## 288. *Polysaccharides. Part XXI. The Constitution and Chain-length of Some Starch Dextrins.*

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The present is one of several investigations on starch dextrins which we hope to communicate. We have studied the graded break-down products of starch because we wished to compare the chain-lengths as found by the gravimetric assay of the end-group (Haworth and Machemer, J., 1932, 2270) and by the viscosity method on the basis of the Staudinger formula. It was important to know also whether the molecules of shorter chain retained the property of undergoing molecular aggregation which is so marked a feature of starch. Moreover, we were concerned to find whether the progressive break-down of starch proceeded regularly, in conformity with the view that its molecule is a continuous but terminated chain of α-glucopyranose units (Hirst, Plant, and Wilkinson, J., 1932, 2375), or whether support could be found for the alternative view, according to which starch would be represented as a continuous loop with side chains of glucose members. In the latter event the scission of the loop at different points might lead to products having end-group values which would show no correlation with the progressive break-down of the starch molecule; for example, it might be possible on such a hypothesis to discover a break-down product giving the same end-group value as the molecule of starch itself. This we have not found to be the case. The proportion of tetramethyl glucose amongst the hydrolysis products from the methylated dextrin increases as it would be expected to do on the first hypothesis, the value for the end-group increasing progressively as the molecule undergoes scission. The loop hypothesis would require that, in the hydrolysis product, tetramethyl glucose should be accompanied by dimethyl glucose, but we have not encountered any appreciable quantity of the latter in the experiments now described.

We now outline the properties of a starch dextrin having 12 glucose units in the chain, and also a second dextrin having a mean chain-length of 8 glucose units. The chemical assay of the end-groups of these products is in agreement with the value of the molecular weight estimated from the viscosities of the methyl derivatives in *m*-cresol solution. It is a matter of special importance to discover whether or not the hexose residues of the dextrin molecules are united exclusively by α-glucosidic links, and in this connexion it is of interest that the rotations of the two methylated dextrins have values which agree closely with those calculated by Freudenberg's optical superposition method (*Ber.*, 1933, **66**, 177) for dextrins of the appropriate chain-length composed of terminated chains of α-glucopyranose units linked through positions 1 and 4. The chain-lengths deduced from various methods are tabulated below:

*Chain-lengths of dextrins.*

| Method. | Viscosity of acetate. | Viscosity of methyl derivative. | Rotation. | End-group. |
|---|---|---|---|---|
| Chain-length (Dextrin I) ..... | 8 | 10 | 10 | 12 |
| Chain-length (Dextrin II) ..... | 5 | 7 | 8 | 8 |

These dextrins show no tendency to undergo molecular aggregation to substances of higher molecular weight. They were prepared by heating starch with glycerol (compare Pictet, *Helv. Chim. Acta*, 1918, **1**, 87), and it is clear that a small amount of glycerol remains combined in the dextrin, probably as the glyceryl glucoside of the first glucose unit of the chain (Berner, *Ber.*, 1933, **66**, 1333). For this reason, iodine numbers cannot be used as a means of determining chain-length. The dextrins gave with iodine in aqueous solution a deep red colour similar to that given by glycogen, and the same colour reaction characterised the dextrins regenerated from the acetates by the action of alkali. It will be recalled that the blue iodine colour of potato starch is retained in a similar way by the starch regenerated from its acetylated derivatives. The dextrins were converted into the acetates, and separation was effected by fractional precipitation; the acetates were methylated, and the methyl dextrins were again submitted to careful fractionation. It is noteworthy that the apparent chain-lengths, determined viscosimetrically, of the dextrin acetates are lower than the corresponding values obtained by the same method for the methylated derivatives. But it is the latter value which is in agreement with the gravimetric determination of the chain-length (compare and contrast Staudinger, *Ber.*, 1934, **67**, 48). We had noticed similar phenomena in other cases, and the problem will be dealt with in subsequent communications. It is not claimed that these dextrins are completely homogeneous in the sense that all the component molecules have precisely the same chain-length, but it seems evident from the properties which we have outlined that the figures given represent a mean chain-length and that sizes of the component molecules vary but little, on either side, from this mean value. Other examples of break-down products of starch, including dextrins prepared by other methods, will be described in later communications.

## Experimental.

*Preparation of Starch Dextrin.*—Pure potato starch (15 g., air-dry, containing 18% moisture) was mixed to a paste with glycerol (80 c.c.) and heated at 190—200° with frequent shaking. The paste swelled, giving a viscid transparent jelly which gradually became mobile. The heating was continued until a sample of the liquid gave in aqueous solution a deep red colour with iodine. After cooling slightly, the clear solution was slowly poured with vigorous stirring into alcohol (2 l.). A flocculent white powder was thus obtained from which the adherent glycerol was removed by protracted trituration with alcohol. After a final trituration with ether, the product was dried in a vacuum (yield, 9 g.); $[\alpha]_D^{22°}$ + 176° in water (*c*, 0·9) (Found: $P_2O_5$, 0·1%). The dextrin was slightly soluble in cold water, and the aqueous solution gave a deep red colour with iodine. It was readily soluble in hot water. It contained a small quantity of combined glycerol (compare Berner, *loc. cit.*), which could not be removed by trituration. The reducing power was negligible.

