652. *An Investigation of the Polysaccharide Components of Certain Fresh-water Algæ.*

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Polysaccharides have been isolated from the fresh-water algæ, *Nitella*, *Oscillatoria*, and *Nostoc*. The polysaccharide content of *Nitella* was composed largely of a cellulose-like polyglucosan. *Oscillatoria* contained an amylopectin-like polyglucosan, whereas *Nostoc* yielded a mucilaginous complex acidic polysaccharide containing at least six different monosaccharide units.

The carbohydrates occurring in marine algæ have been the subject of much investigation because of their widespread occurrence and commercial importance. Little is known, however, concerning the algæ which play a dominant rôle in the vegetation of fresh water. For this reason and because it has been suggested that some fresh-water algæ might be used as a food we decided to investigate the three readily available fresh-water algæ, *Nitella*, *Nostoc*, and *Oscillatoria*.

The *Nitella* (class, Chlorophyceæ; order, Charales; family, Characeæ) was cleaned, washed with alcohol to remove fats and chlorophyll, dried, and milled to a fine brown powder. Preliminary experiments showed that no more than a trace of carbohydrate was extracted by methanol, hot water, cold dilute alkali, or hot dilute alkali.

The dry, powdered *Nitella* was freed from a large quantity of non-carbohydrate material by treatment with hot concentrated alkali, and the residual polysaccharide was isolated (25% yield). The neutral product gave no colour with iodine and was soluble in cuprammonium hydroxide solution; examination of the acid-hydrolysis products of the neutral material on the paper chromatogram showed the presence of glucose. The material was then exhaustively methylated with methyl sulphate and sodium hydroxide in an atmosphere of nitrogen. After de-ionisation by shaking the chloroform solution with a mixture of Amberlite resins IR.120 and IR.400, the methylated material had properties similar to those shown by a normal methylated cellulose, the optical rotation ($[\alpha]_D$ −15°) indicating the β-1 : 4-type of linkage. An examination of the fission products on the paper chromatogram showed the presence of 2 : 3 : 6-trimethyl glucose with a small quantity of dimethyl glucose; no tetramethyl glucose was discernible. Parallel experiments showed that the procedure will detect less than one part of tetramethyl in a hundred parts of trimethyl glucose, and therefore the methylated material probably contains little or no end-group and has a chain length greater than one hundred glucose units.

Separation of the fission products of the methylated polysaccharide by partition chromatography yielded 2 : 3 : 6-trimethyl D-glucose in 93% yield, and a mixture of dimethyl glucoses in 4% yield. The dimethyl sugars probably arise from incomplete methylation and from demethylation during the hydrolysis procedure (cf. Hirst, Hough, and Jones, *J.*, 1949, 928; Barker, Bourne, and Wilkinson, *J.*, 1950, 3027).

Thus, the high yield of 2 : 3 : 6-trimethyl D-glucose, the large chain-length, and the optical rotation of the methylated material, considered together with the properties of the original polysaccharide, show that the main carbohydrate component of *Nitella*, when harvested in November, is a cellulose. Percival and Ross (*J.*, 1949, 3041) have observed that marine-algal cellulose is essentially the same as cotton cellulose.

Both *Nostoc* and *Oscillatoria* are blue-green algæ (class, Cyanophyceæ; order, Nostocales). The free *Oscillatoria* was steeped in alcohol and dried under reduced pressure to a pale green powder. This crude material yielded little polysaccharide on extraction with either hot water or cold dilute alkali, but extraction with hot dilute alkali yielded a mixture of protein and polysaccharide. Examination of the hydrolysis products of this mixture on the paper chromatogram indicated that the main carbohydrate component was glucose, but that it also contained a little rhamnose and xylose. It was thought improbable that the rhamnose and xylose were an integral part of the polyglucosan. This was proved to be the case by the isolation by fractionation of a polyglucosan, free from xylose and rhamnose, from the crude material. The polysaccharide was similar to amylopectin and glycogen in that it was readily soluble in cold water, had a high positive optical rotation, gave a reddish-brown colour with iodine, and on hydrolysis yielded D-glucose only.

