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THE STRUCTURE OF A GALACTOMANNAN FROM THE SEEDS OF *IPOMOEA MURICATA*

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Abstract—The galactomannan from the seeds of *Ipomoea muricata* has been shown to consist of D-galactose and D-mannose in the ratio of 1:1·8. Hydrolysis of the methylated polysaccharide gave 2,3,4,6-tetra-*O*-methyl-D-galactose (3·6 moles), 2,3,6-tri-*O*-methyl-D-mannose (2·8 moles) and 2,3 di-*O*-methyl-D-mannose (3·5 moles). Determination of the end group by periodate method showed, 38·7 per cent which is in fair agreement to that revealed by methylation, 37 per cent. The polysaccharide is highly branched and contains α linked D-galactopyranose end groups combined with 1,4- and 1,4,6-linked β-D-mannopyranose units.

THE seeds of *Ipomoea muricata* were chemically examined for its glycosidic and other constituents by Misra and Tewari.[1] A galactomannan has been isolated as a water soluble component. To the best of our knowledge, it is the first report of a galactomannan from Convolvulaceae.

The polysaccharide was extracted from the ground seeds with cold water containing 1% acetic acid. A thick mucilagenous solution was obtained, from which the galactomannan was separated and purified by repeated precipitation from ethanol. The homogeneity of the polysaccharide was checked by fractional precipitation and via deacetylation of its acetyl derivative. The dry polysaccharide dissolves very slowly in water but easily in alkali; it showed an ash content of 1·2% and had $[\alpha]_D^{25}+56°$. Methoxyl, acetyl and uronide residues were found to be absent.

The purified polysaccharide consisted of galactose and mannose of the molecular proportion 1:1·8. Hydrolysis of the galactomannan with 0·2 N sulphuric acid and chromatographic examination at various intervals indicated that galactose was the first sugar released; mannose was released in detectable amounts only after 30 min. This indicates that the polysaccharide molecule consists of a main chain of mannose and a branched chain containing predominantly galactose units.

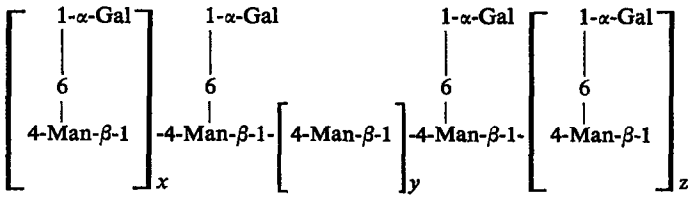
Hydrolysis of the methylated galactomannan yielded 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-mannose and 2,3-di-*O*-methyl-D-mannose which were separated on paper chromatograms and identified by the preparation of crystalline derivatives. Quantitative estimation of the methylated sugars gave a molar ratio of tetra:tri:di, of 3·6:2·8:3·5. The percentage of anhydro-galactose residue calculated from methylation studies was 37 per cent. Thus the galactomannan seems to be highly branched with galactose units occupying the terminal positions, and the branch points on the mannose units at positions 1, 4 and 6.

Determination of the terminal groups by periodate oxidation and subsequent titration of the formic acid liberated, gave a figure of 38·7 per cent which is in close agreement to that revealed by methylation (37 per cent). Oxidized galactomannan showed the presence of

[1] A. L. MISRA and J. D. TEWARI, *J. Indian Chem. Soc.* **30**, 391 (1953).

small amount of D-mannose but no D-galactose. However, prolonged oxidation of the polysaccharide with an excess of sodium metaperiodate resulted in destruction of both hexoses. The considerable differences in rates of oxidation of the galactose and of the mannose are probably attributed to a steric effect resulting from the highly ramified structure of the galactomannan, in which mannose units form all the branching points. Present knowledge, however, indicates that this phenomenon is most likely due to acetal formation.[2] This resistance of some mannose residues to the periodate oxidation has been observed in mannans from guar,[3] fenugreek,[4] lucerne and clover[5] seeds. This indicates that no significant amount of (1–3) linkages are present.

