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Note

Investigations on partial structure of galactomannan "A" from *Cassia renigera* seed

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The endosperm of leguminous seeds is usually mucilaginous, and galactomannan is its main component. The seed polysaccharide of *Cassia renigera* (N.O. Leguminosae), selected for structural investigations, contains 60% of endosperm.

RESULTS AND DISCUSSION

Galactomannan was isolated from the endosperm of *Cassia renigera* seed by extraction with cold water and precipitation with ethanol. Galactomannan "A", the subject of the present study, was recovered as an acid-soluble, major fraction from the barium complex[1,2] of the polysaccharide. It was considered homogeneous because (*i*) almost all of it precipitated from aqueous solution over a narrow concentration-range of ethanol, (*ii*) the precipitated material obtained from its acetyl derivative displayed the same physical properties and had the same sugar ratio as the original pure polysaccharide, and (*iii*) a single spot was observed in electrophoresis.

Complete acid hydrolysis of the purified polysaccharide "A" afforded D-galactose, D-mannose, and xylose in the molar ratio of 1:2.6:0.07.

Methylation analysis of polysaccharide "A" afforded 2,3,6-tri-*O*-methyl-D-mannose (3.2 mol), 2,3-di-*O*-methyl-D-mannose (2 mol), and 2,3,4,6-tetra-*O*-methyl-D-galactose (2.12 mol), together with traces of 2,3,4,6-tetra-*O*-methyl-D-mannose in the main fraction of the hydrolyzate.

The tetra-*O*-methyl-D-galactose indicates a terminal, non-reducing D-galactopyranosyl group. As 2,3,6-tri-*O*-methyl-D-mannose is a major component, the main chain must be composed of (1→4)-linked D-mannopyranose residues. Identification of 2,3-di-*O*-methyl-D-mannose suggests branching at either O-4 or O-6 of the main chain. Traces of tetra-*O*-methyl-D-mannose indicate that some mannose groups also occupy terminal, nonreducing positions.

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The molar ratio of the methylated sugars suggests that the average repeating-unit of the polysaccharide must be seven sugar residues, containing two galactose for every five mannose residues. The *Cassia renigera* galactomannan is thus essentially similar to other seed galactomannans; it is similar to that of fenugreek[3,4], *Gleditsia amorphoides*[5].

Polysaccharide "A" consumed 1.32 mol of periodate with the liberation of 0.26 mol of formic acid. Periodate-oxidized polysaccharide was successively reduced and hydrolyzed[6]. The components were glycerol (2 mol), derived from the terminal, non-reducing hexosyl group, erythritol (4.88 mol) from the non-terminal residues, and traces of mannose from the mannose residues that had escaped periodate oxidation. The results are in substantial agreement with the expected values.

The i.r. spectrum of polysaccharide showed bands at 817 and 874 cm^{-1}, indicating the presence of α-linked D-galactopyranose and β-linked D-mannopyranose residues, respectively[7]. The fact that the galactomannan has mostly β-linkages is further confirmed by the low positive rotation of the polysaccharide and its acetate.

EXPERIMENTAL

General methods. — Unless otherwise stated, all evaporations were performed under diminished pressure and at 45–50°. Melting points are uncorrected and specific rotations are equilibrium values. The i.r. spectra were recorded with a Perkin–Elmer model 137-B instrument, either for chloroform solutions in sodium chloride cells or in potassium bromide pellets. Electrophoresis was conducted with a Laboratorium Felzerclsk Gyara apparatus (Budapest, Hungary), type DE-201. Paper-chromatographic (p.c.) analysis was carried out by the descending method on Whatman No. 1 paper with the non-aqueous phase of the following solvent systems (by volume): (*a*) 167:47:15 benzene–ethanol–water; (*b*) butanone–water (azeotrope); (*c*) 80:20:8:1 butanone– ethyl acetate–water–ammonia (*d*) 2:1:2 ethyl acetate–pyridine–water; and (*e*) 4:1:5 1-butanol–acetic acid–water. Silver nitrate–sodium hydroxide and *p*-anisidine phosphate were used as spray reagents. Sugar mixtures were separated on Whatman No. 3 MM sheets and columns of Whatman cellulose powder. Deionization was performed with Amberlite IR-120 (H^+) and IR-45 (OH^-) resins.

