314. *The Constitution of Damson Gum. Part II. Hydrolysis Products from Methylated Degraded (Arabinose-free) Damson Gum.*

By E. L. Hirst and J. K. N. Jones.

The degraded arabinose-free polysaccharide (A) obtainable from damson gum (Hirst and Jones, J., 1938, 1178) has been converted into the fully methylated derivative, which on hydrolysis gives 2 : 3 : 4 : 6-tetramethyl *d*-galactose (1 part), 2 : 4 : 6-trimethyl *d*-galactose (1 part), 2 : 3 : 4-trimethyl *d*-galactose (1 part), 4 : 6-dimethyl *d*-galactose (1 part), 2 : 3 : 4-trimethyl *d*-glycuronic acid (1 part; provisional estimation), 2 : 3-dimethyl *d*-glycuronic acid (1 part; provisional estimation), and a small quantity of 2 : 3 : 4-trimethyl *d*-xylose (⅛ part), together with an unidentified methylated derivative of *d*-mannose. The methods by which these sugars have been separated and identified, and their relative proportions estimated, are described. The meaning of these results in connection with the mode of linkage of the sugar residues present in the damson gum molecule is briefly considered.

It has been shown (Hirst and Jones, J., 1938, 1174) that graded hydrolysis of damson gum gave a polysaccharide (A), which was free from combined arabinose and contained the following sugars in the proportions indicated: *d*-glycuronic acid (1 part), *d*-mannose (1 part), and *d*-galactose (approximately 2 parts). In addition, a small amount of *d*-xylose (*ca.* 3%) was present. The polysaccharide (A) has now been converted into the fully methylated derivative by use of the thallium hydroxide method of methylation (compare Hirst and Jones, J., 1938, 502). Fractionation of this methylated derivative failed to give portions differing materially from one another and it appeared to be essentially a homogeneous substance.

Like the original polysaccharide, methylated damson gum (A) readily underwent partial hydrolysis on treatment with N-hydrochloric acid, but there remained an acidic portion which was markedly resistant to hydrolysis and could be hydrolysed only with difficulty by boiling 4% methyl-alcoholic hydrogen chloride. Seven different sugar derivatives were recognised as hydrolysis products, namely, 2 : 3 : 4-trimethyl *d*-xylose, 2 : 3 : 4 : 6-tetramethyl *d*-galactose, 2 : 3 : 4-trimethyl *d*-galactose, 2 : 4 : 6-trimethyl *d*-galactose, 4 : 6-dimethyl *d*-galactose, 2 : 3 : 4-trimethyl *d*-glycuronic acid, and 2 : 3-dimethyl *d*-glycuronic acid. A derivative (or derivatives) of mannose must also be present, but the identity of this mannose portion is as yet uncertain.

Proof of the identity of these substances was obtained as follows:

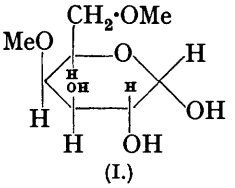
(a) The 2 : 3 : 4-trimethyl *d*-xylose was identified as the crystalline sugar and as the corresponding lactone.

(b) The tetramethyl *d*-galactose was recognised in the form of its crystalline anilide.

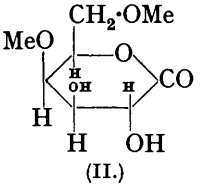
(c) Proof of the identity of the trimethyl galactoses depended on the separation of the anilides of 2 : 3 : 4- and 2 : 4 : 6-trimethyl *d*-galactose and on the isolation of the 2 : 4 : 6-trimethyl β-methylgalactoside. A partial separation of a mixture of 2 : 3 : 4-trimethyl galactose and 2 : 4 : 6-trimethyl galactose can be effected by fractional distillation of the mixed trimethyl methylgalactosides, and in this way we were able to isolate in crystalline form 2 : 4 : 6-trimethyl β-methylgalactoside.

(d) Proof of the identity of the dimethyl galactose (I) depends on the following observations. It crystallises as the monohydrate, and gives a crystalline anilide, m. p. 207°. With cold 1% methyl-alcoholic hydrogen chloride there is no downward change of rotation, the absence of a hydroxyl group on C_4 being thus indicated. The presence of a methoxyl group at C_4 was confirmed by oxidation of the sugar to the corresponding lactone (II), which, from its rate of hydrolysis, specific rotation, and the direction of mutarotation in aqueous solution, was identified as a δ-lactone. The lactone yielded with liquid ammonia a crystalline amide (III), which gave a positive Weerman reaction with sodium hypochlorite, indicating the presence of a free hydroxyl group at C_2. Additional proof of the presence of a hydroxyl group at C_2 was obtained by transformation of the sugar into a crystalline osazone without loss of methoxyl. The sugar must therefore be either 3 : 4- or 4 : 6-dimethyl *d*-galactose. The latter view was shown to be correct, since (1) the osazone of the sugar was identical with one isolated from 2 : 4 : 6-trimethyl *d*-galactose after loss of methoxyl at C_3 (Percival and Somerville, J., 1937, 1618); (2) when the *p*-toluenesulphonyl derivative of the dimethyl methylgalactoside was heated with sodium iodide in acetone, no entry of iodine into the sugar nucleus took place, indicating the presence of a methoxyl group at C_6 (Oldham and Rutherford, *J. Amer. Chem. Soc.*, 1932, **54**, 366); (3) the amide on treatment with sodium hypochlorite gave a product (probably IV), the rotation of which pointed to the formation of a derivative of lyxofuranose rather than to the production of a derivative of lyxopyranose (for comparative rotation values, see Haworth and Hirst, J., 1928, 1221; Bott, Hirst, and Smith, J., 1930, 658); (4) the constants for 3 : 4-dimethyl galactose were kindly supplied to us by Dr. D. J. Bell of Cambridge University, and these were entirely different from those of the above dimethyl *d*-galactose.

