The Pectic Acid from the Pulp of Jackfruit (*Artocarpus Integrifolia*). II

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The constitution of the pectic acid and the associated glucan present in the fleshy pulp of ripe jackfruit has been reported earlier.[1] Additional evidence will be given in the present paper in further confirmation of the proposed structure of the polysaccharide.

When the methylated methyl pectate was subjected to methanolysis, a mixture of methyl glycosides was obtained. Instead of separating the neutral components from the acidic one, we reduced the whole mixture (containing the methyl ester of the acids) with lithium aluminium hydride. The neutral methyl glycosides were then hydrolysed with 1 N hydrochloric acid, and the methyl sugars were repeatedly separated on cellulose columns (vide "Experimental") to give the methyl sugars shown in Table I.

TABLE I. METHYL SUGARS OBTAINED FROM THE METHYLATED PECTIC ACID

Spot No.	R_G	Name of the component	Yield mg.
1	1.00	2,3,4,6-Tetra-*O*-metyl-D-glucose	19
2	0.82	2,3,6-Tri-*O*-methyl-D-glucose	190.5
3	0.76	2,4,6-Tri-*O*-methyl-D-glucose	178.9
4	0.66	2,3,4-Tri-*O*-methyl-D-galactose	42.2
5	0.56	2,3-Di-*O*-methyl-D-glucose	19.3
6	0.49	2,3-Di-*O*-methyl-D-galactose	3718.8

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

The fact that 2, 3-di-*O*-methyl-D-galactose was obtained in large quantities was in agreement with the structure proposed. The reduction of the methyl ester of galacturonic acid left the C_6 position free, and therefore 2, 3-di-*O*-methyl-D-galactose was formed.

Attempts were also made to prepare a neutral polymer from the pectic acid itself. This was achieved through the preparation of ethylene glycol pectate,[2] the pectic acid was treated with ethylene oxide to give the glycol ester, $[\alpha]_D^{29}$ +190° (in water) (anhydrouronic acid 71.6%; equivalent wt. 251).

The ester, on reduction with sodium borohydride, yielded a neutral polysaccharide, mainly a galactan, $[\alpha]_D^{30}$ +240° (in water) (anhydrouronic acid 9.7%). The polysaccharide on hydrolysis yielded 78.3% galactose, 9.75% glucose and a trace of arabinose.

The partially-reduced polysaccharide was methylated, and during methylation the remaining acid portion was reduced with lithium aluminium hydride (vide "Experimental") to yield a methylated galactan, $[\alpha]_D^{29}$ +164° (in chloroform) and $-OCH_3$, 42.1%. The methylated polysaccharide was methanolysed, and the mixture of methyl glycosides was hydrolysed to give a mixture of methyl sugars. This mixture was then separated on a cellulose column in an automatic-fraction collector collecting the 15 ml. in each tube into the following fractions (Table II), from which pure sugars could be isolated and characterised.

The characterisation of tri-*O*-methyl-galactose as 2, 3, 6-tri-*O*-methyl-D-galactose shows that the linkage of the uronic acid units in the original pectic acid is of the 1→4 type. It is evident that the 2, 3, 4, 6-tetra-*O*-methyl-D-galactose originated from the non-reducing end of the galactan and the isolation of a small amount of 2, 3-di-*O*-methyl-D-galactose may be due to the

TABLE II. METHYL SUGARS OBTAINED FROM THE METHYLATED GALACTAN

Fraction No.	Tube No.	Compound	Yield mg.
1	18—19	2,3,4,6-Tetra-*O*-methyl-D-glucose	7.1
2	20—31	2,3,4,6-Tetra-*O*-methyl-D-galactose and 2,3,6-tri-*O*-methyl-D-glucose	79.7
3	32—49	2,3,6-Tri-*O*-methyl-D-glucose, 2,4,6-tri-*O*-metyl-D-glucose and 2,3,6-tri-*O*-methyl-D-galactose	485.0
4	50—61	2,3,6-Tri-*O*-methyl-D-galactose	905.0
5	63—64	2,3-Di-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-galactose	16.1

