489. *Plant Gums of the Genus* Khaya. *Part III.*[1] *The Minor Component of* Khaya senegalensis *Gum*

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The minor polysaccharide component of deacetylated *Khaya senegalensis* gum has been isolated in a homogeneous state. Partial acid hydrolysis of this polysaccharide affords three aldobiouronic acids, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose, and 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose. Hydrolysis of the methylated polysaccharide indicates the presence therein of residues of 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4,6-tetra-, 2,3,4-, 2,3,6-, and 2,4,6-tri-, 2,4- and 2,6-di-, and 2-*O*-methyl-D-galactose, and 2,3,4-tri- and 3,4-di-*O*-methyl-D-glucuronic acid. Degraded polysaccharides have been prepared by mild acid hydrolysis and by degradation of the periodate-oxidised polysaccharide, and the cleavage products from these polysaccharides and their methylated derivatives have been examined by chromatographic techniques.

In Part II [1] it was shown that fractionation of deacetylated *Khaya senegalensis* gum afforded two distinct polysaccharide components, and the main structural features of the major component were established. The fractionation of these polysaccharides has now been re-examined in order to obtain the minor component in a homogeneous state, and structural studies have been carried out on this polysaccharide.

A sample of the gum was saponified, and after removal of sodium ions the resulting solution was chromatographed on diethylaminoethylcellulose.[2] In addition to small quantities of monosaccharides, two acidic polysaccharides were recognised. The minor polysaccharide component (polysaccharide B) was eluted first; it had $[\alpha]_D$ +11°, contained *ca.* 21% of uronic anhydride, and gave arabinose, galactose, and acidic sugars on hydrolysis. The major polysaccharide component (polysaccharide A) was eluted later; it had $[\alpha]_D$ +136°, contained *ca.* 55% of uronic anhydride, and gave rhamnose, galactose, and acidic sugars together with a trace of arabinose on hydrolysis. Further fractionation was required, however, to obtain polysaccharide A in a homogeneous state when arabinose was no longer detected as a hydrolysis product. The large-scale preparation of polysaccharide B was carried out by selective precipitation of polysaccharide A from aqueous solution with ethanol followed by precipitation of polysaccharide B as its insoluble calcium salt. The homogeneity of samples of this polysaccharide was checked by chromatography on diethylaminoethylcellulose, although it was observed subsequently that the absence of rhamnose as a hydrolysis product was adequate evidence for the absence of contamination by polysaccharide A.

Partial acid hydrolysis of polysaccharide B gave a mixture of acidic and neutral sugars. The acids, after separation from neutral sugars by adsorption on an anion-exchange resin, were fractionated by chromatography on filter sheets, to give two acidic oligosaccharide fractions. The first fraction was chromatographically homogeneous and furnished glucuronic acid and galactose on hydrolysis; it was characterised as 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose by conversion into the crystalline methyl glycoside methyl ester hexamethyl ether. The second fraction appeared to be chromatographically homogeneous; hydrolysis gave 4-*O*-methylglucuronic acid and galactose, and reduction of the derived methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave 4-*O*-methyl-D-glucose and D-galactose. The presence in this fraction of 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose was shown by the isolation of the above-mentioned fully methylated derivative of 6-*O*-(β-D-glucopyranosyluronic

[1] Part II, G. O. Aspinall, M. J. Johnston, and A. M. Stephen, *J.*, 1960, 4918.
[2] H. Neukom, H. Deuel, W. J. Heri, and W. Kündig, *Helv. Chim. Acta*, 1960, **43**, 67.

acid)-D-galactose. The optical rotation, $[\alpha]_D$ +48°, of this fraction, however, did not correspond to the values reported for this aldobiouronic acid $[\alpha]_D$ −1°, +6° ±4°,[3,4] and the presence in the fraction of an α-linked isomer was suspected. The presence of such an isomeric aldobiouronic acid containing a linkage other than 1 ⟶ 5 or 1 ⟶ 6 was indicated by the detection of formaldehyde as a product of periodate oxidation of the fraction. A further quantity of the second acidic fraction was methylated and gas chromatography of the methanolysis products from the methylated derivatives indicated the presence of methyl glycosides of 2,3,4-tri-*O*-methylgucluronic acid, and 2,3,4- and 2,3,6-tri-*O*-methylgalactose. It may be concluded that 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose was present as a second component of this acidic fraction.

Polysaccharide B was converted into its fully methylated derivative. From a preliminary examination by gas chromatography of the methyl glycosides formed on methanolysis of the methylated polysaccharide and of the methylated polysaccharide after reduction of hexuronic acid to hexose residues with lithium aluminium hydride, evidence was obtained for the presence in the methylated polysaccharide of residues of 2,3,5-tri-O-methylarabinose, 2,3,4,6-tetra-, 2,3,4- and 2,4,6-tri-, and 2,4-di-*O*-methylgalactose, and 2,3,4-tri-*O*-methylglucuronic acid. The mixture of methyl glycosides formed on methanolysis of the methylated polysaccharide was treated with barium hydroxide to saponify esters of acidic components and the resulting acids were adsorbed on an anion-exchange resin. The mixture of neutral methyl glycosides was hydrolysed and the resulting neutral methylated sugars were fractioned by partition chromatography on cellulose, followed, where necessary, by further fractionation on filter sheets or on charcoal–Celite. The following methylated sugars were characterised by the formation of crystalline derivatives: 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4,6-tetra-, 2,3,4-, 2,3,6-, and 2,4,6-tri-, 2,4- and 2,6-di-, and 2-*O*-methyl-D-galactose, and other evidence was obtained for the presence of traces of 2,3,4-tri-, and 2,3- and 2,5-di-*O*-methylarabinose. The methylated acidic sugars were desorbed from the anion-exchange resin, treated with methanolic hydrogen chloride, reduced with lithium aluminium hydride, and hydrolysed to give a further mixture of sugars, which was fractionated by chromatography on charcoal–Celite followed, where necessary, by chromatography on filter sheets. The following sugars were characterised by derivative formation: 2,3,4-tri-*O*-methyl-D-glucose (from reduction of the corresponding hexuronic acid), 2,3,4- and 2,3,6-tri-, and 2,4-di-*O*-methyl-D-galactose. In addition, the following sugars were identified by optical rotation, paper chromatography of the sugars and their derivatives, and gas chromatography of their methyl glycosides: 3,4-di-*O*-methyl-D-glucose, (from reduction of the corresponding hexuronic acid) 2,6-di- and 2-*O*-methyl-D-galactose.

