Structure of the Polysaccharides from Unripe Mango (*Mangifera Indica* LINN). III

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Crude polysaccharide was isolated in a 7% yield from the mesocarp of unripe mango (*Mangifera Indica* LINN) by extracting it with hot water and precipitating it with ethanol, as has been described in an earlier communication.[1] The material had a $[\alpha]_D^{30}$ +151.3° in 2% sodium hydroxide (*c* 0.5) and uronic anhydride, 12.3%. On hydrolysis, the neutral portion of the above polysaccharide was found to contain glucose (79.3%) and arabinose (6.98%), while the acidic portion was found to contain galacturonic acid. These results show that the percentages of arabinose and uronic anhydride are greater than those present in the crude polysaccharide isolated from ripe mango.[1]

The polysaccharide did not yield any complex with calcium chloride, cetavlon or Fehling's solution. Hence, the purification of the crude polysaccharide was effected by dissolving it in water and precipitating it with ethanol (70%), which has been reported[2] to dissolve the araban preferentially. After four successive precipitations, the uronic anhydride content decreased to 8.78% and the specific rotation changed to +158.8°. On quantitative hydrolysis and estimation, the percentages of anhydro glucose and anhydro arabinose were found to be 85.9 and 3.5 respectively. Further repetition of the fractionation process could not bring about any appreciable change in the percentage composition of the sugar components or in the specific rotation value of the polysaccharide. It was shown in the case of ripe mango polysaccharide that the trace of arabinose which originally appeared in the crude polysaccharide completely disappeared after purification, and that the uronic acid portion originated from an associated uronan component (pectic acid). In the present case also, since the arabinose and uronic anhydride content gradually decrease during fractionation, it is reasonable to believe that the galacturonic acid and arabinose come from galacturonan and araban components, associated as pectic constituents with the glucan.

The purified polysaccharide gives a violet colour with iodine, but it remains unaffected on diastase treatment. This behaviour is similar to that of the glucan from ripe mango.[1]

The purified polysaccharide was methylated according to the procedure of Falconer and Adams.[3,3a] The fully methylated product, obtained in the form of light yellow flakes, was highly soluble in chloroform; OCH_3, 42.3%, $[\alpha]_D^{30}$ +173.5°, in chloroform (*c* 1). The methylated product was methanolised and separated into neutral and acidic components. The neutral sugars were identified as shown in the Table I.

The uronic acid portion was identified as 2, 3-di-*O*-methyl-D-galacturonic acid. The isolation of only 2, 3-di-*O*-methyl-D-galacturonic acid from the hydrolysate of the methylated

1) A. Das and C. V. N. Rao, *Tappi*, **47**, No. 6, 339 (1964).
1a) "The Constitution of the Glucan from Mango," Part II: A. Das and C. V. N. Rao, in press.
2) F. Ehrlich and F. Schuber, *Biochem. Z.*, **203**, 343 (1928).
3) E. L. Falconer and G. A. Adams, *Can. J. Chem.*, **34**, 338 (1956).
3a) G. A. Adams, ibid., **36**, 755 (1958).

TABLE I. NEUTRAL METHYL SUGARS FROM METHYLATED POLYSACCHARIDE

Spot No.	Component	Derivative	R_G*
1	2,3,4,6-Tetra-*O*-methyl-D-glucose	Anilide	1.0
2	2,3,5-Tri-*O*-methyl-L-arabinose	Amide	0.94
3	2,3,6-Tri-*O*-methyl-D-glucose	1,4-Di-*p*-nitrobenzoate	0.82
4	2,4,6-Tri-*O*-methyl-D-glucose	Anilide	0.75
5	2,3-Di-*O*-methyl-L-arabinose	1,4-Di-*p*-nitrobenzoate	0.62
6	2,3-Di-*O*-methyl-D-glucose	Phenylhydrazide	0.55
7	2-*O*-Methyl-L-arabinose	Pheylhydrazone	0.37
8	L-Arabinose		0.14

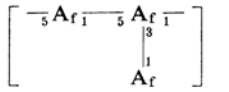
* R_G values are with respect to 2,3,4,6-tetra-*O*-methyl-D-glucose in solvent E.

polysaccharide corroborates the earlier contention that the galacturonic acid originates from the pectic acid component associated with the neutral polysaccharide.

