313. *Polysaccharides. Part XXXII. The Molecular Constitution of Rice Starch.*

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Rice starch, in common with all other starches hitherto examined, contains a repeating unit composed of a straight chain of 24—30 glucose residues. Independently of the mode of preparation of the methyl derivative—whether the starch grain is methylated directly in air or in nitrogen, or whether the methyl derivative is prepared *via* the acetate—and irrespective of the molecular weight of the methylated starch, the percentage of end-group (tetramethyl glucose) obtained on hydrolysis remains unchanged. The observed proportion of end-group cannot therefore be explained by random hydrolysis of long main chains of similarly united residues, and it is concluded that viscous methyl starches are composed of a large number of repeating units joined together laterally, forming side chains. In support of this view, a viscous methyl starch containing some 80 repeating units (mol. wt. approximately 500,000) has been disaggregated by controlled hydrolysis into a non-viscous, essentially homogeneous methyl derivative, which, on further methylation to etherify the hydroxyl groups liberated during the disaggregation process, gives the fully methylated derivative. Osmotic-pressure and ultracentrifuge measurements show that the latter has a molecular weight corresponding to about 3 repeating units (90 glucose residues) and on hydrolysis the methylated disaggregated derivative gives precisely the same amount of tetramethyl glucose as do the viscous derivatives, but the yield of dimethyl glucose is now very small. Consideration of the conditions of the disaggregation process leads to the conclusion that in the starch molecule, the repeating units, each consisting of a chain of 30 glucose residues, are linked to a non-terminal glucose residue of another unit by primary valencies of the glycosidic type. The relationship between viscosity data and molecular weight in the methyl starch series is considered and an empirical method is suggested for the utilisation of viscosity results in the determination of approximate molecular sizes.

This paper is concerned with the nature of the repeating unit present in the rice starch molecule and the manner in which the repeating units are united to form macromolecules containing up to 2000 or more glucose residues. It has been shown previously that in several varieties of starch (for detailed references, see Hirst and Young, this vol., p. 951) there occurs as a repeating unit a chain of 24—30 glucose residues linked glycosidically through their 1—4 positions. It is now demonstrated that a similar repeating unit containing approximately 30 glucose residues is a fundamental feature of the structure of rice starch. That this repeating unit has a real existence has been confirmed by the extensive series of experiments summarised in Table I. The results of these indicate that the chain length of the repeating unit, as determined by the yield of tetramethyl glucose obtained on hydrolysis of methylated rice starch, is independent both of the mode of preparation and of the viscosity of the methylated starch. For instance, the same yield of end group (tetramethyl glucose) is obtainable from methylated rice starch of molecular weight 400,000 and from a disaggregated methylated derivative of molecular weight 20,000. In all cases the methods of purification adopted and the fractionation procedure ensure that no small fragments can be present and it is impossible to reconcile the present results

TABLE I.

Derivatives of Rice Starch.

Operation.	Designation of product in text.	Molecular weight from viscosity.[1]	Molecular weight of repeating unit from end-group assay.[2]
Acetylation (SO_2 and Cl_2 catalysts)	A	250,000	
	B	380,000	
Methylation of A in N_2 at 55°	—	166,000 [3]	
Methylation of B in N_2 at 55°	G (1)	600,000	6900
	G (2)	510,000	
Acetylation in pyridine	C	720,000	
Methylation of C in N_2 at 55°	—	670,000 [3]	
Direct methylation in air at 20°	D (1)	520,000	5900
	D (2)	385,000	
	D (3)	250,000	
Direct methylation in air at 55°	E (1)	190,000	6700
	E (2)	250,000	
	E (3)	205,000	
	E (4)	95,000	
Direct methylation in N_2 at 20°	F (1)	560,000	6100
	F (2)	525,000	
	F (3)	400,000	
	F (4)	175,000	
Disaggregation of F (2)	X	23,000 [3]	
Methylation of X (main fraction)	Y	20,000 [4]	6300

[1] Obtained by the method discussed on p. 1475.
[2] A chain length of 30 units corresponds to a molecular weight of 6100.
[3] Viscosity of unfractionated product.
[4] Confirmed by osmotic-pressure and ultracentrifuge measurements.

with any hypothesis of random hydrolysis taking place during the methylation of an immensely long chain of similarly united glucose residues (compare Richardson, Higginbotham, and Farrow, *J. Text. Inst.*, 1936, **27**, *T*, 131). Again, the yield of tetramethyl glucose is the same whether the methylated derivative has been obtained (a) by direct methylation of rice starch in an atmosphere of nitrogen, (b) by direct methylation in air at room temperature or at 55°, or (c) by simultaneous deacetylation and methylation of an acetyl derivative of rice starch. The last observation is of special importance in that in this particular case acetylation was carried out with the aid of sulphur dioxide and chlorine as catalysts and it is seen, therefore, even when the somewhat drastic conditions of the Barnett method are employed for acetylation, that there is no appreciable breakdown of the glucosidic links of the repeating units. It appears also from the values for molecular weights given in Table I that methylated derivatives can be obtained from acetylated rice starch without diminution of the total particle size in terms of glucose residues. Staudinger and Husemann have reached a similar conclusion in the case of the methylation of potato starch (*Annalen*, 1937, **527**, 195) and of wheat starch (*Ber.*, 1938, **71**, 1057) *via* the acetates. In certain instances, the methyl derivatives obtained in this way have a molecular weight fully as high as that of methylated rice starches obtained by direct methylation in the cold.

The particle sizes of the methylated starches included in Table I are extremely large, fraction F(1), for example, containing some 2800 glucose residues per particle. In general, the milder the methylation treatment the greater the apparent molecular weight, but on the other hand, as is also the case with maize starch (Averill, quoted by Carter and Record, this vol., p. 670), long-continued repetition of the methylation treatment gives products of lower molecular weight and higher methoxyl content. This point will not be considered in detail in this paper, but it may be stated now that these methyl derivatives of lower molecular weight give the same yield of end-group as do those of high molecular weight. It is probable that the particle size of natural rice starch is still greater than the

highest value shown in Table I, but this does not affect the general conclusions arrived at concerning the nature and chain length of the repeating unit.

