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SOLVENTS FOR THE FORMATION AND QUANTITATIVE CHROMATOGRAPHY OF TRIMETHYLSILYL DERIVATIVES OF MONOSACCHARIDES*

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SUMMARY

The use of dimethylformamide or dimethylsulfoxide as the reaction solvent for forming trimethylsilyl ether derivatives with hexamethyldisilazane and trimethylchlorosilane resulted in the formation of a secondary upper phase of hexamethyldisiloxane. The trimethylsilyl ethers of monosaccharides exhibited a high partition for this phase and could thereby be concentrated and removed from the primary reaction solvent. Due to its shorter retention time and less solvent trail, hexamethyldisiloxane was a superior solvent to either dimethylformamide, dimethylsulfoxide or pyridine for gas-liquid chromatography. Additionally, partition into the hexamethyldisiloxane phase afforded specificity for monosaccharide derivatives. Variables such as trimethylsilylation reagent volume, water content of the primary solvents and monosaccharide concentration and proportions were studied and found to be sufficiently definable and reproducible to permit quantitative chromatography of the hexamethyldisiloxane phase.

INTRODUCTION

The use of hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) affords a simple, rapid and quantitative method for preparing trimethylsilyl (TMS) derivatives of monosaccharides suitable for gas-liquid chromatography[14]. Pyridine has been the reaction solvent of choice due, among other reasons, to its compatible solvent properties for reactants and derivatives. However, pyridine trails quite markedly on both polar and non-polar chromatographic liquid phases, often obscuring early emerging components and rendering quantification difficult. Further, certain monosaccharides are of rather limited solubility in pyridine thereby increasing the chromatographic solvent trail to derivative peak ratio.

A number of alternative solvents have been tested and dimethylformamide and dimethylsulfoxide were found to have a number of advantages over pyridine for the chromatography of monosaccharide TMS derivatives.

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EXPERIMENTAL

Chromatographically pure grades of the various monosaccharides (Mann Research Laboratories, New York) were equilibrated in water (20°) for 24 h or by refluxing for 1 h. Water was removed by evaporation at 40° *in vacuo* and the monosaccharides dissolved in sufficient solvent to yield a concentration of 0.2–6.0 mg of individual monosaccharide per ml. Reagent grades of dimethylformamide (DMF), pyridine and dimethylsulfoxide (Matheson, Coleman and Bell, Norwood, Ohio) were used as received after drying over potassium hydroxide pellets.

The trimethylsilyl (TMS) derivatives were prepared initially by the method of SWEELEY *et al.*[13,14]. The final procedure adopted involved 1–3 ml of the solvent containing the monosaccharide mixture in an 8 ml screw-cap test tube to which were successively added 0.3 and 0.2 volumes of HMDS and TMCS, respectively, with intermediate mixing and final capping (plastic-lined cap), and mixing via inversion.

The reaction mixture, or the upper phase when DMSO was the reaction solvent, was chromatographed no sooner than 10 min after trimethylsilylation. The upper phase formed within 16–20 h when DMF was the reaction solvent and an aliquot was removed by the injection syringe for chromatography.

When the reaction solvent was to be removed from the upper phase, this phase was further delineated by centrifugation and transferred by a Pasteur pipette to a second test tube. The upper phase was washed twice with 0.5 ml water and transferred to a screw-cap test tube and dried with anhydrous sodium sulfate.

A Wilkens Gas Chromatograph, Model 600-B, with flame ionization detector was employed. Relatively thick-film columns of nitrile silicone (4 and 16% w/w XE-60; 50% nitrile substitution, General Electric) were used in the intial work and thinner film (1%) columns of higher resolution were used in the later work. Details of column preparation are reported separately[3].

Detector response was initially measured[2,6,8] as the product of peak height and peak retention time (the latter expressed as distance from injection point). Later, response was measured with an electronic digital integrator with an automatic baseline corrector (Infotronics, Model CRS-11 HSB).

