Steroidal Saponins from *Smilax medica* and Their Antifungal Activity

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Three new steroidal saponins (**1**−**3**) were isolated from the roots of *Smilax medica*, together with the known disporoside A (**4**). The structures of the new compounds were elucidated mainly by extensive spectroscopic analysis (1D and 2D NMR, FABMS, and HRESIMS). Compounds **1**, **2**, and **4** demonstrated weak antifungal activity against the human pathogenic yeasts *Candida albicans*, *C. glabrata*, and *C. tropicalis*, with MIC values between 12.5 and 50 μg/mL.

Plants of the family Liliaceae are known as rich sources of steroidal saponins.[1,2] The genus *Smilax* contains 350 species, which are distributed widely mainly in tropical regions of East Asia and South and North America. Several *Smilax* species have already been studied chemically and found to contain steroidal saponins.[3−7] From a biological point of view, some species were documented to exhibit antiinflammatory,[8] NO-modulating,[9] and antileprosic[10] activity, but there has been no detailed report on the biological activity or constituents of *S. medica*. As part of our ongoing search for new antifungal steroid saponins,[11−14] we have investigated the roots of *Smilax medica* Schlecht. et Cham. collected in Mexico. The present paper reports the isolation and characterization of three new steroid saponins (**1**−**3**) along with the known disporoside A (**4**). The structures of **1**−**3** were determined by spectroscopic methods including 1D and 2D NMR experiments and FABMS and HRESIMS. Furthermore, their antifungal activity was tested against three human pathogenic yeasts (*Candida albicans, C. glabrata*, and *C. tropicalis*).

Results and Discussion

The *n*-BuOH-soluble fraction of the MeOH−H_2O (7:3) extract of the rhizomes of *S. medica* was purified by precipitation with diethyl ether to give a crude saponin mixture. This mixture was submitted to multiple chromatographic steps involving vacuum-liquid chromatography (VLC) on reversed-phase C_{18} silica gel and medium-pressure liquid chromatography (MPLC) on normal silica gel to yield compounds **1**−**3** and the known disporoside A (**4**).[15]

Compound **1**, a white amorphous powder, exhibited in the HRESIMS (positive-ion mode) a quasimolecular ion peak at *m/z* 1087.5286 $[M + Na]^+$ (calcd 1087.5301), consistent with a molecular formula of $C_{51}H_{84}O_{23}$. Its FABMS (negative-ion mode) showed a quasimolecular ion peak at *m/z* 1063 $[M - H]^-$, indicating a molecular weight of 1064. Other fragment ion peaks were observed at *m/z* 901 $[(M - H) - 162]^-$ and 739 $[(M - H) - 162 - 162]^-$, corresponding to the successive loss of two hexosyl moieties. On acid hydrolysis, an artifactual aglycon was obtained. Glucose was identified by comparison on TLC with an authentic sample.

The aglycon was identified as smilagenin [(25*R*)-5β-spirostan-3β-ol][16−19] from the 1D and 2D NMR spectra of **1** (see Tables 1 and 2). The A/B *cis*-ring fusion was confirmed by the signals at δ 36.0 (C-5), 39.9 (C-9), and 23.7 (C-19), indicating that the aglycon of **1** is a 5β-steroidal sapogenin.[20,21] The 25*R* stereochemistry of the 27-methyl group was deduced by the resonances of protons and carbons at C-25 (δ_H 1.66/δ_C 30.2), C-26 (δ_{H2} 3.45, 3.56/δ_C 66.6), and C-27 (δ_H 0.64 d, J = 5.7 Hz/δ_C 17.0). The lower field resonance of C-27 (δ 17.0) as compared to the ^{13}C NMR chemical shift of (25*S*)-spirostanes at δ 16−16.5[17,22] and the NOESY correlations between H-11/H_3-19, H-11/H_3-18, H-9/H-14, H-16/H-17, and H-17/H_3-21 confirmed the relative stereochemistry of **1** as having the usual A/B *cis*, B/C and C/D *trans*, D/E *cis*, and C-20α stereochemistry. The ^1H NMR spectrum of **1** displayed signals for four anomeric protons at δ 4.74 (d, J = 7.1 Hz), 5.10 (d, J = 7.9 Hz), 5.20 (d, J = 8.1 Hz), and 5.28 (d, J = 7.9 Hz), which gave correlations, in the HSQC spectrum, with four anomeric carbon signals at δ 100.6, 104.4, 104.1, and 104.2, respectively. The ring protons of the monosaccharide residues were assigned starting from the readily identifiable anomeric protons by means of COSY, TOCSY, NOESY, HSQC, and HMBC experiments (Table 1). Evaluation of spin−spin couplings and chemical shifts allowed the identification of four β-glucopyranosyl units (Glc I, Glc II, Glc III, Glc IV), with three of them being terminal. The absolute configuration of glucose was determined to be D by GC analysis of chiral derivatives of the sugar in the acid hydrolysate (see Experimental Section).

