227. *The Constitution of Mesquite Gum. Part I. Isolation of 6- and 4-Glucuronosidogalactose.*

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Mesquite gum, the neutral salt of an acidic polysaccharide, gives on hydrolysis with dilute mineral acid a mixture of L-arabinose and a degraded mesquitic acid. Hydrolytic cleavage of the latter yields a mixture of two aldobiuronic acids (I) and (II) together with other reducing fragments (galactose and glucuronic acid) of the complex molecule.

Studies of the methylation of the aldobiuronic acids [(I) and (II)] and subsequent hydrolysis revealed their structures. Methylation affords a mixture of the methyl derivatives of 4-glucuronosidogalactose (III) and 6-glucuronosidogalactose (IV). One of these (IV) is convertible directly into the characteristic crystalline amide (V). Methanolysis of the mixture of the methyl derivatives of the two aldobiuronic acids gives almost equal amounts of the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (VI) and a mixture of 2 : 3 : 6- and 2 : 3 : 4-trimethyl methylgalactosides, (XI) and (XIV), respectively. These three products were identified by conversion into characteristic crystalline derivatives, the first being trans-

formed into the amide (VII) of 2 : 3 : 4-trimethyl α-methylglucuronoside and into 2 : 3 : 4-trimethyl glucosaccharo-1 : 5-lactone 6-methyl ester (X); the second into 2 : 3 : 6-trimethyl galactono-γ-lactone (XIII); and the third into the phenylhydrazide of 2 : 3 : 4-trimethyl galactonic acid (XVII).

THE examination of mesquite gum, the first part of which is reported here, was carried out in 1939 (Cunneen, Ph.D. Thesis, Birmingham, 1940) and was a part of an investigation into the constitution of plant gums on which one of us (F. S.) has been engaged for some years.

Gum mesquite exudes from the stem and branches of the mesquite tree, *Prosopis juliflora*, DC, which grows in the semi-arid regions of the South-Western States of the U.S.A., in Argentina, and in Chile. The gum is readily soluble in water and has been shown by Anderson and Otis (*J. Amer. Chem. Soc.*, 1926, **48**, 3172) to be the neutral salt of a complex acidic polysaccharide like damson gum (Hirst and Jones, *J.*, 1939, 1482), cherry gum (Jones, *J.*, 1939, 558), gum arabic (Neubauer, *J. pr. Chem.*, 1854, **62**, 193; Smith, *J.*, 1939, 744), and other plant gums.

Anderson and Otis (*loc. cit.*) also established the fact that mesquite gum contained L-arabinose and D-galactose since these sugars were isolated in the crystalline condition by hydrolysis of the gum with acid. This work also led to the suggestion that a third constituent of the polysaccharide was a monomethyl derivative of D-glucuronic acid (Anderson and Otis, *J. Amer. Chem. Soc.*, 1930, 4461). We have confirmed these observations but as yet there is no proof of the location of the methyl group in the glucuronic acid residue.

Our attention was first directed to a study of the hydrolysis products of this gum, and we have been able to isolate two aldobiuronic acids, 4-glucuronosidogalactose (I) and 6-glucuronosidogalactose (II), the constitutions of which have been proved by the isolation and characterisation of crystalline derivatives.

The gum, or the mesquitic acid obtained by pouring an aqueous acidified solution of the gum into alcohol, undergoes ready hydrolysis when heated with 0·01N-sulphuric acid with formation of L-arabinose and a degraded mesquitic acid. The latter does not appear to contain arabinose and is composed of galactose and glucuronic acid residues only. In this respect degraded mesquitic acid is analogous to degraded arabic acid, which has already been shown to contain galactose and glucuronic acid (Smith, *J.*, 1939, 1724). Further work has been done on the degraded mesquite acid and reference to it will be made in a later communication.

Prolonged hydrolysis of mesquitic acid or degraded mesquitic acid with dilute sulphuric acid (3%) afforded a mixture containing two aldobiuronic acids and reducing fragments. The latter probably consist of galactose and glucuronic acid. The barium salts of the glucuronic and aldobiuronic acids were readily separated from the reducing sugars by precipitation with alcohol.

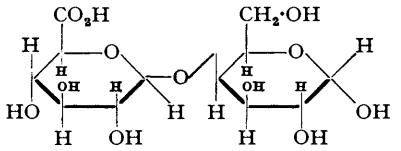
Methylation of the barium salts of the acids, first with methyl sulphate and sodium hydroxide, and then with silver oxide and methyl iodide, gave a product from which a mixture of the two methylated aldobiuronic acids (III) and (IV) was separated.

