

Structural analysis of arabinogalactan from larch

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Introduction

Larch arabinogalactan (LAG) is a polysaccharide obtained from the wood of tamarack larch (*Larix laricina*). LAG is approved by the U.S. Food and Drug Administration (FDA) as a source of dietary fiber [1], but also has potential therapeutic benefits as an immune stimulating agent [2]. Our research tends to structural analysis of LAG as the main component of FiberAid™.

Elementary analysis of LAG has shown that carbon and hydrogen atoms can be found in a 1 : 2 molar ratio. No nitrogen or sulphur atoms could be detected, which indicates that LAG is not attached to a protein moiety. Quantification of neutral sugars by acetylation pointed out a 1 : 5.8 ratio of arabinose (Ara) to galactose (Gal) as main monosaccharides. Determination of uronic acids by specific reduction with deuterium-labelling (NaBD₄) revealed small amounts of glucuronic acid. Linkage type analysis by methylation followed by GLC-MS showed that the main components are 1,3,6-Gal(p) and 1,6-Gal(p), as well as there being minor amounts of 1,3-Gal(p), 1,3-Ara(f) and terminal Ara(f), Ara(p), Gal(p) and GlcA(p). This led to a structural proposal for LAG.

Aim of the study

- ◆ Determination of fine structure of complex polysaccharides obtained from wood of Tamarack (*Larix laricina*).



Results

Monosaccharide composition

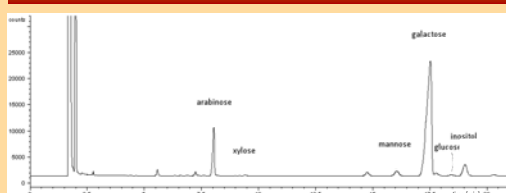


Figure 1: GLC spectrum of LAG after acetylation analysis

From the shown spectrum (fig. 1) it can be concluded that galactose and arabinose are the main monosaccharide components of LAG, accompanied by mannose in small amounts, and xylose and glucose in traces. The total monosaccharide yield from LAG samples is about 84% (m/m). Table 1 shows the amounts (% m/m) of monosaccharides in LAG.

Table 1: Monosaccharide amounts in mass percentage for LAG determined by acetylation analyses

LAG	1a	1b	1c	2a	2b	2c	3a	3b	3c	average	stdev
arabinose	17.5	18.2	17.4	14.9	14.9	14.7	14.9	14.9	15.0	15.8	1.3
xylose	0.5	0.5	0.5	1.1	1.1	1.1	0.8	0.7	0.7	0.8	0.2
mannose	4.8	4.8	4.8	5.1	5.1	5.2	4.3	4.2	4.3	4.7	0.4
galactose	76.2	75.6	76.4	76.4	76.3	76.4	77.9	77.9	77.9	76.8	0.8
glucose	0.6	0.5	0.5	1.5	1.5	1.5	1.1	1.2	1.0	1.0	0.4
yield	90.1	92.6	92.2	76.9	77.8	76.0	83.8	83.8	83.0	84.0	6.1

Polysaccharide structure

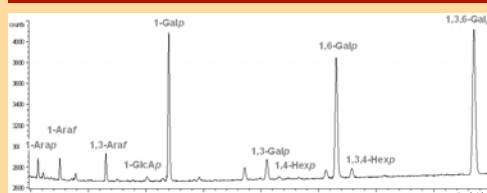


Figure 2: GLC spectrum of LAG after labelling of uronic acids with NaBD₄ and methylation analysis

Galactose as the main monosaccharide component occurred only in its pyranosidic form. Figure 2 shows that the main components are 1,3,6-Gal(p), 1,6-Gal(p) and terminal Gal(p), accompanied by minor amounts of 1,3-Gal(p). Arabinose is found in its pyranosidic form as terminal Ara(p) and in its furanosidic form as 1,3-Ara(f) and terminal Ara(f). Glucuronic acid is detected in its pyranosidic form as terminal GlcA(p). There were two signals which occurred from not further identified pyranosidic hexoses with 1,4- and 1,3,4-linkage.

Table 2: Determination of linkage type by methylation analysis of LAG (amounts in mass %)

LAG	1-Ara(f)	1-Ara(p)	1,3-Ara(f)	1,3-Ara(p)	1-GlcA(p)	Gal(p)	1,3-Gal(p)	1,6-Gal(p)	1,3,6-Gal(p)
1.	5.0	4.5	5.6	1.1	19.4	3.0	25.9	32.7	
2.	5.0	3.9	4.9	0.8	23.4	3.6	23.3	31.8	

Molecular mass

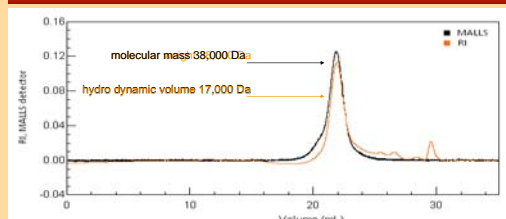


Figure 3: Size exclusion chromatography spectrum of LAG

The molecular mass of the LAG polysaccharide was determined by size exclusion chromatography with MALLS- and RI-detection (fig. 3) and found to be about 38,000 Da. The hydrodynamic volume of the LAG polysaccharide in comparison to standard pullulans was smaller (about 17,000 Da), possibly due to the very compact structure of the molecule.

Smith degradation

Structural characterisation led to the suggestion of a 1,3-linked galactose backbone, such as in an arabinogalactan type II [3], is suggested for LAG. Elimination of 1,6-linked galactose units by periodate degradation was accomplished to proof this model of LAG. Figure 4 shows the suggested backbone which would remain after Smith degradation.