1) U. K. Sen Gupta and C. V. N. Rao, This Bulletin, **36**, 1683 (1963).
2) H. Deuel, *Helv. Chim. Acta*, **30**, 1523 (1947).

incomplete methylation of the reduced polysaccharide. The presence of 2,3,4,6-tetra-, 2,3,6- and 2,4,6-tri-, and 2,3-di-*O*-methyl-D-glucose is in keeping with the structure assigned to the repeating units of the glucan.[1] During the reduction and the methylation, no change in the structure of the methylated polysaccharide takes place.

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) ethyl methyl ketone - water azeotrope ; (C) *n*-butanol : ethanol : water (5 : 1 : 4) upper layer. Unless otherwise stated, the R_G values were calculated with reference to 2,3,4,6-tetra-*O*-methyl-D-glucose in solvent C. Demethylation was carried out with hydrobromic acid.[3] The amounts of individual sugars in a mixture were calculated from the specific rotation of that mixture.

The Methanolysis, Reduction and Hydrolysis of Methylated Methyl Pectate.—Methylated methyl pectate (5.8 g.) in methanolic hydrogen chloride (4%, 125 ml.) was heated (8 hr.) in a sealed tube on a boiling water bath. The solution was then neutralised with silver carbonate, and the precipitate was removed by a centrifuge. The resulting dry sirup was dissolved in dry tetrahydrofuran (160 ml.), and lithium aluminium hydride (2.5 g.) in dry tetrahydrofuran (60 ml.) was added slowly to the boiling solution over a one-hour period. After the mixture had been refluxed for one hour and kept at room temperature for two hours, the mixture was treated with water. The solvent was then removed, and the residue was shaken with acetone and ethanol. The extracts were dialysed against distilled water, deionised with Amberlite IR-120(H) and IR-45(OH), and evaporated to a sirup. N Hydrochloric acid (200 ml.) was added, and the mixture was heated (6 hr.) on a boilng water bath. After neutrarisation (silver carbonate) and deionisation (Amberlite resins IR-120(H) and IR-45(OH)), the resulting solution was evaporated to a sirup (4.39 g.).

The mixture of methyl sugars (ca. 4.35 g.) was separated through a cellulose column (70×3.5 cm.) using solvent B. It was found that only one pure fraction could be collected in a good quantity; most of the other fractions were mixtures of two or more components. The major pure fraction (3.52 g.), with $[\alpha]_D^{30}$ +78.5° (*c* 1.1, water), $-OCH_3$, 29.7% (calcd. for $C_8H_{10}O_6$: $-OCH_3$, 29.8%), gave only galactose on demethylation. It was characterised as 2,3-di-*O*-methyl-D-galactose through the crystalline *N*-phenyl-2,3-di-*O*-methyl-D-galactosylamine, m. p. and mixed m. p. 152—153°C. The remaining fractions were converted to a combined sirup (790 mg.) and separated through a similar cellulose column into eleven fractions, which were individually evaporated to sirups.

3) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, **1950**, 1702.

The Examination of the Fractions and Identification of the Methyl Sugars. — *Fraction 1.*—The chromatographically-pure sirup (17 mg.) had $[\alpha]_D^{30}$ +82° (*c* 0.2, water), $-OCH_3$, 52% (calcd. for tetramethyl hexose : $-OCH_3$, 52.5%), and R_G, 1.0, and on demethylation it gave glucose. It was identified as 2,3,4,6-tetra-*O*-methyl-D-glucose by preparing its crystalline *N*-phenyl-2,3,4,6-tetra-*O*-methyl-D-glucosylamine, m. p. and mixed m. p. 136—137°C.

