810. *Sisal Pectic Acid.*

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Aqueous extraction of sisal flesh yields a mixture of polysaccharides, hydrolysis of which affords L-rhamnose, D-xylose, L-arabinose, D-glucose, D-galactose, and D-galacturonic acid, together with small amounts of 2-*O*-methyl-D-xylose and 2-*O*-methyl-L-fucose. Sisal pectic acid is subsequently extracted with ammonium oxalate. Reduction of the ethylene glycol ester of this pectic acid with potassium borohydride gives a galactan. Methylation studies indicate the presence in the pectic acid of chains of 1 : 4-linked α-D-galacturonic acid residues, but show that residues of L-rhamnose, L-arabinose, and D-galactose are also present.

Pectic substances are usually mixtures of three polysaccharides: pectic acid, containing chains of 1 : 4-linked α-D-galacturonic acid residues, present mainly as methyl esters; a linear galactan containing chains of 1 : 4-linked β-D-galactopyranose residues; and a highly branched araban, containing 1 : 5- and 1 : 3-linked α-L-arabofuranose residues.[1] The proportions of the three polysaccharides vary in different pectins, but the components are very closely associated and it is not yet definitely known whether or not they are linked by covalent bonds. The structure of the acidic polysaccharide is based on methylation studies on pectic acid, which had been previously degraded with mineral acid to hydrolyse the associated neutral polysaccharides, and on the characterisation of the acidic di- and tri-saccharides formed on enzymic hydrolysis.[2] The present paper describes studies of

[1] Hirst and Jones, *Adv. Carbohydrate Chem.*, 1946, **2**, 235.
[2] Jones and Reid, *J.*, 1954, 1361; 1955, 1890.

pectic acid isolated from the fleshy leaves of the sisal plant (*Agave sisalana*) in the mildest possible manner and without recourse to acidic reagents.

Extraction of sisal flesh with hot water gave a mixture of polysaccharides. Although fractional precipitation of this material from aqueous solution with acetone gave fractions differing in optical rotation, the same sugars were detected chromatographically on hydrolysis of the various fractions. One fraction was hydrolysed and the following sugars were identified after chromatographic separation on cellulose: L-rhamnose, D-xylose, L-arabinose, D-glucose, D-galactose, and D-galacturonic acid. In addition, very small amounts of two sugars with high chromatographic mobility were observed. In a separate experiment a larger quantity of the unfractionated water-soluble polysaccharide was hydrolysed, the products were adsorbed on charcoal, unsubstituted monosaccharides were eluted with water, and elution with ethanol–water mixtures followed by chromatography on filter sheets afforded 2-*O*-methyl-D-xylose and 2-*O*-methyl-L-fucose. These two sugars have recently been identified as constituents of plum leaf polysaccharides.[3]

Ammonium pectate was extracted from the sisal residue with hot ammonium oxalate solution, and hydrolysis of this material gave galacturonic acid, rhamnose, galactose, arabinose, and xylose. Regeneration of ammonium pectate after precipitation as the insoluble calcium salt resulted in removal of the xylose-containing moiety. Many subsequent attempts were made to fractionate the ammonium pectate, fractional precipitation from aqueous solution with acetone, precipitation *via* the calcium salt, and precipitation with cetyltrimethylammonium bromide,[4] but in no case was it possible to isolate a polygalacturonic acid devoid of neutral sugars. Indeed, the regenerated ammonium pectates were almost unchanged in optical rotation and uronic anhydride content, and gave on hydrolysis galacturonic acid, together with rhamnose, galactose, and arabinose.

Ammonium pectate was treated with methyl sulphate and sodium hydroxide, the methylated sodium pectate was converted into the silver salt, and treatment with methyl iodide afforded the methyl ester. In order to remove associated neutral methylated polysaccharide the methylated methyl pectate was saponified with cold ethanolic sodium hydroxide, and the precipitated methylated sodium pectate was separated and reconverted into methylated methyl pectate. Hydrolysis of the methylated polysaccharide, together with reduction of uronic acid to hexose residues was carried out in the following manner. Methylated methyl pectate was heated in a sealed tube at 100° with methanolic hydrogen chloride, the methanolysis product was reduced with lithium aluminium hydride in tetrahydrofuran solution, and the reduction product was hydrolysed in aqueous solution. The resulting mixture of sugars was separated on cellulose, to give 2 : 3-di-*O*-methyl-D-galactose as the main component, together with smaller amounts of 2 : 3 : 4-tri- and 3 : 4-di-*O*-methyl-L-rhamnose, and 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4- and 2 : 3 : 6-tri-, 2 : 4-di -,and 2-mono-*O*-methyl-D-galactose. In addition, there was chromatographic evidence for 2 : 3 : 5-tri-*O*-methylarabinose and 3-*O*-methylgalactose.

