344. *Polysaccharides. Part XIV. The Molecular Structure of Amylose and of Amylopectin.*

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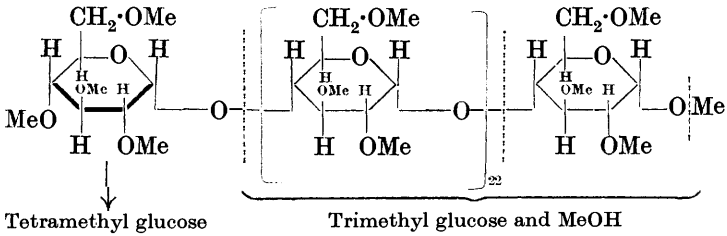
ALTHOUGH it has been generally accepted that starch consists essentially of a mixture of amylose, which dissolves readily in water, giving mobile solutions, and amylopectin, which is insoluble in cold water and gives starch pastes with hot water, the relationship between the two modifications has remained obscure. The work of Samec and his collaborators on the phosphorus content of starch preparations seemed to point to the idea that the soluble amylose portion of starch is free from phosphorus and that the insolubility and the paste-forming properties of amylopectin are traceable to the phosphorus content of the latter. These views are not, however, entirely satisfactory, since it has been demonstrated by Karrer (*Helv. Chim. Acta*, 1929, **12**, 1144; 1932, **15**, 48) that amylose may possess a distinct phosphorus content. The present experiments reveal that a soluble amylose fraction which retains the full phosphorus content of the original starch can be prepared from potato starch. The amylopectin portion, which has the same phosphorus content, differs completely in solubility and paste formation from the amylose.

Haworth, Hirst, and Webb (J., 1928, 2681) pointed out that under suitable (mild) conditions starch reacted chemically as if it consisted entirely of amylose. Further examination of the relationship between amylose and amylopectin has now shown that the chemical similarity is closer even than was anticipated. There is good reason for the belief that each component consists of chains of glucopyranose units which are linked together as in maltose, the number of glucose units in the chain being about 24 both for methylated amylose and for methylated amylopectin. It is probable, therefore, that the unmethylated substances, in the form in which they occur in starch, have a molecular magnitude little if any greater than 24 glucose units. The characteristic properties of starch, as compared with cellulose, would appear to be occasioned by the presence of α-glucosidic links which prevent the formation of long straight molecules. Instead of the thread-like macromolecules of cellulose, starch contains macromolecules which, owing to their particular conformation, possess greater facilities for aggregation and interlocking. As a working hypothesis we picture amylopectin as consisting essentially of aggregates of these interlocked macromolecules which in the presence of water undergo hydration with

the formation of a micellar structure. The micellar character of starch solutions has often been advocated and has been supported recently by the work of Karrer (*loc. cit.*) and of Staudinger and Schweitzer (*Ber.*, 1930, **63**, 2317) on the viscosity of starch solutions.

In amylose solutions, on the other hand, there is found a less interlocked and more heavily hydrated condition of the macromolecules. On this view there should exist a continuous range of products with properties intermediate between those of amylose and amylopectin. This is indeed the case. Free amylose cannot be kept as such and gradually retrogrades through intermediate stages to the amylopectin condition. The experiments now described show that a similar range of acetylated and of methylated derivatives of starch can be prepared. On the basis of this hypothesis concerning the nature of starch it is not surprising that under suitable conditions starch reacts entirely as amylose. One instance of this has already been mentioned (Haworth, Hirst, and Webb, *loc. cit.*). The capacity to undergo retrogradation appears to be connected with the chain-length of the macromolecule. Glycogen, for example, which is structurally exactly similar to starch but consists of macromolecules of shorter chain-length (Haworth and Percival, this vol., p. 2277), does not possess this property. The formulation of the methylated amylose and amylopectin is given in the expression:

