

Targeted functionalization of spruce galactoglucomannans with aid of galactose oxidase (FunMan)

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ABSTRACT

The project focuses on spruce galactoglucomannan (GGM), which is new potential product from forest biorefineries. The key technologies studied are targeted and controlled oxidation of GGM with galactose oxidase (GO), and further chemical functionalization of the oxidized GGM. The usefulness of GGM in various applications is evaluated. The goal is to develop novel innovative applications for a side-product from the processing of spruce.

Pure spruce GGM was prepared from TMP and its fine structure characterized. The oxidation reaction with GO was studied in detail using methyl- α -D-galactopyranoside and D-raffinose as substrates. Reaction products were isolated and their structures analyzed. GGM was oxidized with GO in the optimized conditions and the product analyzed by NMR and GC-MS. The poor solubility of the oxidized product in NMR solvents lead to the investigation of a GC-MS technique utilizing NaBD₄ reduction of the product to result deuterium labelling of the oxidized galactose prior to acid methanolysis and GC-MS analysis. The further chemical modification of oxidized GGM was tested using oxidized methyl- α -D-galactopyranoside as a model compound in a Barbier type reaction.

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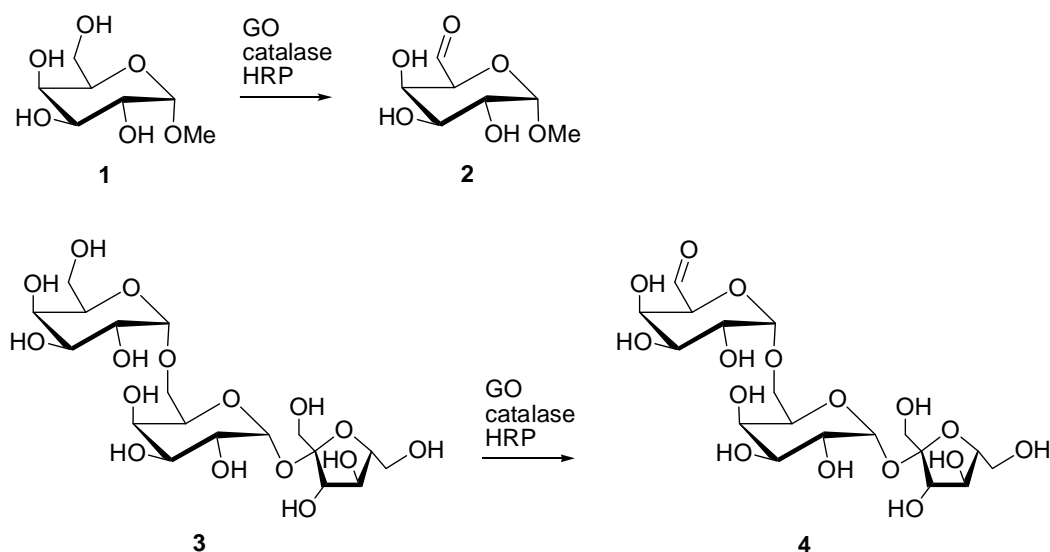
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RESULTS

1. University of Helsinki

Optimization of galactose oxidase catalyzed oxidation

Galactose oxidase (GO) is a copper metalloenzyme which catalyzes the oxidation of primary alcohols to corresponding aldehydes with high regioselectivity for the primary hydroxyl group of galactose and is thus suitable for the specific oxidation of galactose containing carbohydrates. However, the oxidation conditions need to be optimized to obtain pure aldehyde product as well as to control the reaction to the desired oxidation level. During the first year the action of GO alone and in combination with catalase and horse raddish peroxidase in different reaction conditions (solvent, pH, temperature, concentration of starting material) was studied in detail using methyl- α -D-galactopyranoside **1** and D-raffinose **3** as substrates (Scheme 1). This included also setting up analytical methods to follow up the oxidation reaction. Four products after oxidation of methyl- α -D-galactopyranoside were isolated (**2**, uronic acid, dimer, α - β -elimination product) and their structures analysed with the NMR spectroscopy. The reactions were also followed by NMR. The best yields of aldehydes **2** and **4** were obtained using a combination of all the three enzymes and water as a solvent. α - β -Elimination product is formed by the secondary water elimination reaction after the enzymatic oxidation.



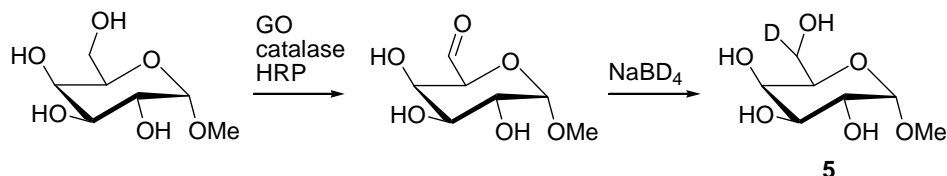
Scheme 1. Oxidation with galactose oxidase.

