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POLYSACCHARIDES OF IRIS

I. GLUCOMANNAN FROM *Iris sogdiana*

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Several fractions of alkali-soluble polysaccharides have been isolated from the seeds of *Iris sogdiana* Bge. The separation of one of the fractions has yielded a glucomannan consisting of glucose and mannose residues in a ratio of 1:1.2. On the basis of the results of oxidation with chromium trioxide of the acetate of the glucomannan, and of periodate oxidation and methylation, it has been established that its molecule consists of a linear chain composed of β-D-glucopyranose and β-D-mannopyranose residues connected by β-1→4 bonds, although the presence of branching is not excluded. The possibility has been shown of isolating D-mannose by the hydrolysis of the seeds.

In Uzbekistan, the genus *Iris* (family Iridaceae) is represented by nine wild-growing species [1]. There are reports in the literature on the study of the monosaccharide composition of the alkali-soluble polysaccharides from the seeds of *I. versicolor* L. and *I. mandschurica* Meissn. [2]. Andrews et al. [3] have studied a glucomannan from the seeds of

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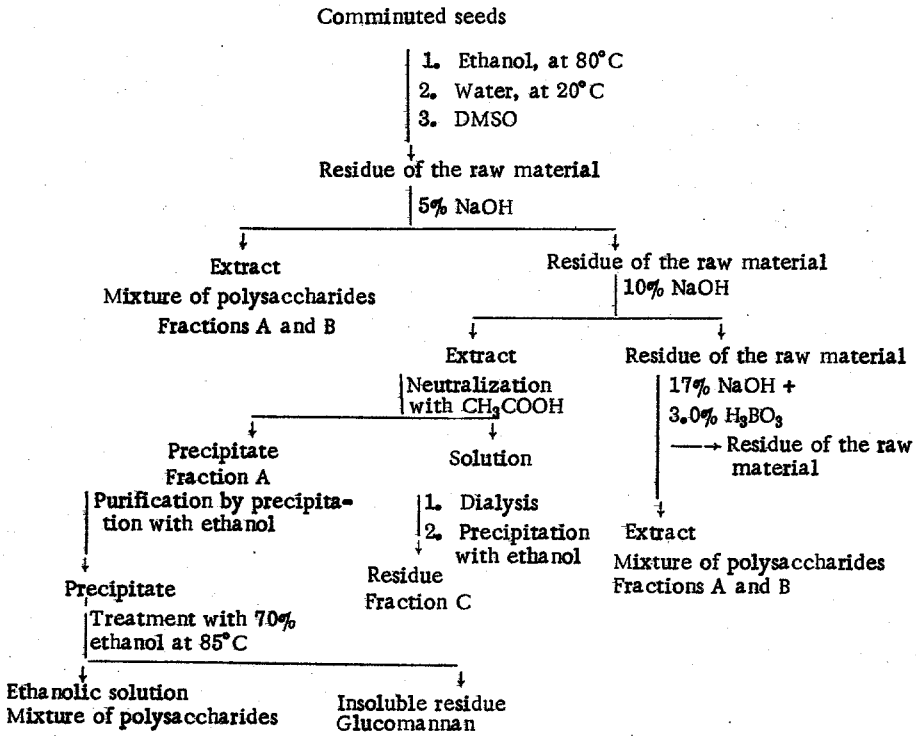
I. ochroleuca and *I. sibirica.*

We have now studied the polysaccharides of the seeds of *I. sogdiana* Bge. (Sogdian iris). The glucomannan was isolated by means of the scheme given below.



Scheme 1. Scheme of the isolation of the glucomannan.

The air-dry raw material was boiled with ethanol to eliminate low-molecular-weight compounds, and the residue was extracted successively with water at room temperature, with dimethyl sulfoxide, with 5% and 10% solutions of NaOH, and then with 17% NaOH containing boric acid. The total yield of alkali-soluble polysaccharides (PSs) was 30.8% on the air-dry raw material. The negative reaction of the PSs with iodine showed the absence of starch from the fractions isolated, and in hydrolysates of them D-mannose, D-glucose, and small amounts of D-galactose were found by CP and GLC.

