822. *Pectic Substances from Lucerne* (Medicago sativa). *Part I. Pectic Acid.*

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Extraction of lucerne with boiling ethanol–water (4 : 1) affords a mixture of mono- and oligo-saccharides, including D-glucose, D-fructose, sucrose, and raffinose. Extraction with water, ammonium oxalate solution, and lime-water gives mixtures of pectic substances, including a polysaccharide of the pectic acid type (from ammonium oxalate extraction) which was isolated in a pure state. Partial hydrolysis of the pectic acid furnishes L-rhamnose, L-arabinose, D-galactose, D-galacturonic acid, traces of L-fucose, 2-*O*-methyl-L-fucose, and 2-*O*-methyl-D-xylose, and a mixture of acidic oligosaccharides including 2-*O*-(D-galactopyranosyluronic acid)-L-rhamnose and oligomers of D-galacturonic acid. Methanolysis of methylated pectic acid, followed by reduction and hydrolysis, affords 2,3,5-tri- and 2,3-di-*O*-methyl-, and 2-*O*-methyl-L-arabinose, 2,3,4,6-tetra-, 2,3,4-tri-, and 2,3-di-*O*-methyl-, and 2- and 3-*O*-methyl-D-galactose, and 3,4-di-*O*-methyl- and 3-*O*-methyl-L-rhamnose. The structural significance of these and other results is discussed.

A method for the reduction of hexuronic acid derivatives with diborane has been studied.

The pectic group of polysaccharides contains three recognised structural species, pectic acid, galactan, and araban, examples of each species having been isolated from appropriate sources.[1] Several recent investigations, however, have suggested that some pectic acids are not polymers of D-galacturonic acid alone and that neutral sugars, especially L-arabinose, D-galactose, and L-rhamnose, are components of the acidic polysaccharides.[2-4] Analytical studies [5] have shown that the leaves and stems of lucerne (alfalfa) contain substantial amounts of pectic substances, and in this paper we describe an examination of some of the carbohydrate constituents of lucerne together with a structural study of the pectic acid.

Oven-dried lucerne was extracted successively with boiling ethanol–water (4 : 1), cold and hot water, hot aqueous ammonium oxalate, and hot lime-water. Extraction with boiling ethanol–water removed colouring matter, and a mixture of soluble sugars, which contained D-glucose, D-fructose, and sucrose as major constituents, together with higher oligosaccharides including raffinose. The cold- and hot-water extracts contained complex mixtures of polysaccharides in which substantial proportions of hexuronic acid residues were present as methyl esters. These extracts were grossly contaminated with inorganic material and protein, and the polysaccharides have not yet been isolated in a pure state. The ammonium oxalate extract was treated with calcium chloride solution, and the precipitated calcium pectate was re-converted into ammonium pectate, which was used for structural studies. Extraction with saturated lime-water gave a mixture of polysaccharides with arabinose as the main component sugar together with galactose, xylose, rhamnose, and unidentified hexuronic acid. This preparation was separated into fractions soluble and insoluble in boiling ethanol–water (7 : 3). The soluble fraction was enriched with respect to araban and had a more negative optical rotation ($[\alpha]_D$ −50°) characteristic of pectic arabans. The insoluble fraction contained xylose as the main component sugar and probably represents a small proportion of the xylan hemicellulose which may be extracted from the plant with aqueous sodium hydroxide.[5]

Lucerne ammonium pectate had a characteristically high positive optical rotation

[1] Hirst and Jones, *Adv. Carbohydrate Chem.*, 1946, **2**, 235.
[2] Aspinall and Cañas-Rodriguez, *J.*, 1958, 4020.
[3] McCready and Gee, *J. Agric. Food Chem.*, 1960, **8**, 510.
[4] Neukom, Deuel, Heri, and Kundig, *Helv. Chim. Acta*, 1960, **43**, 67.
[5] Hirst, Mackenzie, and Wylam, *J. Sci. Food Agric.*, 1959, **10**, 19.

($[\alpha]_D$ +203°) and a uronic anhydride content of *ca.* 50%. Paper chromatography showed that the hydrolysate contained substantial amounts of arabinose, galactose, and rhamnose, together with traces of fucose, xylose, 2-*O*-methylxylose, and 2-*O*-methylfucose. Several attempts were made to fractionate the ammonium pectate further, by precipitation as the calcium salt, precipitation with cetyltrimethylammonium bromide, and extractions of neutral polysaccharides with boiling ethanol–water (7 : 3) and with saturated lime-water, but in each case the regenerated polysaccharide was virtually unchanged in optical rotation and uronic anhydride content. Further, the polysaccharide was chromatographed on diethylaminoethylcellulose by the procedure of Neukom *et al.*[4] Elution with phosphate buffers at pH 6·5 removed only minor traces of polysaccharide and *ca.* 98% of the polysaccharide was eluted in a single broad band with increasing concentrations of sodium hydroxide. Hydrolysis of the recovered polysaccharide showed the presence of all the neutral sugar components in the original ammonium pectate. When the araban-rich polysaccharide (from lime-water extraction) was chromatographed in a similar manner on diethylaminoethylcellulose most of the polysaccharide was eluted from the column with phosphate buffer. By this criterion, therefore, the ammonium pectate was homogeneous, and the neutral sugars were present as components of either a single acidic polysaccharide or a mixture of closely related acidic polysaccharides.

Acid hydrolysis of ammonium pectate afforded neutral monosaccharides, a mixture of D-galacturonic acid and acidic oligosaccharides, and a degraded polysaccharide. The following neutral monosaccharides were characterised as crystalline sugars or as suitable crystalline derivatives: D-galactose, L-arabinose, L-rhamnose, L-fucose, 2-*O*-methyl-D-xylose, 2-*O*-methyl-L-fucose, and xylose (by paper chromatography only). The mixture of acidic sugars was fractionated by gradient elution from anion-exchange resin followed, where necessary, by partition chromatography on filter sheets. The major fractions contained D-galacturonic acid, its dimer and trimer, and the aldobiouronic acid, 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, and minor fractions contained acidic oligosaccharides which were partially characterised. Further quantities of D-galacturonic acid, and its dimer and trimer, were formed when the pectic acid and the acid-degraded pectic acid were degraded with a commercial enzyme preparation.

