216. *The Constitution of Damson Gum. Part I. Composition of Damson Gum and Structure of an Aldobionic Acid (Glycuronosido-2-mannose) derived from it.*

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

Samples of damson gum from several sources have been examined and evidence has been obtained that the gum is essentially a homogeneous polysaccharide. On hydrolysis it gives *d*-glycuronic acid, *d*-galactose, *d*-mannose, and *l*-arabinose. Methods have been developed for the quantitative estimation of these sugars and the composition of the gum has been determined. By graded hydrolysis of the gum, the arabinose residues can be preferentially removed, leaving a *polysaccharide* (*A*), the repeating unit of which contains *d*-glycuronic acid (1 mol.), *d*-mannose (1 mol.), and *d*-galactose (2 mols.). On further hydrolysis the polysaccharide (A) yields an *aldobionic acid* (d-β-*glycuronosido*-2-d-*mannose*) of novel and unexpected constitution. Proof is given of the constitution of this aldobionic acid, which is one of the fundamental units in the structure of the damson gum molecule, and the significance of the occurrence of the linkage through C_2 of the mannose residue is discussed.

THE gum which is exuded on the bark of damson trees as a brownish semi-solid mass is the neutral salt of an acidic *polysaccharide*, which can be transformed readily into a crisp white ash-free powder by precipitation from an acidified aqueous solution by addition of alcohol. Samples of the ash-free gum from several trees and from different districts have been examined, and it appears that damson gum is essentially a homogeneous chemical entity having an equivalent weight of *ca.* 1100 and $[\alpha]_D^{20°}$ — 26° (as sodium salt in water. See Table I). In appearance and properties the substance is a typical gum, resembling gum arabic and cherry gum in the viscosity of its aqueous solutions. The analytical figures for uronic anhydride and for furfural indicate that there are present respectively 16·4% of uronic anhydride and 36·2% of pentosan. Examination of the sugars obtained on hydrolysis of the gum showed that the pentose was almost exclusively *l*-arabinose, and the uronic acid was *d*-glycuronic acid. *d*-Galactose and *d*-mannose are present also, but other sugars have not been encountered.* The mode of attachment of the *l*-arabinose residues, which are combined in the furanose form, and of the galactopyranose residues, will be considered in Part II, where the hydrolysis products obtained from the fully methylated gum will be described. One feature of special interest, which is the main concern of the present paper, is that in damson gum there occurs an entirely novel type of *aldobionic acid*, in which *d*-glycuronic acid is attached by a glycosidic link to the second carbon atom of the *d*-mannose residue. The rate of hydrolysis of the mannosidic link by which the aldobionic acid is joined to the rest of the molecule indicates that the mannose residue is of the pyranose type. It follows that the structure (I) is a fundamental portion of the damson gum molecule. The elucidation of the constitution of the aldobionic acid is therefore a first stage towards the assignment of a detailed structural formula to the gum itself.