Disaggregation of Methylated Rice Starch.—The results considered in the previous section show that by alteration in the conditions of methylation, methyl derivatives of widely different molecular weight are obtainable. We next proceeded to investigate the possibility of transforming methyl derivatives of high molecular weight by methods which involved no alteration in the size or character of the repeating unit. One process whereby this can be effected is to repeat the methylation process a large number of times. For example, methylation of maize starch seventeen times reduced the molecular weight to 38,000 (Averill, *loc. cit.*). This method is, however, tedious, and the extent to which disaggregation can be brought about is limited and is not readily controllable. Further consideration of this process will be postponed except to say that it seems likely that the mechanism of it is similar to that operative in the methods about to be described but, owing to the alkaline conditions, it proceeds at a much slower rate. Two other methods have been found whereby disaggregation can be effected, and both of them are essentially mild hydrolytic reactions, an observation which is of importance in connection with the argument concerning the mode of linking between the repeating units. The first method consists in heating a solution of the highly viscous methylated starch in *m*-cresol containing a little water. The viscosity gradually diminishes to a final value ($\eta_{sp.}^{20^\circ} = 0{\cdot}10$ for *c*, 0·4 g./100 c.c.) which is independent of the molecular weight of the original methylated starch. Isolation of the product from its solution in *m*-cresol is troublesome and in many respects an alternative method offers many advantages. In this the methylated starch is heated in aqueous methyl alcohol containing oxalic acid, and for purposes of comparison the conditions chosen were identical with those employed by Haworth, Hirst, and Percival (J., 1932, 2387) for the hydrolysis of methylated inulin. When this method is used, it is possible to isolate samples at various stages of the reaction and to purify them by precipitation from boiling water. In this way, separation of any small fragments formed by degradation of the repeating units would be effected, but actually no such breakdown products were detected in the experiments described below. It was found that the viscosity of these samples (which are presumably mixtures of polymer-homologues and not homogeneous substances) diminished regularly during the main part of the reaction, and the final product, which was obtained in almost quantitative yield, had a molecular weight of *ca.* 20,000 irrespective of the initial molecular weight. From the method of purification, this final product contained no short-chain break-down products and confirmation of this view was obtained in the following way. The disaggregated methylated starch was submitted to methylation in order to convert into the methyl ethers any hydroxyl groups which had become exposed during the disaggregation procedure. The fully methylated derivative (Y in Table I) was carefully fractionated and it was found that the main portion had a molecular weight very close to 20,000 and was essentially homogeneous. No very small fragments were present and the results of ultra-centrifuge analysis carried out for us by Mr. J. St. L. Philpot, whose report is appended, indicate that no great portion of it can have a molecular weight much different from 20,000. The behaviour towards membranes during osmotic-pressure measurements gives further substantiation of this view (the molecular weight by osmotic pressure being 20,500). The substance (Y) in comparison with (G), (D), (E), or (F) has properties corresponding to its smaller molecular size. It dissolves much more rapidly in organic solvents and in cold water, but, like the viscous methyl derivatives, it is insoluble in boiling water. Because of its higher degree of methylation—a degree not attainable with starch of high molecular weight—its specific rotation ($[\alpha]_D^{20^\circ} + 224^\circ$) is noticeably higher than that of (D), (E), etc. In this connection it is important to note that during the actual disaggregation process the rotation of the material remains unchanged, and only after further methylation to effect closure of hydroxyl groups freed during the hydrolysis does the rotation rise to + 224°. In view of the immediate fall in specific rotation when the glycosidic links in the repeating unit are broken (compare the starch dextrins) we regard this as further strong evidence that no such linkages have been broken during disaggregation. This view is once again substantiated by the fact that on hydrolysis (Y) yields precisely the same percentage of end-

group as do the viscous derivatives (G), (D), (E), and (F). It follows that the disaggregated methylated starch of molecular weight 20,000 contains exactly the same repeating unit as is present in the viscous methyl derivatives, and that the "*disaggregation*" *reaction has not involved any breakdown within the repeating units but is concerned with the separation intact of repeating units from larger aggregates of these units.*

In the experiments described here, the disaggregation process became very slow when the molecule contained three repeating units, but by modification of the conditions it appears to be possible to proceed further without degradative breakdown and to isolate the single repeating unit itself. These experiments will be the subject of a later communication.

The conditions for disaggregation of the methylated starch do not appear to be so favourable for the unmethylated starch, and attempts at disaggregation under similar conditions result in hydrolysis of the repeating units themselves. It would appear that in these circumstances there is little difference in the stability of the side-chain and main-chain glucosidic links. Nevertheless, it is obvious that simplification of the starch molecule, giving products of greater solubility and smaller viscosity, takes place under conditions not dissimilar to those used in the disaggregation experiments during the formation of soluble starch. This view is in accord with the observations of Richardson, Higginbotham, and Farrow (*loc. cit.*), who find a continued increase in the reducing power during the treatment of starch with acids, and with the similar results of the periodic acid oxidation of starch carried out by Caldwell and Hixon (*J. Biol. Chem.*, 1938, **123**, 595).

It is of interest to compare this process of chemical disaggregation with the disaggregating action of the enzyme "amylophosphatase" of Waldschmidt-Leitz and Mayer (*Z. physiol. Chem.*, 1935, **236**, 168). This enzyme causes an immediate fall in the viscosity of starch pastes, and the reducing power rises finally to a value corresponding to a chain length of 36 glucose units (compare Hanes, *New Phytologist*, 1937, **36**, 538). Phosphorus can, however, play no part in the disaggregation of methylated rice starch, in which it is absent.