The structure of the aldobiouronic acid, 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, was established from the following observations: (*a*) hydrolysis of the sugar gave galacturonic acid and rhamnose, and reduction of the derived methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave galactose and rhamnose; (*b*) the absence of a staining reaction with triphenyltetrazolium hydroxide [6] pointed to the presence of a 2-*O*-substituted rhamnose residue; (*c*) methylation with methyl sulphate and sodium hydroxide furnished a methyl glycoside pentamethyl ether as a crystalline dihydrate, which when reduced with diborane in tetrahydrofuran [7] and hydrolysed gave 2,3,4-tri-*O*-methyl-D-galactose and 3,4-di-*O*-methyl-L-rhamnose; (*d*) the α-configuration of the glycosidic linkage was tentatively assigned on the basis of the optical rotation of the acidic disaccharide. A second aldobiouronic acid was an *O*-(galactopyranosyluronic acid)fucose since hydrolysis gave galacturonic acid and fucose; a positive staining reaction with triphenyltetrazolium hydroxide showed that the linkage was other than 1,2. A third aldobiouronic acid was similarly shown to be an *O*-(galactopyranosyluronic acid)galactose. Another acidic oligosaccharide was a trisaccharide containing two parts of rhamnose and one of galacturonic acid. Partial acid hydrolysis gave 2-*O*-(galactosyluronic acid)rhamnose, but no evidence was obtained concerning the mode of linkage of the second rhamnose residue.

The di- and tri-galacturonic acids from acid and enzymic hydrolysis were isolated as calcium salts whose optical rotations ($[\alpha]_D$ +127° and +145°) were indicative of α-glycosidic

[6] Feingold, Avigad, and Hestrin, *Biochem. J.*, 1956, **64**, 351; Bailey, Barker, Bourne, Grant, and Stacey, *J.*, 1958, 1895.
[7] Brown and Subba Rao, *J. Amer. Chem. Soc.*, 1960, **82**, 681.

linkages and were in reasonable agreement with the values quoted for the di- and tri-saccharides formed on enzymic breakdown of apple pectic acid.[8] The digalacturonic acid was treated with cold benzyl alcohol and dry hydrogen chloride, the resulting benzyl ester benzyl glycosides were reduced with lithium aluminium hydride, and the reduction products were hydrogenated over palladium–charcoal, to give galactose and a galactobiose, the latter being chromatographically and ionophoretically indistinguishable from 4-*O*-α-D-galactopyranosyl-D-galactose. In the light of these results and of the subsequent isolation of 2,3-di-*O*-methyl-D-galactose after reduction of the acidic residues in the methylated polysaccharide, there is little doubt that the digalacturonic acid is 4-*O*-(α-D-galactopyranosyluronic acid)-D-galacturonic acid. The trigalacturonic acid was similarly treated with benzyl alcohol and dry hydrogen chloride but only a low yield of benzyl ester glycosides was obtained. Since subsequent reduction of the ester glycosides with lithium aluminium hydride and hydrogenation gave galactose and the same galactobiose, but no galactotriose, it is clear that considerable hydrolysis or alcoholysis took place in the ester glycoside formation. However, only one galactobiose was detected, so it is probable that the trigalacturonic acid contains only one type of linkage.

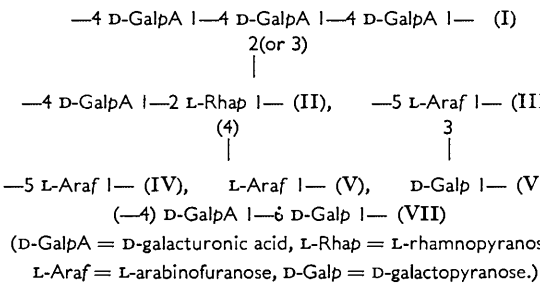
Methylated methyl pectate was prepared from the ammonium pectate, and the methylated polysaccharide was partially depolymerised by heating it with methanolic hydrogen chloride; the methanolysis products were then reduced with lithium aluminium hydride, and the reduction products were hydrolysed. The resulting mixture of methylated sugars was fractionated by partition chromatography on cellulose, giving the following sugars which were either obtained crystalline or characterised as crystalline derivatives: 2,3,4,6-tetra-, 2,3,4-tri-, and 2,3-di-*O*-methyl- and 2- and 3-*O*-methyl-D-galactose, D-galactose, 2,3,5-tri- and 2,3-di-*O*-methyl-L-arabinose, and 3,4-di-*O*-methyl- and 3-*O*-methyl-L-rhamnose. In addition, 2-*O*-methyl-L-arabinose was characterised by optical rotation and by chromatography of the sugar and of the products of demethylation and of periodate oxidation, and a trace of 2,3,4-tri-*O*-methylrhamnose was detected chromatographically. The 2,3,4-tri-*O*-methyl-D-galactose and 2,3-di-*O*-methyl-L-arabinose were obtained in admixture with each other and, although pure samples were obtained by paper-chromatographic separations from which crystalline derivatives were formed, this method was not sufficiently sensitive to detect the presence of other methyl ethers of galactose in the mixture. The derived methyl glycosides were examined by the highly selective gas–liquid partition-chromatographic procedure of Bishop and Cooper.[9] Methyl glycosides of 2,3,4-tri-*O*-methyl-D-galactose and 2,3-di-*O*-methyl-L-arabinose were shown to be the main components, and methyl glycosides of some unidentified sugars and of 2,4,6-tri-*O*-methylgalactose were present as minor components, but methyl glycosides of 2,3,6-tri-*O*-methylgalactose were not detected.

In view of the high uronic acid content of the polysaccharide it is clear that 2,3-di-*O*-methyl-D-galactose, which was the methylated sugar formed in largest amount from the methylated polysaccharide, has arisen, after reduction, from main chains of 1,4-linked α-D-galacturonic acid residues, and it is probable that the mono-*O*-methyl-D-galactoses and possibly also D-galactose have arisen from branching points in the main chains. 2,3,4,6-Tetra-*O*-methyl-D-galactose, 2,3,5-tri- and 2,3-di-*O*-methyl- and 2-*O*-methyl-L-arabinose, and 3,4-di-*O*-methyl- and 3-*O*-methyl-L-rhamnose are clearly derived from D-galactose, L-arabinose, and L-rhamnose residues originally present in the polysaccharide; 2,3,4-tri-*O*-methyl-D-galactose, however, could have originated either from galactose or, after reduction, from galacturonic acid residues. In order to obtain further information on this point the methanolysis products from the methylated polysaccharide were separated into two fractions, soluble and insoluble respectively in chloroform–light petroleum. Hydrolysis of the soluble fraction gave the following sugars, which were

[8] Jones and Reid, *J.*, 1954, 1361.
[9] Bishop and Cooper, *Canad. J. Chem.*, 1960, **38**, 388.

identified by paper chromatography: tetra- and 2,3,4-tri-*O*-methylgalactose, 2,3,5-tri- and 2,3-di-*O*-methyl- and 2-*O*-methyl-arabinose, and 3,4-di-*O*-methylrhamnose. This mixture was fractionated by chromatography on filter sheets, and the sub-fraction containing 2,3,4-tri-*O*-methylgalactose and 2,3-di-*O*-methylarabinose was characterised further by gas–liquid chromatography of the derived methyl glycosides. The insoluble fraction, on hydrolysis, afforded mixtures of acidic sugars (probably mainly oligosaccharides); reduction of the derived methyl ester methyl glycosides with lithium aluminium hydride followed by hydrolysis gave 2,3-di-*O*-methylgalactose and small amounts of 3,4-di-*O*-methylrhamnose and 2,3,4-tri-*O*-methylgalactose; it follows, therefore, that some at least of the 2,3,4-tri-*O*-methyl-D-galactose isolated from the methylated polysaccharide arose from 1,6-linked D-galactose residues, although some could also have been formed from D-galacturonic acid end groups.

