955. *Plant Gums of the Genus* Khaya. *Part II.*[1] *The Major Component of* Khaya senegalensis *Gum.*

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Fractionation of deacetylated *Khaya senegalensis* gum affords two polysaccharide components, and structural studies on the major component are described. Partial acid-hydrolysis of this polysaccharide gives a mixture of aldobiouronic acids, (2-L-rhamnose D-galactopyranosid)uronic acid, and (4-D-galactose 4-*O*-methyl-D-glucopyranosid)uronic acid. Hydrolysis of the methylated polysaccharide indicates the presence therein of residues of 2,3,4,6-tetra- and 2,3,6-tri-*O*-methyl-D-galactose, 3-*O*-methyl-L-rhamnose, 2,3,4-tri-*O*-methyl-D-glucuronic acid, and 2,3-di-*O*-methyl-D-galacturonic acid, and, in smaller amount, residues of 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri- and 2,4-di-*O*-methyl-D-galactose, 2,3,4-tri-*O*-methyl-L-rhamnose, and 3,4-di-*O*-methyl-D-glucuronic acid. The structural significance of these results is discussed.

In Part I [1] it was shown that the gum exudate of the West African tree, *Khaya grandifolia*, was a highly branched polysaccharide containing residues of D-galactose, L-rhamnose,

[1] The paper by Aspinall, Hirst, and Matheson, J., 1956, 989, is regarded as Part I.

D-galacturonic acid, and 4-*O*-methyl-D-glucuronic acid, and the main features of its molecular structure were established. A preliminary examination of the gum exudate from the related species, *Khaya senegalensis*, showed this gum to be composed of the same sugar residues, but in different proportions. Further investigations have now shown that *K. senegalensis* gum contains two polysaccharide components, and structural studies on the major component are described in this paper.

Khaya senegalensis gum, which is partially acetylated in the natural state, was dissolved in N-sodium hydroxide with concomitant deacetylation, and addition of ethanol to the acidified solution then readily precipitated the less soluble major component. After several reprecipitations the isolated polysaccharide had $[\alpha]_D$ +140° (in H_2O), equivalent weight 317 (corresponding to a uronic anhydride content of 55%), and a low, but significant, methoxyl content of 1·8%. Structural investigations reported in this paper were carried out on this major polysaccharide component of the gum. A second polysaccharide was isolated from the supernatant liquors from the above precipitations. Somewhat variable values for optical rotations and equivalent weights were found for the material thus isolated, and it cannot be certain that only one polysaccharide is present. It is clear, however, that there is present in the gum a second polysaccharide with $[\alpha]_D$ *ca.* 5° (in H_2O) markedly different from that of the major component and with a much lower uronic anhydride content (<20%). Structural studies on this second polysaccharide will be reported later.

The two polysaccharide fractions were examined by ultracentrifugation.[2] The fractions sedimented at slightly different rates, but under the same conditions a mixture of the two fractions gave a single broad peak in the ultracentrifuge. Similarly, examination of the gum fractions in borate buffer in an Antweiler micro-electrophoresis apparatus indicated different mobilities, but it was not possible to resolve unambiguously a mixture of the two components.[2] Later, when the present structural investigation had been almost completed, it was possible to examine the gum and its fractions by glass-fibre paper ionophoresis [3] in 2N-sodium hydroxide.* By this criterion, the unfractionated gum contained two components; the minor fraction with a higher mobility was homogeneous, and the major fraction still contained a small proportion (*ca.* 5%) of the minor polysaccharide component.

Partial acid-hydrolysis of the polysaccharide gave a mixture of neutral and acidic sugars. The acids, after separation from neutral sugars by absorption on an anion-exchange resin, were fractionated on cellulose, and an *O*-methylhexuronic acid and a mixture of aldobiouronic acids were obtained in sufficient quantity for further investigation. The *O*-methylhexuronic acid was identified as 4-*O*-methylglucuronic acid since reduction of the derived methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave 4-*O*-methylglucose, characterised by paper chromatography of the sugar and of its periodate oxidation products.[4] Similar reduction and hydrolysis of the mixture of aldobiouronic acids gave galactose, rhamnose, and 4-*O*-methylglucose. The aldobiouronic acids were converted into a mixture of methylated neutral disaccharides by reduction of the acidic residues in the methylated aldobiouronic acids with lithium aluminium hydride followed by further methylation of the reduction products. Hydrolysis of the methylated disaccharides gave 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4,6-tetra- and 2,3,6-tri-*O*-methyl-D-galactose, and 3,4-di-*O*-methyl-L-rhamnose, identified by the formation of crystalline derivatives. A small amount of 2,3,4-tri-*O*-methylgalactose, which was also formed, probably arose from incomplete methylation (after reduction) of galacturonic acid end groups. These results established the mode of linkage of the sugar residues in the aldobiouronic acids, and subsequent examination of the methylated acidic

* We are grateful to Dr. A. Nicolson for performing these experiments.

