Steroidal Saponins of *Yucca schidigera* Roezl.

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Eight steroidal saponins have been isolated from *Yucca schidigera* Roezl. trunk, and their structures were established by spectral (MS and NMR) techniques. These included three novel furostanol glycosides including 3-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl-5*β*(25*R*)-furostan-3*β*,22α,26-triol 26-*O*-*β*-D-glucopyranoside, 3-*O*-*β*-D-glcopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl-5*β*(25*R*)-furost-20(22)-en-3*β*,26-diol-12-one 26-*O*-*β*-D-glucopyranoside, 3-*O*-*β*-D-glcopyranosyl-(1→2)-*β*-D-glucopyranosyl-5*β*(25*R*)-furostan-3*β*,22α,26-triol 26-*O*-*β*-D-glucopyranoside, and five known spirostanol glycosides. On the basis of the extraction efficiency, furostanol glycosides made up only 6.8% of total saponins isolated.

Keywords: *Yucca; Yucca schidigera; steroidal saponins; furostanosides*

INTRODUCTION

Yucca schidigera (Agavaceae) grows in the desert of Baja California, and its extract is commonly used as an additive in the cosmetic and soft drink industries. Yucca extract is also fed to livestock and poultry to improve growth and productivity (*1*) and to reduce ammonia and odors in poultry excreta on poultry farms (*2*). The mode of action of yucca products is believed to be related to their saponins (*2*, *3*), the concentration of which is very high (10% of dry weight). It was documented that the acid-hydrolyzed fraction of *Y. schidigera* mature plant contained three epimeric pairs of saponin aglycons including sarsapogenin, smilagenin, markogenin, samogenin, gitogenin, and neogitogenin (*4*). Sarsapogenin, smilagenin, markogenin, 5*β*-spirost-25(27)-en-3*β*-ol, and 5*β*-spirost-25(27)-ene-2*β*,3*β*-diol were also found as aglycons in six steroidal saponins identified by Tanaka and co-workers (*5*). These six saponins (YE-1...6) were spirostanols, with one sugar chain, composed of three sugar units each, attached at the 3*β*-position. More extensive work by this group resulted in the identification of 14 spirostanol glycosides (*6*) with antiyeast activity. However, no furostanol (bidesmosidic) saponins were identified. Similarly, extensive work on *Yucca gloriosa* afforded 13 saponins (YS-I...XIII), most of them being of spirostanol structure, but some furostanol glycosides were also identified (*7*−*9*).

Preliminary tests of yucca extract performed by thin-layer chromatography (TLC) using specific sprays for visualization of saponins showed that *Y. schidigera* also contains both spirostanol and furostanol saponins. Thus, we deemed it of interest to isolate and characterize the dominant saponins of yucca trunk.

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MATERIALS AND METHODS

Plant Material. Yucca (*Y. schidigera* Roezl.) powder was obtained from Desert King Int., Chula Vista, CA.

Spectroscopic Analysis. Melting points were uncorrected. ESI-HRMS were recorded on a Mariner Biospectrometry workstation (PerSeptive Biosystems). ^{1}H and ^{13}C spectra in CD_3OD solutions were measured on a Bruker DRX-600 spectrometer, operating at 599.19 MHz for ^{1}H and at 150.86 MHz for ^{13}C. Spectra in 2D experimentss (^{1}H−^{1}H DQF-COSY, inverse detected ^{1}H−^{13}C HSQC, and HMBC) were obtained using UX-NMR software. The selective excitation spectra, 1D-TOCSY, were acquired using waveform generator-based GAUSS shaped pulses, with a mixing time of 100 ms and an MLEV-17 spin−lock field of 10 kHz preceded by a 2.5 ms trim pulse.

Extraction and Separation of Saponins. Yucca powder (300 g) was defatted with chloroform in a Soxhlet. After drying, it was extracted three times by boiling with water (0.5 h). The water extract was loaded onto a C_{18} column (70 × 40 mm, 40 μm, Baker) equilibrated with water. The column was washed with water and then with 70% MeOH (F1 fraction) and MeOH (F2 fraction). Fractions F1 and F2 were evaporated to dryness.

Fraction F2 was loaded onto a silica gel column (40 × 170 mm, 40−60 μm, Merck) equilibrated with chloroform/MeOH (8:0.5). The column was washed with chloroform/MeOH (8:0.5 → 8:2), and 5 mL fractions were collected with a fraction collector. Fractions showing identical characteristics [TLC, silica gel, chloroform/MeOH (8:3)] were combined. Three subfractions were obtained, which were further separated on a C_{18} column (40 × 300 mm, LiChroprep RP18, 25−40 μm, Merck) using an MeOH/H_2O (55 → 80% MeOH) gradient. Eight single pure compounds were obtained (Figure 1).