The structural significance of these results may be assessed by considering the various acidic and neutral sugar components. The main chain of the polysaccharide is clearly composed of 1,4-linked α-D-galacturonic acid residues (I). and, although due allowance must be made for a small degree of incomplete methylation of the polysaccharide or for demethylation during hydrolysis, some of these residues probably provide branching points in the structure. The isolation as a partial hydrolysis product of the aldobiouronic acid, 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, and of a trisaccharide containing galacturonic acid and rhamnose residues, provides clear evidence that L-rhamnose residues are components of this pectic acid, and the methylation results suggest that some rhamnose residues may act as branching points (II). It is possible that L-rhamnose residues may be interposed between blocks of 1,4-linked α-D-galacturonic acid residues as in the plant gums of the *Khaya* genus.[10] The methyl ethers of L-arabinose, isolated from the methylated polysaccharide, show that some L-arabinofuranose end-groups are present in the polysaccharide, but they do not define the ring size of the non-terminal residues. If all the L-arabinose residues are indeed present in the furanose form it is noteworthy that the modes of linkage of this sugar are the same (III—V) as those in the arabans from pectic materials.[1] On the present evidence the exact location of the arabinose residues cannot be stated, but the apparent homogeneity of the pectic acid indicates that arabinose is an integral sugar component. The relatively high proportion of the D-galactose residues in



the polysaccharide which are present as end groups (VI) shows that these are probably linked in some way to the framework of D-galacturonic acid residues. The isolation of an *O*-(galactosyluronic acid)galactose as a partial hydrolysis product indicates that non-terminal galactose residues, presumably involved in 1,6-linkages (*e.g.*, in VII), are also components of an acidic polysaccharide. Since no 2,3,6-tri-*O*-methyl-D-galactose was formed on hydrolysis of the methylated polysaccharide it follows that 1,4-linked galactose residues of the type found in the pectic galactan from *Lupinus albus* seeds [11] were absent. Derivatives of the minor components of the original polysaccharide, namely L-fucose and

[10] Hirst and Jones, *J.*, 1947, 1225.
[11] Aspinall, Hirst, and Matheson, *J.*, 1956, 989; Aspinall, Johnston, and Stephen, *J.*, 1960, 4918.

D-xylose, could not be detected in the methylated polysaccharide, and it is possible that these sugars were components of a contaminating polysaccharide present in small amount. Although further criteria of the homogeneity of the main polysaccharide are required, these results confirm the view that pectic acids are a complex group of acidic polysaccharides which may contain substantial proportions of neutral sugar components. New methods of structural investigation need to be developed before their detailed structures can be elucidated.

In the course of this investigation the use of diborane for the reduction of carboxyl groups in hexuronic acid derivatives was explored. Methylated hexuronic acids and aldobiouronic acids are smoothly reduced by this reagent in tetrahydrofuran or 1,2-dimethoxyethane; we used the procedure of H. C. Brown and his collaborators.[7] Similarly the carboxyl groups in a methylated acidic xylan [12] were completely reduced with diborane. Difficulties arose when acetylated gum ghatti was treated with diborane in tetrahydrofuran, because the polysaccharide soon became insoluble, but some reduction was achieved. While this work was in progress Smith and Stephen [13] reported the results of a similar study and it is clear from their results that a high proportion of hexuronic acid residues in acetylated polysaccharides may be reduced with diborane to the corresponding hexose residues. This approach will be of great value in converting acidic polysaccharides into the more readily hydrolysable neutral polysaccharides.

Experimental

Unless otherwise stated, paper chromatography was carried out on Whatman Nos. 1, 3MM, and 31 papers with the following solvent systems (v/v): (A) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer): (B) ethyl acetate–pyridine–water (10 : 4 : 3); (C) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (D) benzene–ethanol–water (169 : 47 : 15, upper layer); (E) ethyl acetate–acetic acid–water (10 : 5 : 6); (F) butan-2-one–acetic acid–water (9 : 1 : 1, saturated with boric acid). R_G Values of methylated sugars refer to rates of movement relative to 2,3,4,6-tetra-*O*-methylglucose in solvent A, and $R_{Gal\ A}$ values to rates of movement of acidic sugars relative to galacturonic acid in solvent C. Demethylations of methylated sugars were performed by the method of Hough, Jones, and Wadman.[14] Paper ionophoresis was in borate buffer at pH 10. Unless otherwise stated, optical rotations were observed for water solutions at *ca.* 18°.

Extractions of Lucerne.—(a) *Boiling ethanol–water* (4 : 1). Oven-dried lucerne (leaves and stem; 2 kg.) was extracted exhaustively with boiling ethanol–water (4 : 1). The extract was concentrated to remove ethanol, water-insoluble material was removed by filtration, the aqueous solution was clarified by the addition of equimolecular amounts of cadmium sulphate and barium hydroxide solutions, the precipitate was filtered off, and the filtrate was concentrated to a syrup (*ca.* 100 g.) containing fructose, glucose, and sucrose, together with smaller amounts of raffinose and higher oligosaccharides. Portions of the syrup were fractionated by elution from charcoal–Celite with water containing 2·5% and 8% of ethanol, followed by partition chromatography on cellulose with butan-1-ol, half saturated with water, and ethyl acetate–butan-1-ol–pyridine–water (5 : 5 : 4 : 3) as eluants, and the following sugars were identified: D-fructose (1,2:4,5-di-*O*-isopropylidene-D-fructose, m. p. and mixed m. p. 119°), D-glucose (2,4-dinitrophenylhydrazone, m. p. and mixed m. p. 120—122°), sucrose (octa-acetate, m. p. and mixed m. p. 70—72°), and raffinose (hendeca-acetate, m. p. and mixed m. p. 98°).

(b) *Cold water.* Ethanol-extracted lucerne was stirred with cold water for 24 hr., the residue was filtered off and washed, the filtrate and washings were concentrated, and the crude polysaccharide (*ca.* 80 g.) was precipitated by the addition of an equal volume of ethanol, redissolved in water, and freeze-dried {Found: $[\alpha]_D$ +70°; uronic anhydride (carbazole method [15]), 11·2; N, 1·3; ash, 25%}. Hydrolysis of the polysaccharides gave galactose, arabinose, xylose, rhamnose, and acidic oligosaccharides.

