CXCV.—*The Constitution of Polysaccharides. Part X. The Molecular Unit of Starch.*

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WE have naturally included the important case of starch as part of our programme of research on the constitution of polysaccharides, applying the same principles and methods as in the analogous examples of cellulose and inulin. The same limitations have also been imposed on the scope of the investigation, which has been confined meanwhile to determining the linkage of the individual glucose residues in starch so as to arrive at the structure of the simplest molecule (or molecules) which, by polymerisation, form the polysaccharide. Our work has not been concerned with the magnitude of these polymerides or with the individual constituents of starch, nor has it included the differences between one variety of starch and another so far as these may be attributed to variations in the nature and amount of extraneous compounds rather than to inherent differences in molecular structure.

Hitherto, the experimental evidence applicable to the constitutional study of starch has been obtained mainly through biochemical agency and, in introduction to the present communication, only two types of such reactions may be selected from the abundant literature on this subject. These are : (*a*) the conversion of starch into a series of simple amyloses in each of which the empirical formula $(C_6H_{10}O_5)_n$ is retained, and (*b*) the formation, through changes which are in part hydrolytic, of maltose, *iso*maltose, and, ultimately, of glucose. An adequate structural representation of starch must accommodate the above reactions and it is evident that the constitution of maltose is of special importance as bridging the gap between the closed-ring structure of the polyamyloses and the C_6-chain of glucose. Generally speaking, the application of ordinary chemical methods has led to few results which bear directly on the constitution of starch, as the processes of substitution and disruption are not sharply differentiated. An exception is provided, however, in the method of methylation, by means of which the hydroxyl groups in starch may be substituted by methoxyl groups which survive hydrolysis, thereby giving a methylated glucose. Work on these lines was commenced in this laboratory 6 years ago, but no detailed account of our results has so far been published, although reference has been made occasionally to some of the conclusions at which we have arrived. The delay has been due, not so much to the fact that the methylation of starch presented severe

experimental difficulties, as to the necessity which arose to suspend the research and devote a series of separate investigations to the constitution of maltose. Meanwhile, other workers have attempted to convert starch into its fully methylated derivative, but have failed, it having been found impossible to replace all the hydroxyl groups by methoxyl. Our experience in this respect was, at first, no different, and consequently we were compelled to subject an incompletely methylated starch to hydrolysis and to separate the mixture of methylated glucoses thus produced. In such constitutional studies, this is unsatisfactory procedure and cannot be regarded as giving an adequate view of structure, but our earlier work revealed that an invariable product of the reaction was a crystalline sugar, which was identified as 2 : 3 : 6-trimethyl glucose. This result was altogether unexpected. Unless the action of diastase on starch is in part synthetic, there can be little reason to doubt that, in the starch molecule, glucose residues are joined in the same manner as in maltose, and it follows that the trimethyl glucose obtainable from starch should be identical with that from maltose. Haworth and Leitch, however, state that the disaccharide gives 2 : 3 : 4-trimethyl glucose, and the formula for maltose advocated by these authors, either in its original form (J., 1919, **115**, 809) or as subsequently modified (Charlton, Haworth, and Peat, this vol., p. 99), does not admit of the formation of 2 : 3 : 6-trimethyl glucose from any compound containing the maltose structure. The evidence before us was thus conflicting. Obvious sources of error, such as the possible presence of hemi-celluloses, had been carefully excluded, but other explanations suggested themselves, one being that the glucose residues in starch might not be symmetrically attached to each other, as one pair might possess the maltose structure and another the linkage characteristic of cellobiose. This idea was expressed in formulæ (*Brit. Assoc. Reports*, 1922, 33; J., 1923, **123**, 898) in which an attempt was made to reconcile our results with those from which the structure of maltose was deduced, but further investigation showed that no satisfactory formula for starch could be constructed on the basis of Haworth's constitution of maltose. This became apparent when we succeeded in methylating starch completely, as, on hydrolysing the product, 2 : 3 : 6-trimethyl glucose was obtained in yields which showed that this sugar must be regarded as the essential and not as an adventitious product of the series of reactions. Concurrently, and by arrangement with Professor Pringsheim, hexa-amylose was subjected similarly to methylation and hydrolysis. The result was equally emphatic, as the same crystalline sugar was obtained as from starch. Finally,

the tri-hexosan prepared by the depolymerisation of starch (Pictet, *Helv. Chim. Acta*, 1922, **5**, 640; 1924, **7**, 932) was similarly treated and was likewise found to give the same variety of trimethyl glucose.

Only two alternative conclusions could be drawn from the above discordant evidence; the first, that maltose has no structural relationship to starch, was rejected as improbable and we were forced to the opinion that the constitution attributed to maltose was incorrect. This we have ascertained to be the case (Irvine and Black, this vol. p. 862), it having been shown that, in common with starch, polyamyloses and cellulose, maltose yields 2 : 3 : 6-trimethyl glucose unmixed with the 2 : 3 : 4-isomeride. The present communication is therefore submitted on the basis of a corrected constitution for maltose.