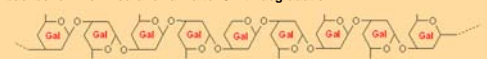


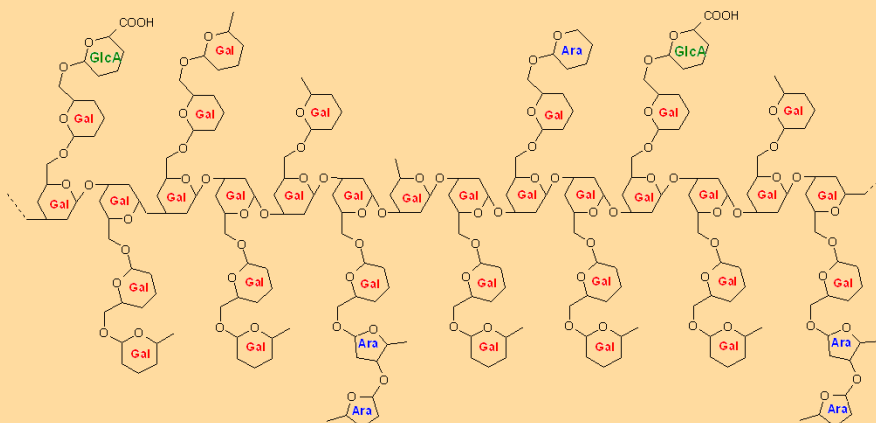
Figure 4: 1,3-linked galactose backbone of the proposed model for the structure of LAG

In table 3 it can be seen that the amount of 1,3-linked galactose extremely increased from 4.0% in unmodified LAG to 69.3% in LAG after Smith degradation. The amount of terminal arabinose and galactose decreased, except for terminal furanosidic arabinose. A decrease in 1,6- and 1,3,6-branched galactose could also be detected. Glucuronic acid was not detectable because the samples were not pretreated by uronic acid reduction and labelling of the corresponding neutral monosaccharides.

Table 3: Determination of linkage type of Smith-degraded LAG by methylation analysis (amounts in mass %)

LAG	1-Ara(f)	1-Ara(p)	1,3-Ara(f)	1,3-Ara(p)	Gal(p)	1,3-Gal(p)	1,6-Gal(p)	1,3,6-Gal(p)
unmodified	5.8	5.9	5.6	15.2	4.0	27.5	33.8	
Smith degradation	6.5	0.0	1.5	7.2	69.3	4.4	11.1	

Proposed structural model for larch arabinogalactan



Material & Methods

- Material:** All LAG samples were supplied by Lonza Inc, Cohasset, MN, USA. LAG is obtained from *Larix laricina* (DuRoi) K.Koch also known as Eastern larch or Tamarack.
- Elementary analysis:** For elementary analysis of elements carbon, hydrogen, nitrogen and sulphur HEKAtech-CHNS Analysator (Co. HEKATECH, Wegberg, Germany) was used.
- Size-exclusion-chromatography (SEC):** By using multi-angle-laser-light-scattering- and RI - detection after size-exclusion- chromatography on three in series connected PL aquagel-OH columns (Polymer Laboratories GmbH, Darmstadt, Germany), the molecular weight of LAG could be determined.
- Acetylation analysis:** Hydrolysis, reduction and acetylation were accomplished by following BLAKENEY et al. (1983). After gas chromatographic separation (WCOT-capillary column, Optima - 225-0.25 µm(L 25 m, ID 0.25 mm), Co. Macherey Nagel, Düren, Germany) the identification and quantification of neutral sugars was performed as alditol acetates.
- Methylation analysis:** Linkage analysis of the LAG samples was performed following the method of HARRIS et al. (1984) by GLC-MS on a Permabond® OV-1701 (25m L, 0.25 mm ID) column (Macherey & Nagel, Düren, Germany). In addition methylation analysis was performed with modified LAG samples with labelled uronic acids.
- Labelling of uronic acids with NaBD₄:** As described by TAYLOR and CONRAD (1972) uronic acids in the LAG polymer in reaction with water-soluble carbodiimides were converted into esters which in turn were reduced with sodium borodeuteride to the corresponding alcohols. Due to this reaction deuterium labelled monosaccharides were approachable to linkage type analysis.
- Smith-Degradation:** The method of GOLDSTEIN et al. (1965) aims at selectively degrading polysaccharides. Degradation was accomplished by oxidation of vicinal hydroxyl groups with sodium periodate and cleavage of the C-C bond between them. Polyalcohols were formed by reduction with sodium borohydride and hydrolysed under mild conditions. During hydrolysis only acetals of oxidised monosaccharides were supposed to be cleaved. The degraded polysaccharides were analysed by methylation analysis.

Acknowledgements:

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Summary

LAG does not contain a protein component and can be declared as pure polysaccharide.

The molecular mass of the LAG polysaccharide is about 38,000 Da and the hydrodynamic volume of the LAG polysaccharide is about 17,000 Da, due to the very compact structure of the molecule.

Galactose and arabinose are the main monosaccharide components in LAG, accompanied by mannose in small amounts and xylose and glucose in traces. The Ara : Gal ratio was 1 : 5.8.

LAG contains small amounts of glucuronic acid.

Main components are 1,3,6-, 1,6- and terminal linked galactose units, accompanied by minor amounts of 1,3-linked galactose. Arabinose could be found as 1,3-linked or terminal unit; glucuronic acids are terminally linked.

Smith degradation pointed out that the backbone of the LAG macromolecule is composed of 1,3-linked galactose units which are branched in position 6 by 1,6-linked galactose as side chains. It also showed that 1,3- and terminal linked arabinose residues were connected to the side chains of the LAG macromolecule.