Further evidence was afforded by an examination of the product obtained by methylation. The methylated material shows a high positive optical rotation, and an examination of the fission products on the paper chromatogram showed the presence of trimethyl glucose and smaller quantities of tetramethyl and dimethyl glucoses. A quantitative estimate of the proportions of these sugars by the method of Hirst, Hough, and Jones (*loc. cit.*) indicated an average chain length of 26 glucose units per non-reducing end group. A further estimate by the method of Brown and Jones (*J.*, 1947, 1344) gave an average chain length of 23 glucose units. 2 : 3 : 4 : 6-Tetramethyl D-glucose and crystalline 2 : 3 : 6-trimethyl D-glucose were isolated from the hydrolysis products by counter-current extraction; the tetramethyl derivative was converted into the characteristic crystalline anilide. Thus, it is clear that the main food-reserve carbohydrate of *Oscillatoria* is a polyglucosan of the amylopectin type.

In distinction to *Oscillatoria* and *Nitella*, *Nostoc* afforded, on extraction with hot water, a mucilaginous complex polysaccharide composed of at least five neutral sugars and, in addition, uronic acids. The purification of the mucilage was difficult and tedious, and the hydrolysis products so complex as to render analysis difficult. The purified mucilage had an equivalent weight of 595 and, on hydrolysis, approximately 60% of the component monosaccharides were rapidly liberated, there remaining a fragment much more resistant to hydrolysis. Examination of the hydrolysis products by paper chromatography suggested

the presence of rhamnose, xylose, an unknown sugar, glucose, and galactose. An examination of the graded hydrolysis products of the mucilage afforded crystalline specimens of D-galactose and D-xylose, along with the degraded mucilage which, on further hydrolysis, afforded crystalline specimens of D-galactose and rhamnose. The analytical results indicate that the xylose residues are readily removed, in contrast to the rhamnose residues which are firmly attached. The uronic acids liberated on prolonged hydrolysis were separated from the neutral sugars by use of ion-exchange resins and when examined on the paper chromatogram gave two spots only, the first corresponding in position to glucuronic acid or galacturonic acid and the second to glucurone, this indicating the presence of glucuronic acid (Partridge, *Biochem. J.*, 1947, **42**, 238). In addition, the uronic acid fraction gave a positive test for galacturonic acid with Ehrlich's reagent (*Ber.*, 1932, **65**, 352), a test not given by glucuronic or mannuronic acids (Stacey, *J.*, 1939, 1529).

The complexity of the mucilage and its stability to acid hydrolysis resulted in such severe analytical difficulties that it is not yet possible to make a precise statement concerning the relative proportion of the component monosaccharides. Nevertheless, as a rough estimate, a consideration of the equivalent weight of the mucilage indicates that it is compounded of some 30% of hexuronic acids, and the paper chromatographic analyses suggest that there is approximately 10% of rhamnose, 25% of D-xylose, and a remainder (35%) composed largely of galactose with smaller amounts of glucose and an unknown sugar.

Experimental

Nitella.—*Treatment of the raw* Nitella. The *Nitella* was harvested in November, 1947, from Lake Windermere; it was a tangled mass of long green filaments, each filament being about 2 mm. in diameter. The crude alga was thoroughly washed in large volumes of cold water to remove small impurities, large foreign bodies being then removed by hand. After being drained free from excess of moisture, it was steeped for 24 hours in each of three successive portions of alcohol (5 l.), then dried under reduced pressure at 40°, and finally milled to a fine brown powder.

Trial extractions. A small portion of the dry powdered *Nitella* was extracted with successive portions of methanol, hot water for 6 hours, 0·5N-sodium hydroxide for 24 hours at 18°, and for 3 hours at 100° with a further portion of the same reagent. Each aqueous extract was poured into an excess of alcohol and only in the case of the hot alkali extract was an appreciable precipitate formed which proved, on hydrolysis, to be largely proteinaceous.

Preparation of the crude cellulose. The remainder of the material (184 g.) was treated with sodium hydroxide solution (25%) for 3 hours at 100°, and the insoluble crude cellulose filtered off, thoroughly washed with water and then alcohol, and dried under reduced pressure (yield 47 g.). The product was largely soluble in cuprammonium hydroxide solution.

Hydrolysis of the crude cellulose. A portion (*ca.* 0·1 g.) was heated with N-sulphuric acid (25 c.c.) at 100° for 12 hours in a sealed tube. The acid, in which was suspended a small amount of flocculent material, was neutralised with barium carbonate, filtered, and the filtrate reduced to a syrup. Examination of this syrup on the paper chromatogram showed it to contain glucose.

