A New Bioactive Steroidal Saponin from Leaves of Furcraea gigantea

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A new bidesmosidic furostanol saponin was isolated from leaves of Furcraea gigantea Vent. Its structure was established as 3-{(O-6-deoxy-α-L-mannopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→3)-O-[O-(β-D-glucopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-3β,5α,15α,22α,25R}-26-(β-D-glucopyranosyloxy)-15,22-dihydroxy-furost-12-one. Its structural identification was performed using detailed analyses of 1H and 13C NMR spectra including 2D NMR spectroscopic techniques (DEPT, COSY, HETCOR and COLOC) and chemical conversions. The steroidal saponin showed no haemolytic effects in the in vitro assays and demonstrated inhibition of the capillary permeability activity.

Key words: Furcraea gigantea, Agavaceae, Steroidal Saponin, anti-Inflammatory Activity

Furcraea gigantea Vent. (Agavaceae) is native to Brazil and is used in the folk medicine as 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 [1, 2]. As part of our programme on the chemical investigation of bioactive steroidal saponins, we have now examined the leaves of F. gigantea. In this paper, we provide an account of the structural determination of a new bidesmosidic furostanol saponin by its spectral data and chemical conversions. The haemolytic property and the anti-inflammatory activity of the isolated saponin were investigated.

Experimental Section

Plant material

Fresh leaves of Furcraea gigantea Vent. were obtained in September 2000 from plants cultivated at the Botanical Garden of Federal University of Rio de Janeiro, Brazil, and a voucher specimen is maintained in the Laboratory of Chemistry of Medicinal Plants at this University.

General procedures

Melting points were determined by an Electrothermal 9200 micro-melting point apparatus and are uncorrected. The optical rotations were measured on a Perkin Elmer 243B polarimeter. IR spectra were measured on a Perkin Elmer 599B spectrometer. The MALDI-TOFMS was conducted 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). NMR spectra were measured in pyridine-d5 (80 mg of 1 in 0.5 ml) at 25 °C with a Varian Gemini 200 NMR spectrometer, with tetramethylsilane (δ = 0.00) used as internal standard. 1H NMR spectra were recorded at 200 MHz and 13C NMR spectra at 50 MHz.

Extraction and isolation

Fresh leaves of F. gigantea (2.0 kg) were extracted with MeOH (8.0 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 (1 : 1 v/v)]. This procedure was repeated and the resulting organic phase was evaporated in vacuum. The
residue (7.3 g) was dissolved in MeOH (100 ml) and roughly chromatographed (365 mg/5 ml each time) on Sephadex LH-20 (3.8 x 65 cm) with MeOH. The fractions were combined based on the TLC profiles to give the saponin mixture (730 mg). Further purification by chromatography on a silica gel column (2.8 x 90 cm) eluted with CHCl₃/MeOH/H₂O (7:3:1, lower phase) provided a TLC homogeneous compound 1 (173 mg, Rf 0.30) which gave a dark blue colour with orcinol/H₂SO₄.