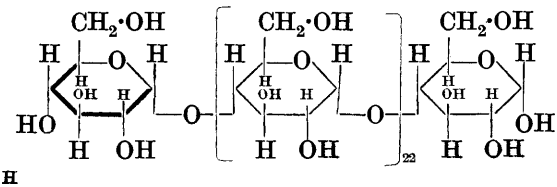


The chain-length of the macromolecule in the starch derivatives was obtained by determining the amount of tetramethyl glucose liberated on hydrolysis of the fully methylated substances, according to the method used by Haworth and Machemer for methylated cellulose (this vol., p. 2270). Since the glucose unit which gives the tetramethyl glucose must have occurred at the end of a chain, the yield of the latter substance enables the chain-length to be calculated. Control experiments by Haworth and Machemer have shown that, under the conditions adopted, tetramethyl glucose can be separated quantitatively from a large excess of 2 : 3 : 6-trimethyl glucose. The relationship between starch and maltose and the yield of 2 : 3 : 6-trimethyl glucose preclude molecular structures

other than continuous chains or large rings and the isolation of tetramethyl glucopyranose demonstrates the presence of a terminated chain and establishes the nature of one of the terminal groups. Some uncertainty still remains concerning the nature of the other terminal group. The amylose and amylopectin used in this work, their acetates, and the regenerated products obtained by deacetylation of the acetates were stable in the presence of alkali, were non-reducing towards Fehling's solution, and gave negligible iodine numbers when tested by the method of Bergmann and Machemer (*Ber.*, 1930, **63**, 316). A terminal reducing group, if present, cannot possess the full reactivity shown by this group in cellodextrins of comparable molecular weight (Haworth and Machemer, *loc. cit.*). It is not impossible that the reactivity is masked on account of the micellar character of the solutions, but the alternative view that the terminal group is of a non-reducing character, *e.g.*, a carboxyl group, is also under consideration.

The separation of amylose and amylopectin was effected by Ling and Nanji's method (J., 1923, **123**, 2666) in which a starch paste is frozen and then extracted with water. Amylose acetate and methylated amylose prepared from the extracted soluble material were identical with products previously described (Haworth, Hirst, and Webb, *loc. cit.*). Special precautions were taken during the preparation of the methylated derivative to ensure the absence of breakdown products and the failure of an attempted fractionation of this substance showed that it contained no degradation products of low molecular weight.

From amylopectin was obtained a continuous range of acetates and methylated derivatives which varied in solubility and in the viscosity of their solutions according to the method of preparation. De-acetylation of the more soluble acetates gave products resembling amylose which had partly retrograded to the amylopectin condition. The methylated derivatives were readily separable into portions differing in solubility and viscosity in solution. Hydrolysis of a highly viscous fraction and of a mobile fraction (the latter being indistinguishable from methylated amylose) gave the same yield of tetramethyl glucose (5%) as was obtained from methylated amylose. The average chain-length was for each derivative 23—24 glucose units, corresponding to a molecular weight of 5000.



Further evidence in favour of the view that the methylated derivatives hitherto referred to contained no degradation products of low molecular weight was obtained from the examination of a sample of methylated material which had been allowed to come into contact with acid for a short time during one stage of the methylation. In this case the methods of fractional extraction and precipitation which were applied to the methylated amylose and amylopectin easily separated the material into a fraction with small average chain-length (8—10 units) and a fraction which closely resembled methylated amylose and like the latter gave on hydrolysis 5% of tetramethyl glucose (average chain-length 24 units). It follows that degraded material, if it had been present in the preparations used in this work, would have been detected by the methods adopted. It is highly probable that methylation under controlled conditions is accompanied by little or no degradation of the starch macromolecule and it would appear that in potato starch both the amylose and the amylopectin components consist of macromolecules of average chain-length about 24 glucose units. Other starches and starch derivatives are being examined in order to discover whether this length of chain is a characteristic function of potato starch only or whether it is found also in starches of different origin.

EXPERIMENTAL.

Separation of Amylose and Amylopectin.—This was effected most conveniently by Ling and Nanji's method (*loc. cit.*). A 5% paste made by heating pure potato starch and H_2O for 30 mins. at 90—100° was stirred at 0° until it solidified, and left over-night at −10° to −15°. The white fibrous mass was extracted several times with H_2O at 60°, the extract concentrated at 50° under diminished press., and EtOH added. The pptd. amylose, after trituration successively with EtOH and Et_2O, was obtained as a finely divided, hard powder (yield, 17% by wt. of the starch used). The material which remained after long-continued extraction of the fibrous mass with warm H_2O was considered to be amylopectin.