Methylation of the crude cellulose. The crude cellulose (20 g.) was repeatedly methylated with methyl sulphate and sodium hydroxide in an atmosphere of nitrogen. After six methylations the product was extracted with chloroform, and the extract filtered, dried (Na_2SO_4), de-ionised with a mixture of Amberlite resin, IR.120 and IR.400, and evaporated to a syrup under reduced pressure (yield, 17·1 g.) (Found: OMe, 44·4; sulphated ash, 0·6%). This had $[\alpha]_D$ −14·9° (*c*, 2·3 in chloroform).

Hydrolysis of the methylated cellulose. A portion (330 mg.) was heated with acetic acid–methanol (1 : 1 v/v) containing hydrogen chloride (3%) in a sealed tube at 100° for 12 hours. The contents were then evaporated under reduced pressure at room temperature to a small volume and heated for a further 12 hours with aqueous hydrochloric acid (3%). The solution was neutralised with silver carbonate, filtered, de-ionised with a mixture of Amberlite IR.120 and IR.400, and evaporated to a syrup (330 mg., 92% yield).

2 : 3 : 6-*Trimethyl* D-*glucose.* A portion of the hydrolysis products (130 mg.) was separated on a sheet-paper chromatogram, butanol–ethanol–water (4 : 1·1 : 1·9 v/v) being used as the mobile phase. After the resultant chromatogram had been dried, the appropriate parts of the paper were extracted with hot methanol in a micro-Soxhlet apparatus and yielded dimethyl

glucoses (*ca.* 5 mg.) and 2 : 3 : 6-trimethyl D-glucose (121 mg.; m. p. 114°) which after two crystallisations from ether had m. p. and mixed m. p. 121°; $[\alpha]_D^{19}$ +71° (equilibrium value; *c*, 6·9 in water) (Found: OMe, 41·1. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

Oscillatoria.—*The raw material.* The crude wet alga was obtained from the Freshwater Biological Association, Ambleside, and was immediately steeped in an excess of alcohol for 24 hours, filtered off, steeped with acetone for 24 hours, again filtered off, and dried under reduced pressure at 40°.

Preliminary examination. A portion of the dried material was stained with a dilute iodine solution; there was a red-brown coloration, which microscopic examination showed to be due to preferential staining of certain granules within the cell.

Small portions of the dry material were extracted with cold water, hot water, cold 0·5N-alkali, and hot 0·2N-alkali, and the extracts examined for carbohydrate content. In the cold water, hot water, and cold alkali extracts appreciable quantities of carbohydrate could not be detected, but hot alkali extracted a considerable amount of material which was precipitated by a large volume of ethanol, removed by centrifugation, and hydrolysed with N-sulphuric acid, whereupon examination of the fission products on the paper chromatogram indicated that it contained glucose with a little xylose and rhamnose.

The isolation of a polyglucosan. The crude material (68 g.) was suspended in 0·2N-sodium hydroxide solution (4 l.) at 18°, and the mixture stirred for 1 hour and filtered. The residue, after being washed free from alkali, was stirred with glacial acetic acid, the mixture filtered, and the residue heated at 100° with 2N-sodium hydroxide solution (1 l.) for 1 hour and then filtered, and the precipitate thoroughly washed with hot water. The combined filtrate and washings were neutralised with glacial acetic acid, copper acetate solution (10%; 100 c.c.) was added, a small precipitate filtered off, and the filtrate, after concentration under reduced pressure to a small volume (*ca.* 200 c.c.), was poured into alcohol (3 l.). The resultant precipitate was filtered off, thoroughly washed with alcohol, and dissolved in water (400 c.c.), and the solution shaken with a mixture of Amberlite resins IR.120 and IR.400. After removal of the resins, the polysaccharide was precipitated by the addition of excess of alcohol, filtered off, washed with alcohol, and dried at 40° under reduced pressure. The neutral, white powder, $[\alpha]_D$ +188° (*c*, 2·1 in water) (Found: N, 0·6; sulphated ash, 3·2%), was readily soluble in cold water and with iodine in potassium iodide solution gave a red-brown colour.

Hydrolysis. A portion (0·37 g.) of the purified polysaccharide was dissolved in 0·5N-sulphuric acid (25 c.c.) and heated for 5 hours at 100°. After neutralisation with barium carbonate and removal of the insoluble barium salts, the solution was evaporated to dryness, extracted with hot methanol containing water (5%), and the methanol removal under reduced pressure, yielding a clear syrup (0·384 g., 93% yield), $[\alpha]_D$ +56° (*c*, 1·7 in water), which showed only glucose on examination on the paper chromatogram. The syrup crystallised to give α-D-glucose hydrate, m. p. 92—93°, $[\alpha]_D$ +110° (*c*, 0·7 in water; initial value) ⟶ +52° (equil. value).

