EFFECT OF MALTOSE ON ACID REVERSION MIXTURES FROM D-GLUCOSE IN RELATION TO THE FINE STRUCTURES OF AMYLOPECTIN AND GLYCOGEN[1]

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ABSTRACT

The products obtained by heating very dilute maltose and D-glucose mixtures in acid solution in a boiling water bath for 8 hours are not markedly different from those produced from D-glucose alone under the same conditions of concentration, acidity, and temperature.

Fragmentation analysis of glycogen and amylopectin by partial acid hydrolysis followed by chromatographic separation of the fragments as their acetates, done in this laboratory, has furnished qualitative evidence for the presence of α-D-(1 $\rightarrow$ 6) (12,17) and α-D-(1 $\rightarrow$ 3) (14,15) linkages in these polymers. During acid hydrolysis, α-D-(1 $\rightarrow$ 3) linkages disappear (16) at a slightly faster rate than α-D-(1 $\rightarrow$ 4) linkages, the α-D-(1 $\rightarrow$ 6) linkages at about one-fourth this rate. Thus a good portion of the α-D-(1 $\rightarrow$ 3) linkages originally present will disappear during the partial hydrolysis.

The criticism that the anomalous linkages may have arisen as a result of reversion of D-glucose during the hydrolysis has been successfully answered by reversion studies on D-glucose (9,10). Beta-gentiobiose

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octaacetate, easily isolable from acetylated reversion mixtures, can serve as an indication of the extent of a reversion reaction. However, Manners (5) has raised a further criticism by stating that the crystalline α-D-(1 $\rightarrow$ 3)-linked β-nigerose octaacetate, isolated in the indicated yields from the fragmentation of amylopectin (0.5%) and glycogen (0.001%) (14,15), may have been produced by "acid-transglucosidation" (8) of maltose which was also present as a hydrolytic fragment. To support his statement he cites the work of Pazur and Budovich (7) in which they claim to have prepared nigerose by acid treatment of a mixture of D-glucose and maltose in greater yield than from an equivalent amount of D-glucose. In the work of Pazur and Budovich the comparative yields of nigerose were determined on crude syrupy products by an indirect analytical procedure. The comparative yields were of the same order of magnitude, and indistinguishable within the accuracy of the method. Moreover, the 30% concentration of carbohydrates used would produce such large quantities of known reversion products that any other possible effect would be eclipsed. Hydrolysis, being a first-order reaction, is independent of initial concentration, but the reverse process, reversion, is bimolecular and therefore highly concentration-dependent.

Materials and Methods

Materials. The D-glucose used in this work was commercial C.P. dextrose. Maltose as it is supplied usually contains substantial quantities of D-glucose and polymeric material. The maltose was specially purified by means of adsorption on a carbon column. Maltose (80 g. of "C.P. maltose") was dissolved in 500 ml. of water and placed on a column (750 $\times$ 75 mm. of Nuchar C Unground, a product of the West Virginia Pulp and Paper Co., Chicago, Ill.). The column was washed with 16 liters of water which was discarded. This was followed by 9 liters of 3% ethanol. The ethanol effluent was evaporated to a syrup which showed upon paper chromatographic examination only traces of D-glucose as impurity. The syrup slowly crystallized from methanol; yield, 40 g.

Acid Treatment of D-Glucose and Maltose Mixture. A mixture of 7.5 g. of D-glucose and 28.5 g. of maltose was dissolved in 9,350 ml. of 0.08*N* hydrochloric acid and held at 99°C. for 10 hours. These conditions were chosen to match those used in previous fragmentation (12,14,15,17) and reversion studies with D-glucose (10) made in this laboratory. The acid-treated sugar solution was cooled and neutralized by passage through a column (500 $\times$ 75 mm.) of Duolite A-4 (OH^-

form) (a product of the Chemical Process Co., Redwood City, Calif.). Four such runs were made using a total of 30 g. of D-glucose and 114 g. of maltose.

Removal of D-*Glucose from the Acid-Treated Mixture.* The neutral solution from each run was percolated in turn through a column (650 × 70 mm.) of Nuchar C Unground, and washed with water after each addition, until the effluent reacted negatively to Benedict solution. The effluent was evaporated under reduced pressure to a syrup and was further dried by distillation with methanol; yield, 132.2 g.

The column was then washed with 10 liters of 25% ethanol, until the effluent reacted negatively to Benedict solution. The ethanolic effluent was evaporated under reduced pressure to a syrup which was further dried by distillation with methanol; yield, 11.1 g.

