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STRUCTURAL STUDIES ON A POLYSACCHARIDE OBTAINED FROM THE CAMBIUM LAYER OF A BAEL (*Aegle marmelos*) TREE

RATAN K. BASAK AND AMAL K. MUKHERJEE*
Department of Macromolecules, Indian Association for the Cultivation of Science, Calcutta-700032 (India)

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ABSTRACT

The purified polysaccharide isolated from the cambium layer of a young bael (*Aegle marmelos*) tree contains galactose, arabinose, rhamnose, xylose, and glucose in the molar ratios of 10.0:9.8:1.4:1.9:1. Methylation analysis and Smith degradation studies established the linkages of the different monosaccharide residues. The anomeric configurations of the various sugar units were determined by oxidation of the acetylated polysaccharide with chromium(VI) trioxide. The oligosaccharides isolated from the polysaccharide by graded hydrolysis were characterized. The structural significance of these results is discussed.

INTRODUCTION

The gummy material surrounding the seeds of bael fruit[1-3], and the exudate gum[4], of the bael (*Aegle marmelos*) tree contain (1→3)-linked galactopyranosyl groups in the backbone chains, but different side chains in these macromolecules. The structures of the polysaccharides of bael-fruit pulp[5] and seed[6] have been investigated. Investigation of a hemicellulose fraction[7] from the trunk of a young bael tree was also conducted. It was, therefore, of great interest to study the polysaccharide present in the cambium layer of bael trees to search for a precursor chain of the exudate bael-gum, and the results of investigations on this polysaccharide are reported herein.

RESULTS AND DISCUSSION

The crude polysaccharide was obtained from the cambium layer of a bael tree by extraction with hot water, and precipitation with ethanol. It had $[\alpha]_D^{24}$ +33.8°, and contained galactose (9.13), arabinose (10.19), rhamnose (1.85), xylose (1.68), glucose (1.15%), and a trace of glucuronic acid. The material, containing 24% of total carbohydrate, was purified as follows; it was dispersed in water, the suspension

*Present address: Department of Chemistry, Indian Jute Industries Research Association, 17, Taratoila Road, Calcutta-700088, India.

TABLE I

METHYL ETHERS OF SUGARS FROM THE HYDROLYZATES OF METHYLATED POLYSACCHARIDE F

Sugars[a]	T[b]		*Approximate mole %*	*Mode of linkages*
	a	*b*		
2,3,4,6-Gal	1.27	1.19	22.09	Gal*p*-(1→
2,3,6-Gal	2.40	2.21	5.38	→4)-Gal*p*-(1→
2,6-Gal	3.67	3.10	4.32	→3,4)-Gal*p*-(1→
3,6-Gal	4.33	—	5.21	→2,4)-Gal*p*-(1→
6-Gal	5.06	—	3.35	→2,3,4)-Gal*p*-(1→
2,3,5-Ara	0.48	0.42	14.31	Ara*f*-(1→
2,3-Ara	—	1.06	26.07	→5)-Ara*f*-(1→
2-Ara	—	1.96	3.58	→3,5)-Ara*f*-(1→
3,4,6-Glc	2.00	1.81	4.13	→2)-Glc*p*-(1→
2,3,4-Xyl	0.63	0.52	3.23	Xyl*p*-(1→
2,3,4-Rha	0.45	0.36	4.59	Rha*p*-(1→
3,4-Rha	0.90	0.86	3.73	→2)-Rha*p*-(1→
2,3,6-Glc	2.49	2.31	trace	→4)-Glc*p*-(1→

[a]2,3,4,6-Gal = 2,3,4,6-tetra-*O*-methylgalactose, etc. [b]Retention times of the corresponding alditol acetates, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, on (*a*) 3% of ECNSS-M column at 170°, and (*b*) 3% of OV-225 column at 170°.

centrifuged at 12,000 r.p.m. for 30 min, and the clear solution passed through a column (95 × 2 cm) of Sephadex G-100 and eluted with water, the elution being monitored with a differential refractometer. The major peak, obtained between 126 and 180 mL, was collected, concentrated, and lyophilized. The polysaccharide (F), recovered as 42% of the material present in the aqueous solution applied to the column, had $[\alpha]_D^{24}$ +15.2°, and contained galactose (42.73), arabinose (36.41), rhamnose (5.23), xylose (6.76), and glucose (4.36%); glucuronic acid was absent, as shown by paper chromatography. In high-voltage electrophoresis, using 0.05M phosphate buffer, pH 7.8, at 38 V.cm^{-1} for 45 min, polysaccharide F moved, as a single spot, a distance of 8.1 cm towards the anode (detected by spray reagent *1*), indicating its homogeneity.

