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THE SEPARATION OF SOME MONOSACCHARIDES BY CAPILLARY COLUMN GAS CHROMATOGRAPHY

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SUMMARY

The conditions for the separation of some pentoses and hexoses, converted to volatile trimethylsilyl ethers, were studied on capillary columns. Relative retention times were determined for arabinose, ribose, lyxose and xylose, and also for rhamnose, mannose, galactose, glucose, fructose, sorbose and tagatose, on silicone phases SE-30, OV-101 and XE-60.

INTRODUCTION

The first papers on the chromatographic separation of sugars in the form of their volatile derivatives were published over ten years ago[1–3]. Most of the subsequent papers were devoted to a search for new or improved methods for the conversion of saccharides to volatile derivatives that would be stable at higher temperatures[4–16]. These studies, except for the work of AVERILL[17], were carried out on packed columns which had an adequate efficiency for the separation of both the anomers of one sugar or even of several sugars. When separating more complex mixtures, peak overlapping occurs, and two columns with different phases must be used, or the contents of each of the anomers in an aqueous or pyridine solution must be considered[6].

As the complete separation of all the components on an efficient column seemed to be possible on the basis of some data measured[4,5], an attempt at this separation was made by using glass capillary columns.

EXPERIMENTAL

The samples of individual pentoses and hexoses were provided by Lachema N. E., Brno, and the Institute of Chemical Technology, Prague.

Trimethylchlorosilane and hexamethyldisilazane were provided by Lachema N. E., Brno and bis(trimethylsilyl)acetamide and trimethylsilylimidazole were obtained from Pierce, U.S.A.

Glass capillary columns were manufactured in the apparatus designed by DESTY *et al.*[18] in the author's laboratory. The surface of the columns was etched in

the gaseous phase[19] and silanized for non-polar silicone phases[20]. The capillaries, having their surface modified in this way, were coated by a dynamic method. SE-30, OV-101 and XE-60 were applied as stationary phases. The concentrations of coating solutions were 3–10%, depending on the consistency of the phases. Some characteristics of the capillary columns used are presented in Table I.

TABLE I

PROPERTIES OF THE GLASS CAPILLARY COLUMNS

Length (m)	*I.D. (mm)*	*Phase*	*No. of theoretical plates*
20	0.18	SE-30	34,000
16	0.17	OV-101	70,000
28	0.17	XE-60	40,000

All the determinations were carried out in a Carlo Erba Model C capillary column gas chromatograph with a flame ionization detector.

Trimethylsilyl ethers of pentoses and hexoses were prepared in small test-tubes having a volume of *ca.* 0.7 ml. The silylation agent in pyridine was added (0.4–0.6 ml) through a serum cap. Most of the sugars dissolved well at room temperature, but the reaction mixture of some hexoses had to be heated in order to effect complete dissolution.

Samples of volume 0.5–1 μl were injected into the stream of nitrogen carrier gas. A glass filler was inserted into the injection block heated at 200–250° so that the time of sample contact with the metal wall was decreased to a minimum. The stream of carrier gas with the sample was split in the ratio 1:150. The operating temperature of the columns was varied over the range 130–170° according to the type of stationary phase and the value of the retention time.

RESULTS AND DISCUSSION

Retention times of arabinose, ribose and lyxose, of xylose, rhamnose, mannose, galactose and glucose aldoses, and of fructose, sorbose and tagatose ketoses were determined on stationary phases SE-30, OV-101 and XE-60.

The retention data of the trimethylsilylated sugars were found to be identical on SE-30 and OV-101, and for this reason only the relative retention times on OV-101 and XE-60 are presented in Tables II and III. In addition, values of the relative volatilities and separation factors, S, according to PURNELL[21] are given in Tables II and III for the pairs with very small differences in their retention times.

It follows from the first column of Tables II and III that not only do both of the anomers of each sugar remain during the silylation but also that further compounds are formed, the existence of which is suggested by one or two smaller peaks. Exceptions are lyxose and rhamnose, each of which gives only one peak on a non-polar phase. For this reason, the notation "name–number", indicating the sequence of the peak in the spectrum of the sugar (*e.g.*, arabinose–4), is introduced in the case of saccharides with several peaks.