Hence, in view of the above results, it is reasonable to believe that the polysaccharide is a mixture of three components, viz., a glucan, an araban and a galacturonan. On the basis of this, structures for the repeating units of the glucan and araban can be assigned.

The methyl sugars of glucose, viz., 2, 3, 4, 6-tetra-, 2, 3, 6- and 2, 4, 6-tri-, and 2, 3-di-*O*-methyl-D-glucose, occur in the ratio of 1 : 8.84 : 9.0 : 1.14, which is the same as that found in the case of the glucan from ripe mango. Also, the behaviour of this polysaccharide towards diastase and iodine is the same. Hence in view of similar arguments,[1,1a] it is rational to assign the same structure to the repeating unit of the glucan portion.

It has already been mentioned that arabinose occurs from an associated araban; otherwise, it is difficult to account for the isolation of three different methyl sugars of arabinose in sufficient quantities. The mole ratio between the methyl sugars of arabinose (2, 3, 5-tri- : 2, 3-di- : 2-*O*-methyl-L-arabinose) was 1 : 1.15 : 0.94. The characterisation of 2, 3, 5-tri-*O*-methyl-L-arabinose shows that the non-reducing ends of the araban are occupied by arabinofuranose residues. The presence of only one kind of dimethyl sugar, viz., 2, 3-di-*O*-methyl-L-arabinose, indicates that the backbone chain is probably constituted of 1→5 linked arabinofuranose residues. That the araban is highly branched and that the arabinose unit at the branch point is linked through C_1, C_3 and C_5 is proved by the isolation of considerable amounts (about 33% of the total araban) of 2-*O*-methyl-L-arabinose. The isolation of a very small amount of arabinose (from the hydrolysate of the methylated polysaccharide) that might have come from a doubly-branched unit in the araban or might have resulted from incomplete methylation and/or demethylation, seems to have no structural significance. Hence, one of the simplest structures for the repeating unit of the araban, one which satisfactorily fits the above facts, is as follows:



where A_f represents an arabinofuranose unit. From the specific rotation of the polysaccharide (a mixture of three components), it is not logical to draw any conclusion about the type of linkage (α or β). However, the glucan and the galacturonan in the polysaccharide from ripe mango have been shown to be predominantly α-linked; arabans of a similar structure isolated from other sources[4,5] were found to contain α-linkage. Hence, in this case also, all the three components of the polysaccharide can be regarded as having predominantly an α-type of linkage. From these results, it appears that the pectic constituents gradually decrease with ripening.

Experimental

Paper chromatography was carried out with Whatman No. 1 filter papers, by the descending method, the solvent being allowed to run against the machine line. The following solvent mixtures (v/v) were used: (A) ethyl acetate : pyridine : water (8 : 2 : 1); (B) *n*-butanol : acetic acid : water (4 : 1 : 5), upper layer; (C) ethyl acetate : acetic acid : water (9 : 2 : 2); (D) ethyl methyl ketone - water azeotrope; (E) *n*-butanol : ethanol : water (4 : 1 : 5), upper layer.

The crude polysaccharide was a white amorphous powder with the following characteristics: moisture, 10.9%; ash, 0.56%, uronic anhydride,[6] 12.3%, methoxyl, 1.3%, nitrogen, traces, lignin,[7] nil, $[\alpha]_D^{30}$ +151.3° in a 2% sodium hydroxide solution (*c* 0.5). A small portion (ca. 0.5 g.) of the above product was hydrolysed (while being followed iodometrically) with N sulphuric acid by heating it (12 hr.) on a boiling water bath. After neutralisation (barium

4) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, **1947**, 1221; **1948**, 2311.
5) E. L. Hirst and J. K. N. Jones, ibid., **1939**, 454.
6) C. Doree, "The Methods of Cellulose Chemistry," 2nd ed., Chapman & Hall Ltd., London (1947), p. 381.
7) C. Doree, ibid., p. 369.

carbonate) and filtration, the solution was concentrated to a small volume (10 ml.). Paper chromatography with solvent A indicated the presence of glucose and arabinose, while that with solvents B and C indicated galacturonic acid in addition to glucose and arabinose.

