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CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY OF STEROIDAL SAPONINS FROM *Furcraea gigantea*

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A new bisdesmosidic furostanol saponin, along with a known spirostanol saponin, furcreastatin, were isolated from Furcraea gigantea *Vent. (Agavaceae). The structure of the new saponin was elucidated as 3-[(*O*-6-deoxy-*α*-L-mannopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-[*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranosyl)oxy]-(3*β*,5*α*,25*R*)-26-(*β*-D-glucopyranosyloxy)-22-hydroxyfurost-12-one. The structural identification was performed using a combination of spectroscopic techniques and chemical conversions. Furcreastatin showed a powerful haemolytic effect in the in vitro assay, but the new bisdesmosidic furostanol saponin demonstrated only a significant inhibition of the capillary permeability activity.*

Key words: *Furcraea gigantea*, steroidal saponins, structural elucidation, biological activity.

Furcraea gigantea Vent. (Agavaceae) is native to Brazil and is cultivated as an ornamental plant [1]. According to the Brazilian herbal description, its root decoction (taken at the rate of two glassfuls each day) is an active diuretic and is claimed to be an effective remedy for venereal diseases, clarifying the urine. A slightly roasted leaf section is applied on any tumor or swelling. The juice of a roasted leaf is extracted and applied on ulcers, fistulas, and wounds [2, 3]. Its fiber is inferior to and more difficult to extract than that of *Agave sisalana*. As part of our programme on the chemical investigation of bioactive steroidal saponins, we have now examined the leaves of *F. gigantea*. This resulted in the isolation of a new bisdesmosidic furostanol saponin (**1**) and the known spirostanol saponin, furcreastatin (**2**) [4]. In this paper, we provide an account of the structural determination of the new saponin by its spectral data and chemical conversions. The hemolytic and anti-inflammatory activities of the isolated saponins were also investigated.

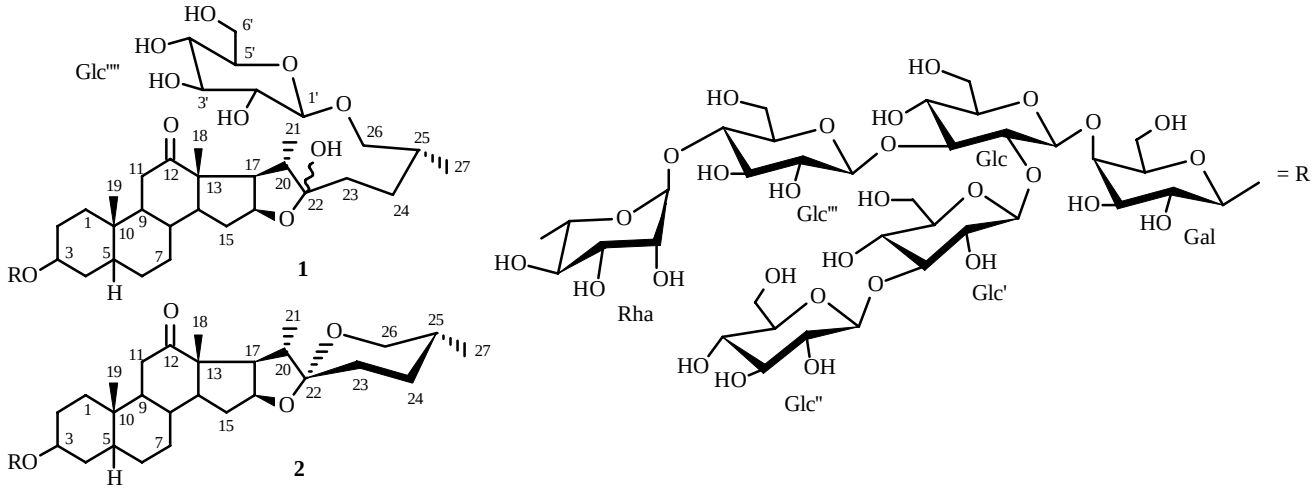

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TABLE 1. Selected ^{1}H NMR Assignments of Compound **1** in C_5D_5N (δ, ppm, J/Hz)

