Structural Characteristics of a Bioactive Polysaccharide from *Sorghum arundinaceum*

Bernadete P. da Silva, Graziela M. Silva, Tatiana P. Mendes, and José P. Parente*

Laboratório de Química de Plantas Medicinais, Núcleo de Pesquisas de Produtos Naturais, Universidade Federal do Rio de Janeiro, PO Box 68045, CEP 21944–970 Rio de Janeiro, Brazil. Fax: +55-21-2562-6791. E-mail: parente@nppn.ufrj.br

* Author for correspondence and reprint requests

Z. Naturforsch. **58 c**, 342–346 (2003); received November 22/January 3, 2003

A polysaccharide, an α-D-glucan with an apparent molecular weight of 6.85×10^4, called PSa glucan, was isolated from fresh seeds of *Sorghum arundinaceum* by fractionation on Sephacryl S-300 HR and Sephadex G-25. Chemical and spectroscopic studies indicated that it has a highly branched glucan type structure composed of α-(1→4) linked D-glucopyranose residues with (1→3), (1→6) branching points, and a significant amount of α-(1→6) branching to α-(1→3) linked D-glucopyranose residues. The anti-inflammatory activity of the polysaccharide was performed using the capillary permeability assay.

Key words: Sorghum arundinaceum, α-D-Glucan, Anti-Inflammatory Activity

Introduction

The occurrence of polysaccharides, α- and β-glucans, in *Sorghum* genus is documented (Ramesh and Tharanathan, 1998). Some species have an ethnopharmacological background, in particular *Sorghum bicolor* which in Curaçao, the seeds are ground, roasted and eaten as remedy for lung ailments (Brenneker, 1961). *Sorghum arundinaceum* (Wild.) Stapf. (Gramineae), known as sorgo-selvagem, is a native species throughout Brazilian subtropical regions. Brazilians drink the seed decoction to relieve coughs, bronchitis and other chest ailments (Cruz, 1965). Nonetheless, no chemical and biological studies have been carried out on the constituents of *S. arundinaceum.* As part of our program of the chemical investigation of bioactive polysaccharides, we have now examined the seeds of this plant. As a result we isolated a polysaccharide, an α-D-glucan, from *S. arundinaceum*, along with an evaluation of its anti-inflammatory properties.

Materials and Methods

Plant material

Fresh seeds of *Sorghum arundinaceum* were obtained from the plant garden of the Federal University of Rio de Janeiro, Rio de Janeiro, in February 2001 and a voucher specimen is maintained in the Laboratory of Chemistry of Medicinal Plants at this University.

General procedures

Carbohydrate content was analyzed by colorimetric assays according to the procedure of Dubois *et al.* (1956), without previous hydrolysis of the sample, and by GC-EIMS of the glucitol acetates (Sawardeker *et al.*, 1965). Protein content was analyzed by the method of Bradford (1976) using ovalbumin as standard. The experimental data were tested for statistical differences using the Student's *t* test. The M_r's of the polysaccharide was estimated from the calibration curve of elution of standard dextrans (average M_r's 2000000, 413000, 282000, 148000, 68000, 37500, 19500 and 9500; Sigma) on Sephacryl S-300 HR (5 × 85 cm; Pharmacia). Dialysis was carried out using tubing with an M_r cut-off 12000. The optical rotation was measured on a Perkin Elmer 243B polarimeter. NMR spectra were measured in D_2O containing sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard with a Varian Gemini 200 NMR spectrometer. ^{1}H NMR spectra were recorded at 200 MHz and ^{13}C NMR spectra at 50 MHz. GC analyses were performed using a Shimadzu GCMS-QP5050A gas chromatograph mass spectrometer using an ionization voltage of 70 eV for EI and CI and an ionization current of 60 μA

and 200 μA for EI and CI, respectively. GC was carried out with FID, using a glass capillary column (0.25 × 25 m, 0.25 micron, J. & W. Scientific Incorporated, Folsom, CA, USA) DB-1. TLC of monosaccharides were performed on silica gel-coated plates (Merck) in *n*-BuOH-pyridine-H_2O (6:4:3), and sugars were detected by spraying with orcinol-H_2SO_4.