It is clear from these results that the main component, 2 : 3-di-*O*-methyl-D-galactose, has arisen from residues of 2 : 3-di-*O*-methyl-D-galacturonic acid, and that sisal pectic acid contains chains of 1 : 4-linked α-D-galacturonic acid residues. It is possible that the 2 : 3 : 4-tri-*O*-methyl-D-galactose originates from a non-reducing D-galacturonic acid end group. The 2 : 3 : 4-tri- and 3 : 4-di-*O*-methyl-L-rhamnose, and 2 : 3 : 4 : 6-tetra- and 2 : 3 : 6-tri-*O*-methyl-D-galactose, however, must arise from L-rhamnose and D-galactose residues originally present as polysaccharide components. Although it was not possible to estimate accurately the amounts of these methylated sugars, the high proportion of both these sugars present as non-reducing end groups can only be explained if these sugars are attached in some way to D-galacturonic acid residues.

In another series of experiments sisal pectic acid was converted into the ethylene glycol

[3] Andrews and Hough, *Chem. and Ind.*, 1956, 1278; Anderson, Andrews, and Hough, *ibid.*, 1957, 1453.

[4] Bera, Foster, and Stacey, *J.*, 1955, 3788.

ester by reaction with ethylene oxide.[5] A similarly prepared propylene glycol ester [5] had a molecular weight (from sedimentation and diffusion measurements) of 37,200, corresponding to a degree of polymerisation of 169. The ethylene glycol ester was reduced with potassium borohydride in aqueous solution in the presence of glycerol, which maintained the pH of the solution at about 8 and minimised alkaline hydrolysis of the ester.[6] Although it was not possible to obtain a completely reduced polysaccharide by this means, after repeated esterification and reduction a polysaccharide containing only 5—6% of uronic anhydride was isolated. Hydrolysis of this material gave galactose (77%), arabinose (6·5%), rhamnose (3%), and glucose (2%). A molecular-weight determination (from sedimentation and diffusion measurements) gave a value of 22,100, corresponding to a degree of polymerisation of 139. A comparison of this value with that obtained for the pectic acid propylene glycol ester shows that potassium borohydride reduction causes very little degradation.

A less completely reduced pectic acid (uronic anhydride, 18%) was methylated and complete reduction of uronic acid groups was effected by treatment of the methylated polysaccharide with lithium aluminium hydride in tetrahydrofuran. Further methylation of the reduction product afforded methylated galactan. Hydrolysis of the methylated polysaccharide gave 2 : 3 : 5-tri-*O*-methyl-L-arabinose, 2 : 3 : 4 : 6-tetra- and 2 : 3 : 6-tri-*O*-methyl-D-galactose, and a mixture of di-*O*-methyl-D-galactoses, the 2 : 4-isomer being definitely characterised. The isolation of 2 : 3 : 6-tri-*O*-methyl-D-galactose as the main product of hydrolysis is consistent with the presence of chains of 1 : 4-linked α-D-galacturonic acid residues in the original pectic acid. Since L-arabinose was found only as non-reducing end groups in the furanose form, this sugar cannot occur as an araban of the type found in other pectic materials [1] and must be present as a constituent of a polysaccharide in which D-galacturonic acid or D-galactose residues form the framework of the molecular structure.