The sequence of the oligosaccharide chain was determined from the HMBC and NOESY spectra. Correlations observed in the HMBC spectrum between the ^1H NMR signal at δ_H 4.74 (d, J = 7.1 Hz, Glc I H-1) and the ^{13}C NMR signal at δ_C 75.0 (Agly C-3) and in the NOESY spectrum between δ_H 4.74 (Glc I H-1) and δ_H 4.20 (m, Agly H-3) proved the Glc I to be linked at C-3 of the aglycon. Furthermore, the correlation in the HMBC spectrum between the ^1H NMR signal at δ_H 5.10 (d, J = 7.9 Hz, Glc II H-1) and δ_C 68.0 (Glc I C-6) and a reverse correlation between signals at δ_H 4.46 (Glc I H-6) and δ_C 104.4 (Glc II C-1) proved the Glc II unit to be linked to Glc I at C-6, which has been often encountered in *Smilax* species.[3,5−7] The correlation in the HMBC spectrum between the ^1H NMR signal at δ_H 5.20 (d, J = 8.1 Hz, Glc III H-1) and δ_C 80.0 (Glc I C-4) and a NOESY cross-peak between δ_H 5.20 (Glc III H-1) and δ_H 4.19 (Glc I H-4) revealed a (1→4) linkage between these two sugars. The linkage of Glc IV to the 2-position of Glc I was deduced by the HMBC correlation observed between δ_H 5.28 (Glc IV H-1) and δ_C

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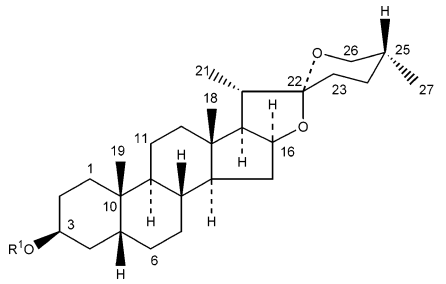

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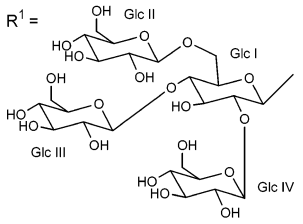

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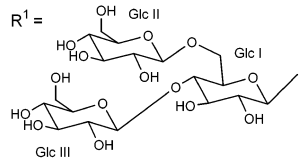

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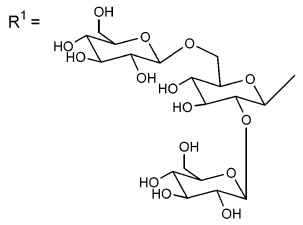

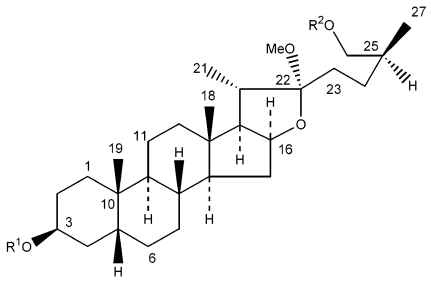

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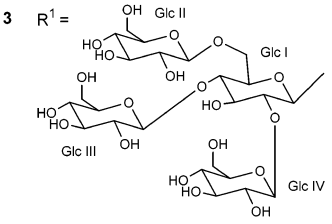

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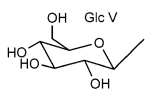

79.7 (Glc I C-2) and the NOESY cross-peak between δ_H 5.28 (Glc IV H-1) and δ_H 4.12 (Glc I H-2). On the basis of the above results, the structure of **1** was established as (25*R*)-5β-spirostan-3β-ol 3-*O*-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside.

