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Hot-water extracts from the inner bark of Norway spruce with immunomodulating activities

Myriam Le Normand[a], Hugo Mélida[b], Bjarne Holmbom[c], Terje E. Michaelsen[d], Marit Inngjerdingen[e], Vincent Bulone[b], Berit Smestad Paulsen[d], Monica Ek[a,*]

[a] Division of Wood Chemistry and Pulp Technology, School of Chemical Science and Engineering, KTH Royal Institute of Technology, Teknikringen 56, SE-10044 Stockholm, Sweden
[b] Division of Glycoscience, School of Biotechnology, KTH Royal Institute of Technology, AlbaNova University Centre, SE-10691 Stockholm, Sweden
[c] Chemistry Centre, Åbo Akademi University, Porthansgatan 3, FI-20500 Turku, Finland
[d] Department of Pharmaceutical Chemistry, School of Pharmacy, University of Oslo, P.O. Box 1068, Blindern, N-0316 Oslo, Norway
[e] Department of Anatomy, Institute of Basic Medical Sciences, University of Oslo, P.O. Box 1105, Blindern, N-0317 Oslo, Norway

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ABSTRACT

The inner bark of Norway spruce (*Picea abies*) was sequentially extracted with hot water at 100 °C, 140 °C and 160 °C. The hot-water extracts (IB 100 °C, IB 140 °C and IB 160 °C) contained pectic polysaccharides and showed immunostimulating activities. Structural analyses of their carbohydrate content, including glycosidic linkage analyses, revealed the presence of pectins with a large rhamnogalacturonan RG-I domain ramified with highly-branched arabinans. IB 100 °C also contained a large amount of terminal glucosyl residues, indicating the presence of highly substituted polymers. IB 160 °C was mainly composed of starch. The hot-water extracts were tested for two biological activities, namely complement fixation and macrophage stimulation. IB 100 °C exhibited the highest complement fixation activity, with a 1.7-times higher IC_H50 than the control pectin, while IB 140 °C and IB 160 °C gave similar IC_H50 values as the control. Macrophages were stimulated by IB 100 °C and IB 140 °C in a dose-dependent manner, but not by IB 160 °C. IB 100 °C presented the highest activity toward macrophages, comparable to the control pectin.

1. Introduction

In Scandinavia, Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) are the most important raw materials used by the forest industry. Manufacturing processes for pulp, paper and timber production from Norway spruce produce large quantities of complex lignocellulosic leftovers, such as bark, which could be recovered and converted into higher-value bio-based products. The quantity of bark from Norway spruce is estimated to represent around 1.5 million tons/year in Sweden alone.

The most studied components of Norway spruce bark are hydrophilic extractives such as tannins (Matthews, Mila, Scalbert, & Donnelly, 1997; Zhang & Gellerstedt, 2009) and stilbenes (Mannila & Talvitie, 1992), and lipophilic compounds (Norin & Winell, 1972). In previous works, we determined the carbohydrate composition of fractions that were sequentially extracted from the bark of Norway spruce (Krogell, Holmbom, Pranovich, Hemming, & Willför, 2012; Le Normand, Edlund, Holmbom, & Ek, 2012). High concentrations of galacturonic acid, arabinose, galactose and rhamnose, indicative of the presence of pectic polysaccharides, were measured in hot-water extracts. The amount of pectins was estimated to represent 9.0% of the outer bark, 12.6% of the inner bark and 10.5% of the bark collected in a paper mill immediately after the debarking process. These values are significantly higher than the concentration values of pectins (~3.5%) in the wood of Norway spruce (Bertaud & Holmbom, 2004; Willför, Sundberg, Hemming, & Holmbom, 2005). Pectins have also been identified in the bark of various conifer species such as *Picea mariana* (Anderson & Pigman, 1947), *Picea glauca* (Painter & Purves, 1960), *Abies amabilis* (Bhattacharjee & Timell, 1965) and, more recently, in the bark of *Pinus pinaster* (Fradinho et al., 2002) and *P. sylvestris* (Valentín et al., 2010). The fine structure and properties of the pectins from the bark of *P. abies* is however scarce, despite the potential economic importance of this raw material.

