797. *Plant Gums of the Genus* Sterculia. *Part II.*[1] Sterculia caudata *Gum*

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Partial hydrolysis of *Sterculia caudata* gum affords a mixture of acidic oligosaccharides including 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, 4-*O*-(β-D-galactopyranosyluronic acid)-D-galactose, 3-*O*-(β-D-glucopyranosyluronic acid)-D-galacturonic acid, *O*-(β-D-glucopyranosyluronic acid)-(1 → 3)-*O*-(α-D-galactopyranosyluronic acid)-(1 → 3)-L-rhamnose, and *O*-(D-galactopyranosyluronic acid)-(1 → 4)-*O*-(D-galactopyranosyluronic acid)-(1 → 2)-L-rhamnose. The cleavage products from the methylated gum have been re-examined, and a number of partial structures are proposed on the basis of these results.

The recognition of both D-glucuronic acid and D-galacturonic acid as constituents of *Sterculia urens* gum ("karaya gum")[1] prompted a re-examination of *Sterculia caudata* (syn. *Brachychiton diversifolium*) gum.[2] Previous studies[2] had shown that this gum consists of a partially acetylated polysaccharide of highly branched structure with residues of D-glucuronic acid, D-galactose, and L-rhamnose. Although Hirst, Williams, and Percival[2] sought evidence that residues of D-galacturonic acid also might be present in the gum, no derivatives of this sugar could be isolated, and they concluded that the sugar, if present, was only a minor constituent. Direct evidence for the presence of D-galacturonic acid as an important constituent of the gum has now been obtained by the characterisation of the sugar itself and of a series of acidic oligosaccharides as products of partial hydrolysis.

The gum, which swells but does not dissolve in water, furnished a water-soluble polysaccharide on deacetylation with dilute ammonia. Chromatography of the acidic polysaccharide on diethylaminoethylcellulose provided evidence for its homogeneity. Partial acid hydrolysis of the polysaccharide afforded a mixture of acidic sugars which were adsorbed on a column of diethylaminoethyl-Sephadex. A partial resolution of the mixture was effected by desorption of the acids by gradient elution with water containing formic acid, and further separations were carried out, as required, by chromatography on filter

[1] Part I, *J.*, 1965, 2710.
[2] E. L. Hirst, E. E. Percival, and R. S. Williams, *J.*, 1958, 1942.

sheets, to give D-galacturonic acid, characterised as the crystalline 2,5-dichlorophenylhydrazone, and six acidic oligosaccharides.

The nature of the constituent sugars and of the reducing sugar residues in each of the acidic oligosaccharides was established by paper chromatography of the products of (a) direct hydrolysis, (b) reduction with sodium borohydride followed by hydrolysis of the derived glycitols, and (c) reduction of the hexuronic acid to hexose residues by treatment of the methyl ester methyl glycosides with sodium borohydride followed by hydrolysis. Oligosaccharide I was characterised as 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose by conversion into the crystalline methyl glycoside pentamethyl ether.[3] Oligosaccharide II was shown to be 4-*O*-(β-D-galactopyranosyluronic acid)-D-galactose by reduction of the methylated derivative with lithium aluminium hydride followed by hydrolysis to give 2,3,4- and 2,3,6-tri-*O*-methyl-D-galactose. Since periodate oxidation of the oligosaccharide gave formaldehyde, a 1,4- rather than a 1,5-linkage was shown to be present, and the tentative assignment of a β-glycosidic linkage may be made on the basis of the optical rotation ($[\alpha]_D$ +64°) of the disaccharide. Oligosaccharide III was characterised as an *O*-acetyl derivative of oligosaccharide II, probably 2-*O*-acetyl-4-*O*-(β-D-galactopyranosyluronic acid)-D-galactose. The nature of the glycosidic linkage was established, as for oligosaccharide II, by methylation and periodate oxidation experiments. Hydrolysis of oligosaccharide III, which surprisingly had a lower chromatographic mobility than oligosaccharide II, gave galacturonic acid, galactose, and a sugar of high chromatographic mobility, further hydrolysis of which gave galactose. Oligosaccharide III and the sugar of high chromatographic mobility both gave hydroxamic acids on treatment with hydroxylamine,[4] and the location of the acyl substituent at C-2 was indicated by the absence of staining reactions with triphenyltetrazolium chloride.[5] The nature of the acyl substituent was established in a parallel investigation of *Cochlospermum gossypium* gum.[6] The identical disaccharide was formed on partial hydrolysis of this gum and furnished acetic acid on saponification. Although the optical rotation ($[\alpha]_D$ +120°) was considerably higher than that of oligosaccharide II, treatment of III with dilute ammonia resulted in a rapid fall in optical rotation with the formation of a component of the same chromatographic mobility as II. The isolation of the acetylated oligosaccharide III indicates that treatment of the gum with aqueous ammonia had not resulted in complete deacetylation and, assuming that no ester migration had taken place, provides evidence for the location of some of the *O*-acetyl substituents in the native gum. The remarkable stability of certain *O*-acetyl groups in polysaccharides has been observed previously by Croon [7] who isolated an *O*-acetylxylobiose during the acid sulphite pulping of birch wood which contains an *O*-acetylxylan.[8]