Aglycone characteristic proton signals			Sugar methyl group and anomeric proton signals		
Position	Compound **1**	^{1}H-^{1}H COSY	Position	Compound **1**	^{1}H-^{1}H COSY
H-3	3.86 m	H-2, H-4	Rha-Me	1.71 (d, J = 6.2)	Rha-H-5
H-5	0.87 m	H-4, H-6	Gal-H-1	4.84 (d, J = 7.8)	Gal-H-2
Me-18	1.08 s		Glc-H-1	5.08 (d, J = 7.8)	Glc-H-2
Me-19	0.67 s		Clc-H-1′	5.50 (d, 7.5)	Glc-H-2′
Me-21	1.38 (d, J = 6.9)	H-20	Glc-H-1‴	5.05 (d, J = 7.5)	Glc-H-2″
Me-27	0.97 (d, J = 6.6)	H-25	Glc-H-1⁗	5.14 (d, J = 8.0)	Glc-H-2‴
			Rha-H-1	4.84 (d, J = 7.8)	Glc-H-2⁗
				5.75 s	Rha-H-2

TABLE 2. ^{13}C NMR Data of the Aglycone and Carbohydrate Moieties of Compound **1** in C_5D_5N[a]

C atom	**1**	DEPT	C atom	**1**	DEPT
1	36.64	CH_2	Glc1	104.40	CH
2	29.89	CH_2	2	80.31	CH
3	77.09	CH	3	87.45	CH
4	34.63	CH_2	4	70.13	CH
5	44.39	CH	5	76.88	CH
6	28.58	CH_2	6	62.43	CH_2
7	31.68	CH_2	Glc1′	103.59	CH
8	34.35	CH	2′	74.23	CH
9	55.53	CH	3′	87.45	CH
10	36.22	C	4′	68.94	CH
11	37.92	CH_2	5′	77.47	CH
12	212.69	C	6′	61.65	CH_2
13	55.29	C	Glc 1″	104.92	CH
14	55.89	CH	2″	75.04	CH
15	31.38	CH_2	3″	77.47	CH
16	79.68	CH	4″	71.09	CH
17	54.43	CH	5″	77.89	CH
18	16.32	CH_3	6″	62.03	CH_2
19	11.76	CH_3	Glc 1‴	104.12	CH
20	42.61	CH	2‴	75.04	CH
21	13.92	CH_3	3‴	76.01	CH
22	110.53	C	4‴	77.47	CH
23	30.15	CH_2	5‴	76.67	CH
24	27.73	CH_2	6‴	60.74	CH_2
25	33.74	CH	Glc 1⁗	105.01	CH
26	74.75	CH_2	2⁗	75.22	CH
27	16.76	CH_3	3⁗	78.61	CH
Gal 1	102.09	CH	4⁗	71.69	CH
2	76.60	CH	5⁗	78.51	CH
3	75.04	CH	6⁗	63.01	CH_2
4	79.60	CH	Rha 1	103.42	CH
5	74.82	CH	2	71.95	CH
6	60.28	CH_2	3	72.14	CH
			4	73.38	CH
			5	69.90	CH
			6	18.00	CH_3

[a]The assignments were made on the basis of DEPT, HETCOR, and COLOC experiments.

Compound **1** was obtained as colorless needles and gave a positive Liebermann-Burchard test for a steroidal saponin. It revealed a quasi-molecular ion peak at *m/z* 1590.6415 $[M+Na]^+$ in the MALDI-TOFMS. In the ^{13}C NMR spectrum (Table 2), of the 69 carbon signals observed, there are five methyls, sixteen methylenes (seven of which were oxygenated), forty-four methines (thirty-seven of which were oxygenated), and four quaternary carbon atoms (two of which were oxygenated). On the basis of the above – mentioned MS and NMR spectral data, compound **1** was assumed to be a saponin with the molecular formula of $C_{69}H_{114}O_{39}$, bearing a chain of seven sugar moieties.

