				
2″	70.09	71.74				
3″	70.54	72.26				
4″	68.75	69.15				
5″	61.63	62.24				
Rha-1‴				99.45	99.46	
2‴				69.8	69.87	
3‴				68.89	68.89	
4‴				70.54	70.56	
5‴				67.36	67.38	
6‴				17.17	17.18	

3β-ol were assigned to the natural products **3** and **4**, respectively.

The most polar fraction of the active fraction 4 (see Experimental Section) was a mixture of two compounds, as indicated by NMR spectral data. Separation of this mixture was achieved by acetylation and further HPTLC separation of the reaction mixture to yield the peracetylated saponins **5a** and **6a**. Compound **5a** was obtained as a white powder. The HRFABMS of compound **5a** indicated the molecular formula $C_{51}H_{76}O_{18}$ (*m*/*z* 977.5139). Positive-ion FABMS displayed prominent fragments at *m*/*z* 703 [M − deoxyhexose] and *m*/*z* 457 [M − deoxyhexose − deoxyhexose]. A peak at *m*/*z* 397 was attributed to the subsequent loss of two deoxyhexose units and a CH_3COO unit. The ^{13}C NMR spectrum of **5a** showed 51 signals, of which 22 were assigned to the saccharide portion and 29 to the acetylated aglycone. Also, in this case, the proton-coupling network within the sugar residue was traced out, using a combination of $^1H-^1H$ COSY, NOESY, TOCSY, HMQC, and HMBC experiments, which indicated that a α-L-rhamnopyranosyl unit was present instead of the β-D-xylopyranosyl unit observed in the disaccharide chain at C-6 of compound **2a** (Tables 1 and 2). Once again, direct evidence for the sugar sequence and the linkage sites was derived from the HMBC experiments. These results established a disaccharide chain made up by a α-L-rhamnopyranosyl unit linked at C-3 of a β-D-quinovopyranosyl unit attached to C-6 of the aglycone. On the basis of the obtained data, the structure of **5** was established as 6-α-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-quinovopyranosyl-(25*R*)-5α-spirostan-3β-ol.

Compound **6a** showed an $[M]^+$ ion at *m*/*z* 1034.5070 in the HRFABMS, corresponding to the molecular formula $C_{53}H_{78}O_{20}$ (Δ −0.0016 mmu of calcd), which was also deduced on the basis of the ^{13}C NMR spectrum combined with DEPT data. The ^{13}C and ^1H NMR spectra of **6a** also suggested it to be a 5α-spirostanol glycoside resembling **5a**. Comparison of NMR data for both compounds indicated identical saccharide chains at C-6 and structural similarity in the aglycone moieties. The main differences were the disappearance of the methylene signals due to C-23 at δ_C 31.3 and δ_H 1.7 (m) and 1.2 (m) and the appearance of additional signals at δ_C 68.6 and δ_H 4.81 (1H, dd, J = 4, 8 Hz), implying an additional acetoxyl group at C-23 in **6a**. This hypothesis was confirmed unambiguously by the HMBC spectrum showing cross-peaks between the proton at δ_H 4.81 (H-23) and the C-25 (δ_C 30.6) and C-22 (δ_C 108.5) signals. The α-configuration of the C-23 acetoxyl group was evident from the chemical shift and the J value of this proton, compared with related compounds.[11−13] Also the NOESY interaction observed between H-23 and H-25 clearly confirmed the α-equatorial position of the C-23 OAc group. The 25*R*-configuration of **6a** was confirmed by the ^1H NMR parameters of the H-26 methylene protons at δ 3.43 (dd, $J_{26eq,26ax}$ = 10.4 Hz, $J_{26eq,25ax}$ = 4.8 Hz) and 3.35 (t, $J_{26ax,26eq}$ = 10.4 Hz, $J_{26ax,25ax}$ = 10.4 Hz). Full assignments for the proton and carbon resonances of the aglycone were secured from the COSY, NOESY, HMQC, and HMBC spectral data (Tables 1 and 2). Moreover, acid hydrolysis of the original mixture (vide supra) afforded rhamnose and quinovose as the sugar components, as well as chlorogenin (**7**) and the new sapogenin (**8**). The mass spectrum of **8** exhibited the molecular ion $[M]^+$ at *m*/*z* 448 ($C_{27}H_{44}O_5$) and intense peaks at *m*/*z* 363, 345, 327, 289, 271, and 253. The $[M - 85]^+$ peak at 363 characterized this compound as a 23-hydroxyspirostane.[13] The ^1H NMR spectrum of **8** showed C-18, C-19, C-21, and C-27 methyl groups at δ 0.74 (s), 0.89 (s) 1.31 (d, J = 7.2 Hz), and 1.31 (d, J = 7.2 Hz), respectively, and 27 carbon signals comprised of those ascribable to the signals of C-3 (70.23), C-6 (66.33), C-16 (80.92), C-17 (61.08), C-22 (110.27), and C-26 (65.34) observed in the ^{13}C NMR spectrum. Proton signals for H-3, H-6, H-23, and H-26 are superimposed between δ 3.35 and 3.64, and H-16 appeared at δ 4.95 (1H, ddd, J = 8, 5.2, 4.4 Hz). Acetylation of **8** afforded a triacetyl derivative (**8a**), in which the proton signals at δ 4.80 (1H, dd, J = 11.6, 4.4 Hz, H-23β), 4.65 (2H, m, H-3 and H-6), 4.43 (1H, dd, J = 14.4, 7.2 Hz, H-16), 3.41 (1H, dd, J = 11.2, 4.8 Hz, H-26β), and 3.34 (1H, t, J = 11.2 Hz, H-26α) were assigned. Thus, the structure of the aglycone moiety of **6** was shown to be (25*R*)-5α-spirostan-3β,6α,23α-triol, which is a new sapogenin in the *Solanum* genus and named chrysogenin. The structure of the disaccharide moiety of **6a** was determined by the following data. A combined use of $^1H-^1H$ COSY and NOESY experiments allowed the sequential assignments of all resonances for each monosaccharide, starting from the anomeric proton signals at δ 4.36 and 4.78 (Table 2). A $^1H-^{13}C$ one-bond chemical shift correlation experiment via a HMQC spectrum correlated all proton resonances with those of corresponding carbons. Comparison of the ^{13}C assignments with those of compound **5a** showed a α-L-rhamnopyranosyl-(1→3)-β-D-quinovopyranoside moiety. In the HMBC spectrum, the anomeric proton signals at δ 4.37

