Carbohydrate Research, 135 (1984) 141–146
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

A NON-IONIC SEED-GUM FROM *Cassia corymbosa*

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(Received April 16th, 1984; accepted for publication, June 28th, 1984)

ABSTRACT

A polysaccharide has been extracted from *Cassia corymbosa* seeds with cold, acidulated water, and purified to give a water-soluble product containing D-galactose and D-mannose in 4:7 molar ratio. Acid-catalyzed fragmentation, periodate oxidation, methylation, and enzymic hydrolysis showed that the seed gum has a branched structure consisting of a linear chain of β-D-(1→4)-linked mannopyranosyl units, some of which are substituted at O-6 by two α-D-(1→6) galactopyranosyl units mutually linked glycosidically. Methylation analysis of the galactomannan afforded 2,3,4-tri- and 2,3,4,6-tetra-*O*-methylgalactose, along with 2,3-di- and 2,3,6-tri-*O*-methylmannose, in the molar ratios of 2:2:2:5. Both the methylation and the periodate-oxidation studies showed ~36.4% of end groups. The significance of these results, together with the findings of partial hydrolysis with acid, are discussed, in relation to ascertaining the structure of the repeating unit of the polysaccharide.

INTRODUCTION

The plants of *Cassia* (Leguminoseae) have been described as being highly medicinal and a rich source of polysaccharides[1,2]. Owing to the high medicinal value and increasing industrial acceptance of the plant mucilages, the seed mucilage from this plant has now been subjected to extensive, structural study.

RESULTS AND DISCUSSION

The polysaccharide was conveniently extracted from the crushed, defatted, and decolorized seeds by extraction with 1.5% acetic acid, and by repeated precipitation from its solution therein with ethanol. It was purified by repeated deproteinization, using chloroform, and by complexation with Fehling solution. The homogeneity of the polysaccharide was verified by fractional precipitation and zone electrophoresis, and *via* acetylation and deacetylation. The white, amorphous polysaccharide was water-soluble, and had $[\alpha]_D^{25}$ +72° (water), an ash content of

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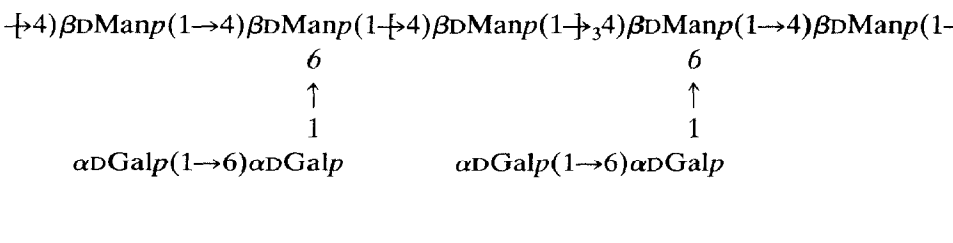
0008-6215/84/$ 03.00

0.2%, and a negligible percentage of methoxyl and acetyl groups, and uronic acid. Upon complete acid hydrolysis, the polysaccharide yielded D-galactose and D-mannose in the molar ratio of 4:7. Graded hydrolysis with 25mM sulfuric acid resulted in the favored removal of galactose, suggesting the presence of α-linked D-galactose units on the periphery, as end groups.

To determine the positions of linkage between the monosaccharide units of the galactomannan, it was exhaustively methylated, first by the Haworth method and then by the Purdie method, to afford a brown, semi-solid, glassy mass that had $[\alpha]_D^{28}$ +45° (chloroform) and was devoid of an absorption band in the hydroxyl region of its i.r. spectrum. Hydrolysis of the methylated polysaccharide gave 2,3,4-tri- and 2,3,4,6-tetra-*O*-methyl-D-galactose, as well as 2,3-di- and 2,3,6-tri-*O*-methyl-D-mannose, in the molar ratios of 2:2:2:5. The identity of these methylated monosaccharides was established on the basis of their R_{TMG} values, optical rotations, and crystalline derivatives. The percentage of terminal groups, calculated from methylation studies, was 36%. The 2,3,4,6-tetra-*O*-methyl-D-galactose present must have arisen from D-galactosyl end-groups on the side chain (confirming the earlier conclusion from graded hydrolysis), and the galactose units yielding 2,3,4-tri-*O*-methyl-D-galactose must be linked through O-1 and O-6. The mannosyl residues that yield 2,3-di-*O*-methyl-D-mannose are linked through O-1, O-4, and O-6, and constitute the branching point in the main chain of the molecule. The formation of 2,3,6-tri-*O*-methyl-D-mannose indicates the presence of (1→4)-linked D-mannosyl residues, forming the main chain. Oxidation of the seed polysaccharide with sodium metaperiodate consumed 0.85 mol of oxidant with the liberation of 226 mmol of formic acid per 100 g of polysaccharide, indicating 36.4% of end groups.

