764. *Structural Studies on the Gum Exudate of* Albizzia zygia (*Macbride*).

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L-Arabinose, D-mannose, D-galactose, L-rhamnose (trace quantity), D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, 3-*O*-β-D-galactopyranosyl-L-arabinose, 3-*O*-β-D-galactopyranosyl-D-galactose, 6-*O*-β-D-galactopyranosyl-D-galactose, 4-*O*-α-(4-*O*-methyl-D-glucopyranuronosyl)-D-galactose, and 2-*O*-β-D-glucopyranuronosyl-D-mannose have been separated and characterised from acid hydrolysates of this gum. From the presence of these constituents and periodate-oxidation studies on the whole gum possible structural features of the gum are discussed.

Albizzia zygia (Macbride), also known as *A. brownei* (Walp.) or West African walnut, is a tree of widespread distribution throughout West and Central Africa. It is particularly abundant in Ghana and Southern Nigeria, where it grows usually 30—40 feet high in the deciduous and secondary forests [1] and it is also grown in plantations to provide timber for building purposes.[2] The genus *Albizzia* containing some twenty-six species is a member of the Mimosaceæ, a family which also includes the gum-bearing genera *Acacia* and *Prosopis*. Only two species of *Albizzia*, *A. zygia* and *A. sassa*, are however, known to produce gum.

The present sample of gum collected by Mr. J. Hotson, of the Soil and Land-use Survey, from the Gold Coast (Ghana), West Africa, was shown to be essentially homogeneous in character by analysis of three separate gum nodules. Each nodule contained the same proportion of acetyl, methoxyl, and ash and each gave on hydrolysis the same mixture of neutral sugars and uronic acids. The presence of acetyl groups is a common feature of unpurified gums, particularly in *Sterculia* spp.[3] and has been reported in the exudates of *Cochlospermum gossypium* [4] and *Fagara xanthoxyloides*.[5]

The present gum partly dissolved in water to yield a pale brown, slightly acidic gel and an insoluble horn-like residue. Treatment with aqueous sodium hydroxide also partly dissolved it, and addition of ethanol to the acidified solution precipitated polysaccharide material (A) in a yield of 58%. A further yield of polysaccharide (B) (16% of weight of crude gum) was obtained by hot aqueous extraction of the alkali-insoluble residue.

Both polysaccharides (A) and (B) had positive rotations and contained D-galactose, D-mannose, and L-arabinose together with traces of L-rhamnose. The molar proportions of these sugars in hydrolysates of the polysaccharides varied with the conditions of hydrolysis; galactose and arabinose were the major sugars and mannose was present only in traces in hydrolysates obtained under relatively mild conditions. On the other hand, under more drastic conditions of hydrolysis these polysaccharides gave hydrolysates containing ~20% of mannose units. The proportion of galactose to arabinose in a mild acid hydrolysate of polysaccharide (A) was 1 : 1·5, and 1 : 0·86 in a more drastic acid hydrolysate. In contrast, polysaccharide (B) gave corresponding hydrolysates in which the molar proportions were galactose : arabinose = 1 : 0·5 and 1 : 0·4, respectively. Both polysaccharides contained about 1% of methoxyl and 1% of ethoxyl. Recent experiments [6] have shown that complete removal of solvent of precipitation from polysaccharides is very difficult and since both polysaccharides had been precipitated with ethanol it appeared probable that the presence of ethoxyl was due to adhering solvent. Hence in a subsequent extraction propanol-2-ol was used as the precipitating solvent, and the resulting

[1] Irvine, " Plants of the Gold Coast," Oxford University Press, London, 1930.
[2] Unwin, West African Forests and Forestry, T. F. Unwin Ltd., London, 1920.
[3] (*a*) Hirst, Percival, and Williams, *J.*, 1958, 1942; (*b*) Beauquesne, *Bull. Soc. Chim. biol.*, 1946, **28**, 895; (*c*) Hirst, Hough, and Jones, *J.*, 1949, 3145.
[4] Hirst and Dunstan, *J.*, 1953, 2332.
[5] Torto, *Nature*, 1957, **180**, 864.
[6] Anderson and Duncan, *Talanta*, 1961, **8**, 241; Anderson and King, *ibid.*, 1961, **8**, 497.

polysaccharide was found to be devoid of ethoxyl but to contain a small quantity of isopropoxy-groups.

The two heteroglycans (A) and (B) differed in their uronic acid content: the former contained 23·5% and the latter 32·6% of uronic anhydride. However, analyses of similar acid hydrolysates revealed that the molar ratio of glucurone to 4-*O*-methylglucuronic acid was approximately 1·8 : 1 in both polymers. Since the small proportion of glucurone which runs on the paper chromatogram as the acid is ignored in this estimation the molar ratio of glucuronic acid to 4-*O*-methylglucuronic acid is probably nearer 2 : 1. In view of the fact that arabinose and rhamnose units are cleaved from polysaccharide (A) by autohydrolysis it is possible that (A) and (B) do not represent different polysaccharides but that under the conditions necessary for the isolation of (B) a number of the more labile sugar linkages are cleaved and a polymer containing a high proportion of the acid-resistant glucuronosyl links remains. As will be seen below, however, oxidation studies indicate other differences in the macromolecular structure of these two materials.