[2] Banks, Greenwood, and Stephen, unpublished results.
[3] Lewis and Smith, *J. Amer. Chem. Soc.*, 1957, **79**, 3929.
[4] Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

oligosaccharides isolated on hydrolysis of the methylated polysaccharide provided evidence for the linking of 4-*O*-methylglucuronic acid and galactose, and of galacturonic acid and rhamnose residues. It follows that the two aldobiouronic acids were (4-D-galactose 4-*O*-methyl-D-glucopyranosid)uronic acid and (2-L-rhamnose D-galactopyranosid)uronic acid, which pair of acidic disaccharides was previously isolated from *Khaya grandifolia* gum.[1]

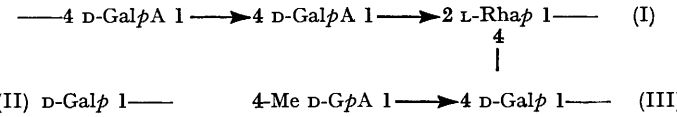
The polysaccharide was converted into its fully methylated derivative. From a preliminary chromatographic examination of the hydrolysis of the methylated polysaccharide, the methylated polysaccharide after reduction with lithium aluminium hydride, and the reduced methylated polysaccharide after remethylation, evidence was obtained for the presence in the methylated polysaccharide of residues of tetra- and tri-*O*-methylgalactose, mono-*O*-methylrhamnose, tri-*O*-methylglucuronic acid, and di-*O*-methylgalacturonic acid. A large-scale hydrolysis of the methylated polysaccharide afforded a complex mixture of neutral methylated sugars and methylated acidic oligosaccharides. By partition chromatography on cellulose the neutral methylated sugars were resolved and fractions containing methylated acids were also obtained. The following methylated sugars were identified by the formation of crystalline derivatives: 2,3,4,6-tetra-, 2,3,4- and 2,3,6-tri-, and 2,4-di-*O*-methyl-D-galactose, and 2,3,4-tri-, 3,4-di-, and 3-*O*-methyl-L-rhamnose. In addition, the following sugars, present in only small amount, were identified by optical rotation, by chromatography and paper ionophoresis of the sugars, and by chromatography of the products of periodate oxidation and of demethylation: 2,3,5-tri-*O*-methyl-L-arabinose, 2,6- and 2,3-di-*O*-methyl-D-galactose, and L-rhamnose. It is possible that some fractions from which 2,3,6-tri-*O*-methyl-D-galactose was characterised also contained another sugar (possibly an isomeric trimethyl ether) which was not identified (see pp. 4925, 4926).

Six fractions containing methylated acidic oligosaccharides were obtained, and small amounts of each of these were converted into methyl ester methyl glycosides and reduced with lithium aluminium hydride and hydrolysed, and the resulting mixtures of methylated sugars were examined by chromatography. Three of the acidic fractions were sufficiently homogeneous for certain broad conclusions to be drawn from the results of these experiments, taken in conjunction with the subsequent identification of the constituent methylated sugars. Fraction (iii), when treated in this way, gave approximately equimolecular proportions of 2,3,4-tri-*O*-methylglucose and 2,3,6-tri-*O*-methylgalactose, indicating that the fraction contained the fully etherified aldobiouronic acid (2,3,6-tri-*O*-methyl-4-D-galactopyranose 2,3,4-tri-*O*-methyl-D-glucopyranosid)uronic acid. Fraction (v), which constituted the major part of the acidic material, furnished 2,3-di-*O*-methylgalactose and 3-*O*-methylrhamnose; it follows that the main chain of the polysaccharide contains 1,4-linked D-galacturonic acid residues and 1,2-linked L-rhamnopyranose residues carrying side-chains through position 4. Fraction (vi), when treated in the same way, gave only 2,3-di-*O*-methylgalactose, this sugar presumably arising from adjacent 1,4-linked D-galacturonic acid residues present in the polymer.

The identity of the methylated hexuronic acid residues in the methylated gum and of neutral methylated sugar residues attached thereto was established by reducing the combined methylated acids (as methyl ester methyl glycosides) with lithium aluminium hydride and hydrolysing the resulting neutral oligosaccharides. The mixture of methylated sugars thus formed was partitioned on cellulose, and the following sugars were characterised by the formation of crystalline derivatives: 2,3,4-tri- and 3,4-di-*O*-methyl-D-glucose, 2,3,4- and 2,3,6-tri- and 2,3- and 2,4-di-*O*-methyl-D-galactose, and 3-*O*-methyl-L-rhamnose. In addition, there were present in small amount 2,3,4-tri-*O*-methylrhamnose, rhamnose, and some unidentified methyl ethers of galactose. Since 2,3,4-tri- and 3,4-di-*O*-methyl-D-glucose were not present, and 2,3-di-*O*-methyl-D-galactose was present only in traces amongst the neutral sugars formed on direct hydrolysis of the methylated polysaccharide, it is clear that these sugars were formed by reduction of the corresponding hexuronic acids.

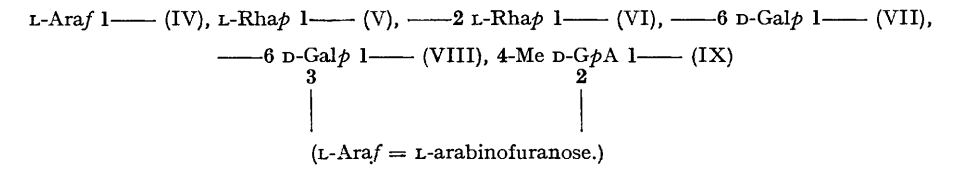
2,3,4-Tri-*O*-methyl-D-galactose could have arisen from either galactose or galacturonic acid residues in the polysaccharide, but since this sugar is also formed on direct hydrolysis of the methylated polysaccharide and hence from galactose residues, it is probable that some if not all of this sugar arises similarly from 1,6-linked galactose units.

