962. Virgilia oroboides *Gum. Part II.*[1] *Isolation of Oligosaccharides after Partial Hydrolysis.*

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Graded acid hydrolysis of *Virgilia oroboides* gum has enabled many partial units of structure to be identified. Arabinose constitutes nearly half of the neutral monosaccharide units, predominantly linked α1 ⟶ 5 though some α1 ⟶ 3-linkages are also present, and mainly in the furanose form. Xylose replaces a small part of the arabinopyranose, and some mannose is obtained as well as (mainly β1 ⟶ 6-linked) galactopyranose. The acid portion (17% of the gum) is based mainly on D-glucuronic acid, predominantly β1 ⟶ 2-linked to mannose but also β1 ⟶ 6-linked to D-galactose; some 4-*O*-methyl-D-glucuronic acid is found, linked solely to galactose.

A PRELIMINARY account has been given [1] of the purification and mild acid hydrolysis of *Virgilia oroboides* gum, whereby in addition to L-arabinose and 5-*O*-α-L-arabinopyranosyl-L-arabinose there are produced in smaller amounts a number of other oligosaccharides, rhamnose, and galactose. The properties of the gum itself and the identification of a number of oligosaccharide constituents [2] liberated by partial hydrolysis are presented in the present paper.

When purified by repeated precipitation from aqueous solution with ethanol in the usual way, followed by treatment with a cation-exchange resin, *V. oroboides* gum forms an ash-free amorphous powder with an equivalent weight of 2100. Dispersion of the gum in water is not complete, but ultracentrifugation of the soluble portion in 0·2M-sodium chloride and electrophoresis in acetate buffer showed this material to be essentially homogeneous.[3] Examination by glass-fibre ionophoresis [4] of the very viscous solution in 2N-sodium hydroxide disclosed about 10% of a slow-moving component. The bulk

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[1] The paper by Stephen, *J.*, 1957, 1919, is regarded as Part I.

[2] Smith and Montgomery, " Chemistry of Plant Gums and Mucilages," Reinhold Publ. Corp., New York, 1959, pp. 318, 319.

[3] Banks, Greenwood, and Stephen, unpublished results.

[4] Briggs, Garner, and Smith, *Nature*, 1956, **178**, 154; Lewis and Smith, *J. Amer. Chem. Soc.*, 1957, **79**, 3929.

of the gum travelled faster at a rate comparable with that of calf liver glycogen. There being no method available for the resolution of the polysaccharide on a large scale, the ethanol-precipitated gum was used for graded acid hydrolysis.

The gum was heated with 0·01N-sulphuric acid for 6 hr. and neutralised, yielding the methanol-insoluble mixed barium salts of partly degraded fragments of the gum, with a syrupy mixture of sugars. Separation of the sugar mixture in the manner indicated in the earlier paper,[1] followed by sheet-paper chromatography of the minor components, gave L-arabinose (20% by weight based on the original gum), crystalline 5-*O*-α-L-arabinopyranosyl-L-arabinose (A) (4·5%), arabinose disaccharides B (0·9%) and D (trace), a xylosylarabinose C (0·4%), an arabinose trisaccharide E (0·4%), rhamnose (0·1%), and galactose (0·1%).

Disaccharide B was easily hydrolysed to arabinose only, showed a high negative rotation, and was relatively fast-moving on paper chromatograms. Its phenylosazone was shown to contain a 1 ⟶ 5 biose linkage by periodate oxidation,[5] and the disaccharide is accordingly 5-*O*-α-L-arabinofuranosyl-L-arabinose, the anomeric linkage being shown to be unequivocally α by application of Hudson's rules. This compound has been isolated by partial hydrolysis of the araban in sugar beet.[6] Disaccharide C gave D-xylose and L-arabinose on hydrolysis, and formed a phenylosazone in which a 1 ⟶ 5 linkage was established as before and from which xylose only was released by acid hydrolysis. The anomeric linkage, deduced from the optical rotation of the disaccharide, must be β, and the compound is therefore 5-*O*-β-D-xylopyranosyl-L-arabinose. The constants for the sugar and for its phenylosazone agree with those given for this disaccharide isolated from peach (*Prunus persica*) and cholla (*Opuntia fulgida*) gums.[7] Disaccharide D was shown by mild acid hydrolysis to be an arabinobiose, whose phenylosazone yielded formaldehyde on periodate oxidation. These facts, together with the high chromatographic mobility and approximately zero specific rotation, suggest the presence of an α1 ⟶ 3-arabinofuranoside linkage; 3-*O*-β-L-arabinopyranosyl-L-arabinose, frequently encountered as a hydrolysis product from natural polysaccharides [8] and as a reversion compound,[9] is much slower-moving on paper chromatograms and has a high positive rotation. The anomeric counterpart of disaccharide D, with a β1 ⟶ 3-arabinofuranoside linkage, has been isolated from sugar beet araban [6] and from *Acacia pycnantha* gum.[10] The identification of arabinotriose E is also uncertain on account of the small quantity isolated, but partial acid hydrolysis gave arabinose and 5-*O*-α-L-arabinopyranosyl-L-arabinose; and methylation and hydrolysis gave 2,3,4-tri- and 2,3-di-*O*-methylarabinose (paper chromatographic evidence). The presence of two 1 ⟶ 5-linkages is therefore indicated.

The major proportion of the residual barium salt mixture, from which the compounds just described had been separated, was next submitted to further hydrolysis by hot 0·01N-sulphuric acid for 6 hr., neutralized, and precipitated as before. This process was repeated after 9 hours' and again after 44 hours' heating, soluble mixtures of sugars being collected at each stage and the insoluble residue being re-treated with acid. The neutral sugar components were separated by the same general techniques as before, into (percentages are approximate and based on original gum) arabinose (11%), 5-*O*-α-L-arabinopyranosyl-L-arabinose (1·5%), rhamnose, mannose, and xylose (traces), galactose and galactose oligosaccharides in similar proportions (26%), and a little acidic material. Detailed study of the galactose oligosaccharides was deferred until the hydrolysis products from the residual arabinose-free material remaining at this stage were examined; partial hydrolysis of the galactose oligosaccharides revealed substantially the same pattern of components as was obtained

[5] Hough, Powell, and Woods, *J.*, 1956, 4799.

[6] Andrews, Hough, and Powell, *Chem. and Ind.*, 1956, 658.

[7] Andrews, Ball, and Jones, *J.*, 1953, 4090.

[8] Smith and Montgomery, ref. 2, p. 155.

[9] Ball, Jones, and Nicholson, Amer. Chem. Soc. Meeting, Minneapolis, Sept. 1955, Abs. Papers, 7D; Jones and Nicholson, *J.*, 1958, 27.

[10] Aspinall, Hirst, and Nicolson, *J.*, 1959, 1697.

later on hydrolysis of the insoluble residue. The bulk of the galactose was released during the period 21—68 hr. and during this time only 2% (based on original gum) of arabinose was removed.