[12] Aspinall and Carter, *J.*, 1956, 3744.
[13] Smith and Stephen, *Tetrahedron Letters*, 1960, No. 7, 17.
[14] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[15] McComb and McCready, *Analyt. Chem.*, 1952, **24**, 1630.

(c) *Hot water.* Cold-water extracted lucerne was stirred with water at 90° for two periods of 3 hr., the extract was concentrated, and the crude polysaccharide (*ca.* 40 g.) was precipitated by the addition of an equal volume of ethanol, redissolved in water, and freeze-dried {Found: $[\alpha]_D$ +38°; uronic anhydride (carbazole method [15]), 22·3; OMe, 1·15; N, 1·8; ash, 4%}. Hydrolysis of the polysaccharide gave galactose, arabinose, xylose, glucose, rhamnose, and acidic oligosaccharides.

(d) *Ammonium oxalate solution.* Hot-water extracted lucerne was extracted twice with 0·5% ammonium oxalate solution at 80—90° for 3 hr. 10% Calcium chloride solution was added to the filtered extract until precipitation of calcium pectate was complete. Calcium pectate was heated in 0·3% ammonium oxalate solution for 0·5 hr. at 90°, calcium oxalate was removed at the centrifuge, and ammonium pectate was precipitated by the addition of acetone, reprecipitated from aqueous solution, dispersed in water and freeze-dried. The ammonium pectate, which was used in subsequent experiments had $[\alpha]_D$ +203° ± 5° (*c* 0·4) [Found: uronic anhydride (by decarboxylation [16]) 50; N, 0·16; ash, 2·8%], and hydrolysis gave galactose, arabinose, rhamnose, small amounts of xylose, fucose, 2-*O*-methylxylose, and 2-*O*-methylfucose, and acidic oligosaccharides.

(e) *Lime-water.* Ammonium oxalate-extracted lucerne was stirred with saturated lime-water at 90° for 2 hr. The filtered extract was deionised by passage through columns of Amberlite resins IR-120(H) and IR-4B(OH), concentrated, and poured into ethanol (8 vol.). The precipitated polysaccharide was dissolved in water and freeze-dried; it had $[\alpha]_D$ −23° (*c* 1·0) [Found: uronic anhydride (carbazole method [15]), 8·8; N, 3·7; ash, 5%], and chromatography of the hydrolysate showed arabinose, galactose, xylose, and rhamnose. Extraction of the polysaccharide (4 g.) with boiling ethanol–water (7 : 3) gave fractions (*a*) soluble and (*b*) insoluble. Fraction (*a*) had $[\alpha]_D$ −50° (Found: uronic anhydride, 4·7; N, 5·9; ash, 3%), and chromatography of the hydrolysate showed arabinose > galactose > xylose and rhamnose. Fraction (*b*) had $[\alpha]_D$ +9° (Found: uronic anhydride, 15·9; N, 2·7; ash, 5%), and chromatography of the hydrolysate showed xylose > galactose > arabinose and rhamnose.

Attempted Fractionations of Ammonium Pectate.—(1) Ammonium pectate was precipitated as calcium pectate as described previously, and the regenerated ammonium pectate had $[\alpha]_D$ +208° ± 5° (*c* 1·2) [Found: uronic anhydride (by decarboxylation), 51%], and chromatography of the hydrolysate showed the same component sugars.

(2) Equal volumes of aqueous solutions of ammonium pectate (2%) and "Cetavlon" (cetyltrimethylammonium bromide) (7%) were mixed, the precipitated complex was removed at the centrifuge and washed free from adhering "Cetavlon," and the polysaccharide was regenerated by decomposing the complex with 5N-acetic acid and precipitation with ethanol. The polysaccharide was dissolved in water, the solution was deionised with Amberlite resins IR-120(H) and IR-4B(OH), and the polysaccharide was isolated by freeze-drying. It had $[\alpha]_D$ +204° ± 5° (*c* 0·9) [Found: uronic anhydride (by decarboxylation), 48%], and chromatography of the hydrolysate showed the same component sugars.

(3) Ammonium pectate was extracted with boiling ethanol–water (7 : 3) for 4 days. The extract contained 0·5% by weight of the original polysaccharide and chromatography of the hydrolysate showed galactose, arabinose, and rhamnose.

(4) Ammonium pectate was treated with saturated lime-water at 90° for 1 hr. The soluble product, isolated after removal of calcium ions with Amberlite resin IR-120(H) and freeze-drying, amounted to less than 1% by weight of the original polysaccharide and gave arabinose, galactose, and rhamnose on hydrolysis. The precipitated calcium pectate was reconverted into ammonium pectate which had $[\alpha]_D$ +206° ± 5° (*c* 1·0) (Found: uronic anhydride by decarboxylation, 51%), and gave the same sugars on hydrolysis.

Fractionation of Polysaccharides on Diethylaminoethylcellulose.—Polysaccharides (*ca.* 0·3—0·6 g.) were dissolved in 0·005M-sodium dihydrogen phosphate buffer (pH 6; 50 ml.) and poured on a column of diethylaminoethylcellulose (phosphate form) (30 × 3 cm.) as described by Neukom *et al.*[4] The column was eluted with 0·025M- (500 ml.), 0·05M- (500 ml.), 0·1M- (500 ml.), and 0·25M-sodium dihydrogen phosphate (pH 6) (500 ml.), and a gradient of sodium hydroxide (0·01—0·5M; 2 l.). Fractions (*ca.* 20 ml.) were collected and analysed for sugars by the anthrone method [17] and for uronic anhydride by the carbazole method.[15] Pectic acid (*ca.* 98%)

[16] Anderson, *Talanta*, 1959, **2**, 73.
[17] Bailey, *Biochem. J.*, 1958, **68**, 669.

was eluted in a single broad band with aqueous sodium hydroxide, and only traces of polysaccharide were detected on elution with phosphate buffer. Hydrolysis of the recovered pectic acid gave the same component sugars. The araban-rich polysaccharide (fraction from lime-water extraction) was mainly eluted in a number of bands with phosphate buffer and only a small proportion (*ca.* 5%) was eluted later with sodium hydroxide.