Nature of the Linkage between the Repeating Units.—On previous occasions (see, for example, Hirst, Plant, and Wilkinson, J., 1932, 2375; Haworth, Hirst, and Isherwood, J., 1937, 577) the view has been expressed that in starch there are units consisting of terminated chains of 24—30 glucose residues and that these units aggregate to form the larger particles present in ordinary starches. Hitherto evidence has been lacking concerning the precise nature of the bond by which the "aggregation" is effected and the question whether it is a glycosidic link (primary valency) or some looser form of association (for example, hydroxyl bond) was left open. Now, however, it is possible from a consideration of observations made during a study of the disaggregation reaction to make certain deductions concerning the nature of this linkage. It is found that the disaggregation reaction, as indicated by the changes in viscosity of the methylated starch, follows a course strictly comparable with the hydrolysis of methylated inulin under the same conditions. The rate of reaction is, in fact, some seven or eight times *slower* than that of methylated inulin and lies between the values for methylated inulin and methylated methyl glucopyranosides. The kinetics of the disaggregatation reaction will be considered in detail in another paper, in which it will be shown that the critical increment for the disaggregation (approximately 25,000 cals.) is closely similar to that for the hydrolysis of glycosides of both the furanose and the pyranose type (compare Moelwyn-Hughes, *Trans. Faraday Soc.*, 1929, **25**, 503). Since in methylated inulin the residues are held together by glycosidic links and in all respects the "aggregation" link in starch has the properties of an ordinary valency link, there seems no reason for supposing that we are here dealing with anything other than a primary valency bond of the glycosidic type. The absence of any evidence of reaggregation in the series of methylated starches is significant in this connection.

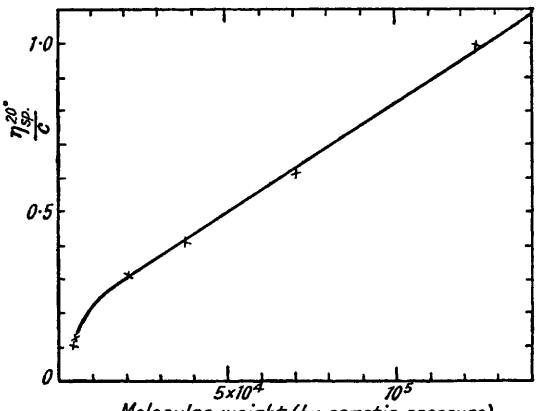
It seems probable, therefore, that in starch there are present extremely large molecules built up of repeating units containing some 30 glucose residues, each unit being joined by a glycosidic link to another such unit, forming branched chains. It follows from this that for highly viscous, fully methylated starches, dimethyl glucose should be isolated in amount equal to that of the tetramethyl glucose. Owing to the difficulties of methylation it is not easy to obtain direct evidence on this point in the case of viscous methyl derivatives, which usually give rise to dimethyl glucose in any case, owing to incomplete methylation, but it is

true that for such derivatives the amount of dimethyl glucose observed is never less than the amount of " tetra." Complete methylation of the disaggregated starch proceeds much more readily and it is significant that in this case the amount of dimethyl glucose is less than with viscous methyl derivatives and indeed is less, as it should be theoretically, than the amount of tetramethyl glucose (for a molecule of three units of 30 glucose residues each, there will be two " dimethyl " residues for every 3 " tetramethyl " residues).

At present there is no definite knowledge concerning the precise positions in the chains of the various repeating units at which the glycosidic group of the next unit is attached, and much further work will be required for the solution of this particular problem. That the attachment does involve a glycosidic group is indicated by the fact that starch has little or no reducing power in its natural state and that there is a definite increase in reducing power, without hydrolysis of the links within the repeating units, during the process of disaggregation.

It is of special interest to find that the side-chain links undergo hydrolysis more readily than do the 1 : 4 links of the main chain, and an analogy for this has recently come to light in the course of work on methylated damson gum (Hirst and Jones, following paper), where a side chain glycosidic link has been found to hydrolyse more rapidly than the glycosidic links of the main chain.

FIG. 1.



Viscosity/Molecular Relationship for Methylated Starches.

Relationship between Viscosity and Molecular Weight of Methylated Starches.—The recent work of Carter and Record (this vol., pp. 660, 664) provides the most accurate osmotic-pressure measurements hitherto recorded for methylated polysaccharides, and enables a critical test to be made of the accuracy of the Staudinger equation ($\eta_{sp.} = k_m cM$) as applied to methylated starches. In Fig. 1 a graph has been drawn showing the relationship between the molecular weight, determined osmotically, and the specific viscosity for unit concentration ($\eta_{sp.}/c$) (c is given in g./100 c.c.). Although it is apparent that the above equation does not apply in its simple form, the fact that the observed points lie regularly on the line shown in the graph provides an empirical method for the calculation of molecular weights from viscosity data in this series. We have accordingly used this graph in order to obtain the molecular weights recorded in Table I and in the experimental section. Certain of the higher values were found by extrapolation. In view of the empirical nature of the relationship employed, the numerical values given are to be regarded as approximate, but it seems probable that the figures are of the correct order of magnitude and that they give an accurate picture of the relative molecular sizes. In the case of the acetates we have no direct osmotic-pressure measurements by Carter and Record's method, and for the few acetates mentioned the molecular weights shown are to be regarded as provisional. They have been obtained by using Staudinger's equation with the value of the constant k_m ($0{\cdot}93 \times 10^{-4}$) given by Staudinger and Husemann (*Annalen*, 1937, **527**, 195) for a different series of acetates.

It will be seen that the viscosity–molecular weight relationship for methylated starches gives an approximately straight line for derivatives of molecular weights from 20,000 to 150,000, for which a modified equation * $\eta_{sp.} = k'_m cM + a$ is valid, where $k'_m = 1{\cdot}3 \times 10^{-4}$. Below 20,000 a different type of relationship holds, k'_m is no longer constant, and the slope of the curve tends towards that observed by Staudinger for the cellulose series, for which $k_m = 10^{-3}$. An interesting possibility is thus revealed in that methylated starch may

* In these instances concentrations are to be expressed in the units employed by Staudinger, namely, g.-mols. of methylated glucose residue per litre, *i.e.*, 0·049 × conc. in g./100 c.c.

represent an intermediate stage between the compact, rigid molecules of glycogen, for which the Staudinger equation breaks down completely, and the long unbranched chains present in cellulose and the cellodextrins. The picture we have given of starch indicates a large molecule with numerous branched chains, which will be more compact in shape and more rigid than the straight chain present in cellulose. The modified Staudinger equation is followed by methylated starch derivatives of molecular weight 20,000 (corresponding to three repeating units) and upwards, but in the course of disaggregation beyond this stage it is to be expected that the shape of the molecule will change abruptly and must then approximate more closely to that of the straight-chain type of cellulose, with the result that the viscosity constants tend to become similar to those observed for the cellulose series. If this explanation proves to be correct, the present observation may point to a possible extension of the use of viscosity data as a guide to molecular shape.

