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An acetylated galactoglucomannan from *Picea abies* L. Karst

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Abstract

A water-soluble galactoglucomannan composed of D-galactose, D-glucose, and D-mannose in 1:3:17 mole proportion has been isolated from the secondary cell walls of *Picea abies* L. Karst. About 33% of the polysaccharide units were substituted by acetyl groups. Structural studies of the polymer indicated a β-(1→4)-linked glucomannopyranosyl backbone with a low content of branch points at O-6 of mannosyl and glucosyl residues. A preference for mannosyl groups indicates the presence of a single D-galactosyl unit side-chain. About half of the mannose residues were *O*-acetylated at C-2 and C-3 in 1.7:1 mole proportion.

Keywords: *Picea abies* L. Karst; Galactoglucomannan; *O*-Acetyl groups; NMR spectroscopy

1. Introduction and discussion

Galactoglucomannans (GGMs) are common constituents of plant cell-walls. As with other plant cell polysaccharides, GGMs show some variations in structural features depending on the plant species and the stage of plant development. GGMs extracted from secondary cell walls of gymnosperms and angiosperms[1] by alkali are characterized by a low content of Gal residues and a Glc:Man ratio ~1:3 or 1:4, whereas water-extractable polymers have roughly equal proportions of Gal and Glc and a Glc:Man ratio between 1:1.4 and 1:3. However, polysaccharides isolated from primary cell-walls, or polymers secreted into extracellular space by suspension cells contained approximately equal proportions of all saccharide constituents, namely Gal, Glc, and Man residues. Polysaccharides of this family, from different tissues of many other plants and seeds of various land-plants, have been reviewed.[2,3] Characteristic features of these secondary cell-wall components are a linear β-(1→4)-linked backbone of D-Man*p* and D-Glc*p* residues, and *O*-acetyl groups. However, in alkali-extracted GGMs the *O*-acetyl groups are released by saponification. The content and position of *O*-acetyl groups in water-extractable GGMs varies from plant to plant.[4–6] Generally, hardwoods contain a higher proportion (3–7%) of *O*-acetyl groups than softwoods[7] (1–2%). In the framework of our studies focused on structure–biological activity relationships of galactoglucomannan-derived oligosaccharides, we described two alkali-extracted GGMs from poplar[8] and spruce.[9] Oligomeric fragments of both polymers showed biological activity in elongation growth induced by auxin, in some morphogenic processes, and in the defense response of plant cells.[10–14] The possible occurrence of GGMs in *Picea abies* wood has been discussed in only a few papers.[15–17] Consequently, this paper deals with the isolation and ^{1}H and ^{13}C NMR characterization of water-soluble GGM isolated by hot-water extraction of *P. abies* L. Karst sawdust.

Defatted and delignified sawdust of *P. abies* was successively extracted twice with hot water, hot 0.5% aqueous ammonium oxalate, dimethyl sulfoxide, aqueous 15% potassium hydroxide, and finally with aqueous 17.5% sodium hydroxide containing 4% boric acid. Ion-exchange chromatography of the hot-water extract, using water as eluent, gave a water-soluble GGM that was homogeneous on free-boundary electrophoresis, and in a yield of 1.0% of defatted and delignified spruce cell-walls. It had M_n 11,300 (dp = 70), an optical rotation of −28° and contained 33% of

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O-acetyl groups. Hydrolysis indicated the presence of D-galactose, D-glucose, and D-mannose in 1:3:17 mole proportion.

Methylation analysis (Table 1) of GGM revealed the presence of two main sugar derivatives, namely 4-linked mannose and glucose units, and indicated a (1→4)-linked glucopyranosyl–mannopyranosyl backbone, characteristic for plant GGMs. The low contents of 2,3-di-*O*-methyl derivatives of glucose and mannose units indicate that the polysaccharide is slightly branched at O-6 of some backbone residues. The substitution of mannosyl residues was about four times higher than for the glucosyl residues. Except from the fact that the glucopyranosyl–mannopyranosyl backbone is slightly branched at O-6, the low proportions of 2,6-di-*O*-methyl derivatives of these sugars indicate branch points also at O-3. The content of 2,6-di-*O*-methyl derivatives was much higher in the mannosyl residues, as with those of alkali-extracted GGM. Mannosyl residues were also found to be branched at 2-position, as indicated by the low content of the 3,6-di-*O*-methyl derivative. However, this derivative was absent in alkali-extracted GGM. The presence of tetramethyl derivatives of Man and Glc residues shows that both sugars occupy the terminal, nonreducing end-positions of the polymeric chains. Galactose was found to occur as single unit stubs at the nonreducing positions terminating the branched points of Glc and Man residues. The content of these short galactopyranosyl side chains is the determining factor for the solubility of this polymer in water.

