The Pectic Acid from the Pulp of Jackfruit (Artocarpus Integrifolia). I. Methylation Studies

By U. K. Sen Gupta and C. V. N. Rao

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The presence of pectic substances in the pulp of jackfruit has been reported earlier by different workers.[1,2] However, no detailed investigations seem to have been carried out on the structure of these pectic substances. The present paper reports some structural studies of the pectic acid isolated from the soft fleshy pulp of the ripe jackfruit.

The polysaccharide obtained from jackfruit pulp by extraction with hot water gave a blue color with iodine. The product obtained after treatment with diastase to remove starch, on hydrolysis gave L-rhamnose, D-xylose, L-arabinose, D-glucose, D-galactose and D-galacturonic acid.

Ammonium pectate was isolated from the water-extracted residue of the jackfruit pulp with a hot ammonium oxalate solution. The polysaccharide was purified by converting it to insoluble calcium salt, and then to ammonium pectate, and finally by treating the material with diastase to destroy any starch. The hydrolysis of the resulting polysaccharide yielded D-galacturonic acid, D-glucose, and traces of arabinose. The polysaccharide was subjected to further purification by different processes, viz., conversion into sodium salt followed by precipitation as calcium salt, complexing with cetyltrimethylammonium bromide[3,4] and repeated fractional precipitation with acetone from an aqueous solution. However, no pure polygalacturonic acid free from neutral sugar components could be isolated by any of these processes, though xylose, rhamnose and galactose containing moieties were removed after repeated purification. Different

1) C. R. Krishnamurti and K. V. Giri, *Proc. Ind. Acad. Sci.*, **29B**, 155 (1949).
2) H. W. Scharpenseel, *Landwirtsch Forsch*, **10**, 191 (1957).
3) A. S. Jones, *Biochim. et Biophys. Acta.*, **10**, 607 (1953).
4) B. C. Bera, A. B. Foster and M. Stacey, *J. Chem. Soc.*, **1955**, 3788.

methods of purification yielded polysaccharides with different specific rotation and uronic acid content values. The hydrolyzates of each material gave different amounts of galacturonic acid, together with glucose and traces of arabinose. It was also found that only galacturonic acid residues were released when ammonium pectate was hydrolyzed under mild conditions using 0.1 N sulphuric acid. As the uronic acid content was highest in sample A, this was used in further investigations.

The purified polysaccharide was dissolved in phosphate buffer and subjected to electrophoresis using a potential gradient of 7.0 V. per cm. Two distinct peaks were observed, one of them migrating towards the anode presumably because of the polyuronic acid constituents. A known mixture of quite divergent groups of polysaccharides has been reported[5] to have been separated by this method.

The results of the electrophoretic experiment, together with the earlier findings, viz., the variation of uronic anhydride content in polysaccharides obtained by different methods of purification and the failure to obtain an aldobiouronic acid by mild hydrolysis, indicate that the material under investigation is a mixture of at least two different polysaccharides, one being a polygalacturonan. Another interesting observation is that glucose was detected in the hydrolyzate of the purified polysaccharide even after the treatment with diastase, showing that glucose may be an artifect of a glucan which is different from starch.

Ammonium pectate was methylated first by treatment with methyl sulfate and sodium hydroxide and then by the addition of methyl iodide to the silver salt. The methylated polysaccharide was purified by adding cold ethanolic sodium hydroxide to remove the associated neutral methylated polysaccharide. The methylated sodium pectate so obtained was, as usual, reconverted into methylated methyl pectate, which showed no hydroxyl absorption band in the infrared spectrum.

The methylated product after methanolysis was saponified with aqueous barium hydroxide. The acid portion was separated from the neutral portion by absorbing the former on an anion-exchange resin. On hydrolysis the neutral portion yielded a mixture of methyl sugars which was separated and estimated[6] by paper chromatography to give 2, 3, 4, 6-tetra- (1 mol.), 2, 3, 6-tri- (10.2 mol.), 2, 4, 6-tri- (9.8 mol.) and 2, 3-di-*O*-methyl-D-glucose (1.1 mol.) ; each of these methyl sugars was identified through a suitable crystalline derivative. As no methyl arabinose was found in the hydrolyzate of the neutral polysaccharide, it is assumed that arabinose was eliminated during the methylation and the subsequent purification processes.