This mixture could not be separated by fractional distillation but, on treatment of it with methyl-alcoholic ammonia, one of its constituents (IV) gave the crystalline amide (V) of hexamethyl 6-β-D-glucuronosido-β-methyl-D-galactopyranoside, obtained previously from gum arabic (Jackson and Smith, *J.*, 1940, 74). This proved that one of the constituents had the constitution (IV), from which it follows that (II) is the aldobiuronic acid from which (IV) is derived.

Confirmation of the structure (II) assigned to one aldobiuronic acid as a result of the isolation of the crystalline amide and proof of the structure (I) of the second one was established as a result of the following observations. Methanolysis (*i.e.*, simultaneous hydrolysis and methylglycoside formation) of the mixture of the two methyl esters of the methylated aldobiuronic acids [(III) and (IV)] with methyl-alcoholic hydrogen chloride (8%) gave the methyl ester of the trimethyl methylglucuronoside (mixture of α- and β-isomers) (VI) and an almost equal amount of a mixture of the two trimethyl methylgalactosides (XI) and (XIV).

The methyl ester of the trimethyl methylglucuronoside (VI) was shown to be the 2 : 3 : 4-trimethyl derivative because upon treatment with methyl-alcoholic ammonia the α-isomer gave the crystalline amide (VII) of 2 : 3 : 4-trimethyl α-methylglucuronoside, identified by comparison with an authentic specimen (Smith, *J.*, 1939, 1732). The structure (VI) assigned to the methylated uronic acid was confirmed as follows. Hydrolysis of (VI) with N-sulphuric acid gave a reducing acid (VIII) which on oxidation with bromine furnished in solution the lactone (IX). Treatment of (IX) with 1% methyl-alcoholic hydrogen chloride effected esterification, and, on distillation in high vacuum, there was obtained the crystalline 2 : 3 : 4-trimethyl glucosaccharo-1 : 5-lactone 6-methyl ester (X). This lactone-ester was identical with an authentic specimen

prepared previously (Charlton, Haworth, and Herbert, *J.*, 1931, 2855; Robertson and Waters, *J.*, 1931, 1709; Smith, *J.*, 1939, 1733).



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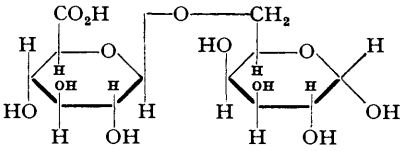

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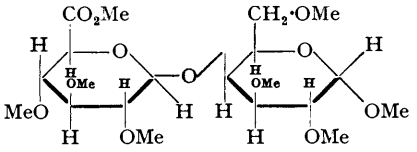

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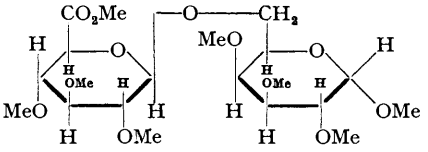

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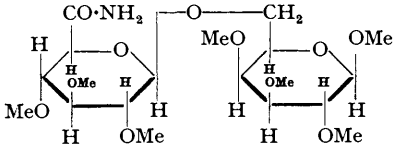

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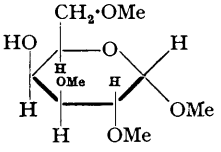

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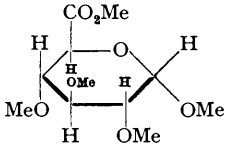

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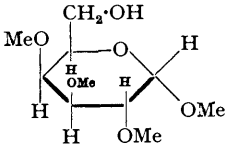

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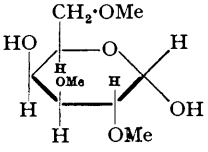

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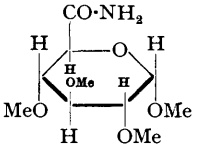

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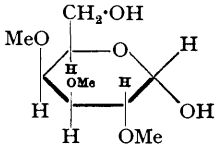

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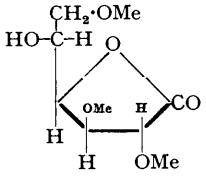

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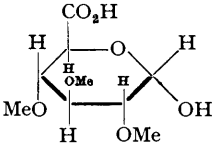

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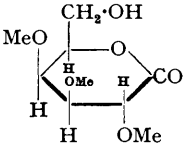

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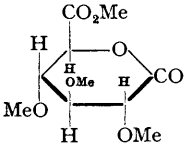

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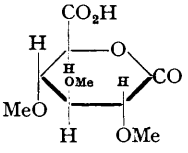

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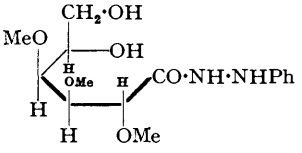

(XVII.)

