518. *Mannose-containing Polysaccharides. Part II.* The Galactomannan of Fenugreek Seed* (Trigonella fœnum-græcum).

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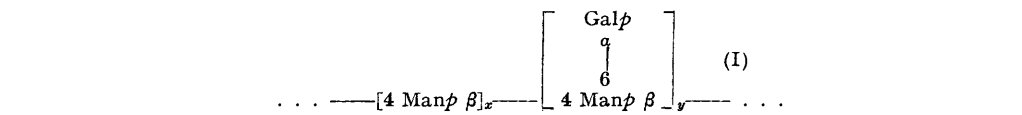
The galactomannan of fenugreek seed has been shown to consist of D-galactose and D-mannose in the approximate ratio 5 : 6. Hydrolysis of the methylated polysaccharide gave 2 : 3 : 4 : 6-tetramethyl D-galactose (5 parts), 2 : 3 : 6-trimethyl D-mannose (1 part), and 2 : 3-dimethyl D-mannose (5 parts). In a consideration of these results, the structure of the galactomannan, and its relation to other galactomannans, is briefly discussed.

The endosperms of many leguminous seeds contain galactomannans (Wise and Appling, *Ind. Eng. Chem. Anal.*, 1944, **16**, 29; Anderson, *Ind. Eng. Chem.*, 1949, **41**, 2887); the proportion of D-galactose to D-mannose in these polysaccharides varies from species to species (Anderson, *loc. cit.*) and possibly within a species (Hirst and Jones, *J.*, 1948, 1278). It is possible that these galactomannans are a mixture of two polysaccharides (*e.g.*, galactan and mannan)—in most cases there is no evidence of homogeneity. Structural investigations of the galactomannans of carob seed (gum gatto) (Hirst and Jones, *loc. cit.*; Smith, *J. Amer. Chem. Soc.*, 1948, **70**, 3249), guar seed (Ahmed and Whistler, *J. Amer. Chem. Soc.*, 1950, **72**, 2524; Rafique and Smith, *ibid.*, 4634; Whistler and Stein, *ibid.*, 1951, **73**, 4187; Whistler and Durso, *ibid.*, p. 4189), clover and lucerne seed (Andrews, Hough, and Jones, *ibid.*, forthcoming publication) suggest that they are not mixtures, and that their structure can be accommodated by the following basic formulation :



(I)

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Man*p* and Gal*p* represent D-mannopyranose and D-galactopyranose residues respectively, linked through the positions specified. The results of *X*-ray diffraction analysis of guar galactomannan (Palmer and Ballantyne, *ibid.*, 1950, **72**, 736) are in agreement with this structure where $x = y$.

If these galactomannans are mixtures of polysaccharides, the only tenable interpretation is that they are composed largely of a branched-chain galactomannan, with a much smaller amount of a linear mannan. This conclusion follows from the observation that, in each case, the terminal units accounted for all the galactose, and that no mannose was detected as end-group. On the balance of the evidence, however, it would appear that the galactomannans of lucerne seed, clover seed, guar seed, and carob seed do not contain mannan. All the galactomannans investigated hitherto contain more mannose than galactose, apart from the lucerne seed galactomannan of Hirst, Jones, and Walder (*J.*, 1947, 1443), which contained twice as much galactose as mannose and was found by these authors to have a structure different from the above (cf. also Andrews, Hough, and Jones, *loc. cit.*). Daoud (*Biochem. J.*, 1932, **26**, 255) has described a galactomannan from fenugreek seed, which was reported to contain the two hexoses in approximately equal amounts. It was of interest, therefore, to investigate the composition and structure of this polysaccharide, since, on the above formulation it would be highly ramified, containing about 50% of terminal galactose units.

The fenugreek (*Trigonella fœnum-græcum*) is a leguminous plant, very similar to clover in appearance, which is grown in N. Africa, Asia Minor, India, and Pakistan. The small brown seeds, which are borne in long pods, are used for various medicinal purposes, and often as a flavouring, *e.g.*, in maize bread.

