# 313. *Polysaccharides. Part XXII. Constitution and Molecular Structure of α-Amylodextrin.*

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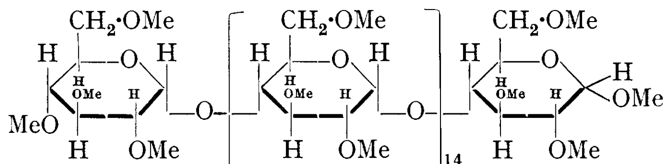
IN the present work we have studied the properties of an α-amylodextrin derived by the break-down of potato starch by means of barley amylase. This product represents an arrested stage in the scission of starch to maltose or glucose. It is composed of α-glucopyranose units united in a chain of about two-thirds the length of the chemical molecule of starch itself. The limiting size of the chemical molecule of the methylated amylodextrin, determined by the gravimetric assay of tetramethyl glucose as the end-group, was 16—17 glucopyranose units.



The α-amylodextrin was prepared from the simplified amylose already described (Baird, Haworth, and Hirst, this vol., p. 1201) and was obtained as a white powder, soluble in cold water when freshly prepared, but soluble only in hot water after being kept and dried (solutions so obtained do not deposit the dextrin on cooling). Iodine gave a red-violet colour with this dextrin, and this is just as characteristic of this product as is the blue colour with starch and the red colour with glycogen. It showed some resemblance to starch and to glycogen in respect of its optical rotation. Acetylation of this substance in the presence of chlorine and sulphur dioxide as catalysts gave an amylodextrin acetate which was soluble in chloroform or acetone and showed an iodine number corresponding to that of the original α-amylodextrin. Its viscosity calculated on the basis of the Staudinger formula corresponded to an apparent molecular weight of 12,000, or about 40 glucose units. On the other hand, acetylation of the α-amylodextrin in the presence of pyridine gave two dextrin acetates. One of these, also soluble in acetone, was similar in all respects to that just described, except that it showed by the viscosity method an apparent molecular weight of 22,000, or 80 glucose units. Viscosity measurements, therefore, appeared to indicate that the latter dextrin acetate had twice the molecular complexity of the former. In addition to the foregoing, there was also formed by the pyridine method a dextrin acetate, representing about half the total product, which was insoluble in acetone, had a smaller iodine number, and in its properties had affinities with a derivative of amylopectin.

It was evident, therefore, that under different methods of acetylation, dextrin acetates of different degrees of aggregation could be prepared, and it was apparent that in the presence of pyridine re-aggregation of a simpler unit seemed to be promoted. Methylation of the dextrin acetate prepared with the aid of sulphur dioxide and chlorine as catalysts gave a methylated amylodextrin which was soluble in chloroform or acetone and showed by the viscosity method an apparent molecular weight of 8,000 (about 40 glucose units). By this method of comparison it would appear that no disaggregation of the physical unit had occurred during the methylation process. The gravimetric assay of the end-group of this specimen of methylated amylodextrin gave a yield of 7·1% of tetramethyl glucose, equivalent to a chain length of 16—17 glucose units or a molecular weight of about 3,500. This result is fully in accordance with other observations on methylated starches which have undergone molecular aggregation. It has recently been shown (Baird, Haworth, and Hirst, *loc. cit.*) to be possible to prepare from starch a methylated amylose the molecular weight of which, determined viscosimetrically, is identical with the value obtained by assay of the end-group. It seems to be clear that the shortened chain of α-amylodextrin retains the capacity to undergo molecular aggregation just as does amylose itself. It will be our endeavour to trace the structural factors which are responsible for the conferment of this property both on amylose and on amylodextrin. Inasmuch as the capacity to undergo

