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Structural of a glucomannan from *Lupinus varius* seed

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Abstract

Polysaccharides extracted from seeds of *Lupinus varius* with hot ethanol 85% (polysaccharide FI) and 0.1 M sodium phosphate buffer, pH 7.5 (polysaccharide FII) were fractionated and purified by ion-exchange and gel-filtration chromatography. According to methylation and hydrolysis analysis, the main chains of FI and FII consisted of (1 → 4)-linked glucomannan; only traces of branched sugar residues were detected. This is the first report on the isolation of glucomannan from *L. varius* seeds.

Keywords: Polysaccharides; Seeds of *L. varius*; Seeds; Glucomannan

1. Introduction

Plants of genus *Lupinus* are known to contain a variety of structural types of alkaloids. They have been used as ornamental plants and as economic plants for fodder and for soil nitrogenation. *Lupinus varius* is an annual herb growing wild in North Africa, South Europe, West Syria, and Palestine. Previous investigations of this species revealed the presence of several quinolizidines, as well as dipiperidine, alkaloids (Bashir & Abdel Halim, 1999; Mohamed & Hassanean, 1997). However, in spite of this extensive exploitation of *L. varius* no information has been published about polysaccharides from the seed of *L. varius*. The purpose of the present study was to contribute further information about isolation and purification of glucomannan from *L. varius* seeds. The result of this study introduces *L. varius* seeds as a possible valuable source for glucomannan, which helps to normalize blood sugar, relieve stress on the pancreas, and discourage blood sugar abnormalities, such as hypoglycemia (Hozumi et al., 1995). Glucomannan acts as a preventative of chronic disease (Vuksan et al., 1999) and as a weight control agent (Reffo, Ghirardi, & Forattani, 1990; Walsh, Yaghoubian, & Behforooz, 1984).

2. Experimental

2.1. General

Solutions of polysaccharides were concentrated at 60 °C under reduced pressure by rotatory evaporation. IR spectra were recorded with a Beckman Acculab 3 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded with a Bruker WM spectrometer (500 MHz) in D_2O (external Me_4Si). Dialysis was carried out in Spectrapor 3 tubes (molecular weight cutoff = 3500) for 80 h with repeated (five times) changes of water. Protein was determined according to the method of Sedmark and Grossberg (1973), with Coomassie brilliant blue reagent and bovine serum albumin as the standard. Total sugar content was determined according to the anthrone method (Morris, 1948) and uronic acid according to the 3-hydroxydiphenyl reagent method of Blumenkrantz and Asboe-Hansen (1973) as modified by Kram (1984). Starch contamination was estimated by treatment with pancreatic R-amylase (Asamizu & Nishi, 1979). Acetyl groups were determined by GLC-MS according to the method of Paulsen,

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Fagherheim, and Overbyl (1978), and were located following the method of using methyl vinyl ether as reagent (De-Belder & Norman, 1968).

2.2. Plant material

Seeds of *L. varius* were collected from Tripoli (Libya) in the flowering season. A voucher specimen, identified by Adel Kermagi, Faculty of Science, El-Fateh University, Tripoli, is deposited in Bio-technology Research Center in Tripoli. Dry seed (2 kg) of *L. varius* was used in this study, since at the dry stage the seeds contain larger amounts of polysaccharides such as glucomannan, galactomannan, and an alkali-soluble heteroxylan (Ishurd, Sun, Xiao, & Ashour, 2002).

2.3. Isolation and purification of the polysaccharide

Dried crushed seeds were extracted successively with light petroleum and ethanol 85% to defat and decolorize and then extracted with aqueous 85% ethanol overnight at room temperature. After centrifugation (6000 rpm, 20 min), the residue was dried and then extracted with 0.1 M sodium phosphate buffer, pH 7.0, for 6 h at 60 °C. After centrifugation (10,000 g, 20 min) and re-extraction of the pellet, the phosphate buffer extracts were combined, concentrated, and dialyzed. The nondialyzable phase was freeze-dried. The yield of crude polysaccharide fraction was 0.14% of the fresh weight. The crude polysaccharide fraction was eluted from a column (2.8 cm diam × 25 cm) of DEAE–Sephacel (PO_4) (Pharmacia), at 50 mL h^{-1} with water (400 mL), followed by 0 → 1 M gradient of sodium phosphate buffer, pH 6.0, and finally by 0.2 M NaOH (400 mL). Anthrone-positive fractions were combined, concentrated, dialyzed against distilled H_2O, and dried. The fractions from gel permeation chromatography was carried out on a column (1 × 30 cm) of Superose™ 12 and Superose™ 6 by elution with 0.1 M NaCl were monitored by the anthrone method. The column (1.6 × 90 cm) of Superose™ 12 was used for larger scale separations. The molecular weight of the high-DP polysaccharide was determined on a column (1.6 × 90 cm) of Sephacryl S-500 (Pharmacia) by elution with 0.1 M NaCl. The column was calibrated as above. After gel permeation chromatography on Superose™ 12 or Superose™ 6, most of the polysaccharides were present in the void volume, but and there was a minor fraction with a molecular weight of 12,000 as determined by using Sephacryl S-500.