The polysaccharide was obtained from the milled seed by extraction with cold water. A thick mucilaginous solution was obtained, from which the galactomannan was isolated *via* its copper complex. The seed yielded 13·6% of galactomannan, which is similar to the amount (15%) estimated by Anderson (*loc. cit.*) to be present in the endosperm in fenugreek seed. It was observed, in preliminary experiments, that the seed material which remained after extraction with cold water still contained small amounts of galactomannan, which was apparently of the same composition as that already extracted. The milled, but otherwise untreated, seeds were also extracted by stirring them with Fehling's solution at room temperature; examination of the extract showed that it did not contain more than a trace of polysaccharide. This is taken as evidence that the seeds do not contain a galactan, since we have found that when *Strychnos nux vomica* seeds, which contain water-soluble polysaccharides composed mainly of galactose and mannose, are extracted with Fehling's solution in a similar way, the solution so obtained yields a polysaccharide which, on hydrolysis, gives chiefly galactose but no mannose (unpublished results). Further evidence that this method is capable of separating a galactan from a mannan was found: when a polysaccharide fraction, which had been obtained from the *nux vomica* seeds and gave only galactose and mannose on hydrolysis, was extracted with Fehling's solution, the soluble polysaccharide gave only galactose on hydrolysis. These experiments are being continued.

Hydrolysis of the fenugreek galactomannan, followed by chromatographic separation of the products, yielded crystalline D-galactose and D-mannose. A quantitative estimate of the molecular proportions of the two sugars indicated that they are present in the galactomannan in the approximate ratio 5 : 6.

The polysaccharide was next exhaustively methylated with methyl sulphate–sodium hydroxide. Fission of the product gave mainly tetramethyl and dimethyl hexoses, with only a small amount of trimethyl hexose. After chromatographic separation, the sugars were identified as 2 : 3 : 4 : 6-tetramethyl D-galactose, 2 : 3-dimethyl D-mannose, and 2 : 3 : 6-trimethyl D-mannose respectively, in the approximate molecular proportions of 5 : 5 : 1. The tetramethyl galactose was characterised as its anilide, and the methyl mannoses as their crystalline lactones and phenylhydrazides. Clearly, the galactomannan is highly branched, with all the galactose units occupying the terminal positions and the points of branching situated on mannose units, linked through positions 1, 4, and 6. Formula (I), therefore, illustrates one of its possible structures, where $5x = y$, and it appears to bear a close structural resemblance to the galactomannans of clover, lucerne, guar, and carob

seeds. The methyl derivatives of all these polysaccharides, on hydrolysis, yield the same three methyl sugars, but in different proportions. This difference is due to the degree of branching or amount of end-group, or, more simply, to the galactose content of each polysaccharide.

Periodate oxidation confirmed the methylation results. Thus, the yield of formic acid from fenugreek galactomannan corresponded to a proportion of end-group (43·6%), which is very close to that revealed by methylation (43·3%). As has been found with other galactomannans (Ahmed and Whistler, *loc. cit.*; Andrews, Hough, and Jones, *loc. cit.*), unless a much larger concentration of periodate is used than is specified by Brown, Halsall, Hirst, and Jones (*J.*, 1948, 27) for the chain-length estimations, some of the mannose residues remain unoxidised; in the presence of large excess of periodate, however, the amount of periodate consumed (1·49 mols.) is approximately that indicated by methylation (1·45 mols.). During this oxidation all the sugar residues are destroyed.

Experimental

Sheet-paper partition chromatography was carried out on Whatman No. 1 filter paper by the descending method (Partridge, *Biochem. J.*, 1948, **42**, 238), with the following solvent systems :

	Mobile phase	Duration of chromatography
For unsubstituted sugars	(*a*) *n*-Butanol–ethanol–water (40 : 11 : 19 parts v/v)	40 hours
	(*b*) Ethyl acetate–acetic acid–water (9 : 2 : 2 parts v/v)	16 hours
	(*c*) *n*-Butanol–pyridine–water (10 : 3 : 3 parts v/v)	40 hours
For methylated sugars	See (*a*) above	16 hours
	(*d*) Benzene–ethanol–water (169 : 47 : 15 parts v/v; top layer, clarified with a little ethanol)	4—6 hours

After separation the sugars and methyl sugars were located with ammoniacal silver nitrate (Partridge, *loc. cit.*) or *p*-anisidine hydrochloride (Hough, Jones, and Wadman, *J.*, 1950, 1702). Solvent mixtures (*a*) and (*b*) give a complete separation of galactose and mannose, which are both clearly distinguished from glucose, and (*b*) separates these hexoses from all the pentoses (cf. Jermyn and Isherwood, *Biochem. J.*, 1949, **44**, 402). The rate of movement of methyl sugars is quoted relative to tetramethyl glucopyranose, *i.e.*, R_G value.

