198. *The Constitution of Cherry Gum. Part II. The Products of Hydrolysis of Methylated Cherry Gum.*

By J. K. N. Jones.

Methylated cherry gum has been hydrolysed and the following products of hydrolysis have been identified: 2 : 3 : 5-trimethyl *l*-arabinose, 2 : 5-dimethyl *l*-arabinose, 2 : 4 : 6-trimethyl *d*-galactose, 2 : 4-dimethyl *d*-galactose, 2 : 3 : 4-trimethyl *d*-glucuronic acid, and 2 : 3-dimethyl *d*-glucuronic acid. Derivatives of *d*-mannose and of *d*-xylose are also present but remain to be identified; other methylated derivatives of *d*-galactose may also be present.

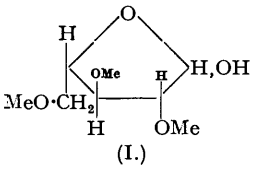
The results indicate the main structural features of the polysaccharide, but owing to the difficulty encountered in effecting the quantitative separation of these sugars it is not possible at this stage to suggest a unique structural formula.

It has been shown (Jones, *J.*, 1939, 558) that cherry gum contains the following sugars in the approximate proportions indicated: *d*-glucuronic acid (1 part), *d*-mannose (1 part), *d*-galactose (2 parts), *l*-arabinose (6 parts), and *d*-xylose (*ca.* 1·5%). The polysaccharide has now been converted into the fully methylated derivative by the thallium hydroxide method of methylation (Menzies, *J.*, 1926, 937; Hirst and Jones, *J.*, 1938, 502). This product is substantially homogeneous since no portions differing materially one from another were obtained on fractionation.

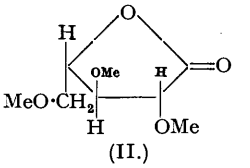
Methylated cherry gum, like methylated damson gum, readily underwent partial methanolysis with methyl alcoholic hydrogen chloride (2%) but there remained a resistant residue which required further prolonged boiling with this reagent before the reaction was complete. Six different sugars were recognised after fractional distillation of the methanolysis products, namely: 2 : 3 : 5-trimethyl *l*-arabinose, 2 : 5-dimethyl *l*-arabinose, 2 : 4 : 6-trimethyl *d*-galactose, 2 : 4-dimethyl *d*-galactose, 2 : 3 : 4-trimethyl *d*-glucuronic acid, and 2 : 3-dimethyl *d*-glucuronic acid. Derivatives of *d*-mannose and of *d*-xylose must be present, but their isolation has not yet been accomplished. It is possible that other methylated galactoses may also be present amonst the products of hydrolysis.

Proof of the identity of these sugar derivatives was obtained as follows:

(*a*) 2 : 3 : 5-Trimethyl *l*-arabinose (I) was identified after oxidation as crystalline 2 : 3 : 5-trimethyl *l*-arabonolactone (II).



(I.)



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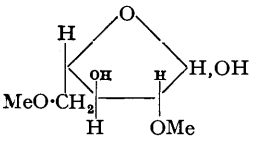

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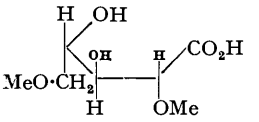

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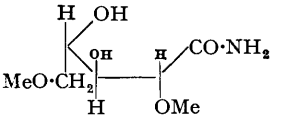

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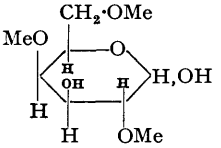

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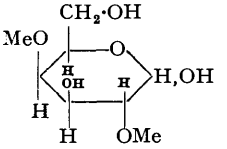

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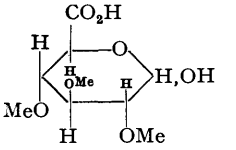

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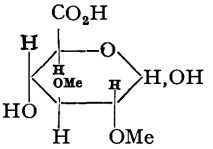

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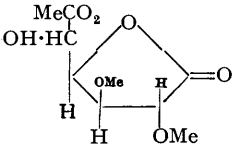

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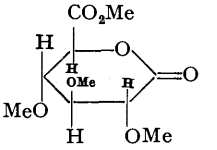


(X.)

(*b*) 2 : 5-Dimethyl *l*-arabinose (III) was recognised after oxidation as the crystalline lactone and amide (V) of 2 : 5-dimethyl *l*-arabonic acid (IV) (Smith, *J.*, 1940, 1035).

