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STRUCTURE OF GALACTOMANNAN FROM SEEDS OF *Gleditsia macracantha*

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The structure of galactomannan from seeds of Gleditsia macracantha *is studied by methylation, periodate oxidation, and partial acid hydrolysis. The principal polyglycoside chain consists of β-1→4-bonded mannopyranose units, where the mannose is substituted in the 6-position by single α-D-galactopyranose units.*

The isolation and purification of galactomannan (GM) from the seeds of *Gleditsia macracantha* have been previously described [1]. The goal of the present work was to study the structure of GM by the chemical methods of methylation, oxidation by sodium periodate, and partial acid hydrolysis.

The water-soluble polysaccharide (WSPS) isolated by us are a mixture of polymeric homologues. Therefore, an aqueous solution of GM was fractionated by alcohol in order to obtain a homogeneous fraction. The number of fractions formed and their properties are given in Table 1.

Fractionation produced three fractions. Of these, fraction 1 contained the bulk of the material. According to ultracentrifugation results, fraction 1 was homogeneous. The hydrolysate contained *D*-galactose and *D*-mannose in a 1.0:4.93 ratio according to paper (PC) and gas—liquid (GLC) chromatographies. Therefore, fraction 1 is the GM.

The GM was methylated according to the literature method [2] in order to determine the bonding type. The resulting permethylate had $[\alpha]_D^{22}$ +27° (*c* 0.1%, $CHCl_3$). The positive specific rotation of the permethylate indicates the presence of an α-glycosidic bond in GM. The permethylate was subjected to formolysis and hydrolysis. According to TLC and GLC, the hydrolysate contained the methylated sugars 2,3-di-O-methyl-*D*-mannose, 2,3,6-tri-O-methyl-*D*-mannose, 2,3,4,6-tetra-O-methyl-*D*-mannose, and 2,3,4,6-tetra-O-methyl-*D*-galactose in a 1.0:18.33:2.58:9.75 ratio with R_f values 0.15, 0.56, 0.85, and 1.0, respectively. The observation of 2,3,4,6-tetra-O-methyl-*D*-galactose and 2,3-di-O-methyl-*D*-mannose suggests that the GM chain may be branched. The presence of 2,3,6-tri-O-methyl-*D*-mannose indicates that the polymer backbone is 1→4-bonded.

The results from methylation are consistent with those from periodate oxidation. Oxidation by sodium periodate consumes 1.44 mole of oxidant and produces 0.45 mole of formic acid for each anhydrohexose unit. The degraded polysaccharide was reduced by $NaBH_4$ [3]. Erythritol and glycerine were detected in a 5.5:3 ratio by PC of the hydrolysate of the resulting polyalcohols. The presence of erythritol is consistent with 1→4 bonds between mannose units in the polymer and with the pyranose isomer. The detection of glycerine argues in favor of a 1→6 bond, which is confirmed by methylation of galactose.

The configuration of the anomeric centers was determined by oxidation of the acetylated GM by CrO_3. The observation of only galactose in last case and IR spectral data (720 cm^{-1}) confirm that the second component of galactose has the α-configuration.

The sequence of hexose units in the GM chain was determined using partial acid hydrolysis. Paper chromatography of the hydrolysate detected mannose, galactose, and four oligosaccharides (A-D) with $R_{f\,gal}$ 0.7 (A), 0.5 (B), 0.31 (C), and 0.1 (D). The oligosaccharides A-D were individually separated by preparative PC. Their structures were studied by total acid hydrolysis before and after reduction by $NaBH_4$, periodate oxidation, and methylation.

Oligosaccharide A, $[\alpha]_D^{20}$ -15° (*c* 0.2%, H_2O). Only mannose was detected after total acid hydrolysis. The hydrolysis products after reduction by $NaBH_4$ contained mannose and mannite in a 1.05:1.0 ratio. The hydrolysate of the permethylate consisted of 2,3,4,6-tetra-O-Me-*D*-mannose and 2,3,6-tri-O-Me-*D*-mannose in a 1:1 ratio. Erythritol and glycerine in a 1:1 ratio

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TABLE 1.