The constitution of the two trimethyl methylgalactosides (XI) and (XIV) obtained by hydrolysis of the methylated aldobiuronic acids (III) and (IV) is based upon the following facts. Hydrolysis of the mixture of the two methylated galactose derivatives with 0·5N-sulphuric acid afforded a mixture of the two sugars (XII) and (XV) which gave rise on oxidation with bromine to the corresponding lactones (XIII) and (XVI) from which 2 : 3 : 6-trimethyl galactono-γ-lactone readily crystallised on nucleation. Purification of the crystalline trimethyl lactone gave a substance identical with an authentic specimen of 2 : 3 : 6-trimethyl galactono-γ-lactone (Haworth, Hirst, and Stacey, *J.*, 1932, 2481).

As much as possible of the crystalline 2 : 3 : 6-trimethyl galactono-γ-lactone was separated from the mixture containing the second trimethyl galactonolactone by extraction with ether–light petroleum. Indications were obtained that the unknown trimethyl lactone was a δ-lactone, although it was contaminated with 2 : 3 : 6-trimethyl galactono-γ-lactone, by reason of the fact

that an aqueous solution of the syrupy lactone displayed relatively rapid mutarotation in aqueous solution in a negative direction, reaching an equilibrium value in 9 hours. For comparison, the crystalline 2 : 3 : 6-trimethyl galactono-γ-lactone (XIII) showed relatively slow mutarotation in a positive direction ($[\alpha]_D$ — 44° changing to — 29° in 14 days). The trimethyl δ-lactone (XVI) present in the syrupy material proved to be 2 : 3 : 4-trimethyl galactono-δ-lactone, for on treatment with phenylhydrazine there was obtained the easily accessible characteristic crystalline phenylhydrazide (XVII) of 2 : 3 : 4-trimethyl galactonic acid (McCreath and Smith, *J.*, 1939, 387).

The presence of 2 : 3 : 4-trimethyl galactose can only be explained by the existence of an aldobiuronic acid in which the uronic acid moiety, in this case glucuronic acid, is joined through its reducing group to C_6 of a galactose unit, *i.e.*, the biose link is of the 1 : 6 type as shown in (II). This is in agreement with the previously established observation of the formation of the crystalline amide (V) of hexamethyl 6-β-D-glucuronosido-β-D-methylgalactopyranoside. This aldobiuronic acid containing the 1 : 6 link is identical with that obtained from gum arabic by prolonged hydrolysis (Haworth, Hirst, and Challinor, *J.*, 1931, 258).

The structure of the second aldobiuronic acid (I), also consisting of a galactose and a glucuronic acid unit, follows from the isolation and identification of the crystalline 2 : 3 : 6-trimethyl galactono-γ-lactone (XIII). This lactone is derivable from either a 2 : 3 : 6-trimethyl galactofuranose or a 2 : 3 : 6-trimethyl galactopyranose unit. Hence the biose link in the aldobiuronic acid may join C_1 of the uronic acid residue either to C_4 of the galactose unit giving a structure for the aldobiuronic acid represented by (I), or to C_5 of the galactose unit in which case the aldobionic acid would be a glucuronosidogalactofuranose. If the aldobiuronic acid was a glucuronosidogalactofuranose, the linking of such an aldobiuronic acid to the rest of the molecule in the mesquite acid would be expected to be cleaved relatively easily by acid hydrolysis. The liberation of the aldobionic acid was found, however, to require rather prolonged hydrolysis. It is therefore designated 4-glucopyruronosidogalactopyranose and assigned the structure (I).

As far as the authors are aware, this is the first occasion on which the isolation of two aldobionic acids from a plant gum has been recorded. Its significance will be discussed in a later communication.

Experimental.