Estimation of the Components in the Polysaccharide.—The polysaccharide (659.5 mg.) was hydrolysed with N sulphuric acid (65 ml.) by heating it (12 hr.) in a sealed tube on a boiling water bath. The hydrolysate was then neutralised (barium carbonate) in the presence of D-ribose (200.8 mg.), filtered, and concentrated (25 ml.). An aliquot portion (0.1 mol.) of the solution was separated on a paper chromatogram (solvent A), and strips corresponding to glucose and arabinose were eluted with water and the sugars therein estimated by the periodate method.[8] Results: anhydro arabinose, 6.98% and anhydro glucose, 79.3%.

Separation and Identification of the Components.—The polysaccharide (1 g.) was hydrolysed (N sulphuric acid, 100 ml.) by heating it (12 hr.) on a boiling water bath. The solution was neutralised (barium carbonate) and deionised through Amberlite IR-120(H).* The acidic component from the resulting solution was absorbed on a column of Dowex 1-X4,** and the neutral solution was evaporated to a syrup (0.86 g.). The latter was put on top of a cellulose column (2.5×60 cm.) and resolved (solvent A) into three fractions. Fraction 1 (63.4 mg.), with $[\alpha]_D^{30}$ +104° in water (*c* 1), R_G, 1.6 (with reference to D-glucose, solvent A) was chromatographically pure and was identified as L-arabinose through the preparation of crystalline *p*-nitro-*N*-phenyl-L-arabinosylamine; m. p. and mixed m. p. 200—201°C; lit.[9] 202°C. Fraction 2 (35.5 mg.) had $[\alpha]_D^{30}$ +63.5° in water (*c* 0.6) and was paper chromatographically found to be a mixture of glucose and arabinose. Fraction 3 (721 mg.), with $[\alpha]_D^{30}$ +52° in water (*c* 1), R_G, 1 (with respect to D-glucose, solvent A), was chromatographically pure and identified as D-glucose through the preparation of crystalline *p*-nitro-*N*-phenyl-D-glucosylamine; m. p. and mixed m. p. 182—183°C, lit.[9] 184°C; $[\alpha]_D^{30}$ −199° in pyridine (*c* 1), lit.[9] −201°.

Characterisation of the Acidic Component.—The acidic component was eluted from the Dowex 1-X4 column after thorough washing with water (20 l.), neutralised (barium carbonate), and deionised (Amberlite IR-120(H)). The resulting solution was evaporated to a syrup (105 mg.) with an equivalent weight, 205 (calcd. for monouronic acid, 194). The syrup (ca. 50 mg.) was then converted to its methyl ester methyl glycoside and reduced with lithium aluminium hydride[10] to the corresponding neutral sugar, which was identified as D-galactose through the preparation of crystalline *p*-nitro-*N*-phenyl-D-galactosylamine; m. p. and mixed m. p. 217—218°C, lit.[9] 219°C; $[\alpha]_D^{30}$ −245°C in pyridine (*c* 0.5), lit.[9] −248°.

