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Chemical properties and biological activity of a polysaccharide from *Cyrtopodium cardiochilum*

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Abstract

A polysaccharide with an estimated weight-average molar mass of 4.6×10^5 was obtained from an aqueous extract of pseudobulbs of *Cyrtopodium cardiochilum* Lindl. by fractionation on Sephacryl S-300 HR and Sephadex G-25. Chemical and spectroscopic studies indicated that it has a backbone of (1→4)-linked β-D-mannopyranosyl residues and β-D-glucopyranosyl residues, and is demonstrated to be composed of D-mannose and D-glucose in 2:1 molar ratios. It was found to contain 4.2% of acetyl groups substituted at *O*-2 of (1→4)-linked β-D-Man*p* and β-D-Glc*p*. The polysaccharide inhibits capillary permeability and demonstrates phagocytosis stimulating property.

Keywords: *Cyrtopodium cardiochilum*; Orchidaceae; Polysaccharide; Glucomannan; Antiinflammatory activity; Phagocytic activity

1. Introduction

Cyrtopodium is a genus of predominantly terrestrial plants, occurring from southern USA to Brazil and most of the species are distributed in tropical regions. It is an orchid with thick pseudobulbs, and sometimes these species are cultivated in landscaping. Some species have an ethnopharmacological background, in particular *Cyrtopodium andersonii* R. Br. and *Cyrtopodium cardiochilum* Lindl. which in Brazil are used for the treatment of chest colds, tuberculosis and haemoptysis (Corrêa, 1975). Polysaccharides are found in orchids, such as glucomannan from tuber salep (*Orchis morio*, *O. mascula*, *Platanthera bifolia*) (Buchala, Franz, & Meier, 1974; Franz & Meier, 1971), and *O*-acetylglucomannan from *Bletilla striata* tubers (Tomoda, Nakatsuka, & Satoh, 1974; Tomoda, Nakatsuka, Tamai, & Nagata, 1973) and from *Dendrobium officinale* stem (Hua, Zhang, Fu, Chen, & Chan, 2004). The present paper is concerned with the isolation, chemical characterization and evaluation of the antiinflammatory and immunological activities of a glucomannan from the fresh pseudobulbs of *C. cardiochilum*.

2. Experimental

2.1. Plant material

Pseudobulbs of *C. cardiochilum* Lindl. were collected from the Riograndina experimental station plantation located in the vicinity of Nova Friburgo (Rio de Janeiro, Brazil) in October 1995.

2.2. Analytical techniques

Carbohydrate content was analyzed by colorimetric assays according to the procedure of Dubois, Gilles, Hamilton, Hebers, and Smith (1956), without previous hydrolysis of the sample, and by gas chromatography–electron impact mass spectrometry (GC–EIMS) of the alditol acetates (Sawardeker, Sloneker, & Jeanes, 1965). Protein content was analyzed by the method of Bradford (1976). The experimental data were tested for statistical differences using the Student's *t*-test. The weight-average molar mass of *C. cardiochilum* polysaccharide (CCP) was estimated from the calibration curve of elution using dextrans of known molecular weight as standards (2000,000, 413,000, 282,000, 148,000, 68,000, 37,500, 19,500 and 9500; Sigma) on Sephacryl S-300 HR (5×85 cm; Pharmacia).

Dialysis was carried out using tubing with an M_r cut-off 12,000. The optical rotation was measured on a Perkin–Elmer 243B polarimeter. Vis and IR spectra were measured on a

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Shimadzu UV-1601 and on a Perkin–Elmer FT-IR 1600 spectrometers, respectively.

^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX-600 NMR spectrometer operating at 600 MHz for δ_H and 150 MHz for δ_C, in D_2O containing sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard. Gas chromatography (GC) was carried out with flame ionization detector (FID), using a glass capillary column (0.31 mm × 25 m) SE-30. GC–EIMS were taken on a VG Auto SpecQ spectrometer operating at 70 eV. Thin-layer chromatography (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.

2.3. Extraction

Fresh pseudobulbs of *C. cardiochilum* (1.5 kg), previously cut into small pieces, were extracted with hot water (3 l) at 80 °C under stirring for 1 h. The aqueous extract was filtered through Whatmann filter paper (4 μm) 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: 18.674 g, 1.24%). A sample of the amorphous powder (2.06 g) was dissolved in 0.01% sodium sulfate (450 ml) and added to 5% cetyltrimethylammonium bromide (90 ml). After centrifugation, the supernatant was poured into two volumes of EtOH and the precipitate obtained was dissolved in water (275 ml), dialyzed and lyophilized to yield a neutral *C. cardiochilum* polysaccharide (CCP) (yield: 2.01 g). It was extracted from pseudobulbs according to Pereira, da Silva, Pereira, and Parente (2000).