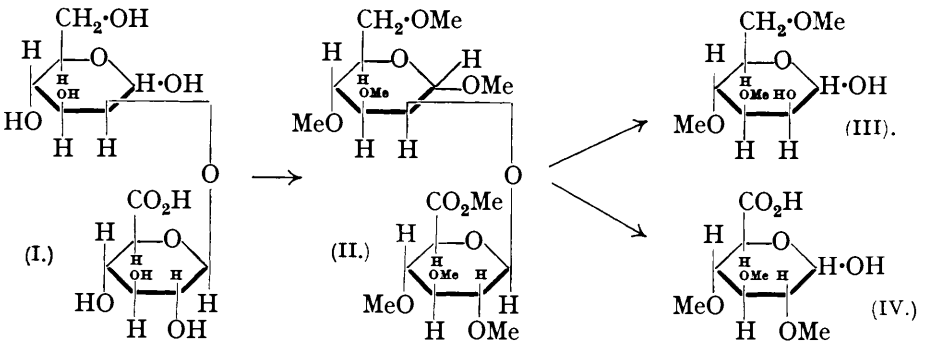
It is of interest to contrast the aldobionic acid present in this gum with the one which plays a corresponding part in the structure of gum arabic. In the latter the aldobionic acid is *d*-glycuronosido-6-*d*-galactose (Challinor, Haworth, and Hirst, J., 1931, 258). In other gums, as, for instance, the specific capsular polysaccharides of Types III and VIII pneumococcus, there occurs an aldobionic acid, β-*d*-glycuronosido-4-*d*-glucose, in which the attachment is *via* C_4 of the glucose residue (Hotchkiss and Goebel, *J. Biol. Chem.*, 1937, **121**, 195), but so far as we are aware, this is the first recorded instance of an aldobionic acid containing a mannose residue, and the first in which the uronic acid is linked to position 2 of the sugar residue. It is, in fact, becoming apparent from recent work that, amongst natural substances, glycosidic linkages involving carbon atoms of a sugar residue other than C_4 or C_6 (which at one time appeared to be specially favoured) are more usual than was supposed to be the case. As further examples may be mentioned linkage through C_3, which occurs in the galactose residues of gum agar (Percival and Somerville, J., 1937, 1615), and, as will appear in a subsequent paper, the same type of linkage is found also in the galactose residues of damson gum. Finally, the only other possible type of linkage, namely, through C_5, is represented in the galactofuranose residue of galactocarolose (Haworth, Raistrick, and Stacey, *Biochem. J.*, 1937, **31**, 640).

The evidence for the structure of the aldobionic acid from damson gum rests on the following observations. The ash-free gum, which is slightly acidic, undergoes autohydrolysis when its aqueous solution is heated at 90—95°, the products being *l*-arabinose, *d*-galactose (a trace), and a polysaccharide (A) composed of *d*-galactose, *d*-mannose, and *d*-glycuronic acid. The quantitative data showed that the repeated unit of the original polysaccharide contained the sugar residues in the following proportions, glycuronic (1 mol.), galactose (2 mols.), mannose (1 mol.), arabinose (3 mols.). All the arabinose residues are removed under conditions which indicate that they are attached to the main portion of the polysaccharide by furanoside links. The mode of attachment of the galactose which is removed during autohydrolysis is not yet clear and is at present under investigation. It is possible that it is derived by hydrolysis of polysaccharide (A).

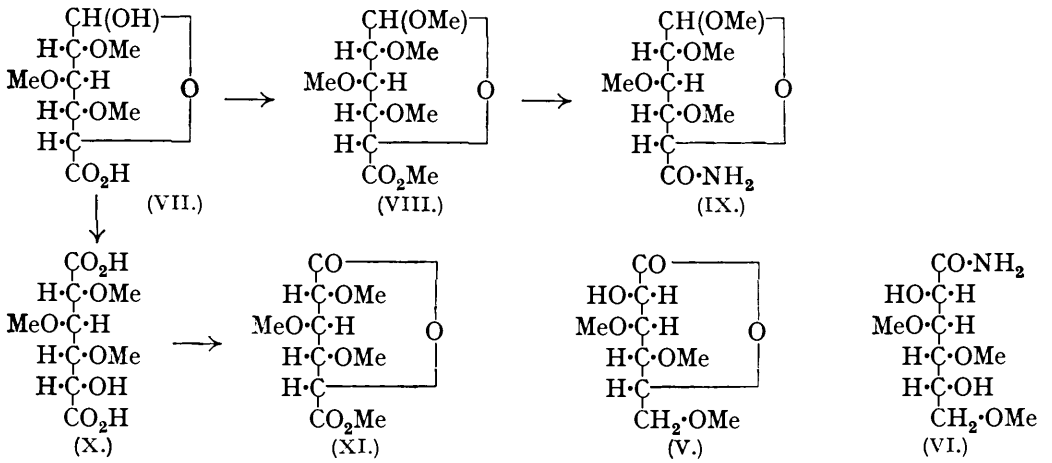
The arabinose-free polysaccharide (A) on further hydrolysis gives rise to the aldobionic acid and galactose. The aldobionic acid resists hydrolysis, but on drastic treatment it is

