Isolation of 1-kestose and nystose by chromatography on a cation exchange resin

In studies of the transformation of sugars in sugar beets during storage it was desirable to determine 1-kestose [O-α-D-glucopyranosyl-(1 → 2)-O-β-D-fructofuranosyl-β-D-fructofuranoside] quantitatively by measuring the density of spots on chromatographic paper sheets developed by alkaline silver nitrate. A supply of 1-kestose was required as a standard because different sugars give different densities upon reaction with alkaline silver nitrate. Kestose is not commercially available, but preparations of several kestoses by paper chromatography and 1-kestose by carbon column chromatography have been described[1]. Recently BINKLEY isolated both 1-kestose and 6-kestose from cane final molasses by column chromatography with several different stationary phases[2]. BINKLEY AND ALTENBURG also isolated a tetrasaccharide fructosyl-1-kestose [O-α-D-glucopyranosyl-(1 → 2)-O-β-D-fructofuranosyl-(1 → 2)-O-β-D-fructofuranosyl-(1 → 2)-β-D-fructofuranoside] which was named nystose[3]. They obtained this sugar from a mixture of oligosaccharides by column chromatography with granular carbon as the adsorbent.

Procedures described for separating sugar mixtures[1] include paper chromatography[4], gas–liquid partition chromatography[5], thin-layer chromatography[6], gel filtration[7], and chromatography on ion exchange resins[8]. Chromatography on ion exchange resins appears to be one of the simplest methods available and it seemed worthwhile to describe the preparation of crystalline 1-kestose and nystose by this procedure.

Experimental

Preparation of oligosaccharide mixture. After the method of GROSS[2], 20 g of sucrose in 80 ml of water and 0.2 *M* phosphate buffer (2 ml) of pH 7 was incubated 48 h with a dialyzed solution of Taka-diastase* (10 g) in water (50 ml) for 24 h at 20°, boiling for 3 min stopped the reaction. The solution was freed from coagulated protein by filtration.

Paper chromatography. Paper chromatography was run in the organic layer of a mixture of 1-butanol, glacial acetic acid, and deionized water (4:1:5, v/v). A sample of 1 μl (about 10% solids) was placed on Schleicher & Schüll No. 2043-B paper sheets and allowed to develop descending for 20 h. Air-dried papers were dipped in an indicator containing 1 ml of saturated silver nitrate in 200 ml of acetone, dried, and dipped in 0.5% sodium hydroxide in ethanol. The sheets were air dried for about 1 h, then dipped first into saturated sodium thiosulfate in 60% alcohol and then into 60% ethanol. The alcohol-washed chromatograms were dried in air.

Separation of oligosaccharides. A column 4.5 cm across × 167 cm high with a coarse fritted disc as a support was prepared. The resin bed (4.5 cm × 122 cm) was formed from slurry of 200–400 mesh Dowex 50W X4 (4% cross-linkage with divinylbenzene in the K^+ form). The resin was conditioned and eluted with 0.2% potassium benzoate to prevent microbial growth and 2.5 mmoles each of glucose, sucrose, and

* Diastase, Pharmaceutical Grade (*Aspergillus oryzae*), Mann Research Laboratory, Inc., New York 6, N. Y. Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

raffinose (5.57 g) in 25 ml was separated at a flow rate of 0.5 ml/min to test the efficiency of the column. Two-ml fractions were collected and assayed by paper chromatography. The recovery of raffinose crystallized from ethanol–water was 99 %. Glucose and sucrose were separated, but no attempts were made to crystallize them.

An amount of 25 ml of oligosaccharide mixture (10 % solids) was added to the top of the column and allowed to drain to the top of the resin. The developing solvent, 0.2 % potassium benzoate at pH 7.3, was then added carefully, and elution commenced at a flow rate of 0.5 ml/min.

Two-ml fractions from 200 tubes were assayed by paper chromatography as described and were grouped as 1-kestose, tetrasaccharide(s), and higher oligosaccharides. Fig. 1 shows a typical fractionation for the preparation of 1-kestose and nystose. The 1-kestose and nystose fractions were combined separately and evaporated to 100 ml each.

