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Note

Improved separation of anomers of saccharides by high-performance liquid chromatography on macroreticular anion-exchange resin in the sulphate form

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Saccharides can be separated into their anomers by gas–liquid chromatography (GLC) following conversion into trimethylsilyl derivatives[1]. Ramnäs and Samuelson[2] achieved such separations by partition chromatography on an anion-exchange resin (SO_4^{2-}) at $-10°$ with aqueous ethanol as eluent. This method needs no prederivatization and is easily applicable to the preparative purpose. It is lengthy, however, more than 6 h being required to separate the α- and β-anomers of D-glucose. This drawback must be overcome if liquid chromatography is to be applied to the kinetic study of mutarotation of saccharides[1,3,4].

This note reports the rapid separation of anomers of several kinds of saccharides, including disaccharides, on a recently developed macroreticular anion-exchange resin (SO_4^{2-}) with a relatively high degree of cross-linking at room temperature using ethanol–water as the eluting solution.

METHOD

A strongly basic macroreticular anion-exchange resin (5–7 μm, Cl^-, CDR-10; Mitsubishi, Tokyo, Japan), which was made by introducing tertiary ammonium groups into a porous polymer based on styrene–divinylbenzene (35%) matrix[5], was treated with 1% sodium sulphate solution to convert it into the sulphate form. The resin was mixed with sodium sulphate–saturated 80% ethanol. The slurry was ultrasonized for 10 min and poured into a 30-ml slurry reservoir assembled to a stainless steel column (250 × 4.0 mm I.D.). The column was packed by pumping 80% ethanol at a flow-rate of 2 ml/min and conditioned by passing 200 ml of the same solution at 1 ml/min.

The chromatographic system included a mini pump (Milton Roy), a bellows-type damper (Umetani Seiki, Osaka, Japan), a 150-kg/cm^2 pressure gauge (Kyowa Seimitsu, Tokyo, Japan) and a syringe-loading sample injection valve (Model 7120; Rheodyne, Calif., U.S.A.) with a 20-μl loop. The effluent was monitored with a high sensitivity differential refractive index detector (RI-2; Japan Analytical Industry Co., Tokyo, Japan). 20 μl of a sample solution containing a concentration of each saccharide of 0.1–0.4% (w/w) was applied to the column. An operating temperature was controlled by circulating thermostated water in a methacrylic resin jacket attached to the column.

MATERIALS

Standard saccharide samples were obtained from Tokyo Kasei Co. (Tokyo, Japan). α-Anomers of D-glucose, D-galactose and D-xylose were prepared by recrystallization of supplied samples from ethanol, and β-anomers from pyridine.

RESULTS AND DISCUSSION

Fig. 1 shows that several saccharides were well separated at 20°. The separation of monosaccharides was enhanced by using an eluent with higher ethanol content (Fig. 1b). These chromatograms, however, were complicated by the presence of anomers of saccharides, as will be mentioned later.

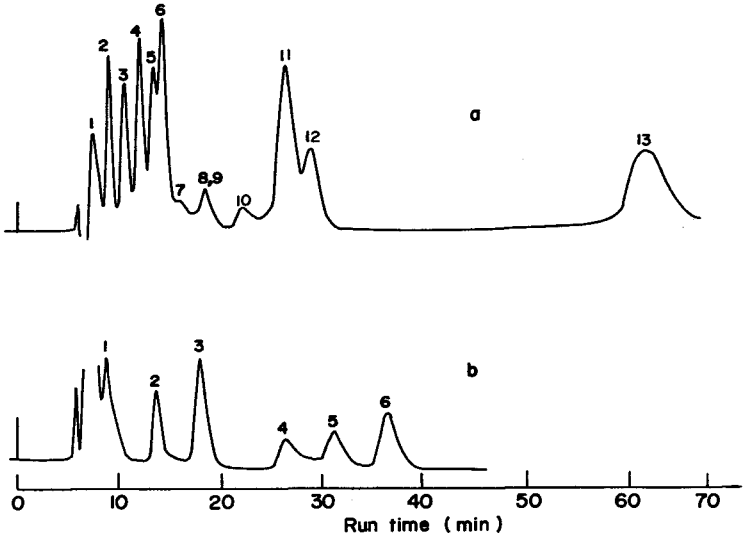

Fig. 1. Chromatogram of saccharide mixture on a macroreticular anion-exchange resin column at 20° with (a) 80% ethanol (inlet pressure, 73 kg/cm²) and (b) 90% ethanol (67 kg/cm²) as eluent. Sample size, 10–80 μg each; flow-rate, 0.9 ml/min; detector, refractive index. Peaks: 1 =2-deoxy-D-Rib; 2 = D-Fuc; 3 = D-Rib; 4 = α-D-Man; 5 = α-D-Gal; 6 = α-D-G; 7 = β-D-Man; 8 = β-D-Gal; 9 = β-D-G; 10 = α-Lac; 11 = β-Lac; 12 = Suc; 13 = Raf.

The effect of temperature on the separation of saccharides with 90% ethanol as eluent is illustrated in Fig. 2. D-Ribose and L-arabinose were not separated at 20° (Fig. 2a), but they were at 50°. However, at the higher temperature D-glucose and D-galactose have a same retention time and anomers were not separated.

With 95% ethanol as eluent, 2-deoxy-D-ribose, L-rhamnose, D-fucose and D-ribose were completely separated, whereas D-mannose, D-glucose and D-galactose were not eluted (Fig. 3).