(e) Proof of the identity of the trimethyl glycuronic acid was furnished by its conversion into crystalline 2 : 3 : 4-trimethyl saccharolactone methyl ester.



(I.)



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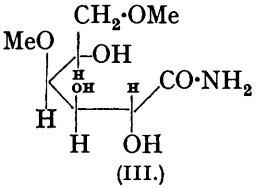

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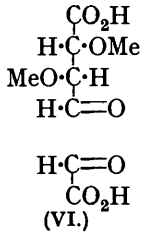
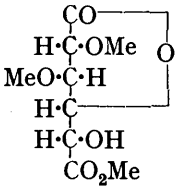
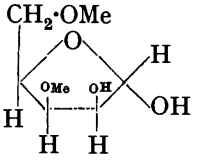


(VI.)



(V.)



(IV.)

(f) The dimethyl glycuronic acid after oxidation, followed by esterification and distillation of the product, gave crystalline 2 : 3-dimethyl *d*-saccharolactone methyl ester (V), which with methyl-alcoholic ammonia gave 2 : 3-dimethyl *d*-saccharamide identical with a specimen previously prepared by Dr. F. Smith of Birmingham University. Further proof

of identity was provided by the observation that the barium salt of the dimethyl saccharic acid reacted with periodic acid, giving glyoxylic acid (VI), thus proving the presence of two adjacent hydroxyl groups, one of which must have been next to a carboxyl group. It follows, therefore, that the two methoxyl groups are attached to C_2 and C_3 of the glycuronic acid molecule.

Because of the number of the substances involved and their similar properties, the quantitative estimation of the sugars obtained after hydrolysis presented no easy problem. A variety of methods, including refractive index and rotation data, isolation of crystalline derivatives, etc., was employed in obtaining the approximate figures given in Table I. The most useful procedure was based on the yields of the crystalline anilides corrected by a factor derived from the yield of anilide obtained from the pure sugar under similar experimental conditions. Some 82% of the distilled methyl glycosides were accounted for, and it appeared that 2 : 3 : 4 : 6-tetramethyl galactose, 2 : 3 : 4-trimethyl galactose, 2 : 4 : 6-trimethyl galactose and 4 : 6-dimethyl galactose are present in approximately equimolecular proportions, and the amount of 2 : 3 : 4-trimethyl xylose is about one sixth of that observed for each of the above sugars. All these sugars must be present in the polysaccharide as pyranose residues.

It has not been possible to identify the mannose residue which was present partly amongst the distilled methyl glycosides and partly as incompletely hydrolysed material in the undistillable residue of the uronic acid portion (fraction D). The experimental difficulty in the latter case lies mainly in the resistance to hydrolysis of the glycosidic link between the glycuronic acid and the mannose residues. The observed yields of tri- and of di-methyl glycuronic acid are therefore low and there is some uncertainty regarding the relative proportions of the tri- and di-methyl derivatives. The observed ratio (1·1 : 1) points to their occurrence in equimolecular proportions and, if it is assumed that the difficulty of hydrolysis affects similarly the portions of the gum molecule which give tri- and di-methyl glycuronic acid respectively (which is probable, in view of previous work on the mode of attachment of the glycuronic acid residues to the mannose) the provisional conclusion is reached that the equimolecular ratio holds for the whole of the glycuronic acid residues. The total uronic acid content of the methylated gum is known accurately and it seems likely, therefore, that residues of 2 : 3 : 4-trimethyl- and 2 : 3-dimethyl glycuronic acid are present in the gum in the same molecular proportion as is found for each of the various methylated galactoses.

One of the glycuronic acid residues, one galactose residue, and the xylose must be present in the repeating unit of the polysaccharide as end groups terminating side chains. The two trimethyl galactoses and the dimethyl glycuronic acid have two points of attachment (C_1C_3, C_1C_6, and C_1C_4 respectively). The dimethyl galactose has three such points ($C_1C_2C_3$) and the mannose is known to be attached at C_1 and C_2 and probably has other points of attachment also. It is obvious that a large number of formulæ can be built up from these data and, although there are limitations imposed by conditions such as the known attachment of glycuronic acid to C_2 of the mannose (Part I), it remains impossible to make a unique decision from the evidence now available. Many more of the potential structures can be eliminated by arguments based on a comparison of the hydrolysis products from the methyl derivative of the undegraded gum with those now described. Accordingly, consideration of structural formulæ will be deferred until examination of the methylated undegraded gum is complete.

Experimental.

