255. *The Galactomannan of Carob-seed Gum (Gum Gatto).*

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Gum gatto (alternative names : locust-bean gum, gum tragon, St. John's bread, carob-seed gum) has been shown to be a branched chain polysaccharide built up of D-mannose residues (*ca.* 84%) and D-galactopyranose residues (*ca.* 16%). The methylated gum yields on methanolysis 2 : 3 : 4 : 6-tetramethyl methyl-D-galactoside (*ca.* 1 part), 2 : 3 : 6-trimethyl methyl-D-mannoside (*ca.* 4 parts), and 2 : 3-dimethyl methyl-D-mannoside (*ca.* 1 part) identified as the crystalline furanolactone of 2 : 3-dimethyl D-mannonic acid which yields a crystalline *phenylhydrazide*. The dimethyl D-mannonic acid on oxidation with periodic acid yields formaldehyde, its constitution being thereby proved.

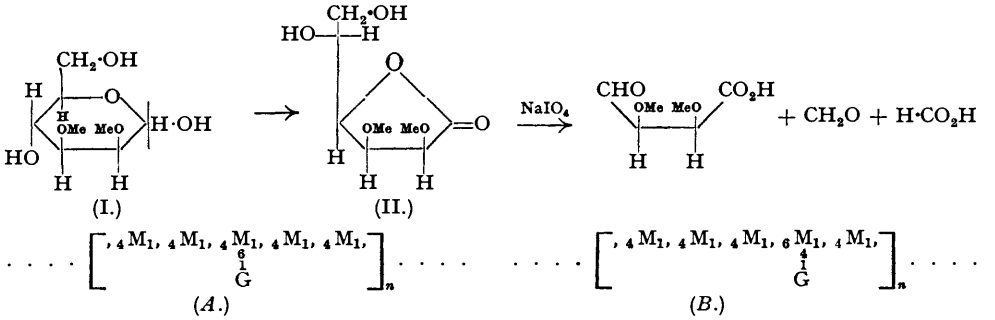
Galactomannans are of common occurrence in Nature. They have been isolated from various seeds such as the fenugreek (*Trigonella foenum-græcum*) (Daoud, *Biochem. J.*, 1932, **26**, 255), the lucerne seed (Hirst, Jones, and Walder, *J.*, 1947, **1443**), the coffee bean (Wise and Appling, *Ind. Eng. Chem. Anal.*, 1944, **16**, 28), and the carob seed (Spada, *Atti Soc. nat. mat. Modena*, 1939, **70**, 20; Lew and Gortner, *Arch. Biochem.*, 1943, **1**, 32). The polysaccharides can be isolated from these sources by extraction with cold dilute sodium hydroxide from which they may be precipitated as a copper complex by addition of Fehling's solution. In this and other respects these galactomannans resemble mannan A of the ivory nut (Klages, *Annalen*, 1934, **509**, 159; 1935, **512**, 185). The galactomannans are utilised in the paper industry since they form thick mucilaginous dispersions which, when added to the cellulose suspension, increase the strength of the resultant paper. A mucilage frequently used for this purpose is the one obtained from carob-seed gum (gum gatto) which forms the subject of the present inquiry. The commercially obtainable material is a white powder which does not dissolve in water, but swells to a thick mucilaginous mass which is soluble in dilute sodium hydroxide with loss of viscosity. The alkaline solution can then be filtered and the purified gum isolated either as the insoluble copper complex by addition of Fehling's solution, or as the pure polysaccharide by precipitation in acidified alcohol. The gum is prepared from the seeds of the bean from *Ceratonia siliqua*, commonly found growing in Crete, Greece, Portugal, the Balearic Islands, and Cyprus.