Pectins are major components of primary cell walls and middle lamellae of all plants (Willats, McCartney, Mackie, & Knox, 2001). They have multiple roles in plant growth (Ridley, O'Neill, & Mohnen, 2001) and are widely used in the food industry as gelling and stabilizing agents (Thakur, Singh, Handa, & Rao, 1997). Although most plant tissues contain pectins, the commercial

* Corresponding author. Tel.: +46 8 790 8104; fax: +46 8 790 6166.
E-mail address: monicaek@kth.se (M. Ek).

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production of this family of polysaccharides is based almost entirely on citrus peel and apple pomace, which fulfill the properties required by the food industry (Willats, Knox, & Mikkelsen, 2006). Pectins are heterogeneous polysaccharides and their structure can vary considerably between plants, tissues and even within a single cell wall. Pectins consist of three main domains: a linear homogalacturonic backbone (HG) alternating with two types of highly branched rhamnogalacturonans regions designated as RG-I and RG-II (Albersheim, Darvill, O'Neill, Schols, & Voragen, 1996). These three domains are typically considered to be covalently linked and form a complex pectic network (Scheller, Jensen, Sørensen, Harholt, & Geshi, 2007). RG-I are substituted with side chains of arabinose and galactose units. Their chain length and sugar composition can be extremely heterogeneous between plants. On the contrary, RG-II has a highly conserved structure, consisting of a HG backbone branched with eleven different monosaccharides, including some rare sugars such as 2-O-methylxylose, 2-O-methylfucose, apiose, aceric acid, 2-keto-3-deoxy-D-manno-octulosonic acid and 3-deoxy-D-lyxo-2-heptulosaric acid (O'Neill et al., 1996). In our previous articles, we reported that pectins from the bark of Norway spruce are rich in arabinose and may have a rather large and branched RG-I region (Le Normand et al., 2012). These structural characteristics often have a positive impact on the pectins' health-promoting capacities (Yamada, 1996; Yamada & Kiyohara, 2007).

Several recent papers have described the immunomodulating activity of hemicelluloses and pectins extracted from a variety of medicinal plants, with effects ranging from complement fixation to macrophage stimulation (Austarheim et al., 2012; Inngjerdingen et al., 2008; Michaelsen, Gilje, Samuelsen, Høgåsen, & Paulsen, 2000; Nergard et al., 2005). Pectins extracted from plants are abundant and relatively nontoxic, thus providing ideal candidates for the development of therapeutics with immunomodulatory action. Although the mechanism of action of plant pectins on the immune system is not fully understood, the therapeutic effects of these polymers are thought to occur *via* modulation of part of the innate immune system such as the complement system and by stimulating macrophages. The complement system consists of more than 30 serum and cellular proteins, linked in two biochemical cascades, that play an important role in the primary defense against bacterial and viral infections. The activation of complement encompasses a series of initiation, amplification and lytic steps (Makrides, 1998). Macrophages play an important role in host defense and inflammation. They have different functions, including surveillance, phagocytosis, destruction of targeted foreign micro-organisms as well as antigen presentation to T lymphocytes (Schepetkin & Quinn, 2006).

In the present study, three hot water extracts of the inner bark of Norway spruce were compared with respect to their carbohydrate composition, structural patterns, complement fixation activity and macrophage activation.

2. Materials and methods

2.1. Extraction of polysaccharides

2.1.1. Bark material

The bark of Norway spruce was sampled from a fresh 30-year-old tree cut in Gävleborg County (Sweden) in July 2009. It was stored in the dark at −20 °C. The inner and outer barks were manually separated using a scalpel. The inner bark was ground with a hand blender to particle sizes not exceeding 5 mm × 2 mm.

2.1.2. Extraction

The ground inner bark was sequentially extracted with acetone at 100 °C, followed by hot water at 100 °C, 140 °C and 160 °C, using an Accelerated Solvent Extractor (ASE) (Dionex, CA). Each extraction step included three cycles of 20 min. In total, 90 g of wet inner bark was extracted with ASE with a load of approximately 10 g in 34 ml-cells. The total dissolved solid (TDS) of all hot-water extracts was determined gravimetrically after freeze-drying 10 ml of each extract.

The fractions were filtered and dialyzed against distilled water in a Spectra/Por dialysis tube with a molecular mass cut-off of 3.5 kDa prior to analyses and bioactivity tests.

