106. *The Nature of the Carbohydrate Residue in Ovomucoid. Part II.*

By Maurice Stacey and John M. Woolley.

Hydrolysis of the methylated carbohydrate residue prepared directly from ovomucoid yields *N*-acetyl 3 : 4 : 6-trimethyl glucosamine (7 mols.), *d*-mannopyranose (2 mols.), 3 : 4 : 6-trimethyl *d*-mannopyranose (1 mol.), and 2 : 3 : 4 : 6-tetramethyl *d*-galactopyranose (1 mol.). The carbohydrate residue is formulated as a hendecasaccharide having a central core of three mannose units to which are attached, by glycosidic links, seven *N*-acetyl glucosamine units and one galactose unit.

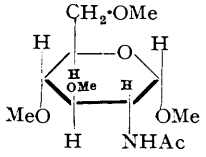
It was mentioned in Part I (Stacey and Woolley, J., 1940, 184) that direct methylation of ovomucoid with sodium hydroxide and methyl sulphate gave the *N*-acetyl methyl derivative of the carbohydrate residue with simultaneous saponification of the peptide constituents. This direct method of methylation has been further developed, and the product shown to be homogeneous and identical with that prepared by an indirect route, namely, by the initial isolation of the carbohydrate residue, followed by its acetylation and methylation.

A suitable method for the hydrolysis of the methylated carbohydrate lay in the use of hot aqueous acid and from the hydrolysate the constituents were separated by appropriate methods. The greater part of the methylated glucosamine constituent was separated by formation of an azomethine with salicylaldehyde. 3 : 4 : 6-Trimethyl glucosamine (Cutler, Haworth, and Peat, J., 1937, 1979) condenses with salicylaldehyde to give an azomethine, from which the trimethyl glucosamine may be regenerated in 95% yield by extracting the chloroform solution of the base with 0·5N-sulphuric acid.

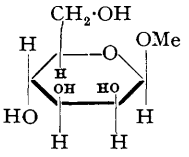
Application of this method to the aqueous hydrolysate of the methylated carbohydrate gave 3 : 4 : 6-trimethyl glucosamine hydrochloride in a yield representing 45% of the amount hydrolysed. The residual glucosamine and the mixture of hexose derivatives in the hydrolysis product were separated by methods in-

volving acetylation, glycoside formation, fractionation by solvents, and fractional distillation in a high vacuum. It was possible to follow the separation of the remaining glucosamine constituent by means of nitrogen determinations and it was identified partly as the α-form and partly as the β-form of 2-acetamido-3 : 4 : 6-trimethyl methyl-*d*-glucoside (I). Despite careful search no partially methylated glucosamine derivative was found.

The presence of derivatives of mannose and galactose in the hydrolysis products was confirmed and these were separated and identified as α-methyl-*d*-mannopyranoside (II), 3 : 4 : 6-trimethyl *d*-mannopyranose (III), and 2 : 3 : 4 : 6-tetramethyl *d*-galactopyranoside (IV).



(I.)



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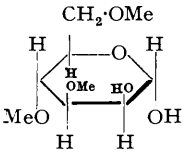

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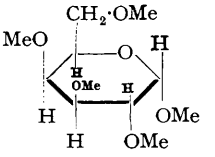
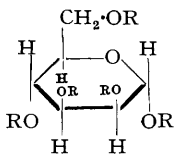


(IV.)

By a process involving the mixing and fractional distillation of corresponding fractions from a number of independent hydrolyses, it was possible to deduce an approximate quantitative estimation of the relative proportions of the constituents. These were : (i) *N*-acetyl 3 : 4 : 6-trimethyl glucosamine, 7 mols. ; (ii) *d*-mannopyranose, 2 mols. ; (iii) 3 : 4 : 6-trimethyl *d*-mannopyranose, 1 mol. ; (iv) 2 : 3 : 4 : 6-tetramethyl *d*-galactopyranose, 1 mol. The total weight of the hydrolysis products isolated amounted to about 90% of the material hydrolysed and in view of the losses inherent in the separation this high yield is taken to indicate the probable absence of any constituent other than those described.

Discussion.