Where only approximate values are given for the relative amount of sugars in the hydrolysates, they were obtained by visual estimation of the colour intensity produced with *p*-anisidine hydrochloride, and by the relative spot sizes (Fisher, Parsons, and Morrison, *Nature*, 1948, **161**, 764; Brimley, *ibid.*, 1949, **163**, 215; Fowler, *ibid.*, 1951, **168**, 1123).

Hydrolyses were performed with N-sulphuric acid at 95—100°, and solutions were concentrated under reduced pressure, unless otherwise stated.

M. p.s are uncorrected. Optical rotations were determined at 20°.

Extraction of Polysaccharide from the Seed.—The milled seeds (20 g.) were four times stirred with cold water (600-c.c. portions; *ca.* 1 hour each time). After each extraction the residual seed material (A) was separated on the centrifuge and Fehling's solution added to a small portion of the yellow supernatant liquor; the first two extracts gave a considerable precipitate, whilst the third gave a little, and the last only a trace. The extracts were combined, and a slight excess of Fehling's solution (30 c.c.) added with stirring. During this process, the viscosity of the mixture increased considerably, then suddenly decreased as the copper complex was precipitated. The copper complex was separated on the centrifuge, and the supernatant solution (B) was retained. The polysaccharide was regenerated from the copper complex by suspending the greenish-blue material in ice-cold water (150 c.c.) with vigorous stirring, and adding N-hydrochloric acid dropwise until solution was just complete. Alcohol (250 c.c.) was then added to the solution, and the precipitated polysaccharide isolated on the filter, washed with alcohol and then ether, and dried under reduced pressure. The product (2·5 g.), a white fibrous material, was incompletely soluble in water; it gave no colour with iodine solution. A small portion (*ca.* 5 mg.) of this product was hydrolysed in a sealed tube for 18 hours. The anions present in the resulting sugar solution were removed on Amberlite IR-4B ion-exchange resin, and the neutral solution concentrated, on a watch-glass, to a syrup on the water-bath. Examination on the paper chromatogram indicated the presence of galactose and mannose only, in approximately equal amounts.

The residual material (A; see above) was further extracted with water (500-c.c. portions) at 70°, until the extract no longer gave a precipitate with Fehling's solution; two treatments

sufficed. The mixtures were centrifuged each time, and the final residue (C) was retained. Fehling's solution (25 c.c.) was added to the combined extracts and the resultant insoluble copper complex, after separation from the residual solution (D), was worked up as described above, to give a white product (0·4 g.). A portion of this polysaccharide, on hydrolysis, gave only galactose and mannose, in approximately equal amounts, as indicated by paper chromatography.

The alkaline solutions (B) and (D), containing Fehling's solution, were combined, neutralised with acetic acid, and dialysed against tap-water until free from copper ions. Addition of an excess of alcohol to a portion of this dialysate produced a precipitate, which was isolated and hydrolysed as above. Examination of the liberated sugars on the paper chromatogram indicated the presence of mainly galactose and mannose, with smaller amounts of arabinose and xylose. When Fehling's solution (100 c.c.) was added to the dialysate, a precipitate was formed after about 5 minutes. This copper complex was isolated on the centrifuge, and worked up to give some polysaccharide (0·3 g.), which yielded approximately equal amounts of galactose and mannose on hydrolysis. The reason why this material escaped precipitation when Fehling's solution was first added to the aqueous solutions is not known.

The residual material (C) was further extracted with 5% sodium hydroxide solution (500-c.c. portions) at 70°. Only the first two extracts gave a precipitate with Fehling's solution; they were combined, and the latter reagent (50 c.c.) was added. The precipitated copper complex was isolated, and the polysaccharide regenerated as described above. The product, a white fibrous material (0·7 g.), yielded galactose, mannose, and xylose, in approximately equal amounts, when hydrolysed.