2.2. Structural analyses of the bark fractions

2.2.1. Carbohydrate composition and Klason lignin content

The freeze-dried fractions were analyzed for carbohydrate content and sugar unit composition in duplicate experiments. Total amounts of carbohydrates in the ground bark, residues and extracts were determined by acid methanolysis, followed by silylation with hexamethyldisilazane and trimethylchlorosilane, and analysis by gas chromatography (Sundberg, Sundberg, Lillandt, & Holmbom, 1996).

Klason lignin was determined after acid hydrolysis following the TAPPI test method T222 om-06 (TAPPI, 2006).

2.2.2. Molecular weight determination of carbohydrates

Size-exclusion chromatography (SEC) in alkaline solvent (NaOH) was used to estimate the molecular weight and polydispersity of the polysaccharides obtained by hot-water extractions. The SEC system consisted of three TSK gel columns (Tosoh Bioscience, Tokyo, Japan) coupled in series (G3000PW, 7.5 mm × 300 mm, 10 μm particle size; GP4000PW, 7.5 mm × 300 mm, 17 μm particle size; G3000PW) and connected to a refractive index detector (Waters 2410). The mobile phase was 10 mM NaOH and the flow rate was of 1 ml min^{-1}. The system was calibrated using pullulan standards of known molecular masses ranging from 320 to 400,000 Da.

2.2.3. Glycosidic linkage analyses

Polysaccharides in the freeze-dried fractions (5 mg) were carboxyl reduced with sodium borodeuteride ($NaBD_4$) (Kim & Carpita, 1992) and methylated (1 mg, four technical replicates) in the presence of NaOH/CH_3I (Ciucanu & Kerek, 1984). The methylation step was repeated five times on each sample to avoid undermethylation. The samples were then hydrolyzed with 2 M TFA at 121 °C for 2 h, reduced and acetylated (Albersheim, Nevins, English, & Karr, 1967). The permethylated alditol acetates were separated and analyzed by GC/EI-MS on a SP-2380 capillary column (30 m × 0.25 mm i.d.; Sigma–Aldrich) with a temperature program increasing from 160 °C to 210 °C at a rate of 1 °C min^{-1}. The mass spectra of the fragments obtained from the permethylated alditol acetates (EI-MS) were compared with those of reference polysaccharide derivatives and to available data (Carpita & Shea, 1989). The quantification was based on the carbohydrate composition and effective carbon response of each compound as detected by GC-MS.

2.2.4. Amylase treatment

Starch was removed from the filtered and dialyzed extracts by treatments with α-amylase from porcine pancreas (Type VI-B, 23 units/mg, Sigma–Aldrich). 50 mg of each extract was dissolved in 30 ml of 0.01 M phosphate buffer (pH 7) and stirred in a water bath at 37 °C. 7 mg of the enzyme was added every 24 h during three days. The solution was then filtered, dialyzed and freeze-dried.

Table 1
Yields of the different extraction steps applied to Norway spruce inner bark (mg g^{-1} bark).

	Extracted material[a]	Non-cellulosic carbohydrates
Starting material	–	434
Acetone	159	33
Water at 100 °C	199	91
Water at 140 °C	267	212
Water at 160 °C	79	47
Residue	–	55

[a] Deduced from the total dissolved solids in each extract.

2.3. Biological activity assessments

2.3.1. Complement fixation assay

The complement fixation activity of the samples was tested in triplicate using an assay based on inhibition of lysis of antibody-sensitized sheep red blood cells mediated by complement present in human sera (Michaelsen et al., 2000). The pectin fraction PMII from the leaves of *Plantago major* L. (Samuelsen et al., 1996) was used as a positive control. The concentration of test sample giving 50% inhibition of lysis (IC_H50) was calculated and the complement fixation activity was expressed as the ratio between the IC_H50 obtained with the PMII control and the IC_H50 measured for the extract. A high ratio indicates a high complement fixing activity of the extract.