Compound **2**, a white amorphous powder, exhibited in the HRESIMS (positive-ion mode) a quasimolecular ion peak at *m/z* 925.4789 $[M + Na]^+$ (calcd 925.4773) consistent with a molecular formula of $C_{45}H_{74}O_{18}$. Its FABMS (negative-ion mode) displayed a quasimolecular ion peak at *m/z* 901 $[M - H]^-$, 162 mass units lower than that of **1**, indicating a molecular weight of 902. Other fragment ion peaks were observed at *m/z* 739 $[(M - H) - 162]^-$ and 577 $[(M - H) - 162 - 162]^-$, corresponding to the loss of two hexosyl moieties. On acid hydrolysis, the same aglycon as that of **1** was obtained. Glucose was identified by comparison on TLC with an authentic sample, and its absolute configuration was determined to be D by GC analysis (see Experimental Section). Scrutiny of the 2D NMR (COSY, NOESY, HMQC) data of compound **2** (Tables 1 and 2) and detailed comparison of the ^1H and ^{13}C NMR chemical shifts with those of **1** showed that **2** differed from **1** only in the absence of Glc IV linked at C-2 of Glc I. This was confirmed by the upfield chemical shift at δ 74.1 (Glc I C-2) instead of at δ 79.7 in **1** and the upfield shift of Glc I C-3 at δ 76.1 instead of at δ 78.0 in **1**. Therefore, the structure of **2** was assigned as (25*R*)-5β-spirostan-3β-ol 3-*O*-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside.

The molecular formula of compound **3** was assigned as $C_{58}H_{98}O_{29}$ on the basis of its HRESIMS (positive-ion mode), which exhibited a quasimolecular ion peak at *m/z* 1281.6103 $[M + Na]^+$ (calcd 1281.6091). Its negative-ion FABMS showed a quasimolecular ion peak at *m/z* 1257 $[M - H]^-$. Other fragment ion peaks were observed at *m/z* 1095 $[(M - H) - 162]^-$ and 933 $[(M - H) - 162 - 162]^-$, corresponding to the loss of two hexosyl moieties. The comparison of NMR data of **3** with literature data allowed the identification of the aglycon as the previously reported (25*S*)-3β,5β,-22α-22-methoxyfurostan-3,26-diol (the aglycon of timosaponin BI).[23] The A/B *cis*-ring fusion was deduced by the signals at δ 35.9 (C-5), 39.8 (C-9), and 23.5 (C-19), indicating that **3** is a 5β-steroidal sapogenin.[20,21] The 25*S* stereochemistry of the Me-27 group was deduced from the resonances of protons and carbons at C-25, C-26, and C-27 in comparison with literature data.[24] The differences observed in ^1H NMR chemical shifts of the geminal protons H-26a and H-26b ($\delta_a - \delta_b = 0.66$) supported a 25*S* furostane-type steroid since this difference is usually >0.57 ppm in 25*S* compounds and <0.48 ppm in 25*R* compounds.[24] The configuration of the 22-methoxy group was deduced to be α by the observed NOESY correlation between the methoxy protons at δ 3.25 (s) and the H-16 proton signal at δ 4.46 (m).[25] The ^1H NMR spectrum of **3** displayed signals for five anomeric protons at δ 4.75 (brd), 5.04 (d, J = 7.4 Hz), 5.28 (d, J = 7.6 Hz), 5.12 (d, J = 7.6 Hz), and 4.72 (d, J = 7.9 Hz), which gave correlations, in the HSQC spectrum, with ^{13}C NMR signals at δ 100.3, 104.0, 103.8, 103.8, and 104.0, respectively. Evaluation of chemical shifts and spin−spin couplings allowed the identification of five β-glucopyranosyl units (Glc I, Glc II, Glc III, Glc IV, and Glc V). The common D-configuration was determined by GC analysis of chiral derivatives of the sugars in the acid hydrolysate. The sugar sequence investigated by extensive 2D NMR experiments revealed that the ^1H and ^{13}C NMR signals of the oligosaccharide chain at C-3 were almost superimposable with those of β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl characterized in **1** (Tables 1 and 2). Furthermore, the correlation observed in the HMBC spectrum between the ^1H NMR signal at δ_H 4.75 (Glc I H-1) and the ^{13}C NMR signal at δ_C 75.8 (Agly-C-3) and in the NOESY spectrum between δ_H 4.75 (Glc I H-1) and δ_H 4.16 (Agly-H-3) confirmed Glc I to be linked at C-3 of the aglycon. The linkage of the fifth remaining sugar at the C-26 position was indicated by long-range coupling (^3J)