* (Added July 19th, 1938.) It now appears that damson gum contains a little combined xylose (*ca.* 3%), since 2 : 3 : 4-trimethyl xylose has been isolated after hydrolysis of the methylated derivative of polysaccharide (A). The significance of the observance will be discussed in Part II.

split up into equal proportions of *d*-glycuronic acid and *d*-mannose. Methylation of the aldobionic acid results in the formation of the fully methylated derivative (II) (*methyl* ester of *heptamethyl* β-d-*glycuronosido*-2-d-*mannopyranose*). This fully methylated derivative ($[\alpha]_D^{20}$ —16°) consists of a mixture of α- and β-forms so far as the mannose residue is concerned. Normally, αβ-mixtures of mannopyranosides are strongly dextrorotatory and it seems highly improbable that the mannose portion on combination with the even more strongly dextrorotatory α-glycuronoside residue would give rise to a total negative rotation. For this reason we tentatively assign the β-glycosidic structure to the aldobionic acid.



On hydrolysis with mineral acid, (II) yields in equimolecular proportion 2 : 3 : 4-trimethyl *d*-glycuronic acid (IV) and 3 : 4 : 6-trimethyl *d*-mannose (III). The latter was identical with the 3 : 4 : 6-trimethyl *d*-mannose described by Bott, Haworth, and Hirst (J., 1930, 1395) and the identification was confirmed by transformation of (III) into the corresponding lactone (V). This was the same as the lactone previously described (Bott,



Haworth, and Hirst, *loc. cit.*), and gave a crystalline *amide* (VI) identical in all respects with an amide we have now prepared from a sample of Bott, Haworth, and Hirst's lactone. Additional confirmatory proof that the methyl groups in the sugar (III) are at positions 3, 4, and 6 was provided by the observation that the amide (VI) gave a strong positive Weerman reaction (*Rec. Trav. chim.*, 1917, **37**, 16) with sodium hypochlorite, indicating the presence of a hydroxyl group in C_2.

The identity of the methylated uronic acid (VII) was established by its conversion on oxidation by bromine water into 2 : 3 : 4-trimethyl saccharic acid (X), which on esterification by methyl alcohol gave the crystalline methyl ester of 2 : 3 : 4-trimethyl saccharolactone (XI), identical with the sample of this substance described by Charlton, Haworth, and Herbert (J., 1931, 2855) and by Robertson and Waters (*ibid.*, p. 1709). By the action

of ammonia on the methylated methyl glycuronoside (VIII) a crystalline ***amide*** was obtained which consisted of the mixed α- and β-forms of the amide (IX). These observations show clearly that the uronic acid residue was the pyranose form of glucuronic acid and that in the aldobionic acid (I) the glucuronic acid is attached by a glycosidic link to position 2 of the mannopyranose residue.

Experimental.

Purification and Properties of Damson Gum.—The gum, as exuded by the damson tree, is a brownish viscid mass, containing bark and other impurities. It gradually darkens and hardens on exposure to air, owing to the removal of water from the nodules of gum. The crude gum is neutral in reaction (salt formation with metallic radicals). Purification was effected, and the gum obtained as the ash-free, slightly acid polyuronide, by dissolving the crude material (100 g.) in warm water (2 l.) and removing the insoluble material on the centrifuge. The clear solution was decanted, and filtered through a fine cloth, and the filtrate (1¾ l.) poured with stirring into absolute alcohol (7 l.) containing concentrated hydrochloric acid (50 c.c.). The gum was ground once with absolute alcohol (250 c.c.) containing concentrated hydrochloric acid (10 c.c.). After filtration, the solid was triturated with absolute alcohol until the filtrate and the gum no longer gave a positive test for chloride ions. The resulting pale cream powder was dried at 50°/12 mm. This procedure gave a gum which was easily soluble in water and in dilute aqueous sodium hydroxide, giving pale yellow, viscous solutions. If the gum was not freed from all traces of water before drying in a vacuum, it gave a horny solid which dissolved exceedingly slowly in water and in alkaline solutions.

It gave a soluble neutral thallium salt, but addition of excess of aqueous thallium hydroxide precipitated an insoluble thallium complex. The gum did not reduce Fehling's solution, and did not give with Fehling's solution an insoluble copper complex (contrast araban and pectic acid; Hirst and Jones, this vol., p. 496). Samples of damson gum from different trees and from widely different sources were purified and were found to have substantially the same constants.