Methylation. A portion of the polyglucosan (9·7 g.) was repeatedly methylated with methyl sulphate and sodium hydroxide, the product extracted with chloroform, the extract dried (Na_2SO_4), and the solvent evaporated under reduced pressure to yield the methylated polyglucosan (yield, 9·3 g.) (Found: OMe, 42·7; sulphated ash, 3·7%), $[\alpha]_D$ +195° (*c*, 3·6 in chloroform).

Fission of the methylated material. A portion (0·115 g.) was heated for 7 hours at 100° with methanolic hydrogen chloride (2%; 25 c.c.) in a sealed tube, after which the contents were concentrated under reduced pressure at room temperature to a small volume, and this dissolved in N-hydrochloric acid (25 c.c.) and heated at 100° for a further 5 hours. The solution was then neutralised by the addition of silver carbonate, filtered, de-ionised with a small amount of mixed Amberlite IR.4B and IR.100 resins, and evaporated under reduced pressure to a syrup (0·116 g.).

Examination of a portion on the paper chromatogram, butanol–ethanol–water (5 : 1 : 4 v/v; top layer) being used as the mobile phase and *p*-anisidine hydrochloride as the spray reagent, indicated the presence of 2 : 3 : 6-trimethyl glucose and smaller quantities of monomethyl, dimethyl, and tetramethyl glucose. In a quantitative experiment, the proportions of these methyl sugars were estimated by the alkaline hypoiodite micro-procedure (Hirst, Hough, and Jones, *loc. cit.*), a phosphate buffer being used (Chanda *et al.*, *J.*, 1950, 1289); these sugars gave, respectively, the following titres with 0·01N-thiosulphate: 6·3, 0·2, 0·9, 0·3 c.c., corresponding to an average chain-length of 26 glucose residues. Another portion (*ca.* 100 mg.) was separated on sheet-paper chromatograms, with benzene–ethanol–water (169 : 47 : 15, v/v; top layer) as the mobile phase, and, after separation, the 2 : 3 : 6-trimethyl glucose was located

by the development of test strips, and the appropriate section of the remainder of the chromatogram extracted with hot methanol in a micro-Soxhlet apparatus. Evaporation of the methanol yielded crystals (73 mg.; m. p. 109°) which, on recrystallisation twice from ether, gave crystalline 2 : 3 : 6-trimethyl D-glucose, m. p. and mixed m. p. 121°, $[\alpha]_D^{19}$ +71° (equilibrium value, *c*, 1·0 in water) (Found : OMe, 42·7. Calc. for $C_9H_{18}O_6$: OMe, 41·9%), which on the paper chromatogram gave only one discrete spot indistinguishable in R_G value and colour reactions from a genuine specimen. In another experiment, the methylated polyglucosan (0·97 g.) was dissolved in 2% methanolic hydrogen chloride (50 c.c.) and heated under reflux for 7 hours. The solution was neutralised with silver carbonate, filtered, and evaporated under reduced pressure at room temperature, and the methyl glycosides (0·971 g.) were dissolved in water (50 c.c.). The fully methylated sugar was isolated from the mixture by Brown and Jones's quantitative procedure (*J.*, 1947, 1344), whereby the aqueous solution is continuously extracted with light petroleum (b. p. 38—40°), two glass continuous extractors being used, one above the other. The first extract obtained after 4½ hours, a mixture (0·101 g.) of " tri " and " tetra," was dissolved in water (50 c.c.) and extracted once again with light petroleum to give tetramethyl methylglucopyranoside (44·2 mg.), the yield corresponding to an average chain-length of 23 glucose residues. The tetramethyl methyglucoside was hydrolysed in N-sulphuric acid for 5 hours, after which the acid was removed on an ion-exchange column to give syrupy 2 : 3 : 4 : 6-tetramethyl D-glucose [26 mg.; $[\alpha]_D$ +83° (*c*, 1·3 in water)] which, when examined on the paper chromatogram, was observed to contain traces of trimethyl glucose. The syrup gave an anilide which crystallised from light petroleum as white needles, m. p. 132° undepressed on admixture of the sample with an authentic specimen of 2 : 3 : 4 : 6-tetramethyl D-glucose anilide.