molecular aggregation does not appear to be shared by glycogen to anything like the same extent, it may be possible to reveal the reason for this difference of behaviour of polysaccharides which are structurally very closely related although they differ in their phosphorus content. In this connexion, it may be pointed out that the α-amylodextrin used in this work was nitrogen-free, but its phosphorus content was unexpectedly high, being more than twice that of potato starch. The phosphorus was retained in large measure during acetylation and methylation, and appeared to be chemically combined. Nevertheless, the dextrin gave no pastes and there was no correlation between phosphorus content and viscosity of derivatives, the viscous and non-viscous varieties of the acetate having similar phosphorus contents. Furthermore, we have not been able to trace any direct connexion between phosphorus content and capacity to undergo aggregation. It may be possible also to determine whether the conformation of the chain is responsible for this property, or whether it is due to the spiral formation of chains and the entanglement of adjoining chains. The extent to which molecular aggregation is brought about by the union longitudinally as well as laterally of several chains, perhaps by a process of co-ordination, will also be studied. Determinations of molecular weight by osmotic pressure methods are being undertaken, but the values so obtained, like those given by the Svedberg ultra-centrifugal method, may represent the particle weights (aggregated molecules) rather than true molecular weights. The direct chemical assay of the end-group of methylated derivatives may be said to give the limiting size of the unit of the chemical molecule.

It is evident that the degree of aggregation of specimens of amylodextrin acetate is reflected also in their chemical behaviour. The acetone-soluble specimen of the dextrin acetate prepared by the pyridine method of acetylation displayed an anomalous behaviour during methylation with methyl sulphate. During the de-acetylation and methylation of the acetate prepared with sulphur dioxide and chlorine as catalysts, the rotation of the methylated product in chloroform rose regularly ($[\alpha]_{5780}$ 149° ⟶ 222°) as the methoxyl content increased. Contrary to this experience, the optical rotation in chloroform of the product from acetate prepared by the pyridine method increased slightly ($[\alpha]_{5780}$ 206° ⟶ 213°) during the early stages of the methylation and then diminished progressively with rise of methoxyl content until the latter reached 40·5%. Thereafter, the rotation rose gradually to the maximum value (222°) which was reached when the methylation was complete. It is probable that this phenomenon is connected with the progressive disaggregation during methylation, and this process of disaggregation appears to be much more marked when methyl iodide and silver oxide are used in place of methyl sulphate. These phenomena will be studied further in order to confirm this interpretation.

In this work we have used viscosity methods with a view to the qualitative differentiation of the derivatives of polysaccharides, inasmuch as the exact quantitative relationship, particularly in the starch series, is not yet clear. For instance, the molecular weight, calculated by the viscosity method, in the case of methylated inulin is only about half that determined by the end-group method. In the case of cellulose derivatives, Staudinger has reported that a methylated cellulose shows a molecular-weight value by the viscosity method which is high in comparison with that of the acetate from which it was prepared. These are anomalies which doubtless will receive an explanation in the near future. Our studies of starch and starch dextrins suggest the view that in native cellulose there may be molecular aggregation, and that the fundamental chemical molecule is probably not much greater than 200 glucose units in length. The reagents employed in preparing derivatives from native cellulose doubtless effect considerable disaggregation, a process which must be distinguished from that of chemical break-down of the molecule.

## Experimental.

Values of $\eta_{sp.}$ refer to 0·02 g. of substance in 5 c.c. of *m*-cresol at 20°.

*Preparation of α-Amylodextrin.*—The barley amylase required was obtained as follows. Ungerminated barley grains (100 g.) were ground to a fine meal and steeped in 20% aqueous alcohol for 6 hours with occasional stirring. The mixture was filtered through muslin and refiltered through paper. The filtrate was poured into 85% alcohol, and after some time the coagulated precipitate was separated on the centrifuge and dissolved in water (40 c.c.). The solution was

heated at 55° for 15 minutes to destroy maltase, and then used for the hydrolysis of 50 g. of starch. An alternative procedure was to heat the 20% alcoholic solution at 55° for 15 minutes and use it directly for the hydrolysis. The action of the enzyme seemed to be unaltered, and the troublesome precipitation was avoided.

