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

A polysaccharide, an α-δ-glucan with an apparent molecular weight of 6.85 × 10⁴, 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 δ-glucopyranose residues with (1→3), (1→6) branching points, and a significant amount of α-(1→6) branching to α-(1→3) linked δ-glucopyranose residues. The anti-inflammatory activity of the polysaccharide was performed using the capillary permeability assay.

**Key words:** *Sorghum arundinaceum*, α-δ-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-selva-gem, 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-δ-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 D₂O containing sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard with a Varian Gemini 200 NMR spectrometer. ¹H NMR spectra were recorded at 200 MHz and ¹³C 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.
Methylsilylation of the glucosyl residues and GC-MS analysis were performed as described by Parente et al. (1985) and Sawardeker et al. (1965), respectively. The main fragments of the products are listed 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 trimethylsilylated (−)-2-butyglucosides (Gerwig et al., 1978).
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 \( [\alpha]^{20}_D + 100^\circ \) \((c 0.1, H_2O)\) and showed 840 cm\(^{-1}\) in the IR spectrum due to an \( \alpha \)-configuration. The average molecular weight of the Psa glucan was estimated to be \( 6.85 \times 10^4 \) \((\pm 1.5 \times 10^3)\) 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-di-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 \((1 \rightarrow 4)\) linked glucosyl residues and branching points at \( O-3 \) and \( O-6 \) of \((1 \rightarrow 4)\) linked glucosyl residues. It possesses exceptional \( \alpha-1,3\)-linked units in addition to the usual \( \alpha-1,4\)-linear linkage. On the other hand, the presence of \( \alpha-1,6\)-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 \(^1\)H NMR spectrum of PSa glucan in D\(_2\)O showed anomeric proton signal at \( \delta 5.38 \) as a broad singlet. Further, the \(^13\)C NMR spectrum showed a signal due to an anomeric carbon of \( \alpha\)-d-glucopyranose at \( \delta 102.26 \) 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.**

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

<table>
<thead>
<tr>
<th>Methylated alditol acetate derivatives</th>
<th>Relative retention times</th>
<th>Molar ratios</th>
<th>Structural features</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-OMe Glc</td>
<td>1.00</td>
<td>2.00</td>
<td>Glc 1 →</td>
</tr>
<tr>
<td>2,3,6-tri-OMe Glc</td>
<td>1.16</td>
<td>12.00</td>
<td>→4 Glc 1 →</td>
</tr>
<tr>
<td>2,3,4-tri-OMe Glc</td>
<td>1.21</td>
<td>12.00</td>
<td>→6 Glc 1 →</td>
</tr>
<tr>
<td>2,6-di-OMe Glc</td>
<td>1.29</td>
<td>12.00</td>
<td>→4 Glc 1 →</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>2,3-di-OMe Glc</td>
<td>1.36</td>
<td>50.00</td>
<td>→4 Glc 1 →</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>2,4-di-OMe Glc</td>
<td>1.42</td>
<td>12.00</td>
<td>→3 Glc 1 →</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

Table II. Methylation analysis of the PSa glucan.

* 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).

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