Acid Hydrolysis of Pectic Acid.—(1) Ammonium pectate (6 g.) was hydrolysed with N-sulphuric acid (250 ml.) on the boiling-water bath for 4 hr. The solution was neutralised with barium carbonate, filtered, deionised with Amberlite resin IR-120(H), and concentrated to a mixture of sugars which was separated on cellulose (60 × 3 cm.) with butan-1-ol, saturated with water, as eluant, which gave five fractions containing neutral monosaccharides. Fraction 1 (12 mg.) contained rhamnose, 2-*O*-methylxylose, and 2-*O*-methylfucose. Fraction 2 (35 mg.) had $[\alpha]_D$ +8° (*c* 1·6) and recrystallised from acetone to give L-rhamnose monohydrate, m. p. and mixed m. p. 95—98°. Fraction 3 (56 mg.) had $[\alpha]_D$ +82° (*c* 0·6) and chromatography showed arabinose as the main component, xylose, and a trace of fucose. Fraction 4 (66 mg.) had $[\alpha]_D$ +103° (*c* 2·1) and was characterised as L-arabinose by formation of the toluene-*p*-sulphonylhydrazone, m. p. and mixed m. p. 155—156°. Fraction 5 (62 mg.) had $[\alpha]_D$ +80° (*c* 1·8); it was chromatographically indistinguishable from D-galactose and on oxidation with nitric acid it afforded mucic acid, m. p. and mixed m. p. 220°.

(2) Ammonium pectate (20 g.) was hydrolysed with N-sulphuric acid (500 ml.) on the boiling-water bath for 3·5 hr. Degraded pectic acid (7·5 g.) was precipitated by the addition of acetone (1 vol.) to the cooled solution, removed at the centrifuge, washed with acetone–water (1 : 1), dissolved in water, and freeze-dried. The supernatant solution and washings were concentrated to remove acetone, neutralised with barium carbonate, filtered, and treated with Amberlite resin IR-120(H) to remove barium ions. The solution, which contained acidic and neutral sugars, was poured on a column of Amberlite resin CG-45 (formate form). Neutral sugars were eluted with water, and acidic sugars were eluted with a gradient of formic acid (0·0—2N) to give fractions A—F.

The neutral sugar solution was concentrated and poured on a column (30 × 3 cm.) of charcoal–Celite (1 : 1). Elution with water gave a mixture of arabinose, galactose, rhamnose, xylose, and fucose, which was not examined further. Elution with water containing 7% of butan-2-one gave a mixture of fucose, rhamnose, 2-*O*-methylxylose, and 2-*O*-methylfucose. This mixture was fractionated on filter sheets with solvent A, to give pure samples of those sugars which had not been previously characterised. L-Fucose (30 mg.) was characterised as the toluene-*p*-sulphonylhydrazone, m. p. and mixed m. p. 169—170°, $[\alpha]_D$ −14° ⟶ −8°. 2-*O*-Methyl-D-xylose (35 mg.), $[\alpha]_D$ +33° (*c* 0·6), after recrystallisation from ethanol was identified by m. p. and mixed m. p. 133—135°, and by *X*-ray powder photograph. 2-*O*-Methyl-L-fucose (10 mg.), $[\alpha]_D$ −80° (*c* 0·5) after recrystallisation from water, was identified by m. p. 148—151° and mixed m. p. 148—149°, and by an *X*-ray powder photograph.

Examination of Acidic Sugars.—Fraction A. This (0·75 g.) contained four components, $R_{Gal\,A}$ 0·80, 0·47, 0·17, and 0·10, which were separated on Whatman No. 31 extra-thick paper with solvent C.

Fraction A (i) (0·41 g.), $R_{Gal\,A}$ 0·80, had $[\alpha]_D$ +84° (*c* 0·41) and gave on hydrolysis galacturonic acid and rhamnose. Reduction of the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave galactose and rhamnose. The sugar gave no stain with triphenyltetrazolium hydroxide. Methyl sulphate (30 ml.) and 30% aqueous sodium hydroxide (60 ml.) were added dropwise during 2 hr. to the sugar (0·35 g.) in water (20 ml.). Two further additions of reagents were made and the solution was heated on the boiling-water bath for 20 min., cooled, and acidified with sulphuric acid. Extraction of the aqueous solution with chloroform (5 × 100 ml.) furnished a syrup (0·12 g.) which crystallised from light petroleum (b. p. 100—120°) to give *methyl* 2-O-(2,3,4-*tri*-O-*methyl*-D-*galactopyranosyluronic acid*)-3,4-*di*-O-*methyl*-L-*rhamnopyranoside dihydrate*, m. p. 67—68°, $[\alpha]_D$ +91° (*c* 1·1 in $CHCl_3$) (Found: C, 47·6; H, 7·6; OMe, 40·1. $C_{18}H_{32}O_{11},2H_2O$ requires C, 47·0; H, 7·8; OMe, 40·4%). The methylated acid (70 mg.) was reduced with diborane in tetrahydrofuran (see below for details), the reduction product was hydrolysed with N-sulphuric acid on the boiling-water bath for 4 hr., and the hydrolysate was neutralised with barium carbonate and concentrated to a syrup. The syrup was separated on filter sheets with solvent A, to yield (*a*) 2,3,4-tri-*O*-methyl-D-galactose (31 mg.), R_G 0·68, $[\alpha]_D$ +114° (*c* 1·0), which was characterised as the aniline derivative, m. p. and mixed m. p. 163—164°, and (*b*) 3,4-di-*O*-methyl-L-rhamnose (29 mg.), R_G

0·85, $[\alpha]_D$ +18° (*c* 0·3), which when recrystallised from light petroleum (b. p. 40—60°)–ether had m. p. and mixed m. p. 95—96°.

Fraction A (ii) (40 mg.), $R_{Gal\ A}$ 0·47, had $[\alpha]_D$ −25° (*c* 0·43) and gave on hydrolysis galacturonic acid and fucose. Reduction of the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave galactose and fucose. The sugar gave an intense stain with triphenyltetrazolium hydroxide.

Fraction A (iii) (20 mg.), $R_{Gal\ A}$ 0·17, had $[\alpha]_D$ −8° (*c* 0·62) and gave on hydrolysis galacturonic acid and galactose.

Fraction A (iv) (70 mg.), $R_{Gal\ A}$ 0·10, had $[\alpha]_D$ +82° (*c* 0·68). Total hydrolysis of the sugar gave galacturonic acid and rhamnose, and on partial hydrolysis 2-*O*-(galactosyluronic acid)-rhamnose was detected. Reduction of the sugar acid with potassium borohydride followed by hydrolysis gave galacturonic acid, rhamnose, and rhamnitol. The apparent equivalent weight (592) from hypoiodite oxidation [18] did not differentiate between a trisaccharide (equiv. wt., 486) and a tetrasaccharide (equiv. wt., 632), but the former is more probable in view of the low consumption of hypoiodite by rhamnose derivatives.[18] Under the same conditions the consumption of hypoiodite by L-rhamnose monohydrate was 83%.