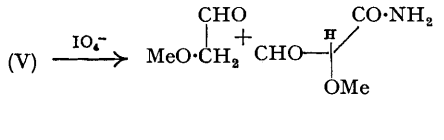
(*c*) 2 : 4 : 6-Trimethyl *d*-galactose (VI) was converted into its characteristic crystalline anilide (Bell and Wilson, *J.*, 1938, 1196; Percival and Somerville, *J.*, 1937, 1615).

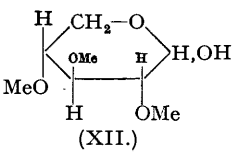
(*d*) 2 : 4-Dimethyl *d*-galactose was isolated as crystalline 2 : 4-dimethyl *d*-galactose (VII) and its crystalline anilide (Smith, *J.*, 1940, 1050).

(*e*) 2 : 3 : 4-Trimethyl (VIII) and 2 : 3-dimethyl *d*-glucuronic (IX) acids were recognised as the corresponding crystalline derivatives of saccharic acid, *viz.* methyl 2 : 3 : 4-trimethyl *d*-saccharolactone ester (X) and methyl 2 : 3-dimethyl *d*-saccharolactone ester (XI).

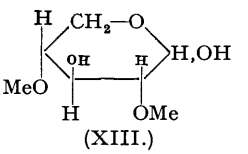
Owing to the difficulty in identifying and separating quantitatively the various glycosides it is not possible to give a precise quantitative estimate of the various sugar derivatives formed on hydrolysis of the methylated polysaccharide. Hence no attempt is made to give a unique formula for cherry gum at this stage. However, a semi-quantitative examination of various sugar residues identified indicates that the ratio of trimethyl *l*-arabofuranose to 2 : 5-dimethyl *l*-arabinose is about 1 : 1 and that the ratio of 2 : 4 : 6-trimethyl *d*-galactose to 2 : 4-dimethyl *d*-galactose is also about 1 : 1.

An inspection of the constants for the pentose fractions shows that derivatives other than 2 : 3 : 5-trimethyl *l*-arabinose and 2 : 5-dimethyl *l*-arabinose must be present in them since both the free sugars and the lactones derived from the pentose fractions had optical rotations too high in the positive sense. 2 : 5-Dimethyl *l*-arabonic acid (IV) and its amide (V) possess hydroxyl groups on adjacent carbon atoms (C_3 and C_4) and should be oxidised by salts of periodic acid. Accordingly, fractions 23, 24, and 25 (see experimental section) were oxidised with periodic acid. From the product a lactone was obtained which must have possessed no α-glycol grouping, and since this lactone behaved as a pyranolactone it must be a derivative of 2 : 4-dimethyl (XIII) or 2 : 3 : 4-trimethyl *d*-xylose (XII) or 2 : 4 : 6-trimethyl *d*-galactose (VI). A pyranose derivative of *l*-arabinose is ruled out as it is known that all the arabinose units are in the furanose form (Jones, *loc. cit.*).





(XII.)



(XIII.)

It has already been shown that 2-(*d*-glucuronosido)-*d*-mannose is part of both the damson gum and cherry gum molecules (Hirst and Jones, *J.*, 1938, 1174), and it is now obvious that both polysaccharides possess other points of similarity. For example, *d*-galactose units which are linked through positions C_3 and C_1 and C_6, C_3, and C_1 occur in both; in addition both molecules contain terminal *l*-arabinose and *d*-glucuronic acid units, and a *d*-glucuronic acid residue linked through C_1 and C_4 is common to both gums. A point of difference, however, is that in cherry gum *l*-arabinose residues occur which are linked through C_1 and C_3 (cf. gum arabic, Smith, *J.*, 1940, 1035) whilst in damson gum the linkage is through C_1 and C_5.

It seems that cherry gum is built up on the same lines as damson gum (Hirst and Jones, *loc. cit.*) and gum arabic (Smith, *loc. cit.*) with probably a main chain of *d*-galactose units to which are attached side chains of aldobionic acids and pentose residues.

A complication arises from the fact that cherry trees may be grown as grafts on different stocks and it remains to be decided whether or not the chemical nature of the exuded gum is dependent upon the type of stock (private communication from F. W. Sansome). Although all the samples of cherry gum which have been examined possess very similar properties, until these points are investigated it cannot be claimed that the results now brought forward necessarily apply to all varieties of cherry gum.

Experimental.