2.4. Determination of sugar composition and linkage

2.4.1. Analysis

Polysaccharides were hydrolyzed with 2 M trifluoroacetic acid for 2 h at 120 °C. The hydrolysate was repeatedly co-concentrated with water to remove the acid and the residue was analyzed by TLC on silica gel 60 (Merck), using acetone/water (86:16 v/v) or ethyl acetate/methanol/boric acid/acetic acid (55:22:15:12, v/v), and detection was made with aniline/diphenylamine/phosphoric acid (Bailey & Bourne, 1960). The monosaccharides in the hydrolysate were converted into the alditol acetates (Blankeney, Haris, Henery, & Stone, 1983) and then analyzed by gas–liquid chromatography at 220 °C for 30 min with a Varian 3500 instrument, fitted with a fused-silica DB 225 (0.25 mm × 30 m) column and a flame ionization detector, with N_2 as the carrier gas at 0.8 mL/min, a split ratio of 1:50, and *myo*-inositol as the internal standard. Methylation analysis was performed according to the method of Harris, Henry, Blankeney, and Stone (1984). Partially methylated alditol acetates were analyzed with a Hewlett-Packard GC 5890A, using a mass-selective detector 5970B, a Durabond fused-silica column DB 225 (0.25 mm × 30 m), and a temperature program of 170–210 °C at 10 °C/min followed by an isothermic phase.

3. Results and discussion

The isolation and structural elucidation of a neutral glucomannan and an acidic arabinogalactan from the powdered dry seeds of *L. varius* is based on extraction with hot ethanol, and with sodium phosphate buffer, pH 7.0, which gave the crude soluble polysaccharides (0.14 %). This polysaccharide fraction contained protein (0.9%) and uronic acid (2%) and had IR bands for ester groups at 1740 and 1250 cm^{-1}.

Hydrolysis of this fraction gave mannose, glucose, arabinose, and galactose in the molar ratio of 54:25:12:12 (GLC of the alditol acetates) and glucuronic acid (identified by TLC). The total hexose content of the crude polysaccharide fraction was 20%, and was resistant to R-amylase, thus proving the absence of starch.

Fractionation of the crude polysaccharides by using DEAE–Sephacel gave neutral and acidic fractions. The acidic fraction (5.8%), eluted with 0.4 M sodium phosphate buffer, pH 6, was composed of arabinose, galactose, rhamnose, glucose, and glucuronic acid (molar ratio 41:41:9:9:3) and protein (0.97%) and had no IR absorption for ester. The molecular weight of the acidic fraction which was composed of arabinose, galactose, rhamnose, glucose, and glucuronic acid was estimated to be 48,000 by gel permeation chromatography on Superose™ 12 (Pharmacia). The neutral fraction (46.9%) was composed of mannose, glucose, arabinose, and rhamnose and uronic acid (molar ratio 84:6:6:4:5) and also contained protein (0.09%). The IR spectrum of this fraction showed absorption for ester groups. After gel permeation chromatography on Superose™ 12 or Superose™ 6, most of the polysaccharides were present in the void volume (MW determined by using Sephacryl S-500, was 1×10^6) but there was a minor fraction with a molecular weight of 12,000. The lyophilized high molecular weight fraction, obtained after gel permeation chromatography, was water soluble, had an IR absorption for ester, and was free from protein and uronic acid. Total hydrolysis yielded only mannose and glucose, at

Table 1
Methylation analysis of the high-DP

Derivatives – partially methylated alditol acetates[a]	*T* (min)	Mol %	Type of linkage
1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol	17.1	1.8	1-Glucose
1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-manitol	24.5	92.0	1,4-Mannose
1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol	25.2	3.7	1,4-Glucose
1,4,5,6-Tetra-*O*-acetyl-2,3-di-*O*-methyl-D-glucitol	29.8	1.2	1,4,6-Glucose

Neutral polysaccharides fraction.
[a] Partially methylated alditol acetates analysed by GLC-MS.

