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α-Maltosyl β-D-Fructofuranoside, a Trisaccharide Enzymically Synthesized from Sucrose[1]

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In the course of an investigation of the action of honey invertase upon sucrose with the objective of comparing such oligosaccharides as may be formed with those occurring in honey, six saccharides other than glucose, fructose and sucrose were demonstrated by paper chromatography.[2]

The synthesis of oligosaccharides during the action of yeast invertase upon sucrose has been described.[3] The most extensive data[4] describe five such compounds, two of which are non-reducing trisaccharides composed of two fructose and one glucose molecules. No data other than R_f values are given. deWhalley[5] has reported further data for one of the trisaccharides, confirming its monosaccharide composition and giving $[\alpha]^{22}D$ +26.61°. He named it kestose.

Since honey invertase differs from yeast invertase in its action on sucrose and other sugars[2,6] it might be expected that the intermediates formed in the action of these enzymes upon sucrose differ.

The principal trisaccharides formed by yeast invertase from sucrose contain two fructose and one glucose molecules[4,5]; the principal trisaccharide formed by honey invertase from sucrose contains two glucose and one fructose molecules.

This sugar has been isolated from a honey invertase digest of sucrose in a yield of 11% of the original weight of sucrose. The structure 4-(α-D-glucopyranosyl)-α-D-glucopyranosyl β-D-fructofuranoside is proposed for this compound. A more convenient name is α-maltosyl β-D-fructofuranoside. The proposed structure is based on the following reactions.

The trisaccharide is non-reducing to Fehling solution and gives glucose and fructose on hydrolysis. Yeast invertase splits the molecule only at the glucose–fructose linkage to give fructose and maltose, toward which the enzyme is inactive. This fixes the glucose–glucose linkage as α-1,4. Honey invertase, which synthesizes the sugar, also can degrade it completely to constituent monosaccharides. However, its mode of action is such that the terminal glucose is first split off, leaving sucrose. There is an accumulation of sucrose during the reaction, which eventually is hydrolyzed completely. This fixes the glucose–fructose linkage as that in sucrose, or β-D-fructofuranosyl α-D-glucopyranoside. Thus, linkages and stereochemical forms of the constituent monosaccharides in the trisaccharide are fixed by identification of maltose and sucrose as degradation products.

Experimental

Preparation of α-Maltosyl β-D-Fructoside.—A honey invertase concentrate was prepared from unheated 1948 fall flower honey by the procedure of Nelson and Cohn.[7] One ml. of the preparation (equivalent to the enzyme content of 32 g. of honey) inverted 0.86 g. of sucrose in 125 minutes at 26°, *p*H 5.8 in 10 ml. of 15% sucrose solution.

A solution of 8.35 g. of sucrose, 2 ml. of 2 *M* acetate buffer at *p*H 5.7 and 5.55 ml. of honey invertase was made to 50 ml. and allowed to stand 128 minutes at 26°. At this time 24% of the original sucrose remained. The solution was heated and subjected to chromatography on a 36 × 160 mm. carbon-diatomaceous earth column as described by Whistler and Durso.[8] Details of the separation are given elsewhere.[2] The fraction eluted with 50% ethanol (0.944 g.) contained all compounds higher than disaccharides, since it followed the 5% ethanol (disaccharide) fraction directly.

Paper chromatography of this fraction showed it to contain principally a non-reducing, ketose-containing material of R_S[9] 0.57 (solvent, butanol 3, pyridine 1, water 1.5)[10]. Small amounts of other materials were present whose migration on the papergram corresponded to that of disaccharides (R_S 1.00) and tetrasaccharides (R_S 0.26).

In a typical purification, 160 mg. of the crude material was freed of these contaminants by chromatography on a powdered cellulose column essentially as described by Hough, *et al.*[11] The solvent used was butanol 41.6, ethanol 47.6, water, 22.5 parts by volume, a single-phase solvent which gives relatively rapid movement of trisaccharides.

