376. *The Constitution of Egg Plum Gum. Part III. The Hydrolysis Products obtained from the Methylated Gum.*

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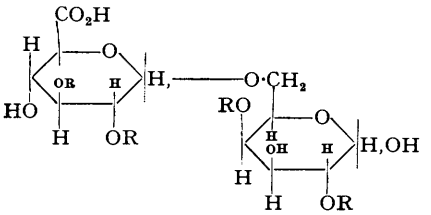
Methylated egg plum gum has been hydrolysed and the following sugars identified amongst the products of hydrolysis : (*a*) 2 : 3 : 4-trimethyl D-xylose, (*b*) 2 : 3 : 5-trimethyl, 2 : 5-dimethyl, and 2-methyl L-arabinose, (*c*) 2 : 4 : 6-trimethyl and 2 : 4-dimethyl D-galactose, and (*d*) 2 : 3-dimethyl D-glucuronic acid. The bearing of these results on the constitution of the gum is discussed.

The products of the hydrolysis of egg plum gum have been identified as D-xylose, L-arabinose, D-galactose, and D-glucuronic acid in the approximate proportions 1 : 3 : 3 : 1 (Part I, *J.*, 1947, 1064). Hydrolysis of the gum with dilute acid results in the removal of the labile L-arabinose and D-xylose residues leaving a more resistant portion of the polysaccharide designated the degraded gum. This material on methylation, followed by hydrolysis, gives a mixture of methylated derivatives of D-galactose and D-glucuronic acid, identified as 2 : 3 : 4 : 6-tetramethyl, 2 : 4 : 6-trimethyl, 2 : 3 : 4-trimethyl, and 2 : 4-dimethyl D-galactose, and 2 : 3-dimethyl D-glucuronic acid (Hirst and Jones, *J.*, 1948, 120). The degraded gum on further hydrolysis yields D-galactose and an aldobionic acid consisting of a D-glucuronic acid residue linked glycosidically to the $C_{(6)}$ of a D-galactose residue (I; R = H). A partly methylated derivative of this substance, 6-(2 : 3-dimethyl D-glucuronosido) 2 : 4-dimethyl D-galactose (I; R = Me) was identified amongst the products of hydrolysis of the methylated degraded gum. The same derivative was very probably present in the products of hydrolysis of the methylated gum itself (see below).

Methanolysis of the methylated gum, followed by fractional distillation of the methyl-glycosides, led to the identification of the following sugar derivatives amongst the products of hydrolysis : (*a*) 2 : 3 : 5-trimethyl L-arabinose (II), identified after oxidation as crystalline 2 : 3 : 5-trimethyl L-arabonolactone and as the crystalline amide thereof, (*b*) 2 : 3 : 4-trimethyl D-xylose (III), isolated as the crystalline sugar, (*c*) 2 : 5-dimethyl L-arabinose (IV), identified after conversion into the crystalline lactone and the derived phenylhydrazide and amide, (*d*) 2 : 4 : 6-trimethyl D-galactose (V), isolated as the crystalline anilide, (*e*) 2 : 4-dimethyl D-galactose (VI) (crystalline), the identity of which was confirmed by its conversion into the corresponding crystalline anilide, (*f*) 2 : 3-dimethyl D-glucuronic acid (VII), identified as the crystalline methyl ester from 2 : 3-dimethyl D-saccharolactone, and (*g*) 2-methyl L-arabinose (VIII). The glycoside of this material was found admixed with the glycoside of 2 : 4-dimethyl D-galactose, the physical properties of which are very similar. It was separated from the latter material by converting it into the 3 : 4-*iso*propylidene compound by treatment with acetone in the presence of hydrochloric acid. 2 : 4-Dimethyl methyl-D-galactoside does not form an *iso*propylidene derivative under these conditions (since it does not possess the requisite grouping of *cis*-hydroxyl groups

on adjacent carbon atoms), and the low boiling point of the *iso*propylidene derivative facilitates separation from the methylated methylgalactoside. The identity of the 2-methyl L-arabinose



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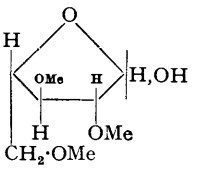

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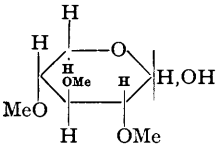

