Isolation and Structure Analysis of a Glucomannan from the Seeds of Libyan Dates

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Polysaccharides extracted from seeds of Libyan dates with hot ethanol 80% (FI) and 0.1 M phosphate solution (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 date seeds.

Keywords: *Polysaccharides; Libyan dates (Phoenix dactylifera); seeds; glucomannan*

INTRODUCTION

Dates are an important fruit in desert regions. There are ∼90 million palm trees all over the world, and according to the statistics the average annual production of dates is nearly 3.411 million tons (*1*, *2*). In Libya many varieties are grown, but the most important varieties are Bikrari, Khadhrai, and Taasfirt, which differ in their color, taste, texture, sugar, protein, and amino acid contents (*3*). Dates and their syrup, locally known as Rub-tamr, are consumed by a large number of Libyans, and in some low-income families they are an important source of carbohydrate. Cook and Furr determined the sugars in 51 tree-ripe, date varieties growing in the United States (*4*). Several investigators have reported on the chemical composition of different date varieties and on the factors that affect their composition. Rygg, Al-Aswad, Jasim, and Al-Delaimy and Hussein et al. investigated the changes that occur during ripening of several date varieties (*5*−*8*). Mostafa and Ahmed reported qualitative and quantitative analyses on the constituents of date, such as carbohydrates, fat, proteins, and amino acids, in Libyan date syrup (*9*). Globbelaar et al. have reported amino acid and oligopeptide contents of California dates at different stages of development (*10*).

The purpose of the present study is to contribute further information about isolation and purification of glucomannan from date seeds. The result of this study introduces date 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 (*11*). Glucomannan acts as a preventative of chronic disease (*12*) and as a weight control agent (*13*, *14*).

RESULTS AND DISCUSSION

We now report the isolation and structural elucidation of a neutral glucomannan and an acidic arabinogalactan from the seeds of Libyan dates. Powdered dry seeds of dates were extracted with hot ethanol, dried, and then extracted with phosphate buffer (pH 7.0) to give the crude soluble polysaccharides (0.13%). This polysaccharide fraction contained protein (0.37%) and uronic acid (1.00%) and had IR bands for ester groups at 1735 and 1245 cm^{-1}.

Hydrolysis of the polysaccharides in this fraction gave mannose, glucose, arabinose, and galactose in the molar ratio of 55:23:10:10 (GLC of the alditol accetates) and glucuronic acid (identified by TLC). The total hexose content of crude polysaccharide fraction was 18%, and it was resistant to α-amylase, thus proving the absence of starch.

Fractionation of the crude polysaccharides by using DEAE-Sephacel gave neutral and acidic fractions. The acidic fraction (4.6%), eluted with 0.4 M phosphate buffer, was composed of arabinose, galactose, rhamnose, and glucose (molar ratio 39:39:11:11). It contained glucoronic acid (3%) and protein (0.87%) and had no IR absorption for ester. The molecular weight of the acidic fraction was estimated to be 48000 by gel permation chromatography on Superose 12 (Pharmacia).

The neutral fraction (45.8%) was composed of mannose, glucose, arabinose, and rhamnose (molar ratio 84:6:6:4) and also contained protein (0.08%) and uronic acid (0.05%). The IR spectrum of this fraction showed absorption for ester groups. After gel permation chromatography on Superose 12 or Superose 6, most of the polysaccharides were present in the void volume, but there was a minor fraction with a molecular weight of 12000. The molecular weight of the fraction in the void volume, determined by using Sephacryl S-500, was 1×10^6.

The lyophilized high molecular weight fraction, obtained after gel permation chromatography, was water soluble, had an IR absorption for ester, and was free of protein and uronic acid. Total hydrolysis yielded only mannose and glucose at the molar ratio of 93:7. The total hexose of this fraction was 70%. The total hexose content of this fraction was 70% (excluding the acetyl content).

The result of methylation analysis, shown in Table 1, agreed with a (1→4)-linked glucomannan; only a trace

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Table 1. Methylation Analysis of the High-DP Neutral Polysaccharides Fraction

derivative	*t* (min)	mol %	type of linkage
1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol	16.1	1.9	1-glucose
1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-mannitol	23.5	93.0	1,4-mannose
1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol	24.2	3.8	1,4-glucose
1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-glucitol	30.8	1.3	1,4,6-glucose

Table 2. ^{13}C NMR Data of the High-DP Neutral Polysaccharides Fraction

assignment	signals
acetyl group (C=O, CH_3)	174.2, 20.9
	174.4, 21.2
	174.4, 21.2
sugar moiety	
C-1	100.9
C-2	70.4
C-3	71.9
C-4	77.5
C-5	75.6
C-6	61.2