The polysaccharides of fraction A, obtained by extraction with 10% NaOH, made up the bulk of the material. In hydrolysates of these polysaccharides the amount of galactose was very small in comparison with the other PS fractions. Consequently, this fraction was subjected to chemical study. It was purified by repeated reprecipitation of an alkaline solution of the PSs in ethanol, and also by heating the polysaccharide with 70% ethanol [4]. A hydrolysate of the PSs so obtained contained only D-glucose and D-mannose in a ratio of 1:1.2. This ratio remained unchanged when the polysaccharide was fractionated with Fehling's reagent. Consequently, the PS was a glucomannan. It formed a fibrous white powder insoluble in water and soluble in aqueous solutions of alkalis with the formation of a viscous solution. Neutralization of the alkaline solution with acid gave a gelatinous precipitate similar to other glucomannans of hemicellulose.

The IR spectrum of the glucomannan contained absorption bands at 820 cm^{-1} (pyranose ring), 890 cm^{-1} (β-glucosidic bond), 1659 cm^{-1} (bound water), and 3200-3400 cm^{-1} (OH).

To determine the configurations of the glycosidic bonds we used the method of oxidizing the acetylated polysaccharides with chromium trioxide [5], in which β-glycosides are subjected to oxidation. Analysis of the composition of the products of the oxidation of the acetate of the glucomannan by the PC method showed the practically complete absence of glucose and mannose residues. It follows from this that the glucose and mannose are linked by β-glycosidic bonds.

To determine the positions of the bonds between the monosaccharide residues in the glucomannan, the latter was methylated first by Haworth's method and then by Purdie's method

[6]. A hydrolysate of the methylated glucomannan was studied by TLC and GLC (in the form of acetates of the corresponding polyols). The ratio of 2,3,6-trimethylglucose and 2,3,6-trimethylmannose proved to be 1:1.2, which agrees with the ratio of the free sugars in a hydrolysate of the initial glucomannan. Below we give the R_f values of the products of the hydrolysis of the methylated glucomannan and the retention times (RT, min) of the acetates of their polyol derivatives:

Methylated hexoses and their polyol acetates	R_f*	RT on GLC
2,3,4,6-Tetra-O-methylglucopyranose	1.0	
2,3,4,6-Tetra-O-methylmannopyranose	0.86	
2,3,6-Tri-O-methylglucopyranose	0.61	
2,3,6-Tri-O-methylmannopyranose	0.43	
Di-O-methylhexoses	0.17	
1,5-Di-O-acetyl-2,3,4,6-tetra-O-methylmannitol and -sorbitol		3.0
1,4,5-Tri-O-acetyl-2,3,6-tri-O-methylmannitol		5.5
1,4,5-Tri-O-acetyl-2,3,6-tri-O-methylsorbitol		6.25
Di-O-methylhexoses		9.25-10.5

*The R_f values were determined for TLC in system 4.

The presence in the hydrolysate of predominating amounts of 2,3,6-tri-O-methyl-D-mannose and -D-glucose indicates that the main chain of the macromolecule of the glucomannan is constructed of D-mannopyranose and D-glucopyranose residues connected mainly by β-1→4 glucosidic bonds. The results of chromatographic (TLC, GLC) analyses of hydrolysates of the products of Smith's degradation showed the presence of erythritol and glycerol. The formation of erythritol shows a 1→4 bond between the glucopyranose and mannopyranose residues. The glycerol was formed from terminal groups.

On the basis of the results of oxidation with chromium trioxide and with sodium periodate and of methylation and the characteristics of the IR spectra it may be assumed that the glucomannan has a branched structure, the main chain of which consists of β-1→4-bound D-glucopyranose and D-mannopyranose residues and differs from the polysaccharides of other species of the genus *Iris* that have been studied [2, 3].

D-Mannose is used in medical practice as a nutrient for the diagnosis of bacteria [7]. Mannose is not much found in the free state in nature. It is obtained mainly by hydrolytic cleavage [8-11]. In order to expand the raw materials basis, we have isolated D-mannose directly from hydrolysates of iris seeds with a yield of 12.5% on the air-dry raw material.

