## 82. *The Constitution of Banana Starch.*

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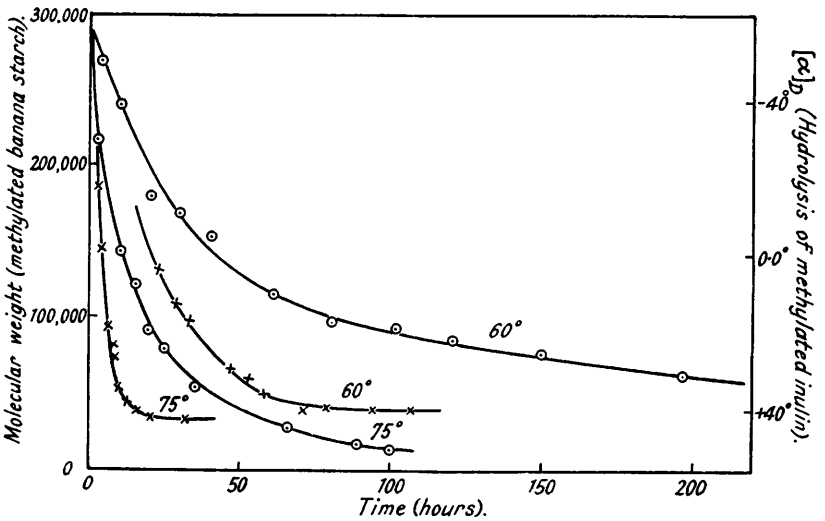
The physical and chemical properties of banana starch resemble closely those of other starches previously examined. The proportion of end-group isolated from the hydrolysis products of the methyl derivative corresponds to a repeating unit of about 24 glucose residues. Hydrolysis of the bonds between the repeating units to give products of lower molecular weight but unchanged chain length (" disaggregation ") proceeds smoothly as in the case of methylated rice starch and gives further evidence of the structural similarity of the starches.

Banana starch, which is one of the main constituents of unripe bananas, appears to be similar in its molecular structure to the other starches previously examined. In general appearance, size of granules, and paste-forming properties it resembles potato starch. Hydrolysis in acid solution proceeds normally, giving finally a solution which, by rotational evidence and from measurement of the reducing power, contains only glucose. From this solution crystalline glucose was isolated in 88% yield. Although the preparation of the fully substituted acetyl and methyl derivatives proceeds with greater difficulty, their properties agree closely with those of the corresponding derivatives of potato starch. The methylated banana starch, whether prepared by direct methylation of the starch or *via* the acetate, had a molecular weight [estimated from viscosity data (Hirst and Young, J., 1939, 1475)] of about 200,000. On hydrolysis, each of these methyl derivatives gave 2 : 3 : 4 : 6-tetramethyl glucose, 2 : 3 : 6-trimethyl glucose, and dimethyl glucoses only. The amount of tetramethyl glucose isolated corresponds to the presence of a repeating unit of about 24 glucose residues.

The similarity of the molecular structure of banana starch to that envisaged for rice starch (Hirst and Young, J., 1939, 1471) is shown by the close resemblance of the course of the disaggregation reactions for the methyl derivatives of the two starches. Treatment with oxalic acid in a mixture of methyl alcohol and water effects hydrolysis of the bonds between successive repeating units in methylated rice starch, causing a gradual decrease in the molecular weight of a sample until finally a product is obtained, the molecule of which is built up of three repeating units linked together by bonds of the glycosidic

type. Throughout this process the proportion of end-group remains unchanged, the solution is non-reducing, and the rotation is undiminished. In all these respects the process is sharply distinguished from normal acid hydrolysis, which results first in the formation of reducing dextrins and finally yields glucose. Fuller consideration of this reaction, termed " disaggregation," has been presented in a previous publication (Hirst and Young, *loc. cit.*), and here we wish to add only that methylated banana starch behaves in a precisely similar manner. The essential identity of the molecular structure of these two starches is thus strikingly confirmed. A consideration of the kinetics of the disaggregation process shows that the type of bond between the repeating units is of the normal glycosidic type. Thus, under identical conditions, the 1 : 6-fructofuranoside linkages in methyl inulin are hydrolysed about seven times more rapidly than the bonds




*Action of* 1% *oxalic acid in aqueous methyl alcohol on methylated banana starch at* 60° *and at* 75° ⊙ ⊙ ⊙ *and on methylated inulin at* 60° *and* 75° × × ×

between the repeating units in methylated starches (see figure). Fuller details of these investigations will be given in another communication.