In the course of the present work it was not found possible to hydrolyse the polysaccharide without some decomposition of the resulting sugars, since it dissolved only slowly in the acid used for hydrolysis. A small quantity of the hydrolysis mixture was examined on the paper

chromatogram by the method of Partridge (*Nature*, 1946, **158**, 270). Galactose and mannose were detected, the latter predominating. A quantitative determination of these sugars by the method described of Hirst, Jones, and Woods (*J.*, 1947, 1048) showed the presence of some 86% of D-mannose and 14% of D-galactose. With a view to determining whether the polysaccharide was of the branched chain type or not a sample of the purified material was oxidised with potassium periodate. The yield of formic acid indicated that hydroxyl groups were present on each of three adjacent carbon atoms in approximately one sugar residue in six, and therefore one sugar residue in six was an end group or was linked through carbon atoms one and six only. The polysaccharide which had been oxidised with potassium periodate was submitted to hydrolysis and the products examined on the paper chromatogram. No sugar other than a mere trace of mannose was detected, and it follows that neither galactopyranose nor mannopyranose residues combined through C_3 or through any two of the carbon atoms C_2, C_3, and C_4 were present (see Halsall, Hirst, and Jones, *J.*, 1947, 1427). Sugar residues linked in any of these ways contain no α-glycol groupings and if present they would have escaped oxidation by the periodate reagent.

The acidic hydrolysis of the polysaccharide was unsatisfactory and attempts were therefore made to hydrolyse the material with enzymes. Some reaction occurred with taka-diastase, but a much more active preparation was the commercial mixture of enzymes known as " Pectinol 10M ", which is used to free fruit juices from pectic acid. This enzyme was found to act on a suspension of gum gatto and to convert it in part into D-mannose, but no D-galactose could be detected. Hydrolysis was slow and incomplete, possibly because the optimum conditions of temperature and pH had not been employed. This enzyme was also capable of degrading the ivory nut mannan A of Klages, but under the experimental conditions employed hydrolysis was again incomplete.

More detailed information about the structure of the polysaccharide was obtained from an examination of its methylated derivative. On hydrolysis the following three sugar derivatives were isolated. (*a*) 2 : 3 : 4 : 6-Tetramethyl D-galactose (*ca.* 1 mol.), identified as its crystalline anilide. (*b*) 2 : 3 : 6-Trimethyl D-mannose (*ca.* 4 mols.) identified as its crystalline anilide. The sugar on oxidation gave crystalline 2 : 3 : 6-trimethyl D-mannonolactone (Haworth, Hirst, and Streight, *J.*, 1931, 1349), further characterised as the corresponding crystalline amide and *phenylhydrazide*. (*c*) A dimethyl hexose (*ca.* 1 mol.) which was mainly, if not entirely, 2 : 3-dimethyl D-mannose (I) since it gave on oxidation a crystalline lactone (II), identical with the product of Goodyear and Haworth (*J.*, 1927, 3136), and yielded when boiled with alcoholic phenylhydrazine a crystalline *phenylhydrazide*. The lactone of Goodyear and Haworth (*loc. cit.*) must be either 2 : 3- or 5 : 6-dimethyl D-mannonolactone from its mode of preparation, and since it differs from the 5 : 6-dimethyl D-mannonolactone which was described by Irvine and Patterson (*J.*, 1914, 898; cf. Wiggins, *J.*, 1946, 13), the lactone of Goodyear and Haworth is almost certainly the 2 : 3-derivative. The constants of (II) were identical with this product, and its identity was placed beyond doubt by a study of its reaction with sodium periodate. This reagent, diagnostic for α-glycols, splits the acid derived from (II) with production of formaldehyde, so proving the presence of hydroxyl groups on C_6 and C_5. Since (II) is a furanolactone it must also possess a hydroxyl grouping on C_4 and is therefore 2 : 3-dimethyl D-mannonolactone.



M and G represent D-mannopyranose and D-galactopyranose residues linked through the numbered carbon atoms.

The isolation of 2 : 3 : 4 : 6-tetramethyl D-galactose, 2 : 3 : 6-trimethyl D-mannose, and 2 : 3-dimethyl D-mannose in the approximate proportions 1 : 4 : 1 proves that all the galactose is

present in the pyranose form as an end group and that it is attached to a D-mannose residue through C_4 or C_6 (contrast Lew and Gortner, *loc. cit.*, who suggest a 1 : 2-linkage). The polysaccharide is highly resistant to acidic hydrolysis, and the mannose residues are therefore probably of the pyranose type. Two main possibilities for the formula of gum gatto are possible. The mannose residues may be in the form of a chain of 1 : 4-linked mannopyranose residues (probably of the β-configuration) with every fifth residue substituted at C_6 by a D-galactopyranose residue (*A*) or, groups of four 1 : 4-β-linked mannopyranose residues may be linked to one another by a D-mannopyranose residue through C_1 and C_6 and a D-galactopyranose residue is linked to C_4 of this sugar residue (*B*). The main chain structure of (*A*) is identical with the structure of ivory nut mannan which was postulated by Klages (*loc. cit.*).