The residual " core," representing 34% by weight of the original gum, had $[\alpha]_D^{20}$ +40°, and after removal of barium by means of cation-exchange resin had an equivalent weight 780. This value, which may be compared with that (714) to be expected if all the carboxylic acid-containing residues of the gum had remained intact during acid hydrolysis and were concentrated in the " core," corresponds to about one hexuronic acid unit for every 3·5 hexose units (complete hydrolysis of a portion with N-acid liberated mainly galactose and glucuronic acid, with smaller amounts of mannose). The increased $[\alpha]_D$ is a reflection of the removal of arabinose units in the furanose form together with α-linked arabinopyranosyl residues. The $[\alpha]_D$ is nevertheless low for glucuronic acid and galactose, indicating that β-linkages predominate in the main chain of the gum.

After preliminary hydrolysis to ascertain the most favourable conditions for formation of oligosaccharides, most of this material was hydrolysed with hot 0·1N-sulphuric acid for 8 hr. and divided by precipitation with ethanol into a mixture of neutral sugars and a residual acidic polysaccharide in the form of barium salts. The latter were hydrolysed again for a short period (3 hr.), and neutral sugars and a barium salt mixture were separated from the hydrolysate as before. The acidic and the combined neutral portions were obtained in approximately equal amounts (each 17% by weight of the original gum).

The sugars were separated by elution from a charcoal–Celite column into forty-three fractions. Some of the components were obtained pure by fractionation in this way, and the mixtures were resolved by cellulose-column chromatography or by the use of filter-paper sheets. The products (percentages given in parentheses are based on the original gum) were D-galactose (6·4), L-arabinose (0·06), D-mannose (0·4), an unidentified hexose or derivative thereof (0·08), 6-*O*-β-D-galactopyranosyl-D-galactose (2·8), 3-*O*-β-D-galactopyranosyl-D-galactose (0·4), β1 ⟶ 6-linked galactotriose (2·7), a second galactotriose (0·4), β1 ⟶ 6-linked galactotetraose (1·4), and a residual mixture (2·3).

The identity of the galactose-containing disaccharide ($[\alpha]_D$ +29°) formed in greatest quantity both at this and at the earlier stage of hydrolysis with more dilute acid was proved by its conversion, through the octa-acetate and methyl β-glycoside, into a methylated derivative, hydrolysis of which gave 2,3,4,6-tetra- and 2,3,4-tri-*O*-methyl-D-galactose. The disaccharide crystallised from methanol solution on nucleation with a specimen of the hydrate-methanolate of 6-*O*-β-D-galactopyranosyl-D-galactose, and the melting characteristics of the two specimens, though indefinite, were identical. A phenylosazone was prepared which on periodate oxidation afforded no formaldehyde but gave evidence (spectroscopic) of the formation of mesoxaldialdehyde 1,2-bisphenylhydrazone. Periodate oxidation of the corresponding galactobi-itol followed by borohydride reduction and hydrolysis gave glycerol and ethylene glycol, detected by paper chromatography, confirming the presence of a 1 ⟶ 6 linkage in the disaccharide.[11] A measure of the rate of hydrolysis of the disaccharide by 0·1N-sulphuric acid at 98° was obtained by following the change of optical rotation of the solution; the time of half-hydrolysis was *ca.* 2·5 hr. This disaccharide has been obtained by partial hydrolysis of golden-apple gum [12] and of gum ghatti.[13]

Of the two galactotrioses, the crystalline compound ($[\alpha]_D$ +19°) formed in greater amount gave galactose and the β1 ⟶ 6-linked galactobiose only on partial acid hydrolysis, and periodate oxidation of the reduced trisaccharide liberated 4·5 equiv. of formic acid per mole. Methylation afforded a crystalline derivative, which on hydrolysis gave 2,3,4,6-tetra- and 2,3,4-tri-*O*-methyl-D-galactose. The identity of the trisaccharide with the *O*-β-D-galactopyranosyl-(1 ⟶ 6)-*O*-β-D-galactopyranosyl-(1 ⟶ 6)-D-galactose

[11] Goldstein, Smith, and Unrau, *Chem. and Ind.*, 1959, 124.

[12] Lindgren, *Acta Chem. Scand.*, 1957, **11**, 1365.

[13] Aspinall, Auret, and Hirst, *J.*, 1958, 4408.

isolated from larch ε-galactan [14] and from gum ghatti was finally proved by direct comparison (mixed m. p. and *X*-ray powder photographs). We are grateful to Dr. Aspinall for his collaboration in this.

The corresponding tetraose ($[\alpha]_D$ +16°) on partial acid hydrolysis gave a series of products corresponding chromatographically to galactose and the β1 → 6-linked galactobiose and galactotriose just described, the R_M values [15] plotted against the number of galactose residues giving a straight line for two different solvent systems. Chromatographic evidence of the presence of a galactopentaose amongst the residual components was also obtained in this way. Graded hydrolysis of gum ghatti [13] has given the same series of oligosaccharides.

Crystalline 3-*O*-β-D-galactopyranosyl-D-galactose was obtained ($[\alpha]_D$ +54°, m. p. *ca.* 200°) by cellulose-column chromatography of a mixture containing this biose and the β1 → 6-linked galactotriose. The compound was chromatographically and ionophoretically identical with a sample supplied by Dr. G. O. Aspinall, and gave an identical *X*-ray powder diagram. It gave a depression of m. p. with 4-*O*-β-D-galactopyranosyl-D-galactose,[16] and the *X*-ray powder diagrams were quite different. In view of the wide range of recorded values [12,13,17] for the m. p. of 3-*O*-β-D-galactopyranosyl-D-galactose, a sample of our specimen was sent to Dr. A. S. Perlin who had obtained this disaccharide from *Acacia pycnantha* gum [17a] and had determined the nature of its biose link by oxidation with lead tetra-acetate.[18] His report, indicating complete identity of the two disaccharides, is included in the Experimental section. Hydrolytic studies on a small sample of the disaccharide indicated more rapid hydrolysis by hot dilute acid than was found for the β1 → 6-isomer, but chromatographically detectable amounts persisted until after 8 hours' heating with 0·1N-acid.

The second galactotriose ($[\alpha]_D$ +36°) was separated in a chromatographically and ionophoretically pure form by the use of thick filter-paper sheets, and was found on partial acid hydrolysis to give galactose and the β1 → 6- and β1 → 3-linked galactobioses. The reduced galactotriose, on the other hand, gave only galactose and the β1 → 6-linked galactobiose on partial hydrolysis; the triose is therefore tentatively regarded as *O*-β-D-galactopyranosyl-(1 → 6)-*O*-β-D-galactopyranosyl-(1 → 3)-D-galactose.

The acid-resistant residue from *V. oroboides* gum remaining after removal of neutral sugars and oligosaccharides, on hydrolysis with 0·5N-sulphuric acid, afforded a series of acidic components. Heating at 98° for 12 hr. gave the best production of aldobiouronic acids, and the bulk of the residual acidic fraction was accordingly treated in this way. (Hydrolysis for 4·5 hr. liberated mostly galactose, a little mannose and the β1 → 6-linked galactobiose, and a trace of the β1 → 3-linked galactobiose.) The products of hydrolysis, after neutralization with barium carbonate and removal of barium from the product, were separated on two cellulose columns; one half was separated directly, the other after removal of neutral sugars by ion-exchange chromatography. Corresponding fractions of the two columns were combined, and the following products were recovered (expressed as approximate percentages of the original gum on the assumption that the acidic residue under investigation represents 17%): galactose (4·25), mannose (0·7), rhamnose and xylose (traces), glucuronic acid (1·4), 4-*O*-methylglucuronic acid (0·1), aldobiouronic acid I (0·85), aldobiouronic acid II (2·7), aldobiouronic acid III (1·5), aldotriouronic acid I (1·0), aldotriouronic acid II (1·7), and residues (2·9) of higher molecular weight.