Properties of Starch, Amylose, and Amylopectin.—The amylose was non-reducing towards boiling Fehling's solution. It was sol. in cold H_2O when first prepared, but became insol. after being dried at 100°. Amylose gradually lost its solubility in cold H_2O when kept for some weeks in the air or in a vac. desiccator. The solution gave a deep blue colour with I. The freshly prepared material had $[\alpha]_D^{20°}$ + 190° in H_2O (*c*, 0·32) and + 151° in 5% NaOH aq. (*c*, 0·6). The P content (estimated by Neumann's method) was 0·20% (calc. as P_2O_5). The moisture content of an air-dried specimen was 10%.

Amylopectin was insol. in cold H_2O, but gave a paste when heated with H_2O at 90°. The solid and the concentrated paste gave a purple colour with I, but on dilution the paste gave a blue colour indistinguishable from that of amylose. The amylopectin was non-reducing and had $[\alpha]_D^{20°}$ + 151° in 5% NaOH aq. (*c*, 0·9). The air-dried material contained about 10% of moisture. The P content was 0·20% (calc. as P_2O_5).

The P content of the starch used was 0·20% (calc. as P_2O_5). The starch was non-reducing and gave the characteristic blue colour with I. Comparative expts. on the enzyme hydrolysis of starch, amylopectin, and amylose failed to disclose any essential difference between the behaviour of the three substances. Under similar conditions the yield of maltose was the same for each. In these expts. both freshly prepared barley diastase and freshly prepared oats diastase were used.

Acetylated Amylose.—(a) Freshly prepared amylose was soaked for 30 mins. in AcOH containing Cl and the mixture was stirred with Ac_2O containing SO_2 for 1 hr. at 15° and for 1 hr. at 55°; a transparent mobile solution was then obtained (for details, see Haworth, Hirst, and Webb, *loc. cit.*). The product was precipitated with H_2O, washed with H_2O until free from acid, then with EtOH and Et_2O and was dried in a vac. desiccator (yield, 98% of the theo.). Amylose acetate was a crisp white powder which contained 2% by wt. of H_2O when air-dried. It charred at 173°. It did not reduce boiling Fehling's solution, gave no colour with I, was sol. in $CHCl_3$ and acetone, giving mobile solutions, and was insol. in H_2O, Et_2O, and EtOH. $[\alpha]_D^{19°}$ + 170° in $CHCl_3$ (*c*, 0·7) (Found: C, 49·9; H, 5·6; CH_3·CO, 44·7. Calc. for $C_{12}H_{16}O_8$: C, 50·0; H, 5·6; CH_3·CO, 44·8%). On removal of the acetyl groups by *N*/2-alc. NaOH a crisp white powder was obtained which was non-reducing and differed from the original amylose only in having a slightly smaller P content (Found: P_2O_5, 0·14—0·18%). The greater part of the phosphorus was retained during acetylation and subsequent deacetylation.

(b) Acetylated amylose, identical in all respects with the above product, was obtained when amylose was acetylated by Ac_2O in the presence of pyridine. The amylose (6 parts) was shaken with pyridine (16 parts) for 24 hrs. at 15° and a mixture of pyridine (40 parts) and Ac_2O (40 parts) was then added with stirring. After 4 days at 60°, the amylose dissolved, giving a transparent mobile solution which gave a flocculent white ppt. when poured into H_2O. The amylose acetate was washed and dried in the usual way.