The results of the methylation analysis cannot be interpreted unambiguously, but it is clear from the quantities of sugars isolated that the following residues are of major structural significance: 2,3,4,6-tetra- and 2,3,6-tri-*O*-methyl-D-galactose, 3-*O*-methyl-L-rhamnose, 2,3,4-tri-*O*-methyl-D-glucuronic acid, and 2,3-di-*O*-methyl-D-galacturonic acid. From this information and from a knowledge of the order of linkage of sugar residues in the aldobiouronic acid units, the major part of the polysaccharide structure may be represented as a main chain (I) of 1,4-linked D-galacturonic acid and 1,2-linked L-rhamnopyranose residues, side-chains being attached thereto through position 4 of the rhamnose residues. It is possible that some 1,4-linked D-galactopyranose residues may be present in the main chain as well as in the side-chains. The polysaccharide contains end groups of D-galactopyranose (II) and 4-*O*-methyl-D-glucuronic acid residues, the latter being linked to position 4 of D-galactose in aldobiouronic acid units (III). The units (II) and (III) are therefore present as side-chains in the polysaccharide and it is probable that they are directly attached to position 4 of L-rhamnopyranose residues in the main chain.



(D-Gal*p* = D-galactopyranose, L-Rha*p* = L-rhamnopyranose, D-Gal*p*A = D-galacturonic acid, and 4-Me D-G*p*A = 4-*O*-methyl-D-glucuronic acid.)

The structural significance of the other methylated sugars formed on hydrolysis of the methylated polysaccharide cannot yet be fully assessed, but it is clear that the polysaccharide sample under examination contains the following sugar residues (IV—IX) in addition to the main structural units (I—III):



(L-Ara*f* = L-arabinofuranose.)

These residues may arise either from units attached in some way to the main backbone (I) of the polysaccharide or from the presence in this material of a small proportion of the minor polysaccharide component of the gum. The second alternative is supported by the demonstration on glass-fibre paper ionophoresis that the major polysaccharide fraction contained some of the second polysaccharide component. Experiments now in progress on the minor component of the gum may throw further light on this problem.

Our present conclusions show that there is present in *Khaya senegalensis* gum a major polysaccharide component whose main structural features are similar to those of the gum from the related species, *K. grandifolia*,[1] and that again two different hexuronic acids are constituents of a single polysaccharide. Although differing in detailed molecular structure, the two gums contain some of the same structural features as *Sterculia setigera* [5] and *Cochlospermum gossypium* [6] gums, which also contain D-galactose, L-rhamnose, and D-galacturonic acid as the main sugar constituents. A further similarity between the *Khaya* gums and *Cochlospermum gossypium* gum has been emphasised by the demonstration

[5] Hirst, Hough, and Jones, *J.*, 1949, 3145; Hough and Jones, *J.*, 1950, 1199.
[6] Hirst and Dunstan, *J.*, 1953, 2332.

that the latter gum contains end groups of D-glucuronic acid,[7] although these residues are not present as the 4-methyl ether.

Experimental

Paper chromatography was carried out on Whatman Nos. 1, 4, and 20 papers with the following solvent systems (v/v): (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) butan-1-ol–acetic acid–water (8 : 2 : 5, upper layer); (C) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (D) benzene–ethanol–water (169 : 47 : 15, upper layer); (E) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (F) butan-1-ol–pyridine–water (9 : 2 : 2). Unless otherwise stated, chromatography of methylated sugars was carried out in solvent C, and R_G values refer to the rate of movement relative to 2,3,4,6-tetra-*O*-methyl-D-glucose in that solvent. Demethylations of methylated sugars were performed by the method of Hough, Jones, and Wadman.[8] Paper ionophoresis was in borate buffer at pH 10. Sugars were normally revealed on chromatograms by spraying with *p*-anisidine hydrochloride in moist butan-1-ol,[8] a little glacial acetic acid being added before spraying of ionophoretograms. Unless otherwise stated, optical rotations were observed for water solutions at *ca.* 20°.

Small-scale Fractionation of the Gum.—Crushed nodules (15 g.) of gum were stirred with N-sodium hydroxide (200 ml.) overnight, and the resulting solution was poured into ethanol acidified with concentrated hydrochloric acid. The precipitate was separated and stirred with water (50 ml.), the insoluble residue was removed at the centrifuge, and the supernatant solution was poured into ethanol to give fraction A; the insoluble residue dissolved with stirring in a larger volume (100 ml.) of water, and the solution when poured into ethanol afforded fraction B.

Fraction A dissolved in water giving a highly mobile solution, which, after passage through columns of cation- and anion-exchangers, gave, when poured into ethanol, a precipitate which was dissolved in water and freeze-dried to furnish the minor component, $[\alpha]_D$ +48° (Found: equiv., 603; ash, <0·4%). This material was dissolved in water, the solution was stirred with mixed cation- and anion-exchangers, concentrated to 19 ml., and poured into ethanol (60 ml.). The precipitate afforded fraction A′ (major component of fraction A), $[\alpha]_D$ +61° (*c* 0·35) (Found: equiv., 545); the solution afforded fraction A″ (minor component of fraction A), $[\alpha]_D$ +7° (*c* 0·75) (Found: equiv., 850), which gave on hydrolysis galactose, rhamnose, a trace of arabinose, and acidic oligosaccharides.

