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ISOLATION OF FIVE OLIGOSACCHARIDES FROM THE GALACTOMANNAN OF *CASSIA ABSUS* SEED*

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Abstract—Three disaccharides and two trisaccharides were isolated from *Cassia absus* seed galactomannan employing an improved method of acid hydrolysis, charcoal–celite chromatography and paper chromatography. One disaccharide, 2-*O*-α-D-galactopyranosyl-D-mannose has not so far been reported in seed galactomannans. The oligosaccharides corroborate the structure assigned earlier to this galactomannan and it is now clear that one galactose branch is attached to main chain at C-2.

INTRODUCTION

IT HAS been shown earlier[1,2] that the *Cassia absus* seed galactomannan is composed of D-galactose (1 mole) and D-mannose (3 moles). Methylation and periodate oxidation results[2] showed that mannose units form the main chain and galactose units are present as branches. During the present investigation a number of oligosaccharides have been isolated and characterized which provide additional information regarding the structure of galactomannan and it has now been possible to locate exactly certain linkages which remained undecided during the earlier work.

RESULTS AND DISCUSSIONS

Four disaccharides and two trisaccharides were isolated from the galactomannan employing an improved method of acid hydrolysis. These oligosaccharides were isolated in pure state by charcoal–celite chromatography followed by paper chromatography. Three disaccharides and two trisaccharides, which formed the major components were identified by their optical rotation, complete acid hydrolysis, degree of polymerization, reduction with sodium borohydride and subsequent hydrolysis (in the case of oligosaccharides containing more than one type of sugar), periodate oxidation and preparation of suitable derivatives wherever possible. These were:

Disaccharides: a. 2-*O*-α-D-galactopyranosyl-D-mannose. This disaccharide has not been isolated in any seed galactomannan. However, it has been recently isolated from a microorganism (*Trichosporon fermentans*) by Gorin and Spencer.[3]

b. 4-*O*-β-D-mannopyranosyl-D-mannose.

c. 6-*O*-α-D-galactopyranosyl-D-mannose.

Trisaccharides: a. 4-*O*-(6-*O*-α-D-galactopyranosyl-β-D-mannopyranosyl)-β-D-mannose.

b. 4-*O*-(4-*O*-β-D-mannopyranosyl-β-D-mannopyranosyl)-β-D-mannose.

* Part II in the series "Galactomannan of *Cassia absus* seed".

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[1] V. P. KAPOOR and S. MUKHERJEE, *Current Sci.* **38**, 38 (1969).

[2] V. P. KAPOOR and S. MUKHERJEE, *Can. J. Chem.* **47**, 2883 (1969).

[3] P. A. J. GORIN and M. B. PERRY, *Can. J. Chem.* **46**, 2299 (1968).

A mixture of two tetrasaccharides was also obtained, which could not be resolved since the yields were so small.

The structural features of the galactomannan[2] are that the main chain consists mostly of 1 → 4 linked D-mannose and that the D-galactose units present as side chains are attached to C-6 and C-2 (or C-3) of the main chain. Isolation of 4-*O*-β-D-mannopyranosyl-D-mannose and 4-*O*-(4-*O*-β-D-mannopyranosyl-β-D-mannopyranosyl)-β-D-mannose corroborates the nature of linkage already determined between D-galactose and D-mannose units. Similarly the 1 → 6 linkage between D-galactose and D-mannose (at one of the side chains) gives rise to 6-*O*-α-D-galactopyranosyl-D-mannose and 4-*O*-(6-*O*-α-D-galactopyranosyl-β-D-mannopyranosyl)-β-D-mannose. The latter must have been derived from the galactose unit present as side chain and two mannose units in the chain. So far it had remained undecided as to whether the other galactose side chain was linked at C-2 or C-3 of the mannose unit in the main chain. Isolation of 2-*O*-α-D-galactopyranosyl-D-mannose conclusively proves that the linkage in the side chain is 1 → 2. It may, therefore, be concluded that some mannose must be attached to other mannose unit in the main chain through 1 → 2.

With the results of methylation and periodate oxidation studies[2] reported earlier and the oligosaccharides now isolated, the structure for the galactomannan can be represented as in Fig. 1.