It is not possible to draw precise conclusions regarding the structure of sisal pectic acid from these experiments. The pectic acid is composed of chains of 1 : 4-linked α-D-galacturonic acid residues, but the rôle of the neutral sugars, D-galactose, L-rhamnose, and L-arabinose, in the polysaccharide structure is not yet clear. In view of the tenacity of the association of these neutral sugars with the D-galacturonic acid residues, it seems probable that they are constituents of acidic rather than neutral polysaccharides. Two possibilities may be suggested on the basis of present evidence: (*a*) that these sugars are constituents of pectic acid and are linked to the galacturonic acid chains; or (*b*) that there is present a mixture of acidic polysaccharides, one composed solely of D-galacturonic acid residues and the other or others containing both neutral sugar and D-galacturonic acid residues. It is possible that pectic substances, like the hemicelluloses, contain a series of closely related molecular species.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems (v/v): (A) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3, upper layer); (B) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (C) butan-2-one, half saturated with water containing 1% of ammonia; (D) ethyl acetate–acetic acid–water (3 : 1 : 3, upper layer); (E) butan-1-ol–acetic acid–water (4 : 1 : 5, upper layer); (F) ethyl acetate–pyridine–water (10 : 4 : 3); (G) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (H) benzene–ethanol–water (169 : 47 : 15, upper layer); (J) butan-2-one–acetic acid–water (9 : 1 : 1), saturated with boric acid. Unless otherwise stated, R_G values of methylated sugars refer to rates of movement relative to tetra-*O*-methyl-D-glucose in solvent B. Chromatography of the periodate oxidation products of methylated sugars was carried by Lemieux and Bauer's

[5] Deuel, *Helv. Chim. Acta*, 1947, **30**, 1523.
[6] Deuel and Zweifel, unpublished results (we are most grateful to Dr. G. Zweifel for furnishing us with experimental details in advance of publication).

method.[7] Methylated sugars were demethylated with hydrobromic acid.[8] Optical rotations were observed at 18° ± 2°.

Isolation and Examination of the Water-soluble Polysaccharides.—Sisal flesh (the fleshy part of the plant from which the fibre has been removed; 1 kg.) was extracted with water (6 l.) at 90° for 3 hr. After filtration and centrifugation the extract was concentrated to 1 l. and poured into an equal volume of acetone to give a crude polysaccharide mixture (*ca.* 20 g.). This material was dissolved in water (400 ml.), and acetone (400 ml.) was added slowly with stirring to the solution to give fraction *a* {14·7 g., $[\alpha]_D$ +74° (*c* 0·11 in H_2O), uronic anhydride (Kaye and Kent's method,[9] 32%}. Addition of further quantities of acetone to the supernatant liquid gave fractions *b* (2·5 g.), $[\alpha]_D$ −19° (*c* 0·21 in H_2O), and *c* (1·3 g.), $[\alpha]_D$ +47° (*c* 0·17 in H_2O). Hydrolysis of samples of the polysaccharide fractions gave galactose, glucose, arabinose, xylose, rhamnose, and traces of two minor components.

Water-soluble polysaccharide (fraction *a*, 1·84 g.) was heated with N-sulphuric acid 100 ml.) at 100° for 4 hr., the cooled solution was neutralised with barium carbonate, and the filtrate was concentrated to a syrup (1·70 g.). The syrup (1·57 g.) was fractionated on a cellulose column with butan-1 ol, half saturated with water, as eluant to give six fractions, and a seventh fraction was obtained by elution with water. Fraction 1 (27 mg.) contained a sugar (R_G 0·42 in solvent B) chromatographically and ionophoretically indistinguishable from 2-*O*-methyl-D-xylose and two minor components with R_G 0·57 and 0·73. Fraction 2 (105 mg.), $[\alpha]_D$ +8·6° (*c* 1·0 in H_2O), was identified as L-rhamnose by conversion into the benzoylhydrazone, m. p. and mixed m. p. 180—181°. Fraction 3 (47 mg.), $[\alpha]_D$ +19·2° (*c* 0·4 in H_2O), was identified as D-xylose by conversion into the di-*O*-benzylidene dimethyl acetal, m. p. and mixed m. p. 210—211°. Fraction 4 (202 mg.), $[\alpha]_D$ +106° (*c* 2·0 in H_2O), was identified as L-arabinose by conversion into the benzoylhydrazone, m. p. and mixed m. p. 185—189°. Chromatography of fraction 5 (300 mg.) showed glucose and galactose, and the presence of D-glucose was confirmed by the formation of the *p*-nitrophenylhydrazone, m. p. 184—185° and mixed m. p. 183—185°. The optical rotation of fraction 5, $[\alpha]_D$ +57·4° (*c* 1·6 in H_2O), corresponded to the presence of D-glucose (236 mg.) and D-galactose (64 mg.). Fraction 6 (130 mg.), $[\alpha]_D$ +81° (*c* 1·3 in H_2O), was identified as D-galactose by conversion into the 1-methyl-1-phenylhydrazone, m. p. and mixed m. p. 187—189°. Fraction 7 (665 mg.), obtained as barium salt, was treated with Amberlite resin IR-120(H) to give D-galacturonic acid, $[\alpha]_D$ +84·5° (*c* 1·7 in H_2O), identified as the 2 : 5-dichlorophenylhydrazone, m. p. and mixed m. p. 179—181°, and by conversion into mucic acid, m. p. and mixed m. p. 205—206°.