Methylation of Damson Gum after Removal of Combined Arabinose (Polysaccharide A).—The polysaccharide A (Hirst and Jones, J., 1938, 1178) (40 g.) was dissolved in water (200 c.c.), and 3·7N-thallous hydroxide (300 c.c.) added with stirring. The precipitated thallium derivative was washed once with alcohol, dried at 60°/12 mm., and boiled with methyl iodide (300 c.c.) for 48 hours. Excess of methyl iodide was removed by distillation, and the residual solid extracted exhaustively with alcohol and then with water. The alcoholic and the aqueous solutions were combined and evaporated under diminished pressure at 50°. N-Thallous hydroxide (100 c.c.) and the filtrate from the original preparation of the thallium complex were then

added, and the evaporation continued. To the dried and finely powdered solid, methyl iodide (300 c.c.) was added, and the mixture was boiled for 48 hours. The methyl iodide was distilled off, and the mixture of methylated polysaccharide and thallium iodide extracted with methyl alcohol. The extracts were concentrated to a thick syrup, thallous ethoxide (200 g.) in benzene (200 c.c.) added, and the resulting semi-solid mass evaporated to dryness under diminished pressure. The powdered product was treated as before with methyl iodide (200 c.c.), giving the methylated polysaccharide A (38·6 g.) as a crisp cream-coloured powder (Found: OMe, 38·7%). This, after one further treatment with thallium ethoxide and after a final methylation with silver oxide and methyl iodide, had OMe, 38·9% (yield, 38·0 g.).

Fractionation of Methylated Polysaccharide A.—Successive additions of light petroleum to a solution of the methyl derivative in chloroform gave three fractions. (I) 3·4 g., $[\alpha]_D^{20°}$ + 2° (*c*, 0·95 in methyl alcohol), equiv. wt. 758 (by titration), $\eta_{sp.}/c$ 0·05 (*c*, 1·0 in *m*-cresol), uronic anhydride 20·3% (calculated from the carbon dioxide evolved on boiling with 12% hydrochloric acid), OMe 41·2%. (II) 27·8 g., $[\alpha]_D^{20°}$ + 1° (*c*, 0·96 in methyl alcohol), equiv. wt. 723 (by titration), $\eta_{sp.}/c$ 0·04 (*c*, 1·0 in *m*-cresol), uronic anhydride (method as for fraction I) 20·7% (Found: C, 51·1; H, 7·7; OMe, 41·4%). (III) 4·1 g., $[\alpha]_D^{20°}$ ± 0° (*c*, 0·87 in methyl alcohol), equiv. wt. 677 (by titration), $\eta_{sp.}/c$ 0·04 (*c*, 1·0 in *m*-cresol), uronic anhydride (method as for fraction I) 22·0%.

Hydrolysis. The methylated polysaccharide (9·55 g. from fraction II) was heated at 90—95° in N-hydrochloric acid (100 c.c.) containing glacial acetic acid (20 c.c.) until the rotation became constant (6 hrs.). $[\alpha]_D^{20°}$ + 16·5° (initial value); + 49·7° (3½ hrs.) and 51·8° (6 hrs., constant value). The cooled solution was neutralised with silver carbonate, filtered before and after passage of hydrogen sulphide, aerated to remove the latter, neutralised with barium carbonate, and again filtered. The solution, which contained the uronic acids as their barium salts, was concentrated to a small volume (100 c.c.) and exhaustively extracted in an automatic apparatus with benzene. The benzene on concentration gave a mixture of sugars (3·4 g., $n_D^{20°}$ 1·4700), which were converted into their methyl glycosides (syrup B) (3·2 g.), $n_D^{19°}$ 1·4505, by boiling for 7 hours with 2% methyl-alcoholic hydrogen chloride (50 c.c.) and were then fractionally distilled (see below).

The aqueous solution after extraction with benzene was concentrated at 50°/12 mm. to a semi-solid mass, which was extracted with acetone, leaving insoluble barium salts. Removal of the acetone gave a syrup (6·0 g.) which still contained some barium salts. It was submitted to simultaneous glycoside formation and esterification by boiling with 4% methyl-alcoholic hydrogen chloride. Hydrochloric acid was then removed with silver carbonate, and the filtered solution evaporated to a syrup (5·50 g.), which was heated at 100° with N-barium hydroxide (13·1 c.c.) for 12 hours. The excess of barium hydroxide (1·2 c.c.) was removed with N-sulphuric acid, and the filtered solution concentrated to a syrup, which was dissolved in acetone (20 c.c.) and the barium salts were precipitated by addition of ether (100 c.c.). The filtered solution was then concentrated to a syrup (C) (4·05 g., $n_D^{21°}$ 1·4672), which was fractionally distilled (see below).

The combined barium salts (4·7 g.) were boiled with 4% methyl-alcoholic hydrogen chloride (100 c.c.) for 12 hours. Hydrochloric acid was removed with silver carbonate, and the filtered solution concentrated to a syrup (D) (2·78 g.; $n_D^{20°}$ 1·4700), which was fractionally distilled.

Fractional Distillation of (B).—Fraction I. Trimethyl methyl-*d*-xylopyranoside and tetramethyl methyl-*d*-galactopyranoside (0·54 g.), b. p. 110—120°/0·003 mm. (bath temp.), $n_D^{20°}$ 1·4418, $[\alpha]_D^{21°}$ + 46° in water (*c*, 1·5), OMe 58·4%.

Fraction II. Tetramethyl methyl-*d*-galactopyranoside and 2 : 4 : 6-trimethyl methyl-*d*-galactoside (1·20 g.), b. p. 120—140°/0·003 mm. (bath temp.), $n_D^{20°}$ 1·4518, OMe 57·8%.

Fraction III. 2 : 4 : 6- and 2 : 3 : 4-Trimethyl methyl-*d*-galactopyranosides (0·74 g.), b. p. 140—154°/0·002 mm. (bath temp.), $n_D^{21°}$ 1·4590, OMe 52·2%.