It may be noted that other workers have found different ratios of galactose to mannose in carob-seed gum, and figures of 27 : 73 (Spada, *loc. cit.*), 20 : 80 (Wise and Appling, *loc. cit.*), and 18 : 82 (Lew and Gortner, *loc. cit.*) have been given. These differences suggest that the galactose/mannose ratio may vary with the origin of the gum and that the glactose is stored by attaching it randomwise to the main chain of mannose residues. The possibility remains, however, that the gum is a mixture of polysaccharides, such as a galactomannan and a mannan, and that the variations in galactose content are due to variations in the amount of the galactomannan component. Fractionation of the methyl ether derivative, however, failed to achieve any obvious separation into components possessing different physical properties, a result which was verified from an examination of the products of hydrolysis of the various fractions of the methyl ether derivative (see experimental section).

Experimental.

(All boiling points recorded in distillation are bath temperatures.)

The sample of gum gatto used in this work was supplied by Messrs. Ellis Jones & Co. Ltd., Stockport. It was a white powder which contained traces of brown material, and had been obtained from the seeds of the bean from *Ceratonia siliqua*. The sample used in these experiments was of Cyprian origin and was free from husks. The crude gum when boiled with 12% hydrochloric acid gave 1·9% of furfuraldehyde, equivalent to the presence of some 3·8% of pentosan (Found : N, 1·1; OMe, 0·3; ash, 1·0%).

The polysaccharide swelled in cold water but did not dissolve. It was purified by dissolving it in dilute aqueous sodium hydroxide, the gum being precipitated by pouring the filtered solution into alcohol acidified with hydrochloric acid. The product, a white powder, was washed with alcohol until it was neutral, then with ether, and dried under diminished pressure. This material was free from ash and nitrogen and gave a negligible yield of furfuraldehyde when boiled with 12% hydrochloric acid. It gave an insoluble copper complex on addition of Fehling's solution to its solution in aqueous sodium hydroxide (cf. ivory nut mannan, *loc. cit.*).

Hydrolysis of Purified Gum Gatto.—The purified gum (3·2 g.) was heated with N-sulphuric acid (50 c.c.) at 93°; it gradually dissolved, leaving a small amount of flocculent impurity. It was not possible to follow completely the change of optical rotation, owing to darkening of the solution. The solution was filtered, and the filtrate neutralised with barium carbonate and again filtered; $[\alpha]_D^{20°}$ + 28° (*c*, 1·1 in water). A sample of the solution was examined on a paper chromatogram (Partridge, *loc. cit.*), and no sugars other than galactose and mannose were detected. Iodimetric titration of the solution showed the presence of some 3·4 g. of reducing sugar, calculated as hexose. A solution containing 80% of D-mannose and 20% of D-galactose would have $[\alpha]_D^{20°}$ + 28° (Found : D-mannose 86%, determined as the phenylhydrazone, and D-galactose 14%, determined as the phenylmethylhydrazone).

The polysaccharide (1 g.) underwent some hydrolysis with loss in viscosity of the solution on standing at pH5 and 35° with " Pectinol 10M ". After 5 days a small amount of insoluble material was removed by filtration and mannose determined as the phenylhydrazone in the residual solution (Found : 0·45 g.; hydrolysis was therefore incomplete).

Oxidation of the Purified Gum with Potassium Periodate.—The gum (1·002 g.) was suspended in water (100 c.c.) contained in a 500 c.c. steamed-out stoppered bottle, and potassium chloride (5 g.) was added. Aqueous sodium metaperiodate (35 c.c.; 0·3M) was then added and the suspension shaken at 20°. At intervals samples were withdrawn and the formic acid content of the solution was determined (Brown, Dunstan, Halsall, Hirst, and Jones, *Nature*, 1945, **156**, 785) [Found : 1010 (170 hours), 970 (193 hours), 965 (220 hours, constant value), recorded as g. of gum yielding one g.-mol. of formic acid]. The last figure indicates the presence of 16·8% of terminal or 1 : 6-linked sugar residues which contain hydroxyl groups on each of three adjacent carbon atoms.