From the above results and by its similarity with the structure of other galactomannans,[3–5] it is postulated that the galactomannan of *Ipomoea muricata* seeds consists of a linear chain of (1–4) β-linked-D-mannopyranose units with D-galactopyranose units attached to 5 out of every 9 mannose units by α-(1–6) linkages. This is in agreement with the specific rotation of the polysaccharide, $[\alpha]_D^{25}+54°$ (NaOH) and its methyl derivative, $[\alpha]_D^{25}+40°$ (chloroform) as compared to other closely related galactomannan of known structures.[3–5] The linkages were further confirmed by i.r. studies[6] of the galactomannan which showed absorption at 815 and 876 cm^{-1}, indicating the presence of α-linked D-galactopyranose units and β-linked D-mannopyranose units respectively.



where Gal = D-galactopyranose, Man = D-mannopyranose

$$x+z=3 \quad \text{and} \quad y=4.$$

A polysaccharide such as that described above should consume 1·4 moles of periodate per mole of anhydrohexose unit. When the galactomannan was oxidized by periodate, the result indicated the consumption of 1·8 mole of periodate per anhydrohexose unit which is in fair agreement to the theoretical values.

EXPERIMENTAL

All specific rotations are equilibrium values and all melting points are uncorrected. Chromatographic separations were carried out at room temperature by descending method using non-aqueous phase of any one of the following solvents: (A) *n*-butanol:ethanol:water (50:10:40); (B) *n*-butanol:acetic acid:water (40:10:50) and (C) Benzene:ethanol:water (169:47:15 part v/v). The spray reagent used was: aniline hydrogen phthalate in *n*-butanol.

[2] A. S. CEREZO, *J. Org. Chem.* **30**, 924 (1965).
[3] R. L. WHISTLER and JOAN Z. STEIN, *J. Am. Chem. Soc.* **73**, 4187 (1951).
[4] P. ANDREW, L. HOUGH and J. K. N. JONES, *J. Chem. Soc.* 2744 (1952).
[5] P. ANDREW, L. HOUGH and J. K. N. JONES, *J. Am. Chem. Soc.* **74**, 4029 (1952).
[6] S. A. BARKER, E. J. BOURNE and D. H. WHIFFEN, *Method of Biochemical Analysis* (Edited by D. GLICK), 3rd ed., p. 213. Interscience, New York (1956).

ISOLATION OF THE GALACTOMANNAN

2 kg of crushed dried seeds of *Ipomoea muricata* were extracted exhaustively with ethanol, and then stirred in 1% acetic acid for 5 hr. This procedure was repeated until no precipitate was obtained when extracts were added to excess of ethanol. The filtered extracts were added slowly to 3 vol. of ethanol with vigorous stirring, and the crude polysaccharide was filtered, washed with ethanol and dried (20 gm).

Purification

300 ml of ethanol was added to the crude product in 1 l of water, and the resinous precipitate filtered. 2 l of ethanol was added slowly with stirring and the solution left overnight. The precipitated polysaccharide was filtered and the process was repeated twice yielding a white amorphous powder (17 gm). $[\alpha]_D^{25}+56°$ (NaOH) slowly soluble in water. The galactomannan is precipitated from aqueous solution with Fehling's solution but does not reduce it.

Homogeneity of the Galactomannan

5 g of the polysaccharide in 300 ml of water was poured into 600 ml of ethanol with continuous stirring. The precipitate was filtered (sample A), and the filtrate diluted with 1 l of ethanol to precipitate the rest of the polysaccharide (sample B). Both samples were hydrolysed and the quantitative estimation of the sugars showed that they were essentially similar.

