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Note

Separation of methyl ethers of xylose, glucose and some other sugars by liquid chromatography

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The separation and identification of the methyl ethers of monosaccharides are important for structural analyses in carbohydrate chemistry. The most reliable method is gas–liquid chromatography combined with mass spectrometry (*e.g.*, refs. 1 and 2). Other common methods are simple gas–liquid chromatography, paper and thin-layer chromatography and electrophoresis. Recently, ion-exchange chromatography of borate complexes has been used successfully for the separation of some methyl ethers of D-arabionofuranose[3]. A modification of this method has been examined in our Institute, and for comparison paper and thin-layer chromatographic separations were carried out.

EXPERIMENTAL

Paper and thin-layer chromatography were carried out as described previously[4]. The solvents used were: A, ethyl acetate–acetic acid–water (18:7:8); B, ethyl acetate–isopropanol–water (100:65:35); C, formic acid–methyl ethyl ketone-*tert*.butanol–water (15:25:35:25); D, *n*-butanol–ethanol–water–ammonia (40:10:49:1); E, *n*-butanol–acetone–water (4:5:1); F, 2-butanone saturated with 3% aqueous ammonia[3]. The sugars were detected with aniline phthalate. In addition, carbonization with 50% sulphuric acid was applied on silica gel thin-layer glass plates and aluminium sheets. For paper chromatography amounts of 20–80 μg, and for thin-layer chromatography 2–20 μg, were applied.

The Aminex A-14 column and the separation conditions were as described in previous papers[5,6]. For additional information on the experimental procedure and its application, see the papers by Sinner[7] and Simatupang *et al.*[8].

RESULTS AND DISCUSSION

Methyl ethers of D-xylose, D-glucose, D-mannose and D-galactose were analyzed by paper chromatography with solvents A and D, with aniline phthalate for detection. One run took 8 and 16 h, respectively. The sugars with the same number of methyl ether groups, *e.g.*, 2- and 3-O-methylxylose or 2,3,4- and 2,3,6-tri-O-methylglucose, were not separated, and the tetra-O-methylhexoses and some tri- and