TABLE II

RETENTION DATA FOR PENTOSES AND HEXOSES ON OV-101

Trimethylsilyl derivative of:	*Relative retention*[a]	*Relative volatility, α*	*Separation factor, S*
Lyxose	0.282		
Arabinose–1	0.284	1.008	
Arabinose–2	0.294	1.036	
Rhamnose	0.302	1.028	
Ribose–1	0.325		
Arabinose–3	0.332	1.022	~ 80,000
Ribose–2	0.337	1.014	
Ribose–3	0.350	1.038	
Ribose–4	0.395		
Arabinose–4	0.399	1.010	
Xylose–1	0.462		
Sorbose–1	0.521		
Xylose–2	0.560		
Tagatose–1	0.665		
Sorbose–2	0.668	1.004	
Mannose–1	0.701		
Fructose–1	0.704	1.004	
Fructose–2	0.721		
Tagatose–2	0.734		
Galactose–1	0.752		
Fructose–3	0.752	1.000	
Tagatose–3	0.767		
Tagatose–4	0.861		
Tagatose–5	0.887		
Sorbose–3	0.891	1.005	
Galactose–2	0.893	1.002	
Tagatose–6	0.966		
Glucose–1	1.000		
Fructose–4	1.020	1.020	
Tagatose–7	1.050		
Galactose–3	1.060	1.009	~500,000
Mannose–2	1.070	1.009	
Glucose–2	1.520		

[a] Glucose–1 = 1.000.

The measured retention values or calculated relative volatilities show that it will not be possible, using the capillary column having an efficiency of about 100,000 theoretical plates, to separate all the components formed by the silylation of the pentoses and hexoses under study. On a non-polar phase, there remain unseparated the lyxose and arabinose–1, arabinose–3 and ribose–2, and ribose–4 and arabinose–4 pairs of pentoses; the galactose–2 and mannose–2 pair of aldohexoses; and the tagatose–1 and sorbose–2; fructose–2 and tagatose–2; and tagatose–5 and sorbose–3 pairs of ketoses.

If a polar phase is used, the separation of some peaks, inseparable on OV-101, is possible; however, some other fractions, not separated owing to the shift of the individual components, arise, as can be seen in Figs. 1 and 2. The rhamnose–1 and lyxose–1, lyxose–2, xylose–2 and arabinose–3, and ribose–3 and xylose–3 pentoses and the fructose–1 and tagatose–2 ketohexoses remain unseparated.

Some sugars studied also show a different number of fractions on the different

TABLE III

RETENTION DATA FOR PENTOSES AND HEXOSES ON XE-60

Trimethylsilyl derivative of:	*Relative retention*[a]	*Relative volatility, α*	*Separation factor, S*
Arabinose–1	0.236		
Rhamnose–1	0.255		
Lyxose–1	0.257	1.008	
Xylose–1	0.272		
Arabinose–2	0.273	1.004	
Rhamnose–2	0.321		
Ribose–1	0.330	1.028	
Lyxose–2	0.345		
Xylose–2	0.345	1.000	
Arabinose–3	0.345	1.000	
Rhamnose–3	0.365		
Ribose–2	0.378	1.036	
Ribose–3	0.421		
Xylose–3	0.425	1.010	~370,000
Xylose–4	0.563		
Sorbose–1	0.586	1.040	
Tagatose–1	0.616	1.052	
Mannose–1	0.657		
Fructose–1	0.706		
Tagatose–2	0.711	1.008	
Galactose–1	0.743	1.045	
Tagatose–3	0.774		
Fructose–2	0.823		
Tagatose–4	0.838	1.021	
Sorbose–2	0.851	1.015	~170,000
Galactose–2	0.912		
Tagatose–5	0.996		
Glucose–1	1.000	1.004	
Fructose–3	1.082		
Mannose–2	1.147		
Galactose–3	1.212	1.057	~ 12,000
Tagatose–6	1.234	1.018	~120,000
Sorbose–3	1.291		
Fructose–4	1.372		
Tagatose–7	1.389	1.013	
Tagatose–8	1.586		
Glucose–2	1.834		

[a] Glucose-1 = 1.000.

phases. Thus lyxose provides only one component on OV-101, but two components on a polar phase. Rhamnose provides three peaks on XE-60 but only one on a non-polar phase. Xylose has four separate fractions on a polar phase, but two peaks on OV-101.