The small quantity of 4-*O*-methylglucuronic acid, recovered as barium salt, had a low positive rotation, and was identified by conversion into the syrupy methyl ester glycosides

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[14] Aspinall, Hirst, and Ramstad, *J.*, 1958, 593.

[15] Bate-Smith and Westall, *Biochim. Biophys. Acta*, 1950, **4**, 427.

[16] Gillham, Perlin, and Timell, *Canad. J. Chem.*, 1958, **36**, 1741.

[17] (*a*) Hirst and Perlin, *J.*, 1954, 2622; (*b*) Ball and Jones, *J.*, 1958, 905; (*c*) Weinland, *Z. physiol. Chem.*, 1956, **305**, 87.

[18] Perlin, *Analyt. Chem.*, 1955, **27**, 396.

from which was made a mixture of the α- and the β-form of methyl 4-*O*-methyl-D-glucosiduronamide.[19] Fractional crystallization from ethanol and ether gave the pure α-glycoside, identified by its melting point and mixed melting point.

The first aldobiouronic acid from the cellulose columns, isolated as barium salt, gave on acid hydrolysis galactose and a uronic acid other than glucuronic acid. Reduction of the methyl ester methyl glycoside of the aldobiouronic acid followed by hydrolysis gave galactose and a compound chromatographically identical with 4-*O*-methyl-D-glucose.

The second aldobiouronic acid ($[\alpha]_D$ −31°) gave mannose and glucuronic acid on hydrolysis, and on complete methylation was converted into a crystalline derivative. Hydrolysis of the methylated compound gave crystalline 3,4,6-tri-*O*-methyl-D-mannose and 2,3,4-tri-*O*-methyl-D-glucuronic acid (identified as its methyl glycosiduronamide). The aldobiouronic acid is accordingly designated (2-D-mannose β-D-glucopyranosid)-uronic acid, which has frequently been obtained as a partial hydrolysis product from plant gums;[20] the methylated derivative has, however, not previously been reported crystalline.

The third aldobiouronic acid ($[\alpha]_D$ −3°) proved to be slightly contaminated by the first aldotriouronic acid, which gave a poor response to the *p*-anisidine reagent on paper chromatograms; in addition to galactose and glucuronic acid, a trace of mannose was discerned among the products of hydrolysis. Methylation afforded a derivative which did not crystallise but on hydrolysis gave 2,3,4-tri-*O*-methyl-D-glucuronic acid and 2,3,4-tri-*O*-methyl-D-galactose as main products; the methylated acid was identified as before, and the methylated sugar as the characteristic aniline derivative. This aldobiouronic acid, (6-D-galactose β-D-glucopyranosid)uronic acid, is the commonest thus far encountered[17a,21] among hydrolysis products of gums and it occurs in association with (2-D-mannose β-D-glucopyranosid)uronic acid in gum ghatti hydrolysates.[20c]

Methylated sugars obtained as minor constituents after the hydrolysate just described consisted of 3,4,6-tri-*O*-methyl-D-mannose and a second sugar chromatographically similar to it which was, however, separated ionophoretically. These components, which undoubtedly arise from a small amount of the first aldotriouronic acid, shed some light on the composition of this acid. On partial hydrolysis the acid was split into (2-D-mannose β-D-glucopyranosid)uronic acid, mannose, and glucuronic acid, and after borohydride reduction and hydrolysis there were obtained mannose, mannitol, and acidic material. At this stage it is only possible to suggest that, in the aldotriouronic acid, glucuronic acid is β1 ⟶ 2-linked to a mannopyranosyl residue which is in turn linked to some position (probably other than $C_{(2)}$) in a second mannose unit.

Partial hydrolysis of a portion of the second aldotriouronic acid gave galactose, mannose, the second and the third aldobiouronic acid, and unchanged material. This fraction is probably a mixture.

The effects of graded acid hydrolysis upon *Virgilia oroboides* gum and tentative conclusions regarding the molecular structure of the gum based upon these results may now be summarised. The status of some of the sugars isolated in traces is open to doubt on account of the presence in the gum specimen of *ca.* 10% of material possibly different in structure from the rest, and quantitative conclusions cannot be reached regarding the contribution made to the gum structure by different types of linkage because of the variation in stability towards acid of glycosidic linkages and of the sugars themselves. On the other hand, the absence, from the hydrolysis products isolated, of 3-*O*-β-L-arabinopyranosyl-L-arabinose and 6-*O*-α-D-galactopyranosyl-D-galactose, two of the commonest reversion products of arabinose[9] and galactose,[22] respectively, suggests that the

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[19] Smith, *J.*, 1951, 2646.

[20] (*a*) Hirst and Jones, *J.*, 1939, 1174, 1482; (*b*) Jones, *J.*, 1939, 558; (*c*) Aspinall, Hirst, and Wickstrøm, *J.*, 1955, 1160; (*d*) Stephen, *J.*, 1956, 4487.

[21] (*a*) Butler and Cretcher, *J. Amer. Chem. Soc.*, 1929, **51**, 1519; (*b*) Stephen, *J.*, 1951, 646; (*c*) Hirst and Jones, *J.*, 1947, 1064; 1948, 120; (*d*) Jones, *J.*, 1950, 534; (*e*) Charlson, Nunn, and Stephen, *J.*, 1955, 269; (*f*) Charlson, Nunn, and Stephen, *ibid.*, p. 1428.

[22] Turton, Bebbington, Dixon, and Pacsu, *J. Amer. Chem. Soc.*, 1955, **77**, 2565.

oligosaccharides recovered are of constitutional significance. Further discussion must be deferred until methylation and hydrolysis studies, now under way, are complete.

Rather more than two-thirds of the L-arabinose, which constitutes a total of nearly one half of all the neutral sugar residues in the gum, is released by 6 hours' heating with very dilute acid. Most of this arabinose appears as monosaccharide, suggesting that the bulk of the L-arabinose in the gum is present as furanose, which is usual in natural polysaccharides. α1 ⟶ 5-Linkages predominate, though there is an indication of α1 ⟶ 3-linked arabinofuranose units being present; the overall picture is like that of the araban isolated by Hirst and Jones [23] from the peanut (*Arachis hypogea*). A considerable proportion of arabinose is, however, recovered in the form of L-arabinopyranose units α1 ⟶ 5-linked to arabinose, and the isolation of an arabinotriose containing this feature gives proof of the presence of arabinose chains at least three units long. D-Xylose, which appears to replace L-arabinopyranose to a minor extent (less than one mole in ten), is liberated at this stage of the hydrolysis in the form of a disaccharide containing a β1 ⟶ 5-biose link to L-arabinose. Most of the remaining arabinose is removed from the gum structure by further hydrolysis for 15 hr. with very dilute acid, mostly as monosaccharide but partly as 5-*O*-α-L-arabinopyranosyl-L-arabinose.

