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Anti-inflammatory and immunologically active polysaccharides of *Periandra mediterranea*

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Abstract

Three polysaccharides, glucans with mean M_r's of 1.5×10^5, 3.6×10^4 and 2.1×10^4, were isolated from dried roots of *Periandra mediterranea* by fractionation on Sephacryl S-300 HR and Sephadex G-25. Chemical and spectroscopic studies indicated that they have a highly branched glucan type structure composed of α-(1 → 4) linked D-glucopyranose residues with both (3 → 4) and (4 → 6) branching points. The polysaccharides enhance phagocytosis in vivo, and exhibit anti-inflammatory activity.

Keywords: *Periandra mediterranea*; Leguminosae; Roots; Neutral polysaccharides; Glucans; Anti-inflammatory; Immunological activity

1. Introduction

Periandra mediterranea Taubert (Syn.: *P. dulcis* Martius) has been widely used in Brazilian ethnomedicine as a cough supressant (Corrêa, 1926); anti-inflammatory, expectorant, diuretic and laxative activities have also been reported (Cruz, 1965). Triterpenes (Hashimoto et al., 1984a, 1984b) and triterpene glycosides (Hashimoto et al., 1980, 1982, 1983) have already been reported from the roots of *P. mediterranea*. The triterpene glycosides named periandradulcins A, B and C were isolated as phosphodiesterase inhibitors from the roots of this plant (Ikeda et al., 1991). Recently, the triterpenoidal saponin, periandrin II (Hashimoto et al., 1980), showed a slight haemolytic effect, inducing a specific and potentially protective humoral response against the FML antigen of *Leishmania donovani* (Santos et al., 1997). Herein, we report the isolation and chemical characterization of three neutral polysaccharides responsible for the anti-inflammatory and immunological activities.

2. Results and discussion

Dried roots of *P. mediterranea* were sequentially extracted in a Soxhlet with methanol and hot water. The aqueous extract was poured into 2 vols. of ethanol, leading to the isolation of the crude polysaccharide fraction (629 mg), which was dissolved in 0.01% sodium sulfate and precipitated with cetyltrimethylammonium bromide. After centrifugation, the supernatant was poured into two volumes of ethanol to yield a crude neutral polysaccharide fraction (530 mg) which contained 90% carbohydrate and 10% protein. A sample of this fraction was fractionated by means of Sephacryl S-300 HR and Sephadex G-25 gel permeation chromatography, leading to the isolation of three neutral, protein-free polysaccharide fractions Pm1, Pm2 and Pm3 (Fig. 1). The fractionation procedures were monitored by carbohydrate content. The sugar molecules Pm1, Pm2 and Pm3 were determined

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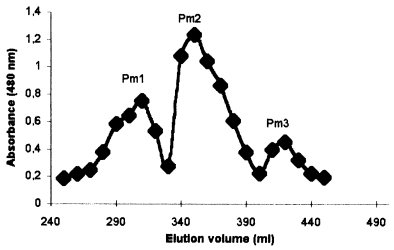

Fig. 1. Elution diagram of Pm1, Pm2 and Pm3 from Sephacryl S-300 HR (0.1 M Tris-HCl).

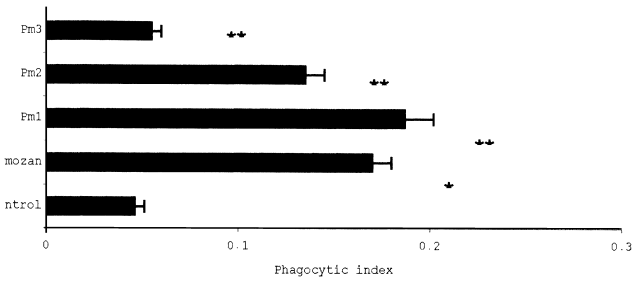

Fig. 2. Effects of Pm1, Pm2 and Pm3 polysaccharides on phagocytic activity. Significantly different from the control group; $* p < 0.05$, $** p < 0.01$.

to be only glucose by the identification on TLC of the acid hydrolysates and by GC of the TMSi methylglucosides derivatives prepared from the monosaccharides. The absolute configuration of the glucose was determined by GC of its TMSi (−)-2-butylglucosides. D-glucopyranose was identified by GC-EIMS of the pertrimethylsilylated methylglucosides. Pm1, Pm2 and Pm3 glucans showed positive specific rotations, $[\alpha]_D^{20} + 130.0^\circ$ (c 0.1, H_2O), $[\alpha]_D^{20} + 80.0^\circ$ (c 0.1, H_2O) and $[\alpha]_D^{20} + 55.0^\circ$ (c 0.1, H_2O), respectively, and showed characteristic absorption at 840 cm^{-1} in the IR spectra due to an α-configuration. The average M_r's of Pm1, Pm2 and Pm3 glucans were estimated to be 1.5×10^5, 3.6×10^4 and 2.1×10^4, respectively, based on the coliaboration curve of the elution volume of standard dextrans from gel filtration on Sephacryl S-300 HR.