1: (0.261 g, 0.17% yield); crystalline; mp 197−198 °C; $[\alpha]_D^{25}$ = −15.2 (MeOH, *c* 0.1); HRMS, *m*/*z* 885.4493 [calcd for $C_{44}H_{69}O_{18}$ (M)$^-$: 885.4478, 753 (M − 132)$^-$; ^{1}H NMR (aglycon) *δ* 4.34 (1H, m, H-16), 4.12 (1H, m, H-3), 3.45 (1H, m, H-26a), 3.30 (1H, m, H-26b), 1.09 (3H, s, Me-19), 1.08 (3H, s, Me-18), 1.04 (3H, d, *J* = 6.0 Hz, Me-21), 0.82 (3H, d, *J* = 6.0 Hz, Me-27), (sugars) signals superimposable to those reported for compound **6**; for ^{13}C NMR see Table 1.

2: (0.832 g, 0.55% yield); in three different solvent systems on TLC this fraction gave one spot, but mass spectrometry and ^{1}H NMR indicated the presence of two different saponins. Thus, the mixture (300 mg) was acetylated (pyridine/acetic

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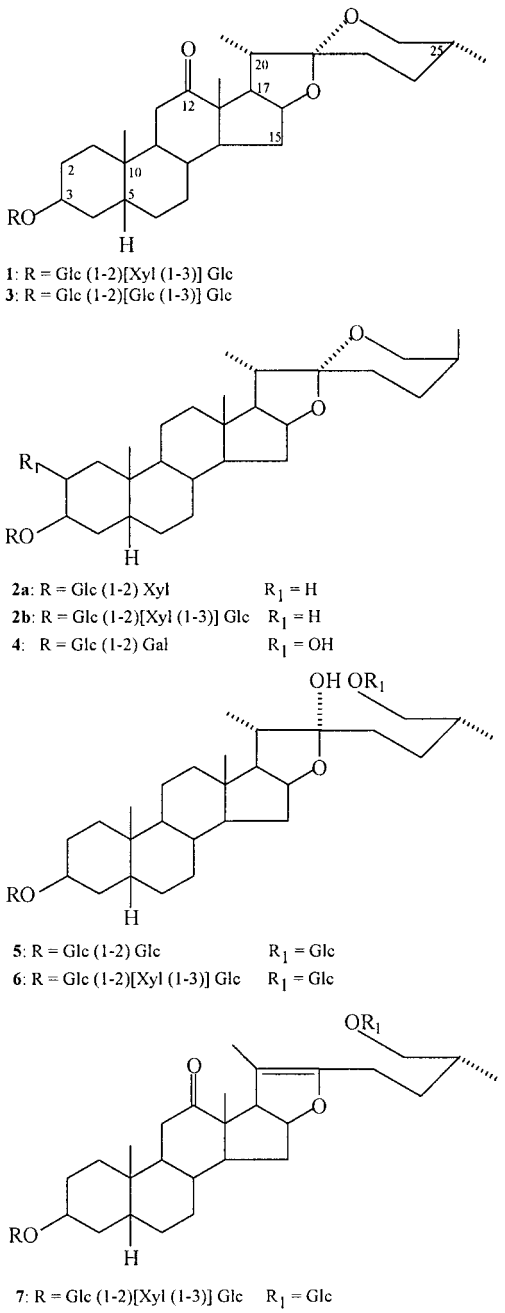

Figure 1. Chemical formulas of identified compounds.

anhydride, overnight at room temperature). Distilled water was added in excess, and saponin acetates were extracted into chloroform. TLC of the chloroform fraction performed on silica gel using chloroform/hexane (90:10) as a solvent revealed two spots. This mixture was loaded onto a silica gel column (LiChroprep Si60, 30 × 1.2 cm, 15−25 μm, Merck). The column was washed with chloroform/hexane (90:10), and 2 mL fractions were collected. This yielded two acetylated compounds, which were then deacetylated by alkaline hydrolysis with 10% NaOH in aquous 50% MeOH. These provided two compounds, **2a** and **2b**.

2a: (0.022 g, 0.04% yield); crystalline; mp 260−261 °C; $[\alpha]_D^{25}$ = −48.0 (MeOH, *c* 0.1); HRMS, *m/z* 739.4295 [calcd for $C_{39}H_{63}O_{13}$ $(M)^-$: 739.4263]; ^{1}H NMR (aglycon) δ 4.42 (1H, m, H-16), 4.12 (1H, m, H-3), 3.95 (1H, m, H-26a), 3.32 (1H, m, H-26b), 1.11 (3H, d, *J* = 6.0 Hz, Me-27), 1.02 (3H, d, *J* = 6.0 Hz, Me-21), 1.02 (3H, s, Me-19), 0.82 (3H, s, Me-18), (sugars) signals superimposable on those reported for compound **5**; for ^{13}C NMR see Table 1.