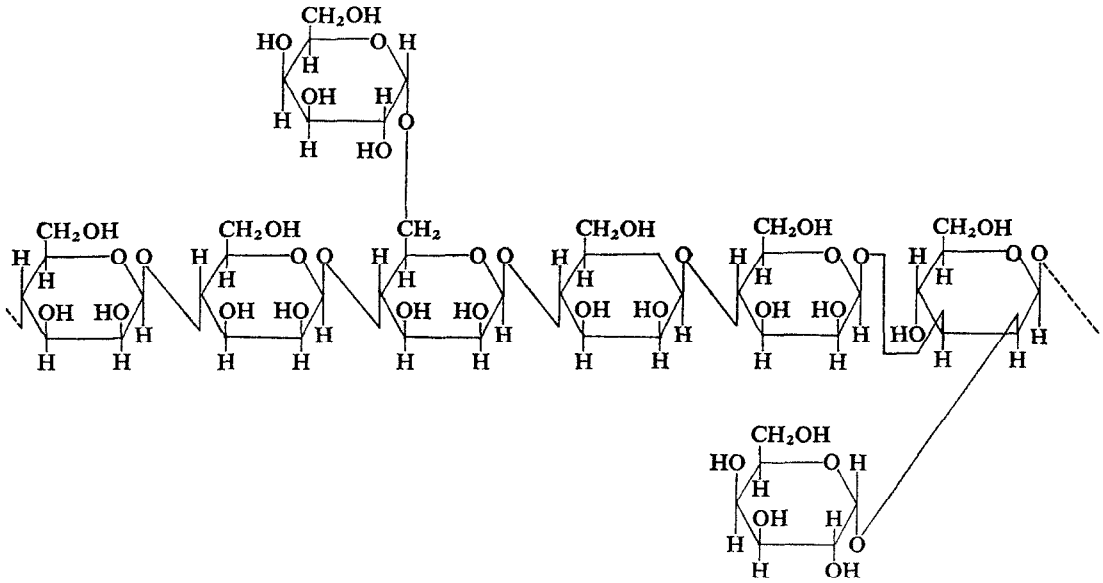

FIG. 1. A PROBABLE STRUCTURE OF *Cassia absus* SEED GALACTOMANNAN.

EXPERIMENTAL

Methods

Unless otherwise stated all evaporations were carried out at 40–50° under reduced pressure. All specific rotations are equilibrium values and m.ps. are uncorrected. Paper chromatography was carried out by descent on Whatman No. 1 paper using non aqueous phases of the following solvents (by vol.): (a) *n*-BuOH–HoAc–H_2O (4:1:5); (b) EtOAc–HOAc–H_2O (9:2:2) and (c) EtOAc–EtOH–H_2O (7:3:2). *p*-Anisidine phosphate[4] was used as spray reagent. Sugar mixtures were separated on columns of charcoal–celite (1:1, w/w) powder and Whatman No. 3MM sheets. Deionization was done with Amberlite 1R-120 (H^+) and 1R-45(OH^-).

[4] S. MUKHERJEE and H. C. SRIVASTAVA, *Nature* **169**, 330 (1952).

Isolation of Oligosaccharides

After a number of trial experiments, the following procedure was found to give maximum yield of oligosaccharides. Conc. H_2SO_4 (50 ml) was added with stirring to ice cold solution of the *Cassia absus* seed polysaccharide (50 g in 1·25 l.), the solution was then heated in a boiling water bath for 10 min and kept immersed in the same bath for another 20 min after removing the source of heat. The hydrolysate was cooled to room temp, neutralized with $BaCO_3$, filtered and concentrated to 400 ml. Material of high degree of polymerization was removed from the hydrolysate by precipitation with alcohol. The degraded polysaccharide was separated from the hydrolysate by filtration and washed with EtOH and acetone. It was dissolved in water (550 ml), added to it conc. H_2SO_4 (22 ml) and hydrolysed under controlled conditions as mentioned above. The reaction mixture was neutralized, filtered and the filtrate was treated with ethanol (500 ml) to precipitate degraded product. The hydrolysates (free from degraded polysaccharide) of both experiments were mixed, deionized by ion exchange resins and concentrated to sirup.

The sugar mixture (in fractions) was separated on charcoal–celite (1:1; w/w) column (60 × 2·5 cm) employing a graded elution method. The column was first eluted with water (2·5 l.) under 6 lbs/in.^{2} pressure to remove monosaccharides. The column was then eluted successively with 2 l. each of 2·5, 5·0, 7·5, 10·0 and 15·0% aq. EtOH (v/v). Each fraction (250 ml) was concentrated and examined by paper chromatography using solvent b or c. It was found that each fraction was a mixture of two or three oligosaccharides. Similar fractions were mixed, concentrated to thin sirups and separated on Whatman No. 3MM sheets using solvent b. In this way four disaccharides, two trisaccharides and one tetrasaccharide were isolated as shown in Table 1.