We find that the methylation of starch displays a number of features which distinguish the polysaccharide from either cellulose or inulin. Methyl groups are introduced fairly readily by repeated action of methyl sulphate and alkali, but the methoxyl content quickly reaches a limit of 36—37%, this value indicating that only seven hydroxyl groups out of nine have undergone alkylation. Methylated starch of this composition appears to be a definite compound possessing a characteristic optical activity, and, in consequence, the conclusion might be drawn that there are not three hydroxyl groups in each C_6 unit. This inference would be unwarranted, however, as the partly methylated starch was capable of acetylation to the exact extent required to substitute the remaining hydroxyl positions. Further, the methylated starch, when regenerated from the acetate, still preserved its resistance to further methylation. Another distinguishing property displayed by starch during methylation is that the reaction is definitely arrested at the dimethyl stage when conducted by means of silver oxide and methyl iodide. When these reagents are applied to a methylated starch containing less than two methoxyl groups per C_6 unit, the methoxyl content is increased to 32—33%, but no further. Similarly, the same reagents have only a limited effect in raising the methoxyl content when applied to methylated starch which has been alkylated by means of methyl sulphate beyond the dimethyl stage. In the above respects, starch resembles the polyamyloses closely and presents features which are also recognisable in the case of maltose. As the methylation of starch proceeded, the colour reaction with iodine vanished, the usual progressive changes in solubility were observed, and the rotation increased in the dextro-sense. Simultaneously, the protein and inorganic constituents were eliminated, particularly when the

silver oxide reaction was employed, as, in this case, they were precipitated in the form of insoluble double compounds with silver iodide. It is important to observe that these precipitates contained combined carbohydrate, thus indicating that nitrogen and phosphorus form part of the polymerised aggregate. In consequence, the same methylated starch was finally obtained, irrespective of the starting material used, but in large-scale experiments we employed only purified rice-starch. Reference to the experimental details will indicate the manner in which the derivatives of hemi-celluloses were removed so as to separate constituents which would otherwise have introduced complications.

Although four successive applications of methyl sulphate and alkali converted the polysaccharide into a derivative containing 37% of methoxyl, this did not represent the limit of the reaction. Subsequent methylations slowly increased the methoxyl content and, after 24 treatments, the value (43·7%) agreed closely with that required for a trimethyl starch. There are thus three distinct stages observable in the alkylation, the products in each case displaying a constancy in composition and properties which warrants the conclusion that they are three consecutive compounds. These are :

		% OMe (found).	Ratio of OMe groups to initial OH groups.	$[\alpha]_D$ in $CHCl_3$.
I.	Dimethyl starch	32·7	6 : 9	+135·7°
II.	Methylated starch	36·3	7 : 9	169·5
III.	Trimethyl starch	43·7	9 : 9	216·5

It will be observed that the specific rotation increased regularly with the rise in methoxyl content, and this behaviour we have verified over a wide range in composition, thus affording powerful evidence that frequent repetition of the methylation process does not cause structural rearrangement.

The hydrolysis of each type of methylated starch was undertaken, the method of heating with methyl alcohol and hydrogen chloride being employed so as to isolate the liberated sugars in the form of the corresponding methylglucosides. Compound I gave essentially a dimethyl methylglucoside, the description of which is deferred to a later communication, while II yielded a mixture from which 2 : 3 : 6-trimethyl methylglucoside (1 mol.) was isolated, together with a dimethyl methylglucoside (2 mols.). In the case of III (trimethyl starch) the reaction furnished a conclusive result, as 2 : 3 : 6-trimethyl methylglucoside melting at 57·5° was obtained in excellent yield. A careful search for the isomeric 2 : 3 : 4-form was made, both by fractional distillation and by fractional crystal-

lisation of the glucoside, but no trace of this compound was detected. These results were confirmed by hydrolysis of the total glucosides with aqueous acid and isolation of crystalline 2 : 3 : 6-trimethyl glucose. All syrupy products were examined, but the 2 : 3 : 4-isomeride was definitely absent.

In the course of this section of our work it was necessary to compare critically the constants of the isomeric 2 : 3 : 6- and 2 : 3 : 4-trimethyl β-methylglucosides, as these compounds, which show a close resemblance, have been utilised in certain structural studies of disaccharides as a means of discriminating between the isomeric trimethyl glucoses. The following values were determined on specimens of the compounds obtained either by the limited action of methyl alcohol and hydrogen chloride or through the agency of the corresponding acetobromo-derivatives.