Injection of α-D-glucose within 30 min of dissolution in the eluting solution gave the chromatogram shown in Fig. 4a at 20°, where a major peak and a minor second component are present. On the other hand, the β-anomer exhibited in its

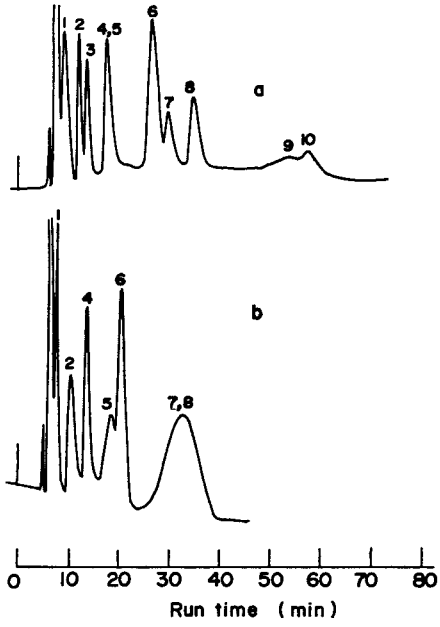

Fig. 2. Chromatogram of saccharide mixture with 90% ethanol as eluent at (a) 20° (inlet pressure, 50 kg/cm²) and (b) 50° (inlet pressure, 35 kg/cm²). Other conditions as in Fig. 1. Peaks: 1 = 2-deoxy-D-Rib; 2 = D-Rha; 3 = D-Fuc; 4 = D-Rib; 5 = L-Ara; 6 = D-Fru; 7 = α-D-Gal; 8 = α-D-G; 9 = β-D-Gal; 10 = β-D-G.

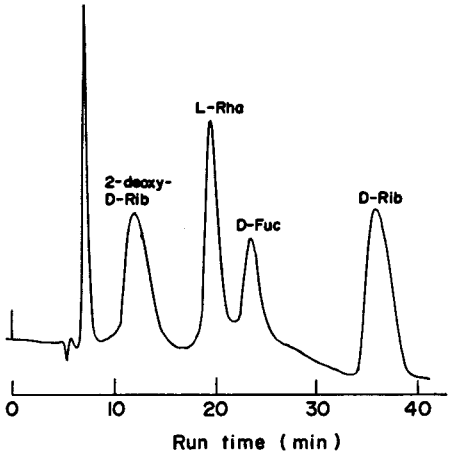

Fig. 3. Chromatogram of a saccharide mixture with 95% ethanol as eluent at 20° (inlet pressure, 50 kg/cm²). Other conditions as in Fig. 1.

chromatogram a major peak corresponding exactly with the minor peak of the α-anomer and a minor peak at the major peak (Fig. 4b). After equilibration (2 days after dissolution), each anomer showed the same chromatogram with two almost equivalent peaks (Fig. 4c). This is also the case for D-galactose and D-xylose (Fig. 5).

The separation of anomers of L-rhamnose, D-fucose, L-arabinose, D-mannose, maltose and lactose (see Table I) was also achieved.

Fig. 4. Separation of the anomers of D-glucose; (a) α- anomer and (b) β-anomer injected within 30 min after dissolution and (c) α-anomer after equilibration in 80% ethanol. Conditions as in Fig. 1.

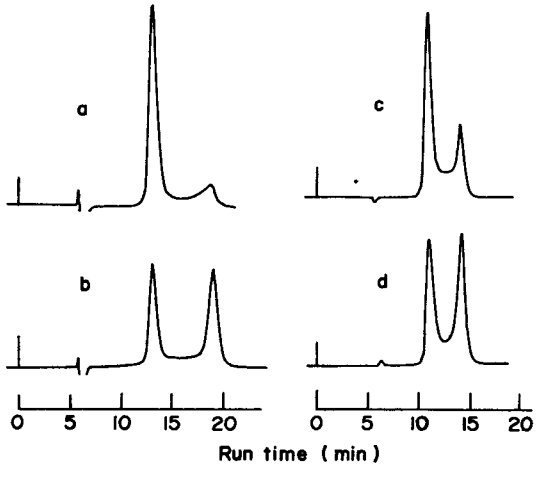

Fig. 5. Separation of the anomers of D-galactose (a, α-anomer injected within 30 min after dissolution and b, after equilibration) and those of D-xylose (c and d correspond to a and b, respectively). Conditions as in Fig. 1.

TABLE I

VALUES OF CAPACITY FACTORS (k') OF SACCHARIDES DETERMINED ON A SULPHATE FORM MACRORETICULAR ANION-EXCHANGE RESIN COLUMN

Eluent, 80% ethanol; flow-rate, 0.9 ml/min; temperature, 20°; detector, refractive index.

*Saccharides**	*Anomer*		
	α	*β*	
2-Deoxy-D-rib			1.29**
D-Rib			1.80**
L-Rha	1.44	1.80	
D-Fuc	1.55	2.10	
L-Ara	1.90	2.74	
D-Xyl	1.94	2.48	
D-Man	2.07	2.85	
D-Fru			2.17**
D-Sor			2.42***
D-Gal	2.42	3.54	
D-G	2.50	3.25	
Lac	3.90	4.63	
Mal	4.04	5.67	
Suc			4.92***
Raf			10.8***
D-GNAc	1.83	2.56	
Mannitol			2.58***
D-GA-lactone			3.96***

* Conventions regarding abbreviations of the names of carbohydrates (*J. Org. Chem.*, 28 (1963) 281) are followed.

** Anomers not discriminated.

*** Anomers not present.

The chromatographic system presented here for the rapid separation of anomers of saccharides may provide an alternative methodology for the study of anomeric effects[6].

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