2b: (0.055 g, 0.11% yield); crystalline; mp 298−299 °C; $[\alpha]_D^{25}$ = −40.6 (MeOH, *c* 0.1); HRMS, *m/z* 871.4704 [calcd for $C_{44}H_{71}O_{17}$ $(M)^-$: 871.4686]; ^{1}H NMR (aglycon) δ 4.42 (1H, m, H-16), 4.15 (1H, m, H-3), 3.95 (1H, m, H-26a), 3.32 (1H, m, H-26b), 1.11 (3H, d, *J* = 6.0 Hz, Me-27), 1.03 (3H, s, Me-19), 1.02 (3H, d, *J* = 6.0 Hz, Me-21), 0.82 (3H, s, Me-18), (sugars) signals superimposable on those reported for compound **6**; for ^{13}C NMR see Table 1.

3: (0.043 g, 0.03% yield); white powder; mp 234−236 °C; $[\alpha]_D^{25}$ = −7.8 (MeOH, *c* 0.1); HRMS, *m/z* 915.5 $(M)^-$, 753.4 $(M - 162)^-$; ^{1}H NMR (aglycon) δ 4.34 (1H, m, H-16), 4.12 (1H, m, H-3), 3.45 (1H, m, H-26a), 3.30 (1H, m, H-26b), 1.09 (3H, s, Me-19), 1.08 (3H, s, Me-18), 1.04 (3H, d, *J* = 6.0 Hz, Me-21), 0.81 (3H, d, *J* = 6.0 Hz, Me-27), (sugars) δ 4.51 (d, *J* = 7.2 Hz, H-1Glc$_1$), 3.72 (dd, *J* = 7.2 and 9.0 Hz, H-2Glc$_1$), 3.76 (dd, *J* = 9.0 and 9.0 Hz, H-3Glc$_1$), 3.38 (dd, *J* = 9.0 and 9.0 Hz, H-4Glc$_1$), 3.32 (ddd, *J* = 2.5, 4.5, and 9.0 Hz, H-5Glc$_1$), 3.68 (dd, *J* = 4.5 and 11.5 Hz, H-6aGlc$_1$), 3.87 (dd, *J* = 2.5 and 11.5 Hz, H-6bGlc$_1$), 4.96 (d, *J* = 7.5 Hz, H-1Glc$_2$), 3.13 (dd, *J* = 7.5 and 9.0 Hz, H-2Glc$_2$), 3.39 (dd, *J* = 9.0 and 9.0 Hz, H-3Glc$_2$), 3.16 (dd, *J* = 9.0 and 9.0 Hz, H-4Glc$_2$), 3.32 (ddd, *J* = 2.5, 4.5, and 9.0 Hz, H-5Glc$_2$), 3.65 (dd, *J* = 4.6 and 11.5 Hz, H-6aGlc$_2$),

Table 1. ^{13}C NMR Spectra of Saponins (1−7)[a]