Fraction 2.—This fraction (25.5 mg.), with $[\alpha]_D^{30}$ +69° (*c* 0.25, water), produced only glucose on demethylation ; it was chromatographically (solvent B) identified as a mixture of 2,3,4,6-tetra- (2 mg.) and 2,3,6-tri-*O*-methyl-D-glucose (23.5 mg.).

Fraction 3.—This chromatographically-pure fraction had $[\alpha]_D^{30}$ +67° (*c* 0.4, water), and R_G 0.82, $-OCH_3$, 41.2% (calcd. for trimethyl hexose: $-OCH_3$, 41.8%), and on demethylation it produced glucose only. It was characterised as 2,3,6-tri-*O*-methyl-D-glucose through its crystalline 2,3,6-tri-*O*-methyl-D-glucose-1,4-di-*p*-nitrobenzoate, m. p. and mixed m. p. 190—191°C.

Fraction 4. — This sirup (40.6 mg.), with $[\alpha]_D^{30}$ +70.2° (*c* 0.45, water), furnished only glucose on demethylation ; it was paper chromatographically identified as a mixture of 2,3,6- (21.2 mg.) and 2,4,6-tri-*O*-methyl-D-glucose (19.4 mg.).

Fraction 5. — This chromatographically-pure fraction (142.2 mg.), with $[\alpha]_D^{30}$ +72° (*c* 0.5, water), R_G 0.76, $-OCH_3$, 41.1% (calcd. for trimethyl hexose : $-OCH_3$, 41.8%), on demethylation gave only glucose ; it was identified as 2,4,6-tri-*O*-methyl-D-glucose through the crystalline *N*-phenyl-2,4,6-tri-*O*-methyl-D-glucosylamine, m.p. and mixed m.p. 162—163°C.

Fraction 6. — The sirup (22.8 mg.), with $[\alpha]_D^{30}$ 82.4° (*c* 0.4, water), produced glucose and galactose on demethylation; it was paper chromatographically identified as a mixture of 2,4,6-tri-*O*-methyl-D-glucose (17.3 mg.) and 2,3,4-tri-*O*-methyl-D-galactose (5.5 mg.).

Fraction 7. — The chromatographically-pure sirup (28.5 mg.) had $[\alpha]_D^{30}$ +110° (*c* 0.42, water), R_G 0.66, $-OCH_3$, 41.05%, and furnished galactose only on demethylation. The sugar was characterised as 2,3,4-tri-*O*-methyl-D-galactose by preparing the crystalline *N*-phenyl-2,3,4-tri-*O*-methyl-D-galactosylamine derivative, m. p. and mixed m. p. 163—164°C.

Fraction 8.—This fraction (10.3 mg.), with $[\alpha]_D^{30}$ +102° (*c* 0.3, water), produced glucose and galactose on demethylation; it was paper chromatographically identified as a mixture of 2,3,4-tri-*O*-methyl-D-galactose (8.2 mg.) and 2,3-di-*O*-methyl-D-glucose (2.1 mg.).

Fraction 9.—The sirup (17.3 mg.) was chromatographically-pure, with $[\alpha]_D^{30}$ +66° (*c* 0.4, water), R_G 0.55, and on demethylation it gave only glucose. The sugar was identified as 2,3-di-*O*-methyl-D-glucose by preparing the crystalline 2,3-di-*O*-methyl-D-gluconophenylhydrazide, m. p. and mixed m. p. 160—161°C.

Fraction 10.—This fraction (42.4 mg.) with $[\alpha]_D^{30}$ +76° (*c* 0.5, water), produced glucose and galactose on demethylation ; it was paper chromatographically identified as a mixture of 2,3-di-*O*-methyl-D-glucose (trace) and 2,3-di-*O*-methyl-D-galactose.

Fraction 11.—The chromatographically-pure sirup (156.4 mg.), with $[\alpha]_D^{30}$ +78° (*c* 1.0, water), and R_G 0.49, $-OCH_3$, 29.3% (calcd. for dimethyl hexose: $-OCH_3$, 29.8%) gave galactose only on demethylation; it was characterised as 2,3-di-*O*-methyl-D-galactose through the crystalline *N*-phenyl-2,3-di-*O*-methyl-D-galactosylamine derivative, m. p. and mixed m. p. 152—153°C.