Acetylated Amylopectin.—Freshly prepared, finely ground and sieved amylopectin reacted slowly with Ac_2O in the presence of SO_2 and Cl. Under the conditions described above, heating for several hrs. was necessary before a clear solution was obtained. With minimum quantities of catalyst, minimum temp. and time of heating, these solutions were viscous and the amylopectin acetate obtained from them had poor solubilities in $CHCl_3$ and acetone, tended to swell in these solvents before dissolution, and gave viscous solutions. It resembled closely the acetate obtained when amylopectin was treated with Ac_2O in the presence of pyridine (see below). By suitable changes in the amount of catalyst and in the time of heating, products with properties intermediate between those of amylose acetate and of the insol. amylopectin acetate could be obtained. Under appropriate conditions material indistinguishable from amylose acetate could be obtained (yield, 98—100%). All these acetates were non-reducing. The following properties are typical and refer to an acetate which gave viscous solutions. $[\alpha]_D^{19°}$ + 170° in $CHCl_3$ (*c*, 0·74) [Found: C, 49·9; H, 5·6; CH_3·CO, 44·7; P (as P_2O_5 calc. for the unacetylated product), 0·14%]. The P content varied with the time required for acetylation, drastic conditions involving loss of P.

De-acetylation of acetylated amylopectin gave products whose solubility in H_2O varied in accordance with the viscosity in solution of the acetylated derivative. Acetates freely sol. in $CHCl_3$, which gave mobile solutions, gave

products completely sol. in cold H_2O and indistinguishable from amylose. Acetates of higher viscosity and smaller solubility gave material partly sol. in H_2O and having properties intermediate between those of amylose and amylopectin, with the exception that the P content was smaller (Found : P_2O_5, 0·14%). The de-acetylation process involved no loss of P. The H_2O-sol. portions had all the properties of amylose. All these products had no action on boiling Fehling's solution.

Amylopectin, pyridine, and Ac_2O gave, after 4 days' treatment at 60—70° or shorter treatment at 100°, a stiff jelly, which after trituration with H_2O gave a crisp white solid. This was washed with H_2O, then with EtOH and Et_2O, and dried in vac. at room temp. This acetylated amylopectin was insol. in org. solvents. It showed marked swelling, without true dissolution, in $CHCl_3$ and acetone. It was non-reducing (Found : $CH_3{\cdot}CO$, 44·3; P_2O_5, calc. for deacetylated product, 0·18%).

Further treatment for 6 days at 60° with pyridine and Ac_2O failed to alter the acetate in any way. Owing to unfavourable solubility it could not be converted into the sol. variety by the action of AcOH containing Cl and SO_2.

The acetylation of starch by Haworth, Hirst, and Webb's method gave rise, according to the experimental conditions, either to viscous products similar to those derived from amylopectin or to the highly sol. non-viscous acetate obtainable from amylose.

Methylated Amylose.—The simultaneous deacetylation and methylation of triacetyl amylose was carried out by the method previously given for methylated starch (*loc. cit.*) with the exception that extra H_2O was added at the end of the reaction and only the solid material insol. in the hot aq. solution was collected. No $CHCl_3$ extract of the aq. portion was made. In this way breakdown products of low mol. wt. were automatically eliminated. The product after 5 methylations was exhaustively washed with boiling H_2O and on pptn. from $CHCl_3$ solution by light petroleum (b. p. 40—60°) it was obtained as a crisp white powder (yield, 80—90%), m. p. 143° after softening, $[\alpha]_D^{21°}$ + 207° in $CHCl_3$ (*c*, 0·4), identical with the methylated amylose previously described (*loc. cit.*). It gave a blue colour with I. Apparent mol. wt. (by Rast's method) 3000—4000 (Found : C, 52·7; H, 8·1; OMe, 44·5; P_2O_5, 0·15. Calc. for $C_9H_{16}O_5$: C, 52·9; H, 7·8; OMe, 45·6%). Attempts to separate methylated amylose into portions differing in physical properties were unsuccessful, fractional extraction of the finely powdered substance by boiling Et_2O giving invariably unchanged methylated amylose. No material of higher methoxyl content was extracted by the Et_2O.