Paper-Electrophoretic and Chromatographic Examination of the Fractions. Analysis of the aqueous fraction by paper electrophoresis in borate buffer at pH 10 showed only D-glucose with silver nitrate and alkali indicator (11). A paper chromatogram developed with 1-butanol-ethanol-water (4:1.1:1.9) and sprayed with silver nitrate and alkali showed principally D-glucose with a small amount of 1,6-anhydro-β-D-glucopyranose and possibly D-fructose. When sprayed with reagents giving specific color tests with ketohexoses (*p*-anisidine and resorcinol reagents) (3), the material was indicated by color and mobility to be fructose, in comparison with a known sample.

Analysis of the 25% ethanol fraction by electrophoresis in borate buffer at pH 10, indicated with silver nitrate and alkali reagent, showed principally maltose, with a small amount of material with the mobility of a (1 → 6)- or (1 → 3)-linked disaccharide.

Paper chromatograms developed with 1-butanol-ethanol-water (4:1.1:1.9) and indicated with silver nitrate and alkali showed principally maltose, with small amounts of D-glucose and 1,6-anhydro-β-D-glucopyranose, a significant amount of (1 → 6)-linked disaccharide, and a trace of nigerose. Other disaccharides were not resolved.

Acetylation of the Polymeric Fraction. The dried syrup, 11.1 g., from the 3% ethanol effluent from the carbon column was acetylated by heating with a mixture of 5.5 g. of sodium acetate and 60 g. of acetic anhydride at the boiling point. The solution was cooled and poured into 500 ml. of ice and water and stirred for 4 hours. The mixture was extracted with chloroform, washed with water, dried with anhydrous sodium sulfate, and evaporated to a syrup; yield, 20 g.

Silicate-Column Chromatography of the Acetylated Polymer Fraction. The acetylated syrup was placed in 5-g. portions on each of four

Magnesol-Celite columns (270 × 75 mm.) and developed with benzene:2-methyl-2-propanol (100:1 by volume) as described by Thompson, Wolfrom, and Quinn (10) for the similarly prepared reversion product from D-glucose alone. The considerable amount of β-maltose octaacetate present made the chromatography difficult, but small amounts of crystalline gentiobiose octaacetate, tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose, and β-maltose heptaacetate were isolated and identified; no octaacetate of nigerose was obtained.

Results and Discussion

A study using maltose and D-glucose (2:1 molar ratio) in a combined concentration of 0.4% in 0.08*N* hydrochloric acid has been detailed. The experiment produces data comparable with those obtained with the use of D-glucose (10) alone. The amounts of reversion material were small. Although paper-chromatographic evidence was found for the presence of traces of nigerose, the quantity was so small that none was isolated as the acetate by silicate chromatography of the acetylated disaccharide fraction.

The term "transglycosylase" (1,2) has been employed to indicate a type of enzyme capable of transferring glycosyl groups from one position to another. The term "transglycosylation" follows as the name of the reaction. The present authors agree with Isbell and Frush (4) that it is desirable to restrict this term to enzymatic processes. The common occurrence of transglycosylation reactions in enzymatic processes makes enzymatic reactions of doubtful value for the elucidation of structure, although there is evidence that low substrate concentrations may minimize this effect.

The conclusion must be reached that there is no evidence to indicate the occurrence of any transfer of D-glycosyl groups from one position to another in aqueous acid which cannot be explained by hydrolysis and reversion. There is no evidence to indicate that the type of linkages found in reversion products is influenced by the nature of the starting material. Therefore, the β-nigerose, α-D-glucopyranosyl-(1 → 3)-β-D-glucopyranose, octaacetate isolated in the fragmentative hydrolysis of amylopectin (14) and glycogen (15), is a true finding and is not an artefact. Indeed, since the relative ease (16) of hydrolytic splitting of the α-D-(1 → 3) linkage is so close to that of the α-D-(1 → 4) bond, the amount of the former type of union preformed in amylopectin and glycogen must be greater than is indicated by the amount actually isolated.

Incidentally to the above work, paper-chromatographic evidence

was obtained for the formation of fructose as an acidic interconversion product of D-glucose, in confirmation of the results of Ohno and Ward (6). Wolfrom and Shilling (13) have established, on an isolative basis, the formation of a small amount of D-glucose in a heated aqueous solution of D-fructose.

A small amount of β-maltose heptaacetate was found in the acetylation products and had been reported (17) earlier in the acetolysis of amylopectin. Its origin from maltose is under further investigation.

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