Pm1, Pm2 and Pm3 glucans were 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 and GC-MS. Pm1 and Pm2 glucans 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, 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl glucitol and 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl glucitol (Table 1). Pm3 glucan furnished the partially methylated alditol acetates (above described) for Pm1 and Pm2 glucans, with the exception of 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl glucitol. The results of methylation analyses indicated that Pm1 and Pm2 glucans contained mainly (1 → 4) linked glucosyl residues and branching points at *O*-3 and *O*-6 of (1 → 4) linked glucosyl residues. Pm3 glucan has no branching point at *O*-3 of (1 → 4) linked glucosyl residues. Their molar ratios and structural features are shown in Table 1.

The ^{1}H-NMR spectra of Pm1, Pm2 and Pm3 glucans in D_2O showed anomeric proton signals at δ 5.40 as a broad singlet, while no acetyl signal was observed in Pm1, Pm2 and Pm3 glucans (Tomoda et al., 1985). Further, the ^{13}C-NMR spectra showed a signal due to an anomeric carbon of α-D-glucopyranose at δ 102.28 ppm (Yamada et al., 1984).

Based on earlier reports of immuno-stimulatory (Tomoda et al., 1994) and anti-inflammatory (Sendl et al., 1993) activities, the pharmacological properties of these polysaccharides were investigated. The effects of Pm1, Pm2 and Pm3 glucans on the reticuloendothelial system were demonstrated by the carbon clearance test (Biozzi et al., 1953). As shown in Fig. 2, the phagocytic indices for Pm1 and Pm2 were significantly increased, suggesting immuno-stimulatory properties. However, Pm3 did not have significant activity. In addition to this, Pm1 and Pm2, but not Pm3, at a dose of 100 μg/g inhibited the increase in vascular per-

Table 1
Methylation analysis of Pm1, Pm2 and Pm3 glucans

Methylated alditol acetate derivatives	Relative retention times[a]	Molar ratios[b]			Structural features
		Pm1	Pm2	Pm3	
2,3,4,6-tetra-*O*Me Glc	1.00	16	10	9	Glc 1 →
2,3,6-tri-*O*Me Glc	1.21	62	86	85	→ 4 Glc 1 →
2,6-di-*O*Me Glc	1.32	5	1	0	→ 4 Glc 1 → 3 ↑
2,3-di-*O*Me Glc	1.45	17	3	6	→ 6 Glc 1 → 4 ↑

[a] Relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.
[b] Calculated from peak areas and molecular weights of derivatives.

meability caused by acetic acid, which is a typical model of first stage inflammatory reaction (Whittle, 1964). The standard drug indomethacin (10 μg/g) also reduced the leakage (Fig. 3).

In conclusion, the highly branched glucans, Pm1 and Pm2, showed immuno-stimulatory and anti-inflammatory properties, in contrast to the simpler Pm3, which demonstrated no significant activity. Consequently, it can be presumed that if humoral amylase can hydrolyse certain (1 → 4) linkages, the residual chains of (1 → 3) linked residues may be long enough to contribute to phagocytic enhancement and inhibition of the inflammatory response (Whistler et al., 1976). The pharmacological results obtained may help explain the use of *P. mediterranea* in Brazilian traditional medicine.

3. Experimental

3.1. Plant material

Roots of *P. mediterranea* were collected in Rio de Janeiro, in November 1966, and identified by Dr. Paulo Occhioni. A voucher specimen (no. 2398) is deposited at the RFA 8068 herbarium, Rio de Janeiro, Brazil.

3.2. General

Carbohydrate content was analyzed by the phenol–H_2SO_4 method (Dubois et al., 1956), without previous hydrolysis of the sample. Protein content was analyzed by the method of Bradford (1976). The experimental data were tested for statistical differences using the Student's *t* test (Fisher and Yates, 1957). The M_r's of Pm1, Pm2 and Pm3 were estimated from the calibration curve of the elution volume of standard dextrans (average M_r's 2,000,000, 413,000, 282,000, 148,000, 68,000, 37,500, 19,500 and 9500) on Sephacryl S-300 HR (5 × 85 cm). Dialysis was carried out using tubing with an M_r cut-off of 12,000. Optical rotations were measured on a Perkin Elmer 243B polarimeter. ^{1}H- and ^{13}C-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 D_2O containing sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an int. standard. GC was carried out with FID, using a glass capillary column (0.31 mm × 25 m) SE-30. GC-MS: recorded at 70 eV. TLC of monosaccharides was performed on silica gel coated plates (Merck) in 1-BuOH–pyridine–H_2O (6:4:3), and sugars were detected by spraying with orcinol–H_2SO_4.

