284. *Polysaccharides. Part XX. The Molecular Size of Amylose and the Relationship between Amylose and Starch.*

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An important fact which has emerged in this series of studies on starch is that the molecular weight of starch derivatives determined by the end-group method (Haworth and Machemer, J., 1932, 2270; Hirst, Plant, and Wilkinson, *ibid.*, p., 2375) differs from the size of the starch molecular aggregates as determined by viscosity methods. This has been observed with derivatives of potato starch (Hirst, Plant, and Wilkinson, *loc. cit.*), waxy maize starch (Haworth, Hirst, and Woolgar, this vol., p. 177), and ordinary maize starch (Averill, Haworth, and Hirst, forthcoming publication); thus the molecular weight of the methylated starch prepared from any of these three sources is about 5,000, corresponding to a chain length of about 25 α-glucopyranose units. Nevertheless, acetylated and methylated derivatives having widely varying viscosities can be prepared from any one of the three sources. These results are not attributable to the break-down of the chemical molecule of the starches, inasmuch as our detailed studies of the various break-down products enable us to recognise them with ease. It has been suggested that the phosphorus content of the starches is intimately connected with the physical properties of their solutions, and throughout the whole of these researches particular attention has been paid to the phosphorus content, and in all the products under examination this factor has been examined and recorded. We find that derivatives such as the acetates and the methyl derivatives can be prepared which still retain phosphorus in combination, in many cases in amounts unimpaired. The stoicheiometric relations of the phosphorus are obscure, for starches of phosphorus contents varying from 0·2% down to 0·01% can be made to give rise to products of very varied viscosity, and it is apparent that the latter property cannot be directly correlated with the phosphorus content.

The hypothesis we have advanced in earlier papers is that the chemical molecule of starch is to be represented on a fairly simple basis as a unit comprising 25—30 α-glucopyranose residues united at positions 1 and 4 in chain-like fashion, and that the viscosity of starch products is dependent upon the degree of aggregation of these chemical molecules. Under a variety of treatments, it is possible to effect disaggregation to simpler units which give less viscous solutions. Amylopectin is, on this reasoning, considered to be a more highly aggregated state of starch than is amylose. Preparations of the latter revert to the aggregated condition of the former. It is understood that micellar aggregates of starches can be represented as bundles or assemblages of the chemical unit attached laterally by light valency forces, and the complementary hypothesis must also be advanced that the molecular unit of starch is capable also of longitudinal extension through the attachment of molecules end to end by processes of co-ordination. In either case the type of valency forces concerned must differ from those recognised in the principal valencies which unite the continuing units of C_6 with one another in a chain-like manner to form the chemical molecule.

We have been able to alter the state of molecular aggregation of starches by various treatments, of which the following is an example. Grains of potato starch were submitted to what appeared to be a process of surface etching by heating at 80° for ½ hour with about an equal weight of ethyl alcohol containing 0·5% of hydrogen chloride. The product retained its original phosphorus content, and contained also a small amount of hydrogen

chloride which was not removed by ordinary washing. Its behaviour in polarised light was similar to that of the original starch, and it gave the same blue colour with iodine. It dissolved readily in hot water giving mobile opalescent solutions, but it no longer gave a paste. After solution in boiling water, it was readily precipitated by the addition of alcohol, and this product, which now contained no hydrogen chloride, was devoid of reducing action towards Fehling's solution, gave the deep blue colour with iodine, which precipitated it from aqueous solution, and was completely soluble in cold water. Again its phosphorus content was unimpaired. The air-dried product contained 10% of water, and when this was removed or when the hydrated product was kept for a few hours, it reverted to a form insoluble in cold water and was similar in every respect to an ordinary specimen of starch such as may be precipitated from an aqueous paste by alcohol. In other words, the specimen previously soluble in cold water had now undergone reaggregation to a higher complex, and it was evident that the disaggregated specimen could not be kept for long without this change intervening.