Polysaccharide F was methylated, first by the Hakomori method[8] and then by the Kuhn method[9], to yield fully methylated product. The permethylated polysaccharide was hydrolyzed with 85% formic acid, and then, after removal of the formic acid, with 0.5M sulfuric acid. The hydrolyzate material was converted into alditol acetates as usual, and these were analyzed by g.l.c. using column *a* and *b*. The results are summarized in Table I. From these results, the modes of linkage of the different sugar residues could be ascertained.

Characterization of 2,3,4,6-tetra-*O*-methylgalactose, 2,3,5-tri-*O*-methylarabinose, 2,3,4-tri-*O*-methylxylose, and 2,3,4-tri-*O*-methylrhamnose indicated their presence as the nonreducing end-units in the methylated polysaccharide. The doubly

TABLE II

SURVIVAL OF SUGARS[a] IN THE OXIDATION OF ACETYLATED POLYSACCHARIDE F WITH CHROMIUM TRIOXIDE

Time (h)	myo-*Inositol*	*Galactose*	*Arabinose*	*Glucose*	*Xylose*	*Rhamnose*
0	100	95.19	52.12	16.35	12.47	15.35
0.5	100	6.20	8.40	1.80	4.40	4.00
1.5	100	0	4.90	0	1.60	0

[a]The sugars were analyzed, and estimated, by g.l.c. using column *a* at 180°.

linked galactose units are (1→4)-linked, and all of the doubly linked arabinose residues are (1→5)-linked, as indicated by the presence of 2,3,6-tri-*O*-methylgalactose and 2,3-di-*O*-methylarabinose. Identification of 6-*O*-, 2,6-di-*O*-, and 3,6-di-*O*-methylgalactose indicated that the galactopyranosyl residues are highly branched. The presence of 2-*O*-methylarabinose indicated that branches at arabinose residues are linked through O-1, O-3, and O-5. Glucosyl residues are present only in the interior part of the macromolecule, and are joined through (1→2) linkages, as indicated by the presence of 3,4,6-tri-*O*-methylglucose in the hydrolyzate of the methylated polysaccharide. In the interior part of the polysaccharide, rhamnosyl residues are present as (1→2)-linked units, as is evident from the presence of 3,4-di-*O*-methylrhamnose. A trace of 2,3,6-tri-*O*-methylglucose was also detected in the hydrolyzate of methylated polysaccharide F. The sum of nonreducing ends (*viz.*, tetra-*O*-methylgalactose, and tri-*O*-methyl-arabinose, -rhamnose, and -xylose) exceeds the branched points (*viz.*, di-*O*-methylgalactose, and mono-*O*-methyl-galactose and -arabinose). This discrepancy could not, however, be explained.

Smith-degradation studies[10] on polysaccharide F indicated the formation of glycerol, threitol, arabinitol, and galactitol in the molar ratios of 20.1 : 3.7 : 1 : 1.6, together with a trace of erythritol. The calculated values are 19.7 : 1.5 : 1 : 3.6. Characterization of these products further supported the results of the methylation analysis.

An attempt was made to determine the anomeric configurations of the different sugar residues by oxidation, with chromium trioxide[11], of the acetylated derivative of polysaccharide F in acetic acid at 50°, using *myo*-inositol as the internal standard. Aliquots were taken at different time-intervals, deacetylated, the products hydrolyzed, and the surviving sugars, as their alditol acetates, estimated by g.l.c. The results (see Table II) indicated that all of the sugars were degraded rapidly, thereby showing that they had the β configuration. But, as acetylated furanoses are nonspecifically oxidized[12] by chromium trioxide, the anomeric configuration of the arabinosyl groups could not be determined by this experiment. However, the high rate at which arabinose residues were released by hydrolysis with 10% formic acid during 2 h at 100° indicated that they probably have the α configuration.