The ^{13}C NMR spectrum of the GGM (Fig. 1(A)) showed six main signals, one in the anomeric and five in the skeletal regions for mannose moieties, at 101.1 (C-1), 70.8 (C-2), 72.4 (C-3), 77.5 (C-4), 75.9 (C-5), and 61.4 (C-6) ppm, and resonances of low intensity for glucose residues at 103.4 (C-1), 74.2 (C-2), 78.2 (C-3), 79.4 (C-4), and 74.8 (C-5) ppm. These chemical shifts were in good agreement with the ^{13}C NMR data of the alkali-extracted GGM[9] and reflect the presence of 4-linked β-glucosyl and β-mannosyl residues in the polymer. In addition to the three signals of lower intensities occurring at 100.5, 100.1, and 99.8 ppm characteristic for anomeric atoms, two signals in the lowest field at 174.3 and 173.9 (C=O) ppm, and two high-field resonances at 21.5 and 21.2 (CH_3) ppm indicate the presence of *O*-acetyl groups in the polysaccharide. In addition to the afore mentioned signals, additional signals at 78.2, 74.5, and 72.7 ppm were present in the ^{13}C NMR spectrum. After deacetylation of the native GGM, these low-intensity signals for acetylated sugar residues disappeared and the ^{13}C NMR spectrum of this polymer (Fig. 1(B)) was identical with that of alkali-extracted polysaccharide (Fig. 1(C)). The main chain of this alkali-soluble polysaccharide is composed of (1→4)-linked β-Glc*p* and β-Man*p* residues, containing segments of mannosyl residues (Man_2, Man_3, Man_4, etc.) interrupted by single glucose residues. Further, the low content of glucosyl and mannosyl residues is terminated at O-6 by single α-D-galactopyranosyl unit side-chains.[9]

Table 1
Methylation analysis data of water-soluble galactoglucomannan

Sugar derivative	Mole (%)	Mode of linkage
2,3,4,6-Me_4-Man [a]	1.0	Man*p*-(1→
2,3,4,6-Me_4-Glc	0.2	Glc*p*-(1→
2,3,4,6-Me_4-Gal	4.1	Gal*p*-(1→
2,3,6-Me_3-Man	77.1	→4)-Man*p*-(1→
2,3,6-Me_3-Glc	14.0	→4)-Glc*p*-(1→
2,6-Me_2-Man	0.3	→3,4)-Man*p*-(1→
2,6-Me_2-Glc	tr.	→3,4)-Glc*p*-(1→
3,6-Me_2-Man	0.3	→2,4)-Man*p*-(1→
2,3-Me_2-Man	2.4	→4,6)-Man*p*-(1→
2,3-Me_2-Glc	0.6	→4,6)-Glc*p*-(1→

[a] 2,3,4,6-Me_4-Man

= 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-mannitol, etc.

In the HSQC spectrum of GGM (Fig. 2), the cross peak at 5.45/72.7 ppm was attributed to the H-2/C-2 atoms as a result of their acetylation in 4-linked β-Man*p* residues. Similarly, the cross peak at 5.10/74.5 ppm is due to H-3/C-3 atoms which are substituted by acetyl groups.[18] The 2D integrals of H-2/C-2 and H-3/C-3 cross peaks indicate that *O*-acetyl groups at C-2 and C-3 are in 1.7:1 mole proportion. From the HSQC spectrum, it is evident that *O*-acetyl groups are situated at only C-2 and C-3 of the mannosyl residues. The mannose units having 2-*O*- and 3-*O*-acetyl groups are randomly distributed in the polysaccharide chains and form some characteristic arrangement of constituent monomers. Only one position can be simultaneously substituted. No signals evidencing *O*-acetylated glucose residues were found. The cross peak of H-1/C-1 atoms of low intensity in the HSQC spectrum at 5.03/99.80 ppm (in alkali-extracted polymer[9] was found at 4.99/99.30 ppm) indicates the presence of terminal α-D-galactose units linked to the glucomannan backbone. In the 1D DEPT spectrum, the very weak signal at 67.00 ppm was attributed to C-6 of mannose residues.

