490. *Plant Gums of the Genus* Sterculia. *Part I. The Main Structural Features of* Sterculia urens *Gum* *

By G. O. Aspinall and Nasir-ud-din

Deacetylation of *Sterculia urens* gum furnishes a polysaccharide containing residues of D-glucuronic acid, D-galacturonic acid, D-galactose, and L-rhamnose. Partial acid hydrolysis of the polysaccharide affords a mixture of acidic oligosaccharides, amongst which 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, 4-*O*-(D-galactopyranosyluronic acid)-D-galactose, and *O*-(D-glucopyranosyluronic acid)-(1 → 3)-*O*-(D-galactopyranosyluronic acid)-(1 → 2)-L-rhamnose have been characterised. Partial acid hydrolysis of the periodate-oxidised polysaccharide (after reduction with borohydride) gives 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose and a higher oligosaccharide containing residues of galacturonic acid and rhamnose. Characterisation of the cleavage products from the methylated polysaccharide indicate the presence therein of residues of 2,3,4-tri-*O*-methyl-D-glucuronic acid, 2,3-di-, and 2- and 3-mono-*O*-methyl-D-galacturonic acid, 2,3,4-tri-, 3,4-di-, and 3-*O*-methyl-L-rhamnose, L-rhamnose, and 2,3,4,6-tetra-, 2,3,6-tri-, and 2,6-di-*O*-methyl-D-galactose. Partial structures for the polysaccharide are advanced.

The commercially important exudate gum from the bark of *Sterculia urens*, a large deciduous tree growing in the dry, elevated areas of North and Central India, is known commonly as "karaya gum." Although some aspects of the structural chemistry of exudate gums from other species of *Sterculia*, *e.g.*, *S. setigera* from tropical West Africa [1] and *S. caudata* (syn. *Brachychiton diversifolium*) from Northern Queensland, Australia,[2] have been studied, little is known of the detailed chemistry of *Sterculia urens* gum.[3] We report here an examination of this gum in which some of the main structural features have been elucidated.

* A summary of this Paper was presented at the Los Angeles meeting of the American Chemical Society, April 1963.

[1] E. L. Hirst, L. Hough, and J. K. N. Jones, *J.*, 1949, 3145; L. Hough and J. K. N. Jones, *J.*, 1950, 1199.

[2] E. L. Hirst, E. Percival, and R. S. Williams, *J.*, 1958, 1942.

[3] F. Smith and R. Montgomery, "Chemistry of Plant Gums and Mucilages," Reinhold Publ. Corp., New York, 1959.

The naturally occurring gum nodules, which swell but do not dissolve in water, consist essentially of a partially acetylated polysaccharide. The sample under investigation had *ca.* 8% of acetyl groups and contained 37% of uronic acid residues. The parent polysaccharide, which is readily soluble in water, was conveniently isolated after deacetylation of the gum with dilute sodium hydroxide or aqueous ammonia, and was purified *via* its insoluble cetyltrimethylammonium complex. The polysaccharide contained 39—40% of uronic acid residues and was homogeneous by the criteria of ultracentrifugation, chromatography on diethylaminoethylcellulose, and by being recovered unchanged on regeneration from insoluble copper and cetyltrimethylammonium complexes.

Partial acid hydrolysis of the polysaccharide gave galactose, rhamnose, galacturonic acid, and a mixture of acidic oligosaccharides. The acidic sugars were adsorbed on anion-exchange resin and then desorbed by gradient elution with aqueous formic acid. After further separation by partition chromatography on cellulose, three discrete acidic oligosaccharides were recognised together with material of higher molecular weight which was not examined in detail. The first acidic disaccharide was characterised as 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose (I) by conversion into the crystalline methyl glycoside pentamethyl ether dihydrate. The second disaccharide, 4-*O*-(D-galactopyranosyluronic acid)-D-galactose (II), was only isolated chromatographically pure in very small amount. Hydrolysis of the disaccharide showed galacturonic acid and galactose to be the component sugars. However, further quantities of this disaccharide were isolated in admixture with an acidic trisaccharide and its structure has been assigned on the basis of experiments carried out on the mixture of sugars, which will be described later. The structure of the third component, an acidic trisaccharide, is based on the following observations. Hydrolysis of the trisaccharide gave rhamnose as the sole neutral sugar together with acids, which were shown to be galacturonic and glucuronic acids since reduction of the methyl ester methyl glycosides followed by hydrolysis gave galactose and glucose. Reduction of the trisaccharide with potassium borohydride followed by hydrolysis gave *inter alia* rhamnitol showing that the rhamnose residue was present as the reducing group. The trisaccharide was methylated, reduced with lithium aluminium hydride, and hydrolysed to give 2,3,4-tri-*O*-methyl-D-glucose, 2,4-di-*O*-methyl-D-galactose, and 3,4-di-*O*-methyl-L-rhamnose. On the assumption that the aldobiouronic acid, 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose (I), is formed on partial hydrolysis of the trisaccharide, the optical rotation ($[\alpha]_D$ +44°) of the sugar indicates that the D-glucopyranosyluronic acid residue has the β-configuration. The trisaccharide, therefore, may be assigned the structure *O*-(β-D-glucopyranosyluronic acid)-(1 ⟶ 3)-*O*-(α-D-galactopyranosyluronic acid)-(1 ⟶ 2)-L-rhamnose (III).