The acidic component was displaced from the resin and reduced with lithium aluminum hydride, and the resulting product on hydrolysis gave a mixture of neutral methyl sugars. The mixture was separated on a cellulose column to give 2, 3, 4-tri- and 2, 3-di-*O*-methyl-D-galactose, which were characterised through their crystalline anilides. The relative amounts of sugars were estimated by alkaline hypoiodite. The molar ratio of the tri- and di-methyl galactose was 1 : 91.

From the above results it is possible to assign structures to the pectic acid and to the glucan associated with it. It is clear from these results that the main component, 2, 3-di-*O*-methyl-D-galactose, has arisen from the residues of 2, 3-di-*O*-methyl-D-galacturonic acid, that the jackfruit pectic acid contains chains of 1 : 4 linked D-galacturonic acid residues, and that the 2, 3, 4-tri-*O*-methyl-D-galactose originates from the nonreducing D-galacturonic acid end groups. As no monomethyl galactose could be isolated, the molecule is considered to be unbranched. From the ratio of the amounts of trimethyl and dimethyl galactose, the average length of each molecules is calculated to be 92 hexouronic acid units. Hence, the structure of the pectic acid is as follows:

$$Ga_pU{=}_1{\longrightarrow}_4\ (Ga_pU)_{90}\ _1{\longrightarrow}_4\ Ga_pU$$

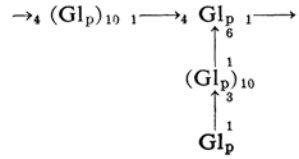
(Ga_pU represents the galactopyranosyl uronic acid residue).

From the methoxyl content of the original polysaccharide, it can be concluded that some of the carboxyl groups are involved in methyl ester linkages. Since the pectic acid and its methylated derivative have high positive rotations, the glycosidic linkages are considered to be of the α-type.

The demonstration of the presence of 2, 3, 4, 6-tetra-*O*-methyl-D-glucose in the tetra-methyl fraction of the neutral hydrolyzate indicates that the non-reducing ends of the glucan are occupied by the glucopyranose residues. The characterization of the two trimethyl sugars, 2, 3, 6-tri- and 2, 4, 6-tri-*O*-methyl-D-glucose, shows the existence of two kinds of linkages, 1→4 and 1→3, in the main a chain. The presence of a considerable amount of 2, 3-di-*O*-methyl-D-glucose suggests that the glucose residues at the branch points are joined through C_1, C_4 and C_6 and that the branches

5) J. R. Colvin, W. H. Cook and G. A. Adams, *Can. J. Chem.*, **30**, 603 (1952).
6) E. L. Hirst, L. Hough and J. K. N. Jones, *J. Chem. Soc.*, **1949**, 928.

originate from certain glucose units linked through the C_1 and C_4 positions and not those through the C_1 and C_3 positions in the chain. On the basis of the above results, one of the possible structures for the repeating unit of the glucan may be given as follow:



(Gl_p represents the glucopyranose unit)
However, it is not possible to stipulate exactly the distribution of 1→3 and 1→4 linked glucopyronose units in the polysaccharide in the light of present knowledge.

Further support for the proposed structure for the pectic acid was obtained from periodic acid oxidation studies of the pectic acid. It was found that one molar proportion of formic acid was liberated for every 30 mol. of anhydrouronic acid residues in 28 hr. and that 0.94 mol. of the oxidant were consumed per anhydrouronic acid residue in 30 hr., the theoretical figure for the consumption of periodate being 0.96 mol. The findings regarding periodate uptake are thus in good agreement with those theoretically expected.

The molecular weight of the methylated pectic acid, as determined by the light-scattering method, is 1.50×10^5.