Methylation.—The polysaccharide (30 g.) was dissolved in sodium hydroxide solution (400 c.c.; 40%) and methylated in an atmosphere of nitrogen by the addition of methyl sulphate (360 c.c.) with vigorous stirring. After the initial reaction was over the solution was evaporated on a steam-bath and the residual slurry re-methylated as described above. A repetition of this process gave methylated gum gatto (30 g.) (Found : OMe; 43%). This product was fractionated from its solution in chloroform by portionwise addition of light petroleum (b. p. 40—60°), giving the following fractions which have almost identical properties : fraction (A) (10 g.), $[\alpha]_D^{20°}$ − 11° (*c*, 0·85 in water) (Found : OMe, 43%); Fraction (B) (12 g.), $[\alpha]_D^{21°}$ − 12° (*c*, 1·1 in water) (Found : OMe, 44·1%); Fraction (C) (7 g.), $[\alpha]_D^{16°}$ − 11° (*c*, 0·1 in water) (Found : OMe, 44·0%). The methylated material was consequently held to be essentially homogeneous or an inseparable mixture. Each fraction was therefore hydrolysed separately and the products of hydrolysis examined in order to detect any differences in the three fractions.

Examination of Fraction (A).—This fraction (3·10 g.) was boiled with methanolic hydrogen chloride (100 c.c.; 2%) for 10 hours until the rotation became constant; $[\alpha]_D^{20°}$ + 60·7°. The solution was neutralised with aqueous sodium hydroxide (30%) and concentrated to a syrup, which was dissolved in water (30 c.c.) and extracted exhaustively first with light petroleum (b. p. 40—60°) and then with ether. The aqueous portion was then evaporated to dryness and the residue was exhaustively extracted with chloroform.

The syrup (1·62 g., $n_D^{16°}$ 1·4575) obtained on evaporation of the light petroleum extract was distilled in a vacuum yielding: fraction (1) (1·06 g.), b. p. 130°/0·1 mm., $n_D^{17°}$ 1·4560 (Found: OMe, 57·7%). The ethereal extract (1·55 g.) was added to the still residue and the distillation was continued yielding: fraction (2) (1·36 g., b. p. 160°/0·1 mm., $n_D^{15°}$ 1·4655 (Found: OMe, 52·6%). The chloroform extract (0·2 g.) was then added to the still and the distillation continued yielding: fraction (3) (0·45 g.), b. p. 160/0·1 mm., $n_D^{17°}$ 1·4715 (Found: OMe, 45·5%). The final still residue weighed 0·4 g. (Found: OMe, 42%).

Fraction (1) (1·05 g.) was hydrolysed by boiling it with N-sulphuric acid (20 c.c.); $[\alpha]_D^{18°}$ + 89° (initial value) ⟶ + 59° (constant value). This figure taken in conjunction with the methoxyl content corresponds to the presence of some 59% of 2 : 3 : 4 : 6-tetramethyl D-galactose ($[\alpha]_D$ + 118°) in fraction (1). The solution was neutralised with aqueous sodium hydroxide (30%) and extracted exhaustively with chloroform. The sugars (0·9 g.) obtained on evaporation of the solvent were converted into their anilides by boiling them with ethyl alcohol containing just under 1 mol. of aniline, the slight deficiency in aniline being found to result in a more stable and less discoloured anilide. Concentration of the alcohol gave 2 : 3 : 4 : 6-tetramethyl D-galactose anilide (total yield, 0·67 g.), m. p. 193°, not depressed on admixture with an authentic specimen. This yield of anilide corresponds to the presence of 0·55 g. of tetramethyl D-galactose in fraction (1). The mother liquor from the preparation of the anilide gave in small yields 2 : 3 : 6-trimethyl D-mannose anilide, m. p. 134°, not depressed on admixture with an authentic specimen (Haworth, Hirst, and Streight, *loc. cit.*).