	Source.	M. p.	$[\alpha]_D$.
2 : 3 : 6-Trimethyl methylglucoside.	2 : 3 : 6-Trimethyl glucose from cellulose, or Trimethyl starch.	57·5°	−29·3° in MeOH
2 : 3 : 4-Trimethyl methylglucoside.	2 : 3 : 4-Trimethyl glucose (synthetic), or Trimethyl glucosan.	93—94	−23·1 in MeOH

The glucosides cannot be distinguished with certainty through their specific rotations, and the melting points, although far apart, are greatly affected by impurities or by the presence of varying proportions of α- and β-forms. It follows that trustworthy identification of these reference compounds is possible only when both the melting point and the specific rotation agree with the standard values. In addition, the polarimetric curve of the hydrolysis of each compound is diagnostic, and in the case of the equilibrium mixture of the α- and β-2 : 3 : 6-isomerides displays two maxima separated by a minimum. The identification of these isomeric glucosides is nevertheless difficult and failure to discriminate between them may lead to erroneous conclusions as to structure. In this connexion, it may be recalled that Haworth and Wylam (J., 1923, **123**, 3125) obtained from gentiobiose a trimethyl methylglucoside melting at 92·5° and showing $[\alpha]_D$ −25·1° in methyl alcohol. This was regarded as the 2 : 3 : 4-variety, a conclusion which is doubtless correct. But in studying the constitution of raffinose (Haworth, Hirst, and Ruell, J., 1923, **123**, 3131) another preparation, possessing nearly the same specific rotation, but melting at 74°, is also claimed to be 2 : 3 : 4-trimethyl methylglucoside. The statements are irreconcilable, as, if the melting point of the latter preparation was depressed by the presence of the stereoisomeric α-form, the specific rotation would

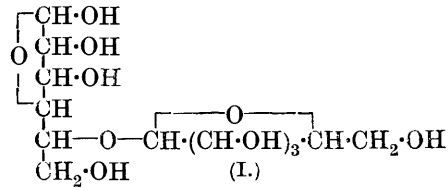
not remain the same, but would be displaced in the dextro-direction. In our examination of these glucosides we have never encountered a form of 2 : 3 : 4-trimethyl methylglucoside melting at 74° and the inconsistency becomes more acute if it be assumed that this figure is a misprint for 94°. We cannot accept, therefore, Haworth's constitution for raffinose, a conclusion further justified by the fact that it contains a melibiose residue based on the formula for maltose recently shown to be incorrect.

Discussion of Results.

As starch is not a uniform homogeneous polysaccharide, limitations must be placed on any discussion regarding constitution, but the results now contributed afford proof that starch consists essentially of a mixture of polymerides which are based on the same molecular unit. The structural similarity displayed by these constituents lies in the fact that there are three hydroxyl groups present in the same positions in each C_6 chain, and it is thus possible to elucidate the constitutional factors which are common to all the components. The expression " starch," as used in this discussion, applies therefore to constituents, amounting to 60—70% of the total, which conform to the above generalisation in that they are convertible into 2 : 3 : 6-trimethyl glucose, but differ in the degree of polymerisation.

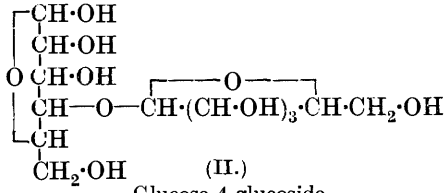
The possibility that starch may be derived from β-glucosan has already been rejected and is again refuted by the results of the present investigation. There remains consideration of the relationship to maltose and to *iso*maltose, but here complications are at once encountered. These difficulties are due in part to inadequate knowledge of *iso*maltose, but are mainly attributable to the confusion which has arisen regarding the constitution of the diglucoses as a class. So far, three disaccharides (maltose, cellobiose, and *iso*cellobiose) have been shown to be convertible into 2 : 3 : 6-trimethyl glucose, and the present research clearly points to the idea that *iso*maltose will be found to give precisely the same result. The formation of the above trimethyl glucose cannot, in consequence, be regarded as final evidence of the linkage of two glucose residues, as such a result fails to discriminate between two alternatives. It follows, also, that the oxydic ring in glucose cannot be restricted to the amylene-oxide linkage alone, as otherwise it would be impossible to formulate all the diglucoses in terms of their properties. For example, the disaccharides postulated below are definitely isomeric and contain glucose residues with different oxygen rings, but each compound would be convertible into 2 : 3 : 6-trimethyl glucose, type I giving in the first place the γ-form of the sugar which

reverts to the stable variety, while type II would give the same final product directly.



(I.)

Glucose 5-glucoside.

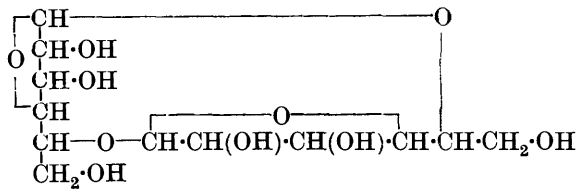


(II.)