At this stage of the distillation, fraction C (see above) was added to the still residue and distillation was continued, giving:

Fraction IV. 2 : 4 : 6- and 2 : 3 : 4-Trimethyl methyl-*d*-galactopyranoside (2·05 g.), b. p. 140—150°/0·002 mm. (bath temp.), $n_D^{20°}$ 1·4625, OMe 50·2%.

Fraction V. 2 : 3 : 4-Trimethyl methyl-*d*-galactoside and 4 : 6-dimethyl methyl-*d*-galactoside (1·08 g.), b. p. 150—160°/0·002 mm. (bath temp.), $n_D^{22°}$ 1·4685, OMe 44·6%.

Fraction VI. Mainly 4 : 6-dimethyl methylgalactoside (0·56 g.), b. p. 160—170°/0·002 mm. (bath temp.), $n_D^{20°}$ 1·4726, OMe 41·7%.

Fraction VII. Mixture of dimethyl and monomethyl methylgalactosides (0·60 g.), b. p. 170—220°/0·002 mm. (bath temp.), $n_D^{20°}$ 1·4790, OMe 34·7%.

The undistillable residue, mainly decomposition products, weighed 0·3 g.

Examination of Fractions I—VII.—Fraction I (0·54 g.) was dissolved in N-hydrochloric acid (30 c.c.) and heated at 95° for 4 hours. Hydrochloric acid was removed with silver carbonate, and the filtered solution concentrated at 40°/12 mm. The sugar was extracted with acetone, and was obtained as a syrup (0·45 g.), $n_D^{21°}$ 1·4585, $[\alpha]_D^{20°}$ + 57° (*c*, 1·0 in water), OMe 50·3%. (In a second hydrolysis experiment this fraction gave some crystalline 2 : 3 : 4-trimethyl *d*-xylose, m. p. 92° after recrystallisation from ether.)

The syrup (0·43 g.) was boiled with alcohol (5 c.c.) containing aniline (0·4 c.c.) for 3 hours. On concentration tetramethyl *d*-galactose anilide (0·20 g.) crystallised, m. p. and mixed m. p. with an authentic specimen, 197°. The filtrate from the crystalline anilide was concentrated to a syrup, which was dissolved in N-hydrochloric acid (10 c.c.) and heated at 90—95° for 30 minutes. Mineral acid was removed with silver carbonate and the filtered solution was extracted with light petroleum to remove aniline and concentrated to a syrup (0·30 g.), $[\alpha]_D^{20°}$ + 24·3° (*c*, 3·0 in water), which was oxidised with bromine (1 c.c.) in water (10 c.c.) at 40° for 8 hours. The excess of bromine was removed by aeration, and the solution neutralised with silver carbonate, filtered before and after passage of hydrogen sulphide, and evaporated to a syrup (0·25 g.; $n_D^{20°}$ 1·4635), which gave on distillation crystalline 2 : 3 : 4-trimethyl *d*-xylonolactone (0·16 g.), $n_D^{17°}$ 1·4620 (superfused solid), m. p. 58° (after recrystallisation from ether–light petroleum), $[\alpha]_D^{20°}$ — 4·0° (*c*, 1·96; initial value in water); — 2·0° (1 hr.); + 2·0° (6½ hrs.); + 4° (10 hrs.); + 15° (50 hrs.); + 20° (71½ hrs.); + 23° (95 hrs., constant value); equiv. wt. 197; OMe 50·2%.

From the known values of the rotations of the free sugars and from the yield of anilide and of lactone, it is estimated that fraction I contained 50% of 2 : 3 : 4-trimethyl *d*-xylose (0·25 g.) and 50% of tetramethyl *d*-galactopyranose (0·25 g.).

Fraction II (1·17 g.) was dissolved in N-hydrochloric acid (40 c.c.) and heated at 90—95° for 5 hours. $[\alpha]_D^{22°}$ fell from + 100° to + 87° (constant value). Mineral acid was removed as silver chloride, and the filtered solution concentrated in a vacuum to a syrup (1·05 g.), $n_D^{19°}$ 1·4650, $[\alpha]_D^{21°}$ + 92° (*c*, 1·2 in water). The sugar (1·0 g.) was boiled with aniline (0·95 c.c.) in alcohol (5 c.c.) for 3 hours. On concentration and cooling, the solution deposited tetramethyl *d*-galactose anilide (0·90 g.) (m. p. and mixed m. p. 196°). The filtrate and washings on further concentration deposited 2 : 4 : 6-*trimethyl* d-*galactose anilide* (0·15 g.), m. p. and mixed m. p. with an authentic specimen prepared from 2 : 4 : 6-trimethyl galactose, 179°. Our anilide was identical with a sample of 2 : 4 : 6-trimethyl galactose anilide which Dr. E. G. V. Percival of Edinburgh University kindly sent us for comparison. $[\alpha]_D^{19°}$ — 92° (*c*, 1·1 in acetone; initial value); — 89° (½ hr.); — 75° (1½ hrs.); — 38° (3 hrs.); + 2° (5½ hrs.); + 25° (8¼ hrs.); + 31° (11 hrs.); + 38° (22 hrs.; constant value) (Found : C, 60·5; H, 7·4; N, 5·0. $C_{15}H_{23}O_5N$ requires C, 60·5; H, 7·8; N, 4·7%). No 2 : 3 : 4-trimethyl *d*-galactose anilide could be detected. Since the observed yields, under the conditions mentioned, of tetramethyl galactose anilide and trimethyl galactose anilide for the pure sugars are respectively *ca.* 80% and *ca.* 70% of the theoretical, it is estimated that fraction II contained 0·9 g. of tetramethyl galactose and 0·17 g. of 2 : 4 : 6-trimethyl galactose.