Fraction (2) (1·35 g.) was hydrolysed by boiling it with N-sulphuric acid (20 c.c.); $[\alpha]_D^{18°}$ + 15·7° ⟶ − 9° (3 hours, constant value). The solution was neutralised with aqueous sodium hydroxide (30%) and extracted exhaustively with chloroform. The syrup {1·16 g., $n_D^{18°}$ 1·4740, $[\alpha]_D^{20°}$ − 10° (*c*, 1·1 in water)} obtained on removal of the chloroform gave, when heated with alcoholic aniline, 2 : 3 : 6-trimethyl D-mannose anilide, in high yield, m. p. 133°, not altered on recrystallisation from benzene–light petroleum (b. p. 40—60°). The sugar (0·4 g.) on oxidation with bromine water was converted into the lactone of 2 : 3 : 6-trimethyl D-mannonic acid (0·4 g., $n_D^{16°}$ 1·4710) which was isolated in the usual manner and purified by distillation; b. p. 180°/0·5 mm. The lactone crystallised in the receiver and had m. p. 79—80° after recrystallisation from ether; $[\alpha]_D^{20°}$ + 73° ⟶ + 67° (200 hours, constant value) (Found: C, 48·9; H, 7·3; OMe, 41·2. Calc. for $C_9H_{16}O_6$: C, 49·1; H, 7·3; OMe, 42·2%). The lactone gave with liquid ammonia a crystalline amide, m. p. 130°, which was not suitable for characterisation, owing to the difficulty encountered in recrystallising it. The *phenylhydrazide* (0·15 g.), prepared by heating the lactone (0·1 g.) with an alcoholic solution of phenylhydrazine (0·1 g.), was more satisfactory as a derivative; m. p. 132° after recrystallisation from alcohol (Found: C, 54·9; H, 6·8; N, 8·2; OMe, 27·4. $C_{15}H_{24}O_6N_2$ requires C, 54·9; H, 7·3; N, 8·5; OMe, 28·4%) (cf. Haworth, Hirst, and Streight, *loc. cit.*).

Fraction (3) and the still residue were combined and the whole (0·8 g.) hydrolysed with N-sulphuric acid (20 c.c.); $[\alpha]_D^{20°}$ + 70° (initial value) ⟶ − 15° (3 hours; constant value). The solution was neutralised with aqueous sodium hydroxide and evaporated to dryness, and the residue extracted exhaustively with chloroform. The syrupy mixture of sugars (0·7 g.) (Found: OMe, 34%) obtained on evaporation of the chloroform was oxidised with bromine water. The mixed lactones (0·5 g.), when heated with alcoholic phenylhydrazine, gave the phenylhydrazide of 2 : 3 : 6-trimethyl D-mannonic acid, m. p. and mixed m. p. 132°, and the *phenylhydrazide* of dimethyl D-mannonic acid, m. p. 170°, which were separated by recrystallisation from alcohol (Found: for the substance of m. p. 170°: C, 53·3; H, 7·6; N, 8·8; OMe, 19·1. $C_{14}H_{22}O_6N_2$ requires C, 53·2; H, 7·0; N, 8·9; OMe, 19·7%).

Hydrolysis of Fraction B.—The methylated gum (5·75 g.) was dissolved in methanolic hydrogen chloride (100 c.c. 3%) and the solution boiled under reflux for 17 hours; $[\alpha]_D^{18°}$ + 14° (initial value), + 56° (2¼ hours), + 60° (17 hours, constant value). The solution was neutralised with N-sodium hydroxide, and the methyl alcohol was removed by distillation. The aqueous solution so obtained was extracted exhaustively with chloroform which removed 5·89 g. of material; it was then evaporated to dryness, and the residue extracted with acetone, evaporation of which yielded a further quantity of syrupy glycosides (0·13 g.).

The material in the chloroform extract was distilled in a vacuum yielding: fraction (4) (1·64 g.), b. p. 125°/0·1 mm., $n_D^{20°}$ 1·4540 (Found: OMe, 59·9%); fraction (5) (0·36 g.), b. p. 150°/0·01 mm., $n_D^{20°}$ 1·4602 (Found: OMe, 54·0%); fraction (6) (1·75 g.), b. p. 150°/0·1 mm., $n_D^{20°}$ 1·4629 (Found: OMe, 51·7%); fraction (7) (1·12 g.), b. p. 150—200°/0·01 mm., $n_D^{17°}$ 1·4740 (Found: OMe, 43·0%); still residue (0·12 g.).