In addition to this, the furostanol glycosidic nature of compound **1** was indicated by the strong absorption bands at 3422 and 1074 cm^{-1} and a 25*R*-furostan steroidal structure (895 and 912 cm^{-1}, intensity 912<895 cm^{-1}) in the IR spectrum [5], confirmed by ^1H and ^{13}C NMR spectra (Tables 1 and 2) [6–8].

The ^1H NMR spectral data (Table 1) contained signals for two hydrogens at δ 3.86 (m, H-3) and 0.87 (m, H-5), two secondary methyl hydrogens at δ 1.38 (δ, J = 6.9 Hz, 3H-21) and 0.97 (δ, J = 6.6 Hz, 3H-27), and two angular methyl hydrogens at δ 1.08 (s, 3H-18) and 0.67 (s, 3H-19). The above ^1H NMR spectral data and a comparison of the ^{13}C NMR signals of the aglycone moiety of 1 (Table 2) with those described in the literature [4, 6] suggest the structure of the aglycone to be 3,22,26-trihydroxy-(3*β*,5*α*,25*R*)-furostan-12-one. In addition to this, the ^1H NMR spectrum of **1** showed seven anomeric hydrogens at δ 4.84 (d, J = 7.7 Hz, 2H), 5.05 (d, J = 7.5 Hz, 1H), 5.08 (d, J = 7.8 Hz, 1H), 5.14 (d, J = 8.0 Hz, 1H), 5.50 (d, J = 7.5 Hz, 1H), and 5.75 (s, 1H) corresponding to Gal-H-1 and Glc-H-1‴″, Glc-H-1″, Glc-H-1, Glc-H-1‴, Glc-H-1′, and Rha-H-1, respectively, indicating the *β*-anomeric configuration for galactose and five glucoses and *α*-anomeric configuration for rhamnose. The ^{13}C NMR spectroscopic data for the sugar moieties indicated that all the monosaccharides were in pyranose form.

In the ^{13}C NMR spectrum of compound **1**, a 1,4-linked inner *β*-D-galactopyranosyl unit, a 1,2,3-linked inner *β*-D-glucopyranosyl unit, a 1,3-linked inner *β*-D-glucopyranosyl unit, a 1,4-linked inner *β*-D-glucopyranosyl unit, two terminal *β*-D-glucopyranosyl units, and a terminal *α*-L-rhamnopyranosyl unit were observed. As shown in Tables 1 and 2, ^1H and ^{13}C NMR chemical shift assignments were made by standard 1D and 2D NMR techniques. Its COLOC spectrum displayed long-range couplings between galactose-H-1 at δ 4.84 and aglycone-C-3 at δ 77.09, between glucose-H-1‴″ at δ 4.84 and aglycone-C-26 at δ 74.75, between glucose-H-1″ at δ 5.05 and glucose-C-3′ at δ 87.45, between glucose-H-1 at δ 5.08 and galactose-C-4 at δ 79.60, between glucose-H-1‴ at δ 5.14 and glucose-C-3 at δ 87.45, between glucose-H-1′ at δ 5.50 and glucose-C-2 at δ 80.31, and between rhamnose-H-1 at δ 5.75 and glucose-C-4‴ at δ 77.47. In addition to this, the methylation analysis of compound **1** furnished 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl rhamnitol, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl glucitol, 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl glucitol, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl glucitol, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl galactitol, and 1,2,3,5-tetra-*O*-acetyl-4,6-di-*O*-methyl glucitol.