The glycosidic linkage attributions between monosaccharide constituents of the polymer were confirmed by an HMBC spectrum (Fig. 2). The dominant cross-peak at 4.74/77.50 ppm represents the most extended sequence of 4-linked non-acetylated manno–mannopyranose units in the polymer. This fact suggests the presence of non-acetylated segments of mannosyl residues of indefinite length. The cross peaks of H-1 and H-2 signals of 2-*O*-acetylated mannose at 4.92/78.24 and 5.45/78.00 ppm, respectively, indicate a β-

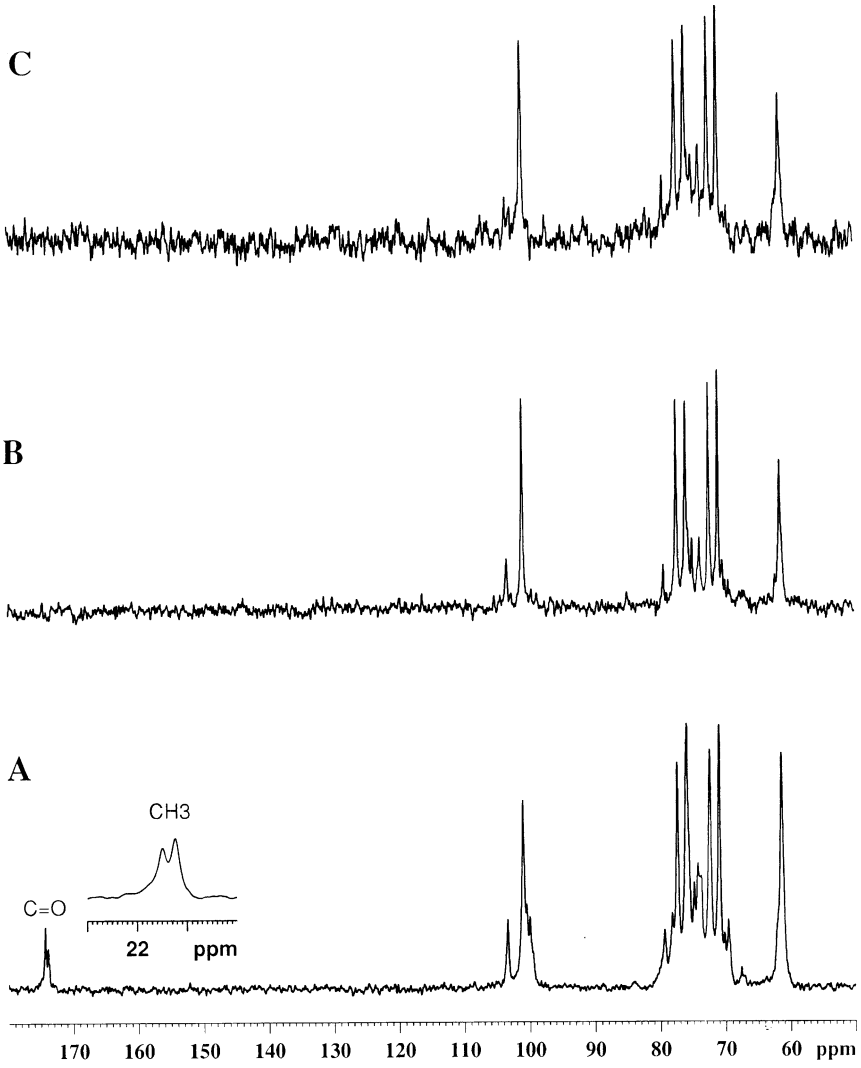

Fig. 1. ^{13}C NMR spectra of water-extracted (A), de-esterified (B), and alkali-extracted (C) GGMs.