Extraction and fractionation

Fresh seeds of *S. arundinaceum* (1 kg) were extracted with hot water (2 l) under stirring for 1 h in a boiling water bath. The aqueous extract was filtered and the filtrate centrifuged. By precipitation with two volumes of EtOH (12 h stirring and 24 h standing at 4 °C), a resulting precipitate was obtained following centrifugation and subsequent lyophilization (yield: 223 mg). The amorphous powder was dissolved in 0.01% sodium sulfate (100 ml) and added to 5% cetyltrimethylammonium bromide (CTAB; 20 ml). After centrifugation, the supernatant was poured into two volumes of EtOH and the precipitate obtained was dissolved in water (100 ml), dialyzed and lyophilized to yield a neutral polysaccharide fraction (100 mg) which was dissolved in 0.1 M Tris (hydroxymethyl)-aminomethane (Tris-HCl buffer at pH 7.0), and applied to a Sephacryl S-300 HR column (5 × 85 cm; 1650 cm^3). The elution was repeated two times in order to remove shoulders with lower and higher molecular weights. Fractions of 10 ml corresponding to the peak PSa (1500–1650 ml) (Fig. 1) were pooled, dialyzed and freeze-dried. The powder corresponding to the peak was dissolved in water (2 ml) and applied to a Sephadex G-25 column (1.5 × 50 cm; 15 g), and 5 ml fractions were collected (100–150 ml). The eluate obtained was concentrated, and lyophilized to yield PSa (51 mg). The fractionation procedures were followed by carbohydrate content.

Molar carbohydrate composition and D, L configuration

Monosaccharides were analyzed as their trimethylsilylated methylglucosides obtained after methanolysis (0.5 M HCl in MeOH, 24 h, 80 °C) and trimethylsilylation (Kamerling *et al.*, 1975). The configurations of the glucosides were established by capillary GC and GC-MS of their trimethylsilylated (–)-2-butylglucosides (Gerwig *et al.*, 1978).

Methylation analysis

PSa glucan *O* was methylated with DMSO-lithium methylsulfinyl carbanion-CH_3I (Parente *et al.*, 1985). The methyl ethers were obtained after hydrolysis (4 N TFA, 2 h, 100 °C) and analyzed as partially alditol acetates by GC-MS (Sawardeker *et al.*, 1965). The main fragments of the products are described in Table I.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated by measuring acetic acid-induced vascular permeability (Whittle, 1964). Male mice (BALB/c, 15–20 g) in groups of five were dosed orally with polysaccharide PSa (100 mg/kg body weight) and with 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 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. The animal was held by a flap of the abdominal wall and the viscera were irrigated with 10 ml of saline (PBS) solution over a petri dish. The washing was filtered through glass wool and transferred to a test tube. To each tube was added 0.1 ml of 1 N NaOH in order to clear any turbidity due to protein, and the absorbance was read at 590 nm.

Results and Discussion

The crude neutral polysaccharide fraction extracted from the seeds of *S. arundinaceum* contained 97.29% carbohydrate and 2.71% protein. A sample of this fraction was fractionated by means of Sephacryl S-300 HR and desalted by means of Sephadex G-25 gel permeation chromatography, leading to the isolation of a neutral polysaccharide, called PSa glucan (Fig. 1). The fractionation procedure was monitored by carbohydrate content. The sugar molecule PSa was determined to be only glucose by the identification on TLC of the acid hydrolysates and by GC of the trimethyl-

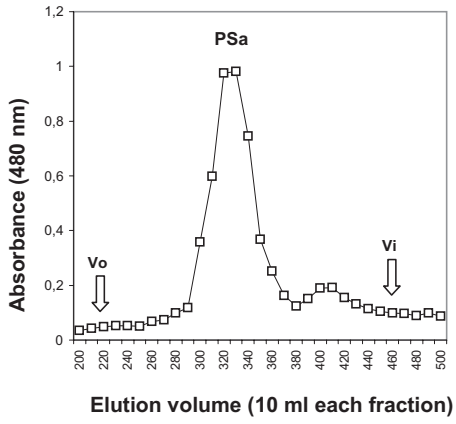

Fig. 1. Elution diagram of polysaccharide PSa from Sephacryl S-300 HR (0.1 M Tris-HCl). Vo: void volume; Vi: inner volume.

silylated methylglucosides derivatives prepared from the monosaccharides. The absolute configuration of the glucose was determined by GC of its trimethylsilylated (–)-2-butylglucosides. D-glucopyranose was identified by GC-EIMS of the pertrimethylsilylated butylglucosides. The PSa glucan exhibited positive specific rotation $[\alpha]_D^{20}$ + 100° (*c* 0.1, H_2O) and showed 840 cm^1 in the IR spectrum due to an α-configuration. The average molecular weight of the Psa glucan was estimated to be 6.85×10^4 ($\pm$ 1.5×10^3) based on the calibration curve of the elution volume of standard dextrans from gel filtration on Sephadex S-300 HR. PSa glucan was methylated by the method of Parente *et al.* (1985). The fully methylated products were hydrolyzed with acid, converted into the alditol acetates, and analyzed by GC-CIMS and GC-EIMS. PSa glucan furnished 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl glucitol, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl glucitol, 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl glucitol, 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl glucitol, 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl glucitol and 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methyl glucitol (Tables I and II).

