Carbohydrate Research, 133 (1984) 157–162
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Separation of carbohydrates by l.c. in an aqueous, silica gel column

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(Received March 20th, 1984; accepted for publication, April 10th, 1984)

For the separation of neutral carbohydrates by liquid chromatography (l.c.), amino[1–4], nitrile[5], and carbohydrate[6] columns have generally been used with a mixture of acetonitrile and water, or acetonitrile, methanol, and ammonium formate buffer as the eluting solvents, but, in general, the low solubility of carbohydrates in these solvent systems may prevent their use for preparative separation.

A column of alkyl-modified silica, with acetonitrile and water as the eluant, was recently reported for use in the separation of oligomers from the hydrolyzates of starch, cellulose, and inulin[7]. However, this system was applied neither to analytical nor to preparative separation of mono- and di-saccharides from those mixtures.

Rocca and Rouchause[8] reported that, using an aqueous solvent system, a silica gel column was applicable for the separation of carbohydrates. Under a special treatment, we prepared a stable, aqueous column[9] of plain silica gel (which is now available under the trade name of "Aquasil") to use for both analytical and preparative separations of water-soluble glycosides by l.c.

Using this system, a new procedure for separation of oligosaccharides and alditols has been developed, without any problem regarding solubility in eluants, which could be applied to the column as an aqueous solution to obtain well defined separations. Monosaccharides can also be well separated by this column, using a solvent system consisting of ethyl formate, methanol, and water. We now describe the analysis of Smith-degradation products of lichen polysaccharides as a practical application of this procedure.

EXPERIMENTAL

Chromatography. — The liquid chromatograph which was used consisted of a degasser ERC 3110 (Erma Optical Works, Tokyo, Japan), an Altex 110A solvent-pumping system, ERC-7520 RI Detector (Erma Optical Works), and a

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0008-6215/84/$ 03.00

TABLE I

COMPONENTS AND THEIR VOLUMETRIC RATIOS IN MOBILE-PHASE SOLVENT-SYSTEM

Solvent system	*HCO_2Et*	*EtOAc*	*MeOH*	*H_2O*
Solvent A	12		3	1
Solvent B		12	3	1
Solvent C		7	3	2

Rheodyne model 7125 injection loop. Chromatograms were recorded on an SIC-7000A integrator (System Instruments Co., Tokyo). Experiments were performed on a Sensyu-Pak Aquasil SS-452N column (25 cm × 4.6 mm i.d.) (Sensyu Scientific Co. Ltd., Tokyo)[9]. All solvents were of "HPLC" grade.

Mobile phase. — The solvent systems used for the separations, and the volumetric ratios of each component, are shown in Table I.

Separation of the Smith-degradation products of polysaccharides. — (a) SJ-2-I, a polysaccharide of Stereocaulon japonicum. An aqueous solution (50 mL) of SJ-2-I (500 mg) and $NaIO_4$ (800 mg) was stirred for 125 h in the dark. The reaction mixture was treated with ethylene glycol (3 mL) for 0.5 h, and dialyzed in cellulose tubing. The inner solution was reduced with $NaBH_4$ (180 mg) for 20 h, treated with acetic acid, and dialyzed. The inner solution was evaporated to dryness, mixed with 0.05M H_2SO_4 (50 mL), and stirred for 6 h at room temperature; the acid was neutralized with Amberlite IRA-47(OH^-) and the solution evaporated to dryness.

(b) PC-3, a polysaccharide of Parmelia caperata. To PC-3 (100 mg) in a 110-mL flask was added 0.04M $NaIO_4$ (100 mL). After the oxidation, the mixture was dialyzed (cellulose tubing). The inner solution was treated with ethylene glycol (20 mg) for 1 h, and dialyzed again (cellulose tubing). The inner solution was reduced with $NaBH_4$ for 10 h, treated with acetic acid, and dialyzed. The products were treated as described for SJ-2-I.

RESULTS AND DISCUSSION

Fig. 1 shows the liquid chromatograms that were obtained by this method for rhamnose, xylose, arabinose, mannose, and glucose. Some disaccharides (sucrose, maltose, and lactose) were separated as well (see Fig. 2). In the case of oligomers of D-glucose, the separation was achieved within 12 min (see Fig. 3), so that this method is applicable to isolation of oligomers from the hydrolyzates of polysaccharides on a preparative scale. By decreasing the proportion of water in the solvent system, glucose, maltose, and maltotriose can be separated, with wide distances between peaks in the chromatogram, as shown in Fig. 4.

