Three New Steroidal Saponins from *Smilax officinalis*

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Z. Naturforsch. **2008**, *63b,* 95 – 100; received August 3, 2007

Three new steroidal saponins were isolated from the roots of *Smilax officinalis* Kunth. Their structures were established as (3β,5β,22α,25*S*)-26-(β-D-glucopyranosyloxy)-22-hydroxyfurostan-3-yl *O*-α-L-arabinopyranosyl-(1→6)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside, (3β,5α,22α,25*S*)-26-(β-D-glucopyranosyloxy)-22-hydroxyfurostan-3-yl *O*-α-L-arabinopyranosyl-(1→6)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside and (3β,5α,6β,22α,25*S*)-26-(β-D-glucopyranosyloxy)-6,22-dihydroxyfurostan-3-yl *O*-α-L-arabinopyranosyl-(1→6)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside. Their structure elucidations were performed using detailed analyses of ^{1}H and ^{13}C NMR spectra including 2D NMR spectroscopic techniques (DEPT, COSY, HSQC, HMBC and HMQC) and chemical conversions.

Key words: Smilax officinalis, Liliaceae, Steroidal Saponins, Furostanol Glycosides

Introduction

This laboratory has been engaged for some time in a comprehensive survey of the plant kingdom in an attempt to find bioactive saponin sources. Steroidal saponins are not widespread. Many plants contain little or no saponin; in others the triterpenoidal saponins predominate. *Smilax* plants (Liliaceae), with about 350 species, are widely distributed in the tropical and temperate zones throughout the world, and especially in the tropical regions of East Asia, and South and North America. Because of the use of *Smilax officinalis* Kunth. in Brazilian folk medicine for the treatment of gout, a chemical investigation on dried roots was carried out, and three steroidal saponins were reported [1]. In this paper, we provide an account of the structure determination of three new bisdesmosidic furostanol saponins by their spectral data and chemical reactions.

Experimental Section

Plant material

Dried roots of *Smilax officinalis* Kunth. were purchased from Flora Medicinal, Rio de Janeiro, Brasil.

General procedures

Melting points were determined by an Electrothermal 9200 micro-melting point apparatus and are uncorrected. The optical rotations were measured with a Perkin Elmer 243B polarimeter. IR spectra were measured with a Perkin Elmer 599B spectrometer. The MALDI-TOFMS were obtained using a Perseptive Voyager RP mass spectrometer. GC-MS analyses were performed with a Shimadzu GCMS-QP5050A gas chromatograph/mass spectrometer using an ionization voltage of 70 eV and an ionization current of 60 μA for EI. GC was carried out with FID using a DB-1 glass capillary column (0.25 × 25 m, 0.25 micron; J. & W. Scientific Incorporated, Folsom, CA, USA). Mass spectra were taken with a VG Auto SpecQ spectrometer. NMR experiments were performed with a Bruker DRX-600 spectrometer at 300 K. All the 2D NMR spectra were acquired in [D$_5$]-pyridine with tetramethylsilane (δ = 0.00) used as internal standard. ^{1}H NMR spectra were recorded at 600 MHz and ^{13}C NMR spectra at 150 MHz. Silica gel columns (230 – 400 mesh ASTM, Merck) and Sephadex LH-20 (Pharmacia) were used for CC. TLC was performed on silica gel plates (Kieselgel 60F$_{254}$, Merck) using the following solvent systems: (A) $CHCl_3/MeOH/H_2O$ (65 : 35 : 10 v/v/v, lower phase) for steroidal saponins **1** – **3**, (B) $CHCl_3$/MeOH (95 : 5 v/v) for pseudosapogenins **1a** – **3a**, and (C) n-BuOH/Me_2CO/H_2O (4 : 5 : 1 v/v/v) for monosaccharides. Spray reagents were orcinol/H_2SO_4 for steroidal saponins **1** – **3** and $CeSO_4$ for pseudosapogenins **1a** – **3a**.