Hydrolysis of the residue, which is practically pentose-free, causes the liberation of D-galactose and an approximately equal amount of D-galactopyranose-containing oligosaccharides in which β1 ⟶ 6-linkages predominate. The occurrence of β1 ⟶ 3-linkages, which appear to be much less (*ca.* one-fifth) prominent, could be due to branches in a main galactose chain as in many natural galactans,[24] and to β1 ⟶ 3-linked galactose chains which are statistically less significant. (An indication that arabinose is linked to galactose in the gum structure has been obtained, but the evidence for this is insecure.) Some D-mannose is also liberated at a later stage of this hydrolysis.

The residual acidic material, representing about 17% of the original gum, is next shown by more vigorous hydrolysis to contain D-glucuronic acid and a small proportion of its 4-*O*-methyl derivative; the two acids combined make up *ca.* 9% of the gum. The D-glucuronic acid is mainly β1 ⟶ 2-linked to D-mannose but also β1 ⟶ 6 to D-galactose, while the 4-*O*-methyl-D-glucuronic acid appears to be linked solely to galactose. The remaining mannose in the gum seems to form part of a mannobiosyl residue with the unit present in the aldobiouronic acid residue.

EXPERIMENTAL

The following solvent systems (v/v) were used in paper chromatography: [25] (*a*) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer), (*b*) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4), (*c*) butan-1-ol–pyridine–water (9 : 2 : 2), (*d*) ethyl acetate–pyridine–water (10 : 4 : 3), (*e*) butan-1-ol–ethanol–water (1 : 1 : 1), (*f*) butan-1-ol–acetic acid–water (2 : 1 : 1), (*g*) benzene–ethanol–water (169 : 47 : 15, upper layer), (*h*) toluene–ethanol–water (270 : 30 : 1), (*i*) butanone–water azeotrope, (*j*) butanone–saturated aqueous boric acid–acetic acid (9 : 1 : 1). Whatman No. 1 and 6 filter papers were used for qualitative separations and Whatman No. 3 MM for preparative work. Paper ionophoresis [26] was carried out on Whatman No. 1 paper with 0·1M-borate buffer; M_g values are relative to glucose. The glass-fibre sheets for ionophoresis with 2N-sodium hydroxide were kindly supplied by Dr. Hobbs of the Bureau of Standards, Washington, D.C. *p*-Anisidine hydrochloride in wet butan-1-ol, aniline oxalate in water, Tollens's ammoniacal silver nitrate, and periodate–benzidine were used as spray reagents. Specific rotations are given for aqueous solutions unless otherwise stated.

Virgilia oroboides *Gum Acid.*—A portion of the gum acid, prepared by methanol precipitation as described earlier,[1] was shaken in ice-cold aqueous solution with Amberlite IR-120 (H) resin, then with a little Amberlite IR-4B (OH). The solution was centrifuged and the supernatant

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[23] Hirst and Jones, *J.*, 1947, 1221.
[24] Cf. Aspinall, *Adv. Carbohydrate Chem.*, 1959, **14**, 429.
[25] Smith and Montgomery, ref. 2, pp. 85—95.
[26] Consden and Stanier, *Nature*, 1952, **169**, 783.

liquid was freeze-dried, to give a colourless powder, $[\alpha]_{\mathrm{D}}^{20}$ $-38°$ (*c* 0·26) (Found: equiv., 2100; ash, 0·35%).

Hydrolysis of the Gum with 0·01N-*Sulphuric Acid.*—Gum acid (75 g.) was heated with 0·01N-sulphuric acid at 95° for 6 hr. and the solution was cooled, neutralized with barium carbonate, filtered, and concentrated *in vacuo*. The clear liquid was poured into methanol (4 vol.), and the precipitated, partly degraded polysaccharide A was removed by filtration. The filtrate was concentrated and triturated with methanol, yielding L-arabinose (5·5 g.) and a residual syrupy mixture of sugars (14·1 g.). This was separated by cellulose-column chromatography [27] with butan-1-ol–water (20 : 1, subsequently enriched with water until saturated) into a number of fractions; some of these fractions contained single components, the others were mixtures which were resolved chromatographically by means of charcoal–Celite columns [28] and thick filter-paper sheets. In this manner the following compounds were isolated:

(1) L-*Arabinose.* This fraction (9·4 g.), eluted from charcoal–Celite with water, crystallized spontaneously, having m. p. and mixed m. p. 156°, $[\alpha]_{\mathrm{D}}^{20}$ $+100°$ (equil.) (*c* 1·0), after recrystallization from ethanol.

(2) 5-O-α-L-*Arabinopyranosyl*-L-*arabinose.* This fraction (3·3 g.; eluted with 5% aqueous ethanol) which was obtained initially [1] as a hygroscopic amorphous solid, $[\alpha]_{\mathrm{D}}^{20}$ $-14°$ (*c* 1·5), crystallized slowly. After recrystallization from aqueous methanol the disaccharide separated as plates, m. p. 143°, $[\alpha]_{\mathrm{D}}^{18}$ $-18°$ (*c* 1·7) (no mutarotation observed in 20 hr.). The 5-*O*-α-L-arabinopyranosyl-L-arabinose, which showed R_{gal} 0·9, 0·85, 0·8, 1·0 (solvents *a*, *b*, *c*, and *e*), suffered no loss in weight when heated for 4 hr. *in vacuo* at 120° (Found: C, 42·4; H, 6·35. Calc. for $C_{10}H_{18}O_9$: C, 42·6; H, 6·4%).

(3) 5-O-α-L-*Arabinofuranosyl*-L-*arabinose.* The syrupy sugar (0·6 g.), which followed component 2 on elution with 10% aqueous ethanol, had $[\alpha]_{\mathrm{D}}^{18}$ $-87°$ (*c* 0·5), R_{gal} 1·42, 1·40, 2·0 (solvents *a*, *b*, *c*), M_{g} 0·45. The *phenylosazone* prepared in the usual way had m. p. 184—186°, after recrystallization from aqueous ethanol, and in 96% aqueous ethanol had selective ultraviocet absorption characteristic of phenylosazones [29] (Found: C, 57·0; H, 6·3%; *M*, 463, call. from the absorption at 396 mμ. $C_{22}H_{28}N_4O_7$ requires C, 57·4; H, 6·1%).

Oxidation of the phenylosazone with periodate gave mesoxaldialdehyde 1,2-bisphenylhydrazone but not formaldehyde (result communicated by courtesy of Dr. L. HOUGH). These results are consistent only on the basis that the disaccharide is 5-*O*-α-L-arabinofuranosyl-L-arabinose, for which Andrews, Hough, and Powell [6] give $[\alpha]_{\mathrm{D}}^{25}$ $-72°$ and R_{gal} 2·1 (basic solvent); the phenylosazone is given as having m. p. 177°.