Fraction No.	Yield, % of total PS weight	Gal:Man ratio	η_{red} (*c* 0.5%, H_2O)	MM
I	81.9	1:4.93	42.42	750000
II	6.9	1:4.08	16	45000
III	1.2	-	-	-

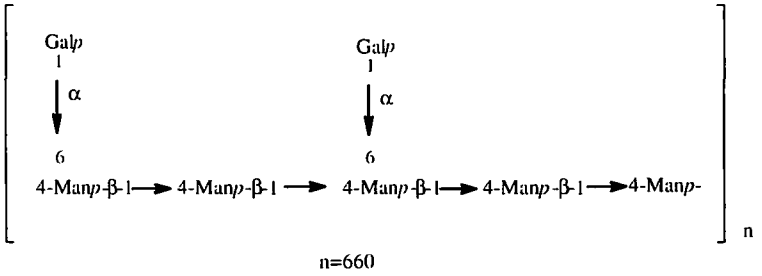
were identified after Smith decomposition. The results indicate that oligosaccharide A is 4-O-β-*D*-mannopyranosyl-*D*-mannose, i.e., mannobiose.

Oligosaccharide B, $[\alpha]_D^{20}$ +119° (*c* 0.1%, H_2O). Mannose and galactose were detected by PC after total acid hydrolysis. The hydrolysate of the reduced product contained galactose and mannite (1.0:1.0). Methylation was performed according to the literature method [2]. The hydrolysate of methylated oligosaccharide B contained 2,3,4,6-tetra-O-Me-*D*-galactose and 2,3,4-tri-O-Me-*D*-mannose. Only glycerine was formed by Smith degradation, indicating the presence of 1→6 glycosidic bonding between mannose and galactose, i.e., oligosaccharide B is 6-O-α-*D*-galactopyranosyl-*D*-mannose.

Oligosaccharide C, $[\alpha]_D^{20}$ -27° (*c* 0.1%, H_2O). Only mannose was detected by PC of the hydrolysate. The hydrolysis products of the reduced oligosaccharide C are mannite and mannose (1.0:2.0). The hydrolysate of the permethylate contained 2,3,6-tri- and 2,3,4,6-tetra-O-Me-*D*-mannose. Erythritol and glycerine were formed by Smith degradation. Thus, oligosaccharide C is a trisaccharide, O-β-*D*-mannopyranosyl-(1→4)-O-β-*D*-mannopyranosyl-(1→4)-*D*-mannopyranose.

Oligosaccharide D, $[\alpha]_D^{20}$ -38° (*c* 0.1%, H_2O). Reduction and subsequent hydrolysis gave mannite and mannose in the ratio 1.0:2.6. The final products of Smith degradation were glycerine and erythritol in the ratio 1.0:3.0. The hydrolysate of the methylated product contained 2,3,6-tri- and 2,3,4,6-tetra-O-Me-*D*-mannose. The degree of polymerization of oligosaccharide D is 4, which means that it is a mannotetraose, i.e., O-β-*D*-mannopyranosyl-(1→4)-O-β-*D*-mannopyranosyl-(1→4)-O-β-*D*-mannopyranosyl-(1→4)-*D*-mannopyranose.

The following repeating sequence was established for GM from seeds of *G. macracantha* based on the data obtained:



n=660

EXPERIMENTAL

GLC of the sugar derivatives was performed on a Tsvet 101 instrument using a flame-ionization detector, steel column (200×0.3 cm), 5% Silicone XE-60 on chromaton NAW (0.200-0.250 mesh), column temperature 210°C, He carrier gas, and 60 ml/min.

PC was carried out using Filtrak FN 1, 7, 11, 15 (Germany) using *n*-butanol—pyridine—water (6:4:3) (System I) in a descending mode.