D-GalpA 1α ⟶ 2 L-Rha (I)
D-GalpA 1β ⟶ 4 (2-*O*-Ac) D-Gal (III)
D-GpA 1β ⟶ 3 D-GalpA 1α ⟶ 2 L-Rha (V)
D-GalpA 1 ⟶ 4 D-GalpA 1 ⟶ 2 L-Rha (VI)

D-GalpA 1β ⟶ 4 D-Gal (II)
D-GpA 1β ⟶ 3 D-GalA (IV)

The structure of oligosaccharide IV as 3-*O*-(β-D-glucopyranosyluronic acid)-D-galacturonic acid was established by reduction of the methylated derivative with lithium aluminium hydride followed by hydrolysis to give 2,3,4-tri-*O*-methyl-D-glucose and 2,4-di-*O*-methyl-D-galactose. The structure *O*-(β-D-glucopyranosyluronic acid)-(1 ⟶ 3)-*O*-(α-D-galactopyranosyluronic acid)-(1 ⟶ 2)-L-rhamnose was assigned to oligosaccharide V since rhamnose was shown to be the reducing sugar residue and hydrolysis of the methylated trisaccharide after reduction afforded 2,3,4-tri-*O*-methyl-D-glucose, 2,4-di-*O*-methyl-D-galactose,

[3] G. O. Aspinall and R. S. Fanshawe, *J.*, 1961, 4215.
[4] M. Abdel-Akher and F. Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859.
[5] D. J. Bell and R. J. Dedonder, *J.*, 1954, 2866.
[6] G. O. Aspinall, R. N. Fraser, and G. R. Sanderson, following Paper.
[7] I. Croon, *Acta Chem. Scand.*, 1962, **16**, 827.
[8] H. O. Bouveng, P. J. Garegg, and B. Lindberg, *Acta Chem. Scand.*, 1960, **14**, 742.

