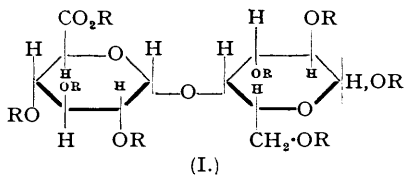
343. *Grapefruit and Lemon Gums. Part I. The Ratio of Sugars present in the Gums and the Structure of the Aldobionic Acid (4-D-Glucuronosido-D-galactose) isolated by Graded Hydrolysis of the Polysaccharides.*

By J. J. Connell, (Miss) Ruth M. Hainsworth, E. L. Hirst, and J. K. N. Jones.

The gums isolated from the grapefruit tree and the lemon tree are shown to be composed of residues of L-arabinose, D-galactose, and D-glucuronic acid (grapefruit gum : arabinose 16, galactose 53, glucuronic acid 31%; lemon gum : arabinose 22, galactose 55, glucuronic acid 22%). The reactions between these gums and the periodate ion have yielded information concerning structure. On hydrolysis the gums give an aldobionic acid, identified as 4-D-glucuronosido-D-galactose, since after methylation and hydrolysis it yields 2 : 3 : 4-trimethyl D-glucuronic acid and 2 : 3 : 6-trimethyl D-galactose. Other aldobionic acids may also be present.

Plant gums hitherto examined have been found to contain residues of D-galactose and a uronic acid. Most of them contain also L-arabinose, present in its furanose form, whilst some contain as well other sugar residues such as D-xylopyranose, L-rhamnopyranose, L-fucopyranose, and D-mannopyranose. In 1936 Anderson, Russell, and Seigle (*J. Biol. Chem.*, 1936, **113**, 683) described a sample of lemon gum which contained L-arabinose, D-galactose and D-glucuronic acid only. It appeared possible, therefore, that the structure of this gum might be relatively simple, in contrast with the complex nature of polysaccharides such as gum arabic, damson gum, and egg plum gum. It was decided, therefore, to investigate, in greater detail, the structure of lemon gum and of grapefruit gum. Through the kindness of Dr. Misener of Toronto and of Professor E. T. Bartholomew of the Agricultural Experimental Station, The University of California, we were able to obtain samples of known origin and previous history. The gums were supplied to us in the form of pale yellow, brittle solids possessing a characteristic aromatic smell. By dissolution in water followed by precipitation with acidified alcohol they were obtained as white, ash-free, water-soluble, acidic powders. The samples of grapefruit gum were characterised by their optical rotation ($[\alpha]_D$ *ca.* +56°) and equivalent weight (*ca.* 590), whilst the lemon gum had a lower rotation ($[\alpha]_D$ *ca.* +20°) and a higher equivalent weight (*ca.* 790). These values were characteristic for the gums and independent of the place in which the tree was growing. It would appear from these and other results (*cf.* Hirst, *J.*, 1942, 70) that the gum produced by a fruit tree is specific to the type of tree.

The pentose residues in both grapefruit and lemon gum are present in the furanose form since they undergo hydrolysis under very mild acid conditions. Further hydrolysis of the polysaccharides with dilute acid (0·1N.) results in the formation of D-galactose and an aldobionic acid (I; R = H), which is converted into D-galactose and D-glucuronic acid when heated with



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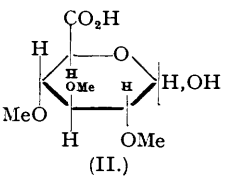

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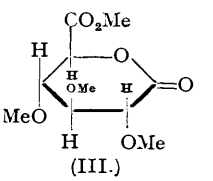

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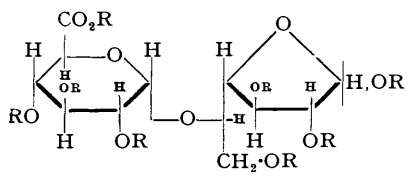

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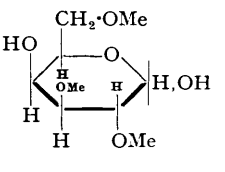