Methylation.—The cherry gum (of English origin) had equiv. 1503, furfuraldehyde 31·8%, and $[\alpha]_D$ −28°. It appeared to be identical with cherry gum obtained from the South of France which had equiv. 1487, furfuraldehyde 31·9%, and $[\alpha]_D$ − 28° (mean values of determinations on seven separate nodules of gum). These figures are closely similar to those given in the literature for other samples of cherry gum isolated from widely different sources. Purified cherry gum (50 g.) (Jones, *J.*, 1939, 558) was dissolved in water (300 c.c.) containing a little N-thallous hydroxide, and a hot concentrated solution of thallous hydroxide (300 c.c. of 4N) was added with stirring. The precipitated thallium complex was rapidly filtered off, washed with methyl alcohol, and dried in a vacuum at 40°. The powdered thallium

derivative (120 mesh) was stirred with methyl iodide, with the exclusion of light and moisture, until the initial reaction had subsided, and the mixture was then boiled (under reflux) until the solid no longer gave an alkaline reaction (50 hours). Excess of methyl iodide was boiled off, the residue was exhaustively extracted with methyl alcohol, and the extracts were concentrated to a cream coloured solid (52 g.). This partly methylated derivative was dissolved in alcohol, and N-thallous ethoxide in benzene (300 c.c.) was added and the mixture evaporated to dryness with the exclusion of carbon dioxide. The solid was powdered (120 mesh), and boiled with methyl iodide until it no longer gave an alkaline reaction to litmus paper. The methylated product was extracted (as above) and the process of methylation with thallous ethoxide twice repeated. A final methylation with Purdie's reagents gave methylated cherry gum (48·0 g.); $[\alpha]_D^{20°}$ — 28° (*c*, 2·1 in methyl alcohol) (Found: equiv., 1650; OMe, 40·7%). This material (42 g.) was submitted to fractionation by precipitation from chloroform solution by addition of light petroleum. Three fractions were obtained as cream coloured solids, soluble in methyl alcohol, benzene, chloroform, acetone, dioxan, and cold water, insoluble in hot water and light petroleum. *Fraction R* (3·0 g.), $[\alpha]_D^{20°}$ — 30° (*c*, 1·4 in methyl alcohol) (Found: uronic anhydride (by boiling with 12% hydrochloric acid under the standard conditions), 10·1%; equiv. (by titration with 0·1N-sodium hydroxide), 1645; OMe, 39·0%). *Fraction S* (37·1 g.), $[\alpha]_D^{20°}$ — 27° (*c*, 2·4 in methyl alcohol) (Found: uronic anhydride, 10·4%; equiv., 1650; OMe, 42·4%). *Fraction T* (1·5 g.), $[\alpha]_D^{20°}$ — 28° (*c*, 0·9 in methyl alcohol) (Found: uronic anhydride, 10·3%; equiv., 1660; OMe, 40·1%. Calc. for fully methylated cherry gum: uronic anhydride, 10·1%; equiv. 1742; OMe, 42·7%).

Hydrolysis.—The methylated gum (Fraction S, 30 g.) was dissolved in 2% methyl alcoholic hydrogen chloride (300 c.c.) and boiled under reflux for 70 hours by which time $[\alpha]_D$ had changed from —27° to + 14°. The cooled solution was neutralised with silver carbonate, filtered, and evaporated to a syrup (34·4 g.), part of which was soluble in ether (A, 27·3 g., $n_D^{19°}$ 1·4545) and part insoluble (B, 7·1 g., $n_D^{19°}$ 1·4810).

The ether-soluble syrup (A, 27·3 g.) was heated to 90° with 0·3N-barium hydroxide (70 c.c.) for 15 hours, cooled, neutralised with carbon dioxide, and filtered, and the filtrate exhaustively extracted with chloroform in an all-glass apparatus. The chloroform extracts were evaporated to a syrup, $n_D^{20°}$ 1·4492, which on extraction with boiling ether gave a fraction soluble in ether (C, 17·45 g., $n_D^{21°}$ 1·4453) and an insoluble residue (D, 3·80 g., $n_D^{21°}$ 1·4624). The aqueous solution of the chloroform extraction was evaporated at 40°/12 mm. to a brown solid (E, 6·4 g.).

Fraction C (17·45 g.) was fractionally distilled in a vacuum, giving: *Fraction* (1), 2 : 3 : 5-trimethyl methyl-*l*-arabofuranoside (7·80 g.), b. p. 110—120°/0·001 mm. (bath temp.), $n_D^{19°}$ 1·4380 (Found: OMe, 60·5%). *Fraction* (2), a mixture of 2 : 3 : 5-trimethyl methyl-*l*-arabofuranoside and 2 : 5-dimethyl methyl-*l*-arabofuranoside (2·11 g.), b. p. 120°/0·001 mm. (bath temp.), $n_D^{19°}$ 1·4438 (Found: OMe, 58·3%). *Fraction* (3), mainly 2 : 5-dimethyl methyl-*l*-arabofuranoside (5·14 g.), b. p. 125—140°/0·001 mm. (bath temp.), $n_D^{20°}$ 1·4532 (Found: OMe, 51·0%).