Table 1. ^{1}H NMR Spectral Data (δ in ppm) of Compounds **1**−**3** (600 MHz, pyridine-d_5, J in Hz)[a]

aglycon	**1**	**2**	**3**	3-*O*-sugar	**1**	**2**	**3**
H-1a, H-1b	1.42, 1.77	1.40, 1.66	1.40, 1.76	Glc I-1	*4.74 d (7.1)*	*4.77 d (7.9)*	*4.75* brd
H-2a, H-2b	nd	nd	1.72, nd	2	*4.12*[b]	3.88[b]	*4.16*[b]
H-3	*4.20* m	*3.90* m	*4.16* m	3	3.84 [b]	4.12[b]	3.86[b]
H-4a, H-4b	1.64 m, nd	1.64 m, 1.72 m	1.62 m, nd	4	*4.19*[b]	*4.28*[b]	*4.16*[b]
H-5	2.10 m	1.93 m	2.10 m	5	3.84[b]	nd	3.88[b]
H-6a, H-6b	1.64 m, 1.83 m	1.64 m, 1.86 m	nd	6	4.46[b], 4.70[b]	4.50[b], 4.76[b]	4.42, 4.68[b]
H-7a, H-7b	1.08 m, nd	1.08 m, nd	1.04 m, 1.40 m				
H-8	1.36 m	1.40 m	1.30 m	Glc II-1	*5.10 d (7.9)*	*5.16 d (7.9)*	*5.04 d (7.4)*
H-9	1.15 m	1.20 m	1.14 m	2	3.94[b]	3.94[b]	3.92[b]
H-11a, H-11b	1.18 m, nd	1.02 m, 1.20 m	1.16 m, nd	3	4.14[b]	4.14[b]	4.10[b]
H-12a, H-12b	0.98 m, 1.60 m	1.00 m, 1.61 m	0.94 m, 1.58 m	4	4.05[b]	4.06[b]	3.98[b]
H-14	0.97 m	1.00 m	0.90 m	5	4.04[b]	3.84[b]	3.80[b]
H-15a, H-15b	1.35 m, 1.95 m	1.33 m, 1.96 m	1.26 m, 1.86 m	6	4.18[b], 4.36[b]	4.18[b], 4.37[b]	4.05[b], 4.30[b]
H-16	4.56 m	4.54 m	4.46 m				
H-17	1.80 m	1.80 m	1.72 m	Glc III-1	*5.20 d (8.1)*	*5.27 d (7.9)*	*5.28 d (7.6)*
Me-18	0.75 s	0.74 s	0.72 s	2	3.90[b]	3.96[b]	3.84[b]
Me-19	0.88 s	0.76 s	0.86 s	3	4.25[b]	4.24[b]	4.21[b]
H-20	1.91 m	1.90 m	2.17 m	4	4.06[b]	4.06[b]	3.98[b]
Me-21	1.08 d (6.9)	1.10 d (6.9)	1.14 d (6.7)	5	4.02[b]	4.04[b]	4.10[b]
H-23a, H-23b	1.42 m, 1.77 m	1.58 m, 1.64 m	2.0 m, nd	6	4.10[b], 4.32[b]	4.10[b], 4.38[b]	4.24[b], 4.47[b]
H-24a, H-24b	1.50 m, 1.64 m	1.58 m, nd	1.72 m, nd				
H-25	1.66 m	1.68 m	nd	Glc IV-1	*5.28 d (7.9)*		*5.12 d (7.6)*
H-26a, H-26b	3.45 m, 3.56 m	3.44 m, 3.55 m	*3.54* m, *4.20* m	2	3.86[b]		3.88[b]
Me-27	0.64 d (5.7)	0.63 d (5.5)	0.92 d (6.7)	3	4.14[b]		4.21[b]
OMe-22			3.25 s	4	4.05[b]		4.04[b]
				5	4.06[b]		3.98[b]
				6	4.30[b], 4.48[b]		4.18[b], 4.40[b]
				26-*O*-sugar			
				Glc V-1			*4.72 d (7.9)*
				2			3.90[b]
				3			4.14[b]
				4			4.01[b]
				5			3.86[b]
				6			4.12[b], 4.32[b]

[a] ^{1}H NMR chemical shifts of substituted residues are italicized. [b] Overlapped with other signals.

in the HMBC spectrum between the anomeric proton of Glc V at δ_H 4.72 (d, J = 7.9 Hz) and δ_C 74.8 (Agly-C-26). On the basis of the above results, the structure of **3** was determined to be (25*S*)-3β,5β,22α-22-methoxyfurostan-3,-26-diol 3-*O*-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl 26-*O*-β-D-glucopyranoside.