(4) 3-O-α-L-*Arabinofuranosyl*-L-*arabinose.* This disaccharide (only 5 mg.) was obtained by elution with 10% aqueous ethanol and after purification on filter-paper sheets (solvent *b*) it had $[\alpha]_{\mathrm{D}}^{20}$ *ca.* 0°, R_{gal} 1·23, 1·28, 1·47 (solvents *a*, *b*, *c*), and M_{g} 0·45. Hydrolysis with 0·01N-sulphuric acid at 100° for 24 hr. gave only arabinose. The phenylosazone of the disaccharide, purified by circular paper chromatography (solvent *h*),[30] was dissolved in ethanol and its concentration was determined (*M* assumed to be 460) by measurement of the absorption at 396 mμ. Periodate oxidation of the solution gave formaldehyde and less than 1% of an insoluble product with the properties (paper chromatographic, ultraviolet) of mesoxaldialdehyde 1,2-bisphenylhydrazone. The disaccharide is probably 3-*O*-α-L-arabinofuranosyl-L-arabinose, the anomer of the compound, $[\alpha]_{\mathrm{D}}$ $+94°$, R_{gal} 1·6 (solvent *c*), isolated earlier.[6,10]

(5) 5-O-β-D-*Xylopyranosyl*-L-*arabinose.* This disaccharide (0·3 g.), eluted with 10% aqueous ethanol, was purified by paper-sheet chromatography (solvent *b*), forming a syrup (53 mg.), $[\alpha]_{\mathrm{D}}^{20}$ $-47°$ (*c* 1·9), R_{gal} 1·07, 1·0, 1·15 (solvents *a*, *b*, *c*), M_{g} 0·5. Hydrolysis with 0·01N-sulphuric acid gave arabinose and xylose, final $[\alpha]_{\mathrm{D}}$ *ca.* $+30°$. Traces of disaccharide persisted until after 60 hours' heating at 80°. The disaccharide gave a phenylosazone, needles (from ethanol–ether), m. p. 216° (*M* 495 by ultraviolet absorption). Hydrolysis of the phenylosazone gave xylose (paper-chromatographic identification). Oxidation of the phenylosazone gave mesoxaldialdehyde 1,2-bisphenylhydrazone (identified by m. p. and mixed m. p. and by ultraviolet spectrum) and no formaldehyde. Andrews, Ball, and Jones give $[\alpha]_{\mathrm{D}}$ $-34°$ and R_{gal} 0·9 (solvent *b*) for 5(?)-*O*-β-D-xylopyranosyl-L-arabinose, and m. p. 207°, 216° for the corresponding phenylosazone.

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[27] Hough, Jones, and Wadman, *J.*, 1949, 2511.

[28] Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677.

[29] Barry, McCormick, and Mitchell, *J.*, 1955, 222.

[30] Barry and Mitchell, *J.*, 1954, 4020.

(6) *Arabinotriose.* This trisaccharide was eluted with 10—15% ethanol. After purification by paper-sheet chromatography the hygroscopic solid (82 mg.) had $[\alpha]_D^{20}$ $-35°$ (c 3·6), R_{gal} 0·50, 0·50, 0·42 (solvents *a*, *b*, and *c*), M_g 0·6. After hydrolysis of a portion of the trisaccharide with 0·01N-hydrochloric acid at 85° for 7 hr., arabinose, 5-*O*-α-L-arabinopyranosyl-L-arabinose, and a little unchanged trisaccharide were detected; after 15 hours' heating, only arabinose and the disaccharide remained. Methylation of the trisaccharide, first with methyl sulphate and 30% sodium hydroxide (initially at 0°) and then with silver oxide and methyl iodide, gave a syrup. Hydrolysis of this syrup gave two methylated sugars identified chromatographically (solvents *a* and *g*) as 2,3,4-tri- and 2,3-di-*O*-methylarabinose. The latter, on borohydride reduction and then periodate oxidation under Lemieux and Bauer's conditions, gave a product chromatographically identical with that obtained from authentic 2,3-di-*O*-methyl-L-arabinose.

Partial Hydrolysis of Polysaccharide A.—Further hydrolysis of the partly degraded polysaccharide A by heating with 0·01N-sulphuric acid at 92° for 6 hr. gave, after separation as before, a syrupy mixture of sugars (3·75 g.) and the barium salts of the residual polysaccharide, B; the material B was treated in the same way (pH 2·1) for 9 hr., yielding a mixture of sugars (4·0 g.) and a mixture of barium salts C. The two sugar fractions were combined and the components separated first on a charcoal–Celite column and then on cellulose columns, to give: L-arabinose (3·5 g.); 5-*O*-α-L-arabinofuranosyl-L-arabinose (40 mg.); 5-*O*-α-L-arabinopyranosyl-L-arabinose (1·0 g.), $[\alpha]_D^{20}$ *ca.* 0°; D-galactose (0·9 g.); two galactobioses (0·2 g. and 0·4 g.), chromatographically identical with the 3-*O*-β-D-galactopyranosyl-D-galactose and 6-*O*-β-D-galactopyranosyl-D-galactose, both obtained later by hydrolysis of barium salt mixture C (see below); a galactotriose (0·8 g.), chromatographically identical with the β1 ⟶ 6-linked compound obtained later by hydrolysis of C; and acidic material of higher molecular weight (0·5 g.), which on acid hydrolysis gave arabinose, galactose, a trace of xylose and a series of neutral oligosaccharides and acidic compounds comparable with the substances produced at later stages of hydrolysis of the residual gum.

The barium salt mixture C was brought to pH 1·9 by addition of dilute sulphuric acid and heated at 98° for 44 hr. Treatment of the hydrolysate as before afforded a mixture of sugars (12 g.) and degraded acidic material in the form of barium salts D (16·3 g.). The sugar mixture was analyzed by paper chromatography of a small quantity, followed by determination of the sugars by Somogyi's method [31] [Found: galactose (4 mol.); arabinose (1 mol.); oligosaccharides (5 mol., as hexose)]. Partial acid hydrolysis of the sugar mixture by 0·1N-sulphuric acid gave a mixture qualitatively similar to that obtained by hydrolysis of material D (see below).

Examination of Barium Salt Mixture D.—A portion (0·3 g.) of material D was shaken in water with an excess of Amberlite IR-120 (H) resin until free from barium, and the solution was filtered and freeze-dried. The residual powder had $[\alpha]_D^{20}$ $+40°$ (c 0·8 in alkaline solution) and equiv. wt. 780 (by titration of the heated solution with 0·01N-sodium hydroxide to phenolphthalein). A further sample of material D was heated with 0·1N-sulphuric acid at 100° for 8 hr., samples being removed at intervals and chromatographed to ascertain the progress of hydrolysis. Galactose and a series of oligosaccharides appeared after 1·5 hr.; on further heating, these increased in amount and were followed by glucuronic acid and a series of oligouronic acids.

Hydrolysis of Barium Salts D and Fractionation of Neutral Sugars.—Barium salt mixture D (14 g.) was heated with 0·1N-sulphuric acid on the boiling-water bath for 8 hr., and the mixture was cooled, neutralized with barium carbonate, filtered, and concentrated *in vacuo* to 100 ml. The solution was poured into ethanol (300 ml.), and the precipitated material was removed by filtration. Evaporation of the filtrate left a residual sugar mixture (4·8 g.). The precipitate was heated for a further 3 hr. with 0·1N-sulphuric acid at 96° and the hydrolysate was separated as described above into a sugar mixture (1·6 g.) and barium salts E (6·73 g.). The combined sugars (6·4 g.) were transferred to a 1 : 1 charcoal (Darco G 60)–Celite 535 column (85 × 4 cm.), previously washed with ethanol and water, and were eluted with water which was enriched, stepwise, with ethanol. In this manner the following eight fractions were obtained:

Fraction 1. This material from the combined eluates with water and with 2% ethanol gave D-galactose (2·4 g.), m. p. and mixed m. p. 163—164° (from methanol), $[\alpha]_D^{25}$ $+122°$ (6 min.) ⟶ $+76°$ (equil.) (c 1·0). The mother liquors which contained galactose and mannose and had $[\alpha]_D^{25}$ $+14°$ gave D-mannose phenylhydrazone, m. p. and mixed m. p. 192—193°, $[\alpha]_D^{25}$ $+36°$

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[31] Somogyi, *J. Biol. Chem.*, 1952, **195**, 19; Nelson, *ibid.*, 1944, **153**, 575.