While some features of the constitution of this novel polysaccharide still remain obscure and detailed measurements of molecular size must await further investigation, it is now possible to draw certain conclusions regarding its structural pattern. It is clear from the fact that the constituent building units are glucosamine, mannose, and galactose, and from the demonstration of the high proportion of terminal units, that the carbohydrate cannot be classified as a linear polymer like starch, glycogen, or cellulose. Indeed the general structural plan more closely resembles that of the plant gums, although the latter contain uronic acid constituents and no amino-sugars. From the acetyl content (10%) of the methylated carbohydrate and from the isolation of (I) in good yield it is apparent that all the amino-groups of the glucosamine constituent are acetylated and thus do not engage in linkage with other residues. This fact, together with the identification of the whole of the glucosamine constituent as the 3 : 4 : 6-trimethyl derivative, proves that it is present in the pyranose form as terminal residues linked glycosidically to other units in the molecular complex. Since the whole of the galactose fragment was isolated as tetramethyl galactopyranose, it too must constitute a pyranose terminal residue linked glycosidically to the rest of the molecule. The attachment of all the terminal residues must in fact be to the mannose units. The existence of such a high proportion of terminal residues is compatible with the isolation of two-thirds of the mannose nucleus in the unmethylated form, the isolation of which from a fully methylated polysaccharide implies that all its hydroxyl groups are engaged in linkage to the other constituents of the saccharide in the manner shown in (V) where R is either *N*-acetylglucosamine, mannose, or galactose.



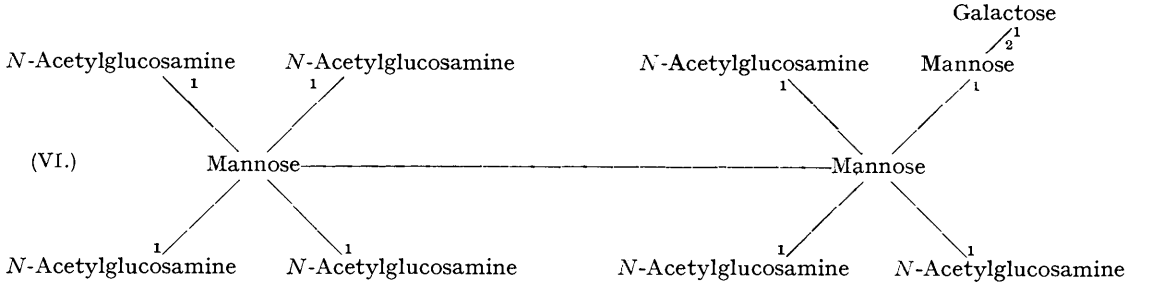
(V.)

An analogy for such an interpretation of the origin of the mannose was forthcoming from contemporaneous work in these laboratories, where *d*-xylose was shown to play a similar rôle in the structure of the β-amylase polysaccharide (Ford and Peat, J., 1941, 856) and where *l*-arabinose forms part of the structure of an araban associated with gum tragacanth (Luckett and Smith, private communication). It is likely, too, that the *l*-arabinose constituent of methylated citrus-araban (Beavan, Hirst, and Jones, J., 1939, 1865) will be shown to originate in a similar way. One-third of the mannose component in the methylated saccharide was isolated as 3 : 4 : 6-trimethyl mannopyranose and clearly is interposed between the nucleus and a terminal group, one of the linkages involved engaging C_2 of this mannose unit. Only one of the terminal units consists of galactose, but its disposition with respect to the three mannose residues has not been determined. Some support for the glycosidic mode of linkage of the *N*-acetylglucosamine constituent has recently been presented by Levene (*J. Biol. Chem.*, 1941, **140**, 279), who has isolated from a " carbohydrate-group of egg proteins " a glucosamine-mannose disaccharide in *ca.* 40% yield. The observation that reduction of this disaccharide converted the mannose moiety into mannitol led to its formulation as glucosaminido-mannose.

Based on the foregoing findings, one of several methods of portrayal of the structure of the carbohydrate residue of ovomucoid is that shown in (VI) in which, by glycosidic attachment, seven *N*-acetylglucosamine units and one galactose unit radiate from a central core of three mannose units. In this formulation the galactose unit could be interchanged with any one of the *N*-acetylglucosamine units.