The point of special importance derived from the ultracentrifuge examination is the evidence concerning the molecular weight and essential homogeneity of the disaggregated methylated starch, but it may be mentioned that other far-reaching problems are implicated by Dr. Philpot's interpretation of the ultracentrifuge results as indicating a spherical shape for the molecule of disaggregated methyl starch. If this is so, it would appear that molecules which according to ultracentrifuge evidence are approximately spherical in shape, behave in their viscosity relationships very similarly to the long rod-shaped particles demanded by Staudinger's theory (for the condition of potentially rod-shaped molecules in solution, compare the views of Kuhn, *Z. physikal. Chem.*, *A*, 1932, **161**, 1; Sakurada, *Naturwiss.*, 1939, **25**, 523; Huggins, *J. Physical Chem.*, 1939, **43**, 439). It is obvious, however, that in the present state of knowledge concerning these starch derivatives it is not possible to discuss usefully the relationship between molecular shape in solution during viscosity determinations and during ultracentrifuge measurements, and we propose at present to confine ourselves to the general observations outlined above.

Experimental.

The rice-starch used showed a purple coloration with aqueous iodine and had the following properties: (a) $[\alpha]_D^{19°}$ + 152° in N-sodium hydroxide (*c*, 0·4); (b) distillation with 12% hydrochloric acid gave no furfural; (c) 0·45% of carbon dioxide was liberated on heating with the same reagent under the conditions used for the estimation of uronic acid groups, but this amount has been shown (Campbell, Hirst, and Young, *Nature*, 1938, **142**, 912) to arise from decomposition of the constituent hexose, and uronic acids are therefore absent; (d) copper number (Schwalbe–Braidy method) *ca.* 0·1 g. of copper per 100 g. of starch; (e) acid number: 1 g. of starch required 0·05 c.c. of 0·1N-alkali for neutralisation (Found: P_2O_5, 0·04; N, 0·05; moisture content of air-dried sample, 13·0; ether-soluble constituents, 0·4%).

Acetylation.—(a) *By acetic anhydride in the presence of sulphur dioxide and chlorine.* Air-dried starch (20 g.) was precipitated from a 3% starch paste by addition of alcohol, and after being washed with alcohol and then ether, was suspended in glacial acetic acid (60 g.) containing a little chlorine. After 30 minutes at room temperature, acetic anhydride (100 g.), through which sulphur dioxide had been bubbled, was added slowly with mechanical stirring. The temperature was kept below 60° by external cooling and the mixture was then stirred at 50° for 3 hours. The clear, viscous liquid was poured into water (3 l.) and the precipitated acetate was washed continuously with water until free from acid, filtered off, and dried at 80° in a vacuum (yield, 89% of the theoretical). The crude acetate (A) was purified by precipitation from chloroform solution (350 c.c. containing 50 c.c. of absolute alcohol) by ether (500 c.c.). $\eta_{sp.}^{20°}$ 0·32 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 250,000 (Found: CH_3·CO, 44·6. Calc. for $C_{12}H_{16}O_8$, 44·8%). Sulphur, chlorine, and nitrogen were absent.

In another experiment, 30 g. of air-dried starch, 180 c.c. of glacial acetic acid, and 300 c.c. of acetic anhydride were used, with a second application of the catalysts. The acetate (B), isolated in 85% yield, had $[\alpha]_D^{19°}$ + 174°, $\eta_{sp.}^{20°}$ 0·494 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 380,000 (Found: CH_3·CO, 44·5%).

(b) *By acetic anhydride and pyridine.* The acetylation was carried out in a similar manner to that described for wheat starch (Hirst and Young, this vol., p. 951). After addition of the reagents, the temperature was kept below 70° by cooling. The solution was then stirred at room temperature for 7 hours, diluted with an equal volume of glacial acetic acid, and poured into cold water (2 l.). The acetate was washed continuously with running water for 36 hours

and dried in a vacuum at 80° (yield, 38 g., 92% of the theoretical) [acetate (C)]. $[\alpha]_D^{20°}$ + 171° in chloroform (*c*, 0·55%). $\eta_{sp.}^{20°}$ 0·93 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 720,000 (Found : $CH_3{\cdot}CO$, 44·7%; iodine number, 0·2 c.c. of 0·1N-iodine for 1 g.).

Methylation of Rice Starch.—(a) *Direct methylation of precipitated rice starch at* 20°. The starch was prepared by precipitation from an aqueous paste by alcohol in the usual manner, and was then methylated at room temperature with methyl sulphate and 30% caustic soda solution as previously described for wheat starch (Hirst and Young, *loc. cit.*), but air was not excluded during the process (yield, 60—70%). The product [30 g., methyl rice starch (D)] was fractionally precipitated from chloroform by light petroleum (b. p. 60—80°), giving fractions with the following properties : (1) 8·0 g., $[\alpha]_D^{20°}$ + 211° in chloroform (*c*, 0·85), $\eta_{sp.}^{20°}$ 1·37 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 520,000 (Found : OMe, 43·0%); (2) 12·0 g., $[\alpha]_D^{20°}$ + 213° in chloroform (*c*, 1·13), $\eta_{sp.}^{20°}$ 1·04 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 385,000 (Found : C, 52·4; H, 7·6; OMe, 44·4. Calc. for a methyl starch of this methoxyl content : C, 52·7; H, 7·8%); (3) 7·4 g., $\eta_{sp.}^{20°}$ 0·70 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 250,000 (Found : OMe, 45·4%).