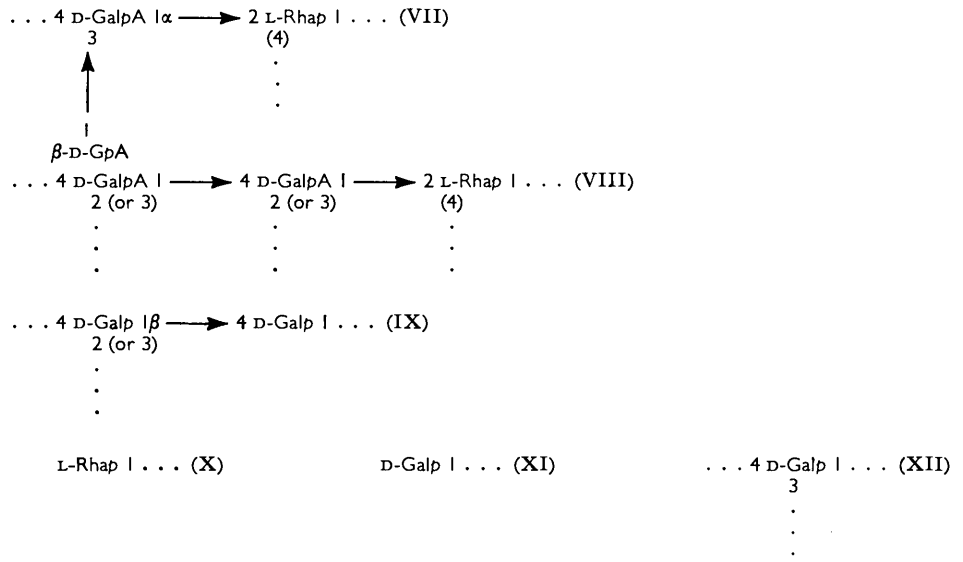
and 3,4-di-*O*-methyl-L-rhamnose. The β-configuration of the D-glucopyranosyluronic acid residue in oligosaccharides IV and V has been assigned tentatively on the basis of the optical rotations of the sugars, it being assumed in the case of the trisaccharide V that the aldobiouronic acid, 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose (I), is formed as a partial hydrolysis product. Oligosaccharide VI was not formed in sufficient quantity to permit characterisation by means of crystalline derivatives, but the structure *O*-(D-galactopyranosyluronic acid)-(1 → 4)-*O*-(D-galactopyranosyluronic acid)-(1 → 2)-L-rhamnose may be proposed on the basis of the following observations. Partial acid hydrolysis gave galacturonic acid, rhamnose, and oligosaccharide I, and reduction with sodium borohydride followed by hydrolysis showed that rhamnose provided the reducing group. The cleavage products of the reduced methylated derivative were examined by paper chromatography of the sugars and gas chromatography of the methyl glycosides, and the presence of 2,3,4-tri- and 2,3-di-*O*-methylgalactose and 3,4-di-*O*-methylrhamnose was indicated.

Acidic oligosaccharides I, II, and V were characterised previously as partial hydrolysis products of *Sterculia urens* gum,[1] and oligosaccharide VI is probably identical with an acidic trisaccharide which was obtained from partial hydrolysis of periodate-oxidised *S. urens* gum. 2-*O*-(α-D-Glucopyranosyluronic acid)-L-rhamnose has been reported as a partial hydrolysis product of *Sterculia caudata* gum,[2] but we have obtained no evidence for such a disaccharide amongst our cleavage products. In view of the similarity of the chromatographic mobility of the oligosaccharide to which this structure was assigned to that of our oligosaccharide V it is probable that the oligosaccharide was in fact *O*-(β-D-glucopyranosyluronic acid)-(1 → 3)-*O*-(α-D-galactopyranosyluronic acid)-(1 → 2)-L-rhamnose.

In previous studies on *Sterculia caudata* gum [2] 2,3,4,6-tetra- and 2,3,6-tri-*O*-methyl-D-galactose, 3,4-di- and 3-*O*-methyl-L-rhamnose, and 2,3,4-tri-*O*-methyl-D-glucose (from reduction of the corresponding hexuronic acid) were characterised as hydrolysis products of the methylated polysaccharide. The cleavage products from the methylated polysaccharide and its reduction product after treatment with lithium aluminium hydride have now been re-examined by gas chromatography of the methyl glycosides formed on methanolysis. In addition, the methylated gum was hydrolysed and neutral and acidic sugars were separated. Paper chromatographic examination of the neutral sugars directly and of the acidic sugars after reduction of their methyl ester methyl glycosides with sodium borohydride followed by hydrolysis permitted the identification of the less substituted sugars whose methyl glycosides were insufficiently volatile to be detected by gas chromatography. The results of this procedure, whose validity was established in studies on *Sterculia urens* gum,[1] provided evidence for the presence in the methylated gum of residues of the following sugars: 2,3,4,6-tetra-, 2,3,6-tri-, and 2,6-di-*O*-methylgalactose (small amounts only), 2,3,4-tri-, 3,4-di-, and 3-*O*-methylrhamnose, 2,3,4-tri-*O*-methylglucuronic acid, and 2,3,4-tri-, 2,3-di-, and 2- and 3-*O*-methylgalacturonic acid.