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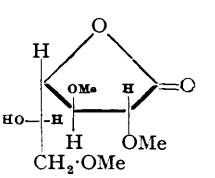

(V.)

more concentrated acid. Methylation of the aldobionic acid gives an octamethyl derivative (I; R = Me) which on hydrolysis yields (*a*) 2 : 3 : 4-trimethyl D-glucuronic acid (II), identified after oxidation and esterification as the methyl ester of 2 : 3 : 4-trimethyl D-saccharopyranolactone (III), and (*b*) 2 : 3 : 6-trimethyl D-galactose (IV), identified after oxidation as the crystalline 2 : 3 : 6-trimethyl D-galactofuranolactone (V). The aldobionic acid is, therefore, either (VI) in which a D-galactofuranose residue is linked through $C_{(5)}$, or (I; R = H) in which a D-galactopyranose residue is linked through $C_{(4)}$. The rotation of the aldobionic acid favours

the pyranose structure which is moreover in accord with the general occurrence of galactopyranose residues in plant gums. The yield of this aldobionic acid was poor and the presence of other aldobionic acid residues in the gum cannot yet be definitely excluded.

Information on the structure of grapefruit gum and lemon gum was obtained from a study of their reactions with periodate. The yield of formic acid obtained by oxidation of a non-reducing polysaccharide with periodate is a measure of the amount of pyranose end group and of hexopyranose residues united through $C_{(1)}$ and $C_{(6)}$ only. By periodate oxidation, grapefruit gum gives 0·5 mole of formic acid per equivalent, whilst lemon gum gives 1 mole per equivalent. Thus grapefruit gum contains a repeating unit of 1180 (equivalent weight, 590) or multiples of 1180, whilst that of lemon gum is 790 or a multiple of this number. The uptake of periodate during the reaction shows that several sugar residues are oxidised. For instance, with grapefruit gum the consumption of periodate is 4 moles per repeating unit of 1180. Now the formation of 1 mole of formic acid requires two moles of periodate and therefore two sugar residues other than the one which yields formic acid are oxidised during the reaction. Lemon gum on the other hand consumes 3·2 moles of periodate per molecule of 790, and in this case one sugar residue other than the one giving formic acid must be oxidised. The results now recorded show that in both lemon gum and grapefruit gum, L-arabinose residues occur which are not oxidised by the periodate ion and must, therefore, be substituted on $C_{(2)}$ or $C_{(3)}$. Grapefruit gum would appear, therefore, to be built on lines as complicated as those of other plant gums.

Both lemon gum and grapefruit gum contain a considerable percentage of D-galactose residues which are not attacked by the periodate ion and therefore possess no contiguous hydroxyl groups. It follows that 1 : 3-linked galactose residues such as are encountered it damson, cherry, and egg plum gum, and in gum arabic, occur also in grapefruit and lemon gum.

Experimental.

The samples of grapefruit and lemon gum were obtained from Dr. T. Misener and the Seminola Co., Florida, and from Professor E. T. Bartholomew of The Agricultural Experimental Station, University of California. They were soluble in water giving viscous light brown solutions, from which they were precipitated by the addition of alcohol or acetone. The purified ash-free materials were prepared by precipitation of the polysaccharide from a solution acidified with hydrochloric acid by the addition of alcohol or glacial acetic acid. The precipitates were washed by decantation with alcohol, filtered off, washed with alcohol, then with ether, and finally dried at 40° under reduced pressure. The gums were isolated as white powders, soluble in water and aqueous sodium hydroxide with the formation of colourless non-reducing solutions. Addition of copper salts, calcium salts, or silver salts to solutions of the neutral sodium salt of grapefruit precipitated the insoluble blue copper, or insoluble white calcium or silver salt. The acidic, ash-free gum could be regenerated by treatment of the salts with alcohol containing hydrochloric acid. Lemon gum also gave an insoluble copper salt.