A portion of the seed residue, after extraction with sodium hydroxide, was washed free from alkali with hot water, and heated in N-sulphuric acid on the boiling water-bath for 24 hours. The hydrolysis products were found to contain mainly xylose, with a little glucose and traces of galactose, mannose, and arabinose.

A larger quantity of galactomannan was prepared from the milled seeds (100 g.) by two successive extractions with cold water (each *ca.* 2·5 l.). The polysaccharide was isolated as the copper complex, and regenerated by addition of N-hydrochloric acid as described above. The product, after two precipitations from its aqueous solution with alcohol, was washed with alcohol and ether, and dried under reduced pressure (yield, 13·6 g.) (Found: sulphated ash, 1·4; N, <0·7%), $[\alpha]_D$ +70°±10° (*c*, 0·22 in water; measurements taken on a filtered solution, and concentration determined after evaporation to dryness, by weighing of the residue). This polysaccharide was used in the following experiments; it was incompletely soluble in water and 2N-sodium hydroxide.

Hydrolysis of the Polysaccharide.—Hydrolysis of a small portion (*ca.* 10 mg.), followed by examination on the paper chromatogram, indicated that it contained galactose and mannose only; pentoses were not detected. A larger amount (0·5 g.) was hydrolysed in N-sulphuric acid (30 c.c.); the optical rotation of the solution was constant after 7 hours ($[\alpha]_D$ +43°). The solution was then neutralised with barium carbonate, filtered, and concentrated to a syrup. The mixture of sugars (*ca.* 0·45 g.) was added in aqueous methanol to a column of hydrocellulose (22 × 2 cm.), on which separation was effected by using *n*-butanol, half saturated with water, as the mobile phase, as described by Hough, Jones, and Wadman (*J.*, 1949, 2511). Fractionation of the eluate, followed by evaporation of the appropriate portions, gave crystalline specimens of D-galactose, m. p. and mixed m. p. 165°, $[\alpha]_D$ +80° (*c*, 0·9 in water; equil. value), and D-mannose, m. p. and mixed m. p. 132°, $[\alpha]_D$ +14° (*c*, 1·1 in water; equil. value); both sugars were crystallised from methanol. Other sugars were not detected.

For a quantitative estimation of the two sugars, the galactomannan (51·5 mg.) was hydrolysed in a sealed tube for 8 hours. The hydrolysate was added, quantitatively, to ribose (25·5 mg.), and the solution, after removal of anions with the minimum amount of Amberlite IR-4B resin, was concentrated to a syrup. The sugars were separated on two sheet-paper chromatograms by elution with the butanol–ethanol–water mixture for *ca.* 90 hours and estimated in duplicate by the periodate oxidation method of Hirst and Jones (*J.*, 1949, 1659) (Found: galactose, 2·86, 2·47; mannose, 3·40, 2·97; ribose, 3·22, 2·77 mg.). Complete recovery of the ribose being assumed, these figures indicate that the galactomannan gave, on hydrolysis, galactose, 20·4, 20·5, and mannose, 24·3, 24·6 mg. (both calc. as $C_6H_{10}O_5$), the corresponding galactose : mannose ratios being 1·00 : 1·19 and 1·00 : 1·20.

Periodate Oxidation of the Galactomannan.—The potassium periodate method of Brown, Halsall, Hirst, and Jones (*J.*, 1948, 28) was used; the galactomannan (286·0 mg.) was dispersed in water (75 c.c.) and potassium chloride (3 g.) added, followed by sodium metaperiodate solution (0·36N; 25 c.c.). The reaction mixture was shaken, the polysaccharide dissolving as the

reaction proceeded. Portions (5 c.c.) were taken at intervals, ethylene glycol (2 c.c.) added, and the formic acid content determined by titration with 0·01N-sodium hydroxide [Found (as g. of polysaccharide yielding 1 g.-mol. of formic acid) : 502 (96 hours), 407 (147 hours), 371 (240 hours), 373 (342 hours)]. The formation of 1 g.-mol. of formic acid from 372 g. of galactomannan corresponds to the presence of 43·6% of hexose residues, calc. as $C_6H_{10}O_5$, possessing three contiguous hydroxyl groups in the polysaccharide.