The disaggregated starch, soluble in cold water, which we shall refer to as simplified amylose, was studied in its freshly prepared state. Acetylation in the presence of pyridine gave an acetate showing no loss of phosphorus content but a greatly increased state of aggregation. As determined viscosimetrically (Staudinger method), this had an apparent molecular weight of 21,000 (*ca.* 73 glucose units), whilst reacetylation of this acetate with chlorine and sulphur dioxide as catalyst gave a product still more viscous having now an apparent molecular weight of 35,000 (120 glucose units).* Under other conditions of acetylation, and with the use of sulphur dioxide and chlorine in traces as catalyst, the simplified amylose gave an acetate having a considerably lower viscosity, and it was evident that the process of reaggregation was slight under these conditions. Deacetylation in alcoholic potassium hydroxide in the cold regenerated what appeared to be the original simplified amylose, and it is significant that almost all the phosphorus in the original starch is retained during the preparation of the amylose, during acetylation, and during the subsequent deacetylation.

The variety of amylose acetate of low viscosity was submitted to the procedure under which deacetylation and methylation proceeded simultaneously by the agency of methyl sulphate and sodium hydroxide in aqueous acetone. This treatment gave rise to a methylated amylose having a unique interest. In common with all derivatives of polysaccharides prepared with the object of determining molecular size, the product was submitted to a careful and prolonged process of fractional purification. The final material had an optical rotation differing little from that of other specimens of methylated starch, a similar methyl content, but a greatly increased solubility, and a remarkably low viscosity in *m*-cresol. The apparent molecular weight according to Staudinger's formula was 4,500—5,000, corresponding to a chain-length of about 25 glucose units, *i.e.*, a value identical with that which we have previously found for all specimens of methylated starch of whatever viscosity or origin. The determination of the molecular weight by chemical assay of the end-group gave identically the same value, *viz.*, a molecular weight of 5,000 corresponding to 25 glucose residues. For the first time, therefore, we have been able to prepare a derivative of starch undegraded in the chemical sense and having the physical properties which would be expected from a simplified chemical unit of the same chain-length as that which has been gravimetrically determined.

The methylated and acetylated starches previously prepared in these laboratories, and of which the properties have been described in earlier papers, have now been examined by viscosity methods. They show apparent molecular weights some 3—4 times the normal value, although their molecular size as determined by chemical methods is again about 25 glucose units. It would therefore appear that pre-treatment of starch in the manner outlined in this communication has resulted in its disaggregation, yielding a form which

* It will, of course, be understood that no special accuracy is claimed for the high molecular weight, since no information is available concerning the applicability of the Staudinger equations to polymerides of this type. The general conclusion that highly polymerised substances are under examination is, however, justifiable, and the relative values recorded are highly significant.

approximates to the chemical molecule of starch, and other results bearing on this problem will be communicated later. The main results of this and previous investigations are given in tabular form at the end of the paper.

Experimental.

Viscosities, $\eta_{sp.}$, were determined in *m*-cresol solution (0·02 g. in 5 c.c.) at 20°.

Preparation of Amylose from Potato Starch.—Potato starch (50 g., air-dried, containing 17% of moisture and 0·2% of phosphorus, calculated as P_2O_5) was ground in a mortar with a little absolute alcohol and then boiled for 30 mins. with 0·5% ethyl-alcoholic hydrogen chloride (50 c.c.). The product was filtered off, and washed with alcohol and finally with ether. The fine granular powder so obtained appeared under the microscope to be identical with the original starch, except that slight cracks were visible across the granules. In polarised light its appearance was the same as that of potato starch. With iodine it gave a deep blue colour. No loss of phosphorus had taken place (Found: P_2O_5, 0·2%). The substance was insoluble in cold water but dissolved readily in hot water, giving mobile opalescent solutions but no pastes. It retained some hydrogen chloride (equivalent to 4 c.c. *N*/10-acid per 10 g. of dry material) which could not be removed by ordinary washing. In the following way, however, this intermediate product was transformed into acid-free amylose completely soluble in cold water. The acid-treated starch (40 g.) was dissolved in boiling water (500 c.c.), and alcohol (2 l.) added. The precipitate was allowed to settle, the supernatant liquid was decanted off, the solid washed with alcohol and ether, and kept in a desiccator. Amylose so obtained was a fine white powder having the properties described on p. 1202; when freshly prepared, it had $[\alpha]_{5780}^{21°}$ + 197° in water (*c*, 1·0), iodine number 0·9, phosphorus content 0·2% (calc. as P_2O_5). When a 2% aqueous solution of freshly prepared amylose was submitted to electrodialysis, flocculation took place after a few hours and about half the starch remained in the clear supernatant solution. The soluble starch was isolated and had P, 0·15%. The insoluble material had a considerably higher phosphorus content (0·24%).