Grapefruit Gum.—Samples of this gum had been obtained from trees growing in California and from one tree growing in Florida. All the specimens had similar properties and this applied also to samples which had exuded from a *Phytophthora*-infected tree. The equivalent weights, determined by direct titration with 0·1N-sodium hydroxide (phenolphthalein) varied from 582 to 596. $[\alpha]_D$, in water, for the various samples lay between +54° and +59°. These values are so close to one another that it is considered that the samples are essentially identical in their physical properties (Found : furfuraldehyde after boiling of the gum with 12% hydrochloric acid, 16·6; uronic anhydride, 31·3; OMe, 5·2%). The polysaccharide on oxidation with nitric acid (*d* 1·2) gave mucic acid in yield equivalent to the presence of at least 40% of galactan. Methyl pentose was not present, since no methylfurfuraldehyde was obtained on distillation with 12% hydrochloric acid, and the sugars obtained on hydrolysis of the gum gave no acetaldehyde on oxidation with periodic acid (method of Nicolet and Shinn, *J. Amer. Chem. Soc.*, 1941, **63**, 1456).

Examination of the mixture of neutral sugars on the paper chromatogram showed the presence of galactose and arabinose only (Found : furfuraldehyde after boiling of the sugars with 12% hydrochloric acid, 16·6; uronic acid anhydride, 31·3%). This percentage of uronic anhydride would yield 6·9% of furfuraldehyde and therefore 16·6 − 6·9 = 9·7% arise from arabinose. This corresponds to the presence of *ca.* 16% of arabinose (calc. as $C_5H_8O_4$) in the molecule. On the basis of the observed analytical figures for arabinose and uronic acid residues, the amount of galactose residues in the gum is (by difference) 52·7%.

Periodate Oxidation of Grapefruit Gum.—(*a*) The potassium salt of the gum (45·4 mg.) in water (8 c.c.) was oxidised with an excess of sodium periodate solution (2 c.c.; 0·25M.) during 7 days. Excess of sodium periodate was then destroyed by the addition of ethylene glycol, and the formic acid was determined by titration [Found : 1·67 mg., equivalent to the formation of 1 mole of formic acid per two equivalents (1012 g.) of gum]. In a second experiment the amount of periodate reduced to iodate in 7 days by the potassium salt of the gum (46·8 mg.) was determined (Found : 1·71 c.c. of M/10-sodium periodate, equivalent to 2 moles of sodium periodate reduced per 549 g. of the ash-free gum).

(*b*) The potassium salt of the gum (0·122 g.) was oxidised with sodium periodate solution (5 c.c.; 0·25M.) for 3 days. (Preliminary experiments had shown that oxidation was complete in this time.) Ethylene glycol was added to destroy excess of sodium periodate and the solution was then dialysed until free from iodate ions. The solution was evaporated to dryness at 50° under reduced pressure and the residual oxidised polysaccharide dried to constant weight (Found : sulphated ash, 7·8%).

The oxidised polysaccharide was hydrolysed at 100° with N-sulphuric acid for 3 hours. The cooled solution was neutralised with barium carbonate and filtered, and the filtrate evaporated to a syrup. Separation on the paper chromatogram showed the presence of arabinose and galactose.

Graded Hydrolysis of Grapefruit Gum.—Purified ash-free grapefruit gum (16 g.) was heated with water (550 c.c.) at 90°, the acidity of the solution being sufficient to bring about slow hydrolysis of the gum. The reaction was followed by polarimetric and iodometric observations: $[\alpha]_D^{20}$ +53° (3 hours, initial value not observable); +56° (7·5 hours); +60° (12 hours); +61° (19 hours); +63° (31 hours); +66° (39 hours); +66° (43 hours); +68° (53 hours). A much slower hydrolysis proceeded beyond this stage. The increase in iodine titre was followed by titration of 2-c.c. portions of the solution with 0·1N-iodine by Baker and Hulton's method (*Biochem. J.*, 1920, **14**, 754): value (in c.c. of 0·1N-iodine, calc. for 1 g. of grapefruit gum), 1·4 (initial); 5·5 (1 hour); 11·0 (3·2 hours); 16·5(7·5 hours); 22·0 (12 hours); 28·2 (19 hours); 33·0 (31 hours); 37·8 (39 hours); 38·5 (43 hours); 40·0 (53 hours). The cooled solution was concentrated, at 40°/12 mm., to 200 c.c. and poured into alcohol; it then gave an alcohol-insoluble polysaccharide (*A*) (5·1 g.) which was washed with alcohol and dried.