(b) *Direct methylation of " prepared " rice starch at* 55°. The " prepared " starch, dissolved in 30% caustic soda solution (360 c.c.), was methylated with six half-hourly additions of methyl sulphate (180 c.c. in all) at 55°. After the last addition, the temperature of the bath was raised to 100° for 30 mins. Otherwise the procedure was similar to that described previously; air was not excluded. The product [methyl rice starch (E)] was fractionally precipitated from chloroform by light petroleum, giving the following fractions : (1) 3·7 g., $[\alpha]_D^{20°}$ + 206° in chloroform (*c*, 0·38), $\eta_{sp.}^{20°}$ 0·55 in *m*-cresol (*c*, 0·4), corresponding to a molecular weight of 190,000, m. p. 200° (decomp.) (Found : OMe, 43·0%); (2) 5·9 g., $[\alpha]_D^{20°}$ + 212° in chloroform (*c*, 0·37), $\eta_{sp.}^{20°}$ 0·70 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 250,000, m. p. 190° (decomp.) (Found : C, 52·9; H, 7·4; OMe, 43·5; P, nil. Calc. for a methyl starch of this methoxyl content : C, 52·6; H, 7·7%); (3) 4·1 g., $[\alpha]_D^{20°}$ + 208° in chloroform (*c*, 0·38), $\eta_{sp.}^{20°}$ 0·58 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 205,000, m. p. 186° (decomp.) (Found : OMe, 43·5%); (4) 5·7 g., $\eta_{sp.}^{20°}$ 0·31 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 95,000 (Found : OMe, 41·6%).

(c) *Direct methylation of native rice starch at* 20° *in an atmosphere of nitrogen.* The starch (granules, 30 g.) was well mixed with cold water (150 c.c.), and 30% caustic soda solution (100 c.c.) added with stirring. A gel formed and this was dispersed by addition of water (50 c.c.). Methylation was then carried out at room temperature in the usual way, in a stream of nitrogen. After eight methylations, a sample had 45·4% OMe. The product [methyl rice starch (F)] was purified as previously described. Yield, 75% of the theoretical. The following fractions were obtained : (1) 0·6 g., $\eta_{sp.}^{20°}$ 1·50 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 560,000 (Found : OMe, 43·3%); (2) 34·0 g., $[\alpha]_D^{20°}$ + 205° in chloroform (*c*, 0·50), $\eta_{sp.}^{20°}$ 1·39 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 525,000 (Found : OMe, 42·9%); (3) 8·2 g., $[\alpha]_D^{20°}$ + 210° in chloroform (*c*, 0·89), $\eta_{sp.}^{20°}$ 1·08 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 400,000 (Found : OMe, 44·5%); (4) 3·5 g., $\eta_{sp.}^{20°}$ 0·51 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 175,000 (Found : OMe, 45·7%).

(d) *Simultaneous deacetylation and methylation of the acetate* (*B*) *at* 55°. The acetate (prepared by using sulphur dioxide and chlorine catalysts, p. 1476) was dissolved in acetone (400 c.c.) and treated at 55° with methyl sulphate (100 c.c.) and 30% caustic soda solution (280 c.c.) added in ten equal portions at 10-minute intervals, in a stream of nitrogen. Otherwise the procedure was similar to that described above. After five methylations, a sample had 44·0% OMe. The following fractions [methyl rice starch (G)] were obtained : (1) 4·5 g., $\eta_{sp.}^{20°}$ 1·57 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 600,000 (Found : OMe, 43·1%); (2) 7·9 g., $[\alpha]_D^{20°}$ + 212° in chloroform (*c*, 0·54), $\eta_{sp.}^{20°}$ 1·34 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 510,000 (Found : OMe, 43·1%).

A small sample of acetate (A) (prepared by using sulphur dioxide and chlorine catalysts, p. 1476) was methylated as above for comparison of the viscosity of the product. After ten methylations the product was isolated in 70% yield; $\eta_{sp.}^{20°}$ 0·487 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 166,000 (Found : OMe, 43·8%).

(e) *Methylation of acetate* (C) *at* 55°. The acetate (16 g., prepared by use of acetic anhydride and pyridine, p. 1476) was methylated as described above (d), in an atmosphere of nitrogen. After ten methylations the methoxyl content was 43·3%. Yield, 11·5 g. (89% of the theoretical). $[\alpha]_D^{20°}$ + 208° in chloroform (*c*, 0·456), $\eta_{sp.}^{20°}$ 1·75 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 670,000.

The Repeating Unit of Methyl Rice Starch.—(1) *Hydrolysis of methyl rice starch (E), prepared by direct methylation of "prepared" rice starch at 55° in air.* The methyl starch (fractions 1, 2, and 3; 10·91 g.) was treated with glacial acetic acid (55 c.c.) and air was removed from the viscous mass by evacuation. 5% Hydrochloric acid (108 c.c.) was added, and the mixture then heated on a boiling water-bath until the rotation became constant (24 hours). The acid was partially neutralised with barium carbonate (19 g.) and the remaining acetic acid was removed by vacuum distillation, with addition of water as required. The residual neutral aqueous solution (200 c.c.) was extracted with chloroform (10 c.c.) twenty times. The chloroform layers were dried over anhydrous magnesium sulphate, which was then filtered off and washed thoroughly with chloroform. The combined chloroform extracts were evaporated to dryness, leaving a syrup (H) (0·7 g.), which contained all the tetramethyl glucose and some trimethyl glucose. The aqueous layer from the chloroform extraction was evaporated to dryness in a vacuum and exhaustively extracted with boiling chloroform. The dried chloroform solution similarly gave syrup (J) (10·0 g.), which crystallised immediately. For convenience in manipulation, a portion of syrup (J) was added to syrup (H). Syrups (J) and (H) were then boiled separately with 2% methyl-alcoholic hydrogen chloride for 7 hours. The glucosides formed were isolated in the usual manner. Syrup (H) (6·3 g.) had a bulk refractive index $n_D^{18°}$ 1·4583; syrup (J) (5·2 g.) had $n_D^{18°}$ 1·4593 (total recovery, 95% of the theoretical).