Acetylation of Amylose.—(a) *With pyridine and acetic anhydride.* Freshly prepared amylose (48 g.) was mixed with pyridine (130 c.c. containing 20 c.c. of water) and shaken at room temperature for 24 hours. Pyridine (320 g.) and acetic anhydride (320 g.) were then added to the viscid mass, and the mixture was heated at 50° for 2 days. The clear colourless solution was poured into water (10 vols.), and the acetylated starch was separated, washed, and dried in the usual manner. It was non-reducing towards Fehling's solution and gave no colour with iodine (yield, 90%); $[\alpha]_{5780}^{18°}$ + 177° in chloroform (*c*, 0·9) [Found: P_2O_5, 0·2% (calc. on weight of starch contained in the acetate); $CH_3{\cdot}CO$, 40·5%]. One further treatment of this acetate (25 g.) with pyridine (125 c.c.) and acetic anhydride (160 c.c.) at 50° for 12 hours was required to complete the acetylation. The acetylated starch then had $[\alpha]_{5780}^{20°}$ + 177° in chloroform (*c*, 0·8) (Found: $CH_3{\cdot}CO$, 44·3. Calc. for $C_{12}H_{16}O_8$: $CH_3{\cdot}CO$, 44·8%). It was non-reducing, and was similar in appearance and solubilities to the partially acetylated material. There was no loss of phosphorus during the acetylation. The apparent molecular weight of the acetate (by viscosity, in *m*-cresol) was 21,000. A sample of this acetate was reacetylated at 50°, with chlorine and sulphur dioxide as catalysts (see below), in the hope that the product would give less viscous solutions. The acetate thus obtained differed little from the starting material but, contrary to expectation, was slightly less soluble in acetone and in chloroform, and its solutions in *m*-cresol were distinctly more viscous (apparent M.W. 35,000).

(b) *With sulphur dioxide and chlorine as catalysts.* Freshly prepared amylose (50 g.) was soaked for 30 mins. in glacial acetic acid (300 g.) through which a gentle stream of chlorine had been bubbled for 5 mins. The mixture was stirred with acetic anhydride (500 g.) containing an equivalent quantity of sulphur dioxide. After 1 hour at room temperature a clear solution was obtained, and the acetylation was completed by heating the solution at 50° for 3 hours. The starch acetate was precipitated, washed, and dried in the usual manner. It was a non-reducing (Fehling's solution), crisp, white powder, which gave no colour with iodine and was soluble in chloroform and in acetone (yield, almost quantitative); $[\alpha]_{5780}^{18°}$ + 179° in chloroform (*c*, 0·5); apparent molecular weight (from viscosity in *m*-cresol) 12,000 [Found: P_2O_5, 0·17 (calc. on weight of free starch); $CH_3{\cdot}CO$, 44·7%; iodine number, 1·0]. A sample of this acetate (3 g.) was digested with *N*/2-alcoholic potassium hydroxide (75 c.c.) at room temperature for 30 mins. The insoluble material was washed with alcohol, and dissolved in water (50 c.c.). The regenerated starch, which was precipitated from the aqueous solution by alcohol, was a non-reducing white powder which appeared to differ from the original amylose only in its slightly smaller

phosphorus content (Found : P_2O_5, 0·15%). A similar product, but one not so readily soluble in cold water, was obtained from the acetate prepared by the pyridine method (Found : P_2O_5, 0·19%). Almost all the phosphorus in the original starch is retained during preparation of the starch, acetylation, and subsequent deacetylation.