Glucose 4-glucoside.

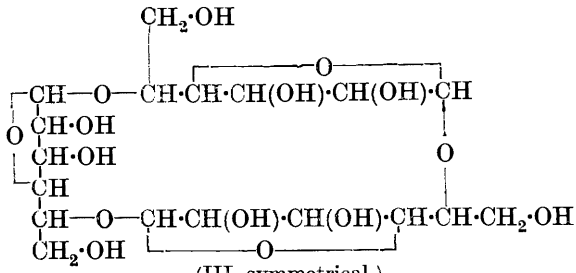
It may be presumed that as cellobiose and *iso*cellobiose form one structural pair, maltose and *iso*maltose will form the other, and the above types can therefore accommodate all four sugars. Pending the completion of further experimental work now in progress, it is inadvisable definitely to assign maltose and cellobiose to their respective types, but, in order to render the present discussion intelligible, formula I may be ascribed arbitrarily to maltose, in which case cellobiose conforms to formula II.

If starch is based on an anhydro-disaccharide unit, the simplest formula for the unpolymerised molecule becomes :

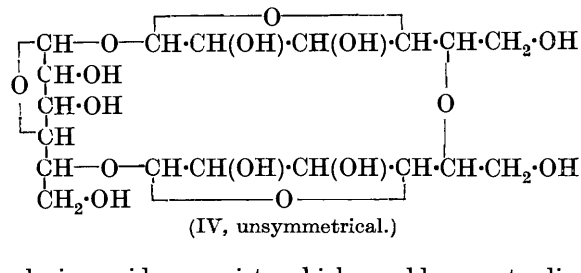


The hydrolysis of the " maltosan " postulated above would give maltose as the only disaccharide, the change including opening of one anhydro-linking followed by adjustment of the unstable oxygen ring in the non-reducing glucose component. Taking into account the difficulty in certifying that any preparation of maltose consists of a single homogeneous chemical individual, it is doubtful if the yield of maltose from starch is as great as that which would be given by a compound of the maltosan type. It would not be justifiable, however, at this stage to exclude maltosan as one of the molecular

units of starch, particularly as polyamyloses exist which contain an even number of C_6 chains. The formula does not, however, admit of the formation, from starch, of a trihexosan or tri-amylose, and consequently consideration must also be given to a constitution based on the union of three glucose residues. Two possibilities exist :



(III, symmetrical.)



(IV, unsymmetrical.)

No conclusive evidence exists which would serve to discriminate between these possibilities. A compound possessing formula (III) could undergo hydrolysis to give only maltose and glucose, whilst, according to formula (IV), maltose, an *iso*maltose and a non-reducing diglucose might result. This is perhaps improbable, as the existence of such hydrolysis products could scarcely have escaped detection, but, in any case, neither of the formulæ reveals the α- or β-configuration of the individual glucose residues. The structures thus fail to account for the fact that starch is convertible into maltose under one set of conditions and into *iso*maltose when the conditions are changed. Similarly, they do not explain why the polyamyloses can be classified in α- and β-series.

We do not share the view that the α- or β-configurations of the methylated glucosides obtained from trimethyl starch afford a true index of the glucose configuration in the starch molecule and purely chemical methods of investigation throw little light on this important aspect of the structural problem. This evidence is, however, rapidly being accumulated in the important researches of Ling and Nanji, and if the results obtained by these workers are not utilised in the

present discussion it is owing solely to the desire to avoid trespass on their field of work.

Limiting the discussion to conclusions drawn from chemical reactions alone, the structures elucidated above may now be tested according to the peculiar behaviour of starch during methylation. The formation of a definite dimethyl starch (*i.e.*, 6 hydroxyl groups out of 9 substituted) is explained in terms of either formula, as the primary alcohol groups are the last to be methylated. Both formulæ also accommodate the production of a trimethyl starch but only formula (IV) offers any explanation as to why methylation can be arrested at the dimethyl stage (*i.e.*, when 6 hydroxyl groups out of 9 have been substituted) and thereafter slows down abruptly when 7 hydroxyl groups out of 9 have been alkylated. For this reason, formula (IV) is retained for further consideration. Much importance must be attached to the fact that methylated starch containing 37% of methoxyl is a definite compound which breaks down on hydrolysis to give exactly 2 molecules of dimethyl glucose and 1 molecule of trimethyl glucose. Reference to the experimental part will show that this compound was obtained under varying experimental conditions on eight successive occasions, so that the composition ascribed to it is unlikely to be fortuitous. Accepting this result, it follows that the molecular unit of starch must contain either nine or a multiple of nine hydroxyl groups. In other words, the simplest unit would be a trihexosan and the next possibility a hexahexosan. In order to explain the existence of polyamyloses containing an even number of C_6 chains, it is, however, necessary to select the hexahexosan as the basal unit, so that the cyclic formulæ given above must be doubled, with a corresponding increase in the structural possibilities involved.