Nostoc.—*The isolation of the mucilage.* The alga was collected in January, 1948, at the Freshwater Biological Association, Ambleside, and obtained as gelatinous nodules containing much impurity. Several nodules were selected and observed to contain on the average 97% of water. The bulk of the material (8·5 kg.) was thoroughly washed with water, steeped in two successive portions of methylated spirits for 24 hours in each, and then in ether for 24 hours. The material was then dried under reduced pressure. Since it contained much extraneous matter, the hard nodules of *Nostoc* were isolated by hand picking. This material (44 g.) was boiled with water (4 l.) for 3 hours, and the resultant mucilaginous mass filtered through a muslin cloth, the residue being boiled with more water (2 l.) for ½ hour and again filtered. The combined extracts were exceedingly viscous, setting to a gel below 50°; the gel was cooled to 20°, made 0·1N with respect to hydrochloric acid, thus reducing the viscosity, and all debris removed by centrifugation. Diatomaceous earth was added to the turbid brown supernatant liquors and the solution filtered on a Buchner funnel. The clear yellow filtrate was neutralised by the addition of ammonia solution, the viscosity increasing so greatly that the solution became a gel. A solution of cupric chloride (25%; 400 c.c.) was added with stirring to this gel and subsequently ammonia solution was added until the solution just became dark blue. The insoluble copper complex and copper hydroxide were removed by filtration under reduced pressure. The filtrate gave negative tests for carbohydrate. The precipitate was washed with ethanol, followed by ethanolic hydrogen chloride (5%) until the washings were copper-free, and finally with ethanol until the washings were chloride-free. The cream-coloured product was dried ($CaCl_2$) under reduced pressure [yield, 7·3 g.; $[\alpha]_D$ +10·6° (*c*, 0·57 in water] [Found : sulphated ash, 4·1; N, 0·2%; equiv. (by alkaline titration), 780, corrected for ash, 580]. The product gave no colour with iodine, but gave a positive naphtharesorcinol test for hexuronic acid.

Purification of the mucilage. Efforts to precipitate the mucilage from aqueous solution by the addition of alcohol or acetone were fruitless, merely resulting in an increased viscosity. The following procedure was found to be of greatest utility. To a solution of a portion (200 mg.) of the mucilage in water (20 c.c.), ethanolic hydrogen chloride (1%; 30 c.c.) was added, followed by ether (30 c.c.), resulting in the formation of a bulky gelatinous precipitate which was removed, with difficulty, by centrifugation. This precipitation was repeated a further five times as above, and then a further six times, the hydrochloric acid then being omitted from the ethanol. On drying the final precipitate, a colourless solid was obtained {yield, 43 mg.; $[\alpha]_D$ +11·8° (*c*, 1·0 in water)} [Found : sulphated ash, 0·03%; equiv. (by alkaline titration), 595; N, 0·2%].

Hydrolysis of the mucilage. The mucilage (237 mg.) was dissolved in 0·5N-sulphuric acid (25 c.c.). Portions (1 c.c.) were sealed into glass tubes which were then immersed in a boiling-water bath. At intervals, the reducing power of the tube-contents was estimated by oxidation with alkaline hypoiodite (Hirst, Hough, and Jones, *loc. cit.*). Conversion of the reducing power, in terms of hexose, into a percentage of the original mucilage gave the following data :

4% (initial value), 24% (16 min.), 35% (38 min.), 43·5% (70 min.), 50% (110 min.), 56% (170 min.), 60% (275 min.), 63% (400 min.), 68% (835 min.), 70% (1270 min.), 88% (2310 min.). It is clear that the rate of hydrolysis decreases sharply after some 400 minutes and, at this stage, the contents of another tube were neutralised with 0·01N-bartya, the barium sulphate removed by centrifugation, and the supernatant liquor allowed to percolate down a column of Amberlite resin IR.100, the effluent being free from barium ions. The solution was concentrated under reduced pressure to *ca.* 0·5 c.c., and a portion transferred to the paper chromatogram, separated, and analysed as described by Hirst, Hough, and Jones (*loc. cit.*). A qualitative examination indicated the presence of rhamnose (R_G 0·31 in butanol saturated with water), xylose (R_G 0·17), an unknown sugar (R_G 0·11), glucose (R_G 0·09), galactose (R_G 0·07), and oligosaccharides. The separation of the galactose, glucose, and the unknown sugar was insufficient to allow of their separate estimation and consequently they were estimated jointly as anhydro-hexose. The quantitative experiment indicates that on partial hydrolysis the mucilage yields 6% of rhamnose, 15% of xylose, and 34% of hexose, calculated as anhydro-sugars.