After 342 hours, an excess of ethylene glycol (10 c.c.) was added to the remaining reaction mixture, and the solution, after dialysis to remove inorganic material, was concentrated to dryness. A portion (*ca.* 10 mg.) of the oxidised material (sulphated ash, 7·0%) so obtained was hydrolysed, and examination of the hydrolysis products on the paper chromatogram showed the presence of a considerable amount of mannose, but no galactose.

The oxidised galactomannan (18·5 mg. ; calc. as ash-free material) was hydrolysed in a sealed tube for 15 hours. Ribose (5·2 mg.) was added to the hydrolysate, and the relative amounts of the sugars estimated by the method of Hirst and Jones (*loc. cit.*) (Found : mannose, 0·45; ribose, 0·53 mg.). On the basis of complete recovery of the ribose, these figures indicate that the oxidised material contained *ca.* 4 mg. of mannose (calc. as $C_6H_{10}O_5$), or 22% by weight.

Two further portions of galactomannan (74·3 and 93·8 mg.) were oxidised with excess of sodium metaperiodate : the galactomannan was dispersed in water (20 c.c.), and sodium metaperiodate solution (0·36N; 10 c.c.) added. The reaction mixtures were periodically shaken and kept in the dark at room temperature. The metaperiodate content of each solution was determined after an interval, by making it just alkaline to phenolphthalein by the addition of sodium hydroxide, adding saturated sodium hydrogen carbonate solution (10 c.c.) and an excess of potassium iodide, and titrating the liberated iodine with 0·47N-sodium arsenite [Found (titres of arsenite) : 2·82, 3·67 c.c.]. These figures correspond to periodate uptakes (quoted as g.-mol. of periodate consumed per 162 g. of galactomannan) of : 1·44 (240 hours), 1·49 (312 hours).

The oxidised material was recovered from the second reaction mixture, after dialysis, and a portion was hydrolysed. When the products were examined on the paper chromatogram, neither galactose nor mannose was detected.

Methylation of the Polysaccharide.—The galactomannan (3 g.) was dispersed in water (100 c.c.), and sodium hydroxide solution (40% w/v; 200 c.c.) was added, followed by methyl sulphate (200 c.c.) with vigorous stirring and ice-cooling. After reaction had ceased, solid sodium hydroxide (80 g.) was added cautiously, and then more methyl sulphate (180 c.c.). The reaction mixture was neutralised by addition of glacial acetic acid and, after dialysis, which removed the bulk of the inorganic material, concentrated to *ca.* 50 c.c. under reduced pressure. Acetone (50 c.c.) was added, and methylation continued by the alternate addition, with continuous stirring, of solid sodium hydroxide to an approximate concentration of 40%, and the equivalent amount of methyl sulphate. After six methylations in this manner, with an intermediate dialysis and concentration, the final reaction mixture was neutralised with acetic acid and heated on the water-bath, enough water being added to dissolve the sodium sulphate. The methylated material, which had separated, was isolated by filtration of the hot mixture and dissolved in hot chloroform, and the solution filtered; removal of the solvent gave a product (2·6 g.) with OMe, 40·7%. This material was dissolved in acetone, and remethylated (six times) with methyl sulphate in the presence of sodium hydroxide as described above. The product (2·3 g.), isolated as before, was a crisp yellow solid (Found : OMe, 43·5%), which was fractionated by boiling mixtures of chloroform and light petroleum (b. p. 40—60°). Two main fractions were obtained : one (0·96 g.), soluble in 80% light petroleum, had OMe, 44·1% and $[\alpha]_D$ $+49° \pm 3°$ (*c*, 1·2 in chloroform), and the other (1·17 g.), soluble in 75% light petroleum, had OMe, 43·9% and $[\alpha]_D$ $+51° \pm 3°$ (*c*, 0·8 in chloroform). The fractions, which appeared to be similar, were combined.