The results of the methylation analysis indicated that PSa glucan contained mainly (1→4) linked glucosyl residues and branching points at *O*-3 and *O*-6 of (1→4) linked glucosyl residues. It possess exceptional α-1,3-linked units in addition to the usual α-1,4-linear linkage. On the other hand, the presence of α-1,6-linked units in PSa glucan is characteristic. In addition to 4,3- and 4,6-branching points, the polysaccharide contains a substantial amount of another 3,6-branching points in the same proportions (Table II, Fig. 2). The ^1H NMR spectrum of PSa glucan in D_2O showed anomeric proton signal at δ 5.38 as a broad singlet. Further, the ^{13}C NMR spectrum showed a signal due to an anomeric carbon of α-D-glucopyranose at δ 102.26 ppm (Yamada *et al.*, 1984).

According to the literature, several polysaccharides were shown to possess the capacity of modulation of the inflammatory and immunological responses (Czarnecki and Grzybek, 1995; Stuelp-Campelo *et al.*, 2002; Whistler *et al.*, 1976). In order to confirm popular informations about the

Table I. Methylation analysis by GC and main fragments (*m/z*) in the MS of partially methylated alditol acetates of PSa glucan.

Methylated alditol acetate derivatives	CIMS: *m/z* $[M + H]^+$ and $[M + NH_4]^+$ (rel. int.)	EIMS: main *m/z* (rel. int.)
2,3,4,6-tetra-*O*Me Glc	323(4), 340(72)	43(100), 45(51), 71(20), 87(27), 101(69), 117(44), 129(47), 145(34), 161(37), 205(8)
2,3,6-tri-*O*Me Glc	351(4), 368(100)	43(100), 45(36), 71(15), 87(36), 99(36), 101(44), 113(32), 117(67), 129(21), 233(10)
2,3,4-tri-*O*Me Glc	351(5), 368(100)	43(100), 45(27), 71(24), 87(57), 99(62), 101(89), 117(83), 129(47), 161(13), 233(6)
2,6-di-*O*Me Glc	379(4), 396(100)	43(100), 45(22), 87(23), 117(77), 129(39), 143(10), 185(4), 203(3), 231(3), 305(2)
2,3-di-*O*Me Glc	379(4), 396(100)	43(100), 85(24), 87(16), 99(24), 101(42), 117(69), 127(27), 161(5), 201(9), 261(6)
2,4-di-*O*Me Glc	379(4), 396(100)	43(100), 87(22), 99(10), 101(9), 117(56), 129(43), 189(11), 201(6), 233(4), 305(1)

Table II. Methylation analysis of the PSa glucan.

Methylated alditol acetate derivatives	Relative retention times[a]	Molar ratios[b] PSa	Structural features
2,3,4,6-tetra-*O*Me Glc	1.00	2.00	Glc 1→
2,3,6-tri-*O*Me Glc	1.16	12.00	→4 Glc 1→
2,3,4-tri-*O*Me Glc	1.21	12.00	→6 Glc 1→
2,6-di-*O*Me Glc	1.29	12.00	→4 Glc 1→ 3 ↑
2,3-di-*O*Me Glc	1.36	50.00	→4 Glc 1→ 6 ↑
2,4-di-*O*Me Glc	1.42	12.00	→3 Glc 1→ 6 ↑

[a] Relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl glucitol.

[b] Calculated from peak areas and molecular weight of derivatives (mol. wt. were obtained by GC-CIMS).

medicinal utilization of this plant against inflammatory conditions, the pharmacological property of the polysaccharide was evaluated using the capillary permeability assay (Whittle, 1964). The polysaccharide showed inhibition of the increase in vascular permeability (64.5% ± 5.5) caused by acetic acid, which is a typical model of first stage inflammatory reaction. The standard drug indomethacin also reduced the leakage (77.5% ± 3.5). The results obtained were significantly different from the control group. This result suggests that the polysaccharide may be the potential therapeutic agent involved in inflammatory disorders justifying the use of this plant in Brazilian traditional medicine.

Acknowledgements

This work was financially supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and Fundação Universitária José Bonifácio (FUJB).