Detector response factors (K) for the TMS derivatives of the various monosaccharides were calculated relative to the detector response for sedopheptulose (K_s) or the first emerging (and principal peak) for mannose presumed to be α-mannopyranose (K_m).

$$K = \frac{\text{summated response for monosaccharide isomers/response of internal standard monosaccharide}}{\text{mg monosaccharide per ml of solvent/mg standard monosaccharide per ml of solvent}}$$

Isomer abundance was calculated as the individual isomer response divided by the summated responses for the monosaccharide isomers resolved by the column and is expressed as a percentage.

RESULTS

When DMF was used as the reaction solvent, oily-appearing droplets became noticeable in the DMF within 8–12 h after addition of trimethylsilylation reagents with a distinct upper phase forming after 12–18 h. The volume of this upper phase increased

rapidly up to 18–24 h and slowly thereafter with new droplet formation in the DMF phase continuing for 4–5 days. Chromatography of the DMF and upper phase over the DMF several days after trimethylsilylation suggested a preferential partitioning of the monosaccharide TMS derivatives into the upper phase. The DMF was only slightly soluble in this upper phase. The upper phase yielded a sharper and earlier emerging chromatographic peak than did DMF. This suggested the possibility of selectively partitioning the monosaccharide TMS derivatives into this phase and the removal of residual DMF by water washing as a means of eliminating the interfering DMF chromatographic peak and trail. The results of such a procedure are illustrated in Fig. 1.

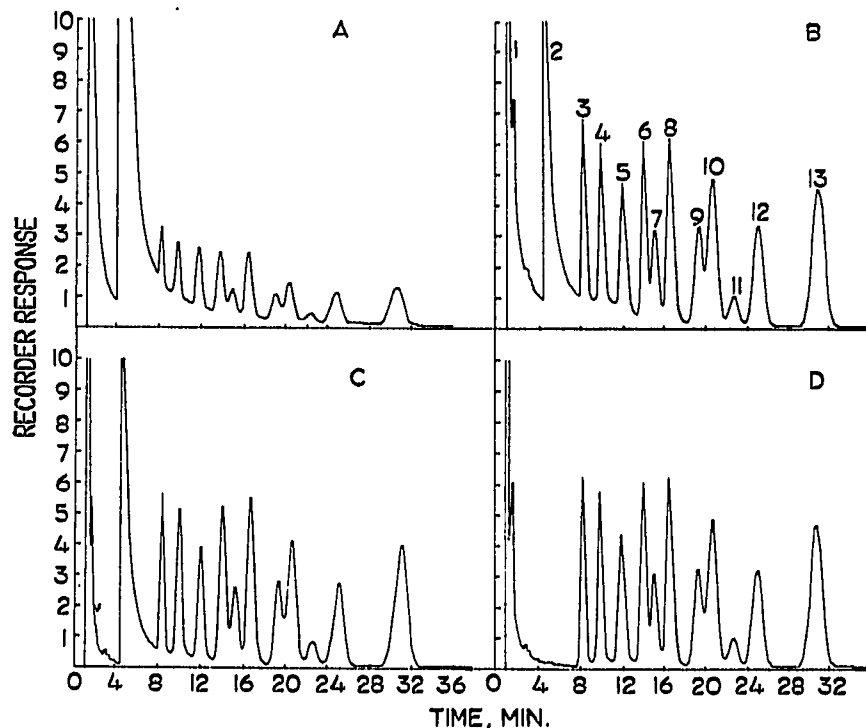

Fig. 1. Distribution of TMS monosaccharides within the DMF reaction mixture and subsequent phases. Chromatogram A: DMF reaction mixture 15 min after trimethylsilylation, 1.5 μl, 10 × 64 attenuation. Chromatogram B: upper phase over DMF 15 h after trimethylsilylation, 1.5 μl, 10 × 64 attenuation. Chromatogram C: upper phase over DMF 33 h after trimethylsilylation, 1.5 μl, 10 × 64 attenuation. Chromatogram D: upper phase 33 h after trimethylsilylation after removal and water extraction, 1.5 μl, 10 × 64 attenuation. Peak identification: upper phase, 1; DMF, 2; TMS arabinose, 3, 4, and 5; TMS xylose, 6 and 8; TMS galactose, 7, 9, 11, and 12; TMS glucose, 10 and 13. Monosaccharide concentration: 5 mg of each monosaccharide/ml DMF.