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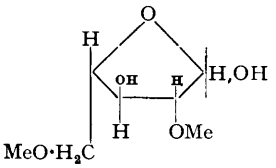

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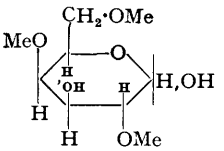

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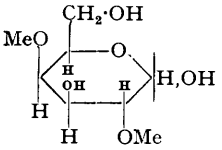

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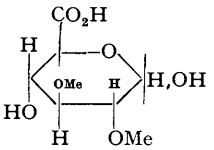

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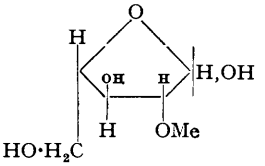

(VIII.)

was confirmed by its conversion into the crystalline toluene-*p*-sulphonylhydrazone (Jones, Kent, and Stacey, *J.*, 1947, 1341).

Quantitative analysis of the mixture of substances (*a*)—(*g*) is a formidable task and is not yet complete, but it is possible to estimate in some cases the molecular proportions. For example, the arabinose derivatives, namely 2 : 3 : 5-trimethyl, 2 : 5-dimethyl, and 2-methyl arabinose, appear to be present in the ratios of approximately 1 : 1 : 1. The proportion of 2 : 3 : 4-trimethyl D-xylose present is, as yet, uncertain, but some indication can be obtained in the following way. In Part I it was shown that potassium periodate attacked the gum with the formation of one mole of formic acid per equivalent of 1200. This formic acid must come from a system in which hydroxyl groups are present on each of three contiguous carbon atoms. Such a grouping is present in an end-group of D-xylopyranose and it is probable, therefore, that all the D-xylose residues are present as end-groups and that the ratio of 2 : 3 : 4-trimethyl D-xylose to 2 : 3 : 5-trimethyl L-arabinose is 1 : 1. Furthermore, in Part I, it was shown that the gum, on oxidation, consumed 5 moles of periodate. The xylopyranose end-group will consume 2 moles of periodate, the arabofuranose end-group will consume one mole, since it contains only one α-glycol grouping, and the uronic acid residue, which contains a similar grouping, will also consume one mole. This accounts for four of the five moles of periodate consumed. The remaining grouping oxidised by the periodate has yet to be identified. The oxidised gum gave no D-xylose on hydrolysis, but it did give some L-arabinose and some D-galactose, though the yields of these sugars were not as high as would have been expected from the weights of 2 : 5-dimethyl and 2-methyl L-arabinose and from the weight of D-galactose derivatives isolated in the present work. The low yields may have been due to the destruction of the sugars under the drastic conditions necessarily employed to hydrolyse the oxidised gum. Estimation of the number of primary hydroxyl groups in the polysaccharide by the method of Lindstedt (*Arkiv Kemi, Min., Geol.*, 1945, **20**, *A*, No. 13) had indicated that four were present per equivalent of 1200. Three of these are supplied by the arabinose end-group (one), the arabinose substituted through $C_{(1)}$ and $C_{(3)}$ (one), and the galactose residue substituted through $C_{(1)}$ and $C_{(3)}$ (one). The position occupied by the fourth triphenylmethyl group is unknown.

Hydrolysis of the methylated degraded gum (arabinose-free) gave 2 : 3 : 4 : 6-tetramethyl D-galactose and 2 : 3 : 4-trimethyl D-galactose. These two sugars do not occur amongst the products of hydrolysis of the methylated gum itself, and it follows that the arabinose and xylose residues in the undegraded gum must be joined through $C_{(6)}$ and $C_{(3)}$ of the D-galactose residues

present in the degraded gum. The isolation of 2 : 3-dimethyl D-glucuronic acid from both the methylated degraded and undegraded gum shows that side-chains of L-arabinose or D-xylose residues are not attached to the acidic residue in the gum. Hydrolysis of the methylated gum left a relatively stable acidic nucleus which on further hydrolysis gave 2 : 4-dimethyl D-galactose and 2 : 3-dimethyl D-glucuronic acid. This is strong indication that an aldobionic acid built up of these two residues is liberated on hydrolysis of the methylated gum. An aldobionic acid containing these two residues is also produced on hydrolysis of the methylated degraded gum indicating that the D-glucuronic acid residue and the D-galactose residue are attached to D-galactose residues and not to pentose residues, since the latter are absent in the degraded material.