Hydrolysis of Methylated Amylose.—Finely powdered methylated amylose (102 g.) was added slowly to fuming HCl aq. (500 c.c.) cooled in a freezing mixture, care being taken to avoid the formation of lumps. The solution was cooled to −17° and saturated with HCl. After 36 hrs. at 0° the excess of HCl was removed by aeration, the solution diluted with an equal vol. of H_2O, and the acid neutralised with $BaCO_3$. These operations were carried out at room temp. The filtered neutral solution (A) was extracted with $CHCl_3$ and the dried $CHCl_3$ solution was evaporated to a thin syrup (105 c.c.), to which light petroleum (500 c.c., b. p. 40—60°) was added with vigorous stirring. The upper layer was then decanted, the syrupy residue was diluted with $CHCl_3$ until the vol. was 105 c.c., and the treatment with light petroleum was repeated. The syrupy residue (B) is referred to below.

The combined petroleum extracts were evaporated to a syrup, which was

boiled for 9 hrs. with MeOH (105 c.c.) containing HCl (1 g.). The acid was neutralised with Ag_2CO_3 and the filtered solution was evaporated in a Claisen flask with a wide side limb to a syrup (6·7 g.), which was heated at 100°/15 mm. for several hrs. to remove all solvent. On distillation under diminished press. a first fraction (C) (5 g.), b. p. 94—95°/0·04 mm., $n_D^{16°}$ 1·4464, was obtained with bath temp. 108—109°. The next portion of the distillate (0·2 g.) had $n_D^{16°}$ 1·4570 (trimethyl methylglucoside). (C) was distilled slowly from a Widmer flask fitted with a fractionating column. The first drop had $n_D^{16°}$ 1·4445. There followed 3·3 g. (D) which distilled with bath temp. 129—130°, b. p. 90°/0·03 mm., $n_D^{16°}$ 1·4445. The next portion (E) (0·30 g.) had $n_D^{15°}$ 1·4470, and the still residue (F) weighed 1·3 g.

(D) was tetramethyl $\alpha\beta$-methylglucopyranoside (Found : C, 52·9; H, 9·0; OMe, 61·0. Calc. for $C_{11}H_{22}O_6$: C, 52·8; H, 8·9; OMe, 62·0%). A portion of it (1·3 g.) was hydrolysed with boiling 5% HCl aq. in the usual manner. The product was a cryst. solid (1·2 g.), which on recrystn. from light petroleum gave almost quantitatively tetramethyl glucopyranose as long needles, m. p. and mixed m. p. 88—89°. $[\alpha]_D^{20°}$ + 83° in H_2O (*c*, 1·02) (equilibrium val.) (Found : C, 50·6; H, 8·4; OMe, 52·8. Calc. for $C_{10}H_{20}O_6$: C, 50·8; H, 8·5; OMe, 52·5%).

The aq. solution (A) after completion of the extraction with $CHCl_3$ was evaporated to dryness at 40° under diminished press. The solid residue was extracted with boiling $CHCl_3$. The $CHCl_3$ solution was evaporated to a thin syrup, which was treated with light petroleum in the manner already described. The petroleum layers from two such treatments contained only 0·5 g. of sugars (G). The pptd. syrup crystallised over-night. The mixture of syrup and crystals was triturated with a large vol. of Et_2O and the material in the ethereal washings was combined with (G) and boiled for 7 hrs. with 1% methyl-alc. HCl (300 c.c.). The product (H) was transferred to the special Claisen flask and subjected to fractional distillation. When 4·7 g. had distilled with bath temp. 105—106°/0·02 mm. the distillation was stopped and the distillate was added to the still residue (F) in the Widmer flask.

On distillation through the column the following fractions were obtained : (a) 1·25 g., bath temp. 134—140°, press. 0·02 mm., $n_D^{16°}$ 1·4480 (Found : OMe, 58·0%); (b) 0·9 g., bath temp. 148°, press. 0·02 mm., $n_D^{11°}$ 1·4568 (Found : OMe, 53%); (c) 1·1 g., bath temp. 153—154°, press. 0·02 mm., $n_D^{11°}$ 1·4588 (time 3 hrs.). (a) and (b) together contained 1·0 g. of tetramethyl methylglucopyranoside. The total yield of tetramethyl methylglucopyranoside contained in fractions (D), (E), (a) and (b) was 4·68 g. It has been shown (Haworth and Machemer, *loc. cit.*) that the experimental loss during the hydrolysis and the separation of tetramethyl glucose from trimethyl glucose is not greater than 10% of the wt. of tetramethyl glucose. The total estimated yield of tetramethyl glucose was therefore 5% by wt. of the methylated amylose.