Fraction B (0·32 g.). This contained galacturonic acid and the oligosaccharide, $R_{Gal\ A}$ 0·10, of fraction A(iv) and was not examined further.

Fraction C (0·50 g.), $[\alpha]_D$ +58° (*c* 0·79). This contained D-galacturonic acid which was characterised as the 2,5-dichlorophenylhydrazone, m. p. and mixed m. p. 180—182°.

Fraction D (0·22 g.). This contained galacturonic acid and a trace of an unidentified sugar, $R_{Gal\ A}$ 0·56, and was not examined further.

Fraction E (0·50 g.), $R_{Gal\ A}$ 0·20 and 0·50 in solvents C and E. This gave a calcium salt which had $[\alpha]_D$ +114° (*c* 1·1 in N-HCl) (Jones and Reid [8] cite $[\alpha]_D$ +119° for calcium digalacturonate). The sugar (300 mg.) was shaken with benzyl alcohol (30 ml.) saturated with hydrogen chloride, at room temperature for 24 hr., ether (35 ml.) was added to the solution, the syrup which separated was dissolved in tetrahydrofuran (20 ml.), and lithium aluminium hydride (0·3 g.) was added. The solution was refluxed for 2 hr., water was added to the cooled solution to destroy the excess of hydride, and the solution was filtered and taken to dryness. The residue was extracted with boiling acetone, and the extract was concentrated to a syrup, which was dissolved in ethanol–water (1 : 4; 20 ml.) and shaken with hydrogen over 10% palladium–charcoal (0·1 g.) at atmospheric pressure. After 24 hr. the solution was filtered and concentrated to a syrup containing approximately equal amounts of galactose and a galactobiose, $R_{galactose}$ 0·57 and 0·29 in solvents B and C, M_G 0·38, which was chromatographically and ionophoretically indistinguishable from 4-*O*-α-D-galactopyranosyl-D-galactose.

Fraction F (0·38 g.), $R_{Gal\ A}$ 0·05 and 0·25 in solvents C and E. This gave a calcium salt which had $[\alpha]_D$ +145° (*c* 0·36 in N-HCl) [Jones and Reid [8] cite $[\alpha]_D$ +154° for calcium trigalacturonate]. When the sugar was treated with benzyl alcohol containing hydrogen chloride only a small proportion dissolved and subsequent reduction with lithium aluminium hydride followed by hydrogenolysis gave only galactose and the same galactobiose.

Enzymic Degradation of Acid-degraded Pectic Acid.—Treatment of acid-degraded pectic acid in aqueous solution (pH 4) at 20° with " Hemicellulase " (L. Light and Co. Ltd.) for 8 hr. resulted in the liberation of galacturonic acid, and its dimer and trimer, but no neutral sugars were detected.

Enzymic Degradation of Pectic Acid.—Ammonium pectate (15 g.) in water (2 l.) was passed through columns of Amberlite resins IR-120(H) and IR-45(OH). " Pectinase " (L. Light and Co. Ltd.) (2 g.) was added to the resulting solution (pH 4) which was kept at 20° for 4 hr. Acetone (1 vol.) was added to the solution, and insoluble material was removed by centrifugation and washed with acetone–water (1 : 1). Hydrolysis of the precipitate gave galactose, arabinose, rhamnose, xylose, fucose, 2-*O*-methylxylose, 2-*O*-methylfucose, 2-*O*-(galactosyluronic acid)-rhamnose, and an unidentified sugar, $R_{galactose}$ 1·2 in solvent C. The acetone–water solution and washings were concentrated to a syrup (9 g.) which contained neutral monosaccharides and galacturonic acid and its dimer and trimer. The syrup (7 g.) in water (200 ml.) was neutralised with calcium carbonate, then filtered, and ethanol (1·5 vol.) was added. The precipitate was isolated by centrifugation, reprecipitated three times from ethanol–water (1·5 : 1) dissolved in water, treated with Amberlite resin IR-120(H) to remove calcium ions, and concentrated to a syrup (H) (2·59 g.). The combined ethanol–water solutions were taken to dryness and the residue was extracted with boiling methanol for 0·5 hr., giving a syrup

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

(1·23 g.) which contained rhamnose, arabinose, galactose, and galacturonic acid. The methanol-insoluble residue was dissolved in water, and the solution was treated with Amberlite resin to remove calcium ions, and concentrated to a syrup G (3·15 g.).

Chromatography of syrup G showed galacturonic acid with small amounts of arabinose, galactose, and digalacturonic acid. The syrup (3·1 g.) was adsorbed on Amberlite resin CG-45 (formate form), which was eluted with water (4 l.) to remove neutral sugars, and with 0·2M-formic acid (4 l.) to give D-galacturonic acid (2·7 g.), m. p. 160—161° (from 95% ethanol), $[\alpha]_D$ +62° (equil.) (*c* 1·2), which was characterised as the 2,5-dichlorophenylhydrazone, m. p. and mixed m. p. 180—181°. Chromatography of syrup H showed galacturonic acid (0·84 g.) and its dimer (0·39 g.) and trimer which were fractionated on Whatman No. 17 paper with solvent E. The digalacturonic acid gave a calcium salt, $[\alpha]_D$ +127° (*c* 0·56 in N-HCl), and reduction of the benzyl ester benzyl glycosides with lithium aluminium hydride followed by hydrogenolysis as described previously gave galactose and a galactobiose, which was chromatographically and ionophoretically indistinguishable from 4-*O*-α-D-galactopyranosyl-D-galactose.

Methylation of Ammonium Pectate.—Ammonium pectate (10 g.) was methylated with methyl sulphate and sodium hydroxide, and the product was isolated as methylated sodium pectate (OMe, 21%). The sodium salt in water was passed through Amberlite resin IR-120(H), and the resulting acid was converted into silver salt by treatment with silver oxide. The dried silver salt (7 g.) was refluxed with methyl iodide (100 ml.) for 2 hr., and silver oxide (8 g.) was added during a further 4 hr. The product was methylated three times with methyl iodide and silver oxide, giving methylated methyl pectate (3·6 g.), $[\alpha]_D$ +133° (*c* 0·85 in $CHCl_3$) (Found: OMe, 39%, not raised on further methylation).

Methanolysis of Methylated Methyl Pectate: Reduction, Hydrolysis, and Separation of Methylated Sugars.—Methylated methyl pectate (3·5 g.) was heated in a sealed tube with methanolic 4% hydrogen chloride (100 ml.) at 100° for 5 hr. After neutralisation with silver carbonate and removal of solvent, the resulting syrup was dissolved in tetrahydrofuran (100 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., and excess of hydride was destroyed by addition of water to the cooled mixture. Insoluble material was removed by centrifugation and extracted with ethanol and acetone. The combined supernatant liquid and extracts were concentrated, deionised with Amberlite resins IR-120(H) and IR-4B(OH), and concentrated to a syrup which was hydrolysed with N-hydrochloric acid (100 ml.) for 5 hr. on the boiling-water bath to a syrupy mixture of sugars (3·0 g.).