Extraction and isolation

Dried roots (2.5 kg) were extracted with MeOH (10.0 L) for 72 h at r. t. The extract was concentrated under reduced pressure to remove most of the MeOH and the resulting aqueous phase was shaken with n-BuOH [water/n-BuOH

0932–0776 / 08 / 0100–0095 $ 06.00

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

		1			**2**			**3**		
Position		^{13}C, δ	^{1}H, δ (J)	DEPT	^{13}C, δ	^{1}H, δ (J)	DEPT	^{13}C, δ	^{1}H, δ (J)	DEPT
Agl	1	30.8		CH$_2$	37.2		CH$_2$	38.8		CH$_2$
	2	26.8		CH$_2$	29.9		CH$_2$	30.0		CH$_2$
	3	74.6	3.91 m	CH	77.5	3.96 m	CH	77.9	3.98 m	CH
	4	30.9		CH$_2$	34.6		CH$_2$	32.8		CH$_2$
	5	36.9	1.94 m	CH	44.6	1.18 m	CH	48.0	1.21 m	CH
	6	26.7	1.75 m	CH$_2$	29.0	1.22 m	CH$_2$	70.9	3.81 m	CH
	7	26.5		CH$_2$	32.4		CH$_2$	40.3		CH$_2$
	8	35.4		CH	35.3		CH	30.7		CH
	9	40.2		CH	54.5		CH	54.6		CH
	10	35.4		C	35.9		C	36.1		C
	11	21.0		CH$_2$	21.3		CH$_2$	21.3		CH$_2$
	12	40.1		CH$_2$	40.2		CH$_2$	40.8		CH$_2$
	13	40.7		C	41.1		C	41.1		C
	14	56.4		CH	56.4		CH	56.3		CH
	15	32.2		CH$_2$	32.4		CH$_2$	32.4		CH$_2$
	16	81.1	4.99 m	CH	81.1	4.98 m	CH	81.0	4.98 m	CH
	17	64.0		CH	64.0		CH	64.0		CH
	18	16.7		CMe	16.7		CMe	16.7		CMe
	19	23.7		CMe	12.3		CMe	16.3		CMe
	20	40.7	2.25 m	CH	40.7	2.24 m	CH	40.7	2.26 m	CH
	21	16.2	1.35 d (7.0)	CHMe	16.4	1.34 d (7.)	CHMe	16.0	1.35 d (7.0)	CHMe
	22	110.7		C	110.6		C	110.6		C
	23	37.1		CH$_2$	37.1		CH$_2$	37.1		CH$_2$
	24	27.7		CH$_2$	27.7		CH$_2$	27.7		CH$_2$
	25	33.6	1.93 m	CH	33.6	1.93 m	CH	33.6	1.93 m	CH
	26	74.8	3.53 m, 4.19 m	CH$_2$	74.8	3.53 m, 4.19 m	CH$_2$	74.8	3.53 m, 4.19 m	CH$_2$
	27	16.7	0.99 d (6.7)	CHMe	16.7	0.99 d (6.7)	CHMe	16.7	0.99 d (6.7)	CHMe
Glc	1	102.0	4.81 d (7.8)	CH	102.0	4.94 d (7.8)	CH	102.1	4.95 d (7.8)	CH
	2	74.7		CH	74.7		CH	74.8		CH
	3	76.5		CH	76.5		CH	76.6		CH
	4	81.1		CH	81.1		CH	81.1		CH
	5	74.9		CH	74.8		CH	74.8		CH
	6	68.4		CH$_2$	68.3		CH$_2$	68.4		CH$_2$
Glc	1′	104.9	5.52 d (7.9)	CH	104.8	5.51 d (7.9)	CH	104.9	5.51 d (7.9)	CH
	2′	75.2		CH	75.2		CH	75.2		CH
	3′	78.5		CH	78.4		CH	78.5		CH
	4′	71.8		CH	71.9		CH	71.9		CH
	5′	78.2		CH	78.1		CH	78.1		CH
	6′	62.6		CH$_2$	62.5		CH$_2$	62.5		CH$_2$
Glc	1″	104.9	4.80 d (7.7)	CH	104.8	4.82 d (7.7)	CH	104.9	4.84 d (7.7)	CH
	2″	75.2		CH	75.2		CH	75.2		CH
	3″	78.6		CH	78.6		CH	78.6		CH
	4″	71.7		CH	71.7		CH	71.7		CH
	5″	78.4		CH	78.4		CH	78.4		CH
	6″	62.8		CH$_2$	62.8		CH$_2$	62.8		CH$_2$
Ara	1	105.6	5.10 d (7.4)	CH	105.7	5.09 d (7.4)	CH	105.6	5.11 d (7.4)	CH
	2	72.5		CH	72.6		CH	72.5		CH
	3	74.6		CH	74.6		CH	74.6		CH
	4	69.7		CH	69.7		CH	69.7		CH
	5	67.1		CH$_2$	67.1		CH$_2$	67.1		CH$_2$