The mother-liquors from the crystallisation of the anilides were heated with N-hydrochloric acid (10 c.c.) at 90—95° for 1 hour. Hydrochloric acid was removed with silver carbonate, and the filtered solution extracted with light petroleum to remove aniline. Concentration of the aqueous solution gave a syrup (0·20 g.), $n_D^{19°}$ 1·4755, which was dissolved in water (5 c.c.) and treated with bromine (1 c.c.) for 6 hours at 60°. The product, isolated in the usual way, was a syrup (0·20 g.), $n_D^{20°}$ 1·4690, which was distilled; b. p. 130—150°/0·002 mm. (bath temp.), $n_D^{20°}$ 1·4688, equiv. wt. 222, OMe 39·5%. The syrup behaved as a δ-lactone : $[\alpha]_D^{22°}$ + 105° (*c*, 3·4 in water; initial value), falling to + 54° (72 hrs.). The lactone on solution in liquid ammonia gave a syrupy amide which gave a negative Weerman test, thereby proving the absence of a hydroxyl group on position 2. This shows the absence of 3 : 4 : 6-trimethyl *d*-galactose, and it follows that mannose derivatives cannot be present in fraction II, since the partly methylated derivatives of mannose must have a free hydroxyl group on C_2 (see Hirst and Jones, *loc. cit.*).

Fraction III (0·74 g.) crystallised partly on standing and was drained on tile, giving 2 : 4 : 6-trimethyl β-methyl-*d*-galactoside (0·05 g.), m. p. and mixed m. p., with an authentic specimen, 102°. The tile was extracted with ether, and the extracts concentrated to a syrup (0·65 g.), which was hydrolysed with N-hydrochloric acid (30 c.c.) at 90—95°; $[\alpha]_D^{20°}$ fell from + 87° to + 70° (constant value) in 4 hours. The product was isolated as before (see fraction II) and concentrated to a syrup (0·52 g.), $n_D^{20°}$ 1·4690, OMe 42·0%, $[\alpha]_D^{20°}$ + 78° (*c*, 1·2 in water), which did not crystallise and was therefore converted into the anilides by boiling with aniline (0·3 c.c.)

in alcohol (10 c.c.). When the solution was cooled, 2 : 4 : 6-trimethyl *d*-galactose anilide (0·20 g.) separated, m. p. and mixed m. p. with an authentic specimen, 179°. Concentration of the mother-liquors gave a further quantity of anilide (0·25 g.), which consisted of 2 : 4 : 6-trimethyl *d*-galactose anilide (0·10 g.) and 2 : 3 : 4-trimethyl *d*-galactose anilide (0·15 g.). These were separated partly by flotation (2 : 3 : 4-trimethyl *d*-galactose anilide is the denser of the two) and partly by recrystallisation from acetone, in which 2 : 3 : 4-trimethyl *d*-galactose anilide is the less soluble. 2 : 3 : 4-Trimethyl *d*-galactose anilide crystallised in tablets, m. p. and mixed m. p. with an authentic specimen prepared by McCreath and Smith (this vol., p. 390), 170°, $[\alpha]_D^{20°}$ — 68° (*c*, 0·82 in acetone) (Found: C, 60·4; H, 7·6; N, 4·9. Calc. for $C_{15}H_{23}O_5N$: C, 60·5; H, 7·8; N, 4·7%). 2 : 4 : 6-Trimethyl *d*-galactose anilide separated in long needles. The presence of a small quantity of either admixed with the other is readily detected by microscopic examination, and a mixture of the two anilides shows a large depression in m. p.

These results show that fraction III contained 0·32 g. of 2 : 4 : 6-trimethyl *d*-galactose and 0·16 g. of 2 : 3 : 4-trimethyl *d*-galactose. The non-crystalline anilides were combined with material from other fractions (see below).

Fraction IV (2·05 g.) was dissolved in N-hydrochloric acid (50 c.c.) and heated at 90—95° for 3½ hours; $[\alpha]_D^{20°}$ 108° (initial value) ⟶ 85° (constant value). Mineral acid was removed with silver carbonate, and the filtered solution concentrated in a vacuum to a syrup (2·0 g.), $n_D^{18°}$ 1·4718, $[\alpha]_D^{20°}$ + 90° (*c*, 1·1 in water), OMe 43·7%, which did not crystallise on nucleation with 2 : 4 : 6-trimethyl *d*-galactose. The anilides were formed by boiling the syrup with alcohol (10 c.c.), containing aniline (1·5 c.c.), for 2 hours. On concentration of the solution 2 : 4 : 6-trimethyl *d*-galactose anilide (0·55 g., m. p. and mixed m. p. 179°) was obtained and on further concentration and addition of ether, mixed anilides (1·10 g.) crystallised; by fractionation from absolute alcohol these were separated into 2 : 4 : 6-trimethyl *d*-galactose anilide (0·17 g.) and 2 : 3 : 4-trimethyl *d*-galactose anilide (0·93 g.), m. p. 170°. The mother-liquors from the anilides were combined with material from other fractions (see below).