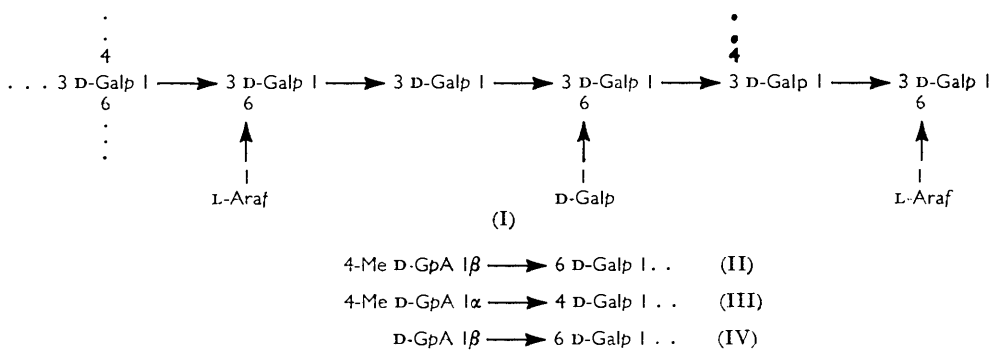
The results of the methylation study indicate that this polysaccharide is highly branched and contains inner chains of D-galactopyranose residues to which are attached a variety of side-chains terminated by residues of L-arabinofuranose, D-galactopyranose, and D-glucuronic acid (in part as the 4-methyl ether). In order to provide further information on the relative disposition of the different types of linkage in the interior chains a degraded galactan was isolated containing those sugar residues which were resistant to cleavage by periodate. Studies on the degradation of periodate-oxidised gum ghatti [5] indicated that the Smith degradation,[6] which involves the sequence of reactions, oxidation with periodate, reduction with borohydride, and mild acid hydrolysis of acyclic acetal linkages, does not necessarily lead to the removal of the fragments arising from the cleavage of hexuronic acid residues. Polysaccharide B was therefore converted into its carboxyl-reduced derivative by treatment of the acetylated polysaccharide with diborane [7] followed

[3] B. O. Lindgren, *Acta Chem. Scand.*, 1957, **11**, 1365.
[4] J. K. N. Jones and J. R. Nunn, *J.*, 1955, 3001.
[5] G. O. Aspinall, V. P. Bhavanandan, and T. B. Christensen, *J.*, 1965, 2677.
[6] I. J. Goldstein, G. W. Hay, F. Smith, and B. A. Lewis, Amer. Chem. Soc. Meeting, Boston, April 1959, Abs. Papers, 3D.
[7] F. Smith and A. M. Stephen, *Tetrahedron Letters*, 1960, No. 7, 17.

by deacetylation. The carboxyl-reduced polysaccharide was submitted to the Smith degradation, and the resulting degraded polysaccharide gave on hydrolysis galactose and a trace of arabinose. The presence of a high proportion of 1,3-linked galactose residues in the degraded polysaccharide was established from the following experiments. Chromatographic examination of the products of partial acid hydrolysis showed the formation of 3-*O*-galactosylgalactose and the polymer-homologous trisaccharide with only small amounts of the 1,6-linked disaccharide, whereas partial hydrolysis of the original polysaccharide gave similar amounts of the two disaccharides. Oxidation of the degraded galactan resulted in the consumption of 0·26 mol. of periodate per sugar residue. Methanolysis of the methylated polysaccharide gave a mixture of methyl glycosides of 2,4,6-tri-*O*-methylgalactose as the major products, together with smaller amounts of methyl glycosides of tetra-, 2,3,4-tri-, and 2,4-di-*O*-methylgalactose, and traces of methyl glycosides of 2,3,5-tri-*O*-methylarabinose. The degraded polysaccharide was submitted to a second Smith degradation and the resulting polysaccharide was shown by a similar series of reactions to approximate yet more closely to a linear 1,3-linked galactan.

The autohydrolysis of polysaccharide B resulted in the release of the majority of the arabinose residues together with a small proportion of galactose residues, and the arabinose-free degraded polysaccharide B contained 28% of uronic anhydride. A sample of the degraded polysaccharide was methylated and the cleavage products of the methylated degraded polysaccharide were compared with those from the undegraded derivative by paper chromatography of the sugars and gas chromatography of their methyl glycosides. The semi-quantitative comparison clearly indicated a decrease in the relative proportion of 2,4-di-*O*-methylgalactose and an increase in that of 2,4,6-tri-*O*-methylgalactose as cleavage products from the degraded polysaccharide. Since the autohydrolysis of polysaccharide does not lead to the release of arabinose residues only, these results do not provide definitive evidence for the site of attachment of these residues. It is probable, however, that the majority of the L-arabinofuranose residues in the polysaccharide are linked to position 6 of 1,3-linked D-galactopyranose residues in the main chain.