Examination of the oxopolysaccharide (obtained after 84 h) showed the absence of D-galactose, but a small proportion of D-mannose was detected. However, prolonged oxidation (96 h) decomposed both hexoses. These results indicated that D-galactopyranosyl end-groups are present in the polysaccharide, and that these protect some of the D-mannopyranosyl units from oxidation.

Acid-catalyzed, partial hydrolysis of the purified galactomannan gave three disaccharides [identified as α-D-Gal*p*-(1→6)-D-Gal*p*, α-D-Gal*p*-(1→6)-D-Man*p*, and β-D-Man*p*-(1→4)-D-Man*p*] and two trisaccharides, namely, α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→6)-D-Man*p* and β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-D-Man*p*, along with the component sugars, mannose and galactose. All of the oligosaccharides were characterized (see the Experimental section). These results corroborated the earlier findings of the methylation studies. The foregoing evidence accords with the following structure.



EXPERIMENTAL

Paper chromatography was conducted at room temperature by the descending technique using the following solvent mixtures (v/v): *A*, 5:1:4 1-butanol–ethanol–water[3]; *B*, 11:6:3 1-butanol–2-propanol–water[4]; *C*, 10:4:3 ethyl acetate–pyridine–water[5]; and *D*, 2:1:2 ethyl acetate–pyridine–water[6], and detection with aniline hydrogenphthalate. All evaporations were conducted under diminished pressure at 35–40°. Melting points are uncorrected, and all specific rotations were measured at equilibrium. Amberlite IR-120 (H^+) ion-exchange resin was used for decationizing the hydrolyzates, and all residues were dried *in vacuo* over anhydrous calcium chloride. The emulsin used was extracted from almonds.

Isolation of the polysaccharide. — Dried, crushed seeds (1 kg) were successively extracted with light petroleum (b.p. 60–80°) and ethanol, and then stirred with 1.5% acetic acid for 24 h at room temperature. The acid extract was slowly added, with stirring, to ethanol (5 vol.) to give a crude product. Dissolution and reprecipitation were repeated, and the polysaccharide was collected, washed with ethanol, and dried.

Purification of the polysaccharide. — The dried polysaccharide was redissolved in water and the solution shaken well with chloroform and then centrifuged, whereupon the denaturated proteins formed, at the water–chloroform interface[7], a gel that was removed. This treatment was repeated 5 times to remove all of the proteins. An excess of Fehling solution was added to the deproteinized, aqueous solution of the polysaccharide, and a copper complex was precipitated[8]. The complex was centrifuged, washed thoroughly with dilute Fehling solution, and suspended in cold water. The complex was decomposed with M hydrochloric acid. The polysaccharide was regenerated by slowly adding the solution to ethanol (5 vol.), with stirring. The pure product was reprecipitated from its solution in 1% acetic acid by ethanol, to yield a nonreducing, white, amorphous material (ash content, 0.2%), $[\alpha]_D^{25}$ +72° (water).

Homogeneity of the polysaccharide. — The polysaccharide (1.5 g) was fractionally[9] precipitated from the aqueous solution (300 mL) by the addition of ethanol (400 and 800 mL). The fractions (*a* and *b*) were collected by centrifugation, washed with ethanol, and dried. Hydrolysis of fractions *a* and *b* gave D-galactose and D-mannose in the molar ratio of 4:7, and both fractions retained the original specific rotation, namely, $[\alpha]_D^{25}$ +72° (water).

The polysaccharide (1 g) was treated with acetic anhydride–sodium acetate[10], and the resulting acetate (600 mg) had $[\alpha]_D^{25}$ +36° (chloroform). Deacetylation[10] regenerated material having $[\alpha]_D^{25}$ +71.5° (water).

The polysaccharide (50 mg) was subjected to conventional zone electrophoresis[9,11] on Whatman No. 1 MM paper in 0.05M sodium tetraborate (pH 9.2) for 6 h at 320 V and 3.7 mA. A plot of the absorbance against segment number showed only a single sharp peak.