2.4. Fractionation

A sample of the neutral polysaccharide (100 mg) was dissolved in 0.1 M Tris–HCl buffer (2 ml; pH 7.0), and applied to a Sephacryl S-300 HR column (5 × 85 cm; 1650 cm^3) with a flow rate of 1 ml/min. The carbohydrate content of each fraction was measured by spectrometry according to the colorimetric method reported by Dubois et al. (1956). Fractions of 5 ml corresponding to the peak CCP (190–390 ml) (Fig. 1) were pooled, dialyzed and freeze-dried. The obtained powder was dissolved in water (2 ml) and applied to a Sephadex G-25 column (1.5 × 50 cm; 15 g) with a flow rate of 1 ml/min and 5 ml fractions were collected (100–150 ml). The obtained eluate was concentrated and lyophilized to yield CCP (8.7 mg). The carbohydrate content of the fractions was measured. This procedure was repeated five times to obtain CCP (42.9 mg). It was obtained according to Pereira et al. (2000).

2.5. Molar carbohydrate composition and D,L configuration of CCP

Monosaccharides were analyzed as their trimethylsilylated methylglycosides obtained after methanolysis (0.5 M HCl in MeOH, 24 h, 80 °C) and trimethylsilylation (Kamerling, Gerwig, Vliegenthart, & Clamp, 1975). The configurations of the glycosides were established by capillary GC and GC–MS of their trimethylsilylated (−)-2-butylglycosides (Gerwig, Kamerling, & Vliegenthart, 1978).

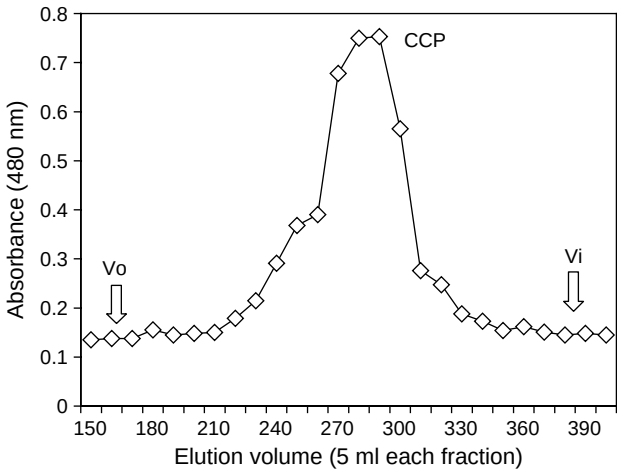

Fig. 1. Elution diagram of CCP polysaccharide from Sephacryl S-300 HR (0.1 M Tris–HCl). Vo, void volume; Vi, inner volume.

2.6. Methylation analysis

CCP was methylated with dimethyl sulfoxide–lithium methylsulfinyl carbanion–methyl iodide (Parente, Cardon, Leroy, Montreuil, Fournet, & Ricart, 1985). The methyl ethers were obtained after hydrolysis (4 N TFA, 2 h, 100 °C) and analyzed as alditol acetates by GC–MS (Sawardeker et al., 1965).

2.7. Deacetylation of CCP followed by periodate oxidation

The sample (25 mg) was dissolved in water (10 ml), then 0.02 N sodium hydroxide (10 ml) was added. After standing at room temperature for 10 min, the solution was neutralyzed with 0.1 N acetic acid and the total volume was adjusted to 25 ml with water. After addition of 0.1 M sodium metaperiodate (25 ml) the reaction mixture was kept at 5 °C in the dark. The periodate consumption was measured by a spectrometric method (Dixon & Lipkin, 1954). The oxidation was completed after 5 days, then 2 ml of the solution was used for the measurement of formic acid liberation by titrating with 0.01 N sodium hydroxide after addition of one drop of ethylene glycol.

2.8. Smith degradation and analysis of products

The residue of the reaction mixture was successively treated with ethylene glycol (0.3 ml) and sodium borohydride (120 mg) at 5 °C for 16 h, then adjusted to pH 5 by addition of acetic acid. The solution was concentrated and applied to a column (5.5 × 72 cm) of Sephadex G-15. Fractions were collected at 50 ml, and the eluates obtained from tubes 10–14 were combined, evaporated and lyophilized. (Tomoda, Satoh, & Ohmori, 1978). The product was hydrolyzed with 1 N sulfuric acid at 100 °C for 6 h, the hydrolyzates were derived to