Fission of the Methylated Galactomannan.—The methylated material (1·05 g.) was dissolved in methyl alcohol (30 c.c.) containing 2% (w/w) of hydrogen chloride, and the solution boiled under reflux for 7 hours. The warm solution was neutralised by addition of a slurry of silver carbonate in methyl alcohol, then filtered, and the filtrate concentrated to a syrup. The mixture of methylglycosides was hydrolysed in N-hydrochloric acid (30 c.c.) at 95—100° for 16 hours. This solution was neutralised with silver carbonate (a slurry in water), filtered, treated with hydrogen sulphide, again filtered, and concentrated to a syrup (1·00 g.).

Examination of the mixture of sugars on the paper chromatogram indicated that it consisted mainly of a tetramethyl and a dimethyl hexose, with a small amount of a trimethyl hexose, and traces of a monomethyl hexose.

Qualitative Examination of the Methyl Sugar Mixture.—The mixture (*ca.* 1·0 g.) was separated

by partition chromatography on a column of hydrocellulose (28 × 4 cm.), with a mixture of benzene (170 parts) and ethanol (50 parts), nearly saturated with water, as the mobile phase. The automatic receiver-changer described by Hough, Jones, and Wadman (*J.*, 1949, 2511) was used to fractionate the effluent into portions of *ca.* 5 c.c. After 250 c.c. had been collected, the eluant was modified by the addition of about one-quarter its volume of methyl alcohol; this mixture served to remove the di- and mono-methyl hexoses from the column. The solvent mixture in each tube was allowed to evaporate, at room temperature, to a volume of 1—1·5 c.c. One or two drops of the liquid from appropriate tubes were then placed in order on two paper chromatograms, which were run in solvent mixtures (*a*) and (*d*) (see above). After consideration of the results obtained by developing these chromatograms, the effluent from the column was divided into four fractions: (1), tubes 19—29, containing tetramethyl hexose; (2), tubes 30—60, containing a little tetramethyl hexose and all the trimethyl hexose; (3), tubes 80—120, containing dimethyl hexose; and (4), tubes 120—150, containing traces of monomethyl hexose.

The solvent was removed from these fractions, and the resultant syrups purified by solution in, successively, water, acetone, and ether or acetone–ether, with intermediate filtration and removal of the solvent. After being dried at 50°/10 mm. for three hours, the fractions were further examined as follows:

Fraction (1) (0·47 g.) (Found: OMe, 51·0. Calc. for tetramethyl hexose: OMe, 52·5%) had $[\alpha]_D$ +105° (*c*, 1·3 in water). Its rate of movement [R_G 0·95 in (*a*); 0·94 in (*d*)] on the paper chromatogram was identical with that of 2 : 3 : 4 : 6-tetramethyl galactose, and it gave the same red colour with the *p*-anisidine spray. A portion of the syrup (0·1 g.) when heated in alcohol with aniline (40 mg.) gave crystalline 2 : 3 : 4 : 6-tetramethyl D-galactose anilide (0·1 g.), m. p. and mixed m. p. 188°, $[\alpha]_D$ −79° (initial) ⟶ +39° (equil. value; *c*, 1·2 in acetone) after recrystallisation from ethanol (Found: N, 4·5. Calc. for $C_{16}H_{25}O_5N$: N, 4·5%).

Fraction (2) (90 mg.), $[\alpha]_D$ +6° (*c*, 0·9 in water), contained approx. 10% of tetramethyl galactose (R_G as above), the remainder being trimethyl hexose [R_G 0·89 in (*a*) and 0·57 in (*d*); corresponding to 2 : 3 : 6-trimethylmannose]. The latter was obtained free from the former by separation on a large sheet paper chromatogram, elution with the benzene–ethanol–water mixture, and Soxhlet-extraction with hot aqueous methanol of the appropriate portion of the paper chromatogram. The syrupy sugar (60 mg.) (Found: OMe, 41·0. Calc. for trimethyl hexose: OMe, 41·8%), obtained in this manner, had $[\alpha]_D$ −4° (*c*, 1·2 in water). After oxidation with bromine water, it gave 2 : 3 : 6-trimethyl γ-D-mannolactone (45 mg.); this was recrystallised from acetone–ether–light petroleum, and then had m. p. 82—83°, undepressed on admixture with an authentic specimen, and $[\alpha]_D$ +62° (initial; *c*, 0·6 in water) ⟶ +58° (40 hours; mutarotation incomplete) (Found: OMe, 40·9. Calc. for $C_9H_{16}O_6$: OMe, 42·2%). A portion of the lactone (25 mg.) was boiled under reflux in alcohol with phenylhydrazine (12 mg.). The resultant phenylhydrazide was separated from coloured by-products on a sheet-paper chromatogram with solvent mixture (*a*); after location with ammoniacal silver nitrate, it was extracted from the paper with hot ethanol. After crystallisation from this solvent, it was identified as 2 : 3 : 6-trimethyl D-mannonic acid phenylhydrazide (15 mg.), m. p. and mixed m. p. 131°, $[\alpha]_D$ −19° (*c*, 0·7 in water) (Found: N, 8·2. Calc. for $C_{15}H_{24}O_6N_2$: N, 8·4%).