The pectic acid obtained from the pulp of the jackfruit is similar to those obtained from apple,[7] strawberry,[8] citrus,[9,9a] and sisal.[10] The pectic acid obtained from these sources is generally associated with a neutral polysaccharide such as araban and galactan. In the case of jackfruit pectic acid, the neutral component is a glucan of a rather unusual structure, one which is similar to that isolated from mangifera indica.[11]

Experimental

All specific rotations are equilibrium values. Unless otherwise stated, all evaporations were carried out in vacuo at 30~40°C. Paper partition chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems (v/v): (A) ethyl acetate: pyridine: water (8: 2: 1); (B) *n*-butanol: acetic acid: water (4: 1: 5) upper layer; (C) *n*-butanol: ethanol: water (40: 11: 19); (D) ethyl methyl ketone-water azeotrope; (E) *n*-butanol: ethanol: water (5: 1: 4) upper layer; (F) ethyl acetate: acetic acid: water (9: 2: 2). The spray reagents used were: (a) aniline hydrogen oxalate (a saturated aqueous solution), (d) *p*-anisidine hydrochloride (a 3% solution in butanol). Unless otherwise stated, the R_G values of methyl sugars refer to rates of movement relative to 2, 3, 4, 6-tetra-*O*-methyl-D-glucose in solvent E. Methyl sugars were demethylated with hydrobromic acid.[12]

The Isolation and Examination of Water-soluble Polysaccharides.—The pulp of jackfruit was freed from the seed and pericarp, and the soft fleshy material was macerated in a blender in the presence of ethanol. After the product had been kept overnight, the slurry was filtered through a cloth and the residue dried in air. The material (100 g.) so obtained was allowed to swell in water (2 l.); it was then heated, while being stirred, on a boiling-water bath for 4 hr. The slurry was diluted with water (2 l.) and filtered through a cloth. The filtrate was further clarified by passing it through a Cepa supercentrifuge, and the polysaccharide was precipitated as a white gelatinous material by adding thrice its volume of acidified ethanol. The precipitate was filtered, washed with ethanol, triturated and dried. The material isolated gave a blue color when iodine was added, indicating the presence of starch which was removed by the action of diastase. The polysaccharide recovered in the usual way was a white amorphous powder; yield, 18 g.; moisture, 8.8%; ash, 1.02%; OMe,[13] 1.1%; nitrogen, nil; equivalent weight, 388.9; anhydrouronic acid,[14] 46.76%; $[\alpha]_D^{29}+168.7°$ (*c* 1.1, in water). The water-extracted polysaccharide (0.8 g.) was heated with 1 N sulfuric acid (50 ml.) for 10 hr. on a boiling water-bath when the hydrolysis, followed iodometrically,[15] was complete. The cooled solution was neutralized, and the filtrate was concentrated to a sirup. On paper chromatography using solvents A, B and F, the sirup was found to contain rhamnose, xylose, arabinose, glucose, galactose and galacturonic acid. A portion of the sirup (460 mg.) was separated on a cellulose column (60×3.5 cm.), using *n*-butanol half-saturated with water as the eluent, to give five fractions and the sugars L-rhamnose, D-xylose, L-arabinose, D-glucose and D-galactose were identified through their specific rotations and the preparation of suitable crystalline derivatives. The sixth fraction, obtained as barium salt by elution with water, was deionised with Amberlite IR-120 (H) to give D-galacturonic acid, which was characterised through its specific rotation and the preparation of crystalline mucic acid.

The Isolation and Purification of Pectic Acid from Jackfruit Pulp.—The residue left (1.5 kg.) after the water-soluble polysaccharide had been removed from the jackfruit pulp was treated with

7) E. L. Hirst and J. K. N. Jones, ibid., **1939**, 454.
8) G. H. Beavan and J. K. N. Jones, ibid., **1947**, 1218.
9) G. H. Beavan, E. L. Hirst and J. K. N. Jones, ibid., **1939**, 1865.
9a) S. Luckett and F. Smith, ibid., **1940**, 1106.
10) G. O. Aspinall and A. Cañas-Rodriguez, ibid., **1958**, 4020.
11) A. Das and C. V. N. Rao, unpublished results.
12) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, **1950**, 1702.
13) E. P. Clark, "Semi-micro Quantitative Organic Analysis," Academic Press, Inc., New York (1943), p. 68.
14) C. Doree, "The Methods of Cellulose Chemistry," 2nd ed., Chapman & Hall Ltd., London (1947), p. 391.
15) F. L. Baker and H. F. E. Halton, *Biochem. J.*, **14**, 754 (1920).