Purification of the Polysaccharide.—The crude polysaccharide (25 g.) was dispersed in water (2 l.) by heating it (4 hr.) on a boiling water bath. The resultant slightly hazy solution was passed through the super centrifuge twice, and the polysaccharide was precipitated by pouring the solution into acidified (pH 4) alcohol whose final volume had been adjusted to 70% with respect to alcohol. The precipitate was collected by centrifugation, followed by the usual process. This product was found to contain uronic anhydride, 9.3%, $[\alpha]_D^{30}$ +152.8° in 2% sodium hydroxide (*c* 0.5). On hydrolysis and quantitative estimation, the material was found to contain anhydro glucose (82.2%) and anhydro arabinose (5.4%). The process of precipitation was repeated three times, after which the final product was found to have the following characteristics: moisture, 11.2%; ash, 0.47%; uronic anhydride, 8.78%; methoxyl, 0.5%; nitrogen, nil; $[\alpha]_D^{30}$ +158.8° in 2% sodium hydroxide (*c* 0.5); anhydro glucose, 85.9%, and anhydro arabinose, 3.5%. Further fractionation could not bring about any substantial change in the relative proportions of the constituent sugars.

Treatment of the Polysaccharide with Diastase.[1]—The polysaccharide (112 mg.) was dissolved in an acetate buffer (25 ml., pH 4.6); diastase (98 mg.) was added and incubated at 35°C. It was found that, even after 24 hr., the iodine-staining power remained intact; paper chromatographic examination of the digest failed to detect the presence of any reducing sugars.

Methylation and Methanolysis of the Polysaccharide.—The polysaccharide (10 g.) was methylated by the procedure of Falconer and Adams[3,3a] to yield a product (5.3 g.) with $-OCH_3$, 42.3% and $[\alpha]_D^{20}$ +173.5° in chloroform (*c* 1). The methylated product (2 g.) was methanolysed by refluxing it with methanolic hydrogen chloride (200 ml., 2%) for 16 hr., whereupon the optical rotation became constant. The solution was neutralised (silver carbonate) and evaporated to a syrup. The syrup was then heated with a saturated solution of barium hydroxide[11] (50 ml.) on a water bath. Baryta was precipitated out by carbon dioxide and the uronic acid portion was absorbed on a column of Dowex 1-X4 after the usual treatments. The neutral sugar glycosides were hydrolysed to the corresponding methyl sugars by heating them (14 hr.) with N hydrochloric acid on a water bath. The hydrolysate was neutralised (silver carbonate), filtered, and deionised (Amberlite IR-120(H) and IR-45(OH)). The resulting solution was evaporated to a syrup (1.78 g.).

The syrup (1.78 g.) was resolved (solvent D) through a cellulose column (3.5×70 cm.) into fourteen fractions, in which the amounts of methyl sugars of arabinose, in the pure state, were 9.3 mg. of 2,3,5-tri-*O*-methyl-L-arabinose, 10.6 mg. of 2,3-di-*O*-methyl-L-arabinose, and 11.2 mg. of 2-*O*-methyl-L-arabinose, along with 3.4 mg. of arabinose, therefore, another lot of the methylated polysaccharide (2 g.)

8) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, **1949**, 1659.

* A product of the Rohm & Hass Company, Pennsylvania, U. S. A.

** A product of the J. T. Baker Chemical Co. Phillipsburg, N. J., U. S. A.

9) F. Weygand, W. Perkow and P. Kuhner, *Chem. Ber.*, **84**, 594 (1951).

10) M. A. Akher and F. Smith, *Nature*, **166**, 1037 (1950).

11) G. G. S. Dutton and F. Smith, *J. Am. Chem. Soc.*, **78**, 2505, 3744 (1956).

was hydrolysed in the same way, and the neutral fraction of methyl sugars was separated on a similar column. Fractions corresponding to pure methyl sugars of arabinose were combined with the previous pure fractions and used in the characterisations. The ratio between the methyl sugars of glucose and that between the methyl sugars of arabinose were determined from the amounts of each sugar obtained from first column chromatography (the amount of a sugar in a mixture was calculated from the specific rotation of the mixture).

Fraction 1 (58.5 mg.), with $[\alpha]_D^{30}$ +81° in water (*c* 1) and $-OCH_3$, 51.8%, was chromatographically (solvent D) homogeneous, and the sugar in it was identified as 2,3,4,6-tetra-*O*-methyl-D-glucose through the preparation of a crystalline aniline derivative, m. p. and mixed m. p. 136—138°C; lit.[12] 136—138°C.

