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Products of the β -Amylolytic of Maltodextrins

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β -Amylase degrades α -1:4-linked polymers of D-glucopyranose by an end-wise action, commencing at the non-reducing chain end, in which alternate linkages are hydrolysed and maltose is liberated in the β -form (Kuhn, 1925; Hanes, 1937; Hestrin, 1949; Bird & Hopkins, 1954). In the linear series of polymers based on maltose (maltodextrins) the smallest molecule which undergoes rapid hydrolysis is maltotetraose (Whelan, Bailey & Roberts, 1953). Maltotriose is attacked very slowly with the production of glucose and maltose. From a study of changes in reducing power during hydrolysis (Whelan *et al.* 1953), and of the priming action in the enzymic synthesis of amylose after treatment of the maltodextrins with β -amylase (Whelan & Bailey, 1954), it was inferred that the rapid β -amylolysis proceeded no further than the conversion of maltotetraose and -hexaose into maltose, and of maltopentaose into equimolar amounts of maltose and maltotriose. The present work affords conclusive proof of the correctness of these deductions by the isolation and characterization of these products.

EXPERIMENTAL AND RESULTS

Maltodextrins. These were prepared from potato amylose as by Whelan *et al.* (1953).

β -Amylase. The crystalline enzyme from sweet potato was used. This was kindly provided by Dr A. K. Balls. Its activity was determined as by Hobson, Whelan & Peat (1950).

Sugar acetates. These were prepared and the maltotriose acetate was purified on Magnesol-Celite as described by Whelan & Roberts (1953).

β -Amylolytic of maltodextrins. The maltodextrin (ca. 100 mg.) was dissolved in water (10 ml.) and Somogyi's (1945*b*) deproteinizing reagents, 5% (w/v) ZnSO_4 (2 ml.) + equiv. amount of 0.4N-Ba(OH)₂, added (cf. Whelan *et al.* 1953). The supernatant solution was divided into a 1 ml. portion used to determine the maltodextrin concentration (Pirt & Whelan, 1951) and a 10 ml. portion to which was added 0.2M sodium acetate-acetic acid buffer (pH 4.8; 6 ml.), β -amylase (60 units; 1 ml.) and water to 50 ml. Measurements of reducing power were made on 1 ml. portions at intervals during incubation at 35° using the Somogyi (1945*a*) copper reagent. The hydrolyses were virtually complete after 30 min. but digestion was allowed

to continue for a total of 21 hr. At this time the percentage conversions of malto-tetraose and -hexaose into maltose were 94.1 and 95.3% respectively; the reducing power of the maltopentaose digest, calculated as an equimolar mixture of maltose and maltotriose, corresponded to a conversion of 99.8%. The enzyme was inactivated by being heated at 100° for 5 min. and the remainder of each digest (ca. 42 ml.) was added to a charcoal-Celite column in which the dimensions of the adsorbent were 55 × 2.0 cm. The columns were washed with water (1 l.) to remove buffer salts. Glucose would also have been removed had it been present but none was detected. The di- and tri-saccharide components were eluted with 7.5 and 15% (v/v) aqueous ethanol respectively, as described by Whelan *et al.* (1953). No trisaccharide products were obtained from malto-tetraose and -hexaose but in all three cases a small amount of optically active material ($\alpha = ca. 0.1^\circ$ in 100 ml. solution; 4 dm. tube) was eluted when the ethanol concentration was increased from 15 to 50%. The same amount of this material was obtained when a control column, containing no added sugar, was similarly eluted. Paper chromatographic analysis of the fractions eluted with 50% ethanol showed them to contain substances reacting with the ninhydrin reagent and sugars of varying R_f values. In the case of the control column the sugar content after acid hydrolysis (Pirt & Whelan, 1951) corresponded to 1.6 mg. (as glucose) in the whole fraction (weight, 14 mg.).

The appropriate fractions containing the disaccharides were combined, as were those containing the trisaccharide. All solutions were evaporated to dryness under diminished pressure and the products acetylated. Each sample of β -maltose octa-acetate from the three maltodextrins melted (over 1°) in the range 157–160° and had $[\alpha]_D^{25}$ in $CHCl_3$ in the range 63.5–65.0 ± 2°. The yields in the acetylation of the maltose specimens varied from 58 to 100%. Authentic octaacetyl β -maltose had m.p. 159–160° and $[\alpha]_D^{25} + 63^\circ$ in $CHCl_3$. The β -maltotriose undeca-acetate (yield, 75%) had m.p. 134.5–136.5° and $[\alpha]_D^{25} + 89 \pm 3^\circ$ in $CHCl_3$. The corresponding values for an authentic specimen were 134–136° and +86°.

DISCUSSION

The results given above prove that the previous deductions as to the course of β -amylase action on the maltodextrins were correct. So far as we are aware this is the first report of a complete chemical identification of all the products of β -amylase action on linear substrates. Amylose and amylopectin, as isolated from starch, have not yet proved amenable

to such accounting because of the presence of branch linkages additional to the chain-forming α -1:4 linkage. This evidence of the course of β -amylase action has provided justification for an enzymic method of determining the average chain length of linear or, if a debranching enzyme is available, branched starch-type polymers based on the quantitative isolation after β -amylolysis of maltose and maltotriose, the latter being a residue from chains containing an odd number of glucose residues. Using R-enzyme in conjunction with β -amylase this method has been used in a determination of the average repeating chain length of waxy maize starch (Thomas, 1952; see Whelan, 1953), and the result was in close agreement with the value obtained by periodate oxidation.

SUMMARY

The β -amylolysis of three maltodextrins has been studied and the products isolated by charcoal chromatography. The yields and the nature of the products are in accordance with the postulated course of action of β -amylase.

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