Preparation of Mesquitic Acid.—Mesquite gum (500 g.), dissolved in cold water (2 l.), was acidified with dilute hydrochloric acid (Congo-red), and the mixture poured slowly with stirring in a thin stream into ethyl alcohol (6 l.). The precipitated solid was washed twice by decantation with fresh alcohol, filtered, and pressed to remove as much alcohol as possible. After a repetition of this process, followed by precipitation of the mesquitic acid by pouring an aqueous solution of it into alcohol to remove mineral acid, the product was filtered off, washed with alcohol and then with ether, and dried in a vacuum. The resulting mesquitic acid was a fine white amorphous powder. It was free of inorganic salts, contained no mineral acid, and showed $[\alpha]_D^{18°}$ + 60° in water (*c*, 1·0) (Found: OMe, 2·9%; equiv., by titration with 0·02N-sodium hydroxide using phenolphthalein as indicator, 1350; iodine number, 2·6).

Hydrolysis of Mesquitic Acid and Formation of the Aldobionic Acids (I) *and* (II).—To a solution of mesquitic acid (300 g.) in water (1800 c.c.) was added concentrated sulphuric acid (36 c.c.) in water (120 c.c.). The solution was heated for 20 hours at 85°, decolourised with charcoal, treated with a slight excess of barium hydroxide (phenolphthalein), neutralised with carbon dioxide, and filtered. The precipitate was washed several times with hot water. The combined filtrate and washings were concentrated to a small volume (700 c.c.) under reduced pressure. When this solution was poured into methyl alcohol (6 l.) a mixture of the barium salts of uronic acids was precipitated. After being washed with alcohol and ether and dried in a vacuum at 40° it was obtained as a white amorphous powder (54 g.) (Found: OMe, 5·82; equiv., 495. Calc. for the barium salt of a mixture of aldobionic acids having one methoxyl group: OMe, 7·1%; equiv., 436). Further hydrolysis was effected by heating the barium salts (50 g.) for 14 hours at 85° with 5% sulphuric acid (360 c.c.). By working up the reaction mixture in the manner used above, the barium salts (20 g.) were isolated as a white powder (Found: OMe, 7·3%; equiv., 426).

The Methyl Esters of Hexamethyl 4- *and* 6-*Glucuronosidomethylgalactoside,* (III) *and* (IV).—A portion of the barium salts (13 g.) was dissolved in water (60 c.c.), and acetone (40 c.c.) was added until a slight turbidity was produced. This solution was then treated with methyl sulphate (200 c.c.) and sodium hydroxide (560 c.c. of 30% solution) at room temperature, the reagents being added gradually during 4 hours with vigorous stirring. After 6 hours' stirring the mixture was non-reducing to Fehling's solution; the temperature was then raised to 50°, and methyl sulphate (50 c.c.) and sodium hydroxide (140 c.c. of 30% solution) added in ½ hour after which time stirring was continued for a further 4 hours. The solution was cooled in an ice-bath, acidified with dilute sulphuric acid, and treated with ethyl alcohol (500 c.c.). The precipitated sodium sulphate was removed and washed with aqueous alcohol (60% ethyl alcohol). The combined filtrate and washings (2 l.) were made alkaline, concentrated almost to dryness under reduced pressure, and the partially methylated compound methylated further at 50° with methyl sulphate (250 c.c.) and sodium hydroxide (700 c.c. of 30% solution). The solution was acidified, the sodium sulphate was precipitated as before, and the solution, after being made just alkaline with dilute sodium hydroxide, was concentrated to about 100 c.c. A third methylation was then applied

using the method described for the second methylation. The resulting solution was concentrated to 700 c.c., acidified, and extracted 5 times with chloroform. The combined chloroform extracts, after being washed with water and dried ($MgSO_4$), gave on concentration a syrup (8·4 g.) consisting of partially methylated aldobionic acids.

A solution of the syrup (8·4 g.) in methyl iodide (25 c.c.) was boiled for 6 hours, and during this time dry silver oxide (10 g.) was gradually added. The residue, after removal of the methyl iodide by distillation, was extracted 6 times with hot acetone. Concentration of these extracts gave a syrup which was methylated twice in the same way. The syrup (7·4 g.), obtained by acetone extraction, furnished on distillation : *fraction* (*i*) (0·86 g.), b. p. 140—200° (bath temp.)/0·03 mm., $n_D^{18°}$ 1·4505; *fraction* (*ii*), a mixture of the methylated aldobionic acids (III) and (IV) (4·0 g.), b. p. 220—230° (bath temp.) /0·015 mm., $n_D^{18°}$ 1·4705, $[\alpha]_D^{20°}$ + 28° in water (*c*, 1·5) (Found : OMe, 50·0; equiv., 468. Calc. for $C_{20}H_{36}O_{12}$: OMe, 52·8%; equiv., 468). The undistilled residue amounted to 2·54 g. Since the mixture of aldobionic acids, fraction (ii) (3·9 g.), had a low methoxyl content, it was re-treated with silver oxide and methyl iodide and the product isolated in the usual way. There was no increase in the methoxyl content and hence the methylation was considered to be complete.