Compound **4** was identified as (25*R*)-5β-spirostan-3β-ol 3-*O*-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside (disporoside A) by comparison of its NMR data with those reported in the literature.[15]

The antifungal activity of saponins **1**−**4** (Table 3) was evaluated at concentrations lower than 200 μg/mL against pathogenic human strains of *Candida albicans*, *C. glabrata*, and *C. tropicalis*. Compounds **1**, **2**, and **4**, having a spirostane skeleton, exhibited antifungal activity against the three yeasts tested. The MIC value for each compound was between 12.5 and 50 μg/mL and suggested a similar spectrum of activity of the three saponins. Compound **3**, having a furostane skeleton, exhibited MIC values above 200 μg/mL and was considered inactive against the yeasts tested. Regarding the aglycon structure, we determined the antifungal activity only for the spirostanol derivatives, whereas none was observed for the furostanol derivative (Table 1).[13,14] This suggests that the E and F rings of spirostane-type steroids play a key role in the mediation of antifungal properties.[13,14,26]

Experimental Section

General Experimental Procedures. Optical rotations were taken with a AA-10R automatic polarimeter. IR spectra ($CHCl_3$) were recorded on a Perkin-Elmer 881 spectrophotometer. The 1D and 2D NMR spectra (^{1}H−^{1}H COSY, TOCSY, NOESY, HSQC, and HMBC) were performed using a UNITY-600 spectrometer at the operating frequency of 600 MHz on a Varian INOVA 600 instrument equipped with a SUN 4 L-X computer system (600 MHz for ^{1}H and 150 MHz for ^{13}C spectra). Conventional pulse sequences were used for COSY, HSQC, and HMBC spectra. TOCSY spectra were acquired using the standard MLEV17 spin-locking sequence and a 90 ms mixing time. The mixing time in the NOESY experiment was set to 500 ms. The carbon type (CH_3, CH_2, CH) was determined by DEPT experiments. All chemical shifts (δ) are given in ppm, and the samples were solubilized in pyridine-d_5 (δ_C 150.3, 155.9, 123.9). FABMS (negative-ion mode, glycerol matrix) was conducted on a JEOL SX 102 spectrometer. The HRESIMS was measured in the positive-ion mode on a Q-TOF-1 micromass spectrometer. GC analysis was carried out on a Thermoquest gas chromatograph using a DB-1701 capillary column (30 m × 0.25 mm, i.d.) (J & W Scientific), with detection by FID, and the initial temperature maintained at 80 °C for 5 min and then raised to 270 °C at the rate of 15 °C/min; carrier gas: He. Compound isolations were carried out using a medium-pressure liquid chromatography (MPLC) system [Gilson M 305 pump, 25 SC head pump, M 805 manometric module, Büchi column (460 × 25 mm and 460 × 15 mm), Büchi precolumn (110 × 15 mm)] and silica gel 60 (Merck, 15−40 μm). Vacuum-liquid chromatography (VLC) was performed on a C_{18} reversed-phase column (Merck, 25−40 μm) (12 × 3 cm). TLC and HPTLC employed precoated silica gel 60 F_{254} plates (Merck). The following TLC solvent systems were used: for saponins (a) $CHCl_3$−MeOH−H_2O (13:7:2, lower phase); for sapogenins (b) $CHCl_3$−MeOH (9:1); for monosaccharides (c) $CHCl_3$−MeOH−H_2O (8:5:1). Spray reagents for the saponins were Komarowsky reagent, a mixture (5:1) of *p*-hydroxybenzaldehyde (2% in MeOH) and H_2SO_4 50%; for the sugars, diphenylaminephosphoric acid reagent.

