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LIQUID PHASES AND SOLID SUPPORTS FOR GAS–LIQUID CHROMATOGRAPHY OF TRIMETHYLSILYL DERIVATIVES OF MONOSACCHARIDES*

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SUMMARY

XE-60 afforded the best resolution between the trimethylsilyl (TMS) derivatives of a monosaccharide mixture containing arabinose, xylose, mannose, galactose and glucose. Critical resolutions involved certain isomers of (a) xylose and mannose, and (b) galactose and glucose. Resolution between the xylose and mannose isomers was related to the liquid phase concentration and complete resolution could be achieved by concentrations of 1.0–1.2% XE-60. Resolution between the galactose and glucose isomers at these liquid phase concentrations was markedly influenced by the solid support and an apparent consequent influence on the number of theoretical plates of the column. This resolution was markedly improved when dimethylchlorosilane-treated Chromosorb W, as compared to a non-silanized Chromosorb W, was used. The relative retention time and approximate isomer abundance for a number of other monosaccharide TMS derivatives is reported.

INTRODUCTION

The use of trimethylsilylation reagents affords a simple, rapid and quantitative method for forming derivatives of monosaccharides suitable for gas–liquid chromatography[7,11]. Chromatography of trimethylsilyl (TMS) derivatives of monosaccharides yields from two to four peaks per monosaccharide, which corresponds to the various structural and anomeric isomers of the monosaccharide. This occurrence of multiple peaks complicates the problem of finding one column which will resolve all the isomers of monosaccharides in complex mixtures such as is often present in biological material.

Alternatively, reduction to the corresponding sugar alcohols followed by trimethylsilation has been proposed[8,11] since only single peaks are yielded by sugar alcohol TMS on chromatography, apparently as the result of destruction of the ring structure. In addition to being more lengthy and lacking in resolution[8], this procedure is of limited value for some biological material which may contain sugar alcohols together with their corresponding monosaccharides.

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The use of derivatives other than TMS has been proposed[8,12]. However, these reactions generally lack the simplicity of the trimethylsilylation reaction using trimethylchlorosilane and hexamethyldisilazane[11] or bis-(trimethylsilyl)-acetamide[4]. The TMS derivatives, or the dimethylsilyl derivatives[10], also have the advantages of higher volatility allowing wider selection of liquid phase, less liquid phase "bleed" and longer column life.

The resolution of multiple peaks for monosaccharide TMS derivatives affords advantages in identification based upon chromatographic retention. The occurrence of other peaks having retention times and areas corresponding to that expected for the isomeric equilibrium mixture of a given monosaccharide TMS adds considerably more evidence confirming identification than does retention time of a single peak.

Complete resolution of all isomers of each monosaccharide TMS within a mixture may not be necessary for qualitative and quantitative analysis since (a) the abundancy of each isomer can be determined, and (b) each isomer TMS derivative is relatively stable[11]. Thus quantitative analysis could be achieved with a minimum resolution of one isomer TMS for each monosaccharide in the mixture[1]. Quantification could then be achieved from a knowledge of the resolved isomer's equilibrium abundance, area and appropriate response factors. Further, when more than one isomer is resolved, calculations of mass could be based on each isomer resolved and compared to provide evidence as to purity of chromatographic peaks.

Thus the TMS derivatives were thought to offer a number of advantages for gas–liquid chromatographic analysis of monosaccharide mixtures derived from complex biological materials and their hydrolysates[2]. Therefore, a number of liquid phases and solid supports were investigated with the objective of finding a combination which would resolve at least one isomer TMS for each monosaccharide likely to be present in most biological materials.

EXPERIMENTAL

Reagents

Chromatographically pure grades of the various monosaccharides (Mann Research Laboratories, New York) were allowed to equilibrate in water either at room temperature for 24 h or by refluxing for 1 h. Water was evaporated *in vacuo* at 40° and the solid sample dissolved in sufficient dimethylformamide (Matheson, Coleman and Bell, Norwood, Ohio) to yield concentrations ranging between 1 and 20 mg per ml. The TMS derivatives were prepared as previously described[3] and either the hexamethyldisiloxane phase over dimethylformamide or the water-washed hexamethyldisiloxane phase was chromatographed within 8 h after being washed.

Liquid phases other than XE-60 and the solid supports were obtained from Wilkens Instrument and Research Company, Walnut Creek, Calif. The Chromosorb W was acid-washed and, unless otherwise specified, was of 70–80 mesh size. The XE-60 was obtained from General Electric and represented a copolymer prepared from 50 mole % of dimethylsiloxane and 50 mole % of β-cyanoethylmethylsiloxane.

Apparatus

A Wilkens Model 600-B gas chromatograph with a flame ionization detector was employed. The injector port was maintained at 215° and contained a Pyrex insert.

Nitrogen was the carrier gas. Other operating conditions are cited in conjunction with appropriate figures and tables.

Procedure

The liquid phase to be tested was weighed into a 300 ml round-bottom flask with ground joint and dissolved in approximately 100 ml of an appropriate solvent (usually methylene chloride). The required amount of solid support was added and the flask connected to a rotary evaporator. Vacuum was applied to the slowly rotating flask at room temperature initially. Subsequent to the initial rapid boil off, the temperature of the flask was elevated to 40–50°. The angle of the evaporator and speed of rotation were varied to distribute the moist solid support evenly over the inner surface of the flask. Rotation was stopped when the moist support adhered to the flask. Vacuum and temperature were maintained until the partially dried support fell free of the upper surface at which time minimal rotation (30–40 r.p.m.) was resumed and the temperature rapidly elevated to 70–80°. Flask rotation was then limited to the time required to obtain a free flowing packing without lumps (1–3 min) at which time rotation was stopped and vacuum and temperature maintained for 20–30 min.