The known structural features of the minor polysaccharide component from deacetylated *Khaya senegalensis* gum may be summarised in the partial structures (I)—(IV). The framework of the polysaccharide is a highly branched galactan (I) in which



D-galactopyranose residues in the 1,3-linked main chains carry side-chains of other D-galactopyranose residues joined by 1,6-linkages. The L-arabinose residues in the polysaccharide are mainly present in the furanose form as end-groups, and it is probable that the majority of these are attached, as indicated in partial structure (I), as single-unit side-chains to the main chains. The D-glucuronic acid residues in the polysaccharide, the majority of which are present as the 4-methyl ether, may be accommodated in the three types of aldobiouronic acid unit (II), (III), and (IV). Since most of the D-glucuronic

acid residues occur as end-groups, these units, (II)—(IV), must be present mainly as side-chains, the length of which and the site of whose attachment to the framework (I) of D-galactose residues is not yet known. It may be noted also that a small proportion of the D-glucuronic acid residues are 2-*O*-substituted, and that some of the D-galactose residues in the aldobiouronic acid units (II)—(IV) are present as branching points since further hydrolysis of the acidic fraction from the methylated polysaccharide, which presumably contained methylated aldobiouronic acids, furnished di- and tri-methyl ethers of D-galactose.

The present results show clearly that this polysaccharide from *Khaya senegalensis* gum belongs to an entirely different structural type from that of the major polysaccharide component.[1] In this respect *K. senegalensis* gum resembles gum tragacanth [8,9] which also contains two structurally unrelated polysaccharides. The minor polysaccharide component of *K. senegalensis* resembles most closely asafoetida gum.[10] The two polysaccharides contain similar arrangements of D-galactopyranose residues in the framework of the molecular structure, both contain L-arabinose residues which are mainly present as single-unit furanose end-groups, and both contain D-glucuronic acid end-groups which are present in part as the 4-methyl ether. The polysaccharide from *K. senegalensis* gum differs from asafoetida gum in that D-glucuronic acid residues are attached to D-galactose by 1 ⟶ 4 in addition to 1 ⟶ 6 linkages. The two polysaccharides also resemble the gums from *Acacia senegal* (gum arabic) [8] and *A. pycnantha* [11] in containing highly branched frameworks of D-galactopyranose residues in which the main chains contain 1,3-linkages and carry other D-galactose residues in side-chains containing 1,6-linkages. In the *Acacia* gums, however, the outer chains of L-arabinose residues are more complex in structure and L-rhamnopyranose residues are also present.

Experimental

Paper chromatography was carried out on Whatman Nos. 1, 3MM, and 31ET 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–acetic acid–formic acid–water (18 : 8 : 3 : 9); (D) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (E) butan-1-ol–acetic acid–water (4 : 1 : 5, upper layer); (F) benzene–ethanol–water (169 : 47 : 15, upper layer); (G) butan-2-one, saturated with water containing a trace of ammonia; (H) 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 D. Demethylations of methylated sugars were performed with boron trichloride.[12] Chromatography of the periodate oxidation products of methylated sugars was carried out by Lemieux and Bauer's method.[13] Unless otherwise stated, optical rotations were observed for water solutions at *ca.* 18°.

Gas chromatography of methylated and partially methylated methyl 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.[14]

Fractionation of Deacetylated Khaya senegalensis *Gum on Diethylaminoethylcellulose.*— Powdered gum (1·2 g.) was allowed to swell in water (35 ml.) overnight, sodium hydroxide

[8] F. Smith and R. Montgomery, " Chemistry of Plant Gums and Mucilages," Reinhold, New York, 1959.
[9] G. O. Aspinall and J. Baillie, *J.*, 1963, 1702, 1714.
[10] J. K. N. Jones and G. H. S. Thomas, *Canad. J. Chem.*, 1961, **39**, 192.
[11] G. O. Aspinall, E. L. Hirst, and A. Nicolson, *J.*, 1959, 1697.
[12] T. G. Bonner, E. J. Bourne, and S. McNally, *J.*, 1960, 2929.
[13] R. U. Lemieux and H. F. Bauer, *Canad. J. Chem.*, 1953, **31**, 814.
[14] G. O. Aspinall, *J.*, 1963, 1676.