The filtrate from (*A*) still contained some acidic material; it was, therefore, evaporated to a syrup at 40°/12 mm., diluted with water, neutralised with barium carbonate, and filtered, and the filtrate poured into alcohol. The precipitated material (10·5 g.) (*B*) was apparently the barium salt of polysaccharide (*A*) which had escaped precipitation. It was filtered off, washed, and dried. The filtrate from (*B*) was evaporated to a syrup (3·3 g.) which was dissolved in water (50 c.c.). An iodine titration at this stage indicated the presence of reducing sugar (2·8 g., calc. as pentose; 3·3 g., calc. as hexose). A furfuraldehyde determination showed the presence of 1·2 g. of pentose, and this was shown to be L-arabinose since quantitative determination with diphenylhydrazine indicated the presence of 1·2 g. of L-arabinose. This value corresponds to the presence in the polysaccharide of some 7% of arabinose, calc. as $C_5H_8O_5$. Oxidation of a portion of the syrup with nitric acid (*d* 1·2) showed the presence of approximately 1·8 g. of galactose. A mixture of 1·2 g. of L-arabinose and 1·8 g. of D-galactose would have $[\alpha]_D^{20}$ +90° and would reduce 360 c.c. of N/10-iodine (Found: $[\alpha]_D^{20}$ +87°; 368 c.c. of N/10-iodine). On the paper chromatogram no sugars other than arabinose and galactose could be detected.

Examination of barium salt (B). This material was isolated as a white powder, easily soluble in water to yield a pale yellow solution, and had $[\alpha]_D^{20}$ +73° (*c*, 2·08 in water). The salt reduced Fehling's solution on boiling and reduced alkaline iodine solution (1 g. reduced 27·5 c.c. of 0·1N-iodine). On heating with 12% hydrochloric acid, carbon dioxide equivalent to the presence of 36% of uronic anhydride, and furfuraldehyde (14·4%) were evolved. The salt contained barium (13·4%) and methoxyl (9·3%; a partly etherified sugar acid may have been present).

The barium salt (6 g.) was dissolved in N-sulphuric acid (100 c.c.) and heated at 95° for 4 hours, the following changes being observed: (*a*) $[\alpha]_D^{22}$ +74° (initial value); +84° (30 minutes); +88° (70 minutes); +89° (2·2 hours); +86° (4 hours); (*b*) iodine titre, in c.c. of 0·1N-iodine per gram of barium salt, 25 (initial value); 36 (30 minutes); 42 (70 minutes); 50 (2·6 hours); 52 (4 hours). The cooled solution was neutralised with barium carbonate and filtered, and the filtrate concentrated to a solid (OMe, 3·1%), which was extracted with alcohol. On concentration the extracts (3·5 g.) gave D-galactose, m. p. 164°, $[\alpha]_D^{21}$ +77°, and a syrup consisting of D-galactose, admixed with a small quantity (*ca.* 0·1 g.) of L-arabinose. The barium salt appeared to be identical with the barium salt isolated after hydrolysis of polysaccharide (*A*) (see below).