Table 2. ^{13}C NMR Spectroscopic Data (δ in ppm) of Compounds **1**−**3** (150 MHz, pyridine-d_5, J in Hz)[a]

aglycon	1	2	3	3-*O*-sugar	1	2	3
C-1	30.5	30.1	30.4	GlcI-1	*100.6*	*102.3*	*100.3*
C-2	26.6	26.6	27.7	2	79.7	74.1	79.7
C-3	75.0	74.6	75.8	3	78.0	76.1	77.8
C-4	30.0	30.1	29.8	4	*80.0*	80.7	79.2
C-5	36.0	36.6	35.9	5	74.6	74.6	74.8
C-6	26.6	26.6	26.4	6	*68.0*	68.3	67.9
C-7	26.4	26.4	26.3				
C-8	35.2	35.2	35.0	GlcII-1	*104.4*	*104.4*	*104.0*
C-9	39.9	39.9	39.8	2	74.6	74.6	74.3
C-10	34.9	34.9	34.7	3	77.7	77.7	77.2
C-11	20.8	20.8	20.6	4	71.0	71.1	70.9
C-12	40.0	40.0	39.8	5	77.7	77.7	77.4
C-13	40.6	40.6	40.8	6	62.1	62.2	61.5
C-14	56.1	56.1	56.0				
C-15	31.8	31.8	31.6	GlcIII-1	*104.1*	*104.2*	*103.8*
C-16	81.0	81.1	81.1	2	74.6	74.8	75.7
C-17	62.7	62.6	63.8	3	77.7	77.6	77.2
C-18	16.3	16.3	16.0	4	71.5	71.1	70.8
C-19	23.7	23.5	23.5	5	77.7	77.7	77.2
C-20	41.7	41.7	40.0	6	61.7	61.9	62.4
C-21	14.7	14.7	15.8				
C-22	109.2	109.2	112.6	GlcIV-1	*104.2*		*103.8*
C-23	31.5	31.5	37.0	2	76.0		74.3
C-24	28.9	28.9	27.7	3	77.7		77.2
C-25	30.2	30.2	33.6	4	71.0		70.8
C-26	66.6	66.6	74.8	5	77.7		77.4
C-27	17.0	17.0	16.7	6	62.7		62.1
OMe-22			47.0				
				26-*O*-sugar			
				Glc V-1			*104.0*
				2			74.3
				3			77.2
				4			71.0
				5			77.8
				6			61.9

[a] ^{13}C NMR chemical shifts of substitued residues are italicized.

Table 3. Antifungal Activity of **1**−**4** against Three *Candida* Species[a]

compound	*C. albicans*	*C. glabrata*	*C. tropicalis*
1	25	12.5	50
2	25	12.5	50
3	>200	>200	>200
4	25	25	50
ketoconazole[b]	0.78	0.78	1.56

[a] Data are presented as MIC values (μg/mL). Compounds with MIC values > 200 μg/mL are considered inactive. [b] Positive control.

Plant Material. The roots of *Smilax medica* were collected in May 2003 near the city of Tuxpan (Vera Cruz Province, Mexico). They were provided by Sedaherb, France, where they were authenticated and authorized to be imported. A voucher specimen (No. 6622) is deposited in the Herbarium of the Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Burgundy, France.

Extraction and Isolation. Dried, powdered roots (130 g) of *S. medica* were refluxed three times with MeOH−H_2O (7:3, 1 L) for 1 h and evaporated to dryness, yielding 30.1 g of a MeOH−H_2O extract. This extract was suspended in water (400 mL) and partitioned successively with hexane and *n*-BuOH (each 3 × 200 mL), yielding after evaporation of the solvents the corresponding hexane (60 mg) and *n*-BuOH (5.7 g) fractions. A 4.5 g aliquot of the *n*-BuOH residue was dissolved in MeOH and purified by precipitation with diethyl ether (3 × 300 mL), yielding a crude saponin mixture (3.4 g). The latter was submitted to VLC on C_{18} reversed phase using as eluents H_2O (100 mL), MeOH−H_2O mixtures (5:5; 4:1, each 100 mL), and finally MeOH (100 mL). After evaporation of the solvents, four fractions were obtained: VLC-F1 (H_2O) (450 mg), VLC-F2 (MeOH−H_2O, 5:5) (170 mg), VLC-F3 (MeOH−H_2O, 4:1) (450 mg), and VLC-F4 (MeOH) (300 mg). VLC-F3 (450 mg) was submitted to MPLC [system a: silica gel (15−40 μm), $CHCl_3$−MeOH−H_2O (13:7:2, lower phase)] to give 13 fractions (1−13). Fraction 13 was concentrated to dryness, yielding the pure compound **3** (16 mg). VLC-F4 (300 mg) was submitted to MPLC (system a) to give 11 fractions (1−11). Fractions 7 and 11 were concentrated to dryness, affording the pure compounds **2** (9 mg) and **1** (40 mg), respectively. Fraction 3 rechromatographed by MPLC (system a) yielded the pure compound **4** (6 mg).

