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STEROIDAL SAPONINS FROM *SMILAX OFFICINALIS*

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Key Word Index—*Smilax officinalis*; Liliaceae; rhizomes; steroidal saponin; spirostanol glycosides.

Abstract—Three new steroidal saponins were isolated from the rhizomes of *Smilax officinalis*. The structures of these saponins were established by extensive spectral data, hydrolysis and chemical correlation as sarsasapogenin 3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside, neotigogenin 3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside and 25*S*-spirostan-6β-ol 3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside. Acid hydrolysis of the latter compound gave a sapogenin which has a new orientation of an hydroxyl on the steroidal skeleton. A route is proposed for the biogenesis of the latter sapogenin which is an uncommon steroidal aglycone. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Plants of the family Liliaceae are known as rich sources of steroidal saponins [1, 2]. The genus *Smilax* contains 350 species, which are widely distributed in the tropical and temperate zones throughout the world, and especially 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, 4]. This paper describes the first chemical study of a Brazilian *Smilax* species, *S. officinalis* Kunth, which is used in folk medicine for the treatment of gout. The present paper describes the isolation and structural elucidation of three steroidal saponins from this species. A biosynthetic scheme for the production of saponin **3** is also proposed.

The structures of the saponins, in particular those of the sugar components, were elucidated by chemical and physical means, ^{1}H, APT and ^{13}C NMR techniques (Tables 1 and 2).

RESULTS AND DISCUSSION

A methanolic extract of the fresh roots of *S. officinalis* Kunth was partitioned between water and *n*-butanol. Chromatographic separations of the organic phase on silica gel and Sephadex LH-20 columns gave compounds **1**, **2**, and **3** according to the classical saponin isolation techniques [5].

Compound **1** gave a positive Liebermann–Burchard test. The spectral data were in favour of a glycoside of the (25*S*)-spirostan steroidal type skeleton. The IR spectrum showed strong absorptions for hydroxyl groups (3450 and 1054 cm^{-1}), and a 25*S*-spirostan structure (852, 897, 922 and 987 cm^{-1}, intensity 920 > 897 cm^{-1}) [6], confirmed by 1H and ^{13}C NMR spectra [7, 8]. The two protons at C-26 of the spirostan aglycone appeared at $\delta = 3.30$ (*d*) and 3.98 (*dd*), respectively, characteristic of a 25*S*-configuration of a steroidal saponin [9]. From the ^{13}C NMR spectrum it was possible to deduce a glycoside linked at C-3 of the aglycone skeleton as well as an A/B *cis*-ring fusion [10]. Acid hydrolysis according to the technique described by Sashida *et al.* [4] gave glucose, arabinose and an aglycone identified as 25*S*-5β-spirostan-3β-ol (sarsasapogenin) by its ^{1}H and ^{13}C NMR spectra and physical data [10, 11]. The ^{13}C NMR spectrum of **1** displayed three anomeric carbon signals (δ 102.0, 104.8 and 105.5), indicating the presence of three sugar units. Comparison of the ^{13}C NMR spectrum of the sugar moiety with that of reference methyl glycosides [12] from the literature leads to the conclusion that glucose was the inner monosaccharide unit linked at C-3 of the aglycone skeleton with two sugar units, glucose and arabinose, linked to it at C-4 and C-6, respectively [3]. The anomeric proton signals in the ^{1}H NMR spectra, δ 5.52 (1H, *d*, $J = 7.9$ Hz, H-1 of Glc), 5.10 (1H, *d*, $J = 7.5$ Hz, H-1″ of Ara) and 4.90 (1H, *d*, $J = 7.5$ Hz, H-1′ of Glc), led to the assignment of the anomeric orientation of the arabinose and glucose units to be α and β, respectively. Thus, the structure of **1** was determined to be sarsasapogenin 3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside.

Compound **2** gave a positive Liebermann–Burchard test and the spectral data revealed it to be of the same chemical nature as saponin **1**. In the ^{1}H NMR spectrum it was possible to assign two secondary methyl proton

