Journal of Chromatography, 179 (1979) 376–380

CHROM. 12,168

Note

Separation of acidic and neutral saccharides by high-performance gel chromatography on cation-exchange resins in the H^+ form with acidic eluents

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(First received May 8th, 1979; revised manuscript received June 29th, 1979)

High-performance liquid chromatography has been applied to the separation of neutral saccharides; anion-exchange chromatography with a borate eluent[1–3], partition chromatography on ion-exchange resins[4–13] or on chemically modified silica gels[14,15], and gel chromatography[3,16–18].

On the other hand, acidic saccharides have been separated by time-consuming anion-exchange chromatography[19–22]. A recent report[23] described separations of uronic acids from neutral saccharides by partition chromatography on cation-exchange resins using *n*-propanol–water as eluent.

Here we describe a high-performance gel chromatographic method for separating neutral and/or acidic saccharides on cation-exchange resins in the H^+ form, based primarily on the difference in molecular size, and its application to the analysis of the acid hydrolysate of plant gum isolated from the sap of the lacquer tree (*Rhus succedanea*).

EXPERIMENTAL

All of the packing materials used were sulphonic acid-type cation-exchange resins based on a styrene–divinylbenzene matrix. A pre-packed column (600 × 7.6 mm) of cation-exchange resins (TSK-gel LS-212, Na^+ form, 12 ± 2 μm, degree of cross-linking 21%; Toyo Soda, Tokyo, Japan) was treated with 500 ml of 1% orthophosphoric acid at a flow-rate of 1 ml/min, and then with 500 ml of 0.1% orthophosphoric acid (column I); when 0.5% acetic acid was used as the eluent, the column was treated with 3% acetic acid and then with the eluting soltuion. A pre-packed column (500 × 8 mm) of cation-exchange resins in the H^+ form (Shodex Ionpak C-811, 12.5 μm, degree of cross-linking 8%; Showa Denko, Tokyo, Japan) (column II) and a home-made column of cation-exchange resins prepared by sulphonation of porous polystyrene gels with a degree of cross-linking of 35% (Hitachi-gel 3011-s, 12.5 ± 2 μm) (column III) were also tested.

The chromatographic instrument was equipped with a Milton Roy mini-pump, a damper (Umetani Seiki, Osaka, Japan), a 150 kg/cm² pressure gauge (Kyowa Seimitsu, Tokyo, Japan) and a sample injector (Model 7120; Rheodyne, Riviera Beach, Fla., U.S.A.). Effluent was monitored by an RI detector (SF-1107, LDC) and a UV detector (UVIDEC-100; JASCO) at 205 nm.

Chromatography was carried out at room temperature at a flow-rate of 0.7–0.9 ml/min and at a pressure *ca.* 45 kg/cm². A 100-μl volume of a sample solution (0.1 · % or less) was applied to a column.

Standard mono- and oligosaccharides were purchased from Tokyo Kasei Kogyo (Tokyo, Japan) or Nakarai Kagaku (Kyoto, Japan) and FITC-dextran was obtained from Seikagaky Kogyo (Tokyo, Japan). The plant gum isolated from the sap of the lacquer tree (*Rhus succedanea*)[24] was hydrolysed with 0.5 *N* trifluoroacetic acid at 100° for 12 h and the hydrolysate was applied directly to a column.

No chemical change in the substrates was detected during passage through the columns.

RESULTS AND DISCUSSION

Neutral saccharides are well separated from each other based on differences in molecular size on column I using 0.1 % orthophosphoric acid as the eluent (Fig. 1). N-Acetyl-D-glucosamine and D-glucuronolactone showed longer retention times than would be expected from their molecular sizes, perhaps because of hydrophobic interaction between substrates and gels.

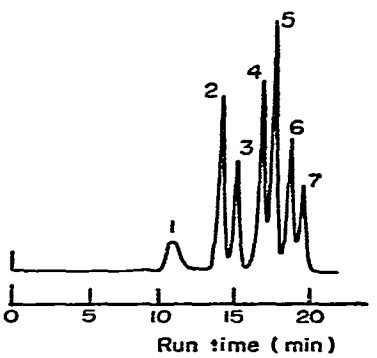

Fig. 1. Chromatogram of neutral saccharides. Column, TSK-gel LS212 (H^+), 600 × 7.6 mm; eluent, 0.1 % orthophosphoric acid; flow-rate, 0.8 ml/min; inlet pressure, 45 kg/cm²; sample size, 50–100 μg for each saccharide; detector, refractive index. Peaks: 1 = dextran (molecular weight 2900); 2 = raffinose; 3 = maltose; 4 = D-glucose; 5 = D-galactose; 6 = L-arabinose; 7 = D-ribose.

Fig. 2 shows the separation of D-glucuronic acid and D-galacturonic acid on column I with a resolution of 1.53. The retention times of uronic acids are close to those of the corresponding neutral saccharides. Use of 1 % orthophosphoric acid as the eluent resulted in a slight increase in the retention times of uronic acids, whereas those of neutral saccharides decreased. Although the molecular size of the substrate is a predominant factor in the separation of uronic acids, electrostatic repulsion between substrates and gels has a substantial effect and enhances the separation of D-glucuronic acid (pK_a 3.20)[25,26] and D-galacturonic acid (pK_a 3.42)[25,26].