Compound 1: white amorphous powder; $[\alpha]^{20}_D$ −22.2° (*c* 0.135, MeOH); IR ($CHCl_3$) ν_{max} 3255 (OH), 2980 (CH), 1232, 1040 (C−O−C), 950, 829 cm^{-1}; ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; negative FABMS (glycerol matrix) *m/z* 1063 [M − H]$^-$, *m/z* 901 [(M − H) − 162]$^-$, *m/z* 739 [(M − H) − 162 − 162]$^-$; HRESIMS (positive-ion mode) *m/z* 1087.5286 [M + Na]$^+$ (calcd for $C_{51}H_{84}O_{23}Na$, 1087.5301).

Compound 2: white amorphous powder; $[\alpha]^{20}_D$ −109.8° (*c* 0.085, MeOH); IR ($CHCl_3$) ν_{max} 3200−3270 (OH), 2960 (CH), 1230, 1030 (C−O−C), 960, 840 cm^{-1}; ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; negative FABMS (glycerol matrix) *m/z* 901 [M − H]$^-$, *m/z* 739 [(M − H) − 162]$^-$, *m/z* 577 [(M − H) − 162 − 162]$^-$; HRESIMS (positive-ion mode) *m/z* 925.4789 [M + Na]$^+$ (calcd for $C_{45}H_{74}O_{18}Na$, 925.4773).

Compound 3: white amorphous powder; $[\alpha]^{20}_D$ −34.3° (*c* 0.333, MeOH); IR ($CHCl_3$) ν_{max} 3265 (OH), 2954 (CH), 1641, 1230, 1033 (C−O−C), 958, 828 cm^{-1}; ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; negative FABMS (glycerol matrix) *m/z* 1257 [M − H]$^-$, *m/z* 1095 [(M − H) − 162]$^-$, *m/z* 933 [(M − H) − 162 − 162]$^-$; HRESIMS (positive-ion mode) *m/z* 1281.6103 [M + Na]$^+$ (calcd for $C_{58}H_{98}O_{29}Na$, 1281.6091).

Acid Hydrolysis. A solution of each saponin (3 mg) in 2 N aqueous CF_3COOH (5 mL) was refluxed on a water bath for 3 h. After extraction with CH_2Cl_2 (3 × 5 mL), the aqueous layer was evaporated to dryness with MeOH until neutral, and glucose was identified by TLC with a standard sugar (solvent system c). Furthermore, a silylated derivative of the sugar was prepared according to the procedure previously described.[27] L-Cysteine methyl ester hydrochloride (0.06 mol/L) and HMDS-TMCS (hexamethyldisilazane−trimethylchlorosilane, 3:1) were added to the aqueous residue. After centrifugation of the precipitate, the supernatant was concentrated and partitioned between *n*-hexane and H_2O, and the hexane layer was analyzed by GC. D-Glucose was detected.

Antifungal Activity. Evaluation of pure saponins was performed using a dilution test.[28] For these bioassays, three human pathogenic yeasts were used: *Candida albicans* (IP 1180-79), *C. glabrata*, and *C. tropicalis*. These clinical isolates were provided by Dr. A. Bonnin, Laboratory of Parasitology and Mycology, Hopital du Bocage, Dijon, France. Minimum inhibitory concentration (MIC) determination was performed by a serial dilution technique using 96-well microtiter plates. Pure saponins were dissolved in 180 μL of malt extract broth (15 g of malt extract and 5 g of peptone in 1 L of distilled water) to achieve final concentrations of 3.12−200 μg/mL and were dispensed in triplicate into the wells. Each well was inoculated with 10 μL of 10^4 CFU/mL fungal suspension. The microplates were incubated at 30 °C for 48 h, and growth was evaluated spectrophotometrically at 630 nm with an ELX 808 automatic microplate reader. The MIC was defined as the lowest concentration of saponin at which no yeast growth was observed after incubation at 30 °C for 48 h. The reference compound ketoconazole (Sigma)[29] was used as positive control.

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