In order to determine the fine structure of a polysaccharide, Smith degradation is often used, and the products have usually been detected by paper partition-chromatography (p.p.c.). The present kind of l.c. can be used preparatively to obtain results more precise than those given by p.p.c. Takeda *et al.*[10] demonstrated

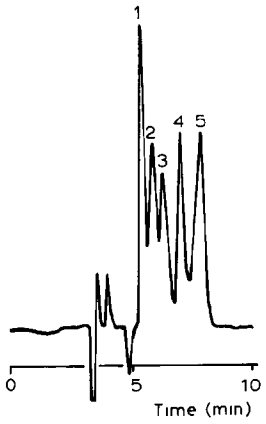

Fig. 1. 7.1-MPa l.c. profile of monosaccharides. [Column: Aquasil SS-452N (25 cm × 4.6 mm i.d.). Eluant: 12:3:1 EtO_2CH–MeOH–H_2O. Flow rate: 1.0 mL/min. Injection solvent: 12:3:1 EtOAc–MeOH–H_2O. Detector: RI. 1, Rhamnose; 2, xylose; 3, arabinose; 4, mannose; 5, glucose.]

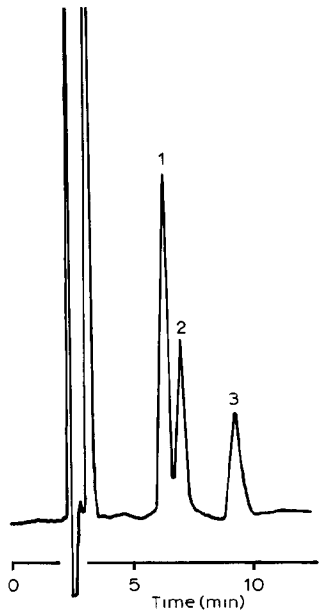

Fig. 2. 11.1-MPa l.c. profile of disaccharides. [Column: Aquasil SS-452N (25 cm × 4.6 mm i.d.). Eluant: 12:3:1 EtOAc–MeOH–H_2O. Flow rate: 1.5 mL/min. Injection solvent: H_2O. Detector: RI. 1, Sucrose; 2, maltose: 3, lactose.]

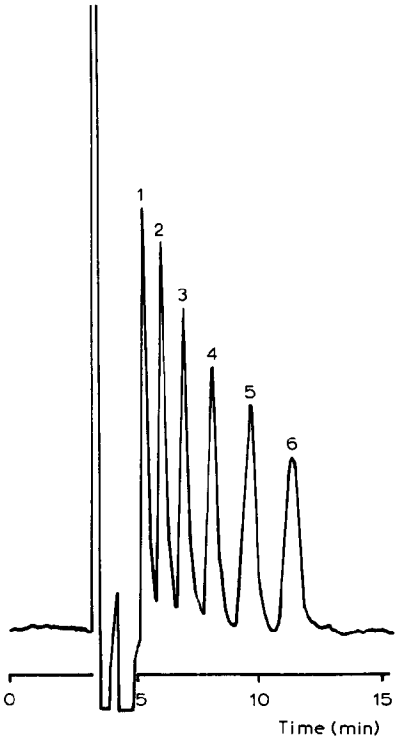

Fig. 3. 8.1-MPa l.c. profile of mono- and oligo-saccharides. [Column: Aquasil SS-452N (25 cm × 4.6 mm i.d.). Eluant: 7:3:2 EtOAc–MeOH–H_2O. Flow rate: 1.0 mL/min. Injection solvent: H_2O. Detector: RI. 1, Glucose; 2, maltose: 3, maltotriose; 4, maltotetraose; 5, maltopentaose; 6, maltohexaose.]

by p.p.c. that glucosylerythritol was the only Smith-degradation product of the polysaccharide, PC-3, of the lichen *Parmelia caperata*, thus proving the presence of alternate α-(1→3) and α-(1→4) linkages in its structure. On re-examination by the present l.c. method, glucosylerythritol was proved to constitute peak 1 (r.t., 7.91 min) of the chromatogram, shown in Fig. 5; it was collected preparatively, acetylated, and identified by e.i.-m.s.: *m/z* 505 ($M^+ - CH_2OAc$). The polysaccharide, SJ-2-I, of *Stereocaulon japonicum*, reported by Yokota and Shibata[11], is a glucan having α-(1→3) and α-(1→4) linkages in the ratio of 2.7:1. The Smith-degradation product of SJ-2-I was chromatographed by the present method, as shown in Fig. 6, and peaks 1 and 2 were collected, acetylated, and examined by e.i.-m.s. The results for peak 1 agreed with nigerosylerythritol, as previously reported[11], and peak 2 was identified as nigerotriosylerythritol. These results supported the structure assigned to SJ-2-I.