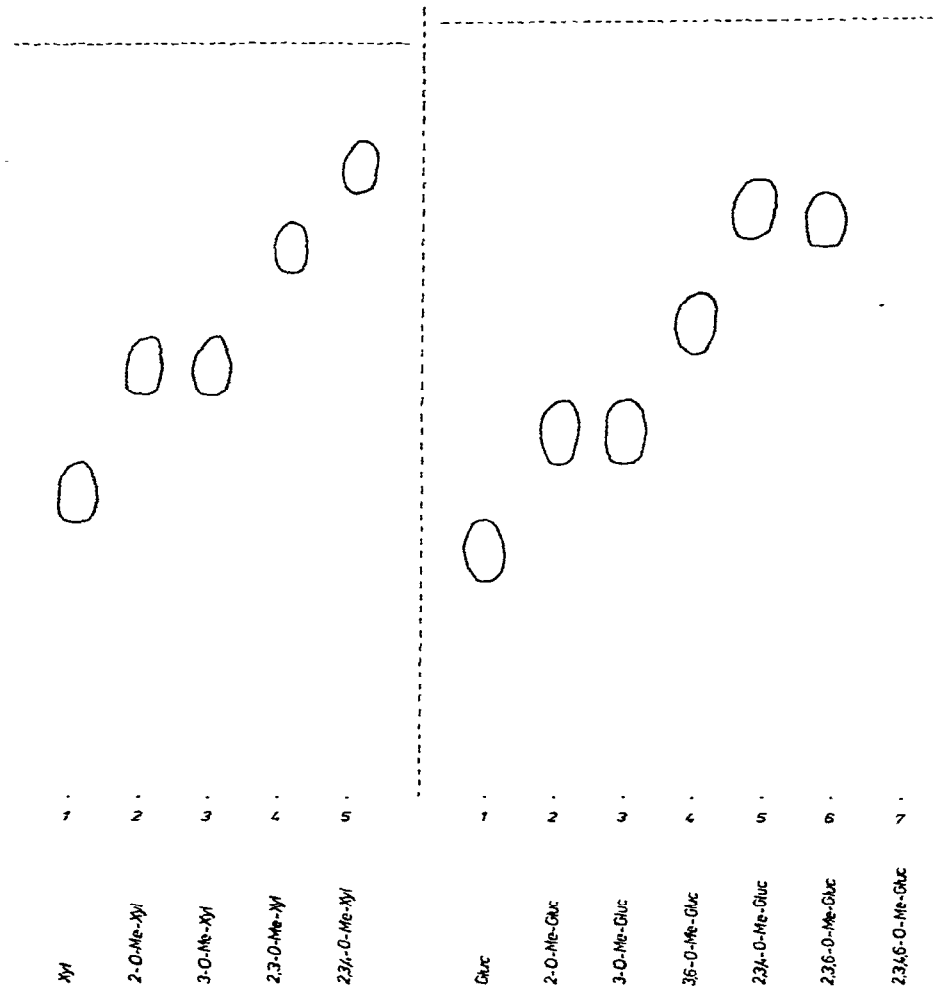

Fig. 1. Paper chromatographic separation of methyl ethers of xylose and glucose. Whatman No. 1 paper; solvent A; aniline phthalate.

di-O-methylhexoses were not detected with aniline phthalate (for solvent A, see Table I, column 2 and Fig. 1).

Separations on cellulose thin-layer plates with the same solvents were achieved in about half the time. The same incomplete resolution of sugars with equal numbers of substituents, and the same incomplete detection, were observed as with paper chromatography (Table I, columns 3 and 4).

The same difficulties were encountered on silica gel thin-layer plates developed with solvent F and sprayed with aniline phthalate. By carbonization with sulphuric acid, however, all methyl ethers could be detected (Table I, column 5; Fig. 2).

The monomethyl ethers of xylose and glucose were well separated on silica gel thin-layer sheets buffered with boric acid, activated at 110° and developed with solvent E. One run took only 2.5 h (Table I, column 6; Fig. 3).

TABLE I

RESULTS OF PAPER, THIN-LAYER AND COLUMN CHROMATOGRAPHIC SEPARATIONS

? = could not be detected (with anilinephthalate).

Substance	*Paper chromatography, solvent A (8 h): R_F (f=36.2 and 37.5 cm)*	*Thin-layer chromatography*				*Column chromatography, Aminex A-14, borate buffer (up to 2½ h)*		