The methylated derivative of such a hendecasaccharide would have N, 4·0 ; OMe, 34·5 ; *N*-acetyl, 12·0%, so that it is of interest to note that the values found for one specimen were N, 4·2 ; OMe, 32·0 ; *N*-acetyl, 10·0%. Having regard to the difficulty of assaying accurately the relative proportions of the constituents, it may well be that (VI) represents a repeating unit which in the polysaccharide is linked polymerically

through its mannose residues. Nevertheless it would appear from the nitrogen contents of the carbohydrate residue (5·5%), of its *N*-acetyl derivative (5·5%), and of its methylated derivative (4·2%), that the proportion



of glucosamine derivative (7 mols.) isolated is a minimum and that the proportion of this constituent actually present is possibly greater than that represented by 7 mols. If (VI) represented a repeating unit, it must show at least one free reducing group. This could only be the case if the maximum number of terminal glucosamine residues were six; in consequence it is considered that the above formulation, that of a hendecasaccharide, depicts the structure of the actual molecule. A number of facts support the view that the carbohydrate is not a polysaccharide in the true sense; *e.g.*, the carbohydrate itself, its acetate, and methylated derivative readily dialysed through parchment and collodion membranes; the methylated derivative showed low relative viscosities in chloroform solution, and both the acetate and the methylated derivative were easily soluble in water. Of significance, too, was the observation that the carbohydrate residue did not reduce Fehling's solution. While this molecule appears to have a structure which is unique in carbohydrate chemistry, it has been shown in these laboratories that methylated derivatives having similar physical properties can be prepared from the carbohydrates of serum mucoid, of pepsin and of gastric mucin. Indeed from the neutral carbohydrate of gastric mucin the presence of glycosidically linked glucosamine residues has been demonstrated.

In regard to the structure of the parent ovomucoid of which the carbohydrate residue forms 20%, it would seem that the peptide constituents (80%) are mainly attached to the terminal residues. Glucosamine would thus form the main connecting link between the saccharide and these peptide constituents. The fact that the latter are split off by saponification suggests that they are attached by ester linkages engaging mainly the 3-, 4-, or 6-hydroxyl groups of the amino-sugar.

Experimental.

(Unless stated otherwise, all solvents were removed by evaporation under diminished pressure.)

Direct Methylation of Ovomucoid.—The method was essentially that described in Part I (*loc. cit.*); it was found necessary, however, before completing the methylation with silver oxide and methyl iodide, to dissolve the partially methylated material in cold water and to clear the solution (centrifuge). Thereby an insoluble fraction, which did not give the Molisch test for carbohydrate and was of a lipoidal character, was separated and discarded. The average yield of methylated carbohydrate residue was 25% of the ovomucoid employed. The product had $[\alpha]_D^{20°} \pm 0°$ in water (*c*, 1·0) (Found: N, 4·2; OMe, 31·0; *N*-acetyl, 10·0%). Fractionation showed that it was essentially homogeneous, and examination of its methanolysis products confirmed its identity with material obtained by methylation of acetylated carbohydrate residue, 2-acetamido-3 : 4 : 6-trimethyl α-methylglucoside (30% yield) together with partially methylated mannose and galactose derivatives (10% yield) being isolated. Further methylation of the unidentified nitrogenous constituent with methyl iodide and silver oxide gave the light brown powder (fraction FF) described in Part I. An aqueous solution of this substance contained ionisable iodine and readily deposited silver iodide on being boiled, while negligible amounts only of crystalline derivatives could be isolated from the aqueous solution (Found: N, 2·1; AgI, 47·1. Calc. for $C_{12}H_{25}O_5NAgI$: N, 2·8; AgI, 48·1%). Its formation can be explained by the probability that methanolysis deacetylates the amino-group, which is methylated by the subsequent treatment, giving a fully methylated glucosamine; the latter then adds one molecule of silver iodide, forming a quaternary ammonium salt.