This l.c. method for efficient separation of carbohydrates showed the following advantages. (*1*) Stable retention-times are reproducible, even after repeated use of the column. (*2*) Water is used as the injection solvent in which a sufficient amount of sugars can readily be dissolved for preparative separations. (*3*) A suita-

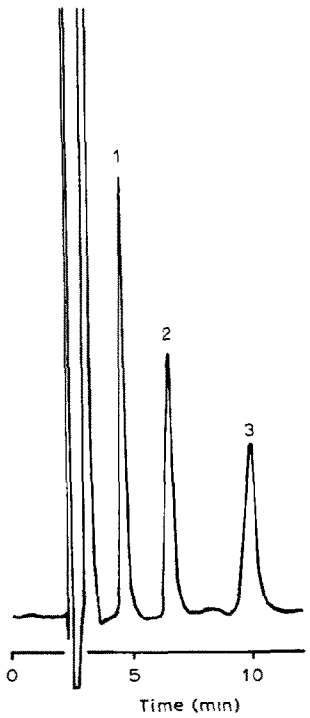

Fig. 4. 11.1-MPa l.c. profile of mono- and oligo-saccharides. [Column: Aquasil SS-452N (25 cm × 4.6 mm i.d.). Eluant: 12:3:1 EtOAc–MeOH–H_2O. Flow rate: 1.5 mL/min. Injection solvent: H_2O. Detector: RI. 1, Glucose; 2, maltose: 3, maltotriose.]

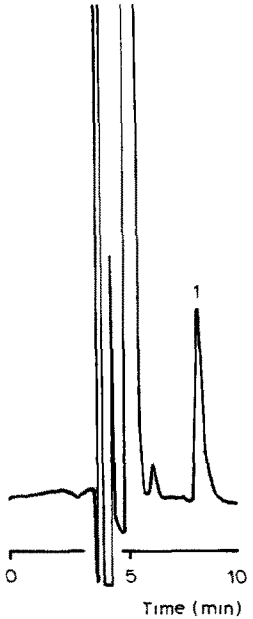

Fig. 5. 7.1-MPa l.c. profile of the degradation product of PC-3. [Column: Aquasil SS-452N (25 cm × 4.6 mm i.d.). Eluant: 12:3:1 EtOAc–MeOH–H_2O. Flow rate: 1.0 mL/min. Injection solvent: H_2O. Detector: RI. 1, Glucosylerythritol.]

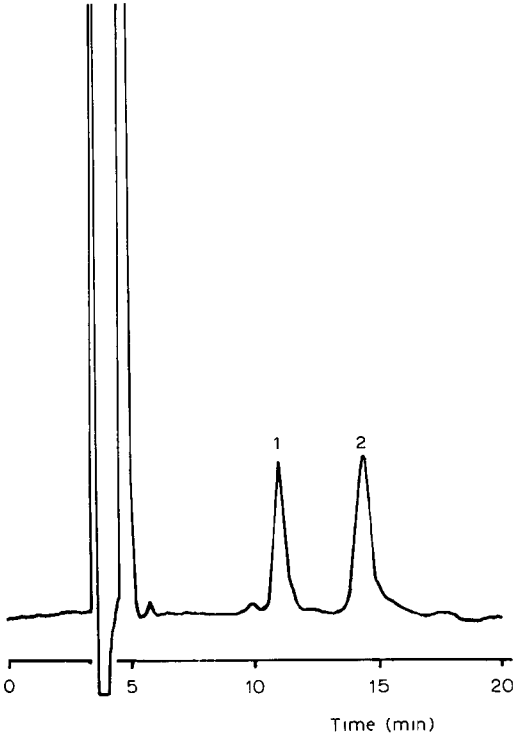

Fig.6. 7.1-MPa l.c. profile of the degradation product of SJ-2-1. [Column: Aquasil SS-452N (25 cm × 4.6 mm i.d.). Eluant 7:3:2 EtOAc–MeOH–H_2O. Flow rate: 1.0 mL/min. Injection solvent: H_2O. Detector: RI. 1, Nigerosylerythritol; 2, nigerotriosylerythritol.]

ble, mobile phase for the l.c. system can be selected from the solvent systems provisionally tested for t.l.c. on silica gel. (*4*) A parallel correlation exists between the elution sequence of carbohydrates in l.c. and their R_F values in t.l.c. when the same solvent system is used in both. (*5*) The aqueous, silica gel column is readily regenerated by washing exclusively with water. (*6*) The column can be readily prepared, and the cost of preparation is much lower than that of any other l.c. column of the same size usually used for the separation of carbohydrates.

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