TLC was performed on Silufol UV-254 plates using $CHCl_3$—CH_3OH (9:1) (System 2).

Sugars were located by spraying with acidic anilinium phthalate, 1% $KMnO_4$ solution, or saturated sodium iodate solution.

Specific rotations of oligosaccharides were determined on a Zeiss polarimeter in a tube of 1 ml volume.

Galactomannans were isolated according to the literature method [1].

Fractional Precipitation by Alcohol. GM (1 g) was dissolved in water (400 ml). Alcohol (75 ml) was added dropwise with vigorous stirring. The resulting white fibrous precipitate (fraction I) was separated by centrifugation, washed with alcohol, dehydrated by an acetone—ether (1:1) mixture, and dried over P_2O_5 under vacuum. Yield of fraction I, 0.819 g. Another

portion of alcohol (75 ml) was added to the supernatant. The resulting fine precipitate was separated and treated as above. Yield of fraction II, 0.069 g. The aqueous-alcohol solution was evaporated. Another portion of alcohol (100 ml) was added, precipitating fraction III (0.012 g).

Periodate Oxidation. GM (0.5 g) was dissolved in water (25 ml) and treated with sodium iodate solution (0.25 M, 5.1 ml). Aliquots (1 ml) were collected every day. The amount of periodate consumed was measured. After 14 days the periodate consumption remained constant. Ethylene glycol was added to destroy the excess of oxidant. The reaction products were reduced with $NaBH_4$ and neutralized with KU-2 (H^+) ion exchanger. The reduced product was hydrolyzed with H_2SO_4 (2 N) for 6 h at 100°C, neutralized with $BaCO_3$, and treated with KU-2 (H^+) cation exchanger to remove the excess of Ba^{+2}. Erythritol and glycerine were detected by PC. The amount of formic acid formed was determined by titration [3].

Methylation of GM. GM (0.1 g) was dissolved in DMSO (12 ml). Powdered NaOH (0.25 g) was added. Freshly distilled CH_3I (2.25 ml) was added. The mixture was stirred by a magnetic stirrer for 2.5 h. Water (4 ml) and $CHCl_3$ (4 ml) were added. The $CHCl_3$ layer was separated, dialyzed, dried over Na_2SO_4, and evaporated to dryness. Methylation was performed three times. Total methylation was confirmed by the absence in the IR spectrum of free-hydroxyl absorption at 3200-3600 cm^{-1}. The methylated polysaccharide was formolyzed (5 ml, 85% HCOOH, 1 h), hydrolyzed (5 ml, 0.5 N H_2SO_4, 100°C, 16 h), and neutralized with $BaCO_3$. The Ba^{2+} ions were removed by treatment with KU-2 (H^+) cation exchanger. Yield of permethylated GM, 0.063 g.

Partial Acid Hydrolysis. GM (2 g) was dissolved in water (200 ml), treated with CF_3COOH (0.5 M, 200 ml), and boiled on a water bath for 7 h. The hydrolysate was evaporated to dryness. The CF_3COOH was removed from the hydrolysate by repeated evaporation with methanol.

Preparative Separation of Oligosaccharides. Oligosaccharides (A-D) were individually isolated by preparative PC (System I). Yields: 0.28 (A), 0.17 (B), 0.12 (C), 0.20 g (D).

REFERENCES

1. M. R. Mirzaeva, R. K. Rakhmanberdyeva, E. L. Kristallovich, and D. A. Rakhimov, *Khim. Prir. Soedin.*, 727 (1998).
2. V. V. Barbakadze, E. P. Kemertelidze, G. E. Dekanosidze, and A. I. Usov, *Bioorgan. Khim.*, **19**, No. 2, 223 (1993).
3. *Methods in Carbohydrate Chemistry* Ed. Roy L. Whistler, M. L. Wolfrom, Acad. Press. New York, London (1962-65) [Russian translation, Mir, Moscow (1967), pp. 476, 469].