## Experimental.

*Preparation of Banana Starch.*—Peeled, unripe bananas were minced and shaken with alcohol. The fibrous material was collected on coarse muslin. Starch settled out from the filtrate and was collected in a fine cloth filter. More starch was obtained by treatment of the fibrous residue again with alcohol and with 1% aqueous sodium sulphite. The crude starch so obtained was separated from fibre by flotation in alcohol, but it still retained a slight discoloration. The granules were large and similar in shape to those of potato starch. In hot water the starch formed a clear paste, which gave a deep blue coloration with iodine. It was non-reducing to Fehling's solution. $[\alpha]_D^{20°}$ + 152° (*c*, 0·34) in N-sodium hydroxide. Hydrolysis with taka-diastase yielded 5% of fibrous residue and the reducing value of the residual solution corresponded to 98% of the theoretical amount of glucose. No furfural was obtained by distillation with 12% hydrochloric acid. With the same reagent, under the conditions used for the estimation of uronic acid groups, 0·53% of carbon dioxide was evolved. 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. After hydrolysis of the starch with 2% sulphuric acid, the reducing power of the product was 97% of the value required if quantitative hydrolysis to glucose had taken place. From this solution, crystalline glucose was isolated in 88% yield. (Found for banana starch : $P_2O_5$, 0·06; N, 0·15; moisture in air-dried sample, 20·6%; acid number, 0·6 c.c. of N/10-alkali required to neutralise 1 g. of starch).

*Acetylation of Banana Starch.*—(a) *With chlorine and sulphur dioxide catalysts.* The starch was first activated by precipitation of a paste by alcohol in the usual manner and the acetylation was carried out as previously described for other starches (cf. Hirst and Young, J., 1939, 1476).

The starch appeared more resistant to acetylation than usual, however, and a good yield of the triacetate was obtained only by prolonged treatment with each catalyst, followed by stirring at 50° for 3 hours. The product (acetate A; yield 87%) was purified by fractional precipitation of a chloroform solution with light petroleum. The main fraction had $[\alpha]_D^{20°}$ + 167° in chloroform (*c*, 0·78) (Found: $CH_3$·CO, 44·5%).

(b) *With acetic anhydride and pyridine.* Here again more drastic conditions than usual were necessary. "Prepared" starch (10 g.) was dissolved in pyridine (120 c.c.), and acetic anhydride (120 c.c.) added with stirring. The clear solution was stirred at 60° for 40 hours and then diluted with glacial acetic acid and poured into water. The precipitate was washed with water and dried in a vacuum at 80° (yield, 80%). The acetate (B) was partially soluble in acetone and chloroform, but dissolved almost completely in dioxan. Fractional precipitation from dioxan solution by water gave a main fraction (96% of the whole), having $CH_3$·CO 39·0%. Even under these drastic conditions, therefore, acetylation is still incomplete.

*Methylation of Banana Starch.*—(a) *Direct methylation of "prepared" banana starch.* The starch was prepared by precipitation in the usual manner, and was methylated at 55° with 30% sodium hydroxide solution and methyl sulphate in an atmosphere of nitrogen (for details of conditions, see Hirst and Young, J., 1939, 953). After 18 treatments, the methoxyl content had risen to 43·1%. The product was fractionally precipitated from a chloroform solution by light petroleum. The main fraction (methyl banana starch C) had the following properties: $[\alpha]_D^{20°}$ + 204° in chloroform (*c*, 0·67); $\eta_{sp.}^{20°}$ 0·64 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 225,000 * (Found: OMe, 41·0%). Small fractions on either side of this main fraction had similar properties.