Approximately equal volumes of the DMF reaction mixture or the upper phases were chromatographed under identical operating conditions. It is apparent from the chromatograms in Fig. 1 that the monosaccharide TMS derivatives were more concentrated in the upper phase compared to the DMF reaction mixture. The detector response to the derivatives per unit volume injected increased in approximate proportion to the ratio between the volumes of the DMF and upper phase (approximately 3.5). This suggested a high partition coefficient in favor of the upper phase.

Detectable amounts of derivatives were found in the DMF phase as long as

TABLE I

RELATIVE DISTRIBUTION OF MONOSACCHARIDE DERIVATIVES WITH TIME AFTER TRIMETHYLSILYLATION IN DIMETHYLFORMAMIDE AND IN SUBSEQUENT PHASES[a]

Solvent chromatographed	*Time after trimethylsilylation, (h)*	*Arabinose*					*Xylose*			*Sedohep.*	
		Monomer (K_m)	*Isomer (mole %)*				*Monomer (K_m)*	*Isomer (mole %)*		*Monomer (K_m)*	*Isomer (mole %)*
			1	*2*	*3*	*4*		*1*	*2*		
DMF	2	1.24[b] ± 0.06[c]	25.6	27.3	38.6	8.5	1.20 ± 0.02	41.6	58.4	0.80 ± 0.08	100
DMF	6	1.21 ± 0.05	26.7	26.9	37.2	9.2	1.22 ± 0.02	41.8	58.2	0.96 ± 0.06	100
HMDSO over DMF	24	1.10 ± 0.04	28.8	30.1	32.7	8.4	1.11 ± 0.01	42.5	57.5	0.78 ± 0.03	100
HMDSO over DMF	96	1.07 ± 0.05	27.4	29.6	34.4	8.6	1.09 ± 0.02	42.5	57.5	0.77 ± 0.03	100
H_2O washed, dried HMDSO	98	0.99 ± 0.08	26.1	33.2	33.73	7.0	1.03 ± 0.03	44.1	55.9	0.38 ± 0.04	100
H_2O washed, dried HMDSO	120	0.95 ± 0.08	26.6	31.8	34.0	7.6	0.91 ± 0.04	44.8	55.2	0.36 ± 0.10	100
H_2O washed, dried HMDSO	192	1.22 ± 0.10	24.7	33.9	36.2	5.2	1.34 ± 0.07	45.2	54.8	0.11 ± 0.20	100

Solvent chromatographed	*Time after trimethylsilylation, (h)*	*Galactose*					*Glucose*		
		Monomer (K_m)	*Isomer (mole %)*				*Monomer (K_m)*	*Isomer (mole %)*	
			1	*2*	*3*	*4*		*1*	*2*
DMF	2	1.00 ± 0.02	14.1	25.4	5.6	55.0	1.22 ± 0.01	41.9	58.1
DMF	6	1.05 ± 0.02	13.2	25.1	5.4	55.8	1.28 ± 0.01	41.9	58.1
HMDSO over DMF	24	0.95 ± 0.01	13.6	26.9	5.8	53.7	1.20 ± 0.01	43.1	56.9
HMDSO over DMF	96	0.96 ± 0.02	13.9	24.4	7.0	55.7	1.21 ± 0.01	43.4	56.6
H_2O washed, dried HMDSO	98	0.90 ± 0.03	13.1	27.9	5.9	53.3	1.14 ± 0.02	45.1	54.8
H_2O washed, dried HMDSO	120	0.90 ± 0.03	13.4	27.4	6.1	53.2	1.12 ± 0.01	44.4	55.6
H_2O washed, dried HMDSO	192	0.83 ± 0.05	13.5	32.0	4.2	50.4	1.09 ± 0.03	44.7	55.3