Examination of the Fractions.—Fraction (4) (1·63 g.) was hydrolysed by boiling it with N-sulphuric acid (20 c.c.); $[\alpha]_D^{20°}$ + 105° (initial value) ⟶ + 77° (constant value). This equilibrium value corresponds to the presence in this fraction of some 68% of 2 : 3 : 4 : 6-tetramethyl D-galactose ($[\alpha]_D$ + 118°) and 32% of 2 : 3 : 6-trimethyl D-mannose. The solution was neutralised with aqueous sodium hydroxide (30%) and then extracted exhaustively with ether. Removal of the ether left slightly impure 2 : 3 : 4 : 6-tetramethyl D-galactose (1·41 g.), identified as the anilide, m. p. 195°, not depressed on admixture with an authentic specimen.

Fraction (5) (0·35 g.) on hydrolysis for 2 hours with N-sulphuric acid (10 c.c.) gave a mixture of sugars having $[\alpha]_D^{20°}$ + 7°. This on conversion into the corresponding anilides gave a very small quantity of 2 : 3 : 4 : 6-tetramethyl D-galactose anilide (30 mg.), m. p. 193°, and a larger quantity of 2 : 3 : 6-trimethyl D-mannose anilide (120 mg.), m. p. and mixed m. p. 133°, separation being effected by recrystallisation from ether–alcohol.

Fraction (6) (1·7 g.) was hydrolysed with boiling N-sulphuric acid (20 c.c.); $[\alpha]_D^{20°}$ + 28° ⟶ − 7° (3 hours, constant value). The solution was neutralised with 30% sodium hydroxide and exhaustively

extracted with chloroform. The material extracted weighed 2·4 g., and a portion of it (0·6 g.), when boiled with alcoholic aniline, gave in good yield (0·6 g.) 2 : 3 : 6-trimethyl D-mannose anilide, m. p. and mixed m. p. 132°. A second portion (0·7 g.) was oxidised with bromine water, and the lactone (0·7 g.) was isolated in the usual manner. The product crystallised. It was purified first by distillation at 140°/0·1 mm. and then by recrystallisation from a large volume of ether; m. p. 80°, not depressed on admixture with an authentic example of 2 : 3 : 6-trimethyl D-mannonolactone.

The optical rotation of this sugar fraction taken in conjunction with its methoxyl content and the yields of anilide and lactone indicate that 2 : 3 : 6-trimethyl D-mannose was the sole constituent.

A portion of fraction (7) (0·98 g.) was hydrolysed by boiling it with N-sulphuric acid (10 c.c.); $[\alpha]_D^{20°}$ + 15° (initial value) ⟶ − 14° (4 hours, constant value). The solution was neutralised with 30% sodium hydroxide, 1 drop of acetic acid added to avoid the development of alkalinity, and the solution evaporated to dryness under reduced pressure. The product (0·71 g.) was extracted from the residue by boiling it with acetone. It was a pale yellow syrup which did not crystallise (Found : OMe, 26·3%). No crystalline anilide or phenylhydrazone could be prepared from it. Accordingly, the sugar (0·5 g.) was oxidised with bromine water and the lactone (which was a syrup) was isolated in the usual manner and converted into the phenylhydrazide by boiling it with an alcoholic solution of phenylhydrazine. The product after recrystallisation from alcohol had m. p. 170°, and was identified as the phenylhydrazide of 2 : 3-dimethyl D-mannonic acid (see below).

Hydrolysis of Fraction C.—This fraction (2·56 g.) was boiled with methanolic hydrogen chloride (50 c.c.; 2%) for 15 hours; the rotation was then constant at $[\alpha]_D^{20°}$ + 60°. The solution (cooled in acetone–solid carbon dioxide) was neutralised with ice-cold diazomethane and evaporated under reduced pressure to a syrup (2·76 g., $n_D^{22°}$ 1·4590). This syrup was separated into fractions by continuous extraction in a special apparatus consisting of 6 extractors in series of the type described by Brown and Jones (*J.*, 1947, 1344). The first fraction, obtained by extraction with purified light petroleum (b. p. 40°), was purified by distillation in a vacuum giving fraction (8) (0·52 g.), b. p. 120°/1 mm., $n_D^{20°}$ 1·4524 (Found : OMe, 62·2. Calc. for tetramethyl methylgalactoside, $C_{11}H_{22}O_6$: OMe, 62·0%). The second fraction, obtained on continuing the extraction with ether, was added to the still residue and the distillation was resumed, yielding fraction (9) (1·50 g.), b. p. 140—160°/1 mm., $n_D^{23°}$ 1·4612 (Found : OMe, 52·0. Calc. for $C_{10}H_{20}O_6$: OMe, 52·3%). Concentration of the aqueous solution remaining in the extraction apparatus left a syrup which was added to the still residue and the distillation continued yielding fraction (10) (0·36 g.), b. p. 180°/1 mm., $n_D^{19°}$ 1·4710 (Found : OMe, 42·6. Calc. for $C_9H_{18}O_6$: OMe, 41·8%). The still residue (0·1 g.) was not further examined.