(b) *Simultaneous deacetylation and methylation of acetate (A).* The methylation was carried out at 55° in an atmosphere of nitrogen, as previously described (Hirst and Young, J., 1939, 953). After 13 treatments the methoxyl content of a sample was 43·0%. Fractionation of the product (methyl banana starch D) gave a main fraction having $[\alpha]_D^{20°}$ + 205° in chloroform (*c*, 0·68); $\eta_{sp.}^{20°}$ 0·59 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 205,000 (Found: OMe, 42·4%).

(c) *Simultaneous deacetylation and methylation of acetate (B).* In this case the acetate was dissolved in dioxan for the first methylation, owing to its insolubility in acetone. Otherwise the procedure was similar to that described above. After 14 treatments the methoxyl content of a sample was 43·6%. The product (methyl banana starch E) was fractionally precipitated from a solution in chloroform by light petroleum. A typical fraction had the following properties: $[\alpha]_D^{20°}$ + 208° in chloroform (*c*, 0·65); $\eta_{sp.}^{20°}$ 0·61 in *m*-cresol (*c*, 0·4), corresponding to an apparent molecular weight of 210,000 (Found: OMe, 42·9%).

*The Repeating Unit in Methylated Banana Starch.—Hydrolysis of methylated banana starch (C), prepared by direct methylation of banana starch.* Simultaneous hydrolysis and glucoside formation was effected by 2% methyl-alcoholic hydrogen chloride in the usual manner. The glucosides, isolated in 93% yield, were fractionally distilled at 0·001 mm., and the following fractions were obtained (for details of the method used, see Hirst and Young, J., 1938, 1247).

TABLE I.

| Fraction. | Wt., g. | $n_D^{16°}$. | Constants used in estimation.* | "Tetra," %. | "Tetra," g. | "Tri," g. | "Di," %. | "Di," g. |
|---|---|---|---|---|---|---|---|---|
| 1 | 0·58 | 1·4462 | (a) 1·4433<br>(b) 1·4565 | 78 | 0·45 | 0·13 | — | — |
| 2 | 0·28 | 1·4538 | (a) 1·4435<br>(b) 1·4575 | 26 | 0·07 | 0·21 | — | — |
| 3 | 0·86 | 1·4572 | — | — | — | 0·86 | — | — |
| 4 | 0·27 | 1·4576 | | — | — | 0·27 | — | — |
| 5 | 6·58 | 1·4584 | — | — | — | 6·58 | — | — |
| 6 | 2·49 | 1·4634 | — | — | — | 1·74 | 30 | 0·75 |
| 7 | 0·86 | 1·4735 | — | — | — | — | 100 | 0·86 |
| Residue | 0·91 | — | — | — | — | — | — | — |
| | 12·83 | | | | 0·52 | 9·79 | | 1·61 |

* (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 relative proportions of the fission products, represented as a percentage of the total recovery of glucosides, were therefore: 2 : 3 : 4 : 6-tetramethyl methylglucoside (end-group),

* For the method of calculation of the molecular weight from viscosity measurements, see Hirst and Young, J., 1939, 1475.

4·0%; 2 : 3 : 6-trimethyl methylglucoside, 76%; dimethyl methylglucosides, 12·5%. This proportion of end-group corresponds to a repeating unit of chain length approximately 25 glucose residues.

*Hydrolysis of methylated banana starch (D), prepared from acetate (A).* The methyl derivative was hydrolysed with 2% methyl-alcoholic hydrogen chloride and the glucosides were isolated in 95% yield. After fractional distillation at 0·001 mm., the following fractions were obtained.

TABLE II.

| Fraction. | Wt., g. | $n_D^{16°}$. | Constants used in estimation.* | "Tetra," %. | "Tetra," g. | "Tri," g. | "Di," %. | "Di," g. |
|---|---|---|---|---|---|---|---|---|
| 1 | 0·50 | 1·4448 | (a) 1·4432 (b) 1·4570 | 88 | 0·44 | 0·06 | — | — |
| 2 | 0·52 | 1·4554 | (a) 1·4433 (b) 1·4572 | 13 | 0·07 | 0·45 | — | — |
| 3 | 4·13 | 1·4573 | — | — | — | 4·13 | — | — |
| 4 | 3·70 | 1·4577 | — | — | — | 3·70 | — | — |
| 5 | 1·44 | 1·4596 | — | — | — | 1·38 | 4 | 0·06 |
| 6 | 0·65 | 1·4680 | — | — | — | 0·25 | 62 | 0·40 |
| Residue | 0·34 | — | — | — | — | — | — | — |
| | 11·28 | | | | 0·51 | 9·97 | | 0·46 |

* See note under Table I.