2.3.2. Nitric oxide assay

The capacity of the extracts to stimulate the production of nitric oxide (NO) by macrophages was investigated as described earlier (Inngjerdingen et al., 2012) The mouse macrophage cell line Raw 264.7 was used. LPS from *Pseudomonas aeruginosa* (Sigma–Aldrich) and the polysaccharide fraction PMII from *P. major* were used as positive controls (Samuelsen et al., 1996). The results were expressed as the mean ± S.D. The difference between the control (medium alone) and the treated samples was tested for statistical significance using the Dunnett's Multiple Comparison Test. A value of $p < 0.05$ was considered as statistically significant.

3. Results and discussion

3.1. Extraction of polysaccharides from the bark of Norway spruce

The polysaccharides from the inner bark of Norway spruce were extracted as previously described for the preparation of polysaccharides from the whole bark (Le Normand et al., 2012). Briefly, the inner bark was treated with a mixture of acetone:water (95:5, v/v) to remove low molecular mass extractives and carbohydrates. The bark material was then exposed to hot water at three different temperatures to allow the extraction of non-cellulosic polysaccharides from the inner bark. The data in Table 1 show that the residue obtained after all extraction steps contained only 55 mg of non-cellulosic carbohydrates per g of starting material, which corresponds to the extraction of almost 90% of the initial carbohydrate content. The highest extraction yield (267 mg g^{-1} bark) was obtained with the treatment at 140 °C (Table 1). More than half of the initial non-cellulosic polysaccharides were extracted during this step. The fraction obtained after the last aqueous extraction step (160 °C) contained 79 mg of material per g of initial inner bark, 47 mg of which corresponded to non-cellulosic carbohydrates (Table 1).

Table 2
Compositional analysis of the hot-water extracts from the inner bark of Norway spruce (%, w/w). The molecular mass distribution (Mn, Mw), the polydispersity (Mw/Mn) of the oligo- and polysaccharides fractions are presented together with monosaccharide and Klason lignin contents.

	IB 100 °C	IB 140 °C	IB 160 °C
Oligo- and polysaccharide content (% of extract)	42.9	78.1	87.8
Monosaccharide composition			
Ara	14.9	32.3	7.8
Rha	3.1	5.8	1.8
GalA	25.2	25.7	4.9
Gal	5.8	10.6	5.6
Xyl	1.9	3.0	6.2
Man	3.1	4.5	7.0
Glc	44.9	17.6	66.3
GlcA	1.1	0.5	0.4
Klason lignin (% of extract)	15.4	4.2	5.6
Mn (kDa)	13.9	14.6	6.0
Mw (kDa)	44.3	54.9	12.3
Mw/Mn	3.1	3.7	2.4

3.2. Carbohydrate composition

The total polysaccharide content, monosaccharide composition and molecular masses of the carbohydrates present in the three hot water extracts were compared (Table 2).

The fraction obtained at 100 °C (IB 100 °C) contained the largest amount of Klason lignin (15.4%) and 43% of carbohydrates, while the two other extracts were characterized by much higher carbohydrate contents, *i.e.*, 80% for IB 140 °C and up to 88% for IB 160 °C. Glucose was the predominant monosaccharide in IB 100 °C and IB 160 °C, whereas IB 140 °C was richer in arabinose and galacturonic acid and contained significantly smaller amounts of glucose. Arabinose and galacturonic acid were also present in relatively high amounts in IB 100 °C. The sum of pectic carbohydrates (GalA, Rha, Ara and Gal) represented 55%, 80% and 25% of the total carbohydrate content of IB 100 °C, IB 140 °C and IB 160 °C. Since the total yield of extraction was much higher for IB 140 °C, it seems that most of the bark pectins were extracted by the treatment at 140 °C. These results were somewhat comparable to those obtained previously using a slightly different extraction protocol (Krogell et al., 2012) and with the whole bark as raw material (Le Normand et al., 2012).

The apparent molecular mass of the hot-water extracts ranged from 12.3 kDa for IB 160 °C to 54.9 kDa for IB 140 °C. This can be explained by the fact that hydrolysis of non-cellulosic polysaccharide chains is more extensive at temperatures around 160 °C, leading to decreased molecular masses. The molecular mass distribution was broad for all extracts, indicating the existence of complex mixtures of polysaccharides with different chain lengths.

3.3. Linkage analyses

Each hot-water extract was subjected to permethylation, hydrolysis and GC analysis for the determination of glycosidic linkages. The results are summarized in Table 3.