[a] ^{13}C (150 MHz) and ^{1}H NMR (600 MHz) in [D$_5$]-pyridine; δ in ppm, J in Hz. Assignments based on ^{1}H-^{1}H COSY, HSQC, HMBC and HMQC experiments. The following convention is used: Agl = aglycone, Glc = β-D-glucopyranosyl, Ara = α-L-arabinopyranosyl.

(1 : 1) v/v]. This procedure was repeated and the resulting organic phase was evaporated *in vacuo* to give a crude material (67.3 g). It was dissolved in MeOH (1.0 L) and roughly chromatographed (673 mg/10 mL each time) on Sephadex LH-20 (3.8 × 65 cm) with MeOH. The fractions were combined based on the TLC profiles to give the saponin mixture

(2.17 g). Further purification by chromatography on a silica gel column (2.8 × 90 cm) eluted with $CHCl_3$/MeOH/n-BuOH/H_2O (10 : 5 : 1 : 4 v/v/v/v) afforded three TLC homogeneous compounds **1** (519 mg, R_f = 0.35), **2** (457 mg, R_f = 0.33) and **3** (311 mg, R_f = 0.29) which gave dark blue colors with orcinol/H_2SO_4.

Compound 1

Amorphous solid. – M. p. $>$ 280 °C. – $[\alpha]_D^{25} = -74$ (c = 0.1, MeOH). – IR (KBr): ν = 3435 (O–H), 2936 (C–H), 1641, 1592, 1513, 1459, 1399, 1312, 1240, 1183, 1054, 984, 916, 847, 816 cm^{-1} [(25S)-furostanol, intensity 916 $>$ 847 cm^{-1}]. – ^1H and ^{13}C NMR data given in Table 1. – MALDI-TOFMS: *m/z* = 1076.2015 (calcd. 1076.2029 for $C_{50}H_{84}O_{23}Na$, $[M + Na]^+$).

Compound 2

Amorphous solid. – M. p. $>$ 280 °C. – $[\alpha]_D^{25} = -20$ (c = 0.1, MeOH). – IR (KBr): ν = 3432 (O–H), 2932 (C–H), 1646, 1450, 1428, 1380, 1260, 1170, 1078, 1048, 915, 849, 815 cm^{-1} [(25S)-furostanol, intensity 915 $>$ 849 cm^{-1}]. – ^1H and ^{13}C NMR data given in Table 1. – MALDI-TOFMS: *m/z* = 1076.2018 (calcd. 1076.2029 for $C_{50}H_{84}O_{23}Na$, $[M + Na]^+$).

Compound 3

Amorphous solid. – M. p. $>$ 280 °C. – $[\alpha]_D^{25} = -36$ (c = 0.1, MeOH). – IR (KBr): ν = 3435 (O–H), 2935 (C–H), 1645, 1449, 1427, 1383, 1262, 1174, 1079, 1049, 918, 851, 817 cm^{-1} [(25S)-furostanol, intensity 918 $>$ 851 cm^{-1}]. – ^1H and ^{13}C NMR data given in Table 1. – MALDI-TOFMS: *m/z* = 1092.2007 (calcd. 1092.2023 for $C_{50}H_{84}O_{24}Na$, $[M + Na]^+$).