Numerous experiments under a variety of conditions were made with pastes made from ordinary potato starch, but the results were variable and the method was unsatisfactory for routine production of dextrin. Consistent results were obtained when use was made of soluble amylose prepared from potato starch by the action of ethyl-alcoholic hydrogen chloride (Baird, Haworth, and Hirst, *loc. cit.*). This freshly prepared amylose is non-reducing and readily gives 10% aqueous solutions which are limpid and mobile. Potato starch (100 g.) was suspended in absolute alcohol (150 c.c.), 5% ethyl-alcoholic hydrogen chloride added (10 c.c.), and the mixture boiled for 30 minutes. The starch was filtered off, washed twice with boiling absolute alcohol, then with ether, and dissolved in hot water (1 l.). The aqueous solution was adjusted to $p_H$ 6·0 by addition of $N/10$-sodium hydroxide. The temperature was then raised to 55°, the appropriate amount (see above) of barley extract added, and the mixture kept for 17 hours. The solution then showed strong reducing power (equivalent to conversion of 60% of the starch into maltose) and gave a reddish-violet colour with iodine; it was diluted with three times its volume of 85% alcohol to precipitate the dextrin, which was coagulated by shaking. A small amount of dextrin (about 5 g.) remained in solution, but on isolation it was found to be much contaminated with strongly reducing substances. The dextrin was then digested with boiling 85% alcohol for 1 hour, and powdered in a mortar under absolute alcohol. Further purification was effected by reprecipitation by alcohol from a 25% aqueous solution.

Attempts were made to fractionate the dextrin by precipitation of an aqueous solution with alcohol, but all the fractions obtained had properties identical with those described below. The α-amylodextrin (yield 40 g.) was a white powder, having the solubility properties described on p. 1299. Its highly concentrated aqueous solutions were viscous but showed no tendency to form a gel. The dextrin gave a reddish-violet colour with iodine, the colour being destroyed above 60°. The iodine number was 1·8—2·0; $[\alpha]_D^{20°}$ + 200° in water ($c$, 1·0); + 144° in 5% aqueous sodium hydroxide ($c$, 0·5) (Found: $P_2O_5$, 0·48%). Solutions of the dextrin in water are not acted upon by barley amylase: no reducing power is developed, no change is observable in the colour with iodine, and the dextrin can be recovered unaltered.

*Acetylation of α-Amylodextrin.*—(a) *By pyridine and acetic anhydride.* The dextrin (30 g.) was soaked for 12 hours with pyridine (120 c.c.). More pyridine (120 c.c.) and acetic anhydride (180 c.c.) were added, and the mixture kept for 4 hours at 15° with frequent stirring. The clear colourless solution was then poured into water, and the precipitated acetate was separated, washed, and dried in the usual way; yield, 40 g. of a crisp white powder. This acetate became sticky on treatment with alcohol but did not dissolve. It was only partly soluble in acetone, but dissolved almost completely in chloroform containing 10% of alcohol, in which it had $[\alpha]_{5780}^{20°}$ + 170° (Found: $CH_3{\cdot}CO$, 45·0. Calc.: $CH_3{\cdot}CO$, 44·8%). About 50% of the acetate was insoluble in boiling acetone. The insoluble portion was fully acetylated (Found: $CH_3{\cdot}CO$, 44·3; $P_2O_5$, 0·22%), but was not sufficiently soluble in *m*-cresol to permit measurements of viscosity; $[\alpha]_{5780}^{20°}$ + 142° in glacial acetic acid ($c$, 0·8); iodine number 0·6.

The portion of the dextrin acetate soluble in acetone was fractionated by precipitation from acetone solution by water. The fractions obtained were, however, indistinguishable, and each had $[\alpha]_{5780}^{19°}$ + 172° in chloroform ($c$, 0·5); + 150° in glacial acetic acid ($c$, 1·0) (Found: $CH_3{\cdot}CO$, 45·0; $P_2O_5$, 0·18%; iodine number 1·2). The acetone-soluble acetate showed $\eta_{sp.}$ 0·306, corresponding with an apparent M.W. of 22,000 (about 80 glucose units).