Samples from the 1-ml. eluate fractions were chromatographed on paper to locate the constituents. The eluate fractions containing only the trisaccharide were combined to yield 123 mg. of material. Since the product has not been crystallized, material from several runs was evaporated and dried for analysis at a pressure of 1.6 mm. at 105° to constant weight. It had $[\alpha]^{25}D$ +121.8° (2.3% in water). No definite melting point was obtained for the amorphous material.

α-Maltosyl β-D-Fructoside Hendecaacetate.—The trisaccharide (100 mg.) was treated with acetic anhydride in pyridine at room temperature by the procedure of Barker and Bourne.[12] The product was dried at pressure of 2 mm. at 60° to constant weight. It was not crystallized. It had $[\alpha]^{25}D$ +86.0° (1.2%, $CHCl_3$).

(1) Report of work carried out under the provisions of the Research and Marketing Act of 1946. Presented at the 122nd Meeting of the American Chemical Society, Division of Sugar Chemistry, Atlantic City, N. J., Sept. 16, 1952.

(2) J. W. White, Jr., and J. Maher, *Arch. Biochem. Biophys.*, in press.

(3) J. S. D. Bacon and J. Edelman, *ibid.*, **28**, 467 (1950); P. H. Blanchard and N. Albon, *ibid.*, **29**, 220 (1950); E. H. Fischer, L. Kohtes and J. Fellig, *Helv. Chim. Acta*, **34**, 1132 (1951).

(4) L. M. White and G. Secor, *Arch. Biochem. Biophys.*, **36**, 490 (1952).

(5) H. C. S. deWhalley, *Internat. Sugar J.*, **54**, 127 (1952).

(6) G. Gorbach and R. Schneiter, *Biochem. Z.*, **296**, 367 (1938).

(7) J. M. Nelson and D. J. Cohn, *J. Biol. Chem.*, **61**, 193 (1924).

(8) R. L. Whistler and D. F. Durso, This Journal, **72**, 677 (1950).

(9) R_S is ratio of travel of spot to travel of sucrose on same paper.

(10) A. Jeanes, C. S. Wise and D. J. Dimler, *Anal. Chem.*, **23**, 415 (1951).

(11) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 2511 (1949).

(12) S. A. Barker and E. J. Bourne, *ibid.*, 209 (1952).

Anal. Calcd. for $C_{40}H_{54}O_{27}$: C, 49.67; H, 5.63. Found: C, 49.40; H, 5.48.

Hydrolysis of α-Maltosyl β-D-Fructoside by Yeast Invertase.—The trisaccharide (15 mg.) was dissolved in 0.1 ml. of a 1% aqueous solution of Wallerstein Blue Label invertase.[13] After 180 minutes a sample was removed to paper, steamed and irrigated. All papergrams were irrigated downward with *n*-butanol 3, pyridine 1, water 1.5.[11] Two products were shown: a reducing, non-ketose-containing disaccharide (R_S 0.75) and fructose (R_S 1.51). For identification, 145.4 mg. of the trisaccharide was dissolved in 1 ml. of water to which was added 1 ml. of 1% Wallerstein invertase. After 45 minutes at 37° it was heated to boiling, cooled, filtered and the filtrate evaporated to dryness under reduced pressure. It was subjected to partitition chromatography on a powdered cellulose–diatomaceous earth (2:1) column using as a solvent the upper phase of a mixture of butanol 4, ethanol 1, water 5.[14] The filtrate was collected in 1-ml. fractions. Fractions 60–80 contained fructose, fraction 75 a trace of glucose, and fractions 85–150 a disaccharide. These last fractions were combined and from them was obtained 92.8 mg. of the disaccharide, 95% of calcd. This material was reducing, contained no fructose, was not split by yeast invertase, was hydrolyzed to glucose by honey invertase, and could not be differentiated from maltose by paper chromatography. It was crystallized from aqueous ethanol; the X-ray powder diffraction pattern of the crystalline product agreed in all respects with that of an authentic sample of maltose hydrate. The disaccharide produced by yeast invertase from the trisaccharide was therefore maltose.