*Acid hydrolysis of **1**–**3***

A solution of each saponin (100 mg) in 1 M HCl/1,4-dioxane (1 : 1 v/v; 10 mL) was heated in a sealed tube for 1 h at 100 °C. After cooling, the reaction mixture was neutralized with 1 M NaOH in MeOH and evaporated to dryness. The salts that deposited on addition of MeOH were filtered off, and the filtrate was passed through a Sephadex LH-20 column with MeOH to give the corresponding hydrolysates free of salts which were chromatographed over silica gel using a discontinuous gradient of $CHCl_3$/MeOH (19 : 1 v/v to 7 : 3 v/v) to yield the pseudosapogenins **1a** (22 mg), **2a** (21 mg) and **3a** (18 mg), and sugar mixtures (57 mg, 55 mg and 52 mg) obtained from **1**–**3**, respectively. The identity of each aglycone was established by comparison with an authentic sample through m. p., IR, ^1H and ^{13}C NMR and EIMS. A sample of each sugar mixture (1 mg) was dissolved in pyridine (100 μL) and analyzed by silica gel-TLC in the above described solvent system (C). After spraying with orcinol/H_2SO_4, arabinose gave a purple spot at R_f = 0.45, and glucose gave a blue spot at R_f = 0.38.

Molar carbohydrate composition and D,L configurations

The molar carbohydrate composition of compounds **1**–**3** was determined by GC-MS analyses of their monosaccharides as their trimethylsilylated methylglycosides obtained after methanolysis (0.5 M HCl in MeOH, 24 h, 80 °C) and trimethylsilylation [2]. The configurations of the glycosides were established by capillary GC of their trimethylsilylated (–)-2-butylglycosides [3].

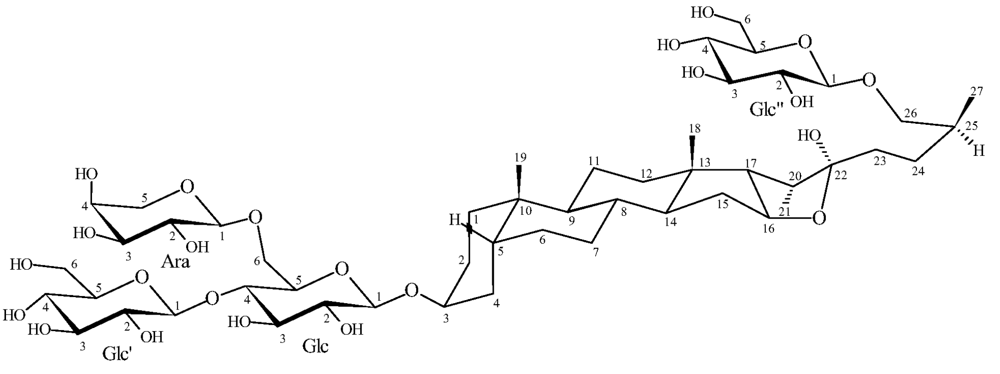
Methylation analyses

Compounds **1**–**3** were methylated with dimethyl sulfoxide/lithium methylsulfinyl carbanion/methyl iodide [4]. The methyl ethers were obtained after hydrolysis (4 N trifluoroacetic acid, 2 h, 100 °C) and analyzed as partial alditol acetates by GC-MS [5].

Result and Discussion

Compound **1** was obtained as amorphous solid and gave a positive Liebermann-Burchard test for a steroidal saponin. It revealed a quasi-molecular ion peak at m/z = 1076.2015 $[M + Na]^+$ in the MALDI-TOFMS. In the ^{13}C NMR spectrum, of the fifty carbon signals observed, there are four methyl groups, fifteen methylene groups (five of which were oxygenated), twenty-eight methine groups (twenty-one of which were oxygenated) and three quaternary carbon atoms (one of which was oxygenated). The number of hydrogens attached to each individual carbon atom was determined by the DEPT spectrum. On the basis of the above mentioned MS and NMR spectral data (Table 1), compound **1** was assumed to be a saponin with the molecular formula of $C_{50}H_{84}O_{23}$, bearing a chain of four monosaccharide moieties.