Table I.

Sample No.	Equiv. wt.*.	$[\alpha]_D^{20°}$.†	Source.
1	1105	− 26·9°	Mixed nodules from tree (a) (Staffs.).
2	1140	− 29·2	,, ,, ,, (b) ,,
3	1073	− 26·7	Single nodule ,, (c) ,,
4	1108	− 24·2	,, ,, ,, (d) ,,
5	1105	− 26·5	,, ,, ,, (e) ,,
6	1082	− 28·0	,, ,, ,, (f) ,,
7	1100	− 25·4	Mixed nodules ,, (g) (Derbyshire).
8	1138	− 24·8	,, ,, ,, (h) (Staffs.).
9	1130	− 27·2	,, ,, ,, several trees.

* By titration with N/10-sodium hydroxide.
† As sodium salt in water (*c*, 1·0).

The work described below was carried out entirely with sample no. 7.

On titration with alkaline iodine under the conditions given by Bergmann and Machemer for determination of iodine numbers, 1·0 g. of damson gum required 3·0 c.c. of N/10-iodine. This small iodine number is probably not significant, particularly as the gum had a small ethoxyl content (3%, probably due to esterification during the purification process) [Found: OMe content of crude gum, nil; N, nil. Furfural (purified gum), 23·5%, estimated both as phloroglucide and as barbiturate, after treatment of the polysaccharide with boiling 12% hydrochloric acid in the usual way. Uronic acid anhydride content calculated from the amount of carbon dioxide liberated on boiling with 12% hydrochloric acid, 16·4%]. (A substance containing 16·4% of uronic anhydride and no other acidic residues should have an equivalent weight of 1140. Found by titration of the gum with alkali, 1100.) This proportion of uronic anhydride accounts for 3·5% of the total furfural (for factor used, see Norris and Resch, *Biochem. J.*, 1935, **29**, 1590), leaving 20·0% of furfural contributed by the pentosan portion of the polysaccharide, and since the only pentose present is *l*-arabinose (see below) the calculated araban content of the gum is 37·6% [Found, 36·2% (from direct estimation of arabinose after hydrolysis. See below].

Graded Hydrolysis of Damson Gum.—(a) *Hydrolysis.* Aqueous solutions of ash-free damson gum are sufficiently acidic to bring about slow graded hydrolysis when the solutions are heated at 90—95° for 24 hours. Damson gum (18·8 g.) was heated with water (300 c.c.) at 90—95°,

the reaction being followed by polarimetric and iodometric observations. $[\alpha]_D^{20°}$ − 26° (c = 6·3 in water, initial value); + 1° (4 hrs.); + 18° (8 hrs.); + 30° (15 hrs.); + 33° (20 hrs.); + 34° (24 hrs.). (A much slower hydrolysis continues beyond this stage.) The increase in reducing power was followed by titration of 1 c.c. portions of the solution with N/10-iodine by the method of Baker and Hulton (*Biochem. J.*, 1920, **14**, 754). Initial value (in c.c. of N/10-iodine, calculated for 1 g. of damson gum) 2·0; 7·0 (2 hrs.); 14·0 (3½ hrs.); 21·0 (5½ hrs.); 30·0 (8½ hrs.); 40·0 (13½ hrs.); 46·0 (19½ hrs.); 48·0 (23½ hrs.). The cooled solution, when poured into alcohol (1½ l.), gave an alcohol-insoluble polysaccharide (A) (11 g.), which was washed with alcohol and dried.