Glucose was the main carbohydrate in the IB 100 °C and IB 160 °C fractions. However, linkage analysis revealed that the glucose residues in IB 100 °C correspond essentially to terminal units (85 mol% of the total glucose content). The terminal glucose residues most likely arise from glucose-substituted polymers, such as condensed tannins and stilbene glucosides, rather than from branched starch only, as suggested earlier (Krogell et al., 2012). Zhang and Gellerstedt (2009) reported the presence of condensed tannins in spruce inner bark and showed that every second flavonoid monomer of the tannins was substituted by a glucose

Table 3
Linkage analysis of the carbohydrates present in the different hot-water extracts of the Norway spruce inner bark (mol%).

	IB 100 °C	IB 140 °C	IB 160 °C
Ara			
t-Araf	4.7	9.5	3.4
5-Araf	5.3	11.2	2.8
3-Araf	2.4	4.4	1.4
3,5-Araf	2.2	4.3	1.1
2,5-Araf	0.9	2.2	0.4
2,3,5-Araf	1.9	4.8	tr[a]
Rha			
2-Rhap	tr	0.8	0.3
4-Rhap	tr	0.6	0.2
2,4-Rhap	3.3	4.7	1.5
GalA			
t-GalpA	3.2	1.6	2.2
4-GalpA	19.4	18.7	2.3
3,4-GalpA	0.6	2.5	tr
Gal			
t-Galp	2.9	4.8	1.0
3-Galp	tr	1.2	0.5
4-Galp	0.3	1.4	tr
6-Galp	1.3	2.0	3.8
3,6-Galp	0.9	0.6	tr
3,4-Galp	0.3	0.1	tr
Man			
4-Manp	2.3	3.9	5.7
4,6-Manp	0.8	0.4	1.1
Xyl			
t-Xylp	1.3	0.7	2.2
4-Xylp	1.0	2.7	5.1
Glc			
t-Glcp	38.8	3.3	4.2
4-Glcp	5.1	10.8	55.7
4,6-Glcp	tr	2.4	4.7
GlcA			
t-GlcpA	0.6	0.3	0.5
4-GlcpA	0.4	0.2	tr

[a] Trace amounts.

unit. The high Klason lignin content combined to the abundance of terminal glucose residues may be related to highly substituted tannins and stilbenes. On the contrary, linkage analysis performed on IB 160 °C revealed the predominance of 4-linked glucose (86 mol% of the total glucose content) and minor amounts of terminal (7%) and 4,6-glucosyl residues (7%). These linkages are all typical of starch (amylopectin). The presence of starch was further confirmed by treating the extract with amylase and repeating the linkage analysis. The permethylated alditol acetate profiles of the amylase-treated and untreated samples are compared in Fig. 1. The decrease of the peak corresponding to the 4-linked glucose residues was extensive, supporting the predominance of starch in the IB 160 °C fraction. IB 100 °C and IB 140 °C were also treated with amylase (not shown). IB 140 °C gave similar results as IB 160 °C whereas the amylase treatment on IB 100 °C did not show any change in glucose composition.

Since IB 160 °C was mainly composed of starch and showed a low pectin content, we focused our linkage analyses of the pectic carbohydrates on IB 100 °C and IB 140 °C. These fractions exhibited comparable proportions of pectic carbohydrates. Galacturonic acid was mostly present as 4-linked residues and represented 83 mol% of the total galacturonic acid content of both IB 100 °C and IB 140 °C. 4-linked galacturonic acid moities are commonly found in the HG, RG-I and RG-II domains of pectic polysaccharides. The HG domain seemed to represent between 25% (IB 140 °C) and 40% (IB 100 °C) of the pectic polysaccharides. 3% of the galacturonic acid residues in IB 100 °C and 12% in IB 140 °C were substituted in position

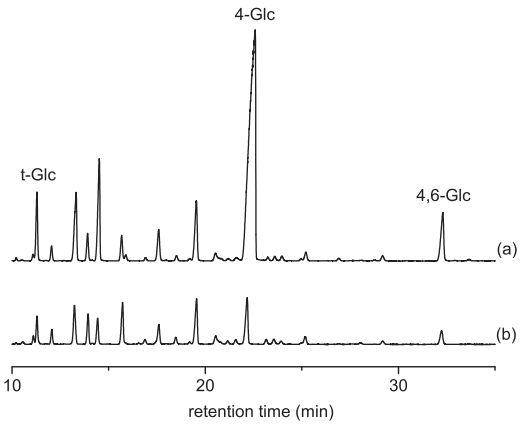

Fig. 1. GC chromatogram of the permethylated alditol acetates obtained from the IB 160 °C fraction before (a) and after (b) treatment with amylase.