In addition to this, the furostanol glycosidic nature of compound **1** was indicated by the strong absorption bands at 3435, 1183 and 1054 cm^{-1} and a 25S furostane steroidal structure (847 and 916 cm^{-1}, intensity 916 $>$ 847 cm^{-1}) in the IR spectrum [2]. The 25S stereochemistry of the Me-27 group was confirmed by resonances of protons and carbons at C-25, C-26 and C-27 in comparison with data reported in the literature [7, 8]. The differences observed in ^1H NMR chemical shifts (Table 1) of geminal protons H-26a and H-26b ($\delta_{Ha} - \delta_{Hb}$ = 0.66) supported a 25S furostane-type steroidal saponin since this difference is usually $>$ 0.57 ppm in 25S compouds and 0.48 ppm in 25R compounds [8]. The A/B *cis*-ring fusion was deduced



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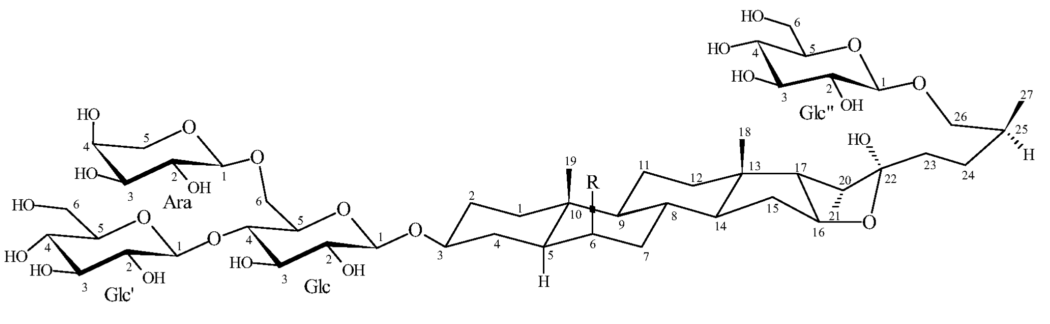

2 R = H
3 R = OH

Fig. 1. Structures of compounds **1** – **3**.

from the signals at δ = 36.9 (CH, C-5), 40.2 (CH, C-9) and 23.7 (CH_3, C-19) indicating that the aglycone of **1** is a 5β steroidal sapogenin [1,9], confirmed by one proton at δ = 1.94 (m, H-5). The ^1H NMR spectrum of **1** displayed signals for four anomeric protons at δ = 4.80 (d, J = 7.7 Hz, 1H), 4.81 (d, J = 7.8 Hz, 1H), 5.10 (d, J = 7.4 Hz, 1H) and 5.52 (d, J = 7.9 Hz, 1H), which gave correlations in the HSQC spectrum with ^{13}C NMR signals at δ = 104.9, 102.0, 105.6 and 104.9, respectively. Evaluation of chemical shifts and spin-spin couplings allowed the identification of three β-glucopyranosyl units (Glc, Glc′ and Glc″), and one α-arabinopyranosyl unit (Ara). The attachments of the sugar moieties to the aglycone moiety were established by ^1H-^1H COSY, HMBC and HMQC experiments. The HMBC and HMQC spectra displayed long range couplings between a terminal glucose-H-1″ at δ = 4.80 and aglycone-C-26 at δ = 74.8, and between an internal glucose-H-1 at δ = 4.81 and aglycone-C-3 at δ = 74.6. In addition to this, long range couplings were observed between a terminal glucose-H-1′ at δ = 5.52 and glucose-C-4 at δ = 81.1, and between a terminal arabinose-H-1 at δ = 5.10 and glucose-C-6 at δ = 68.4, indicating that compound **1** is undoubtedly a bisdesmosidic steroidal saponin.