[a] Three milliliters containing 30 mg of total monosaccharides. Chromatography: 1.2% XE-60 on 70–80 mesh DMCS-treated Chromosorb W. 1/8 in. × 10 ft. stainless steel column, temperature 141°; N, 15 ml/min.
[b] Each value a mean of four separate samples analyzed in duplicate.
[c] Standard deviation, $n = 8$.

droplets continued to coalesce in this phase but were not detectable after 4–5 days when droplets ceased appearing. Thus, it appeared that partition of the monosaccharide TMS derivatives into the upper phase was near completion once coalescing and phasing was completed.

The physical appearance of the upper phase resembled a silicone which was assumed to be hexamethyldisiloxane (HMDSO) formed by excess HMDS and TMCS hydrolyzing to trimethylsilanol which then polymerized to HMDSO[11]. The boiling point of the upper phase was similar to and its chromatographic retention time identical with that of a reference sample of HMDSO (K and K Laboratories). With evaporation of the upper phase at room temperature and addition of HMDSO, chromatography yielded a single major peak with a retention time identical to HMDSO.

The upper phase formed only when both HMDS and TMCS were added to DMF. The infra-red absorption spectra of the water-washed and dried upper phase (formed in the absence of monosaccharides) was qualitatively and quantitatively identical to that of a reference sample of HMDSO, exhibiting regions of absorption characteristic for trimethylsilyl and disiloxane (–O–Si–O–) groupings, and devoid of peaks for chlorosilane and disilazane groupings[9]. Any unreacted HMDS and TMCS were apparently decomposed by the water wash. The infra-red absorption spectra of the upper phase over DMF similarly exhibited regions of absorption characteristic for disiloxane groupings in addition to that for chlorosilane, disilazane and DMF. The upper phase was therefore concluded to be hexamethyldisiloxane (HMDSO).

Chromatography of the HMDSO phase, as compared to a pyridine or DMF solvent reaction mixture, could simplify quantification due to concentration of the derivatives and removal of the interfering trail of the primary reaction solvent. The quantitative application of such a two-stage solvent system would require: (a) reproducible partition coefficients for all TMS derivatives of interest, (b) no hydrolysis of derivatives during washing, and (c) stability of the derivatives in the HMDSO phase. These points were investigated in an experiment, the results of which are presented in Table I.

The relative detector response factors (K_m) for all monosaccharide TMS derivatives were significantly lower in the HMDSO phase over DMF than in the DMF reaction mixture. This indicated that proportionally less of these derivatives, as compared to the mannose isomer TMS, were transferred into the HMDSO phase. The derivatives were of similar stability in the HMDSO phase over DMF as evidenced by the similar K_m values at 24 and 96 h after trimethylsilylation. Although lacking in precision, measurements of volume injected onto the chromatographic column, together with detector response, suggested similar absolute concentrations of all derivatives in the HMDSO phase over DMF at 24 and 96 h after trimethylsilylation.

Washing the HMDSO phase with water resulted in a lowering of the K_m values for all derivatives. This reduction was statistically significant ($P < 0.05$) for only glucose and sedoheptulose, however. The K_m values did not differ significantly 22 h after the water wash but did differ significantly after a longer time interval. The latest emerging isomer TMS for each monosaccharide was significantly ($P < 0.05$) reduced in abundance by the water wash as compared to its abundance in either the HMDSO phase or the DMF reaction solvent.