Action of Salicylaldehyde on 3 : 4 : 6-*Trimethyl Glucosamine.*—2-Acetamido-3 : 4 : 6-trimethyl β-methylglucoside (1 g.), m. p. 195°, was heated on the boiling water-bath with 2N-hydrochloric acid (50 c.c.) ($[\alpha]_D \pm 0° \longrightarrow +95°$ in 5 hours). The acid was neutralised with lead carbonate, and the filtered solution made alkaline with barium hydroxide and shaken for 6 hours with salicylaldehyde. The resulting bright yellow solution was extracted three times with chloroform, which dissolved the azomethine. Isolation of this base was unnecessary, since on shaking the chloroform solution with 0·5N-sulphuric acid it was decomposed and the 3 : 4 : 6-trimethyl glucosamine dissolved in the acid solution. The acid was removed quantitatively as barium sulphate, and the filtered solution acidified with hydrochloric acid and evaporated, giving 3 : 4 : 6-trimethyl glucosamine hydrochloride (0·82 g., 95% yield), $[\alpha]_D^{21°} + 57°$ in methyl alcohol (*c*, 1·0). This method of extraction was applied, as described below, to the hydrolysis products of the methylated carbohydrate residue.

Hydrolysis of the Methylated Carbohydrate Residue with N-*Hydrochloric Acid and Isolation of* 3 : 4 : 6-*Trimethyl Glucosamine Hydrochloride.*—The methylated material (1·0 g.) was dissolved in 4% hydrochloric acid (50 c.c.), and the solution heated at 100°, $[\alpha]_D \pm 0°$; + 20° (1 hour); + 25° (2 hours); + 28° (3 hours); + 31° (4 hours); + 32° (5 hours) (constant value). The solution was neutralised with lead carbonate, filtered, made faintly alkaline with barium hydroxide, and shaken for 6 hours with salicylaldehyde. The yellow solution (SY) was extracted three times with chloroform (50 c.c. portions), and the combined chloroform extracts shaken with N/10-sulphuric acid (100 c.c.). The solution was neutralised with barium carbonate, filtered, and evaporated, giving a syrup, which was dissolved in alcohol containing hydrochloric acid. Evaporation of this solution gave 3 : 4 : 6-trimethyl glucosamine hydrochloride (0·17 g.) identical with an authentic specimen. The aqueous solution (SY) was neutralised with sulphuric acid, and the filtered solution evaporated, giving a syrup fraction FY (0·31 g.). Repetition of this experiment gave an average yield of 3 : 4 : 6-trimethyl glucosamine hydrochloride (34%) and of the syrup fraction FY (65%).

Isolation of α-*Methylmannoside.*—The sample of methylated carbohydrate residue (8 g.) (Found: OMe, 29·1%) used in this experiment had been heated during the initial methylation stages to a higher temperature (80°) than usual.

It was treated as described above and the fraction FY (5·6 g.) (N, 2·8%) was examined. It reduced Fehling's solution, gave a positive Molisch test, and was boiled with 2% methyl-alcoholic hydrogen chloride for 5 hours; the resulting syrupy mixture of glucosides was dissolved in chloroform–ether, and dry hydrogen chloride passed in. An oily deposit, collected by filtering the solution through a cotton wool pad, was dissolved in methyl alcohol, removal of which gave a syrup, fraction FYI (2·1 g.) (N, 3·3%). The filtrate was neutralised with sodium bicarbonate, and the filtered solution evaporated, giving a second syrup, fraction FYII (3·4 g.) (N, 2·6%).

Both fractions contained glucosamine derivatives and accordingly were acetylated by boiling them with acetic anhydride (15 c.c.) and fused sodium acetate (5 g.). *O*-Acetyl groups were then removed by boiling each of the fractions with 2% methyl-alcoholic hydrogen chloride (100 c.c.) for 3 hours (Moggeridge and Neuberger, J., 1938, 748).