3. In pectic polysaccharides, galacturonic acid is usually substituted at this position by xylosyl residues, forming xylogalacturonan (XGA) chains, or by other sugars such as apiofuranose in the RG-II domain. It is difficult to predict how large the RG-II and XGA regions might be, although such domains typically represent minor portions (<10%) of total pectins (Mohnen, 2008).

The extracts contained RG-I-like structures, as evidenced by the relative high amount of 2,4-linked rhamnose units (77–100% of the total Rha content). In RG-I, the 2,4-rhamnosyl residues usually serve as anchorage points for side chains of neutral sugars. The rhamnogalacturonan backbone of RG-I represented about 15% of the pectin structure. The high content of arabinose and galactose in the extracts further supports the occurrence of a large RG-I region.

Approximately 25% of the arabinose residues in the IB 100 °C and IB 140 °C fractions occurred as 5-linked units, 25% corresponded to terminal arabinosyl residues and the rest of the arabinose monosaccharides was represented by 3-, 3,5-, 2,5- and 2,3,5-linked arabinosyl units. These proportions confirmed the occurrence of highly branched arabinans. Part of the arabinose units, especially the terminal residues, may also be associated with galactose units to form arabinogalactan chains. Two types of arabinogalactans are generally encountered in pectic polysaccharides, namely arabinogalactans I (AG-I) and II (AG-II). The occurrence of AG-I was confirmed by the presence of 4-linked galactan backbones substituted at position 3. AG-II was also present in the extracts since 3-, 3,6- and 6-linked galactosyl residues were detected. At this point, it is not possible to conclude whether the arabinan and arabinogalactan chains are covalently bound to the pectins, or if they are present as independent polysaccharides. These chains may also correspond to degradation products of pectins arising from the high temperature treatment used during the extraction procedure. Further fractionations and characterization of IB 140 °C are ongoing to identify the nature and origin of the arabinose-rich chains.

Other minor linkages were detected in all extracts, such as 4- and 4,6-linked mannosyl residues. These linkages are usually combined to 4-linked glucosyl moities and terminal galactosyl residues and arise from galactoglucomannan which is the main hemicellulose (15–20%, w/w) in the wood of Norway spruce (Willför et al., 2005). The IB 160 °C fraction contained relatively high proportions of 4-linked xylosyl (5.1 mol%) and terminal arabinosyl (3.4 mol%) residues. These linkages indicated the extraction of arabinoxylans which also occur in the wood of Norway spruce (5–10%, w/w) (Sjöström, 1981). These data are further supported by the previous finding that galactoglucomannans and arabinoglucoronoxylans are extensively extracted from spruce wood when the samples are treated with water at 160 °C or higher temperatures (Song, Pranovich, Sumerskiy, & Holmbom, 2008).

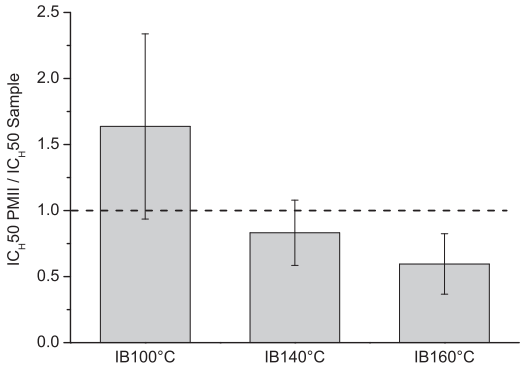

Fig. 2. Complement fixation activity of the hot water extracts. The figure shows the activity of each extract compared to the positive control PMII (dash line). The activity is expressed by the ratios between the IC_H50 obtained with PMII and that of each extract. The error bars represent the standard deviation (S.D.) derived from the average values. Results are expressed as mean ± S.D. based on three independent experiments.