	compounds							
carbon	**1**	**2a**	**2b**	**3**	**4**	**5**	**6**	**7**
1	30.5	30.9	30.7	30.9	40.0	31.4	31.3	30.2
2	27.3	27.3	27.3	27.3	68.1	27.3	27.3	27.1
3	75.7	76.2	76.2	75.7	79.6	76.0	76.1	75.6
4	30.9	31.5	31.4	31.0	31.4	30.9	30.6	30.8
5	36.7	36.7	36.8	36.7	36.8	37.5	37.2	36.6
6	27.2	27.7	27.7	27.2	27.0	27.6	27.6	27.0
7	26.9	26.9	26.9	26.9	27.0	27.7	27.5	26.8
8	35.5	37.5	37.3	35.5	36.8	36.7	36.7	35.4
9	43.1	41.4	41.4	43.1	42.5	41.2	41.3	43.0
10	36.3	36.1	36.2	36.3	37.9	36.1	36.1	36.3
11	38.4	22.0	22.0	38.4	22.2	21.9	21.9	38.4
12	216.1	41.4	41.4	216.1	41.3	41.3	41.2	216.3
13	56.5	41.8	41.8	56.5	41.8	42.2	42.2	58.6
14	57.2	57.6	57.6	57.3	57.5	57.6	57.5	55.6
15	32.2	32.7	32.7	32.2	32.7	32.7	32.7	34.1
16	80.7	82.3	82.4	80.7	82.7	83.4	82.5	83.8
17	54.8	63.7	63.7	54.9	63.7	65.3	65.3	56.8
18	16.3	16.9	16.9	16.3	16.9	16.9	16.5	14.2
19	23.5	24.5	24.5	23.5	24.3	24.5	24.5	23.4
20	43.2	43.4	43.4	43.2	43.4	41.2	41.2	104.3
21	13.5	14.7	14.7	13.5	14.7	16.2	16.2	11.2
22	110.8	111.0	111.0	110.8	111.0	113.9	113.9	153.5
23	32.3	27.7	27.7	32.5	27.7	31.4	31.4	23.8
24	29.5	26.8	26.8	29.4	26.8	28.9	28.9	33.7
25	31.6	28.5	28.5	31.4	28.5	35.0	35.0	31.6
26	67.6	66.1	67.2	67.9	66.1	75.1	75.8	75.4
27	17.3	16.4	16.4	17.3	16.4	17.4	17.4	16.9
1′	100.5	101.0	100.6	100.6	101.7	101.1	100.5	100.5
2′	78.3	80.7	78.7	78.9	78.6	80.6	78.5	78.7
3′	87.7	78.5	87.8	87.6	74.9	77.8	87.7	87.6
4′	69.8	71.7	70.0	69.9	70.2	71.5	69.9	69.9
5′	78.0	78.4	78.2	78.2	76.3	78.2	77.8	77.5
6′	62.5	62.8	62.8	62.7	62.2	62.4	62.5	62.5
1″	103.3	104.4	103.4	103.4	104.5	104.8	103.3	103.3
2″	75.8	76.2	76.2	75.8	76.1	75.9	75.8	75.8
3″	77.6	77.9	78.3	77.9	77.5	77.7	77.8	77.7
4″	72.1	72.0	72.5	72.3	71.7	71.8	72.3	72.3
5″	78.0	77.9	78.6	77.7	78.0	78.0	78.1	78.1
6″	63.4	63.2	63.7	63.4	62.4	62.4	63.4	63.4
1‴	104.6		105.1	104.3			104.8	104.8
2‴	74.7		75.2	75.0			74.9	74.9
3‴	78.1		77.8	77.9			78.2	78.2
4‴	70.4		71.0	71.4			70.8	70.8
5‴	66.6		66.8	77.9			66.8	66.8
6‴				62.8				
1⁗						104.7	104.6	104.6
2⁗						74.9	75.1	75.4
3⁗						77.3	77.5	77.4
4⁗						71.5	71.5	71.5
5⁗						77.8	77.8	77.8
6⁗						62.4	62.4	62.4

[a] Assignments confirmed by HSQC and HMBC experiments.

3.85 (dd, J = 2.5 and 11.5 Hz, H-6bGlc$_2$), 4.68 (d, J = 7.5 Hz, H-1Glc$_3$), 3.31 (dd, J = 7.5 and 9.0 Hz, H-2Glc$_3$), 3.38 (dd, J = 9.0 and 9.0 Hz, H-3Glc$_3$), 3.31 (dd, J = 9.0 and 9.0 Hz, H-4Glc$_3$), 3.38 (ddd, J = 2.5, 4.5 and 9.0 Hz, H-5Glc$_3$), 3.67 (dd, J = 4.5 and 11.5 Hz, H-6aGlc$_3$), 3.87 (dd, J = 2.5 and 11.5 Hz, H-6bGlc$_3$); for ^{13}C NMR see Table 1.

4: (0.045 g, 0.03% yield); white powder; mp 273−274 °C; $[\alpha]_D^{25}$ = −45.8 (MeOH, *c* 0.1); HRMS, *m*/*z* 755.4422 [calcd for $C_{39}H_{63}O_{14}$ (M)$^-$: 755.4212]; ^{1}H NMR (aglycon) δ 4.42 (1H, m, H-16), 4.07 (1H, m, H-3), 3.94 (1H, m, H-26a), 3.30 (1H, m, H-26b), 3.62 (1H, m, H-2), 1.11 (3H, d, J = 6.0 Hz, Me-27), 1.05 (3H, s, Me-19), 1.03 (3H, d, J = 6.0 Hz, Me-21), 0.82 (3H, s, Me-18), (sugars) δ 4.42 (d, J = 7.4 Hz, H-1Gal), 3.92 (dd, J = 7.4 and 9.0 Hz, H-2Gal), 3.71 (dd, J = 4.0 and 9.0 Hz, H-3Gal), 3.83 (dd, J = 2.5 and 4.0 Hz, H-4Gal), 3.54 (ddd, J = 2.5, 2.5, and 4.5 Hz, H-5Gal), 3.70 (dd, J = 4.5 and 12.0 Hz, H-6aGal), 3.77 (dd, J = 2.5 and 12.0 Hz, H-6bGal), 4.69 (d, J = 7.5 Hz, H-1Glc), 3.22 (dd, J = 7.5 and 9.0 Hz, H-2Glc), 3.39 (dd, J = 9.0 and 9.0 Hz, H-3Glc), 3.27 (dd, J = 9.0 and 9.0 Hz, H-4Glc), 3.29 (ddd, J = 2.5, 4.5, and 9.0 Hz, H-5Glc), 3.70 (dd, J = 4.5 and 11.5 Hz, H-6aGlc), 3.88 (dd, J = 2.5 and 11.5 Hz, H-6bGlc); for ^{13}C NMR see Table 1.