Fraction 2 (28.8 mg.) had $[\alpha]_D^{30}$ +64° in water (*c* 0.4) and was chromatographically (solvent E) identified as a mixture of 2,3,5-tri-*O*-methyl-L-arabinose (4.6 mg.) and 2,3,4,6-tetra-*O*-methyl-D-glucose (24.2 mg.).

Fraction 3 (9.3 mg.), with $[\alpha]_D^{29}$ −35.5° in water (*c* 0.5), lit.[18] −39.5°, and $-OCH_3$, 48.2% (calcd. for a tri-*O*-methyl pentose: OMe, 48.4%), was chromatographically (solvent D) pure. On demethylation, it produced arabinose, along with some partially demethylated sugars. The fraction was identified as 2,3,5-tri-*O*-methyl-L-arabinose through the preparation of crystalline 2,3,5-tri-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 134—136°C, lit.[4] 138°C.

Fraction 4 (33.2 mg.), with $[\alpha]_D^{29}$ +58.9° in water (*c* 0.5), gave glucose and arabinose on demethylation; it was identified by paper chromatography as a mixture of 2,3,5-tri-*O*-methyl-L-arabinose (3.2 mg.) and 2,3,6-tri-*O*-methyl-D-glucose (30 mg.).

Fraction 5 (661.7 mg.), with $[\alpha]_D^{29}$ +67° in water (*c* 1), OCH_3, 42% (corresponding to that of a tri-*O*-methyl hexose), was found to be pure 2,3,6-tri-*O*-methyl-D-glucose through the preparation of crystalline 2,3,6-tri-*O*-methyl-D-glucose-1,4-di-*p*-nitrobenzoate, m. p. and mixed m. p. 190°C, lit.[14] 190—192°C; $[\alpha]_D^{27}$ −33° in chloroform (*c* 0.6), lit.[14] −34°.

Fraction 6 (70 mg.), with $[\alpha]_D^{30}$ +69.1° in water (*c* 0.5), on demethylation yielded glucose; it was paper chromatographically (solvent E) identified as a mixture of 2,3,6- (40 mg.) and 2,4,6-tri-*O*-methyl-D-glucose (30 mg.).

Fraction 7 (689.2 mg.), with $[\alpha]_D^{30}$ +72° in water (*c* 1), OCH_3, 41.5%, was identified as pure 2,4,6-tri-*O*-methyl-D-glucose through the preparation of a crystalline aniline derivative, m. p. and mixed m. p. 161—163°C, lit.[15] 162—166°C; $[\alpha]_D^{28}$ −111° in methanol (*c* 0.6), lit.[15] −113°.

Fraction 8 (28.7 mg.), with $[\alpha]_D^{30}$ +74° in water (*c* 0.34), on demethylation produced glucose and arabinose; it was identified by paper chromatography (solvent E) as a mixture of 2,4,6-tri-*O*-methyl-D-glucose (25 mg.) and 2,3-di-*O*-methyl-L-arabinose (3.7 mg.).

Fraction 9 (10.6 mg.), with $[\alpha]_D^{30}$ +88° in water (*c* 0.5), OCH_3, 34.2% (calcd. for a di-*O*-methyl pentose: OCH_3, 34.8%), was chromatographically pure, and on demethylation it gave arabinose. The sugar in it was identified as 2,3-di-*O*-methyl-L-arabinose through the preparation of a crystalline 1,4-di-*O*-*p*-nitrobenzoate derivative, m. p. and mixed m. p. 150—151°C; lit.[16] 150—153°C.

Fraction 10 (13.2 mg.), with $[\alpha]_D^{30}$ +75° in water (*c* 0.45), on demethylation furnished glucose and arabinose; it was identified by paper chromatography as a mixture of 2,3-di-*O*-methyl-L-arabinose (5.4 mg.) and 2,3-di-*O*-methyl-D-glucose (7.8 mg.).