a 0.5% ammonium oxalate solution (12 l.) and the polysaccharide was isolated as usual. A calcium chloride solution (5%) was added to a 2% aqueous solution of this material until no further precipitate was formed. The resulting calcium pectate was suspended in water (1 l.) and heated at 90°C for one hour with ammonium oxalate (6 g.). The calcium oxalate was then filtered off, and the polysaccharide was precipitated by adding ethanol. The above process was repeated once more, after which the solution was dialysed aganst distilled water and then treated with diastase. The purified polysaccharide was isolated in the usual way; yield, 85 g. The hydrolysis of the polysaccharide, followed by a chromatographic examination of the resulting sirup, revealed the presence of galacturonic acid and glucose, together with trace amounts of arabinose. D-Galacturonic acid and D-glucose were characterised through their crystalline derivatives. A portion of the ammonium pectate in the aqueous solution was passed through a column of Amberlite IR-120(H); the pectic acid so obtained had $[\alpha]_D^{29}$ +229° (*c* 0.45, in water) [equiv. 221 corresponds to 79.6% of anhydrouronic acid; found: anhydrouronic acid, 81.5%]; OMe, 1.3%.

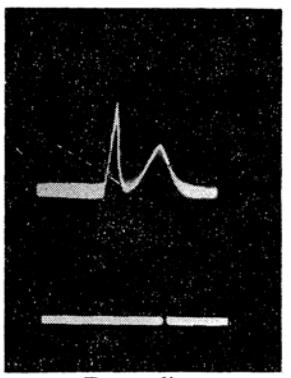
The Attempted Fractionation of Ammonium Pectate.—(1) A suspension of crude ammonium pectate (40 g.) in ethanol (800 ml.) was treated with a sodium hydroxide solution (40%, 50 ml.) and the mixture kept at 2°C for 48 hr. while occasionally being shaken. After filtration the solid was washed with ethanol-water (1 : 1). An equal volume of ethanol was added to the aqueous solution to precipitate sodium pectate. The product, after extraction with hot ethanol-water (1 : 1), was dissolved in water, precipitated as calcium pectate, and finally converted into ammonium pectate. The above process was repeated once more to yield a polysaccharide (29 g., sample A); $[\alpha]_D^{29}$ +213.4° (*c* 0.6, in water), [equiv. 203 corresponds to 86.7% of anhydrouronic acid; found: anhydrouronic acid, 87.5%]. The hydrolysis of a portion of sample A yielded galacturonic acid, glucose, and a trace of arabinose; the amount of glucose was estimated[16] and found to be 9.5%.

(2) A solution of crude ammonium pectate (15 g.) in water (700 ml.) was treated with an aqueous solution of "Cetavlon" (cetyltrimethylammonium bromide) (10%, 200 ml.); the precipitate was then filtered. 5 N-Acetic acid was stirred into an aqueous suspension of the precipitate to decompose the complex. The polysaccharide was precipitated by ethanol, redispersed in water, shaken for 2 hr. with Amberlite IR-120(H) and filtered. The pectic acid was precipitated as usual and subjected to further purification as above to yield a polysaccharide (9.5 g., sample B); $[\alpha]_D^{29}$ +209.5° (*c* 0.5, in water) [equiv. 217 corresponds to 81.1% of anhydrouronic acid; found: anhydrouronic acid, 84.5%]. The hydrolysis of a portion of sample B gave galacturonic acid, glucose, and arabinose.

