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Note

Preparation of 6^3-α-D-glucosylmaltotriose

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In recent research[1] on the hydrolysis of (1→4)-α-D and (1→6)-α-D linkages in starch by glucoamylases (EC 3.2.1.3), model oligosaccharides, including 6^3-α-D-glucosylmaltotriose, were required. The preparation of this tetrasaccharide by the published methods[2–4] is tedious and the yields are small (<1%). A simple preparation of pure 6^3-α-D-glucosylmaltotriose in good yield is now described.

It was considered that 6^3-α-D-glucosylmaltotriose could be prepared by beta-amylolysis of 6^3-α-D-maltotriosylmaltotriose. This hexasaccharide, which was present in maximum amount when pullulan was hydrolysed to ~50% by pullulanase, was completely converted into a mixture of 6^3-α-D-glucosylmaltotriose and maltose by beta-amylase (4 U/mg of hexasaccharide) at 37° and pH 5 for 1 h.

Incubation of pullulan with pullulanase and beta-amylase, in sequence, gave 7% of 6^3-α-D-glucosylmaltotriose. However, it was considered that higher yields could be attained by incubating pullulan with the two enzymes concurrently.

The minimum structure susceptible to hydrolysis by pullulanase is 6^2-α-D-maltosylmaltose, and a (1→6)-α-linked D-glucosyl stub, as present in 6^3-α-D-glucosylmaltotriose, is resistant[5]. Thus, with a sufficient excess of beta-amylase in an incubation mixture, immediately following the cleavage of a (1→6)-α-D linkage in pullulan by pullulanase, maltose would be removed by beta-amylase, leaving a single D-glucosyl residue on the non-reducing side of the (1→6)-α-D linkage, which stabilises the linkage towards pullulanase. The experimental conditions designed to achieve these objectives were as follows. Pullulan (10 g) in 20mM sodium acetate buffer (pH 5, 500 ml) was incubated with small quantities of pullulanase (20 U) and a 10-fold excess (in terms of the relative action of the two enzymes on the hexasaccharide) of beta-amylase (20,000 U) for 20 h at 37°. Excess of pullulanase (200 U) was then added (if necessary) to ensure complete conversion of last traces of material of high d.p. into maltotriose. The solution was incubated at 37° for a further 1 h and then

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at 100° for 10 min to denature the enzymes; the only oligosaccharides present at this stage were 6^3-α-D-glucosylmaltotriose, maltotriose, maltose, and D-glucose. The pH of the solution was then adjusted to 6.9 with sodium glycerophosphate buffer (20mM) containing 5mM calcium chloride, and salivary alpha-amylase (30 ml, 450 U) was added. Incubation was continued until maltotriose had been completely converted into D-glucose and maltose (2 days). Alpha-amylase was inactivated by incubating the solution at 100° for 10 min. Each incubation to this stage was performed in a sealed container and the solution was overlaid with a few drops of toluene to prevent microbial contamination.

The mixture was cooled to room temperature, baker's yeast (*Saccharomyces cerevisiae*, 10 g dry weight before washing with distilled water) was added, and the mixture was shaken at 30° for 24 h. The yeast cells, which utilised the D-glucose and

TABLE I

QUANTIFICATION (BY H.P.L.C.) OF 6^3-α-D-GLUCOSYLMALTOTRIOSE PRESENT IN INCUBATION MIXTURES AT VARIOUS STAGES OF PREPARATION

Stage	*Total carbohydrate (g)*	*6^3-α-D-Glucosylmaltotriose (%)*
Pullulanase plus beta-amylase (8 h)	10	29.4
Pullulanase plus beta-amylase (20 h)	10	40.5
Plus salivary alpha-amylase (48 h)	9.8	36.3
Plus yeast (6 h)	7.8	47.0
Plus yeast (24 h)	4.6	81.8