		Cellulose		*Silica gel*				
		Solvent D (6½ h): R_F (f=12.5 and 12.7 cm)	*Solvent A (4½ h): R_F (f=14.6 and 15.1 cm)*	*Solvent F (7 h): R_F (f=15.1 and 13.8 cm)*	*Solvent E (2½ h): R_F (f=13.6 and 14.4 cm)*	*t_R (distance on recorder in mm)*	*k' (average)*	*Number of analyses*
D-Xylose	0.40	0.25	0.31	0.10	0.40	96.9–101.2	9.2	104
2-O-methyl-	0.56	0.44	0.49	0.35	0.68	33.3– 34.8	2.8	5
3-O-methyl-	0.56	0.44	0.49	0.36	0.46	39.0– 41.0	3.5	9
2,3-di-O-methyl-	0.74	0.68	0.73	0.51	0.78	13.0– 14.0	0.5	11
2,3,4-tri-O-methyl-	0.84	0.87	0.93	0.59	0.84	19.0– 20.0	1.1	10
D-Glucose	0.31	0.19	0.21	0.0	0.31	126.0–131.2	12.3	104
2-O-methyl-	0.46	0.32	0.33	0.10	0.53	26.7– 28.0	2.0	9
3-O-methyl-	0.46	0.32	0.33	0.10	0.44	75.7– 77.0	7.5	7
3,6-di-O-methyl-	0.61	0.54	0.52	0.28	(0.65)*	34.0– 34.5	2.8	7
2,3,4-tri-O-methyl-	0.75	0.79	0.80	0.53	0.79	17.5– 18.0	1.0	8
2,3,6,-tri-O-methyl-	0.75	0.77	?	0.52	0.77	11.4– 12.0	0.3	7
2,3,4,6-tetra-O-methyl-	?	?	?	0.79	0.85	21.0– 23.0	1.6	9
D-Mannose	0.37	0.23	0.25	0.08	0.35	60.1– 62.7	5.3	95
2,3-di-O-methyl-	0.70	0.72	?	0.41	0.65	18.8– 19.5	1.1	9
2,3,6-tri-O-methyl-	0.76	0.72	?	0.43	0.73	10.5– 12.0	0.2	8
2,3,4,6-tetra-O-methyl-	?	?	?	0.61	0.79	23.0– 24.0	1.6	10
D-Galactose	0.29	0.14	0.19	0.04	0.24	85.8– 89.3	8.0	43
2,3,4,6-tetra-O-methyl-	(0.83?)	0.80	?	0.59	0.70	18.0– 18.3	1.0	8