The solvent-washed and dried 0.32 cm O.D. × 3 m stainless steel columns were packed via gravity together with column vibration achieved with a high-speed electric motor-driven eccentric cam. The column was plugged with a minimum amount of glass wool previously treated with dimethylchlorosilane and coiled into an 8 cm diameter spiral.

The packed column was purged with nitrogen and conditioned by the following sequence: 100° for 6–12 h with a nitrogen flow of 1–2 ml/min; 180–225° (depending upon the liquid phase involved) for 6–12 h with 12–16 ml nitrogen per min; and finally, at the anticipated operating temperature and nitrogen flow for 2–4 h before use.

RESULTS

Initially, a number of different liquid phases were coated onto acid-washed 60–80 mesh Chromosorb W and evaluated with a mixture of trimethylsilyl derivatives of arabinose, xylose, mannose, galactose and glucose. The liquid phases investigated included: XE-60, 4%; QF-1, 5%; XF-1150, 5 and 10%; DC-11, 5, 9, 13, 18 and 21%; SE-52, 15%; SF-96, 5, 10 and 20%; DC-200, 5, 9, and 13%; neopenthylglycol isophthalate, 5%; ethylene glycol succinate, 15%; Carbowax 20M, 20%; Carbowax 1849, 10%; Carbowax 6000, 15%; Carbowax 4000, 20%; Carbowax 4000 terminated with terephthalic acid, 20%; and neopentaglycol succinate, 5%.

The most promising resolutions were afforded by the substituted silicones (XE-60, XF-1150 and QF-1) followed by the silicones of lower polarity (DC-11, DC-200 and SF-96). The more polar phases did not provide better resolutions and, with the exception of the Carbowaxes, resulted in poor peak symmetry. Based upon results to be presented later, it appeared that the unsymmetrical nature of peaks might have in part been attributed to interactions with the non-silanized solid support. However, unsymmetrical peaks and low recoveries have been reported for the relatively polar ethylene glycol succinate on HMDS-treated Chromosorb W[7].

Typical chromatograms involving columns packed with XE-60 on Chromosorb

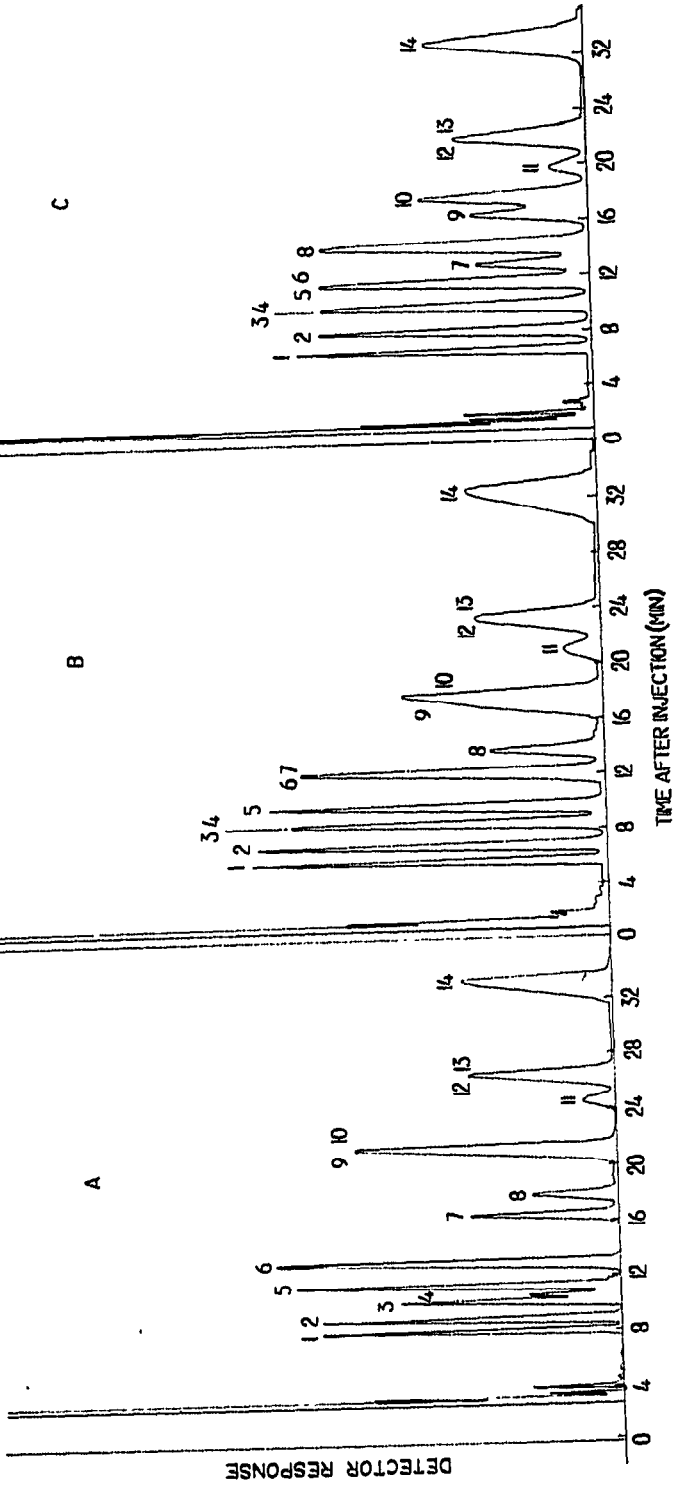


Fig. 1. Separation of a mixture of TMS monosaccharides as influenced by XE-60 concentration on Chromosorb W. Chromatogram A: 1% XE-60, 150°, 11.2 ml N_2/min. Chromatogram B: 4% XE-60, 155°, 12.2 ml N_2/min. Chromatogram C: 16% XE-60, 175°, 18.0 ml N_2/min. TMS arabinose, peaks 1, 2, 3 and 4; TMS xylose, peaks 5 and 6; TMS mannose, peaks 7 and 12; TMS galactose, peaks 8, 9, 11 and 13; TMS glucose, peaks 10 and 14.