D-Gal*p*A 1α ⟶ 2 L-Rha (I) D-Gal*p*A 1 ⟶ 4 D-Gal (II)

D-G*p*A 1β ⟶ 3 D-Gal*p*A 1α ⟶ 2 L-Rha (III)

The main portion of the disaccharide (II) was isolated in admixture with the trisaccharide (III), but no chromatographic solvent was found which would effect a preparative separation of the two components. Hydrolysis of the mixture gave galactose, rhamnose, and acidic sugars. The presence of glucuronic acid, and possibly also of galacturonic acid, residues was indicated by the detection of glucose, galactose, and rhamnose after reduction of the methyl ester methyl glycosides followed by hydrolysis. The presence of two components in the mixture, one with rhamnose and one with galactose as the reducing unit, was shown by reduction and subsequent hydrolysis which furnished galactitol, rhamnitol, and acidic sugars, but no neutral reducing sugars. The mixture of acids was methylated and hydrolysis of the methylated oligosaccharides gave a mixture of acids and two neutral sugars, 3,4-di-*O*-methyl-L-rhamnose and 2,3,6-tri-*O*-methyl-D-galactose, which were characterised by the formation of crystalline derivatives. The methylated hexuronic acids were identified as 2,4-di- and 2,3,4-tri-*O*-methylgalacturonic acid, and 2,3,4-tri-*O*-methylglucuronic acid by gas–liquid chromatography of their methyl glycosides and of

the methyl glycosides of the corresponding methylated hexoses formed on reduction. These results are consistent with the presence of the acidic oligosaccharides (II and III) in the mixture, and this formulation was supported by the results of degradation of the mixture with cold dilute alkali. 3- and 4-*O*-Substituted sugars are degraded under these conditions, whereas 2-*O*-substituted sugars are relatively stable.[4] The mixture of acids was treated with oxygen-free lime-water and chromatography of the product showed the presence of a single acidic oligosaccharide, which was chromatographically indistinguishable from the trisaccharide (III), and a trace of galacturonic acid. Galacturonic acid would be formed on degradation of the disaccharide (II) with alkali and would itself be further degraded slowly with saccharinic acid formation. Chromatography of the hydrolysis products of the alkali-stable sugar and of its reduction products provided further evidence that the undegraded component of the mixture was the acidic trisaccharide (III). This conclusion was substantiated by gas chromatography of the cleavage products from methanolysis of the methylated derivative and its reduction product which showed methyl glycosides of the sugars formed similarly from the trisaccharide (III), namely 2,3,4-tri-*O*-methylglucuronic acid, 2,4-di-*O*-methylgalacturonic acid, and 3,4-di-*O*-methylrhamnose, together with markedly reduced quantities of methyl glycosides of 2,3,4-tri-*O*-methylgalacturonic acid and 2,3,6-tri-*O*-methylgalactose formed from traces of undegraded disaccharide (II).

The highly branched nature of the polysaccharide was indicated by characterisation of the cleavage products from the methylated derivative. In order to facilitate hydrolysis of the methylated polysaccharide and to minimise accompanying degradation, hexuronic acid residues were reduced with lithium aluminium hydride, the carboxyl-reduced methylated polysaccharide was hydrolysed and the resulting methylated sugars were separated chromatographically on cellulose. The following sugars were characterised by the formation of crystalline derivatives: from neutral sugar units, 2,3,4,6-tetra-, 2,3,6-tri-, and 2,6-di-*O*-methyl-D-galactose, 2,3,4-tri-, 3,4-di-, and 3-*O*-methyl-L-rhamnose, and L-rhamnose, and from reduction of hexuronic acid units, 2,3,4-tri-*O*-methyl-D-glucose, 2,3-di-, 2- and 3-*O*-methyl-D-galactose, and D-galactose. Examination of the cleavage products from the methylated acidic polysaccharide by (*a*) gas chromatography of the methyl glycosides and (*b*) paper chromatography of the sugars showed that the latter sugars were absent and had arisen, therefore, only from the reduction of hexuronic acid units. Of the above-mentioned sugars, unsubstituted L-rhamnose and D-galactose were isolated only in small amount and may have arisen from incomplete methylation; 2,6-di-*O*-methyl-D-galactose was also isolated in small amount, but this sugar is assumed to have definite, albeit minor, structural significance since subsequent experiments showed that the polysaccharide contains a small proportion of galactose residues which are resistant to cleavage by periodate.

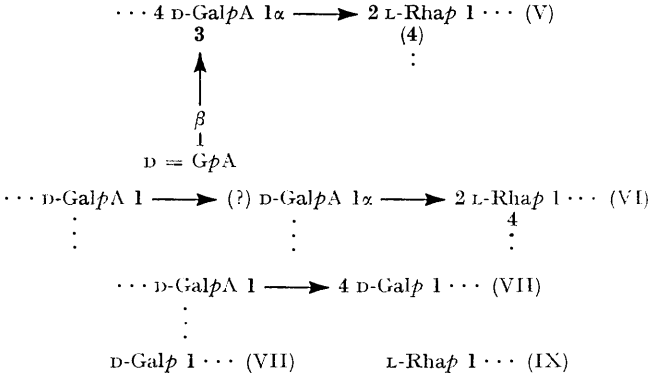
From these experiments it is clear that the majority of the D-galacturonic acid and some of the L-rhamnose residues provide branching points in the polysaccharide structure. These units are also resistant to oxidative cleavage by periodate and further information on their relative disposition was obtained by degradation of the periodate-oxidised polysaccharide by the procedure of Smith and his co-workers.[5] The oxidised polysaccharide was reduced with potassium borohydride and the resulting polyalcohol was treated with cold dilute acid. A degraded polysaccharide was isolated, from the partial hydrolysis of which two acidic oligosaccharides were formed. The first oligosaccharide was characterised as the aldobiouronic acid, 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose (I). The second oligosaccharide was not isolated in sufficient quantity to permit a complete characterisation, but the following evidence indicated that it was a linear trisaccharide containing a rhamnose residue as the reducing group. Partial acid hydrolysis gave

[4] R. L. Whistler and J. N. BeMiller, *Adv. Carbohydrate Chem.*, 1958, **13**, 289.
[5] I. J. Goldstein, G. W. Hay, B. A. Lewis, and F. Smith, Amer. Chem. Soc. Meeting, Boston, April 1959, Abs. Papers, 3D.

galacturonic acid, rhamnose, and the aldobiouronic acid (I). Reduction of the acid with potassium borohydride followed by hydrolysis gave galacturonic acid and rhamnitol. A sample was methylated, the methylated oligosaccharide was reduced with lithium aluminium hydride, and analysis of the cleavage products by paper chromatography of the sugars and by gas chromatography of the derived methyl glycosides showed the presence of 3,4-di-*O*-methylrhamnose, 2,3,4-tri-*O*-methylgalactose, and an unidentified sugar which was probably a di-*O*-methylgalactose. The incomplete structure (IV) may be advanced for this acidic oligosaccharide.

