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Chemical structure and selected biological properties of a glucomannan from the lichenized fungus *Heterodermia obscurata*

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ABSTRACT

Successive aqueous and alkaline extraction of the thallus of the lichenized fungus *Heterodermia obscurata* provided a highly branched glucomannan fraction (GM), whose chemical structure was determined. This was based on monosaccharide composition, methylation, partial acid hydrolysis, and NMR spectroscopic analysis. It consisted of a main chain of (1 → 6)-linked α-D-mannopyranosyl units, almost all being substituted at *O*-2 with α-D-glucopyranosyl, α-D-mannopyranosyl, and 4-*O*-substituted α-D-mannopyranosyl groups. Intra-peritoneal administration of this GM induced a marked and dose-dependent inhibition of acetic acid-induced visceral pain with an ID_{50} of 0.6 (0.2-2.0) mg/kg and inhibition of 88 ± 4%. It also reduced leukocyte migration by 58 ± 4%, but did not alter plasmatic extravasation to the peritoneal cavity. The results suggest that the glucomannan from the *H. obscurata* might have potential for antinociceptive and anti-inflammatory utilization.

1. Introduction

The genus *Heterodermia*, which belongs to class Ascomycetes, order Lecanorales, family Physciaceae (Tehler, 1996), consists of about 80 species, mostly tropical and sub-tropical in distribution (Cohen and Towers, 1995). Approximately 42 species of *Heterodermia* have been described as growing in Brazil (Marcelli, 2006). Among these, the most abundant species is *Heterodermia obscurata* (sect. *Polyblastidium* of Kurokawa, 1973).

The lichenized fungus *H. obscurata* is well-known for its production of anthraquinones (Cohen and Towers, 1995), but its carbohydrates have not yet been investigated. The best known lichen polysaccharides are the α- and β-glucans, isolichenan and lichenan, respectively, as well as heteropolymers including galactomannans, galactomannoglucans, and galactoglucomannans (Woranovicz-Barreira et al., 1999; Carbonero et al., 2001), which can be tools in chemotaxonomic studies (Gorin et al., 1993; Teixeira et al., 1995; Woranovicz-Barreira et al., 1999). Some unusual heteropolysaccharides, also found in lichens were a xylomannan from *Cora pavonia* = *Dictyonema glabratum*; (Iacomini et al., 1987), a rhamnopyranosylgalactofuranan (thamnolan), from *Thamnolia subuliformis*, having a predominant structure of (1 → 3)-linked β-D-galactofuranosyl units with complex rhamnopyranosyl side-chains and terminal xylopyranosyl units (Olafsdóttir et al., 1999). Another was a glucomannan with a main chain formed by (1 → 6)-linked α-Man*p* units, mainly substituted at *O*-2 with side chains of α-Man*p*, with smaller amounts of α-Glc*p*, α-Glc*p*-(1 → 2)-[α-Man*p*-(1 → 4)]-α-Man*p*, and possibly α-Man*p*-(1 → 2)-[α-Man*p*-(1 → 4)]-α-Man*p* groups. This polysaccharide was described as being present in *Tornabenia intricata* (Teixeira et al., 1992).

Lichen species have sometimes been used in traditional medicine (Vartia, 1973), so that a possible beneficial role of their polysaccharides has been suggested (Ingólfsdóttir, 2000). Most of the lichenized fungal species so far investigated contain polysaccharides in considerable amounts (Baron et al., 1991), and many of them have been shown to have antitumor, general stimulating activity on the unspecific immune system, antiviral, memory enhancing effects, as well as other biological activities, accompa-

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nied by low toxicity levels (Olafsdóttir and Ingólfsdóttir, 2001). In some cases, such activities have been enhanced by chemical modification of the polysaccharide structure. As examples, a (1 → 6)-linked β-glucan from *Parmotrema mantiqueirense* (Martinichen-Herrero et al., 2005a) and a galactoglucomannan from *Cladonia ibitipocae* (Martinichen-Herrero et al., 2005b) were submitted to sulfation. This gave respectively to a sulfated glucan and galactoglucomannan with 76.8% and 42.2% sulfate, corresponding to a degree of substitution of 1.95 and 1.29, respectively. Each product was evaluated for its activated partial thromboplastin (APTT) and thrombin time (TT), compared with its corresponding non-sulfated precursor (native), their anticoagulant activity being determined only for sulfated structures (Martinichen-Herrero et al., 2005a,b).

The most active antitumor lichen polysaccharides, found on intra-peritoneal administration to mice, appear to be (1 → 3)-linked β-glucans (Olafsdóttir and Ingólfsdóttir, 2001), as with polysaccharides having a similar structure, although isolated from other sources, mainly basidiomycetes (Bohn and BeMiller, 1995). Immunomodulating effects were found for α-glucans, galactomannan and rhamnogalactan (Olafsdóttir and Ingólfsdóttir, 2001). Even lichen polysaccharides had diverse biological activities, although only limited studies have been carried out. Consequently, these polymers deserve further studies on their biological activities, including those cited above, and others like anti-inflammatory and/or antinociceptive activities. Examples were on polysaccharides isolated from basidiomycetes, such as *Pleurotus pulmonarius* (Smiderle et al., 2008a, 2008b), *Lentinus edodes* (Carbonero et al., 2008), and *Caripia montagnei* (Queiroz et al., 2010).