Products from Fraction FYI.—The non-reducing solution was neutralised with lead carbonate, filtered, and the residue washed well with ethyl alcohol. A semi-solid mass obtained on evaporation of the filtrate and washings was rubbed with a few drops of warm ethyl alcohol; light petroleum was then added to produce a cloudiness, and the solution kept for 24 hours. The crystalline product (0·70 g.) was recrystallised from absolute ethyl alcohol (Found: C, 43·3; H, 7·2; OMe, 15·1. Calc. for α-methylmannopyranoside, $C_7H_{14}O_6$: C, 43·3; H, 7·2; OMe, 16·0%), m. p. 190° alone or in admixture with authentic α-methylmannopyranoside. For confirmatory identification a portion (0·1 g.) of the crystalline material was dissolved in 2N-sulphuric acid (10 c.c.) and heated on the boiling water-bath ($[\alpha]_D$ + 77·0° ⟶ + 15·2° in 4 hours). The acid was neutralised with barium carbonate, and the filtered solution evaporated, leaving a syrup, which was dissolved in water (3 c.c.) and to which were added a few drops of phenylhydrazine acetate solution. On being kept at 20°, there was deposited the characteristic mannosephenylhydrazone, m. p. 192° alone or in admixture with an authentic specimen. The mother-liquors remaining after separation of the α-methylmannopyranoside gave 2-acetamido-3 : 4 : 6-trimethyl α-methylglucoside (1·4 g.), m. p. 148°.

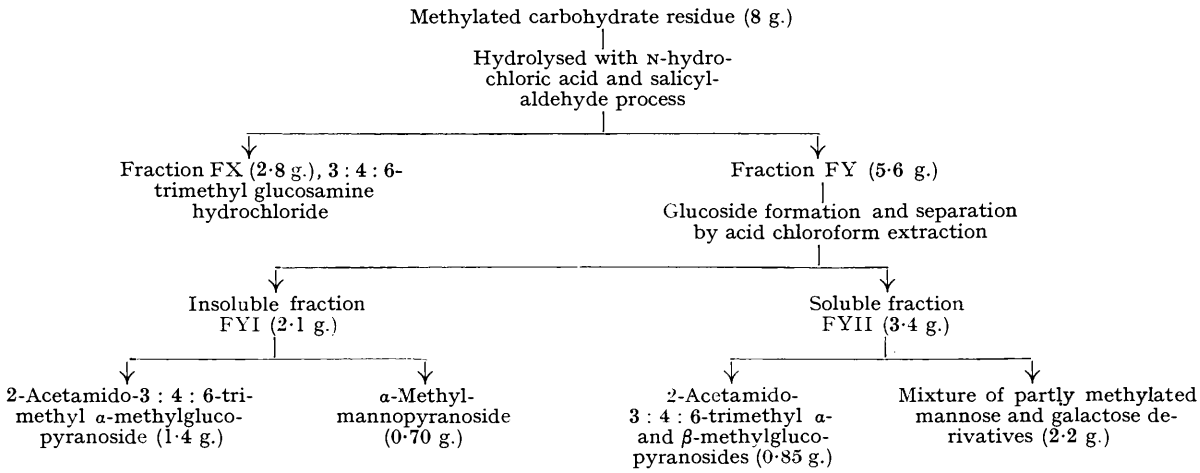
Isolation of 2-Acetamido-3 : 4 : 6-trimethyl β-Methylglucoside.—The syrupy fraction FYII was thoroughly extracted with boiling ether and from the ethereal solution a crystalline deposit (0·85 g.) was collected in four fractions: FYIIa, m. p. 195°; FYIIb, m. p. 170°; FYIIc, m. p. 172°; and FYIId, m. p. 146°. Recrystallisation of FYIIa did not change its m. p. and it was identified as 2-acetamido-3 : 4 : 6-trimethyl β-methylglucoside, $[\alpha]_D$ + 20° in chloroform (*c*, 1·1) (Found: C, 51·7; H, 8·4; N, 5·3; OMe, 44·0. Calc. for $C_{12}H_{23}O_6N$: C, 52·0; H, 8·3; N, 5·1; OMe, 44·8%).

Fraction FYIId on recrystallisation from ether had m. p. 149° alone or in admixture with 2-acetamido-3 : 4 : 6-trimethyl α-methylglucoside, and fractions YIIc and YIId were shown by fractional crystallisation methods to consist of mixtures of the α- and the β-form of 2-acetamido-3 : 4 : 6-trimethyl methylglucoside.