These figures point to the presence of 0·77 g. of 2 : 4 : 6-trimethyl *d*-galactose and 1·0 g. of 2 : 3 : 4-trimethyl *d*-galactose in fraction IV.

Fraction V (1·08 g.) was triturated with ether at −10°, and crystals of 4 : 6-dimethyl α-β-methyl-*d*-galactosides (39 mg.), m. p. 163°, removed by filtration. The non-crystalline syrup (1·00 g.) was hydrolysed with N-hydrochloric acid (30 c.c.); $[\alpha]_D^{22°}$ fell from + 118° to 87° (equilibrium value) in 5 hours. The solution was neutralised with silver carbonate, filtered, and evaporated to a syrup (0·95 g.), $n_D^{20°}$ 1·4752, OMe 33·2%.

The anilides were prepared by boiling the product with aniline (0·7 c.c.) in alcohol (10 c.c.). On removal of the solvent the anilides crystallised (0·55 g.); they were separated by recrystallisation from acetone into 2 : 3 : 4-trimethyl *d*-galactose anilide (0·25 g., m. p. 170°) and 4 : 6-dimethyl *d*-galactose anilide (0·30 g., m. p. 207°) (for proof of structure see below). 2 : 4 : 6-Trimethyl *d*-galactose appeared to be absent from this fraction.

These figures indicate the presence of 0·27 g. of 2 : 3 : 4-trimethyl *d*-galactose and 0·49 g. of 4 : 6-dimethyl *d*-galactose in fraction V.

Search for Methylated Mannose.—The non-crystalline anilides were combined with those from fraction III and fraction IV and hydrolysed with N-hydrochloric acid (50 c.c.) for 3 hours at 95°. The solution was neutralised with silver carbonate, filtered, and extracted with light petroleum to remove aniline. Concentration of the aqueous solution gave a syrup (1·16 g.), $[\alpha]_D^{21°}$ +64·8° (*c*, 11·6 in water), which contained some decomposition products (odour of carbylamine). It was boiled with 2% methyl-alcoholic hydrogen chloride (40 c.c.), giving the mixed methyl glycosides as a syrup (1·12 g.), which on distillation gave fractions V*a* and V*b*.

Fraction V*a* (0·38 g.), b. p. 128—132°/0·002 mm. (bath temp.), $n_D^{20°}$ 1·4592, OMe 50·2%, was hydrolysed with N-hydrochloric acid (20 c.c.) for 3 hours at 95°; $[\alpha]_D^{20°}$ fell from + 92° to + 72° (constant value). Hydrochloric acid was removed with silver carbonate, and the filtered solution evaporated to a syrup (0·34 g.), $n_D^{18°}$ 1·4680, which was converted into the anilides by boiling with aniline (0·12 c.c.) in alcohol (6 c.c.). On concentration of the solution 2 : 4 : 6-trimethyl *d*-galactose anilide (0·18 g., m. p. 179°) was obtained. No evidence of the presence of a crystalline anilide derived from methylated mannose could be obtained.

Fraction V*b* (0·64 g.), b. p. 136—170°/0·002 mm. (bath temp.), $n_D^{20°}$ 1·4685, $[\alpha]_D$ + 86° in water, OMe 44%, was hydrolysed with N-hydrochloric acid (30 c.c.), and the solution worked up as described for fraction VIII, giving a syrup (0·60 g.), $[\alpha]_D^{21°}$ + 81° (*c*, 1·2 in water), $n_D^{18°}$ 1·4740, which was transformed into the anilide, and on concentration of the solution, 2 : 3 : 4-trimethyl *d*-galactose anilide (0·25 g., m.p. and mixed m. p. 170°) was obtained. Again no crystalline derivative of mannose was obtained.

Fraction VI (0·55 g.) was triturated with ether to remove the crystalline 4 : 6-*dimethyl* α-β-*methyl*-d-*galactoside* (46 mg.), m. p. 163° (Found: C, 48·8; H, 8·1; OMe, 41·4. $C_9H_{18}O_6$ requires C, 48·6; H, 8·2; OMe, 41·8%). This material on hydrolysis gave 4 : 6-dimethyl α-*d*-galactose monohydrate, m. p. and mixed m. p. with the material described below, 105°. The syrup (0·50 g.) was hydrolysed with N-hydrochloric acid (30 c.c.) at 90—95° for 5½ hours; $[\alpha]_D^{22°}$ + 103° (initial value) fell to + 66°. Mineral acid was removed with silver carbonate, and the filtered solution concentrated at 50°/12 mm. to a syrup (0·45 g.), which slowly crystallised. Trituration with acetone and filtration gave 4 : 6-dimethyl α-*d*-galactose monohydrate (0·10 g.), m. p. 105°, $[\alpha]_D^{20°}$ + 123° falling to + 82·0° (*c*, 3·7 in water). The acetone solution contained a syrup (0·35 g.), which was isolated and heated with aniline (0·3 c.c.) in alcohol (5 c.c.), giving 0·20 g. of 4 : 6-dimethyl *d*-galactose anilide, m. p. 207°. Since the yield of anilide from the pure sugar under these conditions is 50%, it appears that fraction VI contained in all approximately 0·44 g. of 4 : 6-dimethyl galactose.