After several fractional distillations, a vacuum-jacketed column being used, the following fractions were finally obtained :

TABLE II.

Fraction.	$n_D^{16°}$.	Constants used in estimation.*	Wt., g.	%. "Tetra."	Wt. "tetra," g.	Wt. "tri," g.	%. "Di."	Wt. "di," g.
1	1·4487	(a) 1·4440 (b) 1·4575	0·33	65	0·21	0·12	—	—
2	1·4536	(a) 1·4445 (b) 1·4580	0·13	33	0·04	0·09	—	—
3	1·4560	(a) 1·4450 (b) 1·4580	0·38	15	0·06	0·32	—	—
4	1·4583		0·10	0	—	0·10	—	—
5	1·4607		7·51	—	—	7·51	—	—
6	1·4630		1·27	—	—	0·67	45	0·60
7	1·4717		0·18	—	—	—	100	0·18
Still residue	.....		0·35	—	—	—	—	—
Total recovery	.....		10·25		0·31	8·81		0·78

* (a) and (b) are the $n_D^{16°}$ values of the "tetra" and "tri" portions respectively present in these fractions, as estimated from rotational data (see Hirst and Young, J., 1938, 1247).

The analysis of fission products, expressed as a percentage of the total recovery, was therefore : 2 : 3 : 4 : 6-tetramethyl methylglucoside (end group), 3·3% ; 2 : 3 : 6-trimethyl methylglucoside, 86% ; dimethyl methylglucosides, 7·6%. This amount of end-group corresponds to a repeating unit of chain length of 33 *glucose residues.*

(2) *Hydrolysis of methyl rice starch (D), prepared by direct methylation of precipitated rice starch at* 20°. 10·85 G. off raction D (2) (p. 1477) were hydrolysed in the manner described above. The glucoside formation of the syrups was effected by 3% methyl-alcoholic hydrogen chloride. After successive distillations the following fractions were obtained :

Fraction.	$n_D^{16°}$.	Constants used in estimation.*	Wt., g.	%. "Tetra."	Wt. "tetra," g.	Wt. "tri," g.	%. "Di."	Wt. "di," g.
1	1·4480	(a) 1·4440 (b) 1·4540	0·32	70	0·22	0·10	—	—
2	1·4525	(a) 1·4445 (b) 1·4580	0·33	41	0·14	0·19	—	—
3	1·4571	(a) 1·4450 (b) 1·4585	0·19	12	0·02	0·17	—	—
4	1·4594		0·19	0	—	0·19	—	—
5	1·4600		8·25	—	—	8·25	—	—
6	1·4637		1·18	—	—	0·71	40	0·47
Residue	.....		0·42	—	—	—	—	—
Total recovery	.....		10·88		0·38	9·61		0·47

* See note under Table II.

The analysis of fission products, expressed as a percentage of the total recovery, was therefore : 2 : 3 : 4 : 6-tetramethyl methylglucoside (end-group), 3·5% ; 2 : 3 : 6-trimethyl methylglucoside, 88% ; dimethyl methylglucoside, 4·3%. This amount of end-group corresponds to a repeating unit of chain-length 29 *glucose residues*.

(3) *Hydrolysis of methyl rice starch* (*G*), *prepared by methylation of an acetate at* 55° *in an atmosphere of nitrogen.* This material (fractions 1 and 2; 10·57 g.) was hydrolysed with acetic and hydrochloric acids as above. After neutralisation of the mineral acid with barium carbonate and removal of the acetic acid by distillation in steam, the dried residue was exhaustively extracted with benzene in an attempt to make a preliminary separation of dimethyl glucoses by the method used by Bell (*Biochem. J.*, 1935, **29**, 2031). It was found, however, that the benzene extract contained a considerable amount of dimethyl glucose as well as the higher methyl glucoses. Glucoside formation of the syrups, isolated as before, was effected with boiling 1% methyl-alcoholic hydrogen chloride for 5½ hours.

After fractional distillation at 0·001 mm. pressure, the following fractions, containing tetramethyl methylglucoside, were obtained :

Fraction.	Wt., g.	$n_D^{16°}$.	Constants used in estimation.*	%. "Tetra."	Wt. "tetra," g.
1	0·26	1·4462	(a) 1·4435 (b) 1·4570	80	0·21
2	0·31	1·4541	(a) 1·4440 (b) 1·4570	25	0·08
3	0·14	1·4547	(a) 1·4445 (b) 1·4575	22	0·03
					0·32

* See note under Table II.

This amount of tetramethyl methylglucoside (together with the 10% correction for loss required when calculated on the theoretical yield of glucosides) corresponds to 2·9% of the total, giving a chain length of 34 *glucose residues* for the repeating unit.

(4) *Hydrolysis of methyl rice starch* (*F*), *prepared by direct methylation of native rice starch, in an atmosphere of nitrogen.* The simultaneous hydrolysis of fraction F (3) (7·50 g.) and glucoside formation of the fission products were effected by boiling with 3% methyl-alcoholic hydrogen chloride for 8 hours. The glucosides were isolated in the usual manner, and fractional distillation at 0·001 mm. gave the following fractions :

Fraction.	Wt., g.	$n_D^{16°}$.	Constants used in estimation.*	%. "Tetra."	Wt. "tetra," g.	Wt. "tri," g.	%. "Di."	Wt. "di," g.
1	0·30	1·4506	(a) 1·4440 (b) 1·4575	51	0·15	0·15	—	—
2	0·58	1·4557	(a) 1·4445 (b) 1·4580	17	0·10	0·48	—	—
3	0·27	1·4573	(a) 1·4450 (b) 1·4580	5	0·01	0·26	—	—
4	3·96	1·4585		0	—	3·96	—	—
5	2·27	1·4599		—	—	2·05	10	0·22
6	0·11	1·4648		—	—	0·06	45	0·05
Residue :	0·41							
	7·90				0·26	6·96		0·27

* See note under Table II.