D-Gal*p*A 1 ⟶ (?) D-Gal*p*A 1 ⟶ 2 L-Rha (IV)

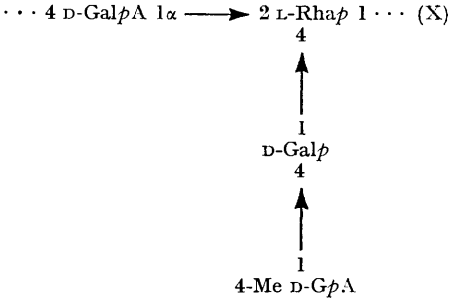
These results provide evidence for the main structural features of the deacetylated polysaccharide from *Sterculia urens* gum and it is possible to put forward a number of partial structures (V—IX). Partial structure (V) shows the mode of attachment of D-glucuronic acid end-groups to the 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose units which are present in the interior chains. Since D-galacturonic acid residues in the polysaccharide are all 4-*O*-substituted, the residues of this sugar in the fragment (V) must be present as branching points; and since some of the 2-*O*-substituted L-rhamnose residues in the polysaccharide also carry substituents at $C_{(4)}$ it is possible that the units in (V) may also be present as branching points. The partial structure (VI) is derived from the incompletely characterised acidic oligosaccharide (IV) which was isolated from the periodate-oxidised polysaccharide and indicates the presence of contiguous D-galacturonic acid residues in the inner chains of the polysaccharide. The partial structures (V and VI) both contain the aldobiouronic acid unit (I), but it is not yet certain if all the units (I) carry D-glucuronic acid residues as side-chains. The structural location of the 4-*O*-(D-galactopyranosyluronic acid)-D-galactose units (II) cannot yet be defined more precisely than in the structural unit (VII) which must arise from inner portions of the molecular structure in which the D-galacturonic acid residues are probably present as branching points. The polysaccharide contains end-groups of D-galactopyranose (VIII) and L-rhamnopyranose (IX) residues whose sites of attachment to other sugar residues are not yet known.



The known structural features of the polysaccharide from *Sterculia urens* gum may be compared with those of the major polysaccharide components of the exudate gums from *Khaya grandifolia*,[6] *K. senegalensis*,[7] *Cochlospermum gossypium*,[8] and *Combretum leonense*,[9] all of which contain residues of D-glucuronic acid (in some cases as the 4-methyl ether) and D-galacturonic acid. With the exception of the last polysaccharide which belongs to a

[6] G. O. Aspinall, E. L. Hirst, and N. K. Matheson, *J.*, 1956, 989.
[7] G. O. Aspinall, M. J. Johnston, and A. M. Stephen, *J.*, 1960, 4918.
[8] E. L. Hirst and S. Dunstan, *J.*, 1953, 2332; G. O. Aspinall, E. L. Hirst, and M. J. Johnston, *J.*, 1962, 2785.
[9] G. O. Aspinall and V. P. Bhavanandan, *J.*, 1965, 2685, 2693.

different structural class, these polysaccharides, which are composed of the same four sugar residues (the two hexuronic acids, D-galactose and L-rhamnose), comprise a group of structurally related polymers, in which several of the sugar residues are involved in the same types of linkage. A general distinction may be made between the locations of the residues of the two hexuronic acids. Residues of D-glucuronic acid (or of its 4-methyl ether) have been found only as non-reducing end-groups, whereas those of D-galacturonic acid have been found only in the interior chains, in some cases as branching points. Some of the D-galacturonic acid residues are attached to L-rhamnose residues since each gum furnishes the aldobiouronic acid, 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose (I), as a partial hydrolysis product. The gums, however, differ in the nature of the branching points and in some of the detailed sequences of sugar residues. The most striking difference so far recognised between the major polysaccharide components of the *Khaya* gums [6,7] on the one hand and of *Sterculia urens* gum (and possibly also of *Cochlospermum gossypium* gum) on the other lies in the site of attachment of the D-glucuronic acid end-groups. In the former gums these are attached to D-galactose residues, probably as in the partial structure (X), whereas in *S. urens* gum they are attached to D-galacturonic acid residues as in partial structure (V).



The polysaccharide from *Sterculia urens* gum contains a number of structural features in common with those of the polysaccharides from the gums of *S. setigera* [1] and *S. caudata* (syn. *Brachychiton diversifolium*).[2] However, only one hexuronic acid constituent in each of these two gums, D-galacturonic acid in *S. setigera* and D-glucuronic acid in *S. caudata*, has been reported. Further examination of these two gums (unpublished results with R. N. Fraser) now indicates that the two hexuronic acids are constituents of both gums. A detailed structural comparison of the *Sterculia* gums will be reported when further investigations have been undertaken.

Experimental

Paper chromatography was carried out on Whatman Nos. 1, 3MM, 31ET, and 541 papers with the following solvent systems (v/v): (*A*) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (*B*) ethyl acetate–pyridine–water (10 : 4 : 3); (*C*) ethyl acetate–acetic acid–formic acid–water (18 : 8 : 3 : 9); (*D*) ethyl acetate–acetic acid–water (10 : 5 : 6); (*E*) ethyl acetate–pyridine–acetic acid–water (5 : 5 : 1 : 3); (*F*) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (*G*) butan-1-ol–acetic acid–water (4 : 1 : 5, upper layer); (*H*) benzene–ethanol–water (169 : 47 : 15, upper layer); (*I*) butan-2-one, half saturated with water; (*J*) 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*-methyl-D-glucose in solvent *F*. Methylated sugars were demethylated with boron trichloride.[10] Chromatography of the periodate oxidation products of methylated sugars was carried out by Lemieux and Bauer's method.[11] Unless otherwise stated, optical rotations were observed for water solutions at *ca.* 18°.