To the still residue was added fraction D (3·80 g.) and the distillation continued. *Fraction* (4), mainly 2 : 4 : 6-trimethyl methyl-*d*-galactoside (2·66 g.), b. p. 140—150°/0·001 mm. (bath temp.), $n_D^{19°}$ 1·4560 (Found: OMe, 50·4%). *Fraction* (5), 2 : 4 : 6-trimethyl methyl-*d*-galactoside (0·85 g.), b. p. 150—160°/0·001 mm. (bath temp.), $n_D^{20°}$ 1·4618 (Found: OMe, 50·4%). This fraction partially crystallised. Still residue (F).

The ether-insoluble syrup B (7·1 g.) and the solid residue E (6·4 g.) were united and boiled with 2% methyl-alcoholic hydrogen chloride (300 c.c.) for 72 hours. The solution was neutralised with silver carbonate, filtered, and concentrated to a syrup which was heated with 0·3N-barium hydroxide (90 c.c.) for 12 hours. The cooled solution was neutralised with carbon dioxide, filtered, and exhaustively extracted with chloroform, and the extracts were concentrated to a syrup (G, 4·78 g., $n_D^{18°}$ 1·4730), which was added to the still residue (F) and the fractionation continued, giving: *Fraction* (6), mainly 2 : 3 : 5-trimethyl methyl-*l*-arabofuranoside (1·278 g.), b. p. 110°/0·001 mm. (bath temp.), $n_D^{19°}$ 1·4372 (Found: OMe, 58·0%). *Fraction* (7), a mixture of 2 : 5-dimethyl methyl-*l*-arabofuranoside and 2 : 4 : 6-trimethyl methyl-*d*-galactoside (0·70 g.), b. p. 120—150°/0·001 mm. (bath temp.), $n_D^{19°}$ 1·4572 (Found: OMe, 52·3%). *Fraction* (8), mainly 2 : 4-dimethyl methyl-*d*-galactoside (4·25 g.), b. p. 160°/0·001 mm. (bath temp.), $n_D^{20°}$ 1·4750 (Found: OMe, 38·4%). *Fraction* (9), dimethyl and monomethyl methyl-hexosides (1·15 g.), b. p. 160—200°/0·001 mm. (bath temp.), $n_D^{18°}$ 1·4845 (Found: OMe, 31·7%). Still residue, (0·38 g.).

The aqueous solution from G was evaporated to dryness and the residual barium salts were boiled with 6% methyl alcoholic hydrogen chloride (200 c.c.) for 24 hours. The solution was neutralised with barium carbonate, filtered, and evaporated to a syrupy mass which was exhaustively extracted with acetone. Removal of the solvent at 40°/12 mm. gave a syrup ($n_D^{21°}$ 1·4722), part of which (3·80 g.) was separated into two fractions by extraction with ether. The ether-soluble material (2·90 g., $n_D^{20°}$ 1·4625) was fractionally distilled in a vacuum, giving: *Fraction* (10), mainly the methyl ester of 2 : 3 : 4-trimethyl methyl-*d*-glucuronoside (1·63 g.), b. p. 120°/0·001 mm. (bath temp.), $n_D^{16·5°}$ 1·4530 (Found: OMe, 55%). This fraction was partially crystalline. *Fraction* (11), mainly the methyl ester of dimethyl methylglucuronoside (0·83 g.), b. p. 150°/0·001 mm. (bath temp.), $n_D^{18°}$ 1·4681 (Found: OMe, 48·7%). To the still residue was added the ether-insoluble portion (0·90 g.) of the glucuronic acid fractions and the distillation was continued, giving: *Fraction* (12), a mixture of the methyl esters of 2 : 3 : 4-trimethyl methyl-*d*-glucuronoside and dimethyl methyl-*d*-glucuronoside (0·60 g.), b. p. 160—200°/0·001 mm. (bath temp.), $n_D^{19°}$ 1·4698 (Found: OMe, 48·8%).