Examination of the Fully Methylated Aldobionic Acids (III) *and* (IV). *Identification of the Amide of Hexamethyl* 6-β-D-*Glucuronosido-β-methyl-*D-*galactopyranoside* (V).—Treatment of the fully methylated aldobionic acid (0·3 g.) with methyl-alcoholic ammonia at 0° for 38 hours followed by evaporation of the solvent gave the amide of hexamethyl-6-β-D-glucuronosido-β-methyl-D-galactopyranose (V) which crystallised from acetone–ether in colourless needles and had m. p. 195°, $[\alpha]_D^{18°}$ − 22° in water (*c*, 0·83) (Found : OMe, 46·3. Calc. for $C_{19}H_{35}O_{11}N$: OMe, 47·9%). A mixed m. p. with an authentic specimen, which had $[\alpha]_D^{20°}$ − 18° in water (*c*, 3·7) and m. p. 196°, showed no depression.

Hydrolysis of the Mixture of Methylated Aldobiuronic Acids (III) *and* (IV).—*Experiment I.* When a solution of a portion (1·27 g.) of fraction (ii) in 3% methyl-alcoholic hydrogen chloride (100 c.c.) was boiled under reflux the following changes in rotation were observed : + 21° (initial value); + 33° (3 hours); + 37° (5 hours); + 39° (7 hours); +43° (9 hours); + 47° (13 hours); + 48° (19 hours). Neutralisation of the acid with silver carbonate, followed by filtration and removal of the solvent, gave a syrupy residue which on distillation afforded : *fraction* (a) (0·47 g.), b. p. 100—118° (bath temp.)/0·01 mm., $n_D^{18°}$ 1·4508—1·4575 (Found : equiv., 390); *fraction* (b) (0·6 g.), b. p. 180—200° (bath temp.) /0·01 mm., $n_D^{18°}$ 1·4680 (Found : OMe, 50·0%; equiv., 450). The undistilled methylated aldobiuronic acid amounted to 0·2 g. It was apparent that hydrolysis was incomplete and that the mixture of the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside and trimethyl methylgalactoside [fraction (*a*)] could not be separated readily by distillation.

Experiment II. When a solution of the mixture of methylated aldobiuronic acid [1·86 g. of fraction (ii)] in 8% methyl-alcoholic hydrogen chloride (100 c.c.) was boiled the rotational changes were as follows : + 23° (initial value); + 63° (1 hour); + 78° (2 hours); + 81° (3 hours); + 88° (5 hours); + 89° (7 hours); + 89° (9 hours). Isolation of the product as in Experiment I gave a mobile liquid (1·83 g.) most of which had b. p. < 160° (bath temp.) /0·01 mm. It was thus evident that hydrolysis could be effected by boiling 8% methyl-alcoholic hydrogen chloride.

The remaining mixture of the methylated aldobionic acids [fraction (ii)] was combined with the products obtained from Experiment I, including the undistilled residue (0·2 g.), and boiled for 10 hours with 100 c.c. of 8% methyl-alcoholic hydrogen chloride. The hydrolytic products were isolated as before and the combined cleavage fragments (3·5 g.) from this experiment and Experiment II were heated for 2 hours at 60° with barium hydroxide (1·5 g.) in water (100 c.c.). The excess of barium hydroxide was neutralised with carbon dioxide. The solution was filtered and evaporated to dryness under reduced pressure giving a syrup consisting of a mixture of the methylated galactosides (XI) and (XIV) and the barium salt of the trimethyl methylglucuronoside. Exhaustive ethereal extraction gave the mixture of galactosides (XI) and (XIV) as a colourless syrup (1·1 g.). The residual barium salt was left as a pale yellow solid (2·5 g.).