It is thus apparent that, approaching the study of starch by strictly chemical methods, conclusions are reached in close agreement with those arrived at in recent studies of enzyme action. It will be equally apparent that both types of investigation are necessary to solve the main factors involved in the starch problem, (1) the α- or β-configuration of adjacent hexose residues, (2) the positions through which these residues are coupled, and (3) the degree of polymerisation undergone by the basal unit.

The research, which we regard only as preliminary, is being continued in various directions.

Experimental.

The starting material used in the research consisted of purified wheaten, potato, or rice starch, but the first-mentioned variety was discarded in favour of the others, which were found to give the same

methylated derivatives. To facilitate description, it may be stated that, throughout the work, all evaporations and distillations were conducted under reduced pressure.

Dimethyl Starch.—With a few modifications, the methylation was carried out as described by Irvine and Steele (J., 1920, **117**, 1474) in the parallel case of inulin, and the following is the account of one typical experiment. 32 G. of starch were mixed with 150 c.c. of water and stirred mechanically whilst 160 c.c. of 12½% sodium hydroxide solution were added. This had to be conducted slowly, as otherwise the starch coagulated to a stiff jelly, a condition which is unsuitable for methylation. Thereafter, 140 c.c. of 50% sodium hydroxide solution and 80 c.c. of methyl sulphate were run in simultaneously, the addition being extended over 3 hours and adjusted so as to maintain the alkaline reaction. The liquid was kept at 35° and was vigorously stirred during the reaction, which was completed by heating at 100° for 40 minutes. After cooling, an equal volume of rectified spirit was added, and carbon dioxide passed through the solution for a prolonged period. The precipitated sodium salts were then removed by filtration through linen, and the filtrate was cautiously neutralised with dilute sulphuric acid. If kept over-night, a crop of sodium sulphate and sodium methyl sulphate separated, but as part of the product was retained by the crystals it was advisable to evaporate the liquid to a thick syrup and repeat the methylation a second time on the total product. In this and similar evaporations it was necessary to add barium carbonate, as otherwise acidity developed and hydrolysis occurred. For the same reason, the temperature was restricted to under 60°.

The aqueous-alcoholic solution of the product, obtained after the second methylation, was evaporated to dryness, and the residue extracted repeatedly with a large excess of boiling chloroform. The inorganic residues were similarly treated and yielded a further quantity of the methylated product showing the same composition (OMe, 22·7—23·6%). A third methylation was conducted on the material extracted by chloroform and thereafter the methylated starch was completely soluble in aqueous alcohol and was no longer occluded by the inorganic salts. The isolation of the product by extraction with chloroform was carried out as already described, but difficulty was experienced in filtering the chloroform solution by ordinary methods, as the methylated starch formed continuous films on the filter-paper. On removal of the solvent, a stiff syrup remained, readily convertible into a white powder; soluble in cold water, hot alcohol, and in chloroform, insoluble in ether. Traces of reducing sugars were present and the aqueous solution gave a

yellow colour with iodine which was not discharged on heating. OMe = 27·2%. Yield 22 g.

The silver oxide reaction was now applied to the product, which was boiled with excess of methyl iodide to which methyl alcohol was gradually added until the solution became comparatively clear. Silver oxide (51 g.) was then added in small amounts, and the mixture kept at the boiling point for 8 hours. Boiling ethyl alcohol was used as the extracting agent, the turbid solution being evaporated and the residue dissolved in chloroform. The silver salts were also extracted in a Soxhlet apparatus with the same solvent, the extracts being united and the product recovered. Repetition of the alkylation in the same manner had very little effect on the methoxyl content, but, apparently owing to depolymerisation, the solubility of the product in methyl iodide increased steadily with each treatment. Concurrently, and contrary to general experience, the experimental loss increased with the solubility in methyl iodide.

No. of silver oxide methylations.	Wt. of methylated starch.	Vol. of MeOH required.	Methoxyl content.
1	21 g.	65 c.c.	27·8%
2	19	39	28·8
3	15	20	28·6
7	6	3	28·9

The loss in yield was due to combination of part of the product with silver iodide as in the case of methylating glucosamine (Irvine and Hynd, J., 1912, **101**, 1128). On extracting the plastic solid with boiling water for several hours the solution contained carbohydrate, a secondary amine and phosphate.