(1→4) linkage to the next mannose unit, similar to those of H-1 of 3-*O*-acetylated mannose at 4.82/78.00 ppm. The low-intensity cross peak of H-1 of mannose at 4.72/74.00 suggests a β-(1→4) linkage to a 3-*O*-acetylated mannose residue, and the cross peaks of H-1 of mannose and glucose residues at 4.73/80.60 and at 4.51/77.14 ppm indicate β-(1→4) linkages to the neighboring glucose and mannose residues, respectively.

Chemical and spectroscopic studies on water-soluble GGM from *P. abies* L. Karst indicated a β-(1→4) glucopyranosyl–mannopyranosyl backbone in which some building units were substituted at O-6 by α-D-galactose units. The frequency of substitution on mannosyl residues was four times higher than for glucosyl ones. About half of the mannosyl units were acetylated at O-2 and O-3 in 1.7:1 ratio, and formed characteristic arrangements of these constituent sugars in the polymer. As may be seen from the HMBC spectrum of the polymer (Fig. 2), the dominant cross peak at 4.74/77.50 ppm confirmed the occurrence of non-acetylated segments of mannosyl and glucosyl–mannosyl residues. Since 50% of the mannose residues are acetylated, it indicates the presence of some definite segments alternating with non-acetylated ones. Although the water-soluble GGM from *P. abies* showed similar structural features of the backbone as those of GGMs isolated from different gymnosperm species,[19] such as *Tsuga canadensis*, *Abies amabilis*, *Picea engelmanni*, *Pinus strobus*, *Thuja occidentalis*, and *Ginkgo biloba*, they differed in the mole proportion of the constituent sugars (GGM of *P. abies* had the lowest content of galactose residues and the highest mannose:glucose ratio, ~6:1), in the degree of branching of mannosyl and glucosyl residues, in molecular weight, and in the content and distribution of *O*-acetyl groups in the molecule.

2. Experimental

Material.—Sawdust was prepared from the trunk of spruce wood (*P. abies* L. Karst) cultivated in Male Karpaty, Slovak Republic.

General methods.—Solutions were concentrated under diminished pressure below 40 °C. Free-boundary electrophoresis of 1% solution of the polysaccharide was effected with a Zeis 35 apparatus, using 0.05 M sodium tetraborate buffer (pH 9.2) at 150 V/cm and 6 mA for 30 min. The number average molecular mass (M_n) was determined osmometrically at 30 °C, using a Knauer vapor-pressure osmometer. Infrared spectra of the methylated products were recorded with a Nicolet Magna 750 spectrometer. Polysaccharides were hydrolyzed with 2 M trifluoroacetic acid for 1 h at 120 °C. Quantitative determination of the neutral sugars was carried out in the form of their trifluoroacetates[20] by gas chromatography on a Hewlett–Packard model 5890 series II chromatograph equipped with a PAS-1701 column (0.32 mm × 25 m), the temperature program of 110–125 (2 °C/min)–165 °C (20 °C/min) and flow rate of hydrogen of 20 mL/min.

The absolute configurations of the monosaccharides were established by the method of Gerwig et al.[21] Gas chromatography–mass spectrometry of partially methylated alditol acetates[22] was effected on a Finnigan mat SSQ 710 spectrometer equipped with an SP 2330 column (0.25 mm × 30 m) at 80–240 °C (6 °C/min), 70 eV, 200 μA, and ion-source temperature of 150 °C.

Isolation of the galactoglucomannan.—The air-dried sawdust (500 g) prepared from the trunk of spruce (*P. abies* L. Karst) was exhaustively extracted with 2:1, w/w benzene–MeOH and delignified by the sodium chlorite method.[23] The holocellulose was extracted twice with boiling water (5 L) for 3 h. The aqueous extracts were combined, concentrated, dialyzed, and freeze-dried. A part of the hot-water extract was dissolved in water and chromatographed on a column of DEAE–Sephacel in the chloride form and eluted with water and NaCl solutions of increasing ionic strength (0.1, 0.25, 0.5, and 1 M). The non-retained fraction, eluting by water, comprised a water-soluble galactoglucomannan and was 16% of the water extract.