The part of FYII (2·2 g.) which remained insoluble in ether contained traces only of nitrogen. A portion (0·2 g.) was methylated with silver oxide and methyl iodide, and the product distilled in a high vacuum. A fraction (0·17 g.) having b. p. (bath temp.) 120—125°/0·03 mm., $n_D^{20°}$ 1·4480, was collected and dissolved in 2N-sulphuric acid (10 c.c.). On heating the solution at 100°, $[\alpha]_D^{20°}$ + 77° ⟶ + 28° (constant value) in 4 hours. The acid was neutralised with barium carbonate, and the filtered solution evaporated, leaving a syrup (0·16 g.) which was strongly reducing to Fehling's solution. The syrup was dissolved in ethyl alcohol containing a little aniline, and the solution boiled for 2 hours. Removal of the alcohol left a crystalline residue, which was extracted with boiling ether. A portion (0·08 g.) which remained undissolved in the ether had m. p. 190° alone or in admixture with 2 : 3 : 4 : 6-tetramethyl galactose anilide, and from the ethereal solution there was obtained on evaporation a substance (0·07 g.) which had m. p. 142° alone or in admixture with 2 : 3 : 4 : 6-tetramethyl mannose anilide.

Thus the previous claim (Part I, *loc. cit.*, cf. Levene, *loc. cit.*) that both mannose and galactose were constituents of the carbohydrate residue was confirmed. A search was now made in the nitrogen-free fractions of the hydrolysate for partially methylated mannose and galactose derivatives.

The fractionation procedure may be briefly summarised diagrammatically as in the table.



Isolation of 2 : 3 : 4 : 6-Tetramethyl Methylgalactopyranoside.—Methylated carbohydrate residue (7·0 g.) was dissolved in N-hydrochloric acid, and the solution heated for 6 hours at 100°. The acid was neutralised with silver carbonate, and the filtered solution evaporated, leaving a syrup (6·5 g.), which was acetylated by being kept in contact with acetic anhydride (35 c.c.) and pyridine (70 c.c.) at 20° for 48 hours. The solvents were removed, and the syrupy product dissolved in chloroform. There remained a small amount of insoluble residue, which was removed (centrifuge) and discarded. Removal of the chloroform gave a syrup, which was boiled with 1% methyl-alcoholic hydrogen chloride. The resulting syrupy glucoside mixture (5·5 g.), isolated in the usual way, was dissolved in water (150 c.c.), and the solution warmed with charcoal, filtered, and extracted thoroughly with chloroform. The dried chloroform extract was taken down to a syrup, fraction FM (2·72 g.); the aqueous solution on evaporation also gave a syrup, fraction FN (2·70 g.). Fraction FM was exhaustively extracted with boiling ether, leaving a residue which crystallised on being rubbed with a few drops of methyl alcohol and was identified as 2-acetamido-3 : 4 : 6-trimethyl α-methylglucoside (0·90 g.). The ethereal solution gave on evaporation a syrup (1·6 g.), which was distilled in a high vacuum, giving a fraction FM_1 (0·57 g.), b. p. (bath temp.) 110—120°/0·04 mm., $n_D^{16°}$ 1·4575. The undistilled residue, fraction FM_2 (0·90 g.), was examined in the manner

described later. Fraction FM_1 (0·38 g.) was dissolved in 2N-sulphuric acid (50 c.c.), and the solution heated on the boiling water-bath; $[\alpha]_D^{20°}$ +47° (initial value); + 42° (30 mins.); + 39° (75 mins.); + 33° (120 mins.); + 27° (240 mins., constant value). The sulphuric acid was neutralised with barium carbonate, and the filtered solution evaporated, leaving a syrup (0·30 g.), which was converted into an anilide (0·1 g.) by the method previously described. When recrystallised from absolute alcohol, the anilide had m. p. 192° alone or in admixture with 2 : 3 : 4 : 6-tetramethyl galactopyranose anilide. Thus 2 : 3 : 4 : 6-tetramethyl galactopyranose was identified as being a constituent of the hydrolysis products of the methylated carbohydrate residue and it must constitute a terminal residue. In a second experiment methylated carbohydrate residue (10 g.) yielded a syrupy fraction FM_1 (0·60 g.), b. p. (bath temp.) 105—110°/0·03 mm., $n_D^{18°}$ 1·4480 (Found: OMe, 57·0. Calc. for a tetramethyl methylgalactoside: OMe, 62·0%), which was hydrolysed and converted into 2 : 3 : 4 : 6-tetramethyl galactopyranose anilide in the manner described above.