In the light of the methylation results it is possible to indicate the location in the polysaccharide structure of some of the fragments which were isolated as partial hydrolysis products. D-Glucuronic acid residues are present only as non-reducing end-groups. Since residues of this sugar were found linked 1 → 3 to D-galacturonic acid in oligosaccharides IV and V, and since 3-*O*-substituted D-galacturonic acid residues in the polysaccharide are also 4-*O*-substituted, partial structure (VII) defines the mode of attachment of D-glucuronic acid residues as single-unit side-chains. With the exception of a small proportion of end-groups, D-galacturonic acid residues, in contrast, are all 4-*O*-substituted and the majority are also 2- or 3-*O*-substituted. Residues of this sugar, therefore, are mainly present in interior chains, frequently as branching points, and may be accommodated in the partial structures VII—IX. L-Rhamnopyranose residues are also present in the sequences VII and VIII, in some of which side-chains may be attached at C-4, and to a small extent as non-reducing end-groups (X). D-Galactopyranose residues

are located mainly as end-groups (XI) and in non-terminal positions in the units (IX), although a small proportion may be present as branching points (XII). The results of the present re-investigation of *Sterculia caudata* gum indicate clearly that the gum bears a close structural similarity to *Sterculia urens* gum.[1]



Experimental

The sample of gum was that used in a previous investigation.[1] Paper chromatography was carried out on Whatman Nos. 1, 3MM, 31ET, and 541 papers with the following solvent systems (v/v): (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (C) ethyl acetate–pyridine–acetic acid–formic acid (5 : 5 : 1 : 3); (D) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (E) butan-2-one–water–ammonia (200 : 17 : 1). R_G Values of methylated sugars refer to rates of movement relative to 2,3,4,6-tetra-*O*-methyl-D-glucose in solvent D, and R_{GalA} values of acidic sugars to rates of movement relative to D-galacturonic acid in solvent B. Demethylations were performed with boron trichloride.[9]

Gas chromatography was carried out on columns of acid-washed Celite coated with (a) 15% by weight of butane-1,4-diol succinate polyester (operating temperature 175°), (b) 10% of polyphenyl ether [*m*-bis-(*m*-phenoxyphenoxy)benzene] (200°), and (c) 3% of neopentyl glycol adipate polyester (150°). Retention times (T) are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside.[10]

Isolation of the Polysaccharide from Sterculia caudata *Gum.*—Finely powdered gum (50 g.) was allowed to swell in water (1·5 l.), concentrated ammonia (375 ml.) was added to the suspension, and the mixture was heated at 60° for 4 hr., with vigorous stirring. A small amount of insoluble material was removed at the centrifuge, and the supernatant was poured into ethanol (4 l.) containing concentrated hydrochloric acid (400 ml.). The precipitated polysaccharide was washed, and redispersed in aqueous 5% ammonia (1 l.) at 60° for 1 hr. The cooled solution was acidified with concentrated hydrochloric acid and poured into ethanol (2 l.), to give precipitated gum acid, which was washed free from chloride ions, redispersed in water, and freeze-dried to give gum acid (35 g.), $[\alpha]_D$ +69° (*c* 0·57 in H_2O containing 1% of ammonia), uronic anhydride (by decarboxylation), 50·2%. A sample of the gum acid was hydrolysed, and chromatographic examination of the hydrolysate in solvents A, B, and C showed the presence of galacturonic acid, glucuronic acid, galactose, and rhamnose. Another portion of the hydrolysate was treated with methanolic hydrogen chloride, reduced with sodium borohydride, and hydrolysed to give galactose, glucose, and rhamnose.

[9] T. G. Bonner, E. J. Bourne, and S. McNally, *J.*, 1960, 2929.
[10] G. O. Aspinall, *J.*, 1963, 1676.

A sample (*ca.* 60 mg.) of the gum acid was chromatographed on diethylaminoethylcellulose (phosphate form) as described by Neukom *et al.*[11] No polysaccharide was detected on elution with phosphate buffer (0—0·5M) at pH 6, but further elution with a linear gradient of potassium chloride (0—1M) furnished polysaccharide in a single band. The polysaccharide, which was recovered after dialysis, deionisation, and freeze-drying, had uronic anhydride, 50·2%, and gave on hydrolysis galacturonic acid, glucuronic acid, galactose, and rhamnose.