Isolation, fractionation, and purification of galactomannan "A". — The seeds were cleaved at low speed in a hand mill. Yellow cotyledons and seed coat were separated by winnowing. The remaining material containing endosperm (200 g) was soaked in water (5 L) and left overnight. The resulting, viscous solution was filtered through muslin, clarified in a centrifuge (25,000 r.p.m.), and white, fibrous polysaccharide (40.6 g) was precipitated with ethanol (8 L). The insoluble barium complex of the crude polysaccharide (15 g) was decomposed with 2M acetic acid (2 L) for 10 h, and galactomannan "A" was recovered by addition of ethanol (4 L) to the acid-soluble fraction. The latter was deionized and alcohol added in stages. The pure polysaccharide had $[\alpha]_D^{20}$ +24.9° (*c* 1.0, water). Nitrogen, sulfur, halogens, uronic acid, and methoxyl were absent; sulfated ash was 0.06%, pentosans 7.24%, and furfural 4.16. The acetylated product had $[\alpha]_D^{24}$ +26.2° (*c* 0.2, acetone) and the deacetylated product $[\alpha]_D^{26}$ +25.3° (*c* 1.0, water).

Electrophoresis. — High voltage electrophoresis was performed on Whatman No. 1 filter paper in borate buffer (0.05M, sodium tetraborate decahydrate) pH 9.2 for 8 h.

Sugar composition. — Sugar components were determined after hydrolyzing the polysaccharide (2 g) with M sulfuric acid (200 mL), followed by p.c. (solvent *e*) of the hydrolyzate. The component sugars were identified as (*i*) D-mannose, m.p. and mixed m.p. 131–132°, $[\alpha]_D^{35}$ +14.4° (*c* 0.2, water); *N*-*p*-nitrophenyl-D-mannosylamine, m.p. and mixed m.p. 217–219°, $[\alpha]_D^{30}$ −326° (*c* 0.6, dry pyridine), (*ii*) D-galactose, m.p. and mixed m.p. 164–165°, $[\alpha]_D^{30}$ +81.2° (*c* 0.6, water); *N*-*p*-nitrophenyl-D-galactosylamine, m.p. and mixed m.p. 213–215°, $[\alpha]_D^{30}$ −246° (*c* 0.5, dry pyridine), and (*iii*) a faint spot of xylose, which moved parallel to an authentic sample.

Methylation. — Polysaccharide "A" (10 g) was methylated 5 times by Haworth's method[8] yielding a light-yellow product (7.32 g), followed once by Shrivastava's procedure[9]; OCH_3, 40.6 (6.6 g), and finally by Purdie's method[10] resulting in a clear yellow, glassy solid (5.9 g); $[\alpha]_D^{26}$ +26.8° (chloroform); OCH_3, 43.6%. The i.r. spectrum of this product in chloroform showed no hydroxyl peak in the 3500 cm^{-1} region. The fully methylated product was extracted with petroleum ether (40–60°) containing various proportions of chloroform.

A portion of the methylated polysaccharide (3 g) was dissolved in methanolic hydrogen chloride (100 mL, 4%), and the mixture was boiled under reflux for 10 h, cooled, made neutral (silver carbonate), filtered, and the filtrate evaporated. The syrupy residue was taken up in M hydrochloric acid (100 mL) and heated for 10 h at 100°. The cooled solution was made neutral (silver carbonate), filtered, deionized, and evaporated to a syrup. P.c. of this syrup (solvents *a*, *b*, and *c*) showed three main spots. When a higher concentration of the syrup was resolved (solvents *a* and *b*), a fourth, faint spot was also observed. Fractions (10 mL) were collected, after performing p.c., and combined into four main fractions. The ratio was determined by the hypoiodite method[13].

Fraction 1 (1.3622 g), $[\alpha]_D^{27}$ −9° (*c* 1.2, water) was identified as 2,3,6-tri-*O*-methyl-D-mannose by p.c. (solvents *a* and *c*), with an authentic sample as reference. Two derivatives were prepared: 2,3,6-tri-*O*-methyl-*N*-phenyl-D-mannosylamine, m.p. 129°, lit.[11] m.p. 127–128°; and 2,3,6-tri-*O*-methyl-D-manno-1,4-lactone, m.p. 82–84°, lit.[12] m.p. 82–83°.

Anal. Calc. for a tri-*O*-methylhexose: OCH_3, 41.8. Found: OCH_3, 41.5.