Gas–liquid partition chromatography of methylated and partially methylated methyl

[10] T. G. Bonner, E. J. Bourne, and S. McNally, *J.*, 1960, 2929.
[11] R. U. Lemieux and H. F. Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

glycosides was carried out on columns of (*a*) 15% by weight of butane-1,4-diol succinate polyester on Celite at 175°; (*b*) 10% by weight of polyphenyl ether [*m*-bis-(*m*-phenoxyphenoxy)-benzene] on Celite at 200°. Retention times (*T*) are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside as an internal standard.[12]

Isolation of the Polysaccharide from Sterculia urens *Gum.*—The gum was received as pale brown nodules and a representative sample of powdered gum had uronic anhydride (by decarboxylation), 37·2, OAc, 8·0, OMe, nil, and OEt, 0·3%. Acetic acid formed on hydrolysis of the gum was characterised as the *p*-nitrobenzyl ester, m. p. and mixed m. p. 77°. Hydrolysis of the gum and chromatography of the hydrolysate showed acidic sugars including galacturonic acid, galactose, and rhamnose, together with traces of arabinose and two unidentified sugars, R_{rhamnose} 1·1 and 1·25 in solvent *B*.

Powdered gum (25 g.) was dispersed in N-sodium hydroxide solution (1·25 l.), mechanical impurities were removed at the centrifuge, and the resulting solution was treated with ethanol (3·75 l.) and concentrated hydrochloric acid (375 ml.). The precipitated polysaccharide was reprecipitated five times from aqueous solution, the final precipitate was dissolved in water, and the solution was treated with Amberlite resins IR-120(H) and IR-4B(OH) and freeze-dried to give polysaccharide acid (18 g.), $[\alpha]_D$ +62° (*c* 1·8 in 1% sodium hydroxide) [Found: uronic anhydride, 39·5%; alkoxyl, nil]. In subsequent experiments polysaccharide acid with the same physical and analytical constants was isolated more conveniently by dispersion of the gum in 1% aqueous ammonia.

Hydrolysis of the polysaccharide with 2N-sulphuric acid for 18 hr. at 100° gave galacturonic acid, galactose, rhamnose, and a trace of arabinose. Another sample of the polysaccharide was hydrolysed with N-sulphuric acid for 6 hr. at 100°, the hydrolysate was treated with methanolic hydrogen chloride, reduced with potassium borohydride and hydrolysed with N-sulphuric acid. Chromatography of the hydrolysate showed galactose, rhamnose, a trace of arabinose, and glucose which arose from the reduction of glucuronic acid residues in the polysaccharide which had not been detected previously. Colorimetric estimations using reagents containing resorcinol [13] and cysteine [14] indicated the absence of ketose sugar residues. Samples of the polysaccharide were precipitated as the insoluble calcium, copper, and cetyltrimethylammonium salts, but in each case the physical and analytical constants of the regenerated polysaccharides were unchanged. A further sample of the polysaccharide was chromatographed on a column of diethylaminoethylcellulose (phosphate form) as described by Neukom *et al.*[15] No polysaccharide was detected on elution with phosphate buffer of increasing concentration and the polysaccharide was eluted in a single band with aqueous sodium hydroxide.

Partial Hydrolysis of the Polysaccharide and Examination of Acidic Sugars.—The polysaccharide (10 g.) was heated in N-sulphuric acid (250 ml.) at 100° for 6 hr., and the cooled solution was neutralised with barium hydroxide and barium carbonate, filtered, treated with Amberlite resin IR-120(H) to remove barium ions, and concentrated to a syrup (6·1 g.) which was poured on a column of Amberlite resin CG-45 (formate form) (70 × 2·5 cm.). The column was eluted with water giving a syrup (3·1 g.) containing galactose, rhamnose, and traces of arabinose and galacturonic acid. Elution of the column with a gradient of formic acid (2 l., 0·0 · · · 2N) gave fractions (total wt. 2·04 g.) all of which contained mixtures of acidic sugars. These mixtures were each separated by chromatography on filter sheets in solvent *A* and fractions of similar mobility were combined to give acidic sugar fractions *A*–*F*.

Fraction A. The sugar (0·315 g.), $[\alpha]_D$ +54° (*c* 2·4), was chromatographically indistinguishable from D-galacturonic acid, and reduction of the derived methyl ester methyl glycosides with potassium borohydride, followed by hydrolysis, gave only galactose. The sugar was characterised as D-galacturonic acid by conversion into the 2,5-dichlorophenylhydrazone, m. p. 178—180° and mixed m. p. 180—181°.

Fraction B. The sugar (0·196 g.), $R_{\text{galacturonic acid}}$ 0·78 in solvent *A*, had $[\alpha]_D$ +90·5° (*c* 3·5) and gave on hydrolysis galacturonic acid and rhamnose. Reduction of the potassium salt with potassium borohydride followed by hydrolysis gave galacturonic acid and rhamnitol. Reduction of the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave galactose and rhamnose. The aldobiouronic acid was characterised as 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose by conversion into the methyl glycoside pentamethyl ether

[12] C. T. Bishop and F. P. Cooper, *Canad. J. Chem.*, 1960, **38**, 388; G. O. Aspinall, *J.*, 1963, 1676.
[13] J. H. Roe, J. H. Epstein, and N. P. Goldstein, *J. Biol. Chem.*, 1949, **178**, 839.
[14] Z. Dische and A. Devi, *Biochim. Biophys. Acta*, 1960, **39**, 114.
[15] H. Neukom, H. Deuel, W. J. Heri, and W. Kündig, *Helv. Chim. Acta*, 1960, **43**, 64.

dihydrate,[16] which was identified by m. p. 67—68° and mixed m. p. 65—66°, and *X*-ray powder photograph.