Methylated egg plum gum, therefore, resembles methylated cherry gum in that it yields 2 : 5-dimethyl L-arabinose on hydrolysis, as well as L-arabofuranose and D-xylopyranose end-groups. It resembles gum arabic in that the D-glucuronic acid residue is combined through $C_{(6)}$ of a D-galactose residue.

The residues which occur in its molecular structure are A1, $5A^1_3$, 3A1, X1, 3G1, $6G^1_3$, 4GL1, where A = an arabofuranose residue linked as indicated, X = a xylopyranose residue, G = a galactopyranose residue, and GL = a glucuronic acid residue. Some insight has been gained, by the method indicated above, of the mode of occurrence of these residues in the gum, but pending the acquisition of exact quantitative data no unique structural formula can be advanced.

Experimental.

Methylation of Egg Plum Gum.—(All boiling points recorded are bath temperatures.) The gum (36·5 g., from one tree) was dissolved in water (200 c.c.) containing N-thallous hydroxide (30 c.c.), and to the solution was added hot 4N-thallous hydroxide (200 c.c.). The precipitated thallium derivative was filtered off, washed with alcohol, and dried under reduced pressure in the absence of light. The dried material was powdered (120 mesh) and the product boiled under reflux with methyl iodide until the thallium derivative no longer reacted alkaline to litmus. Excess of methyl iodide was distilled off, the residue was extracted first with acetone and then with methyl alcohol, and the extracts were concentrated, yielding a solid (30 g.). This product was further methylated by dissolving it in ethyl alcohol containing N-thallous ethoxide (400 c.c.), and the solution evaporated to a solid which was boiled with methyl iodide until the thallium no longer showed an alkaline reaction. Methyl iodide was removed by distillation, and the methylated gum (yield, 30 g.; Found : OMe, 36·6%) isolated from the residue by extraction with acetone. Repetition of the last methylation procedure, followed by methylation of the product with silver oxide and methyl iodide, gave a pale cream-coloured powder (29·4 g.; Found : OMe, 40·4%. A fully methylated gum containing 4 pentose residues, 3 hexose residues, and one uronic acid residue, and with equivalent weight 1470 requires OMe, 42·4%). This *product* was essentially homogeneous since the fractions obtained from chloroform solution by the addition of light petroleum (b. p. 40—60°) possessed the same properties. $[\alpha]_D^{20°}$ −33° (*c*, 1·1 in methyl alcohol).

Hydrolysis of the Methylated Polysaccharide.—A portion of the methylated polysaccharide (8·2 g.) was dissolved in methanolic hydrogen chloride (1%; 250 c.c.), and the solution boiled under reflux. $[\alpha]_D$ was −33° (initial value), −13° (2 hours), −2° (4 hours), +3° (5¼ hours), +5° (6⅓ hours), and +6° (8 hours). The cooled solution was neutralised with an ice-cold ethereal solution of diazomethane, and the solution evaporated to a syrup on the boiling water-bath at atmospheric pressure to avoid loss of fully methylated pentoses. The residual syrup was dissolved in aqueous barium hydroxide and heated at 60° for 3 hours to convert esters of the uronic acids into the corresponding barium salts. The cooled solution was neutralised with carbon dioxide, filtered from barium carbonate, and the filtrate extracted exhaustively with light petroleum (b. p. 40—60°) in an automatic apparatus. Concentration of the extract gave a syrup (*A*; 1·76 g.), $n_D^{17°}$ 1·4361. Further extraction of the aqueous solution with ether yielded first a fraction (*B*) (1·28 g.), $n_D^{21°}$ 1·4505, and then a fraction (*C*) (0·41 g.), $n_D^{17°}$ 1·4570, on further extraction with the same solvent. The aqueous solution was then concentrated under reduced pressure, and the dry residue exhaustively extracted with ether yielding a syrup (*D*) (1·93 g.), $n_D^{26°}$ 1·4709. The residue (3·8 g.) consisted of inorganic material, together with barium salts of oligosaccharides which had escaped hydrolysis with methyl-alcoholic hydrogen chloride. The mixture was extracted with boiling methyl alcohol, yielding a soluble fraction (*X*; 3·10 g.; Found : Ba, 12·8%) and an insoluble inorganic residue (*Y*; 0·70 g.).