Various attempts to purify the methylated starch obtained by the above operations were made, the most successful being boiling in chloroform solution with charcoal, but as this involved loss the material was separated into two portions by allowing an ethyl alcohol solution to stand in the cold. Any insoluble syrup which separated was removed and again methylated by the silver oxide reaction, so that ultimately the entire product was obtained in the soluble form. As the material had now acquired complete solubility in methyl iodide, two further methylations were given without the use of any extraneous solvent. In this way, dimethyl starch was obtained as a white powder, free from mineral matter and soluble in water, the solution giving no colour with iodine (Found : C, 50·4; H, 7·4; OMe, 32·7. Dimethyl starch requires C, 50·5; H, 7·4; OMe, 32·7%). $[\alpha]_D$ in chloroform + 135·7° for $c = 1·916$.

Hydrolysis of Dimethyl Starch.—This reaction was conducted in the first place by heating a dilute solution in 2·5% hydrochloric acid on a boiling water-bath for 3 hours. As the solution remained turbid,

the acid concentration was raised to 4% and heating was continued for an additional 3 hours, the reaction being completed by raising the temperature to 110° for 30 minutes. On filtering, a small quantity of a brown solid remained behind, and as this material still contained methoxyl it apparently consisted of one component of dimethyl starch which is highly resistant to hydrolysis. The clear filtrate, containing dimethyl glucose in solution, was treated so as to remove organic acids and was finally extracted with boiling acetone. The extract contained a thick syrup which when dissolved in ethyl acetate partially crystallised, but the solid could not be isolated in sufficient amount for identification. The solubilities, rotation and analytical composition of the syrup were, however, identical with those ascribed to 2 : 3-dimethyl glucose (Irvine and Scott, J., 1913, **103**, 575). Yield 75%. $[\alpha]_D$ + 50·3° in acetone for $c = 1$ (compare 2 : 3-dimethyl glucose, $[\alpha]_D$ + 50·9° in the same solvent).

Preparation of Methylated Starch.

The expression " methylated starch " is applied to the derivative in which seven hydroxyl groups out of nine have undergone methylation, the formation of this compound being characterised by a sharp cessation in the methoxyl increase. In each experiment, 32 g. of starch were used, the initial procedure being as described in the preparation of the dimethyl derivative. After four successive treatments with methyl sulphate, the insolubility of the product in the alkaline liquor enabled the isolation to be greatly simplified. The liquid was poured away from the coagulated mass of organic material and the latter was dissolved in boiling rectified spirit, the solution being neutralised with dilute sulphuric acid. After removal of the precipitated sodium salts, the filtrate was evaporated in the presence of barium carbonate, and the residue extracted with boiling chloroform. Removal of the solvent left a stiff syrup which was boiled with dry ether to remove traces of solvent, a treatment which converted the product into a white flaky powder (Found in material dried at 100°/15 mm.: C, 51·1; H, 7·6; OMe, 35·5. " Methylated starch " requires C, 51·2; H, 7·5; OMe, 37·0%). Yield 21 g.

When the above methoxyl content had been attained the application of the silver oxide reaction had practically no effect on the composition (Found : C, 51·1; H, 7·5; OMe, 36·3%; $[\alpha]_D$ in methyl alcohol + 186·3° for $c = 1·934$, in chloroform + 168·1° for $c = 2·029$). Methylated starch displayed the same range of solubility as dimethyl starch and similarly had no action upon Fehling's solution. When acetylated by means of acetic anhydride in presence of sodium acetate, it was converted into a granular acetate showing $[\alpha]_D$ in chloroform + 191·7°. The acetyl content (11·8%) agreed

with the value calculated on the basis that two out of nine hydroxyl groups originally present in starch resisted methylation and, on subjecting the acetate directly to the methyl sulphate reaction, the original material was regenerated (OMe = 36%).

Similarly, the use of diazomethane was ineffective in raising the methoxyl value, which remained remarkably steady in eight successive preparations of methylated starch (Found : OMe, 35·6; 35·4; 35·2; 36·3; 35·8; 35·2; 35·4; 35·5%). The specific rotation in chloroform also showed little variation, the extremes being $[\alpha]_D$ + 151·5° and 152·6° for $c = 1·25$. These results point to the idea that methylated starch is a homogeneous definite compound, but it may be emphasised that the above specific rotation is greatly increased if in the preparation of the compound the methyl sulphate reaction is supplemented by treatment with silver oxide and methyl iodide. Typical examples are quoted :

Methylations.				
(*a*) With methyl sulphate.		(*b*) With methyl iodide.	$[\alpha]_D$.	Solvent.
4	followed by	1	+168·1°	Chloroform.
4	,,	3	169·5	,,
5	,,	2	173·9	,,
4	,,	3	186·2	Methyl alcohol.

Reasons exist for the belief that this alteration in rotatory power is attributable to depolymerisation.

Hydrolysis of Methylated Starch.

A 10% solution of methylated starch in methyl alcohol containing 1% of hydrogen chloride was kept at the boiling point until the activity was nearly constant. Initially, the solution was opalescent, but rapidly cleared so that accurate readings were possible. Some typical results are quoted below, the complete series giving a smooth, unbroken curve.