We now describe the structural features of an unusual glucomannan from the thallus of the lichenized fungus of *H. obscurata*, as well as determination of its antinociceptive and potential anti-inflammatory properties, using an inflammatory pain model in mice.

2. Results and discussion

In order to remove lipids, pigments, and other hydrophobic material, the lichen thallus was extracted successively with acetone (12.2 g% yield) and ethanol (3.9 g% yield). The product was then submitted to successive extractions with hot water and hot aq. 2% and 10% KOH, and extracted polysaccharides were recovered by ethanol precipitation (aqueous extract) or after dialysis, after HOAc neutralization of alkaline extracts, giving hot water-soluble fraction W (4.0 g% yield), K2 extracted with 2% KOH (26.0 g% yield), and K10 extracted with 10% KOH (4.0 g% yield). Fractionation of the neutralized extracts (W, K2, and K10) by freezing, followed by gentle thawing (Gorin and Iacomini, 1984) furnished cold water-soluble (SW, 3.3 g%; SK2, 20.7 g%; SK10, 3.1 g%, respectively) and insoluble polysaccharides, which sedimented on centrifugation. The water-soluble fractions were then treated with Fehling solution (Jones and Stoodley, 1965), giving rise to soluble and insoluble Cu^{2+} complexes (FP-W, 0.6 g%; FP-K2, 8.2 g%; FP-K10, 0.2 g%) (Fig. 1).

The polysaccharides derived from insoluble Cu^{2+} complexes (FP-W, FP-K2, and FP-K10 fractions) contained similar monosaccharide components, mainly mannose and glucose (Table 1), and gave rise to homogeneous elution profiles on HPSEC, with M_w 17.2×10^3 g/mol (*dn/dc* 0.144). Comparison of their ^{13}C NMR spectra (Fig. 2A and B), showed that they contained similar structural components, and as fraction FP-K2 was obtained in greater yield, it was selected for further investigation.

Methylation analysis of FP-K2 (Table 2) showed a highly branched structure based on derived partially *O*-methylated alditol acetates (GC–MS), with a high proportion of non-reducing end-units of Man*p* (18%) and Glc*p* (20%). Other Man*p* units were 2-*O*- (2%), 4-*O*- (13%) and 6-*O*- (4%), and 2,6- (42%) and 4,6-di-*O*-substituted (1%).

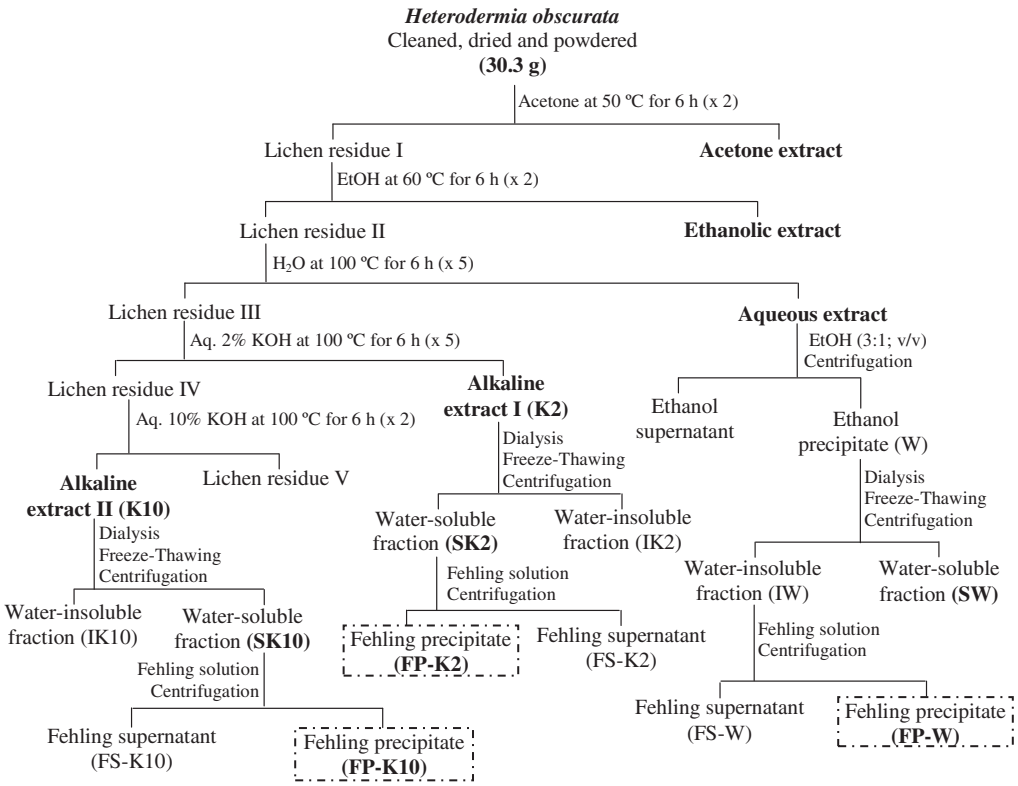

Fig. 1. Scheme of extraction and purification of the glucomannan from the thallus of *H. obscurata*.