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

	T.m.[a]	*T.r.*[b]	*A.n.*[c]	*C.a.*[d]
MeOH extract	8000	8000	10000	>10000
chloroform fraction	4000	4000	8000	10000
3-*O*-*β*-D-sitosteryl glycoside + 3-*O*-*β*-D-stigmasteryl glycoside	200	400	>400	200
fraction 4	400	nt[e]	nt	nt
2	12.5	12.5	100	200
3	100	100	>400	200
4	25	50	>400	200
5	200	50	400	200
6	200	200	200	200
nystatin	nt	nt	nt	8.0
miconazole	4	4	8	nt

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

(inner quinovose) and 4.79 (terminal rhamnose) showed correlations with the carbon signals at *δ* 80.52 (C-6 of aglycone) and 81.88 (C-3 of quinovose), respectively. Accordingly, the structure of the natural product of **6a** was established as 6α-*O*-α-L-rhamnopyranosyl-(1→3)-*β*-D-quinovopyranosyl-(25*R*)-5α-spirostan-3*β*,23α-diol (**6**). All the isolated spirostan saponins here are new natural products.

All the isolated compounds (**2**−**6**) were tested for antimycotic activity against *T. mentagrophytes*, *T. rubrum*, *A. niger*, and *C. albicans* (Table 3). All of tested compounds showed antifungal activity. Compounds **2**, **3**, and **6** inhibited the growth of all the microorganisms tested, with **2** being the most active compound against the dermatophytes *T. mentagrophytes* and *T. rubrum* with MIC values of 12.5 μg/mL each. Nystatin and miconazole were used as positive controls. These results demonstrate that the spirostan saponins described here are responsible for the antifungal activity shown by this plant species.

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 a Varian INOVA-400 at 400 MHz for ^{1}H NMR, ^{1}H−^{1}H COSY, HMQC, HMBC, and ^{1}H−^{1}H TOCSY and 100 MHz for ^{13}C NMR and ^{13}C DEPT in $CDCl_3$. Chemical shifts are reported in ppm relative to TMS. FABMS and HRFABMS were performed using a Hewlett Pakard 5985-B and a JEOL-AX 505 HA mass spectrometer, respectively. HPLC (Merck-Hitachi) was performed using a Merck Lichrospher C_{18} 124 × 4 mm, 5 μm column.

Plant Material. Leaves of *S. chrysotrichum* (2.08 kg dry wt) were collected in the highlands of Chiapas, Mexico, on September 17, 1997, and identified by Biol. Abigaíl Aguilar from the IMSS-Mexico Herbarium (IMSSM), where voucher specimens have been deposited.