Time from start (hours) ...	2	3	5	6	7	8
$[\alpha]_D$	+114·4°	+99·8°	+81·7°	+75·0°	+70·3°	+69·8°

The product, consisting of a mixture of methylated glucosides, was isolated in the usual manner and formed a clear viscous syrup. Practically the whole of the material dissolved in boiling ether and the syrup obtained on removal of the solvent was dried at 100°/1 mm. Yield 21·8 g. from 23 g. of methylated starch.

The two components, *viz.*, trimethyl and dimethyl methylglucosides, were separated by a tedious method which need not be described as it has been superseded by a superior process. The trimethyl methylglucoside was finally distilled at 120°/0·7 mm. as a colourless liquid (n_D 1·4585) which crystallised. When purified

from light petroleum, the crystals melted at 57° and showed $[\alpha]_D$—20° in methyl alcohol (Found: C, 50·6; H, 8·5; OMe, 52·2. Calc. for trimethyl methylglucoside: C, 50·8; H, 8·5; OMe, 52·5%). The glucoside was converted into crystalline tetramethyl glucose by the usual processes, thus showing that it belongs to the stable type, and when hydrolysed with aqueous hydrochloric acid gave crystalline 2 : 3 : 6-trimethyl glucose in 94% yield (mutarotation in methyl alcohol + 83° ⟶ 68·3°. OMe, 41·8. Calc. for trimethyl glucose, 41·9%). A careful search for tetramethyl glucose and for 2 : 3 : 4-trimethyl glucose gave entirely negative results. The dimethyl methylglucoside fraction distilled at 140°/0·4 mm. as a viscous liquid (Found: C, 48·6; H, 8·1; OMe, 42·0. Calc.: C, 48·6; H, 8·1; OMe, 41·9%). As the compound was unknown, it was examined in some detail. On complete methylation, it was converted into tetramethyl methylglucoside, the β-isomeride being present in excess, and when condensed with benzaldehyde it gave a benzylidene derivative. This crystallised imperfectly and could not be characterised definitely as the benzylidene dimethyl methylglucoside described by Irvine and Scott (J., 1913, **103**, 575), although the similarity was very marked. Hydrolysis of the glucoside with aqueous hydrochloric acid gave the corresponding dimethyl glucose as a clear glass. This had the correct analytical composition and showed $[\alpha]_D$ + 56·6° in acetone solution, but the material failed to crystallise. The sugar formed no phenylosazone and did not condense with acid acetone, properties which further suggest that the compound was 2 : 3-dimethyl glucose, but oxidation with nitric acid under the conditions described by Haworth and Leitch (*loc. cit.*) gave a complex mixture. It was, in fact, the study of this reaction which directed our attention to the untrustworthy nature of such oxidations.

With regard to the yields of the glucosides obtained from methylated starch it is important to note that tetramethyl methylglucoside was absent and monomethyl methylglucoside was present to an extent not exceeding 5%. The ratio of the dimethyl to the trimethyl methylglucoside was 1·85 : 1, a result which shows that 2 molecules of the former were produced to 1 molecule of the latter (calc., 1·88 : 1). This was confirmed by repeating the complete series of experiments.

Preparation of Trimethyl Starch.

The methylation of starch may be carried to completion by continuing the methyl sulphate reaction in the manner now described. At the conclusion of the fourth methylation, when the reaction mixture was raised to 100° to destroy excess of methyl sulphate, the

liquid was saturated with salt. The hot liquor was then poured away, leaving the methylated starch adhering to the flask as a plastic mass. On adding hot water, a homogeneous syrup was gradually formed, and this was subjected directly to the next methylation, but at regular intervals the product was separated in a pure condition in order to ascertain with certainty the yields and analytical composition. The homogeneous aqueous syrup was therefore carefully neutralised with sulphuric acid and, after dilution with water, extracted by shaking with chloroform. The residue left on evaporation of the solvent was dried and taken up in dry chloroform, the solution being boiled for 20 minutes with charcoal and filtered hot. A hard, brittle material was left on removal of the chloroform and this, on boiling with ether, was converted into a white, amorphous powder. The treatment with ether eliminated traces of solvent and also removed a small quantity of depolymerised material. The following table indicates the progressive nature of the methylation :

No. of methylations	5	10	14	20	24
% OMe	35·5	39·6	41·4	43·2	43·7
$[\alpha]$ in $CHCl_3$ ($c = 1·7$) ...	+173·9°	+196·8°	+204·8°	+207·2°	+216·5°

At each stage, the carbon and hydrogen values were consistent with the methoxyl content, thus showing that the methylation proceeded without molecular rupture.