5: (0.013 g, 0.01% yield); white powder; mp 207−208 °C; $[\alpha]_D^{25}$ = −38.8 (MeOH, *c* 0.1); HRMS, *m*/*z* 919.4917 [calcd for $C_{45}H_{75}O_{19}$ (M)$^-$: 919.4897]; ^{1}H NMR (aglycon) δ 4.38 (1H, m, H-16), 4.12 (1H, m H-3), 3.82 (1H, m, H-26a), 3.36 (1H, m, H-26b), 1.03 (3H, d, J = 6.0 Hz, Me-21), 1.02 (3H, s, Me-19), 0.98 (3H, d, J = 6.0 Hz, Me-27), 0.84 (3H, s, Me-18), (sugars) δ 4.47 (d, J = 7.3 Hz, H-1Glc$_1$), 3.58 (dd, J = 7.3 and 9.0 Hz, H-2Glc$_1$), 3.30 (dd, J = 9.0 and 9.0 Hz, H-3Glc$_1$), 3.30 (dd, J = 9.0 and 9.0 Hz, H-4Glc$_1$), 3.58 (ddd, J = 2.5, 4.5, and 9.0 Hz, H-5Glc$_1$), 3.68 (dd, J = 4.5 and 11.5 Hz, H-6aGlc$_1$), 3.87 (dd, J = 2.5 and 11.5 Hz, H-6bGlc$_1$), 4.69 (d, J = 7.5 H, H-1Glc$_2$), 3.22 (dd, J = 7.5 and 9.0 Hz, H-2Glc$_2$), 3.38 (dd, J = 9.0 and 9.0 Hz, H-3Glc$_2$), 3.24 (dd, J = 9.0 and 9.0 Hz, H-4Glc$_2$), 3.28 (ddd, J = 2.5, 4.5, and 9.0 Hz, H-5Glc$_2$), 3.68 (dd, J = 4.5 and 11.0 Hz, H-6aGlc$_2$), 3.85 (dd, J = 2.5 and 11.0 Hz, H-6bGlc$_2$), 4.27 (d, J = 7.5 Hz, H-1Glc$_3$), 3.21 (dd, J = 7.5 and 9.0 Hz, H-2Glc$_3$), 3.39 (dd, J = 9.0 and 9.0 Hz, H-3Glc$_3$), 3.31 (dd, J = 9.0 and 9.0 Hz, H-4Glc$_3$), 3.29 (ddd, J = 2.5, 4.5, and 9.0 Hz, H-5Glc$_3$), 3.68 (dd, J = 4.5 and 11.5 Hz, H-6aGlc$_3$), 3.88 (dd, J = 2.5 and 11.5 Hz, H-6bGlc$_3$); for ^{13}C NMR see Table 1.

