Journal of Chromatography, 147 (1978) 491–495

CHROM. 10,482

Note

Separation of acetylated hexopyranoses on silica gel by high-performance liquid chromatography

JOACHIM THIEM, HORST KARL, JENS SCHWENTNER and JOACHIM REIMER

Institut für Organische Chemie und Biochemie der Universität, Martin-Luther-King-Platz 6, D-2000 Hamburg 13 (G.F.R.)

(Received March 17th, 1977)

During the synthesis or the degradation of polysaccharides mixtures of isomeric monosaccharides are obtained, the selective separation and rapid identification of which remains one of the central problems in carbohydrate chemistry. In particular, the separation of epimers and anomers, respectively, causes considerable difficulties. Unblocked carbohydrates as well as carbohydrate derivatives can be separated by use of the methods such as layer and column chromatography. The rapid analysis of carbohydrate trimethylsilyl ethers by gas–liquid chromatography is well documented. Unblocked carbohydrates are mainly separated either by gel-permeation chromatography or by ion-exchange chromatography; however, the preferred method for the separation of protected carbohydrates is adsorption chromatography on silica gel.

Recently, high performance liquid (HPL) ion-exchange chromatography has been successfully applied to free carbohydrates[1–4], and some substituted carbohydrates were separated similarly by use of HPL gel-permeation chromatography[5]. Another recent report on HPL adsorption chromatography described the separation of some perbenzoylated carbohydrates[6]. The present paper describes an improved separation of acetylated carbohydrates by adsorption chromatography on silica gel using HPLC. An advantage of the method is the fact that only the most readily available carbohydrate derivatives are used, *i.e.*, the acetates.

EXPERIMENTAL

Apparatus and materials

An S 100 liquid chromatograph (Siemens, Karlsruhe, G.F.R.) equipped with an MK 00 pumping system (Orlita, Giessen, G.F.R.; maximum pressure, 300 bar) was employed. A pneumatic micro syringe (10 μl) was used for sample injection. The detection was by a UV photometer (Zeiss PLC 2 DLC, Oberkochen, G.F.R.) at 223 or 230 nm. The columns were made of V-4-A steel (25 cm × 3 mm I.D.) and packed with LiChrosorb SI-60, (5 μm) (E. Merck, Darmstadt, G.F.R.). The columns were filled according to a modified balanced slurry procedure[7,8] using dioxane as slurry medium. The packing was achieved by use of an S 15 pumping system (Orlita; maximum pressure, 1000 bar). During the filling process the pump lift was continuously enhanced from 50% to 70% of the maximum pump capacity. The average

filling time was *ca.* 45 min. The eluents used were diethyl ether–pentane (2:1) and (4:1). Solvents were purified before use by careful distillation using a 50 cm Vigreux column. All of the separations were performed at room temperature.

Sample preparations

1,2,3,4,6-Penta-O-acetyl-α-D-gluco- (1)[9], -β-D-manno- (4)[10], and -β-D-allopyranose (8)[11] were obtained from the free monosaccharides by reaction with acetic anhydride in pyridine. 1,2,3,4,6-Penta-O-acetyl-β-D-gluco- (2)[9] and -β-D-galactopyranose (6)[12] were prepared from the free sugars by use of sodium acetate and acetic anhydride. 1,2,3,4,6-Penta-O-acetyl-α-D-manno- (3)[13] and -α-D-altropyranose (7)[14] were prepared by acetolysis with acetic acid and sulphuric acid from mannan or methyl α-D-altropyranoside, respectively. 1,2,3,4,6-Penta-O-acetyl-α-D-galactopyranose (5) [9] was obtained by anomerization of the β-compound 6 with acetic anhydride and zinc chloride. 1,2,3,4,6-Penta-O-acetyl-α-D-ido- (9) and -α-D-talopyranose (10) were synthesized by acetoxonium ion rearrangements from 2,3,4,6-tetra-O-acetyl-β-D-gluco- or -β-D-galactopyranosyl chlorides, respectively[15].

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranose (12) was prepared by treatment of 2,3,4,6-tetra-O-acetyl-α-D-glucosyl bromide with silver carbonate and water[16], the corresponding α-isomer (11) being obtained by solvolysis of N-*p*-tolyl-2,3,4,6-tetra-O-acetyl-D-glucopyranosyl amine[17]. 1,2,3,6-Tetra-O-acetyl-β-D-glucopyranose (13) was synthesized by reduction of 1,2,3,6-tetra-O-acetyl-4-O-nitro-β-D-glucopyranose with zinc in acetic acid[18]. 1,3,4,6-Tetra-O-acetyl-β-D-gluco- (14), -α-D-altro- (15) and -α-D-galactopyranose (16) were obtained by the selective acetylation procedure of Helferich and Zirner[19].