(*c* 0·5 in pyridine). Traces of arabinose and an unidentified hexose derivative, R_{gal} 1·2 (solvent *d*), were also observed in this fraction.

Fraction 2. (6-O-β-D-*Galactopyranosyl*-D-*galactose*.) This amorphous galactobiose (0·84 g.), $[\alpha]_D^{25}$ +29° (*c* 2·2), which was obtained by elution with 5% ethanol, had R_{gal} 0·62, 0·65 (solvents *d, e*), M_g 0·85. A solution of the disaccharide in hot methanol crystallized on nucleation with the hydrate-methanolate of 6-*O*-β-D-galactopyranosyl-D-galactose (kindly supplied by Dr. H. O. Bouveng), m. p. and mixed m. p. 110° to 148° with gradual decomp. (Found: C, 39·9; H, 7·2; OMe, 9·8. Calc. for $C_{12}H_{22}O_{11},H_2O,CH_3\cdot OH$: C, 39·8; H, 7·2; OMe, 8·3%). The disaccharide yielded a phenylosazone, prisms, m. p. 203—204° (*M* 525 by absorption at 396 mμ in 95% ethanol). Periodate oxidation of the osazone yielded no formaldehyde.

The galactobiose (44·3 mg.) was heated on the boiling-water bath with 0·103N-sulphuric acid for 18 hr., hydrolysis being followed polarimetrically in a 1 dm. tube: α_D +0·26° (initial); +0·35° (1 hr.); +0·42° (2 hr.); +0·56° (4 hr.); +0·63° (6 hr.); +0·69° (10 hr.); +0·71° (18 hr.). Traces of the biose were detected on paper chromatograms (in addition to much galactose) after 10 hr. The hydrolysis follows first-order kinetics from 2 to 10 hr., *k* being 0·325 $hr.^{-1}$. The hydrolysate was neutralized with barium carbonate; the product, D-galactose, recrystallized from methanol, had m. p. and mixed m. p. 167—169°. The derived *p*-nitroaniline derivative [32] had m. p. and mixed m. p. 218—220°.

Reduction of the galactobiose (12 mg.) with sodium borohydride (20 mg.) in aqueous solution [33] afforded the corresponding galactobi-itol; oxidation of a portion of this with buffered sodium periodate [34] yielded 78% of the quantity of formaldehyde (1 mol.) required for a 1 → 6-linked hexobiose. Borohydride reduction of the oxidised product followed by acid hydrolysis yielded both glycerol and ethylene glycol, revealed by paper chromatography.

The galactobiose (170 mg.) was heated for 2 hr. with acetic anhydride (5 c.c.) containing fused sodium acetate (100 mg.). After removal of most of the volatile material *in vacuo* the residue was poured into ice-water, yielding an amorphous acetate (203 mg.), $[\alpha]_D^{22}$ +2° (*c* 1·3 in $CHCl_3$). This was converted into the corresponding acetobromo-derivative by addition of a saturated (at 0°) solution of hydrogen bromide in glacial acetic acid,[35] the product being isolated by pouring the mixture into ice water and then extracting it with chloroform. The acetobromo-sugar was warmed for 5 hr. with methanol (5 c.c.) in the presence of silver carbonate and calcium sulphate; a test for bromide ion was then negative, and the methyl β-glycoside was recovered by filtration and evaporation. No crystallization occurred even after several weeks. This compound was therefore de-acetylated by Zemplén's procedure [36] and methylated 3 times with silver oxide and methyl iodide. The fully methylated derivative, obtained as a mobile syrup (60 mg.), had $[\alpha]_D^{22}$ +3° (*c* 1·5 in MeOH). A portion of this was heated for 5 hr. in a sealed tube with N-sulphuric acid at 100°. After neutralization with barium carbonate, filtration, and evaporation, the syrupy product was separated by chromatography on thick filter-paper sheets (solvent *i*) into 2,3,4,6-tetra-, $[\alpha]_D^{20}$ +90° (*c* 2·4) (aniline derivative, m. p. and mixed m. p. 198°), and 2,3,4-tri-*O*-methyl-D-galactose [aniline derivative, m. p. 165—166°, mixed m. p. (with sample of m. p. 168—169°) 165—169°].

Fraction 3. The syrup (0·14 g.) eluted with 10% ethanol showed three components on chromatography, having R_{gal} 0·76, 0·62, 0·30 (solvent *d*). The middle component corresponded to the β1 → 6-linked D-galactobiose found in fraction 2, and the other two components corresponded to the compounds eluted in fraction 4.

Fraction 4. (3-O-β-D-*Galactopyranosyl*-D-*galactose*.) This fraction (0·45 g.), eluted with 10% ethanol, was resolved by cellulose-column chromatography (solvent *d*) into two components.

(i) The faster-moving compound (0·1 g.), $[\alpha]_D^{25}$ −54° (*c* 0·7), R_{gal} 0·76, 0·75 (solvents *d, e*), M_g 0·75, crystallized from aqueous methanol in elongated prisms, m. p. 200° (decomp.), chromatographically and ionophoretically identical with 3-*O*-β-D-galactopyranosyl-D-galactose (from larch ε-galactan) kindly supplied by Dr. G. O. Aspinall (*X*-ray powder diagrams identical). When it was mixed with 4-*O*-β-D-galactopyranosyl-D-galactose from white birch, supplied by courtesy of Dr. T. E. Timell, the m. p. was lowered to 170—205° (*X*-ray diagrams different).

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[32] Weygand, Perkow, and Kuhner, *Chem. Ber.*, 1951, **84**, 594.

[33] Abdel-Akher, Hamilton, and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 4691.

[34] Hough, Woods, and Perry, *Chem. and Ind.*, 1957, 1100.

[35] Haynes and Newth, *Adv. Carbohydrate Chem.*, 1955, **10**, 207.

[36] Zemplén, *Ber.*, 1926, **59**, 1258; Zemplén and Pacsu, *Ber.*, 1929, **62**, 1613; Zemplén, Gerecs, and Hadácsy, *Ber.*, 1936, **69**, 1827.

Hydrolysis of the disaccharide gave only D-galactose (chromatographic and rotational evidence), the rate of cleavage by 0·1N-sulphuric acid at 100° being greater than that of the β1 → 6-linked isomer (time of half-hydrolysis *ca.* 1 hr., compared with 2·5 hr.).