Fraction (3) (0·40 g.) (Found: OMe, 30·1. Calc. for dimethyl hexose: OMe, 29·8%), $[\alpha]_D$ −14° (*c*, 3·2 in water), gave one spot only on the chromatogram [R_G 0·67 in (*a*)], corresponding to 2 : 3-dimethyl mannose. A portion (0·2 g.), when oxidised with bromine water, yielded a crystalline lactone (0·16 g.) which after recrystallisation from acetone–ether was identified as 2 : 3-dimethyl γ-D-mannonolactone, m. p. and mixed m. p. 110°, $[\alpha]_D$ +59° (initial) ⟶ +55° (68 hours, mutarotation incomplete; *c*, 0·8 in water) (Found: OMe, 28·7. Calc. for $C_8H_{14}O_6$: OMe, 30·1%). The lactone (80 mg.) was boiled with phenylhydrazine (35 mg.) in alcoholic solution, and yielded 2 : 3-dimethyl D-mannonic phenylhydrazide (45 mg.), which, after recrystallisation from ethyl alcohol, had m. p. 167° and $[\alpha]_D$ −22° (*c*, 0·9 in water) (Found: N, 9·0. Calc. for $C_{14}H_{22}O_6N_2$: N, 8·9%).

Fraction (4) (*ca.* 7 mg.) had a rate of movement on the chromatogram approximating to that of a monomethyl hexose; it was not further examined.

Quantitative Examination of the Methyl Sugars.—The methylated galactomannan (43·1 mg.) was heated in 2% methanolic hydrogen chloride (10 c.c.) under reflux for 6 hours. Glucose (13·4 mg.) was then dissolved in the solution, which was neutralised with silver carbonate, filtered, and concentrated to a syrup; the glycosides were hydrolysed in N-hydrochloric acid (10 c.c.) on the boiling water-bath for 15 hours. The solution, after cooling, was neutralised by adding the minimum amount of Amberlite IR-4B ion-exchange resin; after filtering, the solution was treated with a small amount of Amberlite IR-100 resin and again filtered, and the

neutral solution concentrated to a syrup (50 mg.). Portions of the syrup, dissolved in acetone, were applied to the base-line of each of two sheet-paper chromatograms, and the acetone allowed to evaporate. The sugars were separated by use of solvent mixture (*d*), and, after location on test strips, were extracted with hot water (6—7-c.c. portions). The methyl sugars were estimated by alkaline hypoiodite (Hirst, Hough, and Jones, *J.*, 1949, 928; with the phosphate buffer recommended by Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289), 0·1N-iodine (2 c.c.) being used in each case, and the reaction mixtures kept for 20 hours (cf. Jones, *J.*, 1950, 3292). After acidification, the excess of iodine was titrated with 0·01N-sodium thiosulphate [Found (results expressed as c.c. of 0·01N-iodine consumed): "tetra," 3·00, 2·58; "tri," 0·58, 0·48; "di," 2·94, 2·50; glucose, 2·52, 2·12]. These figures correspond to tetra : tri : di molecular ratios of 5·00 : 0·96 : 4·90 and 5·00 : 0·93 : 4·86, and the weight of methylated polysaccharide accounted for is, respectively, 39·3, 39·9 mg. (average recovery, 92%).

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