Isolation of 2-O-*Methyl*-D-*xylose and* 2-O-*Methyl*-L-*fucose.*—The unfractionated polysaccharide mixture (10 g.) was hydrolysed with N-sulphuric acid (500 ml.) at 100° for 6 hr., and the cooled solution was neutralised with barium carbonate, filtered, treated with Amberlite resin IR-120(H) to remove barium ions, concentrated, and absorbed on charcoal. The column was eluted successively with water and water containing increasing proportions of ethanol. A fraction (*ca.* 100 mg.), eluted with water containing 15% of ethanol, contained rhamnose, and sugars *a* and *b* with R_G 0·42 and 0·57. Pure samples of these sugars were isolated after chromatography on filter sheets with solvent 5. Sugar *a* (45 mg.) was identified as 2-*O*-methyl-D-xylose by m. p. 132—134° and mixed m. p. 131—133°, $[\alpha]_D$ +34° ± 1° (equil.) (*c* 0·25 in H_2O), and *X*-ray powder photograph. Sugar *b* (18 mg.) was identified as 2-*O*-methyl-L-fucose by m. p. 154—159° and mixed m. p. 155—159°, $[\alpha]_D$ −85° ± 1° (equil.) (*c* 0·36 in H_2O), and *X*-ray powder photograph. Chromatography of the periodate-oxidation products of both sugars showed methoxymalondialdehyde.

Isolation of Sisal Pectic Acid.—Water-extracted sisal flesh (2 kg.) was extracted five times with 0·5% ammonium oxalate solution (10 l.) at 80—90° for 2 hr. The extracts were concentrated to 2 l. and crude ammonium pectate was precipitated by the addition of an equal volume of ethanol. Calcium chloride solution (5%) was added to a 2% aqueous solution of this material until no further precipitate was formed, and the calcium pectate was then separated. A sample of calcium pectate was hydrolysed; chromatography then showed rhamnose, xylose, arabinose, galactose, and galacturonic acid. Calcium pectate was suspended in water and heated at 80° for 1 hr. with a slight excess of ammonium oxalate, calcium oxalate was removed by filtration, and the solution was dialysed against distilled water. Hydrolysis of an aliquot

[7] Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.
[8] Hough, Jones, and Wadman, *J.*, 1950, 1705.
[9] Kaye and Kent, *J.*, 1953, 79.

part gave the same mixture of sugars but no xylose. After a second precipitation of the polysaccharide as calcium salt, it was isolated as ammonium salt by freeze-drying (yield, 180 g.). A sample of ammonium pectate in aqueous solution was passed through a column of Amberlite resin IR-120(H) and the derived pectic acid had $[\alpha]_D$ +238° (*c* 0·57 in H_2O) [Found: uronic anhydride (by decarboxylation), 77·1%; equiv., 233 (corresponds to 75·6% of uronic anhydride)].

Attempted Fractionations of Ammonium Pectate.—(1) Sodium hydroxide (10 g.) in water (20 ml.) was added to a suspension of crude ammonium pectate (20 g.) in ethanol (450 ml.), and the mixture was kept at 0° for 40 hr. with occasional shaking. The solid was filtered off, washed thoroughly with ethanol–water (1 : 1) to remove alkali, and reprecipitated twice from aqueous solution by the addition of an equal volume of ethanol. The resulting sodium pectate was extracted twice for 1 hr. with boiling ethanol–water (1 : 1), dissolved in water, precipitated as calcium pectate, and isolated as above by conversion into ammonium pectate (sample A), $[\alpha]_D$ +213° (*c* 0·83 in H_2O) [Found: uronic anhydride (by decarboxylation), 75·2%]. Hydrolysis of a sample gave rhamnose, arabinose, galactose, and galacturonic acid.