Guided by the results of pilot experiments, the polysaccharide was subjected

to graded hydrolysis with 20% formic acid for 4 h at 100°. A paper chromatogram of the hydrolyzate showed spots for monosaccharides, and a few spots in the region of oligosaccharides. The oligosaccharides were isolated by repeated, preparative paper-chromatography using solvent system *B*. Only three oligosaccharides were obtained pure.

Fraction I, $[\alpha]_D^{24}$ +50°, on hydrolysis, and g.l.c. analysis of the derived alditol acetates gave peaks for galactose. On methylation by the Kuhn method[9], hydrolysis, and analysis of the derived alditol acetates by g.l.c. in columns *a* and *b*, peaks of 2,3,4,6-tetra-*O*-methylgalactose and 2,3,6-tri-*O*-methylgalactose were obtained in the molar ratio of 1:2.1. All of these observations, and the results of chromium trioxide oxidation, indicated that the structure of fraction I is as follows.

β-Gal-(1→4)-β-Gal-(1→4)-Gal

Fraction II, $[\alpha]_D^{24}$ +45.4°, showed only galactose in g.l.c. analysis of the alditol acetates from its hydrolyzate. On hydrolysis, and g.l.c. examination, the fully methylated oligosaccharide yielded 2,3,4,6-tetra- and 3,6-di-*O*-methylgalactose in the molar ratio of 1.9:1. Based on these results, the structure assigned to this oligosaccharide is as follows

β-Gal-(1→4)-Gal
2
↑
1
β-Gal

Fraction III, $[\alpha]_D^{24}$ +64.3°, on g.l.c. analysis of the alditol acetates derived from its hydrolyzate, showed only galactose. On methylation, hydrolysis, and g.l.c. analysis of the product (as already described), it showed peaks for 2,3,4,6-tetra- and 2,3,6-tri-*O*-methylgalactose in almost equimolar proportions (1.08:1). The oligosaccharide therefore appears to be a disaccharide having the following structure.

β-Gal-(1→4)-Gal

From the foregoing experimental results, it is evident that the polysaccharide is dominated by a stable chain of (1→4)-linked galactopyranosyl residues. However, presentation of a repeating unit is not yet feasible, particularly because of the high yields of the tetra-*O*-methylhexose and tri-*O*-methylpentoses in the hydrolyzate of the methylated polysaccharide.

EXPERIMENTAL

General. — Paper partition-chromatography was performed by the descending technique, using Whatman No. 1 chromatographic paper with the following solvent systems: (*A*) 8:2:1 ethyl acetate–pyridine–water, and (*B*) 9:2:2 ethyl acetate–acetic acid–water. Sugars were detected with alkaline silver nitrate. All specific rotations

are equilibrium values, and were recorded with a Perkin–Elmer Model 241 MC spectropolarimeter at 24°. All evaporations were conducted at 40° under diminished pressure. A Shandon high-voltage electrophoresis instrument, model L-24, was used for testing the homogeneity of the material. A Hewlett–Packard Model 5730A gas chromatograph, fitted with a flame-ionization detector and a glass column (1.83 m × 6 mm) containing (*a*) 3% of ECNSS-M on Gas Chrom Q (100–120 mesh) and (*b*) 3% of OV-225 on Gas Chrom Q (80–100 mesh), was used for g.l.c. Infrared spectra were recorded with a Beckman IR-20A instrument.

Isolation of polysaccharide from the cambium layer of a bael tree. — The cambium layer was carefully collected from growing branches of a bael tree (*Aegle marmelos*), and thoroughly washed with ethanol. The dried material (1.239 g) was extracted with water (200 mL), with stirring, for 4 h on a water-bath at 100°. The extract was cooled, and centrifuged, to collect the aqueous solution. The residue was re-extracted as before. Cold ethanol (3 vol., acidified with acetic acid to pH 4–5) was added to the combined, aqueous extract. The precipitate that separated was centrifuged off, dissolved in water, and reprecipitated with ethanol. Finally, the precipitate was washed with dry ethanol, and dried *in vacuo*. The dried material (0.261 g) had $[\alpha]_D^{24}$ +33.8°.

Hydrolysis, and sugar analysis. — The polysaccharide (5 mg) was treated with 77% sulfuric acid[13] (0.1 mL), and kept for 30 min at room temperature. The solution was diluted with water (20 vol.) and then heated for 6 h on a boiling-water bath. The solution was cooled, made neutral with barium carbonate, and centrifuged. Part of the centrifugate was analyzed by paper chromatography using solvents *A* and *B*, and the other part was converted into the alditol acetates as usual. The alditol acetates were analyzed by g.l.c. in column *a*. Neutral sugars were estimated by g.l.c., using *myo*-inositol as the internal standard.

