Separation of carbohydrate derivatives by gas-liquid partition chromatography*

Gas-liquid partition chromatography (G.L.P.C.)[1] has been used extensively for the qualitative and quantitative estimation of a wide variety of highly volatile organic compounds, *e.g.* acids[1], hydrocarbons, alcohols, esters[2]. Compounds of low volatility can also be analysed by this technique if they can readily be converted to derivatives with sufficient volatility. This has been illustrated by the separation of amino acids as their methyl esters[3]. The present communication reports, for the first time, the application of G.L.P.C. to the separation of carbohydrate derivatives. It was found that methyl ethers of the methyl glycosides of the heat-labile monosaccharides were sufficiently stable and volatile to be analysed by G.L.P.C. This report describes the separation of the

TABLE I

FULLY METHYLATED METHYL GLYCOPYRANOSIDES

Compound	*m.p. °C*	*b.p. °C*	$[\alpha]_D$	*Reference*
Methyl 2,3,4-tri-O-methyl-β-D-xylopyranoside	49.5–51.5		—69° (chloroform)	4
Methyl 2,3,4-tri-O-methyl-β-L-arabopyranoside	44.5–46.5		+253° (water)	5
Methyl 2,3,4-tri-O-methyl-α-L-arabopyranoside	45.5–47		+44° (water)	5
Methyl 2,3,4,6-tetra-O-methyl-α-D-mannopyranoside		76–79 at 0.015–0.018 mm	+42° (water)	6
Methyl 2,3,4,6-tetra-O-methyl-α-D-galactopyranoside		76–78 at 0.03 mm	+163° (water)	7
Methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside		100 (bath temp.) at 0.1 mm	+151° (water)	8

fully methylated methyl glycopyranosides listed in Table I. These compounds were prepared by procedures in general use in carbohydrate chemistry and were purified by recrystallization to constant melting points or, for the syrupy products, by fractional distillation.

The observed physical properties recorded in Table I are in good agreement with those reported originally in the references listed. No hydroxyl bands were present in the infrared spectra of these compounds.

Separations of the fully methylated methyl glycopyranosides shown in Fig. 1, A and B, were obtained on a Podbielniak "Chromacon" (series 9475-3V) which was modified to take glass columns and to improve the pressure regulation, temperature control, injection and collection systems. An 8 ft., 7 mm internal diameter column, packed with Apiezon M: Celite 545 (1:4 w/w) prepared as described by JAMES AND MARTIN[9] was used. The operating temperature was 170°, helium was the carrier gas and a katharometer was used as detector. Table II lists the retention volumes of the fully methylated sugars relative to that of quinoline. Quinoline was chosen as a standard for this series because its retention volume was approximately midway between the pentose and hexose derivatives and because it is readily available in a pure form.

Fig. 1B shows the virtually complete separation of methyl 2,3,4,6-tetra-O-methyl-α-D-mannopyranoside from methyl 2,3,4,6-tetra-O-methyl-α-D-galactopyranoside. Fig. 1A shows the separation of methyl 2,3,4-tri-O-methyl-β-D-xylopyranoside, methyl 2,3,4-tri-O-methyl-β-L-arabopyranoside, methyl 2,3,4,6-tetra-O-methyl-α-D-mannopyranoside, methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside, and methyl 2,3,4,6-tetra-O-methyl-α-D-galactopyranoside. In this run, the three hexose derivatives were only partially separated from each other; however, the separation is sufficient to enable quantitative estimation of these components after suitable calibration.