The non-crystalline anilides were converted into the sugars (syrup, $[\alpha]_D$ + 58°) and thence by bromine oxidation into the lactones (syrup, equiv. wt. 201; OMe, 27%; $[\alpha]_D$, equilibrium value, + 31°), the amide from which gave a positive Weerman reaction. Again no definite evidence of the presence of mannose was obtainable.

Identification of 4 : 6-*Dimethyl* d-*Galactose.*—Further quantities of the crystalline sugar were prepared from methylated damson gum in order to establish its constitution.

(a) *Anilide formation.* The sugar (monohydrate, m. p. 105°) (0·18 g.) was heated with aniline (0·5 c.c.) in alcohol (10 c.c.); on removal of the alcohol the *anilide* crystallised (0·12 g.). It was washed with alcohol and acetone; m. p. 207°, $[\alpha]_D^{20°}$ — 174° (*c*, 0·35 in pyridine) (Found: C, 58·9; H, 7·4; N, 5·3. $C_{14}H_{21}O_5N$ requires C, 59·2; H, 7·4; N, 5·0%). The anilide was insoluble in most organic solvents but was soluble in boiling dioxan and methyl alcohol and in cold pyridine and aniline. Hydrolysis of the anilide with N-hydrochloric acid regenerated crystalline 4 : 6-dimethyl *d*-galactose monohydrate.

(b) *Osazone.* The sugar, when heated with aqueous phenylhydrazine acetate solution containing a little sodium sulphite, gave 4 : 6-dimethyl *d*-galactosazone, m. p. and mixed m. p. with authentic samples provided by Dr. D. J. Bell and by Dr. E. G. V. Percival (see J., 1937, 1618), 153° (Found: OMe, 16·0. Calc. for $C_{20}H_{26}O_4N_4$: OMe, 16·5%). The mutarotation of our sample in absolute alcohol solution was identical with that observed with Dr. Percival's material. $[\alpha]_D^{20°}$ + 60° (*c*, 0·12; initial value); + 24° (17 hrs.); + 12° (23 hrs.); — 12° (42 hrs.); — 24° (84 hrs.; constant value).

(c) *Reaction with methyl-alcoholic hydrogen chloride in the cold.* The sugar (0·61 g.) in 1% methyl-alcoholic hydrogen chloride (10 c.c.) had $[\alpha]_D^{20°}$ + 52°, rising slowly to + 58° in 60 hours.

(d) 4 : 6-*Dimethyl* d-*galactonolactone.* 4 : 6-Dimethyl *d*-galactose monohydrate (0·27 g.) was oxidised with bromine (1 c.c.) in water (3 c.c.) at 60° during 24 hours. The solution, worked up in the usual manner, gave the *lactone* (0·25 g.) as a syrup, which was slightly acidic in reaction and had $[\alpha]_D^{20°}$ + 78° (*c*, 2·4 in methyl alcohol); + 91° (*c*, 1·2 in water; initial value, probably containing some acid); + 64° (22 hrs.); + 45° (60 hrs.; constant value) (Found: OMe, 32·2: equiv., 213. $C_8H_{14}O_6$ requires OMe, 30·1%; equiv., 206). Data for the crude undistilled lactone are given because a trial distillation showed that decomposition occurred at the high temperature, *ca.* 230°/0·002 mm., required for distillation. On solution in liquid ammonia, the lactone gave 4 : 6-*dimethyl* d-*galactonamide monohydrate*, m. p. 164° (with sintering at 100°), $[\alpha]_D^{21°}$ + 54° (*c*, 2·0 in water) (Found: C, 40·1; H, 7·8; N, 5·9. $C_8H_{17}O_6N,H_2O$ requires C, 39·9; H, 8·0: N, 5·8%). The amide (22 mg.) on treatment with sodium hypochlorite under the conditions given by Weerman (*loc. cit.*) gave hydrazodicarbonamide (4·0 mg.), m. p. and mixed m. p. 258°, indicating the presence of a hydroxyl group at C_2. When the reaction between the amide and sodium hypochlorite was complete, the rotation of the solution was $[\alpha]_D$ + 30°. Dimethyl lyxoses are unknown, but for qualitative comparison the rotations of trimethyl lyxofuranose (+ 41°) and trimethyl lyxopyranose (— 22°) may be utilised.

Fraction VII (0·56 g.) was hydrolysed with N-hydrochloric acid (30 c.c.) at 95° for 4½ hours; $[\alpha]_D^{20°}$ + 89° (initial value) ⟶ + 55° (constant value). Mineral acid was removed with silver carbonate and the filtered solution was concentrated to a syrup, $[\alpha]_D^{21°}$ + 64° (*c*, 1·1 in water), which was converted into the anilide. Trituration of the partly crystalline product with ether gave 4 : 6-dimethyl *d*-galactose anilide (0·25 g.), m. p. and mixed m. p. with an authentic specimen, 207°. From the yield of anilide it appears that fraction VII contained 0·37 g. of 4 : 6-dimethyl galactose.

Summary of Yields of Sugars in Hydrolysis Product.—From 9·55 g. of methylated polysaccharide, 6·82 g. of distilled methyl glycosides were obtained and of this quantity 5·6 g. have

been accounted for as methylated methylgalactosides and methylated methylxyloside, leaving methylated mannose unidentified. In the table all weights are given as free sugars, not as glycosides.