Preparation of Ethylene Glycol Pectate.[2] — Ethylene oxide (40 ml.) was added to a suspension of pectic acid (sample A,[1] 12.5 g.) in water (250 ml.), and the mixture was shaken (10 days) at room temperature until it became neutral. The resulting glycol ester was precipitated by acetone. The product was redispersed in water and precipitated by ethanol, The glycol ester (9 g.) had $[\alpha]_D^{30}$ +190° (*c* 0.96, water), anhydrouronic acid,[4] 71.6% (≡85.9% anhydrouronic acid in pectic acid), and equivalent wt. 251 (≡70.1% anhydrouronic acid in glycol ester and 84.1% anhydrouronic acid in pectic acid).

Reduction of Ethylene Glycol Pectate.—Ethylene glycol pectate (8 g.) in water (250 ml.) containing glycerol (4 g.) was treated with sodium borohydride[5] (2.5 g.) in water (20 ml.); the mixture was then kept overnight at 2°C. The solution was passed through columns of Amberlite IR-120(H) and Amberlite IR-45(OH), and the partially-reduced polysaccharide (6 g.) was isolated by the addition of acetone. This had $[\alpha]_D^{30}$ +236° (*c* 0.9, water), and was 21.32% anhydrouronic acid. The product subjected to four further reductions when the resulting galactan (2.8 g.), with $[\alpha]_D^{30}$ +240° (*c* 0.8, water) and 9.7% anhydrouronic acid, was obtained. The reduced polysaccharide (0.298 mg.) was hydrolysed, and the amounts of individual sugars were estimated by the periodate method,[6] which showed the presence of galactose (78.3%), glucose (9.75%), and arabinose (trace).

Methylation of Galactan, Hydrolysis and the Separation of Methyl Sugars. — Partially-reduced pectic acid (anhydrouronic acid, 21.32%; 5g.) was methylated[4,7,8] with methyl sulphate and sodium hydroxide. After dialysis, the solution of the partially-methylated polysaccharide was deionised through Amberlite IR-120(H) and then neutralised with silver carbonate to the silver salt. Silver oxide (30 g.) was added in several lots over a 6-hr. period to a boiling solution of dry silver salt in methyl iodide (100 ml.) containing dry methanol (5 ml.). The mixture was refluxed for 4 hr., and product was extracted with chloroform after distilling off the methyl iodide. The above process was repeated once more to yield a methylated polysaccharide (3.1 g., $-OCH_3$, 40.8%).

The methylated polysaccharide (3 g.) was dissolved in boiling tetrahydrofuran (70 ml.), and lithium aluminium hydride (2 g.) in tetrahydrofuran (100 ml.) was slowly added to it with occasional shaking. The mixture was refluxed for one hour. After the removal of the inorganic salts and deionisation, the reduced methylated polysaccharide was twice subjected to methylation with methyl iodide and silver oxide. The resulting methylated galactan (2.1 g.) had $[\alpha]_D^{29}$ +164° (*c* 1.4, chloroform) and $-OCH_3$, 42.1%. A solution of methylated galactan (2 g.) in 5% methanolic hydrogen chloride (125 ml.) was refluxed for 8 hr. The solution was evaporated to a sirup under reduced pressure. N Hydrochloric acid (100 ml.) was added, and the mixture was heated (6 hr.) on a boiling-water bath. The solution was cooled, neutralised (silver carbonate), and filtered. The filtrate was deionised (Amberlite IR-120(H) and Amberlite IR-45(OH)) and evaporated to give a sirup (1.7 g.), which was then, by using solvent B, separated on a cellulose column (60× 3.5 cm.) into five fractions.