(2) A 10% aqueous solution of " Cetavlon " (cetyltrimethylammonium bromide) was added to ammonium pectate (25 g.) in water (1·2 l.), and the precipitated polysaccharide complex was separated at the centrifuge. The complex was decomposed by stirring it with 5N-acetic acid and pouring the mixture into 3 vol. of ethanol. Since the precipitated polysaccharide contained adhering " Cetavlon," it was redispersed in water, shaken for 2 hr. with Amberlite resin IR-120(H), filtered, and freeze-dried, to give pectic acid (sample B; 20 g.), $[\alpha]_D$ +215° (as ammonium salt) (*c* 0·4 in H_2O) [Found: uronic anhydride (by decarboxylation), 79·0%]. Hydrolysis of a sample gave rhamnose, arabinose, galactose, and galacturonic acid.

(3) Crude ammonium pectate (20 g.) was dissolved in water (1 l.) and precipitated by the addition of an equal volume of acetone. After a second reprecipitation the polysaccharide was extracted twice with boiling ethanol–water (1 : 1), dissolved in water, and isolated by freeze-drying. Ammonium pectate (15 g.) had $[\alpha]_D$ +213° (*c* 1·0 in H_2O) [Found: uronic anhydride, 74·0%]. Hydrolysis of a sample gave rhamnose, arabinose, galactose, and galacturonic acid.

Methylation of Ammonium Pectate.—Ammonium pectate (sample A, 20 g.) was methylated with methyl sulphate and sodium hydroxide, and the product was isolated as methylated sodium pectate (OMe, 22·6%). The sodium salt after extraction with methanol to remove neutral polysaccharides was dissolved in water, the solution was passed through a column of Amberlite resin IR-120(H) to remove sodium ions, and the resulting acid was converted into silver salt by treatment with silver oxide. Finely ground silver salt was refluxed with methyl iodide containing 5% of methanol, silver oxide (50 g.) was added during 4 hr., and refluxing was continued for a further 4 hr. After a further treatment with methyl iodide and silver oxide the methylated polysaccharide (10·5—11 g.) was isolated, $[\alpha]_D$ +191° (*c* 0·63 in $CHCl_3$) (Found: OMe, 39·4%).

Purification of Methylated Methyl Pectate.—Light petroleum (b. p. 40—60°) (285 ml.) was added to a solution of the methylated polysaccharide (8·1 g.) in chloroform (135 ml.), and the precipitated material (6·7 g.) was separated. This fraction was dissolved in ethanol (100 ml.), sodium hydroxide (4 g.) in water (4 ml.) was added dropwise with stirring, and the mixture was left at 0° for 8 hr. Ethanol–ether (1 : 1; 100 ml.) was added to the mixture, and the gelatinous precipitate was separated at the centrifuge and washed with ethanol–ether (1 : 1) until free from alkali. The methylated sodium pectate was then converted into the silver salt as described previously, and after treatment with methyl iodide and silver oxide afforded methylated methyl pectate (5 g.), $[\alpha]_D$ +212° (*c* 0·4 in $CHCl_3$) (Found: OMe, 40·0%).

Methanolysis of Methylated Methyl Pectate; Reduction, Hydrolysis, and Separation of Methylated Sugars.—Methylated methyl pectate (5 g.) was heated in a sealed tube with methanolic 4% hydrogen chloride (100 ml.) at 100° for 4 hr. After neutralisation with silver carbonate and removal of solvent, the resulting syrup was dissolved in tetrahydrofuran (150 ml.), and lithium aluminium hydride (2 g.) in tetrahydrofuran (50 ml.) was added dropwise during 1 hr. to the boiling solution. The mixture was refluxed for 1 hr., then set aside at room temperature for 2 hr., excess of hydride was destroyed by addition of water, and the mixture was taken to dryness. The residue was exhaustively extracted with acetone and ethanol, the extracts were diluted with water, de-ionised with Amberlite resins IR-120(H) and IR-4B(OH), evaporated, and hydrolysed with N-hydrochloric acid at 100° for 5 hr., to give a syrupy mixture of sugars (3·66 g.).

The mixture of sugars was separated on cellulose by using solvent B. Only one pure sugar was obtained and the remaining fractions, which all contained several components, were recombined. The pure sugar (2·0 g.) had $[\alpha]_D$ +80° (*c* 0·63 in H_2O) (Found: OMe, 29·9. Calc. for $C_8H_{16}O_6$: OMe, 29·8%) and was characterised as 2 : 3-di-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 154—155°, and into 2 : 3-di-*O*-methyl-D-galactonamide, m. p. and mixed m. p. 139—140°. The remaining fractions (1·23 g.) were refractionated on cellulose (70 × 2·5 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3) saturated with water as eluant to give thirteen fractions.