Methylated Amylose.—Amylose acetate (15 g., prepared by method *b*) was simultaneously deacetylated and methylated in the usual manner by methyl sulphate (180 c.c.) and 30% aqueous sodium hydroxide (500 c.c.) at 55° in the presence of acetone (250 c.c.). Methylated amylose separated from boiling water as a pale yellow solid (Found : OMe, 39%). Five methylations were necessary to raise the methoxyl content to 44% (yield, 70%). Only material which separated from boiling water was collected, and in order to avoid possible contamination with break-down products, no attempt was made to recover any partly methylated starch which remained in the aqueous solution. By this procedure, 30 g. of methylated amylose were collected, and were purified by solution in chloroform, filtration, evaporation of the solvent, and extraction of the solid several times with boiling ether. The methylated amylose was then a colourless glass, $[\alpha]_D^{22°}$ + 215° in chloroform (*c*, 0·5) (Found : OMe, 44·6%).

Fractionation of Methylated Amylose.—Methylated amylose (25 g.) was dissolved in chloroform (60 c.c.), and ether added slowly with stirring. The syrupy precipitate which formed when 800 c.c. ether had been added was dissolved in chloroform, and on removal of the solvent, fraction I was obtained as a colourless glass (5·7 g.). Light petroleum was then added to the mother-liquor of fraction I, giving fraction II (14·7 g.) (Found : C, 52·7; H, 7·7; OMe, 44·5. Calc. for $C_9H_{16}O_5$: C, 52·9; H, 7·8, OMe, 45·6%) which was worked up as before. Evaporation of the solution then gave a pale yellow glass (4·6 g.) which was dissolved in a small volume of chloroform and reprecipitated with light petroleum. This gave a colourless glass (fraction III, 3·2 g.). The properties of the fractions are summarised in the following table.

Fraction.	Weight, g.	$[\alpha]_D^{20°}$ in $CHCl_3$.	OMe, %.	$\eta_{sp.}$.	Apparent M.W.*
I	5·7	+ 214°	44·2	0·109	5,000
II	14·7	+ 216	44·5	0·088	4,500
III	3·2	+ 214	44·0	0·08	4,000

* By Staudinger's formula.

Hydrolysis of Methylated Amylose.—Methylated amylose (22 g.) was boiled with 1·2% methyl-alcoholic hydrogen chloride (900 c.c.) until the rotation was constant (5 hours). After neutralisation of the acid with silver carbonate, the solution was concentrated to a syrup (22 g.), which was dissolved in ether to remove inorganic impurities. After removal of the solvent, the mixture of methylglucosides (A) was fractionally distilled into a Widmer flask until 5 g. had been collected. On distillation † through the column the 5 g. gave (*a*) 0·75 g., b. p. *ca.* 110°/0·03 mm., $n_D^{16°}$ 1·4440; (*b*) 0·5 g., b. p. *ca.* 120°/0·03 mm., $n_D^{16°}$ 1·4560. A further 5 g. of (A) were then distilled, and mixed with the material remaining in the Widmer flask. On distillation through the column, this gave : (*c*) 0·25 g., b. p. *ca.* 115°/0·04 mm., $n_D^{16°}$ 1·4485; (*d*) 4·9 g., b. p. *ca.* 120°/0·03 mm., $n_D^{16°}$ 1·4570. Another 5 g. of (A) were then distilled into the Widmer flask as before, and on redistillation gave a main fraction (*e*) 5 g., b. p. *ca.* 120°/0·03 mm., $n_D^{16°}$ 1·4570.

Fraction (*a*) was pure tetramethyl methylglucopyranoside (Found : OMe, 60·4. Calc. : 62·0%), and on hydrolysis by boiling 7% aqueous hydrochloric acid gave crystalline tetramethyl glucopyranose in good yield, m. p. (and mixed m. p. with an authentic specimen) 89°, $[\alpha]_D^{22°}$ + 83·5°, equilibrium value in water (*c*, 0·9). Fraction (*b*) contained approx. 10% of tetramethyl methylglucoside, *i.e.*, 0·05 g. Fraction (*c*) contained 66% of tetramethyl methylglucoside, *i.e.*, 0·15 g. [The compositions of (*b*) and (*c*) were estimated by comparison of their refractive indices with those of standard mixtures of tetramethyl and trimethyl methylglucoside.] The total yield of tetramethyl methylglucoside was therefore 0·95 g., which requires 10% correction to allow for experimental losses (compare Haworth and Machemer, J., 1932, 2270). This brings the corrected yield of tetramethyl methylglucoside from 22 g. of methylated amylose to 1·05 g., corresponding to a chain length of 26 units.