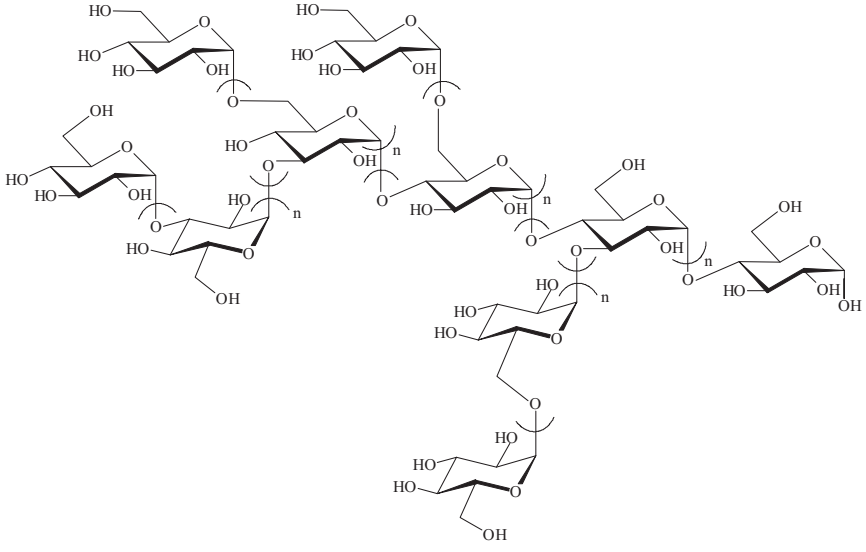

Fig. 2. Proposed structure feature for PSa glucan (*n* = repeating sugar residues).

Bradford M. M. (1976), A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**, 248–254.

Brenneker P. (1961), Jerba-Kruiden van Curaçao en hun gebruik. Boekhandel St. Augustinus, Curaçao.

Cruz G. L. (1965), Livro verde das plantas medicinais e industriais do Brasil. Velloso S. A., Belo Horizonte.

Czarnecki R., and Grzybek J. (1995), Antiinflammatory and vasoprotective activities of polysaccharides isolated from fruit bodies of higher fungi P.1. Polysaccharides from *Trametes gibosa* (Pers.: Fr) Fr. (Polyporaceae). Phytother. Res. **9**, 123–127.

Dubois M., Gilles K. A., Hamilton J. K., Hebers P. A., and Smith F. (1956), Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**, 350–356.

Gerwig G. J., Kamerling J. P., and Vliegenthart J. F. G. (1978), Determination of the D and L configuration of neutral monosaccharides by high-resolution capillary G. L. C. Carbohydr. Res. **62**, 349–357.

Kamerling J. P., Gerwig G. J., Vliegenthart J. F. G., and Clamp J. R. (1975), Characterization by gas-liquid chromatography-mass spectrometry and proton-magnetic-resonance spectroscopy of pertrimethylsilyl glycosides obtained in methanolysis of glycoproteins and glycopeptides. Biochem. J. **151**, 491–495.

Parente J. P., Cardon P., Leroy Y., Montreuil J., Fournet B., and Ricart G. (1985), A convenient method for methylation of glycoprotein glycans in small amounts by using lithium methylsulfinyl carbanion. Carbohydr. Res. **141**, 41–47.

Ramesh H. P., and Tharanathan R. N. (1998), Structural characteristics of a mixed linkage β-D-glucan from sorghum (*Sorghum bicolor*). Carbohydr. Res. **308**, 239–243.

Sawardeker J. S., Sloneker J. H., and Jeanes A. (1965), Quantitative determination of monosaccharides as their alditol acetates by gas liquid chromatography. Anal. Chem. **37**, 1602–1604.

Stuelp-Campelo P. M., de Oliveira M. B. M., Leão A. M. A. C., Carbonero E. R., Gorin P. A. J., and Iacomini M. (2002), Effect of a soluble α-D-glucan from the lichenized fungi *Ramalina celastri* on macrophage activity. Int. J. Immunopharmacol. **2**, 691–698.

Whistler R. L., Bushway A. A., Singh P. P., Nakahara W., and Tokuzen R. (1976), Noncytotoxic, antitumor polysaccharides. Adv. Carbohydr. Chem. Biochem. **32**, 235–275.

Whittle B. A. (1964), The use of changes in capillary permeability in mice to distinguish between narcotic and nonnarcotic analgesics. Br. J. Pharmacol. Chemother. **22**, 246–253.

Yamada H., Kiyohara H., and Otsuka Y. (1984), Characterization of a water-soluble glucan from *Angelica acutiloba*. Phytochemistry **23**, 587–590.