The analysis of the fission products, expressed as a percentage of the total recovery, was therefore : 2 : 3 : 4 : 6-tetramethyl methylglucoside, 3·3% ; 2 : 3 : 6-trimethyl methylglucoside, 88% ; dimethyl methylglucosides, 3·4%. This amount of end-group corresponds to a repeating unit with a chain length of 30 *glucose residues*.

The identity of the 2 : 3 : 4 : 6-tetramethyl methylglucoside and 2 : 3 : 6-trimethyl methylglucoside from the hydrolyses was established in the usual manner by the isolation from the respective fractions of crystalline 2 : 3 : 4 : 6-tetramethyl glucose, m. p. 82—84°, $[\alpha]_D^{20°}$ + 82·5° (equilibrium) in water (OMe, 49·2%), and 2 : 3 : 6-trimethyl glucose, m. p. 113°, $[\alpha]_D^{20°}$ + 69·4° (equilibrium) in water (OMe, 42·1%).

Methylation of the dimethyl methylglucosides from the hydrolysis products by Purdie's method gave tetramethyl methylglucoside in good yield ($n_D^{16°}$ 1·4437; OMe, 61·4%). The possibility of the presence of substituents other than the methyl groups is therefore excluded.

The Disaggregation of Methyl Rice Starch.—Heat treatment in m-*cresol solution.* For these

experiments, the methyl starch [fraction E (3) p. 1477] was dissolved in *m*-cresol (containing a little water) and heated in a flask fitted with an air condenser, in an oil-bath at constant temperature. Air was removed from the flask at the beginning of the experiment by a stream of nitrogen, and the end of the condenser was then closed with a mercury trap. At intervals, samples (about 3 c.c.) were withdrawn, cooled, and filtered through a sintered glass filter, and the viscosity was determined in the usual manner in an Ostwald viscometer at 20° ± 0·05°.

At 100°, little diminution in viscosity was detectable after 2 days. At 150°, however, a regular decrease occurred, the figures being given below. The concentration was 0·4%. The presence of water appears to be essential for the disaggregation, and no disaggregation occurs in water or methyl alcohol in the absence of acid.

Time, hrs.	0	6	11	13	24	29
$\eta_{sp.}^{20°}$	0·59	0·19	0·14	0·13	0·11	0·10
Apparent mol. wt.	205,000	47,000	27,000	24,000	16,000	13,000

Disaggregation by oxalic acid. A more convenient reagent was found in a solution of crystalline oxalic acid (1 g.) in methyl alcohol (75 c.c.) and water (25 c.c.) (p_H *ca.* 1·8 from E.M.F. measurements). In preliminary experiments, a 2·5% solution of the material [fraction D (1)] was boiled in the acid methyl alcohol. At intervals, samples were removed. The product was recovered from the sample by adding excess of alkali, removing the methyl alcohol in a vacuum (with addition of water), and filtering the boiling water from the white precipitate through cloth. The filtrate in all cases was non-reducing to Fehling's solution. The precipitate was dissolved in acetone and dried over anhydrous sodium sulphate, which was then centrifuged off. The acetone solution was concentrated, and the product was precipitated and triturated with excess of light petroleum, giving a flocculent white powder. It was dried in a vacuum at 100°, and the viscosity in *m*-cresol solution (0·4%) determined as usual:*

Time, hrs.	0	10	42	142	212
$\eta_{sp.}^{20°}$ (*c*, 0·4)	1·37	0·63	0·25	0·13	0·13
Apparent mol. wt.	520,000	220,000	78,000	23,000	23,000
$[\alpha]_D^{20°}$ in chloroform	+ 211°	+ 207°	—	—	+ 209°

Investigation of the Disaggregated Material.—The methylated rice starch [fraction F (2)], before disaggregation, had $[\alpha]_D^{20°}$ + 205° in chloroform (*c* = 0·50), $\eta_{sp.}^{20°}$ 1·39 in *m*-cresol (*c* = 0·4), corresponding to an apparent molecular weight of 530,000 (Found: OMe, 42·9%). 33 G. were boiled under reflux with 360 c.c. of 4% aqueous oxalic acid solution and 1500 c.c. of methyl alcohol for 212 hours. The methyl alcohol was then removed in a vacuum and the product was precipitated from the boiling solution after addition of excess of alkali. Neither filtrate nor precipitate reduced Fehling's solution. A sample (X) was removed and purified as described above. It had the following properties: $[\alpha]_D^{19°}$ + 209° in chloroform (*c* = 0·74), $\eta_{sp.}^{20°}$ 0·13 in *m*-cresol (*c* = 0·4), corresponding to an apparent molecular weight of 23,000, copper number (Schwalbe–Braidy method) *ca.* 0·01, iodine number 2·0 (10 c.c. of N-caustic soda and 10 c.c. of N/10-iodine being used) (Found: C, 52·7; H, 7·9; OMe, 43·9; ash, 0·9; moisture, 0·6%. Calc.: C, 52·7; H, 7·8%).

The viscosity of a solution (1%) in chloroform also remained constant during 26 hours. The most marked distinction from viscous methylated starches is in the high solubility in the usual solvents, in which it dissolves very rapidly.

The bulk of the disaggregated material (25 g.) was dried in a vacuum after precipitation from boiling water, and was then dissolved in acetone and methylated with methyl sulphate and 30% caustic soda solution in the usual manner at 55°. After four methylations, the methoxyl value had become constant (OMe, 45·6%), and the viscosity had decreased to $\eta_{sp.}^{20°}$ 0·05 in *m*-cresol (*c*, 0·4). The product (isolated in 75% yield) was purified as usual and fractionally precipitated from chloroform solution by light petroleum (b. p. 60—80°), giving the following fractions, isolated as flocculent white solids:

Fraction.	OMe, %.	$\eta_{sp.}^{20°}$ (*c*, 0·4).	Apparent mol. wt.	$[\alpha]_D^{20°}$ in $CHCl_3$.	Wt., g.
1	44·4	0·26	73,000		1·9
2	45·2	0·17	40,000		1·1
3	45·9	0·13	23,000	+ 226°	3·3
4	45·1	0·12	20,000		1·7
5	45·1	0·12	20,000	+ 224	0·9
6	45·5	0·10	13,000		5·0

* These results are qualitative only, as the temperature was not strictly controlled. The kinetics of the disaggregation reaction will be considered in another paper.