Fraction C. The sugar (18 mg.), $R_{\text{galacturonic acid}}$ 0·39 in solvent *A*, gave on hydrolysis galacturonic acid and galactose. Reduction of the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave galactose only.

Fraction D. The sugar (0·19 g.), $R_{\text{galacturonic acid}}$ 0·27 in solvent *A*, had $[\alpha]_D$ +44° (*c* 3·6) and gave on hydrolysis galacturonic acid, glucuronic acid, and rhamnose. Reduction of the potassium salt with potassium borohydride followed by hydrolysis gave glucuronic acid, galacturonic acid, and rhamnitol (chromatography in solvent *J* showed rhamnose to be absent). Reduction of the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave galactose, glucose, and rhamnose. The sugar (0·13 g.) was methylated with five additions of methyl sulphate (5 ml.) and 30% aqueous sodium hydroxide (11 ml.), and, after precipitation of sodium sulphate with ethanol, the acidified solution was extracted with chloroform. The resulting syrup was further methylated with methyl iodide and silver oxide to give methylated acid (95 mg.). Methylated acid was reduced with lithium aluminium hydride in tetrahydrofuran to give methylated neutral trisaccharide (81 mg.). A sample of the methylated trisaccharide was hydrolysed to give three components with the chromatographic mobilities in solvent *F* of 3,4-di-*O*-methylrhamnose, 2,3,4-tri-*O*-methylglucose, and 2,4-di-*O*-methylgalactose. Another sample of the methylated trisaccharide was heated with methanolic hydrogen chloride and examination of the products by gas–liquid chromatography on column (*b*) showed components having the retention times of methyl glycosides of the three above-mentioned sugars. The methylated trisaccharide (75 mg.) was hydrolysed with N-hydrochloric acid on the boiling-water bath for 6 hr., and, after neutralisation of the cooled solution with silver carbonate, the resulting mixture (68 mg.) of sugars was separated into two fractions by chromatography on filter sheets in solvent *F*. Fraction i (18 mg.), R_G 0·50 and $[\alpha]_D$ +89° (*c* 0·4), gave galactose on demethylation and was recrystallised from ethyl acetate to give 2,4-di-*O*-methyl-D-galactose monohydrate, m. p. 99—100° and mixed m. p. 98—99°. Fraction ii (38 mg.) gave glucose and rhamnose on demethylation and was further fractionated by ionophoresis in borate buffer at pH 10. Fraction ii*a* (16 mg.), R_G 0·91 and $[\alpha]_D$ +22·5° (*c* 0·12), gave rhamnose on demethylation and was recrystallised from light petroleum (b. p. 60—80°) to give 3,4-di-*O*-methyl-L-rhamnose, m. p. 95—96° and mixed m. p. 95°. Fraction ii*b* (17 mg.), R_G 0·91 and $[\alpha]_D$ +69° (*c* 0·17), gave glucose on demethylation and was characterised as 2,3,4-tri-*O*-methyl-D-glucose by conversion into the aniline derivative, m. p. 134—135° and mixed m. p. 135—136°.

Fraction E. The syrup (0·26 g.), $[\alpha]_D$ +49° (*c* 3·9), $R_{\text{galacturonic acid}}$ 0·28—0·39 in solvent *A*, appeared to contain two components but no satisfactory resolution could be achieved by chromatography or ionophoresis and subsequent experiments were carried out on the mixture. Hydrolysis of the syrup gave galacturonic acid, glucuronic acid, galactose, and rhamnose. Reduction of the potassium salts with potassium borohydride followed by hydrolysis gave galacturonic acid, glucuronic acid, galactitol, and rhamnitol, but galactose and rhamnose were absent. Reduction of the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave galactose, glucose, and rhamnose. The mixture (128 mg.) of acids was methylated in the same manner as fraction *D* to give the mixture (102 mg.) of methylated acids. Hydrolysis of a sample gave 2,3,6-tri-*O*-methylgalactose (R_G 0·76), 3,4-di-*O*-methylrhamnose (R_G 0·91) and acidic sugars (R_G 0·09—0·12). Another sample was heated with methanolic hydrogen chloride and examination of the products by gas–liquid chromatography on columns (*a*) and (*b*) showed components having the retention times of methyl glycosides of 2,3,4-tri-*O*-methylglucuronic acid, 2,3,4-tri-*O*-methylgalacturonic acid, 2,3,6-tri-*O*-methylgalactose, and 3,4-di-*O*-methylrhamnose. The methylated acids (91 mg.) were hydrolysed with N-sulphuric acid on the boiling-water bath for 4 hr., and the cooled solution was neutralised with barium hydroxide and barium carbonate, filtered, passed through Amberlite resin IR-120(H), and concentrated. A sample of the hydrolysate was converted into the methyl ester methyl glycosides, reduced with potassium borohydride, and hydrolysed to give 2,3,4- and 2,3,6-tri-*O*-methylgalactose, 2,4-di-*O*-methylgalactose, 2,3,4-tri-*O*-methylglucose, and 3,4-di-*O*-methylrhamnose which were detected by paper chromatography of the sugars in solvent *F* and by gas–liquid chromatography of the derived methyl glycosides on column *b*. The major portion of the hydrolysate from the methylated acids was separated by chromatography

[16] G. O. Aspinall and R. S. Fanshawe, *J.*, 1961, 4215.

on filter sheets using solvent *F* into three fractions. Fraction 1 (14 mg.), R_G 0·91 and $[\alpha]_D$ +22° (*c* 0·1), gave rhamnose on demethylation and was recrystallised from light petroleum (b. p. 80—100°) to give 3,4-di-*O*-methyl-L-rhamnose, m. p. 95—96° and mixed m. p. 94—95°. Fraction 2 (12 mg.), R_G 0·76 and $[\alpha]_D$ +79° (*c* 0·09), gave galactose on demethylation and was characterised as 2,3,6-tri-*O*-methyl-D-galactose by conversion into 2,3,6-tri-*O*-methyl-D-galactonolactone, m. p. 96—97° and mixed m. p. 95—96°. Fraction 3 (46 mg.), which contained methylated acids, was not examined further.

