Note

Separation of neutral sugars and uronic acids in the D-glucuronic acid–D-xylulose (D-*threo*-2-pentulose) pathway

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Chromatography on anion exchangers in the borate[1–6] or acetate[7–12] form has been used for the separation of mixtures of aldoses, ketoses, alditols, uronic acids, and aldonic acids. During the course of our study on carbohydrate metabolism in liver homogenate we required a method of separating mixtures of metabolic intermediates, especially those which are related to the D-glucuronic acid–D-xylulose (D-*threo*-2-pentulose) pathway, on a single column. We have therefore modified the method described by Khym and Zill[1] and that by Ishidate *et al.*[6] for the separation of these carbohydrates.

EXPERIMENTAL

The ion-exchange resin (Dowex 1-X8. 200–400 mesh, Cl^-) was converted to the borate form by washing successively with 5 vol. of 2M hydrochloric acid, with 4 vol. of distilled water, and finally with 0.1M sodium tetraborate until no chloride ion was detected in the eluate. The resin thus prepared was packed in a 1-cm column to a 4-cm bed height. The column was washed with distilled water (30 ml) before application of a sample. A carbohydrate mixture containing 1–50 μmoles of each component was dissolved in 5 ml of 7.5mM sodium tetraborate at 20°. After 15 min, the mixture was adsorbed on the column in the usual method and eluted stepwise with borate buffers of various concentration, at a flow rate of 1.6 ml/min. Fractions (10 ml) were collected, and the sugar content was determined by colorimetric analysis. Ketose was determined by the cysteine–carbazole method of Dische[13]; aldose by the anthrone method developed by Morris[14], sugar alcohol by the method of West and Rapoport[15], and uronic acid was oxidized with periodic acid and the resulting glyoxalic acid was determined by the method described by Ishidate *et al.*[6].

RESULTS

Separation of the following non-phosphorylated intermediates of the D-glucuronic acid-D-xylulose pathway was achieved; D- and L-xylulose, xylitol, L-gulonic acid,

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D-glucuronic acid, ascorbic acid, and D-glucaric acid (Fig. 1). Since tissue preparations usually contain such other sugars as D-glucose and D-fructose, the elution positions