the molar ratio of 94:8. The total hexose content of this fraction was 71% (excluding the acetyl content). The results of methylation analysis, shown in Table 1 concurred with a $(1 \rightarrow 4)$-linked glucomannan structure; only a trace of branched sugar residues was detected. The ^{1}H NMR spectrum showed signals at δ 2.00, 2.02, and 2.08 for acetyl groups, and the signals at δ 4.15 and 5.34 indicated that the acetyl groups in acetylated glucomannans were located at C-6 and C-2, C-3, respectively, *O*-acetyl groups in acetylated glucomannans, previously studied (Kenne, Rosell, & Svensson, 1975; Paulsen et al., 1978) are mainly located at positions 2 and 3. Only one example of an *O*-acetyl group at position 6 has been reported, namely, for the Lilium-A-glucomannan (Paulsen et al., 1978). The acetyl content data shown in Table 3 suggests that *O*-acetyl substituents have different attachment points in individual glycosyl residue types. The anomeric proton signal at δ 4.85 confirmed that the D-sugar residues were linked β-glycosidically (Ishurd, Zahid, Zhou, & Yuanjiang, 2002), which agrees with the presence of an IR band at 870 cm^{-1}. The ^{13}C NMR spectrum (Table 2) showed downfield shifts for the resonances of C-1 and C-4 similar to those of other $(1 \rightarrow 4)$-linked glucomannans (Usui, Mizumo, Tomoda, & Miyajima, 1979). There were distinct signals for OAc groups at δ 20.9–21.7 and 172.8-174.1, and the acetyl content was calculated to be 20%. A chemical method for the determination of acetyl groups gave a value of 20.2% for the acetyl content (Paulsen et al., 1978). The location of the OAc groups was determined by reaction with methylvinyl ether (De-Belder & Norman, 1968), followed by deacetylation, methylation (Harris et al., 1984), hydrolysis, reduction, and acetylation. The methyl groups marked the positions originally occupied by acetyl groups. The result in Table 3 indicated an acetyl content of 22%, corresponding to a degree of substitution (DS) of 1.0, and the acetyl groups were attached to the mannose residues at positions 2, 3, and 6. Similar proportions of mono-, di-, and trisubstituted mannose residues were detected together with 18% of unsubstituted mannose. The neutral fraction with a molecular weight of 12,000 had structural features similar to those of the high molecular weight glucomannan, that is, a mannose/glucose ratio of 93:7, a total hexose content of 70%, and an acetyl content of 18%. Methylation analysis again demonstrated the presence of 4-linked mannose, 4-linked glucose, and a terminal glucose in the molar ratio of 89:5:3.8. Thus, low and high molecular weight glucomannans appear to be produced via the same route of biosynthesis.

Table 2
^{13}C NMR data of the high-DP

Assignment	Signals[a]
Acetyl group (C=O, CH_3)	174.2, 20.9
	174.4, 21.2
	174.4, 21.2
Sugar residue moiety	
C-1	100.8
C-2	70.5
C-3	71.9
C-4	77.5
C-5	75.6
C-6	61.2

Neutral polysaccharides fraction.
[a] In ppm downfield relative to the signal for Me_4Si.

According to the chemotaxonomy viewpoint, glucomannans are typical of the family Liliaceae (Wozniewski, Bleaschek, & Franz, 1990). Differences among species are mainly in the mannose/glucose ratio, in molecular weight, and in patterns of branching and acetyl substitution (Gowda, Neelisiddaiah, & Anjanyalu, 1979). Studies have shown that in diabetic and nondiabetic patients, glucomannan can decrease serum cholesterol by ~10%, without changes in

Table 3
Location of the acetyl groups in the *L. varius* (seeds) glucomannan

Derivatives – partially methylated alditol acetates[a]	*T* (min)	Mol %	Location of acetyl
1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-mannitol	21.3	20.0	2,3,6
1,2,4,5-Tetra-*O*-acetyl-3,6-di-*O*-methyl-D-mannitol	27.0	6.0	3,6
1,4,5,6-Tetra-*O*-acetyl-2,3-di-*O*-methyl-D-mannitol	29.2	25.0	2,3
1,2,3,4,5-Penta-*O*-acetyl-6-*O*-methyl-D-mannitol	29.8	23.0	6
Hexa-*O*-acetyl-D-mannitol	37.9	21.0	–
Hexa-*O*-acetyl-D-glucitol	40.2	5.1	–

[a] Partially methylated alditol acetates analysed by GLC-MS.

diet or exercise (Arvill & Bodin, 1995). The present study suggests a role for *L. varius* seeds as a good supplementary food source for glucomannnan. To the best of our knowledge, this is the first report for the isolation of glucomannans from the seeds of *L. varius*.

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