W are reproduced in Fig. 1. Several modifications were tested in an effort to improve separations particularly between the second emerging xylose isomer (peak 6) and the first emerging mannose isomer (peak 7), and between the second emerging galactose isomer (peak 9) and α-glucopyranose (peak 10). Temperature programming or increasing column length to 6 m resulted in an increased resolution between the galactose and glucose isomers but not between those for xylose and mannose.

The concentration of XE-60 on Chromosorb W was found to have a marked effect upon the elution order for certain isomers as illustrated in Fig. 1. Increasing the concentration of XE-60 from 4 to 16% resulted in (a) the first emerging galactose isomer (peak 8) preceding the second emerging xylose isomer (peak 6) which it followed on the 1 and 4% XE-60 columns and (b) an improved resolution between the second emerging galactose isomer (peak 9) and α-glucopyranose (peak 10). Reducing the concentration from 4 to 1% resulted in (a) the resolution of a fourth emerging isomer for arabinose (peak 4), (b) a complete separation of the first emerging mannose isomer (peak 7) from xylose (peak 6), and (c) deviation from peak symmetry for β-glucose (peak 14).

The influence of concentration of XE-60 on Chromosorb W is further illustrated in Fig. 2. Increasing concentrations of XE-60 had a similar general effect upon the relative retention time for all the isomers except those for the first emerging isomers of galactose and glucose. These two isomers of galactose and mannose reacted in a uniform and uniquely different manner from the other isomers of these monosaccharides by a continued decrease in relative retention time with increasing XE-60 concentrations higher than 2–4%. This interesting interaction could possibly be used to prepare columns for specialized separations involving mannose and galactose and for further confirmatory identification of mannose and galactose.

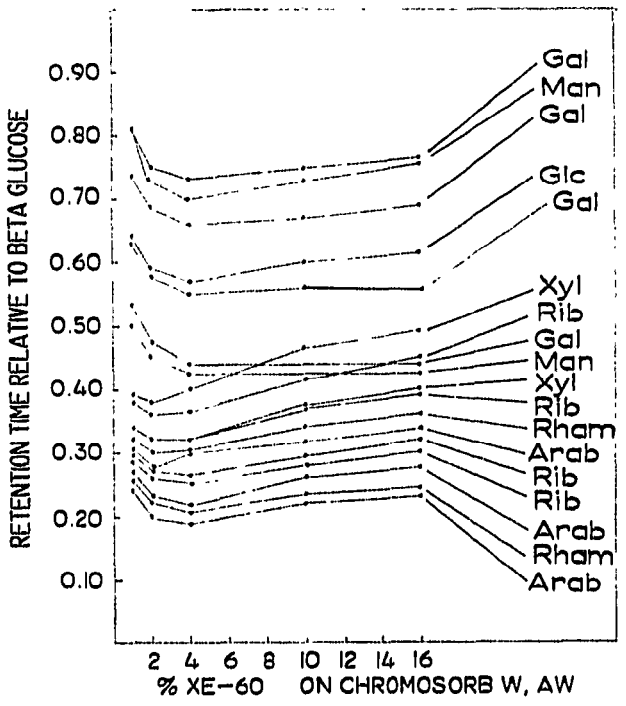


Fig. 2. Influence upon relative retention time of XE-60 concentration.

A typical separation of TMS derivatives when QF-1 was coated onto dimethylchlorosilane (DMCS)-treated Chromosorb W is shown in Fig. 3A. Chromatograms of similar mixtures of monosaccharide TMS were markedly different when the same concentration of QF-1 was coated onto Chromosorb W which had not been treated with DMCS (Fig. 3B). That such differences were attributable to the DMCS treatment was confirmed by their repeatability when additional column packings were prepared using different lots of the two differently treated Chromosorb solid supports. The use of DMCS-treated Chromosorb W as compared to Chromosorb W, resulted in (a) a markedly improved resolution between the second and third emerging isomers (peaks 3 and 4) of arabinose, (b) complete resolution of the isomers (peaks 6 and 7) of xylose, (c) complete resolution of the second emerging xylose isomer (peak 7) from the first emerging (peak 8) galactose isomer, (d) changes in the retention time for the