6: (0.057 g, 0.04% yield); white powder; mp 235−236 °C; $[\alpha]_D^{25}$ = −43.25 (MeOH, *c* 0.1); HRMS, *m*/*z* 1049.5166 [calcd for $C_{50}H_{81}O_{23}$ (M)$^-$: 1049.5163]; ^{1}H NMR (aglycon) δ 4.38 (1H, m, H-16), 4.12 (1H, m, H-3), 3.82 (1H, m, H-26a), 3.36 (1H, m, H-26b), 1.03 (3H, d, J = 6.0 Hz, Me-21), 1.02 (3H, s, Me-19), 0.98 (3H, d, J = 6.0 Hz, Me-27), 0.84 (3H, s, Me-18), (sugars) δ 4.52 (d, J = 7.2 Hz, H-1Glc$_1$), 3.73 (dd, J = 7.2 and 9.0 Hz, H-2Glc$_1$), 3.72 (dd, J = 9.0 and 9.0 H-3Glc$_1$), 3.32 (dd, J = 9.0 and 9.0 Hz, H-4Glc$_1$), 3.39 (ddd, J = 2.5, 5.0, and 9.0 Hz, H-5Glc$_1$), 3.68 (dd, J = 5.0 and 12.0 Hz, H-6aGlc$_1$), 3.90 (dd, J = 2.5 and 12.0 Hz, H-6bGlc$_1$), 4.96 (d, J = 7.5 Hz, H-1Glc$_2$), 3.13 (dd, J = 7.5 and 9.0 Hz, H-2Glc$_2$), 3.39 (dd, J = 9.0 and 9.0 Hz, H-3Glc$_2$), 3.16 (dd, J = 9.0 and 9.0 Hz, H-4Glc$_2$), 3.32 (ddd, J = 2.5, 4.5, and 9.0 Hz, H-5Glc$_2$), 3.65 (dd, J = 4.5 and 11.5 Hz, H-6aGlc$_2$), 3.85 (dd, J = 4.5 and 11.5 Hz, H-6bGlc$_2$), 4.26 (d, J = 7.5 Hz, H-1Glc$_3$), 3.20 (dd, J = 7.5 and 9.0 Hz, H-2Glc$_3$), 3.36 (dd, J = 9.0 and 9.0 Hz, H-3Glc$_3$), 3.31 (dd, J = 9.0 and 9.0 Hz, H-4Glc$_3$), 3.29 (ddd, J = 2.5, 5.0, and 9.0 Hz, H-5Glc$_3$), 3.68 (dd, J = 5.0 and 11.5 Hz, H-6aGlc$_3$), 3.88 (dd, J = 2.5 and 11.5 Hz, H-6aGlc$_3$), 4.60 (d, J = 7.5 Hz, H-1Xyl), 3.30 (dd, J = 7.5 and 9.0 Hz, H-2Xyl), 3.33 (dd, J = 9.0 and 9.0 Hz, H-3Xyl), 3.54 (ddd, J = 4.5, 9.0, and 11.0 Hz, H-4Xyl), 3.28 (t, J = 11.0 Hz, H-5aXyl), 3.94 (dd, J = 4.5 and 11.0 Hz, H-5bXyl); for ^{13}C NMR see Table 1.

Fraction F1was loaded onto a silica gel column (300 × 40 mm, LiChroprep Si60, 40−60 μm, Merck). The column was washed with $CHCl_3$/MeOH (8:2), and 5 mL fractions were collected. These were analyzed with TLC, giving four fractions of which only one showed TLC patterns different from subfractions obtained from fraction F2. It was further separated on a C_{18} column (300 × 40 mm, LiChroprep RP18, 25−40 μm, Merck) using an MeOH/H_2O (50 → 65% MeOH) gradient. One single compound was obtained.

7: (0.015 g, 0.01% yield); white powder; mp 193−195 °C (with browning); $[\alpha]_D^{25}$ = −3.6 (MeOH, *c* 0.1); HRMS, *m*/*z* 1047.5002 [calcd for $C_{50}H_{79}O_{23}$ (M)$^-$: 1047.5007], 915 [M − 132]$^-$, 753 [M − 132 − 162]$^-$, 591 [M − 132 − 162 − 162]$^-$; ^{1}H NMR (aglycon) δ 4.72 (1H, m, H-16), 4.14 (1H, m, H-3), 3.74 (1H, m, H-26a), 3.43 (1H, m, H-26b), 3.27 (1H, s, H-17), 1.61 (3H, s, Me-21), 1.09 (3H, s, Me-19), 0.97 (3H, s, Me-18), 0.96 (3H, d, J = 6.0 Hz, Me-27), 0.84 (3H, s, Me-18), (sugars) signals superimposable on those reported for compound **6**; for ^{13}C NMR see Table 1.

RESULTS AND DISCUSSION

Extraction of yucca powder with water, followed by purification by reversed phase chromatography, resulted in a saponin mixture with an extraction efficiency of ∼10% of dry matter. This mixture was successfully separated using silica gel and then reversed phase columns. In this way eight individual saponins were isolated, and their structures were fully characterized with spectroscopic techniques.