Extraction and Isolation. Leaves were extracted by maceration at room temperature 3× with methanol (10 L) and concentrated to dryness under reduced pressure. The methanol extract (280 g) was suspended in H_2O and partitioned with $CHCl_3$ to yield an active chloroform-soluble fraction and an inactive aqueous-soluble fraction. The chloroform extract (138 g, 6.69%) was fractionated on a vacuum liquid chromatography column (VLC-210 g silica gel) eluting with *n*-hexane−chloroform−methanol mixtures of increasing polarity to yield five fractions: Fr. 1, 3.0 g (100:0:0), Fr. 2, 47.1 g (1:1:0), Fr.3, 42.3 g (0:1:0), Fr. 4, 38.1 g (0:95:5), Fr. 5, 3.7 g (0:9:1). Fraction 4 (16.4 g) was chromatographed on silica gel (500 g) with a gradient mixture of $CHCl_3$−MeOH to give three fractions. Fraction 1, eluted with $CHCl_3$−MeOH (95:5), afforded 35 mg of the mixture of 3-*O*-*β*-D-sitosterol glycoside and 3-*O*-*β*-D-stigmasterol glycoside. Fraction 2, eluted with $CHCl_3$−MeOH (9:1), 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 1.2 mL/min, to afford 15 mg of compound **3** (t_R 6.03 min) and 12 mg of compound **4** (t_R 8.31 min). Fraction 3, eluted with $CHCl_3$−MeOH (4:1), was further chromatographed on a silica gel column with $CHCl_3$−MeOH mixtures to give 60 mg of **2** and 3.5 g of a mixture of two compounds; crystallization from acetone afforded 5 mg of **5** and 40 mg of **6**. An aliquot of this mixture (100 mg) was acetylated with Ac_2O (2 mL) in pyridine (1 mL). The crude acetate was separated by HPTLC (*n*-hexane−AcOEt−MeOH, 5:5:2) to yield 13 mg of **5a** and 25 mg of **6a**.

Acid Hydrolysis of Saponins 2−4. Saponins **2** (5 mg), **3** (5 mg), and **4** (5 mg) in 0.5 M HCl (dioxane−H_2O, 1:1; 5 mL) were refluxed 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.

Acid Hydrolysis of the Mixture of Saponins 5 and 6. The mixture of compounds **5** and **6** (50 mg) was treated as above to give 15 mg of a nonpolar product after precipitation with ice. This product was separated on a preparative HPTLC with *n*-hexane−EtOAc (3:2) to give 5 mg of **7** and 8 mg of **8**.

Sugar Analysis. The sugar fraction (10 mg) in H_2O was dissolved in water (3 mL), to which (*S*)-α-methylbenzylamine (15 mg) and Na[BH_3CN] (24 mg) in EtOH (3 mL) were added, and heated at 40 °C for 5 h, followed by addition of AcOH (0.6 mL) and evaporation to dryness. The reaction mixture was acetylated with Ac_2O (0.6 mL) in pyridine (0.6 mL) at room temperature for 14 h.[14] The crude mixture was suspended in MeOH (5 mL) and filtered to give a mixture of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamine]-1-deoxyalditol acetate derivatives of the monosaccharides, which was then analyzed by GC−MS using an Agilent 6890 gas chromatograph with an Agilent 5973 Netuwarle mass selective detector apparatus, employing a capillary column of 1 m × 0.25 mm i.d., film thickness of 5% phenylmethylsilicone with He as the mobile phase, inlet pressure of 7 psi, temperature program of 40 °C, 3 min, 250 °C final temp, at a rate of 10 °C/min. The diasteromeric alditol derivatives of standard sugars of xylose, quinovose, and rhamnose were used as reference. The derivatives of D-xylose, L-rhamnose and D-quinovose were detected at t_R 12.89 min (*m*/*z* 465), t_R 13.04 min (*m*/*z* 479.5), and t_R 17.69 min (*m*/*z* 479.5), respectively.

Acetylation of Compounds 2−4. Saponins **2** (25 mg), **3** (6 mg), and **4** (6 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 with *n*-hexane−EtOAc, afforded the peracetate derivatives **2a** (25.3 mg), **3a** (5.8 mg), and **4a** (6.1 mg).

Saponin Sc-2 (2): white amorphous powder; mp 239−241 °C; $[\alpha]_D^{25}$ −49° (*c* 1.08, MeOH); IR ($CHCl_3$) ν_{max} 3500−3300 (OH), 2925 (CH) cm^{-1}.

Compound 2a: colorless powder; mp 123−125 °C; $[\alpha]_D^{25}$ −48.2° (*c* 1.16 $CHCl_3$); IR ($CHCl_3$) ν_{max} 2922, 1757 (ester),1452, 1376, 1050 (C−O−C), 757 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; FABMS *m*/*z* 1001 $[M + K]^+$ and *m*/*z* 963 $[M + H]^+$, 962 $[M]^+$, 457 [M − xyl − qui]$^+$, 397 [M − xyl − qui − 60]$^+$; HRFABMS *m*/*z* 962.4901 (calcd for $C_{50}H_{74}O_{18}$, 962.4875).