The mixture (20 mg.) of acidic oligosaccharides was treated with oxygen-free lime-water (5 ml.) for 10 days, the solution was neutralised with Amberlite resin IR-120(H) and concentrated, and examination of the resulting syrup showed a single acidic oligosaccharide, $R_{\text{galacturonic acid}}$ 0·27 in solvent *A*, and a trace of galacturonic acid. Hydrolysis of the degraded product gave galacturonic acid (and probably glucuronic acid) and rhamnose; hydrolysis of the derived glycitol (borohydride reduction) gave galacturonic and glucuronic acids, and rhamnitol; and reduction of the methyl ester methyl glycosides followed by hydrolysis gave galactose, glucose, and rhamnose. A further quantity (70 mg.) of acidic oligosaccharides was degraded with lime water and the product was methylated as described previously to give methylated degraded acids (46 mg.). Hydrolysis of a sample of the methylated degraded acids gave 3,4-di-*O*-methylrhamnose (R_G 0·91) and two acidic sugars (R_G 0·08, 0·12). When the derived methyl glycosides were examined by gas–liquid chromatography on columns *a* and *b*, components were detected which had retention times of methyl glycosides of 2,3,4-tri-*O*-methylglucuronic acid and 3,4-di-*O*-methylrhamnose, and, in traces only, of 2,3,4-tri-*O*-methylgalacturonic acid and 2,3,6-tri-*O*-methylgalactose. Methylated degraded acids were reduced with lithium aluminium hydride and hydrolysed to give 2,3,4-tri-*O*-methylglucose, 2,4-di-*O*-methylgalactose, and 3,4-di-*O*-methylrhamnose, together with traces of 2,3,4- and 2,3,6-tri-*O*-methylgalactose, which were detected by paper chromatography of the sugars in solvents *F* and *H* and by gas–liquid chromatography of the derived methyl glycosides on column *b*.

Fraction F. Chromatography of the syrup (45 mg.), $R_{\text{galacturonic acid}}$ 0·0 and 0·20 in solvents *A* and *D*, showed a single component, but chromatography of the hydrolysates of the syrup and its reduction products indicated the presence of at least two components with galactose and rhamnose reducing groups and containing residues of these two sugars and galacturonic and glucuronic acids.

Preparation and Hydrolysis of Reduced Methylated Acidic Polysaccharide.—The polysaccharide (10 g.) was methylated successively with methyl sulphate and 30% aqueous sodium hydroxide, and methyl iodide and silver oxide to give methylated acidic polysaccharide (2·9 g.), $[\alpha]_D$ +67° (*c* 1·2 in $CHCl_3$) (Found: OMe, 41·3%).

A sample (*ca.* 20 mg.) of the methylated polysaccharide was heated with methanolic hydrogen chloride and examination of the methanolysate by gas chromatography on columns *a* and *b* indicated the presence of components having the retention times of methyl glycosides of 2,3,4-tri-, 3,4,-di-, and 3-*O*-methylrhamnose, 2,3,4,6-tetra- and 2,3,6-tri-*O*-methylgalactose, and 2,3,4-tri-*O*-methylglucuronic acid. The remainder of the methanolysate was hydrolysed and the acidic sugars were adsorbed on diethylaminoethyl–Sephadex A-25 (formate form). Examination of the neutral sugars by paper chromatography in solvent *I* (multiple development) showed the presence of 2,6-di-*O*-methylgalactose as the only dimethyl ether and the absence of monomethyl ethers of galactose. The acidic sugars were desorbed with 0·5N-formic acid, converted into the methyl ester methyl glycosides, reduced with lithium aluminium hydride, and hydrolysed. Paper chromatography of the resulting mixture in solvent *I* indicated the presence *inter alia* of 2,3-di- and 2- and 3-*O*-methylgalactose.

Lithium aluminium hydride (4 g.) in tetrahydrofuran (100 ml.) was added portionwise to methylated acidic polysaccharide (3·3 g.) in tetrahydrofuran (100 ml.). The mixture was allowed to stand for 0·5 hr., and was then heated under reflux for 2 hr. Further lithium aluminium hydride (1·5 g.) in tetrahydrofuran (25 ml.) was added and the mixture was refluxed for 2 hr. The excess of hydride was destroyed by addition of ethyl acetate, and the solution was brought to pH 4 by addition of dilute sulphuric acid. The solution was extracted with chloroform, the extract was dried and concentrated, and reduced methylated polysaccharide (3·1 g.), $[\alpha]_D$ +41° (*c* 0·9 in $CHCl_3$), was precipitated by light petroleum (Found: OMe, 35·8%). Reduced methylated polysaccharide (2·7 g.) was dissolved in 2N-hydrochloric acid (125 ml.), the solution was gradually warmed during 3 days to 100°, and the hydrolysis was completed by heating the mixture on the boiling-water bath for 8 hr. (constant rotation). The cooled solution

was neutralised with silver carbonate and concentrated to a syrup (2·5 g.). Examination of the syrup by paper chromatography of the sugars and gas–liquid chromatography of the derived methyl glycosides showed the presence of 2,3,4-tri-, 3,4-di-, and 3-*O*-methylrhamnose, rhamnose, 2,3,4,6-tetra-, 2,3,6-tri-, di-, and mono-*O*-methylgalactose, and 2,3,4-tri-*O*-methylglucose. The