These data were interpreted to indicate a reproducible partition of TMS derivatives of these monosaccharides into the HMDS phase over DMF in which the

TABLE II

RELATIVE FORMATION, DISTRIBUTION, AND STABILITY OF TRIMETHYLSILYL DERIVATIVES AS INFLUENCED BY SOLVENT, WATER CONTENT OF SOLVENT, AND TIME AFTER TRIMETHYLSILYLATION

Reaction solvent	*Water added (μl/ml)*	*Chromatography*		*Arabinose*				*Xylose*		
		Solvent	*Time (h)*	*Monomer (K_s)*	*Isomer (mole %)*			*Monomer (K_s)*	*Isomer (mole %)*	
					1	*2*	*3+4*		*1*	*2*
Pyridine	0, 5, 10, 20	Pyridine	0.3–0.8	1.011[a] ± 6.6[b]	17.2	33.5	49.3	1.053 ± 7.5	45.5	54.5
	0, 5, 10, 20	Pyridine	24	1.042 ± 7.1	16.2	34.6	48.3	1.070 ± 3.1	45.0	55.0
	0, 5, 10, 20	Pyridine	96	0.918 ± 5.1	17.0	34.4	48.6	0.981 ± 4.6	44.3	55.7
DMF	0, 5, 10, 20	DMF	0.3–0.8	0.908 ± 5.3	28.0	20.1	42.9	0.951 ± 2.1	44.2	55.8
	0, 5, 10	HMDSO over DMF	16–18	1.922 ± 9.4	33.6	32.4	34.0	1.971 ± 5.4	45.4	54.6
	20	HMDSO over DMF	18	2.671 ± 3.0	30.7	31.5	37.8	2.488 ± 3.5	45.9	54.1
DMSO	0, 5, 10	HMDSO over DMF		1.020 ± 14.7	33.3	25.9	40.8	1.050 ± 7.8	37.8	62.2
	20	HMDSO over DMSO	0.3–0.8	1.167 ± 2.6	42.0	26.2	31.8	1.230 ± 1.1	39.3	60.7
	0, 5, 10, 20	HMDSO over DMSO	24	0.978 ± 3.9	32.9	26.2	40.9	1.063 ± 4.1	39.1	60.9
	0, 5, 10, 20	HMDSO over DMSO		0.973 ± 9.1	31.9	26.5	41.5	1.015 ± 4.6	40.9	59.1

Reaction solvent	*Water added (μl/ml)*	*Chromatography*		*Mannose*			*Glucose*		
		Solvent	*Time (h)*	*Monomer (K_s)*	*Isomer (mole %)*		*Monomer (K_s)*	*Isomer (mole %)*	
					1	*2*		*1*	*2*
Pyridine	0, 5, 10, 20	Pyridine	0.3–0.8	1.417 ± 3.4	86.8	13.2	1.434 ± 2.6	41.2	58.8
	0, 5, 10, 20	Pyridine	24	1.406 ± 2.0	86.8	13.2	1.434 ± 0.8	41.4	58.6
	0, 5, 10, 20	Pyridine	96	1.349 ± 3.3	86.5	13.5	1.410 ± 1.7	40.9	59.1
DMF	0, 5, 10, 20	DMF	0.3–0.8	1.324 ± 2.0	88.7	11.3	1.339 ± 1.0	38.5	61.5
	0, 5, 10	HMDSO over DMF	16–18	3.106 ± 4.5	89.7	10.3	2.826 ± 6.5	40.4	59.6
	20	HMDSO over DMF	18	3.410 ± 1.2	90.7	9.3	2.864 ± 3.0	42.0	58.0
DMSO	0, 5, 10	HMDSO over DMF		1.455 ± 2.6	89.3	10.7	1.456 ± 3.1	37.3	62.7
	20	HMDSO over DMSO	0.3–0.8	1.498 ± 2.1	90.7	9.3	1.450 ± 4.5	37.3	62.7
	0, 5, 10, 20	HMDSO over DMSO	24	1.411 ± 3.9	89.7	10.3	1.432 ± 5.1	37.6	62.4
	0, 5, 10, 20	HMDSO over DMSO		1.371 ± 2.7	89.1	10.9	1.379 ± 2.5	37.3	62.7