Fraction 11 (78 mg.), had $[\alpha]_D^{29}$ +66.5° in water (*c* 1) and OCH_3, 29.8% (calcd. for a di-*O*-methyl hexose: OCH_3, 29.8%). The syrup gave glucose on demethylation, and it was identified as 2,3-di-*O*-methyl-D-glucose through a crystalline phenylhydrazide derivative, m. p. and mixed m. p. 159—160°C; lit.[12] 160—162°C.

Fraction 12 (14.2 mg.) with $[\alpha]_D^{28}$ +85.7° in water (*c* 0.5), produced glucose and arabinose on demethylation; it was identified by paper chromatography (solvent D) as a mixture of 2,3-di-*O*-methyl-D-glucose (9.2 mg.) and 2-*O*-methyl-L-arabinose (5 mg.).

Fraction 13 (11.2 mg.), with $[\alpha]_D^{29}$ +98° in water (*c* 0.45) and OCH_3, 18% (calcd. for a mono-methyl pentose: OCH_3, 18.9%), produced arabinose on demethylation. The sugar in it was identified as 2-*O*-methyl-L-arabinose through its crystalline phenylhydrazine derivative, m. p. and mixed m. p. 114—115°C; lit.[4,17] 115—116°C.

Fraction 14 (3.4 mg.), with $[\alpha]_D^{29}$ +103° in water (*c* 0.25), had the same mobility in the paper chromatogram as that of authentic L-arabinose.

Characterisation of Methyl Uronic Acid Portion. —The uronic acid portion was eluted out of the Dowex 1-X4 column with N sulphuric acid (500 ml.) after the column had been thoroughly washed with water. The acid was neutralised (barium carbonate), and the solution after neutralisation (Amberlite IR-120(H)) was evaporated to a syrup (163.8 mg.). This was hxdrolysed with N sulphuric acid and neutralised (barium carbonate), and the resulting solution after deionisation (Amberlite IR-120(H)) was evaporated to a syrup (157 mg.). The syrup (OCH_3, 27.3%, equivalent, 230) was found to be paper chromatographically homogeneous.

The acid (ca. 100 mg.) was converted to its methyl ester methyl glycoside by refluxing it with methanolic hydrogen chloride (2%) and reduced[10] with lithium aluminium hydride in an ethereal solution. The corresponding neutral methyl sugar solution, after hydrolysis and usual treatment, was evaporated to a syrup (88 mg.) which was chromatographically homogeneous and which had $[\alpha]_D^{30}$

12) G. O. Aspinall, E. L. Hirst and W. McArthur, *J. Chem. Soc.*, **1955**, 3075.
13) S. Baker and W. N. Haworth, ibid., **127**, 365 (1925).
14) P. A. Rebers and F. Smith, *J. Am. Chem. Soc.*, **76**, 6097 (1954).
15) H. Granichstädten and E. G. V. Percival, *J. Chem. Soc.*, **1943**, 54.
16) H. C. Srivastava and F. Smith, *J. Am. Chem. Soc.*, **79**, 982 (1957).
17) J. K. N. Jones, P. W. Kent and M. Stacey, *J. Chem. Soc.*, **1947**, 1341.
18) G. O. Aspinall and A. Canas-Rodriguez, ibid., **1958**, 4020.

+80° in water (*c* 0.5), OCH_3, 29.6%. The demethylation of the syrup produced galactose. The sugar in the syrup was finally identified as 2,3-di-*O*-methyl-D-galactose through its crystalline aniline derivative, m. p. and mixed m. p. 152—153°C; lit.[18] 152—154°C.

Summary

The polysaccharide isolated from the mesocarp of unripe mango has been found to be a mixture of three component polysaccharides, viz., a glucan, and araban and a galacturonan. By a methylation study, the glucan has been found to have the same structural pattern as that of the glucan isolated from ripe mango. The araban portion yielded 2, 3, 5-, 2, 3- and 2-*O*-methyl-L-arabinose. On the basis of these results, a probable structure has been assigned to the araban. The isolation of only 2, 3-di-*O*-methyl-D-galacturonic acid shows that the galacturonan portion is 1→4 linked.

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