Examination of Fractions A, B, C, *and* D.—Fraction *A* (1·76 g.) was distilled from a Widmer flask yielding fraction I (1·63 g.), b. p. 150°/13 mm., $n_D^{18°}$ 1·4338, $[\alpha]_D^{20°}$ −55° (in water) (Found : OMe, 60·8%); syrup *B* was then added to the still residue and the distillation continued yielding fraction II (0·25 g.), b. p. 170°/14 mm., $n_D^{17°}$ 1·4468 (Found : OMe, 52·3%), fraction III (0·81 g.), b. p. 125°/0·3 mm., $n_D^{13°}$ 1·4500 (Found : OMe, 49·7%), fraction IV (0·29 g.), b. p. 130°/0·3 mm., $n_D^{28°}$ 1·4470 (Found : OMe, 48·0%), fraction V (0·44 g.), b. p. 160°/0·3 mm., $n_D^{28°}$ 1·4516 (Found : OMe, 47·0%). Fraction *D* was added to the still-residue, and distillation continued, yielding fraction VI (1·90 g.), b. p. 190°/0·1 mm., $n_D^{21°}$ 1·4707, $[\alpha]_D$ +108° (in water) (Found : OMe, 39·4%).

Fraction I. Fraction I was combined with the fractions of high methoxyl content and low refractive index isolated from fractions II, III, and IV (see below). A portion (1·01 g.) of these combined fractions was dissolved in hydrobromic acid (0·04 N.), and the solution boiled under reflux. $[\alpha]_D$ was −55° (initial value), −43° (1½ hours), −26° (5 hours), −24° (7 hours), and −24° (7¼ hours). These conditions caused hydrolysis of all the trimethyl methyl-L-arabofuranoside and of some of the trimethyl methyl-D-xyloside. The cooled solution was neutralised with silver carbonate and filtered, and the filtrate concentrated to

a syrup (0·70 g.). The cooled solution was oxidised with alkaline iodine by the method of Moore and Link (*J. Biol. Chem.*, 1940, **133**, 293). Excess of iodine was removed by the passage of sulphur dioxide, and the solution neutralised with potassium hydroxide. The solution was then concentrated to dryness, and the solid residue (*S*) extracted with light petroleum (b. p. 40—60°), yielding a syrup (0·10 g.), $n_D^{13°}$ 1·4390. Hydrolysis of this syrup with boiling N-sulphuric acid gave 2 : 3 : 4-trimethyl D-xylose, m. p. and mixed m. p. 91°, after recrystallisation from ether.

The solid residue (*S*) was dissolved in water, acidified with dilute sulphuric acid and extracted with chloroform. Concentration of the extracts left a syrup (0·40 g.), which crystallised on distillation and then had m. p. 31°, not depressed on admixture with 2 : 3 : 5-trimethyl L-arabonolactone. Fraction I, therefore, consisted of a mixture of 2 : 3 : 5-trimethyl L-arabinose and 2 : 3 : 4-trimethyl D-xylose.

A portion (0·35 g.) was hydrolysed with boiling N-sulphuric acid, $[\alpha]_D$ −55° → −25° (constant value; 2 hours). The cooled solution was neutralised with barium carbonate and filtered, and the filtrate concentrated under reduced pressure to a syrup, $[\alpha]_D$ −30° (in water) (Found : OMe, 47·2%). The optical rotation indicated that fraction I contained *ca.* 15% of 2 : 3 : 4-trimethyl D-xylose.

Fraction II. The methoxyl value (52·3%) and the refractive index ($n_D^{17°}$ 1·4468) indicated that this fraction was a mixture of trimethyl and dimethyl methylpentoside. The syrup was dissolved in water, and the solution extracted with light petroleum (b. p. 40—60°). The extract (0·08 g.) was added to fraction I (see above), and the residual aqueous solution was evaporated to a syrup (0·17 g.) which was added to fractions III and IV.