Table 1. Selected ^{1}H NMR chemical shifts of compounds **1–3**

	H	1	2	3
Aglycone moiety				
	6	—	—	3.92 *br*
Me	19	0.96 *s*	0.71 *s*	1.25 *s*
Me	18	0.78 *s*	0.80 *s*	0.90 *s*
Me	21	1.12 *d* (7.0)*	1.10 *d* (6.9)	1.13 *d* (7.0)
H_{eq}	26	3.94 *dd* (11, 2.5)	3.92 *dd* (11, 2.5)	—
H_{ax}	26	3.27 *d* (11.0)	3.30 *d* (11)	—
Sugar moiety				
Glc	1	4.90 *d* (7.8)	4.93 *d* (7.8)	4.95 *d* (7.6)
	2	3.87 *dd* (9.3, 7.8)	3.88 *dd* (9.3, 7.8)	3.89 *dd* (9.3, 7.8)
	3	4.21 overlapping	4.22 overlapping	4.22 overlapping
	4	4.45 *dd* (9.3, 9.3)	4.45 *dd* (9.3, 9.3)	4.44 *dd* (9.3, 9.3)
	5	3.96 *m*	3.96 *m*	3.98 *m*
	6	4.65 *br d* (10.8)	4.83 *br d* (10.8)	4.85 *br d* (10.8)
		4.68 *dd* (10.8, 3.6)	4.68 *dd* (10.8, 3.6)	4.68 *dd* (10.8, 3.6)
Glc	1′	5.52 *d* (7.9)	5.51 *d* (7.9)	5.55 *d* (7.9)
	2′	4.05 *dd* (8.8, 7.9)	4.07 *dd* (8.8, 7.9)	4.07 *dd* (8.8, 7.9)
	3′	4.37 *dd* (8.8, 8.8)	4.38 *dd* (8.8, 8.8)	4.38 *dd* (8.8, 8.8)
	4′	4.22 overlapping	4.24 overlapping	4.24 overlapping
	5′	4.15 *ddd* (8.8, 5.6, 2.4)	4.17 *ddd* (8.8, 5.6, 2.4)	4.16 *ddd* (8.9, 5.6, 2.4)
	6′	4.46 *dd* (11.6, 2.4)	4.48 *dd* (11.6, 2.4)	4.48 *dd* (11.6, 2.4)
		4.29 *dd* (11.6, 5.6)	4.29 *dd* (11.6, 5.6)	4.28 *dd* (11.6, 5.6)
Ara	1″	5.10 *d* (7.4)	5.09 *d* (7.4)	5.11 *d* (7.4)
	2″	4.46 *dd* (8.7, 7.4)	4.46 *dd* (8.7, 7.4)	4.47 *dd* (8.7, 7.4)
	3″	4.07 *dd* (8.7, 2.2)	4.06 *dd* (8.7, 2.2)	4.08 *dd* (8.7, 2.2)
	4″	4.22 overlapping	4.23 overlapping	4.24 overlapping
	5″	4.25 overlapping	4.25 overlapping	4.25 overlapping
		3.72 *br d* (11.0)	3.72 *br d* (11.0)	3.73 *br d* (11.0)

Spectral data were measured in pyridine-d_5; TMS was int. standard.
*J values in parentheses are expressed in Hz.

signals (δ 1.00, $J = 7.1$ Hz; δ 1.10, $J = 6.9$ Hz) and two tertiary methyl proton signals (δ 0.81 and 0.70). A comparison of the ^{13}C NMR spectral data for the sugar moiety of **2** with those for **1** permitted the conclusion that the sugar chain was identical in both compounds. Acid hydrolysis of **2** gave glucose and arabinose and one sapogenin identified as 25*S*-5α-spirostan-3β-ol (neotigogenin) by its ^{1}H and ^{13}C NMR spectra and physical data [7, 10]. The ^{13}C NMR spectrum of **2** permitted the assignments of the sugar moiety linked at C-3 of the aglycone (δ 71.6) and also the stereochemistry of the steroidal skeleton (*trans*-A/B ring fusion), (C-5, δ 44.5, and C-6, δ 29.0, respectively), in accordance with the neotigogenin skeleton [8]. Thus, saponin **2** was identified as neotigogenin 3-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-[α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside.