It is difficult to obtain an accurate picture of the overall composition of these polysaccharides owing to the stability of the mannosyl and glucuronosyl links to, and the ready degradation of the arabinose units by, acid. By combining the results from a mild acid hydrolysis of polysaccharide (A) (galactose : arabinose = 1 : 1·5) with those of a more drastic hydrolysis (galactose : mannose : arabinose = 1 : 0·36 : 0·9), and bearing in mind that the polysaccharide contains 23·5% of uronic acid (glucuronic acid : 4-*O*-methylglucuronic acid = 2 : 1), it may be deduced that this polysaccharide comprises D-galactose : D-mannose : L-arabinose : L-rhamnose : D-glucuronic acid : 4-*O*-methyl-D-glucuronic acid in approximately the molar ratios of 4 : 1·5 : 6 : trace : 2 : 1. This, however, can only be regarded as a rough estimate since any difference in the extent to which galactose and mannose, and glucuronic acid and 4-*O*-methylglucuronic acid, are degraded by acid is ignored.

Attempts to fractionate polysaccharide (A) by precipitation with cetyltrimethylammonium bromide or by graded addition of ethanol were unsuccessful. In the latter experiments a plot of the fraction of polysaccharide precipitated against the concentration of ethanol in the solution gave a curve typical of a homogeneous polymer.[7]

L-Arabinose, D-galactose, L-rhamnose, and D-mannose were separated from a partial acid hydrolysate on a cellulose column. The first two were isolated as crystalline sugars and all of them were characterised by their optical rotations and the formation of known crystalline derivatives. From the acidic fraction of the hydrolysate crystalline D-glucurone was separated and characterised by chromatographic mobility, optical rotation, m. p., and mixed m. p. and by the formation of the crystalline *p*-nitroanilide. Syrupy 4-*O*-methyl-D-glucuronic acid was also separated and characterised by chromatographic mobility, rotation, and methoxyl content.

The water washings from the column on which the acidic fraction had been separated contained partly degraded polysaccharide. A portion of this material was re-hydrolysed and chromatography of the resulting syrup revealed the same constituents as were in the original polysaccharide, except that the proportion of arabinose had been reduced to a trace. A second portion of the degraded polysaccharide was converted into the methyl ester methyl glycoside and reduced. From the syrup obtained on hydrolysis of the reduced mixture crystalline D-glucose was separated and characterised. Syrupy 4-*O*-methyl-D-glucose (P) which was chromatographically and ionophoretically identical with an authentic sample was also isolated. This sugar yields, on oxidation with sodium periodate, 2-*O*-methylerythrose, characterised as an orange spot on a paper chromatogram.[8] Chromatographic examination of syrup (P) after oxidation with periodate revealed the presence of this spot, confirming that syrup (P) was indeed 4-*O*-methylglucose and that 4-*O*-methylglucuronic acid is a constituent of the heteroglycan from *A. zygia*.

[7] Bishop, *Canad. J. Chem.*, 1957, **35**, 1010.
[8] Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

From a partial acid hydrolysate, after removal of partly degraded polysaccharide material (Y), a syrup containing neutral mono- and oligo-saccharides was separated. These were fractionated by graded elution on a charcoal–Celite column and, after further fractionation on thick paper, pure samples of a fast-moving material (X) (which will be discussed below), three disaccharides, and two higher saccharides were isolated. 3-*O*-β-D-Galactopyranosyl-D-galactopyranose was obtained in the largest amount and partly crystallised. It gave only galactose on hydrolysis and was characterised by m. p. and mixed m. p., rotation, chromatographic mobility, and by the ionophoretic speed of its *N*-benzylglycosylammonium ion.[9] The amount of periodate it reduced was slightly lower than that reduced by laminaribiose but could only correspond to a 1,3′-linked disaccharide.

3-*O*-β-D-Galactopyranosyl-L-arabinose also partly crystallised after separation and was characterised by m. p. and mixed m. p., rotation, and chromatographic and ionophoretic mobility. Acid hydrolysis before reduction yielded equal amounts of arabinose and galactose, and after reduction only galactose was obtained on hydrolysis. This confirms that it was the arabinose moiety which carried the free reducing group.

A small quantity of syrup which was tentatively identified by rotation and chromatographic mobility as 6-*O*-β-D-galactopyranosyl-D-galactose was also isolated. Hydrolysis of this syrup gave only galactose. Since 6-*O*-α-D-galactopyranosyl-D-galactose is the major reversion product of the action of acid on galactose [10] the presence of 1,6′-linked galactose as a structural unit in the macromolecule of the gum exudate of *A. zygia* cannot be regarded as definitely established.

Finally two samples of syrup which behaved chromatographically as single substances, and of which the *N*-benzylglycosylammonium ions had ionophoretic mobilities corresponding severally to those of a tetraose and a heptaose,[9] were separated. The former of these gave galactose and mannose in the ratio of 3 : 1 on hydrolysis; the latter on hydrolysis yielded mainly galactose with a small proportion of arabinose and on partial hydrolysis gave in addition spots on a paper chromatogram corresponding to 3-*O*-galactosylgalactose and 3-*O*-galactosylarabinose.

Examination of the fast-moving material (X) by infrared analysis revealed the presence of ethoxyl corresponding to a mono-*O*-ethyl sugar. De-ethylation gave a product with the chromatographic colour and mobility of arabinose. It is difficult to explain the formation of an ethoxy-sugar under the present experimental conditions, but it was found that when propan-2-ol replaced the ethanol used in the earlier experiments the material (X) was absent and traces of a substance with a still higher R_{Gal} value were present in the polysaccharide hydrolysates. Until further evidence is available, these substances of high chromatographic mobility cannot be regarded as constituents of the polyuronide and can only be considered as artefacts.