Oxidation of GGM and analysis of GGM oxidation degree

Spruce galactoglucomannan (GGM) was oxidized in the optimized reaction conditions with GO. GC-MS methods were tested for the analysis of the product. Oxidized GGM was degraded by acid methanolysis⁴ and acetylated, or derivatized to a TMS ether, but the very reactive oxidized galactose units were not stable in the procedures. Thus another GC-MS based analysis technique is under investigation at the moment. The oxidized GGM is first reduced with NaBD₄

⁴ Sundberg, A., Sundberg, K., Lillandt, C., Holmbom, B. Nordic Pulp and Paper Research Journal 11 (1996) 216.

back to GGM (deuterated) and then degraded by acid methanolysis and derivatized. The oxidation degree can be calculated from the difference in the mass spectra of unreacted and oxidized galactose (galactose carrying D label). Deuterated methyl- α -D-galactopyranoside **5** was prepared to be used as a standard in the method (Scheme 2).



Scheme 2. Deuteration of methyl- α -D-galactopyranoside.

2. Åbo Akademi

Preparation of GGM

Pure galactoglucomannan (24 g, 90% purity) was prepared from TMP according to a small pilot-scale method. The method used was a modification from the earlier used method.⁵ The carbohydrate composition and the molar mass of the GGM were determined.

Characterization of GGM fine structure

Work has been done for further characterization of the GGM fine structure. Earlier it has been determined that GGM contains ~5 % of galacturonic acid residues, but the connection, if any, between the acidic units and the GGM backbone is unknown. Oligomers were prepared by treating GGM with mannanase, and the neutral oligomers were separated from the charged ones by ion exchange chromatography. The sugar composition of these fractions was analyzed. GGM from untreated spruce wood will be analyzed and compared to the existing results, to rule out the possibility of artefacts caused by the TMP process.

Evaluation of analytical methods

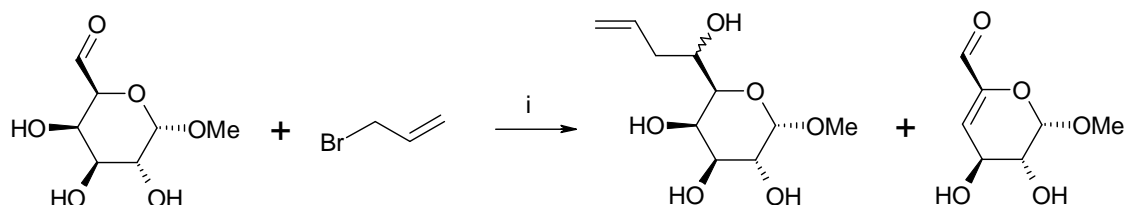
Both oxidized GGM and oxidized methyl galactopyranoside **2** were analyzed by NMR using different solvents. Solvents used were DMSO, D₂O, and a mixture of these. In D₂O, the aldehyde forms a hydrate, thus DMSO seems to be the better alternative. The solubility in DMSO at room temperature is poor, and warming the sample leads to condensation reactions. Work will be continued to find a suitable method for characterization of the substrates.

Chemical modification of oxidized methyl galactopyranoside

The chemical modification of the enzymatically oxidized methyl galactopyranoside **2** was investigated with a metal mediated allylation reaction (Scheme 3). This Barbier type reaction introduces a double bond functionality, which gives possibility to further modification of the molecule. Both zinc and indium have been tested as mediating metals. As expected, there were differences between the reactivity of these two metals. NMR analysis showed that the desired allylated product was formed, but the aldehyde also undergoes α - β -elimination. It seems that the

⁵ Willför, S., Rehn, P., Sundberg, A., Sundberg, K., and Holmbom, B., *Tappi J.* 2 (2003) 27-32.

choice of the mediating metal, as well as the ratio of the reactants and solvents, highly affect the outcome of the reaction. The reaction conditions need to be optimized before the reaction is tried on oxidized GGM.



Scheme 3. Allylation of the oxidized methyl galactopyranoside. (i) Zn, NH₄Cl_{aq}/THF or In, H₂O /THF.

PROGRESS

The research and collaboration between the partners has proceeded as planned.

ACTIVITIES

Participation in the conference 14th EUROCARB in Lübeck, Germany (2.-7.9.2007). Poster presentation.

PUBLICATIONS

Articles

Willför, S., Sundberg, K., Tenkanen, M. and Holmbom, B., Spruce mannans – A remarkable source of hydrocolloids and novel advanced natural materials, *Carbohydr. Polym.*, in press. doi:10.1016/j.carbpol.2007.08.006

Abstracts

Parikka, K., and Tenkanen, M. Oxidation of methyl- α -D-galactopyranoside with galactose oxidase. 14th EUROCARB, Lübeck, Germany (2.-7.9.2007). Poster, abstract p. 246.

Manuscripts

Parikka, K. and Tenkanen, M. Oxidation of methyl- α -D-galactopyranoside and D-raffinose with galactose oxidase.