[a] Each value is a mean of four samples for each water level; each run in duplicate.
[b] Coefficient of variation, % of mean.

derivatives were quite stable for periods in the order of several days. Further, it appeared that the derivatives were sufficiently stable to the water wash that, provided the HMDSO phase was immediately dried, this procedure could be used to remove residual DMF from the HMDSO phase. It should be noted, however, that the variation between duplicate samples was increased as the result of the water wash (Table I).

The formation of HMDSO was presumed to involve the hydrolysis of HMDS and TMS[11] and would therefore require the presence of water. Such water in the DMF reaction mixture was presumably the result of residual water following evaporation of the aqueous monosaccharide equilibrium mixture since the DMF had been dried.

Since variations in water content of the reaction mixture might influence yield rate of HMDSO and compete (via hydrolysis) with the monosaccharides for trimethylsilylation, the influence of water content upon the relative yield of monosaccharide TMS was investigated. This was investigated for reaction mixtures containing either pyridine, DMF, or DMSO and the results are summarized in Table II.

Hexamethyldisilazane was immiscible in DMSO when added alone, but became miscible when TMCS was added. An upper phase formed immediately upon mixing the trimethylsilylation reagents with DMSO with or without monosaccharides. This upper phase was identified as HMDSO upon its chromatographic retention and disiloxane absorption regions in the infra-red.

The level of water added to the pyridine reaction mixture had no significant effect upon the K_s or isomer abundance of samples chromatographed 0.3 to 96 h after trimethylsilylation. The level of water in the DMF solvent had no significant effect when the reaction mixture was chromatographed 0.3 to 0.8 h after trimethylsilylation. However, when the HMDSO phase over DMF was chromatographed, the K_s values for arabinose, xylose, and mannose derivatives were significantly different in the phase derived from the reaction mixture containing 20 μl added water per ml DMF as compared to the other three levels of added water.

Chromatography of the HMDSO phase over DMSO 0.3 to 0.8 h after trimethylsilylation revealed significant differences for phases derived from the reaction mixture containing 20 μl water per ml DMSO as compared to the other levels of water. These differences were not present 24 and 96 h after trimethylsilylation in the DMSO reaction solvent, however.

Thus it appeared that levels of water up to 20 μl/ml had no significant effect upon the formation of derivatives in reaction solvents which did not immediately phase (pyridine and DMF). However, levels of water higher than 10 μl per ml resulted in a derivative distribution in the HMDSO phase which was significantly different from that for HMDSO phases derived from reaction solvents containing 10 μl or less per ml. Thus, water levels higher than 10 μl per ml should be avoided if the HMDSO phase is to be used for quantitative purposes.

Reaction mixtures containing 20 and 10 μl water per ml of DMF phased in the order of 1 and 3 h, respectively. Phasing from DMF containing the lower levels of water occurred within 14–18 h.

The transfer of monosaccharide TMS derivatives into the HMDSO phase represented an increase in their concentration approximately in proportion to the volumes of the HMDSO and reaction solvent. The volume of HMDSO formed was related to the volume of trimethylsilylation reagents used. Therefore, it might be advantageous to minimize the volume of trimethylsilylation reagents in order to maximize

TABLE III

RELATIVE YIELD AND ISOMER ABUNDANCE OF MONOSACCHARIDE TRIMETHYLSILYL DERIVATIVES AS INFLUENCED BY VOLUME OF TRIMETHYLSILYLATION REAGENTS[a]