Fraction B on dispersion in water gave a thick gel, which on treatment with cation-exchanger (causing a rapid lowering of viscosity) and with anion-exchanger, followed by pouring the solution into ethanol, gave a precipitate, which was dissolved in water and freeze-dried to give the major component, $[\alpha]_D$ +143° (Found: equiv., 330; ash, 1·3%). Reprecipitation from aqueous solution with ethanol afforded a precipitate, fraction B′ (major component of fraction B), $[\alpha]_D$ +145° (*c* 0·28) [Found: equiv., 315 (corresponding to a uronic anhydride content of 56%); uronic anhydride, 56% (by decarboxylation)], and a solution from which fraction B″ (minor component of fraction B) was isolated, having $[\alpha]_D$ +129° (*c* 0·32) (Found: equiv., 337).

Large-scale Fractionation of the Gum.—Finely-ground gum (75 g.) was stirred with N-sodium hydroxide (1 l.) for 6 hr., and the resulting solution, after being kept overnight, was poured into ethanol containing concentrated hydrochloric acid (ethanol concentration of resulting solution was 70%). The gel, which separated, was removed at the centrifuge and the supernatant liquid (as also from subsequent reprecipitations) was immediately neutralised by the addition of calcium carbonate. The gel was dispersed in water and reprecipitated five times from aqueous solution by pouring this solution into ethanol (net ethanol concentration was 70% for the first and 60% for subsequent reprecipitations). This procedure furnished polysaccharide I (34 g.), which was used for structural investigations. A sample of polysaccharide I was dissolved in water, the solution was shaken with mixed cation- and anion-exchangers, and the polysaccharide was precipitated, redispersed in water and, freeze-dried [Found (on material dried at 56° *in vacuo*): $[\alpha]_D$ +130° (*c* 0·21); equiv., 321; Found (on material dried at 100° *in vacuo*): $[\alpha]_D$ +140° (*c* 0·21); equiv., 317; OMe, 1·8%; ash, nil].

From the combined supernatant liquors neutralised with calcium carbonate there was isolated (as insoluble calcium salt) polysaccharide II (15 g.), which after reprecipitation had

[7] Aspinall, Hirst, and Johnston, unpublished results.
[8] Hough, Jones, and Wadman, *J.*, 1950, 1702.

$[\alpha]_D$ $\pm 0°$ (*c* 0·91) (Found: equiv., 1040). Evaporation of the supernatant liquid gave polysaccharide III (*ca.* 3 g.), which was subsequently isolated as barium salt, $[\alpha]_D$ $+11°$ (*c* 0·87).

Partial Hydrolysis of the Polysaccharide and Examination of Acidic Fractions.—The polysaccharide (15 g.) was hydrolysed with N-sulphuric acid (400 ml.) at 100° for 6 hr. Part of the polysaccharide remained insoluble, was separated, and was hydrolysed for a further 6 hr. The combined solutions were neutralised with barium hydroxide and barium carbonate, and the solution was filtered, evaporated to small volume, passed through cation-exchanger to remove barium ions, and concentrated to a syrup. Chromatography of the syrup in solvent A showed galactose, rhamnose, a trace of arabinose, and a mixture of acidic sugars. Various unsuccessful attempts were made to fractionate the acidic oligosaccharides by gradient elution from a column of Amberlite resin CG-400 (100—200 mesh) in the acetate form. Neutral sugars were separated by adsorption of acidic sugars on Amberlite resin CG-45 (OH) and elution of the resin with water. Desorption of the acidic sugars from the resin with 15% aqueous formic acid gave a syrupy mixture of acids (2·1 g.). The acids were fractionated on cellulose (60 × 3·5 cm.) with ethyl acetate–acetic acid–water (9 : 2 : 2) as eluent, to give two fractions, which were examined further, and a mixture of higher acidic oligosaccharides.

Fraction 1. The syrup (80 mg.) had $R_{rhamnose}$ 0·95 in solvent B. The acid was converted into methyl ester methyl glycosides, reduced with potassium borohydride, and hydrolysed to give 4-*O*-methylglucose, identified by chromatography of the sugar and of its periodate oxidation products.[4]

Fraction 2. Chromatography of the syrup M (330 mg.) in solvent B suggested the presence of two components, $R_{galactose}$ 0·80—0·88 which were incompletely resolved. Hydrolysis of the syrup and chromatography in solvent A gave rhamnose, galactose, and acidic sugars. Hydrolysis of the syrup after reduction of methyl ester methyl glycosides with potassium borohydride gave rhamnose, galactose, and 4-*O*-methylglucose.