EXPERIMENTAL

Descending chromatography was performed on paper of types FN-7 and 14. Thin-layer chromatography (TLC) was carried out in a fixed layer on KSK silica gel with particle dimensions of about 100 μm containing 5% of gypsum and on Silufol UV-245 (Czechoslovakia). The following solvent systems (ratios by volume) were used for chromatography: 1) butan-1-ol—pyridine—water (6:4:3); 2) propan-1-ol—ethyl acetate—water (7:2:1); 3) chloroform—methanol (9:1); methyl ethyl ketone—1% ammonia (30:4).

The sugars and their derivatives were revealed with: 1) a solution of aniline hydrogen phthalate at 105-110°C; 2) periodate—$KMnO_4$—benzidine; 3) concentrated H_2SO_4 at 115°C, 10 min; and 4) aniline—diphenylamine—phosphoric acid, IR spectra were recorded on a UR-20 instrument in tablets with KBr and in paraffin oil. GLC analysis was carried out on a Tsvet-101 instrument with a flame-ionization detector using a steel column (200 × 0.3 cm) filled with 5% of XE-60 on Chromaton N-AW, 0.200-0.250 mm, column temperature 210°C, air 300 ml/min, hydrogen and helium 60 ml/min each. Solutions were evaporated in vacuum at 40°C. Electrophoresis was performed at pH 9.2 in a 0.05 M solution of borate buffer at a voltage of 400 V for 4 h. The substances obtained were dried in vacuum over P_2O_5.

Isolation of the Polysaccharides. The air-dry seeds collected in September, 1976, at Chimgan (Tashkent oblast) (37 g) were ground in a mortar with the addition of liquid nitrogen and were then boiled with ethanol (1:10) for one hour. The residue of the plant was extracted with 0.6 liter of water for 3 h and filtered off, and treated similarly twice more (0.3 liter of water each time). The extracts were combined, evaporated, and washed with ethanol and with acetone. Yield 0.08 g.

The residue of the raw material was extracted at room temperature with DMSO (600 ml). The extract was dialyzed, and the dialysate was evaporated and precipitated with methanol. A very small amount of polysaccharide deposited. Then the raw material was extracted successively with 5 and 10% NaOH and with 17.0% NaOH containing 3% of boric acid (Scheme 1). The extracts were centrifuged. The alkaline solutions were neutralized with concentrated CH_3COOH to pH 5.0, which led to the deposition of precipitates, which were separated by centrifuging and were washed with aqueous ethanol and with acetone (fraction A). Yields 0.03, 7.24, and 2.6 g, respectively.

The mother solution from fraction A was dialyzed and evaporated, and the polysaccharides were precipitated with three volumes of ethanol and were washed with ethanol and with acetone (fraction C). Yields 1.19, 0.24, and 0.5 g, respectively.

Purification of Fraction A. A solution of 7 g of the polysaccharide of fraction A (from the extract obtained with 10% NaOH) in 300 ml of 8% NaOH was poured into one liter of ethanol. The resulting precipitate was washed with water and with 70% ethanol, and then with 97% ethanol and with acetone. Yield 6.64 g.

Isolation of the Glucomannan. The polysaccharide (5.9 g) was treated with 70% ethanol at 90°C. The insoluble precipitate was separated off by centrifuging (5.8 g). The glucomannan (0.1 g) was hydrolyzed with 2 N H_2SO_4 (98°C, 36 h). The hydrolysate was neutralized with $BaCO_3$ and filtered, the filtrate was evaporated, and glucose and mannose were identified by PC in system 1 and by electrophoresis, and GLC showed their ratio as 1:1.2.

Treatment of the Glucomannan with Fehling's Solution. An equal volume of Fehling's solution was added to a solution of 0.65 g of the glucomannan in 30 ml of 8% NaOH, and the gelatinous precipitate formed was separated off by centrifuging and was washed three times with the same solution. The precipitate was suspended in water, the suspension was cooled with ice-water, 2 N HCl was added with vigorous stirring, and the solution was centrifuged to eliminate insoluble material. The supernatant liquid (100 ml) was treated with 350 ml of fcetone, and the resulting precipitate was washed with a mixture of acetone and water (60:40) containing 2% acetic acid and then with acetone and with ether. The yield of white amorphous powder was 0.52 g. Of this, 0.05 g was dissolved in 2 ml of 72% H_2SO_4 and this solution was then diluted to 0.5 N H_2SO_4 and was heated on the water bath for 8 h. Then it was neutralized with $BaCO_3$ and the filtrate was evaporated to the state of a syrup and was analyzed by PC (system 1) and GLC (in the form of acetates of the aldononitriles). Glucose and mannose were detected (1:1.2).