Compound **3** also gave the positive Liebermann–Burchard colour test. The ^{1}H and ^{13}C NMR spectra indicated the same sugar chain as in **1** and **2**. Acid hydrolysis of **3** yielded glucose, arabinose and a new steroidal aglycone **3a**. The mass spectrum of **3a** showed a molecular ion peak at m/z 432, an increase of 16 mass units in relation to those of **1** and **2**. The ^{1}H NMR of **3a** showed the presence of one additional free hydroxyl group β-oriented at C-6, δ 3.81, *br*, *q*, $J =$ 2.7 Hz. This was confirmed by the ^{13}C NMR spectrum which showed signals at δ 77.9 and 72.0, assigned to the C-3 and C-6 carbons of the aglycone, respectively [13]. Further support for the A/B ring configuration of **3a** came by comparison of its ^{1}H and ^{13}C NMR data with those for steroidal saponins of *Allium sativum* L. (Liliaceae) with the same stereochemical configuration for those rings [13]. On the basis of the above evidence the structure of **3** was deduced as 25*S*-spirostan-6β-ol-3-*O*-β-D-glycopyranosyl-(1 → 4)-*O*-[α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside. A literature survey revealed that the sugar chain found in **1–3** is the same as that of saponins from *S. sieboldii*, a native plant of Japan [3]. Compounds **1–3** are new steroidal saponins. A further comment can be advanced about the β-orientation of the hydroxyl group at C-6 of the aglycone of saponin 3. This uncommon orientation is only found in a few saponins of the Liliaceae and in saponins and free steroids from starfish [13, 14]. This β-orientation contrasts with the rather common α-orientation found in many saponins reported in the literature [15]. From a biogenetic viewpoint, one may assume that this exceptional orientation derives from the opening of the more crowded C-5/C-6β-epoxide in early stages of the biosynthesis of **3**. In order to explain the formation of this epoxide on the more sterically

Table 2. ^{13}C NMR spectral data for compounds **1–3**.

C	1	2	3		
1	31.0	37.2	35.0	Inner-Glc	
2	27.1	30.0	30.3	1	102.0
3	74.7	77.6	77.9	2	74.7
4	3.10	34.7	32.8	3	76.5
5	37.2	44.5	48.0	4	81.0
6	27.2	29.2	72.0	5	75.1
7	26.5	32.3	41.3	6	68.4
8	35.6	35.1	31.0	4-Glc	
9	40.4	54.3	54.6	1′	10.9
10	35.6	36.0	36.1	2′	74.8
11	21.2	21.4	21.2	3′	78.4
12	39.9	39.9	40.5	4′	71.8
13	41.0	40.5	40.6	5′	78.2
14	56.6	56.1	56.3	6′	63.0
15	32.3	32.3	32.0	6-Ara	
16	81.2	81.0	81.0	1″	105.7
17	63.2	62.7	63.0	2″	72.6
18	16.6	16.8	16.7	3″	74.6
19	24.2	12.5	12.5	4″	69.8
20	42.5	42.5	42.4	5″	66.8
21	14.3	14.3	15.0		
22	109.7	110.0	109.9		
23	26.4	26.2	26.3		
24	26.2	26.0	26.2		
25	27.6	28.0	27.8		
26	65.1	65.1	65.1		
27	16.3	16.6	16.5		

Spectra were measured in pyridine-d_5.

hindered side of the steroidal skeleton it can be proposed that its formation from the $\Delta^{5,6}$-double bond occurs before the glycosylation at the C-3β-hydroxy group on the spirostan skeleton. This free hydroxyl group would then permit the interaction of the steroidal skeleton with the epoxidation enzyme, leading to selective epoxidation of the $\Delta^{5,6}$-double bond from the more hindered β-site. Thus, a hypothetical pathway can be proposed for the biosynthesis of **3** as shown in Scheme 1.

A final comment concerns the single *trans*-A/B ring fusion of the steroidal skeleton commonly found in aglycones from eastern *Smilax* saponins as reported in refs. [3, 4]. This contrasts with the *cis*-A/B ring configuration as found in **1**, from a Brazilian plant. Although only one instance of *Smilax* is reported here, this configurational aspect could be of importance for further chemotaxonomic studies.

EXPERIMENTAL

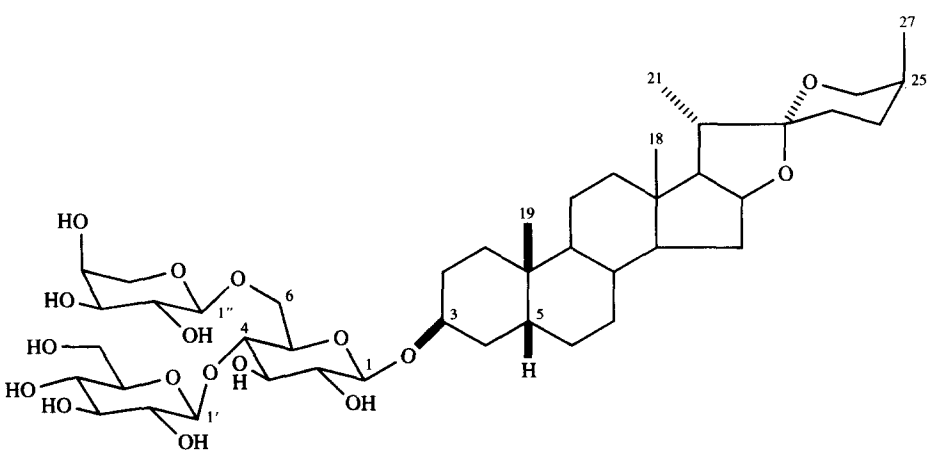
General. ^1H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra were recorded in pyridine-d_5 with TMS as int. standard and chemical shift (δ) reported in ppm. The IR spectra were obtained with a Perkin-Elmer Model 1720 FTIR. The MS were recorded on a VG Spect. mass spectrometer. CC: Silica gel 60 (Merck); Sephadex LH-20 (Pharmacia); TLC: Kieselgel 60 F (0.25 mm thick Merck).