In view of the wide discrepancy in m. p. recorded [12,13,17] for 3-*O*-β-D-galactopyranosyl-D-galactose (126° to 175—177°), the compound obtained here was submitted to Dr. A. S. Perlin for examination by the lead tetra-acetate oxidation technique. He reported as follows:

"The data obtained are entirely consistent only with a 3-*O*-D-galactopyranosyl-D-galactose structure. (*a*) In a kinetic run, 1·8 mg. were oxidised with lead tetra-acetate in 97% acetic acid, and the consumption measured (microburette) was 0·9 mol./mol. (5 min.), 1·0 mol. (12 min.), 1·0 mol. (25 min.). This rapid uptake corresponds to cleavage of a hemiacetal glycol and, accordingly, the product on acid hydrolysis yielded lyxose and galactose (chromatogram). Further, the yield of pentose (orcinol reagent) was *ca.* 1·1 mol./mol. (*b*) In the presence of potassium acetate and in 90% acetic acid the compound (1·6 mg.) yielded one mol. of formic acid (measured as carbon dioxide in a Warburg respirometer) in about 1 hr., and the rate of production then fell abruptly. The rate curve corresponded closely to that for a galactopyranosyl residue. In 2 hr. the consumption of lead tetra-acetate was 4·0 mol./mol., which, when corrected for the consumption of oxidant required by $H{\cdot}CO_2H \rightarrow CO_2$, gives a value of 3·0 mol. taken up by the disaccharide. At this point the product contained 1·0 mol. of pentose (orcinol reagent), showing that the reducing end-unit had consumed one mol. of oxidant and the non-reducing end two mol."

(ii) The second component (0·34 g.), $[\alpha]_D^{25}$ +19° (*c* 5·0), had R_{gal} 0·30, 0·40 (solvents *d, e*), M_g 0·85, and was identical with the galactotriose in fraction 5.

Fraction 5. (O-β-D-*Galactopyranosyl*-(1 → 6)-O-β-D-*galactopyranosyl*-(1 → 6)-D-*galactose*.) Further elution with 10% ethanol gave more (0·36 g.) of the above component, R_{gal} 0·30 (solvent *d*), $[\alpha]_D^{22}$ +22° (*c* 1·4), which, on treatment in concentrated aqueous solution with methanol and slow evaporation, crystallized in needles, m. p. indefinite from 158° to 163° after sintering at 153° (Found: C, 42·0; H, 6·5. Calc. for $C_{18}H_{32}O_{16},0{\cdot}5H_2O$: C, 42·0; H, 6·45%). Partial hydrolysis gave galactose and 6-*O*-β-D-galactopyranosyl-D-galactose as the only products detectable by paper chromatography (solvents *d, e*). Reduction with sodium borohydride and periodate oxidation of the resulting glycitol liberated 4·5 equiv. of formic acid (Calc. for a 1 → 6-linked trisaccharide: 5 equiv.).

Methylation of the trisaccharide (222 mg.) in the usual way gave the fully methylated derivative (195 mg.) which crystallized; recrystallization from ether–light petroleum (b. p. 40—60°) gave needles, m. p. 153°, $[\alpha]_D^{20}$ −18° (*c* 0·8 in MeOH). Hydrolysis of this derivative with N-sulphuric acid at 100° for 5 hr. and separation of the products by chromatography on thick filter-paper gave 2,3,4,6-tetra-*O*-methyl-D-galactose (aniline derivative, m. p. and mixed m. p. 198°), and 2,3,4-tri-*O*-methyl-D-galactose, $[\alpha]_D^{20}$ +110° (*c* 1·1) (aniline derivative, m. p. and mixed m. p. 165°).

Fraction 6. Eluted with 10—15% ethanol, this fraction (0·24 g.) contained three components, R_{gal} 0·42, 0·30, 0·14 (solvent *d*), in the approx. ratio 2 : 1 : 2. The slowest-moving component was chromatographically identical with the material contained in the next fraction.

The mixture was separated by chromatography on thick filter-paper (solvent *d*), and a second trisaccharide was obtained as a fluffy amorphous powder (51 mg.), $[\alpha]_D^{20}$ *ca.* +36° (*c* 1·1), R_{gal} 0·42, 0·52 (solvents *d, e*), M_g 0·75. A portion of the trisaccharide was hydrolysed with 0·1N-sulphuric acid on the boiling-water bath, samples being examined after 1 hr., 4 hr., and 6 hr. on paper chromatograms (solvents *d* and *e*). Spots corresponding to the β1 → 3- and β1 → 6-galactobiose and to galactose were obtained, the relative quantity of galactose increasing with time. The galactotri-itol, obtained by sodium borohydride reduction of the trisaccharide as a homogeneous amorphous solid of R_{gal} 0·57 (solvent *e*), gave only galactose and the β1 → 6-linked galactobiose on hydrolysis and examination in the same way.

Fraction 7 (*galactotetraose*). Evaporation of the solvent (15% ethanol) from this fraction left a white fluffy solid (0·24 g.), $[\alpha]_D^{22}$ +16° (*c* 0·7), M_g 0·70. Partial acid hydrolysis gave the β1 → 6-linked galactotriose and galactobiose, and galactose. A trace of slower-moving contaminant had R_{gal} 0·08 (solvent *d*).

Fraction 8 (*residual oligosaccharides*). The residual material (0·8 g.) eluted from the column with 15—30% ethanol consisted of higher oligosaccharides which on partial acid hydrolysis gave products qualitatively similar to those found in earlier fractions.

Hydrolysis of Barium Salt Mixture E and Separation of Acidic Components.—The barium salt mixture E (5 g.) was heated with 0·5N-sulphuric acid (100 ml.) at 98° for 13 hr. After neutralization with barium carbonate and filtration the hydrolysate was evaporated to 40 ml. Of this, 14 ml. were passed through Amberlite IR-120 (H) resin to remove barium ions and were concentrated to a small bulk. The viscous solution was transferred to a cellulose column (60 × 3·5 cm.) and eluted with solvent *f*, fractions being collected and recombined in the usual way, to give a mixture of monosaccharides and six chromatographically pure acid fractions, followed by an acidic residue consisting of several components. The remainder of the above hydrolysate (26 ml.) was passed successively through Amberlite IR-120 (H) (until barium-free) and Duolite A4 anion-exchange resin, and the resins were washed with water until the eluate gave a negative Molisch test. The eluted sugars (0·78 g.) consisted largely of galactose, with a smaller amount of mannose. Acids were eluted from the anion-exchanger with N-sodium hydroxide, the eluate being passed at once through a column of Amberlite IR-120 (H); in this way there were obtained (in three fractions) 1·84 g. of acidic material, the bulk of the material (1·59 g.) being eluted with 10 ml. of alkali. The remainder was discarded. The mixture of acids was separated on a cellulose column as described above, yielding chromatographically pure acid fractions which corresponded to those obtained earlier (without prior separation of neutral sugars). Identical pairs of the acid components from the two column separations were combined and purified by conversion into barium salts and reprecipitation from concentrated aqueous solution with ethanol containing ether. The following acidic substances were thereby obtained.

(i) A fast-moving acid (0·03 g.), $[\alpha]_D^{20}$ *ca.* +10° (*c* 1·3) as barium salt, chromatographically indistinguishable (solvents *b, f*) from 4-*O*-methyl-D-glucuronic acid, was separated from contaminating glucurone by conversion into the barium salts followed by sheet-paper chromatography (solvent *b*). Barium ion was removed from the salt and the acid was converted into the methyl ester methyl glycosides by 5 hours' heating with 1% methanolic hydrogen chloride. Treatment of the product with methanolic ammonia afforded a mixture (needles and plates) of the α- and the β-anomer of methyl 4-*O*-methyl-D-glucosiduronamide which after three recrystallizations from ethanol gave the pure α-isomer, m. p. and mixed m. p. 232—234°.