The pure polysaccharide (1·0 g) and anhydrous sodium acetate (4·0 g) in acetic anhydride (5·0 ml) was heated 12 hr at 100° cooled and filtered. The acetate (0·8 g) after isolation and purification in the usual manner[2] had acetyl, 39·5%; $[\alpha]_D^{25}+20°$ (chloroform).

A portion (0·5 g) was deacetylated[2] and yielded galactomannan (0·2 g); $[\alpha]_D^{25}+52°$ (NaOH).

Hydrolysis of the Galactomannan

The galactomannan (1·0 g) was hydrolysed at 100° with 50 ml of 2 N H_2SO_4 for 12 hr. The derived syrup, on paper chromatographic analysis, revealed spots with the mobilities of galactose and mannose only (solvents A and B). Elution of the syrup from a cellulose column with solvent A gave two fractions: Fraction 1. Crystalline D-mannose, after recrystallization had m.p. and mixed m.p. 132°, $[\alpha]_D+13°$ (water). Fractions 2 gave D-galactose which was recrystallized from aqueous methanol, m.p. and m.m.p. 164–165°, $[\alpha]_D^{25}+78°$ (water). The molecular proportions of the sugars determined by periodate oxidation[7] after separation on chromatograms using solvent A, was 1·00 (galactose) to 1·18 (mannose).

Graded Hydrolysis

50 mg of the galactomannan in 20 ml of 0·2 N H_2SO_4 was hydrolysed at 100° and the hydrolysate examined chromatographically after 10, 15, 30, 60, 100 and 150 min. Mannose could only be detected after 30 min.

Periodate Oxidation

(a) *Liberation of formic acid.*[8] To the galactomannan, 266 mg in 20 ml of water, was added 3 g of KCl and 25 ml of 0·25 M sodium metaperiodate. The volume was made up to

[7] E. L. HIRST and J. K. N. JONES, *J. Chem. Soc.* 1659 (1949).
[8] F. BROWN, T. G. HALSALL, E. L. HIRST and J. K. N. JONES, *J. Chem. Soc.* 28 (1948).

100 ml with water. The oxidation was conducted in dark at room temperature. 5 ml samples were drawn at various intervals and were titrated by 0·0169 N sodium hydroxide after reduction of the excess periodate by ethylene glycol (48 hr 1·6 ml). The amount of formic acid liberated (48 hr) corresponds to 38·7 per cent of terminal hexose units.

After 60 hr, an excess of ethylene glycol (20 ml) was added, and the solution concentrated. 20 mg of this material was hydrolysed in the usual way and examination of the hydrolysis product on paper chromatogram showed the presence of mannose but no galactose.

(b) *Uptake of periodate.*[9] To the galactomannan, 254 mg, in 25 ml of water was added 25 ml of 0·4 M sodium meta-periodate and the volume was made up to 100 ml with water. 5 ml aliquots were titrated against 0·01 N thiosulphate, at various intervals. The amount of metaperiodate consumed after 60 hr (32·5 mg) corresponds to the consumption of 1·8 moles of meta-periodate per mole of anhydrohexose unit.

After 100 hr, the oxidized polysaccharide was examined similarly for the presence of mannose and galactose, as described above. Neither mannose nor galactose was detected.

Methylation of the Galactomannan

The polysaccharide (5 g) was methylated by the method followed by Andrew *et al.*,[5] when a light yellow crispy powder (3·5 g), $[\alpha]_D^{25}+40°$ (chloroform); MeO, 41·2 per cent was obtained.

Hydrolysis of the Methylated Galactomannan

The methylated galactomannan (2·0 g) was hydrolysed with 90% formic acid and then with N H_2SO_4[2], and the methylated sugars separated on Whatman No. 3 filter paper sheets using solvent C.

Fraction I—(150 mg). R_g in solvent A 0·86 (Found: OMe, 51·0, calculated for tetramethyl hexose: OMe, 52·5%), had $[\alpha]_D^{24}+105°$ (C 1·0 in water), lit.,[10] $[\alpha]_D$ 109·5° (water). It gave red colour with *p*-anisidine spray. 100 mg of the syrup was heated with 40 mg of aniline in alcohol which gave crystalline 2,3,4,6 tetra-*O*-methyl D-galactose anilide, m.p. 185–187°; lit.,[11] m.p. 186–188°.