Fraction (8) (0·5 g.) was hydrolysed by boiling it with N-sulphuric acid (20 c.c.); $[\alpha]_D^{20°}$ + 110° (initial value), + 112° (final value, 4 hours). The sugar (0·41 g.), isolated in the usual manner, gave 2 : 3 : 4 : 6-tetramethyl D-galactose anilide, m. p. and mixed m. p. 189°, in almost quantitative yield. Fraction (9) (1·45 g.) was hydrolysed with boiling N-sulphuric acid (10 c.c.); $[\alpha]_D^{20°}$ − 8° (final value). The trimethyl sugar (1·2 g.) was isolated by continuous extraction of the neutralised solution with chloroform and had $[\alpha]_D^{20°}$ − 10° (*c*, 1·1 in water) (Found : OMe, 41·8%). This product was purified by distillation, and a syrup (1·1 g., b. p. 140°/0·1 mm., $n_D^{20°}$ 1·4720, OMe, 41·9%) was obtained which, on oxidation with bromine water, gave 2 : 3 : 6-trimethyl D-mannonolactone (0·91 g.), m. p. and mixed m. p. 81° (Found : OMe, 42·0%).

Fraction (10) (0·3 g.) was hydrolysed with boiling N-sulphuric acid for 4 hours; $[\alpha]_D$ + 26° ⟶ 70° (constant value). The sugar was isolated after evaporation of the neutralised solution to dryness under reduced pressure by extraction of the residue with boiling acetone (yield, 0·24 g.) (Found : OMe, 30%). It was then oxidised with bromine water giving a lactone which had $[\alpha]_D^{20°}$ + 64° ⟶ + 56° (3 days) (Found : equiv., 208. Calc. for dimethyl hexonic acid $C_8H_{14}O_6$: 206). The crude lactone was converted into the phenylhydrazide which had m. p. 170° (yield, 82%).

The samples of the crystalline phenylhydrazide of m. p. 170° (0·8 g.) from fractions (3), (7), and (10) were combined and hydrolysed by warming them with N-sodium hydroxide (5 c.c.) for 3 hours. Phenylhydrazine was then removed by solution in ether and the residual aqueous solution was acidified with N-sulphuric acid (5 c.c.). The 2 : 3-dimethyl D-mannonic acid (0·3 g.) was removed from the solution by continuous extraction with chloroform. On evaporation of the solvent, crystalline 2 : 3-dimethyl D-mannonolactone remained, m. p. 98°, raised to 107° on recrystallisation from acetone–ether–light petroleum; $[\alpha]_D^{20°}$ + 60° (*c* 1·1 in water, initial value), + 57° (24 hours), thereafter very slow change (Found : OMe, 30·1; equiv., 204. Calc. for $C_8H_{14}O_6$: OMe, 30·1%; equiv., 206). When the lactone was heated with alcoholic phenylhydrazine, the phenylhydrazide, m. p. 170°, was regenerated.

Oxidation of 2 : 3-*Dimethyl* D-*Mannonic Acid with Periodic Acid.*—The lactone (20 mg.) was warmed with N-sodium hydroxide (0·2 c.c.), and a solution of sodium periodate (excess) in N-sulphuric acid added. After 15 minutes at 40°, excess of solid potassium chloride was added, and the top clear aqueous layer decanted from the precipitated potassium iodate and periodate. The clear layer was mixed with an aqueous solution of phenylhydrazine hydrochloride and potassium ferricyanide, and on addition of a few drops of concentrated hydrochloric acid the port-wine colour diagnostic of the presence of formaldehyde was observed. A control experiment with 2 : 3 : 6-trimethyl D-mannonolactone gave no formaldehyde.

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