Purification of the polysaccharide. — The crude polysaccharide (51 mg per lot) was dissolved in water (5 mL). The insoluble material was centrifuged off, and the solution was applied to a column (95 × 2 cm) of Sephadex G-100. The column was eluted with water, and the eluate was collected in 5-mL portions by means of an automatic fraction-collector, and monitored with a differential refractometer. Polysaccharide obtained from the major peak (F) had $[\alpha]_D^{24}$ +15.2° (*c* 0.33, water).

High-voltage electrophoresis. — The purified material (F) was subjected to high-voltage electrophoresis at 38 $V.cm^{-1}$ for 45 min, using 0.05M phosphate buffer, pH 7.8, and the spray reagent. A single spot, at a distance of 8.1 cm towards the anode, was obtained.

Methylation analysis. — Polysaccharide F (4.7 mg) was dissolved in dry dimethyl sulfoxide (4 mL) in a closed vial, and then treated with 2M methylsulfinyl sodium (4 mL) under nitrogen. The solution was stirred overnight, methyl iodide (3 mL) was added dropwise, with external cooling, and the mixture was stirred for 2 h. The product was dialyzed, and then lyophilized. This methylated material was further methylated by the Kuhn method[9]. The i.r. spectrum of this methylated material did not show any band in the region of 3600–3300 cm^{-1}.

The permethylated product, $[\alpha]_D^{24}$ −13.5°, was hydrolyzed with 85% formic acid for 2 h, and then (after removal of the formic acid) with 0.5M sulfuric acid for 18 h at 100°. The hydrolyzate was made neutral, the alditol acetates were prepared as usual, and these were analyzed by g.l.c. using columns *a* and *b*. The results are given in Table I.

Smith degradation[10]. — Polysaccharide F (10 mg) was treated with 0.1M sodium metaperiodate (10 mL) in the dark for 48 h at 10°. The excess of periodate was decomposed by adding ethylene glycol (3 mL, 3 h) and the solution was dialyzed, concentrated, the product reduced with sodium borohydride overnight at room temperature, and the solution acidified with acetic acid, and then dialyzed. After concentration of the solution, the product was hydrolyzed, and the materials were converted into alditol acetates in the usual way. The alditol acetates were analyzed by g.l.c. using column *a*.

Oxidation of polysaccharide F with chromium trioxide[11]. — Polysaccharide F (6.7 mg) with *myo*-inositol (0.5 mg) was dissolved in formamide (1 mL). To this solution were added acetic anhydride (1 mL) and pyridine (1.5 mL), and the mixture was stirred for 16 h at room temperature, concentrated, and the concentrate extracted with chloroform. The extract was washed with water, dried (Na_2SO_4), and evaporated to dryness, and the product was reacetylated.

Powdered chromium trioxide (600 mg) was added to a solution of the acetylated polysaccharide in glacial acetic acid (3 mL), and the mixture was stirred at 50°. Aliquots were withdrawn at 0, 0.5, and 1.5 h, immediately diluted with water, and extracted with chloroform. The extracts were dried (anhydrous Na_2SO_4), and evaporated to dryness. The product was deacetylated with 0.2M sodium methoxide, decationized with Dowex-50W X-8 (H^+) ion-exchange resin, and hydrolyzed, and the alditol acetates were prepared as usual. The alditol acetates were analyzed by g.l.c. in column *a* (see Table II).

Graded-hydrolysis studies. — A solution of polysaccharide F (30 mg) in 20% formic acid (8 mL) was heated on a boiling-water bath for 4 h. The formic acid was removed by codistillation with water under diminished pressure, the oligosaccharides were resolved on Whatman No. 3 MM paper, using solvent *B*, and the separate sugars were isolated by eluting the corresponding strips with water.

The oligosaccharides were hydrolyzed with 0.5M sulfuric acid for 10 h, and, after the usual treatment, the sugars were analyzed by g.l.c. in column *a*. The oligosaccharides were methylated by the Kuhn method[9]. The methylated products were hydrolyzed, the sugars converted into alditol acetates, and these identified, and estimated, by g.l.c. in columns *a* and *b*.

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