(1·4 g.) was added, and the solution was stirred for 8 hr. The resulting solution was filtered through muslin and stirred with Amberlite resin IR-120(H) to pH 6. The sugar concentration of the solution was determined by the phenol–sulphuric acid reagent [15] (26 mg. per ml., estimated as galactose) and a portion (30 ml.) of the solution was poured on to a column of diethylaminoethylcellulose (phosphate form) as described by Neukom *et al.*[2] The column was eluted with 0·025M- (500 ml.), 0·05M- (500 ml.), 0·1M- (500 ml.), and 0·25M-sodium dihydrogen phosphate (pH 6) (500 ml.), and a gradient of sodium hydroxide (0—0·3M; 2 l.). Fractions (*ca.* 10 ml.) were collected and analysed for sugars by the phenol–sulphuric acid method [15] and for uronic anhydride by the carbazole method.[16] The first fraction which was eluted at low phosphate concentration contained sugars (40 mg.) which were absorbed on " Ultrasorb " charcoal [17] to remove salts and then desorbed with water containing 25% of ethanol. Chromatography showed arabinose to be the main constituent together with traces of galactose and unidentified oligosaccharides. The second and third fractions, which were eluted with 0·1M- and 0·25M-phosphate, contained polysaccharide (40 and 80 mg.). The polysaccharide samples were isolated after dialysis, de-ionisation with cation- and anion-exchange resins, and freeze-drying. Both samples had $[\alpha]_D$ +11° (*c* 1·0) and uronic anhydride, 21·5%, and furnished on hydrolysis galactose (42%), arabinose (20%), and acidic sugars, but no rhamnose. The fourth fraction, which was eluted with sodium hydroxide, afforded polysaccharide (400 mg.), which had $[\alpha]_D$ +136° (*c* 0·54) and uronic anhydride, 55% and furnished on hydrolysis rhamnose, galactose, galacturonic acid, 4-*O*-methylglucuronic acid, and a trace of arabinose.

Isolation of Polysaccharide B.—Powdered gum (100 g.) was swollen in water (1·35 l.) overnight, sodium hydroxide (53 g.) was added slowly, and the mixture was stirred for 7 hr. The solution was filtered through muslin and the filtrate was poured into ethanol (4·24 l.) containing concentrated hydrochloric acid (200 ml.). The precipitated polysaccharide A was removed by centrifugation, and neutralisation of the supernatant liquor with calcium carbonate furnished polysaccharide B as its insoluble calcium salt (4 g.). The polysaccharide was regenerated from the calcium salt, and chromatography of a sample on diethylaminoethylcellulose showed the presence of only one polysaccharide component. When further batches of polysaccharide B were prepared the absence of rhamnose from the hydrolysis products provided evidence for the complete removal of polysaccharide A. Examination of samples of the precipitated polysaccharide A by chromatography on diethylaminoethylcellulose revealed the presence of small amounts of polysaccharide B. After two chromatographic separations a homogeneous sample of polysaccharide A was obtained and hydrolysis indicated the absence of arabinose as a constituent sugar.

Partial Hydrolysis of Polysaccharide B and Characterisation of Aldobiouronic Acids.—Polysaccharide B (4 g.) was hydrolysed with N-sulphuric acid (100 ml.) for 4 hr. on a boiling-water bath. The cooled solution was neutralised with barium hydroxide and barium carbonate, barium ions were removed from the filtrate with a cation-exchange resin, and the solution was concentrated and poured on to a column of Amberlite resin CG-45 (formate form) (350 ml.). Elution of the column with water removed neutral sugars (2·3 g.) and elution with a gradient of formic acid removed acidic sugars. The various fractions contained similar mixtures of acidic sugars and from the further fractionation of these mixtures by chromatography on filter sheets using solvent C two fractions containing acidic oligosaccharides were obtained.

Fraction a. The aldobiouronic acid (119 mg.), $R_{\text{galacturonic acid}}$ 0·50 in solvent C, had $[\alpha]_D$ −3° (*c* 2·0) and gave galactose and glucuronic acid on hydrolysis. Reduction of the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave glucose and galactose. The major portion (100 mg.) of the aldobiouronic acid was characterised as 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose by methylation with sodium hydroxide and methyl sulphate, and later with methyl iodide and silver oxide, and conversion into the methyl ester methyl glycoside hexamethyl ether (50 mg.),[18] which was recrystallised from acetone–light petroleum (b. p. 40—60°) and identified by m. p. and mixed m. p. 90°, $[\alpha]_D$ −40° (*c* 0·42 in $CHCl_3$), and *X*-ray powder photograph.

Fraction b. The syrup (533 mg.), $R_{\text{galacturonic acid}}$ 0·63 in solvent C, had $[\alpha]_D$ +48° (*c* 1·0)

[15] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Analyt. Chem.*, 1956, **28**, 350.
[16] E. A. McComb and R. McCready, *Analyt. Chem.*, 1952, **24**, 1630.
[17] R. C. Hughes and W. J. Whelan, *Chem. and Ind.*, 1958, 884.
[18] S. W. Challinor, W. N. Haworth, and E. L. Hirst, *J.*, 1931, 259.

and gave galactose and 4-*O*-methylglucuronic acid on hydrolysis. The syrup (150 mg.) was refluxed with methanolic 3% hydrogen chloride for 4 hr., and the resulting methyl ester methyl glycosides were reduced with sodium borohydride (150 mg.) in water (3 ml.) overnight. Excess of hydride was destroyed, and cations were removed with Amberlite resin IR-120(H), boric acid was removed by evaporation with methanol, and the resulting syrup was hydrolysed with 2N-sulphuric acid for 4 hr. on a boiling-water bath, to give a mixture (100 mg.) of two sugars. The mixture was fractionated on filter sheets using solvent A, to yield (i) 4-*O*-methyl-D-glucose (32 mg.), $[\alpha]_D$ +60° (*c* 0·53), which afforded the phenylosazone, identified by m. p. and mixed m. p. 156°, $[\alpha]_D$ −20° (*c* 0·31 in pyridine), and *X*-ray powder photograph, and (ii) D-galactose (43 mg.), $[\alpha]_D$ +76° (equil.) (*c* 0·4), and m. p. and mixed m. p. 156°. Oxidation of the syrup with sodium metaperiodate solution at pH 8 gave 0·23 mol. of formaldehyde. The syrup (151 mg.) was methylated successively with methyl sulphate and sodium hydroxide, and methyl iodide and silver oxide, and crystallisation of the product from light petroleum (b. p. 40—60°)–acetone furnished the methyl ester methyl glycoside hexamethyl ether of 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose,[18] which was identified by m. p. and mixed m. p. 89°, $[\alpha]_D$ −35° (*c* 1·23 in $CHCl_3$), and *X*-ray powder photograph. A further quantity of fraction *b* was methylated, and a portion of the product was heated with methanolic 2·5% hydrogen chloride in a sealed tube for 2 hr. The resulting methyl glycosides were examined by gas chromatography which indicated the presence of methyl glycosides of 2,3,4-tri-*O*-methylglucuronic acid (as methyl ester), and 2,3,4- and 2,3,6-tri-*O*-methylgalactose. A further portion of the methylated aldobiouronic acids was reduced with lithium aluminium hydride, and the reduction product was heated with methanolic hydrogen chloride. Gas chromatography of the methanolysis products indicated the presence of methyl glycosides of 2,3,4-tri-*O*-methylglucose, and 2,3,4- and 2,3,6-tri-*O*-methylgalactose.