Examination of the Various Fractions.—Fractions (1), (2), and (6) were combined (11·1 g.) and hydrolysed with 0·1N-sulphuric acid (90 c.c.) for 7½ hours at 90°. $[\alpha]_D^{20°}$ — 20° (*c*, 11·1 in 0·1N-sulphuric acid, initial value); — 20° (1¼ hours); — 10° (3¾ hours); ±0° (7½ hours, constant value). The solution was neutralised with barium carbonate, filtered, and evaporated to a syrup at 40°/12 mm., and the sugars were exhaustively extracted with chloroform. Concentration of the extracts gave a syrup (9·28 g., $n_D^{18°}$ 1·4522), a portion (8·68 g.) of which was fractionally distilled in a vacuum giving: *Fraction* (13) (7·40 g.), 2 : 3 : 5-trimethyl *l*-arabofuranose, b. p. 108—120°/0·001 mm. (bath temp.), $n_D^{21°}$ 1·4540; $[\alpha]_D^{20°}$ ±0° (*c*, 0·9 in water) (Found: OMe, 48·0. Calc. for $C_8H_{16}O_5$: OMe, 48·4%).

Fraction (14) (0·83 g.), b. p. 120—140°/0·001 mm. (bath temp.), [$n_D^{19°}$] 1·4668, [α]$_D^{20°}$ + 47° (*c*, 0·55 in water) (Found: OMe, 42·8%). *Residue* (0·25 g.).

Fraction (13) (6·80 g.) was dissolved in water (20 c.c.) and oxidised with bromine (5 c.c.). The lactones (6·40 g.; $n_D^{17°}$ 1·4538) isolated in the usual manner were fractionally distilled giving *Fraction* (15), mainly 2 : 3 : 5-trimethyl *l*-arabonolactone (2·5 g.), b. p. 104°/0·001 mm. (bath temp.), $n_D^{18°}$ 1·4452 (supercooled liquid), m. p. and mixed m. p. with an authentic specimen 27°, [α]$_D^{20°}$ — 28° (initial value in water; *c*, 1·58); — 29° (2 hours); — 32° (4¾ hours); — 35° (6⅔ hours); — 33° (22 hours); — 32° (29½ hours); — 31° (49 hours) (Found: equiv., 198; OMe, 48·3%). With liquid ammonia the lactone gave in good yield 2 : 3 : 5-trimethyl *l*-arabonamide, m. p. and mixed m. p., 138°. *Fraction* (16) (1·93 g.), mainly 2 : 3 : 5-trimethyl *l*-arabonolactone, b. p. 104—124°/0·001 mm. (bath temp.), $n_D^{18°}$ 1·4500. [α]$_D^{20°}$ + 8° (*c*, 1·8 in water, initial value); — 2° (1 hour); — 17° (4 hours); — 19° (6 hours); — 26° (22 hours); — 25° (33 hours); — 24° (49 hours) (Found: equiv., 198; OMe, 51·0%). With liquid ammonia this lactone gave 2 : 3 : 5-trimethyl *l*-arabonamide, m. p. 138° in 70% yield. Fractions (15) and (16) were both contaminated with a lactone which, from its rate of mutarotation, was a δ-lactone and which may have been a derivative of *d*-xylonic or *d*-galactonic acid. The residue (2·0 g.) is considered below.

Fractions (3), (4), and (7) were combined (8·40 g.) and hydrolysed with N-sulphuric acid (100 c.c.) during 7 hours at 90°. [α]$_D^{20°}$ + 21° (initial value); + 55° (¾ hour); + 56° (1½ hours); + 54° (2½ hours); + 52° (4 hours); + 48° (7 hours, constant value). The solution was neutralised with barium carbonate, filtered, and evaporated, and the residual syrupy sugars (7·64 g., $n_D^{17°}$ 1·4740) were isolated in the usual manner and distilled, giving: *Fraction* (17) (2·29 g.), mainly 2 : 5-dimethyl *l*-arabinose, b. p. 128—133°/0·001 mm. (bath temp.), $n_D^{20°}$ 1·4668. [α]$_D^{22°}$ + 60° (*c*, 0·96 in water) (Found: OMe, 41·4%). *Fraction* (18) (3·36 g.), b. p. 135°/0·001 mm. (bath temp.), $n_D^{18°}$ 1·4735. [α]$_D^{22°}$ + 36° (*c*, 1·6 in water) (Found: OMe, 42·0%). *Fraction* (19) (1·07 g.), b. p. 140—170°/0·001 mm. (bath temp.). [α]$_D^{20°}$ + 39° (*c*, 0·88 in water) (Found: OMe, 36·9%). *Residue* (0·73 g.).