Starting from 32 g. of starch, the average yield after ten methylations was 15 g. and thereafter the experimental loss was regular, amounting to less than 0·4 g. per treatment. The final product (10 g., m. p. 143—146°) was a white powder less soluble in hot water than in cold, insoluble in ether, readily soluble in chloroform and in methyl alcohol. The aqueous solution was neutral, had no action upon Fehling's solution and gave no coloration with iodine (Found : C, 52·6; H, 8·0; OMe, 43·75. Calc. for trimethyl starch : C, 52·9; H, 7·8; OMe, 45·6%).

Hydrolysis of Trimethyl Starch.

Method (*a*). *Uncontrolled Hydrolysis.*—An 8% solution of methylated starch in methyl alcohol containing 1% of hydrogen chloride was heated at 100° for 24 hours and thereafter at 130° for 12 hours. The solution, which remained practically colourless, was neutralised with silver carbonate, filtered, taken to dryness, and the residue dissolved in ether. On filtering and evaporating, a colourless syrup remained (yield 94% of the theoretical amount) which was distilled under 0·1 mm. pressure. As the first drops of the distillate showed n_D 1·4551 and had OMe 51·4%, tetramethyl methylglucoside was definitely absent. The main fraction (yield

84%), which showed n_D 1·4583 and OMe 51·9%, consisted of trimethyl methylglucoside, a small, undistilled residue (OMe, 44%) being left in the flask. As under the conditions of the experiment the methylated glucoside was a mixture of α- and β-forms, it failed to crystallise (Found : C, 50·6; H, 8·4; OMe, 51·9. Calc. for trimethyl methylglucoside : C, 50·85; H, 8·5; OMe, 52·5%. $[\alpha]_D$ in methyl alcohol + 69·8° for $c = 1$).

The glucoside was thereafter hydrolysed by heating at 100° in 5% aqueous hydrochloric acid, the progress of the reaction, which was followed polarimetrically, displaying the characteristic double change in activity.

Time from start.	$[\alpha]_D$ for $c = 5$.	Time.	$[\alpha]_D$.
15 minutes.	+69·1°	3 hours.	+69·3°
45 minutes.	79·2	3 hours 45 minutes.	72·4
1 hour 45 minutes.	74·6	4 hours.	76·4
2 hours 30 minutes.	63·9		

The hydrolysis sugar, on isolation, crystallised immediately and completely, the m. p. before crystallisation being 70—80°, and, after purification from ether, 114°. This value remained unaffected on admixture with an authentic specimen of 2 : 3 : 6-trimethylglucose obtained from cellulose. The yield of crystalline sugar was 77% and the mother-liquors contained only a small quantity of uncrystallisable syrup. Contrary to expectation, this was not the isomeric 2 : 3 : 4-trimethyl glucose, as on conversion into the corresponding acetobromide and thereafter into trimethyl β-methylglucoside the product, after recrystallisation, melted at 57·5°. The compound also showed the correct mixed melting point and the standard specific rotation for 2 : 3 : 6-trimethyl methylglucoside.

Method (*b*). *Graded Hydrolysis.*—In this case, the hydrolysis was controlled so as to trace the stages of the reaction. The methylated starch (12 g.) was dissolved in 250 c.c. of methyl alcohol containing 1% of hydrogen chloride and boiled under a reflux condenser until the activity of the solution diminished to a minimum. This point was reached in from 8 to 9 hours and the product was isolated in the usual way. When distilled under 0·8 mm., 10·5 g. of pure trimethyl methylglucoside were obtained, whilst 2·8 g. remained undistilled. The distillate crystallised readily and, after purification from low-boiling petroleum, melted at 57·5° and showed $[\alpha]_D$ −29·3° in methyl alcohol for $c = 1$. When it was hydrolysed with 5% aqueous hydrochloric acid, the specific rotation altered from −25·4° to + 59·5° in 200 minutes, and crystalline 2 : 3 : 6-trimethyl glucose was ultimately obtained, the yield being 82%. A portion of the trimethyl methylglucoside failed to crystallise, but this material, which presumably contained the α-isomeride, was recovered from

the tile and hydrolysed separately. In this case also, 2 : 3 : 6-trimethyl glucose was the only sugar formed.

As already stated, 2·8 g. of non-volatile material were left undistilled when the hydrolysis of methylated starch was conducted as now described. The substance was a hard glass and consisted of depolymerised trimethyl starch (Found: C, 52·5; H, 8·0; OMe, 43%), as the composition remained unaltered but the solubility had increased while the specific rotation had diminished to + 86·9° in chloroform. Hydrolysis took place only when this material was heated for many hours at 130° with methyl alcohol containing 1% of hydrogen chloride, but the essential product was, as before, 2 : 3 : 6-trimethyl methylglucoside (OMe 41·6%; n_D 1·4590). This, in turn, was converted into crystalline 2 : 3 : 6-trimethyl glucose, the identity of which was confirmed by transformation into the corresponding β-methylglucoside. No trace of the 2 : 3 : 4-isomeride could be detected.

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