Isolation of 3 : 4 : 6-*Trimethyl Mannopyranose.*—Fraction FM_2 above (N, 0·5%) (0·80 g.) on distillation in a high vacuum gave mainly a fraction (0·65 g.), b. p. (bath temp.) 115—120°/0·03 mm., $n_D^{18°}$ 1·4592 (Found: OMe, 48·5. Calc. for a trimethyl methylhexoside, $C_{10}H_{20}O_6$: OMe, 52·0%).

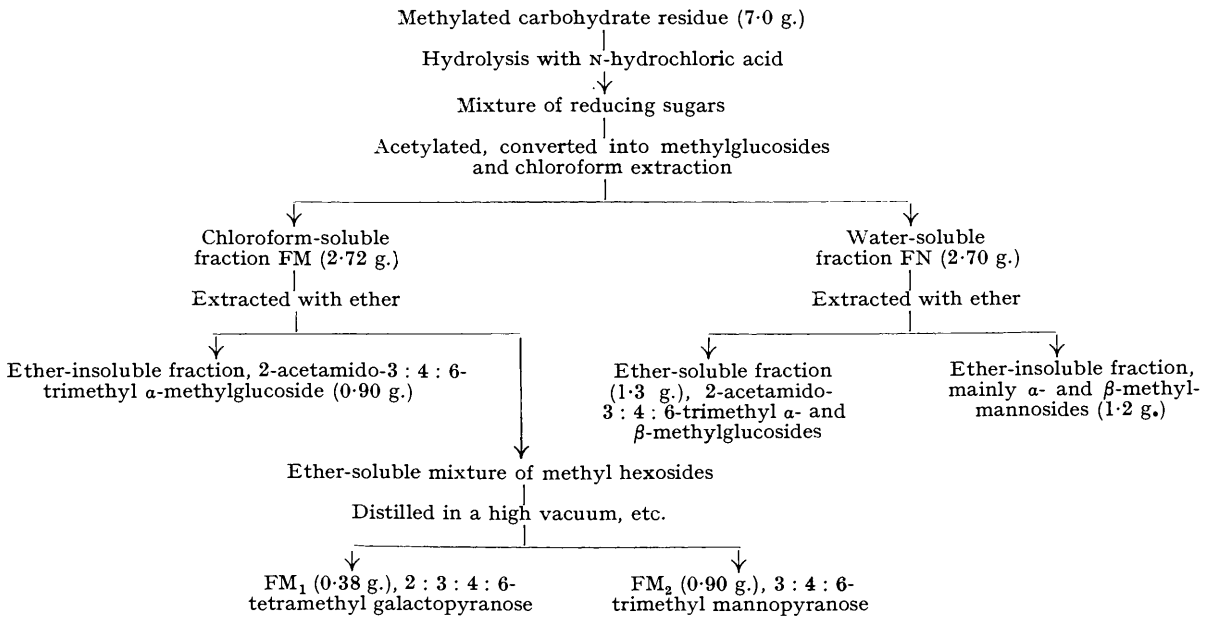
On further methylation with silver oxide and methyl iodide a portion of this syrup (0·10 g.) was converted quantitatively into 2 : 3 : 4 : 6-tetramethyl methylmannopyranoside, which was characterised by formation of 2 : 3 : 4 : 6-tetramethyl mannopyranose anilide, m. p. 142°. No tetramethyl galactose anilide was isolated in this experiment. The trimethyl methylmannoside (0·21 g.) was now hydrolysed by heating it with 2N-sulphuric acid (40 c.c.) on the boiling water-bath ($[\alpha]_D$ + 46° ⟶ + 23°, constant value, in 5 hours). The sulphuric acid was neutralised with barium carbonate, and the filtered solution taken down to a syrup, which was dissolved in ether and a small amount of inorganic material removed by filtration. Removal of the ether by slow evaporation left a crystalline mass (0·15 g.), which after recrystallisation from ether–light petroleum was identified as 3 : 4 : 6-trimethyl mannopyranose, m. p. and mixed m. p. 104°, $[\alpha]_D$ + 10°, equilibrium value in water (*c*, 1·1) (Found: OMe, 41·2. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). Careful fractionation of the mother-liquors from the separation of 2 : 3 : 4 : 6-tetramethyl galactose and 3 : 4 : 6-trimethyl mannose resulted only in the isolation of further amounts of these two compounds. No trimethyl galactose and no tetramethyl mannose fractions were identified.