Fraction 1. The chromatographically pure syrup (23 mg.) had $[\alpha]_D$ +25° (*c* 0·2 in H_2O) and R_G 1·01. Demethylation gave rhamnose. In a separate experiment the sugar was characterised as 2 : 3 : 4-tri-*O*-methyl-L-rhamnose by conversion into 2 : 3 : 4-tri-*O*-methyl-L-rhamnonophenylhydrazide, m. p. 175—177°.

Fraction 2. Chromatography of the syrup (134 mg.) showed two components, R_G 0·95 and 0·90, and the optical rotation, $[\alpha]_D$ +49° (*c* 1·6 in H_2O), was consistent with the presence of 2 : 3 : 5-tri-*O*-methyl-L-arabinose (50 mg.) and 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (84 mg.) in the mixture. Demethylation gave arabinose and galactose.

Fraction 3. The chromatographically pure syrup (19 mg.) had $[\alpha]_D$ +108° (*c* 0·18 in H_2O) and R_G 0·90 and was characterised as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 187—190°.

Fraction 4. Chromatography of the syrup (86 mg.) showed two components, R_G 0·90 and 0·88, together with traces of slower-moving substances. Quantitative chromatography [10] (separation in solvent H) indicated the presence of tetra-*O*-methylgalactose (27 mg.) and di-*O*-methylrhamnose in the mixture. Demethylation gave galactose and rhamnose. 2 : 3 : 4 : 6-Tetra-*O*-methyl-D-galactose was characterised as the aniline derivative, m. p. and mixed m. p. 189—191°. Ionophoresis showed the rhamnose derivative to be the 3 : 4-dimethyl ether. In a separate experiment this sugar was characterised by conversion into 3 : 4-di-*O*-methyl-L-rhamnonolactone, m. p. 76—78°.

Fraction 5. Chromatography of the sugar (137 mg.) showed a main component, R_G 0·78, and a trace of 2 : 3 : 6-tri-*O*-methylgalactose. Demethylation gave galactose. Since further hydrolysis gave 2 : 3-di-*O*-methylgalactose as the major product, it is probable that the main component is a polymer of 2 : 3-di-*O*-methylgalactose resulting from incomplete hydrolysis of the methylated polysaccharide.

Fraction 6. The sugar (18 mg.), $[\alpha]_D$ +111° (*c* 0·35 in H_2O, R_G 0·70) was identified as 2 : 3 : 4-tri-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 163—165°.

Fraction 7. Chromatography of the syrup (47 mg.), $[\alpha]_D$ +85° (*c* 0·61 in H_2O), showed 2 : 3 : 6-tri-*O*-methylgalactose (R_G 0·72) and a small amount of the 2 : 3 : 4-isomer (R_G 0·69). The main component was characterised by conversion into 2 : 3 : 6-tri-*O*-methyl-D-galactonolactone, m. p. and mixed m. p. 98—99°.

Fraction 8. Chromatography of the syrup (54 mg.), $[\alpha]_D$ +86° ± 2° (*c* 0·9 in H_2O), showed two components, R_G 0·76 and 0·64. Quantitative chromatography [10] indicated the presence of 2 : 3 : 6- (24 mg.) and (probably) 2 : 3 : 4-tri-*O*-methylgalactose (29 mg.) in the mixture. Demethylation gave galactose.

Fraction 9. Quantitative chromatography [10] of the syrup (65 mg.) showed two main components, R_G 0·64 (probably 2 : 3 : 4-tri-*O*-methylgalactose) (45 mg.) and 0·50 (di-*O*-methylgalactose) (19 mg.), and a trace of 2 : 3 : 6-tri-*O*-methylgalactose. Chromatography of the periodate oxidation products of the di-*O*-methylgalactose fraction indicated the presence of the 2 : 3- and the 2 : 6-dimethyl ether.

Fraction 10. The syrup (38 mg.) had $[\alpha]_D$ +83° (*c* 0·67 in H_2O) and R_G 0·56. Chromatography in solvent C showed a mixture of 2 : 4- (R_G 0·2) and 2 : 3(and possibly 2 : 6)-di-*O*-methylgalactose (R_G 0·3). Chromatography of the periodate-oxidation products indicated the presence of 2 : 3-, 2 : 4-, and 2 : 6-di-*O*-methylgalactose. The presence of 2 : 4-di-*O*-methyl-D-galactose in the mixture was confirmed by the formation of the aniline derivative, m. p. 208—209·5°.