Reagent (ml)	*Solvent (ml)*	*Arabinose*				*Xylose*		
		Monomer (K_m)	*Isomer (mole %)*			*Monomer* (K_m)	*Isomer (mole %)*	
			1	*2*	*3 + 4*		*4*	*6*
1.0	4	0.64[b] ± 0.06[c]	32.9	39.1	28.1	0.73 ± 0.03	47.2	52.9
1.5	4	1.18 ± 0.05	32.4	39.9	27.8	1.22 ± 0.02	46.8	53.2
2.0	4	0.94 ± 0.04	28.6	36.0	35.4	1.10 ± 0.02	46.3	53.7
2.5	4	0.96 ± 0.03	26.3	33.7	40.1	1.05 ± 0.03	48.2	51.8
3.5	4	1.00 ± 0.03	28.5	31.3	40.2	1.07 ± 0.02	44.0	56.0

Reagent (ml)	*Solvent (ml)*	*Galactose*					*Glucose*		
		Monomer (K_m)	*Isomer (mole %)*				*Monomer* (K_m)	*Isomer (mole %)*	
			5	*7*	*9*	*10*		*8*	*11*
1.0	4	0.86 ± 0.03	24.7	34.6	9.9	30.9	0.95 ± 0.02	42.4	57.6
1.5	4	1.32 ± 0.02	21.2	34.8	6.0	38.1	1.25 ± 0.03	42.5	57.5
2.0	4	0.97 ± 0.03	26.2	29.0	8.7	36.2	1.14 ± 0.02	43.0	57.1
2.5	4	0.96 ± 0.02	22.8	28.9	9.5	38.8	1.08 ± 0.01	42.0	58.1
3.5	4	0.94 ± 0.02	20.7	29.1	8.7	41.5	1.13 ± 0.01	42.7	57.3

[a] Indicated volumes of reagents (HMDS:TMCS ratio 1.5) added to 4.0 ml DMF containing 20 mg of each monosaccharide.
[b] Each value is a mean of four samples.
[c] Standard error.

concentration of, and sensitivity for, the derivatives. The influence of variable volumes of these reagents is summarized in Table III.

The relative yield (K_m) and isomer abundance in the washed HMDSO phase was not significantly influenced by the volume of trimethylsilylation reagents ranging from 0.5 to 0.875 volumes per volume of DMF. In comparison, these were significantly different when the relative volume of the trimethylsilylation reagents was reduced below 0.5 volume per volume DMF. Therefore, 0.5 volume of these reagents was selected for routine use.

The possible influence of concentration and proportion of monosaccharide upon the relative yield of derivatives was investigated. Concentrations of total monosaccharides ranging from 1 to 20 mg per ml had no significant influence upon the relative detector response factors for individual derivatives in either the DMF reaction mixture, the HMDSO phase over DMF, or the washed HMDSO phase. Similarly, the relative detector response factors for individual monosaccharides in all three phases were not significantly different for four different mixtures of monosaccharides. Therefore only the mean detector responses and their coefficients of variation are summarized in Table IV.

The data in Table IV additionally indicate wide differences in sample to sample variation for different derivatives, and for different phases chromatographed. The coefficients of variation were consistently higher for erythritol and sedoheptulose than for the pentoses and hexoses. Erythritol and sedoheptulose were investigated since

TABLE IV

MEAN RELATIVE DETECTOR RESPONSE FACTORS[a] (K_m) FOR DERIVATIVES FORMED FROM DIFFERENT CONCENTRATIONS AND PROPORTIONS OF MONOSACCHARIDES