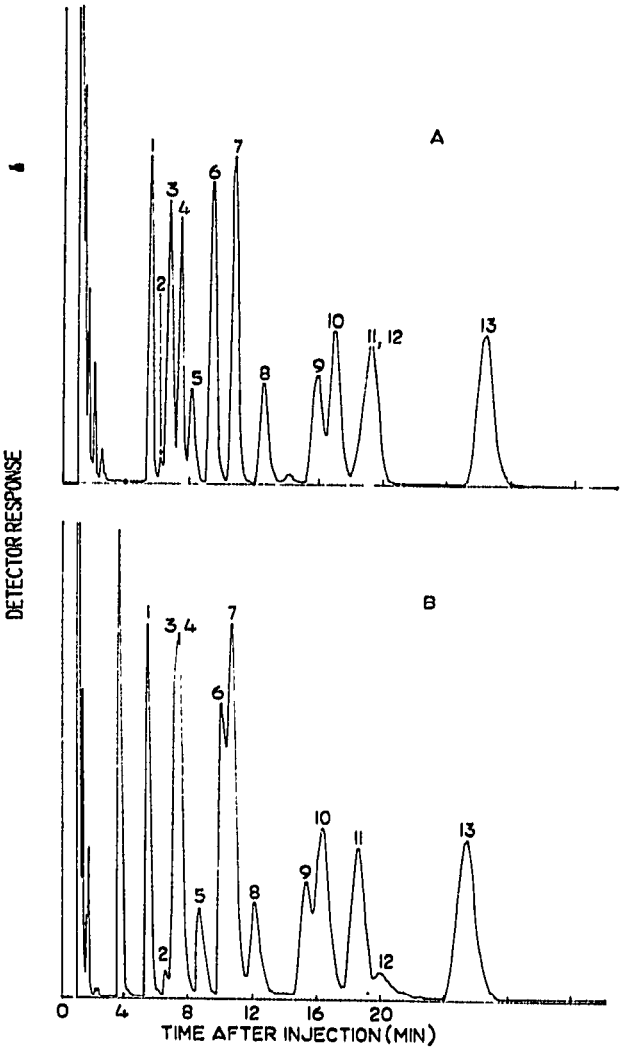


Fig. 3. Separation of a mixture of TMS monosaccharides as influenced by solid support for QF-1. Chromatogram A: 5% QF-1 on 60–80 mesh acid-washed, DMCS-treated Chromosorb W, 150°, 15 ml N_2/min. Chromatogram B: 5% QF-1 on 60–80 mesh, acid-washed Chromosorb W, 150°, 15 ml N_2/min. TMS arabinose, peaks 1, 3, 4 and 5; TMS rhamnose (impurity in arabinose), peak 2; TMS xylose, peaks 6 and 7; TMS glactose, peaks 8, 9, 11 and 12; TMS glucose, peaks 10 and 13. TMS erythritol precedes peak 1 in chromatogram B.

third (peak 11) relative to the fourth emerging isomer (peak 12) of galactose, (e) an inproved resolution between the second emerging isomer for galactose (peak 9) and β-glucose (peak 10), and (f) a reduction in the trail associated with all peaks (note especially peak 12, Fig. 3B).

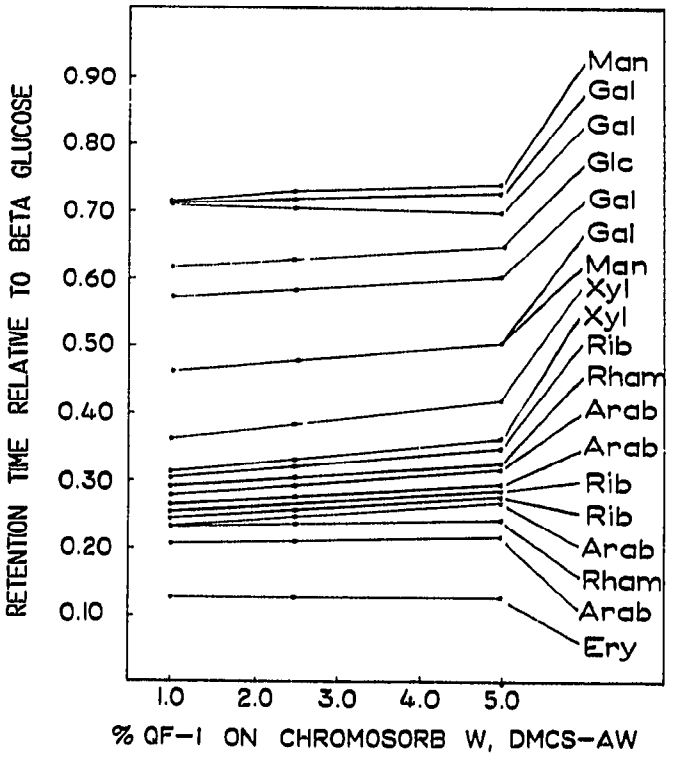
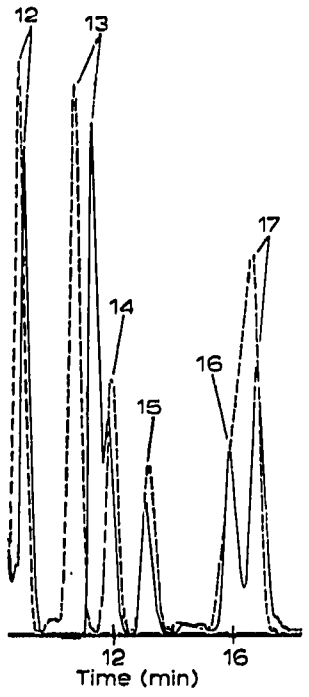


Fig. 4. Influence upon relative retention time of QF-1 concentration.

The influence of concentration of QF-1 on DMCS-treated Chromosorb W upon the relative retention time for a number of monosaccharide TMS derivatives is plotted in Fig. 4. In contrast to XE-60, the concentration of QF-1 had no significant differential effect upon the relative retention time of any isomer. The second emerging mannose isomer was insufficiently resolved from the third and fourth emerging isomers of galactose for qualitative purposes. Thus, the QF-1 phase would be of limited value for most biological applications in which mannose is likely to be a component together with galactose.

Since resolution by the QF-1 phase was improved by the use of a DMCS-treated solid support and the first emerging isomer of mannose was resolved from galactose and xylose by 2% XE-60 phase on Chromosorb W, this concentration of XE-60 was tested on DMCS Chromosorb W. Chromatograms produced from the same concentration (2%) of XE-60 on DMCS-AW Chromosorb W as compared to Chromosorb W are reproduced in Fig. 5 to illustrate the principal differences due to these two solid supports.