The syrup M (300 mg.) was methylated with methyl sulphate and sodium hydroxide, and the mixture was acidified and extracted with chloroform to give methylated aldobiouronic acids (250 mg.). Lithium aluminium hydride (100 mg.) was added to the methylated aldobiouronic acids (250 mg.) in tetrahydrofuran (40 ml.), the mixture was refluxed for 1 hr., further lithium aluminium hydride (50 mg.) was added, and heating was continued for 1 hr. Excess of hydride was destroyed by the addition of water, the resulting precipitate of metal hydroxides was dried, the filtrate was taken to dryness, and both residues were extracted with chloroform to give syrupy partially methylated disaccharides (183 mg.). This mixture (183 mg.) was further methylated with methyl iodide and silver oxide, and the product (110 mg.) was hydrolysed with N-hydrochloric acid at 100° for 4 hr. The resulting methylated sugars were separated on cellulose (45 × 2·5 cm.), with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3), saturated with water as eluent, into four fractions and a trace of a mono-*O*-methylrhamnose (R_G 0·58) which was not examined further. Fraction *a* (19 mg.), R_G 1·0, crystallised when seeded and had m. p. and mixed m. p. (with 2,3,4,6-tetra-*O*-methyl-D-glucose) 81—82°. Fraction *b* contained three sugars and was separated on filter sheets with solvent D into fractions *b*(i) (25 mg.), *b*(ii) (20 mg.), and *b*(iii) (7 mg.); the last was combined with fraction *c*. Fraction *b*(i), R_G 0·89, was characterised as 2,3,4,6-tetra-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 187—188°. Fraction *b*(ii), R_G 0·86, was chromatographically and ionophoretically indistinguishable from 3,4-di-*O*-methyl-L-rhamnose, and furnished 3,4-di-*O*-methyl-L-rhamnonolactone, m. p. and mixed m. p. 70—72°. Fraction *c* (10 mg.), R_G 0·70, was identified as 2,3,6-tri-*O*-methylgalactose by conversion into 2,3,6-tri-*O*-methyl-D-galactonolactone, R_G 1·0 (single component), and m. p. and mixed m. p. 95—96°. Fraction *d* (3 mg.), R_G 0·65, partially crystalline, was chromatographically identical with 2,3,4-tri-*O*-methyl-D-galactose. Reduction of the sugar and of an authentic sample with potassium borohydride, followed by oxidation with periodate, gave in each case 1 mol. of formaldehyde [9] together with a sugar (presumably 2,3,4-tri-*O*-methyl-L-lyxose) with R_G 0·82.

Methylation of the Polysaccharide.—The polysaccharide (25 g.) was treated with methyl sulphate (400 ml.) and 40% w/v aqueous sodium hydroxide (600 ml.) at 0° under nitrogen. Three further additions of the same quantities of reagents were made at room temperature. The final mixture was warmed to 60° to complete reaction, cooled, and neutralised with sulphuric acid to pH 6. The cooled mixture was filtered from sodium sulphate, and the filtrate

[9] O'Dea and Gibbons, *Biochem. J.*, 1953, **55**, 580.

was dialysed until free from inorganic ions. The dialysed solution was neutralised with silver carbonate, filtered, and freeze-dried to give a partially methylated silver salt (*ca.* 30 g.). A portion (2·1 g.) of the silver salt was heated in a boiling mixture of methanol (30 ml.) and methyl iodide (25 ml.), and silver oxide (5 g.) was added slowly. The chloroform-soluble product (1·92 g.) was methylated with methyl iodide and silver oxide and yielded two fractions (*a*) (0·36 g.; OMe, 38·0%), soluble in light petroleum (b. p. 100—120°)–chloroform (4 : 1), and (*b*), insoluble in the solvent mixture. Fraction (*b*) was shaken with dimethylformamide (10 ml.), methyl iodide (10 ml.), and silver oxide (5 g.) for 2 days and afforded fraction (*c*) (1·1 g.) whose methoxyl content (40·1%) was not increased on further treatment with methyl iodide and silver oxide. Fraction (*a*) was remethylated with methyl iodide and silver oxide, and the product was combined with fraction (*c*) to give methylated gum A (0·75 g.), $[\alpha]_D$ +72° (*c* 1·35 in $CHCl_3$) (Found: OMe, 40·1%).

The major portion (15 g.) of the partially methylated silver salt was treated extensively with methyl iodide and silver oxide to give methylated gum B (7·6 g.) (Found: OMe, 42·0%), $[\alpha]_D$ +64° (*c* 2·3 in $CHCl_3$), which was used in the detailed studies described below.

Reduction of the Methylated Gum.—Methylated gum A (0·58 g.) was dissolved in tetrahydrofuran (10 ml.) and lithium aluminium hydride (0·2 g.) in tetrahydrofuran (10 ml.) was added. After 0·5 hr. at room temperature, the mixture was refluxed for 2 hr. Excess of hydride was destroyed with ethyl acetate, and the resulting mixture was shaken with dilute sulphuric acid and extracted with chloroform. The chloroform extract furnished reduced methylated gum C (0·43 g.), $[\alpha]_D$ +35° (*c* 0·9 in $CHCl_3$) (Found: OMe, 37·0%).

Reduced methylated gum C (0·29 g.) was methylated twice with methyl iodide and silver oxide to give methylated reduced gum D (0·27 g.), $[\alpha]_D$ +37° (*c* 2·7 in $CHCl_3$).

Small-scale Hydrolyses of Methylated Gum Samples.—Samples of the methylated gum acid A, and of the reduced methylated gums C and D were each hydrolysed by heating with formic acid at 100° for 4 hr., and after removal of formic acid, with N-sulphuric acid at 100° for 16 hr. The hydrolysates were neutralised with barium carbonate, and the filtrates were examined chromatographically in solvent C. The main hydrolysis products detected in this way are indicated in the Table, together with relative intensities.