*Starch Dextrin Acetate.*—The air-dry dextrin (moisture content 8%) (5 g.) was heated at 70° with pyridine (25 c.c.) until an opalescent limpid paste was formed (45 mins.). Acetic anhydride (17·5 c.c.) was then added, and the mixture stirred and heated at 70°, until a clear solution was obtained (15 mins.). After a further 45 mins.' heating at 70° the clear mobile liquid was poured slowly with vigorous stirring into water (1 l.). The precipitated acetate was washed with water until acid-free, and dried in a vacuum. By repetition of the process, some 100 g. of dextrin acetate were prepared as a crisp white powder soluble in acetone and chloroform, and slightly soluble in alcohol and ether. It gave no colour with iodine and did not reduce boiling Fehling's solution; $[\alpha]_{5780}^{17°}$ + 158° in chloroform (*c*, 0·7) (Found: $CH_3{\cdot}CO$, 44·3. Calc. for $C_{12}H_{16}O_8$: $CH_3{\cdot}CO$, 44·8%). The dextrin acetate contained a small amount of combined glycerol (*ca.* 2%) which was estimated in the usual way after conversion into *iso*propyl iodide. The iodine number was approximately 1·0.

On deacetylation (Hirst, Plant, and Wilkinson, *loc. cit.*), the dextrin acetate gave a white powder which appeared to be identical with the original dextrin. It was non-reducing, gave a deep red colour with iodine, and had $[\alpha]_D^{20°}$ + 174° in water.

*Fractionation of Dextrin Acetate.*—The acetate (90 g.) was dissolved in chloroform (900 c.c.), and on gradual addition of an equal volume of ether to the filtered solution a gummy precipitate was obtained. The supernatant liquor was removed by decantation, and the precipitate dried and powdered (fraction I, yield 22 g.). Light petroleum (400 c.c., b. p. 40—60°) was now added with stirring, causing precipitation of fraction II (35 g.). Further addition of light petroleum (600 c.c.) gave fraction III (17 g.); and fraction IV (13 g.) was obtained on evaporation of the solution decanted from fraction III.

*Properties of the fractions.*

| Fraction. | Yield, g. | $\eta_{sp.}$* | Apparent M.W.† | $[\alpha]_{5780}$ in $CHCl_3$. | Iodine No. |
|---|---|---|---|---|---|
| I | 22 | 0·12 | 3400 | +165° | 0·8 |
| II | 35 | 0·075 | 2200 | 161 | |
| III | 17 | 0·055 | 1500 | 148 | 2·9 |
| IV | 13 | 0·05 | 1400 | 137 | |

* 1% Solution in *m*-cresol at 20°.

† By Staudinger's method, using the constant $K_m = 10^{-3}$.

Fractions III and IV were very similar, and were combined for deacetylation and methylation. Fraction II was examined separately.

*Methylated Dextrin from Acetate Fraction II.*—The acetate (in batches of 13·5 g.) was dissolved in acetone (200 c.c.) and treated in the usual way with methyl sulphate (150 c.c.) and 30% aqueous sodium hydroxide (450 c.c.), the temperature being kept at 52—54°. The mixture was vigorously stirred, and acetone added at intervals to replace evaporation losses. When the reaction was complete, the mixture was heated at 100° for 45 mins., the acetone being allowed to distil away. Methylated dextrin separated from the hot aqueous solution as a yellow gum, from which the aqueous liquid was separated by decantation. This liquor was neutralised with sulphuric acid and evaporated to dryness under diminished pressure. The solid was extracted with boiling acetone to remove water-soluble methylation products. The acetone extract so obtained was used as solvent for the second methylation. The once-methylated gummy product had OMe, 35%. Eight methylations were necessary before the fully methylated dextrin was obtained (yield, 85% of the theo.) (Found : OMe, 45%). The final product separated as a gum from the methylation solution. It was well washed with hot water, and on cooling, set to a hard glass which was almost completely soluble in ether (13·3 g. dissolved out of 16·7 g. crude product, much of the residue being mineral impurity). Only the ether-soluble portion was used in the subsequent experiments. This material (13·3 g.), which was now in the form of a white powder, was extracted twice with boiling light petroleum (b. p. 40—60°). 13·2 G. remained undissolved, and on evaporation of the petroleum a small quantity (approx. 0·1 g.) of viscous syrup remained. This consisted mainly of acetone condensation products formed during the methylations. The purified methylated dextrin was next fractionally precipitated from chloroform solution by addition of light petroleum. Two fractions were obtained, each consisting of approximately half the original material, but these had properties (including viscosities) so nearly identical that they were combined for hydrolysis; $[\alpha]_D^{19°}$ + 199° in chloroform (*c*, 0·6); $\eta_{sp.}$ 0·057 [0·6% solution in *m*-cresol at 20°; apparent molecular weight (Staudinger), approx. 2000] (Found : OMe, 45·5%).