Fraction 11. The syrup (31 mg.), $[\alpha]_D$ +81°, contained a main component (R_G 0·50) and traces of two minor components (R_G 0·72 and 0·42). The main component was identified as

[10] Hirst, Hough, and Jones, *J.*, 1949, 928.

2 : 3-di-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 152—154°.

Fraction 12. The syrup (38 mg.) contained a complex mixture of sugars (R_G 0·88, 0·70, 0·43, and 0·38) and was not examined further.

Fraction 13. Quantitative chromatography in solvent C indicated the presence of 2-*O*-methylgalactose (192 mg.), 3-*O*-methylgalactose (113 mg.), and galactose (14 mg.). Chromatography of the periodate oxidation products showed methoxymalondialdehyde (from 2-*O*-methylaldoses) and a methylated pentose (probably 2-*O*-methyl-lyxose from 3-*O*-methylgalactose). After separation of part of the fraction on filter sheets with solvent J, 2-*O*-methyl-D-galactose, m. p. and mixed m. p. 150—154°, was isolated.

Preparation of Ethylene Glycol Pectate.—Ethylene oxide (100 ml.) was added to a suspension of pectic acid (sample, B; 33 g.) in water (330 ml.), and the mixture was shaken at room temperature for 9 days, until the resulting solution was neutral. The glycol ester was precipitated by the addition of acetone, redispersed in water, and isolated by freeze-drying. The glycol ester (39 g.) had $[\alpha]_D$ +193° ± 3° (*c* 0·59 in H_2O) [Found: uronic anhydride (by decarboxylation), 65% (≡ 78% uronic anhydride in pectic acid); equiv., 263 (≡ 67% uronic anhydride in glycol ester and 80% uronic anhydride in pectic acid); glycol released on saponification, 67·5% (≡ 81% uronic anhydride in pectic acid)].

Preparation of Propylene Glycol Pectate.—Similarly reaction of propylene oxide with pectic acid afforded propylene glycol pectate, $[\alpha]_D$ +168° ± 2° (*c* 0·89 in H_2O) [Found: uronic anhydride (by decarboxylation), 62% (≡ 78% in pectic acid); glycol released on saponification, 63·5% (≡ 80% uronic anhydride in pectic acid)]. Sedimentation and diffusion measurements gave a weight-average molecular weight of 37,200, corresponding to a degree of polymerisation of 169 ($S = 1{\cdot}5 \times 10^{-13}$; $D = 2{\cdot}5 \times 10^{-7}$; $\bar{v} = 0{\cdot}6$).

Reduction of Ethylene Glycol Pectate.—Potassium borohydride (3·5 g.) in water (20 ml.) was added to ethylene glycol pectate (11 g.) in water (250 ml.) containing glycerol (4·5 g.) at 0°. After being kept overnight the solution was de-ionised by passage through columns of Amberlite resins IR-120(H) and IRA-400(OH) and the partially reduced polysaccharide (7·3 g.) was isolated by freeze-drying. The derived ethylene glycol ester had $[\alpha]_D$ +247° (*c* 0·33 in H_2O) [Found: uronic anhydride (Kaye and Kent's method [9]), 18·4% (≡ 19·3% uronic anhydride in polysaccharide acid)]. After five further reductions the resulting galactan (5 g.) had $[\alpha]_D$ +247° (*c* 1·3 in H_2O) [Found: uronic anhydride, 5·0% (by decarboxylation); 5·9% (Kaye and Kent's method [9])]. A sample was hydrolysed and quantitative chromatography [11] showed galactose (77%), arabinose (6·5%), rhamnose (3%), and glucose (2%). Sedimentation and diffusion measurements gave a weight-average molecular weight of 22,100, corresponding to a degree of polymerisation of 139 ($S = 1{\cdot}42 \times 10^{-13}$; $D = 4{\cdot}0 \times 10^{-7}$; $\bar{v} = 0{\cdot}6$).