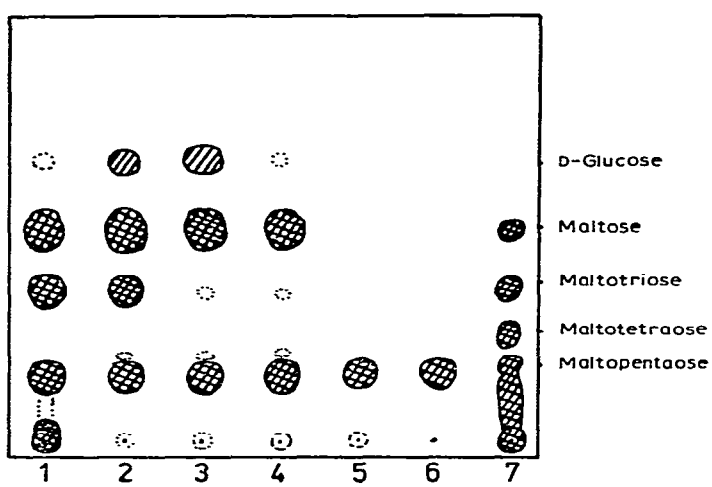

Fig. 1. T.l.c. of oligosaccharide mixtures obtained at different stages during the preparation (see text) of 6^3-α-D-glucosylmaltotriose: 1, pullulan plus beta-amylase plus pullulanase (8 h); 2, pullulan plus beta-amylase plus pullulanase (20 h); 3, hydrolysate plus alpha-amylase (48 h); 4, hydrolysate plus yeast (6 h); 5, hydrolysate plus yeast (24 h); 6, 6^3-α-D-glucosylmaltotriose; 7, malto-oligosaccharides.

maltose but not 6^3-α-D-glucosylmaltotriose[3], were removed by centrifugation (10,000*g*, 10 min) and the supernatant solution was incubated at 100° for 10 min before concentration to a carbohydrate content of 20%. Samples (5 ml) were subjected to chromatography[6] on Bio-Gel P-2 (<400 mesh) to yield 6^3-α-D-glucosylmaltotriose. The details of this purification scheme are shown in Table I and Fig. 1. The oligosaccharide was obtained in 36% yield, with 82% purity, without the aid of chromatography. A single chromatographic run on Bio-Gel P-2 removed the contaminating carbohydrate, which was thought to be partially degraded, yeast cell-walls. The 6^3-α-D-glucosylmaltotriose isolated had chromatographic behaviour which was identical to that of 6^3-α-D-glucosylmaltotriose obtained by alpha-amylolysis of glycogen, and was resistant to pullulanase, beta-amylase, and alpha-amylase. *Aspergillus niger* glucoamylase attacked the compound, but at a rate significantly lower than that for maltotetraose.

EXPERIMENTAL

T.l.c. was performed on silica gel sheets (Eastman, 6061), which were developed twice with 1-propanol–ethanol–water (7:1:2). Detection was effected by charring with sulphuric acid. P.c. was performed on Whatman 3MM paper with ethyl acetate–pyridine–water (2:1:2, upper phase). H.p.l.c. was performed on a Waters Microcarbohydrate column[7] with acetonitrile–water (65:35) at 1,000 p.s.i. and 3.0 ml/min. Pure 6^3-α-D-glucosylmaltotriose was used as the standard.

Materials. — Pullulanase (EC 3.2.1.41) was obtained commercially (Sigma Chem. Co.), and beta-amylase (EC 3.2.1.2) was isolated[8] from sweet potato. Salivary alpha-amylase (EC 3.2.1.1) was prepared by dialysing crude saliva against sodium glycerophosphate buffer (20mM, pH 6.9) containing 5mM calcium chloride. The dialysed enzyme was centrifuged (20,000*g*, 15 min), and the supernatant solution (~120 U of alpha-amylase/ml on soluble starch at 37° and pH 6.9) was used directly. Pullulan (M_n 30 × 10^4, Lot No. P1M 261119) was obtained from Hayashibara Biochemical Laboratories.

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