The trimethyl glucose portions were collected as trimethyl methylglucoside and distilled (yield, 80% of the theo.). A small amount (4 g.) of dimethyl methylglucoside was also obtained, corresponding to the deficiency in methoxyl content of the original methylated amylose.

Methylation of Amylopectin.—(a) When the insol. acetylated amylopectin obtained by the pyridine method of acetylation was submitted to simultaneous de-acetylation and methylation, the product was insol. in H_2O and in org. solvents (Found : OMe, 30—32%). Owing to the lack of solubility in acetone–H_2O further methylation proceeded slowly, at a rate similar to that shown by

ordinary starch when methylation is conducted without preliminary acetylation. The product remained insol. in $CHCl_3$.

(b) Acetylated "amylopectin" which was just sol. in acetone gave a methylated derivative (OMe, 35%) which was slightly sol. in $CHCl_3$. On continued methylation the solubility in $CHCl_3$ gradually increased. Owing to unfavourable solubility methylation proceeded more slowly than with amylose. The product after 10 treatments was freely sol. in $CHCl_3$, and gave a crisp white powder when pptd. by light petroleum from $CHCl_3$ (yield, 90—95%). This had an indefinite m. p., above 160° with previous softening. $[\alpha]_D^{20°}$ + 207° in $CHCl_3$ (Found: C, 52·4; H, 7·9; OMe, 44·7; P_2O_5, 0·14%). It was distinguishable from fully methylated amylose by reason of smaller solubility, greater viscosity in solution, higher m. p., and the feeble colour with I. The apparent mol. wt. by Rast's camphor method was about 5000.

(c) Methylation of readily sol. samples of acetylated amylopectin gave methylated derivatives (yield, 85%) with properties intermediate between those of the substances described above and those of methylated amylose. A typical prep. had m. p. 143° (with previous softening); $[\alpha]_D^{20°}$ + 202° in $CHCl_3$ (c, 0·4). The blue colour with I was less intense than that given by methylated amylose (Found: OMe, 43·5%).

Fractionation of Methylated Amylopectin.—Methylated "amylopectin" (OMe, 43·8%) was prepared by method (b) of the previous paragraph, the pptn. from $CHCl_3$ solution being omitted. The final powdered material (33 g.) was extracted with boiling Et_2O (300 c.c.) for 1 hr. The ether contained 0·6 g. of a viscous syrup (OMe, 5%), mainly adventitious impurities. The extracted amylopectin was dissolved in $CHCl_3$ (300 c.c.) and on the addition of Et_2O (3000 c.c.) with vigorous stirring a syrup was precipitated (18 g.). A further ppt. (8·6 g.) was obtained by adding light petroleum. Evaporation of the mother-liquors gave a third portion (3·4 g.). The fractions did not differ from the methylated amylopectin described above in $[\alpha]_D$ or in composition (C, H, and OMe). They differed markedly in solubility and in the viscosity of their solutions in $CHCl_3$. For instance, the three fractions gave solutions in $CHCl_3$ (1 g. in 12 c.c.) which required respectively 12·5 secs., 9 secs., and 6·5 secs. to flow between the marks on a viscometer. The mobile fraction was indistinguishable from methylated amylose. Refractionation of the viscous portion did not yield material with higher viscosity. By repetition of the above treatment with another batch of methylated amylopectin a further quantity of the non-viscous portion was collected (total, 10·2 g. from 63 g.) together with fractions of intermediate and high viscosity. The separation was not sharp and it was obvious that a continuous range of products was present.