The use of a polar phase is advantageous for the separation of the aldohexoses studied since the only pair which is difficult to separate on OV-101, galactose–3 and mannose–2, is separated on XE-60 on a column with an efficiency of approximately 20,000 theoretical plates (for $k' = 3$), cf. ref. 21.

It is interesting that the chromatographic spectrum is simpler in the case of aldohexoses than in that of ketoses. The aldoses show only two components corresponding to both the anomers; the third small fraction, which leaves the column

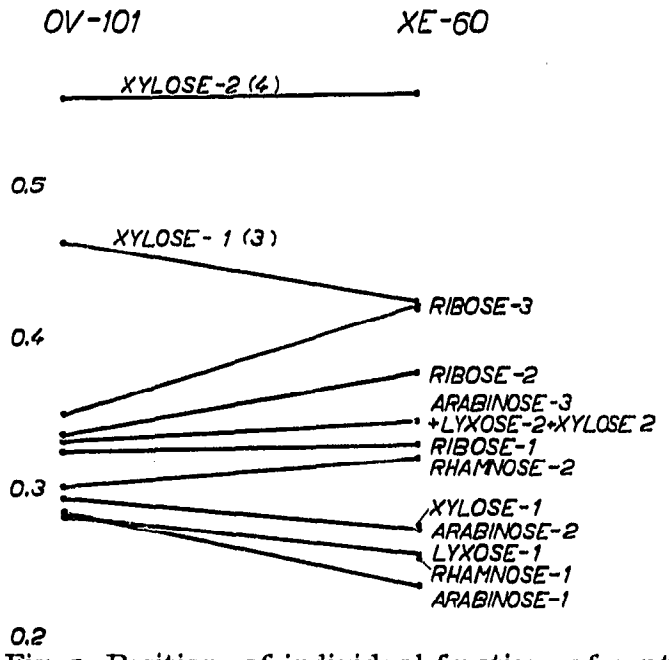

Fig. 1. Positions of individual fractions of pentoses on OV-101 and XE-60.

before both the main peaks, appears only in the case of galactose. In contrast to this, three ketoses provide a total of 14 peaks on OV-101, and 15 peaks on XE-60. Tagatose, with seven or eight components (Fig. 3), has the richest spectrum. This sugar is obviously contaminated with sorbose, galactose and talose, as follows from the almost

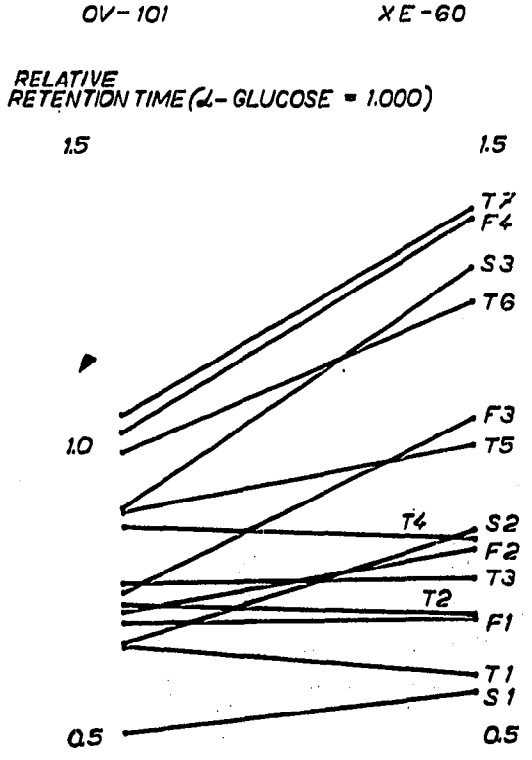

Fig. 2. Positions of individual fractions of ketohexoses on OV-101 and XE-60. Identification of fractions: F = fructose; S = sorbose; T = tagatose.