Polysaccharide (A). This material was a pale buff-coloured powder, easily soluble in water to give a solution which was acidic, reduced Fehling's solution slightly on boiling, and had $[\alpha]_D^{20}$ +25° (in water) [Found: ash, 0·3; OMe, 2·7%; furfuraldehyde (after boiling with 12% hydrochloric acid), 8·1; uronic anhydride (from the weight of carbon dioxide evolved in boiling with 12% hydrochloric acid), 37·7%; equiv. (by titration with 0·1N-sodium hydroxide), 530]. D-Galactose was present since mucic acid was formed on oxidation of a portion of the polysaccharide with nitric acid (*d* 1·2). The polysaccharide (4 g.) was hydrolysed with N-sulphuric acid (50 c.c.) for 5 hours ($[\alpha]_D$ not observable owing to darkening of the solution). The cooled solution was neutralised by titration with 0·33N-barium hydroxide and filtered, and the filtrate concentrated in a vacuum. The residue was exhaustively extracted with methyl alcohol and the extracts were concentrated under reduced pressure to a syrup (1·8 g.), $[\alpha]_D^{20}$ +78°, which crystallised. The crystalline sugar (1·6 g.), separated by trituration with alcohol, had m. p. and mixed m. p. with D-galactose, 165°. The residual barium salt showed $[\alpha]_D^{20}$ +78° in water (Found: Ba, 17·8. Calc. for the barium salt of an aldobionic acid: Ba, 16·0%). This barium salt was very resistant to hydrolysis but was hydrolysed, with much decomposition, by 2N-sulphuric acid. Owing to darkening of the solution it was not possible to observe the change in optical rotation. The sugar, isolated from the hydrolysate in the usual manner, was identified as D-galactose, m. p. and mixed m. p. 164°, $[\alpha]_D^{20}$ +81° (in water, equilibrium value).

It was possible to isolate the barium salt of the aldobionic acid in good yield by the following simplified procedure. The gum (7·2 g.; ash-free) was dissolved in 0·1N-sulphuric acid (50 c.c.), and the solution heated on the boiling water-bath for 20 hours. The cooled solution was neutralised with barium carbonate and filtered and the filtrate concentrated under reduced pressure to a syrup which was poured into alcohol. The insoluble barium salt of the aldobionic acid was collected, washed with alcohol, and dried (yield, 4 g.). The alcoholic filtrate was concentrated and a portion examined on a paper chromatogram which showed the presence of galactose together with a trace of arabinose.

Methylation of the aldobionic acid. The barium salt (4 g.) was dissolved in water (10 c.c.), and methyl sulphate (25 c.c.) was added. Sodium hydroxide (50 c.c.; 40%) was added drop-wise during 12 hours, the solution then not reducing Fehling's solution. The reaction mixture was concentrated to a small volume and re-methylated by dissolving the residue in sodium hydroxide (100 c.c.; 40%) and adding methyl sulphate (70 c.c.) drop-wise. After 24 hours' stirring the solution was concentrated on the steam-bath, cooled, and acidified with sulphuric acid, and the methylated aldobionic acid extracted with chloroform (six times). The extracts were dried and filtered and the solvents removed under reduced pressure. The residual syrup was dissolved in methyl iodide and methylated by the portion-wise addition of silver oxide. The product (3·1 g.; n_D^{20} 1·4678) (Found: OMe, 51·8%) was isolated, in the usual manner,

as a pale yellow syrup. It was purified by fractional distillation. The low-boiling fraction (0·2 g.; n_D^{20} 1·4525) (Found: OMe, 56·5%) was not further examined. The main fraction (2·1 g.), b. p. 160—220°/1 mm., had n_D^{21} 1·4695 and $[\alpha]_D^{20}$ +81° (*c*, 0·7 in methyl alcohol) (Found: OMe, 51·7%).

The methylated aldobionic acid (0·81 g.) was hydrolysed by boiling it with N-sulphuric acid (20 c.c.) for 16 hours ($[\alpha]_D$ not observable). The cooled solution was neutralised with aqueous sodium hydroxide, and the methylated sugar was isolated by exhaustive continuous extraction with chloroform. The product (0·36 g.), $[\alpha]_D^{20}$ +80° (*c*, 0·4 in water), was identified as 2 : 3 : 6-trimethyl D-galactose (Found: OMe, 41·1. Calc. for $C_9H_{18}O_6$: OMe, 41·8%). On oxidation with bromine water in the usual manner the sugar gave 2 : 3 : 6-trimethyl D-galactofuranolactone, b. p. 140°/1 mm., n_D^{20} 1·4565 (super-fused solid), m. p. and mixed m. p. 98° after recrystallisation from ether, $[\alpha]_D^{20}$ being −44° (initial value), −40° (21 hours), −37° (4 days) (Found: Equiv., 211; C, 49·0; H, 7·4; OMe, 42·0%. Calc. for $C_9H_{16}O_6$: equiv., 220; C, 49·1; H, 7·3; OMe, 42·3%).