TABLE 1. NATURE OF OLIGOSACCHARIDES ISOLATED FROM *Cassia absus* SEED GALACTOMANNAN

Fraction No.	R_{gal}* in solvent b	Structure
Disaccharide a	0·78	2-*O*-α-D-galactopyranosyl-D-mannose
b	0·62	4-*O*-β-D-mannopyranosyl-D-mannose
c	0·55	6-*O*-α-D-galactopyranosyl-D-mannose
d	0·51	Unidentified (traces)
Trisaccharide a	0·34	4-*O*-(6-*O*-α-D-galactopyranosyl -β-D-mannopyranosyl)-β-D-mannose
b	0·30	4-*O*-(4-*O*-β-D-mannopyranosyl -β-D-mannopyranosyl)-β-D-mannose
Tetrasaccharide a	0·11	Mixture (traces)

* R_{gal} values are with reference to D-galactose.

Identification of the Oligosaccharides

Disaccharide a, 2-O-α-D-galactopyranosyl-D-mannose. This fraction (142 mg) was purified by dissolving it in aq. MeOH, filtering and evaporating the filtrate to dryness. The sugar having $[\alpha]_D^{25}$ + 81° (C = 1·0, H_2O) was chromatographically pure (lit. +84°).[3] The degree of polymerization, as determined by Timell's method,[5] was 1·76.

The sugar (10 mg) was hydrolysed with N H_2SO_4 (2 ml) in a sealed tube at 100° for 2 hr. On chromatographic examination after usual treatment, D-galactose and D-mannose were found to be present. Estimation of sugar components with phenol-sulphuric acid method[6] showed that disaccharide was composed of galactose and mannose in equimolecular proportions.

The disaccharide (6·8 mg) was dissolved in water (2 ml) and treated with $NaBH_4$ (2%, 1 ml) for 4 hr. The resulting solution was neutralized with N HOAc and hydrolysed with N H_2SO_4 (2 ml) for 2 hr. The hydrolysate was neutralized ($BaCO_3$), filtered and concentrated to sirup. In paper chromatographic examination of the sirup using solvent a only, galactose was detected indicating the reducing end of the disaccharide to be D-mannose.

The disaccharide (98 mg) was refluxed with methanolic HCl (2%, 10 ml) for 15 hr in a boiling water bath. The resulting solution was cooled, neutralized with $AgCO_3$, filtered, concentrated to thin sirup and dried under high vacuum. The sugar glycoside (71·4 mg) was dissolved in water (20 ml) and the solution was

[5] T. E. TIMELL, *Svensk Papperstid.* **63**, 688 (1960).
[6] M. LAMBERT and A. C. NEISH, *Can. J. Chem.* **28(B)**, 83 (1950).

cooled to 0°. A cold solution of sodium metaperiodate (0·2 M, 10 ml) was added to solution and volume was made up to 50 ml. The reaction was carried out at 7° and amounts of periodate consumed and formic acid liberated were estimated[7] at different time intervals. The periodate oxidation was completed in 35 hr corresponding to 3·1 moles of periodate consumed and 1·04 moles of formic acid liberated per mole of disaccharide.

Disaccharide b, 4-O-β-D-Mannopyranosyl-D-mannose

The disaccharide (430 mg) was purified by MeOH as usual and decolourized by charcoal. It was chromatographically pure having $[\alpha]_D^{26}$ − 7·1 → −2·7°, in 17 hr (C = 1·3, H_2O), (lit. −7·7 → 2·2°).[8,9] Acid hydrolysis of the disaccharide produced exclusively D-mannose only and degree of polymerization was found to be 1·8.

The osazone was prepared by dissolving the sugar (50 mg), phenylhydrazine hydrochloride (70 mg) and NaOAc (110 mg) in water (2 ml) and heated for 30 min in a boiling water bath. Upon cooling a yellow crystalline product was precipitated. The derivative was recrystallized with water and had m p. 204–205° (lit. 203–206°).[10]

The periodate oxidation of disaccharide was carried out by previous method at 7° and it was complete in 34 hr It consumed 5·08 moles of periodate liberating 3·06 moles of formic acid.