* No distinct spot (*cf.*, Fig. 2).

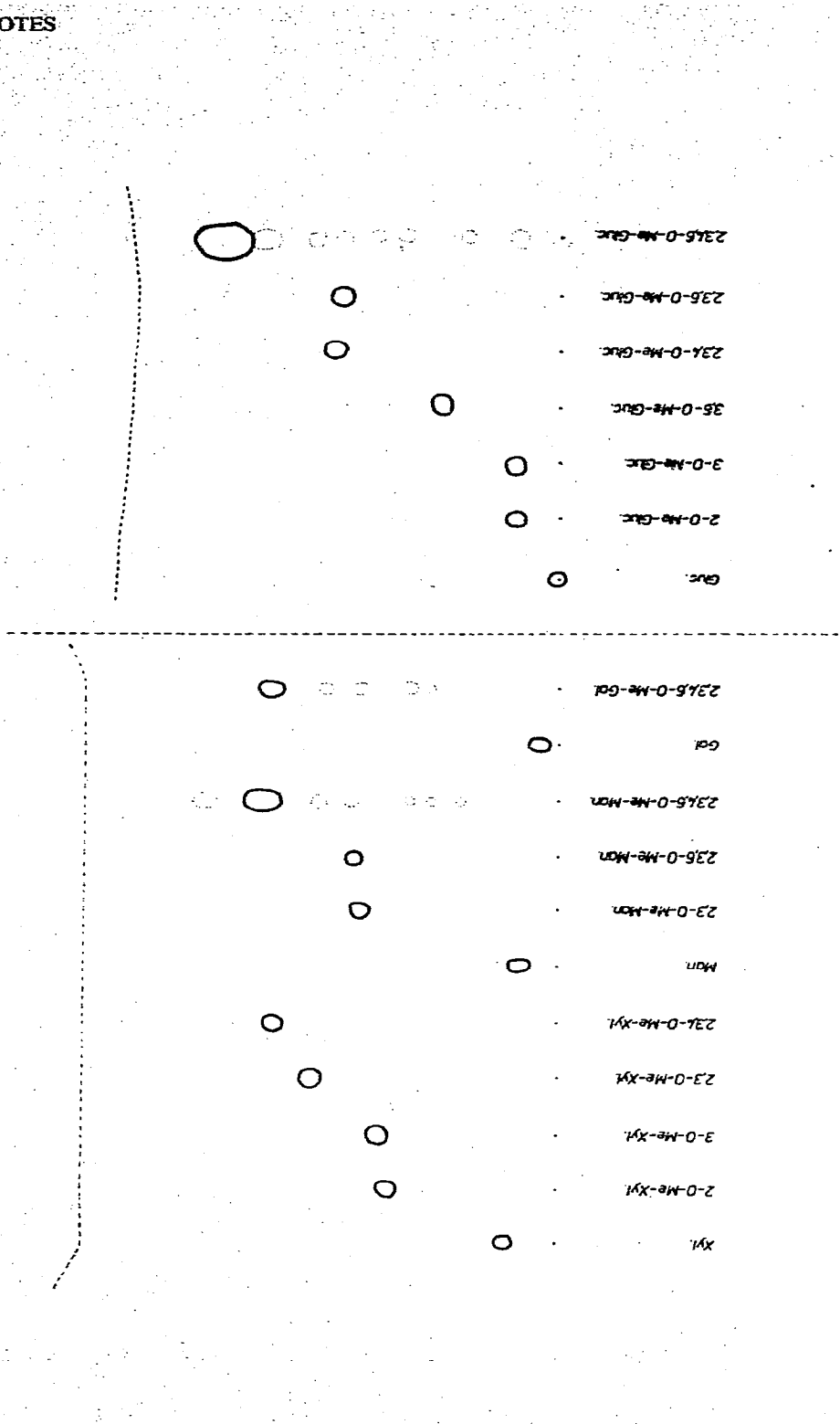

Fig. 2. Thin-layer chromatographic separation of methyl ethers of xylose, mannose and galactose (left) and glucose (right). Silica gel glass plates (Merck, Darmstadt, G.F.R.); solvent F; 50% sulphuric acid.