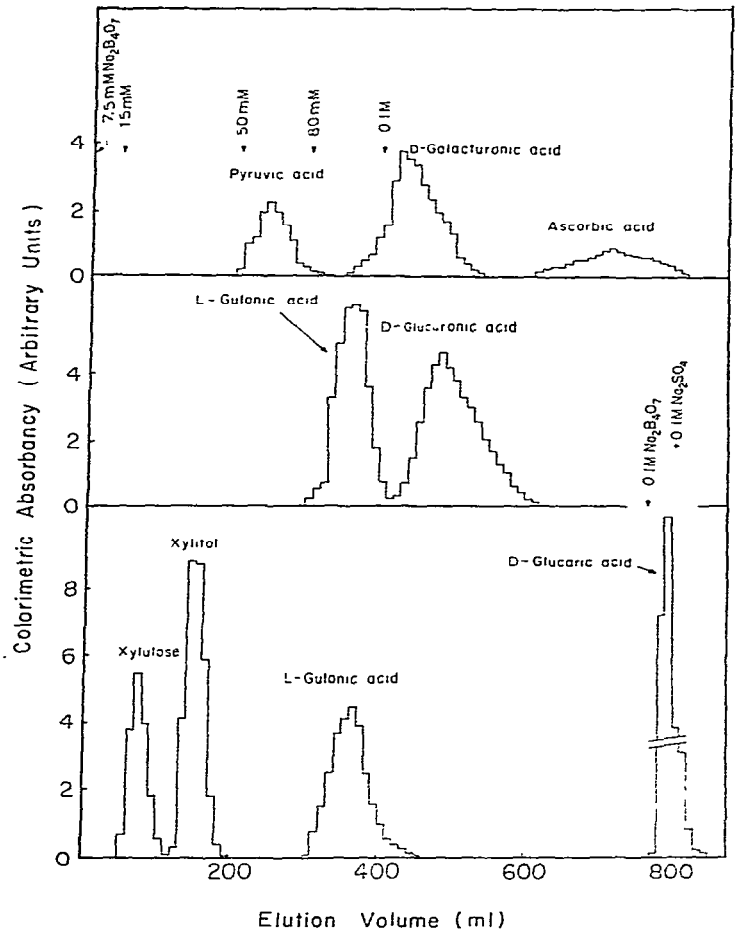

Fig. 1. Chromatography of various carbohydrates on ion-exchange resin. Samples (5 μmoles) were dissolved in 7.5mM sodium tetraborate solution and chromatographed on Dowex 1 ion-exchange resin (X-8, 200–400 mesh, 1 × 5 cm). The sugar concentration of each fraction (10 ml) was measured colorimetrically as described in the Experimental Section.

of various sugars are compared diagramatically in Fig. 2, which also summarizes the results of other workers. Xylulose was eluted immediately after sucrose and was followed by xylitol[16]. The separation of various polyols on an ion-exchange resin was reported by Spencer[4]. Separation of L-gulonic, D-glucuronic, and ascorbic acids was clearly achieved by the stepwise increase in the borate concentration as shown in Fig. 2. D-Glucuronic and L-gulonic acids could not be separated from their respective lactones. D-Glucaric acid, which was reported by Marsch[17] to be derived from D-glucuronic acid, could be eluted from the same column with 50mM sodium-

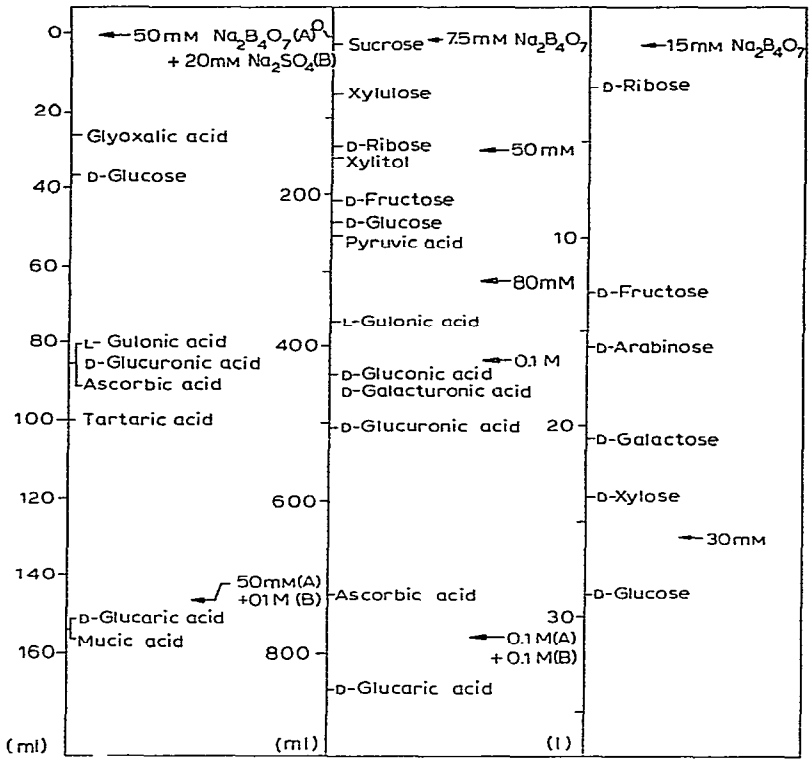

Fig. 2. Comparison of the elution order of various carbohydrates in Dowex-1 column chromatography. Left: Ishidate *et al.*[6] (0.9 × 4 cm column); middle: Present work (1 × 4 cm column); right: Khym and Zill[2] (0.5 × 11 cm column).

tetraborate–0.1M sodium sulfate[6]. D-Gluconic acid and D-galacturonic acid were eluted in the same fraction and could not be separated. Separation of D-galacturonic acid from D-glucuronic acid was reported by Hallén[5] on an anion-exchange resin as borate complexes.

The proportions of each sugar recovered by the present method varied from 96 to 104%. The method was successfully applied to the analysis of guinea-pig liver homogenate[18].

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