To establish that no structural changes occurred in the sugar molecules under the conditions

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TABLE II

RETENTION VOLUMES OF FULLY METHYLATED METHYL GLYCOPYRANOSIDES AT 170° RELATIVE TO QUINOLINE

Methyl 2,3,4-tri-O-methyl-β-D-xylopyranoside	0.528
Methyl 2,3,4-tri-O-methyl-β-L-arabopyranoside	0.785
Methyl 2,3,4,6-tetra-O-methyl-α-D-mannopyranoside	1.468
Methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside	1.594
Methyl 2,3,4,6-tetra-O-methyl-α-D-galactopyranoside	1.708
Quinoline (standard)	1.000

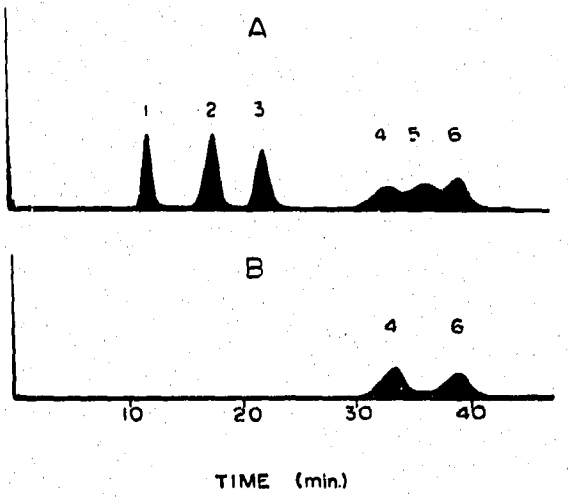

Fig. 1. Separation of fully methylated methyl glycopyranosides on a predominantly paraffin liquid phase. Column length, 8 ft.; stationary phase, Apiezon M vacuum stopcock grease; temperature 170°; flow rate, 130ml helium per minute; inlet gas pressure, 12 p.s.i.; detector at atmospheric pressure. Peaks in order of appearance: (1) methyl 2,3,4-tri-O-methyl-β-D-xylopyranoside, (2) methyl 2,3,4-tri-O-methyl-β-L-arabopyranoside, (3) quinoline (standard), (4) methyl 2,3,4,6-tetra-O-methyl-α-D-mannopyranoside, (5) methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside, (6) methyl 2,3,4,6-tetra-O-methyl-α-D-galactopyranoside.

used, samples of the α and β-methyl glycosides of 2,3,4-tri-O-methyl-L-arabinose were collected from the effluent gas stream of two separate runs and crystallized. Mixed melting points of these with the original samples were not depressed showing that the products were unchanged. Under the conditions used the anomeric methyl glycosides of 2,3,4-tri-O-methyl-L-arabinose and of 2,3,4,6-tetra-O-methyl-D-galactose were not separated.

In view of the results reported above and from work in progress with other carbohydrate derivatives, it is felt that G.L.P.C. will be of considerable assistance in carbohydrate chemistry. Its main advantage lies in the much greater sensitivity and accuracy of quantitative data on very small quantities. A systematic program is now in progress on the separation of other carbohydrate derivatives and on the application of G.L.P.C. to elucidating structures of polysaccharides by separating the products formed by methanolysis of their methyl ethers.

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1 A. T. JAMES AND A. J. P. MARTIN, *Biochem. J.*, 50 (1952) 679.
2 N. H. RAY, *J. Appl. Chem. (London)*, 4 (1954) 21.
3 E. BAYER, in D. H. DESTY, *Gas Chromatography* (preprints), Butterworths Scientific Publications, London, 1958, p. 231.
4 A. CARRUTHERS AND E. L. HIRST, *J. Chem. Soc.*, 121 (1922) 2299.
5 E. L. HIRST AND G. J. ROBERTSON, *J. Chem. Soc.*, 127 (1925) 358.
6 J. C. IRVINE AND A. M. MOODIE, *J. Chem. Soc.*, 87 (1905) 1462.
7 J. C. IRVINE AND A. CAMERON, *J. Chem. Soc.*, 85 (1904) 1071.
8 T. PURDIE AND J. C. IRVINE, *J. Chem. Soc.*, 83 (1903) 1021.
9 A. T. JAMES AND A. J. P. MARTIN, *Biochem. J.*, 63 (1956) 144.

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