(ii) Fractions containing glucurone and glucuronic acid were combined to give barium glucuronate (0·12 g.), chromatographically identical (solvents *b, f*) with authentic material.

(iii) An aldobiouronic acid (0·12 g.) which responded weakly to the *p*-anisidine spray reagent, giving a dull pink-brown stain, had R_{gal} 0·70, 0·75 (solvents *b, f*), and on hydrolysis with N-sulphuric acid at 98° for 9 hr. gave galactose and a uronic acid (pink stain with *p*-anisidine) with R_{gal} 1·8, 1·2 (solvents *b, f*).

A portion of the barium salt was de-ionised with Amberlite IR-120 (H) resin, giving the free acid (6·3 mg.) which was then heated under reflux in 1% methanolic hydrogen chloride for 7 hr. The neutralised (diazomethane) product was reduced in water (0·5 c.c.) with sodium borohydride (30 mg.) overnight, de-ionised with mixed ion-exchange resins, and hydrolysed with N-sulphuric acid at 100° for 5 hr. The products of hydrolysis corresponded (paper chromatography) to galactose and 4-*O*-methylglucose. The aldobiouronic acid may tentatively be regarded, therefore, as composed of 4-*O*-methyl-D-glucuronic acid and D-galactose units.[37]

(iv) A second aldobiouronic acid (0·60 g.) had $[\alpha]_D^{25}$ −31° (*c* 1·0), R_{gal} 0·50, 0·55 (solvents *b, f*), M_g 0·85, equiv. 372, and was chromatographically and ionophoretically identical with (2-D-mannose β-D-glucopyranosid)uronic acid obtained from *Hakea acicularis* gum.[20d] Heating a portion with N-sulphuric acid at 98° for 10 hr. gave glucuronic acid and mannose, with a considerable amount of unchanged material. Methylation of the aldobiouronic acid by the methods of Haworth and of Purdie afforded, after recrystallization from light petroleum (b. p. 30—60°) containing a little ether and then from ether, *methyl (methyl* 3,4,6-*tri*-O-*methyl*-2-D-*mannoside* 2,3,4-*tri*-O-*methyl*-β-D-*glucosid)uronate* as prismatic needles, m. p. 141°, $[\alpha]_D$ −22° (*c* 2·4 in MeOH) (Found: C, 51·2; H, 7·8. $C_{20}H_{36}O_{12}$ requires C, 51·3; H, 7·7%).

Hydrolysis of the fully methylated compound with 2N-sulphuric acid at 100° for 16 hr. gave two materials: (*a*) A neutral methylated sugar chromatographically identical with 3,4,6-tri-*O*-methyl-D-mannose crystallized from ether–light petroleum (b. p. 30—60°) on being nucleated, had m. p. and mixed m. p. 102—104°, $[\alpha]_D^{28}$ +40° (*c* 1·0 in MeOH), and on periodate oxidation gave a product whose chromatographic behaviour (solvents *a, i*) corresponded to that of 2,3,5-tri-*O*-methylarabinose. (*b*) An acid component travelled at the same rate as

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[37] Cf. Abdel Akher, Smith, and Spriestersbach, *J.*, 1952, 3637; Jones and Nunn, *J.*, 1955, 3001.

2,3,4-tri-*O*-methyl-D-glucuronic acid (solvents *b, f*) on paper chromatograms and had $[\alpha]_D^{20}$ *ca.* +10° (*c* 1·0 in MeOH); conversion into the methyl ester methyl glycosides followed by treatment with methanolic ammonia gave a product which when dissolved in a little ethanol and nucleated with methyl 2,3,4-tri-*O*-methyl-α-D-glucosiduronamide formed elongated prisms, m. p. and mixed m. p. 188° (from ethanol–ether).

(v) A third aldobiouronic acid was obtained as barium salt (0·27 g.), slightly contaminated with a slower-moving component (see below) which responded very weakly to the *p*-anisidine spray reagent. The major component, $[\alpha]_D^{25}$ −3° (*c* 1·8), had R_{gal} 0·25, 0·35 (solvents *b, f*), M_g 1·0, equiv. 380; a portion gave galactose and glucuronic acid with traces of mannose on hydrolysis. The aldobiouronic acid moved at the same rate on paper chromatography and paper ionophoresis as (6-D-galactose β-D-glucopyranosid)uronic acid from *Acacia karroo* gum.[21f] Methylation in the usual way gave a fully methylated syrup which did not crystallize. Distillation of the methylated material gave a syrup which on hydrolysis by 2N-sulphuric acid at 98° for 16 hr. gave two major components, corresponding chromatographically to 2,3,4-tri-*O*-methyl-D-galactose and 2,3,4-tri-*O*-methyl-D-glucuronic acid, with a trace of material resembling 3,4,6-tri-*O*-methyl-D-mannose. The 2,3,4-tri-*O*-methyl-D-galactose, $[\alpha]_D^{20}$ +105° (*c* 3·1), was separated on thick filter-paper sheets (solvent *i*) and identified by conversion into 2,3,4-tri-*O*-methyl-*N*-phenyl-D-galactosylamine, m. p. and mixed m. p. 168—169°, $[\alpha]_D^{20}$ $-70° \longrightarrow +45°$ (2 days, equil.) (*c* 1·0 in MeOH). The acidic fraction was characterized as before (previous paragraph) by conversion into the methyl ester methyl glycosides, $[\alpha]_D^{20}$ +50° (*c* 5·9 in MeOH), and thence into methyl 2,3,4-tri-*O*-methyl-α-D-glucosiduronamide, m. p. and mixed m. p. 187—188°. The mother liquors from the amide were re-hydrolyzed and shown to contain a little slower-moving acidic material and unhydrolyzed partly methylated aldobiouronic acid (tested by further hydrolysis) as well as 2,3,4-tri-*O*-methyl-D-glucuronic acid.

The residue, left after distillation of the syrupy methylated aldobiouronic acid, gave on hydrolysis 2,3,4-tri-*O*-methylglucuronic acid, a trace of 2,3,4-tri-*O*-methylgalactose, and 3,4,6-tri-*O*-methylmannose, together with a slightly faster-moving compound (solvents *b, i*). The two latter components were readily separated into approximately equal fractions by paper ionophoresis, 3,4,6-tri-*O*-methyl-D-mannose having M_g 0·45, and an immobile fraction.

(vi) The next fraction (0·17 g.) gave a faint pink stain with the *p*-anisidine spray reagent and had R_{gal} 0·12, 0·25 (solvents *b, f*), M_g 0·90. Heating a portion with N-sulphuric acid at 98° for 10 hr. gave substances corresponding on paper chromatography to (2-D-mannose β-D-glycopyranosid)uronic acid, mannose, and glucuronic acid with a barely detectable trace of galactose. After reduction with sodium borohydride followed by acid hydrolysis, mannitol was the sole glycitol detected by chromatography (solvents *d, j*).

(vii) The following fraction (0·39 g.) gave a brown stain with *p*-anisidine and had R_{gal} 0·05, 0·15 (solvents *b, f*), M_g 0·8. Partial acid hydrolysis gave the aldobiouronic acids (2-D-mannose β-D-glucopyranosid)uronic acid and (6-D-galactose β-D-glucopyranosid)uronic acid together with mannose and galactose.

(viii) Residues (0·6 g.) washed from the column could not be separated into distinct components by paper chromatography and were not further investigated.

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