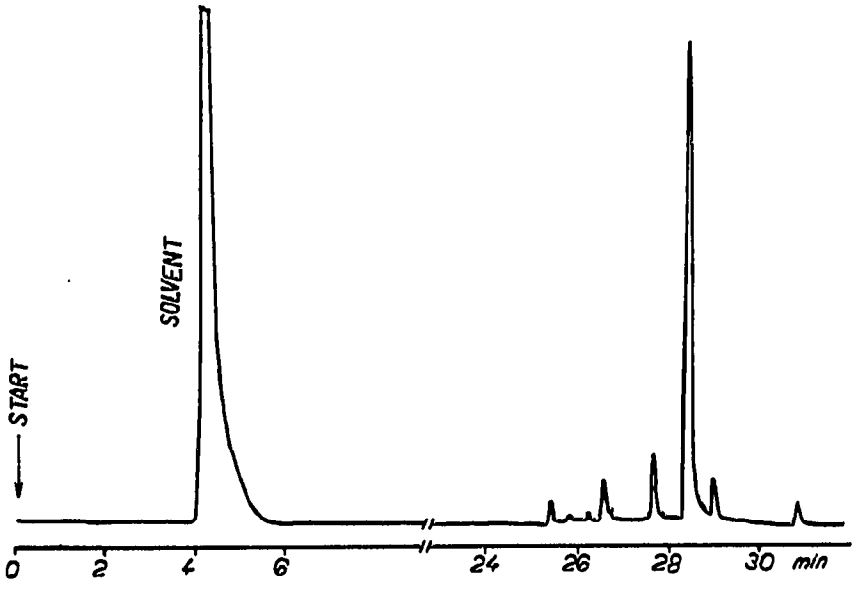

Fig. 3. Chromatogram of tagatose. Capillary column with I.D., 0.17 mm and length, 16 m; stationary phase, OV-101; temperature, 145°.