Fraction.	Weight,[1] g.	2 : 3 : 4 : 6-Tetramethyl galactose, g.	2 : 3 : 4-Trimethyl galactose,[3] g.	2 : 4 : 6-Trimethyl galactose,[3] g.	4 : 6-Dimethyl galactose,[4] g.
I[2]	0·50	0·25	—	—	—
II	1·10	0·90	—	0·17	—
III	0·67	—	0·16	0·32	—
IV	1·85	—	1·00	0·77	—
V	0·97	—	0·27	—	0·48
VI	0·50	—	—	—	0·44
VII	0·51	—	—	—	0·37
Total	6·10	1·15	1·43	1·26	1·29
Molecular ratio	—	0·8	1·0	0·9	1·0

[1] Calculated as reducing sugars.
[2] Fraction I contains approx. 0·25 g. of 2 : 3 : 4-trimethyl xylose.
[3] Estimated from yield of crystalline anilides on the basis that the pure sugars gave under the same conditions a yield of 70% of the theoretical.
[4] Estimated as in 3 but on a yield of 50% recorded for the pure sugar.

Examination of the Uronic Acid Portion of the Hydrolysis Products.—The methylated uronic acids were contained in fraction (D), 2·78 g. (see p. 1485), which on distillation gave : Fraction VIII (0·87 g.), b. p. 127—150°/0·002 mm. (bath temp.), $n_D^{18°}$ 1·4496, OMe 55%, equiv. wt. (on hydrolysis with alkali) 260; and Fraction IX (0·66 g.), b. p. 150—170°/0·002 mm. (bath temp.), $n_D^{18°}$ 1·4655, OMe 48%, equiv. wt. 250. There was an undistillable residue (1·01 g.) which contained combined uronic acid and consisted of incompletely hydrolysed material.

Fraction VIII. This material (0·84 g.) on hydrolysis with N-hydrochloric acid (20 c.c.) at 95° gave 2 : 3 : 4-trimethyl *d*-glycuronic acid (0·7 g.) (containing a little of the dimethyl derivative) as a syrup, $n_D^{20°}$ 1·4678, $[\alpha]_D^{20°}$ + 53° in water (*c*, 0·9), OMe 38·0%, equiv. wt. 235. This syrup (0·65 g.) on oxidation with bromine (1 c.c.) in water (10 c.c.) gave 2 : 3 : 4-trimethyl saccharic acid as a syrup, which was converted into the methyl ester (0·65 g., $n_D^{18°}$ 1·4545) by heating it with 2% methyl-alcoholic hydrogen chloride. The ester gave on distillation (b. p. 150°/0·002 mm.; bath temp.) the methyl ester of 2 : 3 : 4-trimethyl saccharolactone (0·46 g.), which set solid in the receiver; m. p. and mixed m. p. with an authentic specimen 112° (after recrystallisation from ether).

The n_D (compare Pryde and Williams, *Biochem. J.*, 1933, **27**, 1201; Hirst and Jones, *loc. cit.*) and OMe figures, together with the yield of trimethyl saccharolactone methyl ester, show that fraction VIII consisted mainly of the methyl ester of 2 : 3 : 4-trimethyl methyl glycuronide (approx. 0·75 g.) together with some dimethyl derivative (approx. 0·12 g.).

Fraction IX. A portion of this fraction (0·57 g.) was hydrolysed with N-hydrochloric acid (30 c.c.) at 95°. The product (0·55 g.) was a syrup, $[\alpha]_D^{20°}$ + 42° in water (*c*, 1·1), OMe 25%. This (0·52 g.) was oxidised with bromine (conditions as for fraction VIII) and the acid so obtained was esterified by boiling with 2% methyl-alcoholic hydrogen chloride. The solution was neutralised with silver carbonate, filtered, and concentrated at 40°/12 mm. to a syrup (0·51 g., $n_D^{19°}$ 1·4600), which was distilled in a vacuum, giving 0·35 g., b. p. 190—210°/0·002 mm. (bath temp.), $n_D^{21°}$ 1·4711. The distillate crystallised when kept. The solid was separated by trituration with ether, from which it was recrystallised; m. p. 101°, not raised on recrystallisation [Found : OMe, 38·6. *Dimethyl saccharolactone methyl* ester ($C_9H_{14}O_7$) requires OMe, 39·7%].

The ester-lactone (0·10 g.) was dissolved in methyl alcohol, and the solution saturated with ammonia at 0°. After 7 days, the alcohol was removed and the 2 : 3-dimethyl saccharamide obtained was recrystallised from alcohol; m. p. and mixed m. p. with an authentic specimen supplied by Dr. F. Smith of Birmingham University, 155°.

The ester-lactone (0·10 g.) was dissolved in N/3-baryta (2 c.c.), and an excess of aqueous periodic acid added. After 12 hours the solution was neutralised with barium carbonate and filtered. The filtrate reduced Fehling's solution and contained glyoxylic acid, recognised by the purple colour it gave with tryptophan and sulphuric acid.

The n_D and OMe figures for fraction IX and the isolation of 2 : 3-dimethyl saccharic acid from it in good yield show that this fraction consisted mainly of the methyl ester of 2 : 3-dimethyl methyl glycuronide (approx. 0·58 g.), together with a little of the trimethyl derivative (approx. 0·08 g.).

The total yield of trimethyl derivative was therefore 0·83 g. and of dimethyl derivative, 0·70 g. Expressed in molecular proportions, these yields are in the ratio of 1·1 : 1. The yield of distilled methylated methyl glycuronides was 47% of the theoretical (see above for cause of low yield).

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