The use of DMCS-treated Chromosorb W, as compared to Chromosorb W, as the solid support for 2% XE-60 resulted in (a) a marked improvement in the resolution between the second emerging isomer of galactose (peak 16) and α-glucose (peak 17), (b) a reduced resolution between the second emerging isomer for xylose



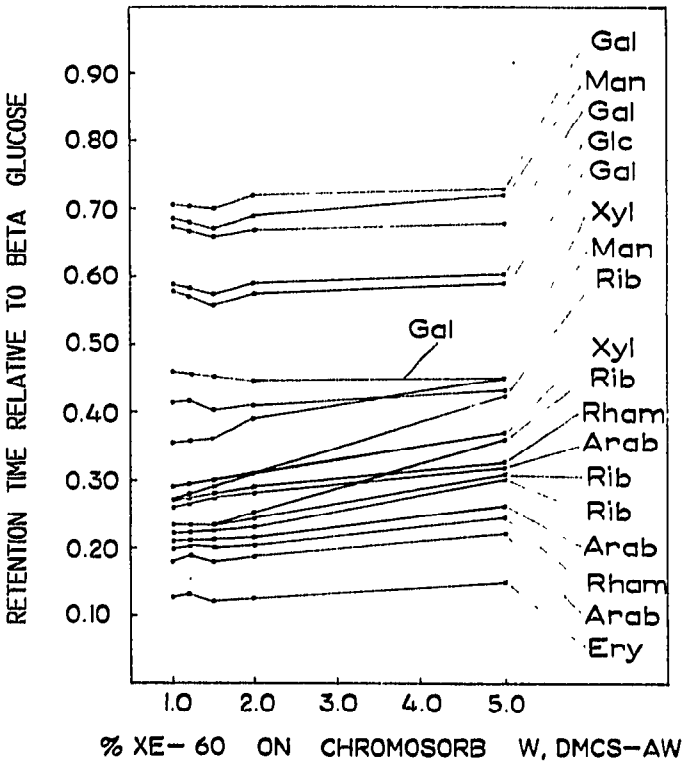


Fig. 5. Influence of solid support upon resolution by XE-60. ———, chromatogram A: 2% XE-60 on 70–80 mesh, acid-washed, DMCS-treated Chromosorb W, 145°, 15.8 ml N_2/min. – – – –, chromatogram B: 2% XE-60 on 60–80 mesh, acid-washed, Chromosorb W, 150°, 10 ml N_2/min. TMS xylose, peaks 12 and 13; TMS mannose, peak 14; TMS galactose, peaks 15 and 16; TMS glucose, peak 17.

Fig. 6. Influence upon relative retention time of XE-60 concentration.

(peak 13) and the first emerging mannose isomer (peak 14), and (c) improved peak symmetry, especially evident for β-glucose.

Much of the improved resolution between α-glucose (peak 16) and the third emerging isomer of galactose (peak 17) appears to have been the result of improved symmetry for the two peaks and an increase in theoretical plates per column (column 5 *vs.* column 2, Table I) when DMCS-treated solid support was used. In contrast, differences in resolution between the second emerging xylose isomer (peak 13) and the first emerging mannose isomer (peak 14) appeared to be largely due to an effect by the solid support upon the absolute retention time of the xylose isomer. The retention time for the xylose isomer was longer when DMCS-treated Chromosorb W was the solid support.

The influence of concentration of XE-60 on DMCS Chromosorb W was then studied to determine if the resolution between xylose and galactose could be improved. Results of these studies are summarized in Fig. 6. The relative retention time for all isomers of hexoses was not greatly influenced by XE-60 concentration. Since retention time was expressed relative to that of a hexose (β-glucose), it appeared that all isomers of the hexoses were similarly influenced by XE-60 concentration. In contrast, the relative retention time for all isomers of the pentoses, and especially those for xylose, increased with increased concentrations of XE-60. Consequently at