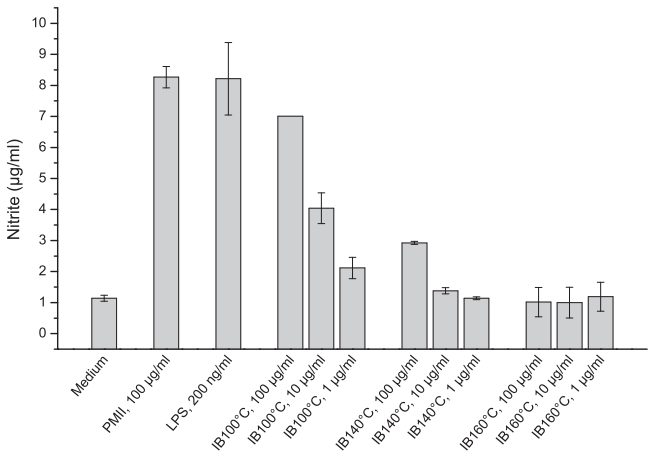

Fig. 3. Stimulation of Raw 264.7 macrophages by the hot-water extracts of the Norway spruce inner bark tested at different dilutions. The activities were quantified by the measurement of nitrite released in the assay medium. LPS and PMII from *Plantago major* L. were used as positive controls.

3.4. Biological activities

The hot-water extracts from the inner spruce bark were tested for two types of biological activities, *i.e.*, complement fixation activity and macrophage stimulation.

As shown in Fig. 2, all extracts exhibited some complement fixation activity. The fraction extracted at 100 °C showed the highest activity, which was approximately 1.7 times stronger than that of the control. This heterogeneous extract contained the lowest amount of polysaccharides. Its high complement fixation activity may be due to a synergistic effect of the polysaccharide mixture and the tannins present in the sample (Wagner, 1999). The fraction extracted at 140 °C, which was mostly composed of pectins, exhibited a complement fixation activity (0.8) comparable to that of the model pectin PMII. It has been previously reported that the biological activities of pectins are mainly due to the side chains of the RG-I and that the HG domains are of little importance for activity (Smestad Paulsen & Barsett, 2005). Therefore, the relatively high activity of IB 100 °C and IB 140 °C may be related to their high arabinose and galactose contents and the existence of specific branching points, such as 3,6-galactosyl and 3,5-arabinosyl residues, which are indicative of the presence of large and highly branched RG-I side chains.

Complement fixation assays indicate the ability of the polysaccharides to interfere with the complement system, but they cannot discriminate between complement activation and complement inhibition. In order to assess such biological activity, another type of test was performed that allow the evaluation of the behavior of macrophages in the presence of the hot-water extracts.

The ability of the hot-water extracts to stimulate the mouse macrophages is shown in Fig. 3. In response to an immune challenge, macrophages become activated and produce pro-inflammatory mediators such as nitric oxide (NO). The production of NO is determined through its stable breakdown product, *i.e.*, nitrite (NO_2^-), which can be measured by UV spectrometry. The activities of the hot-water extracts were compared to those of the bioactive pectin from *P. major* (PMII) and lipopolysaccharide (LPS). LPS is a major component of the outer-membrane of Gram-negative bacteria and is known to induce the activation of the immune system (Sweet & Hume, 1996). The macrophages were stimulated by both IB 100 °C and IB 140 °C in a dose-dependent manner (Fig. 3). IB 100 °C presented the highest activity, comparable to the bioactive pectin control PMII. The extract obtained at 160 °C, which contained starch as a major component (70%), showed no significant effect thereby suggesting that the stimulation of macrophages was closely related to the presence of pectins in the extract. The low activity of this extract may also be explained by the relatively small size of the polysaccharide chains (Mw = 12.3 kDa) compared to the extracts obtained at 100 °C (Mw = 44.3 kDa) and 140 °C (Mw = 54.4 kDa).

4. Conclusions

Here we report the partial structural characterization of the inner bark polysaccharides of Norway spruce, one of the most exploited conifer species in Europe. In addition, our data suggest that bark components extracted with hot water at 100 °C and 140 °C have potential applications as immuno-stimulating agents. Additional structure/activity studies after further purification of individual polysaccharides are being performed on the IB 100 °C and IB 140 °C fractions to determine whether (and which) pectins are responsible for the observed biological activities.

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