Analysis of hydrolysate of reduced methylated acidic polysaccharide

Paper chromatography *

Fraction	Wt. (mg.)	$[\alpha]_D$	R_G	Sugar	Sugars given on demethylation	Other evidence †
1	6		1·10	Unknown sugar		H, J
			1·04	2,3,4-Me_3rhamnose		
2	125	+21°	1·04	2,3,4-Me_3rhamnose	Rhamnose	H, J
3	19		1·04	2,3,4-Me_3rhamnose		H, I, J
			0·93	Me_4galactose		
			0·91	3,4-Me_2rhamnose		
				2,3,4-Me_3glucose		
4	938	+72	0·93	Me_4galactose	Galactose	H, I, J
				3,4-Me_2rhamnose	Rhamnose	
				2,3,4-Me_3glucose	Glucose	
5	43	+48	0·93	2,3,4-Me_3glucose		
			0·88	Unknown sugar		
6	18		0·92	2,3,4-Me_3glucose		
			0·88	Unknown sugar	Glucose	
			0·80	2,3,6-Me_3galactose	Galactose	
7	18		0·86	Unknown sugar	Glucose	
			0·76	2,3,6-Me_3galactose	Galactose	
8	202	+72	0·76	2,3,6-Me_3galactose	Galactose	
9	21	+41	0·76	2,3,6-Me_3galactose	Galactose	
			0·62	3-Me rhamnose	Rhamnose	H, I
10	182	+27	0·61	3-Me rhamnose		
11	21		0·61	3-Me rhamnose	Rhamnose	B, H
			0·54	2,6-Me_2galactose	Galactose	
12	28	+82	0·54	2,6-Me_2galactose	Galactose	H, I, J, P
13	16		0·54	2,6-Me_2galactose	Galactose	A, B, H
			0·51	2,3-Me_2galactose		I, P
14	38	+72	0·50	2,3-Me_2galactose	Galactose	A, H, P, RP
15	19		0·50	2,3-Me_2galactose	Galactose	
			0·44	Unknown sugar		
16	26	+58	0·50	2,3-Me_2galactose	Galactose	
			0·44	Rhamnose	Rhamnose	
17	28	+8	0·42	Rhamnose		
18	28	+38	0·42	Rhamnose	Rhamnose	
			0·33	2-Me galactose	Galactose	
19	129	+82	0·32	2-Me galactose	Galactose	P
20	38		0·32	2-Me galactose		
			0·29	3-Me galactose		
21	198	+104	0·29	3-Me galactose	Galactose	P
22	37		0·29	3-Me galactose		
			0·14	Galactose		
			0·00	Galacturonic acid (*t*)		

* *t* = trace. † A, B, H, I, J = paper chromatography in solvents A, B, H, I, and J, respectively. P = paper chromatography of the periodate-oxidised sugar. RP = paper chromatography of the periodate-oxidised glycitol (from borohydride reduction of the sugar).

syrupy mixture of sugars was separated on cellulose, (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 22 fractions. The annexed Table summarises the results of preliminary examination of the various fractions.

Characterisation of Sugars from Hydrolysis of Methylated Polysaccharide.—Fraction 2. The sugar was characterised as 2,3,4-tri-*O*-methyl-L-rhamnose by conversion into the aniline derivative, m. p. 112—113° and mixed m. p. 111—112°.

Fraction 4. Gas–liquid chromatography of the derived methyl glycosides showed components having the retention times of methyl glycosides of 2,3,4,6-tetra-*O*-methylgalactose, 3,4-di-*O*-methylrhamnose, and 2,3,4-tri-*O*-methylglucose. The mixture (270 mg.) was separated into two fractions by chromatography on filter sheets in solvent *H*. Fraction 4*a* (112 mg.), R_G 0·88 and $[\alpha]_D$ +91° (*c* 1·3), was characterised as 2,3,4,6-tetra-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 197—198°. Fraction 4*b* (126 mg.)

was shown by ionophoresis to contain two sugars and the major portion (120 mg.) was separated by ionophoresis on filter sheets to give fractions 4*b* (i) (58 mg.) and 4*b* (ii) (42 mg.). Fraction 4*b* (i), $[\alpha]_D$ +23° (*c* 2·9) crystallised from light petroleum (b. p. 40—60°) to give 3,4-di-*O*-methyl-L-rhamnose, identified by m. p. 96—97° and mixed m. p. 95—96°, and *X*-ray powder photograph. Fraction 4*b* (ii), $[\alpha]_D$ +68° (*c* 0·84), was characterised as 2,3,4-tri-*O*-methyl-D-glucose by conversion into the aniline derivative, m. p. 129—130° and mixed m. p. 127—128°.

Fraction 5. Examination of the derived methyl glycosides by gas–liquid chromatography showed components having the retention times of methyl glycosides of 2,3,4-tri-*O*-methylglucose (*T* 2·59, 3·69, and 1·38, 1·84 on columns *a* and *b*) and of an unknown sugar (*T* 1·48, 1·79, and 0·75 on columns *a* and *b*). The retention time of the unknown component on column *b* was too low for any known methyl glycoside of a methylated hexose derivative and suggested that the unknown sugar was a 6-deoxyhexose derivative (possibly 2,3-di-*O*-methylrhamnose). Attempts to fractionate the mixture of sugars and to characterise the unknown component failed.

Fraction 8. The sugar was characterised 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—98°. When the methyl glycosides of the sugar were examined by gas–liquid chromatography on column *b* an unrecognised component (*T* 3·41) was detected in addition to methyl glycosides of 2,3,6-tri-*O*-methylgalactose (*T* 1·61, 2·06, 2·21, 2·49). Attempts to detect a second sugar in the fraction, by chromatography of the sugar, the derived aldonolactone, and the periodate oxidation products from the derived glycitol, failed.