The degraded polysaccharide material (Y) was rehydrolysed and partly fractionated on a cellulose column. Neutral sugars were discarded and the acid fractions containing mono- and aldobi-uronic acids were re-separated on thick paper. Two pure aldobiuronic acids were finally isolated. Syrupy 4-*O*-α-(4-*O*-methyl-D-glucopyranonosyl)-D-galactose was characterised by its equivalent weight, rotation of the barium salt, and chromatographic and ionophoretic mobility. Confirmation of this structure was obtained by examination of the derived disaccharide glycoside and of the methylated aldobiuronic acid. Hydrolysis of the former gave equal quantities of 4-*O*-methylglucose and galactose (visual examination, paper chromatography). The reduction of periodate by this disaccharide glycoside was in agreement with a 1,4′-linkage. Chromatographic identification of 2,3,4-tri-*O*-methylglucose and 2,3,6-tri-*O*-methylgalactose in the hydrolysate of the reduced methylated aldobiuronic acid confirmed that the linkage was indeed 1,4′.

The second aldobiuronic acid proved to be 2-*O*-β-D-glucopyranuronosyl-D-mannose.

[9] Barker, Bourne, Grant, and Stacey, *J.*, 1957, 2067.
[10] Turton, Bebbington, Dixon, and Pacsu, *J. Amer. Chem. Soc.*, 1955, **77**, 2565.

The chromatographic mobility [11] and calculated rotation for the barium salt [12] were identical with the values recorded by earlier workers for this acid. It had the correct equivalent and gave on hydrolysis glucurone and mannose. Oxidation of the ester glycoside by periodate was very slow and did not reach the theoretical figure of three moles per mole of ester glycoside. Reduction and hydrolysis of the derived disaccharide glycoside gave glucose and mannose. Final confirmation of the structure was obtained by identification of 2,3,4,6-tetra-*O*-methylglucose and 3,4,6-tri-*O*-methylmannose in the hydrolysate of the methylated disaccharide glycoside.

The possibility of isolating different oligosaccharides after acetolysis of the gum was investigated, but extremely complex mixtures were obtained and complete separation of appreciable quantities of pure fractions was not achieved.

Polysaccharides (A) and (B) reduced approximately the same amount of periodate, although over 50% of (B) remained undissolved after oxidation was virtually complete. The relatively low reduction of periodate (less than one mole for every two anhydro-units) by both polymers indicates that a considerable proportion of the monosaccharides does not contain adjacent free hydroxyl groups and that these units are either 1,3′-linked or triply linked in the polysaccharides. All the uronic acid units had been cleaved in both soluble oxo-polysaccharides, and the insoluble material from (B) still retained a small quantity of glucuronic acid. This may result from under-oxidation due to steric hindrance, and cannot be regarded as definite evidence that a proportion of glucuronic acid is differently linked. However, this difference in behaviour of (A) and (B) towards periodate is a further indication that, although these two fractions of the gum exudate appear to have the same basic constitution, they differ in the fine details of structure.

The relative proportion of galactose in the oxo-polysaccharides compared with that of mannose and arabinose was higher than in the unoxidised materials. This could be explained if one assumes that the galactose comprises the back-bone of the molecule and is to a large extent linked 1,3′- or is triply linked, while a proportion of the arabinose and mannose units are involved in linkages which permit adjacent hydroxyl groups to be cleaved.

These results, together with the large proportion of galactose, the isolation of 1,3′- and 1,6′-linked galactose disaccharides, and the separation of an oligosaccharide containing a number of contiguous galactose units from hydrolysates of the polysaccharide, provide evidence that the framework of the macromolecule is composed of 1,3′- and 1,6′-linked galactose residues (I), although, as already pointed out, in view of the possibility of acid reversion, the presence of the latter is by no means certain. The occurrence of 3-*O*-β-D-galactopyranosyl-L-arabinose (II) units suggests that galactose linked to the 3-position of arabinose is also a major structural unit in the molecule, but whether this is present in the main chain or only in side-chains cannot be decided from the available evidence. At the same time the large proportion of arabinose in the polyuronide and the ready release of arabinose units on autohydrolysis points to the presence of arabinose (III) side-chains. This structure is typical of other members of the Mimosaceæ [13] which comprise mainly 1,3′-linked galactose units with 1,6′-linked galactose occurring to a much smaller extent. Whereas rhamnose is a major constituent in the *Acacia* gums, in the present gum only a small proportion of this sugar is present and it cannot have the structural significance it possesses in, for example, *A. cyanophylla* [14] and *A. verek*.[15]

In contrast, the presence of mannose and of two uronic acids in *A. zygia* is unusual in the Mimosaceæ family. While only some of the mannose units are cleaved by periodate

[11] Aspinall, Hirst, and Wickstrøm, *J.*, 1955, 1160.
[12] Jones, *J.*, 1939, 558.
[13] Smith and Montgomery, " Chemistry of Plant Gums and Mucilages," Reinhold Publ. Corp., New York, 1959.
[14] Charlson, Nunn, and Stephen, *J.*, 1955, 269.
[15] Smith, *J.*, 1940, 1035; Dutton, *Canad. J. Chem.*, 1956, **34**, 406.

practically all the uronic acid units are oxidised and are probably present either as end groups or are 1,2′- or 1,4′-linked. The separation of 4-*O*-α-(4-*O*-methyl-D-glucopyranuronosyl)-D-galactose (IV) and 2-*O*-β-D-glucopyranuronosyl-D-mannose (V) provides evidence for direct union of 4-*O*-methylglucuronic acid to $C_{(4)}$ of galactose, and of glucuronic acid to $C_{(2)}$ of mannose in the macromolecule. At present, there is no indication whether these units are attached directly to the backbone or whether other residues are interposed.

6-*O*-α-D-Galactosyl-D-mannose has been isolated from Guar gum,[16] but although an oligosaccharide containing galactose and mannose was separated in the present experiment it was not completely characterised and the union of mannose to galactose can only be tentatively assumed.