Compound **6** displayed an [M]$^-$ ion peak at *m*/*z* 1049 in the HRMS spectrum. The ^{1}H NMR spectrum showed, for the aglycon moiety, two signals ascribable to tertiary methyl groups at δ 0.84 and 1.02, two doublets typical for secondary methyls at δ 0.98 (d, J = 6.0 Hz) and 1.03 (d, J = 6.0 Hz), and signals indicative of a proton linked to oxygen-bearing carbons at δ 4.12 (1H, m) and 4.38 (1H, m). From the ^{13}C NMR spectrum it was possible to deduce the aglycon structure as 5β,(25*R*)-furostan-3β,22α,26-triol (*10*). The structure of the oligosaccharide unit was determined by 1D-TOCSY and 2D-NMR experiments. Selected 1D-TOCSY spectra obtained by irradiation of the anomeric proton signals at δ 4.26, 4.52, 4.60, and 4.96 yielded the subspectra of each sugar residue with high digital resolution. Thus, by irradiation of the signals at δ 4.26, 4.52, and 4.96 it was possible to identify the spin systems of three β-D-glucopyranose units, whereas irradiation of the signal at δ 4.60 clearly showed the subspectrum typical of a β-D-xylopyranose. The DQF-COSY experiment allowed the complete sequential assignment of all sugar proton resonances, which were correlated by the HSQC experiment to the corresponding carbon signals. The absence of any ^{13}C NMR glycosylation shift for the β-D-glucopyranosyl units with H-1 at δ 4.96 and 4.26 and for the β-D-xylopyranosyl unit suggested that these sugar were terminal units. Glycosylation shifts were observed for C-2 (δ 78.5) and C-3 (δ 87.7) of the glucose unit with H-1 at δ 4.52. The positions of the sugar residues were unambiguously defined by the HMBC experiment. Cross-peaks due to long-range correlations between C-3 of the aglycon (δ 76.1) and H-1Glc$_1$ (δ 4.52), C-2Glc$_1$ (δ 78.5), and H-1Glc$_2$ (δ 4.96), between C-3Glc$_1$ (δ 87.7) and H-1Xyl (δ 4.60), and between C-26 of the aglycon (δ 75.8) and H-1Glc$_3$ (δ 4.26) revealed at C-3 of the aglycon a sugar chain made up of a nodal β-D-glucopyranosyl unit, linking at C-2 a β-D-glucopyranosyl unit and at C-3 a β-D-xylopyranosyl unit, and at C-26 the occurrence of a β-D-glucopyranose. On the basis of these data compound **6** was established as the new 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-5β-(25*R*)-furostan-3β,22α,26-triol 26-O-β-D-glucopyranoside.

The HRMS spectrum of compound **7** showed the [M]$^-$ ion peak at *m*/*z* 1047 and a fragmentation pattern similar to that observed for compound **6**. The ^{13}C NMR spectrum showed 50 signals, of which 23 were assigned to the saccharide portion and 27 to a steroidal aglycon.

The ^{1}H NMR spectrum showed, for the aglycon moiety, two signals ascribable to tertiary methyl groups at δ 0.97 and 1.09, a doublet due to a secondary methyl at δ 0.96, and a signal typical of a methyl linked to an olefinic carbon at δ 1.61. Also evident were signals at δ 4.14 (1H, m) and 4.72 (1H, m) attributable to H-3 and H-16, respectively. The structure of the aglycon was definitely clarified by the HMBC experiment, which showed diagnostic long-range correlations between the methyl signal at δ 0.97 (Me-18) and the carbon resonances at δ 216.3 (C-12), 58.6 (C-13), 56.8 (C-17), and 55.6 (C-14) and between the methyl signal at δ 1.61 and the carbon resonances at δ 104.3 (C-20), 153.5 (C-22), and 56.8 (C-17). On the basis of these finding the aglycon of **7** appeared to be the 5β,(25*R*)-furost-20(22)-en-3β,26-diol-12-one **(***10*). Analysis of 1D-TOCSY and 2D-NMR experiments suggested the same sugar portion as in **6**. Thus, compound **7** was defined as the new 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-5β(25*R*)-furost-20(22)-en-3β,26-diol-12-one 26-*O*-β-D-glucopyranoside.

The ^{1}H NMR spectrum of compound **1** showed the $[M]^-$ ion peak at *m*/*z* 885 and a prominent fragment at 753 corresponding to the loss of pentose. The ^{13}C NMR spectrum suggested a spirostane skeleton characterized by the occurrence of a keto group. In the ^{1}H NMR spectrum, together with the signals typical for H-3 and H-16 at δ 4.12 and 4.34, respectively, signals due to methyl groups at δ 1.09 (s, Me-19), 1.08 (s, Me-18), 1.04 (d, J = 6.0 Hz, Me-21), and 0.82 (d, J = 6.0 Hz, Me-27) were evident. The HMBC spectrum, which showed the long-range correlation between the proton signal at δ 1.08 (Me-18) and the carbon resonances at δ 216.1, 56.5, 57.2, and 54.8, allowed the location of the keto group (δ 216.1) at C-12 and the identification of the aglycon as 5β,(25*R*)-spirostan-3β-ol-12-one (gloriogenin) to be determined (*7*, *9*). 1D-TOCSY and 2D-NMR experiments showed the same sugar chain at C-3 as in compounds **6** and **7**. Thus, compound **1** was identified as 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-5β(25*R*)-spirostan-3β-ol-12-one. This compound was previously identified as a component of the 25*R* and 25*S* saponin mixture, with the trivial name E1 from *Y. schidigera* (*6*).