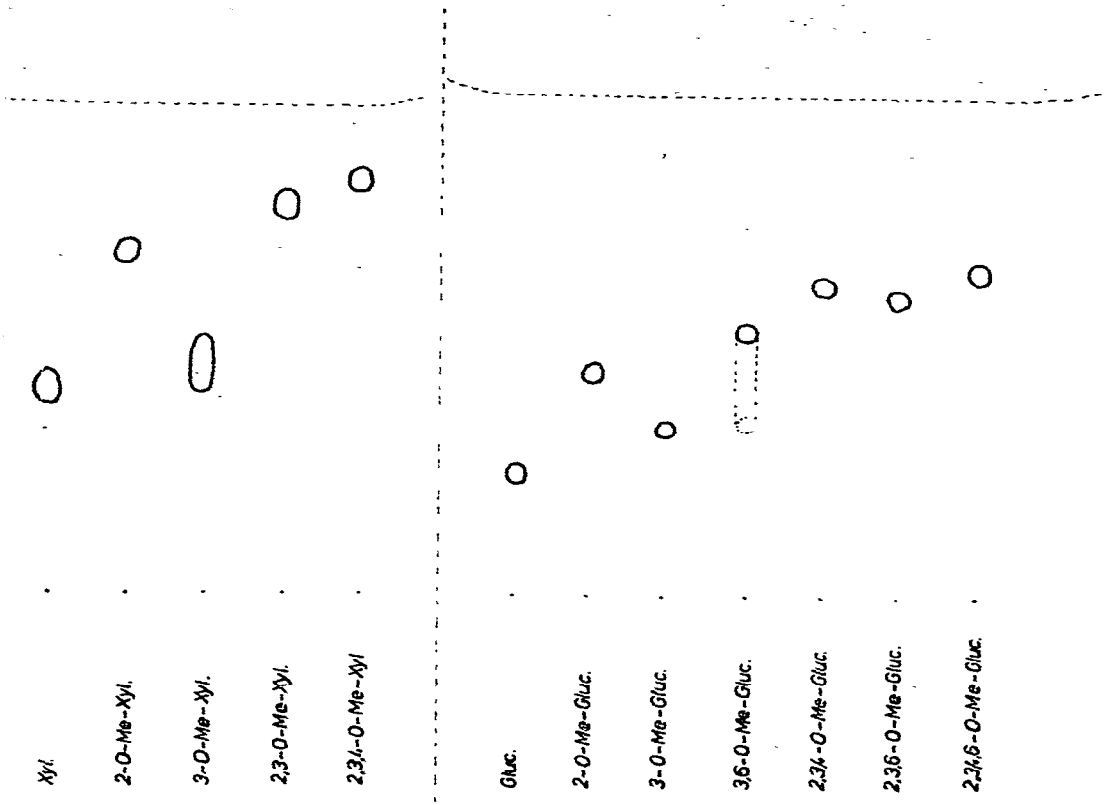

Fig. 3. Thin-layer chromatographic separation of methyl ethers of xylose (left), and glucose (right). Silica gel aluminium sheets buffered with 0.1 *M* boric acid and activated at 110°; solvent E; 50% sulphuric acid.

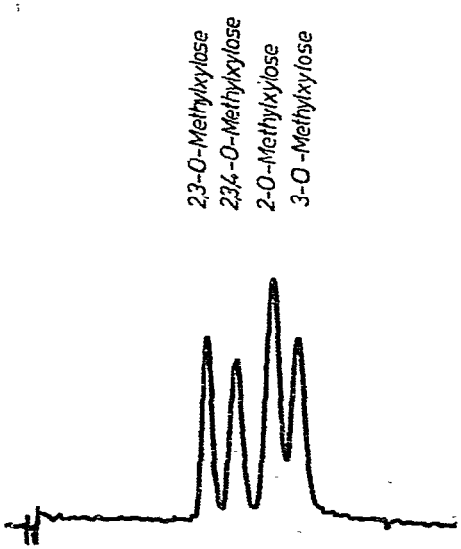

Fig. 4. Column chromatographic separation of methyl ethers of xylose. Ion exchanger, Aminex A-14 (20 μm); glass column, 99 cm × 0.2 cm; mobile phase, 0.11 *M* potassium tetraborate + 0.17 *M* boric acid (pH 8.8); temperature, 55°; detection, 0.1% orcinol in 70% sulphuric acid.

In all paper and thin-layer chromatographic systems tested, the mobility of the methyl ethers was higher than that of the corresponding unsubstituted sugar, and increased with the number of methyl ether groups. The absolute R_F values, however, differed from run to run. Therefore, for each chromatographic system R_F values from only one run are given in the table (compare, for example, values for the methyl ethers of xylose and glucose in columns 3 and 6 with values published earlier[4]).

The column chromatographic separation of the methyl ethers was carried out according to an anion-exchange method, which is commonly used for the separation of unsubstituted mono- and disaccharides[9–15]. The separation unit was conceived according to Floridi[14], using the resin Aminex A-14 and 0.11 *M* potassium tetraborate –0.17 *M* boric acid (pH 8.8) for elution.

The results of this column chromatographic method are given in Table I (columns 7–9). The retention times (t_R) are indicated in millimetres of chart paper and the k' values are average values from 5–104 analyses, calculated according to Kirkland[16].