Hydrolysis of Methylated Amylopectin (Viscous Portion).—Methylated amylopectin (10·0 g.) was hydrolysed by fuming HCl aq. at 0° under the conditions given for methylated amylose. After neutralisation of the acid ($BaCO_3$) the solution was thoroughly extracted with $CHCl_3$ (12 × 60 c.c.). No tetramethyl glucose remained in the aq. portion. This was proved by isolation of the free sugars, conversion into the methylglucosides, and fractional distillation. The $CHCl_3$ extract contained 2·27 g. of mixed tetramethyl glucose and trimethyl glucose. These were converted into the glucosides in the usual way and fractionally distilled from a Widmer flask. This gave (a) 0·56 g., b. p. 87°/0·13 mm., bath temp. 135°, $n_D^{19°}$ 1·4443 (Found: OMe, 56·4%): on hydrolysis this gave tetramethyl glucopyranose, m. p. 85°, $[\alpha]_D^{18°}$ + 84° in H_2O (equilibrium value) (yield, 70%); (b) 0·64 g., b. p. 100—105°/0·14 mm.

(bath temp. 145°), $n_D^{19°}$ 1·4540 (Found, OMe, 53%). From refractive index and OMe content this fraction contained at most 0·10 g. of tetramethyl methylglucoside. The total corrected yield (see above) of tetramethyl glucose was therefore 0·5 g. (*i.e.*, 5%). The corresponding average chain-length of the molecule is 22—24 units. The yield of 2 : 3 : 6-trimethyl methylglucoside was 85%, and a small amount of dimethyl methylglucoside (0·4 g.) was detected.

Hydrolysis of Methylated Amylopectin (Mobile Fraction).—The hydrolysis was carried out exactly as above and only the distillation figures need be recorded. From 10·2 g. of material hydrolysed, the amount extracted by $CHCl_3$ from the aq. solution was 2·59 g. After conversion into the glucosides distillation gave (a) 0·61 g., b. p. 87—90°/0·04 mm. (bath temp. 128°), $n_D^{23°}$ 1·4444 (Found : OMe, 55·9%). On hydrolysis the yield of tetramethyl glucose (m. p. 86°; $[\alpha]_D^{18°}$ + 82° in H_2O, equilibrium value) was 60%. (b) 0·78 G., b. p. 100°/0·04 mm. (bath temp. 135—140°); $n_D^{23°}$ 1·4540 (Found : OMe, 52·4%). The amount of tetramethyl methylglucoside in (a) was 0·4 g. and in (b) at most 0·05 g. From the sugars remaining in the aq. portion there was separated after formation of the glucosides a fraction (0·48 g.), b. p. 94°/0·10 mm., $n_D^{18°}$ 1·4554 (Found : OMe, 53%). This contained approximately 0·05 g. of tetramethyl methylglucoside. The total yield was 0·50 g. of tetramethyl methylglucoside, corresponding to a total corrected yield of 5·1% of tetramethyl glucose (length of chain 22—24 units). The yield of 2 : 3 : 6-trimethyl methylglucoside was 90%. 0·4 G. of dimethyl methylglucoside was shown to be present.

Examination of a Degraded Methylated Amylopectin.—No difficulty was experienced in obtaining colourless methylated products when the experimental conditions were carefully controlled, but during the methylation of a sample of amylopectin a marked colour developed during the heating at the conclusion of the fourth methylation. On this occasion the stirring during the decomposition of the residual Me_2SO_4 was inefficient and the mixture had been allowed to separate into layers. The methylation was completed with the view of testing the possibility of separating degraded material from undegraded methylated starch derivatives. The fully methylated product was a hard glass, more than 70% of which was sol. in Et_2O (Found : OMe, 43%). Systematic fractionation gave eventually two end fractions : (a) $[\alpha]_D^{20°}$ + 148° in $CHCl_3$ (*c*, 0·7) (Found : C, 51·8; H, 8·1; OMe, 41·3%), and (b) a crisp white solid, $[\alpha]_D^{20°}$ + 190° in $CHCl_3$ (*c*, 0·7) (Found : C, 52·3; H, 7·7; OMe, 43·2%). Except for the slight difference in rotation fraction (b) closely resembled methylated amylose. On hydrolysis it gave 5% of tetramethyl glucose (average chain-length 22—24 units). Fraction (a), on the other hand, gave 12% of tetramethyl glucose (average chain-length 8—10 units). It is obvious, therefore, that the separation of degraded from unchanged material can be readily effected.

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