On acid hydrolysis, compound **1** gave a pseudosapogenin, galactose, glucose, and rhamnose. The pseudosapogenin was identified as 3-hydroxy-(3*β*,5*α*,25*R*)-spirostan-12-one (hecogenin). Its identity was established by comparison with an authentic sample through mp, $[\alpha]_D$, IR, ^1H and ^{13}C NMR, and EIMS [6–9]. The molar carbohydrate composition of compound **1** indicated the presence of seven neutral monosaccharides: galactose–glucose–rhamnose (1:5:1). Their absolute configurations were determined by GC of their trimethylsilylated (–)-2-butylglycosides [10, 11]. D-galactose, D-glucose, and L-rhamnose were identified. Consequently, on the basis of IR, ^1H and ^{13}C NMR spectroscopy, MALDI-TOFMS and chemical reactions, the structure of compound **1** was established as 3-[(*O*-6-deoxy-*α*-L-mannopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-[*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranosyl)oxy]-(3*β*,5*α*,25*R*)-26-(*β*-D-glucopyranosyloxy)-22-hydroxyfurost-12-one.

According to the literature, steroidal saponins possess anti-inflammatory properties [12]. However, this activity is sometimes accompanied by an undesirable hemolytic effect [13]. In order to evaluate the pharmacological properties of the isolated steroidal saponins **1** and **2**, it was screened for hemolytic activity *in vitro* [14] and compared to adjuvants commonly used in animal and human experimental models. Generally, steroidal saponins possess powerful hemolytic activity because steroids have higher affinities for cholesterol on erythrocyte membranes [13]. As a consequence of its amphipathic structure, this seems to be the case for compound **2** (Fig. 1). Nevertheless, this is not the case for compound 1, which demonstrated absence of hemolytic effects. This particular behavior can be easily explained by the assumption that saponin **1** possesses sugar units distributed in opposite side of the aglycone moiety, which considerably reduces its hydrophobicity, resulting in the loss of the amphipathic features. In addition to this, compound **1** inhibited an increase in vascular permeability caused by acetic acid, which is a typical model of first-stage inflammatory reaction [15]. As a highly hemolytic substance, compound **2** did not show significant anti-inflammatory activity (Fig. 2). The experimental results obtained may help explain some biological properties attributed to several steroidal saponins reported in the literature [12].

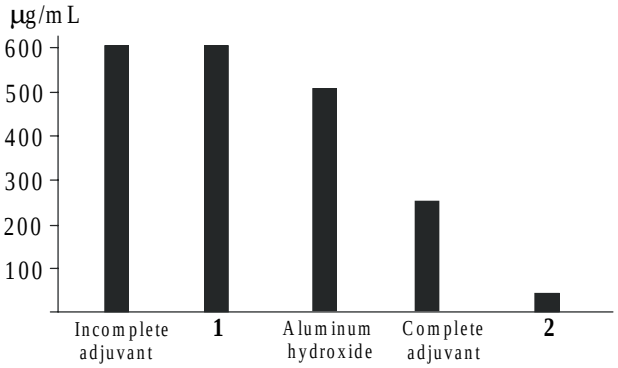

Fig. 1

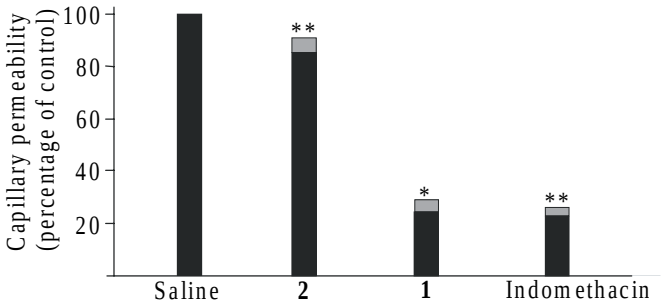

Fig. 2

Fig. 1. 50% Hemolytic dose (μg/mL) of compounds **1**, **2**, and adjuvants.

Fig. 2. Effect of compounds **1** and **2** (100 mg/kg, p.o.) on acetic acid-induced vascular permeability in mice. Standard, indomethacin (10 mg/kg, p.o.). Results are mean +/- S.E.M. (n = 5); *p < 0.05, **p < 0.01 vs. control; Student's t-test.