(b) *By acetic anhydride in presence of sulphur dioxide and chlorine.* The dextrin (70 g.) was suspended in glacial acetic acid (450 c.c.) containing a little chlorine. After $\frac{1}{2}$ hour, acetic anhydride (750 c.c.) containing an amount of sulphur dioxide equivalent to the chlorine was added with stirring. After 2 hours at 50° the dextrin dissolved, giving a clear solution, which was poured into water. The acetate (yield, almost quantitative) was collected in the usual way, and had properties identical with those of the acetate described under (a), with the important exception that it was readily soluble in acetone and in chloroform and that it had $\eta_{sp.}$ 0·165, corresponding with an apparent M.W. of 12,000 (about 40 glucose units) (Found: $P_2O_5$, 0·22%). All samples of the acetylated dextrin gave on deacetylation a dextrin indistinguishable from the original α-amylodextrin, and the regenerated dextrin gave the characteristic red-violet colour with iodine.

*Methylated α-Amylodextrin.*—(a) *From acetate prepared with chlorine and sulphur dioxide as catalysts.* The acetate (20 g.), dissolved in acetone (100 c.c.), was deacetylated and methylated

at 55° by the gradual addition of methyl sulphate (120 c.c.) and 30% aqueous sodium hydroxide (320 c.c.). At the conclusion of the reaction (2 hours), boiling water was added to precipitate the product. After the first methylation, some of the product remained in solution. Accordingly, after neutralisation the solution was evaporated to dryness in the presence of barium carbonate. The mixture of solid and syrup was dissolved in the minimum amount of water and treated as above with methyl sulphate and alkali. In subsequent methylations only the product precipitated by boiling water was collected. Introduction of methyl groups proceeded regularly but sluggishly, and at least 12 methylations were necessary to raise the methoxyl content above 44%. During the methylation the rotation of the product in chloroform rose continuously with the methoxyl content :

| No. of methylations | 3 | 6 | 9 | 12 |
|---|---|---|---|---|
| $[\alpha]_{5780}^{20°}$ in $CHCl_3$ | 149° | 189° | 216° | 222° |
| OMe, % | 28 | 39 | 43 | 45 |

The final product was obtained as a crisp, white froth by evaporation of its solution in chloroform. It was exceedingly troublesome to handle owing to the ease with which its small particles became electrified by friction. It was soluble in chloroform and acetone, insoluble in ether and in hot water, and gave no colour with iodine. It was dissolved in chloroform and separated into fractions by careful addition of light petroleum. No differences between the fractions could be detected, and each showed $[\alpha]_{5780}^{21°}$ + 222° in chloroform (*c*, 0·5) and had $\eta_{sp.}$ 0·17, corresponding with an apparent M.W. 8,000 (about 40 glucose units) (Found : C, 52·7; H, 7·6; OMe, 45·2; $P_2O_5$, 0·19. $C_9H_{16}O_5$ requires C, 52·9; H, 7·9; OMe, 45·5%).

(b) *From acetate prepared with pyridine as catalyst.* The acetate used was the acetone-soluble material described above (the deacetylation and methylation of the acetate insoluble in acetone were not investigated). In this case also methylation proceeded slowly with the additional complication that the rotation of the product was no guide to its methoxyl content (see above). After two treatments with methyl sulphate and alkali, the product had $[\alpha]_{5780}^{19°}$ +206° in chloroform (*c*, 1·0) and OMe, 36·7%. After six methylations the figures were + 213° and 39·3% respectively. After two further methylations the rotation fell to 203° and the methoxyl content rose to 40·5%. Thereafter the rotation rose gradually with the methoxyl content until the final product having $[\alpha]_{5780}^{19°}$ + 222° was obtained (Found : OMe, 45·7%). A still more remarkable rotational change was observed when the methylation procedure was varied slightly. A portion of the material after six methylations ($[\alpha]_{5780}$ + 213°; OMe, 39·3%) was dissolved in methyl iodide and the boiling solution was treated with silver oxide in the usual way. The methoxyl content remained unaltered after this treatment, but the rotation fell by 22° ($[\alpha]_{5780}^{21°}$ + 191° in chloroform). Methylation by methyl sulphate and alkali was then resumed, whereupon the rotation increased steadily along with the methoxyl content, until after the fifteenth methylation the product had $[\alpha]_{5780}^{20°}$ + 222° in chloroform (*c*, 0·5) (Found : OMe, 45·7%). This sample of methylated amylodextrin was indistinguishable from the material described in the previous section : even its viscosity was closely similar ($\eta_{sp.}$ 0·13, whence apparent M.W. 6,500).