		Methylated gums		
Sugar	R_G	*A*	*C*	*D*
Tetra-*O*-methylglucose	1·00	—	—	+
Tetra-*O*-methylgalactose	0·86	+++	+++	+++
Tri-*O*-methylglucose	0·81	—	+	—
Tri-*O*-methylgalactose	0·68	++	++	+++
Mono-*O*-methylrhamnose	0·58	++	++	++
Di-*O*-methylgalactose	0·49	+	+++	++
Acidic sugars	0·00—0·21	+++	—	—

Hydrolysis of Methylated Polysaccharide and Identification of the Neutral Sugars.—Methylated gum B (6·5 g.) was heated at 100° for 1 hr. in formic acid (60 ml.) and water (10 ml.), and, after removal of formic acid at 95—98°, for 14 hr. with N-sulphuric acid (200 ml.). The cooled solution was neutralised with barium carbonate, filtered, and concentrated to a thin syrup which was placed on a cellulose column (120 × 3·8 cm.). The column was eluted with light petroleum (b. p. 100—120°)–butan-1-ol (2 : 1), saturated with water, and with butan-1-ol saturated with water, to give fractions 1—12 containing neutral sugars. Some fractions, which were contaminated with acidic sugars, were purified by extraction with boiling ether, leaving residual barium salts which were combined to give acidic fraction (i). Subsequent elution of the column with ethanol–water and with water gave acidic fractions (ii)—(vi)

Fraction 1. The chromatographically pure syrup (50 mg.), R_G 1·0, $[\alpha]_D$ +21° (*c* 1·3), was identified as 2,3,4-tri-*O*-methyl-L-rhamnose by conversion into the aniline derivative, m. p. and mixed m. p. 115—116°.

Fraction 2. The syrup (60 mg.) had $[\alpha]_D$ −45° (*c* 1·6) and was chromatographically indistinguishable from 2,3,5-tri-*O*-methyl-L-arabinose, R_G 0·98 and 1·02 in solvents C and D. Demethylation gave arabinose.

Fraction 3. Chromatography of the syrup (80 mg.) showed two components, R_G 0·98 and 0·89. The optical rotation, $[\alpha]_D$ +35°, corresponded to that of a mixture of 2,3,5-tri-*O*-methyl-L-arabinose (35 mg.) and 2,3,4,6-tetra-*O*-methyl-D-galactose (45 mg.).

Fraction 4. The syrup (0·75 g.), R_G 0·89, $[\alpha]_D$ +95° (*c* 2·96), crystallised when seeded with 2,3,4,6-tetra-*O*-methyl-D-galactose and had m. p. 67—69°. The derived N-phenylglycosylamine had m. p. and mixed m. p. 191—192°, and $[\alpha]_D$ −88° ⟶ +40° (*c* 0·5 in Me_2CO containing one drop of pyridine) (Found: OMe, 40·2. Calc. for $C_{16}H_{25}O_5N$: OMe, 39·8%).

Fraction 5. Chromatography of the syrup (46 mg.), $[\alpha]_D$ +31° (*c* 1·1), in solvent D showed 2,3,4,6-tetra-*O*-methylgalactose (R_G 0·87) and a sugar (R_G 0·25) which gave a yellow-ochre stain with *p*-anisidine typical of rhamnose derivatives. The latter component (23 mg.), $[\alpha]_D$ +15° (*c* 1·1), was separated on filter sheets by using solvent D. The sugar which was chromatographically and ionophoretically indistinguishable from 3,4-di-*O*-methyl-L-rhamnose, crystallised and gave an *X*-ray photograph identical with that of an authentic sample.

Fraction 6. Chromatography of the syrup (0·59 g.), $[\alpha]_D$ +89° (*c* 3·5), in solvents C, D, and E (R_G 0·72, 0·71, and 0·23) showed only one component. Demethylation gave galactose only. Periodate oxidation [10] gave a negligible amount of formaldehyde (as dimedone derivative). Periodate oxidation [4] of the derived galactitol (from reduction with sodium borohydride) gave a main component (probably 2,3-di-*O*-methyl-L-threose), R_G 0·92, 0·93, and 0·90 in solvents C, F, and D (ochre stain) and a minor component, R_G 0·65, in solvent E, detected with ammoniacal silver nitrate. The main component was identified as 2,3,6-tri-*O*-methyl-D-galactose by oxidation to 2,3,6-tri-*O*-methyl-D-galactonolactone, m. p. and mixed m. p. 99—100° (Found: OMe, 41·4. Calc. for $C_9H_{16}O_6$: OMe, 41·3%). Considerable difficulty was experienced in freeing the crystalline lactone (R_G 1·0 in solvent C; violet stain with hydroxylamine–ferric chloride spray [11]) from an accompanying viscous oil which contained two lactones (R_G 1·0 and 0·80).

Fraction 7. The chromatographically pure syrup (0·20 g.), R_G 0·68, $[\alpha]_D$ *ca.* +100° (*c* 2·92), was identified as 2,3,4-tri-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 162—163° [the m. p. was depressed to 151—153·5° on admixture with the aniline derivative of 2,4,6-tri-*O*-methyl-D-galactose (m. p. 172°)] (Found: C, 60·1; H, 7·9; N, 4·9. Calc. for $C_{15}H_{23}NO_5$: C, 60·6; H, 7·8; N, 4·7%).

Fraction 8. The syrup (0·30 g.), R_G 0·55, crystallised and after recrystallisation from methanol–ether had m. p. and mixed m. p. (with 3-*O*-methyl-L-rhamnose) 113—114° and $[\alpha]_D$ +35° (equil.) (*c* 2·0) (Found: OMe, 17·2. Calc. for $C_7H_{14}O_5$: OMe, 17·4%). Oxidation of the sugar with periodate gave acetaldehyde (dimedone derivative, m. p. and mixed m. p. 137°) in low yield. The derived syrupy mixture of methyl pyranosides (prepared by refluxing the sugar with dry methanolic hydrogen chloride) did not reduce periodate.