Table 3. Location of the Acetyl Groups in the Date (Seed) Glucomannan

derivative	*t* (min)	mol %	location of OAc
1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-mannitol	21.4	20.0	2, 3, 6
1,2,4,5-tetra-*O*-acetyl-3,6-di-*O*-methyl-D-mannitol	28.0	6.0	3, 6
1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-mannitol	29.3	25.0	2, 3
1,2,3,4,5-penta-*O*-acetyl-6-*O*-methyl-D-mannitol	29.9	23.0	6
hexa-*O*-acetyl-D-mannitol	38.9	21.0	
hexa-*O*-acetyl-D-glucitol	41.3	5.1	

of branched sugar residues was detected. The ^{1}H NMR spectrum showed signals at δ 2.00, 2.02, and 2.08 for OAc groups, and the signals at δ 4.15 and 5.34 indicated that the accetyl groups were located at C-6 and C-2,3, respectively. The anomeric proton signal at δ 4.85 confirmed that the sugar residues were linked β-glycosidically (*15*), 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→4)-linked glucomannans (*16*). 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 (*17*).

The location of the OAc groups was determined by reaction with methylvinyl ether (*18*), followed by *O*-deacetylation, methylation (*19*), 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 12000 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.

According to the chemotaxonomy viewpoint, glucomannans are typical of the family Liliaceae (*20*). Differences among species are mainly in the mannose/glucose ratio, in molecular weight, and in patterns of branching and acetyl substitution (*21*). Studies have shown that in diabetic and nondiabetic patients, glucomannan can decrease serum cholesterol by ~10%, without changes in diet or exercise (*12*, *22*). The present study suggests a role for date 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 dates.

MATERIALS AND METHODS

Plant Material. Dry dates (2 kg) of Bikrari variety was used in this study. Uniform fruits in shape, size, and color were obtained from southern Libya at weekly intervals from July 15 to August 15, 2000. Seeds of dry dates were ground after the flesh and calyx had been removed. The maturity of the date fruits selected for this study was in the Tamar stage (full ripeness) as this stage is characterziaed by a larger amount of polysaccharides than the green and yellow stage (*23*).

Isolation of the Polysaccharides. Dried crushed seeds were extracted successively with light petroleum and ethanol (95%) to defat and decolorize and then extracted with aqueous 80% ethanol overnight. After centrifugation (5000 rpm, 15 min), the residue was dried and then extracted with 0.1 M phosphate buffer (pH 7.0) for 7 h at 60 °C. After centrifugation (5000 rpm, 15 min) and re-extraction of the pellet, the phosphate buffer extracts were combined, concentrated, dialyzed. The nondialyzable phase was freeze-dried in a vacuum. The yield of crude polysaccharide fraction was 0.13% of the fresh weight.

The crude polysaccharide fraction was eluted from a column (2.8 × 25 cm) of DEAE-Sephacel (PO_4) (Pharmacia), at 50 mL with (1) water (400 mL), (2) a phosphate buffer (pH 6.0) gradient (0→M, 600 mL), and (3) 0.2 M NaOH (400 mL). Anthrone-positive fractions were combined, concentrated, dialyzed against distilled H_2O, and dried.

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. Fractions were monitored by using the anthrone method. The column (1.6 × 90 cm) of Superose 12 was standard 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.

Determination of Sugar Composition and Linkage Analysis. Polysaccharides were hydrolyzed with 2 M trifluoroacetic acid for 60 min at 120 °C. The hydrolysate was repeatedly coconcentrated from water. The residue was analyzed by TLC on silica gel 60 (Merck), using acetone/water (85:15 v/v) or ethyl acetate/methanol/boric acid/acetic acid (54:21:14:11, v/v), and detection was made with aniline/diphenylamine/phosphoric acid (*24*). The monsaccharides in the hydrolysate were converted into the alditol acetates (*25*) 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 et al. (*19*). Partially methylated alditol acetates were analyzed with a Hewlett-Packard GC 5890A, using a mass

selective detector 5970B, a Durabond fused-silica column (DB225, 0.25 mm × 30 m), and a temperature program of 170–210 °C at 10 °C/min followed by an isothermic phase.

General Procedures. Solutions were concentrated at 60 °C under reduced pressure. 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 (*26*) with Coomassie Brilliant Blue and bovine serum albumin as the standard. Total sugar content was determined according to the anthrone method (*27*) and uronic acid according to the method of Blumenkrantz and Asboe-Hansen (28) as modified by Kram (*29*). Starch contamination was estimated by treatment with pancreatic α-amylase (*30*). Acetyl groups were determined according to the method of Paulsen et al. (*17*) and were located following the method of De-Belder et al. (*18*).

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