Plant material. The rhizomes of *S. officinalis* Kunth. were collected in June 199 in the district of Magdalena, State of Rio de Janeiro, Brazil. The material was dried at 50–60°. A voucher specimen is deposited at the herbarium of the National Museum (Universidade Federal do Rio de Janeiro) herbal number R 187.637.

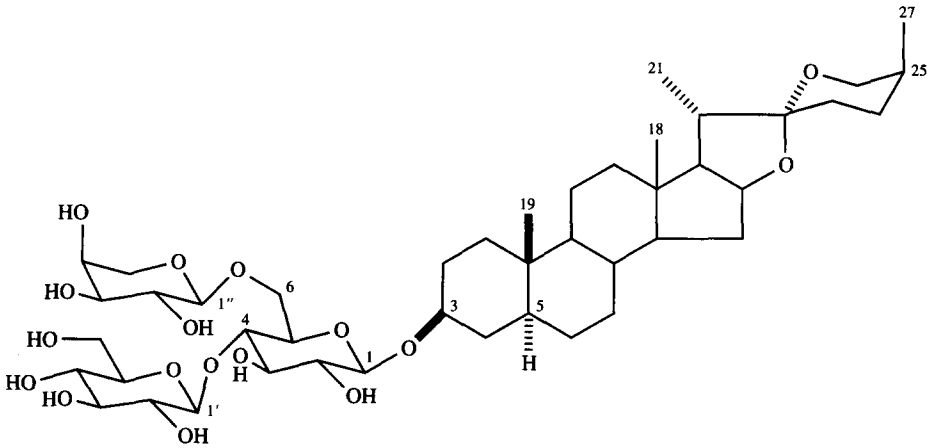
Extraction and isolation. The dried rhizomes (1 kg) were extracted with MeOH and the solvent was evapd under red. pres. After distribution between H_2O and *n*-BuOH, the organic phase was subjected to CC on silica gel with mixts of $CHCl_3$–MeOH–*n*-BuOH–H_2O with increased polarity gradient from (10:5:1:4) to (10:10:1:4), affording 15 frs. The pooled frs 6 and 7, 9 and 10 and 13 and 14 were fractionated on Sephadex LH-20 with MeOH to give **1** (80 mg), **2** (76 mg) and **3** (52 mg), respectively.

Compound **1**. Amorphous powder from MeOH (80 mg), $[\alpha]_D^{25}$ −49.3° (EtOH, *c* 0.30). IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1054 (C-O), 987, 922, 897, 852 (intensity 920 > 897, 25*S*-spirostanol).

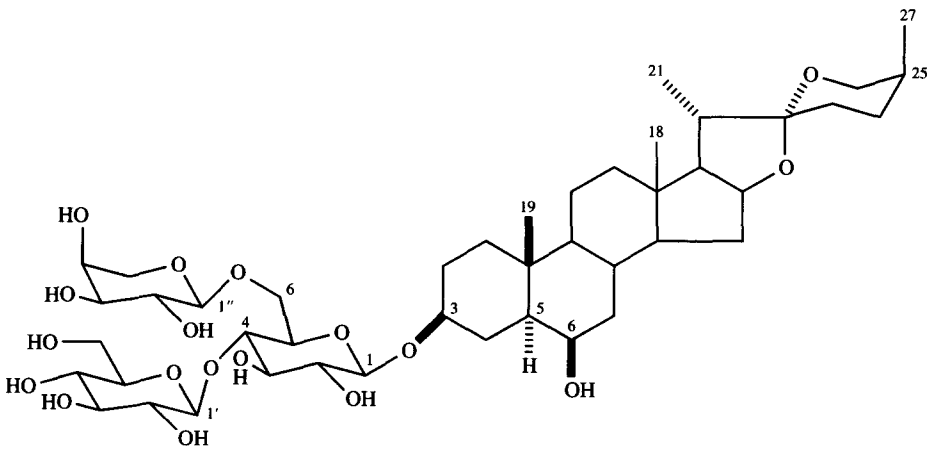
Acid hydrolysis of compound **1**. Compound **1** (10 mg) was hydrolysed with 1 M HCl in dioxane–H_2O (1:1) on a boiling-water bath for 1.5 hr under N_2. The