Fraction 9. The syrup (70 mg.), $[\alpha]_D$ +65° (*c* 0·9), was chromatographically indistinguishable from 2,6-di-*O*-methyl-D-galactose (R_G 0·50). Demethylation gave galactose. Chromatography of the periodate oxidation products [4] showed methoxymalondialdehyde (from 2-*O*-methylaldose) and 3-*O*-methylglyceraldehyde (from 6-*O*-methylaldohexose). An ethyl acetate solution deposited crystals with m. p. 130—131° (cf. 2,6-di-*O*-methyl-β-D-galactose, m. p. 128—130° [12]) which was lowered to 105—108° after recrystallisation from chloroform–light petroleum (b. p. 80—100°) (possibly due to inadvertent partial hydration).

Fraction 10. Chromatography of the syrup (40 mg.), R_G 0·46, and of its periodate oxidation products indicated the presence of 2,3-di-*O*-methylgalactose; demethylation gave galactose. Periodate oxidation of the syrup [10] gave formaldehyde (as dimedone derivative) in good yield.

Fraction 11. Extraction of the crude syrup (0·22 g.), R_G 0·44, $[\alpha]_D$ +80° (*c* 1·0), with ether containing a little methanol gave a product which crystallised from ether–chloroform–methanol as needles, m. p. 100—102°. Demethylation gave galactose. Periodate oxidation [10] gave formaldehyde (as dimedone derivative) in low yield (cf. Bell [13]). The sugar was characterised as 2,4-di-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 207—208° (Found: C, 59·6; H, 7·4; N, 4·8. Calc. for $C_{14}H_{21}NO_5$: C, 59·4; H, 7·5; N, 4·9%).

Fraction 12. The syrup (50 mg.), $[\alpha]_D$ +20° (*c* 1·3), was a mixture of 2,4-di-*O*-methylgalactose (R_G 0·44) and a sugar chromatographically and ionophoretically indistinguishable from L-rhamnose. The latter sugar was separated on filter sheets and on periodate oxidation [10] afforded acetaldehyde (as dimedone derivative).

[10] Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476.
[11] Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859.
[12] Oldham and Bell, *J. Amer. Chem. Soc.*, 1938, **60**, 323.
[13] Bell, *J.*, 1945, 692.

Examination of Acidic Fractions.—Fractions (i)—(vi), isolated as barium salts, were converted into free acids by treatment of their solutions with cation-exchanger. Each fraction was converted into methyl ester methyl glycosides with boiling methanolic 1·6N-hydrogen chloride, the ester glycosides were reduced with lithium aluminium hydride, and the reduction products were hydrolysed with N-sulphuric acid at 100° to syrupy mixtures of sugars which were examined chromatographically.

Fraction (i). This comprised the acid residues which had percolated through the cellulose at earlier stages of the elution; neutral sugars were removed from barium salts by extraction with boiling ether. The barium salts (0·18 g.) had $[\alpha]_D$ +46° (*c* 1·8) and the main acid component had R_G 0·15 and 0·95 in solvents C and E. Reduction, etc., gave 2,3,4-tri-*O*-methylglucose (R_G 0·88) and 2,3,6-tri-*O*-methylgalactose (R_G 0·69) together with small amounts of 3-*O*-methylrhamnose (R_G 0·57) and 2,3-di-*O*-methylgalactose (R_G 0·45).

Fraction (ii). The acids (80 mg. as barium salts) were separated from neutral sugars on filter sheets by using solvent E, and had R_G 0·20 and 0·95 in solvents C and E. Reduction, etc., gave 2,3,4-tri-*O*-methylglucose and 2,3,6-tri-*O*-methylgalactose. The neutral sugar fractions (120 mg.) were heavily contaminated with non-carbohydrate material, but contained 2,4-di-*O*- and 2-*O*-methylgalactose (R_G 0·43 and 0·29), recognised by chromatography of the sugars and of the products of demethylation and of periodate oxidation.[4]

Fraction (iii). The barium salt (0·60 g.) had $[\alpha]_D$ +84° (*c* 3·23) and the free acid had R_G 0·16 and 0·94 (cherry-red stain) in solvents C and E. Reduction, etc., gave 2,3,4-tri-*O*-methylglucose and 2,3,6-tri-*O*-methylgalactose.

Fraction (iv). The barium salts (0·85 g.) afforded free acids which had R_G 0·0—0·15 and 0·65—0·95 in solvents C and E.

Fraction (v). This constituted the major part of the acidic material and was recovered as hygroscopic barium salt (3·06 g.), $[\alpha]_D$ +75° (*c* 0·83); the free acid had R_G 0·0 and 0·65 in solvents C and E. Reduction, etc., furnished 2,3-di-*O*-methylgalactose (R_G 0·48) and 3-*O*-methylrhamnose (R_G 0·57) (the former predominating) together with a trace of 2,3,6-tri-*O*-methylgalactose.

Fraction (vi). The crude residue was extracted with methanol–water and the extract (R_G 0·0 in solvent C) was converted into methyl ester methyl glycosides (0·7 g.), which on reduction, etc., gave a syrup (0·4 g.) containing 2,3-di-*O*-methylgalactose (R_G 0·48) and only traces of other sugars.

Identification of Neutral Sugars from Reduction of Acidic Fractions.—The methylated sugars from reduction, etc., of the acidic fractions (i)—(vi) were combined (3·07 g.) and separated on cellulose as described earlier to give twelve fractions.