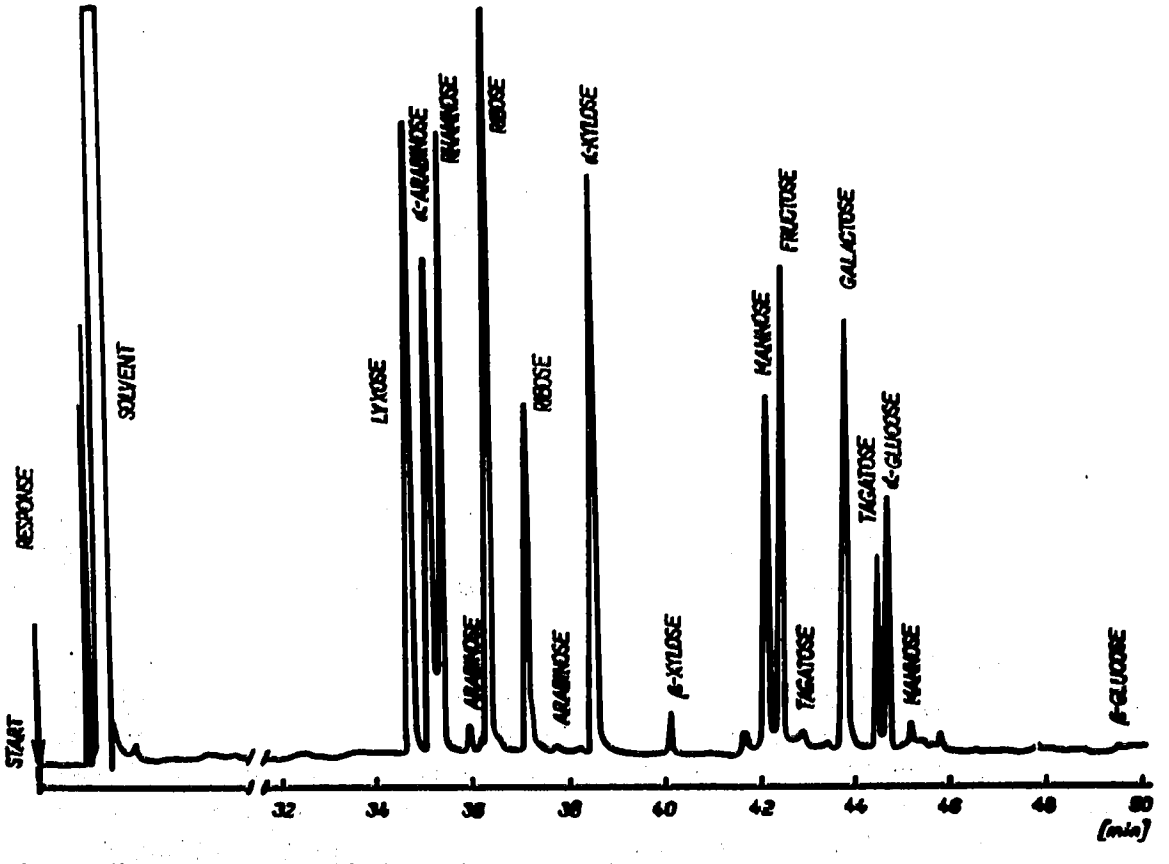

Fig. 4. Chromatogram of the mixture of ketoses. Capillary column with I.D., 0.17 mm and length, 16 m; stationary phase, OV-101; temperature, 160°.

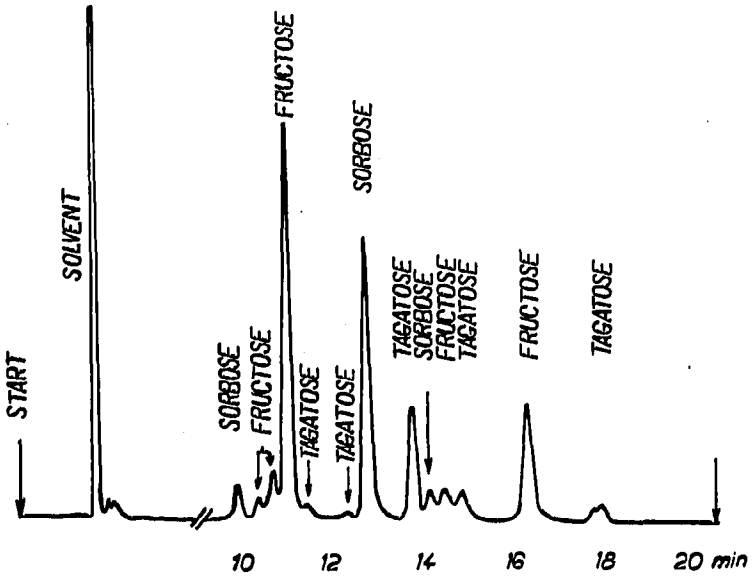

Fig. 5. Chromatogram of the mixture of pentoses and hexoses. Capillary column with I.D., 0.17 mm and length, 16 m; stationary phase, OV-101; temperature-programmed at 3.5°/min (T_R for β-glucose = 195°).

identical relative retention data on OV-101 for tagatose–1 and sorbose–2, tagatose–4 and talose (0.86)[6], and tagatose–5 and sorbose–3 or galactose–2.

The use of a polar phase is generally more advantageous for the separation of sugars since the number of pairs of components that can be separated only with difficulty is lower for both pentoses and hexoses than would be the case on a non-polar phase.

CONCLUSIONS

The use of capillary columns leads to a more complete separation of individual peaks of the sugars studied and allows their accidental contamination, if they are isolated from materials of natural origin, to be determined relatively easily. The use of two columns with stationary phases of different polarity allows the identification of individual sugars in simple mixtures.

The results also show that the silylation reaction is a complicating factor as not only both the anomers remain but also some more peaks appear for the individual monosaccharides. The latter yield a rich spectrum with overlapping peaks which make difficult both qualitative and quantitative analyses of more complex, especially natural, mixtures of sugars (see Figs. 4 and 5).

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