Fraction 1.—The sirup (7.1 mg.) had $[\alpha]_D^{30}$ +81° (*c* 0.25, water) and was identified as 2,3,4,6-tetra-*O*-methyl-D-glucose by paper chromatography and its specific rotation.

Fraction 2. — The sirup (79.7 mg.), with $[\alpha]_D^{30}$ +72.5° (*c* 0.6, water), on demethylation produced glucose and galactose. Paper chromatography (solvent C) of the sirup separated it into two components (R_G 0.87 and 0.84) corrresponding to 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,6-tri-*O*-methyl-D-glucose. The mixture (ca. 50 mg.) was separated on 3MM filter paper with solvent C. Strips corresponding to trimethyl sugar and tetramethyl sugar were eluted with water, and the solutions concentrated. The component which corresponded to 2,3,4,6-tetra-*O*-methyl-D-galactose had $[\alpha]_D^{30}$ +106° (*c* 0.25, water), and the other, which corresponded to 2,3,6-tri-*O*-methyl-D-glucose, had $[\alpha]_D^{30}$ +64° (*c* 0.4, water).

Fraction 3. — This fraction (458 mg.), with $[\alpha]_D^{30}$ +77.9° (*c* 0.8, water), was paper chromatographically (solvent C) found to be a mixture of three components (R_G 0.84, 0.77 and 0.70). Demethylation gave galactose and glucose. The mixture (ca. 200 mg.) was separated on 3MM filter paper. The major component (160 mg.) had $[\alpha]_D^{30}$ +79° (*c* 1.05, water) and was identified as 2,3,6-tri-*O*-methyl-D-galactose through the crystalline 2,3,6-tri-*O*-methyl-D-galactonolactone, m. p. and mixed m. p. 96—97°C. The other two components (about 15 mg. each), corresponding to 2,3,6-tri-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose, had $[\alpha]_D^{30}$ +65° (*c* 0.32, water) and $[\alpha]_D^{30}$ +73° (*c* 0.37, water) respectively.

Fraction 4. — The chromatographically-pure sirup (905 mg.), with $[\alpha]_D^{30}$ +79° (*c* 1.5, water), and $-OCH_3$, 41.6% (calcd. for trimethyl hexose: $-OCH_3$, 41.8%), on demethylation gave galactose only; it was characterised as 2,3,6-tri-*O*-methyl-D-galactose by converting into 2,3,6-tri-*O*-methyl-D-galactonolactone, m. p. and mixed m. p. 96—97°C.

Fraction 5.—Paper chromatography of the sirup, which had $[\alpha]_D^{30}$ +70.5° (*c* 0.3, water), showed it to be a mixture of two components (R_G 0.56 and 0.50). Demethylation gave glucose and galactose. The mixture (ca. 10 mg.) was sepapated on 3MM papers, and the components identified as 2,3-di-*O*-methyl-D-glucose (5 mg.), $[\alpha]_D^{30}$ +65° (*c* 0.25, water) and 2,3-di-*O*-methyl-D-galactose (5 mg.), $[\alpha]_D^{30}$ +77° (*c* 0.2, water).

4) C. Doree, "The Methods of Cellulose Chemistry," 2nd ed., Chapman & Hall Ltd., London (1947), p. 381.
5) G. O. Aspinall and A. Cañas Rodriguez, *J. Chem. Soc.*, **1958**, 4020.
6) E. L. Hirst and J. K. N. Jones, ibid., **1949**, 1659.
7) W. N. Haworth, ibid., **107**, 8 (1915).
8) T. Purdie and J. C. Irvine, ibid., **83**, 1021 (1903).

Summary

The jackfruit pectic acid has been converted into ethylene glycol ester, which, on reduction with sodium borohydride, gives a galactan. Methylation studies of the original polysaccharide and of the reduced pectic acid suggest the presence of chains of 1→4 linked α-D-galacturonic acid residues in the pectic acid which is associated with a glucan.

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