Investigation of the polysaccharide. — *(i) Hydrolysis.* The purified poly-

saccharide (1 g) was hydrolyzed with M sulfuric acid for ~36 h at 100°. P.c. (solvent *C*) of the hydrolyzate then revealed galactose (R_F 0.15) and mannose (R_F 0.21). Identities were confirmed by co-chromatography with authentic samples and preparation of derivatives, and the absolute configuration from the specific rotations: D-galactose, m.p. 164°, $[\alpha]_D^{30}$ +80° (water); D-galactose phenylhydrazone, m.p. 153°; D-mannose, m.p. 131°, $[\alpha]_D^{30}$ +14° (water); D-mannose phenylhydrazone, m.p. 198°.

The polysaccharide (300 mg), together with D-ribose (30 mg) as a reference sugar, was treated with M sulfuric acid for 36 h at 100°. A portion (1 mL) of the hydrolyzate was subjected to p.c. (solvent *B*) on Whatman No. 3 MM paper, and the individual monosaccharides were quantified[9] by periodate oxidation. Assuming 100% recovery of ribose, the molar ratio of galactose to mannose was 4:7.

(ii) Graded hydrolysis. The polysaccharide (200 mg) was hydrolyzed with 25mM sulfuric acid (40 mL) for 4 h at 100°. The hydrolyzate was subjected to p.c. (solvent *B*) after 5, 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min, and galactose was found to be liberated first, followed by mannose.

(iii) Periodate oxidation[12]. To a solution of the polysaccharide (300 mg) in water (30 mL) were added KCl (2.5 g) and 0.25M sodium metaperiodate (25 mL). The volume was made to 100 mL with water, and the mixture was stored in the dark at room temperature. Aliquots (2 mL) were withdrawn at intervals, and, after the excess of periodate had been reduced with ethylene glycol, titrated with 0.01M sodium hydroxide; the formic acid liberated was 226 mmol per 100 g.

After 84 h, an excess (30 mL) of ethylene glycol was added, the solution was evaporated, and the residue was hydrolyzed. P.c. (solvent *B*) then revealed only mannose.

To a solution of the polysaccharide (300 mg) in water (30 mL) was added 0.25M sodium metaperiodate (25 mL), and the volume was made to 100 mL with water. The uptake of periodate was determined at intervals by the procedure of Andrews *et al.*[13]. The uptake became constant in 96 h, and corresponded to 0.84 g per 100 g of the polysaccharide. After 96 h, the oxopolysaccharide was hydrolyzed as already described; neither galactose nor mannose was detected.

(iv) Methylation. The polysaccharide (5 g) was subjected to four Haworth methylations[14], followed by four Purdie methylations[15]. The completely methylated polysaccharide had $[\alpha]_D^{28}$ +45° (chloroform). The methylated derivative (1.5 g) was hydrolyzed with 90% formic acid for 6 h at 100° and then with 0.75M sulfuric acid for 10 h at 100°, and the products were fractionated on Whatman No. 3 paper (solvent *A*), with 2,3,4,6-tetra-*O*-methyl-D-galactose (TMG) as the reference, to give the following products.

(1) 2,3,4-Tri-*O*-methyl-D-galactose: R_{TMG} 0.64; m.p. 85–86°, $[\alpha]_D^{25}$ +152° (water); lit.[16] m.p. 85°, $[\alpha]_D^{25}$ +154°. It was oxidized with barium iodide–iodine solution–barium hydroxide by the Goebel method, and tri-*O*-methyl-D-galactono-1,4-lactone was obtained; this spontaneously changed into tri-*O*-methyl-D-galactonic acid; needles, m.p. 107–108°.

(*2*) 2,3,4,6-Tetra-*O*-methyl-D-galactose: R_{TMG} 0.87; m.p. 72–73°, $[\alpha]_D^{32}$ +120° (water); lit.[17] m.p. 74°, $[\alpha]_D^{32}$ +121°. The anilide had m.p. 192–193°, $[\alpha]_D^{32}$ +43° (acetone); lit.[17] m.p. 194°, $[\alpha]_D^{32}$ +45°.

(*3*) 2,3-Di-*O*-methyl-D-mannose: R_{TMG} 0.53; m.p. 107–108°, $[\alpha]_D^{25}$ −16° (water); lit.[18] m.p. 108°, $[\alpha]_D^{25}$ −15.8°. The anilide had m.p. 136°; lit.[17] m.p. 138°.