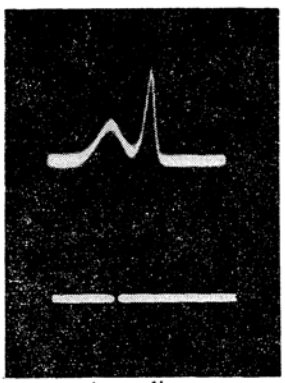
(3) An aqueous solution of crude ammonium pectate (2%, 500 ml.) was precipitated with acetone (600 ml.). The process was repeated several times, and the polysaccharide was extracted with hot ethanol-water (1 : 1) and reprecipitated from the aqueous solution by adding ethanol. The polysaccharide (5 g., sample C) had $[\alpha]_D^{29}$ +202° (*c* 0.7, in water) [equiv. 219 corresponds to 80.3% anhydrouronic acid; found: anhydrouronic acid, 81.7%]. The hydrolysis of a sample yielded galacturonic acid, glucose, and arabinose.

Repeated purification by each of these methods did not increase the uronic acid content.

The Electrophoresis of the Polysaccharide.—The purified polysaccharide (116 mg., sample A) was dissolved in a 0.1 M phosphate buffer (10 ml., pH 7.1). The solution was centrifuged on a high speed centrifuge at 20000 G for 15 min. in order to clarify it. The supernatant liquor was dialysed against the same buffer (500 ml.) for 18 hr. at 15°C. The conductivity of the resultant solution was determined, after which electrophoresis was carried out in a Perkin-Elmer electrophoresis apparatus (Model 38A) at 15°C using a potential gradient of 7.0 V./cm. Photographs of the migrating boundaries were taken at the beginning and at regular intervals. The photographs shown above were taken after one hour interval; the boundaries became diffused afterwards.



Descending



Ascending

Photo. 1

The Mild Hydrolysis of Ammonium Pectate.—Ammonium pectate (5 g., sample A) was heated with 0.1 N sulfuric acid (200 ml.) for 50 hr. at

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

85~90°C. The solution was then neutralized, filtered, and deionised by passing it through Amberlite IR-120(H). The acid was absorbed on a column of Dowex 1X-4 anion-exchange resin, and the column was freed from neutral sugars by washing it with water. The acid was displaced from the column by sulfuric acid, neutralized, and filtered, and the filtrate was deionized and concentrated to a sirup. The paper chromatographic examination of the sirup using solvent F gave a single spot corresponding to galacturonic acid; [6][$[\alpha]_D^{30}$ +80.5°, equiv. 188. The acid was characterised as D-galacturonic acid by converting it to crystalline D-galactose and to mucic acid.

The Methylation of Ammonium Pectate.—Ammonium pectate (22 g., sample A), was methylated by treatment with methylsulfate and sodium hydroxide[17] and then with methyl iodide and silver oxide,[18] according to the method adopted by Aspinall et al.,[10] to yield a methylated polysaccharide (15.3 g.), $[\alpha]_D^{29}$ +196° (*c* 0.8, in chloroform), OMe 39.8%. The methylated polysaccharide (15 g.) was purified by treating it with cold ethanolic sodium hydroxide according to the method of Aspinall et al.[10] The resulting methylated sodium pectate was converted into methylated methyl pectate (9.7 g.), which showed no hydroxyl absorption band in the infrared spectrum, $[\alpha]_D^{29}$ +208° (*c* 1.1, in chloroform) (Found: OMe, 40.5. Calcd. for $C_8H_{14}O_6$: OMe, 42.6%).

The Molecular Weight Determination of the Methylated Methyl Pectate.—All measurements of the intensities of scattered light for the molecular weight determination of the methyl pectate were carried out with a Brice-Phoenix light-scattering photometer (Model OM 1000A) using a semi-octagonal cell. The concentration used was over a range of $(0.4\sim3.0)\times10^{-3}$ g./ml. The molecular weight was found to be 1.503×10^5 as calculated by the dissymmetry method.[19] The value of *r*, corresponding to the root-mean-square end-to-end distances of the polymer, was calculated to be 1998Å for the methylated polysaccharide at the wavelength of 5461Å.