EXPERIMENTAL

General. Melting points were determined by an Electrothermal 9200 micro-melting point apparatus and are uncorrected. The optical rotations were measured on a Perkin–Elmer 243 polarimeter. IR spectra were measured on a Perkin–Elmer 599B infrared spectrometer and GC analyses using an HP5890 gas chromatograph (Hewlett Packard) with an FID using an OV-101 glass capillary column (0.3 mm × 25 m). GC-MS analyses were carried out with a VG Autospec Q mass spectrometer using an electron energy of 70 eV and an ionizing current of 0.2 mA. MALDI-TOFMS was conducted using a Perseptive Voyager RP mass spectrometer using norharmane as matrix. ^{1}H, ^{1}H 2D COSY, ^{13}C, DEPT, HETCOR, and COLOC NMR spectra were obtained on a Varian Gemini 200 NMR spectrometer operating at 200 MHz for δ_H and 50 MHz for δ_C, in C_5D_5N at 25°C. The chemical shifts (δ) for ^{1}H and ^{13}C are expressed in ppm relative to internal tetramethylsilane (δ 0.00 ppm in both cases). Sephadex LH-20 (Pharmacia), and silica gel column (230–400 mesh ASTM, Merck) were used for CC. TLC was performed on silica gel coated plates (Merck) using the following solvent systems: (a) $CHCl_3$–MeOH–H_2O (65:35:10, lower phase) for steroidal saponins **1** and **2**, (b) $CHCl_3$–MeOH (12:1) for pseudosapogenin of **1**, and (c) *n*-BuOH–Me_2CO–MeOH (4:5:1) for monosaccharides. Spray reagents were orcinol/H_2SO_4 for steroidal saponins **1** and **2** and monosaccharides, and $CeSO_4$ for pseudosapogenin.

Plant Material. Fresh leaves of *F. gigantea* Vent. were obtained in September 2000 from plants cultivated at the Botanical Garden of the Federal University of Rio de Janeiro, Brazil, and a voucher specimen (No. 32) is maintained in the Laboratory of the Chemistry of Medicinal Plants at this University.

Extraction and Isolation. Fresh leaves of *F. gigantea* (1.0 kg) were extracted with MeOH (4.0 L) for 72 h at room temperature. The methanolic extract was partitioned between *n*-BuOH and H_2O to afford an *n*-BuOH-soluble portion (3.6 g). The residue was dissolved in MeOH (45 mL) and roughly chromatographed (400 mg/5 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 eluted in the fractions 325–450 mL. The saponin mixture (970 mg) was chromatographed on a silica gel column (2.8 × 90 cm). Elution was with $CHCl_3$–MeOH–H_2O (7:3:1, lower phase) (25 mL each eluent). The fractions 1500–1675 mL exhibiting similar TLC profiles were combined as well as the fractions 1725–1925 mL to afford homogeneous compounds **2** (380 mg, R_f 0.48) and **1** (185 mg, R_f 0.35), respectively. Compounds **1** and **2** gave dark green colors with orcinol/H_2SO_4.

Identification. Compound **1**: 3-[(*O*-6-deoxy-α-L-mannopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→3)-*O*-[*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-(3β,5α,25*R*)-26-(β-D-glucopyranosyloxy)-22-hydroxyfurost-12-one: colorless needles (MeOH), mp > 280°C; $[\alpha]_D^{25}$ – 60° (*c* 0.1, MeOH); Mass spectrum (MALDI-TOFMS, *m/z*): 1590.6415 $[M+Na]^+$ (calcd. for $C_{69}H_{114}O_{39}Na$: 1590.6409); IR spectrum (KBr, ν_{max}, cm^{-1}): 3422 (O-H), 2929 (C-H), 1707 (C=O), 1648, 1453, 1425, 1379, 1314, 1260, 1159, 1074 (C-O), 1038 (C-O), 912, 895, and 587 [(25R)-furostanol, intensity 912<895 cm^{-1}]; for ^{1}H and ^{13}C NMR data, see Tables 1 and 2.