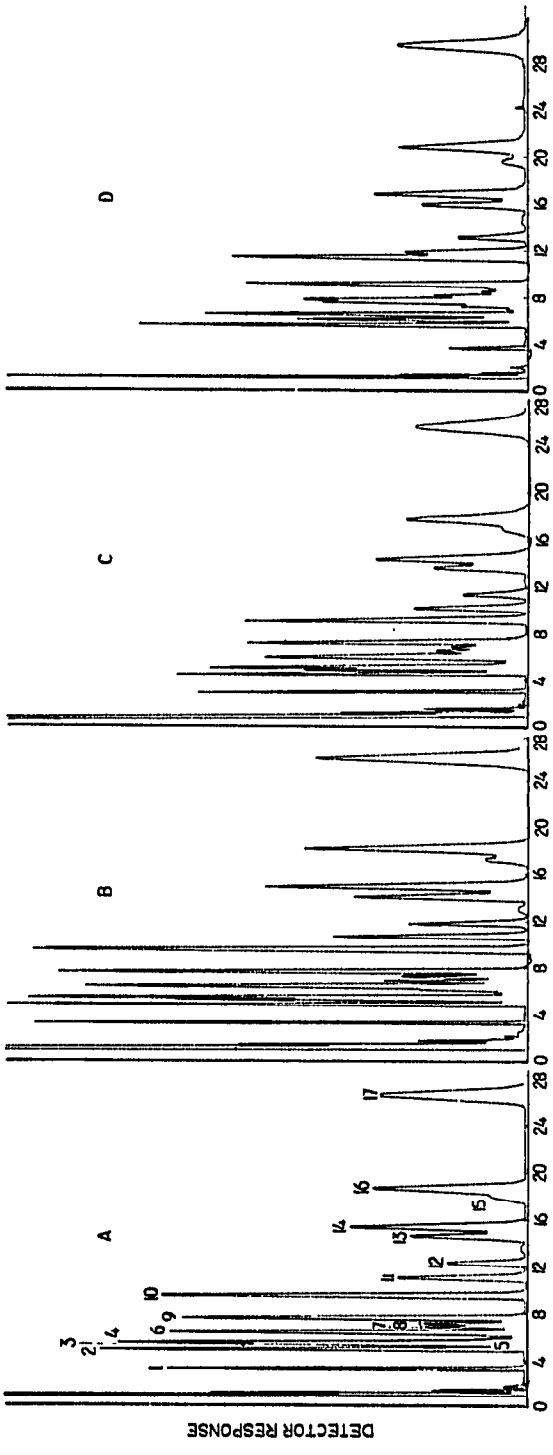


Fig. 7. Separation of a complex mixture of TMS monosaccharides as influenced by XE-60 concentration on acid-washed, DMCS-treated Chromosorb W. Chromatogram A: 1% XE-60, 140°, 16.2 ml N_2/min, 3 m column. Chromatogram B: 1.2% XE-60, 144°, 15.5 ml N_2/min, 3 m column. Chromatogram C: 1.5% XE-60, 144°, 19.5 ml N_2/min, 3 m column. Chromatogram D: 2.0% XE-60, 145°, 15.8 ml N_2/min, 3 m column. TMS erythritol, peak 1; TMS arabinose, peaks 2, 4, 6 and 7; TMS rhamnose, peaks 3 and 8; TMS ribose, peaks 5, 6 and 7; TMS xylose, peaks 9 and 10; TMS mannose, peaks 11 and 16; TMS galactose, peaks 12, 13, 15 and 16; TMS glucose, peaks 15 and 17.

the 5% XE-60 concentration this resulted in retention times for isomers of the earliest emerging hexoses (galactose and mannose) being approximately equal to those for the latest emerging pentose (xylose). This effect was similar to that previously observed for different concentrations of XE-60 on Chromosorb W (Fig. 2). However, the influence of concentration of XE-60 on DMCS Chromosorb W appears to be more uniformly related to the molecular weight of the isomers in that the relative retention time for the first emerging isomers of galactose and mannose were influenced by phase concentration in a manner similar to that of other hexoses. In contrast, the relative retention of these isomers were influenced by increasing concentrations of XE-60 on Chromosorb W in a manner characteristically different from the other isomers of mannose and galactose, the hexoses or the pentoses (Fig. 2).

Only slight changes in concentration of XE-60 on DMCS Chromosorb W resulted in appreciable changes in resolution between xylose and mannose. This is illustrated in the four chromatograms reproduced in Fig. 7. Depending upon the relative concentrations of xylose and mannose, a concentration of XE-60 no greater than 1.2% was required for complete separation of the second emerging xylose isomer (peak 10) from the first emerging mannose isomer (peak 11).

Resolution between the various isomers of arabinose, rhamnose, and ribose was improved by increasing concentrations of XE-60. However, this was insufficient for quantitative purposes. Resolution between the first emerging rhamnose isomer (peak 3) and the second emerging arabinose isomer by the 1.2% XE-60 column was sufficient to qualitatively detect rhamnose. The resolution achieved by the XE-60 columns packed with the same lot of 70–80 mesh DMCS Chromosorb W was reproducible with other columns. Thus the resolution attained with this lot of DMCS Chromosorb W was not an artifact of packing and/or column preparation. However, resolution between the galactose and glucose isomers could not be subsequently reproduced when two other brands of silanized solid supports or five other different lots of DMCS Chromosorb W were employed. The resolution achieved by these other brands and lots of silanized solid supports is typified by the chromatogram reproduced in Fig. 8A. The principal difference in resolution between these and the original solid support is between the second emerging galactose isomer (peak 10) and that of α-glucopyranose (peak 11). In this respect the lack of resolution between these two isomers resembles that achieved by the non-silanized Chromosorb W (Fig. 1A). However, the silanized solid supports employed subsequently were similar to the initial lot of silanized solid support in that, for a given concentration of XE-60, the separation between the second emerging xylose isomer and the first emerging mannose isomer was less for the silanized than for the non-silanized solid supports (Figs. 1, 6, and 8A). Separation between these two isomers was similarly affected by concentration of XE-60 (Fig. 7) on the silanized solid supports subsequently employed.

Resolution between the galactose and glucose isomers could be increased by increasing the column length to 6 m and, as a consequence, increasing the theoretical plates per column (Table I). Resolution between these two isomers adequate for most quantitative purposes required a column containing more than 4,800 theoretical plates (column 4, Table I). Resolution between these two isomers increased in proportion to the number of theoretical plates of the column with excellent resolution being achieved by a column containing 10,050 theoretical plates (column 14, Table I). Tightly packed columns were essential to achieve this as is evident by comparing