Preparation and Hydrolysis of Methylated Polysaccharide B.—Polysaccharide B (10 g.) was methylated successively with methyl sulphate and sodium hydroxide, and methyl iodide and silver oxide, to give methylated polysaccharide B (3·5 g.), $[\alpha]_D$ −26·5° (*c* 0·49 in $CHCl_3$) (Found: OMe, 41·1%, not raised on further methylation). A portion of the methylated polysaccharide was heated with methanolic hydrogen chloride, and gas chromatography of the methanolysis products indicated the presence of methyl glycosides of 2,3,5-tri-*O*-methylarabinose, 2,3,4-tri-*O*-methylglucuronic acid (as methyl ester), 2,3,4,6-tetra, 2,3,4- and 2,4,6-tri-, and 2,4-di-*O*-methylgalactose, and, in small amount, 2,3,4-tri-, and 2,3- and 2,5-di-*O*-methylarabinose.

Methylated polysaccharide B (2·5 g.) was refluxed in methanolic 3% hydrogen chloride (65 ml.) for 16 hr. The cooled solution was neutralised with silver carbonate, filtered, and concentrated, and the resulting methyl glycosides were heated with saturated aqueous barium hydroxide (32 ml.) at 60° for 2 hr. The cooled solution was passed through a column of Amberlite resin IR-120(H) to remove barium ions, and the acidic methyl glycosides were absorbed on Duolite resin A-4 (OH). The neutral methyl glycosides (1·41 g.), which passed through the column, were hydrolysed with N-hydrochloric acid (30 ml.) on a boiling-water bath for 5 hr., to give a mixture of neutral sugars (1·17 g.). The sugars were separated on cellulose (55 × 3 cm.), (i) light petroleum (b. p. 100—120°)–butan-1-ol (3 : 7), saturated with water, and (ii) butan-1-ol, half saturated with water, being used as eluants to give fractions 1—10. The first fraction (231 mg.) from the original separation contained a mixture of sugars, and fractions 1—4 were obtained after re-fractionation on cellulose (55 × 1·8 cm.) using light petroleum–butan-1-ol (4 : 1), saturated with water as eluant.

The acidic methyl glycosides were displaced from the anion-exchange resin with N-sodium hydroxide, and sodium ions were removed by passage through Amberlite resin IR-120(H). The acidic methyl glycosides (0·777 g.) were hydrolysed with N-hydrochloric acid on a boiling-water bath for 5 hr., the cooled solution was neutralised with silver carbonate, silver ions were removed with Amberlite resin IR-120(H), and the solution was concentrated to a syrup (0·535 g.). Unsuccessful attempts were made to separate the methylated acidic mono- and di-saccharide by chromatography in solvents B, D, and E. The major portion (0·425 g.) of the syrup was refluxed with methanolic 2% hydrogen chloride for 4 hr., and the resulting methyl glycosides were reduced with lithium aluminium hydride (400 mg.) in boiling tetrahydrofuran (40 ml.) for 2 hr. The reduction product (265 mg.) was hydrolysed with N-hydrochloric acid (40 ml.) for 6 hr. on a boiling-water bath, to give a syrupy mixture (237 mg.) of sugars, which was separated on charcoal–Celite (35 × 2·5 cm.) by gradient elution with water containing 0·05—5·0% of butan-2-one (2 l.) to give fractions 11—17.

Characterisation of Sugars from Hydrolysis of Methylated Polysaccharide B.—Fraction 1. The chromatographically homogeneous syrup (115 mg.), $[\alpha]_D$ −40° (*c* 0·35) and R_G 0·98, was indistinguishable from 2,3,5-tri-*O*-methyl-L-arabinose, and gave arabinose only on demethylation. The sugar was characterised by conversion into 2,3,5-tri-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 135°.

Fraction 2. The chromatographically pure sugar (113 mg.), $[\alpha]_D$ +100° (*c* 0·5) and 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. 194—195°.

Fraction 3. Chromatography of the syrup (22 mg.) showed the presence of three discrete bands, R_G 0·89, 0·82, and 0·76. Demethylation gave galactose and arabinose. Gas chromatography of the derived methyl glycosides showed components having the retention times of methyl glycosides of tetra- and 2,3,4-tri-*O*-methylgalactose, and 2,3,4-tri- and 2,5-di-methylarabinose.