Analysis of the ^{1}H NMR and ^{13}C NMR spectra of compound **3** in comparison to those of **1** clearly showed that difference between the two compounds should be confined to the sugar portion. In this case 1D-TOCSY spectra obtained by irradiating the anomeric signals at δ 4.51, 4.68, and 4.96 showed the spin system typical of the β-D-glucopyranose. Thus, compound **3** differed from **1** only in the occurrence of a β-D-glucopyranose instead of a β-D-xylopyranose unit. On the basis of these data, compound **3** was assigned the structure 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-5β(25*R*)-spirostan-3β-ol-12-one. This compound was reported in *Y. gloriosa* (*7*) but not in *Y. schidigera*.

The HRMS spectrum of compound **2b** showed the $[M]^-$ ion peak at *m*/*z* 871. Analysis of ^{1}H and ^{13}C NMR data in comparison with those of **1** suggested that difference between the two compounds should be confined to the aglycon. In the ^{1}H NMR spectrum the main differences were the chemical shifts of Me-18 (δ 0.82 in **2b** versus 1.08 in **1**) and Me-27 (δ 1.11 in **2b** versus 0.82 in **1**). On the basis of these findings, together with the ^{13}C NMR resonances, it was possible to establish that **2b** differed from **1** in the absence of the keto group at C-12 and the stereochemistry at C-25, being *S* in **2b** (*10*). Hence, compound **2b** was identified as 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-5β(25*S*)-spirostan-3β-ol, previously reported as saponin D1 identified in *Y. schidigera* (*6*).

Analysis of ^{1}H and ^{13}C NMR data of **2a** and **5** in comparison with those of **2b** and **6** established that **2a** and **5** differed from **2b** and **6**, respectively, only in the absence of the terminal xylose unit. Hence, compound **2a** was identified as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-5β(25*S*)-spirostan-3β-ol, a saponin known as Ys-1 identified in *Y. gloriosa* (*10*) and as a component of the 25*R* and *S* (D5) mixture from *Y. schidigera* (*6*); compound **5** was identified as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-5β(25*R*)-furostan-3β,22α,26-triol-26-*O*-β-D-glucopyranoside, which to the best of our knowledge has not previously been reported in this form but only as a 22-*O*-methyl derivative in *Asparagus africanus* (*11*).

The HRMS spectrum of compound **4** showed the $[M]^-$ ion peak at *m*/*z* 755. Analysis of ^{1}H and ^{13}C NMR data allowed the identification of the aglycon as 5β(25*S*)-spirostan-2β,3β-diol. To identify the two sugar residues, 1D-TOCSY spectra were recorded. Whereas the 1D-TOCSY spectrum obtained by irradiating the anomeric proton at δ 4.60 gave the spin system of a β-D-glucopyranosyl unit, the 1D-TOCSY spectrum obtained by irradiating the anomeric proton at δ 4.42 showed only three protons coupled to the anomeric signal. This situation is typical of β-D-galactopyranose, where, because of the small coupling constant between H-4 and H-5, the distribution of magnetization around the spin system is impeded. The occurrence of a β-D-galactopyranose was confirmed by the HSQC experiment, which also allowed the identification of C-2Gal as the glycosylation site (δ 78.6). Thus, compound **4** was identified as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-5β(25*S*)-spirostan-2β,3β-diol (markogenin glucosylgalactopyranoside), identified previously as (25*S*)-schidigera saponin F2 (*6*).

The above results clearly show that *Y. schidigera* saponin mixture contains both spirostanol and furostanol glycosides. However, on the basis of the extraction efficiency it can be stated that they contain predominantly the spirostanol saponins, which primarily are glycosides of sarsapogenin (66%), gloriogenin (24%), and markogenin (3.5%). Bidesmosidic furostanol glycosides made up only 6.8% of the total. *Y. schidigera* also contains a number of other glycosides that occurr in trace amounts and have not been identified. Thus, the biological activity of yucca saponins (*1*−*3*, *6*) is determined predominantly by spirostanol saponins, the properties of which have been extensively researched (*12*, *13*). However, furostanol saponins, which were regarded as transport forms of saponins, not showing biological activities, may possess a wide spectrum of biological activities as shown for *Allium* deltoside (*14*). Thus, new furostane saponins identified in this work should be more closely researched with respect to their biological properties.

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