11. *The Enzymic Synthesis and Degradation of Starch. Part XXI.* The Dextrins synthesised by D-Enzyme.*

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The dextrins synthesised by the action of D-enzyme on maltotriose have been isolated and shown to be products containing only α-1 : 4-glucosidic linkages. A convenient and economic method of preparing pure maltodextrins is thus provided.

It was shown in Part XX * that potato D-enzyme reversibly disproportionates maltotriose and the higher maltodextrins. The question there left open whether *only* α-1 : 4-linkages are synthesised by D-enzyme, has been re-examined by the isolation of the pure glucose polymers, thus produced from maltotriose.

Maltotriose, prepared from potato starch by α-amylolysis with human salivary amylase, was treated with D-enzyme and the products were separated on charcoal–Celite as described by Whelan, Bailey, and Roberts (*J.*, 1953, 1293). Maltodextrins containing 1—6 glucose units were thus isolated, with higher polymers which were not further examined. The yields are shown in the Table and the low relative yield of maltose should be noted. Paper

Properties of products of D-enzyme action on maltotriose.

Fraction	Yield * (g.)	$[\alpha]_D$ in H_2O †	β-Acetate † M. p.	β-Acetate † $[\alpha]_D$ in $CHCl_3$	Hydrolysis by β-amylase (%) ‡
Monosaccharide ...	1·52	+52·0° (+52·6°)	131° (131°)	+3·1° (+3·8°)	—
Disaccharide	0·143	+137·6° (+136°)	157° (159°)	+62·8° (+63°)	—
Trisaccharide	1·95	+158·9° (+160°)	133° (135°)	+85·2° (+86°)	0
Tetrasaccharide ...	1·48	—	—	—	97·2 (97·5)
Pentasaccharide ...	1·23	—	—	—	102·9 (101·6)
Hexasaccharide ...	0·676	—	—	—	99·7 (99·4)

* From 10 g. of maltotriose.
† Values for authentic specimens are given in parentheses.
‡ Values for the tetra- and the hexa-saccharide are calculated in terms of theoretical maltose, for the pentasaccharide in terms of its conversion into an equimolar mixture of maltose and maltotriose. The values for authentic specimens of the maltodextrins refer to products obtained from amylose as by Whelan *et al.* (*loc. cit.*) and were measured by Dr. P. J. P. Roberts.

chromatography indicated that the mono-, di-, and tri-saccharide fractions contained only glucose, maltose, and maltotriose respectively. Sufficient is now known of the chromatographic behaviour of *iso*maltose, *iso*maltotriose, and the trisaccharides containing both α-1 : 4- and α-1 : 6-linkages, *e.g.*, panose, to be sure that none of these substances was present in detectable amount. In addition, each fraction from the di- to the hexa-saccharide obeyed the linear R_M–molecular weight relation which exists between members of the maltodextrin series (Whelan *et al.*, *loc. cit.*). The mono-, di-, and tri-saccharides were rigidly identified by the $[\alpha]_D$ values of the free sugars and the properties of their crystalline β-acetates. Further, the tetra-, penta-, and hexa-saccharides were pure maltodextrins since each was completely hydrolysed by β-amylase, which is specific for

* Part XX, preceding paper.

α-1 : 4-glucoside scission. The properties of the D-enzyme products and their derivatives are shown in the Table.

These results prove that the digestion of maltotriose with D-enzyme yields a mixture consisting of glucose, unchanged maltotriose, and the higher maltodextrins. Maltose is also produced, but relatively very little, and it is questionable whether it is a true product of D-enzyme action since its occurrence, as revealed by paper chromatographic fractionation of D-treated maltodextrins, is not always observed. The production of maltose by the particular enzyme preparation used here was apparent when the whole maltotriose digest was examined by paper chromatography but the question whether the pure enzyme is capable of producing maltose remains unsettled.

These experiments provide a very convenient means of preparing pure maltodextrins in quantity, better than our earlier preparation by partial acidic hydrolysis of amylose (Whelan *et al.*, *loc. cit.*). The substrate, maltotriose, is more easily prepared than is amylose, which must be carefully purified to avoid the possibility of contamination by amylopectin, for an impure amylose would give maltodextrins contaminated with branched dextrins. Again, only about one-sixth of the maltotriose substrate is converted by D-enzyme into glucose and maltose, whereas the optimum conditions for the liberation of maltotriose and the higher dextrins from amylose result in losses of up to half of the substrate as the unwanted mono- and di-saccharides. Moreover, the possibility of contamination of the maltodextrins from amylose with acid-reversion products (from glucose) is non-existent in the enzymic method. The method is also economical. Untreated saliva is used to prepare maltotriose from starch and the fact that some of the maltotriose is hydrolysed by an enzymic impurity in the saliva is of little consequence since the starch substrate is inexpensive. The D-enzyme preparation required for the experiment is simply the twice-precipitated protein fraction of potato juice soluble in 16%, but insoluble in 30%, ethanol.