Identification of the Methyl Ester of 2 : 3 : 4-*Trimethyl Methylglucuronoside* (VI).—The yellow solid consisting of the barium salt of 2 : 3 : 4-trimethyl methylglucoronoside was converted into the methyl ester by boiling it for 6 hours with 2% methyl-alcoholic hydrogen chloride (100 c.c.). After separation of the precipitated barium chloride from the cooled solution by filtration, the solution was neutralised with silver carbonate and filtered, and the residue was washed well with hot methyl alcohol. Evaporation of the combined filtrate and washings gave a dark brown syrup which was dissolved in acetone. Addition of ether to the solution precipitated some inorganic impurity which was removed. Evaporation of the filtrate gave a clear yellow syrup which on distillation gave the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (VI) (1·0 g.), b. p. 135—155° (bath temp.) /0·02 mm., $n_D^{17°}$ 1·4485, $[\alpha]_D^{15°}$ + 80° in water (*c*, 1·4) (Found : OMe, 57·8; equiv., 263. Calc. for $C_{11}H_{20}O_7$: OMe, 58·6%; equiv., 264). A residue of unhydrolysed methylated aldobionic acids remained (0·76 g.).

The Amide of 2 : 3 : 4-*Trimethyl α-Methylglucuronoside* (VII).—Treatment of the methylated glucuronic ester (VI) (0·1 g.) with methyl-alcoholic ammonia for 2 days at 0° followed by removal of the solvent under reduced pressure gave the crystalline amide (VII) of 2 : 3 : 4-trimethyl α-methylglucuronoside. Recrystallisation from ethyl alcohol–ether–light petroleum yielded colourless needles, m. p. and mixed m. p. 185°, $[\alpha]_D^{17°}$ + 137° in water (*c*, 0·7) (Found : C, 48·5; H, 7·55; N, 5·6; OMe, 48·7. Calc. for $C_{10}H_{19}O_6N$: C, 48·2; H, 7·75; N, 5·6; OMe, 49·8%).

2 : 3 : 4-*Trimethyl Glucosaccharo*-1 : 5-*lactone* 6-*Methyl Ester* (X).—A portion (0·76 g.) of the ester (VI) was heated on the boiling water-bath with N-sulphuric acid (30 c.c.) for 22 hours during which rotation changed from + 57° to + 43°. The solution was neutralised with barium carbonate, filtered, and evaporated to dryness under reduced pressure. Extraction of the resulting solids with methyl alcohol, followed by concentration of the extracts, gave a pale yellow solid (0·76 g.) which reduced Fehling's solution and gave a positive test for barium.

To the yellow solid (0·76 g.) in water (5 c.c.), bromine (1 c.c.) was added and the mixture left (4 days) at room temperature until a portion of the solution, when freed from bromine, no longer reduced Fehling's

solution. The excess of bromine was then removed by aeration, and the solution neutralised with silver carbonate, filtered and the filtrate treated with hydrogen sulphide to remove silver as silver sulphide. The solution was immediately evaporated to dryness and extracted 3 times with hot methyl alcohol. Concentration of the extracts gave the barium salt of 2 : 3 : 4-trimethyl saccharolactone (0·55 g.) which was converted into the methyl ester by boiling it for 8 hours with 1% methyl-alcoholic hydrogen chloride (50 c.c.). After removal of the precipitated barium chloride from the cooled solution, the latter was neutralised with silver carbonate and filtered, the residue being well washed with hot methyl alcohol. Evaporation of the filtrate and washings gave a syrup (0·4 g.) which on distillation gave 2 : 3 : 4-trimethyl glucosaccharolactone methyl ester (X) (0·21 g.), b. p. 133° (bath temp.) /0·03 mm., $n_D^{19°}$ 1·4585; the colourless distillate crystallised spontaneously. After crystallisation from ethyl alcohol–ether the 2 : 3 : 4-trimethyl glucosaccharo-1 : 5-lactone 6-methyl ester (X) had m. p. and mixed m. p. 107°, $[\alpha]_D^{16°}$ + 88° in water (*c*, 0·9) (after 20 minutes), changing in 29 hours to + 35° (equilibrium value) (Found: C, 48·3; H, 6·3; OMe, 50·0. Calc. for $C_{10}H_{16}O_7$: C, 48·4; H, 6·5; OMe, 50·0%).