After removal of the sugars from the hydrolysate (above) the solution was acidified with sulphuric acid and the uronic acid residue (0·45 g.) isolated by further continuous extraction. The product was oxidised with bromine water, and the resultant saccharic acid derivative isolated in the usual manner. This material was boiled with methanolic hydrogen chloride, and the resultant ester was isolated. On distillation the methyl ester of 2 : 3 : 4-trimethyl D-saccharolactone was obtained, having b. p. 140°/0·1 mm., n_D^{20} 1·4570 (Found: OMe, 49·8. Calc. for $C_{10}H_{16}O_7$: OMe, 50·0%). This material crystallised when kept. Recrystallised from ether, it had m. p. 109°, not depressed on admixture with an authentic specimen but depressed to 80—85° on admixture with dimethyl 2 : 3 : 4-trimethyl D-mucate.

Lemon Gum.—Several samples of lemon gum were examined, one from a *Phytophthora*-infected Eureka lemon tree growing on sour orange root-stock and others from lemon trees infected with unknown organisms. In all cases examined the ash-free gum possessed an equivalent weight of 770—800 and $[\alpha]_D$ (as sodium salt in water) between +19·5° to +21·2° (cf. Anderson, Russel, and Seigle, *J. Biol. Chem.*, 1936, **113**, 683, who give a value of +20·7°) (Found: OMe, 4·0; furfuraldehyde, after boiling with 12% hydrochloric acid, 16·6; uronic acid anhydride, from the yield of carbon dioxide after boiling with 12% hydrochloric acid, 22·4%). Methyl pentose was absent.

The amount of uronic anhydride found would give rise to 5·0% of furfuraldehyde and, therefore, 16·6 − 5·0 = 11·6% arises from arabinose. This corresponds to the presence of *ca.* 22% of arabinose (calc. as $C_5H_8O_4$), in the molecule. The difference, *ca.* 55%, is, therefore, galactose (calc. as $C_6H_{10}O_5$).

Examination, on the Paper Chromatogram, of the Sugars produced by Hydrolysis of Lemon Gum.—The ash-free gum (13·04 mg.) was hydrolysed with N-sulphuric acid (0·4 c.c.) containing rhamnose (4·38 mg.), on the steam-bath for 3 hours. The cooled solution was neutralised with barium carbonate, and the sugars present in a portion of the supernatant liquid were separated on the paper chromatogram and determined by the Somogyi copper reagent (Found: 0·222 mg. of rhamnose; 0·0584 mg. of arabinose; 0·104 mg. of galactose). These values correspond to the removal by hydrolysis of 21·2% of arabinose residues and of 38·6% of galactose residues (Calc. as $C_5H_8O_4$ and $C_6H_{10}O_5$ respectively), leaving a resistant core (40·2%) of sugar residues [Calc. for one aldobionic acid unit (consisting of a D-galactose and a D-glucuronic acid residue) per equivalent of polysaccharide, 42·8%].

Oxidation of Lemon Gum with Potassium Periodate.—(*a*) The neutral potassium salt of the polysaccharide (42·8 mg.) in water (10 c.c.) containing potassium chloride (0·3 g.) was oxidised with sodium periodate (0·2 c.c.; 0·25M.) for 150 hours. Ethylene glycol was then added and the formic acid determined by titration [Found: 2·48 mg., equivalent to the formation of approximately 0·98 mole of formic acid per equivalent (775) of the gum].

(*b*) The potassium salt of the gum (42 mg.) was oxidised with sodium periodate (0·2 c.c.; 0·25M.) made up to 10 c.c. with water containing potassium chloride (0·3 g.), for 150 hours. At the end of this time the consumption of periodate was determined (Found: 1·66 c.c. of M/10-solution, equivalent to a consumption of 3·2 moles of periodate per equivalent of ash-free polysaccharide of equiv. wt. 775).