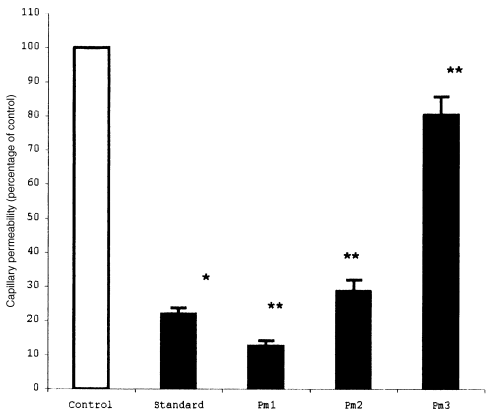

Fig. 3. Anti-inflammatory properties of Pm1, Pm2 and Pm3 polysaccharides. Significantly different from the control group; $* p < 0.05$, $** p < 0.01$.

3.3. Extraction

Dried roots of *P. mediterranea* (100 g), previously cut into small pieces and ground, were extracted in a Soxhlet with MeOH (500 ml, 6 h). The root residue was extracted with hot water (500 ml) under stirring for 1 h in a boiling water bath. The aq. extract was filtered and the filtrate centrifuged. By precipitation with 2 vols., of EtOH (12 h stirring and 24 h standing at 4°C), a crude product was obtained following centrifugation and subsequent lyophilization (yield: 629 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 2 vols., of EtOH and the ppt obtained (as described above) was dissolved in water (100 ml), dialyzed and lyophilized to yield a crude polysaccharide (530 mg).

3.4. Fractionation

A sample of the crude polysaccharide (100 mg) was dissolved in 0.1 M Tris-HCl buffer (pH 7.0), and applied to a column (5 × 85 cm) of Sephacryl S-300 HR, pre-equilibrated and developed with the same buffer. Fractions of 10 ml corresponding to the peaks Pm1, Pm2 and Pm3 were pooled, dialyzed, concd., and freeze-dried. Each fraction was dissolved in water (2 ml) and applied to a Sephadex G-25, column (1.5 × 50 cm), then eluted with water (5) ml fractions were collected. The eluates obtained from each fractionation were concd., and lyophilized to yield Pm1 (25 mg), Pm2 (45 mg) and Pm3 (5 mg). The fractionation procedures were followed by carbohydrate content.

3.5. Molar carbohydrate composition and D, L configuration

Monosaccharides were analyzed as their TMSi methylglycosides obtained after methanolysis (0.5 M

HCl in MeOH, 24 h, 80°C) and trimethylsilylation (Kamerling et al., 1975). The configurations of the glycosides were established by capillary GC and GC-MS of their TMSi (−)-2-butylglycosides (Gerwig et al., 1978).

3.6. Methylation analysis

Polysaccharides Pm1, Pm2 and Pm3 were 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 (Fournet et al., 1978).

3.7. Anti-inflammatory activity

Anti-inflammatory activity was evaluated by measuring acetic acid-induced vascular permeability (Whittle, 1964). Mice (BALB/c, 15–20 g) in groups of five were dosed orally with Pm1, Pm2, Pm3 (100 μg/g body weight) and a positive control, indomethacin (10 μg/g body weight) before the intravenous injection of 4% Evans blue (10 μl/g body weight). After injection of the dye, 0.1 N acetic acid (10 μl/g 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 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.

3.8. Phagocytic activity

Male mice (BALB/c, 15–20 g) were used in groups of five. Pm1, Pm2, Pm3, and a positive control, zymosan, were each dissolved in physiological saline and dosed orally (50 μg/g body weight) once a day, for five days. After 48 h, mice were injected via the tail vein with colloidal carbon (Pelikan drawing ink A.17 black). The ink was diluted eight times with phosphate-buffered saline containing 1% gelatin before use, the amount of the resulting soln, used was 10 μl/g body weight. Blood samples were drawn from the orbital vein at 0, 3, 6, 9, 12 and 15 min. The blood (25 μl) was dissolved in 0.1% sodium carbonate (2 ml) and the absorbance at 660 nm was determined according to Biozzi et al. (1953).

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