This methylated dextrin (11 g.) was dissolved in five times its weight of concentrated hydrochloric acid, the solution cooled to − 15°, and dry hydrogen chloride passed in to saturation. After a few minutes, the viscous liquid became mobile, but the mixture was kept at 0° for a further 24 hours to ensure complete hydrolysis. The greater part of the excess hydrogen chloride was removed by aeration. The solution was then diluted with twice its volume of water, and an excess of barium carbonate added. After filtration, the neutral liquor was extracted four times with chloroform and then evaporated to dryness under diminished pressure. The solid so obtained was separately extracted with boiling chloroform, and the combined chloroform extracts were evaporated to dryness, leaving the mixed hydrolysis products as a partly crystalline paste (yield 10·2 g.). The mixture of methylated sugars was boiled for 12 hours with an excess of 1·5% methyl-alcoholic hydrogen chloride. The mixed methylglucosides (10·1 g.) thereby obtained were isolated in the usual way and were submitted to fractional distillation under diminished pressure. A preliminary separation was effected by slow distillation from an ordinary long-necked distillation flask into a Widmer flask. The 5 g. collected in this operation contained all the materials of lower b. p., together with a considerable proportion of trimethyl methylglucoside. Distillation from the Widmer flask then gave the following fractions : (*a*)

0·1 g., b. p. 90°/0·03 mm., $n_D^{18°}$ 1·4370 (in view of the known small content of combined glycerol in the acetylated dextrin, this fraction probably consisted largely of methylated glycerol; owing to the low b. p. some of this may have been lost during the distillation); (*b*) 0·9 g., b. p. 120°/0·3 mm., $n_D^{18°}$ 1·4450 (Found : OMe, 60·2%); (*c*) 1·59 g., b. p. 130°/0·3 mm., $n_D^{18°}$ 1·4545 (Found : OMe, 52·9%). Afterwards, pure trimethyl methylglucoside distilled over, b. p. 130°/0·3 mm., $n_D^{18°}$ 1·4560 (Found : OMe, 51·9%). A further amount of the last substance was obtained when the liquid which had not been distilled into the Widmer flask was submitted to fractional distillation. Finally, a small non-distillable residue was obtained which was not further examined. Dimethyl methylglucoside was not encountered. The identity of the trimethyl methylglucoside was established by its hydrolysis to 2 : 3 : 6-trimethyl glucose, m. p. 120°, $[\alpha]_D^{20°}$ + 70° in water (equilibrium value). The identity of fraction (*b*) as tetramethyl methylglucoside was confirmed by its hydrolysis by boiling 7% aqueous hydrochloric acid to crystalline 2 : 3 : 4 : 6-tetramethyl glucopyranose, m. p. 90° alone or when mixed with an authentic sample, $[\alpha]_D^{20°}$ + 83° in water (*c*, 1·0) (equilibrium rotation); yield, nearly quantitative. Fraction (*b*) consisted entirely of tetramethyl methylglucoside.

The refractive index of fraction (*c*), together with the analytical results, showed that it contained about 10% of the tetramethyl derivative (0·16 g.). Only a small portion of (*a*) could possibly be tetramethyl methylglucoside. The total estimated yield of tetramethyl methylglucoside was therefore 1·06 g., to which 0·1 g. is to be added to compensate for experimental losses (see Haworth and Machemer, J., 1932, 2270). The over-all yield of tetramethyl glucose from this methylated dextrin was therefore 9·4%, corresponding with a mean chain-length of 12 units.

*Methylated Dextrin from Acetate Fractions III and IV.*—The acetate was methylated exactly as described above for fraction II, and the product was a hard glass which became syrupy in boiling water but did not dissolve (yield 86% of theo.). The methylated dextrin was dissolved in ether to remove some mineral impurities, and, after removal of the solvent, was exhaustively extracted with light petroleum (b. p. 40—60°) which removed a small amount of acetone condensation products. The methylated dextrin, now in the form of a light powder, was fractionally precipitated from chloroform by addition of light petroleum. No differences were observed in the properties of the fractions (including viscosity); $[\alpha]_D$ + 178° in chloroform (*c*, 0·5); $\eta_{sp.}$ 0·039, [*c*, 0·6% in *m*-cresol at 20°, whence (Staudinger) apparent molecular weight = *ca.* 1300] (Found : OMe, 45%). Accordingly the fractions were recombined for hydrolysis, which was carried out by concentrated hydrochloric acid in the manner already described : 13·2 g. of methylated dextrin gave 13·0 g. of mixed methylated sugars, and from the latter 11·3 g. of mixed methylglucosides were obtained. Fractionation of the glucosides gave a first portion, 0·1 g., b. p. 100°/0·3 mm., $n_D^{18°}$ 1·4330 (probably mainly methylated glycerol). The total amount of tetramethyl methylglucoside, recognised by its conversion into crystalline tetramethyl glucose, was 1·95 g., corresponding, after correction by addition of 10% (see above), to a mean chain-length of 8 units. The trimethyl methylglucoside gave on hydrolysis crystalline 2 : 3 : 6-trimethyl glucose.

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