Examination of the Material obtained by the Oxidation of Lemon Gum with Potassium Periodate.—The gum (0·25 g.) was oxidised with potassium periodate (0·75 g.) for 150 hours as described above. The excess of periodate was then removed by addition of ethylene glycol and the solution was dialysed against running water until free from inorganic ions. The dialysed solution was evaporated to dryness and the solid was dried to constant weight (75 mg.). The oxidised polysaccharide was hydrolysed with N-sulphuric acid on the steam-bath for 4 hours. The cooled solution was neutralised with barium carbonate, and the sugars were separated on the paper chromatogram. Arabinose and galactose were the only sugars detected.

Hydrolysis of Lemon Gum.—Lemon gum (5 g.) was dissolved in N-sulphuric acid (11 c.c.) and water (39 c.c.), and the solution was heated on the steam-bath, the following changes being observed: $[\alpha]_D^{20}$ +21·8° (initial value); +34° (½ hour); +42° (1½ hours); +49° (2½ hours); +56° (4 hours); +60° (5 hours); +64° (6 hours); +74° (9 hours); +78° (11 hours); +89° (20 hours). A sugar determination at this stage indicated the presence of *ca.* 3·4 g. of reducing sugar, calculated as hexose. At this stage, a small sample of the solution was examined on the paper chromatogram: galactose and arabinose were the only sugars present.

Isolation of octamethyl 4-D-*glucuronosido*-D-*galactose.* The mixture of reducing sugars was stirred with methyl sulphate (25 c.c.), and sodium hydroxide (25 c.c.; 30%) added drop-wise with vigorous stirring at 25° until the solution was non-reducing to Fehling's reagents. At this stage an excess of sodium hydroxide (50 c.c.; 30%) was added and the methylation continued by the portion-wise addition of methyl sulphate (40 c.c.). After completion of the addition the solution was heated to 60°, cooled, and extracted exhaustively with chloroform to remove methylated sugars. The solution was then made acid with dilute sulphuric acid and the extraction continued. Concentration of the acidic extracts left a syrup (2·35 g.) which was methylated with silver oxide and methyl iodide (twice) and distilled fractionally giving fractions I (0·32 g.), b. p. 160°/1 mm., n_D^{24} 1·4518 (Found: OMe, 58·2%), and II (1·1 g.), b. p. 190°/0·1 mm., n_D^{21} 1·4685 (Found: OMe, 51·1%).

Fraction II (0·94 g.) was hydrolysed by boiling a solution of it in N-sulphuric acid (20 c.c.). The optical rotation was not observable owing to the opalescence of the solution. After 20 hours the solution

was neutralised with barium carbonate and extracted continuously with chloroform, yielding an extract containing 2 : 3 : 6-trimethyl D-galactose and leaving an aqueous solution containing the barium salt of 2 : 3 : 4-trimethyl D-glucuronic acid. The 2 : 3 : 6-trimethyl D-galactose (0·3 g.) had n_D^{20} 1·4710, $[\alpha]_D^{20}$ +78° in water (Found : OMe, 40·6. Calc. for $C_9H_{18}O_6$: OMe, 41·8%). On oxidation with bromine water it yielded crystalline 2 : 3 : 6-trimethyl D-galactonolactone, m. p. 99° (after recrystallisation from ether), not depressed on admixture with an authentic specimen. The aqueous solution of barium 2 : 3 : 4-trimethyl D-glucuronate was acidified with sulphuric acid, and the 2 : 3 : 4-trimethyl D-glucuronic acid (n_D^{20} 1·4700, $[\alpha]_D^{20}$ +50°, in water) isolated by continuous extraction with chloroform. The syrupy acid (0·34 g.) was identified after oxidation with bromine water, followed by esterification, as the methyl ester of 2 : 3 : 4-trimethyl D-saccharopyranolactone, m. p. and mixed m. p. 110°.

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THE UNIVERSITY, BRISTOL.
THE UNIVERSITY, EDINBURGH.
THE UNIVERSITY, MANCHESTER.

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