Acid Hydrolysis of 1. Saponin **1** (100 mg) was hydrolyzed with 2 M HCl–1,4-dioxane (1:1; 15 mL) in a sealed tube for 3 h at 100°C. After cooling, the reaction mixture was diluted with water (45 mL) and extracted with EtOAc (60 mL, twice).

The EtOAc extract was evaporated to dryness, and crystallization from acetone yielded the pseudosapogenin (13.8 mg) as colorless crystals: mp 264–266°C, $[\alpha]_D^{25}$ + 7.8° (*c* 0.1, $CHCl_3$) (lit. mp 264–266°C, $[\alpha]_D$ + 8°) [9]. The identity of hecogenin was established by comparison with an authentic sample (Sigma) through IR, ^1H and ^{13}C NMR and EIMS [6–8]. The aqueous extract was lyophilized (yield: 67 mg) and a portion (1 mg) dissolved in pyridine (1 mL) and analyzed by silica gel-TLC in the above-described solvent system. After spraying with orcinol/H_2SO_4, rhamnose gave a green spot at R_f 0.75, glucose gave a blue spot at R_f 0.70, and galactose gave a purple spot at R_f 0.66.

Molar Carbohydrate Composition and D, L Configurations. The molar carbohydrate composition of compound **1** was determined by GC-MS analysis of its monosaccharides as their trimethylsilylated methylglycosides obtained after methanolysis (0.5 M HCl in MeOH, 24 h, 80°C) and trimethylsilylation [10]. The configurations of the glycosides were established by capillary GC of their trimethylsilylated (–)-2-butylglycosides [11].

Methylation Analysis. Compound **1** was methylated with dimethyl sulfoxide-lithium methylsulfinyl carbanion-methyl iodide [16]. The methyl ethers were obtained after hydrolysis (4 N trifluoroacetic acid, 2 h, 100°C) and analyzed as partially methylated alditol acetates by GC-MS [17].

Animals. Male BALB/c mice, weighing 15–20 g, were used. The animals were housed under standard environmental conditions and fed with standard rodent diet and water *ad libitum*.

Statistical Analysis. The experimental data were tested for statistical differences using the Student's t test.

Hemolytic Activity. Normal human red blood cell suspension (0.6 mL of 0.5%) was mixed with 0.6 mL diluent containing 5, 10, 20, 30, 40, 50, 100, 250, and 500 μg/mL of compounds **1** and **2**, aluminum hydroxide, and 5–500 μL/mL of Freund's Complete Adjuvant (FCA) and Freund's Incomplete Adjuvant (FIA) in saline solution. Mixtures were incubated for 30 min at 37°C and centrifuged at 70 rpm for 10 min. Saline and distilled water were included as minimal and maximal hemolytic controls. The hemolytic percents developed by the saline control were subtracted from all groups. The adjuvant concentration inducing 50% maximum hemolysis was considered the HD_{50} (graphical interpolation). Experiments included triplicates at each concentration [14].

Anti-inflammatory Activity. Anti-inflammatory activity was evaluated by measuring acetic acid-induced vascular permeability [15]. Male mice (BALB/c, 15–20 g) in groups of five were dosed orally with compounds **1** and 2 (100 mg/kg body weight) and a positive control, indomethacin (10 mg/kg body weight), before the intravenous injection of 4% Evans blue (10 mL/kg body weight). After injection of the dye, 0.1 N acetic acid (10 mL/kg body weight) was injected intraperitoneally. Twenty minutes later, the mice were killed with an overdose of ether and the viscera were exposed after a 1 min period to allow blood to drain away from the abdominal wall. The animal was held by a flap of the abdominal wall and the viscera were irrigated with 10 mL of saline over a petri dish. The washing was filtered through glass wool and transferred to a test tube. To each tube 100 μL of 1 N NaOH was added in order to clear any turbidity due to protein, and the absorbance was read at 590 nm.

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