Fraction 10. The sugar crystallised and was identified as 3-*O*-methyl-L-rhamnose by m. p. 114—115° and mixed m. p. 115—116°, and by *X*-ray powder photograph.

Fraction 12. The sugar was recrystallised from chloroform–light petroleum (b. p. 60—80°) to give 2,6-di-*O*-methyl-D-galactose monohydrate, m. p. 86—87° and mixed m. p. 85—86°.

Fraction 14. The sugar was characterised as 2,3-di-*O*-methyl-D-galactose by conversion into 2,3-di-*O*-methyl-D-galactonamide, m. p. 135—136° and mixed m. p. 134°.

Fraction 17. The sugar was recrystallised from acetone–water to give L-rhamnose monohydrate, m. p. and mixed m. p. 91—92°.

Fraction 19. The sugar was recrystallised from acetone–water to give 2-*O*-methyl-D-galactose, m. p. and mixed m. p. 156—157°.

Fraction 21. The sugar was recrystallised from acetone to give 3-*O*-methyl-D-galactose, which was identified by m. p. and mixed m. p. 140—142°, and by *X*-ray powder photograph.

Degradation of Periodate-oxidised Polysaccharide.—The polysaccharide (9·3 g.) was oxidised with 0·1M-sodium metaperiodate solution (750 ml.) for 30 hr. (consumption of reagent was constant, corresponding to 0·87 mol. per sugar residue). Ethylene glycol (25 ml.) was added to destroy excess of periodate and the solution was dialysed for 4 days, concentrated, and treated with potassium borohydride (3·5 g.) for 2 days. Excess of borohydride was destroyed and potassium ions were removed by treatment of the solution with Amberlite resin IR-120(H), and the resulting solution was concentrated and repeatedly evaporated with methanol to remove boric acid as methyl borate. The residue was dissolved in water, and the solution was treated again with cation and anion exchangers and poured into ethanol (5 vol.) to precipitate the polyalcohol (4·8 g.), $[\alpha]_D$ +63° (*c* 0·5) (Found: uronic anhydride, 17%). The polyalcohol (4·7 g.) was dissolved in N-sulphuric acid (100 ml.) at room temperature for 2 hr., and the solution was neutralised by shaking with methyldi-n-octylamine (10% v/v in chloroform), treated with Amberlite resin IR-120(H), concentrated, and poured into ethanol (5 vol.). Degraded polysaccharide (0·81 g.) separated and concentration of the supernatant liquid furnished a syrup (2·8 g.). Chromatography of the syrup showed glycerol, threitol, and a component with $R_{\text{galacturonic acid}}$ 0·14 in solvent but no reducing sugars. Hydrolysis of the syrup gave galacturonic acid, rhamnose, and a trace of galactose in addition to the above-mentioned alcohols.

The degraded polysaccharide (0·65 g.) was hydrolysed with N-sulphuric acid (60 ml.) on the boiling-water bath for 4·5 hr., and the cooled solution was neutralised with barium hydroxide and barium carbonate, filtered, passed through Amberlite resin IR-120(H), and concentrated to a syrup (0·38 g.). The syrup was poured onto a column of Amberlite resin CG-45 (formate form) (30 × 1·5 cm.) and elution of the column with water gave a syrup (105 mg.) which contained rhamnose and traces of galactose and galacturonic acid. Elution of the column with 2N-formic acid furnished a mixture (0·34 g.) of three acidic sugars, $R_{\text{galacturonic acid}}$ 1·00, 0·78, and 0·18 in solvent *A*, which was fractionated by chromatography on filter sheets. The first acidic sugar (58 mg.) was chromatographically indistinguishable from D-galacturonic acid and

reduction of the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave galactose. The second acidic sugar (140 mg.), $[\alpha]_D$ +90° (*c* 2·8), was characterised as 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose by conversion into the methyl glycoside pentamethyl ether dihydrate, which was identified by m. p. 67—68° and mixed m. p. 66—67°, $[\alpha]_D$ +92° (*c* 0·72 in $CHCl_3$), and *X*-ray powder photograph. The third acidic sugar (59 mg.) gave on hydrolysis galacturonic acid and rhamnose, and on partial hydrolysis the aldobiouronic acid, 2-*O*-galacturonosylrhamnose, $R_{\text{galacturonic acid}}$ 0·78 in solvent *A*, was detected. Hydrolysis of the derived glycitol (borohydride reduction) gave galacturonic acid and rhamnitol, and reduction of the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave galactose and rhamnose. The acidic sugar (35 mg.) was methylated as described for other acidic oligosaccharides and reduction of the product with lithium aluminium hydride in tetrahydrofuran gave neutral methylated sugar (18 mg.). Hydrolysis of the methylated sugar gave 3,4-di-*O*-methylrhamnose (R_G 0·91), 2,3,4-tri-*O*-methylgalactose (R_G 0·74), and an unidentified sugar (R_G 0·45, probably a di-*O*-methylgalactose). A sample of the methylated oligosaccharide was heated with methanolic hydrogen chloride and gas–liquid chromatography of the products on column *b* showed components having the retention times of methyl glycosides of 3,4-di-*O*-methylrhamnose (*T* 0·60), 2,3,4-tri-*O*-methylgalactose (*T* 2·64, 2·91), and an unidentified sugar (possibly a di-*O*-methylhexose, *T* 4·16).

The authors thank Professor Sir Edmund Hirst, C.B.E., F.R.S., for interest and advice, the University of Edinburgh for the award of a Postgraduate Studentship (to N.), Messrs. Clarke and Smith (India) Ltd. for supplying the sample of *Sterculia urens* gum, and Mr. R. N. Fraser for assistance.

DEPARTMENT OF CHEMISTRY, UNIVERSITY OF EDINBURGH. [*Received, October 12th,* 1964.]