Fraction 4. Chromatography of the syrup (206 mg.) showed tri-*O*-methylgalactose (R_G 0·72) and a trace of a di-*O*-methylarabinose. Gas chromatography of the derived methyl glycosides showed components having the retention times of methyl glycosides of 2,3,4-, 2,3,6-, and 2,4,6-tri-*O*-methylgalactose (in approximately equimolecular proportions) and, in trace amount, of 2,3-di-*O*-methylarabinose. The syrup was fractionated on charcoal–Celite (45 × 2 cm.) by gradient elution with water containing 1·5—6·0% of butan-2-one, and, after analysis of sugars in the eluate by gas chromatography of their methyl glycosides, the following sub-fractions were obtained: 2a (51 mg.), 2,3,4-tri-*O*-methylgalactose and a trace of 2,3-di-*O*-methylarabinose; 2b (26 mg.) 2,3,4- and 2,4,6-tri-*O*-methylgalactose; 2c (29 mg.), 2,3,6-tri-*O*-methylgalactose; 2d (40 mg.), 2,4,6- and 2,3,6-tri-*O*-methylgalactose; 2e (30 mg.), 2,3,6-tri-*O*-methylgalactose; 2f (7 mg.), 2,3,6-tri- and 2,3,4,6-tetra-*O*-methylarabinose; 2g (5 mg.), 2,3,4,6-tetra-*O*-methylgalactose. Fraction 2a had $[\alpha]_D$ +134° ⟶ +105° (equil.) (*c* 0·51), and the main component was identified as 2,3,4-tri-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 167—168°. Fraction 2c, $[\alpha]_D$ +91° (*c* 0·29), was recrystallised from acetone–ether–light petroleum (b. p. 40—60°), to give 2,4,6-tri-*O*-methyl-D-galactose, m. p. and mixed m. p. 98—100°. The sugar was further characterised as the aniline derivative, m. p. 163° and mixed m. p. (with sample, m. p. 168°) 167°. Fraction 2c, $[\alpha]_D$ +20° ⟶ +81° (equil.) (*c* 0·3), 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—99°.

Fraction 5. The syrup (54 mg.), R_G 0·71 and $[\alpha]_D$ +109° (*c* 0·46), gave galactose only on demethylation. Gas chromatography of the derived methyl glycosides showed the presence of components having the retention times of methyl glycosides of 2,3,4-tri-*O*-methyl-D-galactose and in trace amount of the 2,4,6-isomer. The main component was characterised by conversion into the aniline derivative, m. p. and mixed m. p. 158°.

Fraction 6. Paper chromatography of the sugar (190 mg.) and gas chromatography of the methyl glycosides showed the presence of 2,4-di-*O*-methylgalactose with traces of the 2,6-dimethyl and 2,3,4-trimethyl ethers. The main component was recrystallised from acetone–water, to give 2,4-di-*O*-methyl-D-galactose monohydrate, m. p. and mixed m. p. 94—95°, and $[\alpha]_D$ +104° ⟶ +91° (equil.) (*c* 1·0), and was further characterised by conversion into the aniline derivative, m. p. and mixed m. p. 210°.

Fraction 7. Chromatography of the sugars (112 mg.), R_G 0·53 and 0·58, and of their periodate oxidation products, and gas chromatography of their methyl glycosides showed the presence of 2,4- and 2,6-di-*O*-methylgalactose. The two sugars were separated on filter sheets in solvent G using multiple development. Fraction 7a (37 mg.) was crystallised to give 2,4-di-*O*-methyl-D-galactose monohydrate, m. p. and mixed m. p. 93—94°, $[\alpha]_D$ +103° ⟶ +87° (equil.) (*c* 0·3), and the sugar was further characterised by conversion into the aniline derivative, m. p. and mixed m. p. 212°. Fraction 7b (41 mg.), R_G 0·58 and $[\alpha]_D$ +40° ⟶ +86° (equil.) (*c* 0·41), was recrystallised from chloroform–light petroleum (b. p. 40—60°) to give 2,6-di-*O*-methyl-D-galactose, m. p. and mixed m. p. 113—115°, which was further characterised by conversion into the aniline derivative, m. p. and mixed m. p. 119°.

Fraction 8. Chromatography of the syrup (135 mg.) showed the presence of three components, R_G 0·30, 0·53, and 0·58, which were separated on filter sheets using solvents D and G. Fraction 8a (25 mg.), R_G 0·30 and $[\alpha]_D$ +82° (*c* 0·25), was chromatographically indistinguishable from 2-*O*-methyl-D-galactose, and gave galactose on demethylation and methoxymalondialdehyde on periodate oxidation.[13] Fraction 8b (60 mg.) was 2,4-di-*O*-methyl-D-galactose, and was

identified as the monohydrate, m. p. and mixed m. p. 96°, $[\alpha]_D$ +104° ⟶ +87° (equil.) (*c* 0·5), and as the aniline derivative, m. p. and mixed m. p. 213°. Fraction 8c (37 mg.) furnished 2,6-di-*O*-methyl-D-galactose, m. p. 114—116° and $[\alpha]_D$ +40° ⟶ +80° (equil.) (*c* 0·25).

Fraction 9. The chromatographically homogeneous sugar (35 mg.), R_G 0·30, was recrystallised from acetone–water to give 2-*O*-methyl-D-galactose, m. p. and mixed m. p. 150°, $[\alpha]_D$ +53° ⟶ +82° (equil.) (*c* 0·2).