Partial Hydrolysis of the Gum Acid and Separation of Acidic Sugars.—Gum acid (10 g.) was heated in N-sulphuric acid (500 ml.) on a boiling-water bath for 7 hr., the cooled solution was neutralised with barium hydroxide and barium carbonate, and precipitated barium salts were removed at the centrifuge and washed with water (3 × 500 ml.). The combined centrifugate and washings were concentrated, treated with Amberlite resin IR-120(H) to remove barium ions, and further concentrated to a syrup (6·16 g.). The syrup (6 g.), in water (20 ml.), was added in five portions to a column of diethylaminoethyl-Sephadex A-25 (formate form) (30 g.; 20 × 3 cm.), each portion being washed on to the column with water (100 ml.). The column was allowed to stand for 4 hr. to ensure complete adsorption of acidic sugars, and the column was eluted with water until the eluant gave a negative colour reaction with the phenol–sulphuric acid reagent.[12] Concentration of the aqueous washings gave a syrup (1·38 g.), chromatography of which in solvents A and B showed galactose, rhamnose, and an unknown sugar, R_{rhamnose} 0·82 in solvent A. A portion (300 mg.) of the syrup was separated on filter sheets in solvent A, to give the unknown sugar (23 mg.) which gave a positive ester test with hydroxylamine–ferric chloride,[4] but no colour reaction with triphenyltetrazolium salts.[5] Treatment of the sugar with aqueous 5% ammonia at room temperature for 24 hr., followed by chromatography, showed that galactose only was formed on deacylation. The column was eluted with a gradient of water containing 0—1·5% of formic acid (3 l.), to give fractions 1—5, and with water containing 2% of formic acid to give fraction 6. Further elution with higher concentrations of formic acid yielded only chromatographically immobile material. Fraction 1 (0·94 g.) contained four components, and filter-sheet separation in solvent B furnished D-galacturonic acid (67 mg.), $[\alpha]_D$ +54° (*c* 2·71), which was converted into the 2,5-dichlorophenylhydrazone, m. p. 178—179° and mixed m. p. 180°, oligosaccharide I (194 mg.), oligosaccharide II (61 mg.), and oligosaccharide III (355 mg.). Fraction 2 (1·46 g.) contained chromatographically pure galacturonic acid and was not examined further. Fraction 3 (147 mg.) contained chromatographically immobile material, which gave glucuronic and galacturonic acids, galactose, and rhamnose on hydrolysis, and was not examined further. Fraction 4 (97 mg.) contained oligosaccharide VI and chromatographically immobile material, and filter-sheet separation in solvent B gave pure oligosaccharide VI (31 mg.). Fraction 5 (310 mg.) contained chromatographically immobile material and oligosaccharide V, a pure sample (242 mg.) of which was obtained after filter-sheet separation in solvent B. Filter-sheet separation of fraction 8 (688 mg.) in solvent B furnished oligosaccharide IV (207 mg.) and further quantities (246 mg.) of oligosaccharide V.

Examination of Acidic Oligosaccharides.—*Oligosaccharide* I. The sugar, R_{GalA} 0·79, had $[\alpha]_D$ +89° (*c* 1·8) and gave galacturonic acid and rhamnose on hydrolysis. Reduction of a sample (2 mg.) with sodium borohydride followed by hydrolysis gave galacturonic acid and rhamnitol. A further sample (2 mg.) was converted into the methyl ester methyl glycosides with methanolic hydrogen chloride, reduced with sodium borohydride, and hydrolysed to give galactose and rhamnose. Colorimetric determinations of galacturonic acid (carbazole method [13]) and rhamnose (cysteine method [14]) indicated a molar ratio of 1 : 1. The aldobiouronic acid was characterised as 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose by conversion into the methyl glycoside pentamethyl ether dihydrate,[3] which was identified by m. p. 67—68° and mixed m. p. 67°, and by *X*-ray powder photography.