RESULTS AND DISCUSSION

The ten peracetylated monosaccharides (1–10) were separated at different pressures using diethyl ether–pentane (2:1). The results obtained at 50 bar are shown in Fig. 1. Obviously a base line separation of α- and β-D-manno- (3 and 4) as well as α- and β-D-galactopyranose pentaacetates (5 and 6) can be achieved, whereas a complete separation of α- and β-D-glucopyranose pentaacetates (1 and 2) is not feasible under various conditions. Fig. 1 also demonstrates clearly the remarkable separation of epimers such as the α- and the β-pentaacetates of the gluco, manno and galacto configuration (series 1, 3, 5 and series 2, 4, 6). In addition, the other diastereomeric hexopyranose pentaacetates exhibit different relative retention times.

Fig. 2 shows the chromatogram of six selected partially acetylated monosaccharides taken at a pressure of 90 bar using diethyl ether–pentane (4:1) as eluent. The separation of the gluco isomers, having a hydroxyl group at different positions on the pyranose ring, is complete. Furthermore, tetraacetates of different configurations but acetylated in similar positions can be well separated.

Fig. 3 shows the dependence of the relative retention time k' on the pressure p for pentaacetates 1–10. A maximum in the k' values is observed at a pressure of 80 bar. It is to be noted that the graphs for all of the individual compounds are nearly parallel to each other, indicating that an increase in the pressure has only limited effects on the separation efficiency for this class of compounds. It seems that the most efficient separation will be achieved at pressures between 50 and 80 bar. Further

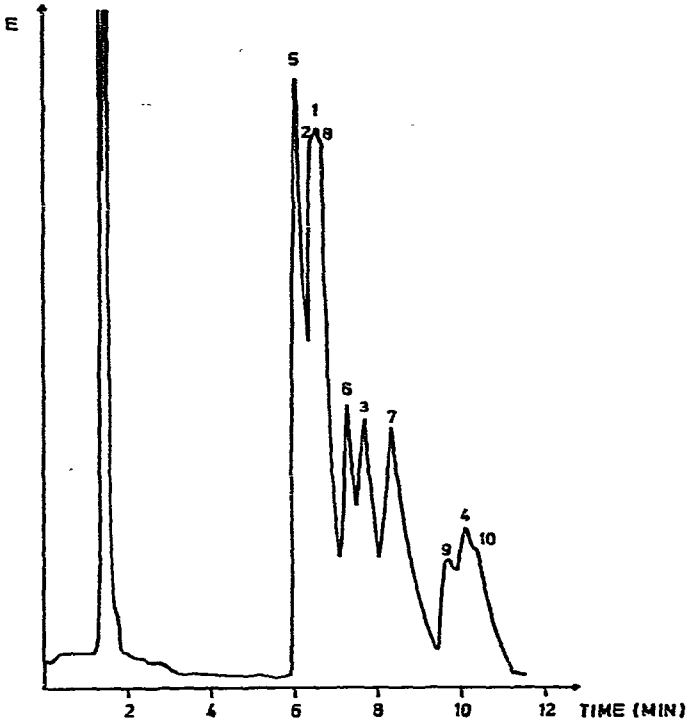

Fig. 1. HPLC of peracetylated hexopyranoses on LiChrosorb SI-60 (5 μm) column at 50 bar. Eluent: diethyl ether–pentane (2:1). Peaks: 1 = 1,2,3,4,6-penta-O-acetyl-α-D-gluco-; 2 = -β-D-gluco-; 3 = -α-D-manno-; 4 = -β-D-manno-; 5 = -α-D-galacto-; 6 = -β-D-galacto-; 7 = -α-D-altro-; 8 = -β-D-allo-; 9 = -α-D-ido-; and 10 = -α-D-talopyranose.

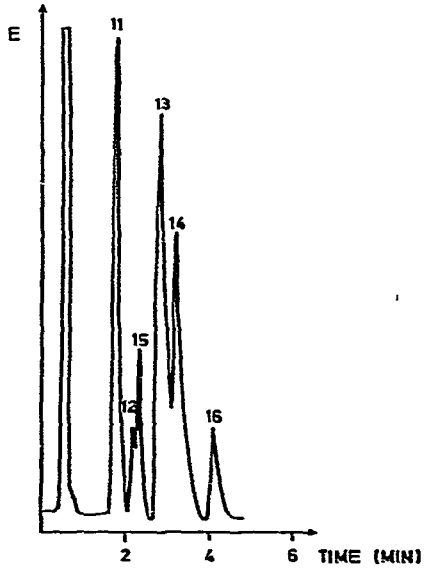

Fig. 2. HPLC of hexopyranose tetraacteates on Lichrosorb SI-60 (5 μm) column at 90 bar. Eluent: diethyl ether–pentane (4:1). Peaks: 11 = 2,3,4,6-tetra-O-acetyl-α-D-gluco-; 12 = -β-D-gluco-; 13 = 1,2,3,6-tetra-O-acetyl-β-D-gluco-; 14 = 1,3,4,6-tetra-O-acetyl-β-D-gluco-; 15 = 1,3,4,6-tetra-O-acetyl-α-D-altro-; 16 = 1,3,4,6-tetra-O-acetyl-α-D-galactopyranose.