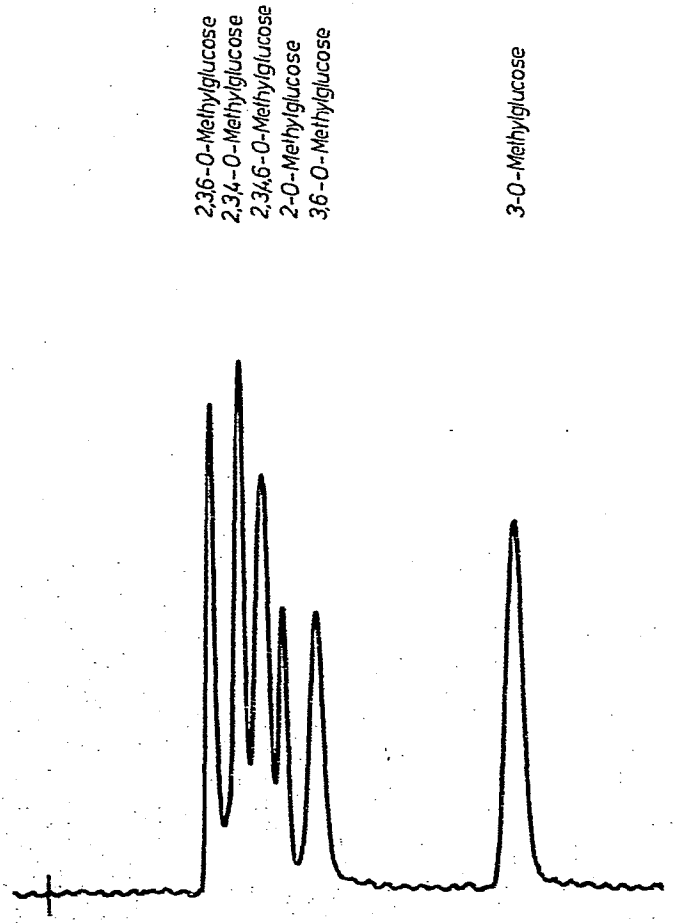

Fig. 5. Column chromatographic separation of methyl ethers of glucose.

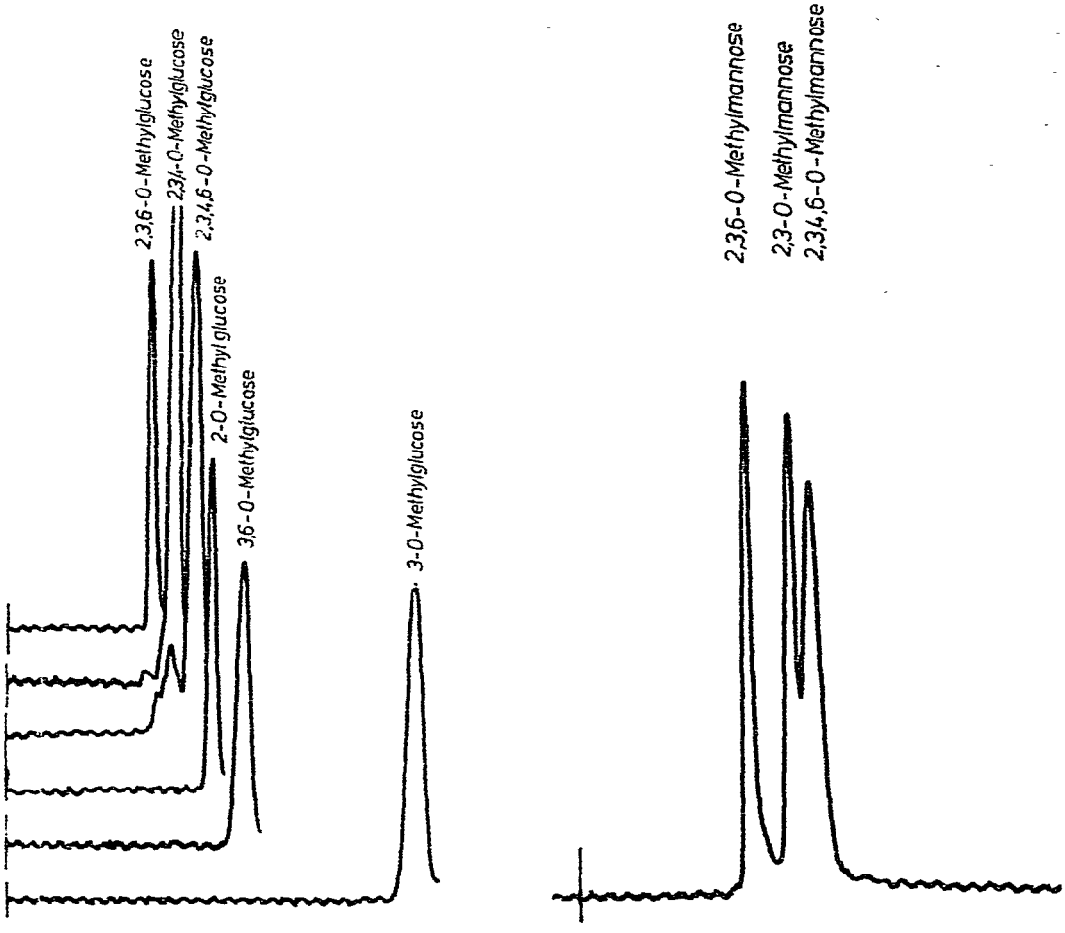

Fig. 6. Column chromatographic separation of the individual methyl ethers of glucose.