The monosaccharide could not be differentiated from fructose by paper chromatography with fructose and by reaction to naphthoresorcinol, TTC and benzidine reagents.

Hydrolysis of α-Maltosyl β-D-Fructoside by Honey Invertase.—The trisaccharide (15 mg.) was dissolved in 0.1 ml. of honey invertase solution. Samples were removed for paper chromatography at 10 and 180 minutes, steamed and irrigated. The papergram of the 10-minute reaction showed in addition to the original trisaccharide (R_S 0.59) a ketose-containing, non-reducing disaccharide (R_S 1.01) and glucose (R_S 1.36). Only glucose and fructose (R_S 1.56) were found on the papergram of the 180-minute reaction.

For identification of the intermediate disaccharide, 110 mg. of the trisaccharide dissolved in 0.77 ml. of a honey invertase preparation. The reaction was stopped by heating at 30 minutes, filtered, evaporated dry and the constituents separated by powdered cellulose column chromatography as above. Fractions 70–85 showed glucose, 80–105 the disaccharide, and 105–191 contained unreacted trisaccharide. The appropriate fractions were combined to yield 5.0 mg. of monosaccharide, 9.6 mg. of disaccharide and 63 mg. of unreacted maltosyl fructoside. Based upon trisaccharide which was not recovered, this is a 38% conversion to disaccharide.

The monosaccharide could not be distinguished from glucose by paper chromatography and reaction to spray reagents. The disaccharide was non-reducing, contained ketose, and travelled on the papergram with sucrose. It was crystallized by repeated evaporation from aqueous ethanol solution. The X-ray powder diffraction pattern of the crystalline disaccharide was identical in all respects with that of authentic sucrose.

When a solution of glucose and fructose is treated with the honey enzyme under these conditions, there is no reaction discernible by paper chromatography.

The intermediate disaccharide produced by action of honey invertase upon α-maltosyl β-D-fructofuranoside is therefore sucrose.

Another honey invertase hydrolysis of the trisaccharide was carried out under conditions which by preliminary experiments were found to produce a better yield of sucrose. Treatment of 145.8 mg. with 1 ml. of a honey invertase preparation and 0.3 ml. of 2 *M* acetate buffer of *p*H 4.05, was carried out at 25° for 2 hours. It was heated to inactivate the enzyme, filtered and subjected to partititon chromatography on the cellulose column. From the column 88.3 mg. of unreacted material was obtained, and also a fraction containing sucrose, glucose and a small amount of fructose. This latter fraction was analyzed for reducing sugar before and after inversion by dilute acid. It was found to contain 27.2 mg. of reducing sugar as glucose before hydrolysis and 62.0 mg. after hydrolysis. The increase, 34.8 mg., corresponds to 33.1 mg. of sucrose.

This is 85% of the theoretical yield of sucrose[15] from the 57.5 mg. of trisaccharide destroyed.

Mild Acid Hydrolysis of α-Maltosyl β-D-Fructoside.—The compound (15 mg.) was dissolved in 1.2 ml. of H_2O and 0.5 ml. of 5 *N* H_2SO_4 added. After heating to 70° it was allowed to stand 24 hours at room temperature. The acid was then neutralized with solid $BaCO_3$, filtered and evaporated dry. Paper chromatography showed that levulose was hydrolyzed from the trisaccharide, leaving maltose, the same disaccharide formed by yeast invertase. The greater sensitivity of the sucrose linkage to acid hydrolysis is well known.

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(13) Mention of trade names does not imply endorsement by the Department over similar products not mentioned.

(14) S. M. Partridge, *Nature*, **158**, 270 (1946).

(15) The remaining 15% of the sucrose was further hydrolyzed to glucose and fructose. Complete conversion of this portion would produce 6.2 mg. of invert sugar, which when added to the 20.6 mg. of glucose, from the hydrolysis of the trisaccharide would total 26.8 mg. of reducing sugar. Actually 27.2 mg. was found.

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