### Compound 1

Colorless needles. – M. p. > 280 °C. – [α]D₂⁰ = −54° (c 0.1, MeOH). – IR (KBr): ν = 3424 (O–H), 2928 (C–H), 1707 (C=O), 1647, 1452, 1425, 1378, 1313, 1260, 1159, 1074 (C–O), 1037 (C–O), 913, 896 and 584 cm⁻¹. – ¹H NMR (200 MHz, pyridine-d₅): δ = 0.70 (s, 3H, 19-Me), 0.99 (d, J = 6.6 Hz, 3H, 27-Me), 1.18 (s, 3H, 18-Me), 1.37 (d, J = 6.9 Hz, 3H, 21-Me), 1.71 (d, J = 6.2 Hz, 3H, Rha-6-Me), 3.85 (br m, 1H, 3-Me), 4.54 (dd, J = 11.0, 4.0 Hz, 1H, 15-H), 4.58 (dd, J = 9.0, 4.0 Hz, 1H, 16-H), 4.84 (d, J = 7.8 Hz, 2H, Gal-1-H and Glc-1”-H), 5.05 (d, J = 7.5 Hz, 1H, Glc-1”-H), 5.08 (d, J = 7.8 Hz, 1H, Glc-1-H), 5.14 (d, J = 8.0 Hz, 1H, Glc-1”-H), 5.50 (d, J = 7.5 Hz, 1H, Glc-1’-H), 5.75 (br s, 1H, Rha-1-H). – ¹³C NMR (50 MHz, pyridine-d₅): δ = 11.83 (C-19, CMe), 13.93 (C-21, CMe), 16.78 (C-27, CMe), 17.35 (C-18, CMe), 18.0 (Rha-C-6, CMe), 27.75 (C-24, CH₃), 28.84 (C-6, CH₃), 29.73 (C-2, CH₃), 30.23 (C-23, CH₂), 32.05 (C-7, CH₂), 33.76 (C-25, CH), 34.65 (C-4, CH₂), 35.21 (C-8, CH), 36.43 (C-10, C), 36.63 (C-1, CH₂), 37.74 (C-11, CH₂), 42.69 (C-20, CH), 44.38 (C-5, CH), 51.83 (C-17, CH), 54.79 (C-13, C), 55.41 (C-9, CH), 60.28 (C-14, CH; Gal-C-6, CH₂), 60.74 (Glc-C-6”, CH₂), 61.65 (Glc-C-6”, CH₂), 62.03 (Glc-C-6”, CH₂), 62.43 (Glc-C-6, CH₃), 63.01 (Glc-C-6””, CH₂), 68.94 (Glc-C-4”, CH), 69.90 (Rha-C-5, CH), 70.13 (Glc-C-4”, CH), 71.09 (Glc-C-4”, CH), 71.69 (Glc-C-4””, CH), 71.95 (Rha-C-2, CH), 72.14 (Rha-C-3, CH), 73.38 (Rha-C-4, CH), 74.23 (Glc-C-2”, CH), 74.26 (C-26, CH), 74.82 (C-5, CH), 75.04 (C-3, CH; Glc-C-2″, CH; Glc-C-2””, CH), 75.22 (Glc-C-2””, CH), 76.01 (Glc-C-3”, CH), 76.60 (Gal-C-2, CH), 76.67 (Glc-C-5”, CH), 76.88 (Glc-C-5, CH), 77.13 (C-3, CH), 77.47 (Glc-C-5”, CH; Glc-C-3”, CH; Glc-C-4””, CH), 77.83 (C-15, CH), 77.89 (Glc-C-5”, CH), 78.51 (Glc-C-5”, CH), 78.61 (Glc-C-3”, CH; Glc-C-3”, CH), 80.31 (Glc-C-2, CH), 87.45 (Glc-C-3, CH; Glc-C-3”, CH), 89.93 (C-16, CH), 102.09 (Gal-C-1, CH), 103.42 (Rha-C-1, CH), 103.59 (Glc-C-1’, CH), 104.12 (Glc-C-1”, CH), 104.40 (Glc-C-1, CH), 104.92 (Glc-C-1”, CH), 105.01 (Glc-C-1””, CH), 110.58 (C-22, C), 212.68 (C-12, C=O). – MALDI-TOFMS: m/z = 1606.6391 [M + Na]⁺ (calcd. for C₉₉H₁₄₁O₃₀Na: 1606.6403).

### Acid hydrolysis of 1

A solution of saponin 1 (100 mg) in 1 M HCl/1,4-dioxan (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 3% KOH 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 with MeOH to give the hydrolysate (91 mg) which was chromatographed on silica gel CC with CHCl₃/MeOH/H₂O (7:3:0.1 v/v/v) to yield the pseudosapogenin (12.7 mg) and a sugar mixture. Identity of aglycone was established by comparison with spectral data through IR, ¹H and ¹³C NMR and EIMS [3]. The sugar mixture was dissolved in pyridine and analyzed by silica gel-TLC in the above described solvent system (C). After spraying with orcinol/H₂SO₄, rhamnose gave a green spot at Rf 0.75, glucose gave a blue spot at Rf 0.70 and galactose gave a purple spot at Rf 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 methanalysis (0.5 M HCl in MeOH, 24 h, 80°C) and trimethylsilylation [4]. The configurations of the glycosides were established by capillary GC of their trimethylsilylated (–)-2-butyglycosides [5].

### Methylation analysis

Compound 1 was methylated with dimethyl sulfoxide/lithium methylsulfinyl carbaniom/methyl iodide [6]. The methyl ethers were obtained after hydrolysis (4 N trifluoroacetic acid, 2 h, 100°C) and analyzed as partially alditol acetates by GC-MS [7].

### 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. IC₅₀ values were obtained from the regression lines.

### Haemolytic 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 compound 1, aluminum hydroxide, and 5 – 50 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 × g for 10 min. Saline and distilled water were included as minimal and maximal haemolytic controls. The haemolytic percents developed by the saline control were subtracted from all groups. The adjuvant concentration inducing 50% of the maximum haemolysis was considered the HD_{50} (graphical interpolation). Experiments included triplicate at each concentration [8].