Fractions (14), (17), (18), and (19) were combined (7·4 g.) and oxidised with bromine water. The lactones (6·60 g.) were isolated, combined with the residue (2·0 g.) from fractions (15) and (16), and fractionally distilled in a vacuum giving *Fraction* (20) (1·15 g.), 2 : 5-dimethyl *l*-arabonolactone admixed with some other lactone of higher positive rotation (2 : 4 : 6-trimethyl *d*-galactonolactone?), b. p. 130—140°/0·001 mm. (bath temp.), $n_D^{18°}$ 1·4562. [α]$_D^{20°}$ + 14° (*c*, 1·25 in water, initial value); + 4° (1¾ hours); — 12° (5¾ hours); — 16° (9 hours); — 15° (22 hours) (Found: equiv., 187; OMe, 38·8%. Calc. for $C_7H_{12}O_5$: equiv., 176; OMe, 35·2%). With liquid ammonia the lactone gave 2 : 5-dimethyl *l*-arabonamide in good yield, m. p. and mixed m. p. with an authentic specimen, 131° (Smith, *J.*, 1939, 751) depressed to 122° on admixture with 2 : 3-dimethyl *l*-arabonamide. 2 : 5-Dimethyl *l*-arabonamide gave a negative Weerman test.

Conversion of 2 : 5-*Dimethyl* l-*Arabonamide into* 2 : 5-*Dimethyl* l-*Arabonolactone*.—The amide (0·4 g.) was heated at 90° with excess of N-sodium hydroxide until ammonia was no longer evolved (2 hours). The solution was acidified with N-sulphuric acid and then extracted exhaustively with chloroform. Concentration of the chloroform solution gave a syrup (0·35 g.) which was distilled in a vacuum; b. p. 160°/0·3 mm. The product crystallised, had m. p. 60°, and was pure 2 : 5-dimethyl *l*-arabonolactone (Found: OMe, 35·0. Calc. for $C_7H_{12}O_5$: OMe, 35%). *Fraction* (21) (5·09 g.) was mainly 2 : 5-dimethyl *l*-arabonolactone, b. p. 150°/0·001 mm. (bath temp.), $n_D^{19°}$ 1·4642. [α]$_D^{20°}$ + 21° (*c*, 1·97 in water, initial value); + 8° (1¾ hours); — 8° (5¾ hours); — 11° (9 hours); — 11° (22 hours); — 10° (38 hours) (Found: equiv., 189; OMe, 35·3%). With liquid ammonia the lactone gave 2 : 5-dimethyl *l*-arabonamide, m. p. 131°, in 60% yield. *Fraction* (22) (1·50 g.), had b. p. 160—180°/0·001 mm. (bath temp.), $n_D^{19°}$ 1·4720 (Found: OMe, 32·8%), and gave no crystalline amide with liquid ammonia.

The non-crystalline amides from Fractions (15) and (16) were combined and heated with barium hydroxide in a stream of nitrogen until the solution was free from ammonia. Excess of barium hydroxide was removed by carbon dioxide and the filtered solution decomposed with the calculated quantity of N-sulphuric acid, filtered, and evaporated to a syrup. In a similar manner lactones were regenerated from fractions (20), (21), and (22). The lactones recovered from (15) and (16) were distilled in a vacuum, giving *Fraction* (23), b. p. 120°/0·001 mm. (bath temp.), $n_D^{19°}$ 1·4550. [α]$_D^{20°}$ + 33° (*c*, 0·84 in water); + 27° (1 hour); + 14° (2 hours); + 7° (3½ hours); — 5° (7 hours); — 11° (11 hours); — 11° (23 hours). (Found: equiv., 200; OMe, 46·4%). With liquid ammonia the lactone gave an amide from which a small amount (50 mg.) of 2 : 3 : 5-trimethyl *l*-arabonamide was isolated; m. p. 138°. The syrupy amide gave a negative Weerman reaction showing the absence of amides with hydroxyl groups on C_2. The lactones from Fractions (20), (21), and (22) were fractionally distilled in a vacuum, giving: *Fraction* (24), b. p. 160°/0·001 mm. (bath temp.), $n_D^{19°}$ 1·4648. [α]$_D^{20°}$ + 43° (*c*, 2·35 in water; initial value); + 32° (1 hour); + 18° (2 hours); + 8° (3½ hours); — 4° (7 hours); — 10° (22 hours) (Found: equiv., 184; OMe, 39·5%). With liquid ammonia this lactone gave a syrupy amide which gave a negative Weerman test. *Fraction* (25), b. p. 160—170°/0·001 mm., $n_D^{19°}$ 1·4740. [α]$_D^{20°}$ + 14° (*c*, 0·85 in water, initial value); + 8° (1½ hours); + 0° (2⅔ hours); — 6° (12⅔ hours); — 11° (25 hours); — 7° (31⅔ hours) (Found: equiv., 186; OMe, 36·4%). This lactone reacted with liquid ammonia giving a syrupy amide which gave a positive Weerman reaction.