Fraction 10. The syrup (29 mg.) contained two components, R_G 0·30 and 0·27, and gave galactose only on demethylation. Chromatography of the periodate oxidation [13] products gave methoxymalondialdehyde (R_G 0·20) and a component (R_G 0·58), which gave a lemon stain with aniline oxalate and was probably a 2-*O*-methyltetrose; these two substances were probably formed from 2- and 4-*O*-methylgalactose, respectively.

Fraction 11. The syrup (26 mg.), which contained three sugars (R_G 0·28, 0·47, and 0·50), gave galactose only on demethylation and was partially fractionated on a filter sheet using solvent D. Fraction 11a (5 mg.) was chromatographically indistinguishable from 2-*O*-methyl-D-galactose and gave methoxymalondialdehyde on periodate oxidation.[13] Fraction 11b (17 mg.) was combined with fraction 12a for further examination.

Fraction 12. The syrup (26 mg.), which contained three sugars (R_G 0·47, 0·50, and 0·65), gave galactose only on demethylation and was partially fractionated on a filter sheet using solvent D. Fraction 12a (7 mg.) in combination with fraction 11b was further fractionated on a filter sheet using solvent G to give (i) 2,4-di-*O*-methyl-D-galactose monohydrate, m. p. and mixed m. p. 93°, $[\alpha]_D$ +100° ⟶ +79° (equil.) (*c* 0·1), and (ii) 2,6-di-*O*-methyl-D-galactose (10 mg.), which was identified by paper chromatography of the sugar (solvents D and G) and of its periodate oxidation products.[13] Fraction 12b (17 mg.), $[\alpha]_D$ +109° (*c* 0·17), was characterised as 2,3,4-tri-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 167°.

Fraction 13. The syrup (13 mg.), $[\alpha]_D$ +102° (*c* 0·13), contained two components with the chromatographic mobilities of 3,4-di-*O*-methyl-D-glucose (R_G 0·54) and 2,3,4-tri-*O*-methyl-D-galactose (R_G 0·65). Gas chromatography of the methyl glycosides showed the presence of components having the retention times of methyl glycosides of these two sugars. Demethylation gave glucose and galactose. The presence of 3,4-di-*O*-methylglucose was further indicated by a colour reaction with triphenyltetrazolium hydroxide [19] (hydroxyl group at C-2) and by the formation of a sugar with the chromatographic mobility of 2,3-di-*O*-methylarabinose on oxidation with periodate.[13]

Fraction 14. The syrup (26 mg.), which contained two sugars (R_G 0·54 and 0·67), gave glucose and galactose on demethylation and was separated on a filter sheet using multiple development with solvent G. Fraction 14a (10 mg.), R_G 0·54 and $[\alpha]_D$ +91° (*c* 0·1), was chromatographically indistinguishable from 3,4-di-*O*-methyl-D-glucose and its methyl glycoside (only one component was detected) had the retention time of the methyl glycoside of this sugar. The sugar, which stained red with triphenyltetrazolium hydroxide, gave glucose on demethylation. Fraction 14b (12 mg.), R_G 0·67, was combined with fraction 15 for characterisation.

Fraction 15. Chromatography of the sugar (25 mg.), which was combined with fraction 14b and gas chromatography of the methyl glycosides indicated the presence of 2,3,6-tri-*O*-methylgalactose. The identity of the sugar was confirmed by conversion into 2,3,6-tri-*O*-methyl-D-galactonolactone, m. p. and mixed m. p. 97—98°.

Fraction 16. Gas chromatography of the methyl glycosides of the sugar (3 mg.) showed components having the retention times of methyl glycosides of 2,3,6-tri-*O*-methylgalactose and 2,3,4-tri-*O*-methylglucose.

Fraction 17. The sugar (89 mg.), R_G 0·84 and $[\alpha]_D$ +75° (*c* 0·89), was characterised as 2,3,4-tri-*O*-methyl-D-glucose by conversion into the aniline derivative, m. p. and mixed m. p. 131°.

Degraded Polysaccharide B.—Polysaccharide B (2·3 g.) in water (115 ml.) was heated on a boiling-water bath for 42 hr. $[\alpha]_D$ +8° ⟶ +32° (const.), the cooled solution was poured into ethanol (600 ml.), and degraded polysaccharide B (1·12 g.), $[\alpha]_D$ 0° (*c* 0·5) and uronic anhydride 28%, was isolated after two re-precipitations from aqueous solution with ethanol. The supernatant liquors were concentrated to a syrup (660 mg.), which was shown by quantitative paper chromatography to contain arabinose and galactose in the molar ratio of 4 : 1.

[19] D. S. Feingold, G. Avigad, and S. Hestrin, *Biochem. J.*, 1956, **64**, 351.

Hydrolysis of degraded polysaccharide B gave galactose and acidic sugars with only a trace of arabinose. Methylated degraded polysaccharide B, $[\alpha]_D$ −18° (*c* 0·5 in $CHCl_3$) (Found: OMe, 43·0%), was prepared by treatment of the parent polysaccharide with methyl sulphate and sodium hydroxide, and methyl iodide and silver oxide. Reduced methylated degraded polysaccharide B, $[\alpha]_D$ −16° (*c* 0·97 in $CHCl_3$) (Found: OMe, 41·2%), was prepared by treatment of the above derivative with lithium aluminium hydride in tetrahydrofuran.