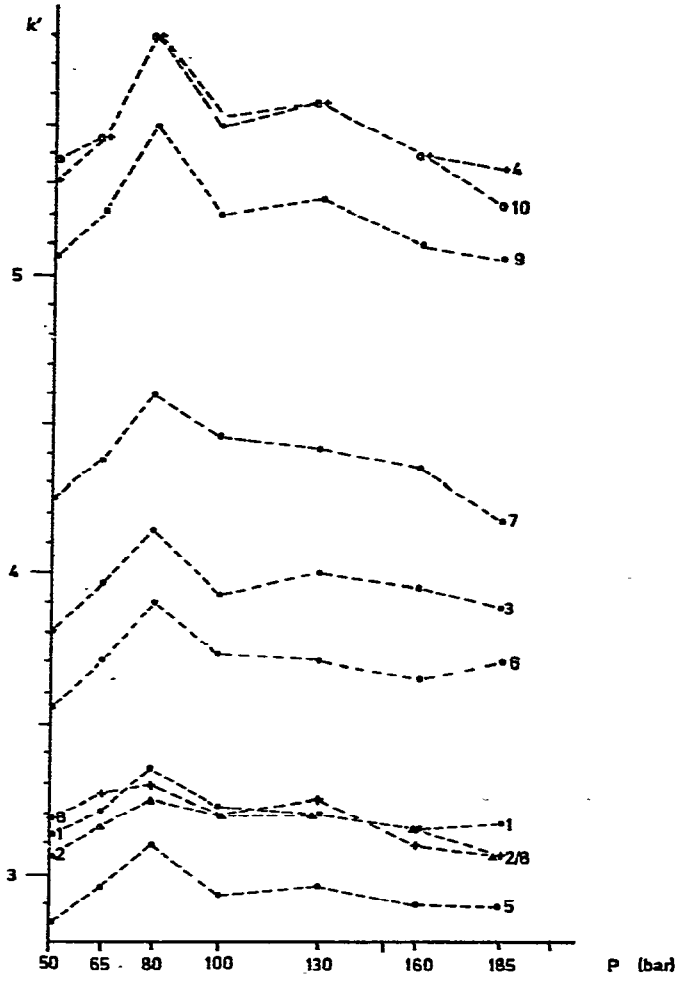

Fig. 3. Dependence of the relative retention time k' on pressure p for pentaacetylhexopyranoses 1–10.

studies of the dependence of the relative retention time k' on the composition of the eluent mixture as well as the temperature are in progress. The present results indicate that a rapid and efficient analysis of oligosaccharides may be achieved by acetolysis and subsequent separation of monosaccharide acetates.

ACKNOWLEDGEMENT

Support for part of this work by the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

REFERENCES

1 J. K. Palmer, *Anal. Lett.*, 8 (1975) 215.
2 J. K. Palmer, *Appl. Polym. Symp.*, 28 (1975) 237.

3 J. G. Lawrence, *Chimia*, 29 (1975) 367.
4 W. Voelter and H. Bauer, *J. Chromatogr.* 126 (1976) 693.
5 G. D. McGinnes and P. Fang *J. Chromatogr.* 130 (1977) 181.
6 J. Lehrfeld *J. Chromatogr.*, 120 (1976) 141.
7 J. J. Kirkland, *J. Chromatogr. Sci.*, 9 (1971) 206.
8 W. Strubert, *Chromatographia*, 6 (1973) 50.
9 M. L. Wolfrom and A. Thompson, *Methods Carbohyd. Chem.*, 2 (1963) 211.
10 J. Conchie and G. A. Levy, *Methods Carbohyd. Chem.*, 2 (1963) 345.
11 J. D. Stevens, *Methods Carbohydr. Chem.*, 6 (1972) 123.
12 M. L. Wolfrom and A. Thompson, *Methods Carbohyd. Chem.*, 1 (1962) 120.
13. J. Thiem, A. Sievers and H. Karl, *J. Chromatogr.*, 130 (1977) 305.
14 N. K. Richtmeyer, *Methods Carbohyd. Chem.*, 1 (1962) 107.
15 H. Paulsen, *Methods Carbohyd. Chem.*, 6 (1972) 142.
16 C. M. McClosky and G. H. Coleman, *Org. Syn. Coll. Vol.*, 3 (1955) 434.
17 J. Honeyman, *Methods Carbohyd. Chem.*, 2 (1963) 98.
18 D. M. Hall, T. E. Lawler and B. C. Childress, *Carbohyd. Res.*, 38 (1974) 359.
19 B. Helferich and J. Zirner, *Chem. Ber.*, 95 (1962) 2604.