Albizzia zygia gum has a high uronic acid content, and is unique in that, with the

. . . Gal I(β)—3 Gal I(β)—3 Gal I(β)—3 Gal I(β)—3 Gal I(β)—3 Gal I(β)—6 Gal I . . .
(I)

Gal I(β)—3 Ara I . . . . (II)

Ar I . . . (III)

4MeGA I(α)—4 Gal I . . . (IV)

GA I(β)—2 Man I . . . (V)

Gal = D-Galactose. GA = D-Glucuronic acid. Ara = L-Arabinose.
4MeGA = 4-*O*-Methyl-D-glucuronic acid. Man = D-Mannose.

possible exception of *Citrus limona* gum,[17] it is the only polysaccharide to contain both D-glucuronic acid and its 4-*O*-methyl ether.* A few other gum exudates such as *Khaya grandifolia* [18] and *K. senegalensis* [18] contain two uronic acids, but in these species the acids are 4-*O*-methyl-D-glucuronic and D-galacturonic acid. Glucuronic is the acid most commonly found in gums of the Mimosaceæ, although *Prosopsis juliflora* (mesquite gum) contains 4-*O*-methyl-D-glucuronic acid.[19] This acid has been found also in gums isolated from a variety of botanical families. In addition to those already mentioned it has been isolated from, for example, *Fagara xanthoxyloides* [5] and *Commphora myrrha* [20] gums.

The aldobiuronic acid, 2-*O*-β-D-glucopyranuronosyl-D-mannose, separated from this gum has also been isolated from *Prunus insitia* and *P. cerasus*.[21] 4-*O*-α-(4-*O*-Methyl-D-glucopyranuronosyl)-D-galactose, the other aldobiuronic acid obtained from *A. zygia*, has also been isolated from *Prosopis juliflora*,[22] *Citrus limona*,[17] and *Khaya grandifolia*.[18] In the last and in the present gum the 4-*O*-methyl-D-glucuronic acid is probably present as end group.

From these investigations it has emerged that, while the gum exudate of *A. zygia* resembles the gums of other members of the Mimosaceæ and has features in common with a variety of other gum exudates, it is also unique in that it differs in major respects from any single gum so far examined, both in its neutral sugars and uronic acids, and in many structural features.

Experimental

The analytical methods used have been described by Hirst, Percival, and Williams.[3a] In addition, solvents (8) ethyl acetate–acetic acid–water (9 : 2 : 2) and (9) ethyl acetate–acetic acid–water (3 : 1 : 3) were used. R_{Gl}, R_{Gal}, R_{GA}, and R_{Glone} are the rates of travel relative to glucose, galactose, glucuronic acid, and glucurone. Acids were converted into the methyl

* See also Gum *Asafoetida*, (Jones and Thomas, *Canad. J. Chem.*, 1961, **39**, 192).

[16] Whistler and Durson, *J. Amer. Chem. Soc.*, 1951, **73**, 4189.
[17] Connell, Hainsworth, Hirst, and Jones, *J.*, 1950, 1696; Andrews and Jones, *J.*, 1954, 1724.
[18] Aspinall, Hirst, and Matheson, *J.*, 1956, 989.
[19] Smith, *J.*, 1951, 2646.
[20] Hough, Jones, and Wadman, *J.*, 1952, 796.
[21] Hirst and Jones, *J.*, 1938, 1174; 1939, 558.
[22] Cunneen and Smith, *J.*, 1948, 1141.

ester methyl glycosides by refluxing them with 4% methanolic hydrogen chloride for 7 hr; the cooled solution, after neutralisation with solid silver carbonate, was filtered and concentrated to a syrup. Reduction with borohydride was achieved by allowing a 2% solution of the sugar in water to stand with an equal weight of solid sodium borohydride for 12—18 hr.; the solution was then neutralised with acetic acid, deionised with resin, and concentrated to a syrup.

Preliminary Examination of the Gum.—The gum, obtained as brown nodules, was contaminated with a little bark and had an odour of acetic acid. Three separate nodules were powdered and analysed. They each had moisture 17; ash, 5·8; N, 0; S, 0; halogen, 0; OMe, *ca.* 1%. Chromatography of an acid hydrolysate of each nodule, after conversion into the free acid by treatment with IR-120(H) resin (solvents 1 and 3), revealed spots corresponding to galactose, mannose, arabinose, glucurone, and 4-*O*-methylglucuronic acid together with a trace of rhamnose.

Purification and Fractionation of the Material.—The crude gum (10 g.), broken in small pieces, was dispersed by stirring it in cold water (500 c.c.) for 30 hr. Concentrated sodium hydroxide solution was added to give a N-solution and stirring was continued at room temperature under nitrogen for 20 hr. The residue was removed by centrifugation and the supernatant liquid cooled to 0° and acidified to pH 2 by addition of 17% w/v hydrochloric acid. Polysaccharide was precipitated from this solution by the addition of ethanol (4 vol.) during rapid stirring. The precipitated material was washed free from chloride with ethanol and dried to constant weight over phosphoric oxide at 60°/10 mm. The product (A) (5·8 g.) was a fine off-white powder. A further yield of polysaccharide (B) (1·6 g.) was obtained by stirring the residue in water (1500 c.c.) at 100° for 24 hr. After removal of insoluble material the polysaccharide was precipitated as for (A).

An aqueous solution of polysaccharide (A) (1·3 g. in 300 ml.) at pH 2 gave successive precipitates (0·75 g., 0·500 g., and 0·020 g.) with 1·5, 1·75, and 2·0 volumes of ethanol. No precipitate was deposited at higher or lower concentrations of alcohol. Each precipitate was removed, washed with ethanol, dried, and weighed. Hydrolysates of each precipitate gave identical chromatograms. Addition of cetyltrimethylammonium bromide to solutions of polysaccharide (A) at pH 4, 7, and 9 [23] resulted in complete precipitation of the polysaccharide in each case.