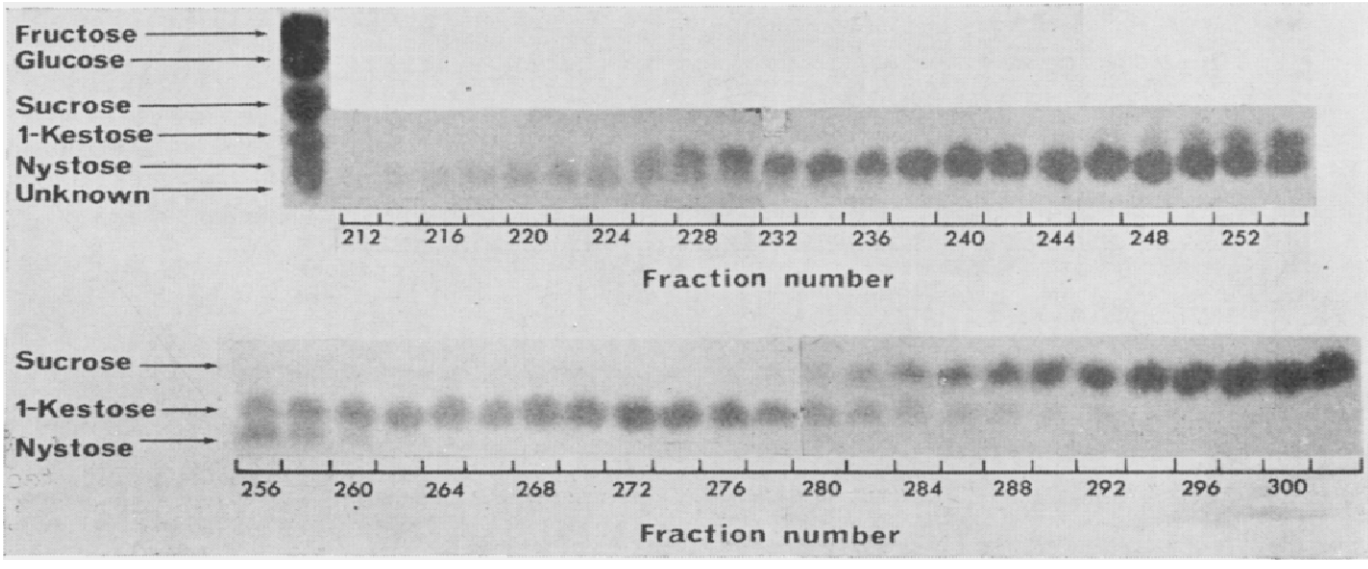

Fig. 1. A typical fractionation of the oligosaccharide mixture on an ion exchange resin and portions analyzed by paper chromatography.

Crystallization of 1-kestose and nystose. Potassium benzoate was removed from the sugar fractions by batch ion exchange in about 30 min at 5°. A magnetic stirrer agitated 1 g of 50–100 mesh Dowex 50W X8 (H^+) with 2 g of 60–80 mesh Permutit A (OH^-). The resin was filtered off and the filtrates containing the samples were adjusted to pH 7.5–8.0 with dilute ammonium hydroxide and evaporated to dryness at 40°. Three runs combined yielded 1.2 g of 1-kestose syrup and 1.3 g of tetrasaccharide syrup. The first syrup taken up in 2 ml of anhydrous methanol and seeded with crystals of authentic 1-kestose crystallized overnight. Upon recrystallization from water and anhydrous methanol, fine white crystals of 1-kestose were obtained. From the second syrup the tetrasaccharide, nystose, crystallized as elongated plates from anhydrous methanol[3]. The compounds gave single spots by paper chromatography. Recrystallized 1-kestose melted at 198–200°, $[\alpha]_D^{25}$ +28.4° (*c* water 2 %), $R_{glucose}$ = 0.187. Recrystallized nystose melted at 130–133°, $[\alpha]_D^{25}$ +9.7° (*c* water 2 %), $R_{glucose}$ = 0.104. Melting points are uncorrected and were measured on a Kofler hot stage. Both 1-kestose and nystose are non-reducing and gave negative RAYBIN tests[9].

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