Disaccharide c, 6-O-α-D-Galactopyranosyl-D-mannose

The chromatographically pure fraction (187 mg) was dissolved in a minimum of water and a large volume of EtOH was added. The solution was heated and filtered. To cold solution was added a volume of *n*-BuOH equal to that of ethanol. The solution was evaporated on a boiling water bath to a slight turbidity. Upon cooling sugar crystallized in the form of cubes. After recrystallization from the same solvent mixture m p. was 203° and $[\alpha]_D^{30}$ + 121·6° (C = 0·6, H_2O) (lit. m.p. 201–203° and $[\alpha]_D$ 120·8 → 124·5).[9]

The degree of polymerization, as determined by Timell's method, was found to be 1·86. The acid hydrolysis of disaccharide afforded D-galactose and D-mannose in equimolecular proportion, as determined by phenol-sulphuric acid method.[6] Reduction of disaccharide with $NaBH_4$ followed by hydrolysis gave galactose only indicating the reducing end to be mannose.

The phenylosazone of sugar (60 mg) was prepared as usual and after recrystallization with 50% aq. EtOH, had m.p. 174–176° (lit. 175–176°).[11] In periodate oxidation at 6°, sugar consumed 6·08 moles of oxidant and liberated 5·10 moles of formic acid per mole of disaccharide after 39 hr.

Trisaccharide a, 4-O-(6-O-α-D-Galactopyranosyl-β-D-mannopyranosyl)-β-D-mannose

The chromatographically pure fraction (179 mg) was dissolved in minimum amount of 50% aq. EtOH by heating, large volume of absolute EtOH was added and it was cooled slowly. The partially crystallized oligosaccharide was obtained which was filtered and when recrystallized from 85% aq. EtOH yielded the sugar in the form of prism. After one more recrystallization from the same solvent m.p. was 227° and $[\alpha]_D^{24}$ + 94·4 → 97·3°, in 15 hr (C = 1·0, H_2O) (lit. m.p. 228–229° and $[\alpha]_D^{25}$ + 93·3 → 98·4°).[9]

The degree of polymerization was found to be 2·78 indicating the sugar was a trisaccharide. Acid hydrolysis of the trisaccharide (12 mg) with N H_2SO_4 produced D-galactose and D-mannose in the molar ratio of 1:1·91, as determined by phenol–sulphuric acid method. Reduction of trisaccharide with $NaBH_4$ followed by acid hydrolysis produced galactose and mannose in approximately equal ratio (by visualization on paper chromatograms), indicating that the one mannose unit which is present at reducing end is removed.

The sugar was partially hydrolysed with 0·1 N H_2SO_4 for 2·5 hr at 100°. After usual treatment, paper chromatographic examination of the sirup, using solvent b revealed the presence of D-galactose, D-mannose, 4-O-β-D-mannopyranosyl-D-mannose, 6-O-α-D-galactopyranosyl-D-mannose and unhydrolysed trisaccharide. In periodate oxidation, reaction was complete after 43 hr when the sugar had consumed 7·05 moles of periodate with concomitant liberation of 3·97 moles of formic acid per mole of trisaccharide.

Trisaccharide b, 4-O-(4-O-β-D-Mannopyranosyl-β-D-mannopyranosyl)-β-D-mannose

The chromatographically pure fraction (30 mg), was purified as usual and it had $[\alpha]_D^{27}$ − 23·8° (C = 0 4, H_2O); (lit −22°).[12] The degree of polymerization of sugar was found to be 2·81 and acid hydrolysis with dil. H_2SO_4 gave only D-mannose. The partial acid hydrolysis of trisaccharide yielded D-mannose, 4-O-β-D-mannopyranosyl-D-mannose and unhydrolysed trisaccharide.

[7] A. N. SHRIVASTAVA, Ph.D. Thesis, p. 93, Agra University, Agra (1959).
[8] R. L. WHISTLER and J. Z. STEIN, *J. Am. Chem. Soc.* **73**, 4187 (1951).
[9] R. L. WHISTLER and D. F. DURSO, *J. Am. Chem. Soc.* **74**, 5140 (1952).
[10] G. O. ASPINALL, R. B. RASHBROOK and G KESSLER, *J. Chem. Soc.* **215** (1958).
[11] R. L. WHISTLER and D. F. DURSO, *J. Am. Chem. Soc.* **73**, 4189 (1951).
[12] M. E. HENDERSON, L. HOUGH and T. J. PAINTER, *J. Chem. Soc.* 3519 (1958).

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