Saponification of the galactoglucomannan.—The polysaccharide (20 mg) was dissolved in 0.05 M KOH (2 mL) and kept in a refrigerator overnight. The de-esterified sample was neutralized, dialyzed, and freeze-dried.

Methylation analysis.—The dry sample of polysaccharide (~ 5 mg) was solubilized in dry Me_2SO (1 mL) and methylated by the Hakomori method.[24] The methylated products were purified using a Sep-Pak C_{18} cartridge (Waters Assoc.) to give fully methylated carbohydrates. The permethylated sample was hydrolyzed with 90% formic acid (1 h, 100 °C) and 2 M trifluoroacetic acid (1 h, 120 °C), reduced with $NaBD_4$, acetylated, and analyzed by GLC–MS.

NMR spectroscopy.—The polysaccharide was dissolved in D_2O (0.5 mL, 99.99 atom%) in 5-mm tubes. Spectra were recorded at 25 °C, on a Bruker DPX Avance 300 spectrometer operating at a 300 MHz for ^1H and 75.46 MHz for ^{13}C. Acetone was used as the internal standard (δ 2.225 ppm for ^1H and 31.07 ppm for ^{13}C). The following pulse programs were used: 2D DQF COSY,[25] HSQC,[26] and HMBC.[27]

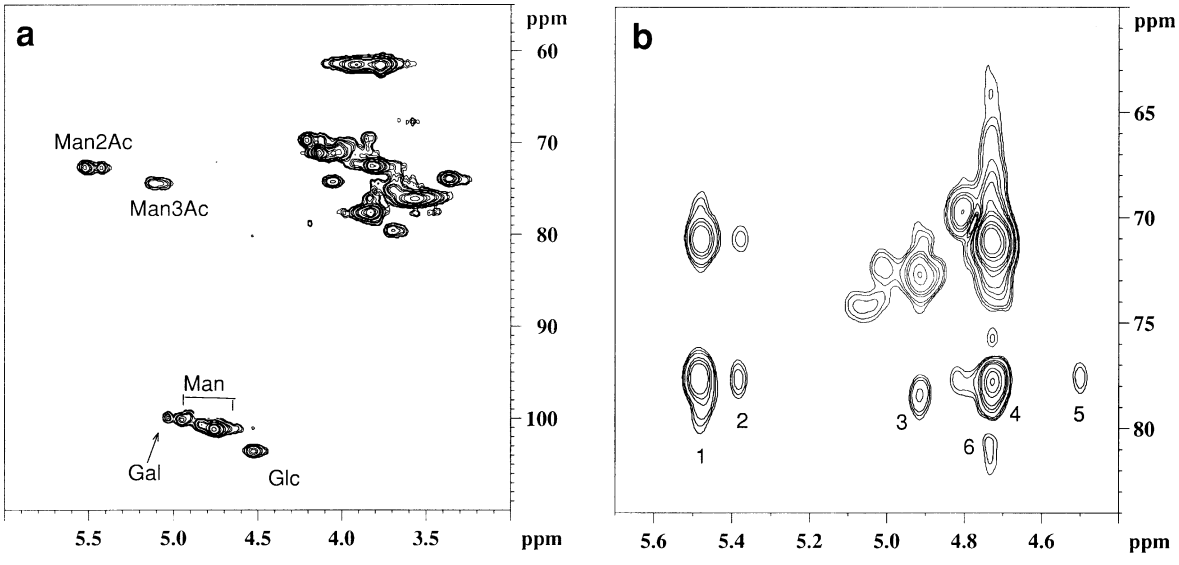

Fig. 2. HSQC spectrum (a) and the part of {^1H, ^{13}C}multi-bond correlation (HMBC) spectrum (b) of GGM. The numbers in the spectrum indicate the following cross-peaks (H-2/C-4): 1, $Man_{2Ac} \rightarrow Man$; 2, $Man_{2Ac} \rightarrow Man_{3Ac}$, and (H-1/C-4): 3, $Man_{2Ac} \rightarrow Man_{2Ac}$; 4, $Man \rightarrow Man$; 5, $Glc \rightarrow Man$; 6, $Man_{2Ac} \rightarrow Glc$ (Man_{2Ac} = 2-*O*-acetylated Man, etc.). All saccharide residues were linked in the backbone by β-(1 → 4) linkages.

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