Partial hydrolysis of the mucilage. A solution of the mucilage (1·35 g.) in 0·5N-sulphuric acid (100 c.c.) was heated on the boiling-water bath for 3 hours. A small precipitate (35 mg.) (protein ?) was removed, the filtrate exactly neutralised with baryta, barium sulphate removed, and the solution concentrated under reduced pressure to a yellow solid (1·37 g.). This was extracted with boiling methanol until the extracts were substantially non-reducing, leaving a residue of the barium salts of the degraded mucilage (*A*; 0·54 g.). The methanol extracts were concentrated under reduced pressure to a syrup (*B*; 0·76 g.).

Examination of syrup B. The reducing power of the syrup {$[\alpha]_D$ +26° (*c*, 2·8 in water)}, as determined by oxidation with alkaline hypoiodite, corresponded to 52% of hexose. Quantitative analysis of the sugars, by using the method described above, gave 22% of "hexose" (galactose, glucose, and the unknown sugar), and 23% of xylose; the chromatogram indicated that the sugars contained no rhamnose, but a considerable amount of oligosaccharides and uronic acid. The mixture of sugars (0·74 g.) was fractionated on a column of cellulose by using *n*-butanol half-saturated with water as the mobile phase. Fraction I (200 mg.) yielded crystalline D-xylose, which was converted into the characteristic dibenzylidene dimethylacetal {m. p. 211—212°, mixed m. p. 211°; $[\alpha]_D$ −7° (*c*, 0·8 in chloroform)}. Fraction II (120 mg.) contained, as indicated by a paper-chromatographic examination, an unknown sugar and glucose, but crystalline derivatives were not obtained. Fraction III (390 mg.) was obtained by eluting the column with water and, when examined on the paper chromatogram, showed the presence of galactose, glucose, and uronic acids. A hot methanolic extract, on concentration to a small volume and being seeded with D-galactose, yielded crystalline D-galactose, m. p. 163°, $[\alpha]_D$ +82° (equilibrium value; *c*, 0·7 in water). The total yield from the column was 710 mg. (96%).

Examination of the barium salts A. The solid, $[\alpha]_D$ +59° (*c*, 2·9 in water), contained 1 mole of reducing group per 990 g., as determined by hypoiodite oxidation. Examination on the chromatogram showed traces of xylose as the only contaminant. The barium salt (535 mg.) was heated with N-sulphuric acid (20 c.c.) at 100° for 16 hours, then filtered, and the filtrate treated with Amberlite IR.100 until it was free from barium salts. The residue (330 mg.), $[\alpha]_D$ +38° (*c*, 2·3 in water), obtained by evaporation of the filtrate to dryness [Found: equiv. (by alkaline titration), 410; reducing group, 1 mole/280 g.] was dissolved in water, the solution allowed to percolate down a column of Amberlite resin IR.4B, the column washed thoroughly with water, and the combined effluents concentrated to a syrup (95 mg.). This was shown by paper chromatogram to contain chiefly galactose and rhamnose with traces of glucose and xylose. On trituration with ethanol, D-galactose crystallised and was isolated on a porous tile. The crystals, m. p. 159°, $[\alpha]_D$ +79° equilibrium value (*c*, 1·1 in water), were converted into D-galactose methylphenyhydrazone (m. p. 179° and mixed m. p. 179—180°). The tile was extracted with hot methanol and the extract concentrated to a small volume and seeded with L-rhamnose hydrate; this gave crystals, m. p. 95° undepressed on admixture with a geniune specimen of L-rhamnose hydrate, indistinguishable from rhamnose on the paper chromatogram. The yield was too low to allow measurement of the optical rotation.

The uronic acids were isolated from the ion-exchange resin by displacement with dilute hydrochloric acid, followed by removal of the chloride ion by the addition of silver carbonate and filtration. Hydrogen sulphide was passed into the filtrate, the insoluble silver sulphide removed, and the acid filtrate concentrated under reduced pressure to a syrup (70 mg.). This was examined on the paper chromatogram alongside galacturonic and glucuronic acids, *n*-butanol–water–acetic acid (6 : 4 : 1 v/v; upper phase) being used as the mobile phase; there were two

strong spots, corresponding to galacturonic and/or glucuronic acid (both showed the same R_F value) and to glucurone, which is always associated with glucuronic acid (Partridge, *loc. cit.*). The syrup gave a brick-red precipitate when warmed with basic lead acetate, thus indicating the presence of galacturonic acid (Ehrlich, *loc. cit.*; Stacey, *loc. cit.*).

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