**anti-Inflammatory activity**

*anti-Inflammatory activity* was evaluated by measuring acetic acid-induced vascular permeability [9]. Male mice (BALB/c, 15 – 20 g) in groups of five were dosed orally with different concentrations of compound 1 (10, 25, 50 and 100 mg/kg body weight) and a positive control, indomethacin (10 mg/kg body weight). After injection of the dye, 0.1 N acetic acid (10 ml/kg body weight) was injected intraperitoneally. 20 min 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 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 was added 100 µl of 1 N NaOH in order to clear any turbidity due to protein, and the absorbance was read at 590 nm.

**Result and Discussion**

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 = 1606.6391 [M + Na]⁺ in the MALDI-TOFMS. In the ¹³C NMR spectrum, 69 carbon signals observed belonged to five methyl groups, fifteen methylene groups (seven of which were oxygenated), forty-five methine groups (thirty-eight of which were oxygenated) and four quaternary carbon atoms (two of which were oxygenated). On the basis of above mentioned MS and NMR spectral data, compound 1 was supposed to be a saponin with the molecular formula C_{69}H_{114}O_{40}, bearing a chain of seven sugar moieties.

In addition to this, the furostan glycosidic nature of compound 1 was indicated by the strong absorption bands at 3424 and 1074 cm⁻¹ and a 25R-furostan steroidal structure (896 and 913 cm⁻¹, intensity 913 < 896 cm⁻¹) in the IR spectrum [10], confirmed by ¹H and ¹³C NMR spectra [3, 11]. The ¹H NMR spectral data contained signals for three hydrogens at δ = 3.85 (br m, H-3), 4.54 (dd, J = 11.0 and J = 4.0 Hz, H-15) and 4.58 (dd, J = 9.0 and J = 4.0 Hz, H-16), two secondary methyl hydrogens at δ = 1.37 (d, J = 6.9 Hz, 3H-21) and 0.99 (d, J = 6.6 Hz, 3H-27) and two angular methyl hydrogens at δ = 1.18 (s, 3H-18) and 0.70 (s, 3H-19). The above ¹H NMR spectral data and a comparison of the ¹³C NMR signals of the above ¹H NMR spectral data and a comparison of the ¹³C NMR signals of the aglycone moiety of 1 with those described in the literature [3] suggest the structure of the aglycone to be 3,15,22,26-tetrahydroxy-(3β, 5α, 15α, 22α, 25R)-furostan-12-one. In addition to this, the ¹H NMR spectrum of 1 showed seven anomic hydrogens at δ = 4.84 (d, J = 7.8 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’ and Rha-H-1, respectively, indicating β-anomeric configuration for galactose and five glucoses and α-anomeric configuration for rhamnose. The ¹³C NMR spectroscopic data for the sugar moieties indicated that all the monosaccharides were in pyranose form.

In the ¹³C 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. The ¹H and ¹³C NMR chemical shift assignments were made by standard 1D and 2D NMR techniques (¹H-¹H-COSY, DEPT, HETCOR and COLOC experiments). Its COLOC spectrum displayed long range couplings between galactose-H-1 at δ = 4.84 and aglycone-C-3 at δ = 77.13, between glucose-H-1”” at δ = 4.84 and aglycone-C-26 at δ = 74.74, 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 Glc-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. These
results indicated that compound 1 is undoubtedly as shown in Fig. 1.

On acid hydrolysis, compound 1 gave a pseudosapogenin, galactose, glucose and rhamnose. The pseudosapogenin was identified as 3,15-dihydroxy-(3β,5α,15α,25R)-spirostan-12-one. Its identity was established by comparison with spectral data through IR, 1H and 13C NMR and EIMS [3]. The molar carbohydrate composition of compound 1 indicated the presence of seven neutral monosaccharides: galactose-glucose-rhamnose (1:5:1) [4]. Their absolute configurations were determined by GC of their trimethylsilylated (−)-2-butylglycosides [5]. D-galactose, D-glucose and L-rhamnose were identified. Consequently, on the basis of IR, 1H and 13C 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→2)-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy}-(3β,5α,15α,22α,25R)-26-(β-D-glucopyranosyloxy)-15,22-dihydroxy-furost-12-one (1).
tivity of compound 1 (Fig. 3), which showed inhibition of the increase in vascular permeability caused by acetic acid, which is a typical model of first stage inflammatory reaction. The results obtained may help explain some biological properties attributed to steroidal saponins reported in the literature [14].

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