Properties of the Purified Polysaccharides.—Polysaccharide (A) had $[\alpha]_D$ +21° (*c* 0·49) [Found: equiv., 723 (by titration); uronic anhydride, 23·5 (by decarboxylation); ash, 0·25; AcO, 0; OEt, 1·2; OMe, 1·3% (by infrared absorption of the gases [24])]. The extraction procedure was repeated on a fresh sample of gum except that propan-2-ol was used to precipitate polyuronide material. (Found: OEt, 0; OPr^i, *ca.* 1%).

Chromatographic analysis of an acid hydrolysate (C) (18 hr., 2N-H_2SO_4 at 100°) of the ethanol-precipitated polysaccharide revealed the same monosaccharides as the hydrolysate of the crude gum, together with a faint spot of R_{Gal} 4·8 (solvent 9). A portion of the hydrolysate, after neutralisation, was separated on 3MM paper into neutral sugars and barium uronates (solvent 1). The latter remained on the starting line and after elution they were converted into the free acids by shaking them with IR-120(H) resin. On concentration a syrup (10 mg.) was obtained. After conversion into the methyl ester methyl glycosides and reduction with sodium borohydride the product was hydrolysed with N-sulphuric acid. Neutralisation was followed by concentration, and the derived syrup on chromatographic examination in solvents (1) and (2) showed the presence of glucose and 4-*O*-methylglucose in addition to the neutral monosaccharides originally detected. The relative molar proportions of the neutral sugars in a second portion of the hydrolysate (C), determined by the methods of Hirst and Jones [25] and of Pridham,[26] were galactose : mannose : arabinose (1 : 0·36 : 0·86 and 1 : 0·36 : 0·90, respectively). Hydrolysis under milder conditions (7 hr., N-H_2SO_4 at 100°) gave a product in which the molar proportions of the respective sugars were 1 : trace : 1·5.[27] In both hydrolysates the proportions of rhamnose were too small to be estimated accurately. The molar proportion of glucurone to 4-*O*-methylglucuronic acid in hydrolysate (C) was found

[23] Scott, *Chem. and Ind.*, 1955, 168; Barker, Stacey, and Zweifel, *ibid.*, 1957, 330.
[24] Anderson and Duncan, *Talanta*, 1961, **8**, 1.
[25] Hirst and Jones, *J.*, 1949, 1659.
[26] Pridham, *Analyt. Chem.*, 1956, **28**, 1967.
[27] Wilson, *Analyt. Chem.*, 1959, **21**, 1977.

to be 1·8 : 1 when determined by application of Wilson's method [27] to uronic acids. Absorbances were measured at 390 mμ and the weights of acids found from calibration graphs. From these results it may be calculated that polysaccharide (A) comprises D-galactose : D-mannose : L-arabinose : L-rhamnose : D-glucuronic acid : 4-*O*-methyl-D-glucuronic acid in approximately the molar ratios of 4 : 1·5 : 6 : trace : 2 : 1 respectively.

Polysaccharide (B), an off-white powder, had $[\alpha]_D$ +38·7° (*c* 0·32 in 0·1N-NaOH) [Found: ash, 0·22; AcO, 0; uronic anhydride 32·6% (by decarboxylation)]. The molar proportions of monosaccharides in a hydrolysate (2N-H_2SO_4, 18 hr., 100°) were found to be galactose : mannose : arabinose = 1 : 0·33 : 0·4, and the proportion of glucurone : 4-*O*-methylglucuronic acid 1·9 : 1.[24] A milder hydrolysis (N-H_2SO_4, 7 hr., 100°) gave galactose : mannose : arabinose = 1 : trace : 0·5.

Autohydrolysis of Polysaccharide (A).—A 1% aqueous solution of polysaccharide (A) (1·5 g.), $[\alpha]_D$ +21°, was heated at 95° for 26 hr. The hydrolysis was followed by measuring the rotation, iodine number,[28] and chromatography (solvent 2) of aliquot parts withdrawn at intervals:

Time (hr.)	α_D	I no.	Sugars released
2	+0·08°	2·0	Arabinose
4	0·11	4·3	,,
6	0·12	6·4	,,
8	0·13	9·2	,,
14	0·14	13·8	Arabinose, rhamnose
24	0·18	21·0	,, ,,
26	0·19	21·5	,, ,,

The residual polysaccharide was precipitated by addition of ethanol and had $[\alpha]_D$ +45·8° (*c* 2·8).

Partial Acid Hydrolysis and Characterisation of the Neutral Sugars and Acids in Polysaccharide (A).—Polysaccharide (A) (4 g.) was heated at 100° for 7 hr. with N-sulphuric acid (500 c.c.). After working up in the usual way the molar ratio [27] galactose : arabinose in a portion of the derived syrup was 1 : 1·4. The residual hydrolysate (3·5 g.) was separated into its constituent sugars on a cellulose column (760 × 37 cm.), and eluted with butan-1-ol two-thirds saturated with water. R_{Gl} values are recorded for solvent 2.

Fraction 1. Syrupy L-rhamnose (79 mg.) had $[\alpha]_D$ +8·6° (*c* 1·2), R_{Gl}, 2·0. The derived benzoylhydrazone [29] had m. p. and mixed m. p. 188°.

Fraction 2. Crystalline L-arabinose (480 mg.), R_{Gl} 1·18, had $[\alpha]_D$ +105° (*c* 2·0), m. p. and mixed m. p. 159° after recrystallisation from methanol. The derived benzoylhydrazone [30] had m. p. and mixed m. p. 203°.