*Hydrolysis of Methylated Amylodextrin.*—The finely powdered methylated dextrin (19·3 g., prepared from the acetate obtained by use of chlorine and sulphur dioxide) was added slowly to hydrochloric acid (110 c.c., saturated with hydrogen chloride at 0°). The clear solution was kept at 0° for 18 hours, the excess hydrogen chloride removed by aeration at 15°, and the solution neutralised with barium carbonate. The filtered solution and washings (A; 300 c.c. in all) were extracted with chloroform, and the solid residue was twice extracted with boiling chloroform. The united chloroform extracts left on evaporation a syrup, which was boiled for 7 hours in 1% methyl-alcoholic hydrogen chloride. After neutralisation of the solution by silver carbonate and removal of the solvent, there remained a non-reducing syrup (B) (13·40 g.), which was slowly distilled into a special flask fitted with a fractionating column. After 6·66 g. (C) had been collected, the refractive index of the distillate had risen to $n_D^{21°}$ 1·4565, indication being thus afforded that the whole of the tetramethyl methylglucoside had distilled over. On distillation of (C) through the column, the following fractions were obtained : (*a*) 1·12 g., b. p. 90°/0·02 mm. (bath temp. 130°), $n_D^{20°}$ 1·4450 (Found : OMe, 59·0%); (*b*) 1·53 g. (bath temp. 145°/0·03 mm.), $n_D^{20°}$ 1·4538 (Found : OMe, 54·5%); (*c*) 3·46 g. (bath temp. 150°/0·02 mm.), $n_D^{19°}$ 1·4561 (Found : OMe, 51·9%). The residue in the distillation flask had $n_D^{19°}$ 1·4572 and weighed 0·5 g. The remainder of (B) was trimethyl methylglucoside, $n_D^{21°}$ 1·4556.

The aqueous solution (A) was evaporated to dryness, and the organic material extracted with boiling chloroform and converted into the methylglucoside by boiling methyl-alcoholic hydrogen

chloride (yield, 7·09 g.). On distillation, this gave 4·1 g. of trimethyl methylglucoside, bath temp. 120°/0·02 mm., $n_D^{21°}$ 1·4556. The still residue (2·8 g.) was a brown glass which did not distil at bath temp. 190°/0·02 mm. It was mainly imperfectly hydrolysed material.

Fraction (*a*) contained 85% of tetramethyl methylglucoside (indicated both by refractive index and by the methoxyl content), *i.e.*, 0·95 g. Fraction (*b*) contained about 22% of the same glucoside, *i.e.*, 0·34 g., the total yield of which was therefore 1·29 g. from 19·3 g. of methylated dextrin. After correction by addition of 10% to allow for experimental losses this amounts to a yield of 7·5%, corresponding to a chain-length of 16—17 glucose units (M.W. *ca.* 3,500).

The identity of the tetramethyl methylglucoside was established by hydrolysis of fraction (*a*) with boiling 6% aqueous hydrochloric acid. This gave in excellent yield tetramethyl glucopyranose, m. p. 87° alone or when mixed with an authentic sample of the same m. p. The trimethyl methylglucoside gave on hydrolysis crystalline 2 : 3 : 6-trimethyl glucose, m. p. 115° alone or when mixed with an authentic sample of the same m. p.

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