The non-crystalline amides (3·50 g.) from the above lactones [Fractions (23), (24), and (25)] were dissolved in water and excess of periodic acid added. The solution become hot, and an odour resembling that of acetaldehyde was detected; this reaction destroyed all the 2 : 5-dimethyl *l*-arabonamide present in the syrupy amides. The solution was neutralised with barium carbonate and filtered, and the filtrate concentrated at 40°/12 mm. The distillate had an aldehydic odour and reduced hot Fehling's solution. The residual syrup was dissolved in water and oxidised with bromine in the presence of barium carbonate until non-reducing to Fehling's solution. Bromine was removed by aeration, the filtered solution evaporated to dryness, and the residue boiled with 2% methyl alcoholic hydrogen chloride for 10 hours. Hydrochloric acid was removed with silver carbonate, and the filtered solution evaporated at 90°/760 mm., to a syrup which was distilled in a vacuum, giving: *Fraction* (26) (1·8 g.), b. p. 135—145°/0·001 mm. (bath temp.), $n_D^{22°}$ 1·4538. [α]$_D^{20°}$ + 50° (*c*, 1·2 in water, initial

value); + 40° (1½ hours); + 23° (4 hours); + 15° (7 hours); + 11° (23 hours); + 11° (28 hours, constant value) (Found: equiv., 175; OMe, 47·9%). With liquid ammonia the distilled lactone gave no crystalline amide. As no dimethyl dimethoxysuccinate was isolated, it is inferred that 2 : 3-dimethyl arabinose and 2 : 3-dimethyl xylose are not present in Fractions (23), (24), and (25). *Fraction* (27) (0·8 g.), b. p. 145—165°/0·001 mm. (bath temp.), $n_D^{21°}$ 1·4628. $[\alpha]_D^{20°}$ + 77° (*c*, 1·0 in water, initial value); + 59° (2 hours); + 33° (5¾ hours); + 23° (24 hours, constant value) (Found: equiv., 190; OMe, 45·7%). This fraction gave no crystalline derivative on conversion into the corresponding amide by solution in liquid ammonia. This amide and the amide from Fraction (26) gave no sodium cyanate with sodium hypochlorite, proving the absence of a hydroxyl group on C_2. 3 : 4-Dimethyl arabinose, 3 : 4-dimethyl xylose, and 3 : 4 : 6-trimethyl *d*-mannose are thus absent from this fraction.

Fraction (5) (0·82 g.) was dissolved in N-hydrochloric acid (20 c.c.) and heated at 90—95° for 3 hours. $[\alpha]_D^{20°}$ + 90° (initial value); + 77° (1 hour); + 69° (2¾ hours, constant value). The solution was neutralised with silver carbonate, filtered and evaporated to a syrup (0·80 g.), $n_D^{20°}$ 1·4738. $[\alpha]_D^{20°}$ + 75° (*c*, 4·0 in water) (Found: OMe, 39·0%). The syrup (0·79 g.) on being refluxed with alcoholic aniline gave 2 : 4 : 6-trimethyl *d*-galactose anilide (0·47 g.), m. p. and mixed m. p. 179°. The non-crystalline anilide was heated with N-hydrochloric acid (10 c.c.) at 70° for 3 hours. The cooled solution was neutralised with silver carbonate, filtered, and exhaustively extracted with light petroleum, and the extracted solution evaporated to a syrup which did not crystallise. The recovered sugar was oxidised with bromine water and the lactone isolated as a syrup which gave with liquid ammonia a non-crystalline amide. The amide gave no sodium cyanate with sodium hypochlorite (Weerman reaction), proving the absence of a hydroxyl group on C_2 and therefore of a mannose derivative in this fraction (see Jones, *loc. cit.*). The yield of 2 : 4 : 6-trimethyl *d*-galactose anilide corresponds to the presence of 0·50 g. of 2 : 4 : 6-trimethyl *d*-galactose in this fraction.