Oligosaccharide II. The sugar, R_{GalA} 0·39, had $[\alpha]_D$ +64° (*c* 1·04) and gave galacturonic acid and galactose on hydrolysis. Reduction of a sample with sodium borohydride followed by hydrolysis gave galacturonic acid and galactitol. Conversion of another sample into the methyl ester methyl glycosides, followed by reduction with sodium borohydride and hydrolysis gave galactose only. A positive colour reaction with triphenyltetrazolium hydroxide indicated the absence of a 2-*O*-substituent. Oxidation of a sample with lead tetra-acetate followed by

[11] H. Neukom, H. Deuel, W. J. Heri, and W. Kundig, *Helv. Chim. Acta*, 1960, **43**, 67.
[12] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Analyt. Chem.*, 1956, **28**, 350.
[13] E. A. McComb and R. McCready, *Analyt. Chem.*, 1952, **24**, 1630.
[14] Z. Dische and L. B. Shettles, *J. Biol. Chem.*, 1948, **175**, 595.

hydrolysis gave threose but no lyxose indicating the presence of a 1,4- rather than a 1,3-linkage.[15] Oxidation of the sugar with sodium metaperiodate solution resulted in the liberation of formaldehyde which was detected by the chromotropic acid reagent.[16] The sugar (50 mg.) was methylated with methyl sulphate and sodium hydroxide, the resulting solution was acidified and extracted with chloroform, and the methylated acid was converted into its silver salt and further methylated with methyl iodide and silver oxide to give methylated oligosaccharide II (37 mg.). The methanolysis products from a sample of methylated oligosaccharide II were examined by gas chromatography on columns a and b, and components with the retention times of methyl glycosides of 2,3,4-tri-*O*-methylgalacturonic acid and 2,3,6-tri-*O*-methylgalactose were detected. The remainder of the methylated aldobiouronic acid was reduced with lithium aluminium hydride in tetrahydrofuran, to give methylated disaccharide (30 mg.). A sample of the methylated disaccharide was heated with methanolic hydrogen chloride, and gas chromatography of the products showed the presence of methyl glycosides of 2,3,4- and 2,3,6-tri-*O*-methylgalactose. The remainder of the methylated disaccharide was hydrolysed with N-hydrochloric acid on a boiling-water bath for 4 hr., and the resulting mixture of sugars (26 mg.) was fractionated on charcoal–Celite (1 : 1; 10 × 1 cm.) with water containing 0—4% of butan-2-one, to give 2,3,4-tri-*O*-methyl-D-galactose (8 mg.), which was characterised as the aniline derivative, m. p. 167—168° and mixed m. p. 168°, a mixture (5 mg.) of the two sugars, and 2,3,6-tri-*O*-methyl-D-galactose (7 mg.), which was characterised by conversion into 2,3,6-tri-*O*-methyl-D-galactonolactone, m. p. 97—99° and mixed m. p. 99°.

Oligosaccharide III. The sugar, R_{GalA} 0·24, had $[\alpha]_D$ +129° (*c* 2·39 in H_2O), +129° ⟶ +77° (4 hr.) (*c* 1·17 in H_2O containing 1% of ammonia), and gave on hydrolysis galacturonic acid, galactose, and a sugar, $R_{rhamnose}$ 0·82, which, like the parent oligosaccharide, gave a violet stain with the hydroxylamine–ferric chloride spray.[4] Reduction of the oligosaccharide with sodium borohydride followed by hydrolysis gave galacturonic acid and galactitol. Reduction of the derived methyl ester methyl glycosides with sodium borohydride followed by hydrolysis gave galactose only. The oligosaccharide gave no colour reaction with triphenyltetrazolium chloride, but treatment with 0·5N-sodium hydroxide overnight at room temperature gave a component with the chromatographic mobility of oligosaccharide II (R_{GalA} 0·39). Oxidation of the sugar with sodium metaperiodate gave formaldehyde. Methylation of the sugar (150 mg.) as for oligosaccharide II yielded methylated oligosaccharide III (111 mg.). The methanolysis products from a sample of the methylated derivative were examined by gas chromatography on columns a and b, and components with the retention times of 2,3,4-tri-*O*-methylgalacturonic acid and 2,3,6-tri-*O*-methylgalactose were detected. The methylated aldobiouronic acid was reduced with lithium aluminium hydride in tetrahydrofuran, to give methylated disaccharide (98 mg.), and examination of the methanolysis products by gas chromatography on column b showed the presence of methyl glycosides of 2,3,4- and 2,3,6-tri-*O*-methylgalactose. Hydrolysis of the methylated disaccharide gave a mixture (79 mg.) of sugars which was fractionated on charcoal–Celite (1 : 1; 23 × 2 cm.) with water containing 0—4% of butan-2-one, to give 2,3,4-tri-*O*-methyl-D-galactose (25 mg.), which was characterised as the aniline derivative, m. p. 167—168° and mixed m. p. 167°, a mixture (9 mg.) of the two sugars, and 2,3,6-tri-*O*-methyl-D-galactose (21 mg.), which was characterised by conversion into 2,3,6-tri-*O*-methyl-D-galactonolactone, m. p. 98—99° and mixed m. p. 98°.