Fractions III *and* IV. Fraction III, which from its physical constants contained some trimethyl methylpentoside, was dissolved in water, and the solution extracted continuously with light petroleum (b. p. 40—60°). The extracts were concentrated and the syrup (0·10 g.) added to fraction I. The aqueous solution, after extraction, was concentrated, and the residue (0·71 g.) was combined with fraction IV (0·29 g.). Hydrolysis of the combined fractions (1·00 g.) was carried out with boiling N-sulphuric acid. $[\alpha]_D$ were −15° (initial value), 32° (1 hour), and 35° (2 hours), the solution then became too dark for further polarimetric readings. After 2½ hours the cooled solution was neutralised with barium carbonate and filtered, and the filtrate concentrated to a syrup, $[\alpha]_D^{20°}$ +43° (in water), $n_D^{23°}$ 1·4690, which did not yield a crystalline anilide on being boiled with alcoholic aniline. A portion (0·30 g.) of the syrup was oxidised with bromine water until the solution was non-reducing. Excess of bromine was removed by aëration, and the solution neutralised with silver carbonate and filtered. After a further filtration, following the passage of hydrogen sulphide, the solution was concentrated under reduced pressure to a syrup which was distilled, b. p. 120°/0·05 mm., $n_D^{18°}$ 1·4480, $[\alpha]_D$ +32° (in water, after ¼ hour), +7·6° (1 hour), ±0° (18¾ hours), −19° (115 hours; constant value) (Found : OMe, 33·0%). Treatment of the lactone with liquid ammonia gave, in small yield, 2 : 5-dimethyl L-arabonamide, m. p. 131°, not depressed on admixture with an authentic specimen. The lactone with alcoholic phenylhydrazine gave 2 : 5-dimethyl L-arabonophenylhydrazide, m. p. and mixed m. p. 162°. This fraction, therefore, contained 2 : 5-dimethyl L-arabinose. Some other sugar, substituted on $C_{(4)}$ by a methoxyl group, must also have been present since the mixture of lactones behaved as if a pyrano-lactone was present. This sugar may have been 2 : 4 : 6-trimethyl D-galactose (see fraction V).

Fraction V. A portion (0·40 g.) of this fraction was hydrolysed with N-sulphuric acid. The change in optical rotation could not be followed owing to the opaqueness of the solution. The reducing sugar was isolated as described above and had $n_D^{27°}$ 1·4717, $[\alpha]_D^{20°}$ 85° (in ethyl alcohol) (Found : OMe, 41·0. Calc. for a trimethyl hexose, OMe, 41·8%). A portion (0·29 g.) of this syrup was boiled for 3 hours with aniline (0·12 g.) in alcohol. Removal of the alcohol left a syrup which rapidly crystallised; m. p. after recrystallisation from a mixture of ether and ethyl alcohol, 175°, alone, or 177—178° admixed with 2 : 4 : 6-trimethyl D-galactose anilide of m. p. 179°. The mixed m. p. with 2 : 3 : 4-trimethyl D-galactose anilide was 135°. This fraction, therefore, consisted mainly, if not entirely, of 2 : 4 : 6-trimethyl D-galactose.

Fraction VI. A portion (0·46 g.) was hydrolysed with boiling N-sulphuric acid (25 c.c.) for 2¾ hours; $[\alpha]_D^{20°}$ +108° → +86°. The reducing sugar was isolated in the usual manner (see above). When this sugar was heated with alcoholic aniline, 2 : 4-dimethyl D-galactose anilide, m. p. 197° (after recrystallisation from water), separated. The optical rotation of the free sugar indicated that an L-arabinose derivative might be present in this fraction. Accordingly a further portion (0·78 g.) of fraction VI was dissolved in acetone (100 c.c.) containing hydrogen chloride (0·45 g.). The reaction, followed polarimetrically, was complete in 1 hour. $[\alpha]_D$ were +108° (initial value), +67° (1 hour; constant value). The solution was poured into aqueous sodium hydrogen carbonate, and, after removal of the acetone on a boiling water-bath, the solution was exhaustively extracted with ether, yielding the fractions (*a*) 0·22 g., $n_D^{17°}$ 1·4588, and (*b*) 0·06 g., $n_D^{18°}$ 1·4628. The aqueous portion was evaporated to dryness and the residue extracted with acetone, yielding fraction (*c*) (0·50 g.).

Fractions (*a*) and (*b*) were combined and hydrolysed with N-sulphuric acid; $[\alpha]_D$ +46° → +71° (2 hours, constant value). The reducing syrup (0·14 g.), isolated as described above, did not crystallise. It was heated at 80° with an aqueous solution of toluene-*p*-sulphonylhydrazide (0·15 g.) in alcohol (10 c.c.) containing acetic acid (1 drop). Removal of the solvent left a syrup which rapidly crystallised. Recrystallisation from aqueous alcohol gave 2-methyl L-arabinose toluene-*p*-sulphonylhydrazone, m. p. 145—147°, not depressed on admixture with an authentic sample (Jones, Kent, and Stacey, *J.*, 1947, 1341).