Acetylation of the Glucomannan. A solution of 0.05 g of the substance in 30 ml of formamide was treated with 15 ml of pyridine, and then 15 ml of acetic anhydride was added dropwise and the mixture was stirred for 48 h. After this, 0.6 liter of ethanol was added to it and the resulting precipitate was separated off by centrifuging and was washed with ethanol and was dried. Yield 0.07 g of a white fibrous powder.

Oxidation with Chromium Trioxide. The acetate of the glucomannan (0.07 g) was oxidized with 0.15 g of CrO_3 in 5 ml of glacial acetic acid (50°C, 3 h) by the method of Hoffman et al. [5]. No glucose was detected in the hydrolysate by PC in system 1.

Methylation of the Glucomannan. The glucomannan (1 g) was methylated twice by Haworth's method and methylation was brought to completion by Purdie's method. Yield 0.67 g of a white fibrous amorphous powder. Soluble in acetone, tetrahydrofuran, and chloroform. TLC in system 3 with revealing agent 3 showed one spot. The IR spectrum of the methylated glucomannan had no absorption from OH groups. $[\alpha]_D^{20}$ −7° (c 1.0; chloroform); OCH_3 − 42.11%.

Hydrolysis of the Methylated Glucomannan. The product (0.1 g) was heated in 2 ml of 85% HCOOH at 100°C for 3 h, and then the reaction mixture was evaporated and the residue was heated on the boiling water bath with 10 ml of 0.25 M H_2SO_4 for 12 h and this mixture was neutralized with $BaCO_3$ and filtered. The residue was washed with aqueous ethanol (50%), the combined filtrates were evaporated, and the methylhexoses were identified by TLC (system 4, revealing agents 1 and 4, see above).

Part of the hydrolysate was reduced with $NaBH_4$, and it was then acetylated and analyzed by GLC.

Periodate Oxidation and Smith Degradation. The glucomannan (0.1 g) was oxidated with 10 ml of a 0.25 M aqueous solution of sodium periodate at room temperature in the dark for 15 days. The excess of periodate was destroyed with ethylene glycol, and the solution was

dialyzed. Then it was treated with 0.25 g of $NaBH_4$, and after 16 h the reaction mixture was acidified with concentrated acetic acid to pH 5.0 and was evaporated with the addition of methanol. The residue was hydrolyzed with 0.5 N H_2SO_4 on the boiling water bath for 8 h, and the monosaccharide composition of the hydrolysate was investigated by PC in system 2 with revealing agents 1 and 2. Erythritol, glycerol, and traces of mannose and glucose were found.

Part of the hydrolysate was evaporated with methanol and acetone. The sugar derivatives for GLC analysis were obtained in the form of aldononitriles by the method of Lance and Jones [12].

Isolation of D-Mannose. A mixture of 20 g of iris seeds and 35 ml of 72% H_2SO_4 was left at 20°C for 3 h and was then diluted with water to 3% and was then heated on the boiling water bath for 6 h. The solution was filtered, the filtrate was boiled with 20 g of activated carbon for 30 min and was neutralized with $BaCO_3$ and centrifuged, the precipitate was washed with 0.5 liter of hot water, the supernatant and the wash-waters were combined and evaporated to 70 ml, and D-mannose was obtained by the method of Stepanenko and Baksova [7]. Yield 2.5 g, mp 128-130°C.

SUMMARY

A glucomannan consisting of D-glucose and D-mannose in a ratio of 1:1.2 has been isolated from the seeds of *Iris sogdiana* Bge.

According to the results of oxidation of chromium trioxide and with sodium periodate and of methylation, the glucomannan has a branched structure the main chain of which is composed of β-1→4-bound D-glucopyranose and D-mannopyranose residues.

The possibility of isolating D-mannose by the hydrolysis of the seeds has been shown.

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