Oligosaccharide IV. The sugar, R_{GalA} 0·29, had $[\alpha]_D$ +45° (*c* 2·07) and gave glucuronic and galacturonic acids on hydrolysis. Reduction of the oligosaccharide with sodium borohydride followed by hydrolysis gave glucuronic acid and galactonic acid. Reduction of the methyl ester methyl glycosides with sodium borohydride followed by hydrolysis gave glucose and galactose. The acidic disaccharide (100 mg.) was successively methylated to give methylated acidic disaccharide (79 mg.), reduced with lithium aluminium hydride in tetrahydrofuran to give methylated disaccharide (64 mg.), and hydrolysed to give a mixture (57 mg.) of sugars whose methyl glycosides had the retention times of those of 2,3,4-tri-*O*-methylglucose and 2,4-di-*O*-methylgalactose when examined by gas chromatography. Separation of the sugars on filter sheets using solvent D gave 2,3,4-tri-*O*-methyl-D-glucose (26 mg.), $[\alpha]_D$ +68° (*c* 1·3), which was characterised as the aniline derivative, m. p. 135° and mixed m. p. 135—136°, and 2,4-di-*O*-methyl-D-galactose (29 mg.), $[\alpha]_D$ +89° (*c* 1·45), which crystallised as the monohydrate, m. p. 99—100° and mixed m. p. 98—99°.

[15] A. S. Perlin, *Analyt. Chem.*, 1955, **27**, 396.
[16] J. F. O'Dea and R. A. Gibbons, *Biochem. J.*, 1953, **55**, 580.

Oligosaccharide V. The sugar, R_{GalA} 0·24, had $[\alpha]_D$ +71° (*c* 2·32), gave no colour reaction with triphenyltetrazolium hydroxide, and furnished glucuronic and galacturonic acids, and rhamnose on hydrolysis. Reduction of the oligosaccharide with sodium borohydride followed by hydrolysis gave glucuronic and galacturonic acids, and rhamnitol. Reduction of the methyl ester methyl glycosides with sodium borohydride followed by hydrolysis gave glucose, galactose, and rhamnose. The acidic trisaccharide (250 mg.) was successively methylated to give methylated acidic trisaccharide (196 mg.), reduced with lithium aluminium hydride in tetrahydrofuran to give methylated trisaccharide (161 mg.), and hydrolysed to give a mixture (153 mg.) of sugars whose methyl glycosides had the retention times of those of 2,3,4-tri-*O*-methylglucose, 2,4-di-*O*-methylgalactose, and 3,4-di-*O*-methylrhamnose when examined by gas chromatography on column b. Separation of the sugars on filter sheets using solvent D gave 2,4-di-*O*-methyl-D-galactose (41 mg.), $[\alpha]_D$ +89° (*c* 2·05), which crystallised as the monohydrate, m. p. 99—100° and mixed m. p. 98—99°, and a mixture (83 mg.) of sugars which was separated by ionophoresis in borate buffer to give 3,4-di-*O*-methyl-L-rhamnose (25 mg.), $[\alpha]_D$ +23° (*c* 1·25), m. p. 96° and mixed m. p. 95—96°, and 2,3,4-tri-*O*-methyl-D-glucose (37 mg.), $[\alpha]_D$ +67° (*c* 1·85), which was characterised as the aniline derivative, m. p. 134—135° and mixed m. p. 135°.