Fraction 3. A syrupy mixture of arabinose and mannose (515 mg.). Syrupy mannose (36 mg.) was separated on Whatman 3MM paper from a portion (135 mg.) of this syrup by elution with solvent 9. The mannose had R_{Gl} 1·16, $[\alpha]_D$ +14° (*c* 1·23; const.), and the derived phenylhydrazone [31] had m. p. and mixed m. p. 188°.

Fraction 4. A syrup (60 mg.) containing arabinose, mannose, and galactose.

Fraction 5. Crystalline D-galactose (763 mg.) after recrystallisation from butan-1-ol had m. p. and mixed m. p. 164°, $[\alpha]_D$ +142° ⟶ +80° (const.) (*c* 2·3). The derived diethyl mercaptal [32] had m. p. and mixed m. p. 141°.

Fraction 6. Water washings which yielded solid barium uronates on evaporation. Treatment with IR-120(H) resin, filtration, and evaporation yielded the free acids as a brown syrup (1·26 g.). Separation of these acids on a Grycksbo filter-paper column eluted with solvent 9 gave the following fractions:

Fraction IA. Crystalline D-glucurone (68 mg.), R_{Gl} 3·0, after recrystallisation from water had m. p. and mixed m. p. 177°, $[\alpha]_D$ +18·8° (*c* 1·2, const.). The derived *p*-nitroanilide after recrystallisation from cold methanol had m. p. and mixed m. p. 130—131.[33]

Fraction IIA. A syrupy mixture of D-glucurone and 4-*O*-methyl-D-glucuronic acid (42 mg.),

[28] Ingles and Israel, *J.*, 1948, 810.
[29] Hirst, Hough, and Jones, *J.*, 1949, 3145.
[30] Hirst, Jones, and Wood, *J.*, 1947, 1048.
[31] O'Donnell and Percival, *J.*, 1949, 2168.
[32] Wolfrom, *J. Amer. Chem. Soc.*, 1930, **52**, 2466.
[33] Hamilton, Spriestersbach, and Smith, *J. Amer. Chem. Soc.*, 1957, **79**, 443.

which was separated on 3MM paper (solvent 9) into D-glucurone (10 mg.) and a syrup (27 mg.), chromatographically identical with 4-*O*-methylglucuronic acid and having $[\alpha]_D$ +42·2° (*c* 1·8) (Found: OMe, 14·0. Calc. for a mono-*O*-methylhexuronic acid: OMe, 14·9%).

Fraction IIIA. A syrup (225 mg.) containing arabinose, mannose, glucurone, and 4-*O*-methylglucuronic acid (paper chromatography). This was not examined further.

Fraction IVA. A syrup (D) (840 mg.), obtained on concentration of the water washings, showed no chromatographic movement in basic or acidic solvents on paper chromatograms developed for 24 hr. A portion of this syrup (540 mg.) was hydrolysed with 90% formic acid (25 c.c.) at 100° for 24 hr. Removal of formic acid by repeated distillation with methanol yielded a dark brown syrup which was hydrolysed with N-sulphuric acid for 2 hr. at 100°. Chromatographic examination of the derived syrup (123 mg.) revealed all the sugars present in the original polyuronide, the arabinose being reduced to a trace.

The remainder of syrup (D) (300 mg.) was converted into the methyl ester methyl glycosides and reduced with potassium borohydride. The neutralised product was hydrolysed with N-sulphuric acid at 100° for 5 hr. After working up in the usual way chromatography of the derived syrup (140 mg.) indicated the presence of glucose, 4-*O*-methylglucose, arabinose, mannose, and galactose (solvents 2, 4, and 9). From this syrup, by separation on 3MM paper, crystalline D-glucose (24 mg.), m. p. and mixed m. p. 146°, and 4-*O*-methyl-D-glucose (18 mg.) were obtained. The 4-*O*-methyl-D-glucose had $[\alpha]_D$ +50·1° (*c* 1·36),[34] was chromatographically identical with an authentic sample in solvents (1, 2, 4, and 9), and gave a single spot on ionophoresis with M_G 0·24, identical with that of an authentic sample. Chromatography of a periodate-oxidised sample showed an orange-brown spot, R_F 0·53 (solvent 3), identical with a spot derived by similar treatment of authentic 4-*O*-methylglucose (aniline oxalate spray).

Periodate Oxidation of Polysaccharides (*A*) *and* (*B*).—Polysaccharides (A) (78 mg.) and (B) (218 mg.) were oxidised with 0·015M-sodium metaperiodate (50 c.c. and 150 c.c., respectively). Oxidation was allowed to proceed in the dark at 2° with frequent shaking. At suitable intervals aliquot parts (0·2 c.c.) were withdrawn and the amount of periodate in the solution was measured.[35]

Time (hr.)		3	5	18	28	48	70
$NaIO_4$ (mole reduced/anhydro-C_6 unit)	Polysac. (A) ...	0·26	0·30	0·44	0·45	0·48	0·50
	,, (B) ...	0·40	0·43	0·49	0·51	0·53	0·57

After 18 hr. the primary oxidation had reached completion and corresponded to 0·44 and 0·49 mole per anhydro-C_6 unit. The oxidation was stopped after 70 hr. by addition of an excess of ethylene glycol. A portion of polysaccharide (B) which remained undissolved was removed and dried (120 mg.). After dialysis of the oxidation solutions for 4 days the soluble oxo-polysaccharides {60 mg. from (A), $[\alpha]_D$ +3·7°; and 20 mg. from (B), $[\alpha]_D$ +21°} were isolated by freeze-drying. Each of the oxopolysaccharides was hydrolysed with sulphuric acid and the relative molar proportion of the sugars determined:

		Galactose	Mannose	Arabinose	Glucurone
Oxopolysaccharide	(A)	1	0·19	0·41	Nil
Soluble oxo-polysaccharide	(B)	1	0·24	0·58	Nil
Insoluble oxo-polysaccharide	(B)	1	0·21	0·33	0·06

Partial Acid Hydrolysis; Separation and Characterisation of Oligosaccharides.—A 0·7% aqueous solution of polysaccharide (A) (25 g.) was heated for 5 hr. at 95° and the residual polysaccharide, $[\alpha]_D$ +44°, was hydrolysed with 0·5N-sulphuric acid (300 ml.) at 95° for 15 hr. After neutralisation, partially degraded barium uronates {10 g.; $[\alpha]_D$ +56° (*c* 2·0)} were precipitated by addition of ethanol (6 vol.). The supernatant solution was concentrated to 100 c.c. and a further yield of barium uronates {2·6 g., $[\alpha]_D$ +61° (*c* 1·8)} was precipitated with ethanol (10 vol.). Evaporation of the supernatant solution yielded a syrup (8·8 g.). This was separated into neutral sugars, disaccharides, and higher oligosaccharides on a charcoal–Celite column (6 × 35 cm.). The neutral sugars (fraction 1; 5·6 g.) were eluted with water

[34] Munro and Percival, *J.*, 1935, 873.
[35] Aspinall and Ferrier, *Chem. and Ind.*, 1957, 1216.

(6 l.) and discarded, the disaccharides contaminated with small quantities of monosaccharides [fraction 2, (*a*) 520 mg. and (*b*) 500 mg.] were eluted with 5, 10, and 12·5% ethanol (4 l.), and the higher saccharides (fraction 3, 980 mg.) with 15 and 17% ethanol (7 l.). From fractions (2*a*) and (2*b*) the following disaccharides were separated on Whatman No. 17 paper by elution with solvent (1) for 4 and 5 days, respectively. Overlap fractions were discarded and similar disaccharides from the two fractions were combined. Syrupy 3-*O*-β-D-galactopyranosyl-L-arabinose (26 mg.) was isolated. This partially crystallised after dissolution in aqueous ethanol and storage at 0° for several days. The crystals had m. p. and mixed m. p. 200—201°. The syrup had $[\alpha]_D$ +60° (*c* 1·85),[36] R_{Gal} 0·62 and 0·46 in solvents (2) and (9), respectively, and M_G 0·68, identical with an authentic sample run as a control. It (3 mg.) gave on acid hydrolysis (N-H_2SO_4, 5 hr., 100°) equal amounts of galactose and arabinose (paper chromatography, visual estimation). Reduction of a sample (6 mg.) with sodium borohydride followed by hydrolysis gave galactose only (paper chromatography).

6-*O*-β-D-Galactopyranosyl-D-galactopyranose (8 mg.) was isolated only as a syrup, $[\alpha]_D$ +26° (*c* 0·67),[37] R_{Gal} 0·22 in solvent (2) and 0·19 in solvent (9) identical with 6-*O*-β-D-galactopyranosyl-D-galactopyranose run as control. Chromatography of an acid hydrolysate gave a single spot with the mobility of galactose.

3-*O*-β-D-Galactopyranosyl-D-galactopyranose (51 mg.) was isolated as a syrup from which needles, m. p. and mixed m. p. 150—151°, were obtained on treatment with aqueous ethanol. The syrup had $[\alpha]_D$ +58° (*c* 3·0),[36] R_{Gal} 0·35 (solvent 2), and gave a spot with the same mobility as 3-*O*-β-D-galactopyranosyl-D-galactopyranose in solvents (2) and (9). Ionophoresis as the *N*-benzylglycosylammonium ion in formic acid buffer gave a spot with mobility of 0·72.[9] The syrup on acid hydrolysis gave only galactose. A portion (10 mg.) was converted into the methyl galactoside and oxidised with sodium metaperiodate in the dark at 2°. Oxidation was complete after 5 hr. and corresponded to the reduction of 1·80 moles of periodate for every disaccharide mole. Methyl laminaribioside reduced 1·95 moles of periodate per mole during the same period in a parallel experiment.

Attempted separation, on thick paper, of pure higher oligosaccharides from fraction 3 was unsuccessful except for a small quantity of a syrupy tetrasaccharide and possibly a heptasaccharide. The former of these (31 mg.) had $[\alpha]_D$ +71·8° (*c* 2·8), R_{Gal} 0·09 in solvent (2), and gave on hydrolysis galactose : mannose in the ratio of .3 : 1 (paper chromatography, visual examination). Ionophoresis as the *N*-benzylglycosylammonium ion in formic acid buffer [9] gave a mobility of 0·5. The higher oligosaccharide (35 mg.) had $[\alpha]_D$ +34° (*c* 3·2) and gave on hydrolysis galactose and arabinose (8 : 1, visual examination of a paper chromatogram). The mobility of its *N*-benzylglycosylammonium ion was 0·3. Chromatography of a partial acid hydrolysate in solvent (2) revealed spots with R_{Gal} 1·5, 1·0, 0·62, and 0·35.

In addition to the above disaccharides a pure syrup (X) (43 mg.) was separated from fraction (2*a*). It had $[\alpha]_D$ +104° (*c* 4·0), R_{Gal} 3·5, R_{Glu} 2·55 in solvent (2) (cf. rhamnose, R_{Gal} 2·5), and R_{Gal} 4·8 in solvent (9), M_G 0·30 [Found: OEt, 23·2 (by infrared absorption measurement of gases [24]). Calc. for a mono-*O*-ethylarabinose: OEt, 25·5%]. Chromatography of a portion after de-ethylation [38] gave a single spot identical with arabinose run as a control. When ethanol was replaced by propan-2-ol substance (X) was absent from the hydrolysates but traces of material which gave a yellow spot, R_{Gal} 4·2 (solvent 2), were present [Found: alkoxyl, nil (infrared measurement)].

