Selective separation of ketoses and aldoses by chromatography on a polyethyleneimine ion exchanger

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Ketoses, as a result of significantly lower retention, can be separated from aldoses by chromatography on a polyethyleneimine ion exchanger eluted with water. Efficient group separation of nine ketoses and fourteen aldoses is demonstrated. A method for characterization of ketoses on the basis of their specific rotation in aqueous solutions of molybdate is also presented.

Хроматографически отделяются кетозы от альдоз при элюировании с водой на полиэтилениминовом ионообменнике вследствие значительно меньшего удерживания. Представлено эффективное отделение девятичленной группы кетоз от группы 14-ти альдоз. Описывается также характеристика кетоз по их удельному вращению в водных растворах молибдата.

One of serious problems of preparative carbohydrate chemistry is the separation of aldoses from ketoses. Of a large number of chromatographic procedures for separation of aldoses and ketoses described in the literature, only the use of anion exchangers in the bisulfite form afforded satisfactory resolution. *Samuelson* and *Sjoström* [1] separated fructose from glucose and mannose on a Dowex 2 ion-exchange resin using a stepwise elution with differently concentrated ethanol. Separation of fructose, sorbose, and tagatose from aldoses was achieved on an ion-exchange resin Amberlit IRA-400 eluted with *n*-propanol [2]. Suitable application of Wofatit SBW in *n*-propanol led to the separation of ribulose from ribose and arabinose [3]. The ion exchangers in the bisulfite form enable effective separation of ketoses and aldoses only when alcohols are used as eluants. In this paper we describe a selective class separation of ketoses and aldoses on a polyethyleneimine ion exchanger in the Cl or OH form eluted with water.

We have investigated chromatographic separation of ketoses from aldoses on an ion exchanger Amberlit IR-120 cycled with hydrazine and using water as eluant. Even though this procedure afforded satisfactory results, the resin in the column lost its efficiency after the first run as a consequence of progressive deprivation of the cycling agent due to its disproportion to ammonia and nitrogen.

The polyethyleneimine ion exchanger (PEI) prepared by cross-linking polyethyleneimine with epichlorohydrin (a modified procedure given in Ref. [4]) was found to be suitable for a selective chromatographic separation of ketoses and aldoses. The efficiency of the separation on a PEI ion exchanger consists in a significantly lower retention of ketoses than aldoses. Contrary to the separation of ketoses and aldoses on ion exchangers in the bisulfite form which requires gradient elution with a mixture alcohol—water, in the case of PEI, water is used as the only eluant, so that the technical difficulties of the gradient elution are avoided. The PEI ion exchanger both in the OH and Cl form selectively separates ketoses and aldoses. However, on the OH form much higher adsorption of aldoses occurs than on the Cl form. Moreover, the PEI in the Cl form is more stable and therefore it can be used repeatedly more than ten times without any changes in the efficiency of the separation. A group of ketoses (D-ribulose, D-xylulose, D-psicose, D-fructose, L-sorbose, D-tagatose, D-glucoheptulose, D-mannoheptulose and lactulose) was effectively separated from a group of aldoses (D-ribose, L-arabinose, D-xylose,

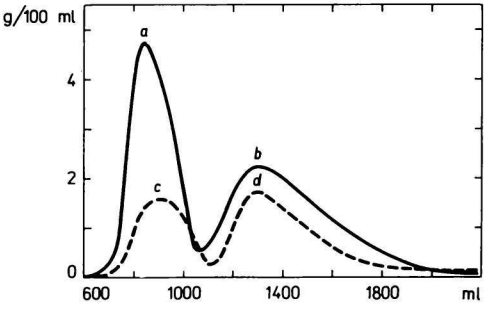

Fig. 1. Chromatographic separation of hydrolysate of saccharose (———) and transformation mixture of D-ribose (– – – –) on a PEI ion exchanger in the Cl form eluted with water. *a*) D-Fructose; *b*) D-glucose; *c*) D-ribulose; *d*) mixture of D-ribose and D-arabinose.

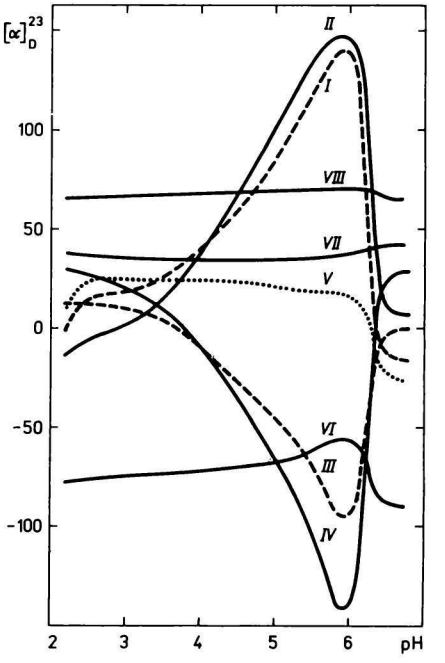

Fig. 2. Dependence of specific rotation of ketoses in aqueous solutions of molybdate on pH. *I.* D-Ribulose; *II.* D-psicose; *III.* D-tagatose; *IV* D-mannoheptulose; *V* D-xylulose; *VI.* D-fructose; *VII.* D-sorbose; *VIII.* D-glucoheptulose.