Fraction A. Although the first litre of eluate contained no sugars which could be detected on chromatograms (*p*-anisidine and silver nitrate sprays), evaporation gave a mobile oil (0·35 g.), $[\alpha]_D$ +13° (*c* 1·5), which was freely soluble in water and ether. Hydrolysis of a portion (50 mg.) with N-hydrochloric acid at 100° for 6 hr., followed by chromatography showed 2,3,4-tri-*O*-methylglucose (R_G 0·88) and small amounts of other sugars, including 2,3-di-*O*-methylgalactose (R_G 0·48).

Fraction B. The syrupy sugar (20 mg.) was chromatographically indistinguishable from 2,3,4-tri-*O*-methyl-L-rhamnose (fraction 1; R_G 1·0).

Fraction C. The sugar (0·31 g.) had $[\alpha]_D$ +74° (*c* 1·23) and R_G 0·87 and 0·20 in solvents C and D. Demethylation gave glucose, and further methylation with methyl iodide and silver oxide followed by hydrolysis gave 2,3,4,6-tetra-*O*-methylglucose. Reduction with sodium borohydride followed by periodate oxidation [4] gave a product with R_G 0·92 and 0·86 in solvents C and D (pink stain, presumably 2,3,4-tri-*O*-methyl-L-xylose). Periodate oxidation [10] of the sugar gave a low yield of formaldehyde (as dimedone derivative) (cf. Bell [14]). The sugar was characterised as 2,3,4-tri-*O*-methyl-D-glucose by conversion into the aniline derivative, m. p. 143—144° and mixed m. p. (with sample of m. p. 137—139°) 139—141°.

Fraction D. The chromatographically pure syrup (0·25 g.) had R_G 0·72 and $[\alpha]_D$ +79° (*c* 2·3). Demethylation gave galactose. Oxidation of the sugar with bromine water gave an aldonolactone which crystallised with difficulty after distillation under reduced pressure; recrystallisation from ether–light petroleum (b. p. 40—60°) gave 2,3,6-tri-*O*-methyl-D-galactonolactone, m. p. 97—98° and mixed m. p. 98—99°. A second crop of crystals had m. p. 70–75°.

[14] Bell, *J.*, 1948, 992.

The pure lactone had R_G 1·0, but the second crop contained two components, R_G 1·0 and 0·80 (cf. fraction 6).

Fraction E. The sugar (70 mg.), $[\alpha]_D$ +88° (*c* 1·3), R_G 0·68, was characterised as 2,3,4-tri-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. 164—165° and mixed m. p. (with sample, m. p. 162—163°) 162—164°.

Fraction F. The syrup (50 mg.), R_G 0·63, contained two components, R_G 0·20 and 0·05 in solvent D. The slower-moving sugar resembled the major component of fraction G and was examined with it; the faster-moving sugar (20 mg.) had $[\alpha]_D$ *ca.* +25° (*c* 0·4) and $[\alpha]_D$ 0° (*c* 0·4 in MeOH). Demethylation gave galactose. Oxidation with periodate [4] gave a sugar with R_G 1·0 and 1·15 in solvents C and D (grey stain).

Fraction G. The syrup (0·12 g.), R_G 0·62, was partly crystalline and contained two components, R_G 0·15 and 0·07 in solvent D. Crystals were separated from syrup (porous tile) and after recrystallisation from ethyl acetate containing a little light petroleum (b. p. 80—100°) gave prisms (18 mg.), m. p. 122—123° (cf. 3,4-di-*O*-methyl-D-glucose,[15] m. p. 110—113°). The mother-liquors were separated on filter sheets (solvent D); the major component (65 mg.), $[\alpha]_D$ +60° (*c* 1·2), R_G 0·07 in solvent D, crystallised. Demethylation gave glucose. Periodate oxidation [4] gave a sugar chromatographically identical with 2,3-di-*O*-methyl-L-arabinose, which was similarly formed from 3,4-di-*O*-methyl-D-mannose. The aniline derivative had m. p. 176° (Bell and Greville [15] give m. p. 177—178° for *N*-phenyl-3,4-di-*O*-methyl-D-glucosylamine).

Fraction H. The sugar (0·22 g.), R_G 0·57, after recrystallisation from methanol–water had m. p. 119° and mixed m. p. (with 3-*O*-methyl-L-rhamnose) 116—117°.

Fraction I. The syrup (15 mg.) was identified as 2,6-di-*O*-methylgalactose by chromatography of the sugar and of its periodate oxidation products.[4]

Fraction J. The sugar (0·56 g.), R_G 0·48, $[\alpha]_D$ +98° (*c* 1·0), constituted the major fraction of the hydrolysate. Demethylation gave galactose. The sugar was identified as 2,3-di-*O*-methyl-D-galactose by conversion into 2,3-di-*O*-methyl-D-galactonamide, m. p. and mixed m. p. 140° (Found: C, 42·9; H, 7·6; N, 6·3; OMe, 28·2. Calc. for $C_8H_{17}NO_6$: C, 43·0; H, 7·7; N, 6·3; OMe, 27·8%).

Fraction K. The sugar (20 mg.) was characterised as 2,4-di-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. 203—204° and mixed m. p. (with sample, m. p. 213—214°) 208—210°.

Fraction L. The syrupy mixture (30 mg.) of sugars contained 2,4-di-*O*-methylgalactose and rhamnose, R_G 0·44 and 0·30.

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[15] Bell and Greville, *J.*, 1950, 1902.