(b) *Reducing sugars obtained by graded hydrolysis.* The filtrate from (A) on concentration under reduced pressure gave a syrup (8·8 g.). An iodometric estimation of this syrup by Baker and Hulton's method showed the presence of a total amount of reducing sugar equivalent to 920 c.c. of N/10-iodine. Some oligosaccharide was present at this stage, since the syrup underwent further hydrolysis with N/2-sulphuric acid. $[\alpha]_D^{21°}$ + 65° (initial value, c = 3·6 in N/2-sulphuric acid); 71° (10 mins.); 92° (1⅔ hrs.); 94° (3½ hrs.) constant value. The iodine titre then indicated the presence of an amount of reducing sugar equivalent to 1110 c.c. of N/10-iodine (equivalent to 8·25 g. of pentose or 9·9 g. of hexose). Furfural determinations now showed the presence of 7·36 g. of pentose (calculated as *l*-arabinose). The solution was neutralised with barium carbonate and filtered. The filtrate on concentration under reduced pressure gave crystalline *l*-arabinose (6·5 g.), m. p. 160°, $[\alpha]_D^{20°}$ + 103° (c = 1·7 in water, equilibrium value). The mother-liquors contained a further quantity of *l*-arabinose (0·7 g.), identified as *l*-arabinose diphenylhydrazone (m. p. and mixed m. p. 198°), together with *d*-galactose (1·4 g.; estimated after oxidation to mucic acid). Xylose appeared to be absent, since no cadmium bromide–cadmium xylonate double salt could be detected on oxidation of a portion of the above-mentioned mother-liquors with bromine in the presence of cadmium carbonate. A polysaccharide of equivalent weight 1058 and containing three molecules of arabinose would yield 42·5% of arabinose on hydrolysis (Found, 39·4%).

(c) *Polysaccharide A.* Polysaccharide (A) was a white powder, easily soluble in water, giving solutions with an acid reaction towards Congo-red. It gave no colour with aqueous iodine. Uronic anhydride, 24·2% (calculated from the amount of carbon dioxide evolved on heating with 12% hydrochloric acid). Furfural, 7·3% (estimated as phloroglucide after boiling with 12% hydrochloric acid under the standard conditions). The polysaccharide on heating with nitric acid (d 1·2) gave mucic acid in a yield of 27·3%, corresponding to 43·7% of galactan [for the factor used (1·6), see Hirst and Jones, J., 1938, 502]. The equivalent weight (by titration with N/10-alkali) was 645. On titration with alkaline iodine by Bergmann and Machemer's method for determination of iodine numbers, 1 g. of polysaccharide (A) required 4·0 c.c. of N/10-iodine. A polysaccharide containing a repeating unit of one molecule of *d*-glycuronic acid, 1 molecule of *d*-mannose, and 2 molecules of *d*-galactose would have an equivalent weight of 662, would contain 48·9% of galactan and 26·6% of uronic anhydride, and on distillation with 12% hydrochloric acid would give 6·7% of furfural.

(d) *Reducing sugars obtained on hydrolysis of polysaccharide A.* The polysaccharide (A) (5 g.) was boiled with 2N-sulphuric acid (80 c.c.) for 6½ hours. The darkening of the solution and the production of a little furfural showed that slight decomposition took place. The rotation fell from $[\alpha]_D^{20°}$ + 29° to + 26°. The cooled solution was neutralised with barium carbonate and filtered. The neutral solution was concentrated at 35°/12 mm. to a small volume and filtered, and the filtrate poured into alcohol. The precipitated barium salt (B) (2·0 g.) was collected, washed with methyl alcohol, and dried. Concentration of the methyl-alcoholic filtrate gave a syrup (3·0 g.), which crystallised. On trituration with alcohol, crystalline *d*-galactose (1·7 g.) was obtained, m. p. 164°, $[\alpha]_D^{20°}$ + 79° (c, 4·5 in water, equilibrium value), from which *d*-galactose-methylphenylhydrazone, m. p. and mixed m. p. 188°, $[\alpha]_D$ − 2·5° (c = 0·6 in pyridine), was prepared. The syrup after removal of crystalline *d*-galactose still contained some galactose, identified as mucic acid after oxidation with nitric acid. In addition, it contained some *d*-mannose, since with phenylhydrazine in the cold it gave *d*-mannose phenylhydrazone, m. p. and mixed m. p. 190°, $[\alpha]_D^{21°}$ + 24° (c = 1·26 in pyridine). A solution of the above sugars was fermentable by baker's yeast.