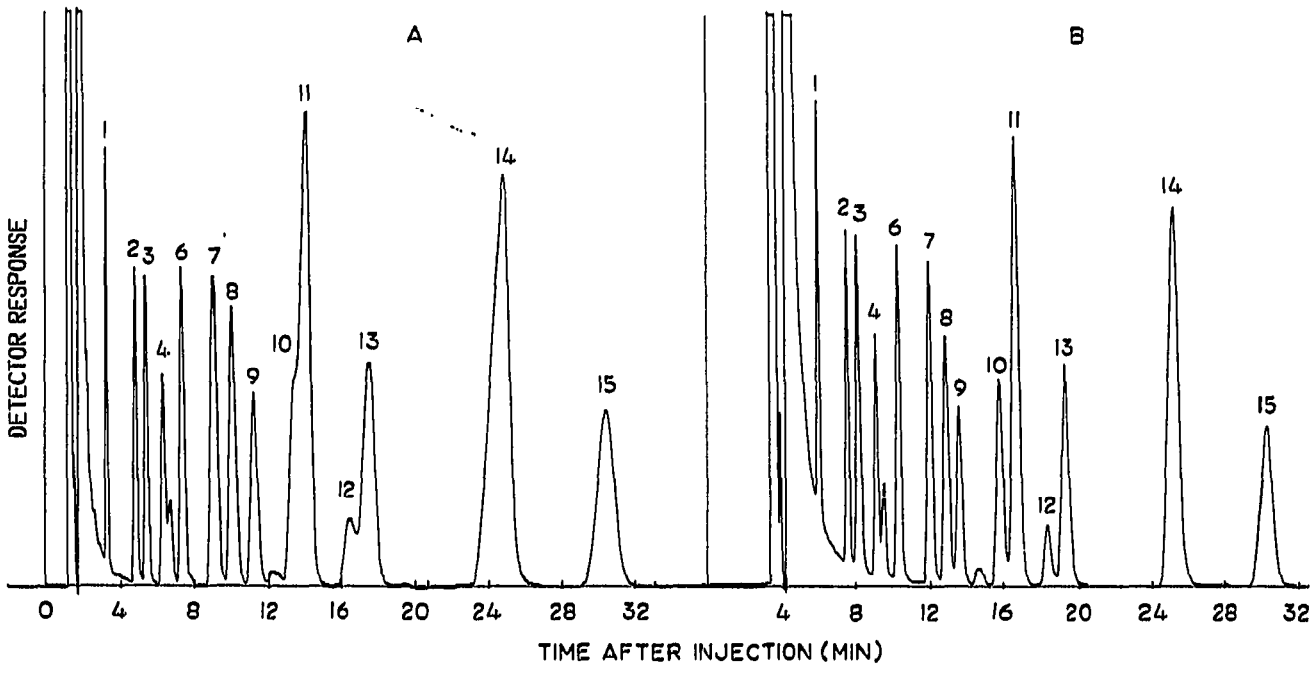


Fig. 8. The influence of column length upon resolution by 1% XE-60 on 70–80 mesh, DMCS-treated Chromosorb W. Chromatogram A: 3 m; chromatogram B: 6 m.

columns 13 and 14, and 9, 11 and 14 in Table I. A minimum of 1.33 g of packing per meter of 0.125 in. O.D. stainless steel tubing appeared essential for good resolution.

The relative retention times for a number of TMS derivatives on the 1.2% XE-60 and 5% QF-1 columns are summarized in Table II.

TABLE I

THEORETICAL PLATES IN CERTAIN COLUMNS PACKED WITH XE-60 ON DIFFERENT LOTS OF CHROMOSORB W

Column No.	*Solid support*	*Lot No.*	*XE-60 conc. (%)*	*Column packing (g)*	*Column length (m)*	*Figure No.*	*Theoretical plates/column*
1	AW	1	1	4.35	3	1A	4 700
2	AW	1	2	4.30	3	5B	2 700
3	AW	1	16	4.10	3	1C	2 400
4	DMCS	2	1.0	4.30	3	8A	4 800
5	DMCS	2	1.2	4.45	3	8B	5 300
6	DMCS	2	2.0	4.05	3	8D	5 900
7	DMCS	3	1.2	4.40	3	—	4 000
8	DMCS	4	1.2	3.50	3	—	3 100
9	DMCS	4	1.2	7.90	6	—	5 900
10	DMCS	5	1.3	4.10	3	—	3 300
11	DMCS	5	1.3	8.00	6	—	7 200
12	DMCS	5	1.0	4.00	3	9A	3 100
13	DMCS	5	1.0	7.25	6	—	3 500
14	DMCS	5	1.0	8.10	6	9B	11 000

TABLE II

RELATIVE RETENTION TIME[a] AND APPROXIMATE ABUNDANCE[b] OF ISOMERS OF TRIMETHYLSILYL DERIVATIVES OF MONOSACCHARIDES AND RELATED POLYHYDROXY COMPOUNDS

TMS derivative	*XE-60, 1.2% on DMCS-treated Chromosorb W*				*QF-1, 5% on DMCS-treated Chromosorb W*			
	1	*2*	*3*	*4*	*1*	*2*	*3*	*4*
Erythritol								
R_{Bg}	0.13	—	—	—	0.14	—	—	—
Arabinose								
R_{Bg}	0.19	0.22	0.26	0.27	0.21	0.25	0.29	0.30
A (%)	25	36	31	8	27	35	28	9
Fucose								
R_{Bg}	0.20	0.26	0.30	0.32	0.23	0.31	0.35	—
A (%)	16	35	8	40	20	35	45	—
Rhamnose								
R_{Bg}	0.21	0.28	—	—	0.26	0.34	—	—
A (%)	88	12	—	—	84	16	—	—
Deoxyribose								
R_{Bg}	0.20	—	—	—	0.17	—	—	—
Lyxose								
R_{Bg}	0.21	0.26	—	—	0.23	0.28	—	—
A (%)	87	13	—	—	83	17	—	—
Ribose								
R_{Bg}	0.23	0.24	0.25	0.28	0.29	0.31	0.37	—
A (%)	5	16	64	15	27	62	11	—
Deoxyglucose								
R_{Bg}	0.26	0.40	—	—	0.32	0.46	—	—
A (%)	54	46	—	—	53	47	—	—
Xylitol								
R_{Bg}	0.27	0.29	—	—	0.33	0.34	—	—
A (%)	26	74	—	—	25	75	—	—
Adonitol								
R_{Bg}	0.28	—	—	—	0.32	—	—	—
Xyloside								
R_{Bg}	0.29	—	—	—	0.36	—	—	—
Xylose								
R_{Bg}	0.30	0.37	—	—	0.35	0.40	—	—
A (%)	48	52	—	—	44	56	—	—
Sorbose								
R_{Bg}	0.34	0.44	0.51	0.67	0.46	0.62	1.00	1.10
A (%)	6	63	4	27	33	44	10	13
α-Methyl mannoside								
R_{Bg}	0.39	—	—	—	0.41	—	—	—
Fructose								
R_{Bg}	0.41	0.42	0.44	0.51	0.46	0.53	—	—
A (%)	22	46	25	7	68	32	—	—
Mannose								
R_{Bg}	0.42	0.69	—	—	0.46	0.74	—	—
A (%)	85	15	—	—	85	15	—	—