Fraction 2 (0.4218 g) had $[\alpha]_D^{26}$ −16.1° (*c* 0.9, water). The compound was identified by treatment with *p*-nitrobenzoyl chloride, which gave 2,3-di-*O*-methyl-1,4,6-tri-*O*-*p*-nitrobenzoyl-D-mannose m.p. 193–194°, lit.[5] m.p. 192–194°.

Anal. Calc. for a di-*O*-methylhexose: OCH_3, 29.8. Found: OCH_3, 29.3.

Fraction 3 (0.5316 g) had $[\alpha]_D^{28}$ +104° (*c* 1.0, water); p.c. with solvents *a* and *b* showed only one spot, but with higher concentration two were visible (solvent *b*). The first component was identified as 2,3,4,6-tetra-*O*-methyl-D-galactose, $[\alpha]_D^{30}$ +118° (*c* 1.0, water). Demethylation gave galactose only. With aniline it gave 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine, m.p. and mixed m.p. 194°; lit.[2] m.p. 194°.

Anal. Calc. for a tetra-*O*-methylhexose: OCH_3, 52.5. Found: OCH_3, 52.0.

The second component gave a faint spot, R_F 0.78 in solvent *b*, and moved parallel to an authentic sample of 2,3,4,6-tetra-*O*-methyl-D-mannose. On demethylation, it gave mannose only.

Periodate oxidation. — A solution of polysaccharide "A" (0.11 g) in water (100 mL) was treated with 0.11 M sodium metaperiodate (100 mL) at 5° in the dark. Periodate consumption was monitored by the Fleury–Lange method[14]. The titer became constant after 150 h. The excess of periodate was removed with 1,2-ethanediol, the solution was dialyzed for five days, and then reduced with sodium borohydride (1.5 g). It was made neutral with acetic acid, dialyzed, and evaporated to dryness. The residue was hydrolyzed with 0.5M sulfuric acid (100 mL) for 12 h at 100°. P.c. (solvent *d*) revealed the presence of glycerol (identified as its tri-*p*-nitrobenzoate, m.p. and mixed m.p. 185–186°, lit.[2] m.p. 185–186°); erythritol (m.p. and mixed m.p. 120–121°, its derivative tetra-*O*-tosylerythritol, m.p. and mixed m.p. 165°, lit.[15] m.p. 165–166°); and traces of mannose. Alcohols were determined by the periodate–chromotropic acid method[16].

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REFERENCES

1 M. H. Ewart and R. A. Chapman, *Anal. Chem.*, 24 (1952) 1460–1464.
2 V. P. Kapoor and S. Mukherjee, *Can. J. Chem.*, 47 (1969) 2883–2887.
3 P. Andrews, L. Hough, and J. K. N. Jones, *J. Chem. Soc.*, (1952) 2744–2750.
4 K. M. Daoud, *Biochem. J.*, 26 (1932) 255–263.
5 A. S. Cerezo, *J. Org. Chem.*, 30 (1965) 924–927.
6 M. Abdel-Akher, J. K. Hamilton, R. Montgomery, and F. Smith, *J. Am. Chem. Soc.*, 74 (1952) 4970–4971.
7 S. A. Barker, E. J. Bourne, and D. H. Whiffen, *Methods Biochem. Anal.*, 3 (1956) 213–243.
8 Z. F. Ahmed and R. L. Whistler, *J. Am. Chem. Soc.*, 72 (1950) 2524–2525.
9 H. C. Shrivastava, P. P. Singh, S. N. Harshe, and K. Virk, *Tetrahedron Lett.*, (1964) 493–498.
10 T. Purdie and J. C. Irvine, *J. Chem. Soc.*, 83 (1903) 1021–1037.
11 W. N. Haworth, E. L. Hirst, and H. R. L. Streight, *J. Chem. Soc.*, (1931) 1349–1354.
12 F. Smith, *J. Am. Chem. Soc.*, 70 (1948) 3249–3253.
13 E. L. Hirst, L. Hough, and J. K. N. Jones, *J. Chem. Soc.*, (1949) 928–933.
14 P. F. Fleury and J. Lange, *J. Pharm. Chim.*, 17 (1933) 107–196.
15 J. K. Hamilton and F. Smith, *J. Am. Chem. Soc.*, 78 (1956) 5907–5909.
16 M. Lambert and A. C. Neish, *Can. J. Res.*, 28 B (1950) 83–89