1

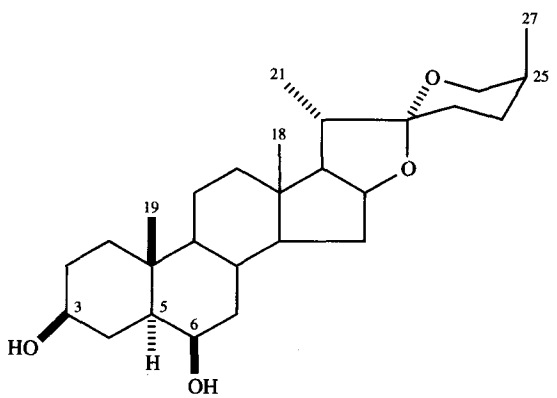


2



3

reaction mixt. was neutralized with $BaCO_3$ and the crude product was subjected to CC on silica gel with gradients of MeOH in $CHCl_3$ (initial solvent, $CHCl_3$–MeOH, 6:1) to yield D-glucose (2.0 mg), L-arabinose (1.0 mg) and sarsapogenin **1a** (3.0 mg). D-Glucose $[\alpha]_D$ +55.0° (H_2O; *c* 0.04); TLC; R_f 0.40 (*n*-BuOH–Me_2CO–H_2O, 4:51). L-Arabinose $[\alpha]_D$ +111.83° (H_2O; *c* 0.012); TLC; R_f 0.45 (*n*-BuOH–Me_2CO–H_2O, 4:5:1) and sarsasapogenin, mp 201–202°; TLC: R_f 0.80, *n*-hexane–EtOH (10:1). EIMS (probe) 70 eV, *m/z* (rel. int.): 416 $[M]^+$ (54), 357 (18), 302 (30), 287 (52), 139 (100), 115 (31) [15].



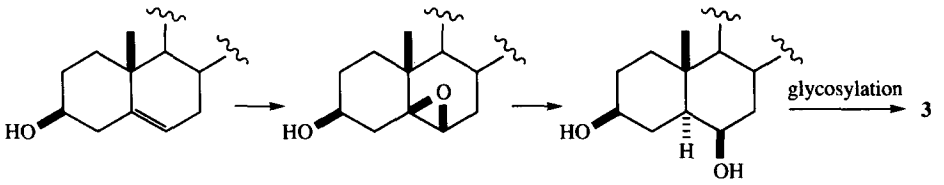
3a

Compound **2**. Amorphous powder form MeOH (76 mg), $[\alpha]_D$ −30.2° (EtOH, *c* 0.30). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1050 (C–O), 985, 920, 900, 850 (intensity 920 > 900, 25*S*-spirostanol).

Acid hydrolysis of compound **2**. Compound **2** (10 mg) was hydrolysed with 1 M HCl (dioxane–H_2O) as for **1**. D-Glucose, L-arabinose and neotigogein, mp 192–194. EIMS (probe) 70 eV *m/z* (rel. int.): 416 $[M]^+$ (6), 401 (3), 398 (40), 357 (4), 347 (10), 344 (15), 302 (34), 287 (24), 273 (56), 255 (7), 176 (15), 147 (7), 139 (100), 115 (36) [7].

Compound **3**. Amorphous powder from MeOH (52 mg); $[\alpha]_D$ −54.2° (MeOH, *c* 0.30).

Acid hydrolysis of **3**. Compound **3** (10 mg) was hydrolysed as for **1** and **2**. D-Glucose, L-arabinose and a new sapogenin 25*S*-6β-ol-spirostan; EIMS (probe)



Scheme 1. Hypothesis for the biosynthesis of compound **3**.

70 eV *m/z* (rel. int.): 432 $[M]^+$; ^1H NMR ($CDCl_3$): δ 0.78 (3H, *s*, 18-Me), 0.97 (3H, *d*, $J = 6.9$ Hz, 21-Me), 1.04 (3H, *s*, 19-Me), 3.81 (1H, *br*, $J = 2.7$ Hz, 6-H).

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