The combined barium uronates (12 g.) were re-hydrolysed with 0·5N-sulphuric acid (300 c.c.) at 100° for 4 hr. After neutralisation the derived syrup (11 g.) consisting of neutral sugars and barium uronates was separated on a cellulose column. The neutral sugars (rhamnose, mannose, and galactose) (3·1 g.) were eluted with butan-1-ol half saturated with water and discarded. The barium uronates were then eluted with butan-1-ol–acetic acid–water (2 : 1 : 1). Similar fractions were combined, evaporated several times with water to remove acetic acid, treated with IR-120(H) resin, and weighed. The first two fractions, (D) (700 mg.) and (E) (1·95 g.), contained glucurone, 4-*O*-methylglucuronic acid, and two aldobiuronic acids. The next fraction (F) (1·7 g.) contained higher oligouronic acids. Fraction (G) (1·14 g.) was partially degraded polyuronide. The first two fractions (D and E) were separated into their constituents on Whatman No. 17 paper by elution with solvent (8) for 36 and 72 hr., respectively.

[36] Aspinall, Auret, and Hirst, *J.*, 1958, 4408.
[37] Lindgren, *Acta Chem. Scand.*, 1957, 1365.
[38] Allen, Bonner, Bourne, and Saville, *Chem. and Ind.*, 1958, **36**, 630.

The chromatograms were freed from acetic acid by drying in air for 36 hr. and the different acids detected by spraying with Bromocresol Green. The appropriate strips were eluted with cold water, and the contaminating spray removed by treatment with charcoal. The two aldobiuronic acids were characterised as follows: Syrupy 4-*O*-α-(4-*O*-methyl-D-glucopyranuronosyl)-D-galactose (295 mg.) had $[\alpha]_D$ +102·8° (*c* 2·17), R_{Ga} 0·70 (solvent 4), M_G 0·67. The barium salt had $[\alpha]_D$ +97° (*c* 1·7) [39] (Found: Equiv., 360. Calc. for a monomethylglucuronosylgalactose: Equiv., 370). A sample (107 mg.) was converted into the methyl ester methyl glycoside and the latter reduced with sodium borohydride: the derived syrup gave on hydrolysis equal amounts of galactose and 4-*O*-methylglucose (paper chromatography). The methyl ester methyl glycoside reduced 1·8 moles of sodium periodate per mole of glycoside. An aqueous solution of the methyl ester methyl glycoside (92 mg. in 30 c.c.) at 5° was treated dropwise with 30% sodium hydroxide solution (15 c.c.) and dimethyl sulphate (6 c.c.) with stirring during 4 hr.; the mixture was then stirred at room temperature for 24 hr.; this methylation was repeated twice; the final solution was heated for 30 min., acidified with sulphuric acid, and sodium sulphate was precipitated by addition of methanol; the filtrate was made slightly alkaline, concentrated to *ca.* 50 c.c., acidified with sulphuric acid, and extracted with chloroform (5 × 50 c.c.); the resulting methylated ester glycoside obtained on evaporation of the combined chloroform extracts was dissolved in dry tetrahydrofuran (5 c.c.) and reduced with lithium aluminium hydride (5 g.); after 1 hour's refluxing, the excess of hydride was destroyed by water (0·1 c.c.), and the solution was diluted with ethyl acetate, filtered, and concentrated; the methylated disaccharide glycoside was extracted from the residue with acetone (2 × 20 c.c.) and after removal of the acetone was hydrolysed with N-hydrochloric acid (1 c.c.) at 100° for 4 hr.; after neutralisation, chromatographic analysis of the derived syrup in solvent (3) showed two spots, R_G 0·87 and 0·74, corresponding to 2,3,4-tri-*O*-methylglucose and 2,3,6-tri-*O*-methylgalactose respectively run as controls (cf. 2,4,6-tri-*O*-methylgalactose R_G 0·67, and 2,3,4-tri-*O*-methylgalactose R_G 0·64).

2-*O*-β-D-Glucopyranuronosylmannose (90 mg.) obtained pure after three separations on Whatman No. 17 paper had $[\alpha]_D$ −32° (*c* 0·83) (Calc. as the barium salt, $[\alpha]_D$ −18° [12]), R_{Gal} 0·46, R_{Glone} 0·23 in solvent (4) [11] (Found: equiv. by titration, 365. Calc. for glucuronosylmannose: equiv., 355). Chromatography (solvent 4) of a hydrolysate (2N-H_2SO_4, 5 hr., 100°) showed spots corresponding to mannose, glucurone, and a faint spot for glucuronic acid. A sample (6 mg.), after conversion into the methyl ester methyl glycoside and reduction with borohydride, gave glucose and mannose on hydrolysis (paper chromatography). The methyl ester methyl glycoside reduced 2·4 moles (30 hr.) and 3·0 moles (70 hr.) of 0·015M-sodium metaperiodate at 2° in the dark. The methyl ester methyl glycoside (35 mg.) was reduced by sodium borohydride, and the product was methylated in the same way as the previous aldobiuronic ester. After extraction with chloroform the methylated disaccharide was hydrolysed and the resulting syrup examined chromatographically. Two spots, R_G 1·0 and 0·77 (solvent 3), identical with those from 2,3,4,6-tetra-*O*-methylglucose and 3,4,6-tri-*O*-methylmannose, respectively, were obtained (cf. 2,3,6-tri-*O*-methylmannose R_G 0·81).

Attempts to isolate pure products from the higher fractions proved unsuccessful.

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[39] Jones and Nunn, *J.*, 1955, 3001.