The sequence of the sugar chain was confirmed by methylation analysis [4] which furnished 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl arabinitol, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl glucitol and 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl glucitol. These results indicated that the sugar-aglycone linkage and the sugar chain sequence of compound **1** was as shown in Fig. 1.

On acid hydrolysis, compound **1** gave a pseudosapogenin, glucose and arabinose. The pseudosapogenin was identified as (3β,5β,25*S*)-spirostan-3-ol (**1a**) by comparison with an authentic sample through m. p., IR, ^1H and ^{13}C NMR and EIMS data. The molar carbohydrate composition of **1** indicated the presence of four neutral monosaccharides: glucose-arabinose (3 : 1) [2]. Their absolute configurations were determined by GC of their trimethylsilylated (−)-2-butylglycosides [3]. D-Glucose and L-arabinose were detected. The ^{1}H NMR spectral data, a comparison of the ^{13}C NMR signals (Table 1) of the aglycone moiety of **1** with those described in the literature [1,7–10], and the pseudoaglycone obtained by hydrolysis from **1** indicated that the structure of the aglycone was (3β,5β,22α,25S)-furostan-3,22,26-triol. Consequently, on the basis of MALDI-TOF MS, IR, ^{1}H and ^{13}C NMR spectroscopy and chemical reactions, the structure of **1** was established as (3β,5β,22α,25S)-26-(β-D-glucopyranosyloxy)-22-hydroxyfurostan-3-yl O-α-L-arabinopyranosyl-(1→6)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside.

Compound **2**, an amorphous solid, revealed a quasimolecular ion peak at m/z = 1076.2018 [M + Na]$^+$. On comparison of the whole ^{13}C NMR spectrum of **2** with that of **1** (Table 1), a set of signals corresponding to the carbons of the A/B *trans*-ring fusion showed variations, while all other signals remained almost unaffected. It was observed that the signals of C-5 and C-9 at δ = 44.6 and 54.5, respectively, were markedly displaced downfield as compared with C-5 and C-9 of **1** at δ = 36.9 and 40.2, respectively. The signal observed at δ = 12.3 for C-19 was markedly displaced upfield as compared with C-19 of **1** at δ = 23.7.

On acid hydrolysis, compound **2** gave a pseudosapogenin, glucose and arabinose. The pseudosapogenin was identified as (3β,5α,25S)-spirostan-3-ol (**2a**) by comparison with an authentic sample through m. p., IR, ^{1}H and ^{13}C NMR and EIMS data. The molar carbohydrate composition, the absolute configuration and the results of the methylation analysis of **2** were similar to those of **1**. The ^{1}H NMR spectral data, a comparison of the ^{13}C NMR signals (Table 1) of the aglycone moiety of **2** with those described in the literature [1,7–10], and the pseudoaglycone obtained from **2** by hydrolysis showed that the structure of the aglycone was (3β,5α,22α,25S)-furostan-3,22,26-triol. Consequently, on the basis of the results described above, the structure of compound **2** was established as (3β,5α,22α,25S)-26-(β-D-glucopyranosyloxy)-22-hydroxyfurostan-3-yl O-α-L-arabinopyranosyl-(1→6)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (Fig. 1).