Concentration of the solution remaining after precipitation of fraction 6 gave 0·5 g. of solid ($\eta_{sp.}^{20°}$ 0·03 in *m*-cresol, *c* 0·4). No syrupy degradation products were found.

Properties of fractions (3), (4), *and* (5). These fractions were so closely similar as to be indistinguishable from one another. M. p. *ca.* 130° with previous sintering (much lower than that of the original viscous methylated starch, which had m. p. *ca.* 200°). The solubilities of the disaggregated material were much greater in cold water and in organic solvents than those of the original methylated starch.

From measurements of the osmotic pressure of aqueous solutions of fractions 3—5 the molecular weight appeared to be about 15,000—20,000. We are indebted to Dr. Carter and Mr. Chambers of the University of Birmingham for determining the molecular weight of a sample of fraction (5) in chloroform. From the observed osmotic pressure, they estimate the molecular weight to be 20,500.

In an appendix to this paper, Mr. J. St. L. Philpot describes the behaviour of fraction (5) in the ultracentrifuge, the results indicating the essential homogeneity of the sample. The sedimentation constants were $2·2 \times 10^{-13}$ and $2·0 \times 10^{-13}$ at concentrations 0·15% and 0·85% respectively, in 0·2M-sodium chloride plus 0·01M-potassium dihydrogen phosphate. The equation $M_{min.} = N[(6\pi\eta s)^{3/2}/(1 - v\rho)]\sqrt{3v/4\pi}$, where $M_{min.}$ is the minimum molecular weight, η the viscosity (0·0101), s the sedimentation constant, ρ the density at 20° (0·9982), N Avogadro's constant ($6·077 \times 10^{23}$), and v the partial specific volume (0·76), gives the minimum molecular weight as 18,700 (see appendix note by J. St. L. Philpot) (Found for fraction 5: C, 53·1; H, 7·8. Calc.: C, 52·9; H, 7·8%).

Hydrolysis of the Remethylated Disaggregated Compound.—Material from fractions 3, 4, 5, and 6 (preceding table) (8·57 g.) was hydrolysed by acetic acid and hydrochloric acid as previously described. Concentration of the more fully methylated glucoses was effected by chloroform extraction of an aqueous solution. The dried syrups were boiled with 3% methyl-alcoholic hydrogen chloride for 5½ hours to form the glucosides, which were then fractionally distilled at 0·001 mm. pressure. The residue from the first fractionation was boiled with 1% methyl-alcoholic hydrogen chloride to complete the hydrolysis. The following fractions were finally obtained:

Fraction.	Wt., g.	$n_D^{16°}$.	Constants used in estimation.*	%. "Tetra."	Wt. "tetra," g.	Wt. "tri," g.	%. "Di."	Wt. "di," g.
1	0·19	1·4435	(a) 1·4435 (b) 1·4570	100	0·19	—	—	—
2	0·51	1·4563	(a) 1·4440 (b) 1·4575	11	0·05	0·46	—	—
3	0·36	1·4567	(a) 1·4450 (b) 1·4570	6	0·02	0·34	—	—
4	5·45	1·4585		—	—	5·45	—	—
5	1·00	1·4601		—	—	0·95	5	0·05
6	0·24	1·4604		—	—	0·23	5	0·01
7	0·17	1·4645		—	—	0·08	50	0·09
Residue:	0·18				0·26	7·51		0·15
	8·10							

* See note under Table II.

The analysis of fission products, expressed as a percentage of the total recovery (88%), is therefore: 2 : 3 : 4 : 6-tetramethyl methylglucoside, 3·2%; 2 : 3 : 6-trimethyl methylglucoside, 92%; dimethyl methylglucosides, 1·9%. This amount of end-group corresponds to a repeating unit of 31 *glucose residues*.

The authors thank Dr. S. R. Carter and Dr. W. T. Chambers of the University of Birmingham for undertaking osmotic-pressure measurements on samples of methylated starch, Mr. J. St. L. Philpot of Oxford University for carrying out the ultracentrifuge examination, the Colston Research Society and Imperial Chemical Industries Ltd. for grants, and the Department of Scientific and Industrial Research for a maintenance grant awarded to one of them (G. T. Y.).

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Addendum: Examination in the Ultracentrifuge. By J. St. L. Philpot (with technical assistance by E. Dodwell).

The specimen of methylated disaggregated starch sent by Professor Hirst was examined at concentrations 0·15% and 0·85% in 0·2M-sodium chloride plus 0·01M-potassium

dihydrogen phosphate in a Svedberg oil-turbine ultracentrifuge at 64,000 r.p.m. The sedimentation constants were $2{\cdot}2 \times 10^{-13}$ and $2{\cdot}0 \times 10^{-13}$ respectively. The accompanying photograph (Fig. 2) was taken by the "Diagonal Schlieren" method, 70 minutes after top speed had been reached in the 0·85% solution. There is only one component, though the curve shows slight irregularities due to convection. The homogeneity, as judged by the spreading of the boundary, seems fairly good; but quantitative tests for it are unsatisfactory with such small molecules. The sedimentation constant $2{\cdot}2 \times 10^{-13}$, when combined with the partial specific volume 0·76 and the molecular weight 20,500, gives a value 1·06 for the ratio of the frictional constants f/f_0 (compare Svedberg, *Proc. Roy. Soc.*, 1939, *B*, **127**, 4). This corresponds to a molecular axial ratio of about 0·4, or even closer to unity if there is hydration. The approximately spherical shape so indicated would explain the smallness of the effect of concentration on the sedimentation constant. Since the determination of the axial ratio is subject to very large errors, these conclusions are only provisional.

FIG. 2.

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