Examination of the Methylated Galactosides.—Identification of 2 : 3 : 6-*trimethyl methylgalactoside* (XI) *and* 2 : 3 : 4-*trimethyl methylgalactoside* (XIV). Slow distillation of the mixture of methylated galactosides gave a colourless liquid (0·7 g.), b. p. 125° (bath temp) /0·01 mm., $n_D^{18°}$ 1·4600, $[\alpha]_D^{16°}$ + 42° in water (*c*, 1·0). For 2 : 3 : 6-trimethyl methylgalactoside $n_D^{19°}$ 1·4550 has been recorded (Haworth, Raistrick, and Stacey, *Biochem. J.*, 1935, **29**, 2668), and 2 : 3 : 4-trimethyl methylgalactoside has $n_D^{19°}$ 1·4640 (McCreath and Smith, *J.*, 1939, 387; Smith, *J.*, 1939, 1726). It appeared that no separation of the galactosides had been effected by distillation (Found: OMe, 52·0. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%).

Hydrolysis of the methylated galactosides. When a solution of the distilled galactosides (0·49 g.) in 0·5N-sulphuric acid (13·5 c.c.) was heated on the boiling water-bath for 8 hours, the rotation changed from $[\alpha]_D^{16°}$ + 41° (initial value) to $[\alpha]_D^{16°}$ + 72° (constant value). The solution was neutralised with barium carbonate, filtered, evaporated to dryness under diminished pressure, and the residue extracted with ether. Concentration of the ethereal solution gave a mixture of the free sugars (XII) and (XV) (0·41 g.) (Found: OMe, 40·5. Calc. for $C_9H_{18}O_6$: OMe, 41·8%).

Oxidation with bromine. The mixture of trimethyl galactoses (0·4 g.) was dissolved in water (5 c.c.), bromine (0·4 c.c.) was added, and the solution left at room temperature for 2 days. After this time a portion of the mixture, when freed from bromine by aeration, was non-reducing to Fehling's solution. By working up in the usual manner there was obtained a mixture of lactones (0·37 g.) which furnished on distillation a syrup (0·22 g.), b. p. 165° (bath temp.) /0·012 mm., $n_D^{19°}$ 1·4660 (Found: OMe, 39·8. Calc. for $C_9H_{16}O_6$: OMe, 42·3%). After being kept for a few days the distillate had undergone partial crystallisation.

Identification of 2 : 3 : 6-*Trimethyl Galactono-γ-lactone* (XIII).—The partially crystalline mixture of lactones was triturated with a little ether–light petroleum; this removed much of the syrup, and the crystals which remained undissolved were freed from the rest of the adhering syrup by means of a porous tile. After crystallisation from ether the 2 : 3 : 6-trimethyl galactono-γ-lactone (XIII) had m. p. and mixed m. p. 101°, $[\alpha]_D^{18°}$ — 44° (initial value in water; *c*, 0·9), changing to $[\alpha]_D^{18°}$ — 29° in 14 days (Found: OMe, 42·45. Calc. for $C_9H_{16}O_6$: OMe, 42·3%). An authentic specimen of 2 : 3 : 6-trimethyl galactono-γ-lactone showed $[\alpha]_D$ — 40° (initial value) in water (*c*, 1·6) changing in 14 days to — 28° (equilibrium value).

Identification of 2 : 3 : 4-*Trimethyl Galactono-δ-lactone* (XVI).—The syrupy portion of the mixture of lactones which dissolved in ether–light petroleum was recovered by removal of the solvent. The syrup so obtained (0·1 g.) had $[\alpha]_D^{17°}$ + 35° in water (*c*, 1·0) changing to $[\alpha]_D^{19°}$ + 10° in 22 hours (Found: OMe, 39·8. Calc. for $C_9H_{16}O_6$: OMe, 42·3%). The lactone syrup (0·1 g.) was heated with phenylhydrazine (0·05 g.) in dry methyl alcohol (1 c.c.) for 15 minutes. The alcohol was then removed and the mixture heated for 3 hours at 95°. After cooling, trituration with ether gave crystalline 2 : 3 : 4-trimethyl galactonic acid phenylhydrazide (XVII) (0·06 g.), m. p. and mixed m. p. 176°, $[\alpha]_D^{17°}$ + 36° in ethyl alcohol (*c*, 0·8) (after crystallisation from ethyl alcohol–ether) (Found: C, 54·8; H, 7·15; N, 8·85; OMe, 27·4. Calc. for $C_{15}H_{24}O_6N_2$: C, 54·9; H, 7·4; N, 8·5; OMe, 28·4%).

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