The syrup on conversion into the glycosides by boiling with methyl-alcoholic hydrogen chloride gave crystalline α-methyl-*d*-mannoside, m. p. and mixed m. p. 193°, $[\alpha]_D^{20°}$ + 89° (c = 0·7 in methyl alcohol).

(e) *Aldobionic acid from polysaccharide A.* The precipitated barium salts (B) consisted of the barium salt of β-*d*-glycuronosido-2-*d*-mannose mixed with a little barium glycuronate.

$[\alpha]_D^{20°}$ — 16° (c = 3·6 in water) [Found: furfural, 9·0; Ba (estimated as barium carbonate), 17·6%. The barium salt of β-*d*-glycuronosido-2-*d*-mannose requires furfural, 10·1; Ba, 16·2%]. Oxidation with nitric acid (d 1·2) under the standard conditions for the detection of galactose gave no mucic acid, showing that galactose was absent from the aldobionic acid.

The barium aldobionate (1·3 g.) was resistant to hydrolysis, but its cleavage could be effected by boiling with 2N-sulphuric acid (20 c.c.) for 22 hours $[\alpha]_D^{20°}$ — 15° (initial value; c = 6·4); + 0° (7 hrs.); + 8° (14 hrs.); + 12° (18 hrs.), the solution then becoming too dark for further observations (slight decomposition). The cooled solution was neutralised with barium carbonate and filtered. The filtrate, when poured into alcohol, gave an insoluble barium salt (0·4 g.). It had $[\alpha]_D^{19°}$ + 15° (in water, c = 1·1) [Found: Ba, 26·0. Calc. for $(C_6H_9O_7)_2Ba$: Ba, 26·4%], and with *p*-bromophenylhydrazine it gave the characteristic yellow derivative identical with a specimen prepared from authentic *d*-glycuronic acid. Definite proof that the substance was glycuronic acid and not mannuronic acid is given below (see methylation experiments). The precipitated barium salt gave no mucic acid on oxidation with nitric acid (d 1·2), showing the absence of galactose and of galacturonic acid. The alcoholic filtrate from the barium *d*-glycuronate gave on concentration a syrup (0·40 g.), which had $[\alpha]_D^{21°}$ + 15° (in water, c = 0·8). This syrup gave *d*-glucosazone, m. p. 212°, with excess of phenylhydrazine and glacial acetic acid on the boiling water-bath and with phenylhydrazine in the cold it gave *d*-mannose phenylhydrazone in 60% yield. Since pure mannose gives the phenylhydrazone in 60—65% yield under these conditions and since the rotation of the sugar is close to that of mannose, it would appear that the syrup consisted almost exclusively of *d*-mannose. The solution was fermented by baker's yeast, but only if all the barium ions were first removed. In control experiments fermentation both of glucose and of mannose was stopped when barium ions were present in the solution.

(f) *Summary of results. Composition of damson gum.* The above results indicate that the repeating unit in damson gum contains the following sugars in the proportions indicated: *l*-Arabinose (3 mols.), *d*-galactose (2 mols.), *d*-mannose (1 mol.), *d*-glycuronic acid (1 mol.).

	Uronic acid anhydride.	Furfural.	Araban.	Galactan.	Mannan.	Equiv. wt.
Calc. %	16·6	23·4	37·4	30·7	15·3	1058
Obs.	16·4	23·5	36·2 [1] 37·6 [2]	31·6 [3]	12·2 [4]	1100

The arabinose-free polysaccharide (A) contains *d*-galactose (2 mols.), *d*-mannose (1 mol.), and *d*-glycuronic acid (1 mol.).

	Uronic anhydride.	Furfural.	Galactan.	Mannan.	Equiv. wt.
Calc.	26·6	6·7	48·9	24·4	662
Obs.	24·2	7·3	43·7	19·5 [4]	645

[1] From yield of arabinose and arabinose diphenylhydrazone.

[2] Estimated from yields of furfural and carbon dioxide.

[3] Based on sum of galactose eliminated during formation of polysaccharide (A) and galactose present in polysaccharide (A), the factor 1·6 being used in mucic acid estimations. Owing to uncertainty as to the value of this factor, the galactose figures are approximate only.