D-lyxose, D-allose, D-altrose, D-glucose, D-mannose, D-galactose, D-talose, D-*glycero*-D-galactoheptose, D-*glycero*-D-taloheptose, D-*glycero*-D-guloheptose, and D-*glycero*-D-idoheptose) on PEI in the Cl form. A satisfactory separation of ketoses and aldoses, *e.g.* D-fructose from D-glucose, or D-ribulose from a mixture of D-ribose and D-arabinose (Fig. 1) can be achieved already at a weight ratio 8 : 1 of the air-dried PEI in the Cl form to the saccharides applied. During chromatographic separation saccharose and alditols do not exhibit a significantly different retention from those of ketoses. Similarly, an attempt to separate lactulose and lactose was not successful.

Table 1

Specific rotation of ketoses

Ketose	$[\alpha]_D^{23}$	
	4% aqueous solution of ammonium molybdate	Water
D-Ribulose	+ 140	− 17
D-Psicose	+ 148	+ 3
D-Tagatose	− 95	− 4
D-Mannoheptulose	− 141	+ 29
D-Xylulose	+ 17	− 33
D-Fructose	− 56	− 92
D-Sorbose	+ 38	+ 43
D-Glucoheptulose	+ 71	+ 66

Specific rotations of ketoses measured in aqueous solutions of molybdate of pH values 2.2—6.8 show extreme values at pH 5.9 (Table 1, Fig. 2) similarly as aldoses [5]. These remarkable changes in specific rotation of some ketoses at pH 5.9 (4% aqueous solution of ammonium molybdate) provide a possibility for a continual polarimetric monitoring of column effluents during large-scale chromatographic separations of some mixtures of ketoses and aldoses.

Experimental

Chromatographic separation of saccharide mixtures on polyethyleneimine ion exchanger was followed by chromatography on Whatman No. 1 paper using solvent systems for pentuloses and pentoses, methyl ethyl ketone—*n*-butanol—water (16 : 2 : 1), for alditols, cyclohexanol—pyridine—water (saturated with boric acid) (6 : 5 : 2) and for other saccharides, *n*-butanol—ethanol—water (5 : 1 : 4). Large-scale separations (hydrolysate of saccharose or D-ribose transformation mixture) were also monitored polarimetrically.

Preparation of polyethyleneimine ion exchanger

Polyethyleneimine (200 g of 50% dry weight, preparation from Fluka, Polyimin P, molecular weight 30 000—40 000) was dissolved in water (200 ml) and under intense stirring at room temperature mixed with epichlorohydrin (20 g). The solution was left to stand for 1—2 h below 50°C. The cross-linked polyethyleneimine was suspended in water and disintegrated in a blender to required magnitude of particles (100—300 mesh).

The product had an exchange capacity of 1—1.5 mval/g and being in the Cl form a bed volume in water 4—4.5 ml/g. It was stored in the Cl form (after being washed with methanol) in dark containers.

Chromatographic separation of saccharides on polyethyleneimine ion exchanger

Chromatographic separation of saccharides was performed on a polyethyleneimine ion exchanger cross-linked with epichlorohydrin, in the Cl form, using two columns having dimensions 2×125 cm (column *A*) and 2.8×150 cm (column *B*) eluted with water at a flow rate of 30 and 50 ml/h, respectively.

Separation of mixtures of aldoses and ketoses (D-ribulose, D-xylulose, D-psicose, D-fructose, L-sorbose, D-tagatose, D-glucoheptulose, D-mannoheptulose, L-arabinose, D-ribose, D-xylose, D-lyxose, D-allose, D-altrose, D-glucose, D-mannose, D-galactose, D-talose, *D-glycero*-D-galactoheptose, D-*glycero*-D-taloheptose, D-*glycero*-D-guloheptose, and *D-glycero*-D-idoheptose) was examined on column *A*. Total amount of applied saccharides was 4 g (one saccharide in amount 400 mg) including saccharose as an internal standard. Column *A* was further used for attempts to resolve a mixture of alditols (ribitol, D-arabinitol, xylitol, D-glucitol, D-mannitol, galactitol) and a mixture of disaccharides (lactose, lactulose, and saccharose). All ketoses (including lactulose), alditols, and the internal standard saccharose were eluted in fraction *1* in elution volume 300—400 ml. All aldoses were eluted in fraction *2* in elution volume 450—1000 ml, whereas lactose emerged in elution volume 250—900 ml.

Column *B* was used for the separation of D-fructose and D-glucose present in the hydrolysate of saccharose (25 g of saccharose, 50 ml of water, 5 ml of acetic acid, 3 h at 90°C) and for the isolation of D-ribulose from a mixture with D-ribose and D-arabinose obtained by transformation of D-ribose in pyridine (25 g of D-ribose, 75 ml of anhydrous pyridine, 5 h at 110°C). Fraction *1*, elution volume 700—1050 ml, contained D-fructose (12.5 g) and D-ribulose (5.3 g), respectively, and fraction *2*, elution volume 1100—2200 ml, contained aldoses (Fig. 1). Chromatographically homogeneous D-ribulose not containing D-xylulose was obtained after rechromatography on an ion-exchange resin Dowex 50 W in the Ba form [6].

Effect of pH on specific rotation of ketoses in molybdate solutions

Specific rotation of ketoses (D-ribulose, D-xylulose, D-psicose, D-fructose, D-sorbose, D-tagatose, D-glucoheptulose, D-mannoheptulose) was measured on a Perkin—Elmer polarimeter, type 141. Effect of pH on their specific rotation at 2% concentration in the

presence of molybdate was followed either in 4% aqueous solutions of molybdenic acid, ammonium molybdate, and sodium molybdate or in solutions prepared by mixing the above molybdate solutions to give desired pH (Table 1, Fig. 2).

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