Compound **3**, an amorphous solid, revealed a quasimolecular ion peak at m/z = 1092.2007 [M + Na]$^+$. The ^{1}H and ^{13}C NMR spectral data (Table 1) of **3** showed that it contains the same sugar structure as found in **1** and **2**, but differs by the aglycone structures of **1** and **2**. The molecular weight of **3** was 16 mass units greater than those of **1** and **2**, indicating that **3** had twenty-four oxygen atoms. The aglycone nature of compound **3** was also manifested by its ^{1}H NMR spectrum which displayed signals for two secondary methyl protons at δ = 0.99 (d, J = 6.7 Hz, Me-27) and 1.35 (d, J = 7.0 Hz, Me-21) and two angular methyl protons at δ = 0.91 (s, Me-18) and 1.24 (s, Me-19). It was observed that the signal of Me-19 at δ = 1.24 was markedly displaced downfield as compared with Me-19 of **2** at δ = 0.85. The IR and ^{1}H NMR spectra of **3** indicated a 25S-furostane-type steroidal saponin as compared with those of **1** and **2**. On comparison of the whole ^{13}C NMR spectrum of **3** with that of **2**, a set of signals corresponding to the carbons C-5, C-6, C-7, C-8 and C-19 at δ = 48.0, 70.9, 40.3, 30.7 and 16.3, respectively, showed variations, while all other signals remained almost unaffected. It was observed that the signals of C-6 and C-19 at δ = 70.9 and 16.3, respectively, were markedly displaced downfield as compared with C-6 and C-19 of **2** at δ = 29.0 and 12.3, respectively. The signals observed in the ^{1}H NMR spectrum at δ = 3.81 (m, 1H) and 1.24 (s, 1H) corresponding to H-6 and Me-19, respectively, confirmed the presence of one additional free hydroxyl group β-oriented at C-6 [11].

On acid hydrolysis, compound **3** gave a pseudosapogenin, glucose and arabinose. The pseudosapogenin was identified as (3β,5α,6β,25S)-spirostan-3,6-diol (**3a**) by comparison with an authentic sample through m. p., IR, ^{1}H and ^{13}C NMR and EIMS data. The molar carbohydrate composition, the absolute configuration and the results of the methylation analysis of **3** were similar to those of **1** and **2**. The ^{1}H NMR spectral data and a comparison of the ^{13}C NMR signals of the aglycone moiety of **3** with those described in the literature [11] and the pseudoaglycone obtained from **3** by hydrolysis showed that the structure of the aglycone was (3β,5α,6β,22α,25S)-furostan-3,6,22,26-tetrol. Consequently, on the basis of spectroscopic methods and chemical reactions, the structure of **3** was established as (3β,5α,6β,22α,25S)-26-(β-D-glucopyranosyloxy)-6,22-dihydroxyfurostan-3-yl O-α-L-arabinopyranosyl-(1→6)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (Fig. 1).

Acknowledgement

This work was financially supported by FUJB, CAPES, FINEP, and CNPq.

[1] R. R. Bernardo, A. V. Pinto, J. P. Parente, *Phytochemistry* **1996**, *43*, 465 – 469.

[2] J. P. Kamerling, G. J. Gerwig, J. F. G. Vliegenthart, J. R. Clamp, *Biochem. J.* **1975**, *151*, 491 – 495.

[3] G. J. Gerwig, J. P. Kamerling, J. F. G. Vliegenthart, *Carbohydr. Res*. **1978**, *62*, 349 – 357.

[4] J. P. Parente, P. Cardon, Y. Leroy, J. Montreuil, B. Fournet, G. Ricart, *Carbohydr. Res*. **1985**, *141*, 41 – 47.

[5] J. S. Sawardeker, J. H. Sloneker, A. Jeanes, *Anal. Chem*. **1965**, *37*, 1602 – 1604.

[6] M. E. Wall, C. R. Eddy, M. L. McClennan, M. E. Klumpp, *Anal. Chem*. **1952**, *24*, 1337 – 1341.

[7] M. Sautour, T. Miyamoto, M. A. Lacaille-Dubois, *J. Nat. Prod*. **2005**, *68*, 1489 – 1493.

[8] P. K. Agrawal, *Magn. Reson. Chem*. **2004**, *42*, 990 – 993.

[9] K. Tori, S. Seo, Y. Terui, J. Nishikawa, F. Yasuda, *Tetrahedron Lett*. **1981**, *22*, 2405 – 2408.

[10] M. Yoshikawa, T. Murakami, H. Komatsu, N. Murakami, J. Yamahara, H. Matsuda, *Chem. Pharm. Bull.* **1997**, *45*, 81 – 87.

[11] H. Matsuura, T. Ushiroguchi, Y. Itakura, T. Fuwa, *Chem. Pharm. Bull*. **1989**, *37*, 2741 – 2743.