The Methanolysis of the Methylated Methyl Pectate and the Separation of Neutral and Acidic Methyl Sugars.—A solution of methylated methyl pectate (3.5 g.) in methanolic hydrogen chloride (4%, 125 ml.) was heated in a sealed tube at 100°C for 8 hr. Neutralization, filtration and evaporation yielded a sirup which was then heated with a barium hydroxide solution (2%, 50 ml.) at 80°C for 4 hr. The solution, after having been neutralized by passing carbon dioxide gas through it, was filtered and passed through a column of Amberlite IR-120(H) and Dowex 1 X-4 (bicarbonate form). The neutral solution was evaporated to a sirup, and the resulting mixture of methyl glycosides was hydrolysed with 1 N hydrochloric acid at 100°C for 8 hr. The hydrolyzate was neutralised (silver carbonate) and filtered, and the resulting solution after deionization was evaporated to a sirup (346 mg.). On paper partition chromatography using solvents D and E, the sirup gave four spots corresponding to 2, 3, 4, 6-tetra-, 2, 3, 6-tri-, 2, 4, 6-tri-, and 2, 3-di-*O*-methyl-D-glucose.

The mixture of neutral methyl sugars (ca. 335 mg.) was separated on Whatman No. 3MM papers using solvent D. The respective zones were eluted with water and evaporated to sirups.

The Identification of 2, 3, 4, 6-Tetra-O-methyl-D-glucose.—The sirup (15.5 mg.) had $[\alpha]_D^{29}$ +82° (*c* 0.4, in water) and R_G 1.0. Demethylation gave glucose. The sugar was characterised as 2, 3, 4, 6-tetra-*O*-methyl-D-glucose by conversion into the crystalline aniline derivative; m. p. and mixed m. p. 136~137°C; lit.[20] 136~138°C.

The Identification of 2, 3, 6-Tri-O-methyl-D-glucose.—The sirup (150.2 mg.) had $[\alpha]_D^{29}$ +67° (*c* 0.8, in water) and R_G 0.82 (Found: OMe, 41.6. Calcd. for trimethyl hexose: OMe, 41.8%). Demethylation gave glucose. The 2, 3, 6-tri-*O*-methyl-D-glucose was identified by preparing crystalline 2, 3, 6-tri-*O*-methyl-D-glucose-1, 4-di-*p*-nitrobenzoate; m. p. and mixed m. p. 190~191°C; lit.[21] 190~192°C.

The Identification of 2, 4, 6-Tri-O-methyl-D-glucose.—The sirup (148.1 mg.) with $[\alpha]_D^{29}$ +71° (*c* 1.0, in water), R_G 0.76 (Found: OMe, 41.1. Calcd. for trimethyl hexose: OMe, 41.8%.) gave glucose on demethylation. The sugar was identified as 2, 4, 4-tri-*O*-methyl-D-glucose by converting it to the crystalline anilide; m. p. and mixed m. p. 162~163°C; lit.[22] 162~166°C.

The Identification of 2, 3-Di-O-methyl-D-glucose.—The sirup (16 mg.) had $[\alpha]_D^{29}$ +65° (*c* 0.3, in water) and R_G 0.58. The sugar was characterised has 2, 3-di-*O*-methyl-D-glucose by conversion into 2, 3-di-*O*-methyl-D-gluconophenylhydrazide; m. p. and mixed m. p. 160~161°C; lit.[20] 160~162°C.

A portion of the mixture of methyl sugars was quantitatively separated by paper chromatography using solvent E, and each component was estimated by alkaline hypoiodite. The molar ratio of 2, 3, 4, 6-tetra-, to 2, 3, 6-tri- to 2, 4, 6-tri- to 2, 3-di-*O*-methyl-D-glucose was 1.0 : 10.2 : 9.8 : 1.1.

The Examination of the Acidic Component of the Methylated Methyl Pectate.—The Dowex 1 X-4 resin, after a thorough washing with water, was eluted with 1 N sulfuric acid (500 ml.). The eluted acid was neutralized ($BaCO_3$) and filtered, and the resulting solution was, after deionization, evaporated to a light yellowish sirup (2.95 g.).