Degraded Galactans B′ and B″.—Polysaccharide B was acetylated in formamide solution with acetic anhydride and pyridine by Carson and Maclay's method,[20] to give acetylated polysaccharide, $[\alpha]_D$ −10° (*c* 1·0 in $CHCl_3$) (Found: CH_3CO, 36·7%). Sodium borohydride (1 g.) was added to the acetylated polysaccharide (5 g.) in 1,2-dimethoxyethane (100 ml.), and diborane was generated *in situ* by the portionwise addition of boron trifluoride diethyl etherate (10 g.) in 1,2-dimethoxyethane (40 ml.) during 1·5 hr. After each addition the stoppered flask was shaken vigorously to break up the gel which separated. The mixture was set aside overnight and was then poured into ice–water (500 ml.). The mixture was made just alkaline and was concentrated under pressure to a thick paste. The paste was dissolved in 0·1N-sodium hydroxide, and the solution was adjusted to pH 9 and heated for 2 hr. at 55°. The resulting solution was dialysed, deionised with cation- and anion-exchangers, and freeze-dried to give reduced polysaccharide B (3 g.). Some samples of reduced polysaccharide, which were prepared in this way, were incompletely deacetylated and deacetylation was repeated using 20% (w/v) aqueous ammonia. Hydrolysis of the reduced polysaccharide gave arabinose, galactose, glucose, and 4-*O*-methylglucose, and no acidic sugars could be detected.

Reduced polysaccharide B (3·2 g.) was oxidised with sodium metaperiodate (13·2 g.) in water (500 ml.) for 32 hr. (uptake of reagent was constant and corresponded to the consumption of 0·82 mole of reagent per sugar residue), ethylene glycol (20 ml.) was added to destroy excess of reagent, and the solution was dialysed for 4 days. Potassium borohydride (1·2 g.) was added to the concentrated solution which was set aside overnight. Excess of hydride was destroyed, potassium ions were removed with Amberlite resin IR-120(H), and the solution was concentrated, methanol being added to facilitate the removal of boric acid as methyl borate. The resulting polyalcohol (2·9 g.) was hydrolysed with N-sulphuric acid (66 ml.) at room temperature for 5 hr. The hydrolysate was neutralised with barium hydroxide and barium carbonate, and the filtrate was poured into ethanol (5 vol.) to give degraded galactan B′ (678 mg.), $[\alpha]_D$ +25° (*c* 1·0). The supernatant liquor was concentrated to a syrup (1·73 g.), which contained glycerol and other unidentified alcohols, but no reducing sugars, and which furnished on hydrolysis small amounts of galactose and arabinose, and a trace of 4-*O*-methylglucose, in addition to non-reducing alcohols.

Hydrolysis of degraded galactan B′ gave galactose and a trace of arabinose. Partial acid hydrolysis of degraded galactan B′ afforded 3-*O*-β-galactopyranosylgalactose ($R_{galactose}$ 0·51 in solvent A), the polymer-homologous trisaccharide ($R_{galactose}$ 0·25), and a relatively small amount of 6-*O*-β-galactopyranosylgalactose ($R_{galactose}$ 0·36). In a parallel experiment partial hydrolysis of degraded polysaccharide B furnished approximately equal amounts of the two galactobioses. Oxidation of degraded galactan B′ with sodium metaperiodate resulted in the consumption of 0·26 mole of reagent per sugar residue. Degraded galactan B′ (150 mg.) was methylated by the procedure of Kuhn and Trischmann,[21] to give methylated degraded galactan B′ (85 mg.), $[\alpha]_D$ −10° (*c* 1·0 in $CHCl_3$) (Found: OMe, 44·1%).

Degraded galactan B′ (400 mg.) was oxidised with sodium metaperiodate, reduced with potassium borohydride, and hydrolysed with cold dilute sulphuric acid, as described above, to give degraded galactan B″ (68 mg.), $[\alpha]_D$ +36° (*c* 1·0). Hydrolysis of degraded galactan B″ gave galactose only and partial hydrolysis gave 3-*O*-β-galactopyranosylgalactose with only a trace of the 1,6-linked isomer. Oxidation of degraded galactan B″ with sodium metaperiodate resulted in the consumption of $<0·05$ mole of reagent per sugar residue. Degraded galactan B″ (50 mg.) was converted into its methylated derivative by Kuhn and Trischmann's procedure.[21]

Analysis of Cleavage Products from Methylated Degraded Polysaccharides.—The cleavage products from methylated degraded polysaccharide B and its reduction product, and methylated degraded galactans B′ and B″ were examined by (*a*) paper chromatography of the hydrolysates in solvent D, and (*b*) gas chromatography of the methyl glycosides formed on methanolysis on columns *a* and *b*. The Table indicates the relative proportions of the cleavage products

[20] J. F. Carson and W. D. Maclay, *J. Amer. Chem. Soc.*, 1946, **68**, 1015.
[21] R. Kuhn and H. Trischmann, *Chem. Ber.*, 1963, **95**, 284.

from the various methylated degraded polysaccharides compared with those from methylated polysaccharide B.

Examination of cleavage products from methylated degraded polysaccharides

Sugar	Methylated polysaccharide B	Methylated degraded polysaccharide B	Methylated degraded galactan B′	Methylated degraded galactan B″
2,3,5-Me_3 arabinose	+++	tr.	tr.	
2,3,4,6-Me_4 galactose	+++	+++	+	+
2,3,4-Me_3 galactose	+++	+++	+	
2,3,6-Me_3 galactose	++	++		
2,4,6-Me_3 galactose	++	+++	+++	++++
2,4-Me_2 galactose	++++	+++	+	+
2,6-Me_2 galactose	++	+		
2-Me galactose	++	+		
2,3,4-Me_3 glucuronic acid	+++	+++		
3,4-Me_2 glucuronic acid	+			

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