Experimental

Preparation of Maltotriose.—Potato starch (125 g., wet) was made into a paste with cold water (500 ml.) and added during 15 min. to boiling 0·1% aqueous sodium chloride (3·3 l.). Vigorous stirring and boiling were maintained for an additional 45 min. and the solution was then cooled rapidly in cold water. 0·2M-Sodium acetate buffer (50 ml.; pH 4·8) was added, followed by the supernatant solution from a mixture of human saliva (25 ml.) and water (25 ml.) which had been allowed to stand for 1 hr. before centrifugation. The digest was diluted to 4 l., covered with toluene, and incubated at 30°. Pirt and Whelan's method (*J. Sci. Food Agric.*, 1951, **2**, 224) showed that 97·2 g. of starch were present. After incubation for 3 days the digest was boiled for a few minutes to destroy the enzyme. (Subsequent experiments have shown that incubation for 1 day is sufficient.) The supernatant liquid was adsorbed on a charcoal–Celite column (100 × 8·5 cm.). The column was irrigated with water, and then 7·5% and 15% ethanol as described by Whelan *et al.* (*loc. cit.*). The optically active material eluted by 15% ethanol contained traces of maltose and glucose; it was therefore refractionated by the same procedure (column size, 80 × 4·5 cm.) and 10·5 g. of chromatographically pure maltotriose were obtained.

Preparation of D-Enzyme.—Charcoal-treated potato juice (preceding paper) (400 ml.) was placed in a bath at −5° and when the juice had cooled to −2° ethanol (76·2 ml.) was added with mechanical stirring during 10 min. After 10 min. at −5° the precipitate was removed on a centrifuge at −5° and to the supernatant solution at −5° were added a further 94·8 ml. of ethanol. The precipitate formed, after being kept for 10 min. at −5°, was recovered by centrifugation and dissolved in 0·01M-citrate buffer (100 ml.; pH 7·0).

Action of D-Enzyme on Maltotriose.—The enzyme solution (45 ml.), 0·2M-citrate buffer (20 ml.; pH 7·0), and maltotriose (10 g.) were mixed and diluted to 100 ml. with water. The digest was incubated at room temperature for 16·5 hr. After inactivation of the enzyme by heat, the cooled digest was passed through a Seitz filter to remove coagulated protein and then transferred to a charcoal–Celite column (80 × 4·5 cm.) which was eluted as described by Whelan *et al.* (*loc. cit.*) except that the ethanol was added by the gradient method (cf. Alm, Williams, and Tiselius, *Acta Chem. Scand.*, 1952, **6**, 826), 33% ethanol being added through a constant-level device to a mechanically stirred reservoir of water (4 l.) which led to the column. By comparison with the stepwise addition of ethanol, this method provides an improved fractionation of the maltodextrin products, separation of mono- to tetra-saccharides being absolute.

The products as far as the hexasaccharide were recovered as described by Whelan *et al.* (*loc. cit.*); the higher dextrins were eluted by passing 50% ethanol through the column. A control experiment in which the enzyme preparation was adsorbed on a charcoal column and eluted with water and aqueous ethanol showed that no sugars were derived from this source.

Characterisation of the Products.—Measurements of $[\alpha]_D$ of the mono-, di-, and tri-saccharides were made by the method employed by Whelan *et al.* (*loc. cit.*); the β-acetates of these sugars were prepared by sodium acetate–acetic anhydride. β-Amylolysis of the tri-, tetra-, penta-, and hexa-saccharides was carried out as follows. Solutions of the dextrins of known concentration were prepared as by Whelan *et al.* (*loc. cit.*). These were incorporated in digests containing dextrin (*ca.* 16 mg.), purified soya-bean β-amylase (1·5 ml.; 35 units; Part XVI, *J.*, 1952, 714), and 0·2M-sodium acetate buffer (1·5 ml.; pH 4·8) in a total volume of 11 ml. Incubation was at 30° and portions (1 ml. each) of the digests were removed after 1·5, 2·5, and 3·5 hr. for determination of reducing power. Digests containing (i) maltose in place of dextrins and (ii) only enzyme and buffer served as controls on the maltase activity and reducing power of the enzyme. The results, corrected for the slight maltase activity, showed that the hydrolysis of the tetra-, penta, and hexa-saccharides was complete after 1·5 hr. and no further change took place. The trisaccharide fraction was not attacked. The digest contents were also examined by paper chromatography in butan-1-ol–acetic acid–water (4 : 1 : 5 by vol.), it being expected that the digests of the tetra- and the hexa-saccharide would show only maltose and that of the pentasaccharide only maltose and maltotriose (cf. Whelan and J. G. Roberts, *Biochem. J.*, 1954, **58**, 569). These inferences were confirmed except that the tetra- and hexa-saccharide digests contained minute traces of tetrasaccharide, and the latter also a very small amount of trisaccharide, agreeing with the appearance of a trace of pentasaccharide in the original hexasaccharide fraction.

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