The sirup (2.9 g.) was refluxed with methanolic hydrogen chloride (4%, 200 ml.) for 8 hr. The solution was neutralised, filtered and evaporated to a sirup. Lithium aluminium hydride (1.5 g.) in dry tetra hydrofuran (100 ml.) was added in portions with occassional shaking to the hot solution of the dry sirup in dry tetrahydrofuran (200 ml.). The mixture was heated for 1 hr., kept at room temperature for 2 hr., and then treated with water. The residue obtained after drying the mixture was extracted with acetone and ethanol;

17) W. N. Haworth, ibid., **107**, 8 (1915).
18) T. Purdie and J. C. Irvine, ibid., **83**, 1021 (1903).
19) P. Debye, *J. Phys. Chem.*, **51**, 18 (1947).
20) G. O. Aspinall, E. L. Hirst and W. McArthur, *J. Chem. Soc.*, **1955**, 3075.
21) P. A. Rebers and F. Smith, *J. Am. Chem. Soc.*, **76**, 6097 (1954).
22) H. Granichstädten and E. G. V. Percival, *J. Chem. Soc.*, **1943**, 54.

the combined extract was diluted with water, deionized, and evaporated. Hydrolysis of the resulting product, followed by the usual treatment, gave a sirupy mixture of sugars (2.36 g.).

The mixture of sugars (ca. 2.35 g.) was separated on a cellulose column (60×3.5 cm.) using solvent D, and the total eluate was separated into three fractions.

Fraction I.—The chromatographically-pure sirup (24.5 mg.) had $[\alpha]_D^{30}$ +112° (*c* 0.4, in water) and R_G 0.69 (Found: OMe, 41.6. Calcd. for trimethyl hexose: OMe, 41.8%). Demethylation gave galactose. The sugar was identified as 2, 3, 4-tri-*O*-methyl-D-galactose by conversion into a crystalline aniline derivative; m. p. and mixed m. p., 163~164°C; lit.[10] 163~165°C.

Fraction II.—The sirup (150.3 mg.) having $[\alpha]_D^{30}$ +82° (*c* 0.5, in water) gave galactose on demethylation. Paper partition chromatography using solvent D indicated that the mixture contained 2, 3, 4-tri-, and 2, 3-di-*O*-methyl-D-galactose.

Fraction III. — The sirup (2.05 g.) having $[\alpha]_D^{30}$ +79° (*c* 1.5, in water) and R_G 0.48 (Found: OMe, 29.6. Calcd. for dimethylhexose: OMe, 29.8%) was chromatographically homogenous and gave galactose on demethylation. The 2, 3-di-*O*-methyl-D-galactose was identified as its crystalline aniline derivative; m. p. and mixed m. p. 152~153°C; lit.[10] 152~154°C.

A small quantity of the mixture of methyl sugars was separated quantitatively by paper chromatrography, and the sugars were estimate by alkaline hypoiodite. The molar ratio of 2, 3, 4-tri- to 2, 3-di-*O*-methyl-D-galactose was 1 : 91.

The Periodate Oxidation of Ammonium Pectate.—The polysaccharide (sample A, 100.2 mg.) was dispersed in water (50 ml.) and oxidised by shaking it with 0.2 M periodic acid (50 ml.) in the dark at 15°C. The liberated formic acid and the amount of periodic acid consumed during the reaction were estimated in the usual way[23] at regular intervals. The liberation of formic acid became constant in 28 hr., corresponding to 30 mol. of anhydrouronic acid residues per mole of formic acid, while the periodate uptake became constant after 30 hr., corresponding to 0.94 mol. of the oxidant per anhydrouronic acid residue.

Summary

Water-soluble polysaccharide extracted from the pulp of jackfruit (*Artocarpus Integerifolia*) has been shown to be composed of L-rhamnose, D-xylose, L-arabinose, D-glucose, D-galactose and D-galacturonic acid. The jackfruit pectic acid has subsequently been extracted with ammonium oxalate and purified. The results of electrophoresis, mild hydrolysis and methylation studies indicate the presence of chains of 1 : 4 linked α-D-galacturonic acid residues in the pectic acid, which is physically associated with a neutral polysaccharide, a glucan. Periodate oxidation studies have also been reported.

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Department of Macromolecules
Indian Association
for the Cultivation of Science
Calcutta-32, India

23) P. Fleury and J. Lange, *J. Pharm. Chim.*, **17**(8), 107 (1933).