Oligosaccharide VI. The sugar, R_{GalA} 0·18, had $[\alpha]_D$ +72° (*c* 1·22), and gave on partial hydrolysis galacturonic acid, rhamnose, and 2-*O*-(galactopyranosyluronic acid)rhamnose. Reduction of the oligosaccharide with sodium borohydride followed by hydrolysis gave galacturonic acid and rhamnitol. Reduction of the methyl ester methyl glycosides with sodium borohydride followed by hydrolysis gave galactose and rhamnose. The oligosaccharide (20 mg.) was methylated and then reduced with lithium aluminium hydride in tetrahydrofuran. Gas chromatography on column b of the methanolysis products from the reduced methylated oligosaccharide showed the presence of methyl glycosides of 2,3,4-tri- and 2,3-di-*O*-methylgalactose, and 3,4-di-*O*-methylrhamnose. Chromatography of the hydrolysis products from the reduced methylated oligosaccharide by multiple development in solvent E readily distinguished 2,3-di-*O*-methylgalactose from the isomeric dimethyl ethers.

Methylation of the Polysaccharide.—The gum acid (15 g.) was methylated with methyl sulphate and aqueous 30% sodium hydroxide as described by Hirst, Percival, and Williams.[2] Further methylation of the partially methylated polysaccharide with methyl iodide and silver oxide afforded methylated polysaccharide (11·9 g.), $[\alpha]_D$ +72° (*c* 1·08 in $CHCl_3$) (Found: OMe, 43·2%). Unless otherwise stated, further experiments were carried out on this sample of methylated polysaccharide.

A further sample (1·5 g.) of gum acid was stirred in suspension in ethereal diazomethane (250 ml.) overnight, and the resulting polysaccharide methyl ester (1·4 g.) was methylated with methyl sulphate (25 ml.) and barium hydroxide (50 g.) in dimethyl sulphoxide (25 ml.) and *NN*-dimethylformamide (25 ml.) as described by Kuhn and Trischmann.[17] Further methylation of the partially methylated polysaccharide (0·67 g.) (Found: OMe, 32·0%) with methyl iodide and silver oxide in *NN*-dimethylformamide furnished methylated polysaccharide (0·31 g.), $[\alpha]_D$ +80° (*c* 0·94 in $CHCl_3$) (Found: OMe, 43·1%).

Samples of both batches of methylated polysaccharide were heated with methanolic hydrogen chloride, and examination of the cleavage products by gas chromatography on columns a and b indicated the presence of methyl glycosides of the following sugars (approximate proportions in parenthesis): 2,3,4-tri- (+), 3,4-di- (++), and 3-*O*-methylrhamnose (+++), 2,3,4,6-tetra- (+++) and 2,3,6-tri-*O*-methylgalactose (++), 2,3,4-tri-*O*-methylglucuronic acid (++), and 2,3,4-tri- (+) and 2,3-di-*O*-methylgalacturonic acid (+).

Methylated polysaccharide (0·2 g.) was refluxed with methanolic 4% hydrogen chloride (10 ml.) for 18 hr. The neutralised solution was concentrated, heated with saturated aqueous barium hydroxide (10 ml.) at 60° for 2 hr., and passed through Amberlite resin IR-120(H) to remove barium ions. The concentrated solution was adsorbed on a column (5 × 1 cm.) of diethylaminoethyl-Sephadex (formate form). Elution with water furnished neutral methyl glycosides, and elution with aqueous 1% formic acid gave acidic methyl glycosides. The neutral methyl glycosides were hydrolysed, and paper chromatography of the resulting sugars in solvents A, D, and E showed 2,3,4-tri- (+), 3,4-di- (++), 3-*O*-methylrhamnose (++), and rhamnose (+) 2,3,4,6-tetra- (+++), 2,3,6-tri- (++), and 2,6-di-*O*-methylgalactose (+).

[17] R. Kuhn and H. Trischmann, *Chem. Ber.*, 1963, **95**, 284.

The acidic methyl glycosides were heated with methanolic hydrogen chloride, reduced with sodium borohydride, and hydrolysed, and paper chromatography of the resulting sugars showed 3,4-di- (+) and 3-*O*-methylrhamnose (+), tri- (+), 2,3-di- (+), 2- (++), and 3-*O*-methylgalactose (++), and 2,3,4-tri-*O*-methylglucose (+++). The dimethyl ethers of galactose were readily distinguished by chromatography in solvent E.

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