(*4*) 2,3,6-Tri-*O*-methyl-D-mannose: R_{TMG} 0.81; syrup, $[\alpha]_D^{25}$ −11° (water); lit.[19] $[\alpha]_D^{25}$ −10°. The derived hydrazide had m.p. 121–131°; lit.[20] 131°.

The methylated polysaccharide (300 mg), together with added D-glucose as a reference, was treated with 0.75M sulfuric acid for 16 h at 100°. The methylated sugars resulting were separated by p.c. (solvent *A*) and quantified by titration with alkaline hypoiodite. The molar ratios of fractions 1 to 4 were 2:2:2:5.

(v) Partial hydrolysis with acid. The polysaccharide (4 g) was hydrolyzed with 0.05M sulfuric acid for 14 h at 100°. The hydrolyzate was subjected to preparative p.c. (solvent *D*), and elution of the fractions with distilled water gave D-galactose, D-mannose, and the following oligosaccharides.

(*1*) Swietenose [α-D-Gal*p*-(1→6)-D-Gal*p*][21]: m.p. 128°, $[\alpha]_D^{25}$ +144° (water); lit.[21] m.p. 230°, $[\alpha]_D^{25}$ +154°. It reduced Fehling solution and the Tollens reagent, and afforded only D-galactose on hydrolysis. Methylation and hydrolysis gave 2,3,4,6-tetra- and 2,3,4-tri-*O*-methyl-D-galactose. It was resistant to emulsin, indicating an α linkage. The octaacetate had m.p. 222–225°, $[\alpha]_D^{25}$ +180° (chloroform); lit.[21] m.p. 224°, $[\alpha]_D$ +180°.

(*2*) Epimelibiose [α-D-Gal*p*-(1→6)-D-Man*p*][22]: m.p. 199°, $[\alpha]_D^{32}$ +120° (water); lit.[22] m.p. 201–202°, $[\alpha]_D^{32}$ +121–124°. Acid hydrolysis gave galactose and mannose, and methylation followed by hydrolysis gave 2,3,4,6-tetra-*O*-methyl-galactose and 2,3,4-tri-*O*-methyl-D-mannose.

(*3*) Mannobiose [β-D-Man*p*-(1→4)-D-Man*p*][23,24]: m.p. 203–205° (from ethanol), $[\alpha]_D^{25}$ −9° (water); lit.[23] m.p. 202–203°, $[\alpha]_D^{25}$ −5.2 to −8.2°. The derived phenylosazone had m.p. 204°; lit.[22] 203–206°. Acid hydrolysis gave mannose only (p.c.), and emulsin hydrolyzed the disaccharide, indicating a β linkage. Methylation followed by hydrolysis gave 2,3,4,6-tetra- and 2,3,6-tri-*O*-methyl-D-mannose.

(*4*) 6^2-*O*-α-D-Galactosyl-6-*O*-α-D-galactosyl-D-mannose [α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→6)-D-Man*p*][21]: m.p. 122°, $[\alpha]_D^{25}$ +129° (water); lit.[21] m.p. 124°, $[\alpha]_D^{25}$ +131°; R_{Glc} (solvent *C*) 0.33 (lit.[21] 0.32). Acid hydrolysis gave galactose and mannose in the molar ratio of 2:1. It could not be hydrolyzed by emulsin, showing α linkages. Methylation, followed by hydrolysis, afforded 2,3,4-tri- and 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,4-tri-*O*-methyl-D-mannose.

(*5*) Mannotriose [β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-D-Man*p*][23,24]: m.p. 211–213° (from ethanol), $[\alpha]_D^{25}$ −13° (water); lit.[24] m.p. 214–215°, $[\alpha]_D^{25}$ −15 to −26°. Acid hydrolysis gave (p.c.) mannose only, and partial hydrolysis with acid gave mannobiose and mannose. Methylation followed by hydrolysis gave 2,3,4,6-tetra- and 2,3,6-tri-*O*-methyl-D-mannose. The trisaccharide was cleaved by emulsin, showing the presence of β linkages.

ACKNOWLEDGMENTS

K.T. is grateful to the Principal, B. B. College, Jhansi, for granting leave, and to the University Grants Commission, New Delhi, for providing a Research Fellowship, under the Faculty Improvement Programme; N.K. and V.S. thank C.S.I.R., New Delhi, for granting them Junior Research Fellowships.

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