Fraction II—(115 mg). R_g in solvent A 0·80 (Found: OMe, 40·9%, calculated for trimethyl hexose: OMe, 41·8%), had $[\alpha]_D^{24}-11°$ (C 1·1 in water), lit.,[12] for 2,3,6 tri-*O*-methyl-D-mannose, $[\alpha]_D-10$ (water). 100 mg was oxidized with bromine water. The product was crystallized from acetone-ether-petroleum ether, m.p. 82–83°, lit.,[5] for 2,3,6 tri-methyl γ-(+)-mannolactone, m.p. 82–83°.

25 mg of this lactone was boiled under reflux in alcohol with phenylhydrazine (15 mg). It was refluxed with little of animal charcoal in ethanol and filtered. On cooling it deposited a crystalline material. After one crystallization from ethanol it melts at 130°, lit.,[5] m.p. for 2,3,6 trimethyl-D-mannoic acid phenylhydrazide, 131°.

Fraction III—(155 mg). R_g in solvent A 0·52 (Found: OMe, 30·5%, calculated for dimethyl hexose: OMe, 29·8%), $[\alpha]_D^{24}-14°$ (C, 2·2 in water), lit.[13] $[\alpha]_D-15·8°$ (water) for 2,3-di-*O*-methyl-D-mannose.

100 mg of dimethyl hexose and 50 mg of *p*-nitrobenzoyl chloride were dissolved in 4 ml

[9] L. HOUGH and D. B. POWELL, *J. Chem. Soc.* 16 (1960).
[10] J. IRVINE and A. CAMERO, *J. Chem. Soc.* **85**, 1071 (1904).
[11] R. JOHNSTON and E. G. V. PERCIVAL, *J. Chem. Soc.* 1994 (1950).
[12] W. N. HAWORTH, E. L. HIRST and M. M. T. PLANT, *J. Chem. Soc.* 1354 (1931).
[13] G. L. ROBERTSON, *J. Chem. Soc.* 330 (1934).

of pyridine. The mixture was refluxed over water-bath for 1 hr, cooled and extracted with 10 ml of 1% sulphuric acid. The crude mass was recrystallized from ether, when 2,3-di-*O*-methyl-D-mannose, 1,4,6-tri-*p*-nitrobenzoate was obtained, m.p. 191–193°, lit.,[14] m.p. 194°.

QUANTITATIVE EXAMINATION OF METHYLATED SUGARS

45·2 mg of the methylated galactomannan was hydrolysed in 2% methanolic HCl (15 ml) and 14·0 mg of glucose was dissolved in it; finally the mixture was completely hydrolysed in 10 ml of 1 N HCl. The methylated sugars were separated by solvent C, according to the method of Andrew *et al.*[5] The sugars were estimated by alkaline hypoiodite,[15] 0·1 N iodine (2 ml) being used in each case and the reaction mixture kept for 20 hr. After acidification, the excess of iodine was titrated with 0·01 N sodium thiosulphate. The recovery of methylated sugars was calculated on the basis of complete recovery of glucose. Found (results expressed as ml of 0·01 N iodine consumed); tetra, 2·28, 2·73; tri, 1·78, 2·13; di, 2·21, 2·72; glucose, 2·67, 3·34. These figures correspond to an average tetra: tri: di, ratio of 3·6: 2·8: 3·5.

[14] F. SMITH and R. MONTGOMERY, *The Chemistry of Plant Gums and Mucilages*, p. 359. American Chemical Society, Monograph Series (1959).
[15] E. L. HIRST, L. HOUGH and J. K. N. JONES, *J. Chem. Soc.* 928 (1949).