TABLE II (*continued*)

TMS derivative	*XE-60, 1.2% on DMCS-treated Chromosorb W*				*QF-1, 5% on DMCS-treated Chromosorb W*			
	1	*2*	*3*	*4*	*1*	*2*	*3*	*4*
Galactose								
R_{Bg}	0.46	0.55	0.67	0.71	0.43	0.55	0.63	0.67
A (%)	21	30	11	39	21	30	9	40
Glucose								
R_{Bg}	0.58	1.00	—	—	0.63	1.00	—	—
A (%)	47	53	—	—	40	60	—	—
Glucosamine								
R_{Bg}	0.70	—	—	—	0.74	—	—	—
Mannitol								
R_{Bg}	0.70	—	—	—	0.78	—	—	—
α-Methyl glucoside								
R_{Bg}	0.71	—	—	—	0.62	—	—	—
Sorbitol								
R_{Bg}	0.71	—	—	—	0.78	—	—	—
Dulcitol								
R_{Bg}	0.73	—	—	—	0.81	—	—	—
Sedoheptulose								
R_{Bg}	1.12	—	—	—	1.14	—	—	—
Mannoheptulose								
R_{Bg}	1.31	—	—	—	1.59	—	—	—
Mesoinositol								
R_{Bg}	1.36	—	—	—	1.82	—	—	—

[a] Retention time relative to β-glucopyranose TMS.
[b] *A* = approximate abundance calculated from peak area expressions determined as the product of peak height times retention time and assuming equal detector response factors for each isomer.

DISCUSSION

Empirically, two factors appear to critically influence resolution of TMS derivatives of the more common monosaccharides (arabinose, xylose, mannose, galactose and glucose) by the XE-60 liquid phase. These are (a) an effect of liquid phase film thickness upon the separation of xylose and mannose isomers, and (b) an effect by the solid support upon separation of galactose and glucose isomers. The two effects appear to be of different origins in that resolution between the second emerging galactose isomer and α-glycopyranose was related to the number of theoretical plates of the columns while resolution between the second emerging xylose isomer and the first emerging mannose isomer was not. Resolution in the latter case appeared to be due to an interaction between thickness of liquid phase and the molecular weight of the derivatives (pentoses *vs.* hexoses). Thus, low liquid phase concentrations were

required for the widest separation between pentose TMS and hexose TMS as groups and, as a consequence of their elution order, the isomers of xylose TMS and mannose TMS.

A similar effect of liquid phase concentration upon the resolution of TMS derivatives has been reported by OATES AND SCHRAGER[5]. Using butane-1,4-diol succinate polyester on alkali- and acid-washed Celite, they reported that "too little" stationary phase resulted in poor resolution of α-glucose and α-galactose peaks whereas "too much" brought the α-fucose and α-mannose peaks closer together.

A similar effect of liquid phase concentration upon the elution order of trifluoroacetyl butyl esters of certain amino acids has recently been reported[9]. Changes in concentration of ethylene glycol adipate on acid-washed Chromosorb W from 0.5 to 2.0% resulted in a complete reversal in the elution order for esters of hydroxyproline and methionine. The retention times of other esters were not affected. These investigators suggested that such selective effects might be attributed to interactions between the polar liquid phase and polar groups of the involved amino acid derivatives. Such effects appear unlikely in the present case due to (a) the highly unpolar nature of the TMS derivatives and (b) the homologous nature of the monosaccharides as compared to amino acids.

A concentration of 1.0% XE-60 on DMCS-treated Chromosorb W packed in a 6 m column appeared to afford the best resolution of TMS derivatives of the more common monosaccharides (arabinose, xylose, galactose, mannose, and glucose) present in plant material. This column will resolve all the isomers of each monosaccharide from the isomers of other monosaccharides with the exception of the second emerging mannose and the fourth emerging isomer of galactose. In this case, the completely resolved, first emerging isomer of mannose represents the major aqueous equilibrium isomer (approximately 90%) and the concentration of mannose can be calculated from a knowledge of the abundance of this isomer. Similarly, a correction can be applied for the contribution of the less abundant mannose isomer to the fourth emerging galactose isomer and the concentration of galactose calculated.

The resolution between the TMS derivatives of α-glucose and isomers of galactose by the 1% XE-60 is superior to that reported for columns packed with ethylene glycol succinate polyester[1,6,11]. Carbowax 20 M has been reported[7] to provide essentially complete separation of TMS derivatives of these two isomers but requires approximately 40 min, as compared to 20 min (Fig. 8) for the 1% XE-60 phase. Further, based upon published retention times[7], the Carbowax 20 phase does not resolve TMS derivatives of certain isomers of xylose from glucose, and certain isomers of arabinose from mannose.

A number of investigators have resolved only three peaks associated with galactose from aqueous solution[5–8,11] whereas others[1,13] have resolved four peaks as reported in the present investigation. Resolution of this fourth isomer (peak 12, Fig. 8) was influenced by the solid support and the number of theoretical plates of the column in a fashion similar to the resolution between the second emerging galactose peak and α-glucose.

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