[4] These values represent the amount of *d*-mannose estimated from the yield of mannose phenylhydrazone. They are minimum figures only, since, as indicated above, the hydrolysis of the aldobionic acid could not be effected without partial decomposition of the molecule.

Methyl Heptamethyl Aldobionate (II).—The mixed barium salts (B) (5·2 g.) were methylated by methyl sulphate and sodium hydroxide (for details, see Challinor, Haworth, and Hirst, *loc. cit.*). The methylated acid (2·8 g.) was then esterified by treatment with methyl iodide and silver oxide in the usual way (yield, 2·8 g.; $n_D^{19°}$ 1·4680. Found: OMe, 49·8%). The product on fractional distillation gave (a) 0·38 g. of a mixture containing methyl tetramethyl *d*-glycuronate, b. p. 140—175°/0·002 mm., $n_D^{20°}$ 1·4490, (b) *methyl heptamethyl aldobionate* (II) (1·74 g.) as a colourless viscid liquid, b. p. 175°/0·002 mm., $n_D^{20°}$ 1·4675, $[\alpha]_D^{20°}$ — 16° in water (c, 0·5) [Found: OMe, 52·6; equiv. wt. (by quantitative hydrolysis), 463. $C_{20}H_{36}O_{12}$ requires OMe, 52·8%; equiv. wt., 470]. (The figures for b. p. refer to bath temperatures.) Hydrolysis of the methyl heptamethyl aldobionate (1·3 g.) was effected by heating its solution in 7% hydrochloric acid at 90—95° for 6½ hours. $[\alpha]_D^{21°}$ — 10° (initial value, c, 4·0); + 7° (20 mins.); + 16° (1 hr.); 26° (2¼ hrs.); 31° (3¼ hrs.); 33° (4 hrs.); 35° (5½ hrs.); 35·5° (6½ hrs., constant value). (The rotation of an equimolecular mixture of 2 : 3 : 4-trimethyl glycuronic acid and 3 : 4 : 6-tri-

methyl *d*-mannose is + 36°.) The solution was neutralised with barium carbonate, filtered, and concentrated to dryness in a vacuum. The product (C) was exhaustively extracted with ether; the ethereal solution gave on evaporation 3 : 4 : 6-trimethyl mannose (III) (0·58 g.) as a syrup, $n_D^{18°}$ 1·4705, which slowly crystallised. $[\alpha]_D^{19°}$ + 10° in water (*c*, 1·1) (Found : OMe, 39·8. Calc. for $C_9H_{18}O_6$: OMe, 41·8%). In 1% methyl-alcoholic hydrogen chloride the sugar had $[\alpha]_D^{20°}$ + 31° (*c*, 1·4), and this value remained constant for several hours, indicating that there was no free hydroxyl group at C_4. After recrystallisation from ether, the m. p. was in agreement with the value (100°) previously recorded (Bott, Haworth, and Hirst, *loc. cit.*). 3 : 4 : 6-Trimethyl mannose (0·5 g.) was oxidised by bromine water at 40° for 6 hours, the solution being then neutralised with silver carbonate and filtered, and the silver removed as sulphide. On evaporation of the solution 3 : 4 : 6-trimethyl mannonolactone (0·44 g.) (V) was obtained. This was purified by distillation, giving an oil (0·4 g.), b. p. 160° (bath temp.) /0·002 mm., which rapidly crystallised to a solid mass. After recrystallisation from ether, the pure lactone was obtained, m. p. 99—100° alone and in admixture with the substance prepared by Bott, Haworth, and Hirst. There was a large depression in m. p. when this lactone was mixed with 2 : 3 : 4-trimethyl mannonolactone. $[\alpha]_D^{21°}$ + 168° (initial value in water, *c*, 3·0); 165° (1¾ hrs.); 156° (5½ hrs.); 149° (8½ hrs.); 124° (24 hrs.); 116° (80 hrs., constant value) (Found : C, 48·8; H, 7·2; OMe, 41·0; equiv. wt. by titration, 219. Calc. for $C_9H_{16}O_6$: C, 49·1; H, 7·3; OMe, 42·3%; equiv. wt., 220).