Fraction FN (2·72 g.) above was thoroughly extracted with boiling ether, from which solution there was obtained a crystalline mass consisting of a mixture of 2-acetamido-3 : 4 : 6-trimethyl α- and β-methylglucosides (1·3 g.).

The syrupy ether-insoluble residue (1·2 g.) was nitrogen-free, had OMe, 14·0%, and appeared to consist mainly of a mixture of α- and β-methylmannosides which failed to crystallise. Part of this material (0·2 g.) was hydrolysed by heating it with 2N-sulphuric acid at 100° ($[\alpha]_D$ + 10°, constant value in 4 hours) and the acid was neutralised with barium carbonate. The filtered solution was strongly reducing to Fehling's solution and from it on suitable treatment with phenylhydrazine acetate small amounts of mannosephenylhydrazone and glucosephenylosazone were isolated. From the results of this and other experiments it was concluded that in order to facilitate the isolation of α-methylmannoside in crystalline form it was necessary to have recourse to methylation of the carbohydrate residue at a higher temperature than was usually employed. It appeared that at this higher temperature small amounts of protein degradation products were rendered insoluble in water, whereas at a lower temperature these substances appeared in the methylmannoside fraction and inhibited its crystallisation.

Quantitative Estimation of the Relative Amounts of the Hydrolysis Constituents.—In order to evaluate the relative proportions of the constituent units, methylated carbohydrate residue (22·5 g.) was hydrolysed and fractionated by the above methods. All unidentified fractions were then analysed for their nitrogen contents and tested for the Molisch reaction (which under the usual conditions is not given by glucosamine derivatives). Similar fractions were then combined and refractionated by solvents or distillation in a vacuum. In this way there were identified 2-acetamido-3 : 4 : 6-trimethyl *d*-glucopyranose (13·6 g.), *d*-mannopyranose (3·1 g.), 3 : 4 : 6-trimethyl *d*-mannopyranose (1·7 g.), and 2 : 3 : 4 : 6-tetramethyl *d*-galactopyranose (1·8 g.). A small amount of dark coloured material (0·5 g.) was not obtained crystalline. Allowing a 10% correction for losses during fractionation methods (Haworth and Machemer, J., 1932, 2270), it is reasonable to assume that no other derivatives are present and that the relative proportions of the constituent units of the methylated carbohydrate residue are 2-acetamido-3 : 4 : 6-trimethyl *d*-glucopyranose (7 mols.), *d*-mannopyranose (2 mols.), 3 : 4 : 6-trimethyl *d*-mannopyranose (1 mol.), and 2 : 3 : 4 : 6-tetramethyl *d*-galactopyranose (1 mol.).

The general fractionation of the hexose derivatives may be summarised briefly as follows:



Molecular Size of the Methylated Carbohydrate Residue.—No satisfactory measurement of the molecular weight of any derivative of the carbohydrate residue has yet been obtained. Initial results from ebullioscopic methods were promising, but the work was interrupted before these could be completed. The carbohydrate residue, its acetylated, and methylated derivatives all dialysed through collodion membranes and molecular weight measurements by osmotic pressure methods were not possible. The ease of dialysis is indicative of a small molecular weight, and further evidence of this was adduced from the fact that the methylated carbohydrate residue had a very low relative viscosity in chloroform or *m*-cresol solution; in addition the acetylated and methylated derivatives were readily soluble in both cold and hot water. The carbohydrate residue did not reduce Fehling's solution.

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