Fig. 7. Column chromatographic separation of methyl ethers of mannose.

All of the methyl ethers of each saccharide tested were separated (*cf.*, Figs. 4–7; for comparison, a separation of unsubstituted sugars is given in Fig. 8). Sugars with several methyl ether groups were detected without difficulty; the methyl ethers of xylose and glucose, which have the same number of substituents, were well resolved (Figs. 4–6). The separation of one series of methyl ethers and the corresponding unsubstituted sugar took 2.5–3 h, including the automatic detection (0.1% orcinol in 70% sulphuric acid). About half an hour was necessary for detection. This time has since been reduced to 3–6 min by using capillary coils and 0.1% orcinol in concentrated sulphuric acid[17]. Amounts of less than 1 μg were detected. With concentrated sulphuric acid and capillary coils, the sensitivity can be increased further.

The resolution of the methyl ethers decreased when a buffer of the same pH value but of lower molarity was used. This was unexpected because for the unsubstituted mono- and disaccharides the resolution increased considerably when the

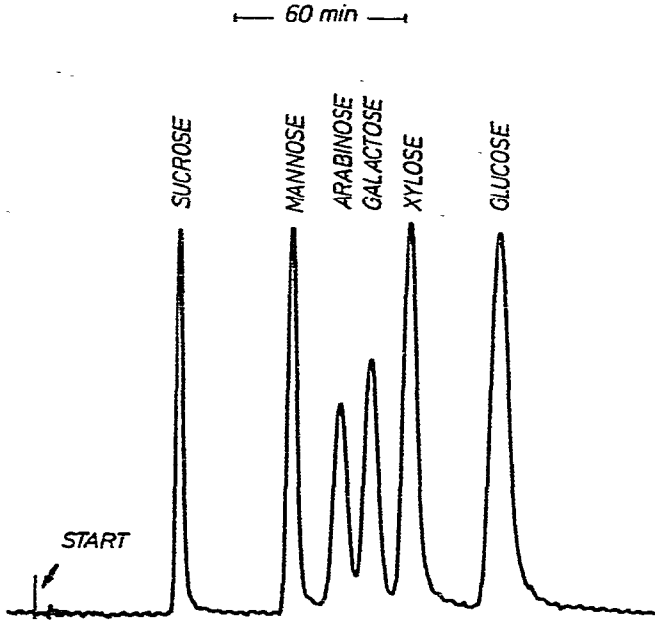

Fig. 8. Column chromatographic separation of some unsubstituted sugars.

molarity of the buffer was decreased. Further, the good results obtained with Aminex A-14 were not achieved with Durrum DA-X4 (column fillings: 24 cm × 6 mm and 30 cm × 4 mm) under similar conditions, although for unsubstituted sugars the same resolution as with Aminex A-14 was achieved in about half the time[17]. Only the monomethyl ethers were always well separated from each other and from the higher methyl ethers. This observation indicates a separation effect with Aminex A-14 which is not due to ion exchange.

Compared with paper, thin-layer and other chromatographic methods, the main advantages of the separation of methyl ethers and unsubstituted sugars by liquid chromatography are the following: the samples are applied to the column without any pre-treatment; several hundred analyses can be conducted with one column filling; the coefficient of variation of the retention time of a sugar is about ± 1 to ± 2% even for series lasting several months; and quantitative evaluation of the chromatograms can be carried out as for unsubstituted sugars[14,17].

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