The trimethyl methylglucoside ($[\alpha]_D^{20°}$ + 36° in water, *c*, 1·0°. Found : OMe, 51·0. Calc. : 52·5%) obtained during the fractional distillation partly crystallised when kept. The crystalline portion was separated and recrystallised from light petroleum, giving 2 : 3 : 6-trimethyl β-methylglucopyranoside, long needles, m. p. 58°, $[\alpha]_D^{20°}$ — 32·7° in water (*c*, 1·0). The liquid portion of the trimethyl methylglucoside, on hydrolysis by 7% aqueous hydrochloric acid at

† The temperatures given are bath temperatures.

100°, gave crystalline 2 : 3 : 6-trimethyl glucose, m. p. 115°; $[\alpha]_D^{20°}$ + 71°, equilibrium value in water (*c*, 0·5). When all the trimethyl methylglucoside had been distilled from (A), there remained a still residue from which a viscid syrup (mainly dimethyl methylglucoside) distilled forward in small amount. The final still residue weighed less than 1 g.

Deacetylation and Methylation of Starch Acetate prepared from Amylose by Acetylation with Pyridine and Acetic Anhydride.—Serious difficulties arose when the simultaneous deacetylation and methylation of the acetate prepared with pyridine as catalyst were carried out by the method described above. The condition of the acetate was such that deacetylation took place very rapidly, and the starch so liberated was thrown out of solution, took no further part in the reaction, and was lost. The following method was more successful. Amylose acetate (20 g.) was digested at 15° for 30 mins. with 470 c.c. of *N*/2-alcoholic potassium hydroxide. The alkali was neutralised with acetic acid, and the solid filtered off and dissolved in hot water (150 c.c.). Addition of alcohol (400 c.c.) to the aqueous solution precipitated the free amylose, which was at once methylated at 60° by methyl sulphate (240 c.c.) and 40% aqueous potassium hydroxide (800 c.c.), the reagents being added in the usual manner. The product (yield 85%), which had OMe 29%, was then methylated four times in succession. The methoxyl content was then 38%, and this rose only to 41·2% after a further 8 methylations with a large excess of reagents. This resistance to methylation is similar to that shown by amylopectin (Hirst, Plant, and Wilkinson, *loc. cit.*), and the resemblance is again exemplified by the high viscosity of solutions of a purified sample of this methylated derivative. The methylated starch was dissolved in chloroform and fractionally precipitated by ether. The main fraction was a pale yellow (nearly colourless) glass, $[\alpha]_D^{20°}$ + 207° in chloroform (*c*, 0·5); $\eta_{sp.}$ 0·37; apparent M.W. 18,000 (Found : C, 51·7; H, 7·6; OMe, 41·7%).

The main fraction was then dissolved in methyl iodide and methylated twice by Purdie's method. The product (yield quantitative) had $[\alpha]_D^{20°}$ + 205° in chloroform (Found : OMe, 41·5%). Its appearance remained unaltered, but it was now rather more easily soluble in chloroform, and in *m*-cresol its viscosity was considerably less ($\eta_{sp.}$ 0·235; apparent M.W. 12,000).

Samples of starch acetate and the corresponding methylated derivative were prepared by Haworth, Hirst, and Webb's method (J., 1928, 2681), and their viscosities in *m*-cresol were determined for comparison with the above figures. The results are collected in the following table.

No.	Starting material.	Derivative.	Apparent M.W. by viscosity.	Apparent M.W. by end-group method.
1.	Amylose (prepared by method on p. 1203.	Acetate (SO_2 and Cl_2 as catalysts).	12,000	—
2.	,, ,,	Methyl derivative from acetate (1).	4,000—5,000	5,000
3.	,, ,,	Acetate (pyridine method).	21,000	—
4.	,, ,,	Acetate [reacetylation of (3) using SO_2 and Cl_2 as catalyst].	34,000	—
5.	,, ,,	Methyl derivative [prepared from acetate (3)].	18,000	—
6.	,, ,,	Methyl derivative [methylation of (5) by Purdie's method].	12,000	—
7.	Potato starch.	Acetate (SO_2 and Cl_2 as catalysts).	35,000	—
8.	,, ,,	Methyl derivative [from acetate (7)].	20,000	5,000

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