Monosaccharide TMS	*Mean[b] for concentrations of 1, 5, 10, 15 and 20 mg per ml*	*Mean[c] for four different monosaccharide mixtures*
DMF reaction mixture		
Erythritol	2.31 ± 9.9[d]	1.76 ± 33.7
Arabinose	1.26 ± 0.9	1.23 ± 1.3
Xylose	1.21 ± 0.6	1.19 ± 3.0
Galactose	1.04 ± 2.5	1.02 ± 4.1
Glucose	1.24 ± 1.4	1.21 ± 4.5
Sedoheptulose	0.89 ± 6.5	0.84 ± 1.8
HMDSO over DMF		
Erythritol	2.46 ± 9.9	2.06 ± 18.2
Arabinose	1.07 ± 2.1	1.08 ± 3.3
Xylose	1.10 ± 2.2	1.07 ± 2.8
Galactose	0.95 ± 1.9	0.93 ± 4.1
Glucose	1.18 ± 1.3	1.15 ± 2.0
Sedoheptulose	0.51 ± 6.3	0.44 ± 8.5
Washed HMDSO		
Erythritol	1.92 ± 14.2	1.61 ± 18.7
Arabinose	1.06 ± 7.3	1.04 ± 4.6
Xylose	1.10 ± 7.7	1.04 ± 4.3
Galactose	0.87 ± 5.1	0.87 ± 12.0
Glucose	1.06 ± 12.7	1.10 ± 11.6
Sedoheptulose	0.34 ± 15.0	0.43 ± 14.4

[a] Detector response determined as product of peak height times retention distance.
[b] $n = 10$.
[c] Mean for mixtures A, B, C, and D at concentrations of 10 and 20 mg/ml.
[d] Coefficient of variation.

their chromatographic retention times made them ideal internal standards. Their use for this purpose is limited by their relatively high coefficient of variation.

The coefficient of variation was lowest for the reaction mixture and progressively increased for the HMDSO phase over DMF and for the washed HMDSO phase.

DISCUSSION

Previous use of HMDS and TMCS for trimethylsilylation of monosaccharides has usually involved pyridine[1,5,7,10,12–15]. Hexamethyldisiloxane does not form from these reagents in pyridine as evidenced by the lack of absorption in the infra-red characteristic of disiloxane groupings. The failure of others to note the formation of an upper phase over DMF[14], even when water was present in the solvent, apparently was due to discarding the reaction mixture before it phased.

The more rapid formation of HMDSO in DMSO than in DMF suggests certain properties of these solvents influence the rate of hydrolysis of the reagents. Trimethylchlorosilane alone is hydrolyzed and forms an upper phase within 5 min in DMSO, whereas it is stable in DMF.

FRIEDMAN AND KAUFMAN[4] have noted the formation of a secondary product

which was formed when DMF and HMDS were refluxed. This secondary product was formed in addition to the TMS ether of a tertiary alcohol. It seems probable that this secondary product may have been HMDSO.

The high partition coefficient for the monosaccharide TMS derivatives favoring the HMDSO is apparently the consequence of a low dielectric constant for the TMS derivatives and wide differences in these constants for the DMF–HMDSO and DMSO–HMDSO biphasic systems. Similarly, little of the primary solvent would be expected in the HMDSO phase.

The speed of the trimethylsilylation of monosaccharides is indicated by the similarity in derivative distribution in the HMDSO phases over DMF to that over DMSO. The phase over DMSO formed very rapidly (1–3 min) yet contained the same monosaccharide TMS distribution as was formed during a much longer period in DMF. Where speed of preparation is an important factor, DMSO would be the preferred solvent.

The use of DMF or DMSO as the primary solvent has a number of advantages over pyridine. Chromatography of the HMDSO phase over the primary solvent materially reduces solvent trail and increases sensitivity, especially for early emerging peaks. The superior solubilizing properties of DMF and DMSO as compared to pyridine result in a further increase in sensitivity.

Due to the selectivity of the HMDSO phase for TMS ether derivatives, added confidence is imparted to derivative identification based on chromatographic retention time. This has been especially helpful in the chromatography of plant extracts and hydrolysates. Peaks are observed in the DMF reaction mixture which interfere with quantitative chromatography of monosaccharides. These same peaks are not observed in the HMDSO phase.

Further, the use of DMF or DMSO affords opportunities for separating the derivatives directly from the reaction mixture. Due to its low boiling point, HMDSO can be allowed to evaporate at room temperature to effect further concentration of the TMS derivatives.

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