Fraction (8) (4·19 g.) was dissolved in N-hydrochloric acid (50 c.c.) and heated at 90—95° for 4 hours. $[\alpha]_D^{20°}$ + 74° (initial value), rising to + 78° (constant value). The solution was neutralised with silver carbonate, filtered, and evaporated to a syrup (3·98 g.) which did not crystallise and had $[\alpha]_D^{20°}$ + 85° (in water) (Found: OMe, 25·2%). The sugar (3·50 g.) on being heated with alcoholic aniline gave 2 : 4-dimethyl *d*-galactose anilide (0·62 g.), m. p. and mixed m. p. with an authentic specimen 212°, and a syrupy anilide (Y). This corresponds to the presence of 1·08 g. of 2 : 4-dimethyl *d*-galactose in this fraction. The crystalline anilide was hydrolysed with N-hydrochloric acid at 90° for 2 hours, the cooled solution neutralised with silver carbonate, and filtered, aniline extracted with ether, and the aqueous solution evaporated to a syrup which crystallised on nucleation with the monohydrate of 2 : 4-dimethyl *d*-galactose; m. p. and mixed m. p. with an authentic specimen 105°. The sugar (0·30 g.) was oxidised with bromine water and the lactone of 2 : 4-dimethyl *d*-galactonic acid (0·20 g.) isolated as a syrup, $[\alpha]_D^{20°}$ + 100° ⟶ + 40° (60 hours, constant value), which on solution in liquid ammonia gave 2 : 4-dimethyl *d*-galactonamide (0·11 g.). $[\alpha]_D^{20°}$ + 58° (*c*, 1·1 in water); m. p. and mixed m. p. 164° (Found: OMe, 27·6. Calc. for $C_8H_{17}O_6N$: OMe, 27·8%).

The filtrate from the anilide (Y) was evaporated to a syrup and hydrolysed with N-hydrochloric acid, and the free sugar (2·35 g., $n_D^{22°}$ 1·4842) isolated in the usual manner. The sugar was boiled in 2% methyl alcoholic hydrogen chloride and the resulting glycosides isolated and distilled; b. p. 160—180°/0·002 mm. (bath temp.), $n_D^{18·5°}$ 1·4700 (Found: OMe, 41·7%). This substance which analysed as a dimethyl methylhexoside failed to give any crystalline products on hydrolysis.

Fraction (9) (0·91 g.) was hydrolysed with N-hydrochloric acid (30 c.c.) for six hours at 95°. $[\alpha]_D^{20°}$ + 60° ⟶ + 85° (constant value). The sugars (0·7 g.) isolated in the usual manner did not crystallise and no crystalline derivative could be isolated from them.

Fraction (10) (1·3 g.) was hydrolysed with boiling N-hydrochloric acid and the resultant 2 : 3 : 4-trimethyl *d*-glucuronic acid isolated. It was converted by oxidation with bromine water into 2 : 3 : 4-trimethyl *d*-saccharic acid which was isolated as follows. Bromine was removed mainly by aeration, the last traces being removed by the passage of sulphur dioxide. The solution containing the methylated derivative was then extracted exhaustively with chloroform and the extracts were concentrated to a syrup (1·1 g.) which was esterified by boiling with methyl alcoholic hydrogen chloride. The resultant dimethyl ester of 2 : 3 : 4-trimethyl *d*-saccharic acid was isolated and converted into the methyl ester of 2 : 3 : 4-trimethyl saccharolactone by distillation in a vacuum. The product (0·9 g.), $n_D^{20°}$ 1·4605, crystallised and was purified by recrystallisation from ether; m. p. and mixed m. p. with an authentic specimen 110°.

Fractions (11) and (12) were combined and a portion of the mixture (0·9 g.) was hydrolysed. The resultant mixture of 2 : 3 : 4-trimethyl *d*-glucuronic acid and 2 : 3-dimethyl *d*-glucuronic acid was oxidised and the product converted into the corresponding dimethyl esters of 2 : 3 : 4-trimethyl and 2 : 3-dimethyl *d*-saccharic acid as described above. Distillation of this mixture (0·6 g.) gave a fraction (0·2 g.), b. p. 140°/0·0 mm., $n_D^{20°}$ 1·4650, which crystallised in part and from which methyl 2 : 3 : 4-trimethyl *d*-saccharolactone, m. p. 110°, was obtained by tiling the crystals followed by recrystallisation of the product from ether. A higher boiling fraction (0·3 g.), up to 200°/0·01 mm., $n_D^{20°}$ 1·4730, crystallised on nucleation with methyl 2 : 3-dimethyl *d*-saccharolactone. The crystals were separated by tiling and were purified by recrystallisation from alcohol–ether; m. p. 101°—not depressed on admixture with an authentic specimen.

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