After solution of the lactone in liquid ammonia and evaporation of the solution (compare Jelinek and Upson, *J. Amer. Chem. Soc.*, 1938, **60**, 356), the *amide* (VI) of 3 : 4 : 6-*trimethyl* d-*mannonic acid* was obtained in quantitative yield, m. p. (after recrystallisation from acetone) 141°, $[\alpha]_D^{21°}$ + 25° in water (*c*, 0·8), the sign of rotation being contrary to that required by the amide rotation rule [compare the corresponding 4 : 6-dimethyl *d*-mannonamide, which also has a free hydroxyl group in position 2 (Harris, Hirst, and Wood, J., 1935, 1658)]. It gave a strong positive Weerman reaction (yield of hydrazodicarbonamide, 40% of the theoretical, similar to that obtained with *l*-arabonamide). The same amide was obtained by treating with liquid ammonia a specimen of the 3 : 4 : 6-trimethyl mannonolactone prepared by Bott, Haworth, and Hirst (Found : C, 45·7; H, 8·0; OMe, 38·5. $C_9H_{19}O_6N$ requires C, 45·6; H, 8·0; OMe, 39·2%).

2 : 3 : 4-*Trimethyl* d-*Glycuronic Acid*.—After removal of the trimethylmannose from (C), the residue consisted of barium trimethyl glycuronate mixed with barium chloride. The solids were dissolved in water and the solution after acidification with N-sulphuric acid (2·2 c.c.) was concentrated to dryness under diminished pressure. The organic matter was extracted (Soxhlet) with boiling ether; the syrup (0·30 g.) obtained on removal of the ether had $n_D^{16°}$ 1·4702, $[\alpha]_D^{20°}$ + 56° in water (*c*, 0·2); OMe, 38·4%; equiv. wt., 246. These figures are in agreement with those required by 2 : 3 : 4-trimethyl glycuronic acid (VII), which has $n_D^{16°}$ 1·4709, $[\alpha]_D^{18°}$ + 58° in water (Calc. : OMe, 39·4%; equiv. wt. 236) (compare Challinor, Haworth, and Hirst, *loc. cit.*). After simultaneous esterification and glycoside formation the syrup gave the *methyl* ester of 2 : 3 : 4-trimethyl *d*-glycuronoside (VIII), b. p. 140°/0·001 mm., $n_D^{21°}$ 1·4471, $[\alpha]_D$ + 31° in water (*c*, 0·7) (Found : OMe, 56·2. $C_{11}H_{20}O_7$ requires OMe, 58·8%), and with methyl-alcoholic ammonia the latter substance gave nearly quantitatively the corresponding *amide* (IX) (mixture of α- and β-forms, which could not be separated by crystallisation), m. p. 158° after recrystallisation from acetone, $[\alpha]_D^{20°}$ + 60° in water (*c*, 5·9) (Found : C, 48·6; H, 7·6; N, 5·7; OMe, 47·2. $C_{10}H_{19}O_6N$ requires C, 48·3; H, 7·7; N, 5·6; OMe, 49·7%). Oxidation of 2 : 3 : 4-trimethyl glycuronic acid (0·29 g.) with bromine water at 60° for 8 hours gave in good yield (0·24 g.) 2 : 3 : 4-trimethyl saccharic acid (X), which was recognised after esterification with methyl-alcoholic hydrogen chloride, as the methyl ester of 2 : 3 : 4-trimethyl saccharolactone (XI), b. p. 140° (bath temp.)/0·002 mm., $n_D^{20°}$ 1·4600. This crystallised immediately to a solid mass, $[\alpha]_D^{21°}$ + 102° (*c*, 0·34 in ethyl alcohol), m. p. 110° alone or when mixed with an authentic specimen prepared by Charlton, Haworth, and Herbert (J., 1931, 2855) from 2 : 3 : 4-trimethyl *d*-glucose. The same substance has been obtained also by Robertson and Waters (J., 1931, 1709) by nitric acid oxidation of both 2 : 3 : 4-trimethyl *d*-glucose and of 2 : 3 : 4-trimethyl *d*-glycuronic acid.

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