The relative proportions of the fission products, expressed as a percentage of the total recovery of glucosides, were therefore: 2 : 3 : 4 : 6-tetramethyl methylglucoside, 4·5%; 2 : 3 : 6-trimethyl methylglucoside, 88%; dimethyl methylglucosides, 4%. This amount of end-group corresponds to a repeating unit of 22 glucose residues.

*Hydrolysis of methylated banana starch (E), prepared from acetate (B).* The methyl derivative (16·5 g.) was hydrolysed with 2% methyl-alcoholic hydrogen chloride. The glucosides (16·5 g.) were fractionally distilled and the following fractions containing tetramethyl methylglucoside were obtained.

TABLE III.

| Fraction. | Wt., g. | $n_D^{16°}$. | Constants used in estimation.* | "Tetra," %. | "Tetra," g. |
|---|---|---|---|---|---|
| 1 | 0·68 | 1·4452 | (a) 1·4435, (b) 1·4560 | 86 | 0·59 |
| 2 | 0·43 | 1·4556 | (a) 1·4445, (b) 1·4565 | 7 | 0·03 |
| | | | | | 0·62 |

* See note under Table I.

This proportion of end-group corresponds to a repeating unit of 26 glucose residues.

In each assay, the identity of the hydrolysis products was proved by the isolation of crystalline 2 : 3 : 4 : 6-tetramethyl glucose and 2 : 3 : 6-trimethyl glucose from the corresponding fractions. The absence of appreciable amounts of other isomers in the syrup from which the latter crystallised was shown as follows: (a) The *p*-toluenesulphonyl derivative was heated with sodium iodide, and the iodine content of the product was estimated by the method of Oldham and Rutherford (*J. Amer. Chem. Soc.*, 1932, **54**, 366). Any free hydroxyl on $C_6$ in the original sugar would under these conditions be replaced by iodine. By this method it was found that less than 1% of the original trimethyl glucose can have had a free hydroxyl group on $C_6$. (b) Oxidation of the trimethyl glucose gave a lactone, the amide of which gave a negative Weermann reaction and hence all hydroxyl groups on $C_2$ must have been substituted.

*Disaggregation of Methylated Banana Starch.*—The reaction was carried out as described for rice starch (Hirst and Young, J., 1939, 1471). A 2·5% solution of the material (molecular weight by viscosity method 290,000) was heated under reflux with a 1% solution of oxalic acid (cryst.) in methyl alcohol (75 parts)–water (25 parts). The temperature was kept constant (± 1°) by means of a thermo-regulator. At intervals, samples were taken, and the methylated starch was recovered by neutralisation with sodium hydroxide and removal of the methyl alcohol in a vacuum, with addition of boiling water. The precipitate was dissolved in chloroform, and the solution dried with anhydrous magnesium sulphate and concentrated. Addition of light petroleum yielded the methyl derivative as a white, ash-free solid. The viscosity of the dried sample in *m*-cresol solution was determined in the usual manner and the mean molecular weight of the sample was derived as previously discussed. The variation of the molecular weight with time of treatment is shown in the figure, for the temperatures 75° and 60°.

The non-reducing character of the solution during disaggregation, the high rotation (+ 200°) of the product, and its viscosity show that the course of the reaction is precisely similar to that of methylated rice starch.

*Hydrolysis of Methylated Inulin.*—For comparison, the rate of hydrolysis of methylated inulin (2·5 g.) in the same reagent [oxalic acid (cryst., 1 g.), methyl alcohol (75 c.c.), and water (25 c.c.)] was measured by the change in rotation of the solution. These results also are shown graphically in the figure.

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