The syrup (*c*) (0·50 g.) was hydrolysed with N-sulphuric acid, and the reducing sugar isolated as before. When kept, the product crystallised and was identified as 2 : 4-dimethyl D-galactose monohydrate, m. p. 104°, not depressed on admixture with an authentic specimen.

Fraction V, therefore, consists in the main of a mixture of 2 : 4-dimethyl D-galactose and 2-methyl L-arabinose in approximately equal amounts.

Examination of Barium Salt (X).—The barium content (12·8%) of this fraction indicated that some oligosaccharide was present. A portion (1·81 g.) of it was, therefore, dissolved in methanolic hydrogen chloride (5%) and the solution boiled under reflux; $[\alpha]_D$ were +15° (initial value), +23° (1 hour), +37° (3¼ hours), +37° (4¼ hours), +41° (5¼ hours), and +38° (7¼ hours; decomposition). After the

mixture had been cooled, barium chloride was removed by filtration, and the solution was neutralised with silver carbonate. The filtered solution was evaporated to a syrup which was warmed (60°) with 0·3N-barium hydroxide (50 c.c.) for 3 hours, to convert esters of the uronic acids into the corresponding barium salts. After the mixture had cooled, the excess of barium hydroxide was removed by carbon dioxide, and the precipitated barium carbonate filtered off. The filtrate was then evaporated to dryness under reduced pressure, and the residue (1·81 g.) was extracted exhaustively with dry ether, yielding fraction (*P*) (0·64 g.), $n_D^{23°}$ 1·4775, and a residue (*Q*) (1·17 g.; Found: Ba, 18·9; OMe, 26·6%). These analytical figures indicate that the barium salt (*Q*) still contained some oligosaccharide.

The methylglycosides (*P*, 0·64 g.) were hydrolysed by boiling N-sulphuric acid; $[\alpha]_D$ +77° → +78° (constant value, 1½ hours). The reducing sugars, isolated in the usual manner, gave, on boiling with alcoholic aniline, 2 : 4-dimethyl D-galactose anilide, m. p. 198°, not depressed on admixture with an authentic specimen. The refractive index of (*P*) indicated that some other sugar of lower methoxyl content was probably also present.

The barium salt (*Q*; 1·17 g.) was converted into the corresponding methyl ester by boiling methanolic hydrogen chloride (1%) (4 hours). The solution was neutralised with silver carbonate, and the filtered solution concentrated under diminished pressure to a syrup which was dissolved in acetone. The purified syrup gave, on distillation, a fraction (0·76 g.), b. p. 140—150°/0·05 mm., $n_D^{18°}$ 1·4582 (Found: OMe, 47·4. Calc. for the methyl ester of dimethyl methylglucuronoside: OMe, 49·6%). The still-residue (0·20 g.) consisted of oligosaccharide which had escaped hydrolysis.

The ester (0·76 g.) was hydrolysed with N-hydrochloric acid; $[\alpha]_D$ +57° → + 41° (constant value after 3 hours). The cooled solution was neutralised with silver carbonate and filtered. Silver was removed by hydrogen sulphide, and the silver sulphide by filtration. Concentration of the filtrate gave syrupy 2 : 3-dimethyl D-glucuronic acid, identified by the following procedure. A portion (0·30 g.) of the hydrolysed syrup was oxidised with bromine water until the solution was non-reducing. Excess of bromine was removed by aëration, and the solution was neutralised with silver carbonate and filtered. The filtrate was treated with hydrogen sulphide to remove silver and was then concentrated under diminished pressure to a syrup, which was esterified by boiling methanolic hydrogen chloride (1%). The cooled solution was neutralised with silver carbonate and filtered. The ester, obtained on concentration of the filtrate, was distilled in a high vacuum, yielding a syrup, $n_D^{15°}$ 1·4598, which slowly crystallised and then had $[\alpha]_D^{20°}$ +14° (in water) and m. p. 101°, alone or admixed with 2 : 3-dimethyl saccharolactone methyl ester. The uronic acid fraction, therefore, contains 2 : 3-dimethyl D-glucuronic acid, and some of this acid is combined with 2 : 4-dimethyl D-galactose. It is not possible from the evidence available to decide whether or not other derivatives of D-glucuronic acid are also present.

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