Fig. 3 shows diagramatically the retention times measured for several saccharides relative to that of raffinose on column I using 0.1 % orthophosphoric acid as the eluent.

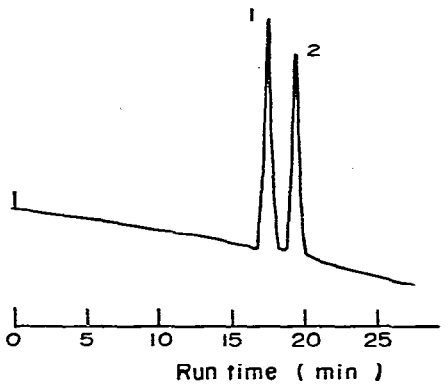

Fig. 2. Chromatogram of uronic acids. Chromatographic conditions as in Fig. 1. Peaks: 1 = D-glucuronic acid; 2 = D-galacturonic acid.

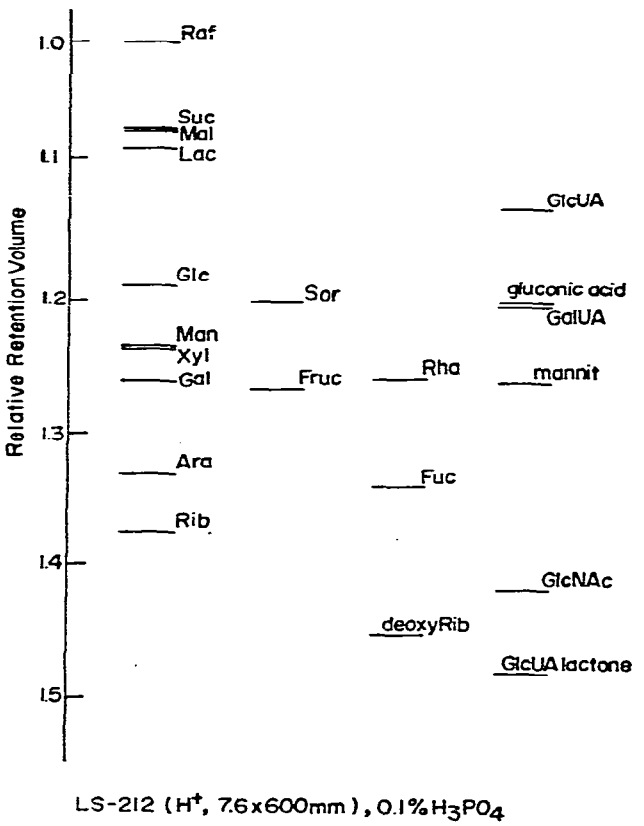

Fig. 3. Retention times of saccharides relative to that of raffinose. Chromatographic conditions as in Fig. 1.

On columns II and III a resolution of only 1.12 was obtained for uronic acids compared with 1.53 on column I. The pore sizes of the gels in columns II and III may be too large for the separation of monosaccharides.

On column III acidic saccharides exhibit considerably shortened retention

TABLE I

RETENTION TIMES (min) OF SACCHARIDES ON COLUMN III (HITACHI-GEL 3011-s 500 × 8 mm) AT A FLOW-RATE OF 1 ml/min

Saccharide	*Eluent*	
	0.5% acetic acid	*0.5% formic acid*
Raffinose	15.7	15.6
Lactose	16.5	16.5
D-Galactose	17.8	17.6
D-Glucuronic acid	13.8	16.7
D-Galacturonic acid	15.9	17.7

times with 0.5% acetic acid as the eluent than with 0.5% formic acid or 0.1% orthophosphoric acid (see Table I). This is advantageous for the identification of eluted saccharides.

In the chromatogram of the acid hydrolysate of plant gum from *Rhus succedanea* (Fig. 4), D-galactose (peak 3) and an aldobiouronic acid (peak 2) can be readily identified based on responses to 205-nm UV light (the detailed identifications of the peaks will be described elsewhere).

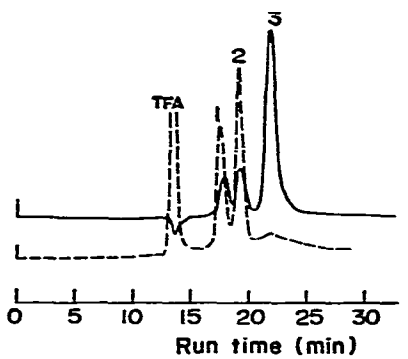

Fig. 4. Chromatogram of the acid hydrolysate of plant gum from the sap of the lacquer tree (*Rhus succedanea*). Chromatographic conditions as in Fig. 1, except for the flow-rate (0.65 ml/min). The solid line represents the separation pattern monitored by a refractive index detector and the broken line that monitored at 205 nm. Peaks 1 and 2 correspond to an acidic tri- or tetrasaccharide and an aldobiouronic acid, respectively, and peak 3 is D-galactose.

ACKNOWLEDGEMENTS

Dr. N. Takai of this Institute is thanked for the preparation of column III. The technical assistance of Mr. Y. Yamauchi of this laboratory with the chromatographic operations is appreciated. Thanks are also due to the instrumental Products Department of Showa Denko for obtaining a chromatogram of uronic acids on a Shodex Ionpak C-811 column.

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