The mixture of sugars was fractionated on cellulose (70 × 3·5 cm.), (i) light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3, later 1 : 1), saturated with water, and (ii) butan-1-ol, half saturated with water, being used as eluants to give twelve fractions.

Fraction 1. The syrup (6 mg.) was chromatographically indistinguishable from 2,3,4-tri-*O*-methyl-L-rhamnose, R_G 1·02.

Fraction 2. Chromatography of the syrup (190 mg.) showed 2,3,5-tri-*O*-methylarabinose, 2,3,4,6-tetra-*O*-methylgalactose, 3,4-di-*O*-methylrhamnose, and a sugar which gave 2,3-di-*O*-methylgalactose on further hydrolysis. The syrup was rehydrolysed with N-hydrochloric acid for 4 hr. on the boiling-water bath, and fractionation of the hydrolysate on cellulose (50 × 1·6 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (9 : 1), saturated with water, as eluant gave fractions 2*a*—2*d*. Fraction 2*a* (61 mg.), R_G 0·96, $[\alpha]_D$ −40° (*c* 1·7), was characterised as 2,3,5-tri-*O*-methyl-L-arabinose by conversion into 2,3,5-tri-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 130—132°. Fraction 2*b* (42 mg.), R_G 0·91, $[\alpha]_D$ +108° (*c* 0·82), was characterised as 2,3,4,6-tetra-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 177—179°. Fraction 2*c* (35 mg.), R_G 0·89, $[\alpha]_D$ +19° (*c* 1·6), was characterised as 3,4-di-*O*-methyl-L-rhamnose by conversion into 3,4-di-*O*-methyl-L-rhamnonolactone, m. p. 76—77°. Fraction 2*d* (40 mg.), $[\alpha]_D$ +79° (*c* 1·1), was chromatographically indistinguishable from 2,3-di-*O*-methyl-D-galactose.

Fraction 3. The syrup (170 mg.) contained some products of incomplete hydrolysis and, after rehydrolysis with N-hydrochloric acid for 4 hr. on the boiling-water bath and fractionation on cellulose (as above) gave the following fractions, all containing sugars which were fully characterised elsewhere: fraction 3*a* (16 mg.), $[\alpha]_D$ +17° (*c* 0·9), R_G 0·89 (3,4-di-*O*-methylrhamnose); fraction 3*b* (86 mg.), R_G 0·71 and 0·68 (2,3,4-tri-*O*-methylgalactose and 2,3-di-*O*-methylarabinose in approximately equal amounts); fraction 3*c* (45 mg.), R_G 0·50, 0·33, and 0·30 (2,3-di-*O*-methyl-, 2- and 3-*O*-methylgalactose in the approximate ratio of 3 : 1 : 1).

Fraction 4. Chromatography of the syrup (264 mg.) showed 2,3,4-tri-*O*-methylgalactose (R_G 0·71), 2,3-di-*O*-methylarabinose (R_G 0·68), and a mixture of sugars (R_G 0·61—0·63). Demethylation with boron trichloride gave galactose, arabinose, and rhamnose. Partial separation of the syrup on filter sheets with solvent B gave fractions 4*a* (170 mg.) and 4*b* (62 mg.). Fraction 4*a* still contained a mixture of two sugars in approximately equal amount, and, although incomplete separation was achieved by chromatography on filter sheets with solvent A, pure samples of both sugars were isolated. 2,3,4-Tri-*O*-methyl-D-galactose, R_G 0·71, $[\alpha]_D$ +110° (*c* 0·2), was characterised as the aniline derivative, m. p. and mixed m. p. 162—163°, and 2,3-di-*O*-methyl-L-arabinose, R_G 0·68, $[\alpha]_D$ +95° (*c* 0·4), by conversion into 2,3-di-*O*-methyl L-arabonamide, m. p. and mixed m. p. 155—156°. The presence of 2,3,4-tri-*O*-methylgalactose and 2,3-di-*O*-methylarabinose as major components of fraction 4*a* together with a number of minor components, including 2,4,6-tri-*O*-methylgalactose but not including 2,3,6-tri-*O*-methylgalactose, was shown by gas–liquid partition chromatography.[9] Fraction 4*b* contained four components, R_G 0·14, 0·11, 0·09, and 0·05 in solvent D. The main component, R_G 0·60 and 0·05 in solvents C and D, $[\alpha]_D$ +33° (*c* 0·27), was isolated after separation on filter sheets in solvent D; chromatography of the sugar and of its periodate oxidation products [19] indicated the presence of 3-*O*-methylrhamnose.

Fraction 5. The syrup (60 mg.), $[\alpha]_D$ +62° (*c* 1·96), contained 2,3-di-*O*-methylarabinose (R_G 0·68), 3-*O*-methylrhamnose (R_G 0·60), and 2,3-di-*O*-methylgalactose (R_G 0·50).

Fraction 6. Chromatography of the syrup (300 mg.) showed 3-*O*-methylrhamnose and 2,3-di-*O*-methylgalactose in the approximate ratio of 1 : 5, and the sugars were separated on filter sheets with solvent A. Fraction 6*a*, $[\alpha]_D$ +78° (*c* 0·68), was chromatographically pure 2,3-di-*O*-methyl-D-galactose. Fraction 6*b*, $[\alpha]_D$ +35° (*c* 0·21), crystallised and had m. p. and mixed m. p. (with 3-*O*-methyl-L-rhamnose) 111—114°.

Fraction 7. Chromatography of the syrup (598 mg.), $[\alpha]_D$ +81° (*c* 2·1), showed 2,3-di-*O*-methylgalactose (R_G 0·50) with a small amount of an *O*-methylarabinose (R 0·45). The main component was characterised by conversion into 2,3-di-*O*-methyl-*N*-phenyl-D-galactosylamine, identified by m. p. 139—140° and *X*-ray powder photograph. The syrup (400 mg.) was separated on charcoal–Celite by gradient elution with water containing 0·0—2·0% of butan-2-one to give chromatographically pure fractions 7*a* (40 mg.) and 7*a* (310 mg.). Fractions 7*a*, R_G 0·45, $[\alpha]_D$ +98° (*c* 0·5), was not stained by triphenyltetrazolium hydroxide. Oxidation with periodate gave 0·79 mol. of formaldehyde.[20] Demethylation gave arabinose. Chromatography of the periodate oxidation products [19] showed methoxymalondialdehyde (from 2-*O*-methylaldose).