Preparation of Methylated Galactan; Hydrolysis, and Separation of Methylated Sugars.—Partially reduced pectic acid (uronic anhydride, 18%; 8 g.) was methylated with methyl sulphate and sodium hydroxide and partially methylated polysaccharide (as sodium salt) was isolated after dialysis. After treatment with Amberlite resin IR-120(H) to remove sodium ions, the acid was converted into the silver salt by neutralisation with silver carbonate. Dry silver salt was dissolved in methyl iodide (150 ml.), and silver oxide (50 g.) was added slowly during 6 hr. to the boiling solution; the mixture afforded methylated polysaccharide (5·2 g.) (Found: OMe, 41·3%). Lithium aluminium hydride (3 g.) in tetrahydrofuran (150 ml.) was added slowly to the methylated polysaccharide (5·2 g.) in boiling tetrahydrofuran (100 ml.) and refluxing was continued for 1 hr. The reduced methylated polysaccharide, after separation from inorganic salts, was methylated twice with methyl iodide and silver oxide to give methylated galactan (3·8 g.), $[\alpha]_D$ +177° ± 1° (*c* 3·0 in H_2O), +158° ± 2° (*c* 3·2 in $CHCl_3$) (Found: OMe, 42·5%).

Methylated galactan (3·5 g.) was refluxed for 5 hr. with methanol (75 ml.) and 2N-hydrochloric acid (75 ml.). Methanol was evaporated under reduced pressure, water was added, and the solution (N with respect to hydrochloric acid) was heated at 100° for 3 hr. The cooled solution was neutralised with silver carbonate, filtered, and concentrated to a syrup (3·12 g.) which was separated on cellulose (75 × 3·9 cm.) by means of light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3) saturated with water as eluant to give four fractions.

Fraction 1. Chromatography of the syrup (759 mg.) showed three components (R_G 1·00, 0·96, and 0·87). A sample was hydrolysed and since the fastest-moving component disappeared

[11] Flood, Hirst, and Jones, *J.*, 1948, 1679.

with the formation of 2 : 3 : 6-tri-*O*-methylgalactose (R_G 0·73) it is probable that either a methyl glycoside or a polymer of 2 : 3 : 6-tri-*O*-methylgalactose was present. The syrup (750 mg.) was re-hydrolysed with N-hydrochloric acid for 5 hr. and quantitative chromatography of the hydrolysate showed 2 : 3 : 5-tri-*O*-methylarabinose (R_G 0·96; 200 mg.), 2 : 3 : 4 : 6-tetra-*O*-methylgalactose (R_G 0·87; 140 mg.) and 2 : 3 : 6-tri-*O*-methylgalactose (R_G 0·73; 410 mg.). Larger quantities of the sugars were separated on filter sheets with solvent H, and 2 : 3 : 5-tri-*O*-methyl-L-arabinose was characterised by conversion into 2 : 3 : 5-tri-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 137—139°, 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose was characterised as the aniline derivative, m. p. and mixed m. p. 194—196°, and 2 : 3 : 6-tri-*O*-methyl-D-galactose was characterised by conversion into 2 : 3 : 6-tri-*O*-methyl-D-galactonolactone, m. p. and mixed m. p. 97—99°.

Fraction 2. The syrup (778 mg.), $[\alpha]_D$ +82° (*c* 3·6 in H_2O), contained 2 : 3 : 6-tri-*O*-methyl-D-galactose (*ca.* 720 mg.) and 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (*ca.* 58 mg.) (approximate quantities calculated from the optical rotation). The main component was identified by conversion into 2 : 3 : 6-tri-*O*-methyl-D-galactonolactone, m. p. and mixed m. p. 98—99°.

Fraction 3. The sugar (R_G 0·73; 759 mg.), $[\alpha]_D$ +79·4° (*c* 4·8 in H_2O), was identified as 2 : 3 : 6-tri-*O*-methyl-D-galactose by conversion into 2 : 3 : 6-tri-*O*-methyl-D-galactonolactone, m. p. and mixed m. p. 97—99°.

Fraction 4. The syrup (420 mg.) contained di-*O*-methylgalactose (R_G 0·51) together with small amounts of other sugars (R_G 0·94, 0·85, 0·72, and 0·24). The di-*O*-methylgalactose fraction was separated by chromatography on filter sheets, and chromatography of the sugar mixture in solvent C and of the periodate oxidation products indicated the presence of the 2 : 3-, 2 : 4-, and 2 : 6-dimethyl ether. The presence of 2 : 4-di-*O*-methyl-D-galactose was confirmed by the formation of its aniline derivative, m. p. and mixed m. p. 207—209°.

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