Saponin Sc-3 (3): white amorphous powder; mp 167−168 °C; IR ν_{max} 3500−3300 (OH), 2925 (CH) cm^{-1}.

Compound 3a: colorless powder; mp 90−92 °C; $[\alpha]_D^{25}$ −41.34° (*c* 1.04, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2920, 1750 (ester), 1455, 1374, 1050 (C−O−C), 757 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; FABMS *m*/*z* 733 $[M + H]^+$, 457 [M − xyl]$^+$, 397 [M − xyl − CH_3COO]$^+$; HRFABMS *m*/*z* 733.4141 (calcd for $C_{40}H_{61}O_{12}$, 733.4163).

Saponin Sc-4 (4): white amorphous powder; mp 194−196 °C; IR ν_{max} 3500−3300 (OH), 2920 (CH) cm^{-1}.

Compound 4a: white amorphous powder; mp 78−79 °C; $[\alpha]_D^{25}$ −53.92° (*c* 1.02, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2922, 1750 (ester), 1451, 1370, 1050 (C−O−C), 756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; FABMS *m/z* 769 $[M + Na]^+$, 747 $[M + H]^+$, 457 $[M - qui]^+$, 397 $[M - qui - CH_3COO]^+$, 273 $[M - C_7H_8O_2]^+$; HRFABMS *m/z* 747.4320 (calcd for $C_{41}H_{63}O_{12}$, 747.4315).

Compound 5a: colorless powder; mp 247−248 °C; $[\alpha]_D^{25}$ −33.89° (*c* 1.18, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2922, 1756 (ester), 1452, 1376, 1056 (C−O−C), 757 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; FABMS *m/z* 999 $[M + Na]^+$, 977 $[M + H]^+$, 976 $[M]^+$, 703 $[M - rha]^+$, 457 $[M - rha - qui]^+$, 397 $[M - rha - qui - CH_3COO]^+$; HRFABMS *m/z* 977.5139 (calcd for $C_{51}H_{77}O_{18}$, 977.5110).

Saponin SC-6 (6): white amorphous powder; mp 198−199 °C; IR ν_{max} 3500−3300 (OH), 2925 (CH) cm^{-1}.

Compound 6a: colorless powder; mp 144−146 °C; $[\alpha]_D^{25}$ −41.2° (*c* 1.48, $CHCl_3$); IR ($CHCl_3$) ν_{max}, 2920, 1760 (ester), 1470, 1050 (C−O−C); ^1H NMR (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; FABMS *m/z* 1073 $[M + K]^+$, 1057 $[M + Na]^+$, 1034 $[M]^+$, 999 $[(M + Na) - CH_3COO]^+$, 710 $[(M + Na) - rha - CH_3COO]^+$, 709 $[(M + Na) - rha - CH_3COO]^+$, 515 $[M - rha - qui]^+$, 457 $[M - rha - qui - CH_3COO]^+$, 395 $[M - rha - qui - CH_3COO \times 2]^+$; HRFABMS *m/z* 1034.5070 (calcd for $C_{53}H_{78}O_{20}$, 1034.5086).

Chlorogenin (7): colorless powder; mp 235−237 °C; identified as (25*R*)-5α-spirostan-3*β*,6α-diol, by spectral data comparison.[7,15]

Chrysogenin (8): colorless powder; mp 158−160 °C; desc; $[\alpha]^{25}_D$ −21.9° (*c* 1.05, MeOH); IR ($CHCl_3$) ν_{max} 3100−3500 (br, OH), 2922, 1461, 1368, 1056 (C−O−C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.74 (3H, s, H-18), 0.83 (3H, s, H-19), 1.31 (6H, d, J = 7.2 Hz, H-21, H-27), 3.36−3.64 (5H, m, H-3, H-6, H-23, H-26), 4.95 (1H, ddd, J = 8, 5, 4.4 Hz, H-16); ^{13}C NMR (50 MHz, $CDCl_3$), see Table 2; EIMS *m/z* 448 $[M]^+$ (30), 363 (100), 345 (52), 327 (25), 289 (37), 271 (20), 253 (12).

Antifungal Activity. Antimycotic evaluation of *S. chrysotrichum* extracts and isolated compounds was performed following the conventional agar dilution assay procedure[16] for activity against *Trichophyton mentagrophytes* (ATCC 28185), *Trichophyton rubrum* (ATCC 28188), *Aspergillus 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, and reference compounds (Nystatin and Miconazole) in DMSO were diluted to give serial 2-fold dilutions in the range 10−1.5 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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