Fraction 8. Chromatography of the syrup (146 mg.) showed 2,3-di-*O*-methylgalactose (R_G 0·50), 2-*O*-methylarabinose (R_G 0·45), and rhamnose (R_G 0·41).

Fraction 9. The sugar (103 mg.), R_G 0·33, crystallised from ethanol and had m. p. and mixed m. p. (with 2-*O*-methyl-D-galactose) 148°, and $[\alpha]_D$ +54° ⟶ +82° (equil.) (*c* 1·74).

Fraction 10. The syrup (52 mg.) was separated on filter sheets with solvent F into fractions 10*a* (25 mg.) and 10*b* (15 mg.). Fraction 10*a*, recrystallised from ethanol, had m. p. and mixed m. p. (with 2-*O*-methyl-D-galactose) 148°. Fraction 10*b*, R_G 0·30, $[\alpha]_D$ +100° (*c* 0·11), crystallised when seeded with 3-*O*-methyl-D-galactose and had m. p. and mixed m. p. 145—147°. Chromatography of the periodate oxidation products [19] gave a mono-*O*-methylpentose (R_G 0·40).

Fraction 11. Chromatography of the syrup (50 mg.) in solvents A and C showed 3-*O*-methylgalactose and arabinose in approximately equal amounts.

Fraction 12. This was chromatographically pure galactose (180 mg.) and gave mucic acid, m. p. and mixed m. p. 215°, on oxidation with nitric acid.

Methanolysis of Methylated Methyl Pectate.—Methylated methyl pectate (130 mg.) was heated in a sealed tube with methanolic 4% hydrogen chloride at 100° for 9 hr., and the resulting solution was neutralised with silver carbonate, filtered, and concentrated to a syrup. The syrup was extracted with chloroform, the extract was diluted with light petroleum (b. p. 100—120°) (6 vol.), the precipitate was separated at the centrifuge (30 mg.), and the supernatant liquid was concentrated to a syrup S (67 mg.). The syrup S was hydrolysed with N-sulphuric acid on the boiling-water bath for 2 hr.; chromatography of the hydrolysate in solvents A and D showed 2,3,5-tri- and 2,3-di-*O*-methyl- and 2-*O*-methylarabinose, 2,3,4,6-tetra- and 2,3,4-tri-*O*-methylgalactose, and 3,4-di-*O*-methylrhamnose. A fraction containing 2,3,4-tri-*O*-methylgalactose and 2,3-di-*O*-methylarabinose was separated chromatographically by using solvent

[19] Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.
[20] Hough, Woods, and Perry, *Chem. and Ind.*, 1957, 1100.

A, and the presence of these two sugars was confirmed by vapour-phase chromatography of the derived methyl glycosides. The precipitate (insoluble in chloroform–light petroleum) and the chloroform-insoluble methanolysis product (38 mg.) were each hydrolysed with N-sulphuric acid on the boiling-water bath for 4 hr., and chromatography of both hydrolysates showed material with R_F 0·55 and 0·06—0·30 in solvent C, both components being immobile in solvent A, and therefore contained no neutral sugars. The combined hydrolysates were separated on filter sheets by using solvent C to give fractions *a* and *b*. Fraction *a*, R_F 0·55 in solvent C, was heated with methanolic 4% hydrogen chloride in a sealed tube at 100° for 3 hr., reduced with lithium aluminium hydride in tetrahydrofuran, and hydrolysed with N-sulphuric acid at 100° for 2 hr.; chromatography of the hydrolysate then showed 2,3-di-*O*-methylgalactose. Similar treatment of fraction *b*, R_F 0·06—0·30, afforded 2,3-di-*O*-methylgalactose together with 2,3,4-tri-*O*-methylgalactose and 3,4-di-*O*-methylrhamnose.

Reduction of Hexuronic Acid Derivatives with Diborane.—Boron trifluoride–ether complex (4·0 g.) in 1,2-dimethoxyethane (10 ml.) was added dropwise with stirring during 1 hr. to lithium borohydride (0·5 g.) in 1,2-dimethoxyethane (10 ml.) through which a slow stream of nitrogen was bubbled, and the liberated diborane was passed into tetrahydrofuran (40 ml.). The solution of diborane in tetrahydrofuran was used in reductions.

(*a*) Diborane in tetrahydrofuran (20 ml.) was added to methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosiduronic acid [21] (0·65 g.) in tetrahydrofuran (20 ml.). The solution was kept at room temperature for 3 hr., excess of diborane was destroyed by the addition of ethanol and water, the solution was taken to dryness, and the residue was repeatedly evaporated with methanol to remove boric acid. The product recrystallised from light petroleum (b. p. 60—80°) to yield methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranoside (0·52 g.), m. p. 92° and mixed m. p. (with sample, m. p. 89°) 90°. A sample of the methyl glycoside was hydrolysed and chromatography of the hydrolysate showed only 2,3,4-tri-*O*-methylglucose. The same procedure was used for the reduction of methyl 2-*O*-(D-galactopyranosyluronic acid)-L-rhamnopyranoside pentamethyl ether.

(*b*) Diborane in tetrahydrofuran (20 ml.) was added to methylated Norway spruce xylan (free-acid form, 0·20 g.) [Found: uronic anhydride (by Kaye and Kent's method [22]), 9%] in tetrahydrofuran (20 ml.), and, after 20 hr. at room temperature, methylated reduced xylan (0·18 g.) [Found: uronic anhydride, *ca.* 0·4%] was worked up as described above. Samples of the methylated acidic xylan and of the reduced methylated xylan were heated with methanolic 2% hydrogen chloride in sealed tubes at 100° for 3 hr., the methanolysis products were hydrolysed with N-hydrochloric acid at 100° for 3 hr., and the hydrolysates were examined chromatographically in solvents A, B, and D. In addition to neutral sugars the methylated acidic xylan afforded a methylated aldobiouronic acid: the reduced methylated xylan furnished the same neutral sugars together with 2,3,4-tri-*O*-methylglucose and an increased proportion of 3-*O*-methylxylose, but no methylated aldobiouronic acid.

(*c*) Acetylated gum ghatti was treated with diborane in tetrahydrofuran in a similar manner; a gel separated from the solution almost immediately. The mixture was kept at room temperature for 20 hr., and the product was isolated. Samples of acetylated gum ghatti and of the reaction product were hydrolysed, and the hydrolysates were examined chromatographically. In addition to neutral sugars and aldobiouronic acids formed from both polysaccharide samples, the reaction product gave glucose, indicating that some glucuronic acid residues had been reduced.

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[21] Challinor, Haworth, and Hirst, *J.*, 1931, 258.
[22] Kaye and Kent, *J.*, 1953, 79.